We have developed a rapid and simple method to diagnose the molecular defects of glucose-6-phosphate dehydrogenase (G6PD) deficiency in Chinese in Taiwan. This method involves the selective amplification of a DNA fragment from human G6PD gene with specific oligonucleotide primers followed by digestion with restriction enzymes that recognize artificially created or naturally occurring restriction sites. Ninety-four Chinese males with G6PD deficiency were studied. The results show that 50% (47 of 94) were G to T mutation at nucleotide (nt) 1376, 21.3% (20 of 94) were G to A mutation at nt 1388, 7.4% (7 of 94) were A to G mutation at nt 493, 7.4% (7 of 94) were A to G mutation at nt 95, 4.2% (4 of 94) were C to T mutation at nt 1024, 1.1% (1 of 94) was G to T mutation at nt 392, and 1.1% (1 of 94) was G to A mutation at nt 487. These results show that the former five mutations account for more than 90% of G6PD deficiency cases in Taiwan. Aside from showing that G to T change at nt 1376 is the most common mutation, our research indicates that nt 493 mutation is a frequent mutation among Chinese in Taiwan. We compared G6PD activity among different mutations, without discovering significant differences between them.

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MATERIALS AND METHODS

Subjects. Ninety-four unrelated male subjects, who were admitted to Taipei Municipal Jen-Ai Hospital and Kaohsiung Medical College Hospital, had G6PD deficiency diagnosed by Sigma Diagnostic Kit with the method described by the manufacturer (Sigma, St Louis, MO).

DNA amplification and restriction enzyme analysis. Total genomic DNA was isolated from peripheral blood leukocytes of the affected subjects as previously described. The strategy of oligonucleotide primer design and restriction enzyme analysis are shown in Table 1. Our main purpose was to design an accurate and affordable method of creating a restriction site in mutations that did not originally have a natural restriction site because the enzyme for natural restriction site is too expensive. For the 1376 G to T mutation, which normally does not generate any restriction site, the mutant T at nucleotide (nt) 1376 with the artificial C (nt 1377) introduced by 3′ mutagenesis primer, created a CTGAG restriction site of enzyme Xho I. However, normal CGGCGAG sequences disrupt the restriction site. Using the same method, a Nde I restriction site was generated to detect G to A mutation at nt 1388, and a Mlu I restriction site for A to G mutation at nt 95. For G to T mutation at nt 392, a C at nt 398 was introduced to create a BstEII restriction site in the unaffected person (Table 1). G to A mutation at nt 487, A to G mutation at nt 493, and C to T mutation at nt 1024 create Alu I, Ava I, and Mbo II restriction sites, respectively. However, there are two additional Alu I restriction sites at nt 487 area and an additional Ava II site at nt 493 area. To overcome these problems, we destroyed the normal Alu I sites and Ava II site by using mutagenesis primers (Table 1). The PCR was performed as described, but with modification of the annealing temperature according to the Tm of the primer pairs respectively. The amplified products were digested with appropriate restriction enzymes followed by electrophoresis on a 3.5% agarose gel. Some cases were confirmed by direct sequencing or allele-specific probe hybridization.

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Table 1. The Primer Sequences, Restriction Enzyme, and Restriction Fragment Sizes of the Mutation in G6PD Deficiency

