Chalisa Louicharoen · Issarang Nuchprayoon

***G6PD Viangchan* (871G>A) is the most common G6PD-deficient variant in the Cambodian population**

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Abstract Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary enzymopathy among Southeast Asians. We studied *G6PD* mutations in 108 migrant Cambodian laborers in Chanthaburi province and cord blood samples from 107 Cambodian newborns at Buriram Hospital. Thirty-one (26.1%) of 119 Cambodian males and three of 96 (3.1%) females were G6PD deficient and were assayed for *G6PD* mutations. *G6PD Viangchan* (871G>A) was identified in most G6PD-deficient Cambodians (28 of 34; 82.4%); *G6PD Union* (1360C>T) and *G6PD Coimbra* (592C>T) was found in one case each. We concluded that *G6PD Viangchan* (871G>A) was the most common mutation among Cambodians. This finding is similar to G6PD-deficient Thais and Laotians, suggesting a common ancestry of people from these three countries.

Keywords Glucose-6-phosphate dehydrogenase deficiency · *G6PD Viangchan* · Cambodian · Thai · Laotian · Myanmar population

(Beutler 1994). The *G6PD* gene consists of 13 exons distributed over approximate 18 kb on the distal long arm of the X chromosome (Xq28) (Martini et al. 1986). At least 442 *G6PD* variants have been described by the biochemical characterization (Xu et al. 1995). To date, more than 130 different mutations in the *G6PD* gene, most of which are nucleotide substitution, have been described (Hamel et al. 2002).

Certain *G6PD* mutations are associated with specific ethnic groups in tropical Asia (Iwai et al. 2001). Epidemiological and molecular studies had previously shown that G6PD deficiency in Southeast Asia is heterogeneous. *G6PD Viangchan* (871G>A; Val291Met) seems to be the most common variant in Thais, Laotians, and Malaysian Malays (Nuchprayoon et al. 2002; Iwai et al. 2001; Ainoon et al. 2003) while *G6PD Mahidol* (487G>A; Gly163Ser) is the most variant in the Myanmar population (Matsuoka et al. 2004). *G6PD* mutations in Cambodians, however, were not known. In this study, we report the prevalence of G6PD deficiency and have identified the G6PD-deficient mutations among Cambodians.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary enzymopathy. The prevalence of G6PD deficiency is high in the Southeast Asian population, which correlated with malaria endemicity (Nuchprayoon et al. 2002; Iwai et al. 2001). G6PD (MIM# 305900) is a housekeeping enzyme that provides NADPH in catalysis of the pentose phosphate pathway (PPP) (Poggi et al. 1990). Inherited deficiency of this enzyme can cause acute or chronic hemolytic anemia, neonatal hyperbilirubinemia, and favism

Materials and methods

Sample collections

One hundred and eight peripheral blood samples were collected from consenting migrant Cambodian laborers in Chanthaburi province, Thailand, as a part of a health screening program for work permit in Thailand between March and April 2002. The immigrants' Cambodian provinces of residence were recorded. Cord blood samples of 107 newborns of consenting Khmer-speaking mothers were collected from the delivery room of Buriram Hospital, Buriram Province, Thailand, between April and May 2003. From each subject, 3 ml ACD blood samples were collected for G6PD activity assay, and 2 ml EDTA blood samples collected for DNA analysis. Blood samples were stored at 4°C until used.

C. Louicharoen · I. Nuchprayoon (✉)
Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Rama IV Rd., Bangkok, 10330, Thailand
E-mail: fmedinp@md2.md.chula.ac.th
Tel.: +66-2-2564949
Fax: +66-2-2564949

G6PD activity assay

G6PD activity assays were performed according to the WHO standard (Betke et al. 1967) within 7 days of collection. In our laboratory, G6PD activity was 7.39 ± 2.57 IU/g Hb (mean $\pm$ SD) in normal males and 6.94 ± 2.51 IU/g Hb in normal females. G6PD activity <1.5 IU/g Hb is defined as G6PD deficiency (Betke et al. 1967).

DNA extraction

Genomic DNA was extracted from G6PD-deficient EDTA blood samples using QIAamp DNA Blood Minikit (Qiagen, Germany) according to manufacturer's instruction.

