Further Molecular Analysis of G6PD Deficiency Variants in Southern Vietnam and a Novel Variant Designated as G6PD Ho Chi Minh (173 A > G; 58 Asp > Gly): Frequency Distributions of Variants Compared with Those in Other Southeast Asian Countries

Fumihiko Kawamoto a,b, Hiroyuki Matsuoka c, Nghiem Minh Pham d, Taeko Hayashi e, Yuichi Kasahara c, Nguyen The Dung e, Yasutoshi Kido b, Toshio Kanbe f, and Indah S. Tantular a,g*

a Institute of Tropical Disease, Airlangga University Campus C, Surabaya 60115, Indonesia,
b Department of Environmental & Preventive Medicine, Oita University Faculty of Medicine, Yufu, Oita 879-5593, Japan,
c Division of Medical Zoology, Jichi Medical University, Shimotsuke, Tochigi 329-0498, Japan,
d Tu Du Hospital, Ho Chi Minh City, Vietnam,
e Vietnam National University School of Medicine, Linh Trung Ward, Ho Chi Minh City, Vietnam,
f Division of Omics Analysis, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan,
and g Department of Parasitology, Airlangga University Faculty of Medicine, Surabaya 60131, Indonesia

We conducted a survey of glucose-6-phosphate dehydrogenase (G6PD) deficiency among newborn babies at Tu Du Hospital, Ho Chi Minh, southern Vietnam. A total of 90 deficient babies were detected, including 85 in the Kinh ethnic group, 4 Chinese, and 1 in the K’Ho minority group. In the Kinh ethnic group, G6PD variants such as G6PD Viangchan (n = 32), Kaiping (n = 11), Canton (n = 8), Chinese-5 (n = 7), Union (n = 5) and Quing Yuan (n = 4) were detected. A variant with silent mutations at 1311 C > T and IVS11 nt 93 T > C was also detected in 17 cases. A novel mutation (173 A > G) in exon 4 with a predicted amino acid change of 58 Asp > Gly was also found in a Kinh newborn girl and her father, and it was designated as G6PD Ho Chi Minh. These findings demonstrated that the Kinh ethnic group in southern Vietnam has 8 different G6PD variants, indicating that the members of this group have many ancestors in terms of G6PD variants from Southeast Asia, China, and Oceania. We compared the frequency distribution of G6PD variants in the Kinh population with those of other Southeast Asian populations, and the Kinh population’s distribution was quite similar to that in the Thai population, but differed from it by the absence of G6PD Mahidol.

Key words: G6PD deficiency, G6PD variant, southern Vietnam, Kinh population, Southeast Asia

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*Corresponding author. Phone: +62-31-599-2445; Fax: +62-31-599-2445
E-mail: indahst99@yahoo.com (Indah S. Tantular)

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The most frequent clinical manifestations of G6PD deficiency are acute hemolytic anemia (MIM #300908), which is usually triggered by taking certain oxidative drugs such as primaquine [2]. Primaquine has been used for the radical treatment of vivax malaria and for gametocytocidal action against falciparum malaria. Primaquine-induced hemolytic crisis is thus a serious problem in chemotherapeutic malaria control efforts. Primaquine should therefore be administered to malaria patients only after normal G6PD activity is confirmed.

We have been surveying malaria and G6PD deficiency in malaria endemic areas in Southeast Asian countries [4-13] by using 2 rapid diagnostic methods for malaria [14, 15] and G6PD deficiency [8, 16]. With these methods, malaria patients can be informed of the diagnostic results, usually within 30 min of a blood examination, and they are prescribed antimalarial drugs on-site, including primaquine, if their G6PD activity is normal.

In southern Vietnam, 2 studies that included molecular analyses of G6PD variants have been reported to date (Table 1). One of the studies was our surveillance [11] in the Bao Loc area, Lam Dong Province (Fig. 1). In that study, we detected 25 G6PD-deficient individuals in three ethnic groups, the Kinh (or Viet), the K’Ho (Ko Ho or Co Ho), and the Nung. G6PD Viangchan (871 G > A, 1311 C > T, IVS11 nt 93 T > C) was the most dominant genotype in the Kinh (a majority group) and in the K’Ho (a minority group), but only the Kinh group possessed many varieties of common Chinese variants, such as G6PD Gaohe (95 A > G), Quing Yuan (or Chinese-4, 392 G > T), Canton (1376 G > T), and Kaiping (1388 G > A).

Because of the strong historical connection between Vietnam and China dating back more than 2,000 years to the Han Dynasty, it is apparent that these variants of Chinese origin had been introduced into the Kinh population. However, in the K’Ho population, these Chinese variants are not seen, implying that the K’Ho may have settled in remote areas, as seen in the present day, and/or they may have rejected relations with other tribes or foreigners.

