Molecular Identification of G-6-PD Mutations in Neonates with G-6-PD Deficiency at Srinagarind Hospital

การวิเคราะห์การกลายพันธุ์ของยีนจี-6-พีดีในทารกแรกเกิดที่มีภาวะพร่องเอนไซม์จี-6-พีดี ที่โรงพยาบาลศรีนครินทร์

Sumalai Dechyotin (สุมาลัย เดชโยธิน)* Dr.Suttiphan Kitcharoen (ดร.สุทธิพรรณ กิจเจริญ)**
Dr.Supan Fucharoen (ดร.สุพรรณ ฟู่เจริญ)***

ABSTRACT

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is the most common genetic enzyme disorder. The most devastating potential complication of G-6-PD deficiency in neonates is an acute hemolytic crisis which may result in severe hyperbilirubinemia and kernicterus. The molecular characterization of the G-6-PD mutations has been reported in various parts of Thailand but there has never been reported in the northeast Thailand. Thus, the aim of this study was to identify G-6-PD mutations in G-6-PD deficiency patients at Srinagarind Hospital. Two hundred and thirty three G-6-PD deficient samples were investigated for G-6-PD mutations commonly found in Thailand by PCR-RFLP. The two most common G-6-PD mutations were G-6-PD Viangchan 54.5% and G-6-PD Canton 14.2%. This study also investigated the relationship between various G-6-PD mutations and the enzymatic activity. The finding would be applicable for setting up molecular diagnosis for G-6-PD mutations in this region.

บทคัดย่อ

ภาวะพร่องเอนไซม์ G-6-PD เป็นความผิดปกติทางพันธุกรรมของเอนไซม์ที่พบมากที่สุด ภาวะแทรกซ้อนที่สำคัญของภาวะพร่องเอนไซม์ G-6-PD ในทารกแรกเกิด คือภาวะเม็ดเลือดแดงแตกเฉียบพลันอย่างรุนแรง ซึ่งอาจนำไปสู่ภาวะตัวเหลืองจัดและสมองพิการอย่างถาวรได้ มีรายงานการกลายพันธุ์ของยีน G-6-PD ในภูมิภาคต่างๆของประเทศไทย แต่ยังไม่มีรายงานในภาคตะวันออกเฉียงเหนือ ดังนั้นการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาการกลายพันธุ์ของยีน G-6-PD ในผู้ที่มีภาวะพร่องเอนไซม์ G-6-PD จากโรงพยาบาลศรีนครินทร์ จำนวน 233 ราย โดยใช้เทคนิค PCR-RFLP ผลการศึกษาพบการกลายพันธุ์ของยีน G-6-PD ที่พบมากที่สุด 2 อันดับแรก คือ G-6-PD Viangchan พบร้อยละ 54.5 และ G-6-PD Canton พบร้อยละ 14.2 นอกจากนี้ได้ศึกษาระดับเอนไซม์ G-6-PD ในการกลายพันธุ์แต่ละชนิดด้วย ผลที่ได้จากการการศึกษานี้จะมีประโยชน์อย่างยิ่งต่อการจัดตั้งการวินิจฉัยระดับโมเลกุลเพื่อตรวจหาการกลายพันธุ์ของยีน G-6-PD ในภูมิภาคนี้

Key Words: G-6-PD deficiency, G-6-PD mutations
คำสำคัญ: ภาวะพร่องเอนไซม์ G-6-PD การกลายพันธุ์ของยีน G-6-PD

* *Student, Master of Science in Medical Science, Faculty of Associated Medical Sciences, Khon Kaen University*
** *Assistant Professor, Department of Clinical Microscopy, Faculty of Associated Medical Sciences, Khon Kaen University*
*** *Associate Professor, Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University*

Introduction

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is the most common genetic enzyme disorder known to affect 300 to 400 million individuals worldwide (Beutler, 1994; Luzzatto, 2006). The most devastating potential complication of G-6-PD deficiency in neonates is an acute hemolytic crisis, causing severe hyperbilirubinemia, which may result in irreversible bilirubin-induced neurologic damage (BIND), known as kernicterus (Kaplan et al.,2010). G-6-PD deficiency is a major risk factor for kernicterus because of both increased bilirubin production (which may or may not have been exacerbated by a hemolytic process) and concurrent diminished bilirubin conjugation. Exacerbation with co-expression of genetic mutations involved with bilirubin conjugation and elimination and/or a triggered increase in bilirubin production may lead to progressive or hazardous hyperbilirubinemia (Bhutani, 2012). The incidence of neonatal hyperbilirubinemia has been shown to be several folds greater in G-6-PD deficient population than in the G-6-PD normal population. However, this incidence is not constant and may vary between population groups and from geographic area to geographic area (Kaplan and Hammerman, 2004).

