RESEARCH NOTE

PREVALENCE AND MOLECULAR CHARACTERIZATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN NORTHERN THAILAND

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Abstract. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common inherited enzymopathies in endemic areas of malaria including Southeast Asia. The molecular features of G6PD deficiency are similar among Southeast Asian population, with differences in the type of the prominent variants in each region. This study determined the prevalence and molecular characteristics of G6PD deficiency in northern Thailand. Quantitative assay of G6PD activity was conducted in 566 neonatal cord blood samples and 6 common *G6PD* mutations were determined by PCR-restriction fragment length polymorphism method on G6PD complete and intermediate deficiency samples. Ninety newborns had G6PD deficiency, with prevalence in male newborns of 17% and that of female newborns having an intermediate and complete deficiency of 13% and 2%, respectively. From 95 *G6PD* alleles tested, G6PD Mahidol, G6PD Kaiping, G6PD Canton, G6PD Viangchan, G6PD Union, and G6PD Chinese-5 was detected in 19, 17, 15, 13, 7, and 2 alleles, respectively. Our study shows that the prevalence of G6PD deficiency in northern Thai population is high and combination of the common Chinese mutations is the majority, a distribution different from central and southern Thailand where G6PD Viangchan is the prominent variant. These findings suggest a higher proportion of assimilated Chinese ethnic group in the northern Thai population.

Keywords: glucose-6-phosphate dehydrogenase deficiency, mutation, prevalence, Thailand

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common inherited enzymopathies (Beutler, 1996). As G6PD deficiency protects against malarial infection, the prevalence is high in endemic areas of malaria, past

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and present, including Southeast Asia (Ruwende *et al*, 1995; Beutler, 1996). G6PD catalyzes the production of nicotinamide adenine dinucleotide phosphate (NADPH) in the pentose phosphate pathway, which is essential for the conversion of glutathione to a reduced form, in order to function in a variety of antioxidant reactions. G6PD-deficient red blood cells are subject to oxidative injury and premature destruction (Beutler, 1994).

G6PD deficiency is an X-linked recessive inherited disorder, and symptomatic patients are mostly hemizygous males, but also the less common homozygous females. A number of heterozygous females may have decreased levels of G6PD in the intermediate-deficient range, due to a skewed inactivation of the X-chromosome (Lyon, 1961). Thus, G6PD-deficient heterozygous females may experience an acute episode of hemolysis and are at increased risk for neonatal hyperbilirubinemia (Meloni *et al*, 1983; Kaplan *et al*, 1999; Herschel *et al*, 2002).

G6PD deficiency is prevalent in the Thai population in the range of 3-18% in males (Flatz and Sringam, 1963; Wasi *et al*, 1967; Tanphaichitr *et al*, 1995). G6PD Viangchan (871G>A) is the most common variant in central and southern Thailand (Nuchprayoon *et al*, 2002; Laosombat *et al*, 2005), but G6PD Mahidol (487G>A) has also been reported to be the most common variant in the south (Panich *et al*, 1972; Ninokata *et al*, 2006). Other common variants found in the Thai population include G6PD Kaiping (1388G>A), G6PD Canton (1376G>T), G6PD Union (1360C>T) and G6PD Chinese-5 (1024C>T) (Nuchprayoon *et al*, 2002; Laosombat *et al*, 2005). The presence of these variants are similar to those found in other Southeast Asian population, although differences in the type of a prominent mutation occur in each region (Ainoon *et al*, 1999; Iwai *et al*, 2001; Ainoon *et al*, 2003; Matsuoka *et al*, 2004; Louicharoen and Nuchprayoon, 2005; Matsuoka *et al*, 2005; Yan *et al*, 2006; Deng *et al*, 2007; Matsuoka *et al*, 2007; Nuchprayoon *et al*, 2008).

Northern Thailand is bordered by Myanmar and Lao PDR, and is close to the southern part of China. In the mountainous areas around Chiang Mai, the major province of northern Thailand, comprising mainly Thais and assimilated Chinese there are several tribal groups including Akha, Hmong, Karen, Lahu, Lisu, Lua, Palong, Tai and Yao (Forbes, 2004). With the different combinations of populations, the prevalence and molecular characteristics of G6PD deficiency in northern Thailand might be different.

Although G6PD deficiency in Thailand has been extensively studied, information from the northern Thai population is still lacking. This study aimed to determine the prevalence of G6PD deficiency and molecular characterization of the G6PD variants in the population of Chiang Mai Province. The information will be crucial for planning screening of affected individuals, for proper health education and surveillance, and for population genetic studies.

