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Original Article

Incidence and Mutation Analysis of Glucose-6-Phosphate Dehydrogenase Deficiency in Eastern Indonesian Populations

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We conducted a field survey of glucose-6-phosphate dehydrogenese (G6PD) deficiency in the eastern Indonesian islands, and analyzed G6PD variants molecularly. The incidence of G6PD deficiency in 5 ethnic groups (Manggarai, Bajawa, Nage-Keo, Larantuka, and Palue) on the Flores and Palue Islands was lower than that of another native group, Sikka, or a nonnative group, Riung. Molecular analysis of G6PD variants indicated that 19 cases in Sikka had a frequency distribution of G6PD variants similar to those in our previous studies, while 8 cases in Riung had a different frequency distribution of G6PD variants. On the other hand, from field surveys in another 8 ethnic groups (Timorese, Sumbanese, Savunese, Kendari, Buton, Muna, Minahasa, and Sangirese) on the islands of West Timor, Sumba, Sulawesi, Muna and Bangka, a total of 49 deficient cases were detected. Thirty-nine of these 49 cases had G6PD Vanua Lava (383T>C) of Melanesian origin. In our previous studies, many cases of G6PD Vanua Lava were found on other eastern Indonesian islands. Taken together, these findings may indicate that G6PD Vanua Lava is the most common variant in eastern Indonesian populations, except for Sikka.

Key words: Glucose-6-phosphate dehydrogenase deficiency, rapid G6PD test, eastern Indonesian population, molecular analysis, G6PD Vanua Lava

In malaria-endemic areas in Southeast Asian countries, we have been introducing 2 rapid diagnostic methods for malaria [1, 2] and glucose-6-phosphate dehydrogenase (G6PD) deficiency [3, 4]. Using these 2 methods, malaria patients can be informed of the diagnostic results within 30 min after the blood examination, and they are prescribed antimalarial drugs on-site, including primaquine. However, if G6PD-deficient individuals take primaquine, a hemolytic attack sometimes occurs by oxidant stress. Thus, primaquine should not be administered to malaria patients before confirming their G6PD condition. In order to transfer these diagnostic techniques to Southeast Asia, we have been conducting field surveys for malaria and G6PD deficiency [5-12] using local staffs as part of their training.

G6PD deficiency is one of the most frequent

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herediary disorders in the world, and is distributed throughout tropical areas in particular. The open reading frame of this gene is 1,545 base pairs in length, encoded in 13 exons. Most G6PD deficiency is caused by a single nucleotide mutation, resulting in one amino-acid change among 515 amino acids. To date, more than 400 G6PD biochemical variants have been described, and at least 140 mutations of G6PD deficiency have been discovered [13, 14].

In Indonesia, malaria endemic areas have been widely eradicated, but malaria is still a serious infectious disease, particularly in eastern Indonesia and Kalimantan. In our previous surveys on Flores Island in eastern Indonesia [5, 9], we detected many G6PD-deficient individuals as well as malaria patients in 3 ethnic groups, the Sikka, Ende (Ende-Lio) and Bajo populations, and it was interesting that these groups had their own frequency distributions of G6PD variants. In Flores Island (and Palue Island), 6 more ethnic groups, *i.e.*, the Manggarai, Bajawa (or Ngada), Riung, Nage-Keo, Palue and Larantuka (or Lamahorot), are present (Fig. 1), and we further surveyed malaria and G6PD deficiency among these groups. In addition, we also surveyed malaria and G6PD deficiency in other eastern Indonesian islands, such as West Timor Island, Sumba Island, the Southeastern Sulawesi and Muna Islands, and the Northern Sulawesi and Bangka Islands (Fig. 2), and compared their G6PD variants molecularly.

Here, we report that G6PD Vanua Lava is the most dominant variant among eastern Indonesian populations, while the Sikka and the Riung groups each possess many G6PD variants not found in the other groups.

