

UGT1A1 gene polymorphisms and jaundice in Indonesian neonates

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Abstract

Background Uridine diphospho-glucuronocyltransferase 1A1 (*UGT1A1*) polymorphisms are a risk factor for unconjugated hyperbilirubinemia in neonates. *UGT1A1* polymorphisms decrease bilirubin conjugation, thus causing hyperbilirubinemia. A variety of polymorphisms have been reported, with *UGT1A1**60 and *UGT1A1**6 especially prominent in the Asian population. Hyperbilirubinemia polymorphism studies are lacking in Indonesian populations.

Objective To identify *UGT1A1**60 and *UGT1A1**6 profiles in Indonesian populations of heterogeneous ethnicity.

Methods We enrolled 42 jaundiced neonates who were born from January to April 2017 and treated in the Neonatal Intensive Care Unit of our national referral center, Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Genetic mutations *60 of exon 1 and *6 of the promoter region were analyzed by polymerase chain reaction – restriction fragment length polymorphism methods, with *DraI* and *AvaII* as restriction enzymes, respectively. Clinical data including total serum bilirubin and racial information were obtained by medical records and interviews with parents.

Results There were no homozygous mutations of *UGT1A1**6, but 4.8% of subjects were heterozygous. As for *UGT1A1**60, 4.8% were heterozygous and 95.2% were homozygous. Racial variations were not observed for *UGT1A1**60, while Betawi descendents were found to have many heterozygous forms of *UGT1A1**6.

Conclusion Polymorphisms of the *UGT1A1* gene were found in Indonesian neonates. Some ethnicities also showed increased tendency towards its incidence, such as the heterozygous form of *UGT1A1**6. [Paediatr Indones. 2019;59:150-6; doi: <http://dx.doi.org/10.14238/pi59.3.2019.150-6>].

Keywords: neonatal jaundice; PCR; polymorphism; RFLP; *UGT1A1*

A physiological increase of bilirubin occurs in 60% of term and 80% of preterm neonates,¹ due to increased heme breakdown and physiological hepatic immaturity.² Hyperbilirubinemia can be observed on the skin and sclera as neonatal jaundice when the serum total bilirubin concentration reaches 5 mg/dL. Hyperbilirubinemia can lead to kernicterus spectrum disorder (KSD) when left untreated, especially in high risk babies. Kernicterus spectrum disorder is a neurologically debilitating condition that may lead to death.^{3,4} Common risk factors for KSD are preterm birth, hemolytic disorders, and other delivery and postnatal conditions such as cephalohematoma, sepsis, and hypoalbuminemia.^{4,5} Genetic factors have been studied extensively in past years. Caucasian and East Asian populations have been shown to have the *UGT1A1**28 polymorphism as a clinically significant risk factor for hyperbilirubinemia.^{6,7}

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Bilirubin conjugation is catalyzed by the uridine diphospho (UDP)- glycosyltransferase (UGT) enzyme, which is encoded by the uridine diphospho-glucuronosyltransferase 1A1 (*UGT1A1*) gene.⁸ Polymorphisms of *UGT1A1* polymorphisms decrease enzyme activity and lead to decreased bilirubin elimination, causing unconjugated hyperbilirubinemia conditions, such as Gilbert's syndrome.^{9,10} One of the genetic variations involved in Gilbert's syndrome is the *UGT1A1**60 (c-3279T>G) polymorphism in the promoter region.¹¹ This polymorphism has been demonstrated to contribute to the incidence of neonatal hyperbilirubinaemia in Malaysian and Taiwanese populations.^{11,12} Another common polymorphism in East Asia is *UGT1A1**6 (c211G>A).¹³ This polymorphism was found to be involved with development of hyperbilirubinemia in the Japanese population. However, in a study on Javanese-Indonesian and Malay-Malaysian populations, Sutomo *et al.* reported that *UGT1A1**6 was found, albeit in small frequencies and with no significant clinical correlation to the high incidence of hyperbilirubinemia.¹⁴

Information on variations of *UGT1A1* and the development of hyperbilirubinemia in Indonesia is still very limited. Racial variations are common in the diverse Indonesian population, but to date, only Javanese and Bengkulu populations have been studied.^{1,15} As such, we aimed to obtain evidence and data on the *UGT1A1**6 and *UGT1A1**60 in the Indonesian population. The national referral hospital, Cipto Mangunkusumo Hospital, was chosen with the assumption that patients make-up may be roughly representative of the racial diversity of the Indonesian population.

