# A Review of Gilbert's Syndrome with Emphasis on the Metabolic Pathway of Bilirubin and Uridinediphosphoglucuronosyl transferase isoform 1A1 (UGT1A1)

Ashley Cravens and Savita Chaurasia*
*Department of Biochemistry & Molecular Biology*
*Bellarmine University, Louisville KY 40205, United States*
*schaurasia@bellarmine.edu

***Received:*** *27.11.2019,* ***Accepted:*** *16.12.2019*

**ABSTRACT**

There are several diseases that prevent the formation of specific enzymes within the human body. One such disease is Gilbert's syndrome, which is an autosomal recessive genetic disorder. In most cases, it does not pose a serious health ricks and can undiagnosed for many years due to its tendency be asymptomatic. The main characteristic of Gilbert's syndrome is the building up of bilirubin in the blood, typically between 2.0mg/dl and 3.5mg/dl. The source of this disease is a UGT1A*28 promoter mutation. The UG1A1 gene encodes the UDP-glucuronosyltransferase-1A1, which is responsible for the conjugation of bilirubin. The purpose of this paper is to explore the normal functioning pathway that leads to the disposal of bilirubin, identify key difference in those who suffer from Gilbert's syndrome, and understand the consequences and treatments for this disease.

**Keywords -** Gilbert's Syndrome, Bilirubin, UDP-glucuronosyltransferase-1A1, UGT1A*28 promoter mutation

## 1. INTRODUCTION

Gilbert's syndrome is a relatively harmless disorder, characterized by excess bilirubin in the blood (U.S. National Library of Medicine, 2019). It was first characterized in 1901 by Augustin Nicolas Gilbert and is less commonly known as Meulengracht disease, constitutional hepatic disfunction, or familial nonhemolytic jaundice. In this disease, an individual has a decreased presence of Uridinediphosphoglucuronosyl transferase isoform 1A1 (UGT1A1) in the endoplasmic reticulum of hepatitis. This enzyme is important in modifying bilirubin with glucuronic acid so that it may be excreted in bile. This disease may go undiagnosed for many years due to its tendency to be asymptomatic (Chandrasekar and Savio, 2019). To understand the implication of Gilbert's syndrome, one must first understand the regulation of bilirubin in a normal individual.

## 2. OVERVIEW OF NORMAL PRODUCTION, DISTRIBUTION, METABOLISM, AND EXCRETION OF BILIRUBIN

### *2.1PRODUCTION OF BILIRUBIN*

Under normal conditions, bilirubin is produced due to normal degradation of heme-containing products, such as senescent red blood cells, cytochromes, and myoglobin. Reticuloendothelial cells remove heme from the blood and break it down into bilirubin in a two-step reaction. The first step is catalyzed by heme oxygenase, which opens the tetrapyrrolic heme ring at the alpha methane bridge. This reaction consumes five NADPH molecules and three oxygen molecules, while releasing an atom of iron and a molecule of

 

carbon monoxide. In this step, heme is converted into intermediate biliverdin. The second step converts biliverdin to bilirubin using biliverdin reductase. In this step, a double bond is broken through the oxidation of NAD(P)H to NADP+. (Wang *et al.,* 2006; Wolkoff, 2014). (Figure 1).

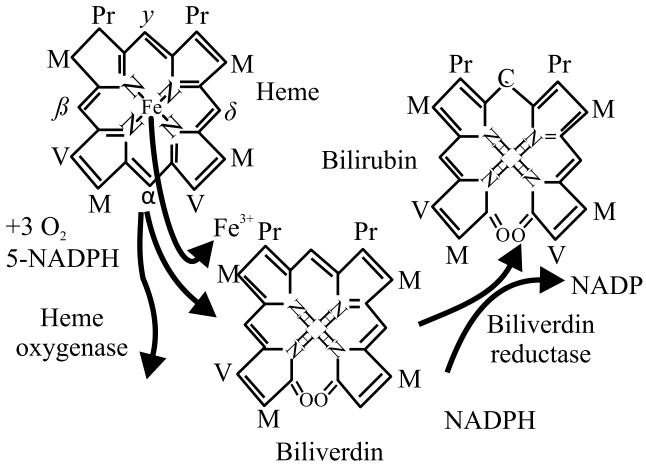


**Figure 1.** Production of Bilirubin from Heme. This figure describes the two-step breakdown of heme into bilirubin, which occurs in reticuloendothelial cells. (Wang *et al.,* 2006).

