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ORIGINAL INVESTIGATION

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Distribution of glucose-6-phosphate dehydrogenase mutations in Southeast Asia

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Abstract Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a heterogeneous enzyme abnormality with high frequency in tropical areas. We performed population screening and molecular studies of G6PD variants to clarify their distribution and features in Southeast Asia. A total of 4317 participants (2019 males, 2298 females) from 16 ethnic groups in Myanmar, Lao in Laos, and Amboinese in Indonesia were screened with a single-step screening method. The prevalence of G6PD-deficient males ranged from 0% (the Akha) to 10.8% (the Shan). These G6PD-deficient individuals and 12 G6PD-deficient patients who had been diagnosed at hospitals in Indonesia and Malaysia were subjected to molecular analysis by a combination of polymerase-chain-reaction-based single-strand conformation polymorphism analysis and direct sequencing. Ten different missense mutations were identified in 63 G6PD-deficient individuals (50 hemizygotes, 11 heterozygotes, and 2 homozygotes) from 14 ethnic groups. One missense mutation (1291 G→A) found in an Indonesian Chinese, viz., G6PD Surabaya, was previously unknown. The 487 G→A (G6PD Mahidol) mutation was widely seen in Myanmar, 383 T→C (G6PD Vanua Lava) was specifically found among Amboinese, 871 G→A (G6PD Viangchan) was observed mainly in Lao, and 592 C→T (G6PD Coimbra) was found in Malaysian aborigines (Orang Asli). The other five mutations, 95 A→G (G6PD Gaohe), 1003 G→A (G6PD Chatham), 1360 C→T (G6PD Union), 1376 G→T (G6PD Canton), and 1388 G→A (G6PD Kaiping) were identified mostly in accordance with distributions reported previously.

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most frequent hereditary enzyme abnormalities. As it is inherited in an X-linked fashion, most of the cases are males. Up until 1997, a total of 122 molecular abnormalities had been identified (Vulliamy et al. 1997). According to WHO criteria, these variants have been grouped into five classes: variants causing chronic nonspherocytic hemolytic anemia (class I), variants with reduced enzyme activity (classes II and III), variants with normal activity (class IV), and those with excessive activity (class V; WHO Working Group 1989). Although the class II–V variants do not cause chronic hemolysis, acute hemolytic attack may occur when the presence of a variant is combined with various types of oxidative stress, including medication and infection. The geographical distribution of G6PD deficiency, especially classes II and III,

