Point Mutations in the Uroporphyrinogen III Synthase Gene in Congenital Erythropoietic Porphyria ( Günther’s Disease)

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Congenital erythropoietic porphyria ( Günther’s disease) is a rare disorder of heme biosynthesis inherited in an autosomal recessive fashion. The molecular abnormality responsible for the characteristic defect in uroporphyrinogen III synthase activity was investigated in two patients. For the first patient, complementary DNA was specifically amplified using the polymerase chain reaction and subsequently cloned and sequenced. Data obtained revealed the coexistence of two distinct point mutations: a T to C change in codon 73 (arginine in place of a cysteine) and a C to T change in codon 53 (leucine in place of a proline). The second case was studied by hybridization with allele specific oligonucleotides and was found to be homozygous for the same mutation in codon 53. These are the first mutations to be recognized in the uroporphyrinogen III synthase gene from congenital erythropoietic porphyria patients.

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RNA Extraction

The lymphoblastoid cell lines were established by Epstein-Barr virus (EBV) transformation of peripheral B lymphocytes. Total RNA was prepared according to Adrian and Hutton.

In Vitro Amplification

Complementary DNA was obtained by reverse transcription of total cellular RNA, as described previously. Sequences of cDNA were amplified in vitro using two specific oligonucleotides as primers: US1 (5'-GTGCCCTATAAGGACTGCCAG-3') and US4 (5'-CAGCGCTAGGGCTGACTCA-3'). These primers allowed the amplification of the cDNA sequence corresponding to the entire coding region of the protein (Fig 1). The amplification process was run following the method of Saiki et al with minor modifications. Briefly, 100 μL of reaction mixture containing the cDNA in 50 mmol/L KCl, 20 mmol/L Tris-HCl pH 8.4, 1.5 mmol/L MgCl₂, 200 μmol/L of each dNTP, 50 pmol of each primer, and 2 units of Taq polymerase (Perkin Elmer-Cetus). The reactions were performed in a DNA thermocycler (Perkin Elmer-Cetus, Norwalk, CT) as follows: 30 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and elongation at 72°C for 2 minutes.

Cloning and Sequencing

The polymerase chain reaction (PCR) products obtained as described above were purified from low gelling temperature agarose with GeneClean (Bio 101, San Diego, CA). The purified DNA was phosphorylated and blunt-ligated into Smal I-digested pGEM7(+) vector (Promega-Biotech, Madison, WI). Different independent clones were then sequenced by the dideoxy chain termination method.

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Fig 1. Amplification of the UROIII-S cDNA by PCR. (A) Schematic drawing of the cDNA for UROIII-S. Arrows indicate the site of hybridization of the two primers (US1 and US4, see Materials and Methods), which allowed amplification of the complete coding sequence. (B) One percent agarose gel electrophoresis of the amplified fragments obtained from CEP patients (lane 2, case 1; lane 4, case 2) and controls (lanes 1, 3, and 5). Arrows indicate molecular weight markers sizes (nt, nucleotides); arrowhead indicates the size of the fragments.

Oligonucleotide Hybridization Analysis

Amplified fragments were slot-blotted on a nylon membrane and then hybridized to allele-specific oligonucleotides (ASO) (Fig 2A) as described previously.10

RESULTS

Determination of the Mutations

Amplified fragments of apparently identical size were consistently obtained, both with patients' and with normal control cDNAs (Fig 1). This ruled out large deletions in the coding sequence of the patients' UROIII-S gene. Subcloning of the amplified fragments was subsequently done to obtain clones from the two different alleles. Eight of these clones were entirely sequenced. Comparison with the normal cDNA sequence1 revealed that six clones carried a T to C change at nucleotide 217, leading to a cysteine to arginine substitution at position 73 in the protein. The two other clones carried a C to T change at nucleotide 158, leading to a proline to leucine substitution at position 53 in the protein (Fig 2). These were the only base changes found in the different independent clones, and we concluded that each of the two groups of clones corresponded to each of the two alleles of the UROIII-S gene, respectively.

Confirmation by ASO Hybridization

Hybridizations were performed on uncloned amplified fragments from the patient cDNA. Four different oligonucleotides (Fig 2A) were used. As expected from the sequencing data above, the amplified fragment from the patient hybridized with all four probes (Fig 2B, line 2, and Fig 3). Amplified fragments from normal controls only hybridized with the oligonucleotides of normal sequence (Fig 2B, lines 1 and 6). Only one of the mutations (53M) was found in the amplified fragment obtained from the mother, and the other mutation (73N) was found in the father, as expected from their heterozygous states (Fig 2B, lines 3 and 4, respectively).

The four probes were further hybridized with cDNA obtained from CEP case 2. The patient cDNA only hybridized with the normal probe at position 53 and with the mutated probe at position 73 (Fig 2B, line 5). These results indicate (Fig 3) that the patient is homozygous for the mutated sequence at codon 73 (arginine instead of cysteine in the protein).
DISCUSSION

The molecular abnormality responsible for the characteristic defect in UROIII-S activity was investigated in two patients with CEP. For the first patient, DNA was specifically amplified using the PCR and subsequently cloned and sequenced. Data obtained revealed the coexistence of two distinct point mutations (Fig 3). The fact that no other base change was found in several independent clones strongly suggested that two distinct point mutations were present in the patient mRNA. As expected, each of the patient’s parents cDNA carried one of the mutations (Fig 2).

The second case was studied by AS0 hybridization and was found to be homozygous for the same mutation in codon 53. This is in agreement with the fact that this patient was born from consanguineous parents.

These are the first mutations recognized so far in the UROIII-S gene from CEP patients. These findings provide strong evidence that the primary defect responsible for congenital erythropoietic porphyria is a structural alteration of the UROIII-S gene, and not a disregulation of the expression of the porphobilinogen deaminase gene as previously suggested. Our results confirm data previously obtained from enzymatic studies in CEP families where UROIII-S defect was found to be inherited as an autosomal recessive trait.

It is noteworthy that both mutations reported here are located in the beginning of the coding sequence and upstream and downstream, respectively, of a previously reported highly conserved sequence that could be involved in binding the pyrrolic D ring of hydroxymethylbilane, which is specifically rearranged by UROIII-S. Studies using expression of the mutated cDNAs are needed to clarify the consequences on the enzymatic activity.

The probes described here will allow the screening of the mutations in other CEP patients. In addition, the nucleotide substitution (C to T) at position 158 suppresses a Fok I restriction site (5'-GGATG[13N]-3'), while the other substitution (T to C) at position 217 introduces a Mae II restriction site (5'-ACGT-3'). Theoretically, this could allow the identification of the mutations by analysis of the restriction fragment lengths, at least on amplified cDNA.

In families with a previously affected child, the rapid determination of the mutation(s) in the proband will now facilitate early antenatal diagnosis by DNA analysis of the trophoblast villosities. Finally, as CEP is an erythropoietic disease and affects animals also, it could be a model for future gene therapy.

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