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# A New Type of Defect in the Gene for Bilirubin Uridine 5′-Diphosphate-Glucuronosyltransferase in a Patient with Crigler-Najjar Syndrome Type I

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**ABSTRACT. Crigler-Najjar syndrome (CN) type I, which is characterized by the complete absence of bilirubin uridine 5′-diphosphate-glucuronosyltransferase (UGT) activity, is inherited as an autosomal recessive trait associated with unconjugated hyperbilirubinemia. Phenobarbital has no effect on the bilirubin concentration in the serum of patients with CN type I. Recently, cDNA for two human liver bilirubin UGT (UGT1A and UGT1D) were isolated, and their genetic organization was determined. The UGT1A (UGT1*1) and UGT1D (UGT1*4) genes each have a unique exon 1, whereas exons 2–5 are common to both genes. It has been predicted that some defect in the exons common to both genes is responsible for the absence of UGT1A and UGT1D activities in CN type I, and five cases with such a mutation have been reported. We describe here a new type of defect in the gene for bilirubin UGT in a patient with CN type I, namely, an abnormality in the exon 1 that is characteristic of the UGT1A gene. This mutation is a single nucleotide substitution, that is, C is changed to A at base position 840 in UGT1A cDNA, and this change results in a stop codon. Our patient is homozygous for the defect, and his nonconsanguineous parents and elder brother, who are clinically normal, are heterozygous for the defective allele. No mutation was detected in any exons of the UGT1D gene. Our results suggest that a homozygous nonsense or deletion mutation is detected not only in the exons common to UGT1A and UGT1D genes but also in unique exon 1 of UGT1A in CN type I. (*Pediatr Res* 35: 629–632, 1994)**

### Abbreviations

**UGT, uridine 5′-diphosphate-glucuronosyltransferase**
**CN, Crigler-Najjar syndrome**
**PCR, polymerase chain reaction**

The UGT (EC 2.4.1.17) form a family of membrane-bound zymes that catalyze the conjugation of endogenous substrates, ch as bilirubin, steroids, or xenobiotics, with uridine diphosphate-glucuronic acid. Bilirubin UGT, one member of this family, is essential for the excretion of bilirubin, a toxic metabolite derived from hemoproteins, into the bile (1).

In 1952, Crigler and Najjar (2) described a syndrome, CN type I, that featured severe chronic nonhemolytic unconjugated hyperbilirubinemia. In such cases, no bilirubin UGT activity can be detected in hepatic cells (3, 4). The patients with CN type I may succumb to kernicterus during the neonatal period (5) unless treated with phototherapy, plasmapheresis, liver transplantation, or some combination. The syndrome is inherited as an autosomal recessive trait and is clinically manifested only in its homozygous state (6). Usually, bilirubin levels reach 342–855 μmol/L in the serum. Traces of unconjugated and monoconjugated bilirubins have been detected in the bile of some patients (6).

CN type II, which is also characterized by marked unconjugated hyperbilirubinemia, was identified by Arias (7) in 1962. Compared with the course of CN type I, the clinical course in CN type II is almost always benign (*e.g.* total bilirubin level in the serum, 137–342 μmol/L) because hepatic bilirubin UGT activity, although at a reduced level, can be detected.

A major distinction between types I and II of this disorder is as follows: in patients with CN type II, administration of phenobarbital results in enhanced excretion of the glucuronide conjugates of bilirubin in the bile (8), as well as abrupt decreases in serum bilirubin levels (3, 8), whereas no effect on the concentration of bilirubin in the serum is observed on administration of phenobarbital to patients with CN type I (3).

