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DO GENE POLYMORPHISMS PLAY A ROLE IN NEWBORN HYPERBILIRUBINEMIA?

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ABSTRACT

Objectives

Polymorphisms of the uridine-diphospho-glucuronosyltransferase 1A1 (*UGT1A1*) gene, hepatic solute carrier organic anion transporter 1B1/B3 (*SLCO1B1/3*) gene, and glutathione S-transferase (*GST*) gene have been associated with significant hyperbilirubinemia in some populations. This study aims to determine whether the variation of *UGT1A1*, *SLCO1B1/3* and *GST* genes play an important role in neonatal hyperbilirubinemia in Turkish newborn infants.

Methods

The study included 61 idiopathic hyperbilirubinemia cases, 28 prolonged jaundice cases, and 41 controls. Ten common polymorphisms in four genes involved in bilirubin metabolism were examined. Polymerase chain reaction-restriction fragment length polymorphism method was used to detect variants of those genes.

Results

No association was found between the variants of *UGT1A1* at nt 211, the *SLCO1B1* gene at nt 388, 463, 521, 1463, the *SLCO1B3* gene at nt 334, 727+118, 1865+19721, and the *GST* gene at nt 313, 341, and neonatal hyperbilirubinemia. There was no difference between the case and control groups in terms of allele frequencies of these genes (except *SLCO1B3* at nt 334) ($p>0.05$ in all comparisons). The presence of the G allele of the *SLCO1B3* at nt 334 variant gene seemed to protect from jaundice in infants with idiopathic hyperbilirubinemia.

Conclusion

These gene polymorphisms currently studied do not seem to modulate the risk of hyperbilirubinemia in Turkish newborn infants.

Key words: Neonatal hyperbilirubinemia, gene polymorphisms, *UGT1A1*, *SLCO1B1/B3*, *GST*

INTRODUCTION

Hyperbilirubinemia is a common finding in the neonatal period and can sometimes lead to serious consequences such as kernicterus. Most cases of neonatal hyperbilirubinemia (NH) are physiological, and approximately 13.4% of the cases are non-physiological [1, 2]. In approximately half of cases of pathological jaundice, there is no identifiable factor [3]. Studies have shown that African newborns have lower serum bilirubin levels and Asian babies develop higher values than their Caucasian counterparts [4]. In a study, the incidence of non-physiological significant hyperbilirubinemia in Turkish newborns was found to be between approximately 10.5% and 25.3% [5]. This may be due to differences in the genetic backgrounds of the populations, suggesting the existence of genetic risk factors for the development of NH [6].

Unconjugated bilirubin is rapidly and selectively taken up across the basolateral membrane of the hepatocyte as a carrier-mediated process involving the partially dissolved carrier organic anion-carrying polypeptide-1B1/B3 (*SLCO1B1* and *SLCO1B3* genes). *SLCO1B1* and *SLCO1B3*

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are sinusoidal transporters that facilitate hepatic uptake of a wide variety of endogenous substrates [1, 2]. In hepatocytes, glutathione S-transferases (*GSTs*) are involved in binding to nonsubstrate ligands, such as unconjugated bilirubin [3]. Unconjugated bilirubin is then conjugated with glucuronate through the enzyme activity of uridine diphosphate glucuronyl transferase (*UGT1A1* gene) [1]. In particular, polymorphisms of four genes specifically related to bilirubin production and metabolism (*UGT1A1*, *SLCO1B1*, *SLCO1B3*, and / or *GST*) may interact with environmental contributors to produce significant hyperbilirubinemia [3, 7].

The aim of this study is to investigate whether polymorphisms in the *UGT1A1*, *SLCO1B1*, *SLCO1B3* and *GST* genes are a contributing factor to idiopathic hyperbilirubinemia or prolonged jaundice with unexplained etiology in Turkish newborns.

