



Should We Consider *UGT1A1* Mutation Analysis in Evaluating the Prolonged Jaundice of Newborn Infants?

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ABSTRACT

Purpose: Uridine diphosphate glucuronosyltransferase 1A isoform 1 (*UGT1A1*) is a crucial enzyme in bilirubin metabolism. Mutations in this gene cause prolonged unconjugated hyperbilirubinemia in infants. This study aimed to investigate the prevalence of *UGT1A1* mutations and their association with prolonged, unexplained, and unconjugated hyperbilirubinemia in infants.

Methods: From July 2019 to March 2023, 74 infants with prolonged jaundice lasting >21 days were enrolled in this study. Diagnostic evaluations, including *UGT1A1* mutation analysis, were performed to identify the underlying causes of hyperbilirubinemia. This retrospective review evaluated the incidence and types of mutations in *UGT1A1*. The clinical and laboratory findings were compared based on the specific mutations detected.

Results: Thirty-three infants agreed to *UGT1A1* mutation analysis, and 30 (90.9%) were positive for *UGT1A1* mutations. Single-nucleotide variants were detected in 20 (66. %) infants. The remaining 10 (33.3%) infants had multiple variants. No significant demographic differences were observed between the group that underwent *UGT1A1* mutation analysis and the group that did not. Among the identified genetic variants, c.211G>A (46.5%) and c.-3275T>G (30.2%) were the two most common variants. The other variants had the following percentages: c.1456T>G, c.-64G>C, and c.1091C>T (4.7% each); and c.-3152G>A, c.189C>T, and c.-41.-40 dup (2.3% each). Among the 20 infants with the c.211G>A variant, eight (40.0%) had a homozygous genotype and 12 (60.0%) had a heterozygous genotype. Infants harboring other variants exhibited heterozygous genotypes. When comparing the group with confirmed *UGT1A1* mutations to the group without detected mutations, breastfeeding was the only significant factor ($P=0.027$). No significant differences were found between the group with single-nucleotide variants and the group with multiple genetic variants or between the homozygous genotype group with c.211G>A and the heterozygous genotype group.

Conclusion: Neonates with prolonged unconjugated hyperbilirubinemia may have a higher chance of *UGT1A1* mutation than expected. Analysis of *UGT1A1* mutations may be beneficial in infants with prolonged unexplained jaundice.

Key Words: *UGT1A1*; Mutation; Hyperbilirubinemia; Jaundice; Infant

Received: 23 December 2023

Revised: 8 February 2024

Accepted: 15 February 2024

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INTRODUCTION

Prolonged jaundice lasting longer than 21 days of life is not an uncommon phenomenon in infants in the aspect of neonatologists. It occurs in approximately 15% of all newborn infants^{1,2}. Although some patients may have hypothyroidism or liver dysfunction as an underlying cause requiring medical or surgical intervention, most other infants have benign unconjugated hyperbilirubinemia, and no specific results have been obtained after routine investigation for prolonged jaundice. The prognosis of unconjugated hyperbilirubinemia is usually good, and bilirubin levels improve within a few weeks if the patient receives the recommended standard of care³. It is necessary for physicians to pay medical attention to prevent prolonged hyperbilirubinemia and resulting bilirubin-induced neurological damage. Primary care providers should monitor the cause of these symptoms if jaundice persists beyond a typical time frame.