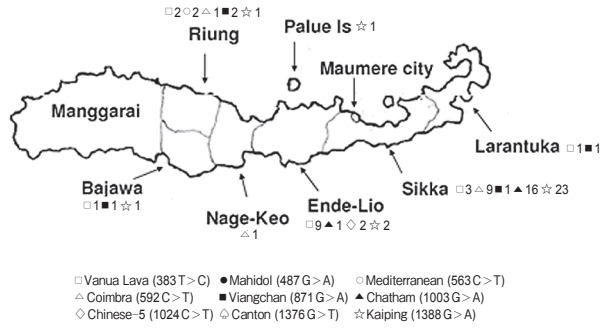

Fig. 1 A detailed map showing the major ethnic groups on the Flores and Palue Islands, and the distribution and frequencies of G6PD variants in each group. Numerals indicate the numbers of G6PD-deficient cases confirmed by sequence analysis.

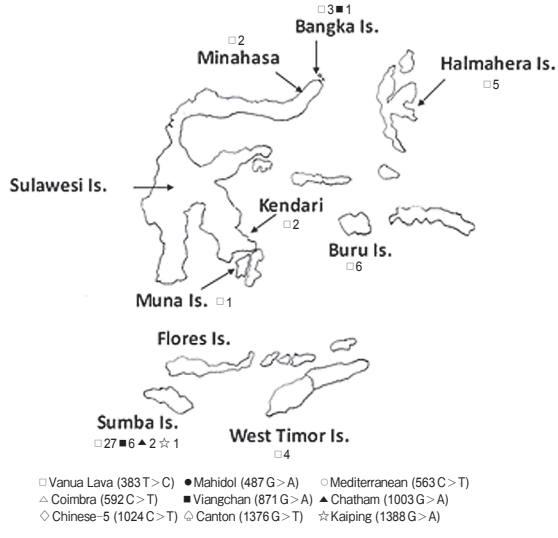

Fig. 2 A map showing our study sites in eastern Indonesia, and the distribution and frequencies of G6PD variants in various populations. Numerals indicate the numbers of G6PD-deficient cases confirmed by sequence analysis.

Materials and Methods

This study was approved by the Ethical Committees of the Health Departments of all regencies and of Airlangga University, Indonesia, and by the Ethical Committees of the Oita University Faculty of Medicine and Jichi Medical University, Japan. The molecular epidemiological data for malaria infection obtained in this study will be reported elsewhere.

Field surveys and mutation analysis. In the East Nusa Tenggara Province, we surveyed malaria and G6PD deficiency at the Longgot and Wae Nakeng villages near Labuan Bajo, the Ngedukelu and Nggelek villages near Lembor (West Manggarai Regency), the Reo village in Riung, the Tiworiwu village in Bajawa (Ngada Regency), the Tonggo village in Nangaroro (Nage-Keo Regency), and the Waimana, Waiklibang and Lewolaga villages in Larantuka (East Flores Regency), Flores Island, and at the Reruwarere village, Palue Island (Sikka Regency) from June 2005 to April 2006, and compared them with the incidence of malaria and G6PD deficiency in a Sikka village (Pruda), 40 km east from Maumere

city. We also surveyed at the Baturutu, Panite and Maiskolen villages near Soe and Oebobo (South Central Timor Regency), West Timor Island, on August 2004. In addition, we surveyed at the Taimanu, Uma Mapu and Protwora villages near Waingapu (East Sumba Regency), Sumba Island, on June 2006. On this island, 2 populations (Sumbanese and Savunese) are distributed.

In the Southeast Sulawesi Province, we surveyed at the Lambuya village near Kendari (Kendari Regency) and Katobu village in Raha (Muna Regency), Southeastern Sulawesi Island, and at the Lamaeo village in Mendo-Wuna (Muna Regency), Muna Island, on August 2004. In the North Sulawesi Province, we also surveyed at the Kaheka, Libas and Kumersot villages, Bangka Island, and at the Apela, Wolaang, Noongan, Gmim Kal and Kalooran villages in Tonbatu (Minahasa Regency), Northern Sulawesi Island, on August 2005 and March 2006.

