BILIRUBIN - URIDINE DIPHOSPHATE GLUCURONOSYLTRANSFERASE (*UGT1A1*) GENE MUTATIONS AMONG NEWBORN BABIES IN THE MALAY POPULATION IN KELANTAN WITH HYPERBILIRUBINAEMIA

by

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Dedication

For my beloved mum and dad (Salehah Mat Hassan and Yusoff Mat Hassan), you are everything in my life. My younger brothers, Zuri, Roslee, Zaidi and Mohd Jailani, I love you all.

Surini 2005

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LIST OF ABBREVIATIONS

ABO	Types of blood group: A, B, AB and O
A	Adenine
Bp	Base Pair
CO	Carbon Monoxide
CO-Hb	Carboxyhaemoglobin
DHPLC	Denaturing High Performance Liquid Chromatography
DNA	Deoxyribonucleic acid
F83L	Phenylalanine to Leucine at codon 83
rpm	Revolutions per Minute
FSM	Full Scheme Milk
g	Gram
G493R	Glycine to Arginine at codon 493
G-6-PD	Glucose-6-phosphate dehydrogenase
G71R	Glycine to Arginine at codon 71
GSH	Reduced Glutathione
GSSG	Oxidized Glutathione
H_2O	Water
H_2O_2	Hydrogen Peroxide
Ig	Anti-immunoglobulin
mg	Miligram
mg/dL	Miligram per deciliter
N	Total Sample Size
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NCBI	National Centre for Biotechnology Information
O_2	Oxygen
P229Q	Proline to Glutamine at codon 229
PBREM	Phenobarbital Response Enhancer Region
PCR	Polymerase chain reaction
PhIP	2-amino-1-methyl-6-phenylimidazo [4,5-f] pyridine
psi	Pound per square inch
SN-38	7-ethyl-10-hydroxycamptothecin
SN-38G	7-ethyl-10-hydroxycamptothecin glucuronide
T	Thymine
T-3279G	Phenylalanine to Cystein at codon -3279
UDPGT	Uridyl diphosphoglucuronyl transferase
UGT1A1	Uridine-diphosphate glucuronosyltransferase 1
UGTs	Uridine-diphosphoglucuronate-glucuronosyltransferases
UV	Ultra Violet
Y486D	Tyrosine to Aspartic Acid at codon 486
α	Alpha
µmol/L	Micromol per litre

MUTASI - MUTASI GEN BILIRUBIN - URIDINE DIPHOSPHATE GLUCURONOSYLTRANSFERASE (*UGT1A1*) DI KALANGAN BAYI – BAYI YANG MENGALAMI HIPERBILIRUBINEMIA DALAM POPULASI MELAYU DI KELANTAN

ABSTRAK

Sindrom Gilbert berpunca daripada keabnormalan pada gen uridine diphosphate-glucuronosyltransferase 1A1 (*UGT1A1*) yang disebabkan oleh mutasi. Mutasi-mutasi ini berbeza bagi setiap populasi dan kebanyakannya menjadi faktor asas bagi jaundis di kalangan neonat. Objektif kajian ini adalah untuk menentukan frekuensi mutasi-mutasi berikut pada gen *UGT1A1*: $A(TA)_7TAA$ (penyebab yang lazim bagi sindrom Gilbert di kalangan orang-orang Kaukasia), G71R (penyebab utama pada populasi Jepun dan Taiwan) dan G493R (dijumpai pada wanita Melayu yang membawa mutasi homozigus bagi sindrom Crigler-Najjar jenis ke-2) di kalangan bayi - bayi Melayu di Kelantan yang mengalami hiperbilirubinemia dan juga di kalangan bayi - bayi normal sebagai kawalan serta membandingkan frekuensi mutasi-mutasi ini di antara kedua-dua kumpulan bayi tersebut. Analisis serpihan penyaringan gen (GeneScan) telah digunakan untuk mengesan varian $A(TA)_7TAA$. Penyaringan mutasi kedua-dua G71R dan G493R telah dijalankan dengan menggunakan kromatografi cecair denaturasi berprestasi tinggi [Denaturing high performance liquid chromatography (DHPLC)]. Seramai 14 daripada 55 orang neonat dengan hiperbilirubinemia (25%) membawa mutasi $A(TA)_7TAA$ (10 heterozigus, 4 homozigus). Seramai 7 daripada 50 kawalan (14%) membawa mutasi ini (6

heterozigus, 1 homozigus). Frekuensi-frekuensi alel bagi bayi hiperbilirubinemia dan kawalan adalah masing-masing 16% dan 8% (p = 0.20). Heterozigositi mutasi G71R adalah lebih kurang sama bagi kedua-dua kumpulan (5.5% bagi bayi hiperbilirubinemia dan 6% bagi bayi yang tidak mengalami hiperbilirubinemia; p = 0.61). Seorang neonat (1.8%) dari kumpulan hiperbilirubinemia dan tiada neonat dari kumpulan kawalan mengalami mutasi heterozigus bagi G493R (p = 0.476). Kesimpulannya, $A(TA)_7TAA$ lebih ketara daripada mutasi G71R dan G493R di dalam populasi Melayu di Kelantan. Walaupun frekuensi alel bagi mutasi $A(TA)_7TAA$ di dalam kumpulan hiperbilirubinemia adalah dua kali ganda daripada kumpulan bukan hiperbilirubinemia, namun perbezaannya adalah tidak signifikan secara statistik.