Uridine diphosphate glucuronosyltransferase 1A isoform 1 (*UGT1A1*) is a key enzyme that helps eliminate bilirubin from the body by adding a glucuronide moiety to substrates^{4,5}. Mutations in *UGT1A1*, which is located on chromosome 2q37, can result in hereditary unconjugated hyperbilirubinemia, specifically Crigler-Najjar syndrome (CN) and Gilbert's syndrome (GS), owing to varying degrees of enzymatic activity reduction⁶⁻¹¹. CN is a rare genetic disorder characterized by a complete (or nearly complete) absence of the ability to eliminate bilirubin from the body by glucuronidation. This disorder has two forms, depending on the degree of elevated bilirubin concentration and reduced *UGT1A1* enzyme activity: CN type 1 and type 2. Mutations in CN type 1 cause severe, life-threatening hyperbilirubinemia with an almost complete absence of *UGT1A1* enzymatic activity, whereas mutations in CN type 2 cause a moderate, less severe form of unconjugated hyperbilirubinemia with a reduced level of enzymatic activity¹²⁻¹⁴. In comparison, GS is a little more common inherited disorder presenting as a mild form of unconjugated hyperbilirubinemia with approximately 30% reduced *UGT1A1* enzymatic activity^{15,16}. The prevalence of GS is approximately 4% to 14% of the global population, with one in three affected patients unaware that they have it¹⁷⁻¹⁹.

To date, more than 100 genotypes of polymorphism of *UGT1A1* have been identified from different countries around the world. They are usually found in the promoter or coding exon regions^{11,15,20,21}. These mutations have been variably associated with GS and CN and appear to differ considerably among ethnic groups

^{22,23}. For example, the insertion of an extra TA into the normal sequence of the promoter TATA box, (TA)₇ TAA (c.-40_-39dupTA or c.-40_-39 insTA), is a common genotype related to GS in Caucasians^{8,22,24}, whereas p.Gly71Arg (c.211G>A), a missense mutation in the coding exon 1 region, is more prevalent in Asian populations^{10,15,25,26}.

Other populations have been extensively studied; however, research on *UGT1A1* mutations in Koreans remains limited and requires further investigation. Previous studies in Korea have focused mainly on adult patients or infants with small sample sizes and examined a few specific single-nucleotide variants²⁷⁻³¹. To the best of our knowledge, there are no national statistics on the prevalence of *UGT1A1* mutations in Korean infants with prolonged jaundice.

This study aimed to identify the types and prevalence of *UGT1A1* mutations in newborn infants with unexplained prolonged unconjugated hyperbilirubinemia and determine the clinical relationship among infants with different mutational variations of *UGT1A1*.

MATERIALS AND METHODS

1. Study participants

The study included 74 infants who visited the neonatal jaundice follow-up clinic of Samsung Changwon Hospital from July 2019 to May 2023. All enrolled infants underwent a thorough examination to identify the underlying cause of prolonged jaundice, including measurement of the peak levels of total and direct serum bilirubin, complete blood count, peripheral blood smear, reticulocyte count, Coombs test, liver function test, thyroid function test, and liver ultrasonography. Mutation analysis of *UGT1A1* was conducted in patients whose parents consented. In this study, unexplained prolonged jaundice was defined as jaundice that persisted beyond 21 days of life in infants who demonstrated no evidence of hemolytic anemia, liver dysfunction, hypothyroidism, or inborn errors of metabolism³². Peak levels of total and direct bilirubin were defined as the highest levels of total and direct bilirubin measured at 21 days of life or later. Patients with gestational age <35 weeks, birth asphyxia, dehydration, cephalohematoma, intracranial hemorrhage, sepsis, immune hemolytic anemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency, medications associated with jaundice, hypothyroidism, hepatobiliary disease, and inborn errors of

metabolism were excluded from the study.

2. Clinical and laboratory data collection

Clinical and laboratory information of the participants was retrospectively reviewed from hospital medical records. Recorded data included demographics (age, sex, date of birth, gestational age, and birth weight), delivery method, breast milk/formula, stool pattern/color, blood type, maternal/family history of jaundice, serum levels of total and direct bilirubin, peak levels of bilirubin, and the presence or absence of liver dysfunction and hypothyroidism. The findings of the mutation analysis of *UGT1A1* were also retrieved.

3. Genetic studies

For sequence analysis of *UGT1A1* gene mutation, the blood samples from the participants were withdrawn and requested from the Department of Laboratory Medicine and Genetics of Samsung Medical Center, Seoul, Korea. Written informed consent for mutation analysis was obtained from the patient's parents before collecting the specimens.

