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Assessment of UGT Polymorphisms and Neonatal Jaundice

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Elevation of the serum bilirubin level is a common, if not universal, finding during the first week of life. This can be a transient phenomenon that resolves spontaneously or can signify a serious or even life-threatening condition. There are many causes of hyperbilirubinemia and related therapeutic and prognostic implications. The diseases in which there is a primary disorder of the metabolism of bilirubin will be reviewed regarding their clinical presentation, pathophysiology, diagnosis, and treatment. These disorders—Gilbert's syndrome and Crigler-Najjar Syndrome—both involve abnormalities in bilirubin conjugation secondary to deficiency of bilirubin uridine diphosphate glucuronosyltransferase. The purpose of this article is to review the current understanding of the genetic polymorphisms that result in these diseases and discuss recent advances in diagnosis and treatment.
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Genetic considerations are very important regarding neonatal jaundice. Whether the jaundice is mild (Gilbert's syndrome) or severe (Crigler–Najjar syndrome), there are polymorphisms of the gene encoding bilirubin glucuronosyltransferase that may be related to the jaundice. The purpose of this article is to review the current understanding of uridine diphosphate glucuronosyltransferase (UGT) polymorphisms as they relate to neonatal jaundice.

Gilbert's Syndrome

Clinical Presentation

Gilbert's syndrome, (GS, OMIM #143500) first described in 1901 by Gilbert and Lereboullet,[1] is characterized by a hereditary chronic or recurrent, mild unconjugated hyperbilirubinemia with otherwise-normal liver function tests (for reviews, see Odell and Gourley[2]; Watson and Gollan[3]; Berk and Noyer[4]; and Bergeron and Gourley[5]). The serum unconjugated bilirubin elevation usually ranges from 1 to 4 mg/dL (17 μmol/L = 1 mg/dL). Frequently, patients are identified when an elevated serum bilirubin is found on screening blood chemistry or mild jaundice (perhaps only scleral icterus) is noted during a period of fasting associated with a nonspecific viral illness.[6] GS is generally associated with no negative implications for health or longevity and may be inherited in either an autosomal dominant[7-10] or recessive[11] fashion.

Although GS is a congenital disorder, it rarely becomes clinically apparent until after puberty. The reasons for this are unknown but have been suggested to be related to the hormonal changes of puberty. Steroid hormones can suppress hepatic bilirubin clearance. Odell[12] speculated that some infants with nonhemolytic neonatal jaundice are manifesting GS. Use of genetic markers (see below section "Pathophysiology") has allowed the investigation of the role GS plays in neonatal jaundice. Individuals carrying such markers have been shown to have a more rapid increase in their jaundice levels during the first 2 days of life,[13] a predisposition to prolonged or severe neonatal hyperbilirubinemia,[14-16] variably increased jaundice when the GS polymorphism occurs with pyloric stenosis[17,18] or is coinherited with hematological abnormalities, such as glucose-6-phosphate dehydrogenase deficiency,[19,20] beta-thalassemia,[19,21-23] or hereditary spherocytosis.[24] Thus, studies from several different parts of the world indicate that GS, as detected by *UGT1A1* analysis, does play some role in neonatal jaundice. Kaplan et al[20] noted that, in their study, neither G6PD deficiency nor the GS type UDPGT1 promoter polymorphism (also known as UGT1A1*28) alone, increased the incidence of hyperbilirubinemia, but both in combination did. They speculated that this gene interaction may serve as a paradigm for the interaction of benign genetic polymorphisms in the causation of

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disease, ie, it may take 2 genetic abnormalities to produce disease symptoms, as shown in subsequent studies.[25,26]

Pathophysiology

GS is a heterogeneous group of disorders, all of which share at least a 50% decrease in hepatic bilirubin UGT activity.[27-30] Some individuals with GS have delayed uptake of bilirubin into the hepatocyte, others have delayed biotransformation, and others demonstrate both abnormalities.[31-34] Immunohistochemical staining for UGT shows a clear reduction throughout the hepatic lobule in specimens from individuals with GS, when compared with normal controls.[35]

The elucidation of the structure of the *UGT1* gene, which encodes human bilirubin, phenol, and other UDP-glucuronosyltransferase isozymes,[36,37] led to the discovery of *UGT1A1* mutations or polymorphisms associated with GS. In white populations, the homozygous finding of an additional TA repeat in the promoter region or, so called TATA box, ie, $(TA)_7TAA$, rather than $(TA)_6TAA$, of the *UGT1A1* gene has been shown to be a necessary, although not sufficient, condition for GS.[38-40] Individuals who are heterozygous for 7 TA repeats have significantly greater serum bilirubin levels than the homozygous wild type 6 repeats.[38] In Asian populations, the $(TA)_7TAA$ mutation is relatively rare,[41] but several different *UGT1A1* mutations have been associated with GS.[10,42,43] These Asian mutations involve exon 1 of the *UGT1A1* gene, rather than the TATA promoter region. One of the most common mutations in Asians, a Gly71Arg mutation in exon 1 (also known as UGT1A1*6), has also linked GS and severe neonatal hyperbilirubinemia.[15] It has been reported that although with white subjects the promoter TA repeat number and bilirubin level are strongly positively correlated, in other ethnic groups, eg, Africans, in whom 2 other variants, $(TA)_5$ and $(TA)_8$, have been identified, there is a negative correlation.[44] Rarely the $(TA)_8$ variant has been reported in white patients[45]; thus, the ethnic implications of these genetic polymorphisms of the *UGT1A1* gene require further analysis.