G-6-PD deficiency is initially characterised biochemically into five classes based on WHO guidelines. To date, about 160 mutations have been reported, most of which are single-base substitutions leading to amino acid replacements (Minucci et al., 2009). The advances in molecular techniques have allowed the molecular characterization of the *G-6-PD* gene in any population. In Southeast Asia, a large number of G-6-PD deficient variants have been reported from various populations and G-6-PD Viangchan (871G>A) seems to be the most common variant in Thais and Laotian (Iwai et al.,2001; Nuchprayoon et al., 2002), whereas G-6-PD Mahidol (487G>A) is reported to be the most common variant in Myanmar (Iwai et al.,2001).

In Thailand, G-6-PD Viangchan is reported to be the most common mutation in the southern and central (31.3% and 54% respectively). In contrast, in the northern Thailand, G-6-PD Mahidol is the most common mutation (20%) in neonates (Charoenkwan et al., 2010). Other mutations have been reported in Thailand including G-6-PD Kaiping, G-6-PD Canton, G-6-PD Union, G-6-PD Chinese-4, G-6-PD Chinese-5 and G-6-PD Gaohe (Iwai et al., 2001; Nuchprayoon et al., 2002; Laosombat et al., 2005; Charoenkwan et al., 2010; Ninokata et al., 2006; Phompradit et al., 2011). In the northeast Thailand, Kittiwatanasarn et al. (2003) reported that the prevalence of G-6-PD deficiency in male was 21.7% but there has been no report on the molecular characterization of G-6-PD deficiency.

Objectives of the study

This study aims to identify G-6-PD mutations in northeast Thai populations which will provide useful data for setting up molecular diagnosis of G-6-PD mutations in this region.

Methodology

Subjects

This study was conducted as a part of the main research project entitled “Genetic polymorphism of *UGT1A1* gene and *G-6-PD* gene in neonates with hyperbilirubinemia” that has been approved by the Institutional Review Board (IRB) of Khon Kaen University, Khon Kaen, Thailand (HE551273). The samples used in this study were leftover ethylene

diamine tetra acetic acid (EDTA) anticoagulated blood from the Diagnostic Microscopy Unit, Clinical Laboratory Division, Srinagarind Hospital, Khon Kaen University. They were screened for G-6-PD deficiency by fluorescent spot test (International Council for Standardization in Hematology recommendation) (Beutler et al., 1979). The G-6-PD deficient samples were recruited for G-6-PD activity assay and DNA analysis.

G-6-PD activity assay

The G-6-PD activity assay was performed using an automated analyzer, Beckman Synchron CX4, according to manufacturer's instruction (G-6-PDH kit, Trinity Biotech, Jamestown, USA). The principle of the assay is as follows:

$$\text{G-6-P} + \text{NADP}^{+} \xrightarrow{\text{G-6-PD}} \text{6-PG} + \text{NADPH}$$

Nicotinamide adenine dinucleotide phosphate (NADP) is reduced by G-6-PD in the presence of G-6-P. The rate of NADPH formation is proportional to the G-6-PD activity and is measeared as an increase in absorbance at 340 nm. The manufacturer reference ranges of G-6-PD activity is 7 – 20.5 U/gHb.

Detection of G-6-PD mutations

All samples were investigated for eight common G-6-PD mutations found in Thai populations by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers and restriction enzymes were designed according to Nuchprayoon et al. (2002). G-6-PD Viangchan was firstly identified in all samples. Then samples negative for G-6-PD Viangchan were subsequently subjected to identification for other mutations, starting from G-6-PD Mahidol and then G-6-PD Canton, G-6-PD Union, G-6-PD Kaiping, G-6-PD Chinese-5, G-6-PD Chinese-4 and G-6-PD Gaohe, respectively.

Statistical analysis

The statistical analysis was performed using Minitab statistical software version 14 (Minitab, PA, USA). Descriptive statistics, mean and standard deviation (SD), were applied as the representative values for each group. Mann-Whitney U test was performed to test significant difference between 2 independent variables. Comparison of difference among three or more independent variables, Kruskal Wallis test was applied. A statistically significant difference was considered at p-value < 0.05.