Methods

This descriptive, cross-sectional study was conducted in neonatal patients with jaundice in the Neonatal Intensive Care Unit (NICU), Division of Perinatology, Department of Child Health, Cipto Mangunkusumo Hospital, Jakarta. Term and preterm neonates born from January to April 2017, as well as whose parents were of Indonesian descent and agreed to participate in the study, were included. Clinical jaundice was assessed by a neonatologist based on Kramer's index between day 3-7 post-natal. Neonates with hemolytic disease, showing clinical signs of sepsis, asphyxiation, and neonates who received blood transfusion or exchange transfusion, were excluded from the study. We used a total sampling method, where the number of samples taken was equal to the number of populations found during the duration of the study. Hence, of 42 jaundiced neonatal patients during the study period, all 42 were included in the study.

Clinical data including birth weight and total serum bilirubin levels were obtained from patients' medical records. Peak bBilirubin level was measured between the 3rd and 7th day of life. Ethnicity was obtained by ethnicity tracing from the parents with the aim of ascertaining their various ethnic or racial variations. Informed consent was obtained from both parents of the neonates. This study was approved by the Health Research Ethics Committee, University of Indonesia and the national referral hospital, Dr. Cipto Mangunkusumo Hospital, Jakarta.

Venous blood samples (1.5 mL) from neonates with jaundice in the NICU were collected into EDTA tubes and stored at -20°C. Genetic confirmation was done using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) analysis. Genomic DNA (g-DNA) was extracted either from fresh or frozen blood specimens using *QIAamp DNA Blood Mini Kit* (Qiagen, Germany).¹⁵ Two microlitre

Table 1. Oligonucleotide primers used in this study

No	Oligonucleotide	Sequence	Target SNP	Variants
1	<i>UGT1A1</i> _promoter Fw	5'-CAC-CAGAACAACTTCTGAG-3'	rs4124874? (c-3279T>G)	<i>UGT1A1</i> *60
2	<i>UGT1A1</i> _promoter Rv	5'-CTGTCCCTTCTG AAT-CATTG-3'	rs4124874? (c-3279T>G)	<i>UGT1A1</i> *60
3	<i>UGT1A1</i> _exon1 Fw /U1F1 forward	5'-AGATACTGT TGATCCCAGTG-3'	rs4148323 (c211G>A)	<i>UGT1A1</i> *6
4	<i>UGT1A1</i> _exon1 Rv /U211R reverse	5'-CTTCAAGGTGTAAAATGCTC-3'	rs4148323 (c211G>A)	<i>UGT1A1</i> *6

SNP: single nucleotide polymorphism

of g-DNA was added to a PCR master mix containing 29.3µL dH₂O, 5µL 10X KOD Hot Start DNA polymerase buffer (Novagen, Germany), 4µL 25 mM MgSO₄, 7µL 2 mM dNTPs (dATP, dCTP, dGTP, dTTP), and 0.7µL KOD Hot Start DNA polymerase (Novagen, Germany). Oligonucleotide primers used for this amplification are listed in Table 1.

The PCR amplification consisted of an initial denaturation for 2 min at 95°C, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, post-extension at 72°C for 10 min, and held at 8°C for 60 min, in a PCR thermocycler TProfessional Standard 96 Gradient (Biometra GmbH, Germany). Amplicons were observed by 2% agarose gel electrophoresis in TAE 1X buffer containing ethidium bromide and visualized by UV transilluminator.

