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Genetic Polymorphisms of Glucose-6-Phosphate Dehydrogenase in Lagos, Nigeria

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ABSTRACT
Glucose-6-phosphate dehydrogenase (G6PD) is an essential enzyme in the pentose phosphate pathway that prevents oxidative damage to cells. This study determined the genotypic and allelic frequencies of G6PD G202A and A376G and also investigated correlation between G6PD polymorphisms and hemoglobin (Hb) phenotypes in children in Lagos, Nigeria. Seventy-eight children [55 with Hb AA (β^A/β^A) and 23 with Hb AS (β^A/β^S) trait] and 65 Hb SS (β^S/β^S) (*HBB*: c.20A>T) subjects in steady state with age range between 5–15 years were recruited for the study. Hemoglobin phenotypes of all study participants were carried out using alkaline electrophoresis and solubility tests. Genomic DNA was extracted from whole blood and restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) was used to determine the G202A and the A376G mutations of the *G6PD* gene. The genotype and allele distributions of G6PD G202A and A376G according to the Hb phenotypes were not statistically significant ($p > 0.05$). The minor allele frequency 202A was 0.15 (15.0%) and 0.14 (14.0%) in cases and controls, respectively. The overall frequency of 376G allele in the case group was 0.35 (35.0%) and 0.38 (38.0%) in the control group. No statistical significance was observed in the genotype and allele distributions of A376G in both the case and control groups ($p > 0.05$). The G6PD A− frequency in Hb SS subjects and the control group were 6.2 and 2.6%, respectively. G6PD G202A and A376G polymorphisms were not associated with Hb phenotypes and the allele distributions of 202A and 376G in this study are typical of West African populations.

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is a significant enzyme in the pentose phosphate pathway that prevents oxidative damage to cells by stimulating detoxification of free radicals. Its deficiency, however, accounts for the most common enzymopathy and affects approximately 400 million people worldwide with varying frequencies depending on the region and their ethnicity [1]. For instance, G6PD deficiency ranges from 15.0-26.0% in tropical Africa and has been estimated to be 15.0% in a suburb of southwestern Nigeria [2,3]. The *G6PD* gene is an X-linked gene with 13 exons that span 18.5 kb; it is highly polymorphic and results in asymptomatic phenotypes until exposed to exogenous oxidizing agents [4]. The public health burden of G6PD deficiency is commonly manifested in hemolytic anemia and neonatal jaundice.

Polymorphisms of the *G6PD* gene at positions 202 (exon 4) and 376 (exon 5) could result in deficient G6PD A− (transition of G>A at nucleotide position 202 resulting in an amino acid change from valine to methionine; and A>G at nucleotide position 376, resulting in an amino acid change asparagine to aspartate: 202A/376G) that is specific to sub-Saharan Africa. The G6PD Mediterranean variant (C>T at nucleotide position 563, resulted from an amino acid change from serine to phenylalanine) is the predominant form in the Mediterranean region and India [5–8].

Given that G6PD deficiency is an X-chromosome linked condition, studies have examined the impact of G6PD deficiency on protection from severe diseases with controversial findings showing presence and/or absence of protective effects in males and females [9–11]. The prevalence of G6PD deficiency in sub-Sahara African populations, where malaria is known to be endemic, supports the geographical background of the deficiency. Effects of G6PD deficiency in malaria endemic region are enormous and the routine screening of G6PD as recommended by the World Health Organization has not yet been integrated into clinical practice in Nigeria. Prevalence of G6PD deficiency has been determined in Nigeria mainly by fluorescent spot test and spectrophotometric G6PD enzymatic activity [3]. There is no data on the polymorphisms of G6PD G202A and A376G in Nigeria. Hence, this study determined the genotypic and allelic frequencies of G6PD G202A and A376G. Given that G6PD A− deficiency and Hb S (*HBB*: c.20A>T) are independently associated with protection from severe malaria, we also investigated the correlation between G6PD polymorphism and hemoglobin (Hb) phenotypes in children in Lagos, Nigeria.

