

Original Article

Prevalence of Glucose 6-Phosphate Dehydrogenase Deficiency and Molecular Genetics of G6PD in Thai Population of Phayao Province

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Background: Glucose 6-phosphate dehydrogenase (G 6-PD) plays an important role in maintenance of cellular redox homeostasis. Mutations in the G6PD gene cause G 6-PD deficiency, which is highly prevalent in malaria endemic area. Currently, there has been no report of G 6-PD deficiency in Phayao province. **Objective:** To determine the prevalence of G 6-PD deficiency and its mutations in Thai population of Phayao province. **Materials and Methods:** Four hundred blood samples from 9 districts of Phayao province were screened for G 6-PD deficiency using fluorescent spot test (FST) and for mutations by PCR-RFLP technique. **Result:** The prevalence of G 6-PD deficiency was 9.1% (24/263) in Phayao males and 5.1% (7/137) in females, highest in Maejai district (6/45, 13.3%), and none in Pong, Chiangkham and Chiangmuan districts. The most common mutations were G6PD Union (c.1360C>T; 7/263; 0.03) and G6PD Kaiping (c.1388G>A; 7/263; 0.03), followed by G6PD Canton (c.1376G>T; 2/263; 0.008), G6PD Viangchan (c.871G>A; 2/263; 0.008), and G6PD Chinese-5 (c.1024C>T; 1/263; 0.004). **Conclusion:** The prevalence of G 6-PD deficiency among Thais in Phayao is lower than other Northern Thai populations. G6PD Union and G6PD Kaiping are distinctly predominant, suggesting an influence from Chinese gene pools.

Keywords : ● G 6-PD deficiency ● G6PD gene ● Phayao province**J Hematol Transfus Med 2015;25:131-7.****Introduction**

Glucose 6-phosphate dehydrogenase (G 6-PD) is an enzyme in the pentose phosphate pathway (PPP). It catalyzes the synthesis of nicotinamide adenine dinucleotide phosphate (NADPH), which is a coenzyme that reduces glutathione for defending red blood cell from oxidation.¹ In people with G 6-PD deficiency, an oxidant stress from certain drugs or fava bean ingestion can cause acute hemolysis.²

G 6-PD deficiency is highly prevalent in Thailand,

which is approximately 11-17%.³⁻⁵ Molecular genetic studies show that the most common mutation in G 6-PD deficient Thais are G6PD Viangchan,⁵ which is similar to Cambodians,⁶ and Laotians.⁷ In Northern Thailand, a report from Chiangmai reveals a prevalence of 17% in males,⁴ with the G6PD Mahidol as the most common mutation.

Phayao is province in Northern Thailand, located between latitude 18°44' and 19°44' North and longitude 99°40' and 100°40' East, and bordered by Chiangrai, Lampang, Phrae, Nan, and Laos. Phayao has 9 districts (Figure 1) and a population of 487,589, mostly farmer (61%),⁸ and speaks Phayap dialect of Eastern Lanna language.⁹ Phayao, 735 km north of Bangkok, is much closer to Myanmar, Laos, and China, and has no previous report

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on G 6-PD deficiency. The authors therefore studied the prevalence and mutations of G 6-PD deficient Thai people in Phayao.

Materials and Methods

Population and Samples

Four hundred unrelated volunteer blood donors, age > 20 years, were invited to participate in the study. Yamane's equation was used to calculate the sample size of each district, which depended on the size of population in each district. Blood donors in each district were randomly selected. The study protocol were provided in Thai language and consented participant were asked to confirm their ethnic group and original district of domicile (Figure 1), and additional 3 mL of blood was collected during blood donation, preserved and stored in EDTA at 2-4°C, and screened for G 6-PD deficiency within 6 hours. The study was approved by the ethics committee of Phayao University, certificate number 5502010017.

G 6-PD screening

Screening for G 6-PD deficiency was performed by a fluorescence spot test (FST, R&D Diagnostics) and visualized under ultraviolet (UV) light. Presence of fluorescence was assessed as normal G 6-PD, while absence of fluorescence was assessed as G 6-PD deficiency.

