Gilbert's Syndrome – Clinical and Pharmacological Implications

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Gilbert syndrome, a mild form of unconjugated hyperbilirubinemia, is a relatively common and often accidental finding in healthy individuals and patients with unrelated diseases. The clinical importance of this frequently encountered entity is unclear. The coexistence of GS with other more clinically significant conditions could interfere with their diagnoses. The genetic variation described as Gilbert syndrome may affect drug glucuronidation and could potentially precipitate unexpected toxicity from therapeutic agents. The purpose of this review is to describe the entity and discuss its clinical and pharmacological implications.

History

Gilbert and Lereboullet first described the syndrome in 1901 as "La cholemie simple familiale" [1]. Meulengracht 'reinvented' the syndrome in 1939 in a published paper titled "Icterus intermittens juvenilis." In the German medical literature GS is referred to as "Morbus Meulengracht." Since then, several authors have described clinical entities similar to the one reported by Gilbert, without relating it to the original syndrome.

Arias [2] described a disorder in which eight patients with chronic non-hemolytic jaundice had glucuronyltransferase deficiency. Sleisenger et al. [3] described Irish kindred in which lifelong jaundice occurred throughout four generations in a dominant pedigree pattern of male-to-male transmission. Hepatic glucuronyltransferase activity was low in the affected individuals.

Epidemiology

It is estimated that 3–10% of the general population have GS, as determined by elevated indirect serum bilirubin levels. Males are more frequently affected than females (12.4 and 4.8% respectively). Moreover, the mean bilirubin concentration in GS is significantly higher in males [4]. This may be explained by either the presence of a greater bilirubin load per kilogram body weight in males, and/or by androgen steroid inhibition of bilirubin enzymatic glucuronidation [5]. Despite the fact that GS is considered an inherited condition, a clear family history is not always apparent.

Pathogenesis

Basically, GS is characterized by impaired bilirubin conjugation in the liver. Bilirubin originates primarily from senescent red blood cells (80%) [Figure 1]. The rest is derived from ineffective erythropoiesis, muscle myoglobin and heme-containing enzymes. Normally, more than 90% of serum bilirubin exists in the form of an unconjugated, non-polar, albumin-bound molecule. Eventually, bilirubin undergoes conjugation with a polar group to allow its excretion through the bile [Figure 2]. This process takes place primarily in the liver [Figure 3]. The co-substrate for bilirubin conjugation is UDP-glucuronic acid. UDP-GlucA is a ubiquitous intracellular substance derived

