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Gilbert's syndrome and jaundice in glucose-6-phosphate dehydrogenase deficient neonates

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ABSTRACT

Background and Objective. The pathogenesis of the hyperbilirubinemia present in approximately 30% of neonates affected by glucose-6-phosphate dehydrogenase deficiency is an unsolved problem. We evaluated the effect of Gilbert's syndrome, the most common defect of bilirubin conjugation, on the hyperbilirubinemia of these neonates.

Design and Methods. One hundred and two neonates affected by glucose-6-phosphate dehydrogenase deficiency were enrolled in this study: 56 had hyperbilirubinemia and 46 had normal bilirubin levels. The analysis of the $A(TA)_nTAA$ motif in the promoter region of the UGT1A gene was performed by means of PCR, followed by separation on 6% denaturing polyacrylamide gel.

Results. The frequency of the three different genotypes of the $A(TA)_nTAA$ motif was similar in the study and control groups. Our results demonstrated no difference in the percentage of homozygotes for the UGT1A (TA)7 variant associated with Gilbert's syndrome.

Interpretation and Conclusions. These findings indicate that Gilbert's syndrome does not account for the hyperbilirubinemia occurring in some neonates with glucose-6-phosphate dehydrogenase deficiency. Furthermore our results suggest that hemolysis is not the major event in the pathogenesis of hyperbilirubinemia in these patients.

Key words: hyperbilirubinemia, Gilbert's syndrome, neonates, glucose-6-phosphate dehydrogenase deficiency, uridine diphosphate glucuronosyltransferase

Glucose-6-phoshate dehydrogenase (G6PD) deficiency is an X-linked genetic abnormality affecting over 200 milion people worldwide. Clinical manifestations of G6PD deficiency are much more common in male homozygotes than in females homozygotes. The condition is usually asymptomatic but ingestion of or contact with fava beans, drugs or chemicals, or bacterial or viral infections may cause hemolytic anemia.[1,2] G6PD deficiency is frequently associated with neonatal hyperbilirubinemia[2,3] and sometimes kernicterus, often in the absence of any trigger or hematologic evidence of hemolysis.[4-6]

The pathogenesis of jaundice in G6PD deficient neonates remains unclear, because increased hemolysis is not always a major factor in its development.[7] Alternative mechanisms for this neonatal hyperbilirubinemia, such as decreased hepatic bilirubin conjugation, have been proposed.[8,9] This hypothesis has recently been confirmed by the demonstration of a decreased di-conjugated bilirubin fraction in many of the G6PD-deficient newborns,[10] and lower serum bilirubin values in infants who received phenobarbital either antenatally[11] or postnatally.[8]

Recently, the molecular bases of Gilbert's syndrome, a chronic mild form of unconjugated hyperbilirubinemia, were elucidated as being the result of molecular alterations in one specific isoform of the UDP-glucuronosyltransferase (UGT1A) gene.[12,13]

The UGT1 gene, which maps in the 2q37 region, is composed of a unique exon 1, specific for a single isoenzyme, and four common exons.[14] Of the two isoforms reported,[14,15] only UGT1A contributes substantially to bilirubin glucuronidation.[16]

The most common genetic alteration of UGT1A is a polymorphic variation in the $A(TA)_nTATAA$ element within the upstream promoter region of the UGT1A gene.[12,17,18] Many patients with Gilbert's syndrome have been found to be homozygous for two extra bases (TA) in the repetitive TATA-box of the gene promoter[12,13] which normally consists of six repeats. The presence of this longer TATAA element $(TA)_7$ causes less efficient binding of transcription regulatory proteins and hepatic glucuronidation activity is reduced to about 30% of normal.

The homozygous form of this $(TA)_7TAA$ element is expressed in 10-16% of the normal population, and is usually associated with high serum bilirubin levels.[12,13] Missense mutations in the UGT1A gene have also been demonstrated.[17,18]

The aim of our study was to analyze whether the

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$(TA)_7$TATA box variant of UGT1A gene plays a role in the pathogenesis of neonatal hyperbilirubinemia in G6PD deficient infants.

