PREVALENCE OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND GENETIC MUTATIONS AMONG KAREN AND LAO POPULATIONS IN THAILAND

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Abstract. Glucose 6-phosphate dehydrogenase (G6PD) deficiency is highly prevalent in Southeast Asia. *G6PD* mutations are often ethnic-specific. Karen is an ethnic minority of Myanmar and resides in both Myanmar and Thailand. A comprehensive molecular characterization of Karen-associated *G6PD* mutations has, hitherto, not been conducted. In addition, heterogeneity of *G6PD* mutations in Laotian has not been defined. Here, we performed G6PD deficiency screening and molecular characterization of associated *G6PD* in Skaw Karens and Laotians in Thailand. Cord blood from 127 Laotian neonates, and peripheral blood from 80 Skaw Karens and 103 migrant Laotians were tested for G6PD deficiency using fluorescent spot test (FST) and G6PD activity assay. *G6PD* mutations were detected by PCR-fragment length polymorphism and direct sequencing of exons. In Skaw Karens, 28% of males were identified as G6PD-deficient and 2% and 14% of females as frank and intermediate G6PD-deficient, respectively. Among Laotians, the prevalence of frank and intermediate G6PD deficiency among neonates was 31% and 5% in males and 8% and 5% in females, respectively. In Laotian migrant laborers, 7% and 4% in males and 4% and 4% in females were frank and intermediate G6PD-deficient, respectively. *G6PD* Mahidol (rs137852314) was the most frequent mutation in Skaw Karens (allele frequency 0.28), whereas *G6PD* Viangchan (rs137852327) was the predominant in Laotians (allele frequency 0.09). Only haplotype rs2230037 (exon 11: nt1311C>T) and rs2071429 (IVS 11: nt93T>C) was found in 16 *G6PD* Viangchan alleles. The other three Chinese-associated mutations, namely, *G6PD* Canton (rs72554665), *G6PD* Union (rs398123546) and *G6PD* Kaiping (rs72554664) were found occasionally in both populations. Thus, G6PD deficiency is highly prevalent in Karens and Laotians, with *G6PD* Mahidol and *G6PD* Viangchan being the predominant alleles.

Keywords: G6PD deficiency, *G6PD* Mahidol, *G6PD* Viangchan, Skaw Karen, Laotian

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INTRODUCTION

Nicotinamide adenine dinucleotide phosphate (NADPH) is involved in protecting erythrocytes from oxidant stress and is re-generated by glucose 6-phosphate dehydrogenase (G6PD) (E.C. 1.1.1.49) (Beutler, 1994). *G6PD* is located on the long arm of X chromosome (Xq28) and comprises 13 exons and 12 introns (Cappellini and Fiorelli, 2008). Mutations in *G6PD* diminish enzyme activity resulting in G6PD deficiency syndrome, which is clinically asymptomatic in female heterozygotes and male hemizygotes, but such individuals are vulnerable to neonatal jaundice and acute hemolytic anemia following exposure to oxidative agents (Beutler, 1994). This enzymopathy is considered one of the most common genetic disorders in humans, affecting more than 400 million people worldwide (Cappellini and Fiorelli, 2008). More than 180 mutations have been identified and are associated with a specific population, which may help deduce their genetic structures (Iwai *et al*, 2001; Minucci *et al*, 2012). In Southeast Asia *G6PD* Viangchan (rs137852327 and rs2230037) is the most common mutation among Kampucheans (Louicharoen and Nuchprayoon, 2005), Laotians (Iwai *et al*, 2001) and Thais (Nuchprayoon *et al*, 2002), while *G6PD* Mahidol (rs137852314) is the predominant mutation among Myanmar and Mon populations (Nuchprayoon *et al*, 2008).

