The Role of UGT1A1*28 Mutation in Jaundiced Infants with Hypertrophic Pyloric Stenosis

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ABSTRACT

Hypertrophic pyloric stenosis (HPS) may be accompanied by jaundice, a condition referred to as the icteropyloric syndrome (IPS). It has long been suspected that the etiology of IPS is an early manifestation of Gilbert's syndrome (GS). Clinical features common to both GS and IPS include jaundice precipitated by fasting and improved with feeding. Prevalence of jaundice in HPS is similar to that of clinically apparent GS in the general population. Discovery of a mutation in the promoter region of the bilirubin uridine diphosphate glucuronosyl transferase gene (UGT1A1*28) as the most common cause of GS has provided a tool to determine the role of GS in IPS. The aims of this study were to determine 1) the prevalence of IPS in a large group of infants with HPS, 2) whether disease severity contributed to the manifestation of IPS, and 3) whether GS played a role in IPS. Radioactive PCR and sequencing were used to determine the presence of UGT1A1*28 mutations. We determined a prevalence

of IPS of 14.3% in HPS. Infants with IPS had significantly higher levels of alkalosis than infants with HPS alone. GS mutations were 4-fold higher in IPS (43.8%) than HPS (10.7%). In conclusion, the frequency of jaundice in HPS is similar to that of clinically apparent GS in the general population. Manifestation of IPS results from a more severe degree of metabolic disturbance and the presence of GS mutations. (*Pediatr Res* 58: 881-884, 2005)

Abbreviations

GS, Gilbert's syndrome

HPS, hypertrophic pyloric stenosis

IPS, icteropyloric syndrome

UDP-GT, uridine diphosphate glucuronosyl transferase

UGT1A1, bilirubin uridine diphosphate glucuronosyl transferase gene

HPS is a common cause of neonatal bowel obstruction, occurring in 1:150 males and 1:750 females. No genetic factor has been identified, although familial incidence occurs in approximately 15%. Some infants with HPS develop jaundice; an association referred to as the IPS (1). The prevalence of IPS ranges from 2% to 8% of infants with HPS (2,3). It has long been suspected that infants with IPS probably represent an early manifestation of GS (4,5). In 1995, a genetic mutation in the promoter region of the human bilirubin uridine diphosphate glucuronosyl transferase gene (UGT1A1) was found to be associated with GS. The defect was the insertion of an extra TA repeat within the TAATA box (UGT1A1*28) (6). Subsequently, other defects in the coding region of the gene have been described (7). Discovery of these genetic mutations has improved the ability to identify GS among suspected individuals.

To date, no controlled study has been done to determine the prevalence of GS in IPS. As such, the role of illness severity as

a contributing factor has not been addressed. A single small study documented the genetic mutation for GS in 3 children with a history of IPS, whereas 10 infants with HPS alone had normal alleles for UGT1A1 (8). Not everyone with the genetic mutation for GS develops clinically apparent jaundice. The prevalence of the genetic mutation for GS in the general population ranges from 10% to 19% (6,9-12), whereas clinically apparent GS ranges from 3% to 10% (13,14). Thus, for GS to be manifest clinically, there must be some complicating factor in addition to a genetic mutation. In infants with IPS the complicating factor could be the degree of stress associated with dehydration, alkalosis, starvation, and/or hypoglycemia. The aims of this study were to determine 1) the prevalence of IPS in infants with HPS in our institution, 2) the role of illness severity in the etiology of IPS, and 3) the role of the common genetic mutation for GS (UGT1A1*28) in the etiology of IPS.

PATIENTS AND METHODS

A computer search was conducted to identify all the children having the hospital discharge diagnosis of hypertrophic pyloric stenosis (ICD 750.5) at the Blair E. Batson Hospital for Children at the University of Mississippi Medical Center from January 1990 through August 2000. A retrospective chart review was conducted and the following demographics were collected: sex, race, and

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age at time of surgery (Ramstedt pyloromyotomy). Also, the following laboratory values from the initial presentation to the hospital were recorded for each patient: serum sodium, potassium, bicarbonate, chloride, glucose, total bilirubin, and venous pH. After August 2000, patients with HPS were identified prospectively. Similar data were collected on these individuals.

