Breit report

Neonatal-onset hereditary coproporphyria with male pseudohermaphrodism

Harue Takeuchi, Masao Kondo, Makoto Daimon, Shintji Susa, Katsuhiro Ueoka, Osamu Uemura, and Hajime Togari

The appearance of hereditary coproporphyria (HCP) before puberty is very rare, and all reported cases of early-onset HCP have been in the homozygous or the compound heterozygous state. Some have been identified as harderooporphyria, which is a rare erythropoietic variant form of HCP. These conditions can be differentiated by molecular analysis because the gene abnormality responsible for harderooporphyria seems to be unique (K404E). Early-onset HCP, not harderooporphyria, is reported with a gene mutation in the heterozygous state and male pseudohermaphrodism. It was shown that adrenal gland hypofunction resulted in neonatal-onset hereditary coproporphyria with male pseudohermaphrodism.

Introduction

Hereditary coproporphyria (HCP) is a hereditary autosomal-dominant disease of heme biosynthesis resulting from a partial deficiency of coproporphyrinogen oxidase (CPO). It is clinically characterized by neurologic dysfunction attacks and occasional photosensitivity.1 HCP is rare before puberty,2 and all reported early-onset cases have been in the homozygous3-8 or the compound heterozygous state.9 Harderooporphyria, a rare erythropoietic variant form of HCP, is characterized by neonatal hyperbilirubinemia and hemolytic anemia, hepatosplenomegaly, and sometimes photosensitivity.6-9 To date, 3 families with harderooporphyria have been reported. Molecular analysis indicates that a CPO gene abnormality, K404E, was unique for the disease.9 Therefore, HCP can be differentially diagnosed from harderooporphyria by both clinical and laboratory examinations and molecular analysis. Here, we describe a first case of neonatal-onset HCP in the heterozygous state with male pseudohermaphrodism.

Study design

Patient history

The patient was born at term to healthy, nonconsanguineous Japanese parents with no family history of porphyria. Perinatal history was normal. The amniotic fluid and placenta were yellow, as occurred in a patient with congenital erythropoietic porphyria (CEP).10 Shortly after birth, the infant was admitted to our hospital for tachypnea and severe jaundice. He displayed icteric skin, petechiae, hypotonia, lethargy, and marked hepatosplenomegaly. Laboratory data showed hypoglycemia (22 mg/dL). After his incubator was wrapped with UV cut-off film, no new skin lesions appeared. The severe hemolytic state, erythroblastosis, and morphologic abnormalities of erythrocyte disappeared, and partial regression of hepatosplenomegaly was observed in a few weeks. In the first week after birth, dark-brown urine was observed. Porphyrins were detected using reverse-phase high-performance liquid chromatography.12 He also had ambiguous genitalia, including severe hypospadias and a bifid scrotum. A vaginal opening was next to the urinary meatus, and he had unilateral cryptorchidism. Several hormonal examinations were performed by standard methods.

Molecular and DNA analysis

Genomic DNA was extracted from peripheral blood leukocytes from 9 family members, including the patient. DNA fragments containing all exons of the patient’s and his parents’ genes were amplified by polymerase chain reaction (PCR) as described previously.13,14 Amplified DNA fragments were analyzed by single-strand conformation polymorphism and nucleotide sequencing analyses. These analyses covered all regions corresponding to the coding region of the CPO cDNA. Nucleotide sequences were analyzed using an ABI Prism 310 Genetic Analyzer (ABI, Foster City, CA) according to the manufacturer’s instructions.

Results and discussion

Hereditary coproporphyria in the heterozygous state

Clinical symptoms, an increase of coproporphyrin III excretion in the stool, and increases of δ-aminolevulinic acid, porphobilinogen, and coproporphyrin III excretion in the urine indicated that the patient had HCP. Mostly, fecal porphyrin excretion showed increased coproporphyrin III (60%-70% in total porphyrin) (Table 1). Genetic molecular analysis confirmed the diagnosis. The nucleotide sequence of the DNA fragment containing exon 6 revealed a heterozygous mutation, a G-to-A transition at the last nucleotide of

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Submitted April 11, 2001; accepted August 13, 2001.

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exon 6 (Figure 1A). This mutation was identical to that reported in another patient with HCP,15 which was responsible for exon 6 skipping. We also examined the nucleotide sequence of all PCR-amplified fragments and found no mutation, indicating that no other abnormality of the CPO gene was responsible for his disease. PCR products were also subjected to restriction analysis with MspI from family members and a healthy control (Figure 1B). These studies showed that the father was heterozygous for the mutation and a normal allele, whereas the mother’s findings were normal. This, however, seemed confusing because the mother showed a slight elevation of coproporphyrin and protoporphyrin (Table 1). Psychological stress might have been the cause of the elevation. It is also possible that she had a mutation of the CPO gene in lariat branch sites in the intron or the promoter region or that she had gene rearrangements because not all of these kinds of gene mutations could be detected by the methods used here. Alternatively, she might have had a gene abnormality responsible for another type of porphyria such as acute intermittent porphyria (AIP), variegate porphyria, or δ-aminolevulinic acid dehydratase–deficient porphyria.