Karen is the largest tribal group living in Karen state and Tenasserim Division in eastern Myanmar and Western Thailand (Karen Buddhist Dhamma Dhutta Foundation, 2011). There are about half of a million Thai-Karens whose ancestral villages are in Thailand, with 140,000 Karen refugees living in camps in Thailand, and more than 7 million Karens living in Myanmar and elsewhere in the world (Karen Buddhist Dhamma Dhutta Foundation, 2011). Many thousands of Karens have migrated to work in Thailand (Ministry of Social Development and Human Security, 2016). Karen people consists of many subgroups, with Skaw Karen (Pwar Kar Nyaw) being the largest. Other subgroups include Pwo Karen (Ploe), Bwe Karen (Bway), Pa-O, and Karenni (cousins of Karen) (Karen Buddhist Dhamma Dhutta Foundation, 2011). Their native language belongs to the Sino-Tibetan family, Tibeto-Burman subfamily, which is used in many countries including Myanmar, India, and China (Lewis and Lewis, 1984). Karens are proposed to be of Tibetan origin and are thought to have entered Myanmar during the 6-7th century AD (Besaggio *et al*, 2007).

Lao PDR, a land-locked country in the eastern region of the Indochina Peninsula with a population of 7 million (Central Intelligence Agency, 2013). More than sixty thousands of Laotians have migrated to work in Thailand (Ministry of Social Development and Human Security, 2016). Their native language is Tai-Kadai, which is used in many ethnic groups including Thais, Phuans and a number of tribes in Southern China (Lewis and Lewis, 1984; Lewis *et al*, 2015). Laotians are proposed to share the same origin with Tai tribes in China (Yue and Ai Lao). Tai migrated into Southeast Asia in the beginning of the 1st millennium, with a large human wave between the 7th and 13th centuries AD (Church, 2006; Edmondson, 2007).

G6PD deficiency in Laotians has rarely been reported and their *G6PD* mutations were thought to be homogenous (Iwai *et al*, 2001). This study reports the G6PD variants in two Laotian populations, namely, migrant Laotian workers in Samut Sakhon Province and newborns

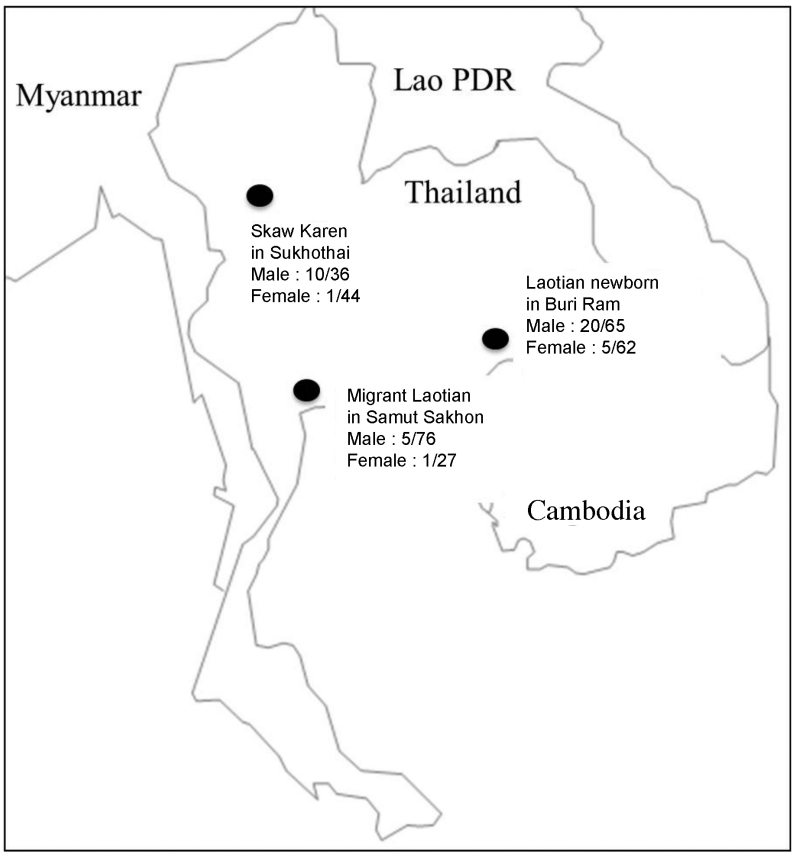

Fig 1–Location of study sites in Thailand, ethnic group, gender and fraction of glucose 6-phosphate dehydrogenase-deficient subjects.

of Laotian mothers in Buri Ram Province, Northeast Thailand. In addition, the prevalence of G6PD deficiency and allele frequency of *G6PD* mutations in Skaw Karen in Sukhothai Province, Thailand are reported.

