Quantitative Trait Analysis of Polymorphisms in Two Bilirubin Metabolism Enzymes to Physiologic Bilirubin Levels in Chinese Newborns

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Objective To explore the effects of variants in *Uridine Diphosphate Glucuronosyl Transferase 1A1* (*UGT1A1*) and *Heme Oxygenase-1* (*HMOX1*) on daily physiological bilirubin levels and bilirubin changes during the first week after birth in Chinese newborns. Both *UGT1A1* and *HMOX1* code rate-limiting enzymes in the bilirubin metabolism pathway.

Study design We conducted a retrospective quantitative trait study to analyze 4154 daily bilirubin values, 3129 bilirubin changes, and 11 polymorphisms of 988 newborns during the natural course of physiological hyperbilirubinemia.

Results For *UGT1A1,* we found minor allele *A* of rs4148323 (G211A, *UGT1A1**6) contributed to higher daily bilirubin levels on days 4-6 (with contributions to variations increasing from 4.8% to 12.3%), minor allele *T* of rs887829 (c-364t) contributed to lower daily bilirubin levels for days 6 and 7 (with contributions to variations increasing from 7.0% to 10.2%) ($P < .03$ for all). In addition, minor alleles of rs887829 and $(TA)_n$ repeat (*UGT1A1**28), and haplotype T-long-G at rs887829-$(TA)_n$-rs4148323 were associated with a decrease in bilirubin levels from day 5 to day 6 ($P < .01$ for all). No contribution from *HMOX1* was found.

Conclusion Bilirubin levels and changes during the middle and late parts of the first week were attributed to variants and haplotypes in *UGT1A1*. This quantitative trait study may provide a more robust statistical method for determining the association of genetic factors and bilirubin kinetics to predict the development of neonatal bilirubin in early postnatal life. *(J Pediatr 2014;165:1154-60)*.

Neonatal jaundice is a common phenomenon, affecting approximately 60% of full-term newborns, and a significant cause of hospital readmission during the first week of life.[1-3] *Uridine Diphosphate Glucuronosyl Transferase 1A1* (*UGT1A1*), a gene coding the key enzyme for bilirubin conjugation, is associated with neonatal hyperbilirubinemia. Heme oxygenase, another key enzyme in the bilirubin metabolism pathway, encoded by the *Heme Oxygenase-1* (*HMOX1*) gene, catalyzes the rate-controlling step of heme degradation and generates biliverdin. The identification of common variants has been studied extensively, but with conflicting results.[4-7] These inconsistent results may be attributed to differing variant frequencies in different ethnic groups or to case-control study designs. Association studies of neonatal hyperbilirubinemia have used varying methods for diagnosing neonatal hyperbilirubinemia and recruiting control groups in different countries and medical centers.

Quantitative trait analysis can significantly increase the statistical power for the detection of genetic factors and avoid ambiguities.[8] Therefore, we quantified daily bilirubin changes and absolute bilirubin values during the natural course of physiological hyperbilirubinemia. Data on the rate of bilirubin increase at different days of life are particularly useful based on current management guidelines of the American Academy of Pediatrics.[9]

In the present study, we investigated the genetic effects of *UGT1A1* and *HMOX1* on bilirubin kinetics during the first week of life. We chose the first week of life as the study period when changes in bilirubin were the greatest, bilirubin reached peak levels, and bilirubin measurements were available.

Methods

Between February and October 2008, we recruited 988 term infants (at ≥37 weeks gestation and birth weight ≥2500 g) from the Municipal Hospital

FDR	False discovery rate
HMOX1	*Heme Oxygenase-1*
LD	Linkage disequilibrium
TcB	Transcutaneous bilirubin
UGT1A1	*Uridine Diphosphate Glucuronosyl Transferase 1A1*

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in Suzhou, China. Eligible infants had no major abnormalities except neonatal jaundice without pathological causes. For each infant, sex, feeding type, delivery mode, date of birth, birth weight, and gestational age at birth were recorded (Table I).

Each morning, transcutaneous bilirubin (TcB) was measured on the forehead of each neonate during the birth hospitalization using a Konica Minolta JM-103 (Konica Minolta, Tokyo, Japan) or a local branded transcutaneous bilirubinometer. Vaginally delivered newborns were discharged at age ≥72 hours, and those delivered by cesarean section were discharged at age ≥120 hours. According to Chinese guidelines,[10,11] hyperbilirubinemia was defined as a TcB level >12.9 mg/dL (221 μmol/L) on day 3 or later before discharge. Hyperbilirubinemic neonates were not excluded from the study, but bilirubin measurements obtained after receipt of phototherapy were not included in our analyses. Infants who exhibited high bilirubin values before day 3 or infants whose pathological causes of hyperbilirubinemia could be diagnosed were transferred to the neonatal unit and excluded from our study. The direct antiglobulin test and glucose-6-phosphate dehydrogenase screening were performed only in jaundiced newborns with symptoms, such as hepatosplenomegaly, anemia, and listlessness, or with suspected pathological hyperbilirubinemia. Neonates with positive results were not included in the study.