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Primer Sequence</th>
<th>Enzyme</th>
<th>Size of Fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>G → T (nt 1376)</td>
<td>5'-ACGTGAAGCTC-CCTGACGC-3'</td>
<td>Xho I (C)</td>
<td>213 bp (N)</td>
</tr>
<tr>
<td></td>
<td>3'-5'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G → A (nt 1388)</td>
<td>5'-the same as for nt 1376</td>
<td>Nde I (C)</td>
<td>227 bp (N)</td>
</tr>
<tr>
<td></td>
<td>3'-5'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A → G (nt 95)</td>
<td>5'-GCITCACAAG-GAGAGGGGTGAC-3'</td>
<td>Mlu I (C)</td>
<td>260 bp (N)</td>
</tr>
<tr>
<td></td>
<td>3'-5'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G → T (nt 392)</td>
<td>5'-GGACTCAAA-GCTCTGCAIGTCC-3'</td>
<td>RsrE II (C)</td>
<td>182 bp (N)</td>
</tr>
<tr>
<td></td>
<td>3'-5'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A → G (nt 493)</td>
<td>5'-GCGTCTGAATG-GAGAGGGGTGAC-3'</td>
<td>Av8 II (C)</td>
<td>142 bp (N)</td>
</tr>
<tr>
<td></td>
<td>3'-5'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C → T (nt 1024)</td>
<td>5'-GTCAAGGTGT-TGAAATACGC-3'</td>
<td>Mbo II (N)</td>
<td>187 bp (N)</td>
</tr>
<tr>
<td></td>
<td>3'-5'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: P, primer; C, created site; N, normal; M, mutant.
Underline = mutagenesis base.

**RESULTS**

The results of direct sequencing of PCR products of site mutagenesis of the two most common mutations in Chinese are shown in Fig 1. The mutagenesis bases and the restriction sites are indicated. Figure 2 shows the digestion pattern of PCR products by restriction enzyme. The size change after digestion is indicated in Table 1. The band would not be visualized in the 3% agarose gel if the size were less than 50 bp. For G to T mutation of nt 1376, a 213-bp fragment was undigested in normal allele, but 192- and 21-bp fragments were formed in mutants after digestion by Xho I. For G to A mutation of nt 1388, the amplified 227-bp fragment remained undigested by Nde I in normal allele. However, the products would be digested to a 205-bp fragment in mutants. Similarly, an undigested 260-bp fragment in normal, and 233-bp fragment in mutant, was found after digestion with Mlu I in the A to G mutation of nt 95. For G to T mutation of nt 392, the PCR products of normal allele were digested to a 182-bp fragment after digestion with BsrEII, whereas the 202-bp products remained uncut in mutants. For mutations of A to G at nt 493, G to A at nt 487, and C to T at nt 1024, the PCR products were digested by Ava II, Alu I, and Mbo II, respectively. There are a 86-bp and a 56-bp fragment in nt 493 mutation, and 142-bp in normal allele. In nt 487 mutation, two fragments (82 and 60 bp) in normal allele and three fragments (the mixed 62- and 60-bp bands and a 20-bp band) in mutant. In nt 1024 mutation, there is a 187-bp fragment in normal allele and two fragments (150 and 37 bp) in mutant.

The clinical data and results of mutations analysis of 94 unrelated G6PD-deficiency patients are shown in Table 2. The results show that 50% (47 of 94) were nt 1376 mutation, 21.3% (20 of 94) were nt 1388 mutation, seven cases were nt 493 mutation, another seven cases were nt 95 mutation, four cases were nt 1024 mutation, one case was nt 392 mutation, and one case was nt 487 mutation. There are no significant differences between these seven mutations and their G6PD activity.

**DISCUSSION**

G6PD deficiency affects approximately 3% of the Chinese in Taiwan and is the main cause of neonatal hyperbilirubinemia and drug-induced hemolytic anemia. 10,11 Although there are more than 30 biochemical variants and restriction sites are indicated. Figure 2 shows the digestion pattern of PCR products by restriction enzyme. The size change after digestion is indicated in Table 1. The band would not be visualized in the 3% agarose gel if the size were less than 50 bp. For G to T mutation of nt 1376, a 213-bp fragment was undigested in normal allele, but 192- and 21-bp fragments were formed in mutants after digestion by Xho I. For G to A mutation of nt 1388, the amplified 227-bp fragment remained undigested by Nde I in normal allele. However, the products would be digested to a 205-bp fragment in mutants. Similarly, an undigested 260-bp fragment in normal, and 233-bp fragment in mutant, was found after digestion with Mlu I in the A to G mutation of nt 95. For G to T mutation of nt 392, the PCR products of normal allele were digested to a 182-bp fragment after digestion with BsrEII, whereas the 202-bp products remained uncut in mutants. For mutations of A to G at nt 493, G to A at nt 487, and C to T at nt 1024, the PCR products were digested by Ava II, Alu I, and Mbo II, respectively. There are a 86-bp and a 56-bp fragment in nt 493 mutation, and 142-bp in normal allele. In nt 487 mutation, two fragments (82 and 60 bp) in normal allele and three fragments (the mixed 62- and 60-bp bands and a 20-bp band) in mutant. In nt 1024 mutation, there is a 187-bp fragment in normal allele and two fragments (150 and 37 bp) in mutant.

