Double Heterozygosity for Glucose-6-Phosphate Dehydrogenase Deficiency

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Erythrocyte glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked hereditary disorder (Brown, 1957). According to Gross, Hurwitz, and Marks (1958) males who are hemizygous for this disorder have low enzyme levels which vary from 0 to 20% of normal, and haemolysis occurs in association with exposure to certain drugs or illness. On the other hand, heterozygous females have intermediate or normal enzyme levels and overt haemolysis is mild or does not occur. However, severe haemolysis may be encountered in females who have low enzyme levels: such subjects may be homozygous for G6PD deficiency, heterozygous but with unusually severe penetrance (Stamatoyannopoulos et al, 1967), or, as in the two patients to be described, doubly heterozygous for two genes for G6PD deficiency.

Methods

Standard haematological techniques were used. Haemolysates and white cell lysates were prepared and assayed for G6PD and 6-phosphogluconate dehydrogenase activities according to previously described methods (Chan, Todd, and Wong, 1965). Methaemoglobin reduction test was performed according to the method of Brewer, Tarlov, and Alving (1962) and the percentage methaemoglobin level by a modified method of Evelyn and Malloy (Tönnz, 1968). Glutathione level and stability were carried out according to the method of Beutler (1957). Vertical starch gel electrophoresis of crude white cell lysate was carried out using tris-borate-EDTA buffer, pH 8.6, with a gradient of 4 volts per cm for 18 hours, and stained for G6PD activity according to methods described in the WHO Report (1967).

Case Reports

Family A. The propositus (II.5), a Chinese female, aged 21, was admitted to hospital with a history and clinical findings compatible with acute viral hepatitis. On the first day of illness, 4 days before admission, she was given 'injections, tablets, and mixtures'. On the third day after admission, menaphthone sodium bisulphite (vitamin K analogue) 40 mg was given intramuscularly. The next day, the haemoglobin level (Hb) fell from 12.7 g/100 ml to 8.5 g/100 ml, with a reticulocytosis of 10%. There was absence of haptoglobin and the presence of methaemalbumin in the serum, but no haemoglobinuria occurred. Heinz bodies were not seen. Direct and indirect Coombs tests were negative and haemoglobin pattern was normal. Red cell G6PD was 100 units; glutathione level was 61 mg/100 ml and after incubation with acetylphenyhydrazine it decreased to 16-5 mg/100 ml. Red cell 6-phosphogluconate dehydrogenase, glutathione reductase, and pyruvic kinase were within normal limits. Vertical starch gel electrophoresis of white cell lysate showed two bands corresponding to G6PD Canton and B (see below). She made an uneventful recovery, and Hb rose to 12.5 g/100 ml, with a reticulocyte count of 2.5% after 4 weeks in hospital. The erythrocyte G6PD level 3 months after discharge was 64 units.

The haematological data of the family members are shown in the Table. Vertical starch gel electrophoresis of white cell lysate from the patient and family members are presented in Fig. 1a and b. These show that her father (I.1) is fully expressed for G6PD deficiency, with a band of G6PD activity similar in electrophoretic mobility to the normal B. This has been designated G6PD B(−), Chinese. Her mother (I.2) has intermediate deficiency and in addition to the normal B band there is a faint faster band corresponding to G6PD (−), Canton (Mccurdy et al, 1966). The male sibs have either a normal G6PD level with a normal B band (II.1, II.3, and II.6) or fully expressed deficiency with G6PD (−), Canton (II.4). This is in keeping with the known X-linked inheritance of the G6PD gene. The female sibs have all inherited the B(−), Chinese gene from the father. The sisters (II.2 and II.7) (have normal enzyme levels but the finding of abnormal methaemoglobin reduction tests of 6.2 and 9.1%, respectively, proved that they were heterozygous for G6PD deficiency. On electrophoresis only one band of G6PD corresponding to B was found, indicating that they inherited the normal B gene from the mother. The propositus has 21% of normal
### Table: Laboratory Findings in Families A and B

