Acta Med. Okayama, 2008
Vol. 62, No. 5, pp. 327–332

Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Original Article

Nine Different Glucose-6-phosphate Dehydrogenase (G6PD) Variants in a Malaysian Population with Malay, Chinese, Indian and Orang Asli (Aboriginal Malaysian) Backgrounds

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The Malaysian people consist of several ethnic groups including the Malay, the Chinese, the Indian and the Orang Asli (aboriginal Malaysians). We collected blood samples from outpatients of 2 hospitals in the State of Selangor and identified 27 glucose-6-phosphate dehydrogenase (G6PD)-deficient subjects among these ethnic groups. In the Malay, G6PD Viangchan (871G > A, 1311C > T, IVS11 nt93T > C) and G6PD Mahidol (487G > A) types, which are common in Cambodia and Myanmar, respectively, were detected. The Malay also had both subtypes of G6PD Mediterranean: the Mediterranean subtype (563C > T, 1311C > T, IVS11 nt93T > C) and the Indo-Pakistan subtype (563C > T, 1311C, IVS11 nt93T). In Malaysians of Chinese background, G6PD Kaiping (1388G > A), G6PD Canton (1376G > T) and G6PD Gaohe (95A > G), which are common in China, were detected. Indian Malaysians possessed G6PD Mediterranean (Indo-Pakistan subtype) and G6PD Namoru (208T > C), a few cases of which had been reported in Vanuatu and many in India. Our findings indicate that G6PD Namoru occurs in India and flows to Malaysia up to Vanuatu. We also discovered 5 G6PD-deficient cases with 2 nucleotide substitutions of 1311C > T and IVS11 nt93T > C, but without amino-acid substitution in the G6PD molecule. These results indicate that the Malaysian people have incorporated many ancestors in terms of G6PD variants.

Key words: Aborigine, Chinese, glucose-6-phosphate dehydrogenase, Indian, Malay

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most frequent hereditary abnormalities. Once a G6PD-deficient person receives an oxidative stress, *e.g.*, eating fava beans or taking an anti-malarial medicine such as primaquine, acute hemolysis occurs. Without sufficient G6PD enzymes, erythrocytes cannot produce a sufficient amount of reduced pyridine nucleotide and reduced glutathione, and cannot prevent oxidative stress [1]. Thus, primaquine should not be administered to malaria patients before confirming their G6PD activity. The *G6PD* gene is distributed in 13 exons on the X-chromosome, and the length of the open reading frame is 1,545 base pairs [2]. Almost all G6PD

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deficiencies are caused by one amino-acid substitution due to a point mutation of the genomic DNA, and about 140 molecular abnormalities of the *G6PD* genotype have been identified [1].

The World Health Organization categorizes G6PD deficient variants into 3 classes according to severity [3]. The Class I variant shows very low G6PD activity (less than 1% of normal individual), and carriers of these genes suffer from heavy anemia because hemolysis occurs chronically. The Class II variant shows low G6PD activity (less than 10% of residual enzyme activity), but is not so serious for the individual's daily life. Hemolysis attack occurs only in response to certain food or medicines. The Class III variant has 10%–60% residual enzyme activity. Hemolysis occurs in rare cases. A female heterozygote of a G6PD variant of Class I or Class II is sometimes categorized in Class III, because a half of her red blood cells lack the G6PD enzyme activity, but the other half have full activity.