Nowadays, it is possible to analyze the genetic background of CN types I and II in molecular level. Ritter *et al.* (9) reported the isolation and characterization of cDNA for two human liver bilirubin UGT, UGT1A [UGT1*1 (10)] and UGT1D [UGT1*4 (10)]. The UGT1A and UGT1D genes belong to the UGT1 locus (11), which is a complex of six overlapping transcriptional units and encodes at least six isoforms of UGT, including phenol UGT (11, 12). The mRNA for two bilirubin UGT, as well as those for the other four UGT, have a common 3′ terminal region composed of four exons (exons 2, 3, 4, and 5), whereas the 5′ half of each is derived from exons 1 that are specific to each isoform (11, 12). These findings suggest that in CN type I a genetic defect exists in both UGT1A and UGT1D in the common exons of the two genes, whereas in CN type II a defect exists in either of the UGT1A and UGT1D, that is to say, in the exon 1 of the UGT1A gene or in that of UGT1D gene or in a partial defect in exons 2, 3, 4, or 5 (13). Indeed, five cases of genetic mutation in the common exons of the UGT1A and UGT1D genes have been found in patients with CN type I (12, 14–16).

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We have analyzed the genetic background of a patient who was diagnosed as having CN type I on the basis of cardinal symptoms. Here we report the new type of mutation form in this patient, namely, a nonsense mutation in exon 1 of the UGT1A gene.

## MATERIALS AND METHODS

*Human subjects.* Blood samples were collected from a 1-y-old male CN type I patient, his parents, elder brother, and two unrelated normal males for DNA analysis. The patient was born after a 40-wk gestation, weighing 2752 g, to clinically normal parents, whose total bilirubin levels in the serum were 21 and 17 μmol/L. According to the census register, the parents of the patient were nonconsanguineous. Jaundice appeared at 4 d of age, and the total bilirubin level in the serum was 359 μmol/L. At 20 d of age, total and direct bilirubin levels were 530 μmol/L and 39 μmol/L, respectively, so this patient received treatment in the hospital. The mother was O positive, and the child was A positive. Results for direct and indirect Coombs' tests were negative for both. Serum bilirubin levels were not lowered by administration of phenobarbital (10 mg/kg/d) for 20 d from d 29 to 48 after birth, and no more than trace quantities of bilirubin conjugates were detectable by HPLC (17) in the blood and duodenal bile of the patient. Serum bilirubin levels in the father and mother of the patient were 21 and 17 μmol/L, respectively.

*Preparation of genomic DNA from lymphocytes.* Genomic DNA was isolated from white blood cells by the method of Poncz *et al.* (18). The DNA, dissolved in 10 mM Tris-HCl buffer (pH 7.5) that contained 1 mM EDTA, was used as a template for PCR.

*Amplification by PCR from genomic DNA.* Four pairs of oligonucleotide primers for PCR (Table 1) and 16 primers for sequencing (Table 2) were designed by modifying the primers described by Bosma *et al.* (12). The unique exons 1 of the UGT1A and UGT1D genes and exon 5 were amplified separately by use of each pair of specific primers. Exons 2, 3, and 4 and the intervening introns were amplified simultaneously with the upstream primer of exon 2 and the downstream primer of exon 4 as described elsewhere (12).

PCR was performed by the method of Yamada *et al.* (19) with slight modification. Genomic DNA (1.5 μg) was mixed with 1 μM of each primer and 2 IU of *Taq* DNA polymerase (AmpliTaq; Perkin-Elmer Cetus, Norwalk, CT) in a final vol of 0.1 mL. Amplification was carried out for 37 cycles (94°C, 1 min; 55°C, 1 min; and 72°C, 2 min).

The sequences of the amplified DNA fragments were determined directly by use of the sequencing primers (Table 2). Only the synthesized DNA fragment corresponding to exon 1 of the UGT1D gene was subcloned into the pUC19 vector (Boehringer-Mannheim, Mannheim, Germany) and sequenced. To exclude the effects of PCR errors, we determined the DNA sequences of nine clones.

*DNA sequencing.* Direct sequencing of DNA was performed by the method of Yamada *et al.* (19). The pUC recombinants were sequenced by the standard protocols provided with Sequenase 2.0 (United States Biochemical Co., Cleveland, OH). Nucleotide sequences were recorded on a personal computer and analyzed with GENETYX, version 8.0, gene analysis software (Software Development Co., Tokyo, Japan).

