Molecular Characterization of Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency in Patients of Chinese Descent and Identification of New Base Substitutions in the Human G6PD Gene


The underlying DNA changes associated with glucose-6-phosphate dehydrogenase (G6PD)–deficient Asians have not been extensively investigated. To fill this gap, we sequenced the G6PD gene of 43 G6PD-deficient Chinese whose G6PD was well characterized biochemically. DNA samples were obtained from peripheral blood of these individuals for sequencing using a direct polymerase chain reaction (PCR) sequencing procedure. From these 43 samples, we have identified five different types of nucleotide substitutions in the G6PD gene: at cDNA 1388 from G to A (Arg to His); at cDNA 1376 from G to T (Arg to Leu); at cDNA 1024 from C to T (Leu to Phe); at cDNA 1024 from C to T (Leu to Phe); and from cDNA 392 from G to T (Gly to Val). These five nucleotide substitutions account for over 83% of our 43 G6PD-deficient samples and these substitutions have not been reported in non-Asians. The substitutions found at cDNA 392 and cDNA 1024 are new findings. The substitutions at cDNA 1376 and cDNA 1388 account for over 50% of the 43 samples examined indicating a high prevalence of these two alleles among G6PD-deficient Chinese. Our findings add support to the notion that diverse point mutations may account largely for much of the phenotypic heterogeneity of G6PD deficiency.

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G6PD deficiency is one of the most common enzymopathies, affecting over 200 million people worldwide.1,2 The deficiency predisposes subjects to neonatal jaundice, drug- or infection-mediated hemolytic crisis, favism, and, less commonly, to chronic nonspherocytic hemolytic anemia.3,4 Clinical and biochemical analyses have identified nearly 400 putative variants of G6PD.1,4 Recent advances in the molecular biology of G6PD suggest that diverse point mutations may cause phenotypic heterogeneity.4,7 Most of the genetic studies of G6PD have been done on samples from African Americans8,8 and people of Mediterranean ancestry.5 The DNA sequence of Asian G6PD variants has not been extensively investigated. There is a regional high frequency (2% to 16%) of G6PD deficiency in Taiwan and southern China.9-11 The clinical presentations of the G6PD-deficient subjects of Asian origin appear to be different from those of African Americans with G6PD deficiency. Approximately 20% to 40% of neonatal jaundice is related to G6PD deficiency in Taiwan and Guangzhou (unpublished observations). In contrast, neonatal jaundice has rarely been attributed to G6PD deficiency in the United States. This may be due in part to the fact that most African Americans with G6PD deficiency are asymptomatic. To further delineate the relationship between the exact genetic defect and phenotypic expression of G6PD deficiency, we sequenced the G6PD gene from 43 G6PD-deficient Chinese subjects whose G6PD has been well defined biochemically.

MATERIALS AND METHODS

Samples and other materials. After obtaining informed consent, blood specimens were collected from normal and G6PD-deficient individuals of Chinese origin residing in the United States, Taiwan, or China. Most of our samples were from Guangdong province. Unless stated otherwise, all biochemicals were purchased from Sigma (St. Louis, MO). Taq polymerase and the polymerase chain reaction (PCR) buffer were obtained from Promega (Madison, WI). The DNA Thermal Cycler used was a Perkin Elmer–Cetus (Norwalk, CT) model.

G6PD activity and characterization. The G6PD activity in fresh red blood cells (RBCs) was quantitatively measured using the kit No. 345-B supplied by Sigma. Physicochemical properties of semipurified RBC G6PD were determined in accordance with the recommendations of the WHO Scientific Group.6 G6PD variants were defined as previously described by Du et al.4 DNA analysis. Genomic DNA was extracted from lymphocyte nuclei by the method of Kunkel et al.13 Direct PCR sequencing of the G6PD gene in the genomic DNA was carried out as previously described.4 The final volume of the PCR mixture was 50 µL containing 50 mmol/L Tris, pH 9.0, 20 mmol/L (NH4)2SO4, 1.5 mmol/L MgCl2, 0.2 mmol/L of all four dNTPs, 0.5 to 1.0 µmol/L of all amplification primers, 1 U of Taq polymerase and 1 µg of DNA template. From the genomic DNA, a DNA fragment of 4.5 kb covering exons 3 through 13 was amplified by a first-round PCR using primers, P1L(5'-GCAAGACAGACATGCTTGGG) and P1R(5'-GCTGGGCTCGGGTAGTACGAGCA). For a higher quality and better yield, a second-round PCR was performed with 2% of the first-round PCR products using internal primers P2L(5'-AGGTATGTATGTAGTATGGTGC) and P2R(5'-AATGTGCAGCTGAGGTCAAT) to generate a 4.2-kb fragment. After 10 minutes of predenaturation at 95°C, 35 cycles of denaturation (94°C, 1 minute), annealing (55°C, 1 minute) and extension (72°C, 5 minutes) were used for both rounds of PCR. Using unequal amounts of PCR primers (0.25 µmol/L and 25 µmol/L, respectively), asymmetric PCR (APCR) was carried out from a mixture containing 2% of the second-round PCR products to gen-
Table 1. DNA “Variants” of 43 Chinese G6PD-Deficient Samples

