Description of a New Mutation in Hepatoerythropoietic Porphyria and Prenatal Exclusion of a Homozygous Fetus

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**Background:** Hepatoerythropoietic porphyria (HEP) is usually a severe form of cutaneous porphyria, characterized biochemically by an increased urinary excretion of polycarboxylated porphyrins. The disease is the result of a profound deficiency (<10% of normal activity) of uroporphyrinogen decarboxylase (UROD) activity. Hepatoerythropoietic porphyria is inherited as an autosomal recessive trait, whereas familial porphyria cutanea tarda is dominant. At least 30 different mutations of the \textit{UROD} gene have been identified in patients with HEP and familial porphyria cutanea tarda, with 1 predominant missense mutation (glycine–to–glutamic acid substitution at codon 281) in Spanish patients with HEP.

**Observation:** A 5-year-old patient with first-degree–related parents presented with HEP and mild symptomatology. We found low levels of UROD enzymatic activity and a new homozygous mutation of the \textit{UROD} gene, a phenylalanine-to-leucine substitution at codon 46 (F46L). Both parents were healthy carriers of the mutation. The mother had reduced UROD activity (50% of normal), whereas the father had normal UROD activity. When the mother started a new pregnancy, a prenatal study showed the absence of F46L mutation in the fetus and no accumulation of porphyrins in the amniotic fluid.

**Conclusions:** A new mutation in the \textit{UROD} gene causes a mild HEP phenotype. A normal UROD enzymatic activity was observed in the father, despite the presence of the heterozygous mutation. To our knowledge, this observation is the first description of a prenatal exclusion of HEP.

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**REPORT OF A CASE**

A 5-year-old boy, with no relevant personal or family history, had a syndrome of skin hyperfragility and photosensitivity since 2 years of age. His urine was dark. Cutaneous lesions appeared as vesicles, blisters, and erosions on the face and the dorsa of the hands. Lesions resolved with superficial scars and milia cysts. He presented with hypertrichosis on the face, limbs, and trunk. In the past 3 years, the patient has not presented with any active lesions, and only some superficial scars and mild hypertrichosis remained visible (Figure 1).

**LABORATORY DATA**

The young boy had a typical profile of phynrin accumulation, ie, an excess of uri-
of additional analyses included a normal reticulocyte count in the father and normal porphobilinogen deaminase catalytic activity in both parents. These findings were consistent with a normal rate of heme biosynthesis, as will be discussed later.

DNA STUDIES

A point mutation in the third exon of the UROD gene was found at codon 46, a phenylalanine-to-leucine substitution (F46L). The boy was homozygous and the parents were heterozygous for the mutation (Figure 2). Prokaryotic expression of the F46L mutation using the pGEX vector confirmed the deleterious effect of the mutation; the mutant pGEX UROD F46L mutation had 1% residual UROD activity (Table 3).

PARENTAL SYNDROME

This report describes a new missense mutation of the UROD gene in a young patient with HEP. The observed mutation was homozygous, a frequent finding in related parents, but it is associated with a mild phenotype. This finding may be related to the structural change in the UROD protein. The amino acid change predicted from the gene sequence is not drastic; phenylalanine is substituted for leucine, and both amino acids are neutral and hydrophobic. Moreover, according to the crystal structure of the UROD protein, position 46 in the amino acid chain is not included in the predicted substrate binding site. The deleterious effect of the mutation is clearly demonstrated by prokaryotic expression of an F46L mutant complementary DNA. The dramatic decrease of UROD catalytic activity in the F46L mutant brings evidence of a rare polymorphism of the UROD gene sequence associated with subnormal catalytic activity of the enzyme. Moreover, we analyzed the overall UROD gene sequence, and the F46L mutation was the only one observed.

This observation is also, to our knowledge, the first description of a prenatal diagnosis in HEP. Porphyrin accumulation in amniotic fluid has been demonstrated in congenital erythropoietic porphyria, and genetic analysis confirmed the prenatal diagnosis. In the present observation, prenatal exclusion of the disease was based on the correlation between the absence of the accumulation of porphyrins in the amniotic fluid and results of UROD genotyping of DNA from a chorionic biopsy sample.