G6PD mutations assay

For G 6-PD deficiency samples, DNA was extracted from the blood sample using Chelex DNA extraction,¹⁰ and assayed for common G6PD mutations including G6PD Mahidol (c.487G>A), G6PD Viangchan (c.871G>A), G6PD Union (c.1360C>T), G6PD Canton (c.1376G>T), G6PD Kaiping (c.1388G>A), and G6PD Chinese-5 (c.1024C>T) using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described previously.^{5,11} In brief, PCR mixture (10 µL) contained 1 µL of DNA (50 ng/µL), 0.4 µM each primer, 200 µM each dNTPs and 1 U Taq DNA polymerase (Invitrogen) in 1X PCR buffer and 1.0 mM MgCl₂. After 10-min initial denaturation



Figure 1 Prevalence of G 6-PD deficiency in Phayao province

of DNA at 95°C, 35 cycles of 95°C for 30 sec, 56°C for 30 sec and 72°C for 30 sec were performed, followed by a final extension at 72°C for 7 min in a Veriti® 96-well thermal cycler (Applied Biosystems). The PCR products were digested with appropriate restriction enzymes and analyzed by 6% polyacrylamide gel-electrophoresis with SYBR Safe DNA gel stain (Invitrogen). Results were visualized under UV light using Gel Doc™ XR+ System (BioRad). Primer sequences and restriction enzymes are shown in Table 1.

Data analysis

Data were analyzed using descriptive statistics, including percentage of G 6-PD deficiency in males and females. Allele frequencies were determined by direct counting and then divided by the total number of male chromosomes only.

Results

Prevalence of G 6-PD deficiency

Of 400 Phayao Thais, 263 were males and 137 were females. The prevalence of G 6-PD deficiency was 9.1% (24/263) in males and 5.1% (7/137) in females. The prevalence was highest in Maejai district (6/45, 13.3%), between 8-12% in Phukamyao, Phuzang, Muang, Dokkhamtai, Jun and none in the easternmost districts

(Pong, Chiangkham and Chiangmuan) (Figure 1 and Table 2).

G6PD deficient mutations among Phayao Thais

Of the 6 mutations assayed in 31 G 6-PD deficient subjects (24 males and 7 females), the common mutations were G6PD Union (7/263 males; allele frequency = 0.03) and G6PD Kaiping (7/263; 0.03), followed by G6PD Canton (c.1376G>T; 2/263; 0.008), G6PD Viangchan (c.871G>A; 2/263; 0.008), and G6PD Chinese-5 (c.1024C>T; 1/263; 0.004). G6PD Mahidol was not identified in any deficient subjects, and G6PD mutation was not identified in 5 deficient males (Table 3). Of 7 deficient females, 2 were heterozygote for G6PD Union, 1 were G6PD Kaiping, and 4 were unknown mutations.

Discussion

G 6-PD deficiency has been recognized as a common inherited enzymopathy among Thai population. This study is the first report of G 6-PD deficiency prevalence and types of mutation among Thais in Phayao province. The prevalence (9.1%) of Phayao Thais in our study was lower than that of Chiang Mai province (17%),⁴ Bangkok (11%),⁵ but was comparable to Laotian (7.2%),⁷ Sikka people of Flores island (8.5%).¹² Similar to most studies, the prevalence of G 6-PD deficiency in males