Informed consent was obtained from each volunteer before diagnoses of malaria and G6PD deficiency. Volunteers were first registered by name, age, gender, and ethnicity, and then, 3 drops of blood were collected from the fingertip [9]: one for malaria diagnosis, one for hemoglobin concentration, and one for G6PD test. Malaria was diagnosed by the acridine orange staining method [1, 2], and hemoglobin concentration was measured using a battery-powered, HemoCue machine (Angelhorm, Sweden).

For screening of G6PD deficiency, we used a modified method in this study by reducing the volume of dye mixture from the G6PD deficiency detection kit (Dojindo Laboratories, Kumamoto, Japan) by half: In the original WST-8 method, the intensities of the developed orange colors between heterozygous female samples and normal samples were not very different by the naked eye, which made it difficult to identify heterozygous female samples. Decreasing the concentration of the dye mixture resulted in slow color development (data not shown). By the naked eye, however, the intensity of orange color development in normal blood samples with the 50% dye mixture was almost equal to that with the 100% dye mixture, and it became much easier to distinguish differences in orange colors between normal and heterozygous blood samples with the 50% dye mixture (data not shown). In fact, all 11 females diagnosed with heterozygosity for G6PD deficiency were later confirmed by sequence analysis (see Tables 1 and 3).

When malaria patients or G6PD-deficient individuals were detected, informed consent was obtained once again, and 0.2～2.0 ml of venous blood was taken for further molecular analysis. G6PD activity in collected blood samples was re-confirmed at a laboratory in Surabaya by another G6PD test [15]. This method is useful for testing hundreds of blood samples per day, although it is very difficult to distinguish heterozygous female samples from normal samples.

G6PD mutation was identified by sequencing both strands of the G6PD gene: Genomic DNA was extracted from 0.1 ml of G6PD-deficient blood samples using a DNA purification kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). Then, DNA was amplified by PCR with 10 sets of primers [6, 10], and the DNA sequences were read by an ABI PRISM 310 (Applied Biosystems, Foster City, CA, USA). Both strands of each exon were sequenced. We also read some introns of G6PD because silent mutations had been found on introns of genomic G6PD; *e.g.* nt 175 C > T on intron 7, nt 163 C > T on intron 8 [16] and nt 93 T > C on intron 11 [17].

Results and Discussion

Field surveys and mutation analysis. On Flores and Palue Islands (Fig. 1), we surveyed 8 major ethnic groups, *i.e.*, the Manggarai, Bajawa, Riung, Nage-Keo, Ende (Ende-Lio), Palue, Sikka and Larantuka populations. These ethnic groups have their own languages. Five (the Bajawa, Nage-Keo, Ende, Palue, Sikka) of these 8 populations are thought to be very close to each other evolutionally. The Riung are thought to be descendant of the Bugis group, whose ancestors transmigrated from the Southwestern Sulawesi Island [18]. Their skin colors is lighter than that of the native Flores or Palue islanders, and they have the characteristic long, straight black hair of the Malayan races.

In our previous studies on Flores Island [5, 9], we reported a high prevalence of G6PD deficiency in 2 native populations, Sikka and Ende, and a nonnative population, Bajo, which is originated from South Mindanao, the Philippines [19]. In the Sikka group, 5 different G6PD variants were identified (these are shown in parentheses in Table 2), and their frequency distribution of G6PD variants was different from that

Table 1 Glucose-6-phosphate dehydrogenase deficiency in eastern Indonesian populations