To determine the polymorphism at rs4148323 of UGT1A1*6 by PCR-RFLP analysis, the restriction enzyme *Ava*II (New England Biolabs, USA) was used to digest the PCR amplicon of the exon 1 region of UGT1A1 gene. Amplification was carried out by employing primer pairs for UGT1A1 exon 1, as reported previously.⁵

To determine the polymorphism at rs4124874 of UGT1A1*60 by PCR-RFLP analysis, the restriction enzyme *Dra*I (New England Biolabs, USA) was used to digest the PCR amplicon of the promoter region of the UGT1A1 gene. Amplification was carried out by employing primer pairs for the UGT1A1 promoter, as reported previously.¹¹

We used STATA version 12 software for MacOS was used for statistical analysis. Only descriptive analysis was used for this study. Distribution of UGT1A1 variants and racial profiles of the UGT1A1 genotype were expressed with numbers and percentages.

Results

The PCR rs4124874 amplicon of UGT1A1*60 was 141 bp, as shown by agarose gel electrophoresis in Figure 1A. The *Dra*I restriction enzyme digestion resulted in two visible bands (Figure 1B). The homozygote type remained uncut, while the heterozygote resulted in three bands, i.e., 141 bp, 120 bp, and 21 bp, as reported by Huang et al.¹¹ However, using a 2% agarose gel, the small fragment of the amplicon cut by *Dra*I could not be visualized.

The PCR rs4148323 amplicon of UGT1A1*6 was 146 bp, as shown in Figure 2A. The *Ava*II restriction enzyme digestion resulted in two bands (Figure 2B). The homozygote remained uncut, while

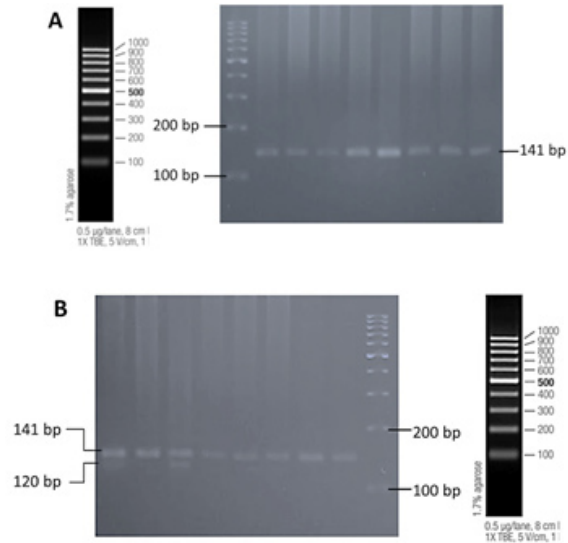


Figure 1. Agarose gel electrophoresis of (A) the PCR amplicon of UGT1A1 rs4124874 (141 bp); and (B) the amplicon after being cut with *Dra*I restriction enzyme (120 bp fragment, in addition to the 141 bp fragment; the 21 bp fragment could not be observed).

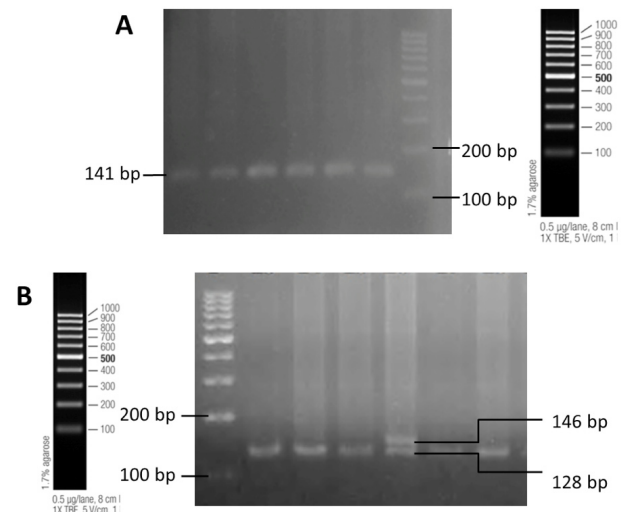


Figure 2. Agarose gel electrophoresis of (A) the PCR amplicon of UGT1A1 rs 4148323 (146 bp); and (B) the amplicon after being cut with *Ava*II restriction enzyme (128 bp fragment, in addition to the 146 bp fragment; the 18 bp fragment could not be observed).

the heterozygote resulted in three bands, i.e., 146 bp, 128 bp, and 18 bp, as reported by Huang *et al.*⁵ However, using a 2% agarose gel, the small fragment of the amplicon cut by *Dra*I could not be visualized.

