

MUTATION IN BRIEF

Glucose-6-Phosphate Dehydrogenase (G6PD) Mutations in Thailand: G6PD Viangchan (871G>A) Is the Most Common Deficiency Variant in the Thai Population

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary disorder in humans. Through a population study for G6PD deficiency using a cord blood quantitative G6PD assay in Bangkok, Thailand, we found that the prevalence of G6PD deficiency is 11.1% in Thai male (N=350) and 5.8% in female (N=172) cord blood samples. Among the neonates with hyperbilirubinemia, the prevalence of G6PD deficiency is 22.1% in males (N=140) and 10.1% in females (N=89). We developed a PCR-restriction enzyme-based method to identify G6PD Viangchan (871G>A), and searched for this and 9 other mutations in DNA from G6PD deficient blood samples. G6PD Viangchan (871G>A) was the most common mutation identified (54%), followed by G6PD Canton (1376G>T; 10%), G6PD Mahidol (487G>A; 8%), G6PD Kaiping (1388G>A; 5%), G6PD Union (1360C>T; 2.6%) and “Chinese-5” (1024C>T; 2.6%). Among 20 neonates with hyperbilirubinemia, G6PD Viangchan was also most frequently identified (60%), followed by G6PD Canton (10%), G6PD Mahidol, G6PD Union, and G6PD Kaiping (5% each). G6PD Viangchan appears from this study to be the most common G6PD mutation in the Thai population, bringing into question previous reports that G6PD Mahidol is most prevalent. G6PD Viangchan, together with G6PD Mahidol and G6PD Canton, are responsible for over 70% of G6PD deficiency in this study of Thais. With the data from other Southeast Asian ethnic groups such as Laotians, G6PD Viangchan (871G>A) is probably the most common variant in non-Chinese Southeast Asian population. © 2002 Wiley-Liss, Inc.

KEY WORDS: glucose-6-phosphate dehydrogenase; G6PD; hyperbilirubinemia; neonatal screening; Thai

INTRODUCTION

Glucose – 6 phosphate dehydrogenase (G6PD, MIM# 305900) is an enzyme in hexose monophosphate pathway. G6PD deficiency is the most common ailment caused by enzyme disorder of human (WHO, 1989). There have been four known clinical syndromes associated with G6PD deficiency, namely: oxidative stress-induced hemolysis (Carson et al., 1956, Beutler 1959), favism, neonatal jaundice, chronic non-spherocytic hemolytic anemia (Beutler, 1994). Neonatal jaundice occurs mostly in the Mediterranean and Asian G6PD deficient infants (Maisel, 1994). In

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Thailand, 65% of severe jaundice in infancy has G6PD deficiency (Sasanakul et al., 1989).

Severe neonatal hyperbilirubinemia is a well known cause of kernicterus and death (Brown and Boon, 1968). In Thailand, 19.7% of hyperbilirubinemia is caused by G6PD deficiency (Tanpaichitr et al., 1995). Phototherapy, exchange transfusion, education and surveillance have been shown to reduce these complications (WHO, 1989).

G6PD has been studied biochemically, and more than 400 variants of the enzyme have been identified. Currently, more than 68 mutants have been characterized at the DNA level (Beutler, 1994). Specific G6PD variants are found among the people of various ethnic groups. In Asia, G6PD Canton has been found to be the most common variant among the Chinese in Taiwan (Huang et al., 1996), China, and Malaysia (Ainoon et al., 1999). There are few population studies published on G6PD mutation in other Asian ethnic groups. In Thailand, G6PD Mahidol (487G>A) was believed to be the most common G6PD variant in Thailand (Panich et al., 1992); however, it has not been confirmed in population studies. In this report, we conducted a population screening for G6PD deficiency from cord blood samples, and identified G6PD deficient mutations in The Thai population as well as in newborns with neonatal jaundice and found G6PD Viangchan (871G>A) to be the most common mutation.