Mutation analysis

To identify *G6PD* mutations, we first screened all G6PD-deficient samples for two mutations, *G6PD Viangchan* (871G>A) and *G6PD Mahidol* (487G>A), which were previously reported to be the most common in the Southeast Asian population (Nuchprayoon et al. 2002; Iwai et al. 2001; Ainoon et al. 2003). For G6PD-deficient samples in which mutation remained unknown, they were assayed for eight common Chinese mutations: *G6PD Canton* (1376G>T), *G6PD Union* (1360C>T), *G6PD Kaiping* (1388G>A), *G6PD Chinese-5* (1024C>T), *G6PD Gaohe* (95A>G), *G6PD Chinese-3* (493A>G), *G6PD Chinese-4* (392G>T), and *G6PD Coimbra* (592C>T) (Nuchprayoon et al. 2002; Ainoon et al. 2003; Tang et al. 1992; Ainoon et al. 1999; Huang et al. 1996; Saha et al. 1994).

For *G6PD* mutation assay, the target gene was amplified using a PCR-based technique with primers that were previously designed to create restriction sites (Nuchprayoon et al. 2002; Huang et al. 1996). The typical PCR reaction was carried out in a 50- μ l reaction containing 1 $\times$ PCR buffer, 1 U of Taq polymerase (Fermentas), 50 ng of each primer, 1.5 mM MgCl₂, 200 μ M of each dNTPs, and approximate 300 ng DNA template. After incubation at 94°C for 5 min, amplification was carried out for 35 cycles with the following temperature cycling parameters: 94°C for 1 min, 56°C for 1 min, 72°C for 1 min, and final extension for 15 min at 72°C. Ten microliters of PCR product were digested with 5 U of an appropriate restriction enzyme digestion

set (Huang et al. 1996) according to manufacturer's protocols (New England Biolabs). The digestion was incubated at 37°C 2–4 h, subjected to electrophoresis on 6% acrylamide gel, and then stained with ethidium bromide.

For nt 1311C>T polymorphism, two primers previously reported to create a restriction site of *BclI* (Vulliamy et al. 1991) were used in a PCR reaction similar to the *G6PD* mutation assays. The PCR amplification was performed on the DNA thermal cycler for 1 cycle of 94°C for 5 min, then 35 cycles of 1 min at 94°C, 1 min at 63°C, 1 min at 72°C, and a final extension at 72°C for 15 min. Ten microliters of PCR product were digested with *BclI* following the technique described above.

Results

Prevalence of G6PD deficiency

From the 215 Cambodian blood samples, we found G6PD deficiency in 26.1% of Cambodian male (31 of 119) and 3.1% of females (3 of 96). Among Cambodian neonates, 21 of 56 males and two of 51 females were G6PD deficient. Among Cambodian adults, 10 of 63 males and one of 45 females were G6PD deficient (Table 1).

Prevalence of *G6PD* mutations

G6PD genotype was examined in both G6PD-deficient neonates and adults. The results are shown in Table 1. Only three *G6PD* mutations were identified in 34 G6PD-deficient Cambodians. *G6PD Viangchan* (871G>A) was found in 28 cases (82.4%, Fig. 1), *G6PD Union* (1360C>T) and *G6PD Coimbra* (592C>T) in one case each. We also screened for *G6PD Mahidol* (487G>A), *G6PD Canton* (1376G>T), *G6PD Kaiping* (1388G>A), *G6PD Chinese-5* (1024C>T), *G6PD Gaohe* (95A>G), *G6PD Chinese-3* (493A>G), and *G6PD Chinese-4* (392G>T) but did not identify any of these mutations in G6PD-deficient Cambodians. In four G6PD-deficient samples, the mutation remained unidentified.

Because *G6PD* mutation 871G>A can be *G6PD Viangchan* (871G>A, nt 1311C>T) or *G6PD Jammu* (871G>A, wild type nt 1311), we assayed for the nt 1311C>T by a PCR-restriction enzyme method. We found that all samples with 871G>A had nt 1311C>T,

Table 1 G6PD mutations in G6PD-deficient Cambodian adults and neonates

Age	Gender	Total	Deficient	Mutation			
				<i>Viangchan</i>	Union	<i>Coimbra</i>	Unknown
Adult	Male	63	10	8	–	–	2
	Female	45	1	1	–	–	–
Neonate	Male	56	21	19	1	1	–
	Female	51	2	–	–	–	2

Fig. 1 Distribution of glucose-6-phosphate dehydrogenase (*G6PD*) variants in Cambodians. Numbers represent number of *G6PD* variant/population tested by province. Only provinces in which *G6PD* variants were found were reported

consistent with *G6PD Viangchan* (Fig. 2). For three *G6PD*-deficient females, all samples were heterozygote for *G6PD Viangchan* (Fig. 3).