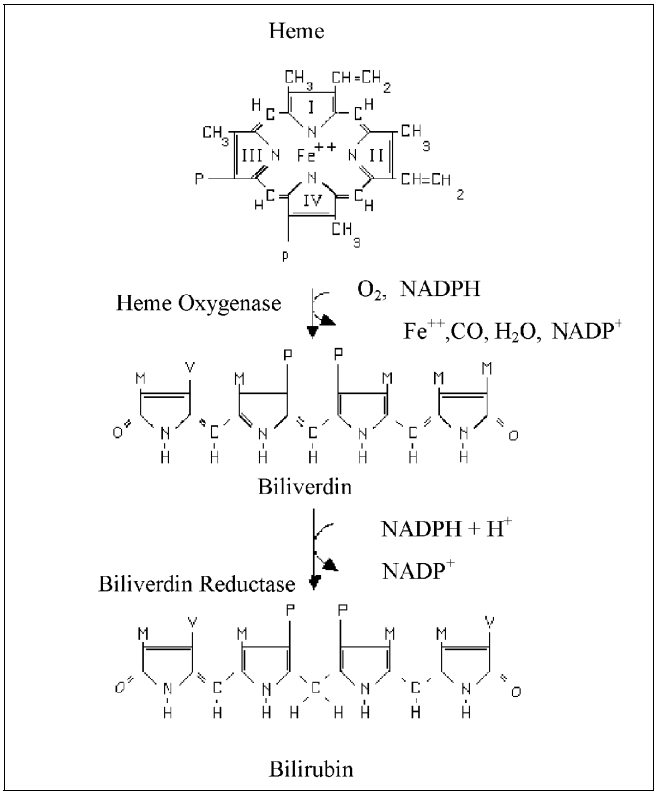

Figure 1. Heme metabolism

GS = Gilbert syndrome

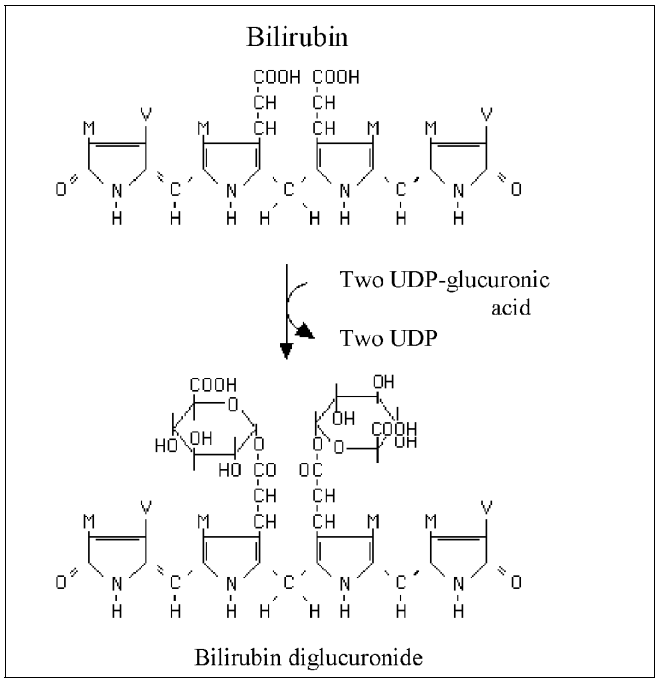

Figure 2. Conjugation of bilirubin

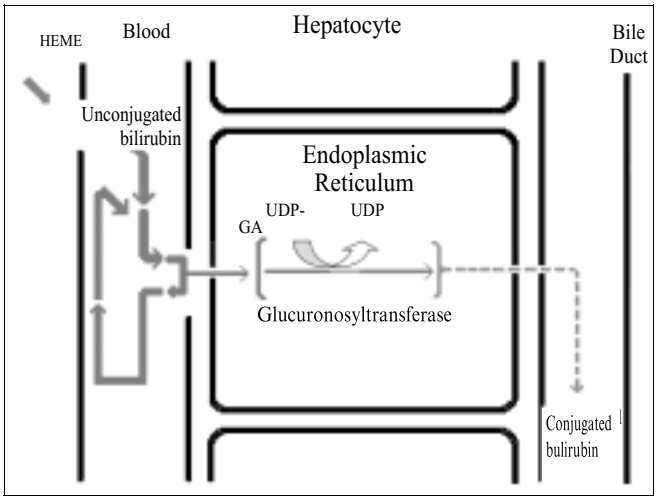

Figure 3. Impaired bilirubin conjugation in Gilbert syndrome

from glucose. It conjugates with various endogenous and exogenous substances, including drugs, to form a group of compounds collectively termed glucoronides. Conjugation of a compound with glucuronic acid produces an acidic, more water-soluble molecule with different metabolic, transport and excretion properties. The availability of UDP-GlucA may decrease following enhanced glucuronidation requirements (e.g., high substrate load) or due to glycogen depletion (e.g., fasting). The conjugation reaction is mediated by a large group of enzymes known as glucuronosyltransferases [6].

In most species, including humans, the major transferase activity is detected in the liver. Glucuronosyltransferases are membrane-bound enzymes, located mainly in the smooth endoplasmic reticulum but also found in cellular and nuclear membranes. Bilirubin conjugation is mediated by one specific isoform: bilirubin uridine diphosphoglucuronate-glucuronosyltransferase, also labeled UGT1A1 according to the UGT superfamily nomenclature proposed by Mackenzie et al. [7].

In individuals diagnosed as having GS, the hepatic bilirubin glucuronidation activity was found to be approximately 30% lower than normal. The genetic and molecular basis of the syndrome has not yet been completely elucidated. A proposed animal model for exploring GS is the Bolivian squirrel monkey (BoSM) [8], which manifests fasting unconjugated hyperbilirubinemia similar to that in humans with GS. A genetic defect in bilirubin-UDPGT was also found in the Gunn rat, in which a single guanosine (G) within the UGT1A1 gene is missing. The defect results in a frame shift and a premature stop codon – the absence of enzyme activity – resulting in hyperbilirubinemia. This animal serves as a model for yet another congenital familial non-hemolytic jaundice – Crigler-Najjar syndrome [9]. In fact, it is argued by some that GS should be considered as a variant of the CN syndrome [10].