Kata kunci: Jaundis neonatal; gen *UGT1A1*; sindrom Gilbert; kromatografi cecair denaturasi berprestasi tinggi; penyaringan gen

ABSTRACT

Gilbert syndrome is caused by defects in the uridine diphosphate-glucuronosyltransferase 1A1 (*UGT1A1*) gene. These mutations differ among different populations and many of them have been found to be genetic risk factors for the development of neonatal jaundice. The objectives of this study were to determine the frequencies of the following mutations in the *UGT1A1* gene: $A(TA)_7TAA$ (the most common cause of Gilbert syndrome in Caucasians), G71R (more common in the Japanese and Taiwanese populations) and G493R (described in a homozygous Malay woman with Crigler-Najjar syndrome type 2) in a group of Malay babies in Kelantan with hyperbilirubinaemia and a group of normal controls and to compare the frequencies of these mutations between these group. The GeneScan fragment analysis was used to detect the $A(TA)_7TAA$ variant. Mutation screening of both G71R and G493R was performed using denaturing high performance liquid chromatography (DHPLC). Fourteen out of 55 neonates with hyperbilirubinaemia (25%) carried the $A(TA)_7TAA$ mutation (10 heterozygous, 4 homozygous). Seven out of 50 controls (14%) carried the mutation (6 heterozygous, 1 homozygous). The allelic frequencies for hyperbilirubinaemia and control patients were 16% and 8% respectively ($p = 0.20$). Heterozygosity for the G71R mutation was almost equal among both groups (5.5% for hyperbilirubinaemia patients and 6.0% for controls; $p = 0.61$). One subject (1.8%) in the hyperbilirubinaemia group and none of the controls were heterozygous for the G493R mutation ($p = 0.476$). In

conclusion, the $A(TA)_7TAA$ seems more common than the G71R and G493R mutations in the Malay population in Kelantan. Even though the allelic frequency of the $A(TA)_7TAA$ mutation in the hyperbilirubinaemia group was twice that of the non hyperbilirubinaemia group, the difference did not reach statistical significance.

Keywords: Neonatal jaundice; *UGT1A1* gene; Gilbert syndrome; denaturing high performance liquid chromatography; GeneScan

CHAPTER I

INTRODUCTION

1.1 Background of the study

Neonatal jaundice is a common phenomenon among newborn infants. In Malaysia most of the neonatal jaundice presents during the first week of life. Many babies develop only mild jaundice which is believed to be physiological. This physiological jaundice is thought to be due to an immaturity of the liver function in the newborn infant and due to the shorter life span of red blood cells carrying foetal haemoglobin.

On the other hand, many babies develop more severe jaundice, putting them at risk of developing bilirubin encephalopathy and kernicterus (American Academy of Pediatrics Subcommittee on Hyperbilirubinemia, 2004). Many causes have been identified for this more severe jaundice. The best known causes include haemolysis (such as rhesus incompatibility, ABO incompatibility or glucose - 6 - phosphate dehydrogenase (G-6-PD) deficiency), liver dysfunction (such as sepsis, Crigler-Najjar syndrome or metabolic disorders) and conditions which increase the enterohepatic circulation of bilirubin (such as breast feeding jaundice). A more detailed overview of the causes of neonatal jaundice and their importance will be given under subheading 1.4.

It is well known that in many cases of moderate or severe neonatal jaundice, the routine screening for common causes (including screening for G-6-PD deficiency, blood grouping, direct Coombs test and full blood picture) does not identify any cause. If in these cases there is neither a history of poor breast feeding, these babies are often labelled as having excessive physiological jaundice or more correctly, idiopathic pathological jaundice (Van Rostenberghe *et al.*, 2004). The term 'idiopathic' implies that the cause is unknown. Recent evidence in literature suggests that causal factors of this type of neonatal jaundice may be due to point mutations in exons or mutations in the promoter region of the uridine-diphosphate glucuronosyltransferase 1 (*UGT1A1*) gene. This gene encodes the enzyme responsible for the rate limiting step for the bilirubin excretion. A thorough literature review about these mutations will be presented under subheading 1.5 and 1.6.

Furthermore it is becoming increasingly clear that haemolysis in G-6-PD deficiency (and also ABO incompatibility) tends to be very mild and there is not much difference between babies developing neonatal jaundice and those not developing neonatal jaundice (Jalloh *et al.*, 2005, Kaplan *et al.*, 2005). Only about 20% of babies with G-6-PD deficiency develop jaundice requiring phototherapy. So then, what is the determining factor of which baby will and will not develop jaundice? It has been shown in some populations that again point mutations or mutations in the promoter region of the *UGT1A1* gene are very important associated factors (Kaplan *et al.*, 1997).

In different populations, different mutations in the *UGT1A1* gene have been identified and allelic frequencies of these mutations were very different depending on the population (Akaba *et al.*, 1998). No population study had been conducted so far regarding these mutations in any South-East Asian population. Since these mutations may be one of the determining factors whether or not a baby with G-6-PD deficiency will develop severe jaundice, it was felt that it is important to know in the Malaysian population whether mutations in this gene are common and whether they are related to the occurrence of jaundice.

That is the reason why this population study was undertaken in a Malay population in Kelantan to determine the frequency of three mutations in the *UGT1A1* gene in a group of jaundiced babies and in a group of babies with non-jaundiced as controls. The first mutation is the $A(TA)_7TAA$ mutation (also referred to as TATA box mutation) which is a mutation in the promoter region of the gene. This mutation is the major cause of Gilbert syndrome in Caucasians. The second mutation is the G71R mutation, a mutation in the first exon, which is common in the Taiwanese and Japanese populations. The third mutation studied is the G493R mutation which had been recently detected in a Malay family with Crigler-Najjar syndrome.

Before going into greater detail on the clinical aspects of neonatal jaundice and its causes, it is essential to have a clear understanding of the bilirubin metabolism itself which is described as follows.

1.2 Bilirubin metabolism

Bilirubin is the end product of heme catabolism. It may play a role through its antioxidative properties in inhibition of lipid peroxidation. Bilirubin formation occurs in mononuclear phagocytes of liver, spleen and bone marrow, previously known as the reticuloendothelial system (Bhagavan, 2002, Ratnavel and Ives, 2005). In some conditions, bilirubin formation may occur in renal tubular epithelial cells, hepatocytes and macrophages.

About 75% of bilirubin is produced through haemolysis and most of the other 25% through ineffective erythropoiesis (Bhagavan, 2002, Shapiro, 2003). Minor contributions to the serum bilirubin are made by the breakdown of myoglobin and the cytochromes. About 34 to 35 mg of bilirubin is produced by the degradation of 1 g of haemoglobin (Thilo, 1999, Zaghloul and Schulze, 2001). One mg/dL of serum bilirubin is equivalent to 17.2 μmol/L of bilirubin (Thilo, 1999). In normal human metabolism, about 250 to 400 mg of bilirubin is generated daily.

