Letter to the Editor
Diagnostic Genetics

Ann Lab Med 2020;40:281-283
https://doi.org/10.3343/alm.2020.40.3.281
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Genetic Spectrum of *UGT1A1* in Korean Patients with Unconjugated Hyperbilirubinemia

Jin Ju Kim, M.D.[1], Joowon Oh, M.D.[2], Yoonjung Kim, M.D. PhD.[2], and Kyung-A Lee, M.D. Ph.D.[2]

[1]Department of Laboratory Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea; [2]Department of Laboratory Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea

Dear Editor,

Uridine diphosphate-glucuronosyltransferase 1A (UGT1A1), an enzyme encoded by the *UGT1A1* gene on chromosome 2q37, is the major bilirubin-conjugating enzyme [1, 2]. Variants in the promoter and coding regions of *UGT1A1* affect the enzyme activity of UGT1A1, leading to unconjugated hyperbilirubinemia disorders, including Gilbert syndrome (GS), Crigler-Najjar syndrome type I (CNI), and Crigler-Najjar syndrome type II (CNII). Variant frequencies show inter-ethnic differences [3, 4]. Reports on the spectrum of variants in Korean patients with hyperbilirubinemia are limited thus far; they are mostly based on a small number of patients evaluating a few mutational hotspots [5, 6]. We retrospectively investigated the spectrum of *UGT1A1* variation in a larger study population to examine the allele distribution in Korean patients with unconjugated hyperbilirubinemia.

We collected clinical data of 81 patients with hyperbilirubinemia who underwent *UGT1A1* genotyping between April 2013 and March 2019 in Gangnam Severance Hospital, Seoul, Korea. For comparison, we retrieved allele frequencies of 1,722 Korean subjects from the Korean Reference Genome database (KRGDB, http://coda.nih.go.kr/coda/KRGDB/index.jsp), and of 80 Chinese and 71 Japanese healthy subjects from published data [7, 8]. For association analysis of genotypes and total bilirubin (TB) levels, we retrieved allele frequencies and laboratory data from a published study [9]. This study was approved by the Institutional Review Board of Yonsei University Health System, Gangnam Severance Hospital (IRB-2019-0188-001). The need for informed consent of the participants for reviewing medical records was waived on the condition that the research involves no more than minimal risk to the patients and the patient's privacy was thoroughly protected.

The promoter region, including the TATA box and all five exons with flanking introns of *UGT1A1*, were PCR-amplified in a 3500 Dx Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using an in-house method. The sequences were compared with a *UGT1A1* reference sequence (accession number: NM_000463.2) registered in the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov). Two independent experts evaluated the variants, and the haplotype was annotated according to *UGT1A1* allele nomenclature guidelines (https://www.pharmacogenomics.pha.ulaval.ca/wp-content/uploads/2015/04/*UGT1A1*-allele-nomenclature.html).

Statistical analyses were conducted using Analyse-it v.5.11 Method Evaluation edition (Analyse-it Software, Ltd., Leeds, UK). Allele frequencies in the control and study groups were compared using Fisher's exact test. Results were considered significant at $P<0.05$.

We detected nine variants in this study (Table 1). All variants

Received: August 7, 2019
Revision received: August 29, 2019
Accepted: November 13, 2019

Corresponding author: Kyung-A Lee, M.D. PhD.
Department of Laboratory Medicine, Yonsei University College of Medicine, 211 Eonju-ro, Gangnam-gu, Seoul 06273, Korea
Tel: +82-2-2019-3531, Fax: +82-2-2019-4822, E-mail: KAL1119@yuhs.ac

Table 1. Variants and frequencies of *UGT1A1* detected in patients with hyperbilirubinemia (N=81) and healthy controls

Location	Nucleotide change	Marker allele	Predicted amino acid change	Minor allele frequency (number of heterozygotes/homozygotes)				$P^{\dagger}$	dbSNP
				Patients (N=81)	Controls				
					Chinese [7] (N=80)	Japanese [8] (N=71)	Korean* (N=1,722)		
Promoter	c.-64G>C	*81	-	0.037 (6/0)	-	-	0.036	0.832	rs873478
Promoter	c.-41_-40dupTA	*28	-	0.438 (31/20)	0.144 (21/1)	0.113 (12/2)	0.200	**<0.0001**	rs34983651
Intron 1	c.864+2T>C	Novel	-	0.006 (1/0)	-	-	0.000	**0.045**	rs772088902
Exon 1	c.182C>G	Novel	p.Ala61Gly	0.006 (1/0)	-	-	0.000	**0.045**	rs1273237448
Exon 1	c.211G>A	*6	p.Gly71Arg	0.315 (31/10)	0.169 (23/2)	0.183 (20/3)	0.173	**<0.0001**	rs4148323
Exon 1	c.686C>A	*27	p.Pro229Gln	0.037 (6/0)	0.013 (2/0)	-	0.014	**0.030**	rs35350960
Exon 4	c.1091C>T	*73	p.Pro364Leu	0.031 (5/0)	0.006 (1/0)	-	0.013	0.077	rs34946978
Exon 5	c.1456T>G	*7	p.Tyr486Asp	0.031 (1/2)	0.000 (0/0)	0.014 (2/0)	0.001	**<0.0001**	rs34993780
Exon 5	c.1352C>T	N/A	p.Pro451Leu	0.006 (1/0)	-	-	0.001	0.241	rs114982090