Bilirubin-UDPGT originates from the same large gene as the phenol UDPGTs and is encoded by a single gene, UGT1, located on chromosome 2 at locus 2q.37 [11]. The UGT1 gene consists of five exons. There are several isoforms of the enzyme gene. Exons number 2 to 5 are common to all isoforms whereas exon number 1 contains one of at least 13 unique variants. A promoter region (5') precedes exon 1, and encodes a unique UGT isoform [Figure 4]. The mRNA encoding each UGT isoform is formed by fusion of one type of exon 1 to the common exons 2–5 region [12].

Bosma et al. [5] found that individuals with GS had a normal coding region of the UGT1A1 gene, but were homozygous for two extra bases (TA) in the TATAA element of the 5' promoter region of the gene (i.e., A(TA)**7**TAA instead of the normal A(TA)**6**TAA configuration). The presence of the longer TATAA element resulted in reduced expression of a reporter gene. The frequency of the abnormal allele was 40% among normal subjects. The subjects in the control group who were homozygous for the longer TATAA element had significantly higher serum bilirubin levels than the other normal subjects. Although (TA)7 was the only mutant allele associated with GS in the Caucasian population, an additional two mutations, (TA)5 and (TA)8, were identified in black people manifesting this syndrome [13].

Akaba et al. [14] evaluated the relationship between bilirubin-UGT genotype and the presence and severity of jaundice in 159 Japanese full-term neonates. They concluded

bilirubin-UDPGT = bilirubin uridine diphosphoglucuronate-glucuronosyltransferase

CN = Crigler-Najjar

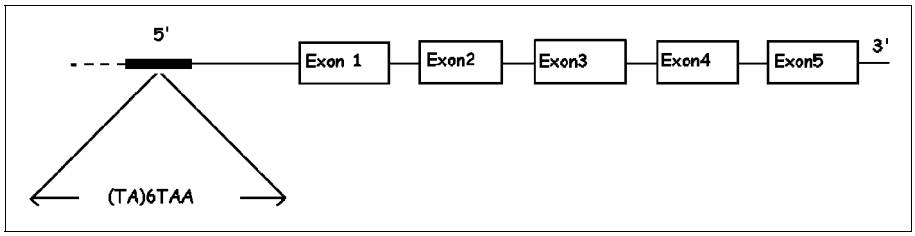

Figure 4. Structure of UGT1A1 gene. Exon 1 differs from isoform variants. Common exon 2–5 region. Exons are separated and flanked by non-coding genomic sequences. 5' – promoter region

that a Gly71Arg (G71A) mutation in the coding **Exon** 1 of the UGT1 gene correlates with the incidence and severity of neonatal hyperbilirubinemia in the Japanese, Korean and Chinese population. It is noteworthy, however, that other authors associate the same G71A mutation with Crigler-Najjar syndrome (type II) [15].

Despite a relatively high frequency of the elongated TATAA element among healthy subjects (40%), the incidence of a clinical or laboratory established GS is surprisingly low (3–10%). Although abnormality in the promoter region is always accompanied by reduced expression of the UGT1A1 gene, the penetrance of this mutation is incomplete, since "overt" Gilbert syndrome is not always evident. This may imply that other – acquired or inherited – factors may also contribute to the actual hyperbilirubinemia of GS: hepatic transport abnormalities, occult hemolysis, and stress-related induction of heme oxygenase [5]. Some observations may indirectly support this assumption. Approximately one-third of individuals with GS have minor abnormalities in plasma clearance of bromsulfophthalein and indocyanine green. These two dyes were formerly used as indicators for hepatic clearance. Neither compound is conjugated in the liver by UDPGT, implying that an undefined defect in hepatic uptake, intracellular transport or excretion of bilirubin may also exist, at least in some cases [16,17]. It was also noted that although overt hemolysis is not a typical feature of GS, the life span of erythrocytes is shorter than normal in about 50% of the affected individuals. This may suggest that a certain degree of a mild, compensated hemolytic state may be present and can further increase the bilirubin load with which UDPGT has to "cope" [18,19].