When haemoglobin is broken down, the globin part is enzymatically hydrolysed into amino acids. On the other hand, heme part of the haemoglobin molecule is bound to the heme oxygenase (a microsomal enzyme) which oxidizes the α-methane carbon of heme to α-hydroxyhemin. Autoxidation to biliverdin (a blue-green pigment) occurs in the presence of oxygen and in the process iron and carbon monoxide (CO) are released (Figures 1.1 and 1.2). The released CO binds strongly to haemoglobin to form carboxyhaemoglobin (CO-Hb).

Measurement of this CO-Hb can give a reliable estimate the heme turn over (Bhagavan, 2002).

Biliverdin is converted to bilirubin which is catalyzed by biliverdin reductase in the presence of NADPH (Bhagavan, 2002). This lipid soluble bilirubin is bound in the serum to albumin. Bound bilirubin-albumin complex is transported to the liver and taken up by hepatocytes. In the liver, bilirubin is conjugated with two glucuronide molecules. This process is catalysed by the enzyme, uridyl diphosphoglucuronyl transferase (UDPGT) and is the rate limiting step of bilirubin excretion (Ives, 1997). Then, the bilirubin diglucuronide is excreted via the bile to the intestine. In the intestine, bilirubin diglucuronide is metabolized to stercobilins by the gut flora and excreted in the stool (Ratnavel and Ives, 2005).

However, bilirubin diglucuronide is cleaved by mucosal beta-glucuronidase in the absence of gut flora or if it stays for a prolonged time in the intestinal lumen. In this condition, bilirubin is reabsorbed through the portal system (the enterohepatic circulation). Free bilirubin is unconjugated bilirubin which is not bound to albumin. This bilirubin has a potential to cross the blood brain barrier and damage neurons (Thilo, 1999). A schematic summary of the bilirubin metabolism pathway is given in Figures 1.1 and 1.2.

