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Association between uridin diphosphate glucuronosylotransferase 1A1 (*UGT1A1*) gene polymorphism and neonatal hyperbilirubinemia

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Objective: To assess the prevalence of UGT1A1*28 and UGT1A1*60 polymorphisms of *UGT1A1* gene and their association with hyperbilirubinemia. Study design: The study was performed at a single centre – at the Department of Obstetrics of the Medical University of Gdansk in Poland. DNA was isolated from Guthrie cards of 171 infants. Only full term newborns (gestational age 38–42 weeks) were included in the study. Fluorescent molecular probes were used for *UGT1A1* promoter variation analysis. The presence of UGT1A1*28 polymorphism was detected with a dual-probe system, and UGT1A1*60 with a SimpleProbe™. Result: Homozygous UGT1A1*28 and UGT1A1*60 genotypes were detected in 14.6% and 20.5% of the newborns, respectively. Homozygous (G/G) genotypes of UGT1A1*60 polymorphism were found in all of the UGT1A1*28 (i.e. $(TA)_7/(TA)_7$) homozygotes. More than 80% (55/66) of the children with "wild" type UGT1A1*28 genotype (where no polymorphism was detected) (i.e. $(TA)_6/(TA)_6$) carried the "wild" (T/T) genotype of UGT1A1*60 as well. The UGT1A1*28 polymorphism was detected more often among neonates with elevated bilirubin. Hyperbilirubinemia was diagnosed more frequently in boys. Conclusion: Polymorphisms of the UGT1A1 gene frequently co-exist in neonates. The presence of UGT1A1*28 polymorphism and male gender seem to predispose to neonatal hyperbilirubinemia.

Key words: *UGT1A1* gene, polymorphism, hyperbilirubinemia, neonates

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Abbreviations: *UGT1A1*, uridin diphosphate glucuronosylotranserase 1A1 gene; PBREM, phenobarbital responsive enhancer module

INTRODUCTION

Hyperbilirubinemia is a relatively frequent condition of neonatal and early infantile period. Neonatal hyperbilirubinemia is defined as a total serum bilirubin level above 5 mg/dL (86 μmol/L) (Porter *et al*., 2002).

Up to 50% of healthy, full term and 80% of preterm neonates demonstrate physiological jaundice (American Academy of Pediatrics Subcommittee on Hyperbilirubinemia, 2004; Scrafford *et al*., 2013). In some cases jaundice can be more severe and prolonged and it can be associated with a potential risk of neurotoxicity (Dore *et al*., 1999; Hammermann *et al*., 1998). Bilirubin monoglucuronide, degraded easier than the diglucuronide, is the only product of bilirubin conjugation in neonates. Moreover, the intestinal concentration of beta-glucuronidase, the enzyme responsible for elimination of glucuronic acid, is markedly higher in newborns than in adults, and only trace amounts of physiological bacterial flora reducing bilirubin to colorless tetrapyrrole compounds are present. As a result, instead of being transformed into urobilinogen, most of unconjugated bilirubin is reabsorbed to the hepato-enteric circulation and can cross the blood-brain barrier causing neuronal injury (Hale *et al*., 2007). This phenomenon is referred to as bilirubin encephalopathy or kernicterus. The penetration of bilirubin into tissues starts with blood concentration exceeding 2.5 mg/dl (Murray *et al*., 2012).

The fact that the evident cause of neonatal or early infantile hyperbilirubinemia cannot be identified in more than half of the cases can suggest the presence of another, unidentified risk factor of this condition.

Racial predisposition suggests potential genetic background of neonatal hyperbilirubinemia (Maruo *et al*., 1999). Conjugation of bilirubin with glucuronic acid is catalyzed by a hepatic enzyme uridin diphosphate glucuronosylotransferase (UDP-glucuronosylotransferase), namely its 1A1 isoform (Clarke *et al*., 1997). The fact that the enzyme is not stable and its determination with classical methods is substantially biased, justifies its testing at a genetic level (Huang *et al*., 2002).

