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Frequency of Two Common Variants in Hyperbilirubinemic Palestinian Patients

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Declaration

I declare that, except where explicit reference is made to the contribution of others, this thesis is substantially my own work and has not been submitted for any other degree at the Arab American University or any other institution.

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Dedication

This research is dedicated to my parents Abdallah Alqadi and Rino Shaaban and my husband, Maher Alkilani.

Ghada Abdallah Mohamed Alqadi

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Frequency of Two Common Variants in Hyperbilirubinemic Palestinian Patients

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Abstract

Elevated bilirubin levels are a common symptom of a condition known as neonatal hyperbilirubinemia (NH). While typically harmless, severe NH can lead to complications like bilirubin encephalopathy (kernicterus), causing brain damage. NH has both genetic and environmental risk factors. The UGT1A1 gene is essential for the metabolism of bilirubin because it encodes UDP-Glucuronosyl transferases, which are enzymes of the glucuronidation pathway that convert small lipophilic molecules like bilirubin into water-soluble metabolites by conjugating bilirubin to glucuronic acid, which is necessary for excretion of bilirubin. Changes in bilirubin levels in different populations have been associated with variations in this gene. Certain syndromes characterized mainly by unconjugated hyperbilirubinemia are caused by different variants within the *UGT1A1*gene including Gilbert's syndrome (OMIM: 143500), Crigelr-Najjar type 1(OMIM: 218800), Crigler-Najjar type 2(OMIM:606785) and Transient familial neonatal hyperbilirubinemia (OMIM: 237900).

The purpose of this study was to investigate the prevalence of two common variants within the *UGT1A1* gene (rs3064744 [(TA)7TAA] and rs4124874 (c.-3279 T > G)) and their association with NH in Palestinian neonates. The study included 70 neonates diagnosed with NH ranging in age from 2 to 64 days. Genotyping was carried out using Sanger's sequencing.

Our analysis revealed an association between [(TA)7TAA] (rs3064744) variant and increased bilirubin levels in the studied group. Moreover, while the c.-3279 T > G (rs4124874) variant was frequent among patients, it did not show a statistically significant correlation with NH.

The study shows that there is a possible genetic contribution of variations in the *UGT1A1* gene to elevated bilirubin levels in the Palestinian population. A better understanding of the genetic background of NH can improve personalized diagnostic and preventive strategies, improving neonatal health outcomes in Palestine.

Keywords: UGT1A1 (uridine diphosphate glucuronosyltransferase); neonatal hyperbilirubinemia; UGT1A1*28 (TA)7TAA variant; c.-3279 T > G variant; jaundice.

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Chapter One: Introduction

1.1 Background of the study

Murekatete et al., 2020 reported that neonatal jaundice is the leading cause of hos pital admission for newborns. Neonatal jaundice is reported in 60% of full-term infants and 80% of premature babies in the American population (Brits et al., 2018). Compared to African populations living in Africa, Americans of African descent had much-reduced prevalence of neonatal jaundice. This suggests that the pathophysiology of newborn jaundice may be significantly influenced by environmental factors. Moreover, compared to Caucasian infants, East Asian infants have a 37% higher diagnosis rate for jaundice (Setia et al., 2002). These findings imply that genetic factors have a role in the higher prevalence of newborn jaundice among East Asians. Thus, the pathophysiology of newborn jaundice may involve both hereditary and environmental variables (Brits et al., 2018).

Neonatal jaundice is known to be caused by hemolytic factors, which include a deficiency of glucose-6-phosphate dehydrogenase (G6PD), Rh incompatibility, and ABO incompatibility. Other causes of neonatal jaundice that are regularly checked in neonates requiring therapy include polycythemia, low birth weight, preterm, positive family history of hyperbilirubinemia, and breastfeeding jaundice. The latter type arises when babies do not get a sufficient amount of milk, which elevates serum bilirubin levels because of increased reabsorption in the intestines (ULLAH et al., 2016).

Several genetic factors may contribute to neonatal jaundice. Variants in a gene known as Uridine Glucuronosyl Transferase 1A1 (UGT1A1) have been identified as a genetic factor potentially contributing to neonatal jaundice. Variants in UGT1A1 may occur in exons, introns, the enhancer region, or the TATA box in the promoter region. An example of a UGT1A1 variant is the UGT1A1*28 [(TA)7TAA] (rs3064744) located in the promoter region; it results in a significantly decreased enzymatic expression and is known to be a risk factor for neonatal jaundice (Yusoff et al., 2006). Another known UGT1A1 variant is the substitution mutation (c211G > A) (rs4148323) in exon 1 of the UGT1A1 gene. It has been linked to 60% enzyme expression and has been found as a risk factor for neonatal jaundice in several East Asian populations (South Korea and Japan) (Amandito et al., 2018). Variants in exon 1 or the promoter lead to phenotypes clinically

known as Gilbert syndrome (GS), Crigler-Najjar syndrome (CNS) type 1 and type 2, transient familial neonatal hyperbilirubinemia, and enzyme deficiency and impaired bilirubin conjugation (ULLAH et al., 2016).

Augustin Gilbert and Pierre Lereboullet originally described the autosomal recessive condition, Gilbert syndrome, in 1901 (Amandito et al., 2018). 3% to 7% of Americans and 5% to 10% of Caucasians are affected by GS, the most common inherited disorder of bilirubin metabolism. It is primarily characterized by intermittent unconjugated hyperbilirubinemia without hepatocellular disease or hemolysis, which manifests clinically during menstruation, physical activity, fasting, or stress (Amandito et al., 2018).

Two types of Crigler-Najar syndrome (CNS) are distinguished based on the total serum bilirubin level. The inheritance pattern for both syndromes is autosomal recessive. Crigler-Najar syndrome type I (CNS-I) is more severe than Crigler-Najar syndrome type II (CNS-II) and characterized by high levels of total serum bilirubin (342–684 μmol/L), whereas the milder form (CNS-II) is characterized by total serum bilirubin (TSB) values ranging from 103 to 342 μmol/L (Sun et al., 2017). CNS-I, first identified by Crigler and Najjar in 1952, is the most severe UGT1A1-associated hereditary disorder. It is characterized by a complete or almost complete absence of UGT1A1 enzyme activity associated with severe jaundice, causing a life-threatening condition with some affected individuals developing a type of brain damage knows as kernicterus. In 1962, Arias described CNS-II, which is marked as a partial loss of UGT1A1 activity, causing a moderate degree of jaundice. (Amandito et al., 2018).

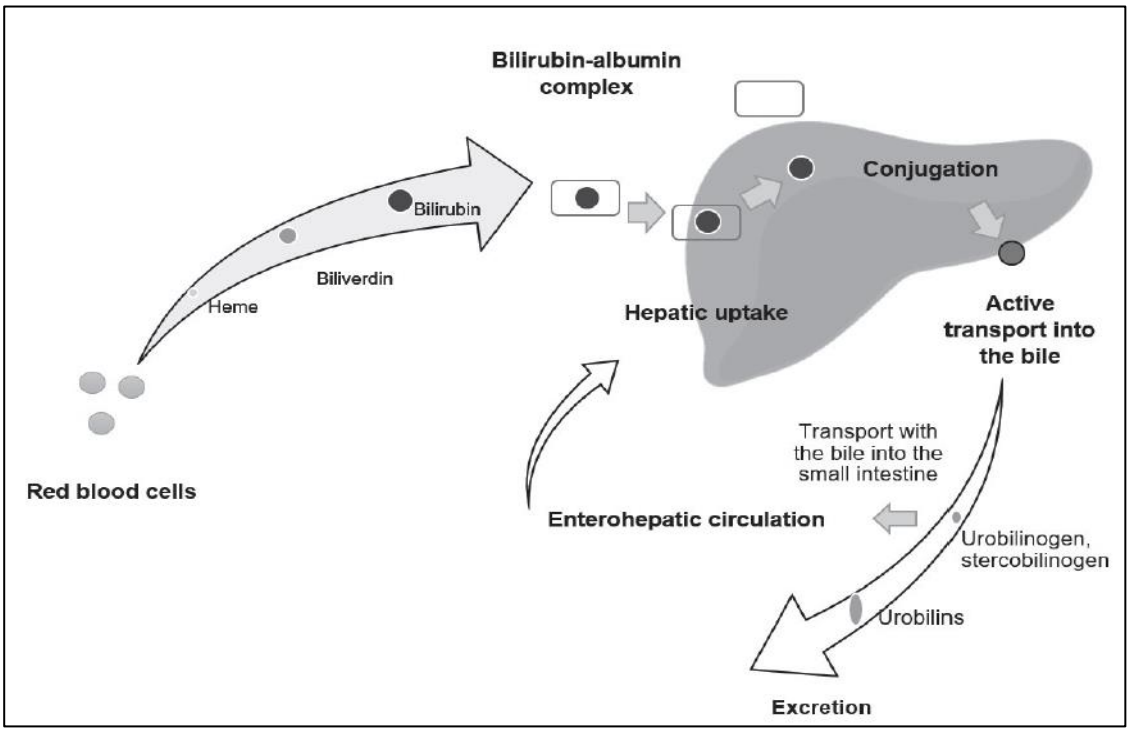
Transient Familial Neonatal Hyperbilirubinemia, also called Lucey Driscol Syndrome, can be either an autosomal dominant or autosomal recessive disorder. It was first described in 1960 by Lucey et al. According to ARIAS et al. (1965), the disorder is a rare familial condition that manifests as severe unconjugated hyperbilirubinemia in the first days of life.

Chapter Two: Literature Review

2.1 Bilirubin Metabolism

Heme is made up of protoporphyrin IX and ferrous iron complexed with a porphyrin ring. It is a part of several different proteins, including red blood cell (RBC) hemoglobin. RBC hemoglobin breaks down to produce the majority of bilirubin. The remaining portion is produced by the breakdown of other heme-containing proteins (Sticova & Jirsa, 2013). Heme oxygenase breaks down heme into biliverdin, which is then reduced by biliverdin reductase to unconjugated bilirubin (UCB).

Unconjugated bilirubin, or UCB, is insoluble in water and is absorbed by the transport protein albumin as soon as it enters the bloodstream. According to Steckova and Jirsa (2013), UCB-albumin complexes are subsequently delivered to the liver. Bilirubin and glucuronic acid get attached to the hepatocyte endoplasmic reticulum to form conjugated bilirubin. The uridine diphosphate glycosyltransferase family 1 member A1 (UGT1A1) enzyme is responsible for the metabolism of this conjugation reaction (Bosma, 2003). Bilirubin's solubility is increased when it is conjugated with glucuronic acid, which is necessary for its excretion in the bile. Active transporters, like the multidrug resistance-associated protein MRP2/cMOAT, excrete conjugated bilirubin into the bile (Erlinger et al., 2014; Jemnitz et al., 2010). Bacterial flora in the small intestine deconjugate bilirubin, converting it into urobilinogen and urobilin, which is eliminated in



the feces (Bosma, 2003). Enterohepatic circulation allows for the partial absorption of urobilinogen. The process of bilirubin metabolism is outlined in **Figure 2.1** (Valávsková & Muchová, 2016).

Figure 2.1 Bilirubin metabolism. The main source of bilirubin is hemoglobin destruction. In the circulation, bilirubin is bound to albumin. Bilirubin-albumin molecules are transported to the liver, where bilirubin is conjugated to glucuronic acid. Conjugated bilirubin is excreted through the intestines. (Figure from Valávsková & Muchová, 2016)

Unconjugated bilirubin can build up as a result of changes in bilirubin metabolism. Although high concentrations of bilirubin are neurotoxic and can have harmful effects, they are also a strong antioxidant that shields cells from oxidative stress (Kapitulnik, 2004). When the levels of UCB exceed the capacity of albumin binding, the levels of unbound (free) bilirubin increase. Free bilirubin is toxic and readily penetrates cells. The central nervous system's cells are the most impacted, as unbound bilirubin can result in severe neurological deficits (Kapitulnik, 2004). Free bilirubin can cross the blood-brain barrier (BBB) in neonates and build up in the brain, resulting in neurological damage and bilirubin encephalopathy (kernicterus), both of which are thought to be irreversible (Brites & Silva, 2021). Due to immature liver function and fetal-enhanced erythrocyte breakdown, which results in poor bilirubin conjugation, neonates typically have elevated plasma unconjugated bilirubin (UCB) levels during the first two weeks of life (Kapitulnik, 2004). values of UCB start to drop at around one month of age, and they eventually reach the typical adult values of 0.2 to 0.8 mg/dL. Neonates can develop critically high bilirubin levels under certain conditions, which can lead to serious complications.