Following our surveillance in the Bao Loc area, Hue et al. (2009) [17] reported a molecular epidemiological study of G6PD variants (Table 1) in the Kinh population in Ho Chi Minh City and the Stieng (or Xieng) population in Binh Phuoc Province, which neighbors Bao Loc.
they found that G6PD Viangchan was also the most dominant in both the Kinh and Stieng populations, and four cases of G6PD Chinese-5 and one case of G6PD Gaohe were detected in the Kinh group. In addition, they discovered 54 cases of silent mutations at 1311 C > T and IVS11 nt 93 T > C (designated here as G6PD 1311 T/93 C) in the Kinh and Stieng groups. Even though Binh Phuoc Province is adjacent to Bao Loc, this variant was not detected in the Kinh or the K’Ho groups in our previous study.

G6PD 1311 T/93 C is a silent mutation that does not change any amino acids in exon 11, but it causes lowered G6PD activity for unknown reasons. This variant was reported first in a Filipino by Silao et al. [18] (but with no mention of the IVS11 nt 93 mutation), and later, many cases of the double silent mutations were reported in populations of the Javanese [19] (also with no mention of the IVS11 nt 93 mutation), Chinese [20-22], southern Thai [23], Malay, Orang Asli (a Malaysian aboriginal group)[12, 24, 25] and Kachin groups (a minority group in Myanmar) [26].

To clarify the precise distribution and features of the G6PD variants in southern Vietnam, we conducted a new surveillance of G6PD deficiency in newborn babies at Tu Du Hospital, Ho Chi Minh City. This hospital is the largest maternity hospital in southern Vietnam, and usually more than 150 babies are born there every day. This hospital covers a wide area including Ho Chi Minh and the surrounding rural areas, so the subjects of this study, even those in the Kinh population, were derived from much more diverse communities than those in the limited area of Bao Loc. Here, we added a total of 85 cases of G6PD deficiency from the Kinh population, 4 from the Chinese population and one from the K’Ho population, and we compared the frequency distribution of G6PD variants with those in other Southeast Asian countries. In this report, we also describe a novel variant of G6PD deficiency, which is tentatively designated as G6PD Ho Chi Minh (173 A > G; 58 Asp > Gly).

Materials and Methods

This study was approved by the Health Ministry of Ho Chi Minh City, and by the Ethical Committees of Airlangga University, Indonesia, Oita University Faculty of Medicine, Japan, and Jichi Medical University, Japan.

During the period from November 2006 to August 2007, we screened for G6PD deficiency in newborn babies at Tu Du Hospital. Informed consent was obtained from the newborns’ parents before the diagnoses of G6PD deficiency. A drop of blood from a leg of each newborn was collected on filter paper. After being dried well, the sample was cut from the filter paper using a hole punch (3 mm dia.). This small, round paper sample was hemolyzed in a 96-microtiter-plate well by adding 100 μl of 10 mM Tris-HCl buffer (pH 7.2), and the plate was shaken by a microtiter-plate shaker for 30 min. G6PD activity was measured by adding 100 μl of the WST-8 test kit solution (the G6PD Assay kit from Dojindo Laboratories, Kumamoto, Japan), and the absorbance was read by a spectrophotometer at 460 nm after a 30-min reaction [16].

When a G6PD-deficient newborn was identified, informed consent was obtained from a parent once again, and 0.5-2.0 ml of venous blood was taken from both the father and mother for molecular analysis. These blood samples were stored at −20°C and brought to Japan; the G6PD activity was then re-confirmed by another G6PD test, the Formazan ring method (Fig. 2), developed by Fujii et al. [27]. 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 [11].

Next, genomic DNA was extracted from 0.1 ml of the deficient blood with a DNA purification kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). G6PD mutation was identified by sequencing both strands of G6PD gene by using the primer sets reported previously [11].

**Results**

We collected and molecularly analyzed 90 G6PD-deficient samples from a parent of 80 boys and 10 girls) (Table 1). These samples included 85 from the Kinh, 4 from the Chinese, and 1 from the K’Ho. In the Chinese deficient individuals, 3 cases of G6PD Canton and one case of G6PD Viangchan were detected. One case of the K’Ho was also G6PD Viangchan.