Discussion

There were few reports on genetic markers in Cambodians because the country was not accessible to international communities until recently. Cambodians speak Khmer, a distinctive Austro-Asiatic language (<http://www.ethnologue.com>). Due to poor living conditions in Cambodia, many Cambodians migrated into Thailand as refugees, migrant laborers, and through marriages with Thais in border provinces. Because of their distinctive language and culture, it has been controversial

Fig. 2 PCR-RFLP for nt 1311C>T. Lane M 100-bp ladder, lane 1 undigested nt 1311C>T showed a 207 band, lanes 2–3, 5–6 digested nt 1311C>T showed 184 bp, lane 4 digested nt 1311C showed 207 bp

Fig. 3 PCR-RFLP for *G6PD Viangchan*. Lane M 100 bp ladder, lane 1 undigested *G6PD Viangchan* showed a 126 bp band, lane 2 digested *G6PD Viangchan* that reduced to 106 bp, lane 3 digested normal, lane 4 digested female heterozygote

whether today's Cambodians share a common ancestry with people of neighboring countries.

G6PD deficiency is highly prevalent in Cambodians. A recent study found *G6PD* deficiency in 13.4% of school boys in central Cambodia (Monchy et al. 2004). In this study, we found a prevalence of 26.1% in Cambodian males, which is comparable to Laotians and northeastern Thais (21.7%, Kittiwatanasarn et al. 2003). The high prevalence of *G6PD* deficiency among Southeast Asians was postulated to be due to selection pressure from malaria, which was hyperendemic in Southeast Asia (Flatz et al. 1963). The mechanism of resistance to malaria infection is still controversial, but it is probable that *G6PD* deficiency reduces NADPH in oxidative stress that affects growth of *Plasmodium* (Beutler 1994).

The objective of our study was to identify as many *G6PD*-deficient cases as possible to characterize their mutations. For this reason, females were included in the study. We used quantitative *G6PD* activity testing in all samples without using *G6PD* screening methods. We also have previously established a histogram of cord blood *G6PD* levels in males and females in our population (Sanpavat et al. 2001). In that study, we found that the cord blood of female babies whose *G6PD* activities are in deficient range are heterozygotes for *G6PD* mutation.

We found that *G6PD Viangchan* was the most common variant in Cambodians, with calculated allele frequency of 0.23. This variant is also the most common

among Thais (Nuchprayoon et al. 2002) and Laotians (Iwai et al. 2001). Our finding is in favor of the theory that proposed a common ancestry of Thais, Laotians, and Cambodians (Church et al. 2003). In contrast, *G6PD Mahidol*, the most common *G6PD* variant in the Myanmar population (Matsuoka et al. 2004), was not found in Cambodians. We screened for seven common Chinese mutations and found only one case each of *G6PD Union* (1360G>T) and *G6PD Coimbra* in Cambodians. These two *G6PD* variants could be attributed to assimilated Chinese immigrants to Cambodia in the recent decades.

Taking all regional evidence together, the *G6PD* mutations of people in the Southeast Asian peninsular can be described. Myanmar in the west of the peninsular are quite homogenous for *G6PD Mahidol* (Iwai et al. 2001) while Laotians and Cambodians in the eastern part of the peninsular are relatively homogeneous for *G6PD Viangchan*. For Thais in the central part of the peninsular, *G6PD* mutations are more heterogeneous but still predominated by *G6PD Viangchan* (Nuchprayoon et al. 2002). *G6PD* mutations become even more heterogeneous in Thais in the south of Thailand (Laosombat et al. 2005) and Malays in Malaysia (Ainoon et al. 2003). Overseas, Chinese immigrants in the recent century contributed Chinese *G6PD* variants to the Southeast Asian gene pools.

In addition to *G6PD Viangchan*, hemoglobin E (HbE) is known to be highly prevalent in Thais, particularly in the Northeastern region (Wasi et al. 1967). A recent study also suggests that HbE is prevalent in Khmer-speaking people in border provinces of Thailand (Fuchareon et al. 2002). This and our finding favors the theory that the Thais, Cambodians, and Laotians were people of the same ancestors living in the Southeast Asia peninsula for some time during the past millennium. Malarial selection pressure may be at work in these indigenous people during the age of agriculture (Tishkoff et al. 2001). Haplotype analysis of the *G6PD* locus could provide further evidence to support this hypothesis.

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