UDP-glucuronosylotransferase is encoded by the *UGT1A1* gene. The *A(TA)$_7$TAA* polymorphism (*UGT1A1*28*), identified as the principal cause of the Gilbert syndrome, is relatively frequently detected in the Caucasian population (11–16%) (Bosma, 2003). The mutated allele is characterized by alterations in its promoter region, namely in the *TATA* box sequence. This sequence constitutes the binding site for transcription factor II D, which is vital for the initiation of the transcription process (Bosma *et al*., 1995). Decreased expression of the *UGT1A1* gene results from the presence of additional TA repeat in the TATA box sequence, i.e. A(TA)$_7$TAA instead of A(TA)$_6$TAA (Bosma, 2003). The presence of this variant is frequently associated with another alter-

ation in the *UGT1A1* promoter, the polymorphism of Phenobarbital Responsive Enhancer Module (PBREM) (Sugatani *et al.*, 2002). This polymorphism, resulting from a single nucleotide substitution (thymine to guanine, *c.-3279T>G*), is referred to as *UGT1A1*60* (Costa *et al.*, 2005; Maruo *et al.*, 2004). Transcriptional activity of *UGT1A1* in $(TA)_7/(TA)_7$ homozygotes is decreased to 60-80%. Also, the presence of c.-3279T>G is associated with a decrease in the transcriptional activity to about 60% of its normal level. However, the co-existence of these two homozygous variants (*UGT1A1*28* and *UGT1A1*60*) can be reflected by a decrease in the transcriptional activity of *UGT1A1* down to 30% (Maruo *et al.*, 2004).

Data on the influence of *UGT1A1* gene polymorphism on the prevalence of hyperbilirubinemia in neonates from a Caucasian population are inconclusive (Kaplan *et al.*, 2003). Bancroft *et al.* (1998), who analyzed the severity of jaundice by means of transcutaneous bilirubinometry, did not observe significant differences in peak bilirubin levels in children with *UGT1A1*28* polymorphism when compared to controls. Nevertheless, they have shown an association between the presence of $A(TA)_7TAA$ variant and the severity of jaundice during the initial two days of life (Monaghan *et al.*, 1999). Another study, involving a group of Scottish newborns, revealed that nearly one-third of the children with prolonged jaundice were $(TA)_7/(TA)_7$ homozygotes. Moreover, presence of the $A(TA)_7TAA$ allele was suggested to predispose to jaundice in breastfed neonates (Monaghan *et al.*, 1999). Also, results of the Italian and Greek studies suggest that newborns who carry the $A(TA)_7TAA$ allele are characterized by a markedly higher prevalence and longer duration of hyperbilirubinemia, despite the lack of significant differences in peak concentrations of bilirubin between the carriers and controls (Laforgia *et al.*, 2002; Roy-Chowdhury *et al.*, 2002). Presence of the *UGT1A1*28* polymorphism seems to predispose to hyperbilirubinemia in patients who are unexposed to other risk factors of this condition (Bancroft *et al.*, 1998). However, opinions in this matter are inconclusive. According to Babaoglu *et al.* (2006), presence of the $A(TA)_7TAA$ allele is not associated with the prevalence of hyperbilirubinemia among Turkish newborns. *UGT1A1*28* was reported as the most common mutation causing the Gilbert's syndrome in Caucasians; in contrast, a markedly lower prevalence of *UGT1A1*28*, corresponding to about 1–2%, was observed in the Asian population (Azlin *et al.*, 2011; Tiwari *et al.*, 2014). This suggests that the role of the *UGT1A1* polymorphism in the pathogenesis of hyperbilirubinemia among newborns of Caucasian heritage is still not completely understood, justifying further research.

Therefore, the aim of this study was to assess the prevalence of UGT1A1*28 and UGT1A1*60 polymorphisms in a randomly selected group of Polish newborns from a single university hospital centre.

METHODS

Study group. The study included 171 newborns consecutively born at term (gestational age 38–42 weeks) at the Department of Obstetrics of the Medical University of Gdansk (University Clinical Center).

Only full term newborns were included in the study because prematurity is one of the risk factors of hyperbilirubinemia.

A detailed medical history, regarding the course of pregnancy (including maternal conditions and used medications), delivery (gestational age, Apgar score), familial history of systemic conditions and liver disorders, was obtained for every newborn. Also, anthropometric measurements at birth (birth weight, body length, head and chest circumferences) were recorded. Serum bilirubin level in all neonates was measured on the 2nd or 3rd day after birth. As the vast majority of subjects were breastfed and only few were subjected to mixed feeding, the effect of diet on bilirubin concentration was not analyzed. Detailed characteristics of the study participants are presented in Table 1.