2.2 Neonatal hyperbilirubinemia (NH)

According to Sawyer and Gleason (2023), the most common reason for hospital readmission in the neonatal period is neonatal hyperbilirubinemia (NH). It is characterized by increased circulating total serum bilirubin (TSB)-above 5 mg/dL (86 μmol per L)-and jaundice (Porter & Dennis, 2002).

Due to insufficient liver maturation and the quick decomposition of red blood cells in neonates, which creates an imbalance in the elimination and synthesis of bilirubin, neonatal hyperbilirubinemia tends to be considered a transient benign syndrome (Sawyer & Gleason, 2023).

Nevertheless, despite its common incidence, some individuals experience noticeably elevated bilirubin levels, which have the ability to cause severe brain damage and are linked to major underlying conditions such as hemolytic disease and metabolic disorders. Jaundice is characterized by a yellow discoloration of the mucous membranes, skin, and the white part of the eye (sclera). It usually manifests only when levels of unconjugated blood bilirubin exceed 90 μmol/L (5.26 mg/dL).

The incidence of neonatal hyperbilirubinemia differs by region and ethnicity. According to Diala et al. (2023), the populations with the highest rates of neonatal hyperbilirubinemia are those in Africa and Southeast Asia. Geographic differences also exist; Setia et al. (2002) and MG et al. (2018) found that Americans of African ancestry had a significantly lower frequency of NH than African populations living in Africa. These results imply that environmental factors might have significant effects on how NH develops. The management and early detection of neonatal jaundice can be affected by environmental factors, including the overall quality of the healthcare system and the availability of prenatal and postnatal care. Variations in incidence rates may be caused by differences in healthcare systems and practices between the United States and different African nations (Kebede et al., 2023).

For instance, dietary habits or nutrient deficits may impact bilirubin metabolism and liver function (Zaitsu et al., 2018). The high prevalence of infections like malaria and other illnesses in various African areas, which worsen jaundice, is another environmental factor. According to Reyburn and Virk (2009), infection-related hemolysis could increase bilirubin levels, which can result in more frequent or severe cases of neonatal jaundice. Furthermore, neonatal jaundice rates may be impacted by the environment, including exposure to severe temperatures or conditions that negatively affect general health. For instance, heat stress and dehydration could worsen jaundice or make managing it more difficult (L. Zhang et al., 2019). Also, there is evidence for an interaction between genetic and environmental factors; while genetics play a role in jaundice, the interaction between genetic predispositions and environmental conditions (such as exposure to certain toxins or pollutants) could influence its prevalence and severity.

Increased bilirubin levels in newborns can be caused by a variety of clinical conditions. Based on the mechanisms beneath bilirubin buildup, these causes can be classified into three main categories, as seen in **Figure 2.2.** The first category includes

conditions associated with increased hepatic bilirubin load and can be classified into hemolytic causes and non-hemolytic causes (Porter & Dennis, 2002; Sawyer & Gleason, 2023). Hemolysis in neonates can occur due to different conditions such as abnormalities in the membrane structure of the red cell such as hereditary spherocytosis. It can also occur due to red blood cell enzyme abnormalities including glucose-6-phosphate dehydrogenase deficiency and pyruvate kinase deficiency (Sawyer & Gleason, 2023).

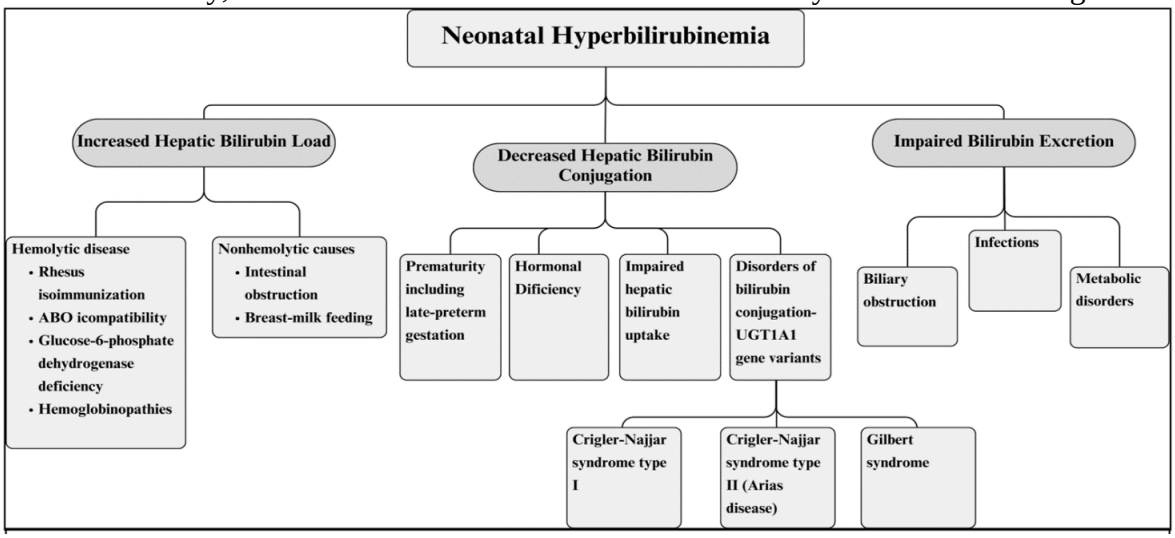
Immune-mediated causes can also result in neonatal hemolysis such as Rhesus isoimmunization and ABO incompatibility. Rhesus isoimmunization has historically been a significant cause of neonatal hyperbilirubinemia, affecting between 3 to 8 per 100,000 patients annually. Before the introduction of anti-D prophylaxis, Rhesus isoimmunization was responsible for fetal loss in approximately 1% of all pregnancies, due to complications such as hyperbilirubinemia (jaundice), hydrops fetalis, and stillbirths. Each year, about 4 million newborns are exposed to jaundice or undiagnosed hyperbilirubinemia, with over 3.5 million being born at 35 weeks of gestation or later in the United States. In sub-Saharan Africa, the lack of affordable anti-D immunoglobulin (Ig) means that RhD isoimmunization remains a major contributor to perinatal morbidity and continues to affect obstetric care. For example, in Ethiopia, the incidence of neonatal jaundice is 26.45% (Aliyo et al., 2023)

Neonatal jaundice associated with ABO incompatibility typically manifests in the first 24 to 48 hours of life (Routray et al., 2023). Hemolysis is caused by a deficit of the RBC enzyme glucose-6-phosphate dehydrogenase, or G6PD (Lee et al., 2022). People from Southeast Asia, Africa, the Middle East, and the Mediterranean region, which includes Thailand and Malaysia, have a high incidence of G6PD deficiency (Q. Li et al., 2015). Because G6PD deficiency is an X-linked recessive condition, the incidence of G6PD deficiency in males is significantly higher than in females. (Alangari et al., 2023).

Non-hemolytic factors that result in elevated bilirubin levels include intestinal blockage, polycythemia, and breastfeeding jaundice, which is associated with a compound (3-α-20β pregnanediol) found in breast milk that can induce neonatal jaundice in 1% to 2% of newborns breastfed. Neonatal jaundice symptoms usually start on day 4 to day 7 and can last between 3 weeks up to 10 weeks, or even longer (Gao et al., 2023).

The second category of neonatal hyperbilirubinemia-causing conditions includes factors that result in decreased hepatic bilirubin conjugation such as hormonal deficiency, prematurity including late preterm gestation because hyperbilirubinemia is more common, severe, and prolonged in preterm infants compared to full-term infants. This is due to the shorter lifespan of their red blood cells (RBCs) and the immaturity of their liver and gastrointestinal systems. Furthermore, delayed enteral feeding is common in preterm newborns, which might worsen bilirubin clearance by reducing intestinal motility and bacterial colonization. Neonatal hyperbilirubinemia in premature newborns is more severe and lasts longer due to these developmental and clinical factors (Aynalem et al., 2020). Another factor that leads to decreased hepatic bilirubin conjugation is the presence of disorders affecting bilirubin conjugation, which are caused by genetic variations in the UGT1A1 gene. The UDP-glucuronosyltransferase enzyme, which is necessary for the glucuronidation pathway, is encoded by this gene. The UDP-GT enzyme helps bilirubin be excreted by converting it into a more water-soluble form. Hereditary unconjugated hyperbilirubinemia can be caused by deficiencies in this enzyme, which may inhibit bilirubin conjugation. This includes conditions such as Gilbert syndrome (GS), Crigler-Najjar syndrome (CNS) type 1 and type 2, and transient neonatal hyperbilirubinemia(Sun et al., 2017).

The last category includes impaired bilirubin excretion which can be caused by biliary obstruction, infections, and metabolic disorders such as alpha1 antitrypsin deficiency. Reduced amounts of the A1AT protein are the result of mutations in the SERPINA1 gene, which causes alpha-1 antitrypsin (A1AT) insufficiency. Normally, this protein protects tissues from white blood cell-released enzymes such as proteases. In A1AT deficiency, the lack of sufficient A1AT allows these enzymes to cause damage to



various organs. Additionally, abnormal A1AT protein can accumulate in liver cells, resulting in liver damage. This damage can present as liver inflammation, fibrosis, and cirrhosis. Consequently, the liver's impaired ability to excrete bilirubin often leads to elevated bilirubin levels(Feldman & Sokol, 2013).

Figure 2.2 Conditions associated with hyperbilirubinemia. Different clinical conditions can contribute to bilirubin increased levels in neonates. These conditions can be classified into three main categories based on mechanisms of bilirubin accumulation. (Porter & Dennis, 2002; Sawyer & Gleason, 2023)

2.2 Uridine Diphosphate glucuronosyltransferase family 1 member A1 (*UGT1A1*) gene

The UGT1A1 gene is located on chromosome 2q37.1 and consists of five exons (**Figure 2.3**.). Its promoter regulates the first exon, which encodes the bilirubin binding site (Sayers et al., 2022). The UGT1A1 gene is approximately 13 kb (13,027 bp). Several UDP-glucuronosyltransferases, which are enzymes of the glucuronidation pathway that convert small lipophilic molecules like steroids, bilirubin, and hormones into water-soluble, excretable metabolites, are encoded by this gene, which is a component of a large locus on chromosome 2 (Sayers et al., 2022). Member A1 of the UDP glucuronosyltransferase family 1 catalyzes the conversion of bilirubin to glucuronic acid, which is necessary for the excretion of bilirubin. Genetic variations in this gene have been documented to be a possible cause of neonatal hyperbilirubinemia since they result in Crigler-Najjar syndromes types I and II and Gilbert syndrome. These variants may be found in the promoter region's TATA box, enhancer region, introns, or exons.

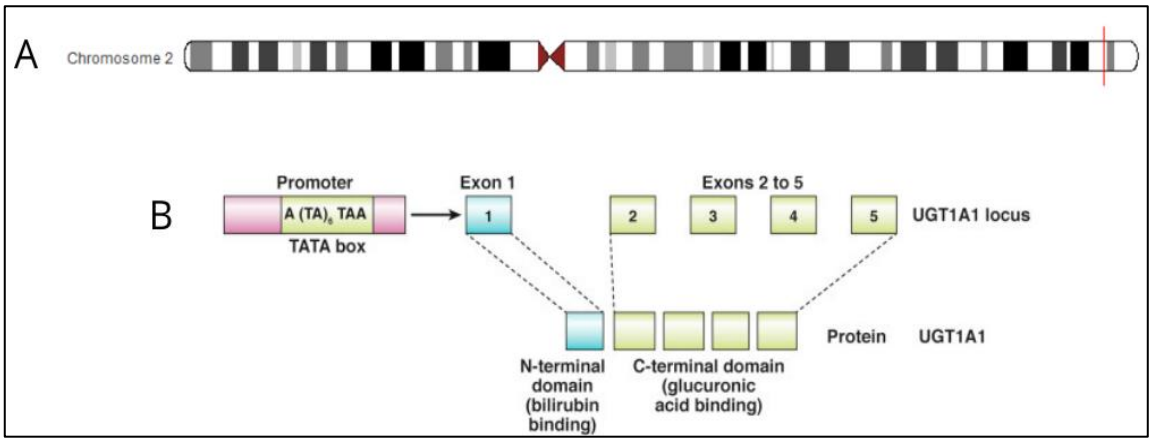


Figure 2.3 Schematic representation of the UGT1A1 genomic locus(A) (Serizawa et al., 2021) and UGT1A1 exons and protein(B) (Erlinger et al., 2014).