Jaundice was defined as a total bilirubin >1.3 mg/dL, and jaundiced infants with HPS served as the study group (denoted IPS). Control subjects were recruited from nonjaundiced infants with HPS (denoted HPS). Subjects identified retrospectively were sent letters explaining the study and requesting that a single blood sample be given. Blood was collected in a tube with sodium-heparin. Samples from prospectively identified patients were collected from residual blood collected as part of their initial evaluation. DNA was extracted from blood samples using a commercial kit (QIAamp DNA Blood Minikit, QIAGEN, Valencia, CA). Radioactive PCR was used to amplify fragments of the promoter region of UGT1A1 gene by the method of Monaghan *et al.* (10). Briefly, amplifications were performed in a 50- μ L reaction mixture containing 0.2 mM dNTPs, 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5 mM MgCl₂, 0.5 mM of each primer, 2.5 unit of Tag polymerase (Promega, Madison, WI), and DNA sample (0.5 μ g). PCR was performed using a thermocycler (GeneAmp PCR System 2400, PerkinElmer, Norwalk, CT) with the following conditions: 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 58°C for 40 s and 72°C for 40 s, followed by annealing at 72°C for 7 min, then dwelling at 4°C. The primer pair used (forward, 5'-AAG TGA ACT CCC TGC TAC CTT-3'; reverse, 5'-CCA CTG GGA TCA ACA GTA TCT-3') resulted in PCR products ranging from 96 bp for the (TA)₅ repeat to 102 bp for the (TA)₈ repeat. One primer was 5' end-labeled with (γ -³²P) ATP (Amersham Biosciences, Piscataway, NJ). The resulting PCR products were resolved on a 5% sequencing gel, exposed to phosphor screen overnight, then read by a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA). Additionally, sequencing was done on random samples (60% of the samples) to confirm the PCR results.

The study was approved by the Institutional Review Board at the University of Mississippi Medical Center. All individuals with jaundice were invited to participate in the study. Control subjects consisted of children with HPS without jaundice that were matched for age, gender, race, form of feeding, and disease severity. Informed consent was obtained from parents before obtaining blood for the purpose of DNA extraction and mutational analysis.

Statistics. To compare the proportions for race, gender, and breast feeding in the two groups a χ^2 analysis was used in the initial, larger comparison of all the patients; Fisher's exact test was used to compare the same demographics in the subset of patients that had genotyping performed. Because of suspected departures from normality, the distributions of laboratory values between the two groups were analyzed using a Mann-Whitney statistic. Due to the small sample sizes, the exact *p* value for the Mann-Whitney test was reported for the subset analysis. A *p* value < 0.05 was considered statistically significant. All values are expressed as average $\pm$ SD unless specified otherwise.

RESULTS

A total of 212 patients were identified in the retrospective review, of which 191 charts (91%) were available for review. Forty-six patients were identified prospectively, thus the total number of patients studied was 237. Overall, 80.6% were male and 63.3% were white. Jaundice was identified in 34 patients (14.3%). Comparisons between infants with IPS and HPS are recorded (Table 1). Infants with IPS were younger (5.0 ± 1.9 wk) than infants with HPS (5.9 ± 2.9 wk, *p* = 0.04). There were no differences in the gender or race between the groups. Laboratory values showed no difference in serum sodium, potassium, glucose, or venous pH between the two groups. IPS subjects had higher serum bicarbonate (29.2 ± 6.1 mEq/L) and lower chloride (95.2 ± 12.4 mEq/L) than the HPS group (26.3 ± 5.2 mEq/L and 98.6 ± 8.2 mEq/L, respectively).

