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Bilirubin Uridine Diphosphate-Glucuronosyltransferase Variation Is a Genetic Basis of Breast Milk Jaundice

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Abstract

Objective—To evaluate the role of bilirubin *UDP-glucuronosyltransferase family 1, polypeptide A1* (*UGT1A1*) gene variations on prolonged unconjugated hyperbilirubinemia associated with breast milk feeding (breast milk jaundice [BMJ]).

Study design—*UGT1A1* gene allelic variation was analyzed in 170 Japanese infants with BMJ with polymerase chain reaction-direct sequencing, and their genotypes compared with serum bilirubin concentrations. In 62 of 170 infants, serum bilirubin concentration was followed after 4 months of life. Genotypes were examined in 55 infants without BMJ.

Results—Of 170 infants with BMJ, 88 (51.8%) were homozygous *UGT1A1*6*. Serum bilirubin concentrations (21.8 ± 3.65 mg/dL) were significantly greater than in infants with other genotypes ($P < .0001$). The Gilbert UGT1A1*28 allele was not detected in infants with BMJ, except in an infant who was compound heterozygous with *UGT1A1*6*. At 4 months of age, serum bilirubin concentration improved to >1 mg/dL, except in 2 infants who were homozygous *UGT1A1*7*. Homozygous *UGT1A1*6* was not detected in the control group.

Conclusion—One-half of the infants with BMJ were homozygous *UGT1A1*6* and exhibited a serum bilirubin concentration significantly greater than other genotypes. This finding indicates that *UGT1A1*6* is a major cause of BMJ in infants in East Asia. Previous finding have demonstrated that 5β-pregnane-3α,20β-diol present in breast milk inhibits p.G71R-UGT1A1

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bilirubin glucuronidation activity. Thus, prolonged unconjugated hyperbilirubinemia may develop in infants with *UGT1A1*6* who are fed breast milk.

Prolonged unconjugated hyperbilirubinemia associated with breast milk feeding (breast milk jaundice [BMJ]) is a phenomenon in infants fed with mother's breast milk.[1] This phenomenon is observed from the late neonatal period to 4 months of age. Hyperbilirubinemia decreases when breast milk is replaced with infant formula, and even if breast milk feeding continues, prolonged unconjugated hyperbilirubinemia improves over time. BMJ causes anxiety in infants, their parents, and pediatricians. In addition, kernicterus (bilirubin encephalopathy) is an occasional risk in severe unconjugated hyperbilirubinemia induced by BMJ.[2] Arias et al[1] andNewman and Gross[3] reported in 1963 that breast milk was a factor in the development of neonatal jaundice. Many substances in breast milk were subsequently suspected to cause BMJ, including pregnane-3α,20β-diol, nonesterified fatty acid, and β-glucuronidase.[4–6] However, a reliable causative agent of BMJ has not yet been conclusively elucidated.[7–9]

In a previous study of BMJ, we showed an association between BMJ and variants of the bilirubin UDP-glucuronosyltransferase (*UDP-glucuronosyltransferase family 1, polypeptide A1* [*UGT1A1*]) gene.[10] Our preliminary study on 17 infants with BMJ showed the c. 211G>A (p.G71R) in the coding region of *UGT1A1* (*UGT1A1*6*) might cause BMJ. From 17 infants with BMJ, 8 with BMJ were homozygous for *UGT1A1*6*, 7 were heterozygous *UGT1A1*6*, and 2 infants did not express the *UGT1A1*6* allele.

UGT1A1 (EC 2.4.1.17) belongs to the UDP-glucuronosyltransferase type 1 (UGT1) family and plays a role in phase II drug metabolism.[11] UGT1A1 catalyzes glucuronidation of many endobiotics and xenobiotics, converting hydrophobic substances to hydrophilic substances as a detoxification.[12] Bilirubin is selectively catalyzed by UGT1A1.[13] Defects in the *UGT1A1* gene generate hereditary unconjugated hyperbilirubinemia, specifically Crigler-Najjar syndrome type I (MIM #21880), type II (MIM #606785), and Gilbert syndrome (MIM #143500).[13–17] Crigler-Najjar syndrome type I and type II are severe and moderate phenotypes of hereditary unconjugated hyperbilirubinemia, respectively, in which the hyperbilirubinemia is life long.[18,19] The clinical diagnosis of Gilbert syndrome occurs in approximately 3%–8.6% of the population.[20–22] Two frequent polymorphisms associated with Gilbert syndrome are a missense mutation in exon 1 c.211G>A generating a p.G71R change (*UGT1A1*6*) and a c.-3279T>G in the promoter region that is linked to the A(TA)7TAA in the TATA box (*UGT1A1*28*).[23]