Genomic DNA was extracted from peripheral blood leukocytes using a Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. The promoter region

and five exons of *UGT1A1* were amplified by polymerase chain reaction (PCR). PCR was performed using a thermal cycler (Applied Biosystems). The PCR products were purified, and direct sequencing was performed using an ABI3730xl Genetic Analyzer (Applied Biosystems). The obtained sequences were compared to the reference sequences NG_002501.2 and NM_000463.2, registered in the National Center for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov>).

4. Data analysis

Statistical analyses were performed using SPSS Statistics for Windows version 23.0 (IBM Corp.). Numerical data were expressed as mean±standard deviation and analyzed using Student's *t*-test and Mann-Whitney test. Chi-square, Fisher's exact, and likelihood ratio tests were used to compare the categorical data, such as sex, delivery method, and breastfeeding. Differences were considered statistically significant at $P<0.05$.

RESULTS

1. Demographics

A total of 65 infants with prolonged unconjugated hyperbiliru-

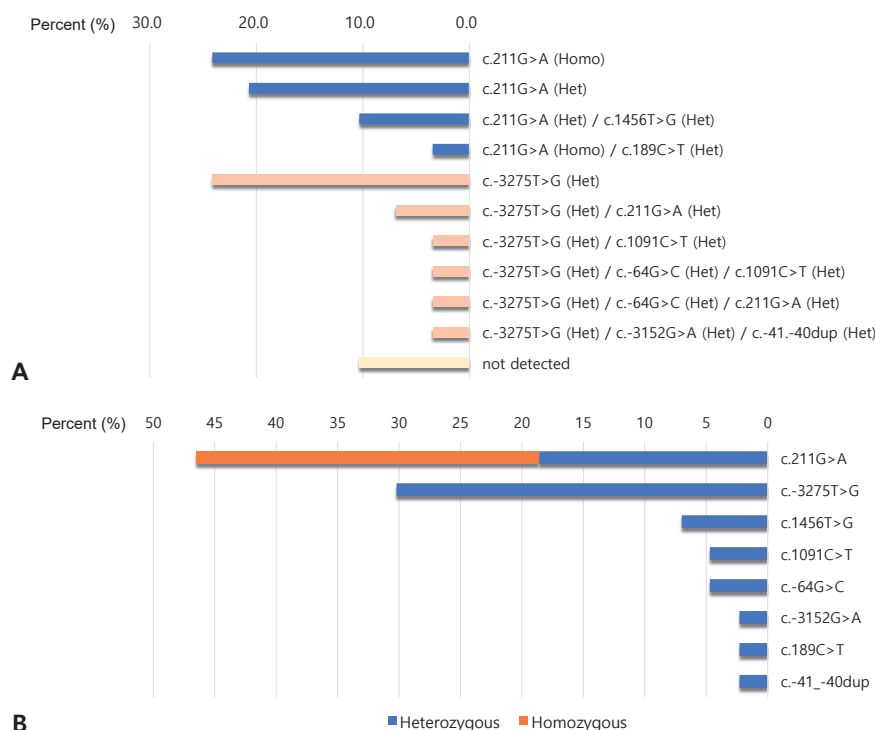


Figure 1. (A, B) Genotype frequencies and distribution of uridine diphosphate glucuronosyltransferase 1A isoform 1 (*UGT1A1*) polymorphisms in studied patients.

binemia were enrolled in this study after excluding nine infants for the following reasons: two infants had biliary atresia, one had hypothyroidism, one had cytomegalovirus hepatitis, and five lacked clinical information. None of the enrolled infants showed evidence of hemolytic anemia, liver dysfunction, hypothyroidism, or inborn metabolic errors. No abnormalities, including heman-gioma, biliary atresia, or hepatic parenchymal disease, were found on liver ultrasonography. Mutation analysis of *UGT1A1* was performed in 33 (50.8%) patients whose parents consented to participate.