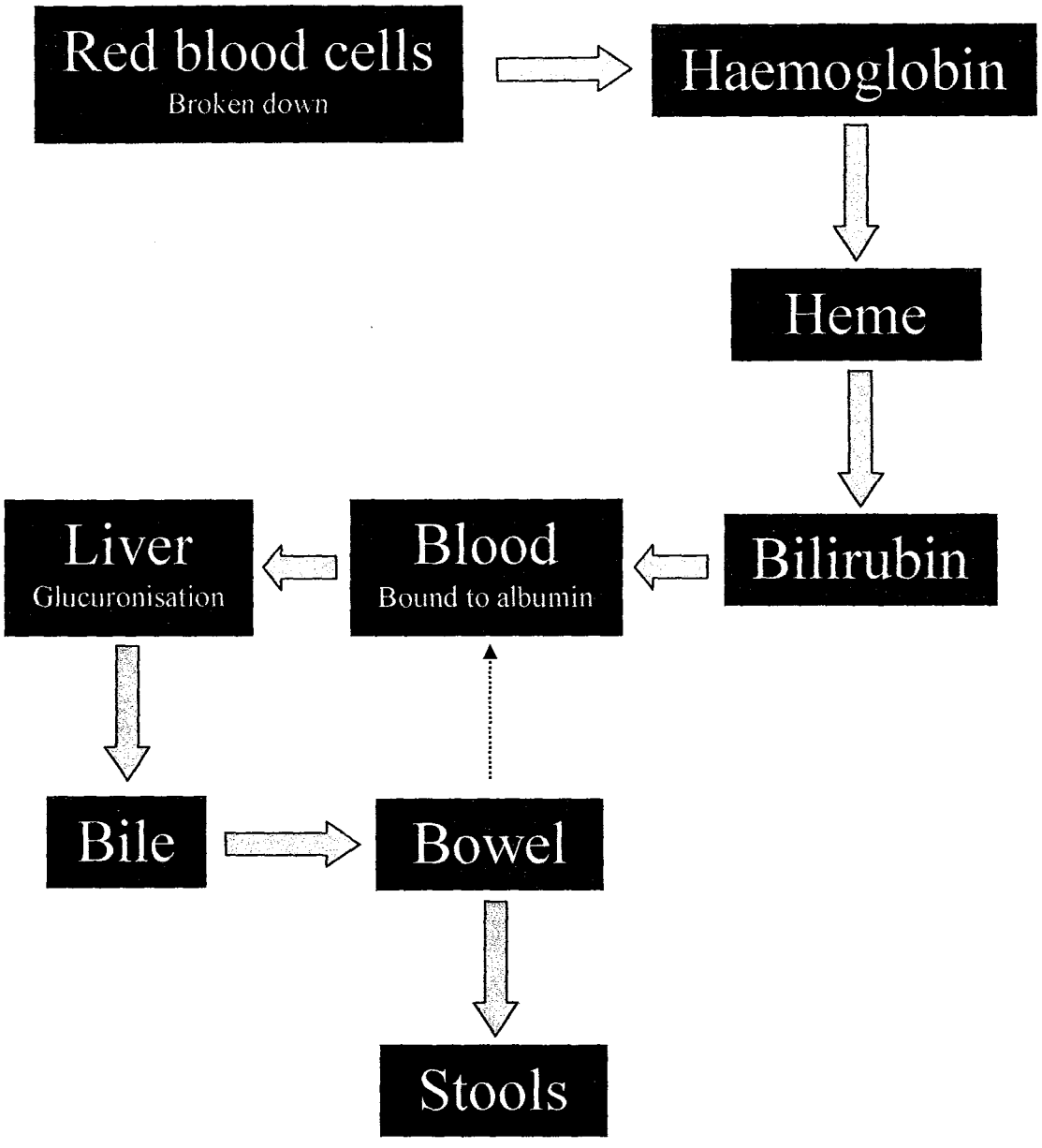

Figure 1.1 Schematic summary of the bilirubin metabolism pathway

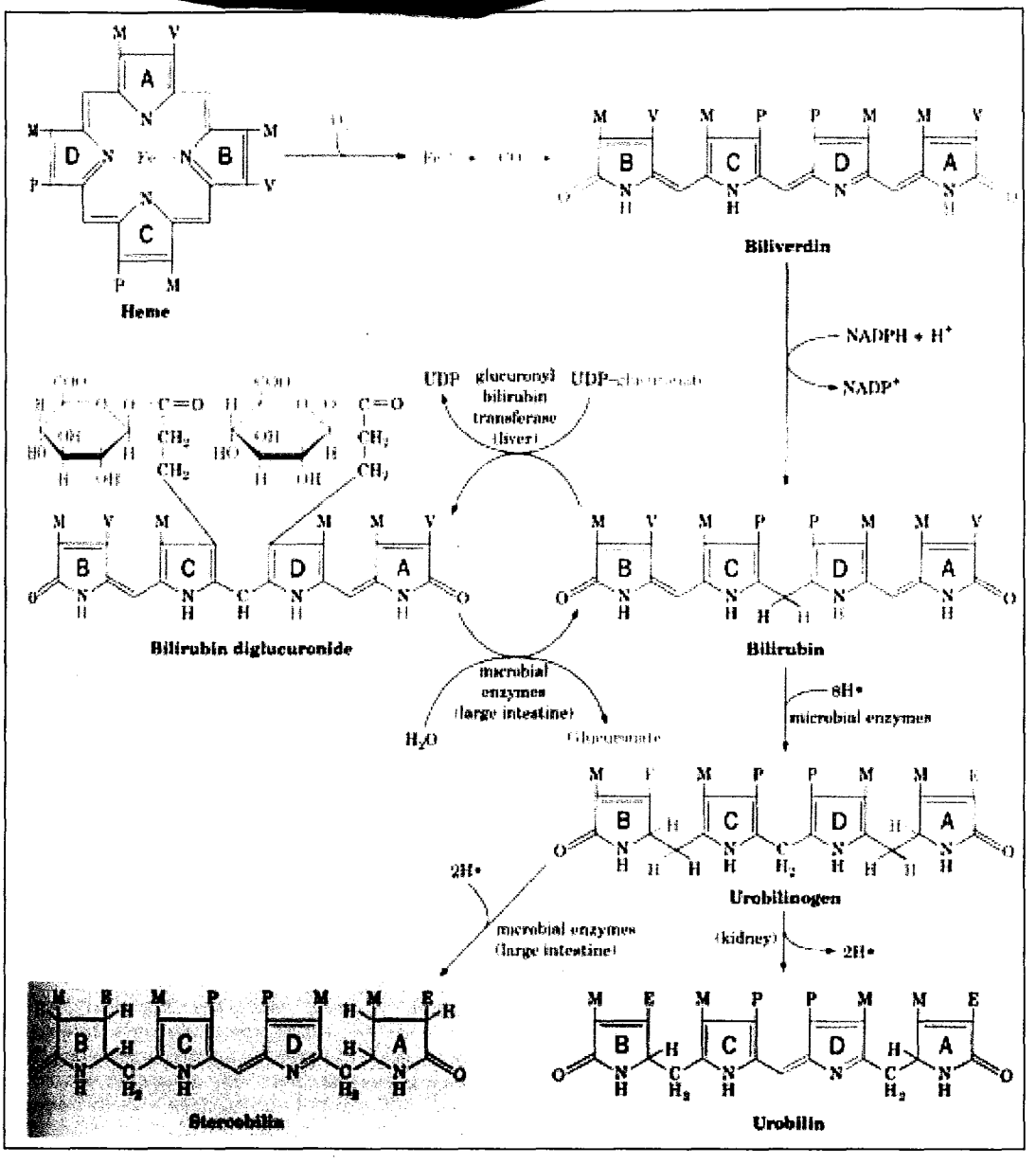

Figure 1.2 Pathway for heme degradation (Voet *et al.*, 2002)

1.3 Clinical aspects of neonatal jaundice

Jaundice or icterus can be defined as a yellowish discoloration of skin and sclera. This phenomenon is an indication of excess bilirubin in the blood. Clinical jaundice appears when the bilirubin level reaches about 90 µmol/L. Initially jaundice tends to appear in the face alone but as the hyperbilirubinaemia gets worse, other areas of the body become yellow as well. Generally it is assumed that jaundice reaching the abdomen indicates that the bilirubin level is about 250 µmol/L. If the legs are jaundiced it means there is severe hyperbilirubinaemia.

Hyperbilirubinaemia can be divided into two types depending on the conjugation of bilirubin to glucuronide molecules: i.e. unconjugated hyperbilirubinaemia and conjugated hyperbilirubinaemia.

1.3.1 Unconjugated hyperbilirubinaemia (Indirect hyperbilirubinaemia)

Unconjugated hyperbilirubinaemia is caused by bilirubin which is not yet conjugated with glucuronide molecules (Thilo, 1999). This is the most common type of hyperbilirubinaemia found in neonates and its causes are listed in Table 1.1. In the next subheading the most important causes of neonatal jaundice will be elaborated on a bit further. In severe hyperbilirubinaemia, unconjugated bilirubin may cross the blood brain barrier and cause neurological problems (kernicterus and bilirubin encephalopathy), renal damage and death (Clarke *et al.*, 1997).

Kernicterus means jaundice of the nuclei in the brain. It was described by Christian Georg Schmorl in 1904. This pathological phenomenon is associated with a clinical entity, bilirubin encephalopathy. It is caused by bilirubin toxicity to the basal ganglia and various brainstem nuclei (Ives, 1997, 2004). This bilirubin toxicity is significantly higher among Asian newborns than among Caucasians (Akaba *et al.*, 1998).

The clinical syndromes of bilirubin encephalopathy are divided into two types; acute bilirubin encephalopathy and chronic bilirubin encephalopathy. Acute bilirubin encephalopathy is associated with lethargy, poor feeding, fluctuating hypotonia and hypertonia, high pitched cry, retrocollis, opisthotonus, impairment of upward gaze, fever, seizures and eventually death (Thilo, 1999, Shapiro, 2003). Chronic bilirubin encephalopathy is the sequel of the acute type. The clinical features of chronic bilirubin encephalopathy range from mild mental retardation and subtle cognitive disturbances to severe athetoid cerebral palsy, deafness and seizures.