We have investigated variants of G6PD deficiency in Asian countries [4–10] (Fig. 1), because this abnormality is prevalent in malaria-endemic areas. We have introduced a rapid diagnosis method for malaria [11] and G6PD deficiency tests [12–15] in malaria-endemic areas. Using these methods, patients are notified of the results of blood examination within 30 min and are able to receive anti-malarial medicine

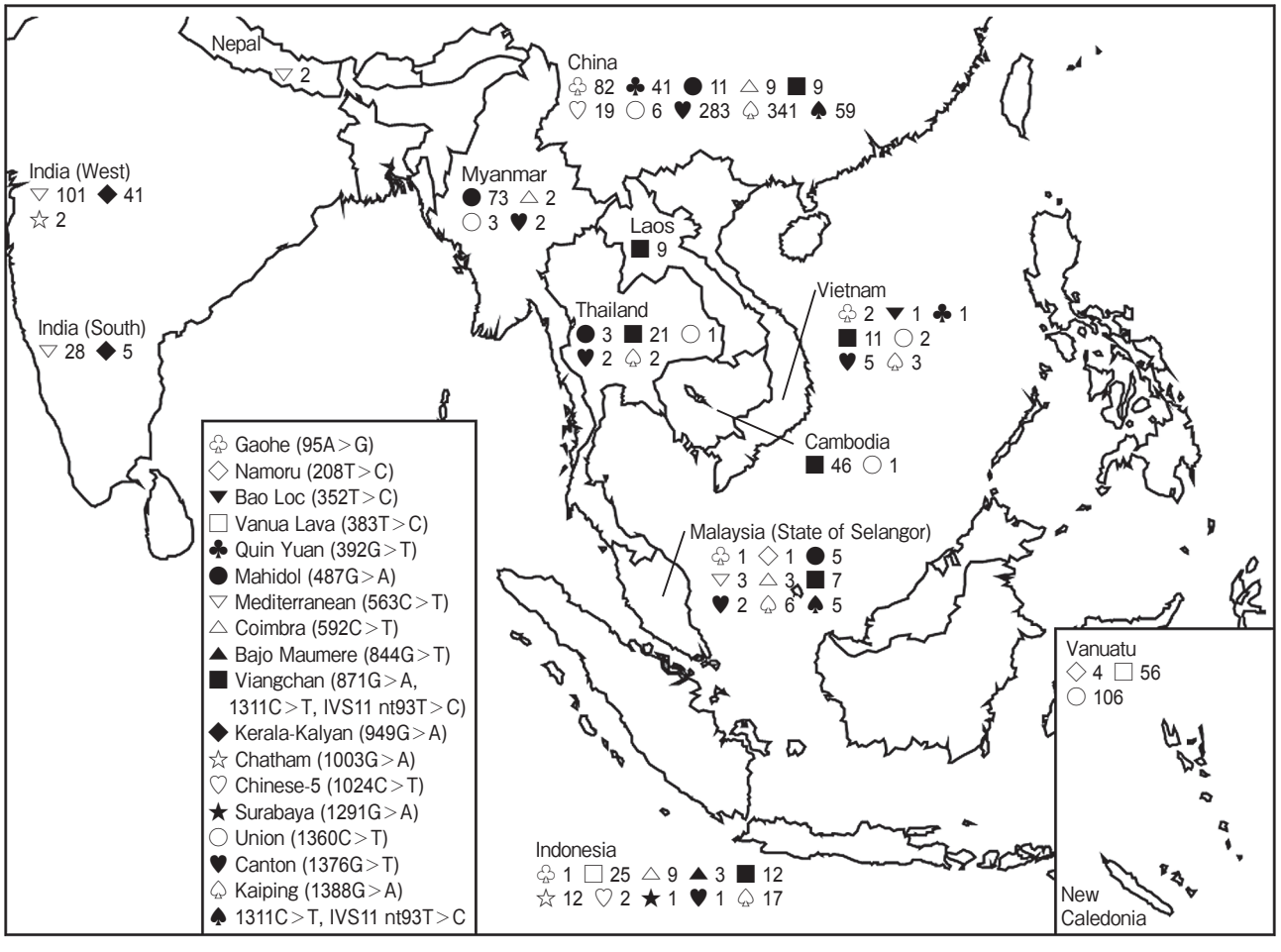

Fig. 1 Distribution and frequencies of glucose-6-phosphate dehydrogenase (G6PD) variants in Asian countries and Vanuatu, Melanesia. Each number indicates the number of G6PD-deficient cases confirmed by sequence analysis. Data of Nepal, Myanmar, Laos, Cambodia, Vietnam and Indonesia are from our previous reports [4–10]. Data of India [28, 30] Thailand [21], China [26] and Vanuatu [29] are from other groups' reports.