2. *UGT1A1* genotypes

Among the participants who underwent mutation analysis, 30 (90.9%) harbored mutations in *UGT1A1*. Single-nucleotide variants were found in 20 (66.7%) infants, and the remaining 10 (33.3%) had multiple variants of *UGT1A1*. Eight different genetic variants were detected by the mutation analysis of *UGT1A1* (Figure 1A). The two most common variants were c. 211G>A (46.5 %) and c.-3275T>G (30.2%) (Figure 1B). The other variants had the following percentages: c.1456T>G (7.0%); c.-64G>C and c.1091C>T (4.7% each); and c.-3152G>A, c.189C>T, and c.-41_-40 dup (2.3% each). Among the 20 infants with the c.211G>A variant, eight (40.0%) had a homozygous genotype and 12 (60.0%) had a heterozygous genotype. Infants harboring other variants exhibited heterozygous genotypes. When comparing the group that underwent mutation analysis with the group that did not, there were no significant differences in demographics.

3. Effects of *UGT1A1* genotypes on clinical and laboratory parameters of patients

Clinical and laboratory data were assessed to identify differences among groups with varying *UGT1A1* mutations. When analyzing the patient data between the groups in which *UGT1A1* mutation was detected (n=30) and not detected (n=3), it was found that breastfeeding was the only significant factor ($P=0.027$). All infants with prolonged unconjugated hyperbilirubinemia without *UGT1A1* mutations were exclusively breastfed, whereas less than one-third of the infants with confirmed mutations were breastfed (Table 1). There were no significant differences in the total and peak bilirubin levels between the two groups.

No significant differences were observed between the group with a single-nucleotide variant and the group with multiple genetic variants, or between the homozygous genotype group with c.211G>A and the heterozygous genotype group. No signi-

ficant differences were observed between the groups of infants with the two most common variants, c.-3275T>G and c.211G>A (Table 2).

Table 1. Comparison of Demographic and Clinical Characteristics of Infants between the Groups Where the Mutation of *UGT1A1* Was Detected and Not Detected

Characteristic	Detected (n=30)	Non-detected (n=3)	P-value
Gestational age (wk)	37.1±2.3	36.6±3.4	0.973
Birth weight (kg)	2.99±0.57	3.09±1.27	0.813
Male sex	17	3	0.312
Vaginal delivery	4	2	0.100
Inborn	23	3	0.557
Singleton	27	3	0.999
Feeding			0.027
Breast milk	8	3	
Formula	9	0	
Mixed	13	0	
PNA at peak total bilirubin level (d)	31.2±9.2	26.0±7.8	0.222
Peak total bilirubin level (mg/dL)	11.5±3.3	11.3±1.1	0.813
Peak direct bilirubin level (mg/dL)	0.9±0.3	0.7±0.4	0.610

Values are expressed as mean±standard deviation.

Abbreviations: *UGT1A1*, uridine diphosphate glucuronosyltransferase 1A isoform 1; PNA, postnatal age.

Table 2. Comparison of Demographic and Clinical Characteristics of Infants with the Two Most Common Variants, c.-3275T>G and c.211G>A Mutation*

Characteristic	c.-3275T>G mutation (n=12)	c.211G>A mutation (n=9)	P-value
Gestational age (wk)	37.2±1.9	36.6±3.0	0.999
Birth weight (kg)	2.99±0.50	2.98±0.74	0.766
Male sex	8	5	0.642
Vaginal delivery	1	1	0.591
Inborn	7	10	0.374
Singleton	10	12	0.999
Feeding			0.635
Breast milk	3	4	
Formula	5	3	
Mixed	4	5	
PNA at peak total bilirubin level (d)	28.1±3.9	36.7±13.5	0.067
Peak total bilirubin level (mg/dL)	12.7±3.2	9.8±3.1	0.095
Peak direct bilirubin level (mg/dL)	1.0±0.3	0.7±0.3	0.131

Values are expressed as mean±standard deviation.

*Mixed/multiple variants of uridine diphosphate glucuronosyltransferase 1A isoform 1 (*UGT1A1*) gene mutation were excluded for statistical purposes.

Abbreviation: PNA, postnatal age.

