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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is associated with asymptomatic malaria in a rural community in Burkina Faso

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PEER REVIEW

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Comments

This is a good study in which the authors showed that the G6PD A- variant associated with protection against a symptomatic malaria in Burkina Faso was probably the most common deficient variant. The results suggested that G6PD deficiency seemed to prevent the normal development of P. falciparum in the body.

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ABSTRACT

Objective: To investigate 4 combinations of mutations responsible for glucose-6-phosphate dehydrogenase (G6PD) deficiency in a rural community of Burkina Faso, a malaria endemic country.

Methods: Two hundred individuals in a rural community were genotyped for the mutations A376G, G202A, A542T, G680T and T968C using TaqMan single nucleotide polymorphism assays and polymerase chain reaction followed by restriction fragment length polymorphism.

Results: The prevalence of the G6PD deficiency was 9.5% in the study population. It was significantly higher in men compared to women (14.3% *vs* 6.0%, P=0.049). The 202A/376G G6PD A- was the only deficient variant detected. *Plasmodium falciparum* asymptomatic parasitaemia was significantly higher among the G6PD-non-deficient persons compared to the G6PD-deficient (P<0.001). The asymptomatic parasitaemia was also significantly higher among G6PD non-deficient compared to G6PD-heterozygous females (P<0.001).

Conclusions: This study showed that the G6PD A- variant associated with protection against asymptomatic malaria in Burkina Faso is probably the most common deficient variant.

KEYWORDS

Polymerase chain reaction, Mutations, Glucose-6-phosphate dehydrogenase deficiency, Asymptomatic malaria, Burkina Faso

1. Introduction

The glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common disease-producing enzymopathy in humans, affecting 400 million people worldwide[1,2]. Its prevalence is highest in malaria endemic areas, because of the selective advantage conferred to carriers against malaria[3,4]. Approximately 140 mutations or combinations of mutations responsible for this deficiency have been described[5-7]. In sub-Saharan Africa, 3 variants occur with polymorphic frequencies above 1%: wild type G6PD B, a non-deficient variant G6PD A and the deficient variant G6PD A-[8,9]. The variant G6PD A results from a point mutation A376G in exon 5 whereas the deficient variant G6PD A- has an A376G mutation and an additional one G202A in exon 4. Other deficient variants associate the mutation A376G and the following mutations: A542T (exon 6), G680T (exon 7) and T968C (exon 9) in the G6PD gene.

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In previous studies carried out in Burkina Faso, the G6PD deficiency has been explored mainly by measuring the enzymatic activities[10]. Furthermore, the genotyping of the G6PD variants have been focused on single 202A/376G G6PD A- variant considered as the most common in Africa[9,11]. However, studies in West Africa have shown the presence of point mutations other than 202A/376G associated with the G6PD deficiency with relatively high frequencies. In fact, it has been shown that the 376G/542T G6PD Santamaria, 376G/968C G6PD Betica Selma and T968C alleles were more frequent in Serer[11] and the general population in the Gambia[12]. In this study four combinations of mutations responsible for the G6PD deficiency were investigated in a rural community in Burkina Faso.

2. Materials and methods

2.1. Settings and type of study

Unrelated participants were recruited in Koubri (a rural community located at 25 km south of Ouagadougou, Burkina Faso), where malaria transmission is perennial because of dams associated with agricultural activities.

2.2. Study population

Two hundred individuals aged from 1 to 79 years were included. The participants were in their great majority from the Mossi ethnic group. They were already involved in another project entitled "Study of fine specific immune responses against *Plasmodium falciparum* (*P. falciparum*) peptides candidate vaccines".

2.3. Blood collection

Venous blood (5 mL of blood per adult and about 3 mL of blood per child) was collected on ethylene diamine tetraacetic acid impregnated tubes. After centrifugation at 15000 r/min for 5 min, plasma was separated from cell pellet. The pellet was stored at −20 °C for DNA extraction.

2.4. DNA extraction and genotyping of G6PD-deficient variants

All DNA samples were extracted using a standard salting-out procedure[13] or the QIAamp DNA Mini Kit from QIAGEN (QIAGEN, Hilden, Germany). DNA purities were estimated spectrophotometrically, and the final concentrations were determined using Biodrop μLITE (Isogen Life Science N.V./S.A, Temse, Belgium).

The mutations A376G, G202A and A542T were genotyped with 20 ng of DNA by the TaqMan assays (ABI, Applera International Inc, Foster City, CA, USA) in a reaction volume of 25 μL, using the ABI 7500 FAST real-time polymerase chain reaction (PCR) systems. Fluorescence curves were analyzed with the 7500 FAST Sequence Detection Software version v.2.1 (Applied Biosystems) for allelic discrimination. The G680T and T968C mutations were genotyped by PCR followed by restriction fragment length polymorphism (RFLP) (PCR/RFLP) as previously described by Beutler *et al*[14].

2.5. Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences 17.0 and EpiInfo version 6 software. The chi square test was used for comparisons. The difference was considered significant for $P<0.05$.

2.6. Ethical considerations

This study was approved by the Ministry of Health and the CERBA/Saint Camille Ethics Committee. Informed consent was obtained from adults and parents or guardians of children under 5 years before blood collection.

3. Results

3.1. Prevalence of G6PD deficiency

The study population consisted of 58% and 42% of women and men, respectively. Nearly three quarters of the subjects (74.5%) were carriers of the G6PD normal alleles; G6PD-heterozygous women represented 16.0% (32/200), while 9.5% (19/200) were 202A/376G G6PD A-. None of the G6PD-deficient variants 376G/542T, 376G/680T and 376G/968C were detected in this study (Table 1). The G6PD deficiency prevalence was significantly higher in men compared to women (14.3% *vs* 6.0%, $P=0.049$).

