

Nucleotide 1376 G→T Mutation in G6PD-deficient Chinese in Malaysia

O Ainoon MBBS, DCP, J Joyce DipMLT, NY Boo FRCPE, FRCPGlasg*, S K Cheong FRCPE, FRCPA, ZA ZAINAL BSc. and NH Hamidah MBChB. DCP.

Departments of Pathology and Paediatrics*, Faculty of Medicine, National University of Malaysia, Kuala Lumpur.

Abstract:

G6PD deficiency is the most common human enzymopathy and affects 200 million people worldwide. To date more than 400 biochemical variants and at least 60 different point mutations in the G6PD locus have been discovered. In Malaysia the overall incidence of G6PD deficiency among males is 3.1%, being more prevalent among the Chinese and Malays and less common among the Indians. As part of our initial effort to characterise G6PD deficiency in the Malaysian population, we investigated 18 G6PD deficient Chinese male neonates for the G6PD mutation G→T at nt 1376, a common mutation seen among the Chinese in Taiwan and mainland China. The mutation was detected by a PCR-based technique using primers that artificially create a site for restriction enzyme *Xho* I. We found 61% (11 out of 18) of the Chinese G6PD deficient male neonates positive for this mutation. Study of enzyme electrophoretic mobility in 7 of the cases positive for this mutation revealed three different patterns of mobility. 107% (5 out of 7), 103% (1 out of 7) and 100% (1 out of 7). This study shows that mutation G→T at nt 1376 is a common allele causing G6PD deficiency in Malaysians of Chinese origin. The finding of different patterns of electrophoretic mobility among the 7 cases positive for 1376 G→T mutation supports the notion that diverse biochemical variants may share the same mutation.

Keywords : G6PD deficiency, molecular variant, Chinese.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) is an important enzyme in the hexosmonophosphate oxidative pathway and plays a key role in the production of NADPH required for the detoxification of the toxic products of oxidative stress. G6PD deficiency is the most common enzymopathy in humans, affecting more than 200 million people worldwide and is a sex-linked disorder. The three geographical areas where this condition is most common are Africa, the Mediterranean area and Southeast Asia. It is prevalent in almost all the populations in Southeast Asia. G6PD deficient individuals are usually healthy but acute haemolysis may occur with ingestion of certain drugs, food, exposure to certain chemicals or accompanying infections. More important is its association with neonatal jaundice and hence a risk to development of kernicterus and mental retardation. This association is especially important among G6PD deficient individuals in Asia making this enzyme defect an important health problem in this part of the world.¹⁻³

Studies have shown the existence of different variants causing G6PD deficiency in different

ethnic groups.^{4,5} These variants may show different enzyme levels in red cells as well as different enzyme properties as measured by biochemical parameters and to date more than 400 biochemical variants have been described worldwide. Since the cloning of the G6PD gene at least 60 different point mutations have been discovered indicating molecular heterogeneity of the G6PD enzyme that causes G6PD deficiency.⁶ These biochemical and molecular parameters may be important determinants of severity of clinical manifestations and perhaps important predictors of severe haemolysis.

Several variants have been described at the G6PD locus among the populations of Asia. Most studies on biochemical characterisation of G6PD variants have been carried out in Thailand, Papua New Guinea, Indonesia (Bali) and China (Guandong province) and Japan, exploring a very high degree of heterogeneity at the Gd locus in these populations.⁷⁻¹¹ At least 30 biochemical variants and seven different types of mutations have been identified among the Chinese from mainland China.¹² Chang *et al.* found that five different mutations account for more than 90% of cases of G6PD deficiency among the Chinese in Taiwan with the mutations

G+T at nt 1376 (50%) and G+A at nt 1388 (21.3%) showing more than 70% occurrence rate.¹³

Malaysia like many countries in Southeast Asia is not spared of the problems of G6PD deficiency, where the overall incidence among males has been found to be 3.1%. Previous reports have been limited to the study of its prevalence and its association with neonatal jaundice.¹⁴⁻¹⁶ There has been no report so far on the study of biochemical or molecular variants in any of the ethnic groups in Malaysia. In this communication we report on the occurrence of a common molecular G6PD variant among the Chinese in Malaysia, a variant which was shown to be common among the Chinese in Taiwan and mainland China. We used the method suggested by Chang *et. al.* to detect the G6PD mutation G→T at nt 1376 in 18 male G6PD deficient Chinese neonates.^{13,17}