2.4 Clinical syndromes associated with variants in the *UGT1A1* gene

According to several studies, reduced activity of the hepatic UDP glucuronosyltransferase family 1 member A1 can result from variants in the UGT1A1 gene, which elevates the levels of unconjugated bilirubin (Leung & Sauve, 2016). Variants in the promoter region of *UGT1A1* reduce gene expression (L. Li et al., 2015; Olusanya et al., 2015), while variants in the coding region cause dysfunction of the UGT1A1 gene and decreased enzyme activity (Olusanya et al., 2015). Different clinical syndromes have been associated with different variants in the UGT1A1 gene

2.4.1 Gilbert syndrome (GS)(OMIM: 143500)

Gilbert syndrome, also known as benign hyperbilirubinemia, is an autosomal recessive syndrome with a clinical significance of conflicting interpretations of pathogenicity. It is characterized by a mild elevation of unconjugated bilirubin levels ranging from 1-6 mg/dL in the absence of abnormalities in liver enzymes. Patients with Gilbert syndrome have reduced activity of UGT1A1 (Bosma et al., 1995). Bosma reported a homozygosity of TA duplication in the TATA box region of the *UGT1A1* gene's promoter to be responsible for GS development (Bosma et al., 1995). Normally, the allele in the TATA box region is characterized by the presence of six TA repeats [A(TA)6TAA]. Two extra bases (TA) in the TATAA element are associated with GS development (Bosma et al., 1995). Rodrigues also reported that homozygosity for this allele is associated with higher bilirubin levels (Rodrigues et al., 2012). Several other variants in the *UGT1A1* gene have been reported to be associated with GS. Maruo and colleagues reported different polymorphisms that affect bilirubin metabolism in GS patients (Maruo et al., 2016). Different genotypes were reported including homozygous UGT1A1*28, UGT1A1*6/UGT1A1*28, and homozygous UGT1A1*6, and UGT1A1*27/UGT1A1*28 (Maruo et al., 2016). Research has shown that different combinations of these variations result in differences in serum bilirubin concentrations (Maruo et al., 2016).

2.4.2 Crigler-Najjar Syndrome Type I (CN I) (OMIM: 218800)

Crigler Najjar syndrome type I is an autosomal recessive syndrome that causes a significant increase in serum bilirubin levels ranging from 20 to 45 mg/dL in the absence of abnormalities in liver enzymes. Severe jaundice appears in the first days of life of the affected neonates and some of them die in the first weeks or months of life with kernicterus. The UGT1A1 enzyme in patients with CN type I is either absent or

nonfunctional. This results in a severe deficiency of the enzyme, leading to a dramatic increase in unconjugated bilirubin levels in the blood. Since unconjugated bilirubin is not water-soluble, it cannot be easily excreted through urine and instead builds up in the blood and tissues.

Reports have linked various variants of the UGT1A1 gene to CN type I. Ritter et al. (1992) found that a patient with CN type I had a homozygous deletion in exon 2. A group of children from consanguineous Pakistani families were found to have multiple variations in this gene, according to research conducted by Khan and colleagues (Khan et al., 2013). Furthermore, a Sudanese newborn with CN type I was also found to have a 22 bp duplication in exon 1 of the UGT1A1 gene (Valmiki et al., 2020). To date, 61 variants in the UGT1A1 gene for Crigler-Najjar syndrome type 1 have been reported on The Human Gene Mutation Database (HGMD).

2.4.3 Crigler-Najjar Syndrome Type II (CN II) (OMIM:606785)

Crigler-Najjar syndrome type II is also an autosomal recessive syndrome characterized by elevated serum bilirubin levels ranging from 6 to 20 mg/dL (*Online Mendelian Inheritance in Man, OMIM®*, 2023) in the absence of abnormalities in liver enzymes. Compared to CN type I, CN type II exhibits milder hyperbilirubinemia due to a partial loss of hepatic UGT1A1 activity, which reduces the liver's ability to convert bilirubin into its conjugated (direct) form. As a result, unconjugated bilirubin levels in the blood are raised, although not as much as in Type 1.

According to Petit et al. (2008), Asian populations reported cases of CN type II more frequently than other populations. Numerous UGT1A1 gene variations were linked to CN type II, including missense variants (rs34946978) c.1091C>T (P364L), c.1456T>G (Y486N), c.686 C>A (P229Q), and c.211G>A (rs4148323) (Ko et al., 2014).

2.4.4 Transient familial neonatal hyperbilirubinemia (OMIM: 237900)

Lucey-Driscoll syndrome, also known as transient familial newborn hyperbilirubinemia, is an autosomal dominant or recessive disorder characterized by elevated blood bilirubin levels that first manifest in the first few days of birth (Online Mendelian Inheritance in Man, OMIM®, 2023). Although the exact cause of the condition is still unknown, Newman and Gross (1963) were the first to propose that it

could be caused by a substance found in breast milk called pregnane-3(alpha),20(beta)-diol, which inhibits the glucuronidation of bilirubin. They also described the condition as benign and associated with jaundice from breast milk (Maruo et al., 2000; NEWMAN & GROSS, 1963). Transient familial neonatal hyperbilirubinemia can be brought on by homozygous or heterozygous mutations in the UGT1A1 (uridine diphosphate-glucuronosyltransferase) gene.

In one study in Japan, 17 breastfed infants with sustained hyperbilirubinemia (TSB >10 mg/dL at 3 weeks to 1 month) were examined (Maruo et al., 2000). All newborns' serum bilirubin levels decreased when their mothers ceased nursing. However, some infants' serum bilirubin levels increased once again when the mothers returned to breastfeeding. When the babies were four months old, their bilirubin levels went back to normal (Maruo et al., 2000). Sequencing of UGT1A1 revealed that eight infants were homozygous and seven heterozygous for the missense mutation 211G-A (rs4148323) which is common in the East Asian population and Japanese population with Gilbert syndrome. In heterozygous patients with the 211G-A (rs4148323) mutation, a different variant was discovered. This variant is an additional TA insertion in the TATA box of the promoter region of the UGT1A1 gene (UGT1A1*28 [(TA)7TAA] (rs3064744).(Maruo et al., 2000).

Therefore variations of UGT1A1 (especially variants seen in GS) are associated with breast milk jaundice, and this suggests that breast milk jaundice is caused by a combination of genetic and environmental factors (i.e., breast milk components) (ARIAS et al., 1964; Maruo et al., 2000).

2.5 Variants in the UGT1A1 gene

The UGT1A1 gene is highly polymorphic, with more than 113 variants reported to be associated with increased bilirubin levels(Strassburg, 2008). Variants of the *UGT1A1* gene can be classified into three groups: (1) Variants that result in reduced production of the enzyme, (2) variants that give rise to a structurally and/or functionally abnormal enzyme, and (3) variants that completely abolish the expression of the enzyme. Variants in the *UGT1A1* gene occur in the coding regions (exons), the non-coding regions (introns), and the promoter region.

Polymorphisms in the number of TA repeats (TA)n in the UGT1A1 gene's promoter influence bilirubin levels. The wildtype form of the *UGT1A1*gene's promoter in Caucasian populations has a TATAA box with 6 TA repeats [(TA)6], this allele is known as the UGT1A1*1 allele (Kaplan et al., 2008). Different variations in the number of TA repeats have been reported. One of the most reported variations is the UGT1A1*28 [(TA)7TAA] (rs3064744) variant which contains an extra thymine-adenine (TA) repeat. This variation results in a decreased rate of transcription initiation of the gene, leading to decreased enzyme activity to about 30% of the wildtype levels (Bosma et al., 1995). People who are homozygous for the UGT1A1*28 allele have a 70% decrease in the transcriptional activity of the gene (Tukey et al., 2002). The UGT1A1*28 allele is associated with Gilbert syndrome (GS) development (Bosma et al., 1995). This variant was reported in 25% percent of 55 Malaysian neonates with hyperbilirubinemia (Yusoff et al., 2006).

Another variant associated with decreased UGT1A1 enzyme activity is the c.211 G > A variant (UGT1A1*6, p.Arg71Gly, rs4148323). This variant was found to be associated with neonatal hyperbilirubinemia in Asian populations (Jinno et al., 2003), and has been identified as the main cause of Gilbert syndrome in East Asian populations (Yang et al., 2015, 2016). Chou and colleagues also showed that neonates who were heterozygous or homozygous for this variant were more susceptible to developing early-onset neonatal hyperbilirubinemia (Chou et al., 2011). Yang has also confirmed that this variant was associated with high conjugated bilirubin levels in Chinese ABO-incompatible newborns (Yang et al., 2021). Interestingly, the compound heterozygous UGT1A1*28 and UGT1A1*6 variants were the most common UGT1A1 variants in Chinese Han GS patients (M. Zhang et al., 2021).

Another major variant related to neonatal hyperbilirubinemia is the c.686C>A variant (UGT1A1*27 p.Pro229Gln, rs35350960), which is also located in exon 1 of the *UGT1A1* gene. Individuals carrying this allele had decreased enzyme activity (Udomuksorn et al., 2007). A heterozygous genotype of this variant was reported to result in GS (Koiwai et al., 1995). Zhang reported that Chinese individuals with compound heterozygous UGT1A1*28+*6+*27 variants were at higher risk of developing hyperbilirubinemia (M. Zhang et al., 2021).

Another variation associated with hyperbilirubinemia is the c.1091C>T in exon 4 (p.Pro364Leu, rs34946978). This variant was shown to cause a significant decrease in enzyme activity (Takeuchi et al., 2004). On ClinVar, this variant is reported to be likely pathogenic and associated with hyperbilirubinemia (RCV000194762.13) (Landrum et al., 2014). This variant was observed in GS and CNS-type patients (L. Li et al., 2015; Takeuchi et al., 2004).

In addition to the abovementioned variants, it has been proposed that the c.1456T>G variant (UGT1A1*7, p.Tyr486Asp, rs34993780) in exon 5 of the *UGT1A1* gene plays a role in neonatal hyperbilirubinemia. It is also reported on ClinVar that this variant is pathogenic and correlated with hyperbilirubinemia (RCV000147900.13) (Landrum et al., 2014). Different studies have reported the association of this variant with GS and CNS type II (Takeuchi et al., 2004; Udomuksorn et al., 2007).

A variant known as c.-3279 T > G (rs4124874) in the UGT1A1 Proximal Promoter Region within the Phenobarbital Responsive Enhancer Module (PBREM) was also correlated with increased hyperbilirubinemia risk. Bilirubin levels are influenced by the presence of this variant (Rodrigues et al., 2012). Additionally, it was documented that this variant reduces transcriptional activity by nearly 60% and results in increased susceptibility to neonatal hyperbilirubinemia (Z. Li et al., 2020). This variant has a conflicting classification, being categorized as a likely pathogenic, variant of uncertain significance, benign or pathogenic on clinVar.

2.6 Problem statement

Hyperbilirubinemia is a common health problem among Palestinian neonates. Various global studies have reported the association of different *UGT1A1* gene variations with hyperbilirubinemia. However, no previous studies have been conducted to identify the frequency of common *UGT1A1* variants among Palestinian neonates suffering from hyperbilirubinemia.

2.7 Significance of the research

Neonatal hyperbilirubinemia can result in devastating consequences including irreversible brain injury. Understanding the underlying pathophysiology and etiology of this condition is crucial for accurate diagnosis and timely intervention. This research

focuses on the identification of the frequency of common variants in the UGT1A1 associated with elevated bilirubin levels within Palestinian neonates. By identifying UGT1A1 alleles related to neonatal hyperbilirubinemia in the Palestinian population, this research holds the potential to improve healthcare outcomes by enabling early diagnosis and risk assessment.

2.8 Research aims

This study aims to determine the frequency of two common variants within the *UGT1A1* gene among Palestinian neonates diagnosed with unconjugated hyperbilirubinemia. The targeted variants are rs3064744 (UGT1A1*28)[(TA)7TAA] in the a promoter TATA box, and c.-3279 T > G (rs4124874) variant which is located in the phenobarbital responsive element (PBREM).

2.9 Research hypothesis

We hypothesized that specific variants in the *UGT1A1* gene are correlated with elevated bilirubin levels among neonates in the Palestinian population.

Chapter Three: Methodology

3.1 Study design and sample selection

This research is a cross-sectional study. The study included 70 neonates ranging in age from 2 to 64 days who had high serum bilirubin in the first 30 days of life, with a median age of 15 (from 1 to 59 days). They were recruited from Jenin Governmental Hospital and from different medical centers related to the Ministry of Health in Ramallah and Hebron between October 2017 and April 2018. Subjects were only included if bilirubin levels were equal to or higher than 3.5 mg/dL. Neonates who had glucose-6-phosphate dehydrogenase (G6PD) deficiency, ABO incompatibility, Rh incompatibility, polycythemia, birth trauma, sickle cell anemia, or other abnormalities of the red blood cells were excluded from the study. Guardians of all patients gave written informed consent for their children to participate in the study.