Blood for mutational analysis was obtained from 17 infants with IPS and 30 age-, gender-, race-, breast fed-, and disease activity-matched HPS subjects. Comparison of demographics and laboratory values between these subgroups is found in Table 2.

The various genotypes found in each group are shown in the Figure 1. There was a 100% correlation between the PCR

Table 1. Demographic data and laboratory results in infants with HPS with or without jaundice

	Jaundiced (n = 34)	Non jaundiced (n = 203)	<i>p</i> Value
Male	82.4%	80.3%	NS
Caucasian	50.0%	65.5%	NS
Age at surgery (wk)	5.0 ± 1.9	5.9 ± 2.9	0.04
Total bilirubin (mg/dL)	6.2 ± 4.5	0.8 ± 0.3	<0.001
Na (mEq/L)	137 ± 5.5	138 ± 3.8	NS
K (mEq/L)	4.9 ± 1.3	4.8 ± 1.0	NS
CO ₂ (mEq/L)	29 ± 6.5	26.0 ± 4.9	0.004
Cl (mEq/L)	95.0 ± 12.6	99.0 ± 8.4	0.024
Venous pH	7.20 ± 0.40	7.10 ± 0.40	NS
Glucose (mg/dL)	82.3 ± 3.4	84.3 ± 1.4	NS

Table 2. Comparison of demographic data and laboratory results in infants tested for the presence of GS by mutational analysis

	Jaundiced (n = 17)	Nonjaundiced (n = 30)	<i>p</i> Value
Male (%)	76.5	73.3	NS
Caucasian (%)	52.9	66.7	NS
Breast-fed (%)	18.2	6.7	NS
Age at surgery (wk)	4.9 ± 1.6	6.1 ± 3.4	NS
Total bilirubin	7.0 ± 5.2	0.9 ± 0.2	< 0.001
Na (mEq/L)	137.9 ± 6.3	137.9 ± 4.1	NS
K (mEq/L)	5.1 ± 1.3	4.8 ± 0.8	NS
CO ₂ (mEq/L)	27.9 ± 5.2	27.9 ± 5.0	NS
Cl (mEq/L)	97.1 ± 11.3	98.6 ± 8.6	NS
Venous pH	7.43 ± 0.12	7.37 ± 0.10	NS
Glucose (mg/dL)	79.4 ± 13.2	86.3 ± 31.5	NS

results and the sequencing. In addition to the common alleles TA₆ and TA₇, we also found the less common alleles TA₅ and TA₈. As discussed below, GS genotypes include TA₇/TA₇, TA₇/TA₈, and TA₈/TA₈. Non-GS genotypes include TA₆/TA₆ and TA₆/TA₇. Other less commonly found genotypes (TA₅/TA₆ and TA₇/TA₅) were not included in the final comparison because their contribution to GS has not been proven. In this study, mutations for GS were found in 43.8% of infants with IPS and 10.7% of infants with HPS. Results of sequencing were consistent with those of PCR.

DISCUSSION

Jaundice complicating HPS has been recognized for nearly 50 y (15), although the pathogenesis remained uncertain until relatively recently. Initially, it was speculated that jaundice in HPS was due to dehydration, mechanical obstruction, decreased carbohydrate intake, decreased hepatic perfusion, or a combination of the aforementioned mechanisms (16). In the early 1970s, two separate studies determined the activity of UDP-GT in liver biopsies of children with pyloric stenosis with or without jaundice; both showed a significant decrease in hepatic UDP-GT activity in jaundiced infants (2,17). The decreased activity was proposed to be due to a maturational delay in the expression of UDP-GT (2). In the early 1980s, GS was proposed as the reason for diminished enzyme activity in IPS (18).

In this series of more than 200 infants requiring pyloromyotomy for hypertrophic pyloric stenosis, 14.3% were jaundiced.