MATERIAL AND METHODS

Five hundred and twenty nine samples of umbilical cord blood were randomly obtained from the delivery room of King Chulalongkorn Memorial Hospital, Bangkok, Thailand. For each sample, 5 ml of cord blood was mixed with acid-citrate-dextrose (ACD) and stored at 4°C until assayed within 3 days from the collection. Peripheral blood samples were taken from jaundiced newborns at the nursery during the first 7 days of life. Serum total bilirubin levels were determined by its optical property using Reichert-Jung unistat bilirubinometer. Newborns whose serum bilirubin above 13 mg/dl were included and assessed for G6PD deficiency.

G6PD activity assay

G6PD activity assays were performed according to the WHO-recommended standard test (Betke et al., 1967) with a minor modification. Two ml of citrated blood was washed with cold normal saline 3 times with removal of buffy coat. The washed red cells were assayed for hemoglobin level, then 50 µl of the washed red cells was lysed by mixing with 950 µl ddH₂O then frozen at -20 °C for 40 minutes. Lysed red cells were centrifuged at 3000 rpm (5000g) for 20 minutes. The hemolysate supernatant was used for G6PD enzyme activity by adding 50 µl of the hemolysate to a 950 µl assay containing buffer (0.1 M Tris-HCl pH 8.0, 10 mM MgCl₂), glucose-6-phosphate (0.6 mM, Sigma), and NADP (0.2 mM, Sigma). The rate of NADPH generation was measured at 340 nm at 30 °C for 10 minutes. The average change of optical density per minute was calculated to determine activity of the G6PD enzyme. The G6PD activity was calculated and reported as IU per gram hemoglobin (g Hb).

In our laboratory, the normal value of the cord blood G6PD activity was 7.39±2.57 IU/g Hb in normal male (mean ± standard deviation, SD), and 6.94±2.51 IU/g Hb in normal female. G6PD deficiency was identified when the activity was less than 1.5 IU/g Hb (WHO, 1967).

DNA extraction

DNA was extracted from G6PD-deficient blood samples by using Qiaquick® Blood DNA extraction kit (Qiagen, Germany) according to manufacturer's recommendation.

Identification of G6PD mutations

For G6PD Viangchan mutation assay, a mutagenic primer pair 871F (5'-TGGCTTTCTCTCAGGTCTAG-3') and G6PD10R (5'-GTCGTCCAGGTACCCTTTGGGG-3') were used in a polymerase-chain reaction (PCR). One microliter of purified DNA from the blood sample was mixed, in 50 µl reaction, with 50 ng of each primer, 200 M each dNTP, 10 mM Tris-HCl pH 8.8, 1.5 mM MgCl₂, 50 mM KCl, 0.1% Triton X-100, 0.5 U of *Taq* polymerase (Promega). The PCR amplification was performed on the DNA thermal cycler for 1 cycle of 95°C for 5 minutes, then 35 cycles of 1 min at 95 °C, 1 min at 56 °C, 1 min at 72 °C, and final extension at 72 °C for 10 minutes. In a 30 µl reaction, 25µl of PCR product was digested with 10 U of *Xba*I (Gibco BRL) for 2 hours, and then resolved on 3% agarose gel (Metaphore, FMC Bioproduct, Rockland, ME) containing ethidium bromide.

For 95A>G, 392G>T, 487G>A, 493A>G, 592C>T, 1024C>T, 1360C>T, 1376G>T, and 1388G>A, 9 oligonucleotides with natural or mutagenic primer sets (Huang et al, 1996) were used for detection of the nine known G6PD mutations

(NSTDA BIOTEC, Bangkok, Thailand). The PCR amplification conditions were similar to G6PD Viangchan, except for the annealing temperature which was 55 °C. The PCR product was then digested with an appropriate restriction enzyme digestion set (Huang et al, 1996) (Gibco BRL) according to the manufacturer's recommendation.