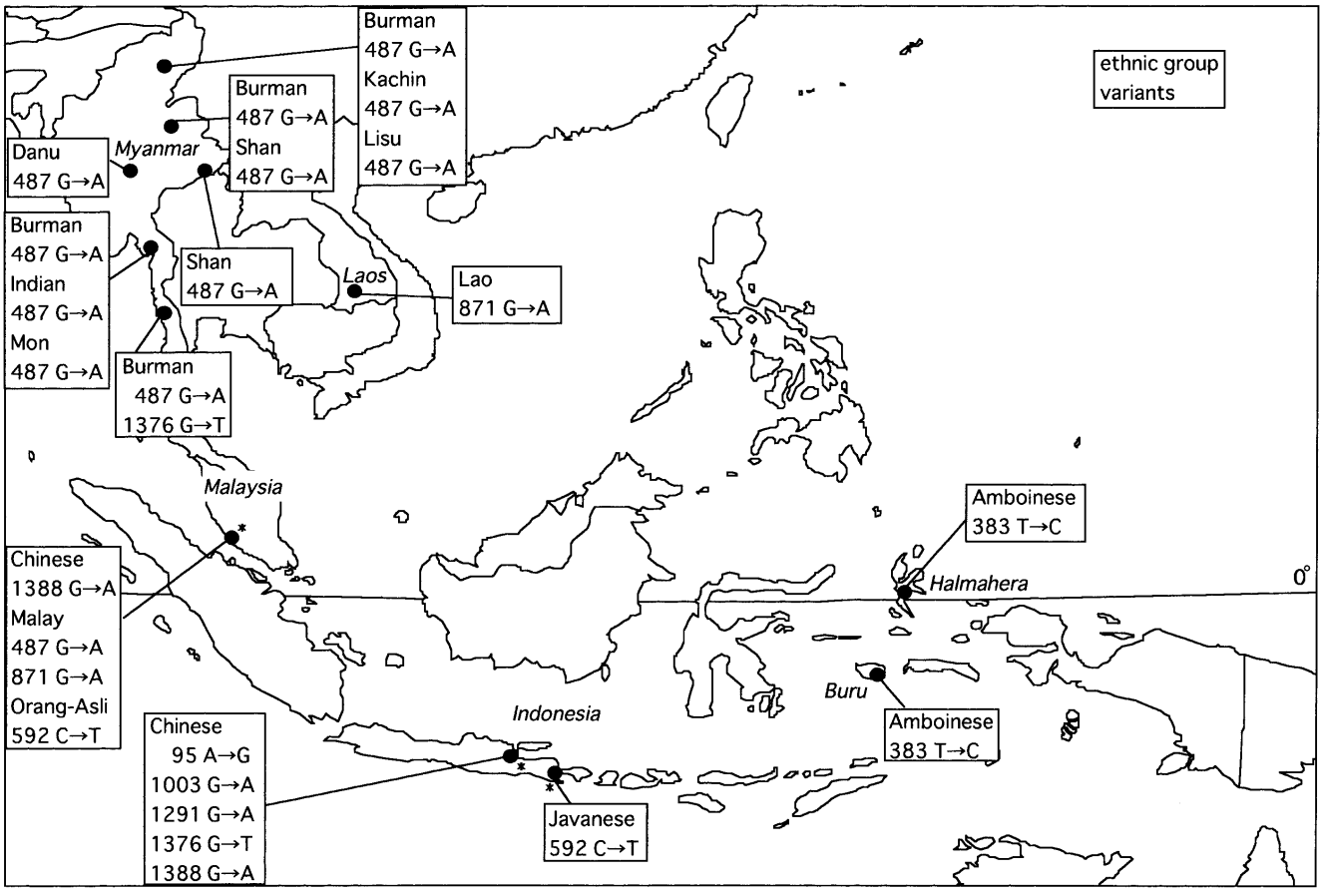

Fig. 1 The region of study and G6PD molecular variants identified in each country. *Asterisks* indicate the cases detected in a hospital-based study or case study; the others are from a population-based study

appears to be correlated with malaria endemicity. The increased resistance of G6PD-deficient erythrocytes to malaria might explain this, at least partially (Ruwende et al. 1995).

The higher frequency and noticeable heterogeneity of G6PD variants among ethnic groups in tropical Asia may help to deduce their genetic relationship (WHO Scientific Group 1967; Panich 1986). Previous studies have established the molecular abnormalities responsible for G6PD deficiency in several ethnic groups in Southeast Asia, including Chinese (Chang et al. 1992; Tang et al. 1992; Chiu et al. 1993; Lo et al. 1994; Saha et al. 1994; Xu et al. 1995; Huang et al. 1996; Chen et al. 1998), aboriginal tribes (the Ami, the Yami, and the Saisiat) in Taiwan (Tang et al. 1995), Javanese (Soemantri et al. 1995), tribes in Papua New Guinea (Wagner et al. 1996; Hirono et al. 1998b), islanders in Vanuatu (Ganczakowski et al. 1995), people in the Solomon Islands (Hirono et al. 1995), and immigrants from the Philippines and Laos (Beutler et al. 1992; Hsia et al. 1993). However, there has been no population-based study on molecular variants in the Indochina Peninsula and the Indonesian islands, except for Java. The purpose of this study has been to clarify the distribution and features of G6PD mutations in various ethnic groups in Southeast Asia. This is the first population-based report concerning G6PD mutations in some tribes in Myanmar and Laos, Amboinese in Indonesia, and aboriginal tribes in Malaysia (Orang Asli) and fills some blank areas in the G6PD mutation survey in Southeast Asia.

