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Original Article

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Molecular Analysis of the *UGT1A1* Gene in Korean Patients with Crigler-Najjar Syndrome Type II

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Purpose: Crigler-Najjar syndrome type II (CN-2) is characterized by moderate non-hemolytic unconjugated hyperbilirubinemia as a result of severe deficiency of bilirubin uridine diphosphate-glucuronosyltransferase (UGT1A1). The study investigated the mutation spectrum of *UGT1A1* gene in Korean children with CN-2.
Methods: Five Korean CN-2 patients from five unrelated families and 50 healthy controls were enrolled. All five exons and flanking introns of the *UGT1A1* gene were amplified by polymerase chain reaction (PCR) and the PCR products were directly sequenced.
Results: All children initially presented with neonatal jaundice and had persistent indirect hyperbilirubinemia. Homozygous p.Y486D was identified in all five patients. Three patients had an associated homozygous p.G71R and two a heterozygous p.G71R. The allele frequency of p.Y486D and p.G71R in healthy controls was 0 and 0.16, respectively. No significant difference in mean serum bilirubin levels was found between homozygous carriers of p.G71R and heterozygous carriers.
Conclusion: The combination of homozygous p.Y486D and homozygous or heterozygous p.G71R is identified. The p.Y486D and p.G71R can be screened for the mutation analysis of *UGT1A1* in Korean CN-2 patients.

Key Words: Crigler-Najjar syndrome, Bilirubin uridine-diphosphoglucuronosyl transferase 1A1, Mutation, Bilirubin

INTRODUCTION

Hepatic glucuronidating activity is essential for efficient biliary excretion of bilirubin. Bilirubin glucuronidation is mediated by the enzyme bilirubin uridine diphosphate-glucuronosyltransferase (UGT-1A1) [1]. UGT1A1 is encoded by five exons of the *UGT1A1* gene. Defects in UGT1A1 cause a non-hemolytic unconjugated hyperbilirubinemia including Crigler-Najjar (CN) syndrome and Gilbert syndrome. CN syndrome is an autosomal recessive disease caused by mutations in *UGT1A1* gene [2-5]. Genetic lesions causing an absence of enzymatic bilirubin glucuronidation result in CN syndrome type I (CN-1), whereas mutations causing incomplete deficiency of the enzyme result in CN syndrome type II (CN-2) [6].

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CN-2 is characterized by intermediate levels of hyperbilirubinemia (7-20 mg/dL). In contrast to CN-1, kernicterus is rare and phenobarbital administration results in induction of the residual UGT1A1 activity with consequent reduction of serum bilirubin levels (>25%). CN-1 is caused by genetic changes that produce premature truncation or critical amino acid residue substitution. CN-2 results from substitution of single amino acid residues that markedly reduce, but do not abolish, enzyme activity [7].

Currently, the spectrum of mutations in Korean patients with CN-2 is not characterized. The aim of this study is to investigate the mutation spectrum of *UGT1A1* gene in Korean children with CN-2.

MATERIALS AND METHODS

Patients

Five Korean CN-2 patients (two males and three females) from five unrelated families were investigated. The median age was 10 years (range: 1-20 years). The diagnosis of CN-2 was based on intermediate levels of hyperbilirubinemia (7-20 mg/dL). The bilirubin was at least 90% unconjugated. Serum alanine aminotransferase and aspartate aminotransferase values were normal. Hemolysis was excluded on the basis of normal hemoglobin and reticulocyte counts. We also studied 50 healthy subjects with no known history of jaundice. This study was approved by the institutional review board of Seoul National University Hospital (IRB No. 1401-108-549). Informed consent was obtained from all of the patients' parents, and patient confidentiality was maintained.

DNA sequencing and mutation analysis of the *UGT1A1* gene

Genomic DNA was extracted from peripheral blood leukocytes using the Wizard genomic DNA purification kit according to the manufacturer's instructions (Promega, Madison, WI, USA). All five exons and flanking introns of the *UGT1A1* gene were amplified using polymerase chain reaction (PCR). PCR was performed using a thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR products were purified and direct sequencing was performed using an ABI3100 Genetic Analyzer (Applied Biosystems). The sequences obtained were compared with the reference sequence NG_002601.2 registered in the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov).