Results

A total of 233 G-6-PD deficient samples were including in this study. They were molecularly identified for eight G-6-PD mutations found in Thai populations. As shown in Table 1, the two most common G-6-PD mutations covering 68.7% of the studied samples were G-6-PD Viangchan (54.5%) and G-6-PD Canton (14.2%). Other G-6-PD mutations were found with lower frequencies and about 9.4% (22/233) remained uncharacterized. Three cases of compound heterozygote (G-6-PD Viangchan/G-6-PD Canton, G-6-PD Viangchan/G-6-PD Chinese-4 and G-6-PD Kaiping/G-6-PD Chinese-5) were also identified in females whereas G-6-PD Gaohe was not found in this study.

One hundred and ninety of G-6-PD deficient blood samples were quantified for G-6-PD activity. G-6-PD activity 0f various G-6-PD mutations were presented in Table 2. The G-6-PD activities in hemizygous males were lower than those of homozygous and heterozygous females, the mean±SD were 1.5±1.2, 2.7±3.2 and 3.8±2.8 U/gHb, respectively.

The statistical analysis, Kruskal-Wallis test and then Mann-Whitney U-test, was test among hemizygotes which showed significant difference in G-6-PD activities among various G-6-PD mutations (p-value < 0.05). In the group of WHO class II (G-6-PD Viangchan, G-6-PD Canton, G-6-PD Union and G-6-PD Kaiping) hemizygous males with G-6-PD Viangchan had significant higher activity than others, except G-6-PD Kaiping and those in the group of WHO class III (G-6-PD Mahidol, G-6-PD Chinese-4 and G-6-PD Chinese-5) G-6-PD Chinese-5 had the highest activity.

Table 1 Frequency of G-6-PD mutations identified in G-6-PD deficient samples.

G-6-PD Mutations	Male (N)	Female (N)		Total (%)
	Hemizygote	Homozygote	Heterozygote	
Viangchan	110	9	8	127 (54.5%)
Canton	29	2	2	33 (14.2%)
Union	9	1	4	14 (6.0%)
Kaiping	11	0	2	13 (5.6%)
Mahidol	10	0	0	10 (4.3%)
Chinese-5	6	0	1	7 (3.0%)
Chinese-4	3	1	0	4 (1.7%)
Compound heterozygote				
-Viangchan/Canton	0	1	0	1 (0.4%)
-Viangchan/Chinese-4	0	1	0	1 (0.4%)
-Kaiping/Chinese-5	0	1	0	1 (0.4%)
Unidentified	17	5		22 (9.4%)
Total	195	38		233 (100%)

Table 2 G-6-PD activities of G-6-PD deficient neonates categorized by G-6-PD mutations.

G-6-PD mutations	G-6-PD activity (U/gHb)						Total
	Male (Hemizygote)		Female (Homozygote)		Female (Heterozygote)		
	N	Mean±SD	N	Mean±SD	N	Mean±SD	
Viangchan	94	1.5 ± 1.1[a]	7	1.3 ± 1.0	4	4.3 ± 2.6	105
Canton	19	0.7 ± 0.7[a]	2	1.3, 7.9	2	1.1, 8.8	23
Union	8	0.4 ± 0.8[a]			4	5.5 ± 4.1	12
Kaiping	8	0.9 ± 0.9[a]			2	0.8, 2.1	10
Mahidol	10	1.7 ± 1.0[a]					10
Chinese-5	6	4.3 ± 0.7[a]			1		7
Chinese-4	2	0.6, 2.2	1				3
Unidentified	14	1.9 ± 1.6	3	3.7±2.6			17
Total	161	1.5 ± 1.2	16	2.7 ± 3.2	13	3.8 ± 2.8	190

Values are presented as mean±SD

[a] Kruskal-Wallis test : $p<0.05$ among 6 common G-6-PD mutations (Viangchan, Canton, Union, Kaiping, Mahidol and Chinese-5)

Discussion and Conclusions

Molecular characterization of G-6-PD mutations has been investigated in different populations of Thailand. The present study is the first report that characterized the molecular basis of *G-6-PD* gene in G-6-PD deficient neonates in the northeastern part of Thailand. Seven mutations namely G-6-PD Viangchan, G-6-PD Canton, G-6-PD Union, G-6-PD Kaiping, G-6-PD Mahidol, G-6-PD Chinese-4 and G-6-PD Chinese-5 were identified. The results showed that the most common variant was G-6-PD Viangchan (54.5%) which is consistent with that has been previously reported by Nuchprayoon et al. (2002) in the central part and Laosombat et al. (2005) in the southern part of Thailand. In contradictory to this study, the four common mutations in northern part are G-6-PD Mahidol (20%), G-6-PD Kaiping (18%), G-6-PD Canton (16%) and G-6-PD Viangchan (14%) (Charoenkwan et al., 2010).