1.3.2 Conjugated hyperbilirubinaemia (Direct hyperbilirubinaemia)

This is an elevation of the serum bilirubin which is conjugated with glucuronide molecules. This water-soluble bilirubin diglucuronide is ready for excretion in bile and may increase when cholestasis occurs (Thilo, 1999). Conjugated hyperbilirubinaemia more commonly occurs after the first week of life, especially when the unconjugated hyperbilirubinaemia has decreased. The causes and the risk factors for conjugated hyperbilirubinaemia are listed in Table 1.2.

Table 1.1 Risk Factors for Neonatal Unconjugated Hyperbilirubinaemia Modified from the Clinical Use of Paediatric Diagnostic Tests (Barness and Barness, 2003)

Maternal Factors	Race or ethnic group -Native American, Asian, Greek Islander Complications during pregnancy -ABO incompatibility, Rhesus incompatibility, Diabetes mellitus Use of oxytocin in hypotonic solutions during labour Breast feeding: Breast-milk contains a competitive inhibitor of hepatic uridine-diphosphate glucuronosyltransferase
Perinatal Factors	Birth trauma -Ecchymoses, Cephalohematoma Infection -Viral -Bacterial -Protozoal
Neonatal Factors	Prematurity Genetic factors Familial disorders of conjugation -Crigler-Najjar syndrome (I and II), Gilbert syndrome Enzymatic defects causing haemolysis -Glucose-6-phosphate dehydrogenase deficiency -Pyruvate kinase deficiency, Hexokinase deficiency -Congenital erythropoietic porphyria Erythrocyte structural defects causing haemolysis -Elliptocytosis, Spherocytosis Polycythaemia Drugs -Chloramphenicol, Benzyl alcohol -Streptomycin sulfate, Sulfisoxazole Low intake of breast-milk (increased enterohepatic circulation)

Table 1.2 Risk Factors for Neonatal Conjugated Hyperbilirubinaemia Modified from the Clinical Use of Paediatrics Diagnostic Test (Barness and Barness, 2003)

Disorder	Supporting information
Infection	Improves with treatment.
Total parenteral nutrition	History of total parenteral nutrition
Biliary atresia	Pale stools.
α_1-Antitrypsin deficiency	-
Alagille syndrome	Characteristic facies congenital abnormalities.
Galactosemia	Cataracts.Urinary reducing substances positive. Hypoglycemia, abnormal clotting.
Tyrosinemia type 1	Serum amino acids increased. Aminoacidemia. Hepatocellular disease. Increased serum α-fetoprotein.
Zellweger syndrome	Hypotonia. Dysmorphic features. Neurologic dysfunction.
Cystic fibrosis	Meconium ileus may occur.

1.4 Common causes of neonatal jaundice

1.4.1 Physiological jaundice

Physiological jaundice most commonly occurs on day 3 to day 5 of life and decreases the first week after birth. It is usually associated with mild to moderate jaundice believed to be caused by a variety of factors: cessation of bilirubin clearance of placenta, immaturity of hepatic enzymatic systems at birth which decreases the ability to conjugate bilirubin, decreased hepatic uptake of bilirubin, larger red cell volume, shortened erythrocyte life span of cells carrying foetal haemoglobin and increased enterohepatic circulation (Hintz *et al.*, 2001).

Physiological jaundice is the most common cause of neonatal jaundice. It tends to be more severe in Asian populations than in Caucasian and Black populations (Zaghloul and Schulze, 2001). Quite often however, the term is used incorrectly to denote severe cases of neonatal jaundice. If screening for common causes does not reveal any cause, neonatal jaundice tends to be labelled as excessive physiological jaundice. A better term for this severe jaundice might be idiopathic pathological jaundice (Van Rostenberghe *et al.*, 2004).

1.4.2 Pathological causes

1.4.2.1 Haemolytic causes

1.4.2.1(a) ABO incompatibility

ABO incompatibility most commonly occurs when a baby with blood group A or B is born to a mother with type O blood group. In these cases antibodies made by the mother against the blood group antigens of the baby may cross the placenta and cause haemolysis. The presence of these maternal antibodies in the baby's blood is tested through the direct Coombs test, using heterologous anti-immunoglobulin (Kaplan and Hammerman, 2003). The Coombs test however has a high false negativity rate that may be related to a relatively low expression of the blood group antigens on the red blood cells of the neonate or to relatively low titres of antibodies. If jaundice occurs in ABO incompatibility, it typically starts on day 1 after birth (Thilo, 1999).

1.4.2.1(b) Rhesus incompatibility

Rhesus incompatibility occurs when a Rhesus negative mother carries a Rhesus positive baby. This condition results in severe anaemia antenatally, causing hydrops fetalis. It is also called erythroblastosis fetalis (Thilo, 1999).

1.4.2.1(c) Glucose-6-phosphate dehydrogenase deficiency

The enzyme glucose-6-phosphate dehydrogenase (G-6-PD) deficiency can be found in every cell in our body. It is used to protect cells (especially red blood

cells) against oxidative damage. The glutathione which is oxidised is used for neutralisation of toxic agents such as hydrogen peroxide and organic peroxidases (Mehta *et al.*, 2000). To maintain the process, oxidized glutathione is directly changed back to the reduced form using hydrogen ions from NADPH. NADPH is formed from nicotinamide adenine dinucleotide phosphate (NADP) and this process is catalyzed by G-6-PD. Therefore, NADPH formation is disturbed in G-6-PD deficiency and this leads to fragility of the red blood cells in the presence of oxidative stresses. This process is illustrated in Figure 1.3. Residual G-6-PD activity and additional antioxidant enzymes such as catalase prevent significant haemolysis under physiological conditions but under oxidant stress conditions, severe haemolysis may occur (Mehta *et al.*, 2000, Kaplan and Hammerman, 2002).