DISCUSSION

This study demonstrates that infants with prolonged unconjugated hyperbilirubinemia are highly likely to have a *UGT1A1* mutation. This unexpected result deviates from our initial expectations. As we were unsure, at the beginning of this study, how many participants would be experiencing prolonged jaundice with genetic backgrounds such as GS or CN, the mutation analysis of *UGT1A1* gene was reluctantly recommended and performed only for those who requested it, seeking the possible underlying disease. As a result, just about half of the recruits received the *UGT1A1* gene mutation analysis. Although the number of participants who underwent mutation analysis was small and limited, this result suggests that a substantial number of newborns who experience prolonged jaundice lasting longer than 3 weeks of life may suffer from hereditary unconjugated hyperbilirubinemia. Therefore, *UGT1A1* mutation analysis may be a reliable option for determining the underlying cause of prolonged jaundice, especially if routine clinical and laboratory investigations, including liver and thyroid function tests, yield no specific results.

This study also demonstrated that c.211G>A in the coding region and c.-3275T>G in the promoter region were the two most common polymorphisms in infants with prolonged unconjugated hyperbilirubinemia. These results are consistent with reports from other Asian populations, including Japanese, Chinese, and Vietnamese^{2,32-34}. However, the prevalence of TA duplications in the TATA box promoter was low in the present study. Previous studies have reported that the frequency of polymorphisms in *UGT1A1* and their association with neonatal hyperbilirubinemia varies among populations by location, race, and ethnicity^{8,25,35,36}. For example, the frequency of the c.211G>A mutation is higher in Asian patients with neonatal hyperbilirubinemia than in European or African individuals. In contrast, mutations in the (TA)_n tandem repeat sequence within the TATA box promoter region are more prevalent in Caucasians and Africans than those in East Asians. Kim et al.³¹ reported that c.211G>A and c.-41_-40 dup TA were the most frequent variants in 81 Korean patients with unconjugated hyperbilirubinemia. The prevalence of TA duplication was higher than that in data from Japanese and Chinese populations, which was also different from the results of this study. Therefore, more participants are needed to clarify the popularity of polymorphisms of *UGT1A1* mutations in the future.

The exact etiology of breast milk jaundice has not yet been determined. Most of the proposed etiologies involve factors present in human breast milk. These factors, including pregnane-3 α , 20 β -diol, interleukin 1 β , β -glucuronidase, epidermal growth factor, and α -fetoprotein, are believed to enhance intestinal resorption of bilirubin and reduce intestinal motility in the neonatal period, leading to increased unconjugated bilirubin levels^{37,38}. Other hypotheses suggest the presence of potential genetic mutations in the affected neonates. Studies have reported different results regarding the prevalence of *UGT1A1* mutations in infants with prolonged jaundice. Maruo et al.³⁹ reported that 16 of 17 breastfed Japanese infants with prolonged jaundice had at least one *UGT1A1* mutation. Yang et al.⁴⁰ also reported that 58.9% of Chinese patients with prolonged jaundice harbored the c.211G>A variant of *UGT1A1*, which was significantly higher than that in the control group. Alaei et al.⁴¹ reported no significant association between prolonged Iranian neonatal jaundice and *UGT1A1* G71R polymorphism. Therefore, more studies with a larger number of participants are needed to reveal the prevalence of *UGT1A1* mutations in infants with prolonged jaundice.

In previous studies, the serum bilirubin levels in the homozygous polymorphism subgroup were significantly higher than those in the wild-type and heterozygous subgroups. This suggests that homozygous *UGT1A1* mutation carriers may have a remarkable decrease in the enzymatic activity of *UGT1A1*, resulting in a more elevated serum bilirubin level^{8,32,42}. Bosma et al.⁸ reported that individuals with homozygous (TA)₇ TA duplications had higher serum bilirubin levels than those with heterozygous or wild-type alleles. Nguyen et al.³² also noted that the serum bilirubin level in the homozygous c.211G>A polymorphism subgroup was significantly higher than those in the wild-type and heterozygous c.211G>A polymorphism subgroup. It is likely that the heterozygous polymorphism only partially impaired the enzymatic activity of *UGT1A1*, and its influence on the conjugation of indirect bilirubin was not noticeable. However, contrary to studies in other countries, we could not find differences in peak total and direct bilirubin levels between the homozygous and heterozygous c.211G>A polymorphism groups. Identical results were obtained when groups with different divisional criteria were compared. This may be partly because our data on peak bilirubin levels were collected from patients beyond 21 days of life and not during their entire lifetime after birth. There might have been a difference if the bilirubin levels were measured consecutively immediately after birth or once after the patient's