MATERIALS AND METHODS

Subjects

Between 2002 and 2003, 127 umbilical cord blood samples (65 males and 62 females) were collected from Laotian mothers who delivered babies at Buri Ram Hospital, Buri Ram, Thailand (Fig 1). In 2004, 103 migrant Laotians (76 males and 27 females, 26 ± 7 years of age), working in Samut Sakhon Province, Thailand, and in 2011, 80 unrelated Skaw Karen individuals (36 males and 44 females, 46 ± 17 years of age), Mae San Village, Sukhothai Province, Thailand were enrolled in the study. Ethnicity of each subject was self-identified by their preferred language during an interview.

Informed consent was obtained from all participants or their parents/guardians. The study was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University, approval no. COA no. 805/2011.

G6PD screening test and G6PD activity assay

Six ml of peripheral blood were collected into acid-citrate-dextrose (ACD) and EDTA for G6PD assay and DNA study, respectively. Blood samples were kept at 4°C until analyzed. For blood samples of Skaw Karen subjects, G6PD activity was determined using a fluorescent spot test (FST) according to the International Council for Standardization in Hematology (ICSH) recommendation (Beutler *et al*, 1979). Fluorescence intensity was classified into three groups: normal (strong fluorescence), intermediate defi-

Table 1
Primers and restriction enzymes used in identification of glucose 6-phosphate dehydrogenase gene (G6PD) mutations.

G6PD mutation (reference)	Primer sequence	Restriction enzyme	Amplicon size (bp)
Mahidol (rs137852314)	F: 5′-GCGTCTGAATGATGCAGCTCTGAT-3′	*Hind* III	N: 104
(Huang *et al*, 1996)	R: 5′-CTCCACGATGATGCGGTTCAAGC-3′		M: 82, 22
Viangchan	F: 5′-TGGCTTTCTCTCAGGTCTAG-3′	*Xba*I	N: 126
(rs137852327)	R: 5′-GTCGTCCAGGTACCCTTTGGGG-3′		M: 106, 20
(Nuchprayoon *et al*, 2002)			
Canton	F: 5′-GTGAAAATACGCCAGGCCTTA-3′	*Afl*II	N: 214
(rs72554665)	R: 5′-GTGAAAATACGCCAGGCCTTA-3′		M: 194, 20
(Huang *et al*, 1996)			
Union	F: 5′-GTGAAAATACGCCAGGCCTTA-3′	*Hha*I	N: 142, 45, 27
(rs398123546)	R: 5′-GTGAAAATACGCCAGGCCTTA-3′		M: 187, 27
(Huang *et al*, 1996)			
Kaiping	F: 5′-GTGAAAATACGCCAGGCCTTA-3′	*Nde*I	N: 227
(rs72554664)	R: 5′-GTGCAGCAGTGGGGTGAACATA-3′		M: 206, 21
(Huang *et al*, 1996)			
Chinese-5 (rs137852342)	F: 5′-GTCAAGGTGTTGAAATGCATC-3′	*Mbo*II	N: 187
(Huang *et al*, 1996)	R: 5′-CATCCCACCTCTCATTCTCC-3′		M: 150, 37
Coimbra	F: 5′-GTCAAGGTGTTGAAATGCATC-3′	*Pst*I	N: 157+83
(rs137852330)	R: 5′-CATCCCACCTCTCATTCTCC-3′		M: 157+63+20
(Huang *et al*, 1996)			
Chinese-4	F: 5′-GGACTCAAAGAGAGGGGCTG -3′	*BstE*II	N: 188+15
(rs137852341)	R: 5′-GAAGAGGCGGTTGGCCGGTGAC-3′		M: 203
(Huang *et al*, 1996)			
rs2230037	F: 5′-TGTTCTTCAACCCCGAGGAGT-3′	*Bcl*I	N: 207
(Vulliamy *et al*, 1991)	R: 5′-AAGACGTCCAGGATGAGGTGATC-3′		M: 184+23
rs2071429	F: 5′-GTGAAAATACGCCAGGCCTTA-3′	*Nla*III	N: 214
(Huang *et al*, 1996)	R: 5′-GTGAAAATACGCCAGGCCTTA-3′		M: 172+42