3.2 Variant Selection

The studied variants are UGT1A1*28 [(TA)7TAA] (rs3064744) and c.-3279 T > G (rs4124874). **Table 2.1** provides detailed information about the two variants. The variants were selected based on previous studies that documented an association between each of the studied variants and hyperbilirubinemia-related disorders such as Gilbert's syndrome, Crigler Najjar syndrome type I, and Crigler Najjar syndrome type II and Transient familial hyperbilirubinemia (Amandito et al., 2018; Huang et al., 2000; Liu et al., 2017; Maruo et al., 2004, 2014; Mi et al., 2019; Sun et al., 2017; Wang et al., 2020).

3.3 DNA Isolation, Qualification, and Quantification

Venous whole blood samples (3-5 ml) were collected in EDTA-containing tubes from 70 neonates suffering from hyperbilirubinemia in the health facilities where they were admitted. The samples were then transferred on ice to the molecular genetics laboratory of the Arab American University. Genomic DNA was isolated using the nuPREP Blood DNA Mini Kit according to the manufacturer’s protocol. First, 200 µl of whole blood was put in a 1.5 ml Eppendorf tube. Then, 200 µl of Lysis buffer (10mMTris-HCl, 400 mM NaCl, and 2mM Na2EDTA, pH 8.2) and 20 µl of proteinase K were added and mixed for 10 seconds. The tubes were incubated for 10 minutes at room temperature. Next, 350 µl of binding buffer was added and mixed using a micropipette. The lysate was

then loaded onto filter tubes and centrifuged at 11.000 xg (~12,000 rpm) for 1 minute. After centrifugation, 400 µl of washing buffer 1 was added, and the tubes were centrifuged at 11,000 x g (~12,000 rpm) for 1 minute. Next, 600 µl of washing buffer 2 was added, and the tubes were centrifuged at 11,000 x g (~12,000 rpm) for 1 minute. The previous step was repeated. The filtrate was discarded, then the spin filter was placed in a new collection tube and centrifuged at a maximum speed for 3 minutes. The spin filter was transferred to a clean elution tube, and 200 µl of elution buffer was added. The tubes were incubated for 2 minutes, followed by centrifugation at 11.000 x g (~12.000 rpm) for 1 minute.

DNA concentration was measured by NanoDrop (Thermo Scientific, USA). All samples had an A260/A280 ratio above 1.7. Finally, the DNA was stored at -20 C until further use.

3.4 DNA Genotyping

Genotyping was performed using DNA sequencing.

3.4.1 Polymerase Chain Reaction

Polymerase chain reaction was utilized to amplify target DNA sequences. The reaction's total volume was 25 µl and included 12.5 µl of 2X GoTaq Green master mix (lyophilized mixture of Taq polymerase, MgCl2, dNTP, and buffer), 1 µl of 10 µM forward primer, 1 µl of 10µM reverse primer, 7.5 to 9.5 µl of ultra-pure water, 4 µl of DNA from samples with DNA concentration ≤ 50 ng/µl, and 2 µl of DNA from samples with DNA concentration ≥ 51ng/µl. The sequences of primers are given in **Table 2.1.** PCR was run on a FlexCycler2 thermocycler (Analytik Jena, Germany) according to the thermal cycling conditions for the two variants provided in **Table 2.2**.

3.4.2 Agarose Gel Electrophoresis

PCR products were examined on a 3% agarose gel with 1X TBE buffer (Bio Basic Inc., Canada). The gel was run at 100 volts for 1.5 hours. Band sizes of PCR products were estimated based on a comparison of a 50 bp DNA ladder (New England BioLabs, UK). The ethidium bromide-stained gels were visualized using a GelDoc-It imaging system (UVP, USA). The resulting bands (for the standard primer PCR products) were approximately sized based on the migration distance of each band.

3.4.3 DNA sequencing analysis

Table 2.1 Variants details, sequences of primers, and method of investigation

Variant reference	Position	Gene region	Variation	Molecular conse-quence	Forward and Reverse primers	Method used
Rs3064744	Chr2(GRCh38/hg38):233760233-233760234	Promoter	NM_019076.5:c.856-6800AT[8]	Promoter variant	5'GTCACGTGACACAG-TCAAAC3' 5'TTTGCTCCTGCCAGAGGTT3'	DNA sequencing
Rs4124874	Chr2(GRCh38/hg38):233757013	Proximal pro-moter PBREM	NM_021027.3:c.856-10021T>G	Promoter variant	5'CAC-CAGAACAAACTTCTGAG3' 5'CTGTCCCTTCTGAATCATTG3'	DNA sequenc-ing

For the CCA(TA)6/7/8TAA (rs3064744) and c.-3279 T > G (rs4124874) variants investigation, Sanger's sequencing was carried out. After DNA amplification, the PCR product was cleaned up by mixing 5 µl of the PCR product with 1µl of clean-up reagent Eppic Fast (A&A biotechnology) and incubated at 37°C for 15 minutes. Samples that required dilution were diluted using distilled water. Sanger's sequencing was then done by the BigDyeTM Direct Cycle Sequencing Kit (Thermo Fisher Scientific, USA) and run on the Applied Biosystems 3500 Genetic Analyzer. Nucleotide variations were examined using the Sequence Scanner Software 2.

Table 2.2 PCR Cycling Profile

Variant	Initial Denaturation (5 min)	Denaturation (45 sec)	Cycling Annealing (45 sec)	Elongation (45 sec)	Final Extension (10 min)	Number of cycles
CCA(TA)5/6/7TAA (rs3064744)	95 ◦C	95 ◦C	60 ◦C	72 ◦C	72 ◦C	35
c.-3279 T > G (rs4124874)	95 ◦C	95 ◦C	55◦C	72 ◦C	72 ◦C	30

3.5 Statistical analysis

Descriptive statistics were employed to summarize the data. For categorical variables, such as alleles and genotypes, frequency tables and percentages were calculated. For continuous variables, including patients' age and total bilirubin levels, measures of central tendency (mean, median) and dispersion (standard deviation) were determined along with the minimum and maximum values. The Kolmogorov-Smirnov test of normality was applied, and since the data did not support parametric assumptions, the Kruskal–Wallis test and the Mann–Whitney U test were performed when applicable.

Bonferroni correction was applied for multiple group comparisons, and the results were evaluated accordingly. The Pearson Chi-square test was performed to evaluate the associations between categorical variables. The level of significance was defined as $\alpha = 0.05$. The SPSS (Statistical Package of Social Sciences Demo Version 22.0) program was used for calculations and analyses.

Chapter Four: Results

This study investigated the prevalence of rs3064744 and rs4124874 variants in the *UGT1A1* gene and their association with neonatal hyperbilirubinemia in the Palestinian population. The studied variants were previously reported to be associated with neonatal hyperbilirubinemia in different populations around the world.

To determine the prevalence and association of the mentioned variants with hyperbilirubinemia, 70 neonates suffering from hyperbilirubinemia were enrolled in the study. Out of the 70, 43.5% (31/70) were males and 56.5% (39/70) were females. Participant's ages ranged from 2 to 64 days with a median age of 15 days. The demographic characteristics of the sample are shown in **Table 4.1**.

Table 3.1 Demographic characteristics of the enrolled sample

Characteristics	**N (n=70)**	**%**
Gender		
Male	31	43.5
Female	39	56.5
Age (Mean ±SD)	19.07± 15.77	
Total bilirubin (Mean(SD))	11.68 ± 4.27	

4.1 Statistical analysis of possible correlation between patient's age and total bilirubin levels

Pearson correlation coefficient (r) was calculated to evaluate the correlation between participant age and total bilirubin. The coefficient ranges from -1 to 1, with a value closer to 0 indicating a weaker correlation and values closer to -1 or 1 indicating a stronger negative or positive correlation, respectively. As shown in **Table 4.1**, Our analysis showed that for all ages, the relationship was low negative and statistically significant (r = -0.242, P = 0.045). However, when stratified by age category, the results varied. For participants younger than 30 days, a significant moderate negative relationship between bilirubin and the participant's age was found (r = -0.424, P = 0.001). In contrast, participants older than 30 days showed a weak positive but statistically insignificant correlation (r = 0.262, P = 0.387). Furthermore, the test showed a strong negative and statistically significant relationship for participants aged less than 15 days. At 16-30 days, there was a strong negative but statistically insignificant relationship.

Regression analysis was also calculated to predict total bilirubin concentration based on the participant's age. It revealed a significant association between the participant's age

and total bilirubin concentration (F(3,65) = 3.56, P = 0.019). The model explained 14.1% of the variance in bilirubin levels (R^2 of 0.141). The regression equation predicted that total bilirubin concentration decreased by 0.076 mg/dL with each day increase in participant age.

Table 4.2 Correlation between age and total bilirubin levels.

Age (days)	Mean Total Bilirubin	Mean Participant age (days)	R-value	P- value	Number
All Ages	11.62± 4.26	11.68 ± 4.27	-0.242*	0.045	70
≤ 15	12.7± 4.95	9.18 ± 4.0	-0.302	0.050	40
16-30	10.05± **2**.38	20.41± 4.2	-0.449	0.71	17
<30	11.86± 4.51	12.2 ± 6.2	-0.424**	0.001	57
≥30	10.56 ± 2.84	48.6 ± 8.3	0.262	0.387	13

Statistical significance at $p \leq 0.05$.

4.2 Genotyping and statistical analysis of $(TA)_n$ repeat polymorphism (rs3064744)

The PCR product of the UGT1A1 (TA) promoter region containing rs3064744 revealed a distinct single band with a size of 98 bp as expected, given that the ladder size is 50 bp. This is depicted in **Figure 4.1**. Representative Sanger sequencing results are displayed in **Figure 4.2**. The homozygous state of rs3064744 TA(6/6) is displayed in **Figure 4.2.A**, and the homozygous mutant state of rs3064744 TA(7/7) is displayed in **Figure 4.2.B**.

Our statistical analysis showed that the most common allele in the samples was TA(7), with a frequency of 0.52, followed by TA(6), which had a frequency of roughly 0.29. **Table 4.3** displays the genotype frequencies and **Table 4.4** shows the allele frequency in our sample and in gnomAD database. As seen in **Table 4.5**, the Mann-Whitney U test revealed that the presence of the (TA)7 allele raises total bilirubin levels.

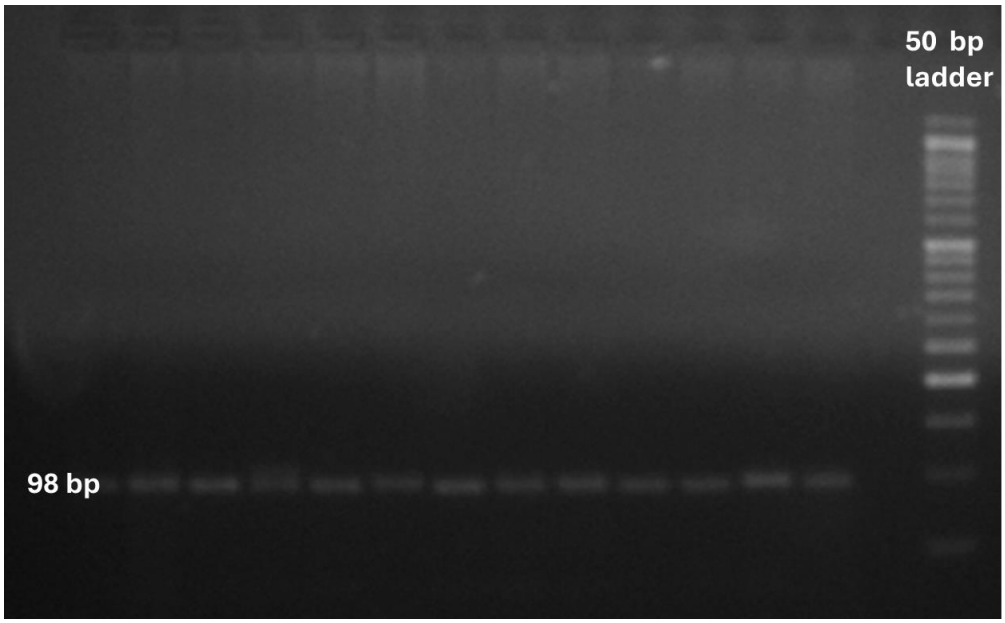


Figure 4.1 Representative image of rs3064744 PCR gel electrophoresis

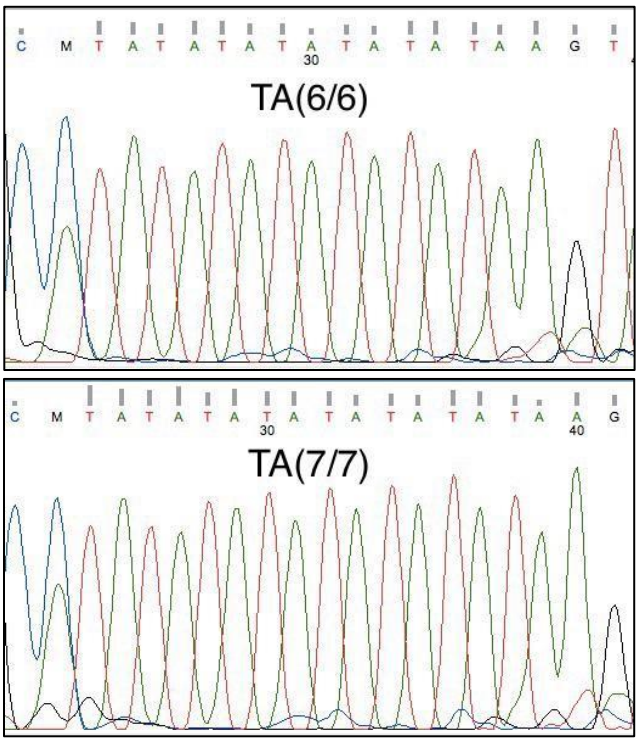


Figure 4.2 Representative images of rs3064744 sequencing results

Table 4.3 (TA)n variant genotype frequencies and their effect on total bilirubin level± std deviation (mg/dl)