MATERIALS AND METHODS

Eighteen Chinese male G6PD deficient newborns delivered in the Maternity Hospital Kuala Lumpur were studied. Initial diagnosis of G6PD deficiency was made by fluorescent spot test carried out on dried blood samples collected on Whatman filter paper in the Hospital. 5 ml of blood samples were collected for repeat fluorescent spot test, determination of G6PD enzyme activity, electrophoretic mobility, reticulocyte count, full blood picture and DNA analysis.

Total genomic DNA were prepared from peripheral blood leucocytes of affected subjects by standard method. The detection of G6PD mutation G→T at nt 1376 was carried out by selective amplification of exon 12 by PCR using oligonucleotide primer sequences suggested by Chang *et. al.* which were designed to create a restriction site for enzyme XhoI.¹³ The reaction condition was optimized by using the following concentrations: MgCl₂ 1.5 mmol/l, DNTP 200 μmol/l, each primer 0.1 μmol/l and Taq polymerase 1.5 u. The PCR was performed on a DNA Thermal Cycler 480 (Perkin Elmer) using annealing temperatures calculated according to T_m of primer pairs and run at 30 cycle with a further 10 minutes extension at 72° C. The amplified products were digested with restriction enzyme Xho I followed by electrophoresis on a 3.5% agarose gel.

The samples were assayed following the WHO standardised method for G6PD assay of haemolysates on the same day of collection.¹⁸

Enzyme electrophoresis was carried out on cellulose acetate gel in TBE buffer pH 8.6 in 7 of the cases found to be positive for the mutation.

RESULTS

Fig. 1 shows a photograph of the pattern of PCR products which have been digested by restriction enzyme Xho I and run on a 3.5% agarose gel. Alleles carrying the G6PD mutation G+T at nt 1376 produced 2 DNA fragments of sizes 192 bp and 21 bp (this fragment is not detectable in agarose gel) after digestion with XhoI. Normal alleles produced an undigested DNA fragment of 213 bp.

Eleven (61%) out of the 18 G6PD deficient samples examined were positive for this mutation. Electrophoretic mobility studies in 7 of the cases demonstrated three different patterns of mobility with 6 of them being fast bands out of which 5 showed 107% and one showed 103% mobility. The remaining case showed normal electrophoretic mobility. Fig. 2 illustrates the different patterns of electrophoretic mobility in 2 of the cases positive for mutation 1376 G→C. The red cell enzyme activities of these cases range from 0.18 iu/g Hb to 0.91 iu/g Hb. 8 cases positive for this mutation had severe enzyme deficiency (G6PD activity <10% of normal), and 3 had moderate to mild enzyme deficiency (G6PD activity 10% - 60% of normal).¹⁹ All the 11 cases had neonatal jaundice but none with severe hyperbilirubinaemia.

DISCUSSION

In this study we have examined the DNA of 18 G6PD-deficient Malaysian Chinese male neonates for the G6PD mutation G→T at nucleotide 1376 and found the frequency to be 61%. This finding is comparable to the findings by Chang *et. al.* and Xu W *et. al.* when they studied the frequency of this mutation in the Chinese in Taiwan and mainland China respectively. Chang *et. al.* in a study in Taiwan found five different mutations that accounted for 90% of the G6PD deficiency among the Chinese, the most common mutation being G→T at nt 1376, making up 50% of the cases studied.¹⁷ Xu W *et. al.* studied 21 cases of G6PD deficient Chinese from South China and found 10 cases (50%) positive for mutation G+T at nt 1376.¹⁹ The high frequency of this mutation seen in our G6PD deficient Chinese population is not unexpected as the majority of Malaysian Chinese are descendents of immigrants from southern mainland China.

