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COMMENTARY

Genetics and the Risk of Neonatal Hyperbilirubinemia

Commentary on the article by Huang et al. on page 682

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In this issue, Huang and coworkers provide provocative new evidence underscoring the clinical importance of genetics in the development of marked neonatal hyperbilirubinemia (1). Their study of Taiwanese newborns investigated the effects of several risk factors including among others i) breast feeding, ii) premature birth, iii) glucose-6-phosphate dehydrogenase deficiency, iv) a coding sequence gene polymorphism for the hepatic bilirubin conjugating enzyme uridine diphosphate glucuronosyl transferase 1A1 (UDP-GT1A1), and v) a variant gene polymorphism for the organic anion transporting protein OATP-2, purported to be involved in the hepatic uptake of unconjugated bilirubin. Of particular interest is their novel finding that the genetic polymorphism for the organic anion transporter protein OATP-2 (also known as OATP-C, liver specific transporter-1 [LST-1] and *SLC21A6*) at nucleotide 388 correlates with a 3-fold increased risk (95% confidence interval 1.30–6.99) for developing marked neonatal jaundice (peak total serum bilirubin level of $\geq$ 20 mg/dL [342 μM]) when adjusted for co-variates in their study cohort. Moreover, the combination of the OATP-2 gene polymorphism with the variant UDP-GT1A1 gene at nucleotide 211 further increased this risk to 22 fold (95% CI: 5.5–88.0) and when these two genetic variants were combined with breast milk feeding the risk for marked neonatal hyperbilirubinemia increased still further to 88 fold (95% CI: 12.5–642.5). This constellation of statistically significant variables points toward genetically defined impairments in hepatic bilirubin uptake and conjugation as clinically important contributors to marked neonatal hyperbilirubinemia. What do we know about OATP-2 and its mutations, other genetic factors that might predispose to the development of neonatal jaundice, and how breast-milk feeding might impact the phenotype of an individual genotype?

ORGANIC ANION TRANSPORTER PROTEINS

The OATPs are a family of multispecific pumps mediating the sodium-independent uptake of bile salts and a broad range of amphipathic organic compounds. In humans, three liver-specific OATPs have been identified: OATP-A, OATP-2, and OATP-8 (2). OATP-2 is selectively expressed at the basolateral membrane of hepatocytes (2) where it plays a role in the hepatic uptake of various substrates including taurocholate, conjugated steroids, thyroid hormones and peptides. The hepato-specific expression of OATP-2 is unique amongst the OATP transporters, all others of which are found in multiple tissues in addition to the liver (3). Of particular interest in relation to the work of Huang and associates is evidence that unconjugated bilirubin may be a substrate for human OATP-2. Cui and colleagues reported high affinity uptake of unconjugated [^{3}H]-bilirubin by OATP-2 in the presence of albumin in human embryonic kidney cells (HEK293) permanently expressing human recombinant OATP-2 (4). Their *in vitro* results suggest carrier mediated hepatocyte bilirubin uptake occurs *in vivo*. Presumably, mutations in the gene encoding OATP-2 could enhance unconjugated hyperbilirubinemia by impairing its hepatic uptake. A distinction between carrier mediated hepatic bilirubin uptake and that attributed to passive diffusion would be supported by the identification of specific functional consequences of OATP-2 mutations as suggested in the current report. In this regard, investigators have characterized several OATP-2 gene mutations that affect both protein maturation and organic anion transport [although bilirubin transport was not examined] (5,6). Complicating this picture, however, is a recent report that failed to confirm unconjugated bilirubin transport by OATP-2 in either OATP-2 stably transfected HeLa or HEK293 cells (7) challenging the notion of OATP-2 mediated hepatic bilirubin transport. Nevertheless, one cannot discount the results of the current study by Huang and colleagues demonstrating an associated 3-fold increased risk of marked neonatal hyperbilirubinemia in infants with an OATP-2 gene polymorphism at position 388 (1). Clearly, this area of investigation merits further study to more completely define the functional role of OATP-2 in hepatocytes and clarify how mutations in OATP-2 increase ones risk for neonatal

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hyperbilirubinemia. There is precedent for transporter mutations leading to disorders in hepatic bilirubin handling, most notably a deficiency of the canalicular multispecific organic anion transporter (cMOAT), also known as multidrug resistance associated protein 2 (MRP-2), which is critical to the hepatic excretion of conjugated bilirubin and the cause of cholestasis in the Dubin-Johnson syndrome (8) and in TR^{-}/GY and Eisai (EHBR) hyperbilirubinemic rat strains (9).