Table 1 Primers and restriction enzymes in G6PD mutations assay

G6PD mutation	Primer sequence	Restriction enzyme	Product size (bp)
Mahidol ¹¹ (c.487G>A)	F: 5'-GCGTCTGAATGATGCAGCTCTGAT-3'	<i>Hind</i> III	N: 104
	R: 5'-CTCCACGATGATGCGGTTCAAGC-3'		M: 82, 22
Viangchan ⁵ (c.871G>A)	F: 5'-TGGCTTTCTCTCAGGTCTAG-3'	<i>Xba</i> I	N: 126
	R: 5'-GTCGTCCAGGTACCCTTTGGGG-3'		M: 106, 20
Canton ¹¹ (c.1376G>T)	F: 5'-GTGAAAATACGCCAGGCCTTA-3'	<i>Afl</i> II	N: 214
	R: 5'-GTGAAAATACGCCAGGCCTTA-3'		M: 194, 20
Union ¹¹ (c.1360C>T)	Same as Canton	<i>Hha</i> I	N: 142, 45, 27 M: 187, 27
Kaiping ¹¹ (c.1388G>A)	F: Same as Canton	<i>Nde</i> I	N: 227
	R: 5'-GTGCAGCAGTGGGGTGAACATA-3'		M: 206, 21
Chinese-5 ¹¹ (c.1024C>T)	F: 5'-GTCAAGGTGTTGAAATGCATC-3'	<i>Mbo</i> II	N: 187
	R: 5'-CATCCCACCTCTCATTTCTCC-3'		M: 150, 37

N, normal sequence; M, mutant sequence

Table 2 Prevalence of G 6-PD deficiency in 9 districts

District	Male		Female	
	Number of samples	G 6-PD deficient cases	Number of samples	G 6-PD deficient cases
Maejai	45	6	14	1
Phukamyao	33	4	31	3
Phuzang	9	1	1	0
Muang	81	8	45	2
Pong	15	0	2	1
Dokkhamtai	43	4	28	0
Jun	12	1	7	0
Chiangkham	18	0	4	0
Chiangmuan	7	0	5	0
Total	263	24	137	7

Table 3 G6PD gene mutations in Thai population of Phayao province

G6PD mutations	Nucleotide change	Allele frequency	Number identified	
			Hemizygote	Heterozygote
Union	1360C>T	0.03	7	2
Kaiping	1388G>A	0.03	7	1
Canton	1376G>T	0.008	2	0
Viangchan	871G>A	0.008	2	0
Chinese-5	1024C>T	0.004	1	0
unknown			5	4

was higher than in females. It could be explained by several reasons. First, G 6-PD deficiency is an X-linked disorder. Random X chromosome inactivation enhances mosaicism in heterozygous females leading to diversity of phenotypes; normal, intermediate or grossly deficient G6PD activity. Second, FST is a semi-quantitative technique in screening for G 6-PD deficiency. Therefore, intermediate phenotype of heterozygous females may be misclassified as normal.

The three most common mutations among Thais of Phayao are G6PD Union, Kaiping, and Canton. G6PD Union has been reported most commonly in Italians,¹³⁻¹⁶ and were the less common G6PD deficient alleles in Chinese,¹⁷⁻¹⁸ Thais,¹⁹ Filipinos,²⁰ Myanmar,⁷ Southwest Pacific Islander,²¹ Singaporean Chinese,²² Vietnamese,²³ and Malaysian Chinese.²⁴ The occurrence of G6PD Union in diverse population and the lack of relationship between Italians and Asian people suggest independent

origins of G6PD Union, the allele of which is located in CpG dinucleotide.¹⁶

G6PD Canton and G6PD Kaiping are the most common mutations among Chinese,^{18,25} and Thais in the southern part,²⁶⁻²⁷ but are the less common G6PD deficient alleles among Thais in Bangkok,⁵ Chiang Mai,⁴ Malaysian Chinese,²⁴ Malays,²⁸ and Singaporean Chinese.^{22,29} G6PD Chinese-5 has similar distribution in these ethnic groups. While G6PD Canton is more common than G6PD Kaiping among Chinese, G6PD Kaiping is more common in our studied population. Similarly, Sikka people of Flores island in Indonesia has a high prevalence of G6PD Kaiping with no G6PD Canton,¹² suggesting founder effect of Chinese people who settled in Flores island. It is likely that founder effect is at work with Phayao people. This was supported by a historical record from 1788 when a Naan head of state of Naan moved Tai people from Shan state (Burma) and Tai Lue people

from Southern China into Nan and Phayao.³⁰⁻³¹ Despite similar G 6-PD deficiency profile, there is no evidence to suggest a relationship between Phayao people and Flores islanders.

