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A Homozygous Mutation in *UGT1A1* Exon 5 May Be Responsible for Persistent Hyperbilirubinemia in a Japanese Girl with Gilbert's Syndrome

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ABSTRACT

The *UGT1A1* gene encodes a responsible enzyme, UDP-glucuronosyltransferase1A1, for bilirubin metabolism. Many mutations have already been identified in patients with inherited disorders with hyperbilirubinemia, Crigler-Najjar syndrome and Gilbert's syndrome. In this study, we identified a *UGT1A1* mutation in an 8-year-old Japanese girl with persistent hyperbilirubinemia who was clinically diagnosed as having Gilbert's syndrome. For the mutational analysis of *UGT1A1*, we performed a full sequence analysis of the gene using the patient's DNA. She was homozygous for a T to G transversion at nucleotide position 1456 in *UGT1A1* exon 5 (c.1456T>G), leading to the substitution of aspartate for tyrosine at position 486 of the UGT1A1 protein (p.Y486D). In conclusion, the homozygous mutation of *UGT1A1* may be responsible for persistent hyperbilirubinemia in this patient.

INTRODUCTION

UDP-glucuronosyltransferases (UGTs) are membrane-bound enzymes that are localized in the endoplasmic reticulum and that catalyze the glucuronidation of many substrates. Glucuronidation is a major process for the detoxification and excretion of endogenous and xenobiotic compounds in human and other mammals (Miners and Mackenzie, 1991). UGTs are known to exist as a superfamily of independently regulated enzymes that can be divided into two subfamilies, namely UGT1A and UGT2B (Mackenzie et al., 1997). The UGT1A subfamily, derived from a single gene locus on chromosome 2q37, comprises at least 13 promoters and unique first exons that are separately spliced to shared exons 2-5, resulting in transcripts that encode enzymes with unique amino termini preceding the identical carboxyl terminus (van Es et al., 1993; Gong et al.,2001). The amino termini of the UGTs, determined by the promoter and the first exon of the UGT-coding genes, are substrate-specific. The UGT2B subfamily consists of 5 similar genes located in a cassette on chromosome 4q13 (Jedlitschky et al., 1999).

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UGT1A1, a member of UGT1A family, is mainly expressed in the liver, where it is the main glucuronidation enzyme for bilirubin (Ritter et al., 1991; Senafi et al., 1994; Watanabe et al., 2003). More than 60 genetic variants, mutations or polymorphisms in the *UGT1A1* gene have been reported, and many of them were found in patients with Crigler-Najjar syndrome (CN) and Gilbert's syndrome (GS) where they hamper bilirubin-glucuronide formation (Sato et al., 1996; Aono et al., 1993; Maruo et al., 1999). The most common genetic variant in the Caucasian population is a dinucleotide repeat polymorphism in the atypical TATA box region of the *UGT1A1* promoter (the TA-7 polymorphism). However, the variant promoter (TA-7) is infrequent in East Asian populations. A c.211G>A transition (glycine to arginine at position 71 of the UGT1A1 protein, p.G71R) is the most common variant in this population (Akaba et al., 1999; Maruo et al., 2000).

In this study, we identified a homozygous mutation, c.1456T>G, in *UGT1A1* exon 5 in an 8-year-old Japanese girl with persistent hyperbilirubinemia who was clinically diagnosed as having GS. The mutation may lead to the substitution of aspartate for tyrosine at position 486 of the UGT1A1 protein (p.Y486D). In addition, the mutation in *UGT1A1* exon 5, which is common to the UGT1A subfamily, would affect the activity of all enzymes belonging to the subfamily and would also disturb the metabolisms of other compounds than bilirubin.

MATERIALS AND METHODS

The Patient

The patient was an 8-year-old Japanese girl with Gilbert's syndrome. She was born to nonconsanguineous parents in good health. She had a history of phototherapy for her neonatal hyperbilirubinemia. She also showed prolonged neonatal hyperbilirubinemia and recurrent hyperbilirubinemia after the neonatal period. Icteric sclera was the only finding when she was referred to the Department of Pediatrics, Kobe University Hospital in 2009 at the age of 8 years. Laboratory examination at that time revealed hyperbilirubinemia with a high concentration of total bilirubin (3.7 mg/dL) (Table I). Hemoglobin and serum transaminase levels, serum AST (aspartate aminotransferase) and ALT (alanine aminotransferase), were within normal limits, suggesting that she had neither hemolysis nor hepatocellular damage. After obtaining informed consent from the patient and her parents, DNA analysis was performed. DNA analysis was approved by the ethics committee of Kobe University Hospital.