Table 2 shows the distribution of *UGT1A1* variants of both the exon 1 *UGT1A1**6 mutation and the *UGT1A1**60 promoter mutation. Most of the exon 1 mutation (*UGT1A1**6) did not occur, but all subjects had either homozygous or heterozygous promoter region mutation (*UGT1A1**60). In our study, no subjects had the homozygous *UGT1A1**6 A/A genotype, only 4.8% of subjects had the heterozygous G/A genotype, and 95.2% of subjects had the G/G genotype (wild type) or did not have any SNP form of *UGT1A1**6. With regards to *UGT1A1**60, 95.2% had the homozygous G/G genotype, and 4.8%

had the heterozygous T/G genotype. No subjects had the wild type genotype (TT).

Table 3 shows that the *UGT1A1**6 heterozygous subjects (G/A genotype) came from parents of Betawi-Betawi and Betawi-Sundanese ethnicities. Whereas for *UGT1A1**60, the T/T homozygous mutation, occurred in neonates of many ethnicities. In contrast with the *UGT1A1**6 variants, there was no specific ethnic tendency for the *UGT1A1**60 mutations based on our limited study.

Discussion

Based on the detection of *UGT1A1* mutations in this study, as shown in **Table 2**, the *UGT1A1**6 mutations may not affect the incidence of hyperbilirubinaemia in Indonesian neonates. Similarly, Sutomo *et al.* showed that *UGT1A1**6 was rarely found in Javanese and Malaysian populations.¹⁴ In our previous study of a Bengkulu population, interestingly we found over 60% of the neonates had SNP of *UGT1A1**6 in both healthy and jaundiced neonates.¹⁵ This finding shows that even within Indonesian population, there exists an intra-ethnicity variation related with *UGT1A1*. The meta-analysis of *UGT1A1**6 studies in Southeast Asian populations including Malaysia and Thailand by Yu *et al.* showed the same results of 0% A/A genotype

Table 2. Distribution of *UGT1A1* variants

Genotype	(N=42)
<i>UGT1A1</i> *6, n (%)	
G/G (wild type)	40 (95.2)
G/A (heterozygote)	2 (4.8)
A/A (homozygote)	0 (0)
<i>UGT1A1</i> *60, n (%)	
T/T (wild type)	0 (0)
T/G (heterozygote)	2 (4.8)
G/G (homozygote)	40 (95.2)

Table 3. Genotypes of *UGT1A1* polymorphisms by ethnicity in Indonesia

Ethnicity	<i>UGT1A1</i> *6 (n=42)			<i>UGT1A1</i> *60 (n=42)		
	A/A homozygous	G/A heterozygous	G/G wild type	T/T homozygous	T/G heterozygous	G/G wild type
Batak-Batak, n (%)	0(0)	0(0)	1 (2.4)	1 (2.4)	0(0)	0(0)
Betawi-Betawi, n (%)	0(0)	1 (2.4)	6 (14.2)	8 (19)	0(0)	0(0)
Betawi-Sundanese, n (%)	0(0)	1 (2.4)	5 (11.9)	5 (11.9)	1 (2.4)	0(0)
Javanese-Javanese, n (%)	0(0)	0(0)	10 (23.8)	8 (19)	1 (2.4)	0(0)
Javanese-Lampung, n (%)	0(0)	0(0)	1 (2.4)	1 (2.4)	0(0)	0(0)
Javanese-Minangkabau, n (%)	0(0)	0(0)	1 (2.4)	1 (2.4)	0(0)	0(0)
Javanese-Sundanese, n (%)	0(0)	0(0)	3 (7.1)	3 (7.1)	0(0)	0(0)
Minangkabau - Minangkabau, n (%)	0(0)	0(0)	3 (7.1)	3(7.1)	0(0)	0(0)
Sundanese -Sundanese, n (%)	0(0)	0(0)	7 (16.7)	7(16.7)	0(0)	0(0)
Indonesian Chinese – Indonesian Chinese, n (%)	0(0)	0(0)	1 (2.4)	1 (2.4)	0(0)	0(0)
Bima-Bima, n (%)	0(0)	0(0)	1 (2.4)	1(2.4)	0(0)	0(0)
Javanese – Maluku, n (%)	0(0)	0(0)	1 (2.4)	1(2.4)	0(0)	0(0)