Genotype	Frequency	Total bilirubin
(TA)6/6	0.24	11.91±2.78
(TA)6/7	0.10	11.39±3.24
(TA)7/7	0.40	11.83±6.70
(TA)7/8	0.14	11.54±5.23
(TA)8/8	0.11	13.39±4.14

Table 4.4 (TA)n variant (rs3064744) allele frequencies of Palestinian neonates and in gnomAD in the middle east.

Allele	Frequency in our study	Frequency in gnomAD database in the middle east
(TA)6	0.292	0.685
(TA)7	0.521	0.314
(TA)8	0.185	0.002

Table 4.5 rs3064744 allele and its relation to bilirubin concentration

Allele (TA)n rs3064744	Total bilirubin		
	Presence Mean ±SD	Absence Mean ±SD	P value
(TA)6	12.14±3.03	11.35 ±4.79	0.16
(TA)7	11.19±4.67	12.38 ±3.37	0.06
(TA)8	12.77±6.57	11.22 ±3.09	0.86

Data were presented as mean and standard deviation and were compared using the Mann-Whitney U test.
*Statistically significant at $p \leq 0.05$.

4.3 Genotyping and statistical analysis of rs4124874

Amplification of the rs4124874 containing sequence using PCR yielded a 396 bp product as shown in **Figure 4.3** which shows a representative image of the yielded amplicon as qualified using gel electrophoresis, next to a 50 bp ladder. Sanger sequencing was used for rs4124874 genotyping, **Figure 4.4** shows representative images of sequencing results. **Figure 4.4.A** shows the homozygous variant G/G, **Figure 4.4.B** shows the homozygous wild type T/T, and **Figure 4.4.C** shows the heterozygous genotype G/T.

According to statistical analysis, the homozygous mutant genotype (G/G) had a frequency equal to 0.32 and the heterozygous genotype (G/T) was the most common, with a frequency equal to 0.45. Conversely, the homozygous wild type (T/T), which is the least common genotype, had a frequency of 0.18. The genotype frequencies from our study and from gnomAD database are displayed in **Table 4.6**. A one-way ANOVA test revealed

that there was no significant relationship between any genotype and the level of total bilirubin.

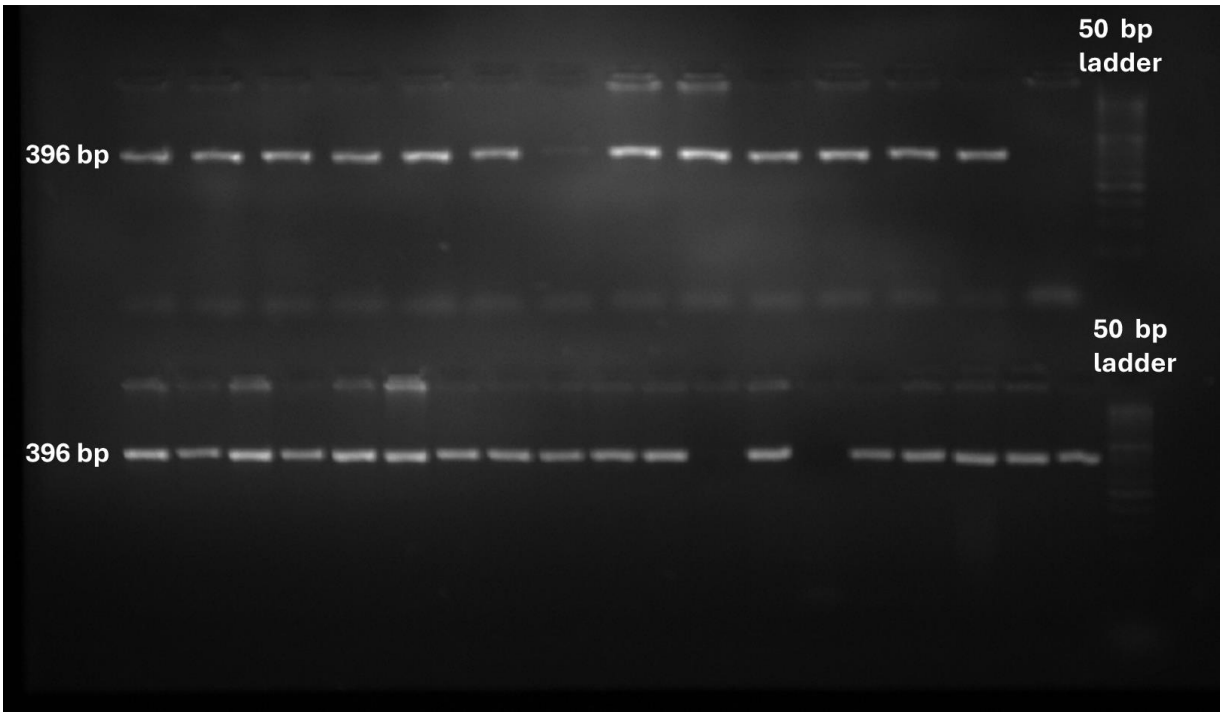
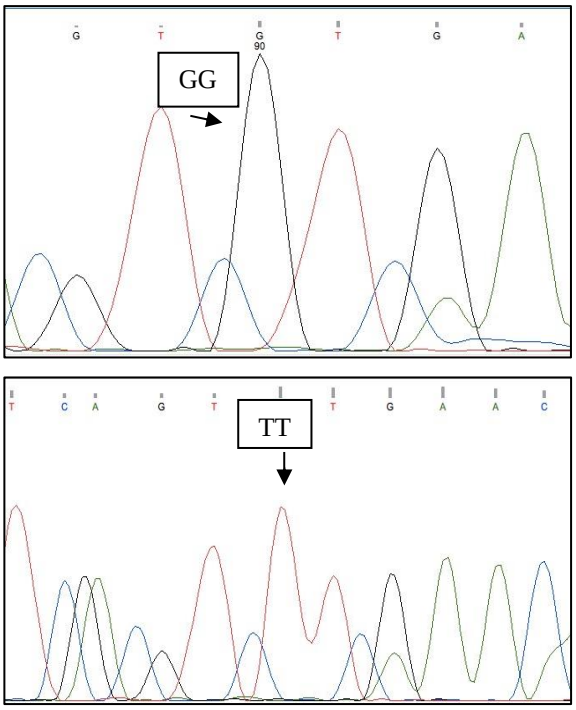


Figure 4.3 Representative image of rs4124874 PCR gel electrophoresis



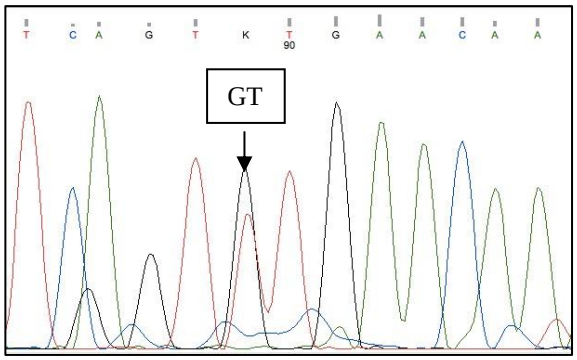


Figure 4.4 Representative images of rs4124874 sequencing results

Table 4.6 rs4124874 variant genotype frequencies and their effect on total bilirubin level ±std deviation (mg/dl)

Genotype	Frequency in our study	Frequency in gnomAD in the Middle East	Total bilirubin
G/T	0.471	0.50	12.79±4.93
T/T	0.200	0.25	10.97±3.04
G/G	0.328	0.25	10.48±3.53

The mean difference is considered significant at the 0.05 level.

Table 4.7 rs4124874 variant allele frequencies of Palestinian neonates and in gnomAD in the Middle East.

Allele	Frequency in or study	Frequency in gnomAD in the Middle East
G	0.564	0.50
T	0.435	0.50

Chapter Five: Discussion

5.1 Discussion

The exact pathogenesis of neonatal hyperbilirubinemia is still not fully understood. Different risk factors had been identified to contribute to neonatal hyperbilirubinemia such as congenital malformations, G6PD deficiency, ABO incompatibility, and genetic variants in different genes including the *UGT1A1* gene. This study aimed to investigate the frequency of two common variants in the UGT1A1 gene among hyperbilirubinemic Palestinian patients. These variants are two promoter variants, UGT1A1*28 (TA)7TAA (rs3064744) variant and c.-3279 T > G (rs4124874) variant.

The UGT1A1 gene encodes the enzyme bilirubin UDP glucuronosyltransferase, which is responsible for bilirubin glucuronidation. Detoxification of endogenous and external lipophilic substances, including bilirubin, depends on glucuronidation. Hyperbilirubinemia is a result of bilirubin buildup brought on by decreased UGT1A1 activity. Three different hereditary UGT1A1 deficiencies: Gilbert's syndrome, Crigler-Najjar syndrome type 1, and Crigler-Najjar syndrome type 2 are caused by different variants in this gene. These conditions can present with varying degrees of neonatal hyperbilirubinemia, with Crigler-Najjar syndrome type 1 being the most severe form and Gilbert’s syndrome exhibiting the mildest phenotype.

A variant in the TATA box in the *UGT1A1* promoter, particularly a TA insertion A(TA)7TAA (UGT1A1*28)(rs3064744), has been identified in patients with Crigler-Najjar syndrome type 1, Crigler-Najjar syndrome type 2, and Gilbert's syndrome (Bosma et al., 1995; Ciotti et al., 1998; Monaghan et al., 1996). This variant is associated with conflicting evidence of pathogenicity based on the ClinVar database (Variation ID:12275, Accession: VCV000012275.56) (Landrum et al., 2014). The variant is frequently observed in individuals of Caucasian and African ancestry but is relatively uncommon in Asian populations (Beutler et al., 1998). In the Middle East, a study conducted in Saudi Arabia reported a frequency of 25.7% for this variant, which is lower than in Africans but higher than in Asian populations (Alkharfy et al., 2013).

In normal individuals, the UGT1A1*28 variant is associated with increased bilirubin levels (Bosma et al., 1995). Homozygosity for UGT1A1*28 is correlated with prolonged neonatal hyperbilirubinemia (Bancroft et al., 1998; Pasha et al., 2017). Various

studies have demonstrated that this variant reduces transcriptional activity dramatically in contrast to the wild type six repeats allele (Bosma et al., 1995; Ciotti et al., 1998). The UGT1A1*28 variant has a reduced affinity to transcription factor II D-binding protein, thus resulting in less *UGT1A1* transcription (Hsieh et al., 2007). An inverse relationship between the number of TA repeats and the promoter's activity within the range of 5–8 Ta repeats has been observed (Beutler et al., 1998). Additionally, Yu et al. (2015) showed that UGT1A1*28 markedly increases the risk of hyperbilirubinemia in neonates.