MATERIALS AND METHODS

Patients

This study was conducted at Dr. Sami Ulus Maternity and Children's Research and Training Hospital between April 2011 and May 2012. Of the 130 newborn babies included in the study, 89 jaundiced babies consisted of the study group (61 babies with idiopathic hyperbilirubinemia, 28 babies with prolonged jaundice of unexplained etiology), and 41 healthy babies without jaundice consisted of the control group. NH refers to newborn babies with serum total bilirubin levels above 17 mg/dl in the first 7 days of life. Prolonged jaundice is a condition in which serum total serum bilirubin (TSB) is above 10 mg/dl, which persists after the 14th day of life in newborns. The control group consisted of healthy newborns with peak serum TSB level ≤12.9 mg/dl in the first week of life. All newborns were born at 38-42 weeks of gestation and weighed over 2500 grams. Neonates with known risk factors such as major congenital malformations, sepsis, perinatal asphyxia, maternal diabetes, polycythemia, glucose-6-phosphate dehydrogenase (G6PD) activity deficiency, cephalic hematoma, dehydration, hypothyroidism, liver disease, Rh/subgroup and/or direct Coombs (DC) positive ABO incompatibility, or hemolysis for any reason were excluded from the study. Newborns in the control group were followed up for the development of jaundice, and in addition to determining the STB concentration, complete blood count, peripheral blood smear, blood group typing, DC and thyroid function tests were examined. In hyperbilirubinemia and prolonged jaundice groups, in addition to the above parameters, serum direct and indirect bilirubin levels, reticulocyte count, G6PD, liver function tests, thyroid function tests, urine culture and, if necessary, C-reactive protein (CRP) were examined. The study was approved by the Hacettepe University Faculty of Medicine Ethics Committee with Decision number 08/II dated 02 May 2011.

Genotyping procedure

Blood samples were taken from all cases and then placed in EDTA vacuum containers. Genomic DNA was isolated from peripheral leukocytes using the standard phenol-chloroform procedure. DNA was isolated using the QIAamp DNA blood kit (Qiagen, Hilden, Germany).

- **Variants of the genes *UGT1A1* (nucleotides 211), *SLCO1B1* (nucleotides 388, 463, 521, 1463) and *SLCO1B3* (nucleotides 334, 727+118, 1865+19721)**

  The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied to detect variants of *UGT1A1* at nucleotide 211, *SLCO1B1* at nucleotides 388, 463, 521, 1463 and *SLCO1B3* at nucleotides 334, 727+118, 1865+19721. PCR mixture (25 µL) consisted of 200 ng DNA, 0.2 mM of each dNTP, 120 nM of primer, 2.5 µL of 10 x buffer, and 1 µL of 50 mM MgCl2 solution. The final concentration of the PCR mixture was 1.5 mM MgCl2 in a volume of 100 µl of working solution. PCR amplification was performed in a DNA thermal cycler for 35 cycles of initial denaturation for 5 min, denaturation for 1 min at 94 °C, annealing for 1 min at 55 °C, primer extension for 1.5 min at 72 °C, and final extension for 7 min at 72 °C. The PCR product was digested with appropriate restriction enzymes and analyzed on 3% agarose gel (variants of the *UGT1A1*53 gene were examined for 53 subjects with idiopathic hyperbilirubinemia, 27 subjects with prolonged hyperbilirubinemia and 37 healthy subjects. Variants of the *SLCO1B1* / *SLCO1B3* genes were investigated in 61 subjects with idiopathic hyperbilirubinemia, 28 subjects with prolonged hyperbilirubinemia and 41 healthy subjects).

- **Variant of *GST* (*GSTP1*) gene**

  PCR-RFLP method was applied to detect the *GST* variant (*GSTP1*). PCR mixture (25 µL) consisted of 200 ng DNA, 0.5 µL of 10 mM dNTP, 0.6 µL of 10 mM *GSTP* primer, 0.12 µL of 10 mM albumin, 2.5 µL of 10 x buffer, 1.25 µL of 50 mM MgCl2 solution. The final concentration of the PCR mixture was 1.5 mM MgCl2 in a volume of 100 µl of working solution. PCR amplification was performed in a DNA thermal cycler for 35 cycles of initial denaturation for 5 minutes, denaturation for 1 minute at 94°C, annealing for 1 minute at 64°C, primer extension for 1 minute at 72°C and final extension for 7 minutes at 72°C. The PCR product was digested with appropriate restriction enzymes and analyzed on a 3% agarose gel as previously described (variants of