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Study participants and methods

The study participants comprised 143 children (55 with Hb AA (β^A/β^A) and 23 with Hb AS (β^A/β^S) trait) and 65 Hb SS (β^S/β^S) with ages ranging between 5–15 years. Hb SS subjects in steady state were recruited from the Sickle Cell Foundation Nigeria during their routine visit to the Centre for Laboratory Investigations at Lagos, Nigeria. The control subjects were recruited from the places of religious worship. Participants who were ill or had had a blood transfusion in the last 3 months prior to study enrolment were excluded. Participants classified as being in steady state Hb SS were those who had no history of an acute painful episode that required hospitalization for at least 4 successive weeks after a previous painful crisis, no history of hospital admission 2–3 days after the point in time in question, no history of illness such as infection, inflammation during the previous 4 weeks, and no treatment with medications such as antibiotics that may affect the blood counts during the previous 3 weeks [12]. Participants older than 7 years gave assent and their guardians/parents gave written informed consent to participate in the study. The study protocol was approved by the Health and Research Ethics Committee of the College of Medicine, University of Lagos (CM/HREC/PHM/09/16/051).

Five milliliters of blood was obtained by venous puncture and some socio-demographic data (gender and age) were also obtained. Hemoglobin phenotypes of all study participants were carried out using alkaline electrophoresis and solubility tests. DNA samples were obtained immediately from the blood after collection.

Genomic DNA was extracted from whole blood sample using the Jena Bioscience DNA Kit (Jena Bioscience GmbH, Jena, Thuringia, Germany) following the manufacturer's instructions. The purity of DNA obtained was between 1.8–2.0 using Nanodrop Spectrophotometer 1000 (Thermo Fisher Scientific, Wilmington, DE, USA).

All samples were subjected to polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) to screen for the G202A mutation on exon 4 and the A376G mutation on exon 5 of the *G6PD* gene [13]. Polymerase chain reaction was performed in a 25 µL reaction volume using 50.0–100.0 ng DNA, 5 µL 5 × PCR Master Mix, 1 µL each of forward and reverse primers and 16 µL PCR grade water. Primers synthesized by Jena Bioscience were used. Thermal cycling (9800 systems; Applied Biosystems, Foster City, CA, USA) involving initial denaturation at 94 °C for 2 min., followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 30 seconds and elongation at 72 °C for 1 min., followed by a final elongation step at 72 °C for 2 min. for the G202A mutation; and initial denaturation at 94 °C for 2 min., followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 62 °C for 30 seconds and elongation at 72 °C for 1 min., followed by a final elongation step at 72 °C for 2 min. for the A376G mutation.

For RFLP, 10 U of the restriction enzymes *Nla*III (New England Biolabs, Ipswich, MA, USA) and *Fok*I (Jena Bioscience) was incubated with 15 µL of PCR products for 2 hours. Digested products together with the 50 bp DNA ladder was separated using 2.5% agarose gel electrophoresis, stained with ethidium bromide, and visualized using ultraviolet trans illumination (gel documentation system, Alpha Imager; Alpha Innotech, San Leandro, CA, USA). G202A revealed fragment sizes for GG: 109 bp; GA: 109, 63, 46 bp; AA: 63 and 46, while A376G showed AA: 90 bp; AG: 90, 58, 32 bp; GG: 58 and 32 bp.

Statistical analysis

Statistical analysis involved use of the Statistical Package for the Social Sciences software (version 15; SPSS Inc., Chicago, IL, USA) and GraphPad prism 5.00 (GraphPad Software Inc., San Diego, CA, USA; www.graphpad.com). Genotype and allele frequencies for G202A and A376G were determined and compared using Fisher's exact test and odds ratio (OD) and 95% confidence interval (95% CI). Hardy-Weinberg equilibrium was also calculated and a p value of <0.05 was considered to be statistically significant.

Results

The genotype and allele distributions of G6PD G202A and A376G according to the Hb phenotypes were not statistically significant (Table 1). The frequency of the heterozygous genotype GA of G202A was lower in Hb SS subjects compared to the control group (18.5 *vs.* 23.0%, OR: 0.79, 95% CI: 0.35–1.80), while the mutant genotype AA had higher frequency in Hb SS subjects compared to the control group (6.2 *vs.* 2.6%, OR: 2.37, 95% CI: 0.42–13.49). The minor allele frequency 202A was 0.15 (15.0%) and 0.14 (14.0%) in cases and controls, respectively. However, the genotype and allele distributions of G202A between the cases and control group did not show any statistical significance ($p > 0.05$) (Table 2). The overall frequency of the 376G allele in the case group was 0.35 (35.0%) and 0.38 (38.0%) in the control group. No statistical significance was observed in the genotype and allele distributions of A376G in both the case and control groups ($p > 0.05$).

The 202A allele frequency of females in Hb SS subjects was 20.0% compared to the control group, 13.0%, while

Table 1. Genotype and allele distribution of glucose-6-phosphate dehydrogenase G202A and A376G polymorphisms according to hemoglobin phenotypes.