DNA was isolated from surplus filter paper blood spots with ethanol. The study was approved by both the hospital's Reproductive Medicine Ethics Committee and the Ethics Committee of the institute, with a waiver of written consent.

Genotyping

Eight variants in *UGT1A1* and 3 variants in *HMOX1* were evaluated. Among these, $(TA)_n$ repeat (*UGT1A1**28) and rs4148323 (G211A, *UGT1A1**6) in *UGT1A1* and $(GT)_n$ repeat in *HMOX1* were functional variants.[12-14] Rs887829 (c-364t) in *UGT1A1* was associated with adults' bilirubin level.[15] The other 7 variants were tagging single nucleotide polymorphisms located within 5 kb upstream and 2 kb downstream of each gene, selected from the HapMap Han Chinese population based on r^2 >0.8 and a minor allele frequency of >0.1. Selected variants were able to capture 18 of 22 polymorphisms of *UGT1A1* and 7 of 9 polymorphisms of *HMOX1*. Genotyping of each variant was done following the methodology of Zhou et al.[16] In brief, $(TA)_n$ and $(GT)_n$ repeats were amplified and analyzed on a Genetics Analyzer 3730xl (Applied Biosystems, Foster City, California). Nine single nucleotide polymorphisms were genotyped with the SNPstream Genotyping System (Beckman Coulter, Fullerton, California). To ensure the reliability and the reproducibility of the genotyping, roughly 3%-5% of the samples were reanalyzed for each assay. The overall genotyping call rate was 95%, and the concordance rate was >99%.

Table I. General characteristics of the study population (n = 988)

Characteristics	Value
Male sex, n (%)	535 (54.1%)
Feeding, n (%)*	
Breastfeeding	953 (99.1%)
Formula milk	2 (0.2%)
Mix	7 (0.7%)
Delivery mode, n (%)	
Vaginal	518 (52.4%)
Cesarean	419 (42.4)
Forceps	51 (5.2%)
Birth season, n (%)	
Winter (February, March)	117 (11.8)
Spring and Autumn (April, May, September, October)	591 (59.8)
Summer (June, July, August)	280 (28.3)
Birth weight, g, mean ± SD†	3420 ± 430
Gestational age, d, mean ± SD	277 ± 9

*Twenty-six participants had missing data.
†Ninety-three participants had missing data.

Statistical Analyses

Hardy-Weinberg equilibrium for the $(GT)_n$ repeat was performed using an exact test.[17] Hardy-Weinberg equilibrium for other variants were examined by Fisher exact test. Haplotypes and their frequencies were inferred using PHASE version 2.1.1 (http://stephenslab.uchicago.edu/software.html#phase). Haploview (www.broad.mit.edu/haploview/haploview) was used to identify block structures and to calculate $|D'|$ and r^2 to measure linkage disequilibrium (LD).

Daily TcB and daily TcB change according to different genotypes and haplotypes were compared using the Student *t* test and adjusted using ANCOVA. For multiple hypothesis testing, a Benjamini-Hochberg false discovery rate (FDR) was applied to reduce the number of false-positives results and increase the chance of identifying true positives.[18] Furthermore, stepwise linear regression models using a criterion of <0.05 were applied to identify a subset of polymorphisms significantly associated with absolute bilirubin values or changes after adjustment for the effects of other polymorphisms. A partial R^2 value was calculated to denote the proportion of the variation in bilirubin explained by the polymorphism. Candidate covariates in stepwise selection included all of the polymorphism effects and the covariates of sex, feeding, delivery mode, birth season, birth weight, and gestational age. A general (additive) model was assumed for each polymorphism. All analyses were performed with SPSS version 16.0 (SPSS, Chicago, Illinois). A 2-sided *P* value <.05 was considered statistically significant.

Results

TcB Measurements

Daily TcB measurements and daily TcB changes from day 1 to day 7 during the birth hospitalization stay are summarized in Table II. A total of 4154 daily TcB measurements and 3129 daily bilirubin changes were recorded. In general, TcB increased greatly from day 1 to day 3, increased moderately from day 3 to day 5, and then changed little from day 5 to day 7. Average daily TcB peaked on day 5.