Island and Ethnicity	Deficiency detected (%) Male	Female	Total No. (%)
Flores Island			
Manggarai	0/157	0/69	0/226
Bajawa	3/72 (4.2)	0/32	3/104 (2.9)
Riung	6/54 (11.1)	2/48 (4.2)	8/102 (7.8)
Nage-Keo	1/49 (2.0)	0/41	1/90 (1.1)
Palue	1/83 (1.2)	0/142	1/225 (0.4)
Larantuka	2/146 (1.4)	0/111	2/257 (0.8)
Sikka (Pruda village)	18/132 (13.6)	1/119 (0.8)	19/251 (7.6)
[Balinese	1/2	-	1/ 2]*
West Timor Island			
Timorese	3/94 (3.2)	1/102 (1.0)	4/196 (2.0)
Sumba Island			
Sumbanese	26/549 (4.7)	5/186 (2.7)	31/735 (4.2)
Savunese	3/23 (13.0)	2/16 (12.5)	5/39 (12.8)
Southeastern Sulawesi Island & Muna Island			
Kendari	1/45 (2.2)	0/32	1/77 (1.3)
Buton	1/10 (10.0)	0/8	1/18 (5.6)
Muna	1/61 (1.6)	0/43	1/104 (1.0)
Northern Sulawesi Island & Bangka Island			
Minahasa	2/158 (1.3)	0/161	2/319 (0.6)
Sangirese	4/22 (18.1)	0/10	4/32 (12.5)
Buru Island & Halmahera Island [22]			
Ambonese	42/654 (6.4)	-	-

*A Balinese detected at a village near Labuan Bajo.

Table 2 Glucose-6-phosphate dehydrogenase variants detected in major ethnic groups on Flores and Palue Islands and comparison with Javanese

Nucleotide Change	Name of variant	No. of identified									
		Sikka	Ende	Bajo#	Bajawa	Riung	Nage-Keo	Palue	Larantuka	Total	Javanese†
383T>C	Vanua Lava	1 (2)*	(9)*	(3)*	1	2			1	19	
487G>A	Mahidol										2
563C>T	Mediterranean					2				2	5
592C>T	Coimbra	2 (7)		(1)		1	1			12	1
844G>T	Bajo Maumere			(3)						3	
871G>A	Viangchan	(1)		(11)	1	2			1	16	
1003G>A	Chatham	7 (9)	(1)							17	
1024C>T	Chinese-5		(2)**							2	
1376G>T	Canton										3
1388G>A	Kaiping	9(14)	(2)		1	1		1		28	
Total		19(33)	(14)	(18)	3	8	1	1	2	99	11

*Numbers in parentheses are reported in our previous studies [5, 9].
#The Bajo group is of Mindanao origin. **Heterozygous females with a Chinese father.
† [22, 24]

Table 3 Glucose-6-phosphate dehydrogenase variants detected in eastern Indonesian populations and comparison with Sikka, Ende, Riung and Javanese

Nucleotide Change	Name of variant	No. of identified												
		Sikka	Ende	Riung	Timorese	Sumbanese	Savunese	Kendari	Buton	Muna	Minahasa	Sangirese	Ambonese†	Total	Javanese†
383T>C	Vanua Lava	3*	9*	2	4	24	3	1	1	1	2	3	11	64	
487G>A	Mahidol														2
563C>T	Mediterranean			2										2	5
592C>T	Coimbra	9		1										10	1
871G>A	Viangchan	1		2		5	1					1		10	
1003G>A	Chatham	16	1			2								19	
1024C>T	Chinese-5		2											2	
1376G>T	Canton														3
1388G>A	Kaiping	23	2	1			1							27	
Total		52	14	8	4	31	5	1	1	1	2	4	11	134	11

*Total numbers reported in our previous studies [5, 9] and this study †[22, 24]

of the Ende group, which possessed the most dominant variant of G6PD Vanua Lava. In addition, these 2 frequency distributions were also different from those of the Bajo group. In the present study, we further surveyed in other major ethnic groups on Flores and Palue Islands, and compared the results with those of the Sikka collected at a new study site in Sikka village, Pruda.

As shown in Table 1, no deficiency was detected in the Manggarai population, but one case of deficiency was found in a trans-immigrant Balinese near Labuan Bajo. Deficiency rates in the Bajawa, Nage-Keo, Palue and Lalantuka populations were apparently very lower than that of a Sikka village, ranging between 0.4% to 2.9%.