Materials and methods

Sample collection

Samples were obtained from G6PD-deficient individuals and some non-G6PD-deficient individuals in Southeast Asian countries, including Myanmar, Laos, Indonesia, and Malaysia during 1997 and 1998 (Fig. 1). The G6PD-deficient individuals were detected by a single-step screening method (Hirono et al. 1998a). In Myanmar and Laos, the G6PD-deficient individuals were screened in the context of population-based field trials for the quick detection and treatment of malaria (Tantular et al. 1999). In Indonesia, we collected samples from G6PD-deficient individuals who were detected in two population-based studies and from the patients who visited a hospital because of acute hemolytic attack. In Malaysia, the G6PD-deficient individuals were detected by only a hospital-based study. The blood samples were transported at 4°C or at –20°C and were kept at –20°C until DNA purification.

Mutation analysis

Genomic DNA was purified from 100 µl thawed whole blood by using a genomic blood DNA purification kit (GFX; Amersham Pharmacia Biotech, Buckinghamshire, UK), following the manufacturer's instructions. Polymerase-chain-reaction-based single-

strand conformation polymorphism (PCR-SSCP) analysis was performed by our previously published method (Hirono et al. 1994, 1997). We amplified the entire coding region (from exon 2 to exon 13) and the partial intron sequences adjacent to the exons in three segments (exons 2, 3–6, and 6–13) by using three pairs of oligonucleotide primers. The nested PCR for SSCP analysis was performed in 10 μl reaction mixture containing 1/50 volume of first PCR product. The exons that showed mobility shifts were directly sequenced by an automated DNA sequencer (ABI PRISM 310; PE Biosystems, Conn., USA) with the same synthetic oligonucleotide primers as in the PCR-SSCP analysis. The mutation was verified either by sequencing both strands or by detecting an altered restriction site. If the sample showed no mobility shift by SSCP, exons 2–13 were sequenced by the same method. The position of each mutation was expressed by using the cDNA numbers identified (Vulliamy et al. 1997).

Results

We screened overall 4317 participants (2019 males, 2298 females) with identified ethnic origins in our population-based study; 2058 (1032 males, 1026 females) individuals belonged to 16 ethnic groups in Myanmar, 678 to the Lao ethnic group (291 males, 387 females) in Laos, and 1581 to the Amboinese group (696 males, 885 females) in Indonesia. In Myanmar, Burmans inhabited all study areas, whereas other ethnic groups tended to cluster in smaller areas (Fig. 1). In Indonesia and Laos, the participants originated within the same Provinces.

Table 1 shows the prevalence of G6PD-deficient males in the various ethnic groups. We did not include the data from females, because G6PD activities in heterozygous females are highly variable, and it may be difficult to detect all heterozygotes by the present screening procedures. The prevalence of G6PD-deficient males in the population-based study ranged from 0% (the Akha) to 10.8% (the Shan). No case of G6PD deficiency was found in the Akha, an isolated ethnic group in Shan State and whose prevalence was previously unknown.

All mutations, except 1388 G→A (G6PD Kaiping), showed a clear mobility shift on SSCP gels. The het-

Table 1 Prevalence of G6PD deficiency in males among various ethnic groups (*Others* includes the ethnic groups Ghurkha, Intha, Karen,Lahu, Naga, Rakhine, and Wa)

Country	Ethnic group	Non-deficient	Deficient	Prevalence (%)
Myanmar	Shan	33	4	10.8
	Indian	59	5	7.8
	Burma (Burman)	490	39	7.3
	Danu	26	2	7.1
	Mon	42	3	6.7
	Kachin (Chingpaw)	120	8	6.3
	Chinese	86	4	4.4
	Lisu	37	1	2.6
	Akha	45	0	0.0
	Others	28	0	0.0
Laos	Lao	270	21	7.2
Indonesia	Amboinese	654	42	6.0