Figure 1. Representative gel of PCR demonstrating the genotypes found in infants with hypertrophic pyloric stenosis with or without jaundice. The figure depicts representative genotypes for infants with HPS and hypertrophic pyloric stenosis and jaundice (IPS). Controls consisted of an adult with known GS and an adult with no history of jaundice. PCR in both individuals was confirmed with sequencing. The markers are for 96 bp to 100 bp products. Mutation is included to show one of the less commonly found genotypes. In this study, four different alleles varying in the number of TA repeats from five to eight. The percentage of the various genotypes found are reported above the samples. In addition, the IPS group had one patient each with a 7/5 genotype (5.9%) and a 7/8 genotype (5.9%), whereas the HPS group had one patient each with a 5/6 genotype (3.3%) and a 7/5 genotype (3.3%).

The higher prevalence of jaundice in our study is explained by defining jaundice at a lower level of bilirubin (1.3 mg/dL) than previous studies (2 mg/dL) (2,17,18). Our rationale was to include all infants that were >2 SD above the mean, increasing the sensitivity of identifying all infants with GS. If jaundice were defined as 2 mg/dL as in previous studies, the prevalence in our study (10.5%) would be more in line with those previous studies (2–8%) (2,3).

In the present study, infants with IPS were younger than infants with HPS, suggesting a possible role for physiologic jaundice. Yet, in physiologic jaundice, bilirubin typically reaches a peak value on the third day of life, decline relatively rapidly by the fifth day of life, then slowly decline to adult levels by the second week of life (19). The duration is affected by the gestational age of the infant, being more prolonged in preterm infants (19). The role of GS in prolonged physiologic jaundice has been studied and shows mixed results. In one study, jaundiced neonates with a GS genotype had a slower decrease in bilirubin levels than jaundiced infants with a non-GS genotype (20). However, in two other studies, GS genotypes were not associated with prolonged physiologic jaundice (21,22). Although prolonged physiologic jaundice could represent an early manifestation of GS, it does not persist into the second month of life and therefore could not explain the difference in jaundice between infants with HPS and IPS in this study.

What has not been previously addressed is the role of illness severity as a contributing factor to jaundice in pyloric stenosis. In the present study, a number of biochemical markers were assessed to determine whether degree of illness contributed to the development of jaundice. No difference was seen between the groups comparing serum levels of glucose, sodium, and potassium. However, jaundiced infants had significantly higher serum bicarbonate and lower serum chloride levels than non-jaundiced infants. These differences suggest that infants with IPS experienced greater losses of hydrochloric acid, possibly due to more severe or more prolonged vomiting. Interestingly, the degree of alkalosis as measured by venous pH was no different between the two groups. This discrepancy between serum bicarbonate levels and venous pH is likely explained by respiratory compensation. Although a difference was noted in the serum bicarbonate between the two groups, there was not a correlation between level of serum bilirubin and bicarbonate.

Thus, it may be that metabolic stress potentiates the manifestation of jaundice in children with IPS. Yet, in addition to metabolic stress, other contributing factors have a role in the phenotypic expression of GS. Potential factors include hepatic levels of residual enzyme activity, bilirubin load, and duration of fasting.