<table>
<thead>
<tr>
<th>Pedigree and Designation</th>
<th>Relation</th>
<th>Age (yr)</th>
<th>Hb (g/100 ml)</th>
<th>Red Cell G6PD (ΔOD/min/100 ml)</th>
<th>MHB Reduction Test (%MHb)</th>
<th>White Cell G6PD (ΔOD/min/10^9)</th>
<th>G6PD Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Family A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I.1</td>
<td>Father</td>
<td>59</td>
<td>13-0</td>
<td>42</td>
<td>91.0</td>
<td>6.9</td>
<td>GdB-Chinese</td>
</tr>
<tr>
<td>I.2</td>
<td>Mother</td>
<td>55</td>
<td>12-7</td>
<td>167</td>
<td>35.0</td>
<td>15.4</td>
<td>GdB/Gd Canton</td>
</tr>
<tr>
<td>II.1</td>
<td>Brother</td>
<td>34</td>
<td>16-2</td>
<td>270</td>
<td>0.0</td>
<td>48.5</td>
<td>GdB/...</td>
</tr>
<tr>
<td>II.2</td>
<td>Sister</td>
<td>30</td>
<td>13-0</td>
<td>250</td>
<td>6.2</td>
<td>28.3</td>
<td>GdB/...</td>
</tr>
<tr>
<td>II.3</td>
<td>Brother</td>
<td>25</td>
<td>14-5</td>
<td>367</td>
<td>0.0</td>
<td>44.0</td>
<td>GdB/...</td>
</tr>
<tr>
<td>II.4</td>
<td>Brother</td>
<td>22</td>
<td>13-7</td>
<td>0</td>
<td>97.0</td>
<td>11.3</td>
<td>GdB/...</td>
</tr>
<tr>
<td>II.5</td>
<td>Propositus</td>
<td>21</td>
<td>8-5</td>
<td>100</td>
<td>96.0</td>
<td>10.0</td>
<td>GdB/...</td>
</tr>
<tr>
<td>(3 mths later)</td>
<td>Brother</td>
<td>18</td>
<td>13-7</td>
<td>283</td>
<td>0.0</td>
<td>39.6</td>
<td>GdB/...</td>
</tr>
<tr>
<td>II.6</td>
<td>Sister</td>
<td>12</td>
<td>13-7</td>
<td>200</td>
<td>9.1</td>
<td></td>
<td>GdB/...</td>
</tr>
<tr>
<td><strong>Family B</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I.1</td>
<td>Father</td>
<td>47</td>
<td>14-9</td>
<td>10</td>
<td>100.0</td>
<td>11.5</td>
<td>GdB Hong Kong</td>
</tr>
<tr>
<td>I.2</td>
<td>Mother</td>
<td>42</td>
<td>11-8</td>
<td>217</td>
<td>19.5</td>
<td>15.0</td>
<td>GdB/...</td>
</tr>
<tr>
<td>II.1</td>
<td>Propositus</td>
<td>13</td>
<td>12-0</td>
<td>50</td>
<td>100.0</td>
<td>16.8</td>
<td>GdB/...</td>
</tr>
<tr>
<td>II.2</td>
<td>Brother</td>
<td>12</td>
<td>13-3</td>
<td>267</td>
<td>2.0</td>
<td>33.0</td>
<td>GdB/...</td>
</tr>
<tr>
<td>II.3</td>
<td>Brother</td>
<td>7</td>
<td>12-0</td>
<td>50</td>
<td>94.0</td>
<td>37.5</td>
<td>GdB/...</td>
</tr>
<tr>
<td><strong>Normal Controls</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>n = 43</td>
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**Fig. 1a.** Family A. Electrophoretic pattern of G6PD at pH 8-6 from white cell lysate. I.2, GdB/Gd Canton; II.5, GdB Canton; I.1, GdB-Chinese.

**Fig. 1b.** Family A. Electrophoretic pattern of G6PD at pH 8-6 from white cell lysate. II.2, GdB/GdB-Chinese; II.3, GdB; II.4, GdB Canton; II.7, GdB/GdB-Chinese.
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G6PD activity, and on electrophoresis besides the B band there is a Canton band of about equal intensity, indicating that she has inherited the abnormal Canton gene from the mother. Thus her low enzyme level is explained by a double heterozygous state for G6PD (−), Canton and B (−), Chinese.

