Association between UGT 1A1 Gly71Arg (G71R) Polymorphism and Neonatal Hyperbilirubinemia

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Background: *Neonatal hyperbilirubinemia is a common problem in neonates and affects 60% of Asian newborn babies which is twice that found in Caucasians. These findings suggest that a genetic factor might be involved. Recently, a relationship between polymorphisms of the bilirubin uridine 5-diphosphate-glucuronosyltransferase (UGT1A1) gene and neonatal hyperbilirubinemia has been reported. It was demonstrated that the genetic variations cause a decrease in UGT1A1 activity in neonates, leading to an accumulation of unconjugated bilirubin in serum. However, in Asians the G to A missense mutations in the UGT1A1 at nucleotide 211 (known as G71R), were the predominant findings. Therefore, the impact of this polymorphism on serum bilirubin in healthy Thai neonates is of interest.*
Objective: *The aim of the present study was to investigate the frequency of UGT1A1 allele in healthy Thai neonates and to determine its role in neonatal hyperbilirubinemia.*
Material and Method: *A cross sectional study was conducted to investigate an association between the UGT1A1 G71R polymorphism and neonatal hyperbilirubinemia. Cord blood of 291 neonates was obtained to determine the gene frequency of UGT1A1 G71R by PCR-restriction fragment length polymorphism method. During the first 48 to 72 hours of the infants' life, the infants' blood was collected to measure their microbilirubin.*
Results: *PCR-RFLP analysis revealed the UGT1A1 G71R polymorphism in 42 infants (14.4%). In addition, six of this group (14.3%) had a microbilirubin level more than 95th percentile which was approximately 2.5 times more than those with the wild type allele (5.6%). The maximum microbilirubin level of infants in the R71 allele group was significantly higher than those in the G71 allele group, (11.79 $\pm$ 3.34 mg/dL and 9.53 $\pm$ 2.34 mg/dL, respectively, $p < 0.01$).*
Conclusion: *In the present study, the UGT1A1 G71R allele was found to be one of the risk factors for neonatal hyperbilirubinemia in Thai neonates.*

Keywords: *neonatal hyperbilirubinemia, UGT1A1, G71R, microbilirubin*

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Neonatal hyperbilirubinemia is the most common problem of the newborns, with approximately half of all babies demonstrating clinical jaundice. It can cause neurologic dysfunction, particularly kernicterus, which is seen more often when jaundice is caused by hemolytic disease, severe illness, or hepatic dysfunction[1]. To date, abnormal hepatic bilirubin conjugation cannot be detected by routine jaundice work up.

The bilirubin uridine 5-diphosphate-glucuronosyltransferase (UGT1A1) is the key enzyme for bilirubin conjugation. Because UGT1A1 is too labile to be measured by classic biochemical methods, it is ideally studied at the genetic level[2].

The peak serum levels of unconjugated bilirubin in full-term Asian (Japanese, Korean, or Chinese) and American Indian neonates are double those in Caucasian and black populations[3]. These findings suggest that genetic factors are involved in the development of neonatal hyperbilirubinemia. In Caucasians[4,5], Sephardic Jews[6], Americans[7], Italians[8,9] and British[10], neonatal hyperbilirubinemia is associated with the coinheritance of homozygous $A(TA)_7TAA$ variation in the UGT1A1 gene. However the results of recent studies indicate that carriage of homozygous 211 G to A variation (G71R) within the coding region in the UGT1A1 gene is an additive risk factor for neonatal hyperbilirubinemia in Asian populations (Japanese[11,12] and Taiwanese[13-17]). In the

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present study, the UGT1A1 G71R polymorphism in Thai neonates, their bilirubin levels and the subsequent hyperbilirubinemia were determined for their relationship.

Material and Method

A cross sectional study was conducted to investigate the effects of predetermined risk factors for neonatal hyperbilirubinemia. The present study protocol was approved by the Ethics Committee on Human Experimentation of Faculty of Medicine, Thammasat University, Thailand. Informed consents were obtained from the parents. The subjects were 320 term newborn infants who were born between August 1st, 2006 and November 30th, 2008 at Thammasat Hospital, Pathumthani, Thailand. Infants with congenital infection, hypothyroidism, neonatal hepatitis, biliary atresia or gastrointestinal obstruction were excluded from the present study.

Cord blood samples were collected in EDTA-containing tubes to analyze the UGT1A1 gene. Blood samples were taken to measure serum microbilirubin level and to screen phenylketonuria and hypothyroidism when the infants were 48-72 hours of age or when they developed jaundice. Serum microbilirubin was measured by BR-501 bilirubin meter (APEL Co LTD, Japan).

Definition

Significant hyperbilirubinemia was defined as a serum microbilirubin level more than 95th percentile for age-specific in hours according to the Bhutani normogram[18].

