J Hum Genet (2003) 48:541–544
DOI 10.1007/s10038-003-0073-8

ORIGINAL ARTICLE

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Five different glucose-6-phophate dehydrogenase (G6PD) variants found among 11 G6PD-deficient persons in Flores Island, Indonesia

Received: 24 July 2003 / Accepted: 12 August 2003 / Published online: 23 September 2003

Abstract We conducted a survey for malaria diagnosis and treatment in four primary schools in Flores Island, one of the Indonesian Islands with an area of 17,000 km^2 and a population of 1.8 million. Of those examined, 24.4% were diagnosed as having malaria (90/363) and administered medicine immediately. A glucose-6-phophate dehydrogenase (G6PD) test was performed at the same time, and 16 persons (4.4%) were diagnosed as G6PD deficient. Eleven persons consented to analysis of the *G6PD* genome. We analyzed these subjects and found one case of G6PD Vanua Lava (383T > C), five cases of G6PD Coimbra (592C > T), one case of G6PD Viangchan (871G > A), one case of G6PD Chatham (1003G > A), and three cases of G6PD Kaiping (1388G > A). These were unexpected findings because five different G6PD variants were found in such a small population. This suggests that people of Flores Island are derived from various ancestries.

Keywords Glucose-6-phophate dehydrogenase deficiency · Flores · Indonesia · Malaria · Primaquine

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most frequent hereditary abnormalities. *G6PD* exists on the X-chromosome distributed in 13 exons (Chen et al. 1991). Almost all G6PD deficiencies are caused by one amino-acid change caused by a point mutation of the genomic DNA, and more than 100 molecular abnormalities of the *G6PD* genotype have been identified (Fujii and Miwa 1998).

We introduced the rapid diagnosis method for malaria (Kawamoto and Billingsley 1992), a disease that kills more than 2 million people world-wide annually, and G6PD deficiency test (Hirono et al. 1998) in malaria endemic areas. Using these methods, patients are notified of the results of blood examination within 60 min and are able to receive antimalarial medicine, including primaquine (Tantular et al. 1999). Primaquine can kill gametocytes, the sexual stage of malaria parasites, which are the cause of malaria transmission to mosquitoes. However, when G6PD-deficient persons take primaquine, a hemolytic attack can occur. Without G6PD, erythrocytes cannot prepare a sufficient amount of reduced pyridine nucleotide and reduced glutathione, and cannot prevent oxidant attack by primaquine. Thus, primaquine should not be administered to malaria patients before confirming their G6PD activity.

We have visited malaria endemic areas in Asian countries to introduce these quick methods of malaria diagnosis and the G6PD test. By these activities, we have rediscovered two types of human malaria, *Plasmodium minuta* and *P. tenue* (Kawamoto et al. 2002) and also described ten G6PD variants including one new variant, G6PD Surabaya (1291G > A, V431 M) (Iwai et al. 2001). By the investigation of these G6PD variants in Asian countries, we can estimate peoples' origin. For instance, G6PD Viangchan (871G > A, V291M) spreads in Laos and Thailand but not in Myanmar. G6PD Mahidol (487G > A, G163S) distributes in Myanmar and Thailand but not in Laos. Thus, Myanmar people

and Lao people are different in terms of G6PD variation. The*G6PD* genome is a good marker for studying human origin among these countries.

In Indonesia, we previously visited Halmahera Islands (Ambon) and found malaria patients and G6PD-deficient persons with the same variant type–G6PD Vanua Lava (383T > C, L128P) (Iwai et al. 2001)–that was previously found in Vanuatu, one of the Melanesian countries (Ganczakowski et al. 1995). Recently we visited Flores Island, which was a part of the Sunda Archipelago, to carry out a survey of malaria diagnosis and treatment in primary schools. We detected eleven G6PD-deficient persons in Flores Island consisting not of the same G6PD variant but of five different G6PD variants.