Design and Methods

We examined 102 male neonates originating from the same geographic area of Sardinia, in which there is a known high incidence of G6PD deficiency. Babies found to be enzyme deficient through a screening program had a confirmatory quantitative test performed. All the subjects had the Mediterranean variant of G6PD deficiency. Only healthy full-term infants free of any factors known to exacerbate jaundice, such as cephalohematoma, maternal diabetes, positive Coombs' test result or sepsis, were included in this study.

Serum total bilirubin was measured from capillary heel-stick blood in the jaundiced G6PD deficient neonates. The direct bilirubin fraction was not routinely estimated unless clinically indicated. For the purpose of this study, physiologic jaundice was defined as a total serum bilirubin level up to 13.9 mg/dL (230 mM/L) after the first day of life, in the absence of any identifiable risk factor.

According to the bilirubin level the examined G6PD deficient neonates were divided into two groups: the study group comprised 56 subjects with evidence of neonatal jaundice, whereas the control group (46 subjects) was composed of similar neonates without neonatal hyperbilirubinemia.

The average bilirubin level in the study group was 16±1.62 mg/dL, whereas in the control group it was 6.8±3.2 mg/dL.

Genomic DNA was obtained by standard methods from peripheral blood leukocytes.[19] The analysis of the $A(TA)_n$ TAA motif in the promoter region of the UGT1A gene was performed by means of PCR with the primers described by Bosma, followed by separation of the amplified products on 6% denaturing polyacrylamide gel.

Results

A total of 102 G6PD deficient infants were enrolled in this study: 56 were jaundiced and 46 were not.

According to the autosomal recessive inheritance of Gilbert's syndrome two groups of subjects were identified: the first was composed of 88 infants with either the 6/6 genotype [homozygous for the common allele bearing the $(TA)_6$TAA sequence] or the 6/7 genotype (heterozygous). The second group was composed of 14 individuals with the 7/7 genotype (homozygous for the rarer allele with the $(TA)_7$TAA sequence (Table 1). The frequency of the three different genotypes of the A(TA)nTAA motif was similar both for the study and control group. In the 46 unjaundiced control infants, one subject with the $(TA)_7/(TA)_7$ genotype had a significantly higher bilirubin level (6 mg/dL) than that of subjects with $(TA)_6/(TA)_7$ or $(TA)_6/(TA)_6$ genotypes.

Table 1. Correlation between G6PD deficiency and length of TATA element in the promoter region of UGT1 gene.

	UGT1 promoter		
	$(TA)_6/(TA)_6$	$(TA)_6/(TA)_7$	$(TA)_7/(TA)_7$
Jaundiced G6PD-deficient infants	26/56 (46%)	24/56 (43%)	6/56 (11%)
Unjaundiced G6PD-deficient infants	19/46 (41.5%)	19/46 (41.5%)	8/46 (17%)

The bilirubin values of the heterozygotes with the expanded $A(TA)_n$TAA motif were in the range of those found in neonates with the $(TA)_6/(TA)_6$ genotype.

Discussion

The incidence and intensity of neonatal jaundice in various populations with G6PD deficiency differ.[20] These findings suggest interaction of additional genetic or exogenous factors with G6PD deficiency, predisposing certain neonates to neonatal hyperbilirubinemia and conversely protecting others.

In one report from northern Sardinia neonatal jaundice occurred in 29.9% of G6PD deficient male neonates, in 25% of homozygous G6PD deficient female neonates and in 14.5% of heterozygous G6PD deficient female newborns.[21]

The etiology of this kind of jaundice is obscure. Acute hemolytic anemia with neonatal jaundice may be triggered by direct exposure to hemolytic agents such as naphthalene,[20] or, in the case of breastfed infants whose mothers eat fava beans, via breast milk,[22] or even, as reported in one case, by transplacental passage of divicine and isouramile from a mother who ingested fava beans five days before her child's delivery.[23] However, even in the absence of all known triggers, jaundice can occur. An alternative factor influencing the development of jaundice may be defective glucuronidation of bilirubin in the liver. Since serum bilirubin level is dependent on the rate of bilirubin production minus the rate of bilirubin excretion, it seemed useful to study the contribution of bilirubin conjugation to the pathogenesis of the G6PD deficiency-associated neonatal jaundice.