symptoms appeared. Again, the number of participants and time to measure the bilirubin levels should be upgraded to interpret their clinical significance correctly.

We did not differentiate between the GS and CN groups in this study. Usually, the distinction between CN type 1 or 2 and GS is based on the severity of serum bilirubin levels, *UGT1A1* enzymatic activity, and responsiveness to phenobarbital administration in neonatal jaundice⁴³. However, it is challenging to differentiate between them in clinical practice because many patients in this study had a similar range of serum bilirubin levels (intermediate between the two syndromes) measured after 3 weeks of life. All these forms of hereditary unconjugated hyperbilirubinemia are caused by mutations in *UGT1A1*. They are considered part of the spectrum of a single disorder with varying grades of severity and altered glucuronidation that can extend to fatal CN⁴⁴⁻⁴⁶. Nevertheless, all participants in this study were monitored and followed for the persistence of jaundice, and their symptoms gradually disappeared by approximately 8 to 10 weeks of age without any sequelae.

As the prognosis is generally good, GS does not require dietary therapy or specific management. However, mutation analysis of *UGT1A1* could not only confirm the diagnosis but also alleviate patients' parents' concerns and provide genetic counseling to prevent repeated examinations or hospitalization of patients. Although no specific therapy is required for patients with GS, they should be educated to avoid exacerbating conditions and increasing the risk of toxicity with specific medications. For example, irinotecan, an anticancer drug used to treat colon and small cell lung cancers, can increase the risk of toxicity in patients with GS, such as diarrhea and neutropenia^{47,48}. Several antiviral medications, including atazanavir, ribavirin, and pazopanib, can induce hyperbilirubinemia in patients with GS by inhibiting bilirubin-uridine diphosphate glucuronosyltransferase (UGT) activity⁴⁹⁻⁵¹. Additionally, GS is associated with an increased risk of cholelithiasis in adults and children^{52,53}.

This study had some limitations. First, the number of participants was small and needs to be increased to determine the statistical significance of the clinical relationships among groups with various *UGT1A1* genetic variations. Second, although no significant demographic differences were found between the groups that underwent mutation analysis and those that did not, there may still be selection bias in the recruitment process. Finally, as a single-center study that cannot guarantee reproducibility and generalizability, multicenter research with more cases

is warranted to better understand the prevalence of *UGT1A1* mutations and their clinical relationships.

In conclusion, this study showed that infants with prolonged unconjugated hyperbilirubinemia have a high likelihood of *UGT1A1* mutations if the possibility of specific liver or thyroid disorders is excluded. Therefore, *UGT1A1* mutation analysis may be beneficial and should be considered an evaluation tool for identifying the underlying cause of prolonged jaundice in infants. It will also be helpful to ease the patient's parents' anxiety and provide information as to why their child had jaundice for a long time.

ARTICLE INFORMATION

Ethical statement

This study was approved by the Institutional Review Board (IRB) of Samsung Changwon Hospital (IRB No. 2022-06-008). The requirement for signed informed consent from the parents was waived as the research involved no more than minimal risk to the subjects.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

Author contributions

Conception or design: Y.D.K.

Acquisition, analysis, or interpretation of data: Y.D.K.

Drafting the work or revising: Y.D.K.

Final approval of the manuscript: Y.D.K.

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Funding

None

Acknowledgments

The author thanks the support from Prof. Ja-Hyun Jang, Department of Laboratory Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, for providing information about *UGT1A1* gene mutation analysis.

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