Severe Neonatal Hyperbilirubinemia and UGT1A1 Promoter Polymorphism

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Objective To assess whether UGT1A1 promoter polymorphisms associated with Gilbert Syndrome (GS) occur with a greater frequency in neonates with severe hyperbilirubinemia.

Study design In a case-control study performed at a single hospital center in Italy, 70 case subjects with severe hyperbilirubinemia (defined as bilirubin level ≥20 mg/dL or 340 μmol/L) and 70 controls (bilirubin level <12 mg/dL or 210 μmol/L) were enrolled. Both case and control subjects were full term newborns. Polymerase chain reaction analysis on blood spot was performed to determine the frequency of UGTA1A1 promoter polymorphisms in cases and controls.

Results No statistical difference in the prevalence of UGTA1A1 gene variants was found between cases and controls (P = 1). Thirteen infants homozygous for (TA)7 polymorphism associated with GS were in the case group (18.6%) and 14 in the control group (20.0%). A heterozygous group was also equally distributed between cases (44.3%) and controls (42.9%). No (TA)8 repeat was found in the 2 groups.

Conclusions In our study population, GS polymorphism alone does not appear to play a major role in severe neonatal hyperbilirubinemia in neonates without signs of hemolysis. *(J Pediatr 2014;165:42-5)*.

Jaundice occurs in more than 60% of healthy term newborns.[1] Although it is a benign phenomenon in the vast majority of infants, in rare cases, total serum bilirubin (TSB) can reach very high and dangerous concentrations.[2,3] The main feature in neonatal jaundice may be the imbalance between increased bilirubin production and decreased conjugation rates. Bilirubin conjugation is performed by uridine diphosphate-glucuronosyl-transferase, and particularly by the UGT1A1 isoenzyme, which is the main enzyme in bilirubin conjugation in humans.[4] Reduction of UGT1A1 isoenzyme activity, leading to mild intermittent elevation of unconjugated serum bilirubin, is known as Gilbert Syndrome (GS), a common condition occurring in 3%-13% of the adult population.[5,6] Several polymorphisms in the promoter and coding region of the UGT1A1 gene complex that reduce transcription rate of the gene have been described in association with GS. Of these, the (TA)7 allelic variant within the A(TA)nTAA repeat element of the UGT1A1 TATAA box promoter is the most common in the Caucasian population where it has an estimated allelic frequency of 0.387.[5,7,8] In previous studies, gene variants associated with GS have been associated with increased incidence and severity of hyperbilirubinemia in combination with ABO incompatibility and with glucose-6-phosphate dehydrogenase (G6PD) deficiency.[9-11] The role of these gene variants in nonhemolytic jaundice is incompletely defined. At least in theory, causing decreased bilirubin conjugation, GS may enhance the severity of neonatal jaundice in a small, but not negligible group of newborns.

The aim of this study was to assess whether UGT1A1 promoter polymorphisms associated with GS are more frequent in neonates with severe hyperbilirubinemia than in nonjaundiced controls in the absence of signs of hemolysis. We tested the hypothesis that the frequency of subjects with UGT1A1 promoter polymorphism associated with GS, namely (TA)7/(TA)7, would not differ between cases and controls.

Methods

This was a case-control study conducted in the well-baby nursery of a single hospital (Institute of Maternal and Child Health, 'Burlo Garofolo,' Trieste, Italy). The Ethics Committee of the Institute approved the study, and a written informed parental consent was obtained before patient enrollment.

Severe neonatal hyperbilirubinemia was defined as a TSB concentration above 20 mg/dL (340 μmol/L).[3] Inclusion criteria were gestational age ≥37 weeks, birth weight >2500 g, postnatal, and age <7 days. Exclusion criteria were positive Coombs test on cord blood, asphyxia, infection, liver disease, and hemolytic anemia. Patient data included gestational age, birth weight, sex, ethnicity, type of feeding, and percent of weight loss. Case subjects were 70 term infants with TSB above 20 mg/dL enrolled consecutively between November 2004 and July 2008. Control infants were 70

G6PD	Glucose-6-phosphate dehydrogenase
GS	Gilbert Syndrome
TSB	Total serum bilirubin

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healthy term infants with no clinical jaundice or with a TSB <12 mg/dL (210 μmol/L).[12-14]

A convenience sampling approach was used. Both case subjects and controls were assessed for jaundice according to the institutional protocol based on the 2004 clinical practice guidelines by the American Academy of Pediatrics.[12] During the study period, the direct antiglobulin test on cord blood was performed, according to the same protocol, on every newborn baby irrespective of maternal blood type. The decision to start phototherapy was based on the above mentioned American Academy of Pediatrics guidelines. Formula supplementation was given when weight loss exceeded 10% of birth weight or if mothers chose not to breastfeed.