including primaquine [16, 17]. In Southeast Asian countries, we have tested more than 3,000 individuals for malaria and G6PD activity and found more than 200 G6PD-deficient cases. These G6PD variants were all categorized as Class II. We read the G6PD gene of those deficient samples and found 15 G6PD genotypes including 3 new genotypes, which were named G6PD Surabaya (1291G > A; 431Val > Met) [4], G6PD Bajo Maumere (844G > T; 282Asp > Tyr) [9], and G6PD Bao Loc (352T > C; 118Tyr > His) [10].

In the Malaysian population, the incidence rates of G6PD deficiency are reported as 5.1% in the Malay and 5.5% in the Chinese [18]. Some genetic studies on G6PD deficiency have been done [4, 19, 20]. A total of 13 G6PD variants among 127 cases of G6PD-deficient individuals are described in these reports. We reported 6 cases of G6PD deficiency of Malaysians including people of Chinese, Malay and Orang Asli background [4]. Ainoon *et al.* reported 121 G6PD-deficient cases including 35 Chinese Malaysians [19] and 86 Malay Malaysians [20]. In this report, we add 27 cases of G6PD deficiency in the Malaysian people including 5 G6PD-deficient Indian Malaysians, the first such reported cases. We also describe a new type of G6PD deficiency categorized in Class III but having no amino-acid substitutions in the open reading frame of the G6PD molecule.

Materials and Methods

This study was approved by the National Ethical Committee, Malaysia, and the Ethical Committees of China Medical University and Jichi Medical University. We collected glucose-6-phosphate dehydrogenase (G6PD)-deficient samples at 2 hospitals, the Kajan District Hospital and the Hospital Orang Asli Gombak. Both are in the State of Selangor, Malaysia. We asked outpatients of these hospitals for permission to test G6PD activity when their venous blood was taken for other purposes, *e.g.* RBC count, WBC count, blood sugar level, *etc.* We received informed consent and recorded name, age, gender and ethnicity for all samples. We used 5 μl of blood for the G6PD test. When the blood showed G6PD deficiency, we further took another 50 μl of blood on a filter sheet, which was dried and then stored at 4℃ for DNA analysis.

To test G6PD activity, we adopted Hirono's method [13]. Briefly, 5 μl of test blood was added to a 1.5 ml microcentrifuge tube containing 200 μl of DAEA-Sephadex A-50 (Sigma, St Louis, MO, USA) equilibrated in 0.1 mM Tris-HCl, 10 mM $MgCl_2$, pH 6.5, and 600 μl of substrate mixture of 2.5 mM glucose-6-phosphate (G6P: Sigma), 0.2 mM nicotinamide adeninl dinucleotide phosphate ($NADP^+$: Sigma), 0.01% of 3 (4, 5 dimethylthiazolyl 1–2) 2, 5 diphenyltetrazolium bromide (MTT: Dojin, Kumamoto, Japan) and 0.01% of phenazine methosulfate (PMS: Sigma). The tube was shaken vigorously to destroy red blood cells and left to stand for 20 min in a dark place. A blue color developed on the Sephadex gel when the sample contained full activity of G6PD. In the case of G6PD deficiency, the Sephadex gel did not change color. Thus, participants could receive the results of the G6PD test before they went home.

