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SHORT COMMUNICATION

G6PD deficiency assessment in Freetown, Sierra Leone, reveals further insight into the molecular heterogeneity of G6PD A-

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Abstract Glucose-6-phosphate dehydrogenase (G6PD) deficiency in Africa is of high prevalence, although precise data are lacking in many individual nations. We investigated 129 unrelated subjects (71 male subjects, 58 female subjects) visiting a teaching hospital in Freetown, Sierra Leone, to collect baseline data on the distribution of G6PD deficiency among respective ethnic groups in the country. We confirmed eight G6PD-deficient male subjects by two formazan-based blood tests (11.3% of the male subjects examined), and also detected the common 376A > G mutation in 11 male subjects and eight female subjects by sequencing exons 3–5 of the G6PD gene. Selected samples were further sequenced for exons 2–13 and introns 5, 7, 8, and 11. Among the deficient male subjects, six were G6PD A- carrying the double mutations (202G > A and 376A > G), all of whom were in the Temne and Mende ethnic groups. Others included A- Betica, and a novel variant having double mutations in exon 5 (311G > A and 376A > G forming 104 Arg > His and 126 Asn > Asp, respectively), which we designate as *G6PD Sierra Leone*. Subsequent haplotype analysis linked this novel variant to the G6PD A- "family".

Keywords Glucose-6-phosphate dehydrogenase A- · Malaria · Molecular heterogeneity · Sierra Leone · Variant

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy, affecting about 400 million people worldwide, and more than 130 molecular variants have been described at the DNA level (Beutler and Vulliamy 2002). The distribution of G6PD deficiency is closely correlated with a history of or current malaria endemicity, although there are also sporadic variants. Apart from the variants associated with concomitant chronic hemolytic anemia, G6PD-deficient individuals are generally healthy unless otherwise subjected to oxidant stress, e.g., the antimalarial primaquine.

In African populations, the most common G6PD variants described to date are the A-, A, and B variants. The A variant differs from the B (wild type) by one mutation at nucleotide position 376 (A > G) with amino acid change (126 Asn > Asp), whereas the common A- variant (also sharing this 376A > G) carries an additional mutation at nucleotide 202 (G > A) with amino acid change (68 Val > Met). Although prevalence rates have been documented in some African countries, precise data of G6PD deficiency are still lacking in many areas, including Sierra Leone. The aim of this study was to collect baseline data on G6PD deficiency distribution in Sierra Leone through analyses in its capital, Freetown, as well as with ethnic groups. We also describe a novel type of G6PD A-.

Materials and methods

Study site

This study was conducted at one of the teaching hospitals of the University of Sierra Leone, the Princess Christian Maternity and Children's Hospital (PCMH), which is located in an overcrowded area of the capital Freetown. Sierra Leone comprises about 20 native ethnic (tribal) groups, with the Mende (predominantly southerners) and Temne (predominantly northerners) accounting for the majority (each 30–35%). The rest include, among others, the Limba, Creole (Krio), Kono, and Fullah (Fulani). With perennial transmission in Freetown, malaria continues to be a major public health problem in Sierra Leone and may account for up to 40% of hospital visits. Whereas presumptive diagnosis followed by self-treatment is not uncommon, use of the antimalarial primaquine is not the norm in Sierra Leone.

Sample collection and G6PD-deficiency screening

In September–October 2006 and in April–May 2007, outpatients presenting for investigative blood tests [e.g., red blood cell (RBC) count, white blood cell (WBC) count, malaria, blood sugar level, etc.) at the PCMH microbiology laboratory were screened for G6PD deficiency as described previously (Tantular and Kawamoto 2003; Jalloh et al. 2004). Filter-paper blood samples were collected from all consenting patients, air-dried, and later brought to Jichi Medical University in Tochigi, Japan, for confirmatory screening using Fujii's spot test (Fujii et al. 1984), and for molecular analyses.