For nt1311 polymorphism, three primers, G6P10F2 (5'-ATGATGACCAAGAAGCCGGGC-3'), 1311TR (5'-CGTCCAGGATGAGGCGCTCA-3') and G6P12R (5'-CTGCCATAAATATAGGGGATGGG-3') were used in a PCR following the technique described above. The PCR amplification was performed on the DNA thermal cycler: 1 cycle of 95 °C for 5 minutes, then 35 cycles of 95 °C for 1 min, 68 °C for 1 min, 72 °C for 1 min, then the final extension at 72 °C for 10 minutes. Twenty-five µl of PCR product was resolved on 3% agarose gel (Gibco BRL, Grand Island, NY) containing ethidium bromide. Presence of a 200-bp band indicates 1311C>T. Presence of 400-bp but not 200-bp band indicates wild-type nt1311.

DNA Sequencing

PCR product from G6PD exon 9 was amplified using G6PD9F (5'-AGCTGCAGGCCAACAATGTGGT-3') and G6PD10R. The 360 bp amplicon was used as template and the DNA sequence was determined using ABI prism 310 Genetic Analyser (Perkin-Elmer, Norwalk, CT) following manufacturer's recommendation using G6PD9F as primer.

RESULTS

Prevalence of G6PD deficiency

From the 522 cord blood samples, we identified G6PD deficiency in 11.1% of Thai male (N=350) and 5.8% of female (N=172). Among neonates with hyperbilirubinemia, the prevalence of G6PD deficiency was 22.1% in male (N=140) and 10.1% in female (N=89).

Prevalence of G6PD mutations

The G6PD deficient DNA samples were assayed for the known G6PD mutations. The first mutation assessed in this study was G6PD Mahidol (487G>A), because it was thought to be the most common variant among Thai G6PD-deficient individuals (Panich et al., 1992). Out of 39 G6PD-deficient male cord blood samples, only 3 (7.7%) was found to be G6PD Mahidol (Table 1). We then searched for eight other mutations and identified 4 (8%) cases of G6PD Canton (nt1376 mutation), 2 (4%) cases of G6PD Kaiping (nt1388 mutation), 1 case (2%) of G6PD Union (nt 1360 mutation), and 1 case (2%) of "G6PD chinese-5" (nt 1024 mutation). No case of G6PD Gaohe (95A>G), "Chinese-4" (392G>T), "Chinese-3" (493A>G), or G6PD Coimbra (592C>T) was identified.

To search for unknown mutation, we then performed a PCR amplification of exon 9 on one of the unidentified G6PD-deficient DNA sample and determined the DNA sequence. A mutation at nt871 from G to A, known as G6PD Viangchan, was found. We then developed a PCR-based assay for G6PD Viangchan using a mutagenic 5'-primer (871F) and a 3'- primer (G6PD9R) to amplify exon 9. A 126-bp amplicon from this PCR assay was then digested by restriction enzyme *Xba*I digestion, which can cleave 871G>A mutant, but not wild-type, amplicon to 106-bp (Figure 1). Using this PCR-based assay, G6PD Viangchan was identified in 21 of 39 male cord blood samples (53.8%) as well as 12 of 20 peripheral blood samples from jaundiced newborn (60%). (Table 1) Approximately half of G6PD Viangchan had undetectable cord blood G6PD activities. To distinguish G6PD Viangchan from G6PD Jammu, which differs at a non-coding nt 1311, two allele-specific oligonucleotide primer sets were used. We found that in all samples with 871G>A, nt 1311 was T, consistent with G6PD Viangchan (data not shown).

From 10 G6PD-deficient female cord blood samples, 6 were G6PD Viangchan, while 4 remained unidentified. All female samples were most likely heterozygote since residual G6PD activity were found, ranging from 0.57 to 1.60 IU/g Hb and amplicon was partially digested (Figure 1). Similarly, among 7 samples from G6PD-deficient females with neonatal jaundice, 4 were G6PD Viangchan.