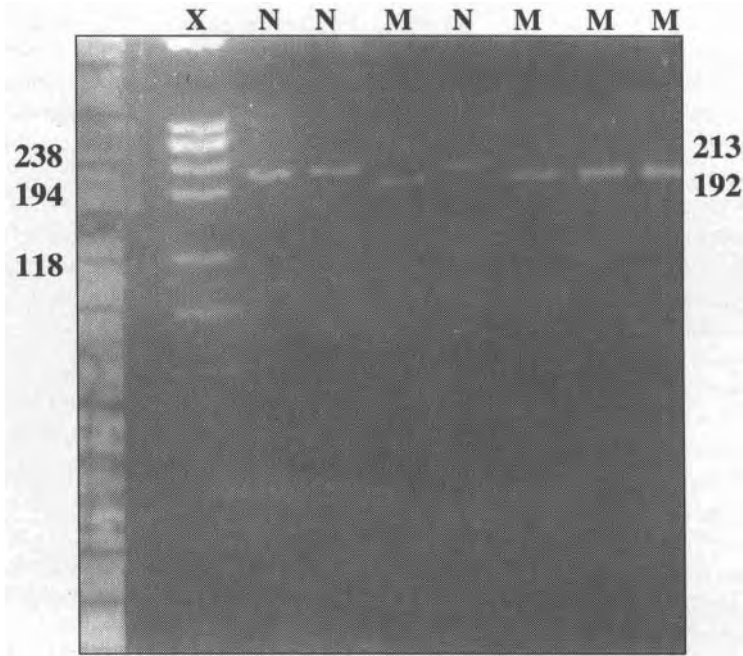


FIG. 1: The results of PCR products digested with restriction enzyme Xho 1 for the 1376 G→T mutation. X represents the X174 DNA size marker. Rows marked N represent individuals negative for mutation 1376 G-T (normal allele) showing a 213 bp DNA fragment. Rows marked M represent individuals positive for mutation 1376 G-T (mutant allele) showing a 192 bp DNA fragment.

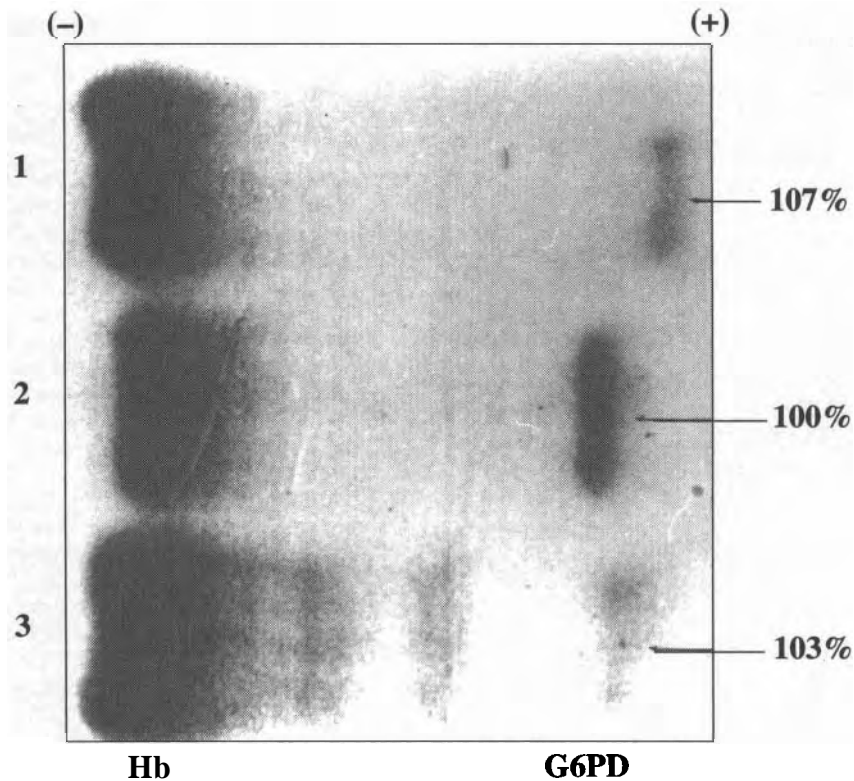


FIG. 2: The results of G6PD enzyme electrophoretic mobilities in 2 cases positive for mutation G→T at nt 1376. Rows 1 and 3 represent patients with mutation at nt 1376 (107% and 103% mobilities, respectively); row 2 is the normal control (100% mobility).