TSB values were measured by direct multiwavelength spectrophotometry (ABL 700, Radiometer, Copenaghen, Denmark) and confirmed by laboratory testing (Modular P; Roche Diagnostic, Milan, Italy) in case subjects and by ABL 700 in controls.

DNA Collection and Analysis

DNA material was isolated from dried blood spot obtained at the time of the routine metabolic screen between 36 and 72 hours of life. UGT1A1 promoter genotype was determined by using the Kit Gilbert Syndrome-FL (Experteam, Margherа-Venezia, Italy). Fluorescent amplicons have been run on the ABI 3100 Genetic Analyzer (Applied BioSystems, Foster City, California) and the size of the amplicons determined by using the software GeneMapper 4.0 (Applied BioSystems). UGT1A1 promoter genotypes were then double checked by polymerase chain reaction amplification (using the following primers, forward 5′-CTACCTTTGTGGACTGACAGCTT-3′; reverse 5′-AGTGGCTGCCATCCACTG-3′) and direct sequencing with the Big Dye Terminators Sequencing kit and a ABI 3100 automatic sequencer (Applied BioSystems). The Gilbert Syndrome-FL kit, can only detect the presence of the (TA)6 and (TA)7 alleles. Because Iolascon et al[15] described the occasional presence of a (TA)8 allele in the Italian population, our results were verified by direct sequencing after polymerase chain reaction amplification with UGT1A1 promoter specific primers. According to the UGT1A1 promoter TATA box genotypes, we divided the study population into 3 groups: homozygous for (TA)7, heterozygous (TA)6/(TA)7, and homozygous for (TA)6 (wild type).

Outcome Measures

The primary outcome was the frequency of the (TA)7 promoter polymorphism of the UGT1A1 gene associated with GS in infants with severe hyperbilirubinemia compared with infants with bilirubin levels <12 mg/dL. Secondary outcomes were peak TSB level and duration of phototherapy in infants with severe hyperbilirubinemia according to the different genotypes.

Statistical Analyses

Categorical data are presented as absolute frequencies and percentages, continuous data are presented as means and SDs. Differences in categorical variables were evaluated using Fisher exact test. Differences in continuous variables between the 2 groups were analyzed with Mann–Whitney nonparametric test. When more than 2 groups were compared, Kruskall–Wallis test was used.

To assess the independent relationship between 1 group and UGT1A1 polymorphisms, a logistic regression model was built taking into account potential risk factors for jaundice. The independent variable has been "case" or "control"; a step down procedure retaining only those variables with a P value of <.05 was used. UGT1A1 genotype was forced into the model. A P value of <.05 was considered to be statistically significant. Data were analyzed in STATA11 software (StataCorp, College Station, Texas).

Results

Case subjects (n = 77) defined as infants with TSB > of 20 mg/dL and 80 controls were enrolled in the study. Of the patients enrolled as case subjects, 5 were excluded because of incomplete data collection and 2 were excluded because blood samples were not adequate for DNA analysis. Of the 80 infants in the control group, 10 subjects were excluded: 3 of them developed TSB >12 mg/dL and 7 had incomplete data collected. A total of 140 newborns were evaluated for statistical analyses. Basic demographic characteristics of cases and controls are summarized in Table I. There were no differences between case subjects and control subjects with respect to birth weight and sex. Only Caucasian infants were observed. Mean age was 38.8 (±1.2) weeks for the case infant group and 39.2 (±1) weeks for the control infant group.

Hyperbilirubinemic Cases vs Controls

The frequencies of different genotypes in the 2 groups are reported in Table II. The proportion of infants with UGT1A1 promoter variant genotypes associated with GS, namely $(TA)_7/(TA)_7$, in case subjects was not statistically different from the proportion in control subjects (18.6 % vs 20%; $P = 1$). The frequency of $(TA)_7$ allele did not differ between

Table I. Basic characteristics of study groups

	Cases (70)	Controls (70)	*P* value
Sex†			
N (%)			
Female	28 (40%)	33 (47%)	.49
Male	42 (60%)	37 (52%)	
Birth weight,* g	3370 (2600-4250)	3290 (2560-4250)	.116
Median (IQR)			
Maximum TSB level mg/dL	21.3 (±1.37)	8.69 (±2.43)	<.01
Mean (SD)			
Weight loss, % BW*	7.5 (6.3-9.0)	6.7 (5.3-8.0)	.009
Median (IQR)			
Feeding modalities†			
N (%)			
Breast fed	33 (47%)	49 (70%)	.012
Breast and formula	36 (51%)	20 (28%)	
Formula fed	1 (1%)	1 (1%)	

BW, birth weight.
*Mann–Whitney.
†Fisher exact test.