Genotype	Hb AA ($n = 55$) n (%)	Hb AS ($n = 23$) n (%)	Hb SS ($n = 65$) n (%)	p Value
G202A				0.574
GG	42 (76.4)	16 (69.6)	49 (75.4)	
GA	11 (20.0)	7 (30.4)	12 (18.5)	
AA	2 (3.6)	–	4 (6.2)	
HWE (p value)	0.263	0.389	0.019	
G allele	95 (0.86)	39 (0.85)	110 (0.85)	0.924
A allele	15 (0.14)	7 (0.15)	20 (0.15	
A376G				0.314
AA	27 (49.1)	13 (56.5)	37 (56.9)	
AG	10 (18.2)	7 (30.4)	10 (15.4)	
GG	18 (32.7)	3 (13.0)	18 (27.7)	
HWE (p value)	<0.0001	0.232	<0.0001	
A allele	64 (0.58)	33 (0.72)	84 (0.65)	0.253
G allele	45 (0.42)	13 (0.28)	46 (0.35)	

Hb AA: β^A/β^A (normal); Hb AS: β^A/β^S (trait); Hb SS: β^S/β^S (sickle cell anemia); HWE: Hardy-Weinberg equilibrium.

Table 2. Genotype and allele distribution of glucose-6-phosphate dehydrogenase G202A and A376G polymorphisms between the case and control groups.

Genotype	Cases	Controls	OR (95% CI)	*p* Value
G202A				
GG	49 (75.4)	58 (74.4)	Reference	1.00
GA	12 (18.5)	18 (23.0)	0.79 (0.35–1.80)	0.679
AA	4 (6.2)	2 (2.6)	2.37 (0.42–13.49)	0.417
GA + AA	16 (24.6)	20 (25.6)	0.95 (0.44–2.02)	1.00
HWE (*p* value)	0.019	0.675		
G allele	110 (0.85)	134 (0.86)	Reference	1.00
A allele	20 (0.15)	22 (0.14)	1.11 (0.57–2.13)	0.867
A376G				
AA	37 (56.9)	40 (51.3)	Reference	1.00
AG	10 (15.4)	17 (21.8)	0.64 (0.26–1.56)	0.374
GG	18 (27.7)	21 (26.9)	0.93 (0.43–2.01)	1.00
AG + GG	28 (43.1)	38 (48.7)	0.80 (0.41–1.54)	0.614
HWE (*p* value)	<0.0001	<0.0001		
A allele	84 (0.65)	97 (0.62)	Reference	1.00
G allele	46 (0.35)	59 (0.38)	0.90 (0.56–1.46)	0.713

OR: odds ratio; 95% CI: 95% confidence interval.

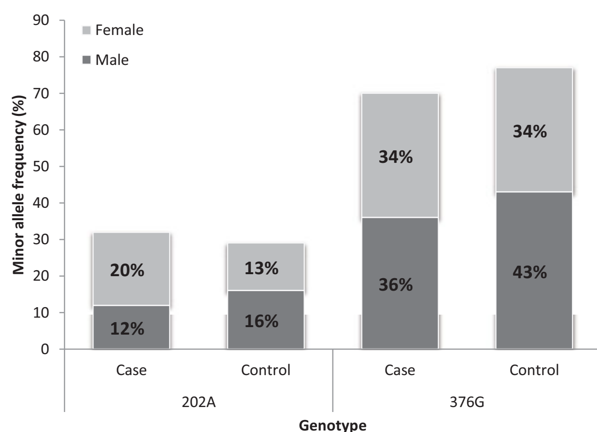

Figure 1. Minor allele frequency of 202A and 376G according to gender distribution.

12.0% was observed in males in the case group compared to 16.0% in the control group. These differences were not statistically significant ($p = 0.768$). Also, the 376G allele frequency according to gender distribution was not statistically significant ($p = 0.438$) (Figure 1). Our findings show that the combined G6PD G202A/A376G genotype distribution between the case and control groups had no statistical significance (Table 3).

Table 3. Glucose-6-phosphate dehydrogenase G202A/A376G genotype combinations.

