**Red Cell Disorders**

Two new glucose-6-phosphate dehydrogenase mutations causing chronic hemolysis

We describe two new missense mutations in the glucose-6-phosphate dehydrogenase (G6PD) gene associated with chronic hemolytic anemia: mutation 1205C→A in exon 10 predicts the amino acid change 402Thr→Asn in the β-sheet M of the polypeptide chain, within the dimer interface (G6PD Covão do Lobo); mutation 1366G→A in exon 12 predicts the amino acid substitution 456Asp→His in the α-helix N, at the protein surface (G6PD Figueira da Foz).

The majority of individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency are usually asymptomatic, with normal hematologic parameters, but may develop acute hemolysis in response to oxidative stress induced by several drugs, infection or fava bean ingestion (classes II to IV G6PD variants). A small number of G6PD-deficient patients have rare class I molecular variants responsible for chronic non-spherocytic hemolytic anemia of heterogeneous severity; this, too, can be further exacerbated by oxidative stress. To date more than 60 G6PD class I mutations have been identified, the great majority being missense mutations present in and around exon 10, with the corresponding amino acids located within or close to the subunit interface of the G6PD dimer. Structure-function studies have verified that class I mutations mainly occur at conserved amino acids.

In this report we present the molecular characterization of three severe G6PD-deficient Portuguese patients with chronic hemolysis from two unrelated families.

**Case #1.** A 25-year old Portuguese male presented at the emergency room complaining of weakness, jaundice and dark urine for the last 3 days. His spleen was palpable 15 cm below the left costal margin. He had had neonatal jaundice, requiring phototherapy, and chronic yellowish sclera and had had one severe hemolytic episode in infancy. His hemoglobin concentration was 70 g/L, his mean cell volume 101 fl, reticulocytes 12%, unconjugated serum bilirubin 58.1 μmol/L (normal range: 3-20); a peripheral blood smear showed some erythrocytes with oxidative stress. Out of the hemolytic episode, he has a just palpable spleen and moderate macrocytic anemia (Table 1).

**Case #2.** At the age of 3 years old, a Portuguese boy presented with a febrile episode and jaundice. His spleen was not palpable. His hemoglobin concentration was 86 g/L and unconjugated serum bilirubin: 51 μmol/L. He had a history of neonatal jaundice, requiring phototherapy, but no other important hemolytic episodes. A family study revealed a maternal uncle (Case 3) with chronic jaundice and a palpable spleen who had had several episodes of hemolytic anemia (data on Table 1). To exclude other causes of chronic non-spherocytic hemolytic anemia, the samples were screened for abnormal hemoglobins and common glycolytic enzyme deficiencies.

**Figure 1.** Human G6PD dimer showing mutated residues Thr402 (shown as red spheres) within the dimer interface, and Asp456 (shown as pink spheres) located within alpha helix N. The structural NADP⁺ molecules are shown as stick models and the catalytic Lys205 is highlighted in yellow. G6PD subunits are colored in green and blue. The figure was prepared with PyMOL using the co-ordinates of the human G6PD Canton mutant 1QKI (Protein Data Bank (http://www.rcsb.org/pdb/)).

---

**Table 1.** Hematologic parameters, G6PD activity out of the hemolytic crisis and molecular data.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (Yr)</th>
<th>Hb (g/L)</th>
<th>MCV (fl)</th>
<th>Retics (% of RBC)</th>
<th>Unc Bilir (μmol/L)</th>
<th>LDH (U/L)</th>
<th>Serum Ferritin (ng/mL)</th>
<th>G6PD (μmol/L)</th>
<th>(TA) in UGT1</th>
<th>G6PD Mutation</th>
<th>Amino acid substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>26</td>
<td>112</td>
<td>108</td>
<td>7</td>
<td>35.3</td>
<td>416</td>
<td>1290</td>
<td>0.9</td>
<td>TA/TAe</td>
<td>1205C→A</td>
<td>402Thr→Asn</td>
</tr>
<tr>
<td>Patient #2</td>
<td>10</td>
<td>131</td>
<td>101</td>
<td>5.2</td>
<td>57.3</td>
<td>512</td>
<td>120</td>
<td>2.9</td>
<td>TA/TAe</td>
<td>1366G→A</td>
<td>456Asp→His</td>
</tr>
<tr>
<td>Mother</td>
<td>131</td>
<td>97</td>
<td>94</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1366G→A</td>
<td>ND/NF</td>
</tr>
<tr>
<td>Patient #3 (Maternal uncle)</td>
<td>41</td>
<td>137</td>
<td>110</td>
<td>4.9</td>
<td>39.1</td>
<td>ND</td>
<td>ND</td>
<td>1.2</td>
<td>TA/TAe</td>
<td>1366G→A</td>
<td></td>
</tr>
</tbody>
</table>

Hb: hemoglobin (normal values: adult females 120-150 g/L; adult males 135-170 g/L; 6-12 years 115-150 g/L); MCV: mean cell volume (normal values adults 92±9 fl; 6-12 years 75-95 fl); Retics: reticulocytes (normal values: 0.2%-); Unc Bilir: unconjugated bilirubin (normal values: 3-20 μmol/L); LDH: lactate dehydrogenase (normal values: 100-190 U/L); Serum Ferritin (normal values: 4 months to 16 years 30-400 ng/mL; adult males 50-400 ng/mL); ND: not determined; NF: not found. Screening for hemoglobinopathies detected a heterozygosity for the Ha variant City of Hope (CD.69 GGT→AGT) in patient 1; this variant has not been associated with any phenotypic changes. None of the patients has detectable hemosiderinuria between the hemolytic episodes.
A single-institution fifteen-year experience

During the last years, liver disease has emerged as a major cause of mortality in patients with thalassemia major (TM). In spite of its clinical relevance, TM-associated liver damage has been insufficiently characterized. Therefore, we performed this retrospective study in order to evaluate the prevalence and severity of liver disease in TM patients of our Department who underwent liver biopsy since 1990.

All patients were being regularly transfused in order to maintain the posttransfusion hemoglobin level at approximately 9.5 g/dL. Chelation therapy with deferoxamine 40–60 mg/kg/day for 5–6 days/week was initiated as soon as the patients were old enough.

Funding: This work was performed in part with the support of the Fundação para a Ciência e a Tecnologia (FCT) and the Fundação para a Ciência e Tecnologia (FCT/SFRH/BPD/19081/2004).