

Genetic factors in neonatal hyperbilirubinemia and kernicterus

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Although the relationship between hyperbilirubinemia and genetic factors has long been questioned, the role of genetic factors in the development of severe hyperbilirubinemia and kernicterus has been investigated in detail in the last decade with the rapid progression in molecular medicine.

Although the first historical data gathered about genetical tendency to neonatal hyperbilirubinemia dates back to description of the Crigler-Najjar syndrome in 1952, a substantial interest is currently focused on coding and promoter region mutations of uridine diphosphoglucuronate glucuronosyltransferase 1A1 gene.

In this article, the role of uridine diphosphoglucuronate glucuronosyltransferase gene mutations in neonatal significant hyperbilirubinemia and kernicterus is reviewed together with the clinical presentations of the most common syndromes of bilirubin conjugation, such as Gilbert and Crigler-Najjar syndromes.

Genetic counseling and investigation may be useful and necessary in newborns presenting with severe, unexplained familial hyperbilirubinemia. In these various syndromes where enzymatic and genetic deficiencies are present, studies about treatment with gene replacement, though currently experimental, are ongoing, especially in type 1 Crigler-Najjar.

Key words: neonatal hyperbilirubinemia, uridine diphosphoglucuronate glucuronosyltransferase gene, Gilbert syndrome, Crigler-Najjar syndrome, kernicterus.

Although the relationship between hyperbilirubinemia and genetic factors has long been questioned, the role of genetic factors in the development of severe hyperbilirubinemia and kernicterus has been investigated in detail in the last decade with the rapid progression in molecular medicine.

The bilirubin molecule is conjugated to bilirubin monoglucuronides and diglucuronides taking glucuronosyl moiety under the enzymatic catalysis of uridine diphosphoglucuronate glucuronosyltransferase (UDPGT) enzyme in the endoplasmic reticulum of hepatocytes. Bilirubin diglucuronide is the major pigment excreted in the bile.

The glucuronizing enzyme complex, including bilirubin-UDPGT, is located in the endoplasmic reticulum and nuclear membranes, and has a role in glucuronidation of several endogenous and exogenous substances. In mammals, 47 different genes in the UDPGT family are located in the subfamilies of UDPGT 1, UDPGT 2 and

UDPGT 8. UDPGT 1 enzyme group consisting of four subfamilies provides glucuronidation of bilirubin, estriol, estradiol, quinols and phenols, whereas UDPGT 2 enzyme group, which can be induced by phenobarbital and has its gene locus on chromosome 4, provides glucuronidation of endogenous steroids and biogenic amines. The UDPGT 8 enzyme group, also encoded on chromosome 4, takes a role in the galactosylation of ceramide. Gene encoding subfamily of UDPGT 1A is on chromosome 2 (2q37), and the most important enzyme in glucuronidation of bilirubin is UDPGT 1A1, although only two members (UDPGT 1A1 and UDPGT 1A4) of this family constituted of 13 variable exons accept bilirubin as substrate. In the UDPGT 1A gene complex, there exist 13 variable exons (coding sequence: 1A1, 1A2, 1A3, ..., 1A12, 1A13), 13 promoter regions (nucleotide sequence; region controlling gene expression), each located adjacent to each of the variable exons, and four common coding exons²⁻⁵ shared by 13 variable coding exons in

the neighborhood of UDPGT 1A1^{1,2} (Fig. 1). Thus, the most important enzyme in the bilirubin glucuronidation, UDPGT 1A1, is constituted of five variable and common coding exons and a promoter region (Fig. 1).

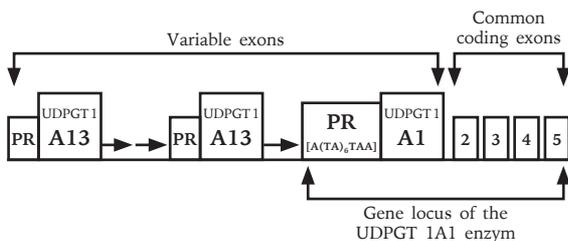


Fig. 1. Gene locus of the uridine diphosphoglucuronate glucuronosyltransferase (UDPGT) 1A enzyme.