Clinical features and diagnosis

Schmid [20] pointed out that Gilbert syndrome is an entirely benign and clinically inconsequential entity that requires neither treatment nor long-term medical attention. Its clinical importance lies in the fact that the mild hyperbilirubinemia may be mistaken for a sign of occult, chronic, or progressive liver disease. Since the diagnosis is largely one of exclusion, clinicians sometimes find it difficult to dispel lingering fears of serious liver disease, causing patients unwarranted anxiety.

GS is usually diagnosed between the ages of 15 and 30 years. Typically mild, asymptomatic jaundice is first noticed in adolescence. Upper abdominal pain, fat intolerance, and fatigue have been reported infrequently [21].

The principal diagnostic criterion for GS is an increased level of serum unconjugated ("indirect") bilirubin, sometimes found accidentally in routine laboratory tests. If accompanied by jaundice, serum bilirubin levels usually exceed 34–43 μmol/L (2.0–2.5 mg/dl), or about twice the upper limit of the normal range. Serum bilirubin concentrations characteristically fluctuate daily. Mean values are lower in women than in men. Since the fluctuating bilirubin levels often fall within accepted normal limits, it is argued by some that individuals with GS actually represent a distinct normal subpopulation of bilirubin values within the upper end of the normal distribution curve [22]. Bilirubin concentrations may rise significantly during fasting, physical exercise, stress, intercurrent illness or menstruation. Elevation of bilirubin levels during fasting is used, in fact, as the most common diagnostic tool for GS. Following reduced calorie intake of 400 kcal/day for 24–48 hours, a significant increase in bilirubin blood levels is usually observed, and total bilirubin blood levels tend to be higher than the acceptable normal ranges [21]. Routine liver function tests are characteristically normal, and there is no evidence of biliary tract obstruction or structural abnormalities in liver scans or hepatic histology [23]. A nicotinic acid provocation test has also been suggested for the diagnosis of GS. Following intravenous administration of nicotinic acid there is a significant rise in unconjugated bilirubin. However, this test is non-specific and may be positive in unconjugated hyperbilirubinemia regardless of the cause and, therefore, is probably of no value in differentiating GS from other entities including chronic liver diseases [24].

Although the genetic polymorphism in the promoter region of UGT1A1 gene may be identified, it is not done routinely for clinical purposes. This method could help in establishing the contribution of this polymorphism in individuals with jaundice due to multiple causes [25].

An immunohistochemical assay, using polyclonal antibodies raised to UDP-glucuronosyltransferase, has also been developed, primarily as an investigational rather than a diagnostic tool. In the normal human liver there is a diffuse pattern of specific staining for UGT noted in all hepatocytes, and particularly accentuated in zone 3 of the hepatic tissue. This zone is considered to play a role in the excretion of bile acids rather than being involved in biliary excretion of organic anions. In GS, staining is significantly reduced throughout the

G6PD = glucose-6-phosphate dehydrogenase

hepatic lobule. Only faint residual staining has been detected in zone 3 [26].

The differential diagnosis of GS from other unconjugated hyperbilirubinemic states is important when considering further management of a patient. These conditions may be inherited or acquired. The hereditary unconjugated hyperbilirubinemias include Gilbert syndrome, Crigler-Najjar syndrome type I (CN-I) and Crigler-Najjar syndrome type II (CN-II). The latter may be clinically identical to Gilbert syndrome. Table 1 outlines some causes for hyperbilirubinemia due to UDPGT gene defect, and their distinct features. It is noteworthy that some known syndromes, e.g., Dubin-Johnson syndrome, Rotor syndrome, and several forms of intrahepatic cholestasis are characterized by a predominantly conjugated rather than unconjugated hyperbilirubinemia. Acquired unconjugated hyperbilirubinemias have also been described in newborns. These include the Lucey-Driscoll syndrome in which an inhibitor of the UGT enzyme activity is acquired from the mother's serum, and breast milk jaundice in which the inhibitor of the enzyme activity is transmitted through the mother's milk.

Clinical significance

The clinical significance of moderately high indirect serum bilirubin levels or asymptomatic jaundice with normal liver function tests remains unclear. Currently GS is regarded as a benign, non-progressive condition, requiring neither treatment nor long-term medical attention. It has been shown that small doses of phenobarbital (0.05–0.15 g daily) reduce bilirubin levels to normal, this effect lasting only for the period of the drug administration.