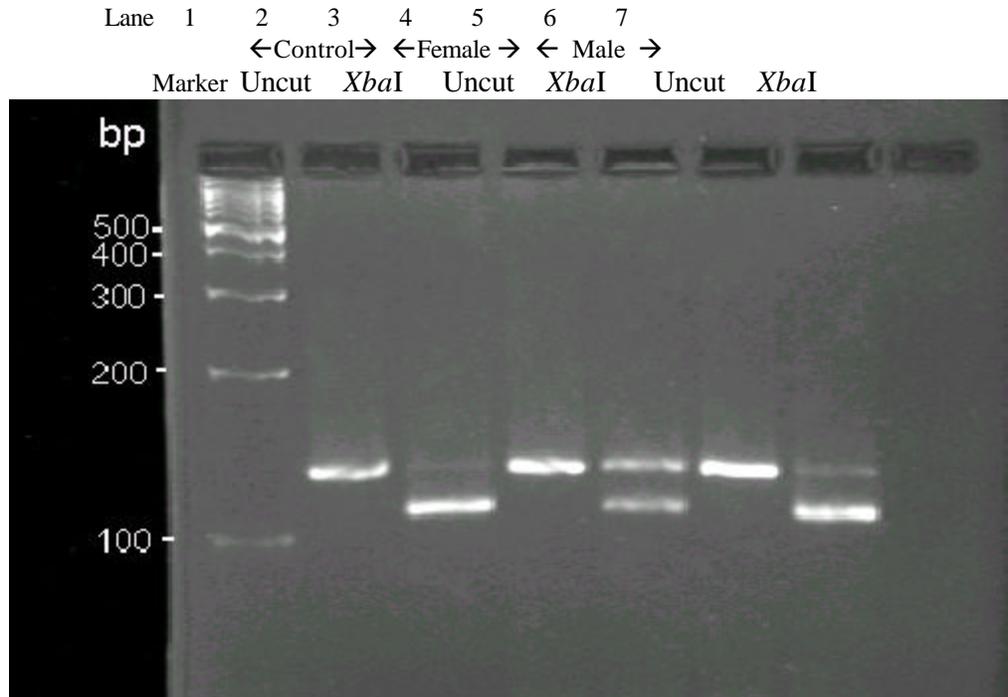


Figure 1. PCR-restriction enzyme assay for G6PD Viangchan. PCR was performed on genomic DNA from normal known G6PD Viangchan (control) showed a 126-bp band (uncut) that reduce to 106 bp after *Xba*I digestion (*Xba*I). Female heterozygote show both 126- and 106-bp bands after *Xba*I digestion.

Table 1. Prevalence of G6PD mutations and their activity from male G6PD-deficient cord blood samples or peripheral blood sample from male neonatal jaundice

Mutation	Cord blood			Neonatal jaundice		
	Number (%)	G6PD activity (IU/ g Hb)		Number (%)	G6PD (IU/ activity g Hb)	
		Median	Range		Median	Range
871G>A (G6PD Viangchan)	21 (53.8%)	0.12	0.00 - 1.03	12 (60.0%)	0.14	0.00 -1.50
1376G>T (G6PD Canton)	4 (10.3%)	0.39	0.16 - 1.44	2 (10.0%)	0.29	0.21, 0.37
487G>A (G6PD Mahidol)	3 (7.7%)	0.11	0.00 - 0.44	1 (5.0%)	0.09	-
1388G>A (G6PD Kaiping)	2 (5.1%)	0.09	0.00, 0.17	1 (5.0%)	1.13	-
1360C>T (G6PD Union)	1 (2.6%)	0.00	-	1 (5.0%)	0.00	-
1024C>T (G6PD “chinese-5”)	1 (2.6%)	0.50	-	0	-	-
Unknown	7 (17.9%)	0.00	0.00 - 1.05	3 (15.0%)	0.00	0.0 - 0.17
Total	39 (100%)			20 (100%)		

DISCUSSION

We have identified G6PD Viangchan as the most common variant among the Thai population. With 21 cases identified among 350 male cord blood samples, the gene frequency of G6PD Viangchan among the Thai population was calculated to be 0.06. Consistent with this finding, heterozygous deficient female was also found in 6 out of 172, indicating that some of the female G6PD Viangchan heterozygotes was not in deficient range.