To analyze the *G6PD* gene, filter sheets soaked with blood were sent to Jichi Medical University. DNA was eluted from the filter paper by heating at 80℃ for 10 min in DNA extraction buffer. DNA extraction and purification were done according to the manufacturer's instructions (Amersham Pharmacia Biotech, Buckinghamshire, UK). Since genomic *G6PD* consists of 13 exons, we prepared primers for these exons [10], amplified each exon by PCR, and read the DNA sequence (ABI PRISM 310; Applied Biosystems, Foster City, CA, USA). Both strands of each exon were sequenced. To indicate the mutation point, the nucleotide number of the cDNA sequence (No. 1–1, 545) was used.

Results and Discussion

We collected and analyzed 27 G6PD-deficient samples (20 male, 7 female). There were 12 samples from the Malay, 7 from the Chinese, 5 from the Indian and 3 from the Orang Asli ethnic groups (Table 1). Each ethnic group had the respective characteristic genotype. In the Malay, we found G6PD Mahidol (487G > A), which is common in Myanmar [7], and G6PD Viangchan (871G > A, 1311C > T, IVS11 nt93T > C), which is common in Cambodia [8]. As these 2 variants are common in Thailand [21], which borders Malaysia, ancestors of the Malay must have intercommunicated with the Thai people. We also found the 2 subtypes of G6PD Mediterranean

Table 1 G6PD variants in each ethnic group in the Malaysian populations

	Malay	Chinese	Indian	O. Asli	Total
Gaohe 95A > G		1			1
Namoru 208T > C			1		1
Mahidol 487G > A	3 + (2)				3 + (2)
Mediterranean 563C > T, 1311C, IVS11 nt93T	1		1		2
Mediterranean 563C > T, 1311C > T, IVS11 nt93T > C	1				1
Coimbra 592C > T	1			(2)	1 + (2)
Viangchan 871G > A, 1311C > T, IVS11 nt93T > C	3 + (1)	1		2	6 + (1)
Canton 1376G > T		2			2
Kaiping 1388G > A		3 + (1)	2		5 + (1)
1311C > T, IVS11 nt93T > C	3		1	1	5
Total	12 + (3)	7 + (1)	5	3 + (2)	27 + (6)

Numbers in parentheses were data from our previous report (Iwai *et al*. 2001).

in the Malay: the Mediterranean subtype (563C > T, 1311C > T, IVS11 nt93T > C), which may have come from the Mediterranean people, and the Indo-Pakistan subtype (563C > T, 1311C, IVS11 nt93T), which may have come from India [22]. It is of value to note that both subtypes were found from one ethnic group of the Malay. Two gene flows, one from the Mediterranean countries and another from India, might have come into the Malaysian peninsula in the past. In the literature, there is a paper describing these 2 subtypes of G6PD Mediterranean in the Kuwait population [23]. Both Kuwait and Malaysia may have received these 2 gene flows in the past. We detected one case of G6PD Coimbra (592C > T) [24] in the Malay. This variant is reported in low frequencies in the aboriginal Taiwanese [25], the aboriginal Malaysians [4], the Malay Malaysians [20], the Indonesians [6], and Myanmar populations [7]. This variant is suspected to have spread in Asian countries in very old times: more than several thousand years ago [25]. These results indicate that the Malay Malaysians has been influenced by several gene flows from Thailand, Mediterranean countries, and Indo-Pakistan areas as well as the aboriginal Malaysians.

In the Chinese Malaysians, we found G6PD Kaiping (1388G > A), G6PD Canton (1376G > T) and G6PD Gaohe (95A > G). These are commonly reported in the Chinese population [26], and are frequently detected in Singapore [27] and Vietnam [10], suggesting that these variants spread from China to these countries in the past. Historically, the Chinese people migrated and settled in the surrounding Asian counties. When we compare G6PD variants in the mainland of China to those in other areas, we notice gene flows between Chinese and other ethnicities. For instance, G6PD Mediterranean (563C > T), which is common in Mediterranean countries and Indo-Pakistan areas, has been detected in Chinese populations living in Singapore [27] and Malaysia [19], but not on the mainland of China.