Among white, black, and west Asian subjects, homozygous *UGT1A1*28* is associated with the clinical diagnosis of Gilbert syndrome.[16,17] However, in east Asian patients (Japanese, Koreans, and Chinese), *UGT1A1*6* is an important cause of adult hyperbilirubinemia, which is described clinically as Gilbert syndrome.[24] In this study, we demonstrate that during neonatal development, the role of the UGT1A1*6 allele predominates over the other polymorphisms in its contribution towards the onset of breast milk induced neonatal hyperbilirubinemia.[25] Thus, the *UGT1A1*6* allele is predicted to be a risk factor for breast milk–induced jaundice during neonatal development.

Methods

We studied 170 Japanese infants (95 male and 75 female) with prolonged unconjugated hyperbilirubinemia associated with breast milk feeding. Infants had a gestational age greater than 35 weeks (range, 35 weeks, 1 day to 41 weeks, 3 days; mean, 38 weeks, 5 days ± 10.6 days). Birth weights were greater than 2300 g (2305–3902 g; mean, 3003 ± 385 g). All infants showed apparent prolonged unconjugated hyperbilirubinemia beyond 3 weeks of life. Total and indirect bilirubin concentrations at diagnosis ranged from 7.3 mg/dL to 32.5 mg/dL (124.8–555.7 μmol/L), and more than 95% of bilirubin was indirect. Except for jaundice, the infants were healthy and did not show signs of hemolytic anemia, liver dysfunction, or hypothyroidism. Serum bilirubin concentrations were within the normal range (<1mg/dL) or jaundice disappeared visually for all infants at 4 months of age even if breast milk feeding continued, except for infants with a particular genotype [homozygous p.Y486D (*UGT1A1*7*)].[26] We followed serum bilirubin concentrations after 4 month of age in 62 cases and checked the association between genotypes and final serum bilirubin concentrations. The project was approved by the ethics committee of Shiga University of Medical Science.

The control group comprised 55 term infants (21 male and 34 female). All infants were fed breast milk. At an obligatory neonatal health check at 1 month of age, they showed no visible evidence of prolonged jaundice. Mean gestational age was 39 weeks 4 days ± 7 days (range, 37 weeks, 4 days to 41 weeks), and birth weights were greater than 2300 g (mean, 3050 ± 337 g; range, 2386–3900 g). After informed consent fromthe parents was received, genomic DNA was extracted from lymphocytes in stored cord blood.

Sequence Analysis of *UGT1A1*

For sequence analysis of *UGT1A1* polymorphisms, genomic DNA was isolated from the leucocytes of infants, with parental informed consent. We amplified exons, the promoter region, and phenobarbital responsive enhancer module (gtPBREM) of UGT1A1 from genomic DNA by using polymerase chain reaction (PCR). In brief, approximately 100 ng of total genomic DNA was amplified with pairs of oligonucleotide primers. Exons 2, 3, and 4 and their intervening introns were simultaneously amplified as a single DNA fragment using a primer pair of 5′-CTCTATCT CAAACACGCATGCC-3′/ 5′-TTTTATCATGAATGCCATG ACC-3′. The 5′ region of UGT1A1, including the TATA box to exon 1, exon 5, and gtPBREM, was amplified separately with primer pairs of 5′-AAGTGAACTCCCTGC TACCTT-3′/5′-GCTTGCTCAGCATATATCTGGG-3′ (5′-region to exon 1), 5′-GAGGATTGTTCATACCACAGG-3′/ 5′-GCACTCTGGGGCTGATTAAT-3′ (exon 5), and 5′-CTGG GGATAAACATGGGATG-3′/5′-CACCACCACTTCTGGAAC CT-3′ (gtPBREM), respectively. Conditions for PCR were as follows: initial denaturation for 2 minutes at 94°C, followed by 1 minute at 94°C, 1 minute at 60°C, and 2 minutes at 72°C for 30 cycles with aMinicycler (MJ Research, Inc,Watertown, Massachusetts). A final extension for 10minutes at 72°C was performed to ensure complete extension of PCR products.

