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Brief communication (Original)

Molecular characterization of *G6PD* mutations in the Phuan tribe in Thailand

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Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an enzymopathy with high frequency in Southeast Asians. Phuan is a minority tribe in Thailand. The prevalence of G6PD deficiency and its molecular heterogeneity in this population is yet unknown.
Objectives: To characterize molecular heterogeneity of *G6PD* in Phuan people and investigate whether the heterogeneity of *G6PD* could be used to delineate the origin of Phuan people in Thailand.
Methods: Cord blood samples from 202 Phuan neonates were tested for G6PD deficiency using a G6PD activity assay. *G6PD* mutations were determined in G6PD deficient blood samples by polymerase chain reaction-restriction fragment length polymorphism analysis and sequencing.
Results: G6PD deficiency was found in 12 (12.2%) of 98 males and 8 (7.7%) of 104 females in the study population. Molecular analysis was performed on 12 males and 8 females to identify G6PD mutations. G6PD Viangchan (871G→A, 1311C→T) (25.0%) was the most dominant mutation followed by the G6PD Canton (1376G→T) (15.0%), G6PD Union (1360C→T) (10.0%), one case each of G6PD Kaiping (1388G→A) and G6PD Mediterranean (563C→T, 1311C) (5%), and eight G6PD deficient unidentified mutations.
Conclusions: G6PD deficiency in Phuan is highly frequent and G6PD Viangchan (871G→A, 1311C→T) is the most common mutation. Our study suggests that Phuans have coevolved with Thais, and were influenced by gene flow from Chinese and Indian mutations.

Keywords: G6PD deficiency, G6PD Mediterranean, G6PD Viangchan, Phuan, Tai-Kadai

Glucose-6-phosphate dehydrogenase (G6PD) (E.C. 1.1.1.49; MIM#305900) catalyzes the first step of the pentose phosphate pathway (PPP) to generate nicotinamide adenine dinucleotide phosphate (NADPH) [1]. Mutations in the *G6PD* gene can lead to G6PD deficiency, an X-linked hereditary enzymopathic disorder [2]. The disorder causes neonatal jaundice and oxidative stress induced hemolytic anaemia after exposure to oxidative agents, including antimalarial drugs. More than 400 million people worldwide carry *G6PD* mutations and at least 140 molecular mutations have been defined [2]. Two common mutations associated with specific ethnic groups have been described. G6PD Viangchan (871G→A; Val291Met and nt 1311C→T) is the most common mutation among Thais [3], Cambodians [4], and Laotians [5]. G6PD Mahidol (487G→A; Gly163Ser) is the most common mutation among Burmese and Mon [6].

In Thailand, there are several minorities that emigrate from neighboring countries. This includes Phuan people, also known as "Tai Phuan" or "Lao Phuan" whose native language is Tai-Kadai, which is used in many ethnic groups including Thais, Laotians, Indians, and Chinese [7]. The majority of Phuan lives in northeastern and central Thailand and Xieng Khouang province of Laos (U.S. Center for World Mission. Joshua Project). In addition to molecular characterization of *G6PD* in Phuan people, this study also investigate whether the heterogeneity of *G6PD* could be used to delineate the origin of Phuan people in Thailand.

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Materials and methods

During April 2006 to June 2006, newborns of mothers whom self-identified as Phuan in Lopburi Hospital and Banmi Hospital were recruited

for the study. Cord bloods from 202 Phuan babies (94 males and 108 females) were collected in the delivery room, into acid citrate dextrose (ACD) and ethylenediaminetetraacetic acid (EDTA) tubes and stored at 4 C for assay of G6PD enzyme activity and molecular typing, respectively [8]. Interview by questionnaire of each parents generated the demographic information and ethnic. Informed consent was individually obtained from all their parents. This study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University (COA No. 401/2010 IRB No. 235/53). All samples were measured for G6PD activity using a standardized method from the WHO recommended standardized test [8]. The G6PD activity less than 1.5 IU/g Hb was classified as deficient. Genomic DNA was extracted from G6PD deficient blood samples using a QIAamp DNA blood mini kit (Qiagen, Germany). The DNA of patients were genotyped for five different *G6PD* mutations previously reported in Southeast Asian; G6PD Viangchan (871G→A), G6PD Mahidol (487G→A), G6PD Canton (1376G→T), G6PD Union (1360C→T), and G6PD Kaiping (1388G→A) using PCR-RFLP. A subset of those with the Viangchan and Mediterranean variants, were further investigated for the silent mutation (1311 C→T) (rs2230037). PCR-RFLP was performed using our previously published method [6]. For individuals showing an absence of those six mutations, we then directly sequenced all coding regions by modified primer sets as previously described [6, 9].

