# GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY: MOLECULAR HETEROGENEITY IN SOUTHEAST ASIAN COUNTRIES

Masafumi Matsuo[1], Kaoru Nishiyama[2], Taku Shirakawa[2], Carmencita David Padilla[3], Lai Poh San[4], Purnomo Suryantoro[5], Narazah Mohd Yusoff[6], Nguyen Thi Ngoc Dao[7]

[1]Division of Molecular Medicine, Kobe University Graduate School of Medicine, Japan; [2]Faculty of Health Science, Kobe University Graduate School of Medicine, Japan; [3]Department of Pediatrics, College of Medicine, University of the Philippines Manila, Philippines; [4]Department of Pediatrics, National University of Singapore, Singapore; [5]Faculty of Medicine Pediatrics, Gadjah Mada University, Indonesia; [6]Department of Pathology, School of Medical Sciences, University Science of Malaysia, Malaysia, and [7]National Center for Natural Science & Technology, Vietnam

**Abstract.** Glucose-6-phosphate dehydrogenase (G6PD) deficiency is common in malaria endemic regions and is estimated to affect more than 400 million people worldwide. Deficient subjects are mostly asymptomatic but clinical manifestations range from neonatal jaundice due to acute hemolytic anemia to chronic non-spherocytic hemolytic anemia. To date, biochemical parameters allowed more than 400 different G6PD variants to be distinguished thereby suggesting a vast genetic heterogeneity. So far, only a small portion of this heterogeneity has been confirmed at the DNA level with the identification of about 90 different point mutations in the G6PD coding sequence. To determine the molecular background of G6PD deficiency in Southeast Asian countries, we conducted molecular analyses of G6PD patients from the Philippines, Malaysia, Singapore, Vietnam and Indonesia. The most prevalent mutation identified differs from country to country, thus suggesting independent mutational events of the G6PD gene.

## INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common enzymopathies affecting erythrocyte metabolism. It affects more than 400 million people worldwide, and is common in malaria endemic regions such as Southeast Asia. The gene responsible for G6PD deficiency is located on chromosome Xq28 and consists of 13 exons spanning about 18 kb of genomic DNA. The G6PD promoter region is extremely GC-rich with an atypical TATA box and extends from −1200 bp to within the first intron. The entire coding sequence of 1545 bp is transcribed downstream from exon 2 but a number of other alternative sites for the initiation of transcription have been identified.

Main clinical presentations include neonatal jaundice, severe chronic non-spherocytic hemolytic anemia and acute hemolytic anemia triggered by drugs, infections or fava bean ingestion. However, most individuals may clinically be asymptomatic and may never have experienced a hemolytic episode.

Biochemical characterization based on properties such as G6PD enzyme activity and electrophoretic mobility was initially used to identify the variants in G6PD deficient individuals and the presence of over 400 biochemical variants of G6PD points to great genetic heterogeneity. To date, there are about 90 known molecular variants. Most molecular variants identified so far have been missense mutations indicating that severe mutations such as nonsense or frameshift mutations would completely abolish the function of this housekeeping gene and thus be lethal. Some of the biochemical variants have been shown to bear identical molecular mutations, while distinctly different mutations have been reported to result in a phenotypically identical variant. Although molecular characterization is now more commonly used to identify variants rather than biochemical characterization, the latter still provides useful information about the functional abnormalities of the variant enzymes, and relationships between genetic mutations with structure and function. In particular, interesting information is also beginning to emerge on the convergent evolution of malarial resistance with the selective advantage conferred by polymorphic G6PD variants.

A mutation scanning method of the G6PD gene called MPTP (multiplex PCR using multiple tandem forward primers and a common reverse primer) has recently been established (Shirakawa *et al*, 1997). MPTP is based on the principle that a very short primer can recognize a single nucleotide difference on the target sequence, and thus, there is failure to hybridize. Tandem forward primers were designed to cover the entire target region resulting in the amplification of a ladder of multiple fragments. A nucleotide change in the primer annealing region results in the disappearance of one or two amplified products. The significant advantage of this method is that all sequence variations in a target region can be localized to a narrow region after two PCR steps. Its usefulness has been demonstrated when mutations in exon 12 of the G6PD gene of Singaporean G6PD deficient patients were identified (Shirakawa *et al*, 1997). Although G6PD deficiency has been extensively studied in Southeast Asian countries, these previous reports have mainly been on the biochemical characterization of enzyme properties with some limited molecular characterization on a few common variants. This present study aimed to determine the molecular genetic heterogeneity of G6PD deficiency and the gene frequency in this region.