The sequences of the amplified DNA fragments were determined directly using the following sequencing primers. Sequence primers used for the determination of gtPBREM,

TATA box, and coding region are as follows: for sequencing of gtPBREM: 5′-TGAGTTTATATAACCTC-3′; for the TATA box and exon 1: 5′-CTATTTCATGTCCCCTCTGC-3′, 5′-GT CTTTTGTTAGTCTCGGGC-3′, 5′-TTGTTGTGCAGTAAG TGGGA-3′, 5′-CCATTCTCCTACGTGCCCAG-3′, and5 ′ -AA GGGTTGCATACGGGGAATA-3′; for exon 2: 5′-GGAAGCT GGAAGTCTGGG-3′; for exon 3: 5′-CTAGTTAGTATAGCA GAT-3′; for exon 4: 5′-CAGCTGTGAAACTCAGAG-3′; and for exon 5: 5′-TGCTGACAGTGGCCTTCATC-3′ and 5′-GG TAGCCATAAGCACAACAT-3′. The sequences of the amplified DNA fragments were determined directly using a BigDye Terminator v1.1 Cycle Sequencing Kit and Genetic analyzer ABI Prism 3130×l (Applied Biosystems, Carlsbad, California).

Statistical Analyses

Serum bilirubin concentrations for the different genotypes detected were analyzed by ANOVA and the Scheffé test for pairwise comparisons using JMP9 (SAS Institute Inc, Cary, North Carolina) and Statview 4.5 (Abacus Corporation, Baltimore, Maryland). The analysis was performed among 5 frequently observed groups. Homozygous *UGT1A1*6* (encodes the p.G71R variant), compound heterozygous for *UGT1A1*6* and *UGT1A1*60* (promoter c.-3279T>G in the gtPBREM region), heterozygous *UGT1A1*6*, and homozygous *UGT1A1*1* (the normal common allele). All DNA samples were screened for mutations in the gtPBREM region, promoter and TATA box, exons, and exon-intron boundaries of the *UGT1A1* gene.

Results

Genotype in the BMJ Group

Homozygous *UGT1A1*6* was the most frequent genotype detected in the BMJ group (Table I). Approximately one-half the patients had this genotype (88 cases, 51.7%). Other frequent genotypes were heterozygous *UGT1A1*6* (26 cases, 15.2%) and compound heterozygous for *UGT1A1*6* and *UGT1A1*60* (23 cases, 13.5%). Four cases were homozygous *UGT1A1*60*. The other groups included less common genotypes such as heterozygous *UGT1A1*60* (5 cases), compound heterozygous for *UGT1A1*6* and *UGT1A1*28* (1 case), homozygous polymorphisms that encode the p.Y486D variant in exon 5 (*UGT1A1*7*) (3 cases), compound heterozygous for UGT1A1*6 and UGT1A1*7 (3 cases), compound heterozygous for UGT1A1*60 and c.-3279T>G+p.P364L (1091C>T in exon 4: UGT1A1*63) (2 cases), compound heterozygous for UGT1A1*6 and UGT1A1*63 (1 case), heterozygous for p.A471V (c.1412C>T) (1 case), homozygous for *UGT1A1*63* (1 case), *UGT1A1*6* /p.[G71R;Y486D] (2 cases), and *UGT1A1*7*/p.[G71R;Y486D] (1 case). In 8 cases, no mutation was detected in the gtPBREM, promoter, exons, and exon-intron boundaries of *UGT1A1* (*UGT1A1*1*). In this study, no infants were homozygous for the *UGT1A1* allele encoding the p.[G71R;Y486D]-UGT1A1 protein, the typical genotype of the Japanese patient with Crigler-Najjar type II.[15]

