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**Kazuo Yamamoto · Yoko Soeda · Toshinori Kamisako
Hiroo Hosaka · Miya Fukano · Hiroshi Sato
Yoshihide Fujiyama · Yukihiko Adachi · Yuzuru Satoh
Tadao Bamba**

Analysis of bilirubin uridine 5′-diphosphate (UDP)-glucuronosyltransferase gene mutations in seven patients with Crigler-Najjar syndrome type II

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Abstract Crigler-Najjar syndrome (CN) type II is caused by a reduction in hepatic bilirubin uridine 5′-diphosphate (UDP)-glucuronosyltransferase activity. Recently, there has been progress in mutation analysis of patients with CN type II. Here, we analyzed both the coding and the promoter regions of the gene in seven Japanese patients with CN type II from five unrelated families. The mutations found in this study were classified into three types. The first type was composed of double homozygous missense mutations (Gly71Arg and Tyr486Asp) in exons 1 and 5. These mutations, which were detected in five patients from three unrelated families, were the commonest. The second type, which was detected in one patient, consisted of a single homozygous missense mutation (Arg209Trp) in exon 1. The third type, which was detected in one patient and was a new type of mutation combination, was composed of a homozygous insertion mutation of the TATAA element and a heterozygous missense mutation (Pro229Gln) in exon 1. Although the first and the second type of mutations are recessive, the third type appears to be dominant with incomplete penetrance, since the allele frequency of the insertion mutation of the TATAA element is very high (40%).

Key words Crigler-Najjar syndrome type II · Mutation · Inheritance

H. Sato (✉)
Department of Biology, Shiga University of Medical Science, Seta Tsukinowa, Otsu, Shiga 520-21, Japan
Tel.: +81-775-48-2121; Fax: +81-775-48-2415
e-mail: satoh@bellebsd.shiga-med.ac.jp

K. Yamamoto · M. Fukano · Y. Fujiyama · T. Bamba
Second Department of Internal Medicine, Shiga University of Medical Science, Shiga, Japan

Y. Soeda · Y. Satoh
Department of Obstetrics and Gynecology, Meiji College of Oriental Medicine, Kyoto, Japan

T. Kamisako
Second Department of Internal Medicine, Kinki University School of Medicine, Osaka, Japan

H. Hosaka
Internal Medicine, Saiseikai-Yokohama-City-Nanbu Hospital, Yokohama, Kanagawa, Japan

Y. Adachi
Third Department of Internal Medicine, Mie University School of Medicine, Tsu, Japan

Introduction

Crigler-Najjar syndrome (CN) type II, which was first described by Arias et al., is characterized by chronic nonhemolytic unconjugated hyperbilirubinemia (Arias 1962). The jaundice in a patient with CN type II is caused by a reduction in hepatic bilirubin uridine 5′-diphosphate (UDP)-glucuronosyltransferase (B-UGT) activity (to less than 10% of normal). Recently, the *UGT1*1* gene which encodes the enzyme was identified (Ritter et al. 1992), and there has been progress in mutation analysis of CN type I, CN type II, and Gilbert's syndrome.

In recent studies on mutation analysis of patients with CN type II, five types of mutations which exist exclusively in the coding region have been reported: a single homozygous missense mutation (Arg209Trp) (Bosma et al. 1993), a single homozygous missense mutation (Gln331Arg) (Moghrabi et al. 1993), double homozygous missense mutations (Gly71Arg and Tyr486Asp) (Aono et al. 1993), heterozygous missense (Leu175Glu) and frame shift mutations (Seppen et al. 1994), and a single heterozygous nonsense mutation (Gln331Stop) (Koiwai et al. 1996).

On the other hand, it has been revealed that Gilbert's syndrome is caused by both missense mutations in the coding region (Aono et al. 1995) and a homozygous insertion mutation of the TATAA element in the promoter region (Bosma et al. 1995). It is hypothesized that the abnormality of the promoter region would also be one of the contributing factors in CN type II. To test this hypothesis, we analyzed both the coding and the promoter regions of the gene in seven Japanese patients with CN type II, and discuss the characteristics of their mutations.