Although the first historical data gathered about genetical tendency to neonatal hyperbilirubinemia originated with the first description of Crigler-Najjar (CN) syndrome in 1952, a substantial interest is currently focused on coding and promoter region mutations of the UDPGT 1A1 gene³⁻⁹. These mutations result in constitutional and functional deficiency in the enzyme; bilirubin conjugation is disturbed as a result, and hyperbilirubinemia evolves. Gilbert genotype is formed when an extra two (thymine-adenine)-base-pair is added to the promoter sequence of UDPGT 1A1 gene, and the normal form of “Adenine, [Thymine-Adenine]₆, Thymine, Adenine, Adenine” (Fig. 1) is replaced with (A[TA]₇TAA). Proper message transcription is disturbed and UDPGT 1A1 enzyme activity is decreased with the addition of the extra two-base-pair³. This mutation is more common in whites. Patients with Gilbert syndrome are homozygous for A[TA]₇TAA (variant) promoter, and they have mild and benign jaundice. Nine percent and 42% of the whole population are homozygous and heterozygous, respectively, for the variant/mutant promoter, and one of every two persons has Gilbert promoter (polymorphism) at least at one allele⁴. However, not all of the adults with variant UDPGT 1A1 promoter homozygous polymorphism have clinical signs of Gilbert syndrome. An additional independent and genetically determined factor in bilirubin uptake, such as a hepatic transport mechanism defect¹⁰, is necessary to cause a full phenotypic clinical expression.

On evaluating the significance of Gilbert genotype in neonatal hyperbilirubinemia, the risk of severe hyperbilirubinemia and kernicterus does not increase in the presence of variant homozygous or variant heterozygous UDPGT 1A1 polymorphism alone, but it severely increases if there are conditions associated with increased bilirubin production or increased enterohepatic circulation, such as glucose-6-phosphate dehydrogenase (G-6PD) deficiency⁵, hereditary spherocytosis⁶ and ABO hemolytic disease⁷ together with UDPGT 1A1 polymorphism. Gilbert syndrome and UDPGT 1A1 polymorphism have been deemed responsible for prolonged hyperbilirubinemia in newborns fed breast-milk¹¹⁻¹³. However, it should be kept in mind that in addition to the disturbance in bilirubin conjugation, there is also an increase in heme catabolism (bilirubin production) in homozygous Gilbert genotype^{2,14}. As a probable result, slightly higher serum bilirubin levels (≤ 15 mg/dl), though not reaching kernicteric levels, have been reported in newborns with homozygous Gilbert genotype when compared to newborns with normal genotype or heterozygous Gilbert genotype¹⁵⁻¹⁷. Thus, in the absence of additional icterogenic factors, homozygous Gilbert genotype may be associated with higher than normal serum bilirubin levels; however, severe hyperbilirubinemia is not the rule. It has been suggested that UDPGT 1A1 promoter polymorphism demonstrates ethnic variations, and this flexible polymorphism causes a mild serum bilirubin increase to a degree that prevents against early oxidative reaction but does not lead to kernicterus¹⁸. According to this hypothesis, UDPGT 1A1 promoter polymorphism is much more common in African origins, and severe hyperbilirubinemia and kernicterus are rarely seen in blacks¹⁸.

Different rates of homozygosity for normal (wild-type) allele of UDPGT 1A1 gene (A[TA]₆TAA) have been reported in the Turkish population (19,20). Babaoglu et al.¹⁹ investigated the prevalence of homozygous and heterozygous A[TA]_{6/7}TAA (variant) promoter in newborns with early hyperbilirubinemia and prolonged jaundice and in healthy newborns. They reported similar rates (around 60%) of homozygosity for the normal allele in each of the three groups. In a similar study from the Aegean region of the country, Ulgenalp

et al.²⁰ reported a lower frequency of normal A[TA]_{6/6}TAA homozygosity (around 45%) in similar study subgroups. However, neither of these studies was able to demonstrate an association between the (variant) promoter polymorphism of UDPGT 1A1 gene and early or prolonged neonatal jaundice although the gene polymorphism is quite prevalent^{19,20}.

