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Short Communication

THREE JAPANESE PATIENTS WITH CRIGLER-NAJJAR SYNDROME TYPE I CARRY AN IDENTICAL NONSENSE MUTATION IN THE GENE FOR UDP-GLUCURONOSYLTRANSFERASE

Osamu KOIWAI,[1] Yoshihiro YASUI,[1] Kayo HASADA,[2]
Sachiko AONO,[3] Hiroshi SATO,[4] Morihiko FUJIKAKE,[5]
and Tsugutoshi AOKI[6]

[1]*Departmept of Biochemistry and* [2]*Ultrastructure Research, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya 464, Japan*
[3]*Department of Perinatology, Institute for Developmental Research, Aichi Prefecture Colony, Kasugai 480–03, Japan*
[4]*Department of Biology, Shiga University of Medical Science, Ohtsu 520–21, Japan*
[5]*Department of Pediatrics, Seirei Hospital, Showa-ku, Nagoya 466, Japan*
[6]*Department of Pediatrics, Toho University School of Medicine and Ohashi Hospital, Ohashi, Meguro-ku, Tokyo 153, Japan*

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Crigler-Najjar syndrome type I (CN-I) is a severe disorder caused by chronic nonhemolytic unconjugated hyperbilirubinemia due to the complete absence of hepatic bilirubin UDP-glucuronosyltransferase activity (UGT; Crigler and Najjar, 1952; Arias *et al.*, 1969). The disease is inherited as an autosomal recessive trait (Roy-Chowdhury *et al.*, 1982). Bilirubin levels in the serum reach more than 342 μmol/liter and the patients succumb to kernicterus during the neonatal period unless treated with phototherapy, plasmapheresis or liver transplantation (Wolkoff *et al.*, 1979). Treatment with phenobarbital has no effect on the bilirubin level of CN-I.

Bilirubin UGT exists as a tetramer on the luminal surface of the endoplasmic reticulum in liver cells (Peters *et al.*, 1984) and it catalyzes the conversion of insoluble bilirubin to a water-soluble form by glucuronidation. Recently, cDNAs for rat and human bilirubin UGT were isolated (Sato *et al.*, 1990; Ritter *et al.*, 1991) and the structure of the gene for human bilirubin UGT in terms of exon-

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intron organization was determined (Ritter *et al.*, 1992; Bosma *et al.*, 1992a). The gene is located on chromosome 2 (van Es *et al.*, 1993) and consists of five exons (Bosma *et al.*, 1992a, 1994). On the basis of the structure of the gene for human bilirubin UGT, the genetic backgrounds of patients with CN-I have been elucidated. While almost all reported patients with the disease have homozygous nonsense or deletion mutations in the coding region of the gene (Brierley and Burchell, 1993), a few cases with homozygous missense mutations were found recently (Bosma *et al.*, 1992b; Erps *et al.*, 1994). By screening, we found four patients who suffered from CN-I in Japan. We already reported that one of them, patient A, carried a homozygous nonsense mutation in exon 1 of the gene for bilirubin UGT (Aono *et al.*, 1994). We have now analyzed the genetic backgrounds of the other three patients, B, C, and D, and, as we report herein, all three patients have a mutation in exon 1 identical to that found in patient A.

The diagnosis of CN-I was based on a markedly elevated level of unconjugated bilirubin in the serum that did not respond to treatment with phenobarbital and on the absence of hepatic bilirubin UGT activity. Liver tissues were obtained from the patients by biopsy and bilirubin UGT activities were assayed. No bilirubin UGT activity was detected in patient B at 14 days after birth or in patient C at 5 months after birth by HPLC method (Kawade, 1980). In patient D, no enzymatic activity was detected at 51 days after birth by the method of Heirwegh

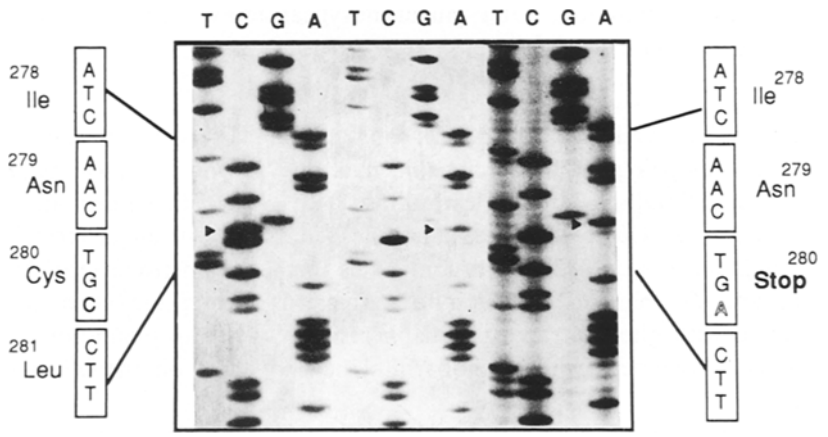

Fig. 1. Mutations detected in patients with CN-I. Nucleotide sequences of the gene for bilirubin UGT of patients B, C, and D were determined. Products of PCR were sequenced directly. The sites of mutations detected in the three patients were confirmed by sequencing of the subcloned products of PCR in pUC vectors. Since the mutation detected in patient B and his elder sister C were identical to each other, only the typical pattern from patient B is depicted. The wedges indicate the sites of mutations.