RESULTS

All children initially presented with neonatal jaundice requiring phototheray. The median age at the time of jaundice onset was 3 days of birth (range: 1-14 days) (Table 1). They had persistent indirect hyperbilirubinemia. Serum total bilirubin decreased with the administration of phenobarbital. Median follow up periods were 2 years (range: 1-17 years).

We identified a missense mutation (p.Y486D produced by the nucleotide substitution c.1456T>G in exon 5) and a polymorphism (p.G71R produced by the nucleotide substitution c.211G>A in exon 1). Homozygous p.Y486D was found in all five patients while p.Y486D was not found in healthy controls (p <0.01). Three patients had an associated homozygous p.G71R and two a heterozygous p.G71R. The allele frequency of p.G71R in CN-2 patients and con-

Table 1. Clinical Features and Genotypes of Crigler-Najjar Syndrome Type II Patients

Patient No.	Sex	Age at the time of jaundice onset (d)	Mean serum total bilirubin (mg/dL)	p.G71R	p.Y486D
1	Male	1	9.6	Homozygous	Homozygous
2	Female	14	14.2	Homozygous	Homozygous
3	Female	3	9.1	Homozygous	Homozygous
4	Male	1	9.5	Heterozygous	Homozygous
5	Female	5	10.8	Heterozygous	Homozygous

trols was 0.88 and 0.16, respectively ($p<0.01$). No significant difference in mean serum bilirubin levels was found between homozygous carriers of p.G71R and heterozygous carriers.

DISCUSSION

This study presents the molecular characterization of *UGT1A1* in Korean CN-2 patients. The homozygous p.Y486D missense mutation was observed in all five CN-2 patients. Four patients with CN-2 from Japan have been found to be homozygous for p.Y486D [8]. The p.Y486D mutation has also been reported in a Tunisian CN-2 patient [9]. Relative UGT1A1 activity of a homozygous p.Y486D expression model was 7.6% of the normal level [10]. Enzyme activity of a patient with CN-2 is generally less than 10% of normal level. Premature terminating mutations in *UGT1A1* were more commonly observed among CN-1 patients, while missense mutations were more frequently associated with a CN-2 phenotype. This finding explains the milder phenotype observed in CN-2 and the inducibility of the residual enzyme activity by phenobarbital administration [7].

We found that all CN-2 patients had an associated homozygous or heterozygous p.G71R. Combination of homozygous p.Y486D and heterozygous p.G71R has been previously reported in a Taiwanese CN-2 patient [11]. A Japanese study revealed that three of the four CN-2 patients had an associated homozygous p.G71R [8]. It has been suggested that a homozygous p.Y486D associated with a homozygous or a heterozygous p.G71R always expresses CN-2 phenotypes in Asian populations.

In the present study, the allele frequency of p.G71R was 0.16 in healthy controls. p.G71R is considered as a polymorphism, which is any sequence variant present at a frequency $>1\%$ in a population. It has been reported that -c.3279T>G, TATA box, and p.G71R are three common polymorphisms in the Korean population, and that the allele frequency of p.G71R is 0.21. Serum bilirubin in homozygous carriers of p.G71R was reportedly significantly higher than those in heterozygous carriers or homozygous carriers of wild type allele [12]. A mutant expression model also showed that a heterozygous p.G71R and a homozygous p.G71R reduced transferase activity to 60% and 30% of the normal level, respectively [10]. Serum total bilirubin was markedly elevated when homozygous p.Y486D was present in combination with homozygous p.G71R [8]. In this study, no significant difference in serum bilirubin was found between homozygous carriers of p.G71R and heterozygous carriers. Further larger studies are helpful to investigate whether p.G71R additionally increases serum bilirubin level in homozygous p.Y486D carriers with CN-2. The p.G71R polymorphism has been identified as a risk factor for Gilbert syndrome [13,14] and neonatal breastfeeding jaundice [15,16] in East Asia.

In summary, the combination of homozygous p.Y486D and homozygous or heterozygous p.G71R is identified in CN-2 patients. The p.Y486D and p.G71R allele can be screened for the mutation analysis of *UGT1A1* in Korea. Further larger genetic studies are needed to confirm our findings.

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