G-6-PD deficiency is a major cause of neonatal jaundice (Mehta *et al.*, 2000) and is common in many developing countries with limited access to modern medical care. Half of the morbidity and mortality which is associated with neonatal jaundice may be caused by G-6-PD deficiency (Kaplan *et al.*, 2001a).

Classically it was believed that the neonatal jaundice in babies with G-6-PD deficiency is due to haemolysis. However in the majority of cases there is no identifiable oxidative stress and haemolysis has been found to be very mild and similar in G-6-PD deficient babies developing jaundice and those not developing jaundice (Jalloh *et al.*, 2005).

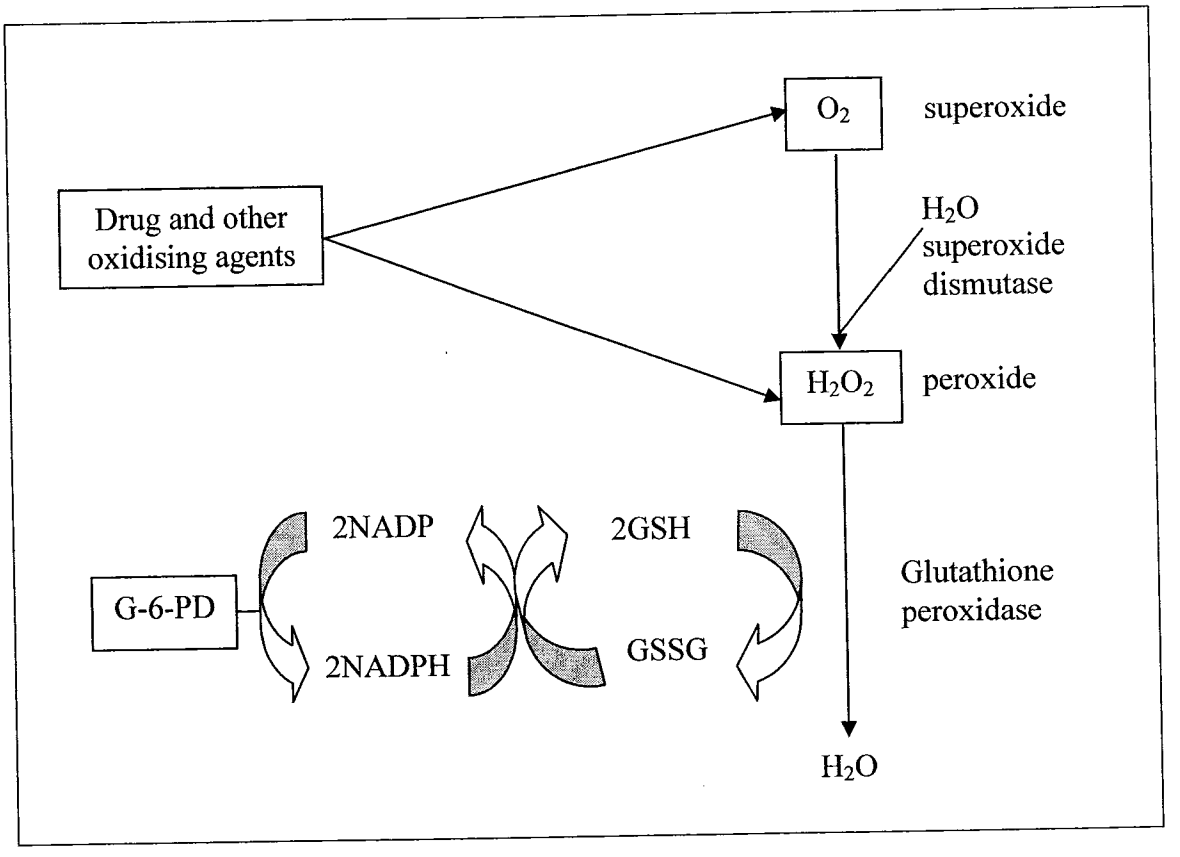

Figure 1.3 Diagram for the regeneration of NADPH (reduced form) from NADP in the presence of G-6-PD enzyme. This is a part of the antioxidant defence mechanism. GSH: Reduced Glutathione; GSSG: Oxidized Glutathione (Kaplan and Hammerman, 2002).

The presence of other risk factors for jaundice in combination with G-6-PD deficiency may become the crucial factor determining whether or not a baby with G-6-PD deficiency gets jaundiced. Such factors may include a decrease in bilirubin conjugation that may be related to promoter polymorphism in the gene which controls the bilirubin conjugating enzyme UDP-glucuronosyltransferase (Kaplan *et al.*, 2001b). These findings were also supported by other studies (Bosma *et al.*, 1995, Beutler *et al.*, 2002).

Total serum bilirubin levels in G-6-PD deficient neonates rise gradually and eventually leading to jaundice. This gradual onset jaundice may cause the jaundice to be missed in babies discharged early from the postnatal ward. This is the reason that in Malaysia, all babies are screened and the G-6-PD deficient babies are monitored for 5 days in the ward, so phototherapy can be started early so as to prevent the need for exchange transfusion.

1.4.2.1(d) Other causes of haemolysis

Another common cause of haemolysis is the extravasation of blood due to birth trauma. Extravasated blood is rapidly broken down and may cause severe hyperbilirubinaemia. Other less common causes of haemolysis can be found in Table 1.1.

1.4.2.2 Non-haemolytic causes of neonatal jaundice

The common causes of jaundice without haemolytic disorder can be classified as follows:

a) Increase of bilirubin production which is related to polycythaemia and swallowed blood.

b) Decrease of bilirubin clearance, which is caused by UDPGT deficiency, resulting in Crigler-Najjar and Gilbert syndrome. A more detailed literature review on its relation to neonatal jaundice will be given in section 1.5. Decreased bilirubin clearance is also seen in hypothyroidism and breast feeding which is associated with jaundice.

c) Unknown or multiple pathogeneses include altitude, racial differences, prematurity and bowel obstruction.

1.5 Clinical syndromes associated with mutations in the *UGT1A1* gene

Decreased bilirubin clearance is attributed to Crigler-Najjar and Gilbert syndrome, due to lack of UDPGT enzyme in the liver.