G202A/A376G genotypes	Cases ($n = 65$) *n* (%)	Controls ($n = 78$) *n* (%)
GG/AA	37 (56.9)	36 (46.2)
GG/AG	3 (4.6)	11 (14.1)
GG/GG	9 (13.8)	11 (14.1)
GA/AA	–	4 (5.1)
GA/AG	7 (10.8)	6 (7.7)
GA/GG	5 (7.7)	8 (10.3)
AA/AA	–	–
AA/AG	–	–
AA/GG	4 (6.2)	2 (2.6)

Discussion

The prevalence of G6PD deficiency is high in sub-Saharan Africa [3], the Arabian Peninsula and across west Asia. The overall G6PD deficiency allele frequency across malaria endemic countries is estimated to be 8.0%, corresponding to approximately 220 million males and 133 million females [14]. The predominance of 202A/376G (G6PD A−) and Betica-Selma 376G/968C contributed significantly to the G6PD deficiency, especially in the West African sub-region [15–17]. Studies have demonstrated that the 202A mutation reduces G6PD activity to 12.0% compared to the wild-type G6PD allele, while the 376G mutation exhibits 83.0% of the normal enzyme activity [18,13].

The 202A/376G allele frequencies differ significantly based on the geographical background and ethnic groups. The 202A allele frequency of approximately 0.6% and 7.7–11.9% were reported among Fulani and Dogon ethnic groups in Mali [19,20], 6.0–14.9% in Burkina Faso [20], 20.0% in apparently healthy Tanzanian individuals (Hb AA, Hb AS trait) [10].

Studies from West African countries have reported significantly lower frequencies of the 202A/376G allele than the rate of G6PD deficiency, suggesting that other G6PD polymorphisms may account for the enzyme deficiency [9,17]. The 376G allele however, has higher frequencies in most populations than the 202A allele, in keeping with our findings [9,10,18]. This study showed comparable 202A and 376G allele frequencies in subjects with Hb AA, Hb AS trait and Hb SS. Comparable genotype and allele frequency distributions of G202A and A376G were also observed between Hb SS subjects and Hb AA, Hb AS trait subjects (classified as a control).

Hemolytic anemia is common to both G6PD deficiency and sickle cell disease and is predominant in sub-Saharan Africa malaria endemic regions. G6PD deficiency was not associated with the severity of vaso-occlusive crisis and the risk of infection, although controversial reports have been demonstrated in relation to stroke events in persons with sickle cell disease [22–24]. However, the deficiency may heighten chronic hemolytic anemia in sickle cell disease individuals.

The allele frequencies of 202A and 376G in our control subjects were similar to the Tanzanian healthy subjects with 16.3 and 37.4%, respectively [10]. Furthermore, 2.8 and 31.9% for 202A and 376G were observed in the mixed ethnic groups of Gambians [9]. The 202A and 376G alleles in Malian subjects were 7.7 and 35.3% for 202A and 376G alleles, respectively [18]. These heterogeneous allele frequencies may be due to the complexity of G6PD haplotype signature in the tropical African region.

Frequencies of the G6PD A− allele in the sub-Saharan African populations ranges from 3.0 to 19% [25]; this is comparable to a frequency of 6.2% reported in this study in subjects with Hb SS, and a frequency of 2.6% observed in the control group is the same as the frequency reported in a previous study [9]. It should be noted that the G6PD deficiency reported in this study only focuses on the 376G/202A genotype, which may underestimate overall G6PD deficiency in our population because the high prevalence of the Betica (376G/968C) variant reported in the Seerer population of Senegal and the Republic of Guinea [17,26] was not investigated in this study. In conclusion, G6PD G202A and A376G polymorphisms were not associated with Hb phenotypes and

the allele distribution of 202A and 376G in this study are typical of West African populations.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

[1] Ademowo OG, Falusi AG. Molecular epidemiology and activity of G6PD variants in a homogeneous Nigerian Population. East Afr Med J. 2002;79(1):42–44.

[2] Nkhoma ET, Poole C, Vannappagari V, *et al.* The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. Blood Cells Mol Dis. 2009;42(3):267–278.

[3] Williams O, Gbadero D, Edowhorhu G, *et al.* Glucose-6-phosphate dehydrogenase deficiency in Nigerian children. PLoS One. 2013;8(7):e68800.

[4] Persico MG, Viglietto G, Martini G, *et al.* Isolation of human glucose-6-phosphate dehydrogenase (G6PD) cDNA clones: primary structure of the protein and unusual 5′ non-coding region. Nucleic Acids Res. 1986;14(6):2511–2522.

[5] Takizawa T, Yoneyama Y, Miwa S, Yoshida A. A single nucleotide base transition is the basis of the common human glucose-6-phosphate dehydrogenase variant A(+). Genomics. 1987;1(3):228–231.