Difference in Serum Bilirubin Concentration among the Genotypes in BMJ Group

The relationship between genotype and serum bilirubin concentration was compared between 5 genotypes (Figure) of 149 infants in the BMJ group (homozygous *UGT1A1*6*,

compound heterozygous *UGT1A1*6* and *UGT1A1*60*, heterozygous *UGT1A1*6*, homozygous *UGT1A1*60*, and homozygous *UGT1A1*1*). Results of ANOVA revealed significant differences among the genotypes (degrees of freedom 4/144, $F = 19.456$, $P < .0001$; Table II). The severity of hyperbilirubinemia was as follows: homozygous *UGT1A1*6* > compound heterozygous for *UGT1A1*6* and *UGT1A1*60* > heterozygous *UGT1A1*6* > homozygous *UGT1A1*1* > homozygous *UGT1A1*60* (Table II). Comparison of the 5 groups showed that the serum bilirubin concentration in homozygous *UGT1A1*6* was significantly greater than in compound heterozygous *UGT1A1*6* and *UGT1A1*60* and heterozygous *UGT1A1*6* ($P < .0001$ and $P < .0113$, respectively; Figure).

In the other groups, the severity of elevation in serum bilirubin concentration differed considerably depending on genotype. Infants with the *UGT1A1*7* or p.[G71R:Y486D] allele showed serum bilirubin concentrations >25 mg/dL.

Follow-Up of Serum Bilirubin Concentration in 62 Patients with BMJ

In all, serum bilirubin concentrations of 62 patients with BMJ were followed up after 4–6 months for life (Table III). In most infants, serum bilirubin concentration was reduced <1 mg/dL (17.1 μmol/L), except for infants with 1 genotype (homozygous *UGT1A1*7*). Serum bilirubin concentration of 32 infants with homozygous *UGT1A1*6* was reduced from 21.1 ± 3.8 mg/dL to 0.81 ± 0.31 mg/dL. Two infants with homozygous *UGT1A1*7* showed prolonged unconjugated hyperbilirubinemia of Gilbert syndrome, (3.3 and 3.4 mg/dL, respectively) after 6 months of age.

Genotypes in the Nonhyperbilirubinemic Group with Breast Milk Feeding

Genotype analysis of infants in the control group showed the normal common allele, homozygous *UGT1A1*1* (17 cases, 30.9%), heterozygous *UGT1A1*6* (15 cases, 27.2%), heterozygous *UGT1A1*28* (7 cases, 12.7%), heterozygous *UGT1A1*60* (7 cases, 12.7%), compound heterozygous for *UGT1A1*6* and *UGT1A1*28* (3 cases), compound heterozygous for *UGT1A1*6*/*UGT1A1*60* (2 cases), *UGT1A1*28*/*UGT1A1*60* (2 cases), homozygous *UGT1A1*60* (1 case), and homozygous *UGT1A1*28* (1 case). No infant was homozygous *UGT1A1*6* (Table I).

Discussion

UGT1A1 mutations are a known cause of unconjugated hyperbilirubinemia in patients with type I and type II Crigler-Najjar syndrome and in Gilbert syndrome.[14–17,23] In the neonatal period, infants with Crigler-Najjar syndrome show severe-to-moderate unconjugated hyperbilirubinemia, which continues throughout life. In contrast, mutations in the coding region, but not the regulatory region of *UGT1A1* in Gilbert syndrome, are a risk factor for neonatal hyperbilirubinemia and BMJ.[10,24] We show that mutations in *UGT1A1* in infants also were associated with BMJ. The causes of BMJ can be classified into 2 categories: agents in the breast milk and infant diathesis. The causative agents in breast milk have not yet been determined. However, in an in vitro expression study, we recently showed that 5α-pregnane-3α, 20β-diol inhibits p.G71R UGT1A1, which is encoded by *UGT1A1*6*.[27] Although genetic factors in neonatal hyperbilirubinemia have been reported,[28,29] no

significant association between genetic factors and BMJ has yet been demonstrated. In this study, we show a causal relationship between *UGT1A1*6* polymorphism and BMJ.

These studies selected infants from across Japan who showed prolonged neonatal hyperbilirubinemia while nursing on breast milk. The most frequent genotype in the BMJ group was homozygous *UGT1A1*6*, occurring in 88 of 170 infants (51.7%) (Table I). Other frequent genotypes were heterozygous *UGT1A1*6* (26 cases, 15.2%) and compound heterozygous for *UGT1A1*6* and *UGT1A1*60* (23 cases, 13.5%). In the Japanese population, the allelic frequency of *UGT1A1*6* is 0.16 (Table IV; available at www.jpeds.com), and the estimated incidence of homozygous carriers of *UGT1A1*6* is 2.56%.[25] The high incidence of homozygous *UGT1A1*6* in Japanese infants with BMJ implicates *UGT1A1*6* is a genetic cause of BMJ.