N, normal; M, mutant.

cient (moderate fluorescence), and frank deficient (no fluorescence) (Procedure 203-A, Trinity Biotech, Bray, Ireland). G6PD status in Laotian subjects was determined by a quantitative enzyme assay, based on increase in absorbance of NADPH at 340 nm after addition of G6-P and NADP (Betke *et al*, 1967). G6PD activity <1.5 and ≥1.5-<2.8 IU/g Hb is classified as frank and intermediate G6PD deficient (Hsia *et al*, 1993).

Determination of *G6PD* mutation and haplotype

Genomic DNA was extracted from G6PD deficient and randomly selected normal blood samples using Nucleospin® Blood Kit (Macherey-Nagel, Duren, Germany). For G6PD-deficient samples, DNA was genotyped for eight common *G6PD* mutations: Canton (rs72554665), Chinese-4 (rs137852341). Chinese-5 (rs137852342), Coimbra (rs137852330), Kai-

Table 2
Prevalence of glucose 6-phosphate dehydrogenase (G6PD) deficiency in Karen and Laotian test populations.

Ethnic group	Gender	Number of samples	Frank deficiency	Intermediate deficiency	Prevalence (%)
Skaw Karen	Male	36	10[a]	0	28
	Female	44	1[a]	6[a]	16
Laotian neonate	Male	65	20[b]	3[b]	35
	Female	62	5[b]	3[b]	13
Laotian migrant worker	Male	76	5[b]	3[b]	11
	Female	27	1[b]	1[b]	7

[a]Fluorescent spot assay. [b]Enzymatic assay.

ping (rs72554664), Mahidol (rs137852314), Union (rs398123546), and Viangchan (rs137852327). In brief, PCR was carried out in 10 μl mixture containing 1 μl of 50 ng/μl DNA, 400 nM of each primer set (Table 1), 200 μM dNTPs, 1.5 mM $MgCl_2$, 20 mM Tris-HCl pH 8.4, 50 mM KCl, and 0.25 U *Taq* polymerase (RBC Bioscience, New Taipei City, Taiwan). Thermocycling was performed in a Veriti® 96-well thermal cycler (Applied Biosystems, Foster city, CA) as follows: 94°C for 5 minutes; 35 cycles of 94°C for 45 seconds, 60°C for 45 seconds and 72°C for 45 seconds; with a final step at 72°C for 7 minutes. Amplicons were digested with appropriate restriction enzymes (Table 1) and separated by 6% polyacrylamide gel-electrophoresis, stained with Novel Juice (GeneDireX, Las Vegas City, NV) and recorded using Gel Doc™ XR+System (BioRad, Hercules, CA). In samples of *G6PD* Viangchan, rs2230037 (exon 11: nt1311C>T) and rs2071429 (IVS 11: nt93T>C) also were genotyped.

Statistical analysis

G6PD activity is expressed as mean ± range of experiments conducted in duplicate. Statistical significance of G6PD deficiency prevalence is evaluated by chi-square test, with p-value <0.05 considered significant.

RESULTS

Prevalence of G6PD deficiency

Among the Skaw Karen group, 10/36 (28%) of males were identified as G6PD-deficient and 1/44 (2%) and 6/44 (14%) of females G6PD frank and intermediate deficient, respectively (Table 2). Among Laotians, the prevalence of G6PD frank and intermediate deficiency among neonates was 20/65 (31%) and 3/65 (5%) in males and 5/62 (8%) and 3/62 (5%) in females, respectively. Among migrant laborers, 5/76 (7%) and 3/76 (4%) of males and 1/27 (4%) and 1/27 (4%) of females were G6PD frank and intermediate deficient, respectively.