The clinical features of our patient were significantly different from those of a reported Czech patient,15 who repeatedly had neurologic symptoms including paresis. In contrast, our patient never exhibited neurologic symptoms. Except for the ambiguous external genitalia and adrenal hypofunction, the clinical features of our patient resembled those of hardero (K404E) (Figure 1C). Therefore, it was considered that our patient had HCP in the heterozygous state, not hardero.

Steroid hormone abnormalities may aggravate porphyria

We examined the pathogenesis of his male pseudohermaphroditism. His karyotype was 46,XY and his testosterone level was low for his age (45 ng/dL). Administration of 5000 U human chorionic gonadotropin intramuscularly for 3 days was ineffective in elevating serum testosterone and 5α-dehydrotestosterone levels. Hepatic 5α-reductase deficiency was ruled out by a urinary steroid profile (5αTHF/5βTHF = 0.66).16 Concerning adrenal function, the patient’s adrenocorticotropic hormone (ACTH) level was high (1800 pg/mL), whereas his cortisol level was within normal range (7.6 g/dL) (Table 2). We found no significant change in levels of serum steroid metabolites before and after a rapid ACTH test done at 16 months of age. Abdominal magnetic resonance imaging and ultrasonography showed no adrenal tumor or hypertrophy. Therefore, he had adrenal gland hypofunction with an abnormality in testosterone biosynthesis. Skin pigmentation was markedly decreased after corticosteroid substitution therapy. He had no problems except for chronic anemia, requiring periodic blood transfusions, until he was 8 months old. At age 2, despite having mild anemia and photosensitivity, he showed normal growth and mental

Table 1. Laboratory data

<table>
<thead>
<tr>
<th>Erythrocytes</th>
<th>Urine</th>
<th>Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porphyrins and precursors</td>
<td>Copro I + III (μg/dL RBC)</td>
<td>Proto IX (μg/dL RBC)</td>
</tr>
<tr>
<td>Father</td>
<td>25 y</td>
<td>---</td>
</tr>
<tr>
<td>Mother</td>
<td>25 y</td>
<td>---</td>
</tr>
<tr>
<td>Patient (1 mo)</td>
<td>77.2</td>
<td>287.6</td>
</tr>
<tr>
<td>Patient (3 mo)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Patient (5 mo)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Porphyrin concentration of patient and his father showed tremendous excretion of coproporphyrin III in feces and urine.

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**Table 2. Hormonal examination**

<table>
<thead>
<tr>
<th>Hormones (11-18 d)</th>
<th>Normal findings (8-14 d) (range)</th>
<th>Urinal steroid metabolites (11 d)</th>
<th>Normal findings (8-14 d) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (TR-FIA)</td>
<td>0.36 mIU/mL</td>
<td>Mean, 0.403 (0.083-1.950)</td>
<td>An/E1 (&lt;5a5b)</td>
</tr>
<tr>
<td>FSH (TR-FIA)</td>
<td>0.74 mIU/mL</td>
<td>Mean, 0.684 (0.293-1.645)</td>
<td>5αTHF/5βTHF</td>
</tr>
<tr>
<td>ACTH</td>
<td>1800.0 pg/mL</td>
<td>146.0 ± 22.6</td>
<td>—</td>
</tr>
<tr>
<td>Cortisol</td>
<td>7.6 μg/dL</td>
<td>12.7 ± 5.5 (4.9-22.8)</td>
<td>—</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>236.0 ng/mL</td>
<td>21.5 ± 16.8</td>
<td>—</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>120.0 ng/dL</td>
<td>65.3 ± 56.9</td>
<td>—</td>
</tr>
<tr>
<td>Testosterone</td>
<td>45.0 ng/dL &gt; 100</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Hormonal examination of the patient showed hypofunction of adrenal glands. LH indicates luteinizing hormone; TR-FIA, time-resolved fluoroimmunoassay; An, androsterone; Et, etiocholanolone; FSH, follicle-stimulating hormone; THF, tetrahydrocortisol; DHEA-S, dehydroepiandrosterone-sulfate.

development. No skin pigmentation, hypertrichosis, or symptoms of rickets were observed. The patient is now receiving oral β-carotene\(^{17,18}\) and cortisone acetate daily.

The natural history of AIP provides strong suggestive evidence of the significant interplay of endocrine and genetic factors in the clinical expression of porphyria.\(^{19,20}\) Savage et al\(^{21}\) reported successful treatment of active AIP with a testosterone implant in women, suggesting that an androgenic environment partially protects against porphyria attacks. They also speculated that androgens might act later in the porphyrin-heme pathway to stimulate heme production, which is necessary to maintain intracellular levels of cytochromes. Steroid hormone metabolism depends on the heme enzyme cytochrome P450, especially in the liver. It is unclear why this heterozygous mutation presents such an early onset and severe clinical features as in homozygous patients. Our patient exhibited male pseudohermaphroditism. This indicates that a relative lack of androgen and estrogen of maternal origin may aggravate the dysfunction of heme biosynthesis caused by porphyria. Further investigations are necessary to clarify the cause of steroid hormone abnormalities, which, in our patient, may be linked with the clinical expression of HCP.

**Acknowledgments**

We thank Dr K. Honma, Prof N. Matsuo (Department of Pediatrics, Keio University) for urinary steroid profile analyses, Dr T. Yanase (The Third Department of Internal Medicine, Kyushu University), and Dr Y. Horie (The Second Department of Internal Medicine, Tottori University) for valuable comments.

**References**

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