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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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).

PRENATAL EXCLUSION OF HOMOZYGOSITY

A year later, the patient’s mother started a new pregnancy. She asked for a prenatal study for HEP. Analysis of amniotic fluid showed no accumulation of porphyrins. Analysis of the UROD gene on the chorionic biopsy sample showed that the fetus was a healthy homozygote with no F46L mutation (Figure 2). A healthy infant girl was delivered. Later clinical, biochemical, and enzymatic studies (Table 2) confirmed that she remained healthy.

COMMENT

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,9 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,20,21 and genetic analysis confirmed the prenatal diagnosis.23 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 catal-

### METHODS

**PORPHYRIN MEASUREMENTS**

Porphyrin levels were quantitated by means of spectrofluorometry (excitation at 405 nm; emission at 595 nm) on an F4500 fluorescence spectrophotometer (Hitachi, Ltd, Tokyo, Japan). Porphyrin profiles were identified by means of high-performance liquid chromatography as described elsewhere.17

**UROD ASSAY**

Enzymatic activity of UROD was measured in hemolyzed blood, using high-performance liquid chromatography with pentacarboxyl porphyrinogen I as a substrate.18 Results are expressed as nanomoles of coproporphyrinogen III in feces (Table 1). Enzymatic assay of the parents showed surprising values (Table 2). The mother had the expected reduced UROD activity (50% of normal), whereas the father had a normal level. This result was confirmed by iterative analyses at an interval of a few months. Results
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 in the father.

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>Proband</td>
<td>16 (5.6)</td>
<td>2</td>
</tr>
<tr>
<td>Mother</td>
<td>69 (23.8)</td>
<td>3</td>
</tr>
<tr>
<td>Father</td>
<td>174 (31.6)</td>
<td>3</td>
</tr>
<tr>
<td>Sister</td>
<td>165</td>
<td>1</td>
</tr>
<tr>
<td>Controls</td>
<td>153 (23.8)</td>
<td>18</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 artifac-
tual 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. Illegiti-
macy was excluded by HLA haplotype findings, but UROD
gene mutation analysis was not reported.

In our report, the transmission pattern of the mut-
ant allele excluded the hypothesis of illegitimacy and the
occurrence of maternal isodisomy (the presence of 2 ho-
moelogous chromosomal fragments from the same parent).

We hypothesized about the presence of an overex-
pressed 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 ex-
tracted from lymphoblastoid cells established from
peripheral leukocytes.

In our observation, the normal UROD catalytic
activity may result from other regulatory factors, not nec-
essarily genetic, as observed in familial porphyria cuta-
nea tarda, especially in the area of iron regulation.26,27

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Table 3. Expression of GST-UROD Fusion Proteins*

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Specific Activity†</th>
<th>No. of Assays</th>
<th>Residual Activity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>pGEX</td>
<td>0.28 (0.03)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>pGEX-UROD</td>
<td>28.93 (5.67)</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>pGEX-UROD-F46L</td>
<td>0.57 (0.17)</td>
<td>10</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Bacterial lysates were obtained after an overnight culture followed by induction for 3 hours with 2mM isopropylthiogalactoside (IPTG), and uroporphyrinogen decarboxylase (UROD) specific activity (SA) was measured using pentaacetate porphyrin I as a substrate. Residual activity was determined by dividing 100 × (SA – SA [pGEX]) by (SA [pGEX-UROD] – SA [pGEX]). GST indicates glutathione S-transferase; F46L, phenylalanine-to-leucine substitution at codon 46. pGEX-UROD is a GST-UROD gene fusion vector.
† Data are given as nanomoles of coproporphyrin I formed per hour and per milligram of protein.