*2.2 DISTRIBUTION OF BILIRUBIN*

Once bilirubin is produced in the reticuloendothelial cells, it is released into the blood where it quickly binds to albumin due to its hydrophobic nature. The albumin-bilirubin complex is transported through the blood until it reaches the liver sinusoid (Figure 2), which blood capillaries located in the liver. Albumin-bilirubin complexes pass through fenestrations (small openings) in the hepatic sinusoidal endothelium and enter the space of the Disse. The Disse is a perisinusoidal space where plasma proteins can be absorbed by the liver. A small fraction of bilirubin interactions with a transport protein on the hepatocyte surface and the albumin-bilirubin complex is broken. Albumin is released back into the blood and the bilirubin is brought into the hepatocyte (Wolkoff, 2014).

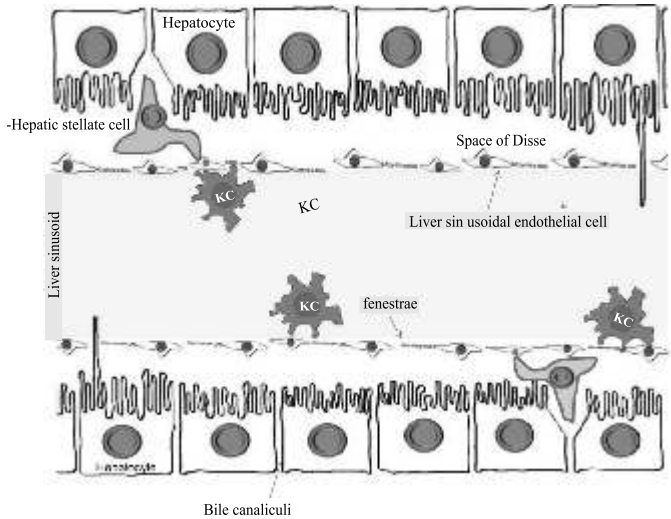


**Figure 2:** Liver Sinusoid and surrounding tissue (Bogdanos *et al,* 2013).

*2.3 METABOLISM AND EXCRETION OF BILIRUBIN*

In the hepatocyte, the freed bilirubin begins to form intramolecular hydrogen bonds. To prevent this bilirubin is bound to glutathione S-transferases (GSTs) and travels to the endoplasmic reticulum of the cell (Figure 3). The endoplasmic reticulum contains Uridinediphosphoglucuronosyl transferase isoform 1A1 (UGT1A1). This enzyme catalyzes glucuronidation of bilirubin. In this reaction bilirubin is conjugated with glucuronic acid, which breaks the intermolecular hydrogen bonds that make bilirubin insoluble in water. The conjugated bilirubin is transported out of the cell through ATP-dependent multidrug resistance associated protein 2 (MRP2) and stored in bile. Bile is released into the small intestine during digestion and most of the conjugated bilirubin is excreted from the body waste (Wolfoff, 2014).