In the Indian Malaysians, we found one case of G6PD Mediterranean, whose subtype was Indo-Pakistan (563C > T, 1311C, IVS11 nt93T). This is a common subtype in Indian populations [28]. We also found a case of G6PD Namoru (208T > C), which was first found in the Vanuatu Archipelago but only in small numbers [29]. Recently, many cases of G6PD Namoru have been found in south India [30], suggesting that G6PD Namoru originally occurred in India. Interestingly, we have read of more than 80 G6PD deficient-subjects in Indonesia [4, 6, 9], but we have never encountered G6PD Namoru in Indonesia. This variant may occur in India, flow to Malaysia and finally to the Vanuatu Archipelago but not spread in Indonesia. We also detected 2 cases of G6PD Kaiping among the Indian Malaysians. This variant is common in China [26] and is distributed in Vietnam [10], Thailand [21] and Indonesia [6, 9]. According to reports from India, G6PD Kaiping has not so far been detected in the mainland of India [28,

30, 31]. We concluded from this finding that intermarriage must have occurred between Indian and Chinese in the Malaysian community in the past.

We found G6PD Viangchan in Orang Asli (aboriginal Malaysians). This mutation is frequently found in Southeast Asian countries. This shows that the Orang Asli intermarried with people from the Southeast Asian peninsula in the past.

We found 5 cases of a genotype with 2 substitutions of 1311C > T and IVS11 nt93T > C from the Malay, the Indian and the Orang Asli ethnic groups. All other open reading frame showed wild type. According to enzyme activity, this variant showed 30–50% of residual enzyme activity, which was categorized in Class III. This genotype is also reported in Chinese populations [26]. Interestingly, 1311C > T is a non-sense mutation because both TAC and TAT code the same amino-acid, tyrosine. According to Gene Bank data, codon usage of TAC and TAT in *Homo sapiens* are 15.3% and 12.1%, respectively. Among the G6PD gene of the wild type, the frequency of TAC was 14 and that of TAT was 7. Thus, there is no bias for codon usage [32] between TAC and TAT for carrying tyrosine. We cannot explain, so far, the low activity of G6PD in this variant. Furthermore, we cannot explain why 1311C > T is accompanied with IVS11 nt93T > C. It is worth noting that the G6PD Viangchan and the Mediterranean subtypes of G6PD Mediterranean also accompany 1311C > T and IVS11 nt93T > C. Further studies are necessary, in particular, to search for promoter areas of the G6PD gene.

Acknowledgments. We thank Miss Maiko Nakamura and Miss Tomoko Yoshitomi for their technical assistance. This work was supported by a grant-in-aid from the Japanese Ministry of Education, Science, Culture and Sports (14406026 and 17406025 to HM) as well as a grant to JW and EL from the US-Japan Cooperative Medical Science Program for encouraging Asian scientists.