Table 1

Prevalence of four combinations of mutations responsible for G6PD deficiency.

G6PD genotypes	Number	Percentage
Male	84	-
202A/376G hemizygous	12	14.3
376G/542T	0	0.0
376G/680T	0	0.0
376G/968C	0	0.0
G6PD normal	72	85.7
Female	116	-
202A/376G homozygous	7	6.0
202A/376G heterozygous	32	27.6
376G/542T	0	0.0
376G/680T	0	0.0
376G/968C	0	0.0
G6PD normal	77	66.4

3.2. Asymptomatic malaria and G6PD

The presence of the *P. falciparum* parasite was investigated in 15 out of the 19 G6PD-deficient individuals. The absence of parasitaemia was observed in 53.3% of the G6PD-deficient and 57.0% of the non-deficient persons, respectively. Among the G6PD-heterozygous women, *P. falciparum* asymptomatic malaria was found in 48.1% (13/27) of them.

No statistically significant difference was found by comparing the prevalence of *P. falciparum* infection between deficient and non-deficient persons on one hand, and between non-deficient and heterozygous individuals on the other hand. However, the geometric mean of parasites in infected individuals from the 3 groups were significantly higher in the non-deficient compared to the deficient persons (1104 *vs* 204 parasites/μL, $P<0.001$) and also in the non-deficient compared with the individuals who are heterozygous (1104 *vs* 628 parasites/μL, $P<0.001$).

4. Discussion

In this study, we investigated four combinations of mutations (202A/376G; 376G/542T; 376G/680T; 376G/968T) responsible for G6PD deficiency in individuals living in a malaria endemic area, such as Burkina Faso. The G6PD deficiency prevalence was 9.5% in our study population. This prevalence is comparable to that of 9.0% reported by Carter *et al*[8], in six African countries, but lower than the prevalence of 19.6% and 16.3% respectively reported by Modiano *et al*[15], and Simpore *et al*[10], in Burkina Faso. These variations could be explained not only by the small size of our population sample, but also by the diagnostic methods used for the detection of the G6PD (real-time PCR, PCR/RFLP versus measurement of enzyme activity) deficiency. Furthermore, these differences could also be due to the difficulty of distinguishing deficient and non-deficient by genotyping heterozygous women[16].

Indeed, in a previous study it was shown that among 81 G6PD heterozygous women, 53% had a normal enzyme activity, while 33% had an intermediate activity and 14% had a biochemical deficiency[17].

Four deficient genotypes were sought in this study, and only the genotype 202A/376G G6PD A- was found. These results are similar to those obtained by Carter *et al*[8], in six African countries including Burkina Faso, and confirms the results shown by previous studies that the 202A/376G G6PD A- is the most common deficient variant in sub-Saharan Africa[9,18]. The prevalence of G6PD deficiency was significantly higher among men (14.3%) compared to women (6.0%). These results are comparable to that found by Simpore *et al*. who observed 20.5% of deficiency among men and 12.3% among women[10]. The prevalence of parasitaemia in infected individuals was significantly higher among those who are not deficient with respect to those who are deficient ($P<0.001$). In addition, heterozygous females had a significantly high prevalence of parasitaemia ($P<0.001$).

Our results are comparable to those of a study in Gabon which has shown that there is an association between the G6PD deficiency and the protection against asymptomatic malaria[19]. Indeed, G6PD deficiency seems to prevent the normal development of *P. falciparum* in the body.

The protection mechanism of the G6PD deficiency against malaria remains hypothetical and various mechanisms are developed[20]. This study shows that the variant 202A/376G G6PD A- is associated with the protection against asymptomatic malaria in Burkina Faso. However, other genotyping studies are needed to confirm the absence of other deficient variants, and to determine more accurately the 202A/376G mutation frequency in the general population and specific ethnic groups.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The G6PD deficiency is the most common disease-producing enzymopathy in humans. Its prevalence is highest in malaria endemic areas, because of the selective advantage conferred to carriers against malaria. Approximately 140 mutations or combinations of mutations responsible for this deficiency have been described.

Research frontiers

In this study, four combinations of mutations responsible for the G6PD deficiency were investigated in a rural community in Burkina Faso. This study showed that the 202A/376G G6PD A- variant associated with protection against a symptomatic malaria in Burkina Faso is probably the most common deficient variant.

Related reports

Approximately, 140 mutations or combinations of mutations

responsible for this deficiency have been described. The G6PD deficiency has been explored by the enzymatic activities in Burkina Faso. The genotyping of the G6PD variants have been reported on single 202A/376G G6PD A- variant in Africa, and also on 376G/542T, 376G/968C and T968C in the Gambia.

Innovations and breakthroughs

Four deficient genotypes (202A/376G, 376G/542T, 376G/680T and 376G/968T), responsible for G6PD deficiency were searched in Burkina Faso, but only the genotype 202A/376G G6PD A- was found. Results showed that there was an association between the G6PD deficiency and the protection against asymptomatic malaria.

Applications

The authors investigated mutations which are responsible for G6PD deficiency in individuals living in a malaria endemic area. It may be significant to know the genotyping of the G6PD variants. Accumulation of the additional information may lead to the solution of the protection mechanism of the G6PD deficiency against malaria.

Peer review

This is a good study in which the authors showed that the G6PD A- variant associated with protection against a symptomatic malaria in Burkina Faso was probably the most common deficient variant. The results suggested that G6PD deficiency seemed to prevent the normal development of *P. falciparum* in the body.

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