Materials and methods

Flores Island is one of the Indonesian islands, with an area of 17,000 km^2 and a population of 1.8 million. On Flores Island, we selected four primary schools: Two were in Maumere City and two were in Talibura subdistrict 50 km east of Maumere City. Students ranging from 7–12 years old were the target group because the incidence of malaria is higher in younger age groups than in older groups (Matsuoka et al. 1987). Three drops of blood were collected from a fingertip of each student–one for malaria diagnosis, one for hemoglobin concentration, and one for the G6PD test. Malaria was diagnosed by acridine orange staining methods (Kawamoto and Billingsley 1992). Hemoglobin concentration was measured using a battery-powered, HemoCue machine (Angelhorm, Sweden). The G6PD test was carried out using a newly developed, rapid method (Tantular and Kawamoto, 2003). Results of G6PD activity were obtained in 20 min after taking blood.

When a G6PD-deficient student was detected, we contacted his/her parent and explained the purpose of the investigation of the *G6PD* genome. When the student and the parent agreed, we received informed consent from them and collected 0.2 ml of peripheral blood from the student. Blood samples were stored at 4°C and brought back to Japan. G6PD activity of each sample was confirmed by another G6PD test developed by Fujii et al. (1984). Then the DNA sequence of *G6PD* was identified. This study was approved by the Health Department, Sikka District, Flores Island, East Nusa Tenggara Province; and the Ethical Committees of Jichi Medical School and Nagoya University Graduate School of Medicine.

Genomic DNA was extracted from 0.1 ml of whole blood and purified with a DNA purification kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). Since genomic *G6PD* consists of 13 exons, we prepared primers for each exon (Hirono et al. 1994), amplified the exon by PCR, and read the DNA sequence (ABI PRISM 310; PE Biosystems, CT, USA). Both strands of each exon were sequenced. To indicate the mutation point, the nucleotide number of cDNA sequence was used. We also read some introns of *G6PD* because silent mutations had been found on introns of genomic *G6PD*; e.g., nt 175C > T on intron 7, nt 163C > T on intron 8 (Vulliamy et al. 1991), and nt 93T > C on intron 11 (Beutler et al. 1992).

Results and discussion

Among 363 students examined, 90 were malaria positive (24.8%). They received malarial medicine within 60 min after taking blood. Besides the malaria diagnosis, 11 persons among 177 males and five among 186 females were diagnosed as G6PD deficient (4.4%). Three students were malaria positive in 16 students of G6PD deficiency (18.8%) (Table 1). None of the sixteen students with G6PD deficiency showed anemia. Their hemoglobin levels were from 10.1 to 13.6 g/dl. This indicates that G6PD deficiency did not cause chronic anemia in these subjects from Flores Island.

Eleven parents of 16 students agreed to undergo molecular analysis of G6PD. We first read exon 5 (nt 268–485 of *G6PD* cDNA) for all eleven samples because all G6PD-deficient people showed the same genotype—G6PD Vanua Lava (383T > C, L128P)–in our previous investigation on Halmahera Islands near Flores

Table 1 List of students with glucose-6-phophate dehydrogenase (G6PD) deficiency in Flores Island, Indonesia

Place of study	Age/gender	Hb (g/dl)	G6PD[a]	Malaria[b]	Nucleotide change[c] (exon no.)	Amino-acid change	Name of variant
Maumere	7/M	13.0	–	–	871G > A (ex 9)	V291M	Viangchan
	7/M	13.6	–	–	1388G > A (ex 12)	R463H	Kaiping
	8/M	11.6	–	–	ND[d]		
	9/M	11.0	–	–	383T > C (ex 5)	L128P	Vanua Lava
	12/F	11.8	±	–	ND[d]		
Talibura	7/M	11.4	–	Pv+	592C > T (ex 6)	R198C	Coimbra
	7/M	10.8	–	Pf+	592C > T (ex 6)	R198C	Coimbra
	7/F	10.6	±	–	1388G > A/G (ex 12)	R463H	Kaiping
	7/F	10.1	±	–	1388G > A/G (ex 12)	R463H	Kaiping
	7/F	12.9	±	Pf+	592C > T/C (ex 6)	R198C	Coimbra
	8/M	10.4	–	–	592C > T (ex 6)	R198C	Coimbra
	9/M	10.7	–	–	ND[d]		
	9/M	12.5	–	–	ND[d]		
	10/M	11.1	–	–	ND[d]		
	11/M	11.8	–	–	1003G > A (ex 9)	A335T	Chatham
	12/F	10.2	±	–	592C > T/C (ex 6)	R198C	Coimbra