Discovery of a genetic marker for GS has improved the diagnostic ability of researchers. Individuals can be classified as having GS genotypes or non-GS genotypes based on the number of TA repeats in the promoter region of the UGT1A1 gene. Non-GS genotypes include the wild-type (TA_6/TA_6) and the TA_6/TA_7 heterozygous state; GS genotypes include TA_7/TA_7 , TA_7/TA_8 , and TA_8/TA_8 (9). Despite being considered a non-GS genotype, TA_6/TA_7 heterozygous state has UDP-GT activity intermediate between that of the TA_6/TA_6 and TA_7/TA_7 homozygous states (23). The mean serum bilirubin level in the TA_6/TA_7 heterozygotes is higher than that of TA_6/TA_6 and lower than that of TA_7/TA_7 (6,24). There are limited and conflicting data regarding the heterozygous states TA_6/TA_5 and TA_7/TA_5 . *In vitro* experiments using a reporter gene in hepatoma cells showed an inverse relationship between the number of TA repeats and the activity of the promoter (9), implying that a TA_6/TA_5 genotype would have normal-to-low serum bilirubin levels. However, the TA_6/TA_5 genotype has been associated with mild hyperbilirubinemia in a previous study (21) and no evidence of jaundice in our series. Theoretically, the TA_7/TA_5 genotype contains one allele associated with increased serum bilirubin (TA_7) and a second allele associated with decreased serum bilirubin (TA_5), each allele possibly negating the effect of the other (9). In a previous study, TA_7/TA_5 was associated with jaundice (21), whereas in our study it was seen both in an infant with jaundice and an infant without jaundice. The number of individuals described with these genotypes is too small to determine whether or not they represent GS genotypes. Of interest, the TA_5 and TA_8 alleles have previously only been described in individuals of African descent (9). However, in our series, the TA_7/TA_5 genotype was found in two Caucasian infants; the TA_6/TA_5 and TA_7/TA_8 genotypes were found in African American infants.

In the general population, the prevalence for genetic mutation of GS is estimated to be 10–19% (6,9–12), whereas clinically apparent GS ranges from 3% to 10% (13,14). Thus,

manifestation of GS requires an additional factor that increases bilirubin load, impairs hepatic uptake of bilirubin, or impairs efficiency of glucuronidation (6). Classical teaching is that GS is first clinically apparent following puberty, however, more recent data would suggest manifestation in the neonatal period. Infants with GS genotypes have a more rapid rise in transcutaneous jaundice index over the first 2 d of life than infants with normal genotypes. Despite the more rapid rise, the maximum bilirubin index is no different in GS and normal infants (12).

In our study, we controlled for age, gender, race, disease severity, and breast-feeding, when comparing the prevalence of GS genotypes in IPS and HPS. Controlling for the age of the patients when assessing the genotypes, any contribution from physiologic jaundice was removed. Controlling for race removed the differences in bilirubin levels seen among the various races. Controlling for disease severity removed the effect of metabolic derangement in the manifestation of GS. Controlling for breast-feeding when assessing the genotypes negated any effect it may have on unmasking GS. A GS genotype was found in 10.7% of the infants with HPS alone, within the known range for the general population. A GS genotype was found in 43.8% of infants with IPS. A previous study documented the presence of GS genotypes in 67% of individuals with IPS and a normal genotype in all the HPS subjects (8). However, it was a small study (3 IPS, 10 HPS) and did not control for demographic differences or illness severity (8).

In our series, the risk of having a GS genotype was 4.1-fold higher in IPS than HPS. These results suggest that GS plays a role in infants with IPS. The mechanism accounting for the phenotypic manifestation of GS cannot be stated with certainty, however, an attractive theory is that persistent vomiting in HPS is in essence a "forced fast." This is similar, but to a greater extent, to the overnight fast used to clinically diagnose GS. The reason for jaundice in those infants with IPS and non-GS genotypes remains uncertain. Possibly the true prevalence of GS was underestimated by only testing for the most common genetic abnormality accounting for GS. However, abnormalities in the promoter region of the UGT1A1 gene account for the vast majority of GS (6,9).

In conclusion, the prevalence of IPS was 14.3% for all infants with HPS seen over a 13-y period at our institution. It appears that severity of metabolic stress, as measured by elevated bicarbonate and decreased chloride, plays a role in the manifestation of jaundice in infants with HPS. When all demographic data and biochemical tests are controlled for, the risk of having a mutation for GS is 4.1-fold higher in IPS than HPS. These data support the notion that GS is in part responsible for IPS and further supports the concept that GS can present during infancy if significant physiologic stress is experienced.

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