DNA extraction and sequencing

DNA was extracted from blood samples of 17 male subjects and 18 female subjects (Table 1) using the GFX^{TM} Genomic Blood DNA purification kit (GE Health Care, UK). In the initial phase, all 35 samples were sequenced for exons 3–5 of the G6PD gene using the method previously described (Matsuoka et al. 2007). In the subsequent step, exons 2–13 and introns 5, 7, 8, and 11 of the gene were sequenced for all 17 male samples as well as selected female samples using the ABI PRISM 310 Genetic Analyzer, where primers were made as described (Hirono et al. 1994).

This study was approved by the Ethical Committees of the PCMH, University of Sierra Leone, and Jichi Medical University, Tochigi, Japan.

Table 1 G6PD variants with respective nucleotide mutations found among different ethnic groups in Freetown

Ethnicity	G6PD variant						Total
	A 376A > G	A hetero 376A/G	A- 202G > A 376A > G	A- hetero 202G/A 376A > G	Sierra Leone 311G > A 376A > G	B 202G 376A	
Male subjects							
Creole	(1)[a]						1
Kono–Mende					1		1
Loko	1					1	2
Mende			2			1	3
Sherbro	1						1
Temne	1		4			4	9
Subtotal	3(1)[a]		6		1	6	17
Female subjects							
Fullah		1		1		2	4
Kono						2	2
Limba	1					1	2
Loko		1					1
Sherbro						1	1
Temne	1	2		1		3	7
Yoruba						1	1
Subtotal	2	4		2		10	18

Results are based on sequencing of exons 3–5 of the G6PD gene for 35 selected DNA samples

Hetero heterozygote

[a] The DNA sample of the Creole boy, here categorized as G6PD A, was later confirmed to be G6PD A- (Betica) when all exons were sequenced

Results

G6PD-deficiency prevalence

Overall, 129 subjects (71 male subjects and 58 female subjects) were screened for G6PD deficiency from the two surveys by two formazan-based blood tests, and we found eight male subjects (8/71 = 11.3%) as well as seven female subjects, including one 4-year-old severely deficient Temne girl and six mild or borderline cases of deficiency.

Table 1 summarizes the results obtained from sequencing exons 3–5 of 35 selected samples. Of the eight phenotypically deficient male subjects, six (two Mende, four Temne) had the common A- variant (202G > A, 376A > G), one with a novel variant (311G > A, 376A > G), and one had only the 376A > G mutation, thus apparently showing G6PD A. However, subsequent sequencing of other exons revealed that the latter was indeed A- Betica, as described below. Three other G6PD-normal male subjects had the 376A > G mutation. One severely deficient (i.e., the 4-year-old Temne) girl was homozygous for the 376A > G mutation and heterozygous for the 202 mutation (i.e., 202G/A). Of the six mild or borderline female cases, two (one Temne and one Limba) were homozygous for the A allele (376A > G), one (Fullah) was A- heterozygous (202G/A; 376A > G), and three had no mutations in exons 3–5. Four G6PD-normal female subjects were heterozygous for the A allele (376A/G).

The novel variant identified, hereby designated *G6PD Sierra Leone* (311G > A, 376A > G), was from a 15-year-old Kono–Mende boy observed with a very low residual enzyme activity and an Hb value of 14.8 g/dl. Surprisingly, two point mutations occurred in the same exon: the common 376A > G mutation resulted in 126 Asn > Asp, and a novel 311G > A mutation resulted in 104 Arg > His (Fig. 1).

G6PD haplotypes

As we only screened a couple of exons for common G6PD variations in the initial phase, we might be underestimating the G6PD situation in our samples. Thus, exons 2–13 as well as introns (known to contain polymorphic sites, i.e. introns 5, 7, 8, and 11) of the G6PD gene were sequenced for all 17 male samples together with three representative female samples, and their haplotypes were compared (Table 2). This approach further identified two unique A- variants: (1) G6PD Betica (376A > G and 968T > C, Vives-Corrons and Pujades 1982) in a Creole boy, who had temporally been assigned as G6PD A by sequencing of only exons 3–5 (Table 1); and (2) G6PD Santamaria (376A > G, 542A > T) in the severely deficient 4-year-old-Temne girl. This girl was actually a compound heterozygote with two distinct variants (ID:P-093 in Table 2): the common G6PD A- (202G > A, 376A > G) as well as G6PD Santamaria (376A > G, 542A > T).