Physiologically the enzyme bilirubin uridine diphosphate glucuronosyltransferase catalyzes the transfer of glucuronic acid to the bilirubin molecule to form bilirubin monoglucuronide and subsequently, diglucuronide. In conditions of partial enzyme deficiency, such as Gilbert's syndrome[24] or in situations in which the bilirubin load is high relative to the conjugating capacity[25] decreased serum concentrations of diglucuronide have been found.[7]

Gilbert's syndrome is a benign unconjugated hyperbilirubinemia characterized by episodes of mild intermittent jaundice. It is the most common inherited

disorder of hepatic bilirubin metabolism, occurring in 2-13% of the general population.[12,13] A correlation between homozygosity for a 2 bp insertion in the promoter region of the UDP-glucuronosyltransferase 1 (UGT1) gene and Gilbert's syndrome has been demonstrated. However, some individuals with normal bilirubin levels have also been found to be homozygotes for the $(TA)_7$ motif, indicating that this element is necessary but not always sufficient for the production of hyperbilirubinemia. Other inherited or acquired factors affecting bilirubin metabolism may cause the hyperbilirubinemia of Gilbert's syndrome.

Gilbert's syndrome has been related to hyperbilirubinemia in heterozygous β-thalassemia[26] and adult subjects affected by G6PD deficiency.[27] This latter report investigated whether the variation of the $A(TA)_nTAA$ motif in the promoter region of the UGT1A gene could contribute to the pathogenesis of neonatal jaundice in G6PD deficient infants. The results of our study indicate that homozygosity for the UGT1A $(TA)_7$TATA box variant did not correlate with neonatal hyperbilirubinemia or neonatal jaundice. These findings suggest that the presence of Gilbert's syndrome does not usually play a role in the pathogenesis of neonatal hyperbilirubinemia in G6PD deficient neonates.

We recently demonstrated that jaundice in newborn infants affected by hereditary spherocytosis (HS)[28] is exacerbated by the interaction between hemolysis of spherocytes and the genetic variation in bilirubin UGT1A gene promoter associated with Gilbert's syndrome.[29] The lack of this synergistic effect in the neonates with G6PD deficiency and Gilbert's syndrome strongly suggests, in accordance with previously reported data,[5] that increased bilirubin production due to hemolysis is not a frequent event in these patients.

A similar study performed on Sephardic Jewish neonates demonstrated an interaction between the variant UGT1 promoter and G6PD deficiency in producing elevated bilirubin levels. The authors concluded that this was the consequence of the combined effect of an increased bilirubin load, due to excessive hemolysis, and decreased bilirubin conjugation.[30] These completely contrasting data might be, at least partially, explained by the extremely different genetic makeup of the populations investigated. We examined Sardinian subjects whereas Kaplan studied a particular population which has lived for 2000 to 2500 years in relative isolation, with consequent marked consanguinity.[6] The different genetic background may also explain the finding of a higher percentage of jaundice in Sardinian G6PD deficient neonates than in Sephardic Jewish ones (50% versus 23%).

In Sardinian G6PD deficient neonates, jaundice might be the result of impaired liver function due to hepatic enzyme deficiency, as suggested by Beutler,[2] rather than being a manifestation of accelerated erythrocyte destruction.

Contributions and Acknowledgments

AI formulated the design of the study, and analyzed and interpreted the data. MFF did some of the DNA assays and wrote the paper. SP took part in the DNA assays. GFM and GR took part in assessment of patients. EM analyzed the clinical data and commented on the draft of the paper.

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Disclosures

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Manuscript processing

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