1.5.1 Crigler-Najjar syndrome

Defects of the *UGT1A1* gene may occur in any of its five exons and contribute to Crigler-Najjar syndrome, either type I or II (Kadakol *et al.*, 2000). Crigler-Najjar syndrome type I is the severe form and was described by Crigler and

Najjar in 1952 as a potentially lethal hyperbilirubinaemia (serum bilirubin 20–50 mg/dL) without liver disease or overt haemolysis (Crigler and Najjar, 1952).

Crigler-Najjar syndrome type II is an intermediate form with moderate elevations of the level of bilirubin (7–20 mg/dL). It is also known as Arias syndrome as it was described by Arias in 1962. It is commonly caused by a severe, but incomplete lack of *UGT1A1* activity in the liver (Seppen *et al.*, 1994).

1.5.2 Gilbert syndrome

Gilbert syndrome was first described by Augustin Gilbert and Pierre Lereboullet in 1901. Patients with Gilbert syndrome commonly have mild and chronic unconjugated hyperbilirubinaemia with normal liver function and without overt haemolysis (Sugatani *et al.*, 2002). It is an inherited disorder of bilirubin metabolism.

Gilbert syndrome is considered to be a harmless disease, but it may be a risk factor for neonatal jaundice, especially in combination with haemolytic disorders, such as G-6-PD deficiency and ABO incompatibility (Sutomo *et al.*, 2002, Kaplan *et al.*, 2001a, Beutler *et al.*, 2002). There is still controversy whether Gilbert syndrome is inherited as a recessive or dominant trait. Schmid, (1995) assumed that it was inherited as an autosomal dominant trait while a study by Bosma *et al.*, (1995) suggested an autosomal recessive trait.

There may be different degrees of severity of the enzyme defect depending on the particular mutation. Some mutations are known to cause Crigler-Najjar syndrome type II in the homozygous state and Gilbert syndrome in the heterozygous state (Koiwai *et al.*, 1995). Phenobarbital treatment has been found to increase the enzyme activity resulting in a decrease in the serum bilirubin level (Black and Sherlock, 1970).

The incidence of Gilbert syndrome in the general population is about 3% to 10% (Iyanagi *et al.*, 1998, Yamamoto *et al.*, 1998). Hsieh and colleagues found that the TATA box mutation and G71R underlies the molecular background of Gilbert syndrome in the Taiwanese population.

Simultaneous occurrence of more than one mutations were also found in this population which resulted in higher level of bilirubin and caused a more severe form of Gilbert syndrome (Hsieh *et al.*, 2001). Since the *UGT1A1* gene is the focus of this study, a more in depth review on the *UGT1A1* gene is presented below. Several genotypes associated with Gilbert syndrome are listed in Table 1.3.

Table 1.3 Genotypes causing Gilbert syndrome (Bosma *et al.*, 2003)

	Mutation protein	Mutation DNA	Mutation no.	Diagnosis	Remarks
Allele 1		(TA)7TAA	UGT1A1*28	Gilbert syndrome	
Allele 2		(TA)7TAA	UGT1A1*28		
Allele 1	G71R	(TA)7TAA	UGT1A1*28	Gilbert syndrome	
Allele 2		211G > A	UGT1A1*27		
Allele 1		(TA)7TAA	UGT1A1*28		
Allele 2	Y486D	1456T > G			
Allele 1	G71R	211G > A	UGT1A1*27	Gilbert syndrome	
Allele 2	G71R	211G > A	UGT1A1*27		
Allele 1	Y486D	1456T > G		Gilbert syndrome	
Allele 2	Y486D	1456T > G			
Allele 1	G71R	211G > A		Gilbert syndrome	
Allele 2	Y486D	1456T > G			
Allele 1		(TA)7TAA structural mutation	UGT1A1*28	Gilbert syndrome	Crigler-Najjar syndrome carrier
Allele 2					
Allele 1	G71R	211G > A structural mutation	UGT1A1*27	Gilbert syndrome	Crigler-Najjar syndrome carrier
Allele 2					
Allele 1	Y486D	1456T > G structural mutation		Gilbert syndrome	Crigler-Najjar syndrome carrier
Allele 2					

1.6 The *UGT1A1* gene

The Uridine-diphosphoglucuronate-glucuronosyltransferases (UGTs) belong to the enzyme group of glycosyltransferases. UGTs in mammals have been classified into three families, based on the sequence similarity and structure of the gene: the UGT1, UGT2 and UGT8 (Meech and Mackenzie, 1997, Kadakol *et al.*, 2000). Within these families there are several isoforms. Only two isoforms in the UGT1A family (*UGT1A1* and *UGT1A4*) have bilirubin as a substrate but in humans only *UGT1A1* plays a significant role in bilirubin glucuronidation (Bosma *et al.*, 1994).

UGT1A1 is located on chromosome 2q37 (Kiang *et al.*, 2005) as represented in Figure 1.4 and is the focus of the current study. It consists of 5 exons. Exons 2 to 5 are common exons of other isoforms within the UGT1A group (*UGT1A1-13*) and exon 1 is a unique exon for *UGT1A1* (Figure 1.5). The four common exons at 3' end encode the carboxyterminal domain for all isoforms of UGT which bind to UDP-glucuronic acid. The unique exon for each isoform at 5' end encodes the N-terminal domain for the enzyme which specifies the substrate for the isoform. At least 13 exons (for *UGT1A1* until *UGT1A13*) are located upstream of exon 2 (Ritter *et al.*, 1992, Mackenzie *et al.*, 1997, Watchko *et al.*, 2002).

A decrease in enzyme activity caused by defects or lesions in the *UGT1A1* gene results in unconjugated hyperbilirubinaemia (Sutomo *et al.*, 2002). Several mutations in *UGT1A1* gene have been reported to be associated with Gilbert

syndrome (e.g. the TATA box mutation ($A(TA)_7TAA$), G71R and P229G) (Hsieh *et al.*, 2001). For the purpose of this study three mutations were studied.