***G6PD* mutations**

G6PD Mahidol was the only mutation found in the 10 Skaw Karen G6PD-deficient males, and among females, *G6PD* Mahidol was predominant (6/7) with *G6PD* Canton being carried the remaining female (Table 3). Only one female was

Table 3
Glucose 6-phosphate dehydrogenase gene (*G6PD*) mutation and allele frequency in Karen and Laotian test populations.

Ethnic group	Allele frequency of *G6PD* mutation											
	Mahidol		Viangchan		Canton		Union		Kaiping		Unknown	
	M	F	M	F	M	F	M	F	M	F	M	F
Skaw Karen	0.28	0.07	0	0	0	0.01	0	0	0	0	0	0
Laotian	0	0	0.09	0.02	0.02	0.01	0.01	0	0.01	0	0.04	0.01

F, female; M, male.

homozygous for *G6PD* Mahidol.

Only *G6PD* Canton (4/31; 13%), *G6PD* Kaiping (2/31; 6%), *G6PD* Union (1/31; 3%), and *G6PD* Viangchan (15/31; 48%) were found among the G6PD-defient Laotian subjects (neonates and adults) (Table 3). Only one female was homozygous for *G6PD* Mahidol. In 9 G6PD-deficient Laotian subjects (3 male and 3 female neonates, and 3 male adults), mutations could not be identified in the sequenced exons using primers described by Nuchprayoon *et al* (2008) (data not shown). Only haplotype rs2230037 (exon 11: nt1311C>T) and rs2071429 (IVS 11: nt93T>C) was found in 16 alleles of *G6PD* Viangchan.

DISCUSSION

This is the first study of the prevalence of G6PD deficiency among Skaw Karen population, albeit a small cohort, and confirms the previous observation that *G6PD* Mahidol is highly prevalent (allele frequency of 0.24) in Pwo Karens (Louicharoen *et al*, 2009). In that report, the other *G6PD* mutation detected is *G6PD* Viangchan. Our study identified the existence of *G6PD* Canton.

This study is, to date, the largest survey of Laotians for the prevalence of G6PD deficiency and indicated that almost one-third of Laotians are G6PD-deficient, although there is a significant difference in prevalence between Laotian neonates in Buri Ram Province and migrant workers in Samut Sakhon Province ($p < 0.001$). This may be due to that Laotians in Buri Ram Province are from the southern part of Lao PDR while migrant workers come from various regions of that country. The prevalence of G6PD deficiency in Laotian migrant workers in Samut Sakhon Province was similar to the previous report (7%) (Iwai *et al*, 2001), which represent people from northern and central Lao PDR, while people from southern Lao PDR have a higher prevalence, similar to that of neighboring northeastern Thais (22%) (Kittiwatanasarn *et al*, 2003) and Kampucheans (26%) (Louicharoen and Nuchprayoon, 2005).

The prevalence of frank and intermediate G6PD deficiency in male Skaw Karens and Laotian neonates are similar to that observed in male Kampucheans (26.1%) (Louicharoen and Nuchprayoon, 2005), while frank and intermediate G6PD deficiency in male migrant Laotian workers is similar to other male ethnic groups

in Thailand and neighboring countries in Southeast Asia, *viz*. 6.6-11% in Burmese (Nuchprayoon *et al*, 2008; Phompradit *et al*, 2011), 10.8% in Shan (Iwai *et al*, 2001), 6.7-12% in Mon (Nuchprayoon *et al*, 2008), 6.3% and 7.1% in Kachin and Danu, respectively in Myanmar (Iwai *et al*, 2001), and 7.2% in Laotians (Iwai *et al*, 2001).

G6PD Mahidol,' the dominant mutation among Skaw Karens, also is the major mutation in other ethnic groups in Myanmar, *viz*. Burmese (71-96.2%) (Nuchprayoon *et al*, 2008; Phompradit *et al*, 2011) and Mon (63%) (Nuchprayoon *et al*, 2008). *G6PD* Mahidol is predominant but to a lesser extent among northern Thais (20%) (Charoenkwan *et al*, 2014), but occasionally is found among Thais from the central region (Nuchprayoon *et al*, 2002), and is not present in Laotians (Iwai *et al*, 2001) or Kampucheans (Louicharoen and Nuchprayoon, 2005). Karens are thought to have migrated down from Tibet and entered Myanmar 1,500 years ago, and at that period *G6PD* Mahidol evolved under the selective pressure of vivax malaria (Louicharoen *et al*, 2009), and was brought along with the gradual migrated southwards into the current Thai-Myanmar border region.