Enzyme electrophoretic studies in 7 cases positive for G+T mutation at nt 1376 revealed three different patterns of mobility reflecting phenotypic heterogeneity of this mutation. It is known that the same type of G6PD mutation can result in different biochemical variants.²⁰ There appears to be a high occurrence (5 out of 7) of a variant that shows a mobility of 107% associated with severe enzyme deficiency. It is most likely that this variant belongs to the group G6PD Canton, an electrophoretically fast G6PD variant belonging to class II (WHO classification), reported to be common in Asia.^{21,22} G6PD Canton was first described in 5 Chinese males who lived in the United States and Canada by Mc Curdy *et al.*²¹ and was later found in four Chinese males from Hong Kong and a Thai male.^{23,24} Stevens *et al.* initially discovered the mutation G+T at nt 1376 in a patient with G6PD Canton.²⁵ At the same time Chiu *et al.* independently identified the same mutation in association with three other Chinese G6PD variants (G6PD Taiwan-Hakka, Gifu-like and Agrigento-like) in Guangdong, China.²³ The Taiwan Hakka variant is also a fast variant but with mobility slower than the Canton variant. It is most likely that one of our cases who demonstrated electrophoretic mobility of 103% belong to the Taiwan-Hakka variant. Complete biochemical characterisation was not carried out in this study as it was not possible to collect large amounts of blood from the neonates.

In our previous observation we have found that 66.7% of our G6PD deficient male Chinese had severe enzyme deficiency (unpublished data). In this study we found all (including all those with mutations uncharacterised) except three cases who were positive for mutation G+T at nt 1376 had severe enzyme deficiency. In the latter three cases with moderate enzyme activity the electrophoretic mobilities were not determined. Whether these represent different biochemical variants or whether there was a false high estimation of the red cell enzyme activity due to contamination with buffy coat in severely G6PD-deficient individuals needs to be verified. It is known that G6PD-deficient samples contaminated with buffy coat or with high white cell count may give a false high red cell enzyme activity because expression of activity is less severe in white cells.

We have found that all the cases positive for the mutation G+T at nt 1376 had neonatal jaundice within the first few days of life, but none with severe hyperbilirubinemia. Further studies are required to determine whether there

is an association between the nature of this mutation and the severity of neonatal jaundice.

In conclusion, we have found that mutation G+T at nt 1376 is a common allele causing G6PD deficiency in Malaysian Chinese. Our observation that there are three different patterns of electrophoretic mobility in G6PD deficient patients carrying the mutation G+T at nt 1376 supports the notion that diverse biochemical variants may share the same point mutation.

ACKNOWLEDGMENT

This project was full funded by the IRPA grant no. 03-07-03-082 from the Ministry of Science, Technology and the Environment, Malaysia.