The presence of G-6-PD Viangchan at the highest frequency in northeastern Thais reflects a common ancestral origin of the Thais with Laotians, and Cambodians (Iwai et al., 2001; Laosombat et al., 2005). In Laos 100% of G-6-PD deficient cases (9 cases) and in Cambodians 82.4% of G-6-PD deficient cases (28/34 cases) are G-6-PD Viangchan (Iwai et al., 2001). Furthermore, geographic proximity among northeastern Thailand, Laos and Cambodia also explains the genetic similarity of these populations. While G-6-PD Mahidol, the most common mutation in Burmese, were found in a smaller number in this study. These result consistents with other reports in the central and southern part of Thailand (Nuchprayoon et al., 2001; Laosombat et al., 2005). In Myanmar, more than 90% of cases are G-6-PD Mahidol and there is no case of G-6-PD Viangchan (Matsuoka et al., 2004). This finding supports the assimilation of the Burmese to various part of Thailand.

G-6-PD Canton, the most prevalent among the Chinese who live in southern China (Matsuoka et al., 2007) was the second most common G-6-PD mutation (14.1%) followed by other Chinese mutations namely G-6-PD Union (6.0%), G-6-PD Kaiping (5.6%), G-6-PD Chinese-5 (3.0%) and G-6-PD Chinese-4 (1.7%). These finding consistents with the migration of the Chinese to Thailand and assimilation to northeastern region. Moreover, the pattern and the proportion of G-6-PD mutations from this finding are similar to the central part of Thailand (Nuchprayoon et al., 2002). However, these are different from those of the northern part (Chiang Mai) with the higher proportion of G-6-PD Mahidol (Charoenkwan et al., 2010). These data also support that Thais are derived from various ancestries which is different from Burmese who are from homogeneous ancestries (Watchko et al., 2013).

Compound heterozygous state was also found reflecting the complexity of G-6-PD mutations in northeast Thai populations. In Thailand, there has been a few reports of compound heterozygote. Tanphaichitr et al. (2011) reported compound heteozygotes of G-6-PD Bangkok (Class I)/G-6-PD Vanua Lava (Class II) in two females. Nantakomol et al. (2012) recently reported two cases of G-6-PD Viangchan/G-6-PD Mahidol and one case of G-6-PD Viangchang/G-6-PD Union. In this study, there are three cases of compound heterozygote between G-6-PD Viangchan and Chinese mutations, G-6-PD Viangchan/G-6-PD Canton and G-6-PD Viangchan/ G-6-PD Chinese-4, and one case compound heterozygote between Chinese mutations, G-6-PD Kaiping/G-6-PD Chinese-5. This observation may imply the intermarriage between Thais and Chinese.

In this study, there were 22 cases (9.4%) whose mutations could not identify. This proportion is similarly to the southern (Laosombat et al., 2005) but less than those of the northern (Charoenkwan et al., 2010) and central (Nuchprayoon et al., 2002) populations. It is possible that these 22 cases may have one of less common mutations which is not investigated in this study. DNA sequencing should be done to identify the mutation.

According to the WHO classification, G-6-PD Viangchan, G-6-PD Canton, G-6-PD Union and G-6-PD Kaiping are classified as class II (G-6-PD activities less than 10% of normal activity) and G-6-PD Mahidol, G-6-PD Chinese-4 and G-6-PD Chinese-5 are classified as class III (10-60% of normal activity). This results show that G-6-PD activities of the class II mutations are lower than those of the class III mutations. Therefore, G-6-PD activity assay by automated analyzer gives a reliability for diagnosis of G-6-PD deficiency.

In Conclusion, this study demonstrates the molecular heterogeneity of G-6-PD mutations among northeastern Thais and shows that G-6-PD Viangchan is the most common mutation. This data support that G-6-PD Viangchan is a hallmark of Thai populations, similar to the hemoglobin E allele in this population. (Wasi et al., 1967). The results provide a basic knowledge and useful epidemiology of G-6-PD mutations in this populations which will be applicable for setting up molecular testing of G-6-PD mutations in northeastern populations.

Acknowledgement

The study was supported by the Khon Kaen University's Graduate Research Fund, Khon Kaen University, Khon Kaen, Thailand.

References

Beutler E, Blume KG, Kaplan JC, Lohr GW, Ramot B, Valentine WN. 1979. International committee for standardization in Haematology: recommended screening test for glucose-6-phosphate dehydroganase deficiency. Br J Haematol. 43:469-77.

Beutler E. 1994. G-6-PD deficiency. Blood. 84(11): 3613-36.

Bhutani VK. 2012. Jaundice due to glucose-6-phosphate dehydrogenase deficiency. American academy of pediatrics. 13: 166-77.