<table>
<thead>
<tr>
<th>DNA “Variant” (cDNA no.) (exon no.)</th>
<th>Base Change</th>
<th>Predicted Amino Acid Substitution</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1388 (exon 12)</td>
<td>G to A</td>
<td>Arg to His</td>
<td>12</td>
<td>25.6</td>
</tr>
<tr>
<td>1376 (exon 12)</td>
<td>G to T</td>
<td>Arg to Leu</td>
<td>12</td>
<td>27.9</td>
</tr>
<tr>
<td>1024 (exon 9)</td>
<td>C to T</td>
<td>Leu to Phe</td>
<td>2</td>
<td>4.6</td>
</tr>
<tr>
<td>392 (exon 5)</td>
<td>G to T</td>
<td>Gly to Val</td>
<td>3</td>
<td>7.0</td>
</tr>
<tr>
<td>95 (exon 2)</td>
<td>A to G</td>
<td>His to Arg</td>
<td>8</td>
<td>18.6</td>
</tr>
<tr>
<td>Undefined*</td>
<td>—</td>
<td>—</td>
<td>7</td>
<td>16.2</td>
</tr>
</tbody>
</table>

* The exons of these samples could not be completely sequenced.

...generate an excess of single-stranded DNA. Three pairs of APCR primers were predominantly used to cover exons 3 through 13 of the G6PD gene. The sequences of these primers have been previously described by Beutler et al. Routine, 5 μL of the APCR products were electrophoresed on agarose gel for both quality and quantity analysis. APCR products were cleaned by the following steps: After the volume was adjusted to 100 μL with dH2O, 50 μL of 7.5 mol/L ammonium acetate and 150 μL of cold 100% ethanol were added, mixed, and incubated at room temperature for 5 minutes; the mixture was centrifuged in an Eppendorf centrifuge for 5 minutes at room temperature. The supernatant was removed by aspiration and the pellet was washed with 150 μL of 70% ethanol. It was then centrifuged for 3 minutes, followed by removal of the ethanol by aspiration. The DNA pellet was dried in a speed vacuum and then resuspended in water to a concentration of 0.5 to 1.0 μg/μL. Usually, 2 μL of the above samples was used for sequencing by the dideoxy-chain termination method. T7 DNA polymerase was used for the sequencing reaction. To sequence the G/C-rich regions of exon 2, the dGTP was replaced by deoxy-7-deaza-guanosine triphosphate (dc7GTP) in our PCR reactions. The pair of primers used for the PCR reaction to generate the 1.4-kb fragment was PE1(5'-GGCGAAAGCTCAGTATAGTAAGC3') and PE2(5'-GCCTGCAAAATTAGTTGGAA3'). An internal primer (PE2-5'-TGTCACCCCTGTTGAGACCCC3') for asymmetric PCR was used.

RESULTS

The majority of the 43 G6PD-deficient samples examined (36 of the 43) are associated with one of the five different single base changes. The details of the nucleotide substitutions in the G6PD gene in our sample population are listed in Table 1. All these base substitutions have been found only among ethnic Chinese and have not been reported in any other ethnic group. Substitutions at cDNA 1376 and 1388 are by far the most common base changes and account for over 50% of the nucleotide substitutions associated with our G6PD-deficient samples.

Substitutions at cDNA 392 and 1024 are new findings and have not been previously reported except in an abstract form. The DNA sequence abnormality for substitutions at cDNA 392 and 1024 identified by the direct PCR sequencing technique is shown in Figs 1 and 2, respectively. The G to T base substitution at cDNA 392 creates a Dra III restriction endonuclease site, whereas the C to T base substitution at

![Fig 1. Sequencing gels for normal and G6PD variant (cDNA 392). The normal panel shows the partial sequence of exon 5 of the human G6PD gene from an individual with G6PD B (wild type). The patient panel shows the partial sequence of exon 5 of the human G6PD from an individual with G6PD Quing Yuan. The arrows point to the positions of nucleotide substitution. *The nucleotide that has been changed in the G6PD variant. The numbers represent the position of the amino acids in human G6PD.](image-url)
cDNA 1024 abolishes a Mnl I restriction endonuclease site (data not shown).

The characteristics of the Chinese G6PD variants studied are summarized in Table 2. Electrophoretic mobility of the variants examined is generally consistent with the predicted amino acid change based on the nucleotide substitution found in the G6PD gene. For instance, the G to T substitution at cDNA 1376, causing a predicted amino acid change of Arg to Leu, is reflected by a faster electrophoretic mobility as compared with normal, whereas the A to G substitution at cDNA 95, causing a predicted change in amino acid from His to Arg, is reflected by a slower electrophoretic mobility as compared with normal. Substitutions involving charged amino acids appear to affect the enzyme activity more severely than those only involving neutral or hydrophobic amino acids. The deamino NADP utilization rate exhibited by the variant with the DNA 1376 substitution is distinctly different from that exhibited by the wild type and that exhibited by the variants with other nucleotide substitutions (Table 2).