[6] Hirono A, Beutler E. Molecular cloning and nucleotide sequencing of cDNA for human glucose-6-phosphate dehydrogenase variant A(–). Proc Natl Acad Sci USA. 1988;85(11):3951–3954.

[7] Hirono A, Kawate K, Honda A, *et al.* A single mutation 202G>A in the human glucose-6-phosphate dehydrogenase gene *(G6PD)* can cause acute hemolysis by itself. Blood. 2002;99(4):1498–1499.

[8] Sarkar S, Biswas NK, Dey, B. A large, systematic molecular-genetic study of G6PD in Indian populations identifies a new nonsynonymous variant and supports recent positive selection. Infect Genet Evol. 2010;10(8):1228–1236.

[9] Clark TG, Fry AE, Auburn S, *et al.* Allelic heterogeneity of G6PD deficiency in West Africa and severe malaria susceptibility. Eur J Hum Genet. 2009;17(8):1080–1085.

[10] Manjurano A, Sepulveda N, Nadjm B, *et al.* African glucose-6-phosphate dehydrogenase alleles associated with protection from severe malaria in heterozygous females in Tanzania. PLoS Genet. 2015;11(2):e1004960.

[11] Toure O, Konata S, Sissoko S, *et al.* Candidate polymorphisms and severe malaria in Malian population. PLoS One. 2012;27(9):e43987.

[12] Ballas SK. More definitions in sickle cell disease: steady state **v** base line data. Am J Hematol. 2012;87(3):338.

[13] Beutler E, Kuhl W, Vives-Corrons JL, *et al.* Molecular heterogeneity of glucose-6-phosphate dehydrogenase A. Blood. 1989; 74(7):2550–2555.

[14] Howes RE, Piel FB, Patil AP, *et al.* G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model based map. PLoS Med. 2012; 9(11):e1001339.

[15] Town M, Bautista JM, Mason, *et al.* Both mutations in G6PD A− are necessary to produce the G6PD deficient phenotype. Hum Mol Genet. 1992;1(3):171–174.

[16] Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet. 2008;371(9606):64–74.

[17] De Araujo C, Migot-Nabias F, Guitard J, *et al.* The role of the G6PD AEth376G/968C allele in glucose-6-phosphate dehydrogenase deficiency in the Seerer population of Senegal. Haematologica. 2006;91(2):262–263.

[18] Battistuzzi G, Esan GJ, Fasuan FA, *et al.* Comparison of GdA and GdB activities in Nigerians. A study of the variation of the G6PD activity. Am J Hum Genet. 1977;29(1):31–36.

[19] Maiga ID, Dolo A, Campino S, *et al.* Glucose-6-phosphate dehydrogenase polymorphisms and susceptibility to mild malaria in Dogon and Fulani, Mali. Malaria J. 2014;13:270.

[20] Dolo A, Maiga B, Guindo A, *et al.* Frequency of glucose-6-phosphate dehydrogenase deficiency (A-376/202) in three Malian ethnic groups. [in French]. Bull Soc Pathol Exot. 2014;107(3): 165–170.

[21] Ouattara AK, Bisseye C, Bazie BV, *et al.* Glucose-6-phosphate dehydrogenase (G6PD) deficiency is associated with asymptomatic malaria in a rural community in Burkina Faso. Asian Pac J Trop Biomed. 2014;4(8):655–658.

[22] Bernaudin F, Verlhac S, Chevret S, *et al.* G6PD deficiency, absence of α-thalassemia, and hemolytic rate at baseline are significant independent risk factors for abnormally high cerebral velocities in patients with sickle cell anemia. Blood. 2008;112(10):4314–4317.

[23] Rees DC, Lambert C, Cooper E, *et al.* Glucose 6 phosphate dehydrogenase deficiency is not associated with cerebrovascular disease in children with sickle cell anemia. Blood. 2009;114(3): 742–743.

[24] Benkerrou M, Alberti C, Couque N, *et al.* Impact of glucose-6-phosphate dehydrogenase deficiency on sickle cell anemia expression in infancy and early childhood: a prospective study. Br J Haematol. 2013;163(5):646–654.

[25] Tishkoff SA, Varkonyi R, Cahinhinan N *et al*: Haplotype diversity and linkage disequilibrium at human G6PD: recent origin of alleles that confer malarial resistance. Science. 2001; 293(5529):455–462.

[26] Millimono TS, Loua KM, Rath SL, *et al.* High prevalence of hemoglobin disorders and glucose-6-phosphate dehydrogenase (G6PD) deficiency in the Republic of Guinea (West Africa). Hemoglobin. 2012;36(1):25–37.