In contrast to most Thais,⁵ Khmer,⁶ and Laotians,⁷ G6PD Viangchan is rarely found in Phayao people who speaks Thai language. Likewise, G6PD Mahidol, the common alleles in Burmese,³² Mon,³² and Karen people,³³ is not identified in people of Phayao. Taken together with the predominance of G6PD Union, Kaiping, Canton and Chinese-5, Phayao people are likely assimilated Chinese in Thai speaking society. All G6PD Union individuals found in Phayao people are of Chinese origin. Even though G6PD Union was not common in Chinese, the few Chinese with this allele may have migrated and became the majority group in Phayao province, which gave rise to G6PD Union allele and reduce G6PD Viangchan allele.

Conclusions

The prevalence of G 6-PD deficiency among Thais in Phayao is lower than other Northern Thai populations. G6PD Union and G6PD Kaiping are distinctly predominant, suggesting an influence from Chinese gene pools.

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ความชุกของภาวะพร่องเอนไซม์กลูโคส 6-ฟอสเฟต ดีไฮโดรจีเนส และความหลากหลายทางพันธุกรรมของยีนจีซีพีดีในคนไทยจังหวัดพะเยา

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บทคัดย่อ

ที่มา เอนไซม์กลูโคส 6-ฟอสเฟต ดีไฮโดรจีเนส (G 6-PD) ช่วยรักษาสสมดุลรีดอกซ์ของเซลล์ ภาวะพร่องเอนไซม์ G 6-PD เกิดจากการกลายพันธุ์ของยีน G6PD ซึ่งพบได้บ่อยในบริเวณที่มีมาลาเรียระบาด โดยเฉพาะภาคเหนือของประเทศไทย ปัจจุบันยังไม่พบรายงานภาวะพร่องเอนไซม์ G 6-PD ในจังหวัดพะเยา **วัตถุประสงค์** เพื่อศึกษาความชุกของภาวะพร่องเอนไซม์ G 6-PD และการกลายพันธุ์ของยีน G6PD ในคนไทยจังหวัดพะเยา **วัสดุและวิธีการ** ทดสอบภาวะพร่องเอนไซม์ G 6-PD ในคนไทยจังหวัดพะเยาทั้งหมด 9 อำเภอ จำนวน 400 ราย ด้วยวิธี fluorescent spot test (FST) และศึกษาการกลายพันธุ์ของยีน G6PD ด้วยวิธี PCR-RFLP **ผลการศึกษา** พบภาวะพร่องเอนไซม์ G 6-PD ร้อยละ 9.1 ในเพศชาย (24/263 ราย) และร้อยละ 5.1 ในเพศหญิง (7/137 ราย) โดยอำเภอแม่ใจ พบภาวะพร่องเอนไซม์ G 6-PD ในเพศชายมากที่สุด คิดเป็นร้อยละ 13.3 (6/45 ราย) ส่วนอำเภอปง เชียงคำ และเชียงม่วน ไม่พบภาวะพร่องเอนไซม์ G 6-PD นอกจากนี้ยังพบการกลายพันธุ์ G6PD Union (c.1360C>T; 7/263 ราย; 0.03), G6PD Kaiping (c.1388G>A; 7/263 ราย; 0.03), G6PD Canton (c.1376G>T; 2/263 ราย; 0.008), G6PD Viangchan (c.871G>A; 2/263 ราย; 0.008) และ G6PD Chinese-5 (c.1024C>T; 1/263 ราย; 0.004) **สรุป** คนไทยจังหวัดพะเยามีภาวะพร่องเอนไซม์ G 6-PD ต่ำกว่าคนไทยชาวเหนือและภูมิภาคอื่นๆ และพบ G6PD Union และ G6PD Kaiping เป็นชนิดที่พบบ่อยต่างจากประชากรไทยส่วนใหญ่ ซึ่งอาจมาจากพันธุกรรมของชาวจีน

Keywords : ● ภาวะพร่องเอนไซม์ G 6-PD ● ยีน G6PD ● จังหวัดพะเยา

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2558;25:131-7.