## RESULTS

*Identification of mutation in UGT genes.* We determined the DNA sequence of all the exons in the UGT1A and UGT1D genes. DNA analysis of exons 2, 3, 4, and 5 and of their flanking regions in the patient revealed no abnormal sequences, point mutations, or deletions as compared with the sequences for the normal controls (data not shown) and the published cDNA sequences (9). However, direct determination of the DNA sequence of exon 1 of the UGT1A gene revealed that the patient was homozygous for a single nucleotide substitution of C by A at position 840 (9) in UGT1A cDNA (Fig. 1). The observed point mutation (C → A) changes the codon for cysteine at amino acid position 280 to a stop codon (TGA). We performed further analysis of the DNA sequence of exon 1 in the gene for the UGT1D for our patient, but no other mutation was detected.

*Familial genetic analysis.* The DNA sequence of exon 1 in the UGT1A gene was determined for each member of the patient's family. His parents (Fig. 1) and elder brother (not shown) were found to be heterozygous with respect to the mutation at base position 840.

## DISCUSSION

From the clinical symptoms, such as severe unconjugated hyperbilirubinemia, trace levels of bilirubin glucuronides in the bile, and the absence of any effect on the total bilirubin level in the serum of the administration of phenobarbital, our patient was diagnosed as having CN type I.

We expected that a mutation would be detected in the common exons of the UGT1A and UGT1D genes, as found in other cases. Ritter *et al.* (14), Bosma *et al.* (12), and Moghrabi *et al.* (15) found a deletion of 13 nucleotides in exon 2 and a point mutation (at base position 1069 or 1021 of the UGT1A cDNA) that resulted in a stop codon in exon 3, respectively, in their patients with CN type I. Bosma *et al.* (16) also reported two patients with a missense mutation in exon 4 and with a nonsense mutation in exon 2, respectively. Moreover, in the hyperbilirubinemic Gunn rat (20), a model for CN type I (21), a -1 frame shift mutation in the common 3′ termini of cDNA for both bilirubin UGT (22) and phenol UGT (23) has been reported (24–26). However, our patient with CN type I has a nonsense mutation in the unique exon 1 of the UGT1A gene for bilirubin UGT protein, which is constitutively expressed (Fig. 1). This is the first case of the identification of a mutation other than a mutation in the common exons (exons 2–5) of UGT genes in a case of CN type I.

It has been recognized that, among patients with CN type I, some show a reduction in UGT activity toward phenolic sub-

Table 1. *Oligonucleotide primers for amplification of all exons of the UGT1A and UGT1D genes**

| Exon | Primer | | |
|---|---|---|---|
| | Name | Nucleotide sequence | |
| 1 | B1E1A | 5′-AGG AGC AAA GGC GCC-3′ | F |
| (UGT1A) | B1E1B | 5′-GCT TGC TCA GCA TAT ATC TGG G-3′ | R |
| 1 | B2E1A | 5′-AGT CAG CTG TCG GTG GCT TCT G-3′ | F |
| (UGT1D) | B2E1B | 5′-CAT TGA TTG GAT GAA GGC ACC A-3′ | R |
| 2-4 | BE24A | 5′-CTC TAT CTC AAA CAC GCA TGC C-3′ | F |
| | BE24B | 5′-TTT TAT CAT GAA TGC CAT GAC C-3′ | R |
| 5 | BE5A | 5′-GAG GAT TGT TCA TAC CAC AGG-3′ | F |
| | BE5B | 5′-GCA CTC TGG GGC TGA TTA AT-3′ | R |

* F, sense primer; R, antisense primer.

Table 2. *Oligonucleotide primers for sequencing of all exons of the UGT1A and UGT1D genes**