Molecular analysis of variants in 19 samples detected from the Sikka village (Table 2) indicated that this village had a frequency distribution of G6PD variants similar to those in our previous studies [5, 9], and the most dominant variant was also again G6PD Kaiping, followed by G6PD Chatham. In the Bajawa, Nage-Keo, Palue and Lalantuka populations, 2 cases of G6PD Vanua Lava, one case of G6PD Coimbra, 2 cases of G6PD Viangchan and 2 cases of G6PD Kaiping were identified (Table 2; Fig. 1). However, it was difficult to clarify the characteristics of their genotypes because of the limited numbers of deficient individuals among these groups. One case found in a Balinese was identified as G6PD Murcia (209A>G), a variant of Spanish origin with a substitution from Cys to Arg [20]. This was the first report of this variant in Asia.

On the other hand, the Riung are nonnative islanders, and we therefore expected to discover a unique G6PD variant or unique combination of G6PD variants in this group. And in fact, we did find a combination pattern of G6PD variants distinct from the patterns in the Sikka and the Ende groups, with one of the largest differences being that the Riung possessed the G6PD Mediterranean and G6PD Viangchan variants. G6PD Viangchan is the most common variant in continental Southeast Asia, but is very rare or entirely absent in the Sikka and the Ende groups [5, 9]. These results may suggest that the Riung group is absolutely different from native Flores islanders.

In our survey on West Timor Island, the deficiency rate in Timorese was 2.0%, and all 4 cases had the G6PD Vanua Lava (Tables 1 and 3; Fig. 2). Furthermore, 27 cases of G6PD Vanua Lava were detected in the Sumbanese and Savunese populations (Tables 1 and 3). On the Southeastern Sulawesi and Muna Islands, 3 cases of deficient individuals were detected from Kendari, Buton and Muna, and all of them were also identified as G6PD Vanua Lava. In addition, on the Northern Sulawesi and Bangka Islands, 4 cases of Sangirese were identified as carrying the G6PD Vanua Lava (n=3) and G6PD Viangchan variants. In the Minahasa group, deficiency incident was very low. However, 2 cases of deficient individuals were detected in this survey, and both were also G6PD Vanua Lava. The origin of the Minahasa group is unknown, but it is thought to have migrated from the Philippines, or from more northern areas, including Taiwan [21]: The Minahasa differ largely from other Indonesian peoples in that their skin has a light brown or yellow tint like Chinese.

Therefore, the incidence rate of G6PD deficiency seems to be very low in the Minahasa, as observed in Mongolian, Korean and Japanese.

We anticipated that our analysis of G6PD variants in the Timor, Sumba and Sulawesi islanders would reveal many different variants, as detected in the Sikka and the Riung groups. Surprisingly, however, almost all of variants detected were G6PD Vanua Lava only, except for 7 cases of G6PD Viangchan, 2 cases of G6PD Chatam and one case of G6PD Kaiping (Table 3). This tendency was also observed in the Ende group. Considering these results together along with our previous findings for the Ambonese on the Halmahera and Buru Islands [22], in whom G6PD Vanua Lava was found to be the only variant (n=11), G6PD Vanua Lava may be widely distributed as the most common variant in eastern Indonesian populations, except for the Sikka: In fact, among 134 G6PD-deficient cases detected from the eastern Indonesian islands, nearly half were identified as G6PD Vanua Lava (Table 3). Statistical analyses by Chi-square test for Sikka vs. Ende and Sikka vs. eastern Indonesian islanders (Timorese+Sumbanese+Savunese+Kendari+Buton+Muna+Minahasa+Sangirese+Ambonese) showed statistically significant differences at $P<0.05$. G6PD Vanua Lava was first reported in Vanuatu [23], where it is one of the most common variants, along with G6PD Union (1360C>T).