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MOLECULAR CHARACTERIZATION OF G6PD DEFICIENCY

Fig 2. The results of PCR products digested with restriction enzymes for their different mutations. The Xho I is for the 1376 G → T mutation, Nde I for the 1388 G → A mutation, Mlu I for the 95 A → G mutation, BstE II for the 392 G → T mutation, Mbo II for the 1024 C → T mutation, Ava II for the 493 A → G mutation, and Alu I for the 487 G → A mutation. M represents the pGem marker, and the N and M in the upper portion of the figure indicate the normal and mutant allele, respectively.

Table 2. The Clinical Data and Results of Mutations Analysis

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Case No.</th>
<th>Range Mean</th>
<th>G6PD Activity (IU/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G → T (nt 1376)</td>
<td>47</td>
<td>0.1-4.1</td>
<td>1.65 ± 1.00</td>
</tr>
<tr>
<td>G → A (nt 1388)</td>
<td>20</td>
<td>0.1-4.2</td>
<td>1.74 ± 0.93</td>
</tr>
<tr>
<td>A → G (nt 95)</td>
<td>7</td>
<td>0.9-4.5</td>
<td>2.51 ± 1.20</td>
</tr>
<tr>
<td>A → G (nt 493)</td>
<td>7</td>
<td>0.9-3.1</td>
<td>1.57 ± 0.89</td>
</tr>
<tr>
<td>C → T (nt 1024)</td>
<td>4</td>
<td>1.5-2.6</td>
<td>1.68 ± 0.24</td>
</tr>
<tr>
<td>G → A (nt 487)</td>
<td>1</td>
<td>3.57</td>
<td></td>
</tr>
<tr>
<td>G → T (nt 392)</td>
<td>1</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>7</td>
<td>0.1-6.5</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>51</td>
<td>8.0-11.2</td>
<td>9.61 ± 1.63</td>
</tr>
</tbody>
</table>

Seven mutations have been reported, no definite gene frequency has been studied until now.5,6,12-14 In this report, we find that five mutations account for more than 90% of cases of G6PD deficiency in Taiwan. This condition is similar to β-thalassemia in that four mutations are very common, whereas several mutations are rare.15,16 The G to T mutation at nt 1376 (50%) and G to A mutation at nt 1388 (21.3%) have more than 70% occurrence rate, and the A to G mutation at nt 493 is specific to Chinese in Taiwan. This finding is in accordance with Chiu et al.6 In studying the relationship between the mutations and the G6PD activity, we did not find any meaningful difference among these seven mutations.

There are several methods to detect the known point mutations and small deletions, such as allele-specific hybridization,24 direct sequencing of the PCR products,17 ligase-mediated allele detection,18 and cleavage mismatch detection.19,20 In this study, we used the natural and amplification-created restriction sites to molecularly characterize the G6PD deficiency. We found this is a very useful method not only to detect the genetic lesion of G6PD deficiency, but also to provide a screening method for further investigation of new mutations. Similar strategies have been used to diagnose PKU mutations,21 β-thalassemia in Mediterraneans,22 ras oncogene mutations,23 G6PD variant in the Middle East,24 cystic fibrosis, and retinitis pigmentosa.25 All of the studies show that the approach provides a simple, rapid, and accurate screening method. Our experience has confirmed this for us and we recommend its continued use.

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REFERENCES


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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

JG Chang, SS Chiou, LI Perng, TC Chen, TC Liu, LS Lee, PH Chen and TK Tang

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