Nevertheless, the presence of GS as a contributory factor to other prevailing diseases or conditions should be considered:

- **Newborn jaundice:** A genetic predisposition to develop prolonged neonatal hyperbilirubinemia in breast-fed infants is associated with TATA box polymorphism of the same UGT1A1 gene related to GS in adulthood [27]. GS actually accelerates the development of neonatal jaundice. Although peak jaundice levels did not differ among groups, jaundice progressed more rapidly during the first 2 days of life in neonates carrying the molecular markers for GS [28].
- **Thalassemia:** Hyperbilirubinemia in heterozygous beta-thalassemia seems to be related to co-inherited GS. In fact, homozygosity for the promoter (TA)7 motif, which constitutes the typical genetic configuration of GS, is one of the predisposing factors of hyperbilirubinemia in heterozygous beta-thalassemia individuals [29,30]. Alpha-thalassemia could also be accompanied by GS. Apparently the hyperbilirubinemia in this case results from impaired bilirubin clearance rather than increased erythrocyte destruction [31].
- **Glucose-6-phosphate dehydrogenase deficiency:** The association between G6PD deficiency and GS in newborns is unclear. Thirty percent of newborns with G6PD deficiency encounter jaundice, sometimes severe or fatal. The pathogenesis of the hyperbilirubinemia is uncertain in these cases. Hemolysis does not seem to be the major cause of hyperbilirubinemia, and GS is not necessarily present in all individuals. Kaplan and co-workers [19] concluded that neither G6PD deficiency nor the variant UDPGT1 promoter increases the incidence of hyperbilirubinemia on their own, but that the combination of both may have some effect. It has been reported that GS contributes to the severity of hyperbilirubinemia in G6PD-deficient adult subjects during an episode of acute hemolytic anemia (fabic crisis) [32].
- **Spherocytosis:** Some authors have reported GS coexisting with hereditary spherocytosis. A common complication of hereditary spherocytosis is the precocious formation of bilirubinate gallstones that may warrant preventive splenectomy. It was observed that co-inheritance of GS increases the risk for developing gallstones in patients with hereditary spherocytosis [33]. The presence of GS should therefore be taken into account when considering preventive splenectomy in these patients.
- **GS and liver transplant:** There is no contraindication for using liver transplants from donors with Gilbert syndrome [34]. Persistent unconjugated hyperbilirubinemia has occasionally been observed in liver transplant recipients with otherwise normal liver function tests. When the UGT1A-gene TATAA-box was investigated in the DNA of the organ donors, it was shown that some recipients with persistent unconjugated hyperbilirubinemia had received a liver from a donor with an abnormal TATAA-box in the bilirubin-UGT1A-gene promotor region [35].
- **Crigler-Najjar syndrome:** One case report noted that an adult who was heterozygous for Crigler-Najjar syndrome and homozygous for the Gilbert-type genetic defect developed severe hyperbilirubinemia. This case demonstrates how

Table 1. Differential diagnosis of hyperbilrubinemic states due to glucoronyltransferase gene defect (predominantly unconjugated hyperbilirubinemia)

Entity	Transmission	Gene defect	Type of hyperbirubinemia	Kernicterus	Bile	Phenobarbital
C-N I	Autosomal-recessive	Structural (exon 2–5): P387R(73m)(57m)	Severe	+	Colorless	No influence
C-N II	Autosomal-recessive	Structural (exon 2–5): Y486D(49m)	Mild-Severe	±	Variable	Partial influence
GS	Autosomal-dominant	Controller gene (TATA promoter region)	Mild	–	Pigmented	Prompt response

seemingly benign genetic defects, when combined, may result in a serious clinical condition [36]. In kindred with a history of CN syndrome type II, only the heterozygous carriers, who had a longer TATAA element on the structurally normal allele, had mild hyperbilirubinemia characteristic of GS.

Pharmacological implications

Since the UDP-GT system plays an important role in the elimination not only of endogenous metabolites but also of xenobiotics such as drugs that undergo glucuronidation, their metabolism could be impaired in patients with genetic hyperbilirubinemias. Many drugs are direct substrates of the various isoforms of UDPGT: paracetamol (UGT1A6, UGT1A9), morphine, oxazepam, temazepam, (UGT2B7), amitryptiline (UGT1A4), ritodrine, diflunisal, zidovudine and others. Most of these drugs also contain phenol acceptor groups and could, therefore, undergo sulfation rather than glucuronidation. Other agents such as ibuprofen and probenecid contain carboxylic acid residues, which exclusively bind to glucuronic acid. Some compounds such as diflunisal and 6-hydroxy bile acids have both phenolic and carboxylic acceptor groups. In addition to these directly binding substrates, many other drugs undergo biotransformation by phase-I reactions to generate acceptor groups for glucuronic acid.