Despite the wealth of research supporting the association between this TATA box variant and neonatal hyperbilirubinemia, some studies indicated the absence of such an association. For instance, Alexandrino et al. (2001) documented no correlation between this TATA box variant and hyperbilirubinemia in Portuguese neonates. Similarly, A study on Turkish neonates reported similar distributions of TA(6/6), TA(6/7), and TA(7/7) genotypes among subjects with neonatal hyperbilirubinemia and healthy subjects (Babaoglu et al., 2006). Moreover, a systematic meta-analysis concluded that the UGT1A1*28 variant is not related to an increased risk of neonatal hyperbilirubinemia (H. Li & Zhang, 2021). In our study, the homozygous wild type (7/7) had the highest rate with a frequency of 0.4, followed by the homozygous wild type (6/6), and the heterozygous genotype (6/7) had a frequency equal to 0.1.

Data from the database (gnomAD) which was taken on 18th of the of August, 2024, from version 4.0.1, showed that the rs3064744 variant has a frequency of 0.314 in the Middle Eastern population, 0.038 in its homozygous state (7/7) and 0.275 in its heterozygous state (6/7) (Chen et al., 2024). It should be noted that data from gnomAD does not exclude people who had had neonatal hyperbilirubinemia.

The impact of *UGT1A1* promoter TATA box polymorphisms on UGT1A1 activity and bilirubin levels varies across populations, potentially explaining the controversial research results regarding the relationship between the UGT1A1*28 variant and bilirubin levels. Additionally, other factors such as age and gender may contribute to the discrepancies observed in different studies.

Another variant examined in this study is the c.-3279 T > G (rs4124874) variant, which has a conflicting classification of pathogenicity, likely pathogenic, uncertain significance, and benign in ClinVar. This variant is located in an enhancer element located

in the *UGT1A1* promoter, referred to as the upstream phenobarbital-responsive element module (PBREM). PBREM functions as a binding site for several transcription factors such as constitutive androstane receptor (CAR) and pregnane X receptor (PXR)(Sugatani et al., 2004). The c.-3279 T > G (rs4124874)variant has been associated with a decrease in *UGT1A1* transcription and increased risk for Gilbert's syndrome (Sugatani et al., 2002). Various studies have demonstrated the role of this variant in neonatal hyperbilirubinemia development in different populations, including Indians (D'Silva et al., 2014), Malay (Yusoff et al., 2010), and Japanese (Yanagi et al., 2017). For instance, in Indian neonates with hyperbilirubinemia the c.-3279 T > G (rs4124874) variant was significantly higher among the patients than the controls(D'Silva et al., 2014). Li et al. (2020) conducted a meta-analysis and showed that this variant increases susceptibility to neonatal hyperbilirubinemia. In agreement with other studies, our study showed that the heterozygous (G/T) and homozygous mutant (G/G) genotypes were higher in the patients than the wild-type (T/T) genotype, with frequencies of 0.45 and 0.32 respectively. Data taken from the database (gnomAD), version 4.0.1 on the 18th of August, 2024, showed that the homozygous variant genotype G/G has a frequency of 0.267 in all populations which is higher than the heterozygous G/T which has 0.232 frequency(Chen et al., 2024). High levels of bilirubin were seen mostly in the homozygous genotype in our study. Despite that, our analysis revealed no significant correlation between any of the genotypes and total bilirubin level.

This study also examined the relationship between age and bilirubin levels among different age categories (≤15, 16-30, <30, and ≥30 days old). The results indicated a low negative and statistically significant correlation between age and bilirubin levels across all ages. This suggests that as age increases, total bilirubin levels tend to decrease slightly, which aligns with the established understanding that bilirubin levels peak shortly after birth and then start decreasing as the liver matures and the neonatal physiology stabilizes. This decrease may indicate that the enzymatic activity or transcriptional activity of the *UGT1A1*gene increases with age, which coincides with other reports that showed that gene transcription is an important factor in the development of hyperbilirubinemia (Nie YL. et al., 2017) and that gene transcription and consequently enzymatic activity is modulated with age (Neumann E. et al., 2016).

A limited sample size of only 70 neonates in our study reduces the statistical power to identify significant relationships, especially for variations that may have an effect on

NH. Furthermore, the research is limited in its generalizability to other communities due to its focus on Palestinian neonates, a community peculiarity, distinct genetic backgrounds or ethnic groups that may have distinct consequences from genetic variants. The fact that this study only looks at two specific genetic variants inside the UGT1A1 gene presents another limitation due to its narrow variant analysis. Environmental Factors are also considered a limitation because this study does not provide detailed information about the environmental factors that can also influence bilirubin levels, such as feeding practices, birth weight, gestational age, or maternal health.

Also, the neonatal age which ranged from 2 to 64 days, may introduce variability in bilirubin levels due to the natural progression of jaundice over time. In addition, even though the Sanger sequencing methodology is accurate, it is also labor-intensive and may not detect all types of genetic variations, such as large insertions, deletions, or other complex rearrangements, which could be relevant to NH. In the end, this study is a cross-sectional study that assesses bilirubin levels at a single point in time. This design limits the ability to establish a causal relationship between the identified variants and NH.

5.2 Conclusion

This study is the first to investigate the frequency of variants in the *UGT1A1*gene commonly associated with neonatal hyperbilirubinemia in the Palestinian population. Our results demonstrated an association between the promoter variant UGT1A1*28 [(TA)7TAA](rs3064744) and increased bilirubin levels in Palestinian neonates, consistent with some previous research conducted on other ethnicities. This finding suggests that UGT1A1*28 may influence bilirubin metabolism in Palestinian neonates, highlighting the potential genetic contribution to neonatal hyperbilirubinemia. Further research is required to explore the frequency of various UGT1A1variants among the Palestinian population and their association with disease development. This knowledge would enhance management and risk assessment strategies for neonatal hyperbilirubinemia.

References

Alangari, A. S., El-Metwally, A. A., Alanazi, A., Al Khateeb, B. F., Al Kadri, H. M., Alshdoukhi, I. F., Aldubikhi, A. I., Alruwaili, M., & Alshahrani, A. (2023). Epidemiology of Glucose-6-Phosphate Dehydrogenase Deficiency in Arab Countries: Insights from a Systematic Review. *Journal of Clinical Medicine*, *12*(20). https://doi.org/10.3390/jcm12206648

Aliyo, A., Ashenafi, G., & Abduselam, M. (2023). Rhesus Negativity Prevalence and Neonatal Outcomes among Pregnant Women Delivered at Bule Hora University Teaching Hospital, West Guji Zone, South Ethiopia. *Clinical Medicine Insights. Pediatrics*, *17*, 11795565221145598. https://doi.org/10.1177/11795565221145598

Alkharfy, K. M., Alghamdi, A. M., Bagulb, K. M., Al-Jenoobi, F. I., Al-Mohizea, A. M., Al-Muhsen, S., Halwani, R., Parvez, M. K., & Al-Dosari, M. S. (2013). Distribution of selected gene polymorphisms of UGT1A1 in a Saudi population. *Archives of Medical Science : AMS*, *9*(4), 731–738. https://doi.org/10.5114/aoms.2013.37012

Amandito, R., Putradista, R., Jikesya, C., Utaminingsih, D., Rusin, J., Rohsiswatmo, R., & Malik, A. (2018). UGT1A1 gene and neonatal hyperbilirubinemia: A preliminary study from Bengkulu, Indonesia. *BMC Research Notes 2018 11:1*, *11*(1), 1–6. https://doi.org/10.1186/S13104-018-3284-Y

Arias, I. M., Gartner, L. M., Seifter, S., & Furman, M. (1964). Prolonged Neonatal Unconjugated Hyperbilirubinemia Associated With Breastfeeding And A Steroid, Pregnane-3(Alpha), 20(Beta)-Diol, In Maternal Milk That Inhibits Glucuronide Formation In VITRO. *The Journal of Clinical Investigation*, *43*(11), 2037–2047. https://doi.org/10.1172/JCI105078

ARIAS, I. M., WOLFSON, S., LUCEY, J. F., & MCKAY, R. J. J. (1965). TRANSIENT FAMILIAL NEONATAL HYPERBILIRUBINEMIA. *The Journal of Clinical Investigation*, *44*(9), 1442–1450. https://doi.org/10.1172/JCI105250

Aynalem, S., Abayneh, M., Metaferia, G., Demissie, A. G., Gidi, N. W., Demtse, A. G., Berta, H., Worku, B., Nigussie, A. K., Mekasha, A., Tazu Bonger, Z., McClure, E. M., Goldenberg, R. L., & Muhe, L. M. (2020). Hyperbilirubinemia in Preterm Infants Admitted to Neonatal Intensive Care Units in Ethiopia. *Global Pediatric Health*, *7*, 2333794X20985809. https://doi.org/10.1177/2333794X20985809

Babaoglu, M. O., Yigit, S., Aynacioglu, A. S., Kerb, R., Yurdakok, M., & Bozkurt, A. (2006). Neonatal jaundice and bilirubin UDP-glucuronosyl transferase 1A1 gene polymorphism in Turkish patients. *Basic & Clinical Pharmacology & Toxicology*, *98*(4), 377–380. https://doi.org/10.1111/j.1742-7843.2006.pto_341.x

Bancroft, J. D., Kreamer, B., & Gourley, G. R. (1998). Gilbert syndrome accelerates the development of neonatal jaundice. *Journal of Pediatrics*, *132*(4), 656–660. https://doi.org/10.1016/S0022-3476(98)70356-7

Beutler, E., Gelbart, T., & Demina, A. (1998). Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: A balanced polymorphism for regulation of bilirubin metabolism? *Proceedings of the National Academy of Sciences of the United States of America*, *95*(14), 8170–8174. https://doi.org/10.1073/pnas.95.14.8170

Bosma, P. J. (2003). Inherited disorders of bilirubin metabolism. *Journal of Hepatology*, *38*(1), 107–117. https://doi.org/10.1016/s0168-8278(02)00359-8

Bosma, P. J., Chowdhury, J. R., Bakker, C., Gantla, S., de Boer, A., Oostra, B. A., Lindhout, D., Tytgat, G. N. J., Jansen, P. L. M., Elferink, R. P. J. O., & Chowdhury, N. R. (1995). The Genetic Basis of the Reduced Expression of Bilirubin UDP-Glucuronosyltransferase 1 in Gilbert's Syndrome. *New England Journal of Medicine*, *333*(18), 1171–1175. https://doi.org/10.1056/NEJM199511023331802

Brites, D., & Silva, R. F. M. (2021). Bilirubin neurotoxicity: A narrative review on long-lasting, insidious, and dangerous effects. *2021*, *4*. https://pm.amegroups.org/article/view/6295

Brits, H., Adendorff, J., Huisamen, D., Beukes, D., Botha, K., Herbst, H., & Joubert, G. (2018). The prevalence of neonatal jaundice and risk factors in healthy term neonates at National District Hospital in Bloemfontein. *African Journal of Primary Health Care & Family Medicine*, *10*(1), e1–e6. https://doi.org/10.4102/phcfm.v10i1.1582

Chen, S., Francioli, L. C., Goodrich, J. K., Collins, R. L., Kanai, M., Wang, Q., Alföldi, J., Watts, N. A., Vittal, C., Gauthier, L. D., Poterba, T., Wilson, M. W., Tarasova, Y., Phu, W., Grant, R., Yohannes, M. T., Koenig, Z., Farjoun, Y., Banks, E., Genome Aggregation Database Consortium. (2024). A genomic mutational constraint map using variation in 76,156 human genomes. *Nature*, *625*(7993), 92–100. https://doi.org/10.1038/s41586-023-06045-0

Chou, H.-C., Chen, M.-H., Yang, H.-I., Su, Y.-N., Hsieh, W.-S., Chen, C.-Y., Chen, H.-L., Chang, M.-H., & Tsao, P.-N. (2011). 211 G to a variation of UDP-glucuronosyl transferase 1A1 gene and neonatal breastfeeding jaundice. *Pediatric Research*, *69*(2), 170–174. https://doi.org/10.1203/PDR.0b013e31820263d2

Ciotti, M., Chen, F., Rubaltelli, F. F., & Owens, I. S. (1998). Coding defect and a TATA box mutation at the bilirubin UDP-glucuronosyltransferase gene cause Crigler-Najjar type I disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1407*(1), 40–50. https://doi.org/10.1016/S0925-4439(98)00030-1

Diala, U. M., Usman, F., Appiah, D., Hassan, L., Ogundele, T., Abdullahi, F., Satrom, K. M., Bakker, C. J., Lee, B. W., & Slusher, T. M. (2023). Global Prevalence of Severe Neonatal Jaundice among Hospital Admissions: A Systematic Review and Meta-Analysis. *Journal of Clinical Medicine*, *12*(11). https://doi.org/10.3390/jcm12113738

D'Silva, S., Colah, R. B., Ghosh, K., & Mukherjee, M. B. (2014). Combined effects of the UGT1A1 and OATP2 gene polymorphisms as major risk factors for unconjugated hyperbilirubinemia in Indian neonates. *Gene*, *547*(1), 18–22. https://doi.org/10.1016/j.gene.2014.05.047

Erlinger, S., Arias, I. M., & Dhumeaux, D. (2014). Inherited disorders of bilirubin transport and conjugation: New insights into molecular mechanisms and consequences. *Gastroenterology*, *146*(7), 1625–1638. https://doi.org/10.1053/j.gastro.2014.03.047

Feldman, A., & Sokol, R. J. (2013). Alpha-1-Antitrypsin Deficiency: An Important Cause of Pediatric Liver Disease. *Lung Health Professional Magazine*, *4*(2), 8–11.