Table 2 Distribution of G6PD mutations found in each country and ethnic group. Heterozygotes are in parentheses

Nucleotide change	Amino acid change	Name of variant	Myanmar							Laos	Indonesia			Malaysia			Total
			Shan	Burma (Burman)	Danu	Mon	Kachin (Chingpaw)	Lisu	Indian	Lao	Amboinese	Javanese	Chinese	Unknown	Malay	Orang-Asli	Chinese	
95 A→G	32 His→Arg	Gaohe											[a]1					1
383 T→C	128 Leu→Pro	Vanua Lava									11 (3)							11 (3)
487 G→A	163 Gly→Ser	Mahidol	2	14 (1)	1	2	5	2	2						2			30 (1)
592 C→T	198 Arg→Cys	Coimbra										1				2		3
871 G→A	291 Val→Met	Viangchan								9 (6)					1			10 (6)
1003 G→A	335 Ala→Thr	Chatham											1 (1)[a]	1				2 (1)
1291 G→A	431 Val→Met	Surabaya											1[a]					1
1360 C→T	454 Arg→Cys	Union		1														1
1376 G→T	459 Arg→Leu	Canton		1									1[a]					2
1388 G→A	463 Arg→His	Kaiping											1[a]				1	2
Total			2	16 (1)	1	2	5	2	2	9 (6)	11 (3)	1	5 (1)[a]	1	3	2	1	63 (11)

[a]Samples obtained from patients who had a history of acute hemolytic attack

erozygous females for the 487 G→A (G6PD Mahidol) mutation were confirmed by the creation of another *Alu*I cleavage site. Because most of the 1003 G→A (G6PD Chatham) and the G6PD Kaiping mutations showed minimal mobility shift, the final identification of these mutations often required sequencing analysis. A novel mutation, 1291 G→A (G6PD Surabaya), was found in an 18-year-old Chinese male who visited a hospital in Surabaya because of an episode of acute hemolytic attack without chronic hemolysis. The biochemical properties of his G6PD were not identified because of the difficulty of obtaining an additional blood sample. All other mutations were those reported previously.

As shown in Table 2, 10 missense mutations were identified in a total of 63 G6PD-deficient individuals (50 hemizygotes, 11 heterozygotes, and 2 homozygotes), 51 (39 hemizygotes, 10 heterozygotes, and 2 homozygotes) were detected by the population-based study, and the others were patients visiting hospitals for hemolytic crises. In Malaysia, two Orang Asli, a Temiar, and a Jakun were found to be G6PD-deficient. The Orang Asli came from an aboriginal group living on the Malay Peninsula. They consist of three main tribes; the Negritos, the Senoi, and the Proto-Malays (Halin 1990). The Temiar is a subtribe of the Senoi, and the Jakun belongs to the Proto-Malays.

Mutation 383 T→C (G6PD Vanua Lava) was specifically found in the Amboinese. G6PD Mahidol was the predominant mutation in various ethnic groups in Myanmar and was also found in Malay. Mutation 592 C→T (G6PD Coimbra) was found in the Orang Asli and in a Javanese who was originally from the eastern coast of Java Island. Mutation 871 G→A (G6PD Viangchan) was found in the Lao and a Malay. A Burman in Mon State had 1360 C→T (G6PD Union). Other mutations (95 A→G, 1003 G→A, 1376 G→T, and 1388 G→A) were observed exclusively in Chinese, except for the 1376 G→T (G6PD Canton) mutation, which was found in a Burman living in the Taninthayi Division of Myanmar (Fig. 1).

Discussion

Our screening results show that the frequency of G6PD deficiency in Southeast Asia is highly variable (Table 1), as previously documented in some ethnic groups in this area (WHO Scientific Group 1967; Panich 1986).