[a] – = G6PD deficient, severe; ± = G6PD deficient, mild
[b] – = No malaria parasites in peripheral blood; Pv+ = vivax malaria positive; Pf+ = falciparum malaria positive
[c] Nucleotide numbers are from cDNA sequence. Variants of four female students were heterozygotes
[d] ND = Sequence analysis was not done

Island (Iwai et al. 2001). Unexpectedly, we could find only one case of G6PD Vanua Lava. We read the other exons one by one and found four other different G6PD variants (Fig. 1). These were five cases of G6PD Coimbra (592C > T, R198C), one case of G6PD Viangchan (871G > A, V291M), one case of G6PD Chatham (1003G > A, A335T), and three cases of G6PD Kaiping (1388G > A, R463H) (Table 1). All four samples from female students were heterozygotes.

Compared to our previous results, distribution of G6PD variants was quite unique in Flores Island (Fig. 1). We obtained five different G6PD variants from such a small sample pool. We have read more than 60 persons' *G6PD* genomes and described ten molecular abnormalities of the *G6PD* genotype in Asian countries (Iwai et al. 2001), but we have not previously found such a wide variation of G6PD variants from such a small population. In Myanmar, we visited more than ten villages across the country and analyzed 30 G6PD-deficient samples. The result was 28 cases of C6PD Mahidol (487G > A, G163S), one case of G6PD Union (1360C > T, R454C), and one case of G6PD Canton (1376G > T, R459L). Thus, we concluded that Myanmar people have similar ancestry in terms of G6PD variation (Iwai et al. 2001).

In contrast, Ainoon et al. (2003) demonstrated ten G6PD variants among 80 G6PD-deficient persons in Malaysian Malays. They concluded that Malaysian Malays had various ancestral contributions. Data from Flores Island may similarly indicate a diverse ancestry. Flores Island belongs to the Sunda Archipelago where people might come from Eurasian countries, African countries, Philippine Islands, and Pacific Islands. Historically Flores Island might have accepted many tribes from different origins.

We found one case of G6PD Viangchan (871G > A, V291 M). This case had a silent C > T mutation at nt 1311 on exon 11 (nt 1288–1364 of *G6PD* cDNA) and another silent mutation at nt 93 T > C on intron 11. We examined these points in the other ten G6PD variants from Flores Island, but all showed the wild type (nt 1311C on exon 11 and nt 93T on intron 11). Beutler et al. (1992) described these triple mutations in G6PD Mediterranean (563C > T, S188F) and G6PD Viangchan (871G > A, V291 M). Our findings concur with their