Values in bold indicate statistical significance.
*Korean Reference Genome database (http://coda.nih.go.kr/coda/KRGDB/index.jsp); †*P* values for comparison between Korean patients with hyperbilirubinemia and Korean control subjects were calculated based on Fisher's exact test.

Table 2. Genotyping of *UGT1A1* in hyperbilirubinemia patients with clinical data (N=46)

Genotype	Promoter TATA box	Coding region	Patients (N=46)		Controls [9] (N=324)	
			N	TB (μmol/L) (mean±SD)	N	TB (μmol/L) (mean±SD)
1	Wild	Wild	HS (1)	69.08	148	13.34±3.42
2	TA6/7	p.Gly71Arg +/- ; p.Ala61Gly +/–	GS (1)	20.52	-	-
3	Wild	p.Gly71Arg +/+	GS (3)	25.65±2.91	18	23.60±6.16
4	TA6/7	p.Pro229Gln +/–	GS (2)	28.22±1.20	-	-
5	TA7/7	p.Pro229Gln +/+	GS (1)	30.78	-	-
6	TA6/7	Wild	GS (3)	34.54±15.56	49	17.27±5.64
7	TA7/7	p.Pro364Leu +/–	GS (2)	35.91±7.18	-	-
8	Wild	p.Gly71Arg +/–	GS (4)	34.71±12.31	83	15.90±3.25
9	TA6/7	p.Gly71Arg +/–	GS (14)	40.01±24.45	19	24.62±5.13
10	TA7/7	Wild	GS (12)	46.68±17.10	7	33.17±4.10
11	Wild	p.Gly71Arg +/– ; p. Pro364Leu +/–	GS (1)	61.56	-	-
12	Wild	p.Gly71Arg +/– ; p.Tyr486Asp +/+	CNII (1)	75.24	-	-
13	Wild	p.Gly71Arg +/+ ; p.Tyr486Asp +/+	CNII (1)	79.86	-	-

Abbreviations: +/–, heterozygous variant; +/+, homozygous variant; GS, Gilbert's syndrome; CNII, Crigler-Najjar syndrome II; HS, hereditary spherocytosis; TB, total bilirubin.

identified in exons were missense variants, and two novel variants (c.864+2T>C and p.Ala61Gly) were detected. The most frequent allele was *UGT1A1*28*, with a frequency of 43.8% (71/162), followed by the *UGT1A1*6* allele, with a frequency of 31.5% (51/162). The frequency of the wild-type allele was 4.9% (8/162) in the 81 hyperbilirubinemia patients. The frequencies of the *UGT1A1*28*, *UGT1A1*6*, *UGT1A1*27*, and *UGT1A1*7* alleles were significantly different between patients and the Korean control group.

Table 2 shows the *UGT1A1* genotypes detected in 46 patients with available clinical data. Homozygous *UGT1A1*7* alleles with homozygous or heterozygous *UGT1A1*6* alleles were detected in two CNII patients only, and these patients showed the highest TB levels (79.87 μmol/L and 75.24 μmol/L, respectively). Forty-three GS patients were grouped into 10 different *UGT1A1* genotypes. Heterozygous or homozygous *UGT1A1*28* (TA6/7, TA7/7)

https://doi.org/10.3343/alm.2020.40.3.281

(58.1%, 50/86) and heterozygous or homozygous *UGT1A1**6 (30.2%, 26/86) were frequently identified in GS patients.

In this study, *UGT1A1**28 and *UGT1A1**6 were the most frequent alleles. While *UGT1A1**6 is the most frequent allele in East Asian hyperbilirubinemia patients [3], *UGT1A1**28 was the most frequent allele (44.3%, 51/81) in this study. Two CNII patients were homozygous for the *UGT1A1**7 allele in combination with the *UGT1A1**6 allele. Homozygous genotype of the *UGT1A1**7 allele has been previously reported in CNII cases [5-8]. Homozygous *UGT1A1**7 allele has a strong association with CNII in the Asian population [6], in line with our results.