Family B. The propositus (II.1), a Chinese female, aged 13, was found to be G6PD deficient on routine screening for this disorder. She has no symptoms of anaemia and no previous history of jaundice. The haematological data of this family are shown in the Table. Vertical starch gel electrophoresis of white cell lysate from the patient and family members are presented in Fig. 2. These show that her father (I.1) is fully expressed for G6PD deficiency with a band of G6PD activity slower in electrophoretic mobility than the normal B. This has been designated G6PD (−), Hong Kong. Her mother (I.2) has normal enzyme levels and a single band corresponding to B. However, the finding that one of her sons (II.2) is G6PD normal with a B band and the other (II.3) is G6PD deficient with B(−), Chinese, indicates that she is heterozygous for G6PD B(−), Chinese. This is confirmed by the finding of 19% methaemoglobin in the methaemoglobin reduction test. The propositus has 18% G6PD activity and two bands on electrophoresis corresponding to B and Hong Kong. This could be explained by a heterozygous state for G6PD (−), Hong Kong with severe penetrance. However, the finding that her father is G6PD deficient and her mother is heterozygous for G6PD deficiency is also compatible with her being doubly heterozygous for G6PD B(−), Chinese, and G6PD (−), Hong Kong. This latter assumption is supported by the finding of 100% methaemoglobin in the methaemoglobin reduction test.

Discussion

In the two propositi, both females, the low enzyme levels of 21% and 18% normal, respectively, were in each instance due to a double heterozygous state for two G6PD deficient variants. In family A, the propositus inherited the G6PD B(−), Chinese gene from the father and the G6PD (−), Canton gene from the mother. In family B, the propositus inherited the G6PD (−), Hong Kong gene from the father and the G6PD B(−), Chinese gene from the mother. The finding of abnormal methaemoglobin reduction tests in three heterozygous females (II.2, II.7 of family A, and I.2 of family B) with normal enzyme levels confirmed the findings of Stammatoyannopoulos et al (1967) that the former is a superior test.

Severe haemolysis in females with G6PD deficiency is unusual but may occur if they have very

![Fig. 2. Family B. Electrophoretic pattern of G6PD at pH 8.6, from white cell lysate. II.3, GdB-Chinese; II.2, GdB; II.1 GdB-Chinese/GdHong Kong; I.2, GdB/GdB-Chinese; I.1, GdBHong Kong.](image-url)
low enzyme levels. This may result from homo-
zygosity for G6PD deficiency, heterozygosity with
severe penetrance, or as in the two patients de-
scribed double heterozygosity for two deficient
variants. As far as we are aware this is the first re-
port of the latter condition. Characterization of
enzymes from G6PD deficient Chinese have shown
a heterogeneous group (Wong et al, 1965) and the
major variant with electrophoretic mobility 105% of
the normal B enzyme at pH 8.6 was named Canton (McCurdy et al, 1966). In the course of
our study on G6PD deficient Chinese in Hong Kong
we have found two other variants in addition to
Canton: B(−), Chinese, and Hong Kong as in the
families described. All these variants are X-linked. G6PD (−), Hong Kong which on electrophoresis at pH 8.6 and 7.0 runs slower than B may be identical to G6PD (−), Panay (McCurdy et al, 1970).

The provoking factor for haemolysis in the first
patient described is probably viral hepatitis. This
association has been reported in Negroes (Burka,
Weaver, and Marks, 1966; Salen et al, 1966), Mediterraenean people (Choremis et al, 1966), and
Chinese (Wong, 1966). The difficulty is to know
whether viral hepatitis or drugs given to these
patients was responsible since many drugs have
been incriminated (WHO Report, 1967). This
patient was given various undetermined drugs on
the first day of illness. That vitamin K was not re-
sponsible can be concluded from the study of
Zinkham (1963) and our unpublished observations
have confirmed his result.

Summary

Two Chinese females with low erythrocyte
G6PD levels were reported. Family studies con-
firmed that in both instances this was due to a
double heterozygous state for two different G6PD
deficient variants. In one the two variants were
Canton and B(−), Chinese, while in the other
they were B(−), Chinese and Hong Kong.

One of the propositi presented with acute haemo-
lysis during the course of viral hepatitis and the
other was not anaemic.

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