REFERENCES

1. Tan KL. Glucose-6-phosphate dehydrogenase - status and neonatal jaundice. *Arch Dis Child* 1981; 56: 874-7.
2. Boon Wh. Singapore kemicterus. *Singapore Med J* 1986; 21:556-67.
3. Fok T, Lau S. Glucose-6-phosphate dehydrogenase deficiency: A preventable cause of mental retardation. *Br Med J* 1986; 292: 829.
4. Yoshida A, Beutler E, Motulsky AG. Human glucose-6-phosphate dehydrogenase variants, *Bull WHO* 1971; 45: 243-53.
5. Yoshida A, Beutler E. Human glucose-6-phosphate dehydrogenase variants: A supplementary tabulation. *Ann Hum Genet Lond* 1978; 41: 347-55.
6. Beutler E. G6PD deficiency - A review article. *Blood* 1994; 84: 3613-36.
7. Miwa S, Nakashima K, Ono J, Fuji H, Suzuki K. Three glucose-6-phosphate dehydrogenase variants found in Japan. *Human Genet* 1977; 36: 327-34.
8. Panich V, Na-Nakom. G6PD variants in Thailand. *J Med Assoc Thailand* 1980; 63: 537-43.
9. Cockalingam K, Board PG, Brequet G. Glucose-6-phosphate dehydrogenase variants of Bali Island (Indonesia). *Hum Gen* 1982a; 60: 60-2.
10. Cockalingam K, Board PG, Nurse GT. Glucose-6-phosphate dehydrogenase in Papua New Guinea. The description of 13 new variants. *Hum Genet* 1982b; 60: 189-92.
11. Du CS, Du YK, Hua XY, Wu QL, Liu LB. Glucose-6-phosphate dehydrogenase variants and their frequency in Guangdong, China. *Hum Genet* 1988; 80: 385-8.
12. Beutler E. The genetics of glucose-6-phosphate dehydrogenase deficiency. *Semin Hematol* 1990; 27: 137-64.
13. Chang JG, Chiou SS, Peng LI, Chen TC, Liu TC, Lee LS, Chen PH, Tang TK. Molecular characterization of glucose-6-phosphate dehydrogenase (G6PD) deficiency by natural and amplification created restriction sites: five mutations account for most G6PD deficiency cases in Taiwan. *Blood* 1992; 80: 1079-82.

14. Lie-Injo LE, Virik HK, Lim PW, Lie AK, Ganesan J. Red cell metabolism and severe neonatal jaundice in West Malaysia. *Acta Haematol* 1977; 58: 152-60.
15. Tang TH, Balakrishnan, Zamri Ahmad. Hyperbilirubinemia and erythrocytic glucose-6-phosphate dehydrogenase deficiency in Malaysian children. *Med J Malaysia* 1989; 44: 30-4.
16. Singh H. Glucose-6-phosphate dehydrogenase deficiency - a preventable cause of mental retardation. *Br Med J* 1986; 292: 397-8.
17. Chang JG, Peng I, Chiou SS, Chen TC, Liu TC, Lee L, Chen PH, Tang TK. Three mutations account for most affected chinese with G6PD deficiency in Taiwan: Detection by natural and artifactually created restriction recognition sites. *Blood* 1991; 78: 87a (abstr, suppl 1).
18. WHO Scientific Group Standardization of procedures for the study of glucose-6-phosphate dehydrogenase. WHO Tech Rep Ser 1967; 366: 4-53.
19. Xu W, Westwood B, Bartsocas CS, Aspiazu JJM, Indrak K, Beutler E. Glucose-6-phosphate dehydrogenase mutations and haplotypes in various ethnic groups. *Blood* 1995; 80: 257-63.
20. Beutler E. Glucose-6-phosphate dehydrogenase deficiency. *Current Concepts. N Engl J Med* 1991; 324: 169-71.
21. Mc Curdy PR, Kirkman HN, Naiman JL, Jim RTS, Pickard BM. A Chinese variant of glucose-6-phosphate dehydrogenase. *J Lab Clin Med* 1966; 67: 374-85.
22. Mc Curdy PR, Blackwell RQ, Todd D, Tso SC, Tuchinda S. Further studies on glucose-6-phosphate dehydrogenase deficiency in Chinese subjects. *J Lab Clin Med* 1970; 75: 788-97.
23. Chiu DTY, Zuo L, Chen E, Chao L, Louie E, Lubin B, Liu TZ, Du CS. Two commonly occurring nucleotide base substitution in Chinese G6PD variants. *Biochem Biophysics Res Commun* 1991; 180: 988-93.
24. Chiu DTY, Zuo L, Chao E, Chen E, Lubin B, Liu TZ, Du CS. Molecular Characterization of Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency in Patients Descent and Identification of New base substitutions in human G6PD Gene. *Blood* 1993; 81: 2150-4.
25. Stevens, DJ, Wanachiwanawin W, Mason PJ, Vulliamy TJ, Luzzatto L. G6PD Canton a common deficient in South East Asia caused by a 459 Arg→Leu mutation. *Nucl Acids Res* 1990; 18: 7190.