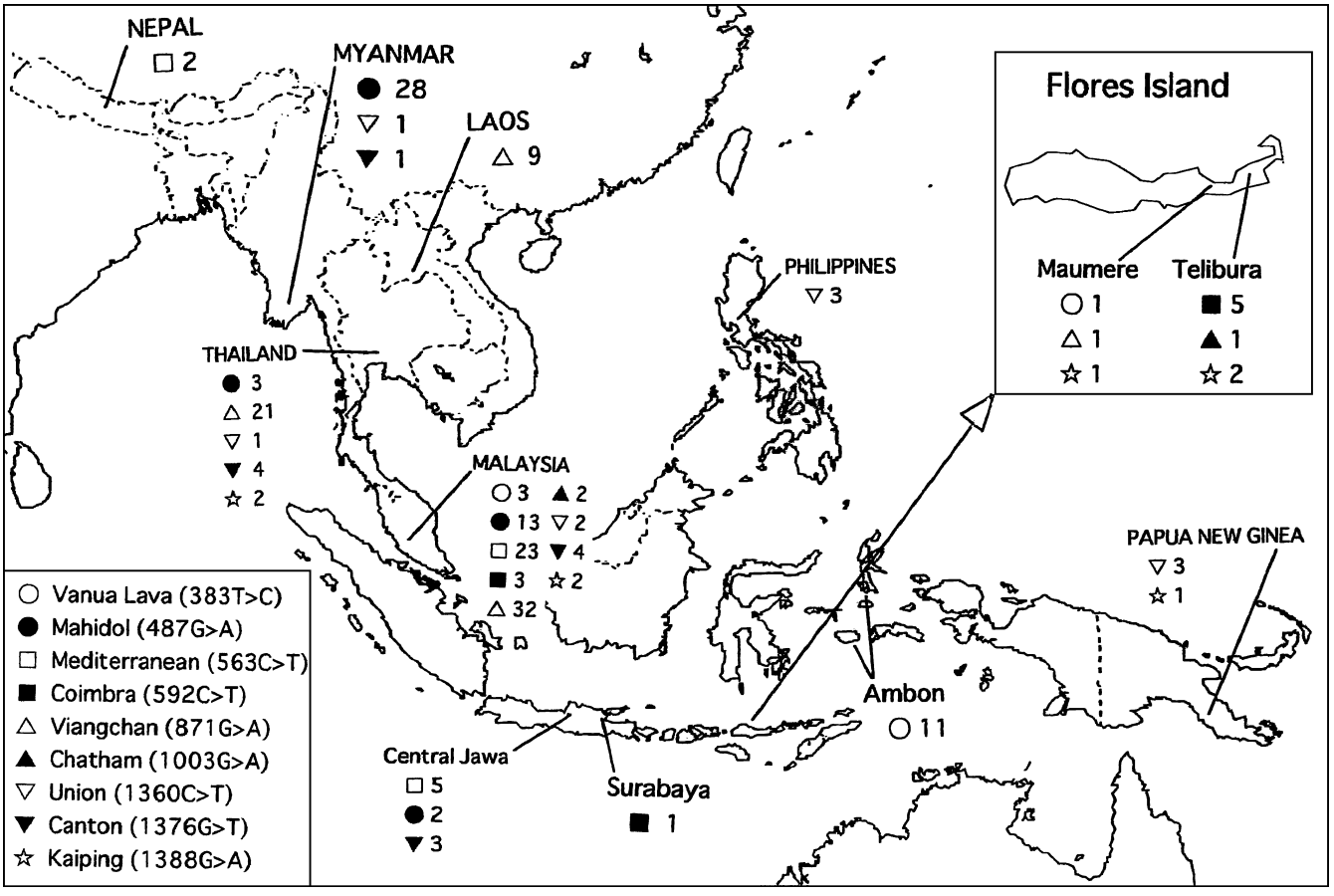

Fig. 1 Distribution of glucose-6-phophate dehydrogenase (G6PD) variants in Asian countries. Each number indicates the number of G6PD-deficient cases confirmed by sequence analysis. Data of Nepal, Myanmar, Laos, Surabaya, and Ambon (Halmahera Islands) are from our previous reports (Iwai et al. 2001; Matsuoka et al. 2003). Data of Thailand, Malaysia, Central Java, Philippines, and Papua New Guinea are from reports by Nuchprayoon et al. (2002), Ainoon et al. (2003), Soemantri et al. (1995), Silao et al. (1999) and Wagner et al. (1996), respectively. Data from Chinese origin are omitted

results. We previously obtained the same silent mutations as G6PD Mediterranean (563C > T, S188F) with a silent mutation of nt 1311C > T on exon 11 and another silent mutation at nt 93 T > C on intron 11 in Nepal (Matsuoka et al. 2003). Mutations of G6PD Mediterranean (563C > T, S188F) and G6PD Viangchan (871G > A, V291 M) might occur in the Asian peoples having two mutations, 1311C > T on exon 11 and nt 93 T > C on intron 11.

Acknowledgements We thank Miss Maiko Nakamura for her technical assistance. This work was supported by a grant-in-aid from the Japanese Ministry of Education, Science, Culture, and Sports (14406026 to HM).

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