Two patients carried novel variants. A 57-year-old female patient had a c.864+2T>C variant combined with a heterozygous *UGT1A1**28 allele. c.864+2T>C is a variant at the splice-donor site at the 5′ end of intron 1. An adjacent splice-donor site variant, c.864+1G>C, has been reported in a CNI patient in a homozygous genotype, and this variant is expected to induce splicing at a cryptic site [10]. Therefore, we believe that the c.864+2T>C variant contributed to the medical condition of the patient. A five-year-old male patient had a c.182C>G variant combined with compound heterozygous *UGT1A1**6 and *UGT1A1**28 alleles. c.182C>G is a missense variant in exon 1. Further evidence based on more patient cases and investigation of the effects of these variants on enzyme activity are needed to assign a pathogenic role to these two novel variants; we interpreted these variants as variant of uncertain significance according to American College of Medical Genetics guidelines [11].

In conclusion, from the spectrum of *UGT1A1* variations, *UGT1A1**28 and *6 alleles tend to be prevalent in Korean patients with unconjugated hyperbilirubinemia and the *UGT1A1**28 allele tends to be more prevalent than in other Asian populations.

ACKNOWLEDGEMENTS

We appreciate all laboratory technicians involved in the molecular genetic test part for their support.

AUTHOR CONTRIBUTIONS

J Kim analyzed the data and wrote the draft; K Lee designed the study and finalized the draft; Y Kim and J Oh reviewed the draft and commented on it. All authors have accepted responsibility for the entire content of the manuscript and approved its submission.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

RESEARCH FUNDING

None declared.

ORCID

Jin Ju Kim https://orcid.org/0000-0001-9166-1848
Joowon Oh https://orcid.org/0000-0002-8449-3660
Yoonjung Kim https://orcid.org/0000-0002-4370-4265
Kyung-A Lee https://orcid.org/0000-0001-5320-6705

REFERENCES

1. Mackenzie PI, Miners JO, McKinnon RA. Polymorphisms in UDP glucuronosyltransferase genes: functional consequences and clinical relevance. Clin Chem Lab Med 2000;38:889-92.
2. Bosma PJ, Seppen J, Goldhoorn B, Bakker C, Elferink RO, Chowdhury JR, et al. Bilirubin UDP-glucuronosyltransferase 1 is the only relevant bilirubin glucuronidating isoform in man. J Biol Chem 1994;269:17960-4.
3. Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (*UGT1A1*) promoter: a balanced polymorphism for regulation of bilirubin metabolism? Proc Natl Acad Sci 1998;95:8170-4.
4. Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. Pharmacogenomics J 2003;3:136.
5. Kim SY, Lee SH, Koh H, Lee ST, Ki CS, Kim JW, Chung K. A case of Crigler-Najjar syndrome type 2 diagnosed using genetic mutation analysis. Korean J Pediatr Gastroenterol Nutr 2008;11:219-22.
6. Ko JS, Chang JY, Moon JS, Yang HR, Seo JK. Molecular analysis of the *UGT1A1* gene in Korean patients with Crigler-Najjar syndrome type II. Pediatr Gastroenterol Hepatol Nutr 2014;17:37-40.
7. Li L, Deng G, Tang Y, Mao Q. Spectrum of *UGT1A1* variations in Chinese patients with Crigler-Najjar syndrome type II. PLoS One 2015;10:e0126263.
8. Takeuchi K, Kobayashi Y, Tamaki S, Ishihara T, Maruo Y, Araki J, et al. Genetic polymorphisms of bilirubin uridine diphosphate-glucuronosyltransferase gene in Japanese patients with Crigler–Najjar syndrome or Gilbert's syndrome as well as in healthy Japanese subjects. J Gastroenterol Hepatol 2004;19:1023-8.
9. Ki CS, Lee KA, Lee SY, Kim HJ, Cho SS, Park JH, et al. Haplotype structure of the UDP-glucuronosyltransferase 1A1 (*UGT1A1*) gene and its relationship to serum total bilirubin concentration in a male Korean population. Clin Chem 2003;49:2078-81.
10. Gantla S, Bakker CTM, Deocharan B, Thummala NR, Zweiner J, Sinaasappel M, et al. Splice-site mutations: a novel genetic mechanism of Crigler-Najjar syndrome type 1. Am J Hum Genet 1998;62:585-92.
11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.