Table 1. Demographic characteristics of the study population

	Idiopathic Hyperbilirubinemia (n:61)	Prolonged jaundice (n:28)	Control (n:41)	*P*
Gestational age (weeks, mean± SD)	38.1 ± 1.0	38.5 ± 1.0	38.8 ±1.0	>0.05
Birth weight (g, mean ± SD)	3145 ±397	3154 ±390	3322 ±416	>0.05
Gender (Male/Female)	30/31	13/15	20/21	>0.05
Peak STB* level (mg/dl)	19.3 ± 1.9	-	7.1 ±2.4	0.0001
Peak time of STB (days)	5.2 ±1.9	-	5.1 ± 1.5	>0.05
Feeding pattern (breast milk/breast milk+formula)	56/5	26/2	37/4	>0.05

*, Serum total bilirubin

the *GST* gene were examined for 55 subjects with idiopathic hyperbilirubinemia, 28 subjects with prolonged hyperbilirubinemia and 40 healthy subjects.

Statistical analysis

All means are presented as means ± standard errors of the means. The weights and gestational ages of the groups were compared with one-way ANOVA. The Chi-square test was used to determine whether there were differences in gender distribution and genotype distribution between groups. Whether the quantitative data conformed to normal distribution was tested with the Shapiro-Wilk test or the Kolmogorov Smirnov test. Since the STB values of the patients showed a normal distribution, the data are given as mean ± standard deviation values. Differences were considered statistically significant when the *P* value was less than 0.05.

RESULTS

Demographic characteristics of the three groups are shown in Table 1. There was no difference between the groups in terms of mean gestational age and birth weight. There was no difference between the groups in terms of feeding patterns, gender and mode of delivery. There is a significant difference between the groups in terms of bilirubin levels.

The genotype distributions on the basis of nucleotides in these three groups are shown in Table 2. There was no significant difference in genotype distribution between the case and control groups (*P*>0.05 in all comparisons). There were no statistically significant differences in the prevalence of the variant of *UGT1A1* gene at nt 211, variants of *SLCO1B1* gene at nt 388, 463, 521 and 1463 variants of

Table 2. Frequency of allelic and genotypic polymorphisms in *UGT1A1*, *SLCO1B1*, *SLCO1B3* and *GSTP1* genes in case and control groups

	Genotype frequency n (%)				Allele frequency n (%)		
UGT1A1 rs4148323 G71R c.211A>G	**A/A**	**A/G**	**G/G**	**Total**	**A**	**G**	**Total**
IH	49 (92.4)	2(3.8)	2(3.8)	53 (100)	100 (94)	6(6)	106(100)
Prolonged jaundice	21 (77.8)	6 (22.2)	0	27(100)	48 (89)	6(11)	54(100)
Control	35 (92.1)	3 (7.9)	0	38(100)	73 (96)	3(4)	76(100)
P				0.14			0.26
SLCOIBI rs2306283 c.388A>G	**A/A**	**A/G**	**G/G**	**Total**	**A**	**G**	**Total**
IH	17(27.8)	35 (57.3)	9(14.9)	61(100)	69(57)	53 (43)	122(100)
Prolonged jaundice	7(25)	13 (46.4)	8 (28.6)	28(100)	27(48)	29 (52)	56(100)
Control	19 (46.3)	16 (39)	6(14.7)	41(100)	54(66)	28 (34)	82(100)
P				0.09			0.11
rsl1045819 c.463C>A	**C/C**	**C/A**	**A/A**	**Total**	**C**	**A**	**Total**
IH	47 (77)	13 (21.3)	1 (1.7)	61(100)	107 (88)	15 (12)	122(100)
Prolonged jaundice	20 (71.4)	7(25)	1 (3.6)	28(100)	47 (84)	9(16)	56(100)
Control	32 (82)	7(18)	0	39(100)	71(91)	7(9)	78 (100)
P				0.59			0.46

