Crigler-Najjar Syndrome Type 2

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Crigler-Najjar syndrome is a rare disorder of bilirubin metabolism with two distinct forms: type 1 and type 2. We report three patients with Crigler-Najjar syndrome type 2 (CN-2). All patients had serum bilirubin values higher than 171 μmol/L and deep yellow skin color. The results of other liver function tests, glucose-6-phosphate dehydrogenase activity and hematology tests were normal, and immunologic tests for hepatitis A, B and C were negative, although one patient had slightly elevated alanine aminotransferase level (45 IU/L). Polymerase chain reaction and sequence analysis of the UDP-glucuronosyltransferase 1A1 (*UGT1A1*) gene revealed a novel homozygous T > A mutation at nucleotide 479 in exon 1 ($Val^{160}Glu$) of patient 1, a novel homozygous A > G mutation at nucleotide 610 in exon 1 ($Met^{204}Val$) of patient 2, and a homozygous T > G variation at nucleotide 1456 in exon 5 ($Tyr^{486}Asp$) plus a heterozygous G > A variation at nucleotide 211 in exon 1 ($Gly^{71}Arg$/normal) of patient 3. Two of these mutations were novel and variations identified within the coding region of the *UGT1A1* gene were considered the cause of CN-2 in all three patients. [*J Formos Med Assoc* 2006;105(11):950–953]

Key Words: Crigler-Najjar syndrome, glucuronosyltransferase, novel mutation

Three types of nonhemolytic unconjugated hyperbilirubinemia have been classified according to their clinical severity, Crigler-Najjar syndrome type 1 (CN-1: severe, serum bilirubin 340–850 μmol/L) and type 2 (CN-2: moderate, serum bilirubin 85–340 μmol/L), and Gilbert's syndrome (mild, serum bilirubin normal to 85 μmol/L). These three diseases, reported in 1992, 1993 and 1995, respectively, are caused by a defect in UDP-glucuronosyltransferase 1A1 (UGT1A1) because glucuronidation is essential for biliary elimination of bilirubin, and UGT1A1 is the only physiologically relevant isoform in bilirubin glucuronidation.[1] Single nucleotide polymorphism may alter the activity of UGT1A1, and such activities are absolutely absent, about 10%, and about 30% of normal in patients with CN-1, CN-2, and Gilbert's syndromes, respectively.[1,2] Unlike CN-1, kernicterus is rare and phenobarbital treatment results in induction of residual UGT1A1 activity, with consequent reduction of bilirubin concentrations > 25% in CN-2 patients.[1,3]

The results of some studies showed that all CN patients carried mutations (variations) within the coding region of the *UGT1A1* gene.[1–11] Recently, we and other authors discovered that, compared to Caucasians, the variation rate within the coding region of the *UGT1A1* gene is much higher in Asians.[4,5,12] These findings may indicate that the prevalence of CN in Asians is higher than in Caucasians (1 per million).[1] However, the only report of CN from Taiwan was in a CN-2 patient with a novel compound heterozygous variation (heterozygous C>T at nucleotide 625 plus heterozygous G deletion at nucleotide 1186) of the *UGT1A1* gene.[6] Two Chinese patients with CN-1

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involving a homozygous nonsense mutation (R341X) and a novel homozygous nonsense mutation (C>T at nucleotide 715) of the *UGT1A1* gene have also been reported.[7,8] Here, we report three variations in the *UGT1A1* gene in one Indian patient born in Singapore and two Taiwanese CN-2 patients. Two of the three mutations are novel, with the mutation in the remaining Taiwanese patient similar to that previously determined for Japanese CN-2 patients.[1,4,5]

Methods

The first patient was a 7-year-old Indian girl who was born full-term in Singapore in 1998. Severe hyperbilirubinemia was diagnosed on the 4th day of life. She was the only child of consanguineous parents. Medical records showed a peak total bilirubin of 491 μmol/L, with a conjugated bilirubin level of 5.1 μmol/L at 1 month after birth. She was severely icteric without hepatosplenomegaly. The results of other liver function tests, glucose-6-phosphate dehydrogenase (G6PD) activity and hematology tests were normal. Immunologic tests for hepatitis A, B and C were negative. Serum bilirubin was reduced to a minimal level of 115 μmol/L at 2 months of life after phototherapy treatment. In the nursery, she spent 10–14 hours a day sleeping under a phototherapy light and showed good intellectual development. Phenobarbital treatment was given for 1 week, but serum bilirubin level was not apparently different from when she was being treated with phototherapy only, so this drug therapy was discontinued.