Charoenkwan P, Sirichotiyakil S, Taweehpol R, Sanguansermsri T. 2010. Neonatal hyperbilirubinemia in northern Thailand: roles of G-6-PD deficiency and alpha-thalassemia. Prevalence and molecular characterization of glucose-6-phosphate dehydrogenase variants in northern Thailand. Chiang Mai: Chiang Mai University.

Iwai K, Hirono A, Matsuoka H, Kawamoto F, Horie T, Lin K, et al. 2001. Distribution of glucose-6-phosphate dehydrogenase mutations in Southeast Asia. Hum Genet. 108: 445-9.

Kaplan M, Hammerman C. 2004. Glucose-6-phosphate dehydrogenase deficiency: a hidden risk for kernicterus. Semin Perinatol. 28: 356-64.

Kaplan M, Hammerman C. 2010. Glucose-6-phosphate dehydrogenase deficiency and severe neonatal hyperbilirubinemia: a complexity of interactions between genes and environment. Semin Fetal Neonatal Med. 15(3): 148-56.

Kittiwatanasarn P, Sukkapan P, Nuchprayoon I. 2003. Glucose-6-phosphate dehydrogenase deficiency in Northeastern Thailand: prevalence and relationship to neonatal jaundice. Chula Med Journal. 47: 471-9.

Laosombat V, Sattayasevana B, Janejindamai W, Viprakasit V, Shirakawa T, Nishiyama K, et al. 2005. Molecular heterogeneity of glucose-6-phosphate dehydrogenase (G-6-PD) variants in the south of Thailand and identification of a novel variant (G-6-PD Songklanagarind). Blood Cells Mol Dis. 34: 191-6.

Luzzatto L. 2006. Glucose 6-phosphate dehydrogenase deficiency: from genotype to phenotype. Haematologica. 91: 1303-6.

Matsuoka H, Thuan DT, van Thien H, Kanbe T, Jalloh A, Hirai M, et al. 2007. Seven different glucose-6-phosphate dehydrogenase variants including a new variant distributed in Lam Dong Province in southern Vietnam. Acta medica Okayama. 61: 213-9.

Matsuoka H, Wang J, Hirai M, Arai M, Yoshida S, Kobayashi T, et al. 2004. Glucose-6-phosphate dehydrogenase (G-6-PD) mutations in Myanmar: G-6-PD Mahidol (487G>A) is the most common variant in the Myanmar population. Journal of human genetics. 49: 544-7.

Minucci A, Giardina B, Zuppi C, Capoluongo E. 2009. Glucose-6-phosphate dehydrogenase laboratory assay: How, when, and why?. IUBMB Life. 61: 27-34.

Nantakomol D, Palasuwan A, Chaowanathikhom M, Soogarun S, Imwong M. 2012. Red cell and platelet-derived microparticles are increased in G-6-PD deficient subjects. Eur J Haematol. 89: 423-9.

Ninokata A, Kimura R, Samakkarn U, Settheetham-Ishida W, Ishida T. 2006. Coexistence of five G-6-PD variants indicates ethnic complexity of Phuket islanders, Southern Thailand. J Hum Genet. 51: 424-8.

Nuchprayoon I, Sanpavat S, Nuchprayoon S. 2002. Glucose-6-phosphate dehydrogenase (G-6-PD) mutations in Thailand: G-6-PD Viangchan (871G>A) is the most common deficiency variant in the Thai population. Hum Mutat. 19: 185.

Phompradit P, Kuesap J, Chaijaroenkul W, Rueangweerayut R, Hongkaew Y, Yamnuan R, et al. 2011. Prevalence and distribution of glucose-6-phosphate dehydrogenase (G-6-PD) variants in Thai and Burmese populations in malaria endemic areas of Thailand. Malar J. 10: 368.

Tanphaichitr VS, Hirono A, Pung-amritt P, Treesucon A, Wanachiwanawin W. 2011. Chronic nonspherocytic hemolytic anemia due to glucose-6-phosphate dehydrogenase deficiency: report of two families with novel mutations causing G-6-PD Bangkok and G-6-PD Bangkok Noi. Ann Hematol. 90: 769-75.

Wasi P, Na-Nakorn S, Suingdumrong A. 1967. Studies of the Distribution of Haemoglobin E, Thalassemias and Glucose-6-Phosphate Dehydrogenase Deficiency in North-eastern Thailand. Nature. 214:501-2.

Watchko JF, Kaplan M, Stark AR, Stevenson DK, Bhutani VK. 2013. Should we screen newborns for glucose-6-phosphate dehydrogenase deficiency in the United States?. J Perinatol. 33: 499-504.