In contrast, homozygous *UGT1A1*1* was the most prevalent (30.9%) genotype in the randomized control group (Table I). Other frequent genotypes in this group were heterozygous *UGT1A1*6* (27.2%), heterozygous *UGT1A1*28* (12.7%), and heterozygous *UGT1A1*60* (12.7%). One neonate was homozygous for *UGT1A1*28*. No infants were homozygous *UGT1A1*6*. The incidence of *UGT1A1*6* and *UGT1A1*28* in the control group was similar to that previously reported in the Japanese population (0.16 and 0.15, respectively).[24] The *UGT1A1*28* allele was not detected in the BMJ group, except in infants who were compound heterozygous. All results confirm our previous finding that the *UGT1A1*28* allele does not induce hyperbilirubinemia in the neonatal period.[25] Conversely, it might have a protective effect against the development of hyperbilirubinemia in the neonatal period, because the frequency of *UGT1A1*28* in the BMJ group was significantly lower compared with the reported frequency in the normal Japanese population and control group (Table IV).

In our studies, serum bilirubin concentration in infants with BMJ differed among the genotypes. The serum bilirubin concentration in the infants with homozygous *UGT1A1*6* was significantly greater than in other genotypes (Figure). The serum bilirubin concentration in compound heterozygous for *UGT1A1*6* and *UGT1A1*60* was greater than that in heterozygous *UGT1A1*6*, but the difference was not significant. Homozygous *UGT1A1*60* showed the lowest concentration, but in combination with *UGT1A1*6*, it contributed significantly to hyperbilirubinemia.

In 62 cases that were followed beyond 4 months of age, serum bilirubin concentration in most cases decreased below 1 mg/dL (Table III). Concentration in the homozygous carriers of *UGT1A1*6* was 0.81 ± 0.31 mg/dL. A previous report has suggested that homozygous *UGT1A1*6* is not characteristic of Gilbert syndrome but mimics Crigler-Najjar syndrome type II.[30] However, the present study confirms that homozygous *UGT1A1*6* carriers do not develop Crigler-Najjar syndrome type II. UGT1A1 concentrations at birth are >1% of the concentration at adulthood, which appears to be reached by 3–4 months of age.[31] BMJ is usually observed before 3 months of age and disappears even if breastfeeding is continued. Serum bilirubin concentrations of infants who are homozygous for *UGT1A1*6* is reduced to <1 mg/dL. Those infants may develop Gilbert syndrome after puberty.[10,32] BMJ with *UGT1A1*6* should be a phenotype of Gilbert syndrome in infants. Two infants with

homozygous p.Y486D (*UGT1A1*7*) showed mild elevation of serum bilirubin concentration after 4 months of life (3.3 and 3.1 mg/dL, respectively). Infants with *UGT1A1*7* (10% of wild type) mutations showed a larger reduction in UGT1A1 activity than *UGT1A1*6* (30%–80% of wild type) mutations.[27,33] Therefore, carriers of homozygous *UGT1A1*7* may continue to show mild hyperbilirubinemia even after 4 months of age.

Approximately 50% of the infants with BMJ were *UGT1A1* genotypes other than homozygous *UGT1A1*6*. This finding implicates other potential mechanisms may contribute towards BMJ. A recent study using a humanized *UGT1* mouse model clarified that expression of intestinal UGT1A1, but not hepatic UGT1A1, correlated with glucuronidation of bilirubin in the neonatal period.[34] Inhibition of hepatic UGT1A1 expression was mediated in part by pregnane X receptor suppression of the *UGT1A1* gene.[35] Although the lack of hepatic UGT1A1 would be expected to produce dramatic hyperbilirubinemia, delayed expression of intestinal UGT1A1 prevented the onset of bilirubin induced neurological defects. Even though the *UGT1A1*6* genotype was not evaluated in the *UGT1A1*1* and *UGT1A1*28* mouse models, breast milk was shown to play an important role in suppression of intestinal UGT1A1 expression. These findings implicated an important regulatory role for the I-κB kinase/nuclear factor kappa B inflammatory pathway in controlling expression of intestinal UGT1A1.[36]