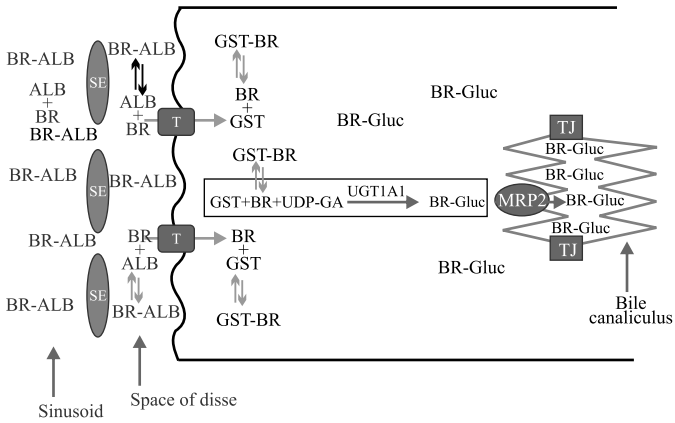


**Figure 3*:*** Metabolism and Excretion of Bilirubin in Hepatocyte (Wolkoff, 2014).

## 3. MOLECULAR IMPLICATIONS

Patients with Gilbert's syndrome lack Uridinediphosphoglucuronosyl transferase isoform 1A1 (UGT1A1), so glucuronidation of bilirubin in hepatocytes does not occur. (U.S. National Library of Medicine). Mature human UDP-glucuronosyltransferase enzymes are approximately 500 – 510 amino acids in length and contain two domains (Figure 4). The amino terminal domain binds aglycones and the carboxyl-terminal binds to UPD glucuronic acid (Patana, 2009).

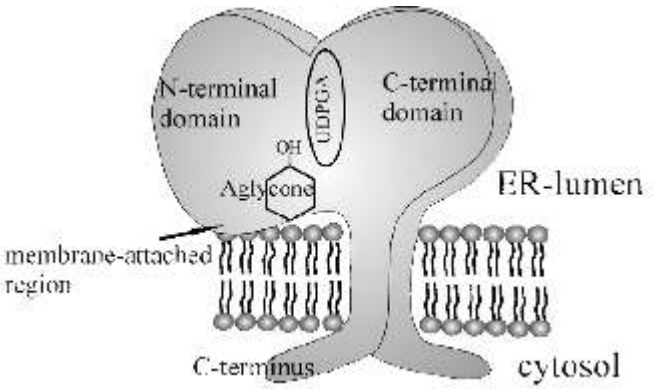


**Figure 4:** Mature Human UDP-Glucuronosyltransferase Enzyme (Patana, 2009).

 

This enzyme brings the substrates to the catalytic site, which is generally between amino acids 300 – 400 and contains a histidine residue. Once in this location, aglycones are acted upon by UPD glucuronic acid in an $S_N2$ reaction mechanism (Figure 5). In the case of bilirubin, the -OH of the carboxyl group acts as the nucleophile. It is important to note that the UGT1A1 enzyme contains a catalytic histidine residue, which increasing the nucleophilic action of the -OH through deprotonation (Patana, 2009).

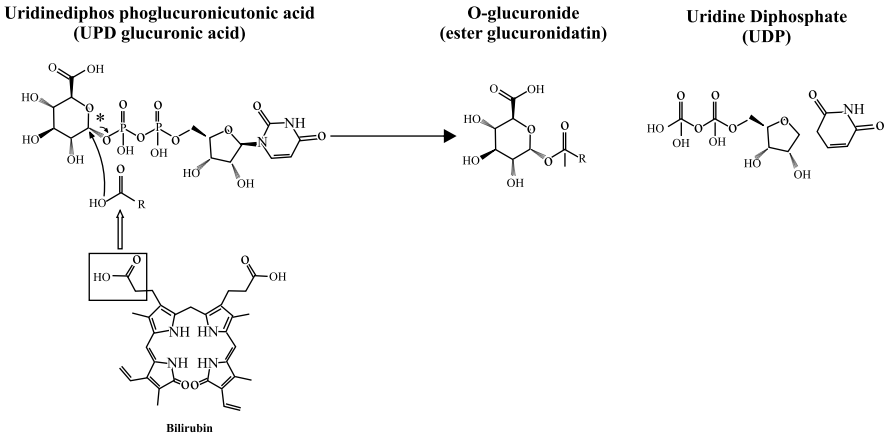


**Figure 5:** $S_N2$ Reaction Mechanism of Ester Glucuronide. The nucleophile attacks an electrophilic carbon from the back side relative to the location of the leaving group. This is known as a "back side attack" (Patana, 2009).

The glucoronidation of bilirubin is known as ester glucuronidation, due to the formation of ester substituents. Bilirubin contains two carboxyl groups which undergo the reaction, respectively. This leads to the consumption of two UPD glucuronic acids (Figure 6) The lack of UDP-glucuronosyltransferase-1A1 in a patient with Gilbert's syndrome can understood when examining the UGT1A1 gene.