Contrary to the previous report that only *G6PD* Viangchan is present among Laotians (Iwai *et al*, 2001), our study demonstrates that this mutation accounted for only half of the *G6PD* mutations detected in the Laotian test population. Likewise, *G6PD* Viangchan is found in 54% of Thais (Nuchprayoon *et al*, 2002) and 25% of Phuans (25%) (Cheepsunthorn and Nuchprayoon, 2013), but is by far more common in Kampucheans (82-98%) (Louicharoen and Nuchprayoon, 2005). The latter is likely due to selective pressure by malaria (Louicharoen *et al*, 2009).

This difference in *G6PD* mutation types supports the previous report that Burmese and Laotians have different ancestries (Nuchprayoon *et al*, 2008). Laotians are thought to have descended from Tai and entered Chao Phraya valley between the 8th-10th centuries (Church, 2006; Edmondson, 2007; Pittayaporn, 2014). From there, they took advantage of the waning Khmer Empire and emerged as an independent country. The Lao people believe the beginning of their national history to this time (Cœdès, 1921). Some of Tai tribes came to the area of present-day Thailand (Charles, 1997).

This is the first report of *G6PD* Viangchan haplotype in Laotians and that the only haplotype found, rs2230037 (exon 11: nt1311C>T) and rs2071429 (IVS 11: nt93T>C), is consistent with previous reports in Cambodians and Thais (Nuchprayoon *et al*, 2002; Matsuoka *et al*, 2005). Our result supports the notion that *G6PD* Viangchan allele in Laotian, Cambodian, and Thai have a common ancestry.

We also identified *G6PD* Canton, *G6PD* Kaiping and *G6PD* Union among the Laotian test population. This is not surprising as northern Lao PDR borders southern China. *G6PD* Canton is most prevalent (24%) in southern China (Yan *et al*, 2006) and among Malaysian Chinese (42%), as well as *G6PD* Union and *G6PD* Kaiping (0.8% and 39.4%, respectively) (Ainoon *et al*, 2004). The occurrence of *G6PD* Canton, *G6PD* Kaiping and *G6PD* Union among Laotians suggests assimilation of the Chinese into Laotian population, as was observed among northern Thais from Phayao (Cheepsunthorn and Nilsri, 2015) and Chiang Mai (Charoenkwan *et al*, 2014), and less commonly in Thais in the central region (Nuchprayoon *et al*, 2002). On the other hand, these

Chinese-associated *G6PD* mutations were not identified in Skaw Karens in our study, nor were they reported among the Burmese or Mons (Nuchprayoon *et al*, 2008).

This study is handicapped by three factors. Firstly, it was difficult to carry out quantitative enzyme assay or gold standard testing in the villages, which necessitated the use of two different methods in different populations. Secondly, genotyping was not conducted on samples from female subjects with low normal G6PD activity; it is well known that G6PD deficiency heterozygotes can manifest such levels (Domingo *et al*, 2013). Thirdly, there were several G6PD deficient samples in which *G6PD* mutations were not detected in the coding regions, and future studies on *G6PD* promoter and introns regions of *G6PD* may provide explanations for alterations in G6PD function alteration.

In summary, this study shows G6PD deficiency was highly prevalent in Karen and Laotian populations, with *G6PD* Mahidol and *G6PD* Viangchan being predominant alleles, similar to findings in other ethnic groups of Southeast Asia.

ACKNOWLEDGEMENTS

The authors thank all volunteers and staff of Buri Ram and Samut Sakhon Hospital. This research was supported in part by grants from Rachadapisek Sompot Fund, Faculty of Medicine, Chulalongkorn University (RA57/006) and the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund).

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