References

1. Cappellini MD and Fiorelli G: Glucose-6-phosphate dehydrogenase deficiency. Lancet (2008) 371: 64–74.
2. Chen EY, Cheng A, Lee A, Kuang WJ, Hillier L, Green P, Schlessinger D, Ciccodicola A and D'Urso M: Sequence of human glucose-6-phosphate dehydrogenase cloned in plasmids and a yeast artificial chromosome. Genomics (1991) 10: 792–800.
3. WHO working group: Glucose-6-phosphate dehydrogenase deficiency. Bull World Health Organ (1989) 67: 601–611.
4. Iwai K, Hirono A, Matsuoka H, Kawamoto F, Horie T, Lin K, Tantular IS, Dachlan YP, Notopuro H, Hidayah NI, Salim AM, Fujii H, Miwa S and Ishii A: Distribution of glucose-6-phosphate dehydrogenase mutations in Southeast Asia. Hum Genet (2001) 108: 445–449.
5. Matsuoka H, Wang J, Hirai M, Yoshida S, Arai M, Ishii A and Baral MP: Two cases of glucose-6-phophate dehydrogenase deficient Nepalese belonging to the G6PD Mediterranean-type, not India-Pakistan sub-type but Mediterranean-Middle East sub-type. J Hum Genet (2003) 48: 275–277.
6. Matsuoka H, Arai M, Yoshida S, Tantular IS, Pusarawati S, Kerong H and Kawamoto F: Five different glucose-6-phophate dehydrogenase (G6PD) variants found among 11 G6PD-deficient persons in Flores Island, Indonesia. J Hum Genet (2003) 48: 541–544.
7. Matsuoka H, Wang J, Hirai M, Arai M, Yoshida S, Kobayashi T, Jalloh A, Lin K and Kawamoto F: Glucose-6-phosphate dehydrogenase (G6PD) mutations in Myanmar: G6PD Mahidol (487G > A) is the most common variant in the Myanmar population. J Hum Genet (2004) 49: 544–547.
8. Matsuoka H, Nguon C, Kanbe T, Jalloh A, Sato H, Yoshida S, Hirai H, Arai M, Socheat D and Kawamoto F: Glucose-6-phosphate dehydrogenase (G6PD) mutations in Cambodia: G6PD Viangchan (871G > A) is the most common variant in the Cambodian population. J Hum Genet (2005) 50: 468–472.
9. Kawamoto F, Matsuoka H, Kanbe T, Tantular IS, Pusarawati S, Kerong HI, Damianus W, Mere D and Dachlan YP: Further investigations of glucose-6-phosphate dehydrogenase variants in Flores Island, eastern Indonesia. J Hum Genet (2006) 51: 952–957.
10. Matsuoka H, Thuan DTV, van Thien H, Kanbe T, Jalloh A, Hirai M, Arai M, Dung NT and Kawamoto F: Seven different glucose-6-phosphate dehydrogenase variants including a new variant distributed in Lam Dong Province in southern Vietnam. Acta Medica Okayama (2007) 61: 181–185.
11. Kawamoto F and Billingsley PF: Rapid diagnosis of malaria by fluorescence microscopy. Parasitol Today (1992) 8: 69–71.
12. Fujii H, Takahashi K and Miwa S: A new simple screening method for glucose 6-phosphate dehydrogenase deficiency. Acta Haematol Jpn (1984) 47: 185–188.
13. Hirono H, Fujii H and Miwa S: An improved single-step screening method for glucose-6-phosphate dehydrogenase deficiency. Jpn J Trop Med Hyg (1998) 26: 1–4.
14. Tantular IS and Kawamoto F: An improved, simple screening method for detection of glucose-6-phosphate dehydrogenase deficiency. Trop Med Int Health (2003) 8: 569–574.
15. Arai M, Kosuge K, Kawamoto F and Matsuoka H: Reactivity of blood samples spotted onto filter papers in the WST-8 method for screening of G6PD deficiency. Acta Med Okayama (2006) 60: 127–134.
16. Tantular IS, Iwai K, Khin-Lin, Basuki S, Horie T, Htay HH, Matsuoka H, Marwoto H, Wongsrichanalai C, Dachlan Y P, Kojima S, Ishii A and Kawamoto F: Field trials of a rapid test for G6PD deficiency in combination with a rapid diagnosis of malaria. Trop Med Int Health (1999) 4: 245–250.
17. Jalloh A, Tantular IS, Pusarawati S, Kawilarang AP, Kerong H, Lin K, Ferreira MU, Matsuoka H, Arai M, Kita K and Kawamoto F: Rapid epidemiologic assessment of glucose-6-phosphate dehydrogenase (G6PD) deficiency in malaria-endemic areas in Southeast Asia using a novel diagnostic kit. Trop Med Int Health (2004) 9: 615–623.
18. Ainoon O, Alawiyah A, Yu YH, Cheong SK, Hamidah NH, Boo NY and Zaleha M: Semiquantitative screening test for G6PD defi-