**DISCUSSION**

In the present study, we have characterized the molecular defects of the G6PD gene and the biochemical abnormalities of the G6PD in a large group of G6PD-deficient patients of Chinese descent. We have identified five unique nucleotide substitutions in the G6PD gene associated with G6PD deficiency in our patient population (Table 1). These five sub-

<table>
<thead>
<tr>
<th>Genomic Variant (cDNA no.)</th>
<th>Activity*</th>
<th>EPM†</th>
<th>$k_m$ for G6P</th>
<th>Analogue Utilization Rate</th>
<th>Representative Biochemical Variant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2'dG6P</td>
<td>Gal-6-P</td>
</tr>
<tr>
<td>1376</td>
<td>4.9</td>
<td>104</td>
<td>28.9</td>
<td>12.3</td>
<td>7.2</td>
</tr>
<tr>
<td>1388</td>
<td>8.0</td>
<td>102</td>
<td>55.3</td>
<td>7.2</td>
<td>3.5</td>
</tr>
<tr>
<td>95</td>
<td>6.1</td>
<td>93</td>
<td>44.7</td>
<td>6.7</td>
<td>3.7</td>
</tr>
<tr>
<td>392</td>
<td>20.0</td>
<td>100</td>
<td>56.3</td>
<td>4.7</td>
<td>2.5</td>
</tr>
<tr>
<td>1024</td>
<td>12.0</td>
<td>91</td>
<td>65.0</td>
<td>6.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Wild type</td>
<td>100.0</td>
<td>100</td>
<td>54.0</td>
<td>3.0</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* Activity is expressed as % of normal.
† Electrophoretic mobility (EPM) is expressed as the percentage of normal.
‡ 2-Deoxy-glucose-6-phosphate (2'dG6P).
MOLECULAR DEFECTS IN CHINESE G6PD VARIANTS

Substitutions have thus far only been found in ethnic Chinese and have not been reported in other ethnic groups.

Substitutions at cDNA 392 and at cDNA 1024 are new findings (Figs 1 and 2). It is interesting to note that both substitutions involve only neutral and hydrophobic amino acids (Table 1). The substitution at cDNA 392 creates a Dra III restriction endonuclease site, whereas the substitution at cDNA 1024 abolishes a Mlu I restriction endonuclease site. These two substitutions account for about 11% of the G6PD-deficient samples examined in our study.

Of the nucleotide substitutions identified in our samples (Table 1), substitutions at cDNA 1376 and 1388 are the two most common nucleotide changes associated with G6PD deficiency, and they have been previously reported by our group.16,17 and others.18 These two nucleotide substitutions account for over 50% of our G6PD-deficient individuals, suggesting that these two alleles are common among Chinese with G6PD deficiency. It is rather interesting that both substitutions are located in exon 12 of the G6PD gene and are only 12 nucleotides apart. Moreover, both substitutions result in a replacement of an arginine residue by other amino acids.

The high frequency of cDNA 1376 and cDNA 1388 substitutions among Chinese with G6PD deficiency (Table 1) may explain the apparent difference in the clinical presentations between G6PD-deficient American blacks and G6PD-deficient Chinese. Beutler18 has recently stated that the more severe form of G6PD deficiency is due to mutations clustering near the putative NADP binding site of G6PD, whereas milder forms of G6PD deficiency are mostly caused by mutations near the amino end of the enzyme. Although both cDNA 1376 and cDNA 1388 substitutions are not in the immediate vicinity of the putative NADP binding domain,8 their close proximity to this region may alter the kinetics of G6PD in such a way that individuals with either of these two variants would be more susceptible to chemical-induced hemolytic crisis. In contrast, the mutations giving rise to the A or the A' variants among African Americans are located far away from the putative NADP binding site toward the amino end. It also should be noted that although the nature of the mutations may be a major determinant of clinical severity in G6PD deficiency, other factors such as nutrition, environment, and additional genetic factors may play a role in the pathophysiology of G6PD deficiency.17

Aside from a previously reported three-base deletion in exon 2,19 the nucleotide substitution at cDNA 95 is the only base change that has been reported in this exon of the G6PD gene.1 This substitution accounts for almost one fifth of the G6PD-deficient samples examined in our study. Our findings suggest that this allele is quite common among Chinese with G6PD deficiency and that the amino end of G6PD may play some functional role for this enzyme.

Interestingly, we have also found that 4 of the 43 samples examined have a substitution at cDNA 1311 from C to T causing no amino acid change (data not shown). This finding confirms the notion postulated by other investigators20-23 that this third-position mutation is a worldwide polymorphism in the G6PD gene.

In conclusion, we have found that the nucleotide substitutions associated with G6PD deficiency in Chinese are distinctly different from those associated with G6PD deficiency in other ethnic groups. Most importantly, our findings support the postulate that G6PD deficiency is mainly caused by diverse point mutations2,4,23 and that this wide spectrum of point mutations may contribute to the heterogeneity in the pathophysiology of this disease.

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Molecular characterization of glucose-6-phosphate dehydrogenase (G6PD) deficiency in patients of Chinese descent and identification of new base substitutions in the human G6PD gene

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