DNA extraction and analysis of the UGT1A1 gene

DNA was extracted from white blood cells by Genomic DNA Mini Kit (Geneaid®, Geneaid Biotech Ltd., Taipei, Taiwan). The PCR-restriction fragment length polymorphism technique was applied to detect the variants of UGT1A1 at nucleotides 211. The primers used for PCR were 5′AGATACTGTTGATCCCAGTG3′ (forward primer) and 5′CTTCAAGGTGTAAAATGG TC3′ (reverse primer)[16]. The PCR mixture (25 mL) consisted of 50 ng of DNA, 50 mM KCl, 20 mM Tris-HCl pH 8.0, 0.05% Tween 20, 1.5mM $MgCl_2$, 60 μM dNTPs, 0.2 μM each primer and 0.625 units taq DNA polymerase (Fermentas, USA). The PCR amplification was performed in a DNA thermal cycler, initial denaturation 3 min at 95°C, denaturation for 1 min at 94°C, annealing for 1 min at 53°C, primer extension for 1 min at 72°C for 40 cycles and a final extension for 5 min at 72°C. The PCR product was digested with the AvaII restriction enzymes (New England Biolabs, USA) and analyzed by electrophoresis on 6% polyacrylamide gel in TBE Buffer.

Statistical analysis

Demographic data was demonstrated as percentage, mean, and standard deviation. Genotypic distributions and frequencies of the G71R mutation were compared by the Chi-square test, whereas microbilirubin levels were compared using the Student's t-test. The p-value of less than 0.05 was considered to be a significant difference.

Results

Of 320 term newborn infants, 29 infants were excluded from analysis due to absence of either DNA or serum samples. Therefore, 291 infants (152 males and 139 females) were the subjects of the present study. Mean birth weight was 3,121.87 $\pm$ 369.97 g (range: 2,215-4,550 g) and a mean gestational age was 38.5 $\pm$ 1.1 weeks (range: 37-41 weeks).

Twenty newborn infants developed significant hyperbilirubinemia. Of these, 14 (70%) newborn infants underwent phototherapy. In half of them, the cause of neonatal hyperbilirubinemia was unexplained, 2 (14.3%) had ABO blood group incompatibility and 5 (35.7%) had breast feeding jaundice.

The genotype frequencies of wild type, heterozygous and homozygous variants of UGT1A1 G71R were 85.6, 14.1 and 0.3%, respectively. The allele frequency of the variant R71 was 0.07. In the heterozygous (G71/R71) group, 6 of 41 infants (14.6%) had hyperbilirubinemia, whereas 14 of 235 infants (5.6%) in the wild type (G71/G71) group had hyperbilirubinemia ($p < 0.01$). The allele frequency of the G71R mutation in the hyperbilirubinemia group was 0.15, significantly higher than the value of 0.07 in the non-hyperbilirubinemia group (Table 1).

When the babies with heterozygous and homozygous variants were combined and analyzed, the maximum microbilirubin level was significantly higher (11.79 $\pm$ 3.34 mg/dL) than wild type group (9.53 $\pm$ 2.34 mg/dL) ($p < 0.01$).

Discussion

Increased bilirubin formation and decreased bilirubin conjugation play an important role in the pathogenesis of neonatal jaundice. Insufficient conjugation of bilirubin by the liver due to the relative

Table 1. Comparison of genotypic distributions between hyperbilirubinemia infants and normal newborn infants

Newborn infant	UGT1A1 nt 211			p-value
	GG (%)	GA (%)	AA (%)	
Hyperbilirubinemia	14 (5.6)	6 (14.6)	0	< 0.01*
Normal	235 (94.4)	35 (85.4)	1 (100)	
Total	249 (100)	41 (100)	1 (100)	

immaturity of the liver enzyme system, including bilirubin UDP-glucuronosyltransferase (UGT1A1), may lead to the development of neonatal jaundice[19]. In the *in vitro* expression study by Yamamoto et al[20], the G211 to A mutation in exon 1 of UGT1A1 at nucleotide number 211 in exon 1 (G71R) in the homozygote and heterozygous decreased the UGT1A1 enzyme activities to 32.2% and 60.2% of normal, respectively.

Yamamoto et al[21] found that genotypic distribution for the G71R mutation in the Japanese newborn infants was 0.22. The G71R mutation was observed more frequently in the hyperbilirubinemia group (0.39) than in the non-hyperbilirubinemia group (0.17). Sun et al[22] reported that 19 of 48 (39.6%) Chinese neonates had the G71R polymorphism and the frequency of hyperbilirubinemia and prolonged neonatal jaundice in this group was significantly higher than that in the G71 ($p = 0.016$). Huang et al[16] revealed that the prevalence (20%) of the G71R variant in the normal Taiwanese infant that is similar to the healthy Taiwanese adults (21.4%; 62 of 290)[17] and the variation at nucleotide 211 of the UGT1A1 gene is a risk factor for severe hyperbilirubinemia in Taiwanese neonates. This observation is in concordance with our findings, where the R71 allele frequency in the hyperbilirubinemic group was significantly higher compared to the control group. In the present study, the prevalence (14.4%) and the allele frequency (0.07) of this allele in Thai neonates was less than in Japanese, Taiwanese and Chinese neonates, but similar to our previous report[23].

