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Gly71Arg Mutation of the Bilirubin UDP-Glucuronosyltransferase 1A1 Gene is Associated with Neonatal Hyperbilirubinemia in the Japanese Population

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The serum bilirubin level of Japanese newborn infants in their first few days is significantly higher than that in Caucasian newborn infants, suggesting that there might be genetic risk factors for the development of neonatal hyperbilirubinemia in the Japanese population. Recently, it has been reported that a variant TATA box in the promoter region of the bilirubin UDP-glucuronosyltransferase 1 (*UGT1A1*) gene is associated with the development of neonatal hyperbilirubinemia. This finding led us to the idea that a mutation, glycine to arginine at codon 71 (G71R), in the coding region of the *UGT1A1* gene can cause neonatal hyperbilirubinemia. In this study, we determined the genotypic distribution of the G71R mutation in 72 Japanese newborn infants: 23 infants with hyperbilirubinemia and 49 infants without hyperbilirubinemia. In the hyperbilirubinemia group, 15 of 23 newborn infants had the G71R mutation (3 homozygotes and 12 heterozygotes), whereas in the non-hyperbilirubinemia group 16 of 49 newborn infants had the G71R mutation (1 homozygote and 15 heterozygotes). Therefore, the G71R mutation was present significantly more frequently in the hyperbilirubinemia group than in the non-hyperbilirubinemia group. This finding strongly suggests that the presence of the G71R mutation contributes to the development of neonatal hyperbilirubinemia in the Japanese population.

The serum bilirubin level of Japanese newborn infants in their first few days is significantly higher than that in Caucasian newborn infants [1]. This may be due to a difference between the genetic backgrounds of the Japanese and Caucasian populations, and suggests the presence of genetic risk factors for the development of neonatal hyperbilirubinemia in the Japanese population.

Recently, it has been reported that a variant TATA box in the promoter region of the gene responsible for Gilbert's syndrome, bilirubin UDP-glucuronosyltransferase 1 gene (*UGT1A1*), is associated with the development of neonatal hyperbilirubinemia. Bancroft et al. [2] showed that newborn infants with a variant TATA box have a greater increase in the jaundice index during the first 2 days of life. This finding led us to the idea that a mutation in the

coding region of the *UGT1A1* gene could be a risk factor for neonatal hyperbilirubinemia, since according to our previous study a variant TATA box *per se* does not contribute to the neonatal hyperbilirubinemia in the Japanese population [3].

The most frequent mutation in Japanese patients with Gilbert's syndrome is a missense mutation, G-to-A at nucleotide 211, causing the amino acid change of glycine to arginine at codon 71 (G71R) [4]. The G71R mutation has been proved to decrease enzyme activity [5]. To clarify whether the G71R mutation contributes to the development of neonatal hyperbilirubinemia in the Japanese population, we performed genotyping analysis of the G71R mutation in Japanese newborn infants with and without hyperbilirubinemia.

PATIENTS AND METHODS

Patients. Seventy-two Japanese newborn infants were recruited into this study after obtaining informed consent from their parents: 23 infants with hyperbilirubinemia (hyperbilirubinemia group) and 49 infants without hyperbilirubinemia (non-hyperbilirubinemia group) (Table I). The definition of newborn infants with hyperbilirubinemia in this study is 'newborn infants with a total bilirubin level of more than 15 mg/dl serum in the first 7 days of life'. All infants were born at 37-42 weeks gestation and weighed more than 2500g. They showed no blood incompatibility, negative direct Coombs test, no significant antenatal or intrapartum complications, and no clinically detectable pathology except for hyperbilirubinemia. There were no significant differences between the hyperbilirubinemia and non-hyperbilirubinemia groups in gestational age, birth weight, sampling day and ratio of infants fed with breast milk to those fed with formula milk.

All of the newborn infants with hyperbilirubinemia enrolled in this study underwent phototherapy, while any of the newborn infants without hyperbilirubinemia enrolled in this study did not undergo phototherapy.

Table I. Clinical data for the newborn infants enrolled in the study.

	hyperbilirubinemia	non-hyperbilirubinemia	P
Number	23	49	
total bilirubin (mg/dl serum)	19.2±2.5	9.9±2.3	p<0.0001
gestational week (w)	38.1±1.8	38.9±1.4	NS
birth weight (g)	2965±400	2998±376	NS
sampling day (th)	5.2±2.0	5.0±1.6	NS
breast milk/bottle milk	12/11	24/25	NS

PCR amplification of *UGT1A1* exon 1. Genomic DNA was extracted from 0.2 ml of whole blood by use of the Gene Trapping by Liquid Extraction Kit (Takara Biomedicals, Kyoto, Japan) and used as a template for PCR. The *UGT1A1* exon 1 was amplified with the primer set B1E1A (5'-AGG AGC AAA GGC GCC-3') and B1E13 (5'-TTG TTG TGC AGT AAG TGG GA-3') [6].

The 30µl reaction mixture contained 200 ng genomic DNA in 1 x PCR buffer with 10mM Tris-HCl (pH 8.3), 50mM KCl, 1.5mM MgCl₂, 200µM dNTPs, 0.001% (w/v) gelatin, 0.15µM each primer, and 1 unit of *Taq* DNA polymerase (AmpliTaq Gold; Perkin-Elmer Applied Biosystems, Foster City, CA, USA). The PCR process was as follows: an initial denaturation step at 95°C for 4 min, 37 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min, and a final extension step at 72°C for 10 min. The PCR products were electrophoresed in a 3% agarose gel and stained with ethidium

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bromide.