Gao, C., Guo, Y., Huang, M., He, J., & Qiu, X. (2023). Breast Milk Constituents and the Development of Breast Milk Jaundice in Neonates: A Systematic Review. *Nutrients*, *15*(10). https://doi.org/10.3390/nu15102261

Hsieh, T.-Y., Shiu, T.-Y., Huang, S.-M., Lin, H.-H., Lee, T.-C., Chen, P.-J., Chu, H.-C., Chang, W.-K., Jeng, K.-S., Lai, M. M. C., & Chao, Y.-C. (2007). Molecular pathogenesis of Gilbert's syndrome: Decreased TATA-binding protein binding affinity of UGT1A1 gene promoter. *Pharmacogenetics and Genomics*, *17*(4), 229–236. https://doi.org/10.1097/FPC.0b013e328012d0da

Huang, C. S., Luo, G. A., Huang, M. L., Yu, S. C., & Yang, S. S. (2000). Variations of the bilirubin uridine-diphosphoglucuronosyl transferase 1A1 gene in healthy Taiwanese. *Pharmacogenetics*, *10*(6), 539–544. https://doi.org/10.1097/00008571-200008000-00007

Jemnitz, K., Heredi-Szabo, K., Janossy, J., Ioja, E., Vereczkey, L., & Krajcsi, P. (2010). ABCC2/Abcc2: A multispecific transporter with dominant excretory functions. *Drug Metabolism Reviews*, *42*(3), 402–436. https://doi.org/10.3109/03602530903491741

Jinno, H., Tanaka-Kagawa, T., Hanioka, N., Saeki, M., Ishida, S., Nishimura, T., Ando, M., Saito, Y., Ozawa, S., & Sawada, J.-I. (2003). Glucuronidation of 7-ethyl-10-hydroxycamptothecin (SN-38), an active metabolite of irinotecan (CPT-11), by human UGT1A1 variants, G71R, P229Q, and Y486D. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, *31*(1), 108–113. https://doi.org/10.1124/dmd.31.1.108

Kapitulnik, J. (2004). Bilirubin: An endogenous product of heme degradation with both cytotoxic and cytoprotective properties. *Molecular Pharmacology*, *66*(4), 773–779. https://doi.org/10.1124/mol.104.002832

Kaplan, M., Slusher, T., Renbaum, P., Essiet, D. F., Pam, S., Levy-Lahad, E., & Hammerman, C. (2008). (TA)n UDP-Glucuronosyltransferase 1A1 Promoter Polymorphism in Nigerian Neonates. *Pediatric Research*, *63*(1), 109–111. https://doi.org/10.1203/PDR.0b013e31815b8e7e

Kebede, C., Fentie, B., & Tigabu, B. (2023). Treatment Outcome of Jaundice and Its Associated Factors Among Neonates Treated in Neonatal Intensive Care Unit of Comprehensive and Specialized Hospitals of Southern Nations Nationalities and Peoples Region, Ethiopia 2022. *Pediatric Health, Medicine and Therapeutics*, *14*, 237–247. https://doi.org/10.2147/PHMT.S405453

Khan, S., Irfan, M., Sher, G., Zubaida, B., Alvi, M. A., Yasinzai, M., & Naeem, M. (2013). UGT1A1 gene mutations in Pakistani children suffering from inherited nonhemolytic unconjugated hyperbilirubinemias. *Annals of Human Genetics*, *77*(6), 482–487. https://doi.org/10.1111/AHG.12039

Ko, J. S., Chang, J. Y., Moon, J. S., Yang, H. R., & Seo, J. K. (2014). Molecular Analysis of the UGT1A1 Gene in Korean Patients with Crigler-Najjar Syndrome Type II. *Pediatric Gastroenterology, Hepatology & Nutrition*, *17*(1), 37–40. https://doi.org/10.5223/pghn.2014.17.1.37

Koiwai, O., Nishizawa, M., Hasada, K., Aono, S., Adachi, Y., Mamiya, N., & Sato, H. (1995). Gilbert's syndrome is caused by a heterozygous missense mutation in the gene for bilirubin UDP-glucuronosyltransferase. *Human Molecular Genetics*, *4*(7), 1183–1186. https://doi.org/10.1093/hmg/4.7.1183

Landrum, M. J., Lee, J. M., Riley, G. R., Jang, W., Rubinstein, W. S., Church, D. M., & Maglott, D. R. (2014). ClinVar: Public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Research*, *42*(Database issue), D980-985. https://doi.org/10.1093/nar/gkt1113

Leung, A. K. C., & Sauve, R. S. (2016). Breastfeeding and Breast Milk Jaundice: *Http://Dx.Doi.Org/10.1177/146642408910900615*, *109*(6), 213–217. https://doi.org/10.1177/146642408910900615

Li, H., & Zhang, P. (2021). UGT1A1*28 gene polymorphism was not associated with the risk of neonatal hyperbilirubinemia: A meta-analysis. *The Journal of Maternal-Fetal & Neonatal Medicine : The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, *34*(24), 4064–4071. https://doi.org/10.1080/14767058.2019.1702962

Li, L., Deng, G., Tang, Y., & Mao, Q. (2015). Spectrum of UGT1A1 Variations in Chinese Patients with Crigler-Najjar Syndrome Type II. *PloS One*, *10*(5), e0126263. https://doi.org/10.1371/journal.pone.0126263

Li, Q., Yang, F., Liu, R., Luo, L., Yang, Y., Zhang, L., Liu, H., Zhang, W., Fan, Z., Yang, Z., Cui, L., & He, Y. (2015). Prevalence and Molecular Characterization of Glucose-6-Phosphate Dehydrogenase Deficiency at the China-Myanmar Border. *PloS One*, *10*(7), e0134593. https://doi.org/10.1371/journal.pone.0134593

Li, Z., Song, L., & Hao, L. (2020). The role of UGT1A1 (c.-3279 T > G) gene polymorphisms in neonatal hyperbilirubinemia susceptibility. *BMC Medical Genetics*, *21*(1), 218. https://doi.org/10.1186/s12881-020-01155-2

Liu, W., Chang, L., Xie, M., Li, W., Rong, Z., Wu, L., & Chen, L. (2017). Correlation between UGT1A1 polymorphism and neonatal hyperbilirubinemia of neonates in Wuhan. *Current Medical Science*, *37*(5), 740–743. https://doi.org/10.1007/s11596-017-1797-6

Maruo, Y., D'Addario, C., Mori, A., Iwai, M., Takahashi, H., Sato, H., & Takeuchi, Y. (2004). Two linked polymorphic mutations (A(TA)7TAA and T-3279G) of

UGT1A1 as the principal cause of Gilbert syndrome. *Human Genetics*, *115*(6), 525–526. https://doi.org/10.1007/s00439-004-1183-x

Maruo, Y., Morioka, Y., Fujito, H., Nakahara, S., Yanagi, T., Matsui, K., Mori, A., Sato, H., Tukey, R. H., & Takeuchi, Y. (2014). Bilirubin uridine diphosphate-glucuronosyltransferase variation is a genetic basis of breast milk jaundice. *The Journal of Pediatrics*, *165*(1), 36-41.e1. https://doi.org/10.1016/j.jpeds.2014.01.060

Maruo, Y., Nakahara, S., Yanagi, T., Nomura, A., Mimura, Y., Matsui, K., Sato, H., & Takeuchi, Y. (2016). Genotype of UGT1A1 and phenotype correlation between Crigler-Najjar syndrome type II and Gilbert syndrome. *Journal of Gastroenterology and Hepatology*, *31*(2), 403–408. https://doi.org/10.1111/jgh.13071

Maruo, Y., Nishizawa, K., Sato, H., Sawa, H., & Shimada, M. (2000). Prolonged unconjugated hyperbilirubinemia associated with breast milk and mutations of the bilirubin uridine diphosphate- glucuronosyltransferase gene. *Pediatrics*, *106*(5), E59. https://doi.org/10.1542/peds.106.5.e59

Mi, X.-X., Yan, J., Ma, X.-J., Zhu, G.-L., Gao, Y.-D., Yang, W.-J., Kong, X.-W., Chen, G.-Y., Shi, J.-P., & Gong, L. (2019). Analysis of the UGT1A1 Genotype in Hyperbilirubinemia Patients: Differences in Allele Frequency and Distribution. *BioMed Research International*, *2019*, 6272174. https://doi.org/10.1155/2019/6272174

Monaghan, G., Ryan, M., Seddon, R., Hume, R., & Burchell, B. (1996). Genetic variation in bilirubin UPD-glucuronosyltransferase gene promoter and Gilbert's syndrome. *Lancet (London, England)*, *347*(9001), 578–581. https://doi.org/10.1016/s0140-6736(96)91273-8

Murekatete, C., Muteteli, C., Richard, N., & Chironda, G. (2020). Neonatal Jaundice Risk Factors at a District Hospital in Rwanda. *Rwanda Journal of Medicine and Health Sciences*, *3*, 204–213. https://doi.org/10.4314/rjmhs.v3i2.10

NEWMAN, A. J., & GROSS, S. (1963). HYPERBILIRUBINEMIA IN BREAST-FED INFANTS. *Pediatrics*, *32*, 995–1001.

Olusanya, B. O., Osibanjo, F. B., & Slusher, T. M. (2015). Risk factors for severe neonatal hyperbilirubinemia in low and middle-income countries: A systematic review and meta-analysis. *PloS One*, *10*(2), e0117229. https://doi.org/10.1371/journal.pone.0117229

Online Mendelian Inheritance in Man, OMIM®. (2023). https://www.omim.org

Online Mendelian Inheritance in Man, OMIM® 143500 (MIM Number: {143500}). (2023). https://omim.org/

Online Mendelian Inheritance in Man, OMIM® 218800 (MIM Number: {218800}). (2023). https://omim.org/

Online Mendelian Inheritance in Man, OMIM® 606785 (MIM Number: {606785}). (2023). https://omim.org/

Pasha, Y. Z., Kacho, M. A., Niaki, H. A., Tarighati, M., & Alaee, E. (2017). The Association between Prolonged Jaundice and TATA Box Dinucleotide Repeats in Gilbert's Syndrome. *Journal of Clinical and Diagnostic Research : JCDR*, *11*(9), GC05–GC07. https://doi.org/10.7860/JCDR/2017/19376.10597

Petit, F. M., Bézieau, S., Gajdos, V., Parisot, F., Scoul, C., Capel, L., Stozinic, V., Khrouf, N., M'Rad, R., Koshy, A., Mollet-Boudjemline, A., Francoual, J., & Labrune, P. (2008). The Tunisian population history through the Crigler-Najjar type I syndrome. *European Journal of Human Genetics : EJHG*, *16*(7), 848–853. https://doi.org/10.1038/sj.ejhg.5201989

Porter, M. L., & Dennis, B. L. (2002). Hyperbilirubinemia in the term newborn. *American Family Physician*, *65*(4), 599–606.

Reyburn, A. M., & Virk, A. (2009). A 22-year-old woman with fever and jaundice after travel to Ghana. *Mayo Clinic Proceedings*, *84*(5), 461–464. https://doi.org/10.1016/S0025-6196(11)60565-X

Ritter, J. K., Yeatman, M. T., Ferreira, P., & Owens, I. S. (1992). Identification of a genetic alteration in the code for bilirubin UDP-glucuronosyltransferase in the UGT1 gene complex of a Crigler-Najjar type I patient. *The Journal of Clinical Investigation*, *90*(1), 150–155. https://doi.org/10.1172/JCI115829

Rodrigues, C., Vieira, E., Santos, R., de Carvalho, J., Santos-Silva, A., Costa, E., & Bronze-da-Rocha, E. (2012). Impact of UGT1A1 gene variants on total bilirubin levels in Gilbert syndrome patients and in healthy subjects. *Blood Cells, Molecules & Diseases*, *48*(3), 166–172. https://doi.org/10.1016/j.bcmd.2012.01.004

Routray, S. S., Mishra, D., Kanungo, G. N., & Behera, R. (2023). Hemolytic Disease of Newborn due to ABO Incompatibility between B Blood Group Mother and A Blood Group Neonate. *Journal of Laboratory Physicians*, *15*(1), 146–148. https://doi.org/10.1055/s-0042-1750071

Sawyer, T., & Gleason, C. A. (2023). *Avery's Diseases of the Newborn* (11th ed.). Elsevier.