Another remarkable finding in this study was the confirmation, by molecular analyses, that G6PD Viangchan, G6PD Mahidol, G6PD Mediterranean, and G6PD Canton (of Chinese origin), the most common variants detected in continental Southeast Asian populations (probably including Javanese; see Tables 2–3), were very rare or entirely absent in eastern Indonesian populations [9]. However, G6PD Kaiping, another common variant of Chinese origin in Southeast Asia, is widely distributed among native Flores and Palue islanders for unknown reasons.

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References

1. Kawamoto F: Rapid diagnosis of malaria by fluorescence microscopy using light microscope and interference filter. Lancet (1991) 337: 200–202.
2. Kawamoto F and Billingsley PF: Rapid diagnosis of malaria by fluorescence microscopy. Parasitol Today (1992) 8: 69–71.
3. 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.
4. Arai M, Kosuge K, Kawamoto F and Matsuoka H: Reactivity of blood samples onto filter papers in the WST-8 method for screening of G6PD deficiency. Acta Med Okayama (2006) 60: 127–134.
5. 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.
6. 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.
7. 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.
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, Tantular IS, Pusarawati S, Kerong HI, Wera D, Mere D, Kanbe T and Dachlan YP: Further investigations of glucose-6-phosphate dehydrogenase variants including a novel variant in Indonesia. J Hum Gene (2006) 51: 952–957.
10. Matsuoka H, Thuan DTV, Thien HV, 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 Med Okayama (2007) 61: 213–219.
11. Wang J, Luo E, Hirai M, Arai M, Abdul-Manan EAS, Mohamed-Isa Z, Hidayah NI and Matsuoka H: Nine different glucose-6-phosphate dehydrogenase (G6PD) variants in a Malaysian population with Malay, Chinese, Indian and Orang Asli (Aboriginal Malaysian) backgrounds. Acta Med Okayama (2008) 62: 327–332.
12. Wang J, Matsuoka H, Hirai M, Mu L, Yang L and Luo E: The first case of a class I glucose-6-phosphate dehydrogenase deficiency, G6PD Santiago de Cuba (1339 G>A), from Chinese population as found in a survey for G6PD deficiency in northeastern and central China. Acta Med Okayama (2010) 64: 49–54.
13. Beutler E and Vulliamy TJ: Hematologically important mutations, glucose-6-phosphate dehydrogenase. Blood Cells Mol Dis (2002) 28: 93–103.
14. Cappellini MD and Fiorelli G: Glucose-6-phosphate dehydrogenase deficiency. Lancet (2008) 371: 64–74.
15. 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.

16. Vulliamy TJ, Othman A, Town M, Nathwani A, Falusi AG, Mason PJ and Luzzatto L: Polymorphic sites in the African population detected by sequence analysis of the glucose–6–phosphate dehydrogenase gene outline the evolution of the variants A and A-. Proc Natl Acad Sci USA (1991) 88: 8568–8571.
17. Beutler E, Westwood B and Sipe B: A new polymorphic site in the G6PD gene. Hum Genet (1992) 89: 485–486.
18. Muller K: Flores; in East of Bali from Lombok to Timor, Muller K and Pickell D eds, Periplus, Hong Kong (1997) pp 119–145.
19. Fox JJ: History and people, The flow of life in Nusa Tenggara; in East of Bali from Lombok to Timor, Muller K and Pickell D eds, Periplus, Hong Kong (1997) pp 30–35.
20. Rovira A, Vulliamy T, Pujades MA, Luzzatto L and Vives Corrons JL: Molecular gentics of glucose–6–phosphate dehydrogenase (G6PD) deficiency in Spain, identification of two new point mutations in the G6PD gene. Br J Hematol (1995) 91: 66–71.
21. Wallace AR: Celebes; in The Malay Archipelago, Periplus, Hong Kong (2000) pp 185–207 (original book was published in 1890)
22. 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.
23. 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.
24. Soemantri AG, Saha S, Saha N and Tay JS: Molecular variants of red cell glucose–6–phosphate dehydrogenase deficiency in Central Java, Indonesia. Hum Hered (1995) 45: 346–350.