Crigler-Najjar syndrome type II is inherited both as a dominant and as a recessive trait

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Crigler-Najjar syndrome type II (CN-II) is caused by a severely reduced hepatic activity of bilirubin UDP-glucuronosyltransferase (UGT). Recently, by the analysis of the genetic background of CN-II patients, it has been clarified that the patients carry homozygous missense mutations or nonsense plus missense mutations on the gene for UGT, and CN-II was inherited as an autosomal recessive trait. We encountered a new case which had a nonsense mutation caused by a single nucleotide substitution on one allele. This indicates that CN-II is also inherited as a dominant trait as well as a recessive trait. Expression study in vitro strongly suggests that the disease in this case is caused by a dominant negative mutation by forming a heterologous subunit structure.

INTRODUCTION

In 1962 Arias described a marked unconjugated hyperbilirubinemia different type from Crigler-Najjar type I (CN-I), in which the serum bilirubin level ranges between 60 and 340 µmol/l and the bilirubin UDP-glucuronosyltransferase (UGT) activity is <10% of normal levels (1,2). In this syndrome, Crigler-Najjar type II (CN-II), a slight increase in levels of bilirubin monoconjugates (>30% of total conjugates) are detected in the bile compared to normal controls (27%) (3). Treatment with phenobarbital causes an increase in the enzymatic activity and reduces at least 25% of the serum bilirubin level in these patients (4).

Recently, cDNA clones for rat, human and mouse bilirubin UGT were isolated (5–8) and the unusual genomic structure determined; it is composed of at least 11 exons, four (second to fifth exons) of which are common to all UGTs, and seven are isoforms of exon 1 which are unique for each UGT isoform (5,9,10). Each first exon encodes the amino-terminal half of the polypeptide, which determines the substrate specificity of the particular UGT. At first it was considered that two forms of bilirubin UGT for human liver cDNAs, phenobarbital inducible HUG-Br1 and constitutively expressed HUG-Br2, existed. However, Bosma et al. (11) reported that the Br1 product was the only relevant bilirubin-glucuronidating isoform from the results of expression of both clones in COS cells.

From the structure of the gene for bilirubin UGT, the genetic basis for three types of unconjugated hyperbilirubinemia, namely, CN-I, CN-II and Gilbert’s syndrome has been clarified (12–15). Among them, patients with CN-II, who have ~10% normal activity, have homozygous missense mutations (14,16,17). From genetic analyses of the members of patients’ families, it was demonstrated that both CN-I and CN-II were inherited as autosomal recessive traits (14). However, by screening patients with marked unconjugated hyperbilirubinemia, we encountered a unique case of a 64 year old male with CN-II that was inherited as an autosomal dominant trait. We report here the results of molecular and biochemical analysis of one patient with CN-II with a heterozygous nonsense mutation at base position 991 [nucleotides numbered in accordance with the report by Ritter et al. (7)]. The manifestation of CN-II may be caused by a dominant negative nature of the mutation.

RESULTS AND DISCUSSION

In the course of the molecular analysis of hyperbilirubinemia, we encountered a unique case of CN-II having heterozygous nonsense mutation. We collected a blood sample from the patient and directly determined the DNA sequences of the promoter region and all the exons of the genes for bilirubin UGT by PCR. The patient had a heterozygous nonsense mutation at position 991 (Gln331Stop; C to T at nucleotide 991) (Fig. 1) on the second exon. No mutation was found in the promoter region or other first (Br-2) and third to fifth common exons. Extra TA nucleotides in the TATA-box, suggested by Bosma et al. (18) as a cause of another type of mild hyperbilirubinemia, Gilbert’s syndrome, were not found.

We investigated whether the detected nonsense mutation at base position 991 might result in no activity of bilirubin UGT in vitro. The pcDCT expression vector that carried the gene with the nonsense mutation was transfected to COS cells by the DEAE-dextran method and we found that no UGT activity was expressed (Fig. 2). This result shows that CN-II in our patient is caused by...
from the intact allele, we isolated poly(A)+ RNA by biopsy and a heterozygous nonsense mutation and, consequently, that CN-II is inherited, in this case, as an autosomal dominant trait.

To investigate whether bilirubin UGT is normally expressed from the intact allele, we isolated poly(A)+ RNA by biopsy and performed Northern analysis. The total mRNA (non-mutated plus mutated mRNA) for bilirubin UGT from the patient was the same level as that from normal (data not shown). The cDNAs synthesized from the mRNA contained equal amounts of normal and mutated cDNAs of bilirubin UGT (by directly sequencing the several cDNA clones for Br1), indicating that the gene for bilirubin UGT was expressed normally from the intact allele.