Our results from the molecular analysis suggest that the distribution of G6PD mutations varies with geographical areas and/or ethnic groups (Fig. 1, Table 2). In Myanmar, G6PD Mahidol was the dominant variant in all ethnic groups analyzed in our study. This indicates that, in spite of their different cultural backgrounds, these populations share the same origin or there has been long-lasting genetic interaction among them. We have also found G6PD Mahidol in two Indian males in Myanmar. Although this mutation has been reported in an Indian immigrant in Mauritius (Kotea et al. 1999), it has never been documented on the Indian subcontinent (Kaeda et al. 1995). We consider that the G6PD Mahidol mutation in the Indians in Myanmar may have occurred independently in India, because intermarriage between Indians and other ethnic groups in Myanmar is rare. The G6PD Mahidol mutation has also been documented in Laotian immigrants in Hawaii (Beutler et al. 1992; Hsia et al. 1993), Javanese in central Java (Soemantri et al. 1995), a Thai immigrant in Japan (A. Hirono, unpublished), and Chinese in Taiwan (Chang et al. 1992; Tang et al. 1992; Lo et al. 1994; Huang et al. 1996), suggesting the ancient origin of this mutation.

In Laos, G6PD Viangchan was the only mutation found in the current study. It is interesting that we have found totally different mutations in Myanmar and Laos, in spite of their geographical continuity. This suggest the different origin of ethnic groups in Myanmar and in Laos (Lao). G6PD Viangchan has been identified in Thai immigrants in Japan (A. Hirono, unpublished) and Filipino immigrants in Hawaii (Beutler et al. 1992; Hsia et al. 1993). G6PD Mahidol and G6PD Viangchan appear to be the most important variants as genetic markers in the Indochina peninsula. A more detailed population survey may clarify the origin of populations in this area.

G6PD mutations in Chinese are heterogeneous. At least 10 mutations have so far been reported (Chang et al. 1992; Tang et al. 1992; Chiu et al. 1993; Lo et al. 1994; Saha et al. 1994; Xu et al. 1995; Huang et al. 1996; Chen et al. 1998). We have identified five different mutations, including a novel one. Of our five mutations, 95 A→G (G6PD Gaohe), G6PD Canton, and G6PD Kaiping have previously been identified in Chinese. A novel missense mutation, 1291 G→A causing 431 Val→Met, has been found in a Chinese male with an overt hemolytic episode. The mutation is located in a region in which many severe class I mutations cluster. Val 431 is not a phylogenetically conserved residue. This may partially explain the relatively mild nature of this mutation. G6PD Chatham has never been described in Chinese.

The Amboinese are characterized by G6PD Vanua Lava, which has primarily been reported in the Vanuatu archipelago in the Western Pacific (Ganczakowski et al. 1995). This suggests a genetic relationship between the populations in Maluku and the islanders in Vanuatu. Most of the inhabitants in Vanuatu are Melanesians. The dominant G6PD mutation in Melanesians is reported to be G6PD Union (Ganczakowski et al. 1995; Hirono et al. 1995). It is somewhat surprising that we found no G6PD Union in the Amboinese, despite their possible connection with Melanesians. A possible reason for the lack of G6PD Union in the Maluku population is the genetic drift caused by their long-term isolation.

With respect to the Orang Asli (the Malaysian aborigines), we could not draw any significant conclusion because of the insufficient sample size. A previous study of the peptidase B variants in these people concluded that the Semai, a sub-tribe of Senoi, might be a separate group from Proto-Malays including the Jakun (Welch 1973). In contrast to this study, we have found G6PD Coimbra in both Temiar and Jakun individuals. G6PD Coimbra has

also been found in a Javanese male in the present study. This mutation has also been reported in the Ami, an aboriginal tribe in Taiwan (Tang et al. 1995).

Genetic analysis of G6PD deficiency may help to clarify the genetic relationship among Southeast Asian populations and to infer their historical movements. However, the use of G6PD mutations as population markers is limited, because very few haplotypes have been established, particularly in Mongoloids. Further efforts, including a survey of non-coding polymorphic mutations, are necessary for the precise interpretation of the accumulated data.

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