Table 1. Clinical and obstetrical characteristics of the studied neonates (n=171)

Variable	Value
Birth weight (g)	3379±520.66 (2490–4875)
Gestational age (weeks)	40.0±2 (38–42)
Males (n)	91 (53.2%)
Females (n)	80 (46.3%)
Natural delivery (n)	142 (83.0%)
Cesarean section (n)	29 (17.0%)
Apgar score (points)	9.12 ±1.9 (7–10)
Total serum bilirubin (mg/dl, 2nd or 3rd day after birth)	8.6 ± 6.5 (0.8–19.7)

Data presented as arithmetic means ± S.D. (ranges) or numbers (%)

Ethics. The protocol of the study was approved by the Local Bioethical Committee of the Medical University of Gdansk (decision no. NKEBN/87/2008). Parents of all children gave their informed consent for their participation in the project. All of the samples were collected during a routine medical examination and the newborns participating in the study were not exposed to any additional invasive procedures.

Material. The examined material included 171 so-called Guthrie cards (Whatman, UK) with the dried samples of capillary blood obtained by pricking the heel. Moreover, the serum level of bilirubin was determined by means of the diazo method (Architect Systems™, USA) on the 2nd or 3rd day after birth.

The data was collected between November 2010 and January 2011.

Genetic tests. Genomic DNA was isolated from the Guthrie cards with an aid of Sherlock AX set (A&A Biotechnology, Poland). The methodology of detecting the studied *UGT1A1* polymorphisms was described previously (Romanowski *et al.*, 2009). *UGT1A1* gene fragments of interest were amplified by means of asymmetric PCR. The presence of *UGT1A1*28* polymorphism was detected with two molecular probes, a fluorescein-labeled anchor probe and a quencher-labeled (IowaBlackFQ; Integrated DNA Technology, USA) sensor probe, and the presence of *UGT1A1*60* polymorphism was determined with a single fluorescent SimpleProbe™ designed with LightCycler Probe Design Software 2.0 (Roche Diagnostics, Germany). Genotyping was based on the differences in the binding stability (melting temperatures) of the probes and various allelic variants. Melting temperature profiles of the probes hybridized to PCR products were determined with the LightCycler 2.0 instrument (Roche Diagnostic, Germany) by increasing temperature (T) while measuring the sample fluorescence (F). Fluorescence was further expressed as the first negative derivative of temperature (-dF/dT). The examples

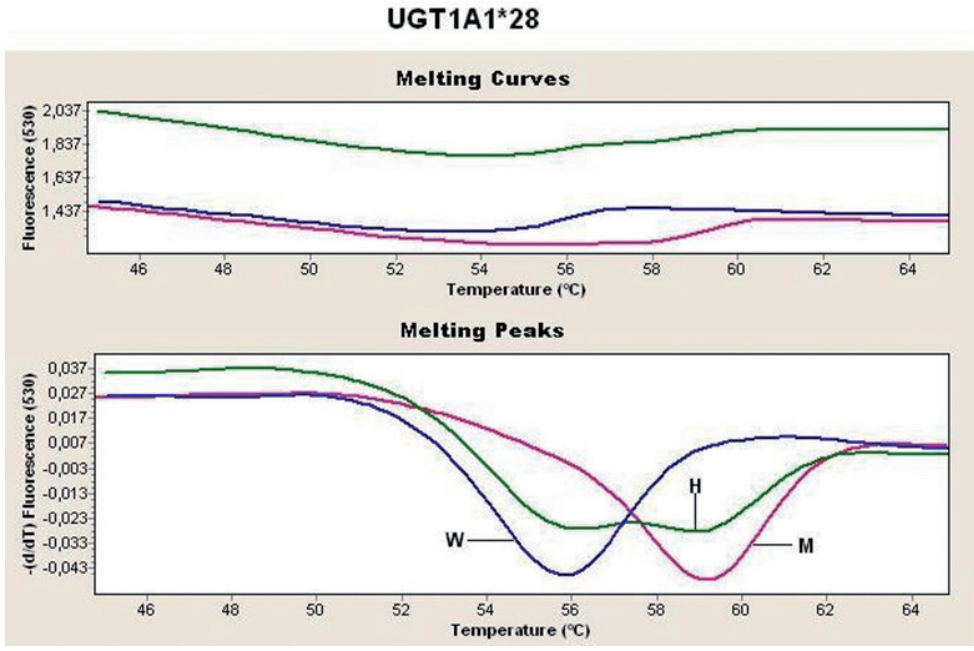

Figure 1. Melting temperature analysis of PCR products for the UGT1A1*28 genotype.
W, wild type homozygote; H, heterozygote; M, mutant homozygote