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rsl1045819 c.521T>C	T n	**T/C**	**C/C**	**Total**	**T**	**C**	**Total**
IH	38 (62.3)	21 (34.4)	2 (3.3)	61(100)	97(80)	25 (20)	122(100)
Prolonged jaundice	21 (75)	6(21.4)	1 (3.6)	28(100)	48 (86)	8 (14)	56(100)
Control	31(75.6)	9(21.9)	1 (2.5)	**41**(100)	71(87)	11(13)	82(100)
P				0.27			0.35
rs59502379 c. 1463G>C	**G/G**	**G/C**	**C/C**	**Total**	**G**	**C**	**Total**
IH	61 (100)	0	0	61(100)	122(100)	0	122(100)
Prolonged jaundice	28(100)	0	0	28(100)	56(100)	0	56(100)
Control *P*	40 (100)	0	0	40 (100)	80 (100)	0	80(100)
SLC01B3 rs4149117 c.334T>G	**T/T**	**T/G**	**G/G**	**Total**	**T**	**G**	**Total**
IH	2(3.4)	13 (22.4)	43 (74.2)	58(100)	17(15)	99 (85)	116(100)
Prolonged jaundice	0	6(21.4)	22 (78.6)	28(100)	6(11)	50 (89)	56(100)
Control	0	3 (7.5)	37(92.5)	40(100)	3(4)	77 (96)	80(100)
P				0.20			**0.03**
rsl7680137 c.727+118C>G	c/c	**C/G**	**G/G**	**Total**	**C**	**G**	**Total**
IH	46 (75.4)	11(18)	4 (6.6)	61(100)	103 (94)	19(16)	122(100)
Prolonged jaundice	19(67.8)	7(25)	2 (7.2)	28 (100)	45(80)	11(20)	56(100)
Control	33 (80.5)	7(17)	1 (2.5)	41 (100)	73 (89)	9(11)	82(100)
P				0.49			0.36
rs2117032 c. 1865+19721C>T	**C/C**	**C/T**	**T/T**	**Total**	**C**	**T**	**Total**
IH	17 (27.8)	29 (47.5)	15 (24.7)	61(100)	63 (52)	59 (48)	122(100)
Prolonged jaundice	6 (21.4)	13 (46.4)	9 (32.2)	28 (100)	25 (45)	31(55)	56(100)
Control	4 (9.7)	21(51.2)	16(39.1)	41 (100)	29 (35)	53 (65)	82(100)
P				0.07			0.07
GSTP1							
rsl695 c.313A>G	**A/A**	**A/G**	**G/G**	**Total**	**A**	**G**	**Total**
IH	28 (50.9)	18 (32.7)	9(16.4)	55 (100)	74(67)	36 (33)	110(100)
Prolonged jaundice	12(42.8)	13 (46.4)	3 (10.8)	28(100)	37(66)	19 (34)	56(100)
Control	18(45)	19(47.5)	3 (7.5)	40 (100)	55(69)	25 (31)	80 (100)
c				0.74			0.94
rsl138272 c.341C>T	**C/C**	**C/T**	**T/T**	**Total**	**C**	**T**	**Total**
IH	48 (87.3)	7(12.7)	0	55 (100)	103 (94)	7(6)	110(100)
Prolonged jaundice	21 (75)	7(25)	0	28(100)	49(88)	7(12)	56(100)
Control	29 (72.5)	11(27.5)	0	40 (100)	69(86)	11(14)	80(100)
P				0.15			0.19

IH, Idiopathic hyperbilirubinemia

SLCO1B3 gene at nt 334, 727+118, 1865+19721 variants of *GSTP1* gene at nt 313 and nt 341, and allele frequency of these genes (except *SLCO1B3* at nt 334) among the three groups ($P>0.05$ in all comparisons, Table 2). The G allele frequency of the variant of *SLCO1B3* gene at nt 334 of the infants in the idiopathic hyperbilirubinemia group was 85%, which was significantly lower than that in the control group (96%, $P=0.03$; Table 2). There was no statistically significant difference in the frequency of the G allele of the *SLCO1B3* variant at nt 334 between infants in the prolonged jaundice and control groups ($P=0.16$). No variant of the *SLCO1B1* gene was found at nt 1463 (rs59502379).