In the Kinh group, G6PD Viangchan was the most frequent variant, and many variants of Chinese origin such as G6PD Kaiping, Canton, Chinese-5 (1024 C>T), and Quing Yuan were detected again in a total of 30 cases (35.3%). G6PD Union (1360 C>T), which is dominantly found in countries in the Pacific Ocean or Melanesia such as the Philippines, Papua New Guinea, the Solomon Islands, and the Vanuatu archipelago [11], was also detected again in 5 individuals (5.9%) of the Kinh group. Seventeen cases (20.0%) of silent mutations at 1311 C>T and ISV11 nt 93 T>C were found in the Kinh group, as reported by Hue et al. [17].

In addition to the above seven G6PD variants, we found a novel variant with a single nucleotide change at 173 A>G (Fig. 3). A newborn Kinh girl showed a heterozygous deficiency, and her father was found to be hemizygous-deficient, as shown in Fig. 2. Our DNA sequence analysis indicated a mutation at 173 A>G,
and an amino-acid change was predicted at 58 A > G (Fig. 3). This variant was estimated to be categorized in Class III (10-60% residual activity by the World Health Organization criteria), and we designated it as G6PD Ho Ch. Minh (173 A > G, 58 A > G).

Discussion

Our present findings confirm the presence of a frequency distribution of G6PD variants in southern Vietnam that is similar to those identified in previous study [11] and by Hue et al. [17].

Table 2  G6PD variants reported from Southeast Asian countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Ethnicity</th>
<th>Vietnam</th>
<th>Laos</th>
<th>Cambodia</th>
<th>Thailand</th>
<th>Malaysia</th>
<th>Myanmar</th>
<th>Philippines</th>
<th>Indonesia</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Kinh</td>
<td>K’Ho</td>
<td>Stieng</td>
<td>Lao</td>
<td>Khmer</td>
<td>O. Asli</td>
<td>Burman</td>
<td>Javanese</td>
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<tr>
<td></td>
<td></td>
<td>58</td>
<td>6</td>
<td>13</td>
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<td>69</td>
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<td>2</td>
</tr>
<tr>
<td>Vietnam</td>
<td>Mahidol</td>
<td>2</td>
<td>60</td>
<td>18</td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>2</td>
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<tr>
<td></td>
<td>Vanua Lava</td>
<td>1</td>
<td>25</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>5</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
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<td>1</td>
<td>1</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
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<td>3</td>
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<tr>
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<td>Canton</td>
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<td>95#</td>
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<td>35</td>
<td>18</td>
<td>72</td>
<td>5</td>
<td>318</td>
<td>101</td>
</tr>
</tbody>
</table>

Gothic numerals indicate the most dominant variant in each ethnic group. Data of Laos are reported from [5] and from Lao ethnic immigrants in Hawaii [28]. Data of Cambodians are reported from [9, 29]. Data of Thailand are reported from [23, 30-35]. Data of Malaysia are reported from [12, 24, 25, 36]. Data of Myanmar are reported from [5, 7, 26, 35, 37]. Data of Philippines are reported from [18, 28, 38]. Data of Javanese are reported from [5, 19] and Eastern Indonesia from [5-6, 10, 13]. Minorities of Vietnam include: 1) Minorities of Rakhine (14 Mahidol), Danu (1 Mahidol), Indian (2 Mahidol), Mon (14 Mahidol, 1 Viangchan, 1 Kaiping, 1 Mediterranean, 1 other [94 C > G]), Lisu (2 Mahidol), Kayin (1 Mahidol), Shan (2 Mahidol and 1 Canton), and Yakine (1 Quing Yuan) are included. 2) G6PD Songkla (196 T > A). 3) G6PD 1311 T > G and 4) G6PD Vietnam 3 (219 C > T). #Totals of 24 and 2 cases of Mahidol/1311 T > GC and Kaiping/1311 T > GC, respectively, were detected.
excluding 95 cases of silent mutation 1; in Table 2). Therefore, the Thai population, like the Kinh, may have been easily accepting marriages with members of other tribes from Cambodia, Laos, Myanmar, China, and Oceania.

In contrast, Myanmar tribes seem to be quite homogeneous population in comparison with the Thai or Kinh populations: G6PD Viangchan and other variants derived from China and Oceania are very rare (11.3%, 33/292). The absence of G6PD Mahidol in the Kinh population may be the result of no close relation with Myanmar tribes, since their territory is a faraway country.

The Malaysian Malay peoples also have ancestral sources that are similar to those of the Thai population, possessing the 2 of the most common variants in Southeast Asia, i.e., G6PD Viangchan and Mahidol, but the high prevalence of the Mediterranean, Chatham, Coimbra, and Vanua Lava variants are apparently different from the frequency distribution observed in the Thai population. This evidence may indicate that the Malay peoples intermarried with peoples from Europe, Middle East, India, and eastern Indonesia or Melanesia rather than with the Chinese.