The components of breast milk clearly contribute to the onset of neonatal jaundice.[37] Our in vitro investigation showed the inhibitory effect of 5α-pregnane-3α, 20β-diol on UGT1A1 activity.[26] 5α-pregnane-3α, 20β-diol does not inhibit transcriptional activity of either the *UGT1A1*1* wild-type enhancer-promoter or the c.-3279T>G+A(TA)7TAA enhancer-promoter (*UGT1A1*28*). 5α-pregnane-3α, 20β-diol also does not inhibit UGT1A1 that is encoded by the *UGT1A1*1* allele. However, glucuronidation activity of p.G71R UGT1A1, encoded by the UGT1A1*6 allele, is inhibited by 5α-pregnane-3α, 20β-diol. Although our analysis of *UGT1A1* expression using HepG2 cells showed no inhibitory effect on expression of RNA, recent reports using humanized *UGT1* mice demonstrate that breast milk reduces expression of intestinal UGT1A1.[36] Thus, breast milk may not only inhibit glucuronidation activity of p.G71R-UGT1A1 directly by 5α-pregnane-3α, 20β-diol but also decrease activity by inhibiting expression of intestinal *UGT1A1*.

In conclusion, we report that a homozygous *UGT1A1*6* mutation is an important cause of BMJ in infants in East Asia, although the role of UGT1A1 polymorphisms for developing BMJ is in other ethnic groups is unknown. In infants with homozygous *UGT1A1*6* fed with breast milk, 5β-pregnane-3α, 20β-diol may inhibit p.G71R-enzyme activity. In addition to the inhibitory effect of breast milk on intestinal induction of UGT1A1, these infants may exhibit prolonged BMJ up to 4 months of age.

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Glossary

BMJ	Breast milk jaundice
gtPBREM	Phenobarbital responsive enhancer module
PCR	Polymerase chain reaction
UGT1	UDP-glucuronosyltransferase type 1
UGT1A1	Bilirubin UDP-glucuronosyltransferase 1 family, polypeptide A1

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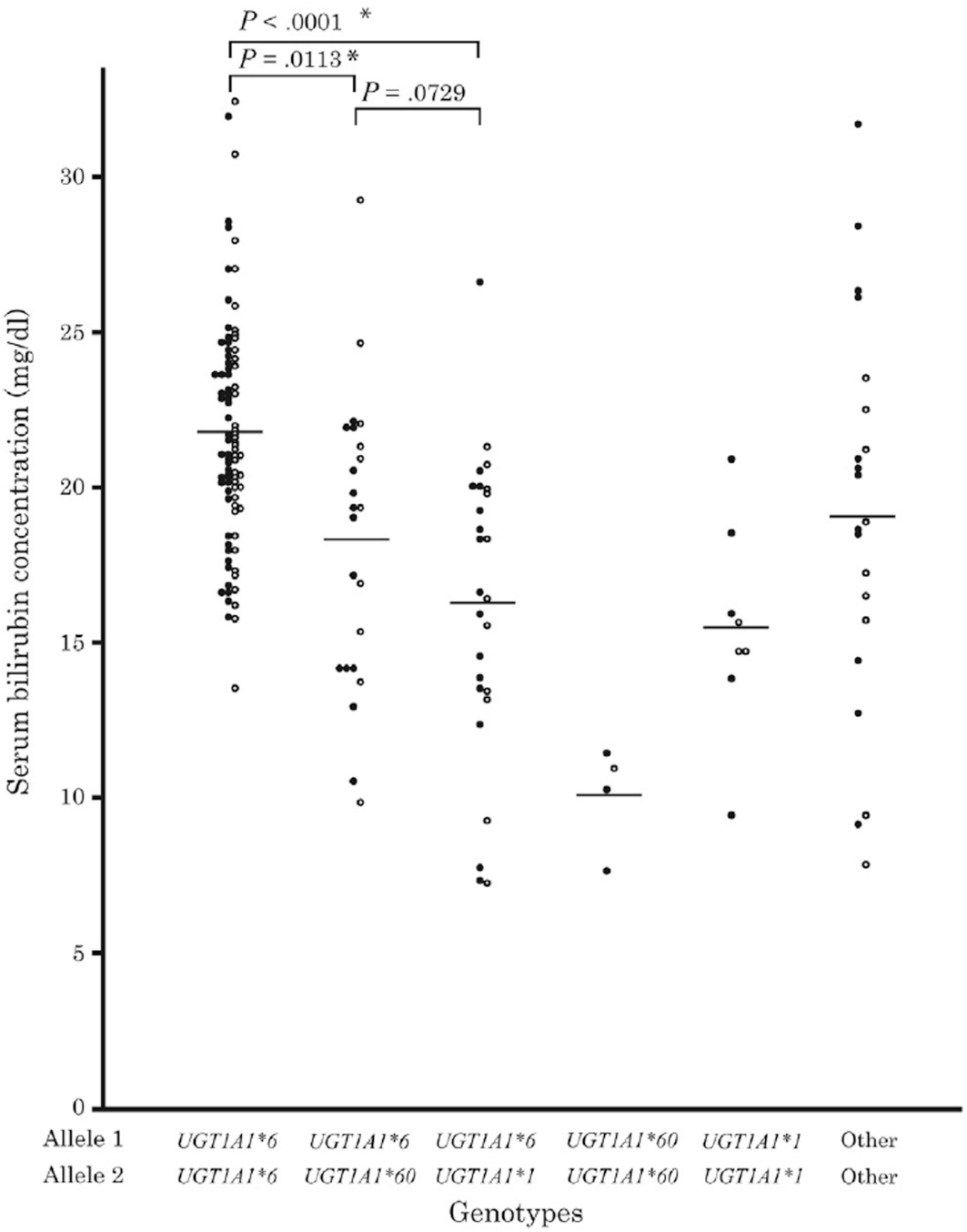