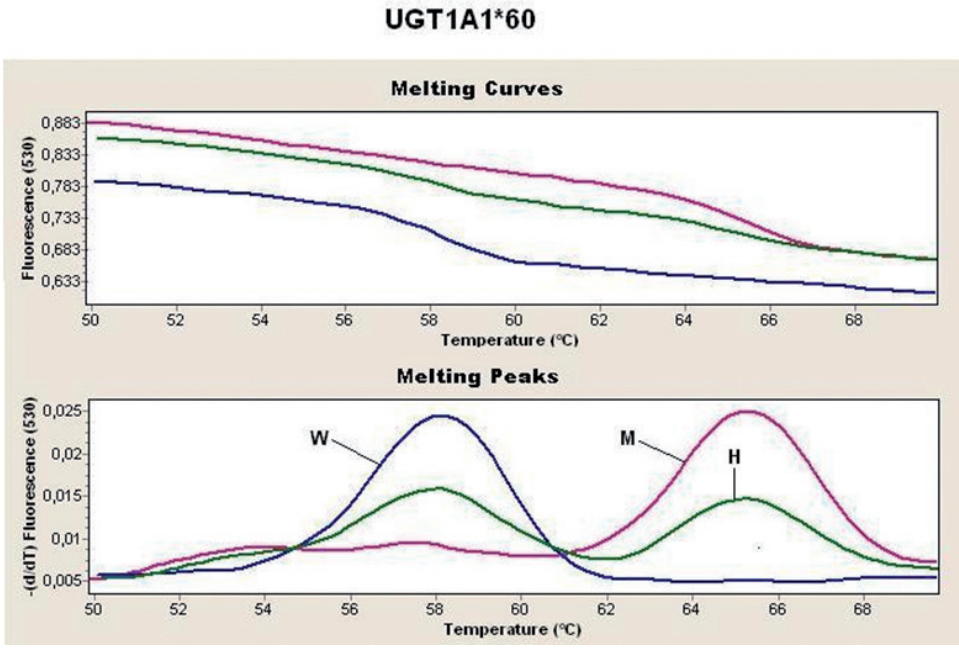

Figure 2. Melting temperature analysis of PCR products for the UGT1A1*60 genotype.
W, wild type homozygote; H, heterozygote; M, mutant homozygote

of melting curve analyses of the *UGT1A1* genotypes are shown in Figs. 1 and 2.

Statistical analysis. Normal distribution of continuous variables was verified with the Kolmogorov-Smirnov test, and their statistical characteristics were presented as mean values and standard deviations (S.D.), or medians and ranges. Statistical characteristics of continuous variables of the neonates with hyperbilirubinemia and normal bilirubin level were compared with the Mann-Whitney U-test. The Pearson's chi-square test and the exact Fisher test were used for the intergroup comparisons of qualitative variable distributions (i.e. presence of the studied polymorphisms and general characteristics of the neonates). The results of all tests were considered significant at $p < 0.05$ All analyses were performed with the Statistica 10 software (Stat Soft. Inc., USA).

RESULTS

Polymorphism of the *UGT1A1* gene

Homozygous *UGT1A1*28* and *UGT1A1*60* genotypes were detected in 25/171 (14.6%) and 35/171

Table 2. Prevalence of UGT1A1*28 and UGT1A1*60 polymorphisms among the studied neonates (n=171)

UGT1A1*28			UGT1A1*60		
Genotype	n	%	Genotype	n	%
$(TA)_6/(TA)_6$	66	38.6	T/T	55	32.1
$(TA)_7/(TA)_6$	80	46.8	G/T	81	47.4
$(TA)_7/(TA)_7$	25	14.6	G/G	35	20.5
Total	171	100.0	Total	171	100.0

Table 3. Coexistence of UGT1A1*28 and UGT1A1*60 polymorphisms in the studied neonates (n=171)

UGT1A1*28	UGT1A1*60		
	T/T (n=55)	G/T (n=81)	G/G (n=35)
$(TA)_6/(TA)_6$ (n=66)	55	9	2
$(TA)_7/(TA)_6$ (n=80)	0	72	8
$(TA)_7/(TA)_7$ (n=25)	0	0	25

(20.5%) studied newborns, respectively. The presence of homozygous (G/G) genotype of *UGT1A1*60* polymorphism was identified in all *UGT1A1*28* (i.e. $(TA)_7/(TA)_7$) homozygotes. In contrast, more than 80% (55/66) of the children with "wild" (i.e. $(TA)_6/(TA)_6$) *UGT1A1*28* genotype carried the normal (T/T) genotype of *UGT1A1*60* as well (Tables 2 and 3).