Clinical features among genotypes based on nucleotides

There was no significant difference in the mean peak bilirubin levels and the onset time of hyperbilirubinemia

Table 3. Peak bilirubin levels and peak time of hyperbilirubinemia in the idiopathic hyperbilirubinemia group according to genotypic distributions

	Genotype distribution			*P*
UGT1A1 rs4148323 G71R C.211A>G	**G/G**	***AJG***	**G/G**	
Mean peak bilirubin levels (mg/dL)	19.4 ±2.1	20 ±2.8	19.7 ±1.3	0.87
Peak time of hyperbilirubinemia (day)	5.1 ± 1.9	6 ± 1.4	8.5 ±3.5	0.06
SLCOIBI rs2306283 c.388A>G	**A/A**	***AJG***	**G/G**	
Mean peak bilirubin levels (mg/dL)	18.8 ± 1.2	19.5 ±2.3	19.2 ±1.5	0.42
Peak time of hyperbilirubinemia (day)	5.0 ±2.0	5.2 ±2.2	5.4 ± **1.1**	0.89
rsl 1045819 c.463C>A	**C/C**	**C/A**	**A/A**	
Mean peak bilirubin levels (mg/dL)	19.2 ± 1.6	19.9 ± 2.9	17 ±0	0.28
Peak time of hyperbilirubinemia (day)	5.1 ± 1.9	5.6 ± **2.4**	5.0 ±0.0	0.74
rsl 1045819 c.521T>C	**T/T**	**T/C**	**C/C**	
Mean peak bilirubin levels (mg/dL)	19.3 ± 2.2	19.3 ± 1.5	18.1 ±0.9	0.67
Peak time of hyperbilirubinemia (day)	5.5 ±2.1	4.7 ± 1.8	4.5 ±2**.1**	0.27
rs59502379 c.I463G>C	**G/G**	**G/C**	**C/C**	
Mean peak bilirubin levels (mg/dL)	19.4 ± 1.5	-	-	-
Peak time of hyperbilirubinemia (day)	5.2 ± 1.8	-	-	-
SLC01B3 rs4149117 c.334T>G	**T/T**	**G/T**	**G/G**	
Mean peak bilirubin levels (mg/dL)	20.3 ±2.1	18.8 ± 1**.1**	19.4 ±2.2	0.49
Peak time of hyperbilirubinemia (day)	6.0 ± 1.4	4.8 ±2.1	5.2 ±2.0	0.69
rsl7680137 e.727+118C>G	**C/C**	**C/G**	**G/G**	
Mean peak bilirubin levels (mg/dL)	19.3 ± 2.0	18.9 ± 1.4	19 ±2.4	0.77
Peak time of hyperbilirubinemia (day)	5.3 ± 2.2	4.9 ± 0.9	5.2 ±2.6	0.84
rs2117032 c.I865f 19721C>T	**C/C**	**C/T**	**T/T**	
Mean peak bilirubin levels (mg/dL)	19.4 ± 2.3	19.8 ± 2.0	18.7 ± 1.1	0.19
Peak time of hyperbilirubinemia (day)	5.0 ± 1.8	5.5 ± 2.3	4.8 ± 1.5	0.47
GSTP1 rsl695 c.313A>G	**A/A**	***AJG***	**G/G**	
Mean peak bilirubin levels (mg/dL)	19.8 ±2.1	19.2 ± 1.8	18.5 ± 1.6	0.25
Peak time of hyperbilirubinemia (day)	5.5 ± 1.9	5.3 ± 2.4	4.4 ± 1.3	0.40
rsl 138272 c.341C>T	**C/C**	**C/T**	**T/T**	
Mean peak bilirubin levels (mg/dL)	19.4 ± 2.0	18.9 ± 1.4	-	0.50
Peak time of hyperbilirubinemia (day)	5.2 ± 1.9	5.2 ± 2.6	-	0.98

among the newborn infants with the different genotypes based on the *UGT1A1* gene at nt 211, variants of *SLCO1B1* gene at nt 388, 463, 521 and 1463, variants of *SLCO1B3* gene at nt 334, 727+118 and 1865+19721, variant *GSTP1* gene at nt 313 and 341 in the idiopathic hyperbilirubinemia group (Table 3). Since all newborn babies with hyperbilirubinemia were given phototherapy, no conclusion could be reached about the duration of hyperbilirubinemia in this study (Table 3).