Interestingly, haplotypes of the common A- variant (202G > A, 376A > G) were identical in all samples, irrespective of ethnicity (Table 2), consistent with previous findings (Beutler and Kuhl 1990; Vulliamy et al. 1991). All sequences had a guanine and none had an adenine at the 1116G > A silent mutation position in exon 10. However, the 1311C > T silent mutation in exon 11, thought to be linked with nt93T > C (intron 11) in non-African populations, i.e., one X chromosome can either have both mutations (++) or have none (−), appears not to be the case in our samples (−+ or ++). Whereas two haplotypes were detected for the B (wild type) alleles, the A alleles had a uniform haplotype. The rare A- variants (G6PD Sierra Leone, Betica, and Santamaria) had slightly different haplotypes from the common A- allele.

Discussion

Unlike other malaria-endemic areas—e.g., Southeast Asia, where G6PD deficiency mutations are being widely investigated (Kawamoto et al. 2006; Nuchprayoon et al. 2008; Matsuoka et al. 2007)—G6PD data for African nations are scanty. Presented here is the first attempt to study the prevalence of G6PD deficiency at the population level in Freetown, Sierra Leone, with both enzyme and

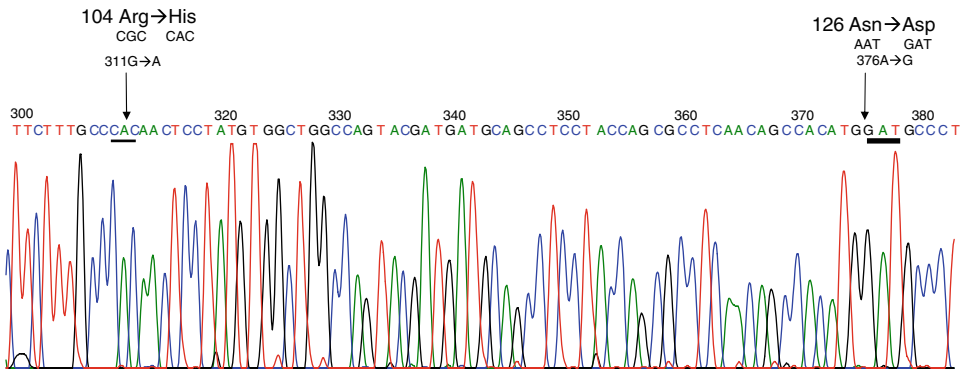

Fig. 1 Exon 5 electrophoregram (forward direction) of the new G6PD variant *G6PD Sierra Leone* (311G > A, 376A > G), with corresponding nucleotide and amino acid substitutions