Sayers, E. W., Bolton, E. E., Brister, J. R., Canese, K., Chan, J., Comeau, D. C., Connor, R., Funk, K., Kelly, C., Kim, S., Madej, T., Marchler-Bauer, A., Lanczycki, C., Lathrop, S., Lu, Z., Thibaud-Nissen, F., Murphy, T., Phan, L., Skripchenko, Y., Sherry, S. T. (2022). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, *50*(D1), D20–D26. https://doi.org/10.1093/nar/gkab1112

Setia, S., Villaveces, A., Dhillon, P., & Mueller, B. A. (2002). Neonatal jaundice in Asian, white, and mixed-race infants. *Archives of Pediatrics & Adolescent Medicine*, *156*(3), 276–279. https://doi.org/10.1001/archpedi.156.3.276

Sticova, E., & Jirsa, M. (2013). New insights in bilirubin metabolism and their clinical implications. *World Journal of Gastroenterology*, *19*(38), 6398–6407. https://doi.org/10.3748/wjg.v19.i38.6398

Strassburg, C. P. (2008). Pharmacogenetics of Gilbert's syndrome. *Pharmacogenomics*, *9*(6), 703–715. https://doi.org/10.2217/14622416.9.6.703

Sugatani, J., Yamakawa, K., Tonda, E., Nishitani, S., Yoshinari, K., Degawa, M., Abe, I., Noguchi, H., & Miwa, M. (2004). The induction of human UDP-glucuronosyltransferase 1A1 mediated through a distal enhancer module by flavonoids and xenobiotics. *Biochemical Pharmacology*, *67*(5), 989–1000. https://doi.org/10.1016/j.bcp.2003.11.002

Sugatani, J., Yamakawa, K., Yoshinari, K., Machida, T., Takagi, H., Mori, M., Kakizaki, S., Sueyoshi, T., Negishi, M., & Miwa, M. (2002). Identification of a defect in the UGT1A1 gene promoter and its association with hyperbilirubinemia. *Biochemical and Biophysical Research Communications*, *292*(2), 492–497. https://doi.org/10.1006/bbrc.2002.6683

Sun, L., Li, M., Zhang, L., Teng, X., Chen, X., Zhou, X., Ma, Z., Qi, L., & Wang, P. (2017). Differences in UGT1A1 gene mutations and pathological liver changes between Chinese patients with Gilbert syndrome and Crigler-Najjar syndrome type II. *Medicine*, *96*(45), e8620. https://doi.org/10.1097/MD.0000000000008620

Takeuchi, K., Kobayashi, Y., Tamaki, S., Ishihara, T., Maruo, Y., Araki, J., Mifuji, R., Itani, T., Kuroda, M., Sato, H., Kaito, M., & Adachi, Y. (2004). Genetic polymorphisms of bilirubin uridine diphosphate-glucuronosyltransferase gene in Japanese patients with Crigler-Najjar syndrome or Gilbert's syndrome as well as in

healthy Japanese subjects. *Journal of Gastroenterology and Hepatology*, *19*(9), 1023–1028. https://doi.org/10.1111/j.1440-1746.2004.03370.x

Tukey, R. H., Strassburg, C. P., & Mackenzie, P. I. (2002). Pharmacogenomics of human UDP-glucuronosyltransferases and irinotecan toxicity. *Molecular Pharmacology*, *62*(3), 446–450. https://doi.org/10.1124/mol.62.3.446

Udomuksorn, W., Elliot, D. J., Lewis, B. C., Mackenzie, P. I., Yoovathaworn, K., & Miners, J. O. (2007). Influence of mutations associated with Gilbert and Crigler-Najjar type II syndromes on the glucuronidation kinetics of bilirubin and other UDP-glucuronosyltransferase 1A substrates. *Pharmacogenetics and Genomics*, *17*(12), 1017–1029. https://doi.org/10.1097/FPC.0b013e328256b1b6

ULLAH, S., RAHMAN, K., & HEDAYATI, M. (2016). Hyperbilirubinemia in Neonates: Types, Causes, Clinical Examinations, Preventive Measures and Treatments: A Narrative Review Article. *Iranian Journal of Public Health*, *45*(5), 558.

Valá\vsková, P. & Muchová. (2016). *Metabolism of bilirubin and its biological properties*. https://api.semanticscholar.org/CorpusID:40541078

Valmiki, S., Mandapati, K. K., Miriyala, L. K. V., Kelgeri, C. C., Rela, M., Shanmugam, N. P., & Vegulada, D. R. (2020). A case report of a novel 22 bp duplication within exon 1 of the UGT1A1 in a Sudanese infant with Crigler-Najjar syndrome type I. *BMC Gastroenterology*, *20*(1), 62. https://doi.org/10.1186/s12876-020-01192-4

Wang, J., Yin, J., Xue, M., Lyu, J., & Wan, Y. (2020). Roles of UGT1A1 Gly71Arg and TATA promoter polymorphisms in neonatal hyperbilirubinemia: A meta-analysis. *Gene*, *736*, 144409. https://doi.org/10.1016/j.gene.2020.144409

Yanagi, T., Nakahara, S., & Maruo, Y. (2017). Bilirubin Uridine Diphosphate-glucuronosyltransferase Polymorphism as a Risk Factor for Prolonged Hyperbilirubinemia in Japanese Preterm Infants. *The Journal of Pediatrics*, *190*, 159-162.e1. https://doi.org/10.1016/j.jpeds.2017.07.014

Yang, H., Lin, F., Chen, Z.-K., Zhang, L., Xu, J.-X., Wu, Y.-H., Gu, J.-Y., Ma, Y.-B., Li, J.-D., & Yang, L.-Y. (2021). UGT1A1 mutation association with increased bilirubin levels and severity of unconjugated hyperbilirubinemia in ABO-incompatible newborns of China. *BMC Pediatrics*, *21*(1), 259. https://doi.org/10.1186/s12887-021-02726-9

Yang, H., Wang, Q., Zheng, L., Lin, M., Zheng, X., Lin, F., & Yang, L.-Y. (2015). Multiple Genetic Modifiers of Bilirubin Metabolism Involvement in Significant Neonatal Hyperbilirubinemia in Patients of Chinese Descent. *PloS One*, *10*(7), e0132034. https://doi.org/10.1371/journal.pone.0132034

Yang, H., Wang, Q., Zheng, L., Zheng, X.-B., Lin, M., Zhan, X.-F., & Yang, L.-Y. (2016). Clinical Significance of UGT1A1 Genetic Analysis in Chinese Neonates with Severe Hyperbilirubinemia. *Pediatrics and Neonatology*, *57*(4), 310–317. https://doi.org/10.1016/j.pedneo.2015.08.008

Yusoff, S., Takeuchi, A., Ashi, C., Tsukada, M., Ma'amor, N. H., Zilfalil, B. A., Yusoff, N. M., Nakamura, T., Hirai, M., Harahap, I. S. K., Gunadi, Lee, M. J., Nishimura, N., Takaoka, Y., Morikawa, S., Morioka, I., Yokoyama, N., Matsuo, M., Nishio, H., & van Rostenberghe, H. (2010). A polymorphic mutation, c.-3279T>G, in the UGT1A1 promoter, is a risk factor for neonatal jaundice in the Malay population. *Pediatric Research*, *67*(4), 401–406. https://doi.org/10.1203/PDR.0b013e3181d22f78

Yusoff, S., Van Rostenberghe, H., Yusoff, N. M., Talib, N. A., Ramli, N., Ismail, N. Z. A. N., Ismail, W. P. W., Matsuo, M., & Nishio, H. (2006). Frequencies of A(TA)7TAA, G71R, and G493R mutations of the UGT1A1 gene in the Malaysian population. *Biology of the Neonate*, *89*(3), 171–176. https://doi.org/10.1159/000088844

Zaitsu, M., Yoshihara, T., Nakai, H., & Kubota, S. (2018). Optimal Thermal Control with Sufficient Nutrition May Reduce the Incidence of Neonatal Jaundice by Preventing Body-Weight Loss Among Non-Low Birth Weight Infants Not Admitted to Neonatal Intensive Care Unit. *Neonatology*, *114*(4), 348–354. https://doi.org/10.1159/000491817

Zhang, L., Liu, W., Hou, K., Lin, J., Song, C., Zhou, C., Huang, B., Tong, X., Wang, J., Rhine, W., Jiao, Y., Wang, Z., Ni, R., Liu, M., Zhang, L., Wang, Z., Wang, Y., Li, X., Liu, S., & Wang, Y. (2019). Air pollution exposure is associated with an increased risk of neonatal jaundice. *Nature Communications*, *10*(1), 3741. https://doi.org/10.1038/s41467-019-11387-3

Zhang, M., Wang, H., Huang, Y., Xu, X., Liu, W., Ning, Q., Chen, T., & Qi, J. (2021). Compound heterozygous UGT1A1*28 and UGT1A1*6 or single homozygous UGT1A1*28 are major genotypes associated with Gilbert's syndrome in Chinese Han people. *Gene*, *781*, 145526. https://doi.org/10.1016/j.gene.2021.145526

تواتر متغيرين شائعين لدى المرضى الفلسطينيين الذين يعانون من فرط بيليروبين الدم

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ملخص

فرط بيليروبين الدم عند حديثي الولادة هو حالة شائعة عند الأطفال حديثي الولادة حيث يتم تمييزه بمستويات مرتفعة من البيليروبين في الدم. بشكل عام يعتبر فرط بيليروبين الدم عند حديثي الولادة غير ضار, إلا أنَه في حال لم يتم معالجته وارتفعت مستويات البيليروبين إلى حد كبير يمكن أن يؤدي إلى مضاعفات مثل اعتلال الدماغ مما يسبب تلفاً في أنسجة الدماغ. يتضمن هذا الوضع الصحي أسباب جينية وأسباب غير جينية. أحد الجينات الذي يلعب دوراً مهماً في استقلاب البيليروبين هو الجين المسمى ب UGT1A1. وقد تم الربط العديد من التغيرات في تسلسل هذا الجين بتغير مستويات البيليروبين في جنسيات وعرقيات مختلفة حول العالم. أيضاً, هناك متلازمات مرضية تتميز بشكل رئيسي بفرط بيليروبين الدم تنتج عن طفرات مختلفة في هذا الجين بما في ذلك متلازمة جيلبرت, وكريجيلر نجار النوع 1 والنوع 2وفرط الدم الوليدي العائلي العابرز هدفت هذه الدراسة لمعرفة مدى انتشار مجموعة من الطفرات الجينية في هذا الجين في الشعب الفلسطيني ومدى علاقتها بـ فرط بيليروبين الدم عند حديثي الولادة. شملت الدراسة 70 طفل من حديثي الولادة الذين كانوا يعانون من فرط بيليروبين الدم وتراوحت أعمارهم بين 2 إلى 64 يوماً. تم دراسة طفرتين تم الإبلاغ عنها في دراسات سابقة بأنها مرتبطة بـ فرط بيليروبين الدم عند حديثي الولادة تشمل(rs4124874 و rs3064744). تم تحديد الأنماط الجينية بواسطة Sanger sequencing

كشف تحليلنا عن وجود ارتباط بين متغير (rs3064744) وزيادة مستويات البيليروبين في المجموعة المدروسة. في حين أن متغير (rs4124874) كان شائعًا بين المرضى بشكل ملفت ، إلا أنه لم يُظهر وجود علاقة ذات دلالة إحصائية مع فرط بيليروبين الدم عند حديثي الولادة.

تلقي هذه الدراسة الضوء على المساهمة الوراثية للتغيرات في جين UGT1A1 في ارتفاع مستويات البيليروبين لدى السكان الفلسطينيين. إن الفهم الأفضل للخلفية الوراثية لـ فرط بيليروبين الدم عند حديثي الولادة يمكن أن يحسن الاستراتيجيات التشخيصية والوقائية، مما يحسن النتائج الصحية لحديثي الولادة في فلسطين.

الكلمات الافتتاحية: UGT1A1 (يوريدين ثنائي فوسفات جلوكورونوزيل ترانسفيراز)؛ فرط بيليروبين الدم عند الأطفال حديثي الولادة؛ متغير UGT1A1*28 (TA)7TAA؛ متغير c.-3279 T > G؛ اليرقان .