Materials and methods

Patients and samples

The diagnosis of CN type II was based on the elevation of serum bilirubin concentration from 60 μM to 340 μM, with no evidence of hemolytic jaundice or other liver disease. Patient 1 was a 19-year-old male; his serum bilirubin concentration was 86 μM. Patients 2, 3, and 4 were sisters. Their ages were 58, 60, and 74 years old, respectively. Their serum bilirubin concentrations were 332, 154, and 282 μM, respectively. Patient 5 was a 53-year-old female with a serum bilirubin concentration of 238 μM. Patient 6 was a 74-year-old male; his serum bilirubin concentration was 258 μM. Patient 7 was a 51-year-old male with a serum bilirubin concentration of 333 μM. Bile analysis revealed 75.6% of bilirubin monoglucuronide and 5.3% of bilirubin diglucuronide. A liver biopsy specimen revealed no liver disease. Phenobarbital treatment reduced serum bilirubin concentration to 91 μM.

Two pairs of parents, those of patients 2–4 and 5, were consanguineous. It was unclear whether or not the parents of patient 1, 6, and 7 were consanguineous.

Polymerase chain reaction (PCR) and DNA sequencing

Genomic DNA was extracted from white blood cells. The promoter and coding regions of the *UGT1*1* gene were amplified as described elsewhere (Aono et al. 1994; Soeda et al. 1995). Sense and anti-sense primers used for PCR to amplify the TATAA element of the gene were 5′-GCCATA-TATATATATATA-3′ and 5′-GCTTGCTCAG-CATATATCTGGG-3′. Direct sequencing was performed three times for each patient as previously described (Aono et al. 1994).

Results

Patient 1 was heterozygous for a C to A transversion at codon 229 (position 686) in exon 1 (Fig. 1, Table 1). This nucleotide shift resulted in a substitution of glutamine for proline. He was also homozygous for an insertion mutation of the TATAA element (which contained two extra nucleotides (TA), a "long TATAA element") in the promoter region (Bosma et al. 1995).

Patient 2 was homozygous for a G to A transition at codon 71 (position 211) in exon 1 (Fig. 2A) and homozygous for a T to G transversion at codon 486 (position 1456) in exon 5 (Fig. 2B). The first mutation resulted in a substitu-

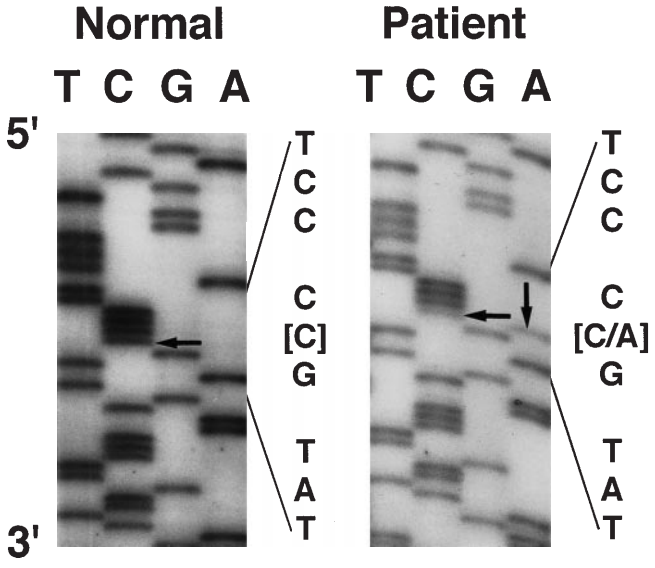
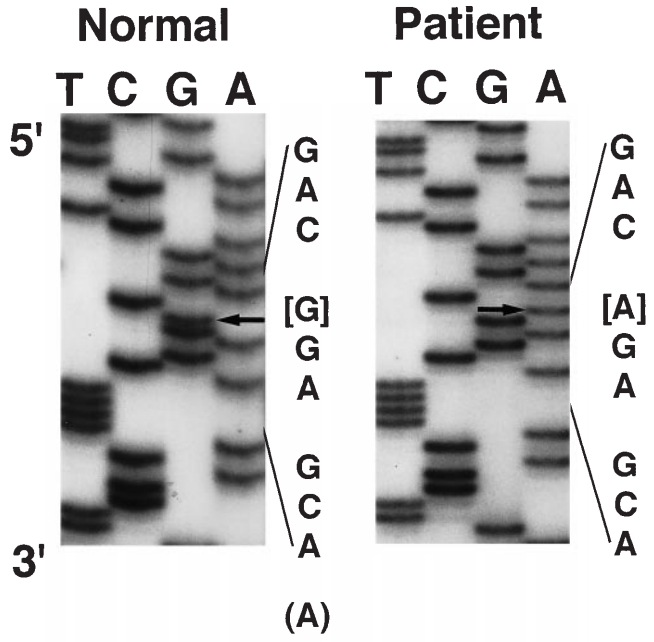