Table 2 Comparison of G6PD haplotypes detected among 20 individuals in Freetown

ID	Ethnicity	New	Known mutation points or polymorphic sites in the G6PD gene of Africans											G6PD variant
		Exon 5 311G > A	Exon 4 202G > A	Exon 5 376A > G	*Intron* 5 *nt*611C > G	Exon 6 542A > T	Exon 7 680G > T	*Intron* 7 *nt*175C > T	*Intron* 8 *nt*163C > T	Exon 9 968T > C	Exon 10 1116G > A	Exon 11 1311C > T	*Intron* 11 *nt*93T > C	
P-090	Kono–Mende	+[a]	−	+	+	−	−	+	+	−	−	−	+	A- (Sierra Leone)
P-116	Creole	−	−	+	−	−	−	−	+	+	−	−	+	A- (Betica)
P-104	Temne	−	+	+	+	−	−	+	+	−	−	−	+	A-
P-067	Temne	−	+	+	+	−	−	+	+	−	−	−	+	A-
P-050	Mende	−	+	+	+	−	−	+	+	−	−	−	+	A-
P-006	Temne	−	+	+	+	−	−	+	+	−	−	−	+	A-
P-034	Mende	−	+	+	+	−	−	+	+	−	−	−	+	A-
P-112	Temne	−	+	+	+	−	−	+	+	−	−	−	+	A-
P-089	Sherbro	−	−	+	−	−	−	−	+	−	−	−	+	A
P-012	Temne	−	−	+	−	−	−	−	+	−	−	−	+	A
P-036	Loko	−	−	+	−	−	−	−	+	−	−	−	+	A
P-038	Temne	−	−	−	−	−	−	−	−	−	−	+	+	B
P-134	Temne	−	−	−	−	−	−	−	−	−	−	−	+	B
P-004	Temne	−	−	−	−	−	−	−	−	−	−	+	+	B
P-007	Temne	−	−	−	−	−	−	−	−	−	−	+	+	B
P-019	Mende	−	−	−	−	−	−	−	−	−	−	−	+	B
P-085	Loko	−	−	−	−	−	−	−	−	−	−	+	+	B
P-093 (F)	Temne	−	±	+	±	±	−	±	+	−	−	−	+	A- (Santa[b])
P-135 (F)	Temne	−	−	−	−	−	−	−	−	−	−	+	+	B
P-136 (F)	Fullah	−	−	−	−	−	−	−	−	−	−	+	+	B

F female

[a] The presence (+) or absence (−) of the respective mutations in the G6PD gene or the presence of both nucleotides (±), as in heterozygotes, are shown

[b] A- (Santa) is actually a compound heterozygote with two variants: the common G6PD A- and G6PD Santamaria

nucleotide sequence analyses. Although the effects of sampling bias cannot be excluded, it is interesting to note that the two largest ethnic groups (i.e., Mende and Temne) of the Sierra Leonean population accounted for 75% (6/8) of the male deficiencies observed (Table 1). In African populations, the common A- variant is reportedly widespread, a trend we also confirmed. In addition, two other unique A- variants (G6PD Betica, Santamaria) as well as a novel variant, were observed. Taken together, this is suggestive of a molecular heterogeneity of G6PD A- in the general Sierra Leonean population, which comprises diverse ethnic groups, each having distinct cultural characteristics.

G6PD Sierra Leone (311G > A, 376A > G) was categorized into the World Health Organization (WHO) Class III (WHO Working group 1989), as the enzyme activity was similar to that of the common A- variant (202G > A, 376A > G), and no anemia was seen on diagnosis (Hb = 14.8 g/dl). The single substitution of Asn with Asp, a structurally similar amino acid, as seen with the A variant may not influence the enzyme activity, but the additional change with a structurally different amino acid in the new variant may explain reduction of the activity. The common G6PD A- variant has been shown to demonstrate a similar structure–function relationship (Gomez-Gallego et al. 2000). Due to the existence of a "family" of A- variants (Beutler et al. 1989), all with the 376A > G mutation and an additional mutation at either position 202, 542, 680, or 968, we named our new variant *G6PD Sierra Leone* (311G > A, 376A > G). Although subsequent haplotype analysis (Table 2) supports a rather unique G6PD A-, it remains to be established whether G6PD Sierra Leone is a rare coincidental finding or is at polymorphic frequencies among any population group in the country or in Africa.

Whereas the G6PD deficiency situation in Africa is probably underestimated, the molecular heterogeneity of G6PD A- documented here further emphasizes the rather unique distribution pattern of G6PD-deficient genotypes in African populations and provides additional, albeit speculative, evidence for the malaria-G6PD-deficiency selection hypothesis. In a country where malaria is rampant, malnutrition and anemia among children is of high prevalence, it would be of great value for local health care practitioners to be more aware of G6PD deficiency as one of the possible causes of acute hemolytic anemia or in neonatal jaundice, or when administering oxidant drugs, particularly in patients of Mende and Temne ethnic backgrounds.

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