Determinants of hyperbilirubinemia

Hyperbilirubinemia (total bilirubin level >5 mg/dl) was diagnosed in 66 (38.6%) of the examined neonates (44 boys – 48.35% – percentage of the whole group of boys, and 22 girls – 27.5% – percentage of the whole group of girls).

We did not find a significant correlation between presence of the UGT1A1*60 polymorphism and hyperbilirubinemia. In contrast, the UGT1A1*28 polymorphism was detected significantly more often among neonates with hyperbilirubinemia (p=0.047). Furthermore, hyperbilirubinemia was diagnosed significantly more frequently in boys than in girls (p=0.005). We noted no impact of the mode of delivery or birth weight on the prevalence of hyperbilirubinemia (p=0.449, p=0.07 respectively) (Table 4).

Table 4. Prevalence of hyperbilirubinemia and normal serum bilirubin levels stratified according to obstetrical and genetic factors

Variable	Hyperbilirubinemia	Normal bilirubin	p
Mode of delivery			
natural delivery	53	89	0.449
cesarean section	13	16	
Gender			
male	44	47	0.005
female	22	58	
Birth weight (g)	3379 ± 520.66	3252 ±328.5	0.070
UGT1A1*28 polymorphism			
yes	14	11	0.047
no	19	47	
UGT1A1*60 polymorphism			
yes	18	17	0.062
no	15	40	

DISCUSSION

Presence of the $(TA)_7/(TA)_7$ genotype was documented in 14.6% of the examined newborns. This value is similar to others previously reported in the European studies. Prevalence of the $(TA)_7/(TA)_7$ homozygous genotype in the Caucasian population is estimated at several percent, amounting to 9.7% in Sweden, 13.6% in Slovenia, 11.6% in Germany, and 12.6% in the Netherlands (Borlak *et al.*, 2000; Mercke Odeberg *et al.*, 2006; Ostanek *et al.*, 2007; Peters *et al.*, 2003). However, data from a Turkish study documented a markedly lower percentage, amounting to7.5% prevalence of this genotype. This has underlined that *UGT1A1*28* is the most common mutation causing the Gilbert's syndrome in Caucasians. In contrast, its prevalence in the Asian population was estimated at about 1–2%. The most common mutation of the UGT1A1 gene in this population was nt211 G>A, with the prevalence up to 10–20% (Azlin *et al.*, 2011; Tiwari *et al.*, 2014; Liu *et al.*, 2013).

Frequency of the $(TA)_7/(TA)_7$ genotype in our group seems relatively high as compared to the abovementioned investigations, although even higher prevalence of this genotype (16.3%) was documented by an Italian study (Biondi *et al.*, 1999). Moreover, it should be noted that we have analyzed newborns only from one hospital, and therefore our findings are not representative for the whole Polish population.

Presence of the G/G genotype of *UGT1A1*60* was identified in 20.5% of the newborns in our group. This value is higher than the prevalence of the *UGT1A1*28* polymorphism. The prevalence of the *UGT1A1*60* polymorphism has not been studied extensively thus far (Bosma *et al.*, 1995; Sugatani *et al.*, 2002; Maruo *et al.*, 2004). Nevertheless, previous studies dealing with the problem in question documented a tendency to the co-existence of both analyzed genetic variants (e.g. *UGT1A1*28* and *UGT1A1*60*) (Costa *et al.*, 2005, Maruo *et al.*, 2004). The co-existence of *UGT1A1*28* and *UGT1A1*60* polymorphisms was documented in our group of newborns as well. The homozygous form of *UGT1A1*28* was detected in more than 70% of the neonates carrying *UGT1A1*60*. The co-existence of both mutated homozygous genotypes is postulated to decrease the translational activity of *UGT1A1* down to 30% (Maruo *et al.*, 2004).