## MATERIALS AND METHODS

**Patient.** Patients diagnosed with G6PD deficiency from Singapore, the Philippines, Malaysia, Indonesia and Vietnam were enrolled in this study (Lai *et al*, 2001; Silao *et al*, 1999; Suryantoro *et al*, 2001; Yusoff *et al*, 2001). The diagnosis of G6PD deficiency was done by doctors in their respective ways.

**Mutation analysis.** Total genomic DNA was extracted from peripheral blood leukocytes of these affected individuals using standard methods. A two-step PCR amplification named MPTP was carried using previously described primers and conditions (Shirakawa *et al*, 1997). DNA samples of 122, 96, 24, 23, 19 and 17 were collected from Singapore, Malaysia, the Philippines, Indonesia and Vietnam, respectively. Twelve different types of missense mutations known to be responsible for G6PD deficiency were screened using MPTP. G6PD Gaoha (95 A>G),

Table 1. Distribution of 12 Major G6PD mutations in Southeast Asia by MPTP.

| Variant | Mutation (122) | Singapore (96) | Malaysia (17) | Vietnam (24) | Phil (19) | Thai (23) | Indonesia (276) | Total |
|---|---|---|---|---|---|---|---|---|
| Gaoha | 95 A>G | 5 | 0 | 0 | 0 | 0 | 0 | 5 |
| Orissa | 131 C>G | 3 | 3 | 0 | 0 | 0 | 0 | 6 |
| Vanua Lava | 383 T>C | 14 | 13 | 0 | 4 | 0 | [redacted] | 38 |
| Mahidol | 487 G>A | 5 | 6 | 0 | 0 | 3 | 0 | 14 |
| Taiwan-Hakka | 493 A>G | 1 | 0 | 0 | 0 | 1 | 0 | 2 |
| Mediterranean | 563 C>T | 8 | 18 | 0 | 0 | 0 | 0 | 26 |
| Coimbra | 592 C>T | 3 | 5 | 0 | 0 | 0 | 1 | 9 |
| Viangchan | 871 G>A | 22 | [redacted] | 3 | [redacted] | | 0 | [redacted] |
| Chatam | 1003 G>A | 2 | 2 | 0 | 4 | 0 | 2 | 10 |
| Union | 1360 C>T | 0 | 0 | [redacted] | 4 | 3 | 0 | 15 |
| Canton | 1376 G>T | [redacted] | 2 | 3 | 0 | 3 | 3 | 40 |
| Kaiping | 1388 G>A | 15 | 6 | 0 | 0 | 2 | 4 | 27 |
| New mutation | 196 T>A | - | - | - | - | 1*1 | - | 1 |
| unknown | 13*2 | 13 | 3 | 5 | 2 | 6 | 29 | |
| Identified ratio by MPTP | | 85 (88%) | 83 (86%) | 14 (82%) | 19 (79%) | 16 (84%) | 17 (75%) | 234 (85%) |

*1: New mutation was detected by sequencing

*2: Three mutations (G6PD Chinese 4 and 5 ; 1 and 2 samples) were identified by sequencing

G6PD Orissa (131 C>G), G6PD Vanua Lava (383 T>C), G6PD Mahidol (487 G>A) G6PD Taiwan-Hakka (493 A>G), G6PD Mediterranean (563 C>T), G6PD Coimbra (592 C>T), G6PD Viangchan (871 G>A), G6PD Chatam (1003 G>A), G6PD Union (1360 C>T), G6PD Canton (1376 G>T) and G6PD Kaiping (1388 G>A) were analyzed.

## RESULTS AND DISCUSSION

In Singapore, the most common mutation was G6PD Canton, found in 29 of 122 samples (Table 1). The most common mutation in Malaysia, the Philippines and Thailand was G6PD Viangchan, while it was G6PD Vanua Lava and G6PD Union in Indonesia and Vietnam, respectively. These results indicated that each country has its own spectrum of mutations. In each country nearly 80% of samples were found to have mutations by scanning 12 different mutations. This suggested that most mutations were common in these countries.

MPTP has been reported to be quite useful for rapidly searching various known and unknown mutations in the G6PD gene with high sensitivity (Shirakawa *et al*, 1997). Our results showed that MPTP screening is advantageous for screening known mutations of the G6PD gene and is also an ideal screening method for handling many samples.

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