Since GS occurs quite frequently among the general population, it would seem conceivable that drugs that undergo glucuronidation will display a different pharmacokinetic profile in affected individuals. Moreover, these patients are at a potentially higher risk for certain drug toxicities. It is difficult, however, to directly correlate drug metabolism to UGT expression and activity due to marked inter-individual and intra-individual (age-related) variability. Contributing factors are genetic and environmental and have a variable ontogenetic activity. Hall et al. [37] showed a great variability in the TA repeats of the UGT1A1 gene promoter TATA box present across ethnic groups. Populations of African origin display four different alleles while non-African populations appear to have only two alleles. Moreover, the same authors noted that there also seems to be a phylogenetic variability throughout the primate evolutation. In nine sequential primate species, the number of TA repeats has increased during the evolution, achieving the largest number in humans [37].

The fact that other organs are capable of glucuronidation with organ-specific activity and/or organ-specific ontogeny, and the availability of alternative metabolic pathways, further complicate the picture. It is therefore not surprising that only a small amount of conclusive data are available on the pharmacological implications of GS.

The best known example of drug toxicity related to GS is that of CPT-11 (irinotecan). CPT-11, a semi-synthetic analog of the cytotoxic alkaloid camptotecin, is used for the treatment of metastatic colorectal cancer. It is biotransformed by tissue and serum carboxyl esterase into a highly active metabolite, SN-38 (7-ethyl-10-hydroxycamtothecin), which is glucuronidated by hepatic uridine diphosphate glucuronosyltransferases (UGTs). The major dose-limiting toxicity of irinotecan therapy is diarrhea, believed to be secondary to the biliary excretion of SN-38, the extent of which is determined by SN-38 glucuronidation [38]. A wide intersubject variability in SN-38 glucuronide formation rates was found in humans. Patients with low UGT1A1 activity, such as those with GS, are at increased risk for irinotecan toxicity.

There is no clear suggestion in the data that therapeutic doses of paracetamol (acetaminophen) are associated with increased risk for hepatic or systemic toxicity in subjects with GS. This may be explained by the fact that UGT1A6 (and to a lesser extent UGT1A9) are the isoenzymes that are primarily involved in paracetamol metabolism, rather than UGT1A1 [39].

In individuals who are poor metabolizers of propafenone through cytochrome P4502D6, glucuronidation is the major metabolic pathway for elimination. The possibility of increased toxicity of this drug in patients who are poor metabolizers and also display concomitant GS has been investigated. This hypothesis was not substantiated by the study, and it seems that patients with GS who are also slow metabolizers of propafenon are not at a higher risk for toxicity. Alternatively, propafenone may be a substrate for other UGT isoforms and not necessarily UGT1A1 [40].

Conclusions

It has been known for many years that mild to severe deficiency of bilirubin UDP-glucuronosyltransferase in the liver is the cause of two types of familial unconjugated hyperbilirubinemias: Crigler-Najjar syndromes types I and II, and Gilbert syndrome. Since the gene encoding for bilirubin UDP-glucuronosyltransferase was elucidated, a number of mutations affecting the expression of this gene has been identified. These mutations can be classified into three groups: mutations that result in a reduced production of the normal enzyme, mutations that give rise to the synthesis of a structurally abnormal and dysfunctional enzyme, and mutations that completely abolish the expression of the affected allele. The combination of mutations affecting the coding region of the gene and of promoter mutations that reduce the expression of the gene accounts for the wide clinical spectrum of familial unconjugated hyperbilirubinemias, ranging from the clinically negligible Gilbert syndrome to the severe and often fatal Crigler-Najjar type I syndrome.

A better understanding of the genetics of these conditions and the availability of molecular diagnosis will improve the diagnostic efficiency and afford better informed genetic counseling, not only for Crigler-Najjar and Gilbert syndromes, but also for several acquired conditions characterized by unconjugated hyperbilirubinemia.

Surprisingly, only a small amount of data are available on the pharmacological implications of GS. The fact that at least one drug toxicity is almost definitely related to Gilbert syndrome warrants further investigation of other drugs.

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