It is worth to note, that the UDP-glucuronosylotransferase activity is only one of numerous mechanisms contributing to the pathophysiology of jaundice in newborns.

Recent studies had shown that 388G>A, 521 T>C and 463 C>A mutations of the *SLCO1B1* (solute carrier organic anions transporter family membrane 1B1) gene may predispose to neonatal hyperbilirubinemia by limiting the hepatic bilirubin uptake (Liu *et al.*, 2013; Biondi *et al.*, 1999).

Glucose-6-phosphate dehydrogenase deficiency has been also reported as a risk factor for severe neonatal hiperbilirubinemia. The prevalence of this defect in newborns with indirect hyperbilirubinemia is estimated at about 4% (0.5–20%, depending on geographical and ethnic group) (Watchko *et al.*, 2010; Celik *et al.* 2013). These

important risk factors for neonatal jaundice, not assessed in our study, need further investigation.

We have documented a significantly higher prevalence of hyperbilirubinemia in male newborns. This observation is consistent with the results of previous research, however, the mechanism of this phenomenon is not clear (Tioseco *et al*., 2005). We did not reveal a statistically significant association between presence of the *UGT1A1*60* polymorphism and the prevalence of hyperbilirubinemia in our group. However, we have observed a significant relationship between presence of the *UGT1A1*28* polymorphism and an elevated level of bilirubin in our samples. This finding is consistent with the results of previous studies conducted in the Caucasian population (Tiwari *et al*., 2014; Borlak *et al*., 2000; Mercke Odeberg *et al*., 2006; Ostanek *et al*., 2007; Peters *et al*., 2003). We have also analyzed the effect of birth weight on the prevalence of hyperbilirubinemia but did not find a significant association between these two parameters. According to some authors, low birth weight is a risk factor of jaundice (Scafford *et al*., 2013; Tioseco *et al*., 2005; Okwundu *et al*., 2013; Yang *et al*., 2013). Indeed, the immaturity of enzymatic systems in preterm newborns constitutes a potent factor modulating the severity of jaundice, and most preterm babies are delivered with low birth weight (Huang *et al*., 2002).

To minimize the number of invasive procedures, we have determined the serum bilirubin level only once, on the 2nd or 3rd day after birth. Presently, transcutaneous measurement is suggested to be a potential non-invasive method for serum bilirubin level determination, not requiring an extra blood sample. This method enables multiple determination of bilirubin level and is optimal for monitoring of jaundice in newborns (Scafford *et al*., 2013).

Interestingly, authors from Nepal had relied only on visual determination of skin and sclera color while defining risk factors of hyperbilirubinemia in newborns (Scafford *et al*., 2013).

The use of dried blood spot testing used to determine the *UGT1A1* polymorphism had enabled us to analyze very small amounts of biological material, without unnecessary exposure of the studied babies to additional venipuncture. Blood collected onto the Guthrie card can be stored for a long period of time and reused if any additional analysis is required. The quality and purity of genetic material isolated from the cards is sufficient to obtain melting temperature profiles of the fluorescent probes and the PCR products, and to identify genetic profile of the probands accurately.

CONCLUSION

In conclusion, our study revealed that assessment of the *UGT1A1* gene polymorphism is an important test in neonatal patients.

We have found co-existence of the mutated homozygous genotypes of *UGT1A1*28* and *UGT1A1*60*.

*UGT1A1*28*, but not *UGT1A1*60* polymorphism correlated with presence of hyperbilirubinemia. Neonatal hyperbilirubinemia occurs more frequently in the male gender.

These preliminary findings should be verified in a larger cohort of neonates. Furthermore, other potential modulators of bilirubin level should be included in the risk analysis.

Conflict of interest

The authors declare no conflict of interest.