The second patient was a 21-year-old Taiwanese woman with a history of severe hyperbilirubinemia since early childhood. Examination revealed deep yellow skin and yellow irises. The results of other liver function tests, G6PD activity and hematology tests were normal. Hepatitis B surface antigen (HBsAg) and anti-hepatitis C antibody (anti-HCV) were negative. Analysis of a blood specimen for *UGT1A1* gene analysis revealed serum bilirubin level of 217 μmol/L per 20.5 μmol/L (total/conjugated). Phenobarbital therapy led to a decreased serum bilirubin concentration (110 μmol/L) 10 days later.

The third patient was a 28-year-old Taiwanese woman with a history of severe hyperbilirubinemia since infancy. At the time of presentation at our clinic and *UGT1A1* gene analysis, serum bilirubin level was 173 μmol/L per 18 μmol/L (total/conjugated) and alanine aminotransferase was slightly elevated (45 IU/L; normal, <35). Deep yellow skin and yellow irises were noted. G6PD activity and hematology tests were normal. HBsAg and anti-HCV antibody tests were negative. Phenobarbital treatment was given and total serum bilirubin level was reduced to 103 μmol/L 2 weeks later.

The Blood DNA Isolation Kit (Maxim Biotech Inc., San Francisco, CA, USA) was used to isolate total genomic DNA from whole blood cells of all three patients after obtaining written consent. The promoter area of the *UGT1A1* gene, exons 1–4, the coding region of exon 5, and their flanking intronic regions were amplified by polymerase chain reaction (PCR) using the primers as described previously.[12]

Results

The PCR results for the *UGT1A1* gene showed a homozygous T > A substitution at nucleotide 479 in exon 1 in the Indian girl. No other variation was detected in the *UGT1A1* gene. Theoretically, the UGT1A1 enzyme in this girl is Val^{160}Glu. A heterozygous T > A substitution at nucleotide 479 was found in the *UGT1A1* gene of both her parents. The PCR product for the *UGT1A1* gene showed a homozygous A>G substitution at nucleotide 610 in exon 1 in the first Taiwanese patient. No other variation was detected in her *UGT1A1* gene. Theoretically, the UGT1A1 enzyme is Met^{204} Val in this patient. The PCR results for the *UGT1A1* gene in the second Taiwanese patient indicated that there was a homozygous T > G substitution at nucleotide 1456 in exon 5 plus a heterozygous G > A substitution at nucleotide 211 in exon 1. No other variation in the *UGT1A1* gene was found. Theoretically, the UGT1A1 enzyme in

this patient is $Tyr^{486}Asp$ and half of the enzyme is $Gly^{71}Arg$.

Discussion

Our review of previous reports indicate that the homozygous 479T > A ($Val^{160}Glu$) and the homozygous 610A > G ($Met^{204}Val$) variations of the first and second CN-2 patients in this report are novel mutations. The compound variation of heterozygous 211G > A ($Gly^{71}Arg$/normal) and homozygous 1456T > G ($Tyr^{486}Asp$) in our third CN-2 patient is not a new finding as homozygous 1456T > G ($Tyr^{486}Asp$) and double homozygous 211G > A plus 1456T > G variations ($Gly^{71}Arg$ plus $Tyr^{486}Asp$) have previously been reported in Japanese CN-2 patients.[1,4,5] A previous *in vitro* study found that the UGT1A1 enzyme activity of the homozygous $Tyr^{486}Asp$ model was 7.6 ± 0.5% of normal, at the level necessary for CN-2.[2] That finding might explain the highly elevated bilirubin value in our third CN-2 patient. Although patients with CN-2 are distinguished from those with CN-1 by a