GENETICS OF BILIRUBIN CONJUGATION AND NEONATAL HYPERBILIRUBINEMIA

The results of Huang and coworkers lend further credence to the important role UDP-GT1A1 gene mutations play in the genesis of neonatal jaundice, the so called indirect hyperbilirubinemia syndromes (10,11). These include the well-characterized UDP-GT1A1 coding sequence mutations of the Crigler-Najjar type I [nonsense or stop mutations] and II [missense mutation] (Arias) syndromes, as well as the UDP-GT1A1 promoter sequence mutations of Gilbert's syndrome. Infants of East Asian populations also evidence coding sequence mutations [missense mutation] of the UDP-GT1A1 gene, the most common of which is a guanine to adenine transition at nucleotide 211, the polymorphism examined in the current study (also known as G71R) (11). The results of Huang *et al.* 's work confirm the importance of the 211 polymorphism in enhancing the risk of developing marked neonatal hyperbilirubinemia, a 3.36 fold increase (95% CI: 1.54–7.35). Indeed, Huang and colleagues have previously reported that the G71R mutation alone in Taiwanese (12) neonates is sufficient to exert a serum bilirubin increasing effect without additional jaundice risk factors, a phenomenon documented in Japanese infants as well (13). The superimposition of G6PD deficiency on homozygosity for the G71R mutation further increases the risk of neonatal hyperbilirubinemia in this population. Of great interest in the current study is the dramatic effect combined OATP-2 and UDP-GT1A1 G71R mutations has on neonatal jaundice and provides another compelling example of dose dependent genetic interactions that enhance the development of marked neonatal hyperbilirubinemia (10,11). These phenomena are analogous to that observed in Western infants with 1) combined Gilbert genotype and G6PD deficiency, 2) combined Gilbert genotype and hereditary spherocytosis, and 3) compound heterozygosity for the Gilbert genotype and UDP-GT1A1 coding sequence mutations, in increasing the risk for neonatal hyperbilirubinemia (10,11) and even kernicterus (14). This growing body of literature underscores the importance of increasing our understanding of the biology of neonatal hyperbilirubinemia at the molecular level.

BREAST MILK FEEDING, GENE POLYMORPHISM, AND MARKED NEONATAL JAUNDICE

It is notable that breast milk feeding was again shown to be a significant risk factor for the development of neonatal hyperbilirubinemia marking a 4.64 fold increased risk (95% CI: 2.25–9.57) in the current study cohort. Of particular interest was that the risk of developing a bilirubin level of $\geq$20 mg/dL (342 μM) associated with breast milk feeding was significantly enhanced when combined with genetic polymorphisms for OATP-2 and/or UDP-GT1A1 genes –indeed the combination of all three factors resulted in the greatest risk of all at 88 fold. Thus, breast milk feeding may act as a modifier that for an individual genotype may predispose to the development of marked neonatal jaundice. In this respect, it is likely no mere coincidence that almost every reported case of alleged kernicterus in the past two decades has been in breast fed infants (15). How breast milk feeding, or more likely lactation failure, enhances the risk for marked neonatal jaundice ("breast nonfeeding jaundice" (16)) is not entirely clear (16,17), but may relate in part to a "caloric deprivation effect" that enhances heme oxygenase activity (increased bilirubin production), and enterohepatic bilirubin circulation (increased bilirubin load), conditions that could overtax a genotype wherein hepatic bilirubin uptake and conjugation capacities are limited. This hypothesis too merits clinical study.

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