Table I. Laboratory data (8 years old)

Complete Blood Count			Chemistry Data					
WBC	8900	/μL	GOT	27	IU/L	BUN	12	mg/dL
RBC	492	×10⁴/μL	GPT	18	IU/L	Cre	0.44	mg/dL
Hb	13.4	mg/dL	LDH	225	IU/L	UA	4.7	mg/dL
Ht	39.5	%	γGTP	14	IU/L			
MCV	80	fL	ALP	748	IU/L	T-bil	3.7	mg/dL
MCH	27.2	pg	LDH	225	IU/L	D-bil	<0.1	mg/dL
MCHC	33.9	%	CPK	131	IU/L	Haptoglobin	110	mg/dL
Plt	26.6	×10⁴/μL	ChE	377	IU/L	T-Chol	181	mg/dL
			Amy	148	IU/L	BS	83	mg/dL
			Lipase	31	IU/L			
Serological Test						TSH	1.571	μU/mL
CRP	<0.1	mg/dL	Na	138	mEq/L	fT4	1.20	ng/dL
			K	4.3	mEq/L			
IgG	1210	mg/dL	Cl	104	mEq/L			
IgA	125	mg/dL	Ca	10.5	mg/dL			
IgM	149	mg/dL	P	5.2	mg/dL			

DNA extraction and mutation screening for TA-7 and c.211G>A

Genomic DNA was extracted from the leukocytes of the patient with a QuickGene DNA whole blood kit S (FUJIFILM, Tokyo, Japan). Extracted DNA was stored in several tubes at -20°C until analysis. Some of the extracted DNA was sent to the genetics laboratory at the Mitsubishi Chemical Medience Corporation (Tokyo, Japan), where mutation screening for the variant promoter (TA-7) and for the c.211G>A mutation (p.G71R) in *UGT1A1* was done using the Invader® UGT1A1 Molecular Assay (Sekisui Medical Co., Ltd, Tokyo, Japan) (Nagano et al., 2002). The variant promoter (TA-7) contains seven repeats of TA, while the normal one contains six repeats of TA (TA-6).

Amplification of UGT1A1 exons

All exons of the *UGT1A1* gene were amplified using the primer sets described by Aono et al. (Aono et al., 1994). The PCR was carried out using a Mastercycler Gradient PCR machine (Eppendorf, Hamburg, Germany). The 30 µL reaction mixture contained 200 ng genomic DNA in 1 × PCR buffer with 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.15 µM primers and 1 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, USA). The conditions for PCR included initial denaturation at 95°C for 4 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 2 min, extension at 72°C for 2 min and final extension at 72°C for 10 min. The PCR products were electrophoresed in a 1.5% agarose gel and stained with ethidium bromide.

Nucleotide sequencing of UGT1A1

To locate the mutation in the *UGT1A1* gene, the PCR products were directly sequenced using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems), following the manufacturer's protocol. The primers used for sequencing analysis were those described by Aono et al. (Aono et al., 1994). The products from the cycle sequencing reaction were purified by ethanol precipitation and applied to a genetic analyzer ABI PRISM 310. The nucleotide sequences were determined using the DNA Sequencing Analysis Software (Applied Biosystems).

RESULTS

Mutation screening for TA-7 and c.211G>A in UGT1A1

Mutation screening for the variant promoter (TA-7) and the c.211G>A mutation (p.G71R) in *UGT1A1* was done using an Invader method. The mutation screening indicated that the patient and her mother had neither a variant promoter (TA-7) nor a c.211G>A mutation (p.G71R) in *UGT1A1*. The father, however, was heterozygous for the c.211G>A mutation, while the variant promoter was absent.

Identification of an intraexonic mutation of UGT1A1

To search for an intraexonic mutation in the *UGT1A1* gene of the patient, direct sequencing analysis of the PCR products for all the exons was performed. Homozygosity of a T to G transversion (T>G) was identified at nucleotide position 1456 in exon 5 (c.1456T>G) (Figure 1). The nucleotide change is predicted to cause the substitution of aspartate for tyrosine at amino acid position 486 (p.Y486D). No mutations were detected in any of the other exons.

Haplotype analysis of the family members

To screen for the c.1456T>G mutation in the parents, we amplified the DNA fragment of *UGT1A1* exon 5 and found that the parents were heterozygous for the mutation (Figure 1). Taken together with the screening results for the variant promoter (TA-7) and for the c.211G>A mutation (p.G71R), the haplotype of the family members was determined (Figure 2).