Table. Reported genetic defects in Crigler-Najjar syndrome type 2

Nucleotide change	Amino acid substitution	Reference	Origin/patients (*n*)
44T > G	$Leu^{15}Arg$	1	Holland/2
111C > A, 1207C > T	$Pro^{34}Gln$, $Arg^{403}Cys$	11	Italy/1
211G > A, 1456T > G	$Gly^{71}Arg$, $Tyr^{486}Asp$	1, 4	Japan/6
211G > A/normal, 1456T > G	$Gly^{71}Arg$ /normal, $Tyr^{486}Asp$	This report	Taiwan/1
479T > A	$Val^{160}Glu$	This report	India/1
508–510 del TTC	Phe^{170} del	11	Italy/1
524T > A	$Leu^{175}Gln$	1	Holland /1
524T > A/973 del G	$Leu^{175}Gln$/frameshift	1	Holland /1
576C > G, 1130G > T, 6/7	$Tyr^{192}Stop$, $Gly^{377}Val$	11	Italy/1
610A > G	$Met^{204}Val$	This report	Taiwan/1
625C > T	$Arg^{209}Trp$	1 4	Holland /1, Japan/1
625C > T/1186 del G	$Arg^{209}Trp$/frameshift	6	Taiwan/1
674T > G, 717–718 del AG, 7/7	$Val^{225}Gly$, frameshift	9	Italy/1
674T > G/722–723 del AG	$Val^{225}Gly$/frameshift	1	America/1
674T > G, 878–890 del, 6/7	$Val^{225}Gly$, $Tyr^{293}Leu$, frameshift	11	Italy/1
674T > G, 1130G>T, 7/7	$Val^{225}Gly$, $Gly^{377}Val$	11	Italy/1
686C > A/normal, 7/7	$Pro^{229}Gln$/normal	4	Japan/1
717–718 del AG, 1381T > C, 6/7	Frameshift, $Trp^{461}Arg$	11	Italy/1
865–1G > A, 1007G > T, 7/7	Splicing, $Arg^{336}Leu$	11	Italy/1
877T > A, 878–890 del, 1060T > C, 6/7	$Tyr^{293}Met$, frameshift, $Trp^{354}Arg$	11	Italy/1
928A > G, 1292T > C	$Met^{310}Val$, $Ile^{431}Thr$	3	Africa/1
991C > T/normal	$Gln^{331}Stop$/normal	1	Japan/1
992A > G	$Gln^{331}Arg$	1	Ireland/1
1006C > T, 1130G > T	$Arg^{336}Trp$, $Gly^{377}Val$	11	Italy/1
1006C > T, 1304+1G > T, 6/7	$Arg^{336}Trp$, splicing	11	Italy/1
1213A > G, 8/8	$Asn^{400}Asp$	10	Morocco/1
1391A > C/normal, 7/7	$Glu^{464}Ala$/normal	1	America/1
1433C > A, 7/7	$Ala^{478}Asp$	11	Italy/1
1456 T > G	$Tyr^{486}Asp$	1, 5	Japan/5

6/7, 7/7 and 8/8 indicate $A(TA)_6TAA/A(TA)_7TAA$, $A(TA)_7TAA/A(TA)_7TAA$ and $A(TA)_8TAA/A(TA)_8TAA$ instead of $A(TA)_6TAA/A(TA)_6TAA$ in the promoter area of the UGT1A1 gene, respectively.

response to either phenobarbital treatment or phototherapy, phenobarbital treatment sometimes has to be repeated several times to successfully reduce serum bilirubin levels in CN-2 patients.[1,3] Since the serum bilirubin level in the first patient of this report was reduced after phototherapy, the diagnosis of CN-2 as opposed to CN-1 is suggested. Repeat phenobarbital treatment for this patient, and the measurement of UGT1A1 enzyme activity in this patient and the second CN-2 patient in this report were indicated but not performed during follow-up. Including our patients, at least 40 CN-2 cases with confirmed mutations (variations) in the *UGT1A1* gene have now been reported (Table): 18 Asians, 20 Caucasians, and two Africans. Since the prevalence of CN in Asians may be higher than in Caucasians, and the results of recent research showed that variation of the *UGT1A1* gene is the predominant hereditary defect in Taiwanese patients with unconjugated hyperbilirubinemia,[6,13–15] more Taiwanese patients with CN as well as mutant (variant) *UGT1A1* gene are likely to be found in the future.

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