Fig. 1 Nucleotide sequence of region around position 686 of the *UGT1*1* gene from a normal control and patient 1. A heterozygous C to A transversion at position 686 is shown

Table 1 Clinical features and mutations

Patient number	Sex	Age	Bilirubin (μM)	TATAA element (zygosity)	Mutations in the coding region (zygosity)	Exon
1	M	19	86	Long[a] (homo)	Pro229Gln (hetero)	1
2	F	58	332	Normal[b] (homo)	Gly71Arg (homo) Tyr486Asp (homo)	1 5
3	F	60	154	Normal (homo)	Gly71Arg (homo) Tyr486Asp (homo)	1 5
4	F	74	282	Normal (homo)	Gly71Arg (homo) Tyr486Asp (homo)	1 5
5	F	53	238	Normal (homo)	Gly71Arg (homo) Tyr486Asp (homo)	1 5
6	M	74	258	Normal (homo)	Gly71Arg (homo) Tyr486Asp (homo)	1 5
7	M	51	333	Normal (homo)	Arg209Trp (homo)	1

[a] Long, a long TATAA element caused by insertion mutation: ATATATATATATATATAA.
[b] Normal, indicates a normal TATAA element: ATATATATATATATAA.



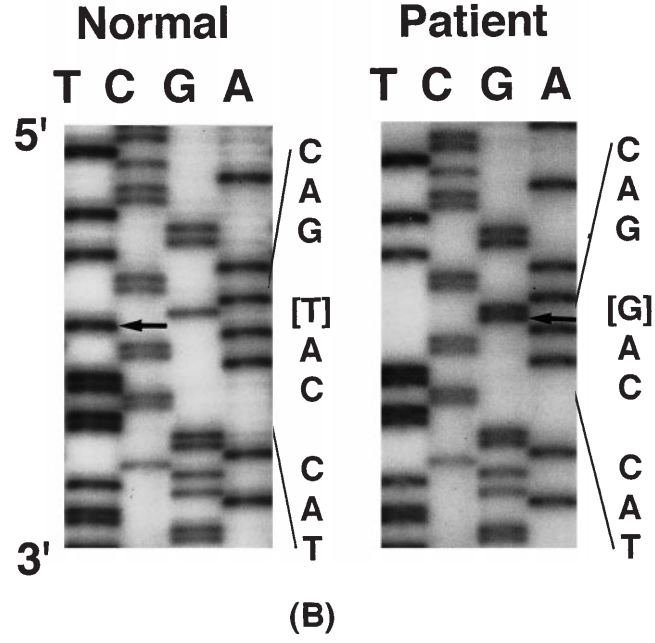


Fig. 2 **A** Nucleotide sequence of region around position 211 of the *UGT1*1* gene from a normal control and patient 2. A homozygous G to A transition at position 211 is shown. **B** Nucleotide sequence of region around position 1456 of the *UGT1*1* gene from a normal control and patient 2. A homozygous T to G transversion at position 1456 is shown

tion of arginine for glycine, and the second mutation resulted in a substitution of aspartate for tyrosine. He was homozygous for a normal TATAA element. Patients 3–6 had mutations which were identical to those of patient 2.

Patient 7 was homozygous for a C to T transition at codon 209 (position 625) in exon 1 (Fig. 3), resulting in a substitution of tryptophan for arginine. He was homozygous for a normal TATAA element.

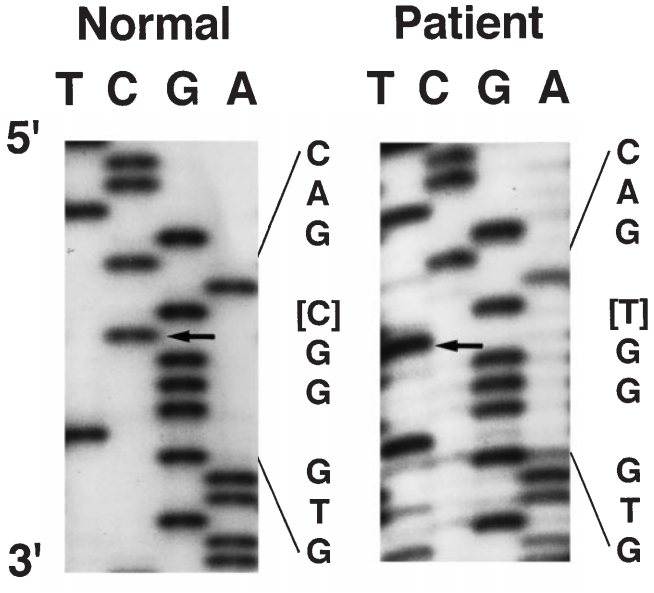


Fig. 3 Nucleotide sequence of region around position 625 of the *UGT1*1* gene from a normal control and patient 7. A homozygous C to T transition at position 625 is shown