The enzymatic profile observed in the parents of the affected child remains intriguing. The parents have been characterized as healthy carriers of the F46L mutant allele. However, the father demonstrated normal UROD catalytic activity...
lytic activity instead of the expected 50% decrease, as observed in the mother. As described previously, a primary erythrocytic disorder can be accompanied by increased porphobilinogen deaminase levels (porphobilinogen deaminase is the rate-limiting enzyme in heme biosynthesis) and urodecarboxylase enzymatic activities. The father had a normal reticulocyte count (1.6%) and a normal level of porphobilinogen deaminase catalytic activity (126 pmol/h UROD per milligram of hemoglobin compared with the reference range of 120-280 pmol/h UROD per milligram of hemoglobin). These data reflected a normal rate of heme biosynthesis and no clinical signs or symptoms of porphyria.

Table 1. Porphyrin Levels in the Proband

<table>
<thead>
<tr>
<th>Sample</th>
<th>Porphyrins</th>
<th>Level (Normal Level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine, µg/24 h</td>
<td>Coproporphyrin</td>
<td>30 (&lt;283)</td>
</tr>
<tr>
<td></td>
<td>Uroporphyrine</td>
<td>3425 (&lt;40)</td>
</tr>
<tr>
<td>Feces</td>
<td>Isocoproporphyrin</td>
<td>Presence (absence)</td>
</tr>
<tr>
<td>Erythrocytes, µg/L</td>
<td>Protoporphyrin</td>
<td>520 (&lt;400)</td>
</tr>
</tbody>
</table>

Table 2. Enzymatic Analyses in Family Members

<table>
<thead>
<tr>
<th>Patient</th>
<th>UROD Activity*</th>
<th>No. of Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td>Proband</td>
<td>16 (5.6)</td>
<td>12-20</td>
</tr>
<tr>
<td>Mother</td>
<td>69 (23.8)</td>
<td>51-96</td>
</tr>
<tr>
<td>Father</td>
<td>174 (31.6)</td>
<td>144-207</td>
</tr>
<tr>
<td>Sister</td>
<td>185</td>
<td>11-27</td>
</tr>
<tr>
<td>Controls</td>
<td>153 (23.8)</td>
<td>110-190</td>
</tr>
</tbody>
</table>

*Data are given as nanomoles of coproporphyrin I formed per hour and per milligram of hemoglobin. UROD indicates uroporphyrinogen decarboxylase.

Figure 1. A and B, The patient, aged 5 years, with mild hypertrichosis of the forehead. C and D, Superficial scars, after blisters and erosions, on the dorsum of the hands.

Figure 2. Sequencing profiles for a mutation of the UROD gene (phenylalanine-to-leucine substitution at codon 46 [F46L]) in the different family members. TTT indicates the normal allele (phenylalanine) present in the control sample and the healthy fetus; TTA, the mutant allele (leucine) is homozygous in the affected child and heterozygous (TTW) in both parents; and W, A, and T when both TTA and TTT alleles are present.
biosynthesis and excluded the hypothesis of an artificial increase in UROD activity secondary to a red blood cell disease in the father.

A similar observation has been described in an Italian family.25 A mild phenotype was observed in a 32-year-old woman with related parents. The UROD catalytic activity was normal in the father and was reduced to 9% and 50% in the proband and the mother, respectively. Illegitimacy was excluded by HLA haplotype findings, but UROD gene mutation analysis was not reported.

In our report, the transmission pattern of the mutant allele excluded the hypothesis of illegitimacy and the occurrence of maternal isodisomy (the presence of 2 homologous chromosomal fragments from the same parent).

We hypothesized the presence of an overexpressed normal allele in the father. However, we had no evidence of a higher expression of the normal RNA in the father compared with the mother, using RNA extracted from lymphoblastoid cells established from peripheral leukocytes.

In our observation, the normal UROD catalytic activity may result from other regulatory factors, not necessarily genetic, as observed in familial porphyria cutanea tarda, especially in the area of iron regulation.20,27

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REFERENCES