Figure.

Difference in serum bilirubin concentration among the genotypes in BMJ groups. *Closed circles* and *open circles* represent male and female infants, respectively. The relationship between genotypes and serum bilirubin concentration was compared between the 5 genotypes. Results of ANOVA revealed significant differences among the genotypes (degrees of freedom: 4/144, $F = 19.456$, $P < .0001$).

Table I

Differences in UGT1A1 genotypes between infants with and without BMJ

Genotype		Infants with BMJ, n = 170	%	Infants without BMJ, n = 55	%
Allele 1	**Allele 2**				
*UGT1A1*6*	*UGT1A1*6*	88	51.7	0	0
*UGT1A1*6*	*UGT1A1*60*	23	13.5	2	3.6
*UGT1A1*6*	*UGT1A1*1*	26	15.2	15	27.2
*UGT1A1*6*	*UGT1A1*28*	1	0.58	3	5.4
*UGT1A1*28*	*UGT1A1*28*	0	0	1	1.8
*UGT1A1*28*	*UGT1A1*60*	0	0	2	3.6
*UGT1A1*28*	*UGT1A1*1*	0	0	7	12.7
*UGT1A1*60*	*UGT1A1*60*	4	2.3	1	1.8
*UGT1A1*60*	*UGT1A1*1*	5	2.9	7	12.7
*UGT1A1*1*	*UGT1A1*1*	8	4.7	17	30.9
Other	Other*	15	9.4	0	0

*UGT1A1*1*, wild-type allele; *UGT1A1*6*, pG71R; *UGT1A1*28*, c.-3279T>G+A(TA)7TAA; *UGT1A1*60*, c.-3279T>G.

* Other/other genotype in the BMJ group includes homozygous for p.Y486D (UGT1A1*7) (3 cases), compound heterozygous for *UGT1A1*6* and UGT1A1*7 (4 cases), compound heterozygous for *UGT1A1*60* and c.-3279T>G+p.P364L (*UGT1A1*63*) (2 cases), compound heterozygous for *UGT1A1*6* and *UGT1A1*63* (1 cases), heterozygous for p.A471V (c.1412C>T) (1 case), homozygous *UGT1A1*63* (1 case), *UGT1A1*6*/p.[G71R;Y486D] (2 cases), and *UGT1A1*7*/p.[G71R;Y486D] (1 case).