The results of the molecular analysis of our patient with CN-II strongly support our hypothesis that severe manifestation of jaundice in this case might be caused by a dominant-negative mutation since four UGT monomers combine to a tetramer which is normally the active form of the enzyme in the endoplasmic reticulum (ER) (15,19). To investigate this possibility, we expressed the truncated UGT, normal UGT or truncated plus normal UGT in COS cells. As shown in Figure 2, only 6% of the normal level of enzymatic activity was detected after cotransfection with the cDNA with the nonsense mutation and the normal cDNA. No UGT activity was expressed after transfection with the cDNA that included the mutation. This result clearly indicates that CN-II in this case was caused by a dominant-negative mutation in one allele.

Catalytically inactive subunits produced as a result of the nonsense mutation in the second exon still retain the ability to form a complex with active subunits but the resultant total UGT activity is less than 10% of normal. Thus, severe CN-II syndrome might be caused by the formation of heterologous complexes of intact and truncated subunits. The value (6%) obtained from our study in COS cells is close to the value of 4.3% of normal for the activity in a homogenate of a liver biopsy from the patient and also to the theoretical value of 6.3% (1/16) predicted from the dominant-negative model. Reports published about other patients with CN-II who have homozygous missense mutations suggest that CN-II can be inherited both as a dominant and as a recessive trait. Since the initial report by Arias (1), conflicting reports that CN-II is inherited as a dominant and as a recessive trait have been presented (12). In this report, we assert that the inheritance of CN-II can be explained in terms of both dominant and recessive traits, depending on the nature of the particular mutation and its effect on the activity of the UGT tetramer.

MATERIALS AND METHODS

Human subjects

The patient is a 68 year old male. The bilirubin UGT activity and serum bilirubin level were 0.009 nmol/min/mg protein and 116 µmol/l, respectively. Approximately 53% of the bile bilirubin was composed of monoglucuronide. The patient’s deceased father and sister had both suffered from jaundice (tunica conjunctival icterus; serum bilirubin level >195 µmol/l). Phenobarbital (total 540 mg for 5 days) was injected into the patient and brought a decrease of total serum bilirubin level to 65 µmol/l.

PCR amplification from genomic DNA and DNA sequencing

Genomic DNA was isolated from white blood cells according to the method of Poncz et al. (20). Four pairs of oligonucleotide primers for PCR and sixteen primers for DNA sequencing were designed, based on the primer structures described by Bosma et al. (9). The DNA sequence of the amplified DNA fragments was determined directly by use of the sequencing primers.
Expression of UGT cDNA clones in COS cells

The experimental procedures were essentially the same as described previously (15). The cDNA was inserted into the XbaI restriction site of the pcDL expression vector and the resultant vector (30 μg per plate) was cotransfected with the plasmid (10 μg per plate) encoding the gene for chloramphenicol acetyltransferase (CAT) into COS7 monkey kidney cells by the DEAE-dextran method. The enzymatic assay of bilirubin UGT was performed by the method of Jackson et al. (21). UDP-glucuronic acid ([glucuronyl-14C(U)]-UDP; 10.55 GBq/mmol) was purchased from Dupont-NEN (Wilmington, DE). Reaction mixtures were incubated at 37°C for 2 h. A quantity of 250 μg of each sample were subjected to TLC on a plastic sheet that had been precoated with silica gel 60 at a thickness of 0.2 mm (E. Merck, Darmstadt, FRG). Labeled conjugated bilirubins after TLC were detected with an Imaging Analyzer (BAS 2000; Fuji Film, Tokyo) with exposure for 12 h. The spots on the TLC plate were then cut out and radioactivity was quantitated in a scintillation counter. CAT activity was assayed by the method of Neumann et al. (22). ([1–14C] butyryl coenzyme A; 148 MBq/mmol) was purchased from Dupont-NEN.

Bilirubin UGT expressed in COS cells was detected by polyclonal rabbit antibodies (anti-37-mer). 37-mer from amino acid position 83 to 119 [amino acids numbered in accordance with the report by Ritter et al. (7)] were chemically synthesized and polyclonal antibodies against 37-mer were raised in rabbit. Immunoblotting was performed according to the Amersham protocol (Amersham, UK).

REFERENCES