Maruo et al[12] reported that the mean peak transcutaneous jaundice index in babies heterozygous for G71R was significantly higher than that of normal babies. Sun et al[22], also found that babies with G71R missense allele had a significant increase in jaundice index at 48 and 96 hours post-natally. In addition their findings, as well as our results, demonstrated that the maximum microbilirubin levels were significantly higher in neonates with R71 than with those in the G71 allele group. This finding further supports the relationship between UGT1A1 gene polymorphisms and the development of neonatal jaundice.

Conclusion

The bilirubin UDP-glucuronosyltransferase (UGT1A1) gene polymorphism at nucleotide 211 (G71R) is an important risk factor for neonatal hyperbilirubinemia in Thai neonates.

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Potential conflicts of interest

None.

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ความสัมพันธ์ระหว่างความผิดปกติของจีน UGT1A1 Gly 71 Arg (G71R) กับภาวะตัวเหลืองจากบิลิรูบินสูงในทารกแรกเกิด

ศริยา ประจักษ์ธรรม, พรเพ็ญ ก่ำนารายณ์, ศศิชัย กังสดาลอำไพ

ภูมิหลัง: *อาการตัวเหลืองจากบิลิรูบินในเลือดสูงเป็นปัญหาหนึ่งที่พบบ่อยในทารกแรกเกิด ทารกเกิดครบกำหนดเชื้อสายเอเชียมีอาการตัวเหลืองร้อยละ 60 ซึ่งมากกว่าทารกเชื้อสายคอเคเซียนถึง 2 เท่า แสดงให้เห็นว่าปัจจัยทางพันธุกรรมน่าจะมีส่วนเกี่ยวข้องกับการเกิดภาวะตัวเหลืองที่ผิดปกติในทารกแรกเกิด มีหลายการศึกษาพบว่าการเปลี่ยนแปลงทางพันธุกรรมของจีน UGT1A1 ซึ่งควบคุม uridine 5-diphosphate-glucuronosyltransferase enzyme ให้ทำหน้าที่คอนจูเกตบิลิรูบิน เป็นปัจจัยเสี่ยงในการเกิดภาวะตัวเหลืองผิดปกติในทารก ในเชื้อสายเอเชียส่วนใหญ่มีการเปลี่ยนแปลงทางพันธุกรรมของยีน UGT1A1 gene ในตำแหน่ง nucleotide 211 (G71R) ผู้วิจัยจึงสนใจที่จะศึกษาการเปลี่ยนแปลงทางพันธุกรรมของยีน UGT1A1 ซึ่งอาจจะเป็นสาเหตุหนึ่งของทารกที่มีภาวะตัวเหลืองผิดปกติจากบิลิรูบินสูงได้*

วัตถุประสงค์: *1) ศึกษาหาความชุกของการเปลี่ยนแปลงทางพันธุกรรมของยีน UGT1A1 gene ในตำแหน่ง nucleotide 211 ในเด็กไทย, 2) ศึกษาหาความสัมพันธ์เกี่ยวกับการเปลี่ยนแปลงทางพันธุกรรมของยีน UGT1A1 gene ในตำแหน่ง nucleotide 211 ที่มีผลต่อการเกิดภาวะตัวเหลืองจากบิลิรูบินสูงในทารกแรกเกิด*

วัสดุและวิธีการ: *ทำการศึกษาแบบ cross sectional study มีทารกเข้าร่วมการศึกษา 291 คน ทารกจะได้รับการเก็บเลือดจากสายสะดือเพื่อส่งตรวจ gene variation ของ UGT 1A1 ตำแหน่ง nucleotide 211 ด้วยวิธี PCR–restriction fragment length polymorphism method และได้รับการตรวจเลือดวัดระดับ microbilirubin ที่อายุ 48-72 ชั่วโมง*

ผลการศึกษา: *ทารก 42 คน (ร้อยละ 14.4) มีการเปลี่ยนแปลงทางพันธุกรรมของยีน UGT1A1 ในตำแหน่ง nucleotide 211 (G⟶A) ทารกกลุ่มนี้จำนวน 6 คน (ร้อยละ 14.3) มีระดับบิลิรูบินมากกว่าหรือเท่ากับเปอร์เซ็นต์ไทล์ที่ 95 ซึ่งมากกว่ากลุ่มทารกที่มียีน UGT1A1 แบบ wild type (14 คน คิดเป็นร้อยละ 5.6) อย่างมีนัยสำคัญทางสถิติ และเมื่อเปรียบเทียบระดับ microbilirubin ที่สูงที่สุดจะพบว่ากลุ่มทารกที่มีการเปลี่ยนแปลงทางพันธุกรรมของจีน UGT1A1 มีระดับ microbilirubin 11.79 $\pm$ 3.34 mg/dL ซึ่งสูงกว่ากลุ่มทารกที่ไม่มีการเปลี่ยนแปลงของยีน UGT1A1 (microbilirubin 9.53 $\pm$ 2.34 mg/dL) อย่างมีนัยสำคัญทางสถิติ*

สรุป: *ในการศึกษานี้พบว่าการเปลี่ยนแปลงทางพันธุกรรมของยีน UGT1A1 gene ในตำแหน่ง nucleotide 211 เป็นปัจจัยเสี่ยงที่ทำให้ทารกเกิดภาวะตัวเหลืองผิดปกติ*