MATERIALS AND METHODS

Subjects

Neonatal cord blood screening for G6PD deficiency was conducted between December 2007 and January 2009. Full-term newborns (gestational age 37-42 weeks) of mothers with uneventful pregnancy at Chiang Mai University Hospital, Thailand were enrolled in the study. After delivery of the newborn, 7 ml of umbilical cord blood were collected in acid-citrate-dextrose (ACD) solution for G6PD assay

and 5 ml in EDTA for DNA study. Blood samples were kept at 4ºC until analysis. Samples that were G6PD deficient were further tested for *G6PD* mutations. The study protocol was approved by the Institutional Ethics Committee. Written informed consent was obtained from the mothers.

G6PD assay

G6PD assay was performed according to the World Health Organization (WHO) method within 7 days of blood collection (Betke *et al*, 1967). The mean G6PD level in cord blood from male newborns was 12.5 ± 2.3 IU/g Hb. Complete and intermediate deficiency of G6PD was defined as the level of < 1.5 and 1.5-8.0 IU/g Hb, respectively.

In G6PD deficient samples, six common *G6PD* mutations previously reported in Thailand, namely, G6PD Viangchan (871G>A), Mahidol (487G>A), Kaiping (1388G>A), Canton (1376G>T), Union (1360C>T) and Chinese-5 (1024C>T) were detected using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis (Table 1) as described previously (Huang *et al*, 1996; Nuchprayoon *et al*, 2002). In brief, genomic DNA was extracted from peripheral blood leukocytes by Chelex method (Walsh *et al*, 1991). PCR mixture (50 l) contained 1 l of DNA, 0.4 M each primer, 200 M each dNTPs and 1 U *Taq* DNA polymerase (Qiagen, Hilden, Germany) in 1X PCR buffer and 1.0 mM $MgCl_2$. After 3 minute initial heating at 94ºC, 35 cycles of 94ºC for 1 minute, 56ºC for 1 minute and 72ºC for 1 minute were performed, followed by a final heating at 72ºC for 10 minutes in a GeneAmp PCR system 9700 (Perkin Elmer, San Jose, CA). PCR amplicons were digested with appropriate restriction enzymes and analyzed by 1.5% agarose gel-electrophoresis containing 0.5 g/ml of ethidium bromide. DNA bands were visualized under UV light and documented using a Biorad Gel Doc 1000 system. Primer sequences and restriction enzymes are shown in Table 1.

RESULTS

Mothers of 566 newborns consented to the study, and 90 (15.9%) newborns had G6PD deficiency. The prevalence in male newborns was 17% (48/289) and that of female newborns with intermediate and complete G6PD deficiency was 13% (37/277) and 2% (5/277), respectively.

The majority (43%) of *G6PD* mutations detected included the 4 common Chinese mutations (G6PD Kaiping, G6PD Canton, G6PD Union, and G6PD Chinese-5), although G6PD Mahidol and G6PD Viangchan were also prominent in the population studied (Table 2).

DISCUSSION

The prevalence of G6PD deficiency in northern Thai population was higher compared with those in other parts of Thailand and with other previous studies (Table 3). Given the high prevalence of G6PD deficiency in the total Thai population, G6PD deficiency should be investigated in both male and female newborns with neonatal hyperbilirubinemia or acute hemolysis, and universal neonatal screening for G6PD deficiency should be established.

G6PD Kaiping, G6PD Canton, G6PD Union, and G6PD Chinese-5 are common in the population of southern China (Yan *et al*, 2006; Deng *et al*, 2007), Taiwan (Chiu *et al*, 1993) and Malaysia (Ainoon *et al*, 1999), confirming the assimilation of the Chinese ethnic group to northern

Table 1
Primers, restriction enzymes and amplicon used in PCR assays.

G6PD mutation	Primer sequence	Restriction enzyme	Amplicon (base pairs)
Mahidol[a]	F: 5′GCGTCTGAATGATGCAGCTCTGAT3′	*Hin*d III	N 104
487G>A	R: 5′CTCCACGATGATGCGGTTCAAGC3′		M 82+22
Viangchan[b]	F: 5′TGGCTTTCTCTCAGGTCTAG3′	*Xba* I	N 126
871G>A	R: 5′GTCGTCCAGGTACCCTTTGGGG3′		M 106+20
Chinese-5[a]	F: 5′GTCAAGGTGTTGAAATGCATC3′	*Mbo* II	N 187
1024C>T	R: 5′CATCCCACCTCTCATTCTCC3′		M 150+37
Union[a]	F: 5′GTGAAAATACGCCAGGCCTTA3′	*Hha* I	N 142+45+27
1360C>T	R: 5′GTGAAAATACGCCAGGCCTTA3′		M 187+27
Canton[a]	Same as Union	*Afl* II	N 214
1376G>T			M 194+20
Kaiping[a]	F: Same as Union	*Nde* I	N 227
1388G>A	R: 5′GTGCAGCAGTGGGGTGAACATA3′		M 206+21

[a]Huang *et al*, 1996. [b]Nuchprayoon *et al*, 2002. N, normal; M, mutant.