| Exon | Primer | | |
|---|---|---|---|
| | Name | Nucleotide sequence | |
| 1 | B1E11 | 5'-CTA TTT CAT GTC CCC TCT GC-3' | R |
| (UGT1A) | B1E12 | 5'-GTC TTT TGT TAG TCT CGG GC-3' | F |
| | B1E13 | 5'-TTG TTG TGC AGT AAG TGG GA-3' | R |
| | B1E14 | 5'-CCA TTC TCC TAC GTG CCC AG-3' | F |
| | B1E15 | 5'-AAG GGT TGC ATA CGG GGA ATA-3' | R |
| 1 | B2E11 | 5'-ACC ACC AAC ACC TTT CCA CT-3' | R |
| (UGT1D) | B2E12 | 5'-TTA CGC TGG GCT ACA CTC AAG-3' | F |
| | B2E13 | 5'-CAG TAG CTC CAC ACA ACA CCT A-3' | R |
| | B2E14 | 5'-CGA CCA ATT CAG ACC ACA TG-3' | F |
| | B2E15 | 5'-ACT GAC ACC TCT CTC TGA AA-3' | R |
| | B2E16 | 5'-CAT CCG TGT GGC TGT TCC GA-3' | F |
| 2 | BE241 | 5'-GGA AGC TGG AAG TCT GGG-3' | R |
| 3 | BE242 | 5'-CTA GTT AGT ATA GCA GAT-3' | F |
| | BE243 | 5'-GCT GTG CTT AAG CCA TTT-3' | R |
| 4 | BE244 | 5'-CAG CTG TGA AAC TCA GAG-3' | F |
| 5 | BE51 | 5'-TGC TGA CAG TGG CCT TCA TC-3' | F |

* F, sense primer; R, antisense primer.

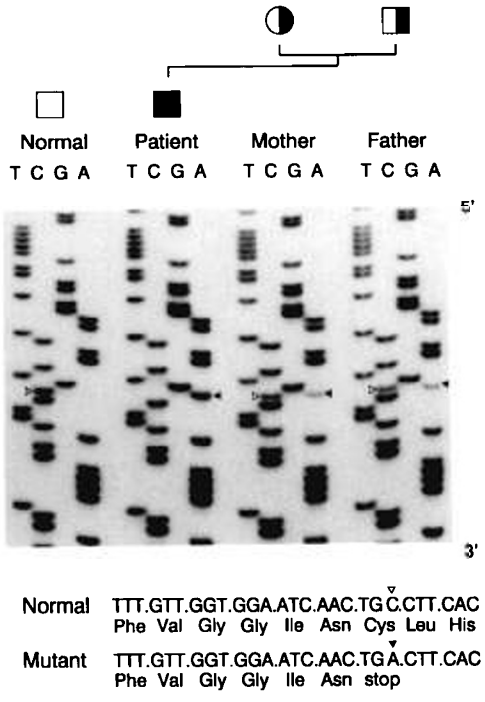


Fig. 1. Nucleotide sequences of part of exon 1 of the UGT1A gene amplified from the genomic DNA of a control, a patient with CN type I, and his parents. The mutation, a single nucleotide substitution of C (▽) by A (▼) at base position 840 in UGT1A cDNA, generates a stop codon. The nucleotide position refers to the position in the previously reported sequence of UGT1A cDNA (8).

strates, as expected, but some have normal enzymatic activity in this respect (27). This observation indicates that some patients with CN type I have some defect in the exons 1 characteristic of each isoforms of UGT, in addition to patients with a mutation in the common exons. In this study, we confirmed this possibility by analyzing the DNA sequences of the UGT1A and UGT1D genes of a 1-y-old male.

Predictably, the UGT1A protein of our patient lacks the carboxyl-terminal 253 amino acids of the wild-type protein as a result of the point mutation and is completely, or almost completely, devoid of UGT1A enzymic activity. The origin of the trace amounts of conjugated bilirubin detected in bile and serum of the patient is presently unknown. Also puzzling is why the UGT1D gene, which was apparently normal, was unable to provide enough enzyme to catalyze formation of significant amounts of bilirubin conjugates, particularly after phenobarbital treatment.

On the other hand, Bosma *et al.* (28) described unexpected findings that patients with CN type II had a mutation not only in exon 1 of the UGT1A but also in exon 1 of the UGT1D, which is consistent with the report of Aono *et al.* (29). On the basis of the study of the kindred, Bosma *et al.* (28) suggest that UGT1D do not significantly contribute to the physiologic disposition of bilirubin. Our result that only the defect in the exon 1 of the UGT1A gene is responsible for CN type I may be consistent with their notion.

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