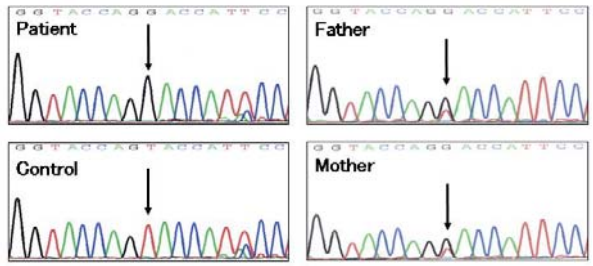

Figure 1.
DNA sequence analysis of family members. A trace of the nucleotide sequencing data showing the T>G mutation at position 1456 (indicated by the arrow) in exon 5 of *UGT1A1*. Other exons and promoter region were normal.

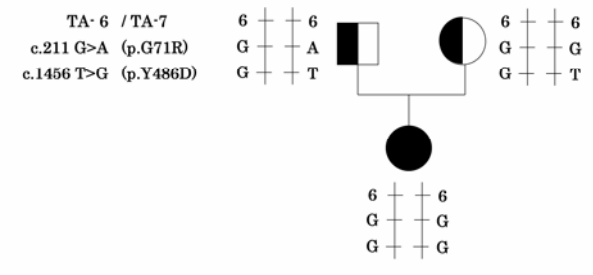

Figure 2.
UGT1A1 haplotype analysis of the patient and her parents. The circles represent the patient and her mother and the square represents the father.

DISCUSSION

We found that the patient with GS had a homozygous mutation, c.1456T>G (p.Y486D), in the *UGT1A1* gene. Previous reports from Japan had identified the p.Y486D mutation in *UGT1A1* in CN and GS patients, but many of the CN patients were double homozygotes for p.G71R and p.Y486D mutations (having an allele with both p.G71R and p.Y486D mutations) (Aono et al., 1993; Yamamoto et al., 1998a; Yamamoto et al., 1998b). Our GS patient was a single homozygote for the p.Y486D mutation. The haplotype analysis of the parents showed that the father carried the p.G71R mutation in one of his alleles but did not have the p.Y486D mutation on the same allele. These findings indicate that our patient could not have inherited an allele with both the p.G71R and the p.Y486D mutations.

Yamamoto et al. expressed normal and mutant *UGT1A1*s in COS-7 cells to clarify the contribution to the CN and GS of each of these *UGT1A1* mutations (Yamamoto et al., 1998a). According to their data, the relative UGT1A1 activity of the single homozygous model of p.G71R was 32.2 ± 1.6% of normal, that of the single homozygous model of p.Y486D was 7.6 ± 0.5%, and that of the double homozygous model of p.G71R and p.Y486D was 6.2 ± 1.6%. The decreased activities of the single homozygous models, p.G71R and p.Y486D, and of the double homozygous model of p.G71R and p.Y486D were at an appropriate level to be diagnosed as GS or CN. Therefore, prolonged neonatal hyperbilirubinemia and recurrent hyperbilirubinemia after the neonatal period, which are compatible with the symptoms of GS, can in our patient be explained by the homozygous mutation, p. Y486D, in *UGT1A1*.

We predicted that the p.Y486D mutation in exon 5, one of the shared exons, may disturb the disposition of a widely used antipyretic, acetaminophen, because it may affect the activity of UGT1A1, UGT1A6 and UGT1A9. About 85% of acetaminophen is metabolized by conjugation, mainly glucuronidation via the UGTs (Gelotte et al., 2007). Acetaminophen glucuronidation is mainly catalyzed by UGT1A1, UGT1A6 and UGT1A9 (Court et al., 2001). Because the GS patient in this study was homozygous for p.Y486D, we expected her to exhibit disturbed acetaminophen disposition. We have a plan to study the acetaminophen metabolism in the patient.

In conclusion, we identified a homozygous mutation, c.1456T>G, in *UGT1A1* exon 5 in an 8-year-old Japanese girl with persistent hyperbilirubinemia who was clinically diagnosed as having GS. The homozygous mutation of *UGT1A1* may be responsible for persistent hyperbilirubinemia in this patient. The mutation in *UGT1A1* exon 5 would affect the activity of all enzymes belonging to the subfamily and would also disturb the metabolisms of some xenobiotic compounds including acetaminophen.

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