Results

A total of 202 cord blood samples of Phuan people were assayed for G6PD deficiency. The hemoglobin levels of all G6PD-deficient cases were in the normal range for cord blood (13.0–19.0 g/dL) (**Table 1**). We found G6PD deficiency in 12.2% of males (12 of 98) and 7.7% (8 of 104) females. DNA analysis of 20 G6PD deficient samples as described revealed that G6PD Viangchan (871G→A, 1311C→T) was detected in 5 (25.0%), G6PD Canton (1376G→T) in 3 (15.0%), G6PD Union (1360C→T) in 2 (10.0%), and G6PD Kaiping (1388G→A) in 1 (5.0%) individuals. None of the samples has G6PD Mahidol (487G→A). We then sequenced all coding regions of the *G6PD* gene in the remaining 9 samples to search for unknown mutations. We found a heterozygous G6PD Mediterranean (563C→T) with homozygous 1311C in one female, while the mutations of 8 samples remained unidentified.

Table 1. Prevalence of G6PD deficiency in Phuan

Mutation	N (%)	Sex (n)	Cord blood hemoglobin g/dL (mean ± SD)	G6PD activity IU/gHb (mean ± SD)
G6PD Viangchan	5 (25)	M (2)	17.90 ± 0.85	0.67 ± 0.62
(871G→A,		F^a (1)	18.80	1.48
1311 C→T)		F^b (2)	17.05 ± 2.47	0.96 ± 1.03
G6PD Canton	3 (15)	M (3)	15.93 ± 1.69	0.30 ± 0.19
(1376G→T)		F (0)	–	–
G6PD Union	2 (10)	M (2)	17.10 ± 0.71	0.19 ± 0.07
(1360C→T)		F (0)	–	–
G6PD Kaiping	1 (5)	M (1)	16.90	0.13
(1388G→A)		F (0)	–	–
G6PD Mediterranean	1 (5)	M (0)	–	–
(563C→T)		F^a (1)	16.50	1.30
Unknown	8 (40)	M (4)	14.13 ± 5.22	0.58 ± 0.49
		F (4)	16.68 ± 1.68	0.61 ± 0.26
Total number	20	M (12)		
		F (8)		

F^a = heterozygous female, F^b = homozygous female

Discussion

G6PD deficiency was highly prevalent in Phuan males (12.2%), which is comparable to other ethnic groups in Southeast Asia; 7.2% in Laotians [5], 11.1% in Thais [3], 12.6%–26.1% in Cambodians [4], 7.3%–11% in Burmese [6], 6.7%–12.0% in Mon [6]. The overall high prevalence of G6PD deficiency in Phuan people and other tribes in Southeast Asia is probably the result of the selective advantage against malaria in endemic areas [10].

In this study, cord blood was collected from all births from Phuan people during the study period. The result is considered a good representation of Phuan population as samples were collected from asymptomatic population without selection.

In current study, only 5 nonsynonymous *G6PD* mutations were found and the most common mutations was G6PD Viangchan (871G→A, 1311C→T), which was also the most frequent mutation in Thais [3] and Laotians [5]. It is believed that Phuan is a descendant of Tai people in southwestern China. During immigration into Thailand and Laos in the thirteenth century, Tai received cultures from many tribes along the way. From 1827 to 1890, a group of Phuan people were transplanted from Laos to Thailand after they were defeated in war (U.S. Center for World Mission. Joshua Project). Therefore, their language (Tai-Kadai) is closely related to Thai Dam and the Thai Loei. Moreover, their culture is very similar to Tai tribes and Lao people. Thus, our finding that there is high gene frequency of G6PD Viangchan without G6PD Mahidol (487G→A) in Phuan population is consistent with their ancestral origin, sharing with Thai and Laotian, and linguistic affiliations of Phuan people as described [7]. However, the allele frequency is of G6PD Viangchan (871G→A, 1311C→T) in Phuan people is less than in Thais (31.0%–54.0%) [3] and Laotians (100.0%) [11]. This finding suggests a higher degree of genetic admixture among Phuan people.

The Chinese *G6PD* mutations; G6PD Canton (1376C→T), G6PD Union (1360C→T), and G6PD Kaiping (1388G→A) were also found in Phuan people. The finding supports the effect of ethnic integration with the Chinese who migrated to Southeast Asia.

We also reported the presence of G6PD Mediterranean with 1311C in a Phuan female, which is most likely caused by gene flow of Mediterranean variant from India throughout Asia. G6PD Mediterranean (563CT) was firstly described in Fuscaldo, south of Italy [12] and subsequently found in Indians (37.2%) [13]. Polymorphism of nt 1311 C→T was then defined in *G6PD* Mediterranean. G6PD Mediterranean, with 1311T is found in Mediterranean and Middle Eastern people while 1311C is predominantly observed in the Italians and Indians [14, 15].

Although, previous studies of Y chromosome haplotypes suggest a genetic continuum throughout greater Southeast Asia and China irrespective of linguistic affiliations; be they Hmong, Mien, Austronesian, Austroasiatic, or Sino-Tibetan [16, 17]. The heterogeneity of *G6PD* mutations in Phuan people as described here is coincides with the distribution of Tai-Kadai linguistic ethic groups, which could be the origin of Phuan people in Thailand.

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