G6PD Vanua Lava is the most common variant in populations of eastern Indonesian islanders [6,10,13] and Melanesians in Papua New Guinea, the Solomon Islands, and Vanuatu. The frequency distribution of G6PD variants in eastern Indonesian populations is basically similar to that of the Malaysian Malay, but the high prevalence of G6PD Vanua Lava and the absence of G6PD Mahidol are remarkable features in the eastern Indonesians. In addition, the frequency distribution is much different from those of the Javanese and other populations in mainland Southeast Asia. The high prevalence of G6PD Union in the Philippine population reveals a distinct feature, also differing from those in the mainland Southeast Asian populations and Indonesian populations. With respect to the Javanese, we could not draw any significant conclusion because of the insufficient sample size, but the high prevalence rates of G6PD Mediterranean and G6PD 1311 T/93 C imply a resemblance to the frequency distribution of the Malay population.

G6PD 1311 T/93 C has been reported to be the most dominant in the Orang Asli (or "Original People") in Malaysia (Table 2). The Orang Aslis are known as one of the ancient ethnic groups in Southeast Asia, and thus this variant may have existed since antiquity. G6PD Coimbra, another one of the oldest variants, is also detected with high frequency in the Orang Asli. The Orang Asli now inhabit Peninsular Malaysia, but a recent study [39] revealed evidence of gene flow between the Austroasiatic-speaking Orang Aslis and populations from Southeast Asia and South China, suggesting a widespread presence of these people in Southeast Asia and South China.

It is well known that South China, bordering northern parts of Vietnam, Myanmar, Laos, and Thailand, is the "mother-land" for all ethnic groups in Southeast Asia, and from there, all of these tribes are thought to have migrated southward with this variant together. However, as shown in Table 2, this variant is not detected in all ethnic groups. The number of ethnic groups possessing this variant seems to be very limited. For example, in many ethnic groups in Myanmar, G6PD Mahidol is the most common variant without exception, but only the Kachin group possesses G6PD 1311 T/93 C. It is quite interesting that among the 95 Kachin females who were shown to have the 1311 T/93 C mutation (Table 2), 24 and 2 cases were also found to carry the Mahidol and Kaiping mutations, respectively [26]. One case of Mahidol/1311 T/93 C was also reported in a Chinese subject in Yunnan Province, South China [20]. These mutations may be designated as G6PD "new" Mahidol (487 G > A, 1311 C>T, nt 93 T>C) or "new" Kaiping (1388 G>A, 1311 C>T, nt 93 T>C).

The occurrence of these “new” mutations may be reminiscent of G6PD Viangchan (871 G > A, 1311 C > T, nt 93 T > C) or G6PD Mediterranean (the Mediterranean subtype; 563 C > T, 1311 C > T, nt 93 T > C) since the silent mutations of 1311 T/93 C always accompany both variants. The high prevalence of 1311 T/93 C in ancient ethnic population(s) around South China may be closely linked with the occurrence and spreading of G6PD Viangchan into many ethnic groups in Southeast Asia, such as the Orang Asli, Kinh, K’Ho, Stieng, Khmer, Lao, Thai, and Malay groups, but not into the populations in Myanmar.

G6PD Ho Chi Minh reported in this paper is the fifth novel variant, followed by G6PD Bao Loc [14] and G6PD Vietnam 1-3 [17], discovered from the Kinh population in southern Vietnam. As shown in Table 2, however, only 2 cases of novel variants have been found from Southeast Asian countries other than Vietnam; G6PD Songklanagarind (196 T > A) from a southern...
Thai population [23], and a novel mutation, 94 C>G, from the Mon population [37]. It is thus surprising that so many novel variants appeared in the Kinh population in southern Vietnam, for unknown reasons.

Several cases of novel variants in other tribes that are not included in Table 2 were revealed by our previous surveys in Indonesia [5,10] and a clinical follow-up study in Thailand [40]; G6PD Surabaya (1291 G>A) from a Chinese man at Surabaya, Java Island, Indonesia [5], G6PD Bajo Maumere (844 G>T) from a Sea Gipsy family settled at Maumere town, Flores Island, eastern Indonesia [10], and G6PD Bangkok (825 G>C) and G6PD Bajo Maumere (844 G>T) from a Sea Gipsy family settled at Maumere town, Flores Island, eastern Indonesia [5,10], G6PD Surabaya (1291 G>A) from two Chinese-Indonesian families, and G6PD Bangkok Noi (1502 T>G) from two Chinese-Thai families in Bangkok, Thailand [40].

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