G6PD VIANGCHAN AND G6PD MEDITERRANEAN ARE THE MAIN VARIANTS IN G6PD DEFICIENCY IN THE MALAY POPULATION OF MALAYSIA

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Abstract. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked red blood cell enzymopathy common in malaria endemic areas. Individuals affected by this disease show a wide variety of clinical signs of acute hemolytic anemia. Mutations of the G6PD gene in the Malay population with G6PD deficiency in Kelantan, a state in North East Malaysia were studied. Ninety-three individuals with G6PD deficiency were subjected to mutation analysis of the G6PD gene using polymerase chain reaction based techniques of multiplex PCR. Of the ninety-three DNA samples studied, molecular defects were identified in 80 cases (86%). Variants were heterogeneous - 28.7% were found to have a G to A nucleotide change at nucleotide 871 of the G6PD gene (G871A), corresponding to G6PD Viangchan. The other major mutations were G6PD Mediterranean, G6PD Vanua Lava, G6PD Coimbra, G6PD Kaiping, G6PD Orissa, G6PD Mahidol, G6PD Canton and G6PD Chatham. These results showed that there are heterogeneous mutations of the G6PD gene associated with G6PD deficiency and that G6PD Viangchan and G6PD Mediterranean account for the main variants in G6PD deficiency among the Malay population in Malaysia.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common inherited disorders of mankind with more than 400 million people being affected worldwide. There is evidence that high frequencies of deficient alleles have arisen because they confer a selective advantage against malaria (Luzzatto, 1979; Ruwende *et al*, 1995). The vast majority of affected individuals are asymptomatic. However, deficient subjects are at risk for severe acute hemolytic anemia following infections and ingestion of certain drugs or fava beans.

G6PD deficiency is a major health problem in countries where this disease is common. In Malaysia, neonatal screening has been carried out since 1985 with the objective of screening for G6PD deficiency in all newborns to reduce morbidity and mortality from kernicterus related to G6PD deficiency.

The cloning and sequencing of the human G6PD gene has enabled the molecular basis of G6PD deficiency to be defined. There are more than 130 known G6PD mutations and at least 34 of these are polymorphic, that is, they are present in high frequencies in some human populations (Luzzatto *et al*, 2001).

In this study, the mutations of the G6PD gene in G6PD deficient patients in Malaysia were analyzed.

MATERIALS AND METHODS

Whole blood samples collected in EDTA were taken from unrelated 89 Malay patients and 4 Chinese patients with G6PD deficiency after consent was given. There were 73 male patients and 20 female patients. Their ages ranged from neonates to twelve years old. Clinical manifestations of these patients included acute hemolysis, favism, hyperbilirubinemia or neonatal jaundice. Screening of the G6PD enzyme was done using the semi-quantitative fluorescent spot test (Beutler and Mitchell, 1968). DNA samples were extracted using the standard phenol chloroform method. In order to screen mutations of the G6PD gene, multiplex polymerase chain reaction using tandem primers (MPTP) was employed essentially as described (Shirakawa *et al*, 1997; Silao *et al*, 1999). Exons 3 - 12 of the G6PD gene were subjected to mutation studies starting first with the possible candidate mutations and the other exons

Table 1. Summary of G6PD Mutations in Malays and Chinese in Malaysia.

Mutation	No. of males	No. of females	Prevalence %	Allele frequency
Orissa 131 C-G	2	-	3.08	0.03
Vanua Lava 383 A-G	12	1	18.46	0.18
Mahidol 487 G-A	6	-	9.23	0.09
Mediterranean 563 C-T	11	7	16.92	0.17
Coimbra 592 C-T	4	1	6.15	0.06
Viangchan 871 G-A	23	4	35.38	0.35
Chatham 1003 G-A	1	-	1.54	0.02
Canton 1376 G-T	1	1	1.54	0.02
Kaiping 1388 G-A	5	1	7.69	0.08
Uncharacterized	8	5	-	-
Total	73	20	100.00	1.00

systematically. Automated sequencing was performed as appropriate to confirm mutations some samples.

RESULTS

Our results showed that for all the patients (Malay and Chinese), mutations were identified in 80 (86%) of the samples while the mutation was uncharacterized in the other 13 patients (14%). Although all of these were missense mutations, the identified nucleotide changes were heterogeneous. The major mutation was a G to A nucleotide change at nucleotide 871 of the G6PD gene (G871A), corresponding to G6PD Viangchan. The allele frequency in males is 0.35 (Table 1). In the Malays, the other mutations were G6PD Mediterranean (C563T), G6PD Vanua Lava (T383C), G6PD Coimbra (C592T), G6PD Kaiping (G1388A), G6PD Orissa (C131G), G6PD Mahidol (G487A) and G6PD Canton (G1376T) (Table 1). In Chinese patients, the mutations identified were G6PD Kaiping (2 patients) and G6PD Viangchan (1 patient). In the remaining sample, the mutation was uncharacterized.

DISCUSSION

This study showed that G6PD Viangchan, G6PD Mediterranean and G6PD Vanua Lava are the main G6PD mutations in Malays. G6PD Viangchan was first described in Laos (Beutler *et al*, 1991), G6PD Mediterranean (Vulliamy *et al*, 1988) and G6PD Coimbra (Corcoran *et al*, 1992) were first described in the people of the Mediterranean countries but since then have been reported in several parts of the world. This is the first study to show that G6PD Viangchan, G6PD Mediterranean and G6PD Vanua Lava were the main G6PD mutations in Malays. G6PD Viangchan, G6PD Mediterranean and G6PD Vanua Lava have not been reported as major mutants in Malays in Indonesia. Thus our findings suggest that Malays in Malaysia have a different genetic background to Malays in Indonesia (Soemantri *et al*, 1995).

G6PD Vanua Lava was first described in the Southwestern Pacific of the Vanuatu Archipelago and Papua New Guinea (Ganczakowski *et al*, 1993). In view of the fact that Melanesians may have migrated to Vanuatu from Southeast Asia via Papua New Guinea (Serjeantson *et al*, 1989), it is interesting to note that we found G6PD Vanua Lava in the Malays in Malaysia.

This study showed that the molecular basis of G6PD deficiency in the Malays in Malaysia is heterogeneous. Considerable heterogeneity of G6PD has been documented in various Asian populations. In the Malays of Indonesia, for example, the mutations were G6PD Mediterranean, G6PD Kaiping and G6PD Mahidol (Soemantri *et al*, 1995).

The sequential approach that we performed is fast and efficient as MPTP detected up to 86% of mutations and this method can be applied to other populations where G6PD is prevalent.

For samples in which no mutation was identified, further investigations are being conducted. For the future,

we would like to study more cases so as to characterize these samples. Lastly, we are also studying phenotypic genotypic correlation in all the samples studied.

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