The ages of the patients, and their mutations and serum bilirubin concentrations, are summarized in Table 1. The serum bilirubin concentrations of patients 2–6, who had identical double homozygous mutations, were at almost the same level. The serum bilirubin concentration of patient 1, who had a homozygous long TATAA element and heterozygous missense mutation, was the lowest of the seven. Every patient had mutations in exon 1, which encodes the N-terminal half of the B-UGT.

Discussion

Following identification of the *UGT1*1* gene (Ritter et al. 1992), there has been progress in mutation analysis of patients with CN type II. Until now, five types of mutations which exist exclusively in the coding region have been reported, as described earlier.

In this study, an additional, new type of mutation combination was found in patient 1. Gene analysis of patient 1 showed that he had both a homozygous insertion mutation of the TATAA element and a heterozygous missense mutation (Pro229Gln) in exon 1. Recently, we reported two patients with Gilbert's syndrome who were heterozygous for the same missense mutation (Pro229Gln), and the mutated enzyme had 25% of normal activity in the heterozygous state based on an in vitro expression study (Koiwai et al. 1995). Furthermore, the insertion mutation of the TATAA element was reported to reduce the transcriptional level of the gene to about 30% of normal in the homozygous state during in vitro expression experiments (Bosma et al. 1995). However, the reduction of activity caused by each mutation was not sufficient to express the phenotype of CN type II, because the enzyme activity of a

patient with CN type II is generally less than 10% of normal. Therefore, the phenotype as CN type II of this patient would appear to be caused by a combination of the heterozygous missense and homozygous insertion mutations.

Double homozygous missense mutations (Gly71Arg and Tyr486Asp) in different exons were detected in patients 2–6. These mutations were identical to those found in another patient reported earlier (Aono et al. 1993). We could not clearly indicate which mutation contributed to the phenotype as CN type II in the previous report. However, following that study, we found a patient with Gilbert's syndrome who was a single homozygote for Gly71Arg (Soeda et al. 1995). Furthermore, double homozygotes for Gly71Arg and Tyr486Asp always express the phenotype of CN type II, so both of these homozygous missense mutations would additionally contribute to elevation of serum bilirubin concentration to the level found in CN type II. A total of six patients with CN type II who had these double homozygous mutations, from four unrelated families, were detected, including one case reported by Aono et al. (1993) These mutations were the commonest of those encountered in our study of Japanese patients. Patients 2–4 (they were sisters) and patient 6 resided in Kanagawa prefecture. Patient 5 and the patient reported by Aono et al. (1993) resided in Kyoto and Aichi prefectures, respectively. An obvious concentration of the patients with the double mutation could not be demonstrated in a specific local area.

In patient 7, a single homozygous missense mutation (Arg209Trp) was found in exon 1. This mutation was identical to one reported earlier (Bosma et al. 1993). The serum bilirubin concentration of patient 7 (333 μM) was comparable to that of the previously reported case (340 μM). This mutation is the first of its type in a Japanese patient with CN type II.

Based on pedigree analysis, the inheritance of CN type II has been postulated to be autosomal recessive (Blaschke et al. 1974; Hunter et al. 1973) or autosomal dominant with incomplete penetrance (Arias et al. 1969). Gene analyses have shown both patients having recessive traits with homozygous missense mutations and a patient having dominant traits with a heterozygous nonsense mutation (Aono et al. 1993; Bosma et al. 1993; Koiwai et al. 1996). In our study, inheritance in patients 2–7 was recessive, in agreement with findings reported earlier; however, patient 1 appears to show complicated inheritance. We could not analyze the pedigree of patient 1, but it is speculated that the apparent inheritance of the patient would be detected as autosomal dominant with incomplete penetrance because the allele frequency of the insertion mutation of TATAA element is very high (40%; Bosma et al. 1995).

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