Table 2
Molecular characterization of glucose-6-phosphate dehydrogenase (G6PD) deficiency in northern Thailand.

Level of G6PD deficiency	Number of cases	Number of alleles	G6PD mutations						
			Mahidol 487G>A	Kaiping 1388G>A	Canton 1376G>T	Viangchan 871G>A	Union 1360C>T	Chinese-5 1024C>T	Unknown
Complete deficient male	48	48	10	10	6	5	5	2	10
Intermediate deficient female	37	37	9	6	6	4	2	0	10
Complete deficient female	5	10	0	1	3	4	0	0	2
Total, *n* (%)	90	95 (100)	19 (20)	17 (18)	15 (16)	13 (14)	7 (7)	2 (2)	22 (23)

Thailand. These findings differ from the other regions of Thailand where G6PD Viangchan and G6PD Mahidol are the prominent variants (Table 3). G6PD Viangchan is the most common variant in Laotian, Vietnamese, Cambodian population and Malaysian Malays (Iwai *et al*, 2001; Ainoon *et al*, 2003; Louicharoen and Nuchprayoon, 2005; Matsuoka *et al*, 2005,2007). Mahidol is the most common variant in Myanmar and Malaysia (Iwai *et al*, 2001; Matsuoka *et al*, 2004; Nuchprayoon *et al*, 2008).

Approximately 25% of the *G6PD* mutations were not identified, implying a greater heterogeneity than anticipated,

Table 3
Prevalence and molecular characteristics of G6PD deficiency in Thailand, neighboring countries and southern China.

Region	Myanmar	Lao PDR	Cambodia	Malaysia	Southern China	Central Thailand	Southern Thailand	Southern Thailand (Phuket island)	Northern Thailand
Reference	Matsuoka *et al*, 2004 Jalloh *et al*, 2004	Iwai *et al*, 2001	Nuchprayoon *et al*, 2008	Ainoon *et al*, 2003	Deng *et al*, 2007	Nuchprayoon *et al*, 2002	Laosombat *et al*, 2005	Ninokata *et al*, 2006	Current study
Number tested for G6PD deficiency	855	678	215	5,362	-	522	-	345	566
Prevalence	Males 11.6% Females 9.6%	Males 7.2%	Males 26.1% Females 3.1%	Males 5.3% Females 1.05%	-	Males 11.1% Females 5.8%	-	Males 9.8% Females 10.4%	Males 16.6% Females 15.2%
Number tested for *G6PD* mutations	80	9	34	86 (Malaysian Malays)	240	39	134	35	90 (95 alleles)
DNA analysis method	DNA sequence analysis	PCR-SSCP and DNA sequence analysis	PCR-RFLP (10 mutations)	PCR-SSCP and DNA sequence analysis	Mutation-specific PCR (3 mutations) and DNA sequence analysis	PCR-RFLP (10 mutations)	PCR-RFLP (7 mutations) MPTP and DNA sequence analysis	PCR-RFLP (2 mutations) and DNA sequence analysis	PCR-RFLP (6 mutations)
Viangchan 871G>A	0	100%	82.40%	37.20%	0	53.80%	31.30%	31.40%	13.70%
Canton 1376G>T	2.50%	0	0	4.70%	20.00%	10.30%	9.70%	0	15.80%
Mahidol 487G>A	91.25%	0	0	15.10%	0	7.70%	17.20%	40%	20.00%
Kaiping 1388G>A	0%	0	0	3.50%	79.20%	5.10%	20.10%	2.90%	17.90%
Union 1360C>T	3.75%	0	2.90%	2.30%	0	2.60%	2.20%	0	7.40%
Chinese-5 1024C>T	0	0	0	0	0.80%	2.60%	0%	0	2.10%
Gaohe 95A>G	0	0	0	0	0	0	1.50%	5.70%	Not tested
Mediterranean 563 C>T	0	0	Not tested	26.70%	0	Not tested	0.70%	0	Not tested
Other mutations	Coimbra 592C>T: 2.5%	0	Coimbra 592C>T: 2.9%	Coimbra 592 C>T: 3.5% Vanua Lava 383 T>C: 3.5% Chatham 1003 G>A: 2.3% Orissa 131 C>G: 1.2% Andalus 1361 G>A: 1.2%	0	0	Quing Yuan 392G>T: 0.7% Songklanagarind 196T>A: 0.7% Silent mutation 1311C>T: 6.7%	Kerala-Kalyan (949G>A): 2.9%	Not tested
Uncharacterized	0	0	11.80%	0	0	17.90%	9%	17.10%	23.10%

G6PD, glucose 6 phosphate dehydrogenase; MPTP, multiplex polymerase chain reaction by multiple tandem forward primers and a common reverse primer assay, PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SSCP, single-strand conformation polymorphism.

and further studies of these possible novel variants will be needed.

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