Nucleotide sequencing of UGT1A1 exon 1. To detect mutations in the UGT1A1 gene, the PCR products were directly sequenced with a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). The primers used for sequencing analysis were the same as described above. The products from cycle sequencing were purified by ethanol precipitation, and then applied to an ABI 310 Genetic Analyzer. The nucleotide sequences were determined with DNA Sequencing Analysis Software (Applied Biosystems).

Statistics. Clinical data (total bilirubin level, gestational age, birth weight, sampling day) were compared between the hyperbilirubinemia and non-hyperbilirubinemia groups by Student's t test. Genotypic distributions and frequencies of the G71R mutation were compared by use of a chi-square test. A P-value less than 0.05 was considered to indicate a significant difference.

RESULTS

Genotypic distribution for the G71R mutation in the 72 Japanese newborn infants was as follows: G/G 41 cases (57%), G/R 27 cases (38%) and R/R 4 cases (5%). The genotype frequencies were compatible with the Hardy-Weinberg equilibrium ($\chi^2 = 0.026$, $df = 1$, $p = 0.87$) (Table II). Allele frequency of the G71R mutation was 0.22.

Table II. Genotypic distributions of the newborn infants in this study.

	G/G	G/R	R/R
observed value (n = 72)	41	27	4
expected value	41.3	26.5	4.3
$\chi^2 = 0.026$	$df = 1$	$p = 0.87$	

To clarify whether the presence of the mutation contributes to the development of hyperbilirubinemia, the newborn infants enrolled in the study were classified into two groups: infants with total bilirubin level more than 15 mg/dl serum in the first 7 days of life (hyperbilirubinemia group, n = 23) and those with total bilirubin level less than 15mg/dl serum in the first 7 days of life (non-hyperbilirubinemia group, n = 49).

The genotypic distributions differed significantly between the hyperbilirubinemia and non-hyperbilirubinemia groups ($\chi^2 = 8.26$, $df = 2$, $p = 0.016$) (Table III). The allele frequency of the G71R mutation in the hyperbilirubinemia group was 0.39, significantly higher than the value of 0.17 in the non-hyperbilirubinemia group ($\chi^2 = 8.07$, $df = 1$, $p = 0.0045$).

Table III. Genotypic distributions of the hyperbilirubinemia and non-hyperbilirubinemia group.

	G/G	G/R	R/R
hyperbilirubinemia (n = 23)	8	12	3
non-hyperbilirubinemia (n = 49)	33	15	1
$\chi^2 = 8.26$	$df = 2$	$p = 0.016$	

When the heterozygous and mutant homozygous cases were combined and analyzed, in the hyperbilirubinemia group 15 of 23 infants (65%) had the G71R mutation (3 homozygotes

and 12 heterozygotes), whereas in the non-hyperbilirubinemia group 16 of 49 infants (33%) had the G71R mutation (1 homozygote and 15 heterozygotes). Therefore, the G71R mutation was present significantly more frequently in the hyperbilirubinemia group than in the non-hyperbilirubinemia group ($\chi^2 = 6.77$, $df = 1$, $p = 0.0092$) (Table IV).

Table IV. G71R mutation of the hyperbilirubinemia and non-hyperbilirubinemia group.

	G/G	G/R and R/R
hyperbilirubinemia (n = 23)	8	15
non-hyperbilirubinemia (n = 49)	33	16
$\chi^2 = 6.77$	$df = 1$	$p = 0.0092$

DISCUSSION

We showed a higher frequency of the G71R mutation of the *UGT1A1* gene in Japanese newborn infants with hyperbilirubinemia than in those without hyperbilirubinemia. This finding was consistent with the experimental finding reported by Koiwai *et al.* [5]. They performed an expression study using pcDCT expression vector that carried the *UGT1A1* cDNA with the G71R mutation. In their study, the COS cells transfected with the G71R mutation-carrying vector showed less than 14% of normal BUGT activity.

Akaba *et al.* [7] and Maruo *et al.* [8] also reported that the frequency of the G71R mutation in newborn infants who required phototherapy (i.e., those with severe hyperbilirubinemia) was significantly higher than that in neonates who did not require therapy. Their findings, as well as our results, strongly suggest that the presence of the G71R mutation is one of the genetic risk factors associated with neonatal hyperbilirubinemia in Japanese newborn infants. In addition, since the G71R mutation is common in Gilbert's syndrome in Japan [4], neonatal hyperbilirubinemia with the G71R mutation may be an infantile phenotype of Gilbert's syndrome.

The frequency of the G71R mutation has been reported to be very rare in the Caucasian population [9], although the data of the G71R mutation in Caucasian newborn infants with hyperbilirubinemia are not available. Therefore, the higher frequency of the G71R mutation in Japanese newborn infants may partly account for the fact that serum bilirubin level in Japanese newborn infants is significantly higher than that in Caucasian newborn infants [1].

In conclusion, the presence of the G71R mutation of the *UGT1A1* gene contributes to the development of neonatal hyperbilirubinemia in the Japanese population. However, it should also be noted that more than 30% of our newborn infants with hyperbilirubinemia showed the G/G genotype, i.e., no G71R mutation. This finding suggested that other genetic and/or environmental factors than the G71R mutation of the *UGT1A1* gene may also contribute to the development of neonatal hyperbilirubinemia.

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