DISCUSSION

Neonatal jaundice is a good example of a complex condition in which the usual clinical factors examined alone cannot reveal the real cause. Although a few studies have been conducted to define the mechanisms involved in NH, more attention has recently begun to be paid to genetic factors as a contributing cause [8]. This study aimed to elucidate the contribution of multiple genetic modifiers affecting bilirubin metabolism to the development of pathological hyperbilirubinemia in Turkish newborns.

UGT is the key enzyme of bilirubin conjugation. 211G > A (Gly71Arg), one of the most common *UGT1A1* gene polymorphisms in the coding region, may cause unconjugated hyperbilirubinemia by reducing enzymatic activity [9]. Recently, in a meta-analysis of 21 studies including 4738 newborns, it was reported that *UGT1A1* Gly71Arg variation may increase the risk of NH in Asian and African children [10]. There are two studies investigating the relationship between hyperbilirubinemia and the G71R variant in Turkish newborns. Kilic et al. [11] showed the frequency of heterozygous G71R variants in pathological jaundice, prolonged jaundice and control groups as 8.7%, 20.8% and 4.3%, respectively; however, these differences were not statistically significant. Narter et al. [12] reported a frequency of 33.3% and 27.1% for heterozygous G71R variants and 7.7% and 5.7% for homozygous G71R variants in the hyperbilirubinemia and control groups, respectively, and no statistically significant difference was found between these frequencies. Our study showed that the frequency of heterozygous G71R variants in idiopathic hyperbilirubinemia, prolonged jaundice, and control groups was 3.8%, 22.2%, and 7.9%, respectively. But these differences were not statistically significant. The frequency of homozygous G71R variant in idiopathic hyperbilirubinemia was 3.8%. However, no homozygous G71R variant was found in the prolonged jaundice and control groups.

SLCO1B1/B3, the gene encoding the hepatic solute transporter organic anion transporter 1B1, a putative bilirubin transporter, may also be associated with increased susceptibility to NH by limiting bilirubin uptake [6, 13]. In our study, *SLCO1B1* A388G in both the heterozygous and homozygous variants was found 72.2%, 75% and 53.7% in the idiopathic hyperbilirubinemia, prolonged jaundice and control groups, respectively. Other studies of *SCLO1B1* in different countries showed similar findings to our study in terms of the incidence of polymorphism. In studies conducted on newborns with hyperbilirubinemia, the *SLCO1B1* A388G variant was found at a rate of 77.7% in India and 87.7% in Malaysia [14, 15]. All these studies show that more than half of the studied population has the A388G variant. Our study showed that the *SLCO1B1* A388G variant was not a significant risk factor for idiopathic and prolonged hyperbilirubinemia. There is tentative evidence from different studies in different populations regarding the association between *SLCO1B1* A388G and high bilirubin levels in newborns. In a study conducted on Taiwanese newborns in 2004 by Huang et al. [16], it was reported that A388G was seen more frequently in babies with hyperbilirubinemia. Liu et al. [17] found that the A388G variant in Chinese newborns was associated with hyperbilirubinemia in the Guangdong population, but not in the Yunnan population. In our country, Büyükkale et al. [18] reported that polymorphic forms of 388 nucleotides of the *SLCO1B1* gene were risk factors for idiopathic hyperbilirubinemia. Other studies conducted in Malaysian, Brazilian, American or Thai populations also failed to prove the association of the A388G variant with NH [15, 19-21].