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REFERENCES

American Academy of Pediatrics Subcommittee on Hyperbilirubinemia (2004) Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics* **114**: 297–316

Azlin I, Wong FL, Ezham M, Hafiza A, Ainoon O (2011) Prevalence of uridine glucuronosyl transferase 1A1 (UGT1A1) mutations in Malay neonates with severe jaundice. *Malays J Pathol* **33**: 95–100

Babaoglu MO, Yigit S, Aynacioglu AS, Kerb R, Yurdakok M, Bozkurt A (2006) Neonatal jaundice and bilirubin UDP-glucuronosyl transferase 1A1 gene polymorphism in Turkish patients. *Basic Clin Pharmacol Toxicol* **98**: 377–380. http://dx.doi.org/10.1111/j.1742-7843.2006.pto_341.x

Bancroft JD, Kreamer B, Gourley GR (1998). Gilbert syndrome accelerates development of neonatal jaundice. *J Pediatr* **132**: 656–660

Biondi ML, Turri O, Dilillo D, Stival G, Guagnellini E (1999) Contribution of the TATA-box genotype (Gilbert syndrome) to serum bilirubin concentrations in the Italian population. *Clin Chem* **45**: 897–898

Borlak J, Thum T, Landt O, Erb K, Hermann R (2000) Molecular diagnosis of a familial nonhemolytic hyperbilirubinemia (Gilbert's syndrome) in healthy subjects. *Hepatology* **32**: 792–795. http://dx.doi.org/10.1053/jhep.2000.18193

Bosma PJ (2003) Inherited disorders of bilirubin metabolism. *J Hepatol* **38**: 107–117

Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, Lindhout D, Tytgat GN, Jansen PL, Oude Elferink RP (1995) The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med* **333**: 1171–1175. http://dx.doi.org/10.1056/NEJM199511023331802

Celik HT, Günbey C, Unal S, Gümrük F, Yurdakök M (2013) Glucose-6-phosphate dehydrogenase deficiency in neonatal hyperbilirubinaemia: Hacettepe experience. *J Paediatr Child Health* **49**: 399–402. http://dx.doi.org/10.1111/jpc.12193

Clarke DJ, Moghrabi N, Monaghan G, Cassidy A, Boxer M, Hume R, Burchel B (1997) Genetic defects of the UDP-glucuronosyltransferase-1 (UGT1) gene that cause familial non-haemolytic unconjugated hyperbilirubinaemias. *Clin Chim Acta* **266**: 63–74

Costa E, Vieira E, Dos Santos R (2005) The polymorphism c.-3279T>G in the phenobarbital-responsive enhancer module of the bilirubin UDP-glucuronosyltransferase gene is associated with Gilbert syndrome. *Clin Chem* **51**: 2204–2206. http://dx.doi.org/10.1373/clinchem.2005.055681

Dore S**,** Takahashi M, Ferris CD, Zakhary R, Hester LD, Guastella D, Snyder SH (1999) Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury. *Proc Natl Acad Sci USA* **96**: 2445–2450

Hale TW, Hartmann PE (2007) *Hale & Hartmann's Textbook of Human Lactation*. Hale Pub., Amarillo, TX

Hammermann C, Goldstein R, Kaplan M, Eran M, Goldschmidt D, Eidelman AI (1998) Bilirubin in the premature: toxic waste or natural defense? *Clin Chem* **44**: 2551–3255

Huang CS, Chang PF, Huang MJ, Chen ES, Chen WC (2002) Glucose-6-phosphate dehydrogenase deficiency, the UDP-glucuronosyl transferase 1A1 gene, and neonatal hyperbilirubinemia. *Gastroenterology* **123**: 127–133

Kaplan M, Hammerman C, Maisels MJ (2003) Bilirubin genetics for the nongeneticist: hereditary defects of neonatal bilirubin conjugation. *Pediatrics* **111**: 886–893

Laforgia N, Faienza MF, Rinaldi A, D'Amato G, Rinaldi G, Iolascon A (2002) Neonatal hyperbilirubinemia and Gilbert's syndrome. *J Perinat Med* **30**: 166–169. http://dx.doi.org/10.1515/JPM.2002.021]

Liu J, Long J, Zhang S, Fang X, Luo Y (2013) Polymorphic variants of SLCO1B1 in neonatal hyperbilirubinemia in China. *Ital J Pediatr* **39**: 49–53. http://dx.doi.org/10.1186/1824-7288-39-49

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. *Hum Genet* **115**: 525–526. http://dx.doi.org/10.1007/s00439-004-1183-x

Maruo Y, Nishizawa K, Sato H, Doida Y, Shimada M (1999) Association of neonatal hyperbilirubinemia with bilirubin UDP-glucuronosyltransferase polymorphism. *Pediatrics* **103**: 1224–1227