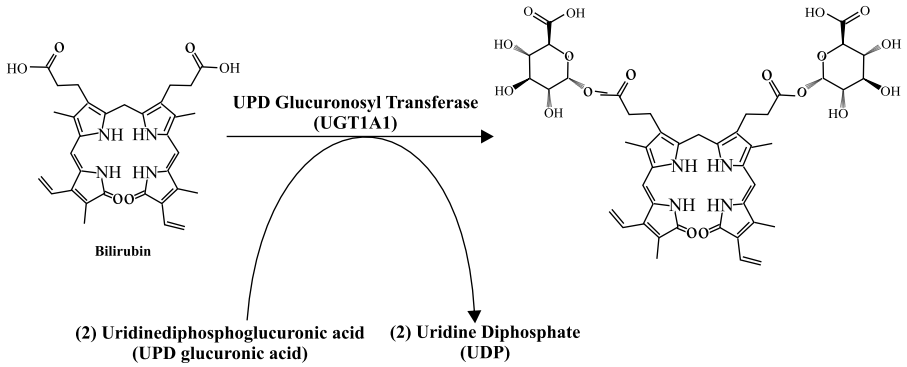


**Figure 6:** Glucuronidation of Bilirubin catalyzed by the UGT1A1 enzyme. The addition of the acid prevents intermolecular hydrogen bonding, making the conjugated bilirubin hydrophilic.

## 4. GENETIC IMPLICATIONS

The UDP-glucuronosyltransferase-1A1 is encoded by the UGT1A1 gene located on the long arm of chromosome 2 (Roy-Chowdhury, 2015). Like many eukaryotic genes, UGT1A1 contains a promoter region with a TATAA element. In this gene, the TATAA element is the binding site for transcription factor IID, which is important in the initiation of transcription. The normally functioning TATAA region for this gene is $A(TA)_6TAA$ (Bosma *et al.*, 1995). Patients with Gilbert's syndrome have an extra 'TA' in the

TATAA sequence, which is expressed as A(TA)$_7$TAA. In literature, this mutation is denoted as UGT1A1*28 promoter mutation. The decrease in enzyme produced lead to approximately 30 percent more bilirubin left in the bloodstream (Mölzer *et al.,* 2016).

For this mutation to have a noticeable effect in hepatocytes, both copies of chromosome two must have the mutation. This means that Gilbert's syndrome is inherited in an autosomal recessive fashion. Only in 3-7 percent of the general population is found to have this condition, due in part to the pattern of inheritance (Roy-Chowdhury, 2015).

## 5. SYMPTOMS

The most common indicator of this disease is recurrent episodes of jaundice during periods of increased stress, such as strain, dehydration, fasting, infection, or exposure to cold. Jaundice is the yellowing of the skin or eyes as a result excess bilirubin in the blood. If there is excess bilirubin in the blood, not all of it can complex to albumin. When unbound to albumin, the yellow-tinted bilirubin floats in the bloodstream due to its hydrophobic nature (Wang *et al,* 2006).

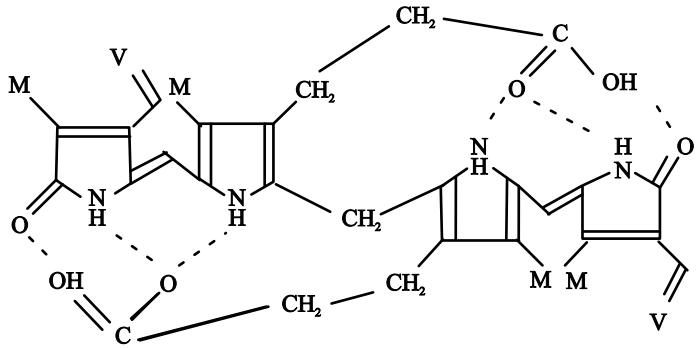


**Figure 7:** Intermolecular Hydrogen Bonding of Bilirubin

Despite its large number of hydrogen donating and accepting groups, bilirubin is a hydrophobic molecule (Figure 7). This is due to its tending to form intramolecular hydrogen bonds instead of intermolecular bonds.