Diagnosis and Treatment

Generally, GS can be diagnosed when there is a mild, fluctuating unconjugated hyperbilirubinemia, the rest of the liver function tests are normal, and there is no hemolysis. Hemolysis can add confusion because it can result in similar findings and it is not unusual in GS. Hence other tests are sometimes used to aid in diagnosis.

One diagnostic test involves the intravenous administration of nicotinic acid (niacin) with assessment of the subsequent rise in serum bilirubin concentration.[46] Nicotinic acid is usually administered to adults in a dose of 50 mg[47-49] over 30 seconds. Nonconjugated serum bilirubin is then measured every 30-60 minutes for the next 4-5 hours. In individuals with GS the bilirubin rise is greater and clearance is delayed longer than in normals.[46,50-53] Nicotinic acid causes increased osmotic fragility and hemolysis of red blood cells with sequestration in the spleen. Splenic heme oxygenase is also induced with rapid conversion of heme to bilirubin.[52] Hence, the prolonged serum bilirubin levels are related to delayed hepatic clearance of bilirubin. Nicotinic acid infusion has been suggested to be a better method to diagnose GS than a 400-kcal fast because delayed bilirubin clearance was seen after nicotinic acid in GS subjects who otherwise had normal serum bilirubin levels.[47] The nicotinic acid test is not useful in differentiating GS from chronic liver disease as both groups showed positive tests.[54]

Rifampin, given to fasting or nonfasting adults in one oral dose of 900 mg, increases total serum bilirubin levels in normal controls and those with GS, although there is an exaggerated increase in GS (fasting: >1.9 mg/dL increase in bilirubin concentration 2-6 h after rifampin, nonfasting: >1.5 mg/dL increase 4-6 h after rifampin).[55] This exaggerated rise in serum bilirubin enabled differentiation of 10 healthy control patients and 15-GS patients with high sensitivity and specificity.

Fractionation of the total serum bilirubin using alkaline methanolysis and thin-layer chromatography can also aid in diagnosing GS.[56,57] This allows precise measurement of the conjugated and unconjugated bilirubin levels and has shown that in GS ~6% of the total serum bilirubin was conjugated compared with approximately 17% in healthy patients and those with chronic hemolysis. Individuals with chronic persistent hepatitis had 28% of their total bilirubin present as conjugates. Fasting did not change the percentage of conjugates in GS, despite the increase in total serum bilirubin concentration. An overlap of only 3 individuals was seen among the 77 with GS and 60 normal subjects.[57] Other studies support these findings.[58] In patients with GS, fractionation of the total serum bilirubin by high-performance liquid chromatography (HPLC) showed significantly decreased bilirubin monoglucuronides (1.1% vs 6.2% in healthy patients) and increased unconjugated bilirubin (98.8 vs 92.6 in healthy patients).[59]

Genetic testing can be useful in the investigation of prolonged neonatal jaundice. The UGT1A1 TA repeat can be determined for non-Asian subjects and the Gly71Arg mutation for Asian subjects. Although this is a not a widely available test, it is performed by many genetic laboratories around the world (see http://genetests.org).

GS has no significant negative implications regarding morbidity or mortality. In general, drug metabolism studies have revealed no major dangers,[58,60] although there appears to be an increased incidence of slow acetylators,[61,62] and lorazepam clearance is 20%-40% decreased.[63] Concurrent genetic deficiencies in other xenobiotic pathways may put individuals with GS at increased risk of drug toxicity to such compounds as acetaminophen,[64,65] mycophenolate mofetil,[66] cancer chemotherapeutic agents CPT-11 (irinotecan),[67] or TAS-103[68] or the viral protease inhibitor, indinavir.[69,70] Therefore, Bosma[70] suggests that screening for GS can be of clinical importance. No specific treatment is necessary for GS, although phenobarbital has been shown to lower serum bilirubin levels in these patients.[71] An Italian study of cholestasis in thalassemia major demonstrated greater rates of gallstones at younger ages in patients with GS and recommended early biliary ultrasonography in patients who have both thalassemia and Gilbert's.[72] If the well-documented antioxi-