ciency detects severe deficiency but misses a substantial proportion of partially-deficient females. Southeast Asian J Trop Med Public Health (2003) 34: 405–414.
19. Ainoon O, Joyce J, Boo NY, Cheong SK, Zainal ZA and Hamidah NH: Glucose-6-phosphate dehydrogenase (G6PD) variants in Malaysian Chinese. Hum Mutat (1999) 14: 352.
20. Ainoon O, Yu YH, Amir Muhriz AL, Boo NY, Cheong SK and Hamidah NH: Glucose-6-phosphate dehydrogenase (G6PD) variants in Malaysian Malays. Hum Mutat (2003) 21: 101.
21. Nuchprayoon I, Sanpavat S and Nuchprayoon S: Glucose-6-phosphate dehydrogenase (G6PD) mutations in Thailand: G6PD Viangchan (871G > A) is the most common deficiency variant in the Thai population. Hum Mutat (2002) 19: 185.
22. Beutler E and Kuhl W: The NT 1311 polymorphism of G6PD: G6PD Mediterranean mutation may have originated independently in Europe and Asia. Am J Hum Genet (1990) 47: 1008–1012.
23. Samilchuk E, D'Souza B and Al-Awadi S: Population study of common glucose-6-phosphate dehydrogenase mutations in Kuwait. Hum Hered (1999) 49: 41–44.
24. Corcoran CM, Calabrò V, Tamagnini G, Town M, Haidar B, Vulliamy TJ, Mason PJ and Luzzatto L: Molecular heterogeneity underlying the G6PD Mediterranean phenotype. Hum Genet (1992) 88: 688–690.
25. Tang TK, Huang WY, Tang CJ, Hsu M, Cheng TA and Chen KH: Molecular basis of glucose-6-phosphate dehydrogenase (G6PD) deficiency in three Taiwan aboriginal tribes. Hum Genet (1995) 95: 630–632.
26. Jiang W, Yu G, Liu P, Geng Q, Chen L, Lin Q, Ren X, Ye W, He Y, Guo Y, Duan S, Wen J, Li H, Qi Y, Jiang C, Zheng Y, Liu C, Si E, Zhang Q, Tian Q and Du C: Structure and function of glucose-6-phosphate dehydrogenase-deficient variants in Chinese population. Hum Genet (2006) 119: 463–478.
27. Saha S, Saha N, Tay JS, Jeyaseelan K, Basair JB and Chew SE: Molecular characterisation of red cell glucose-6-phosphate dehydrogenase deficiency in Singapore Chinese. Am J Hematol (1994) 47: 273–277.
28. Sukumar S, Mukherjee MB, Colah RB and Mohanty D: Molecular basis of G6PD deficiency in India. Blood Cells Mol Dis (2004) 33: 141–145.
29. Ganczakowski M, Town M, Bowden DK, Vulliamy TJ, Kaneko A, Clegg JB, Weatherall DJ and Luzzatto L: Multiple glucose 6-phosphate dehydrogenase-deficient variants correlate with malaria endemicity in the Vanuatu archipelago (southwestern Pacific). Am J Hum Genet (1995) 56: 294–301.
30. Chalvam R, Mukherjee MB, Colah RB, Mohanty D and Ghosh K: G6PD Namoru (208 T-- > C) is the major polymorphic variant in the tribal populations in southern India. Br J Haematol (2007) 136: 512–513.
31. Kaeda JS, Chhotray GP, Ranjit MR, Bautista JM, Reddy PH, Stevens D, Naidu JM, Britt RP, Vulliamy TJ, Luzzatto L and Mason PJ: A new glucose-6-phosphate dehydrogenase variant, G6PD Orissa (44 Ala-- > Gly), is the major polymorphic variant in tribal populations in India. Am J Hum Genet (1995) 57: 1335–1341.
32. Comeron JM and Aguade M: An evaluation of measures of synonymous codon usage bias. J Mol Evol (1998) 47: 268–274.