Some individuals report fatigue, weakness, nausea, abdominal discomfort, and diarrhea; however, many researchers believe that these symptoms are a result of anxiety rather than the accumulation of bilirubin in the blood (Roy-Chowdhury, 2015). Gilbert's syndrome does have some interesting advantages and can offer some protection against cancer and heart disease due to the anti-oxidant effects of bilirubin. Studies have shown a decreased risk of ischemic heart disease, Hodgkin lymphoma, endometrial cancer, and cancer-related mortality (Chandrasekar and Savio, 2019).

## 6. DIAGNOSIS AND PROGNOSIS

Gilbert's syndrome is easily diagnosed through blood tests that analysis the percent of bilirubin in the blood. Normal individuals will have between 1.0mg/dl of bilirubin present in the blood, while patients with Gilbert's syndrome will have between 2.0mg/dl and 3.5mg/dl of bilirubin present in the blood (Wolkoff, 2014). In many cases, the individual is diagnosed after a routine blood draw.

Individuals diagnosed with this Gilbert's have a very bright outlook. In most cases, patients are

 

asymptomatic, and no treatment is required (Roy-Chowdhury, 2015). If a patient displays jaundice, this can be treated with lifestyle changes such as physical stress reduction. For individuals with coexisting conditions that increase bilirubin levels, such as hemolytic anemia, may be prescribed phenobarbital.

## 7. CONCLUSION

Those who experience Gilbert's syndrome have one key difference in the promoter region of the UGT1A1 gene located on the long arm of chromosome 2. This mutation results in an extra 'TA' in the TATAA sequence, which is expressed as $A(TA)_7TAA$. Failure to express this gene results in the lack of UDP-glucuronosyltransferase-1A1. Without this enzyme, bilirubin is not conjugated and remains in the bloodstream. While most people are asymptomatic, the most common visible symptom is jaundice, which yellowing of the skin or eyes as a result excess bilirubin in the blood. This does not tend to pose a serious health risk and can be addressed through lifestyle changes.

## 8. REFERENCES

Bogdanos, D. P., Gao, B., Gershwin, M. E. 2013. Liver immunology. *Comprehensive Physiology*, *3*(2), 567–598.

Bosma, P. J., Chowdhury, J. R., Bakker, C. Gantla, S., de Boer, A. Oostra, B. A., Lindhout, D., Tytgat, G. N., Jansen, P. L., Elferink, R. O., Chowdhury, N. R. 1995. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *The New England Journal of Medicine, 333*(18), 1171-1175.

Chandrasekar V. T., Savio, J. 2019. Gilbert Syndrome. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing. < https://www.ncbi.nlm.nih.gov/books/NBK470200/>

Mölzer, C., Wallner, M., Kern, C., Tosevska, A., Schwarz, U., Zadnikar, R., Doberer, D., Marculescu, R., Wagner, K. H. 2016. Features of an altered AMPK metabolic pathway in Gilbert's Syndrome, and its role in metabolic health. *Scientific reports*, *6* (30051). doi:10.1038/srep30051

Patana, A. S. 2009. The human UDP-glucuronosyltransferases : studies on substrate binding and catalytic mechanism. Accessed 01 November 2019, https://pdfs.semanticscholar.org/7005/9cc239e1065388aac8651bc2df51f4fed4ff.pdf

Roy-Chowdhury, N., and Roy-Chowdhury, J. 2015. Gilbert Syndrome. *National Organization for Rare Disorders.* accessed 20 October 2019, < https://rarediseases.org/rare-diseases/gilbert-syndrome/>

U.S. National Library of Medicine. 2019. Gilbert syndrome, National Institutes of Health, accessed 20 October 2019, < https://ghr.nlm.nih.gov/condition/gilbert-syndrome>

Wang, X., Chowdhury, J., Chowdhury, N. 2006. Bilirubin metabolism: Applied physiology. *Current Paediatrics, 16* (1), 70-74.

Wolkoff, A. W. 2014. Bilirubin Metabolism. In: *Gastrointestinal Anatomy and Physiology : The Essentials*. Reinus, J. F. (Ed.) Retrieved from https://ebookcentral-proquest-com.libproxy.bellarmine.edu