Mercke Odeberg J, Andrade J, Holmberg K, Hoglund P, Malmqvist U, Odeberg J (2006) UGT1A polymorphisms in a Swedish cohort and

a human diversity panel, and the relation to bilirubin plasma levels in males and females. *Eur J Clin Pharmacol* **62**: 829–837. http://dx.doi.org/10.007.s00228-006-0166-3

Monaghan G, McLellan A, McGeehan A, Li Volti S, Mollica F, Salemi I, Din Z, Cassidy A, Hume R, Burhell B (1999) Gilbert's syndrome is a contributory factor in prolonged unconjugated hyperbilirubinemia of the newborn. *J Pediatr* **134**: 441–446

Murray RK, Bender DA, Botham KM, Kenelly PJ, Rodwell VW, Weil PA (2012) *Harper's Illustrated Biochemistry*. McGraw-Hill Medical: Columbus, OH

Okwundu CI, Okoromah CA, Shah PS (2013) Cochrane Review: Prophylactic phototherapy for preventing jaundice in preterm or low birth weight infants. *Evid Based Child Health* **8**: 204–249. http://dx.doi.org/10.1002/14651858.CD007966.pub2

Ostanek B, Furlan D, Mavec T, Lukac-Bajalo J (2007) UGT1A1(TA) n promoter polymorphism – a new case of a (TA)8 allele in Caucasians. *Blood Cells Mol Dis* **38**: 78–82. http://dx.doi.org/10.106/jbcmd.2006.10.160

Peters WH, Te Morsche RH, Roelofs HM (2003) Combined polymorphisms in UDP-glucuronosyltransferases 1A1 and 1A6: implications for patients with Gilbert's syndrome. *J Hepatol* **38**: 3–8

Porter ML, Dennis BL (2002) Hyperbilirubinemia in the Term Newborn. *Am Fam Physician* **65**: 599–607

Romanowski T, Sikorska K, Bielawski KP (2009) UGT1A1 gene polymorphism as a potential factor inducing iron overload in the pathogenesis of type 1 hereditary hemochromatosis. *Hepatol Res* **39**: 469–478. http://dx.doi.org/10.1111/j.1872-034X.2008.00487.x

Roy-Chowdhury N, Deocharan B, Bejjanki HR, Roy-Chowdhury J, Koliopoulos C, Petmezaki S, Valaes T (2002) Presence of the genetic marker for Gilbert syndrome is associated with increased level and duration of neonatal jaundice. *Acta Paediatr* **91**: 100–101. http://dx.doi.org/10.1111/j.1651-2227.2002.tb01650.x

Scrafford CG, Mullany LC, Katz J, Khatry SK, LeClerq SC, Darmstadt GL, Tielsch JM (2013) Incidence of and risk factors for neonatal jaundice among newborns in southern Nepal. *Trop Med Int Health* **18**: 1317–1328. http://dx.doi.org/10.111/tmj12189

Sugatani J, Yamakawa K, Yoshinari K, Machida T, Takagi H, Mori M, Kakizaki S, Sueyoshi T, Negishi M, Miwi M (2002) Identification of a defect in the UGT1A1 gene promoter and its association with hyperbilirubinemia. *Biochem Biophys Res Commun* **292**: 492–497. http://dx.doi.org/10.1006/bbrc.2002.6683

Tioseco JA, Aly H, Milner J, Patel K, El-Mohandes AA (2005) Does gender affect neonatal hyperbilirubinemia in low-birth-weight infants? *Pediatr Crit Care Med* **6**: 171–174. http://dx.doi.org/10.1097/01.PCC.0000154961.37833.79

Tiwari PK, Bhutada A, Agarwal R, Basu S, Raman R, Kumar A (2014) UGT1A1 gene variants and clinical risk factors modulate hyperbilirubinemia risk in newborns. *J Perinatol* **34**: 120–124. http://dx.doi.org/10.1038/jp.2013.140

Watchko JF, Lin Z (2010) Exploring the genetic architecture of neonatal hyperbilirubinemia. *Semin Fetal Neonatal Med* **15**: 169–175. http://dx.doi.org/10.1016/j.siny.2009.11.003

Yang WC, Zhao LL, Li YC, Chen CH, Chang YJ, Fu YC , Wu HP (2013). Bodyweight loss in predicting neonatal hyperbilirubinemia 72 hours after birth in term newborn infants. *BMC Pediatr* **13**: 145. http://dx.doi.org/10.1186/1471-2431-13-145