Table II

Genotypic profile of infants with BMJ

Genotype									Serum bilirubin concentration	
Allele 1	Allele 2	No. (total = 170)	Male	Female	Birth weight, g*	Mean ± SD	Gestational age, (weeks, days)*	Mean ± SD	mg/dL	Mean ± SD
*UGT1A1*6*	*UGT1A1*6*	88	47	41	2305–3902	3041 ± 385	35 w, 4 d-41 w, 3 d	38 w, 5 d ± 9.4 d	13.6–32.5	21.8 ± 3.65
*UGT1A1*6*	*UGT1A1*60*	23	13	10	2374–3408	2944 ± 316	35 w, 1 d-41 w, 2 d	39 w, 1 d ± 12.2 d	9.9–29.3	18.3 ± 4.70
*UGT1A1*6*	*UGT1A1*1*	26	15	11	2320–3818	3016 ± 390	35 w, 1 d-41 w, 1 d	38 w, 4 d ± 11.3 d	7.3–26.7	16.3 ± 4.69
*UGT1A1*60*	*UGT1A1*60*	4	3	1	3066–3430	3220 ± 188	38 w, 4 d-39 w, 4 d	39 w, 0 d ± 3.5 d	7.7–11.5	10.1 ± 1.68
*UGT1A1*1*	*UGT1A1*1*	8	5	3	2315–3400	3031 ± 452	37 w, 0 d-41 w, 3 d	38 w, 6 d ± 12.6 d	9.5–21.0	15.5 ± 3.37
Other	Other†	21	12	9	2306–3375	2889 ± 390	36 w, 4 d-40 w, 5 d	39 w, 2 d ± 7.7 d	7.9–31.8	19.3 ± 6.19

*Birth weight and gestational age are not statistically different among the each group.

†Other/other genotype group in infants with BMJ include the following genotypes: heterozygous *UGT1A1*60* (5 cases), compound heterozygous for *UGT1A1*6* and *UGT1A1*28* (1 cases), homozygous for *UGT1A1*7* (3 cases), compound heterozygous for *UGT1A1*6* and *UGT1A1*7* (4 cases), compound heterozygous for *UGT1A1*60* and *UGT1A1*63* (2 cases), compound heterozygous for *UGT1A1*6* and *UGT1A1*63* (1 cases), heterozygous for p.A471V (1 case), homozygous *UGT1A1*63* (1 case), *UGT1A1*6*/p.[G71R;Y486D] (2 cases), and *UGT1A1*7*/p.[G71R;Y486D] (1 case).

Table III

Differences in serum bilirubin concentration depending on the type of mutations in infants with BMJ

Genotype			Mean serum bilirubin concentration, mg/dL	
Allele 1	**Allele 2**	**No. (total = 62)**	**At diagnosis**	**4 months later**
*UGT1A1*6*	*UGT1A1*6*	33	21.2 ± 3.8	0.81 ± 0.31
*UGT1A1*6*	*UGT1A1*60*	8	16.3 ± 2.9	0.85 ± 0.42
*UGT1A1*6*	*UGT1A1*1*	9	14.9 ± 4.5	0.82 ± 0.73
*UGT1A1*60*	*UGT1A1*60*	1	11.5	0.58
*UGT1A1*7*	*UGT1A1*7*	2	15.1	3.35
Other	Other*	6	19.3 ± 7.8	0.83 ± 0.34
*UGT1A1*1*	*UGT1A1*1*	3	17.6 ± 2.9	0.61 ± 0.22

* Other/other genotype group includes a rare combination of UGT1A1 mutations; compound heterozygous for *UGT1A1*6* and p.[G71R:Y486D] (1 case), homozygous *UGT1A1*63* (1 case), compound heterozygous for *UGT1A1*6* and *UGT1A1*7* (2 cases), and heterozygous for *UGT1A1*60* (2 cases).

Table IV

Allelic frequencies of UGT1A1 polymorphism in the BMJ group, non-BMJ group, and Japanese population

	BMJ group		Non-BMJ group		Previous report*
	n = 340	Frequency	n = 110	Frequency	Frequency
*UGT1A1*1*	48	0.141	63	0.573	
*UGT1A1*6*	236	0.694	20	0.182	0.151–0.16
*UGT1A1*28*	1	0.003	14	0.127	0.121–0.15
*UGT1A1*60*	34	0.100	13	0.118	0.115
Other alleles	21	0.062	0	0	

*Allelic frequencies in Japanese population as determined in previous reports.[25]