G6PD Viangchan (MIM# 305900.0026) was first characterized biochemically in 1988 from a Laotian G6PD-deficient patient in Canada (Poon et al, 1988). This G6PD variant was categorized as a WHO class 2, or severely deficient, variant (WHO, 1989). G6PD Viangchan was subsequently defined at a DNA level to be a nucleotide substitution at nt871 from G to A, predicting aa substitution of an amino acid 291 from Val to Met (Beutler et al., 1991). Nucleotide substitution 871G>A was also found in G6PD Jammu (Beutler et al., 1991) which was found in a patient from India. These two variants differ at a nucleotide 1311 polymorphism, where it was C in G6PD Jammu, and T in G6PD Viangchan.

G6PD Viangchan was reported as a common variant among Laotian people (5 of 9 G6PD-deficient subjects) based on a small transplanted population in Hawaii (Hsia et al., 1993). Our finding that the gene frequency of G6PD Viangchan is high among the Thais and Laotians suggested a common ancestry of the people from this region. In contrast, G6PD Viangchan was found in 10% among the Filipinos (6 of 53) (Hsia et al., 1993), and rarely (1 in 112 G6PD-deficient male neonates) among the Chinese population (Huang et al, 1996).

In contrast to the previous study (Panich et al., 1972), we did not find that G6PD Mahidol was the most common G6PD variant in Thailand. Historically, G6PD Mahidol was named after the university where it was identified biochemically in 1972, and assessed as a mild (WHO class 3) variant. Among 22 patients with acute hemolysis, G6PD Mahidol was identified in most cases. Subsequently, DNA analysis identified the point mutation at nucleotide 487 with substitution of G with A, which changed translation of amino acid 163 from glycine to serine (Vulliamy et al., 1989). In our study, we identified G6PD Mahidol in less than 10% of G6PD-deficient population. The difference between our finding and Panich (1972) may be explained by the different population surveyed and the technique used in mutation analysis. Our report, which is based on a relatively large population of unselected individuals through cord blood samples at the DNA level, would better represent the general Thai population, while the Panich study used biochemical assays to study patients with acute hemolysis. It remains possible that G6PD Mahidol, but not Viangchan, is associated only with episodic hemolysis. The ease of PCR-based assay would allow us to study this mutation in hemolytic patients in a subsequent study.

In contrast to multi-ethnic Malaysia and Singapore, the majority of the Thai population consists of native Thai and assimilated Chinese. Similar to Malaysia and Singapore, Chinese immigrants were mostly from Fushan and Guangdong province of two earlier generations. Estimated at 20%, the proportion of ethnic Chinese in Thailand was uncertain because of assimilation with the Thai population. G6PD Canton was the most prevalent (50%) among the Chinese, and was found to be the second most common variant (10%) in our study. This subpopulation could be the descendant of Chinese immigrants into the Thai population. The other less common Chinese variants, G6PD Kaiping, G6PD Union, and G6PD "chinese-5" were also identified in the proportionately smaller number.

In contrast to G6PD Canton, which had been shown to be related to severe hyperbilirubinemia (Huang et al., 1996), there is no trend toward a relationship between G6PD Viangchan and hyperbilirubinemia. The proportion of this to other mutations in G6PD-deficient jaundiced newborn is similar to that found in general population, implied by cord blood study. Similar to G6PD deficiency at large, G6PD Viangchan contributes to a relatively late onset of hyperbilirubinemia. The level of bilirubin and date of onset is indistinguishable from other mutations.

In summary, our findings suggest that G6PD Viangchan is a hallmark of Thai ethnicity, similar to the hemoglobin E allele in this population (Wasi, 1967). The high prevalence of G6PD Viangchan and hemoglobin E alleles could be a result of malarial selection pressure in this region.

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