Apart from mutations in the promoter sequence, homozygous missense mutations in the coding area of UDPGT 1A1 gene also cause Gilbert syndrome. The most common of these, especially encountered in some East Asian populations, is G→A transition at nucleotide 211, which causes arginine to replace glycine at position 71, of the corresponding protein product^{12,21}. Pro229Gln, Tyr486Asp, Arg209Trp and Arg367Gly mutations are the others especially reported in Asian newborns^{9,22,23}. Although homozygous mutations in both coding and promoter areas of the UDPGT 1A1 gene are associated with clinically apparent Gilbert syndrome, these two polymorphisms behave differently with regard to their ability to influence the development of neonatal hyperbilirubinemia. An additional icterogenic risk factor is necessary in promoter area mutations to cause severe neonatal hyperbilirubinemia, whereas neither homozygous nor heterozygous mutations of the coding sequence (G71R) require any additional jaundice-provoking factors to produce their effects^{9,12}.

The CN syndromes are caused by one or more mutations in any one of the five (1A1 variable and 1A2-5 common) exons of the gene coding for the UDPGT 1A1 enzyme, and CN type 1 and type 2 syndromes result from complete to severe impairment of bilirubin conjugation. Type 1 CN syndrome (inherited autosomal recessively) is characterized by almost complete absence of UDPGT 1A1 enzyme activity and there is no response to phenobarbital treatment. Serum bilirubin levels are between 20 to 50 mg/dl and kernicterus may develop in the neonatal period. Type 2 CN syndrome has autosomal recessive or autosomal dominant inheritance, and enzyme activity is severely reduced but can be induced by phenobarbital administration. Although decrease in serum bilirubin levels by 30% to 80% and increase in biliary-conjugated bilirubin fraction concentrations in response to phenobarbital treatment are suggestive of type 1 CN, the definitive diagnosis of the CN

syndrome is only possible by high-performance liquid chromatography analysis of bile or tissue enzyme assay from a liver biopsy (24). Kernicterus is rarely seen in type 2 CN. There are more than 50 UDPGT 1A1 mutations reported that cause CN syndromes¹.

A heterozygous mutation in the coding area (sequence) of the UDPGT 1A1 gene does not normally lead to neonatal hyperbilirubinemia². However, there is kernicterus risk in very rare conditions where Gilbert genotype (promoter mutation) coexists with a structural mutation in the coding area of the UDPGT 1A1 gene^{4,8,25,26}. In these instances, serum bilirubin levels are expected to be higher than in Gilbert syndrome but lower than in type 1 CN, respectively. When the gene frequency of this rare condition in the population is calculated, at least one in 3,300 newborns is expected to have a mixed heterozygosity for Gilbert genotype and UDPGT 1A1 coding gene mutations and to be at a great risk of kernicterus^{4,27}. These cases usually have serum bilirubin levels above 30 mg/dl^{28,29}. It should be considered that some of the cases presenting with idiopathic and severe hyperbilirubinemia and even kernicterus may simultaneously have Gilbert syndrome, UDPGT 1A1 coding gene sequence variants and/or Gilbert promoter heterozygosity, and structural mutations of the UDPGT 1A1 gene^{2,27,30}.

In another study investigating the genetic effects on neonatal hyperbilirubinemia and kernicterus, genetic polymorphism in organic anion transporter protein-2 (OATP-2), which has a role in the entry of unconjugated bilirubin into the hepatocyte, increased the risk of severe hyperbilirubinemia to three-fold³¹. The combination of OATP-2 gene polymorphism and UDPGT 1A1 promoter polymorphism, and the addition of breast-feeding to these factors, increase the risk of severe hyperbilirubinemia to 22-fold and 88-fold, respectively³¹.

In another study investigating the relationship between genetical effects and kernicterus in newborns, polymorphism in p-glycoprotein (P-Gp) transporter gene demonstrated a tendency to kernicterus³². P-Gp is a member of the ATP-binding family of membrane transporters encoded by ABCB1 (also called multidrug resistance) gene. Free bilirubin is substrate for P-Gp. In the P-Gp polymorphism, deposition of free bilirubin at the cell membrane and influx of it into the cell increase^{33,34}.

In conclusion, genetic counseling and investigation may be useful and necessary in newborns presenting with severe, unexplained familial hyperbilirubinemia. In these various syndromes where enzymatic and genetic deficiencies are present, studies about treatment with gene replacement, though experimental currently, are ongoing, especially in type 1 CN³⁵⁻³⁷.

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