In our study, there was no statistically significant difference in NH risk between *SLCO1B1* T521C allele carriers (T/C+C/C) and T/T allele carriers; the same result was observed in the *SLCO1B1* T521C variant when the T allele was compared with the C allele. A meta-analysis including five case-control studies (637 subjects with hyperbilirubinemia and 918 control subjects) from three countries examined the association between the *SLCO1B1* T521C variant and NH. This meta-analysis reported that the *SLCO1B1* T521C variant conferred protection for NH in the Chinese population but not in the Malaysian, Taiwanese, Brazilian, or American populations; when the T allele was compared with the C allele, the same situation was observed in the *SLCO1B1* T521C variant [17].

Analysis of the SLCO1B1 C463A variant in our study showed a crossover variant in 14 of 61 (23%) neonates with idiopathic hyperbilirubinemia (13 heterozygous and one homozygous) and in 8 of 28 (28.6%) neonates with prolonged jaundice (7 heterozygous and one homozygous), and 7 of 39 (24.75%) control subjects (all heterozygous). The *SLCO1B1* C463A variant was not a significant risk factor for idiopathic and prolonged hyperbilirubinemia in the present study. A meta-analysis of three case-control studies involving 286 cases of hyperbilirubinemia and 456 controls from three countries examined the association between the *SLCO1B1* C463A variant and NH [17]. In studies conducted on Taiwanese and Thai newborns, carriage of the C to A substitution at nucleotide 463 was not detected [16, 21]; however, the study conducted by Watchko et al. [20] including American subjects showed that in those 31 of 153 newborns (20.26%) with hyperbilirubinemia (one homozygous and 30 heterozygous) and 74 of 299 control subjects (24.75%) (nine homozygous and 65 heterozygous). In that study (20), no statistically significant difference was found in the risk of NH between allele carriers (C/A+A/A) and (C/C) in the *SLCO1B1* C463A variant; the same situation was observed when the A allele in the SLCO1B1 C463A variant was compared with the C allele; the same situation was observed when the A allele was compared with the C allele in the *SLCO1B1* C463A variant.

There was no mutant allele in *SLCO1B1* at Nt 1463 G>C. However, according to our research, no study investigating this variant has been found in the literature.

Similar to *SLCO1B1* polymorphisms in this study, SLCO1B3 polymorphisms did not show statistical differences in genotype distribution. It was found in the present study that only the G allele at nt 344 of *SLCO1B3* may be a protective factor for idiopathic NH. However, it does not

protect against prolonged jaundice. No study investigating the relationship between *SLCO1B3* nt 334 variant and NH has been found in the literature. However, there are very few studies on the C727+118G and C1865+19721T polymorphisms of this gene. Alencastro de Azevedo et al. [19] reported that the allelic and genotypic frequencies of *SLCO1B3* gene C727+118G and C1865+19721T variants did not differ between idiopathic hyperbilirubinemia and control groups. They stated that only the T allele at nt 1865+19721 could be protective against NH in those without ABO incompatibility.

During the bilirubin conjugation process in hepatocytes, bilirubin binds to GST, also known as ligandin [22]. GST binds both bilirubin and bilirubin conjugates and reduces hepatocyte reflux into plasma [23]. The present study found that there is no statistically significant difference in the genotype and allele frequencies of *GSTP1* A313G and C341T among the idiopathic hyperbilirubinemia, prolonged jaundice, and control groups. Muslu et al. [24], from Türkiye, reported that the frequencies of *GSTM1* and *GSTT1* were similar in newborns with hyperbilirubinemia and control groups.

In this study, no significant difference was found in terms of peak total bilirubin levels and onset time of hyperbilirubinemia in newborns in the idiopathic hyperbilirubinemia group with different genotypes.

The limitation of the present study is that the need for phototherapy was taken as a criterion, which does not have a strong discriminatory feature in determining genetic variability differences. Another limitation of our study is the small number of cases.

CONCLUSION

The role of *UGT1A1*, *SLCO* and *GST* polymorphisms in neonatal jaundice is still controversial and deserves further attention. Although they do not have the power to modulate neonatal jaundice, other genes that play a role in bilirubin metabolism (heme oxygenase and biliverdin reductase) other than the genes mentioned above can be investigated.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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