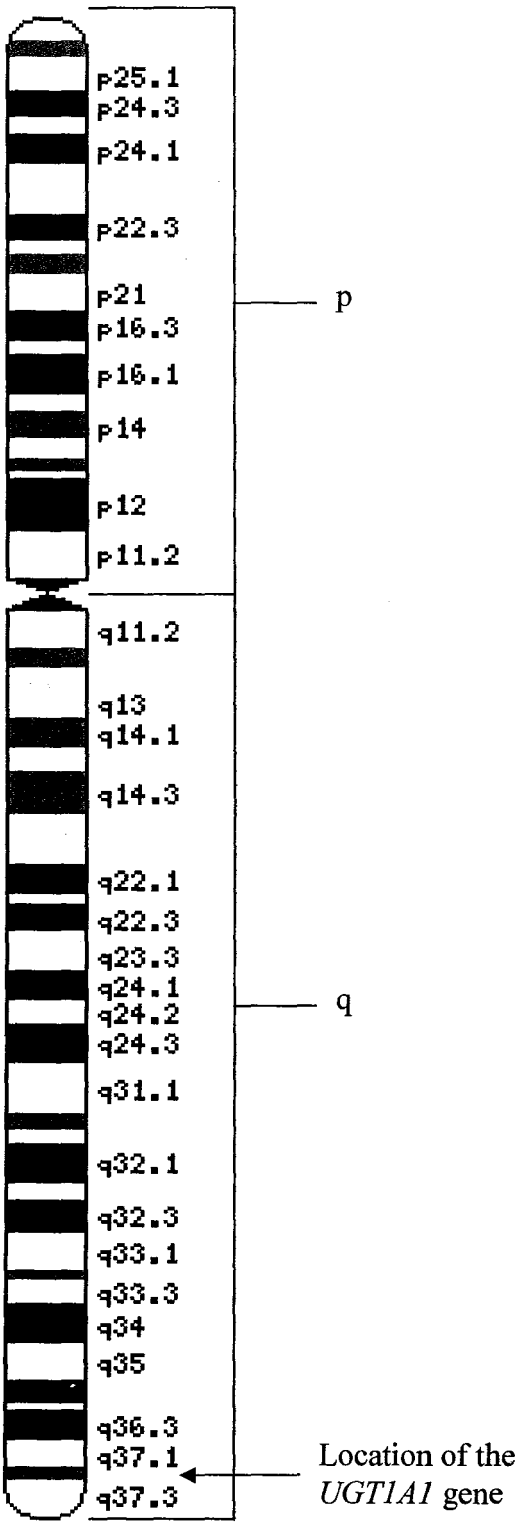

Figure 1.4 Human chromosome 2
(http://www.ensembl.org/Homo sapiens/mapview?chr=2)

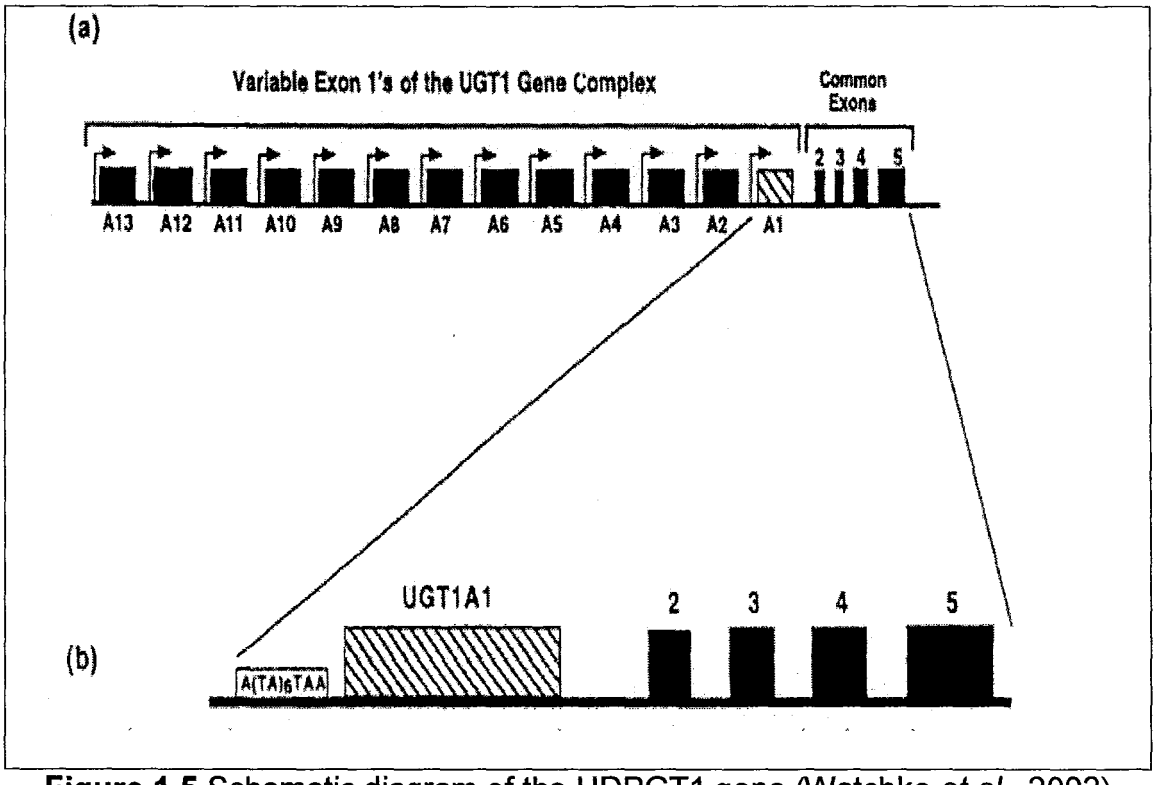

Figure 1.5 Schematic diagram of the UDPGT1 gene (Watchko *et al.*, 2002)

a) The entire UGT1 gene complex (*UGT1A1–UGT1A13*) and exons 2–5 of the *UGT1A1* gene

b) The *UGT1A1* gene which consists of a normal TATA box in the promoter region and exons 2-5 of the gene