et al. (Van Roy and Heirwegh, 1968; Mizutani *et al.*, 1980). The total bilirubin level in the serum of each of the three patients B, C, and D exceeded 342 μmol/liter unless the patients were treated with phototherapy and the level was not lowered by administration of phenobarbital as much as 5 mg/kg/day. The bilirubin level in the serum of each patient is being maintained below 171 μmol/liter by the phototherapy for 12 hr. The results of other liver function tests (ALT, AST, and albumin levels, ICG and BSP) and liver histological findings were normal in each patient.

Blood samples were collected from patients B (9-year-old male), C (17-year-old female), D (15-year-old male) and their parents, and from three normal males for DNA analyses. Patient C was an elder sister of patient B. The entire first to fifth exons of the gene for bilirubin UGT of the patients and normal controls were amplified by PCR with specific primers (Aono *et al.*, 1994) and the products of PCR were sequenced directly. Since the family of patient B (C) was independent

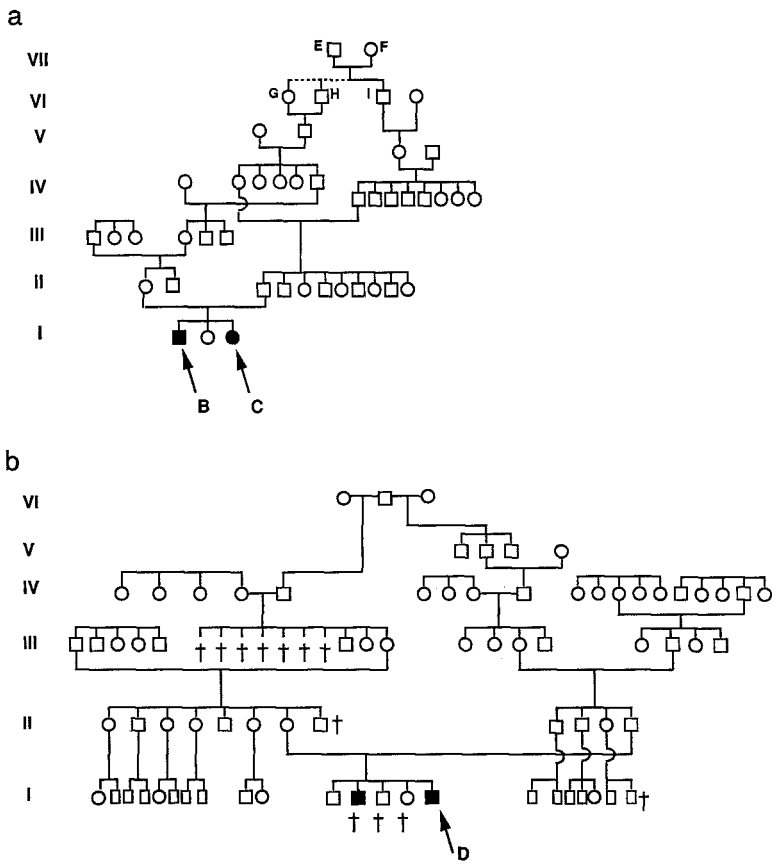

Fig. 2. Pedigrees of patients. (a), pedigree of patient B (C); (b), pedigree of patient D. It is not clear which child (G or H) was produced by E and F. A cross indicates that a child died at birth.

of patient A, we first expected that a novel nonsense or missense mutation at a position different from the mutation in patient A would be found in the gene of patient B (C). Unexpectedly, we found a mutation from C to A at position 840 (Fig. 1), which was identical to the mutation in patient A (Aono *et al.*, 1994). Next we analyzed the DNA sequence of the gene for bilirubin UGT of patient D. To our surprise, we found that patient D with CN-I also had the identical mutation at position 840 (Fig. 1).

We promptly performed pedigree analysis of patients B (C) and D (Fig. 2). From the analysis of patient B (C) (Fig. 2a), it was determined that the mother's grandfather was the elder brother of the father's mother. In the case of patient D, the mother's and father's ancestor were related at more than five generations ahead of patient D (Fig. 2b). More than one hundred years ago, a point mutation might have occurred at position 840 in the pedigree of patient D. The man carrying the mutation married twice and the mutated allele passed for four generations in two independent pedigrees (Fig. 2). Unfortunately, without their knowledge of the relationship, the parents married and produced children with the homozygous nonsense mutation. While a clear relationship between the families of patient B (C) and patient D was not elucidated from the pedigree analysis, the two families have the same last name, suggesting that they might be distantly related. Since we could not gain the informed consent of patient A's family for pedigree analysis, the relationships between patient A and patient B (C) and that between patient A and patient D have not been clarified. However, it is noteworthy that all three families come from a small local area, the Mikawa district, in Japan. This fact strongly suggests that the three Japanese families with CN-I are distantly related.

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