frequency, which differed from other Asian populations such as India, Japan, and China.¹⁶ In certain conditions with co-existing risk factors, UGT1A1*6 could still increase the risk for hyperbilirubinaemia. In one Japanese study, UGT1A1*6 was shown to be a cause of prolonged unconjugated hyperbilirubinaemia.¹⁷ In another Japanese study, UGT1A1*6 was a risk factor for inadequate breastfeeding instead of breastmilk jaundice.¹⁸ Therefore, despite the low incidence of UGT1A1*6 in the Indonesian population, further study into multiple co-existing risk factors may prove UGT1A1*6 to be a risk factor in Indonesia.

Due to the high incidence of UGT1A1*60 in our study, this polymorphism could be a risk factor for the occurrence of unconjugated hyperbilirubinemia in Indonesian newborns. Yusoff *et al.* and Amandito *et al.* had similar results in Malaysian neonatal populations.^{12,15} The Malaysian population is ethnically closer to the Indonesian population and located in Southeast Asia. Sutomo *et al.* found a similarity in UGT1A1*60 mutation profiles in Javanese-Indonesian and Malay-Malaysian populations, which may be related to the anthropological proximity of Java-Indonesia and Malay-Malaysian populations.¹⁴ In the population of neonates with hyperbilirubinemia in Malaysia, UGT1A1*60 mutations play a role in causing hyperbilirubinemia.¹² However, Amandito *et al.* reported no significant correlation between the incidence of UGT1A1*60 and total serum bilirubin level. This finding indicates that despite its high incidence, UGT1A1*60 is not a clinically significant risk factor for hyperbilirubinemia.¹⁵ In future studies, genetic mutations or other genes related to bilirubin conjugation or excretion should be included. It is also possible however, that clinical risk factors are more profound in the Indonesian population compared to genetic factors.

The incidence of mutations are related to ethnicity and race. Kanai *et al.* reported that the UGT1A1*60 mutation was not significantly associated with neonatal hyperbilirubinemia in Japan.¹⁹ Although the UGT1A1*6 mutation did not contribute to the incidence of hyperbilirubinemia in the Javanese-Indonesian population, the UGT1A1*6 frequency is high in the Japanese population. This may be due to the genetic differences between populations and other possible factors that may be significantly involved in the development of hyperbilirubinemia.¹⁴

We can divide the Indonesian population into three major sub-racial groups: protomalay, deuteromalay, and melanesoid.²⁰ Despite the fact that both East Asian and Southeastern Asian are of Mongol racial descent, their sub-ethnicity differs from those of Malays, which could explain why the genetic mutation patterns differ.

In our study, there may be a tendency of the Betawi ethnicity to dominate the heterozygous mutation results, suggesting that ethnicity might influence UGT1A1*6 incidences in our limited population (Table 3). This finding was in agreement with that of Zhang *et al.*, who noted an influence of intraethnic differences in certain ethnic groups on UGT1A1 genetic variation.²¹ The study was conducted on three Chinese sub-ethnics of Dong, Han, and She, which showed significant differences in genotypic frequencies between sub-ethnicities. The Han sub-ethnicity group carried the highest G/A genotype of UGT1A1*6 frequency compared to sub-ethnicities of Dong and She.²¹ In our study, Betawi ethnicity had the highest G/A genotype frequency compared to other ethnicities.

However, no ethnicity had a UGT1A1*60 or is a wild type, as shown in Table 3. Therefore, the UGT1A1*60 mutation in neonatal patients with unconjugated hyperbilirubinemia in Indonesia may not be influenced by a particular ethnicity or by racial diversity. In other racial groups, sub-ethnicity may affect the occurrence of a particular polymorphism, as noted by Zhang *et al.*²¹ The frequency of UGT1A1*60 was relatively higher in the Dong and Han ethnic groups than in the She group.²¹

Polymorphism UGT1A1*60 may be a risk factor for neonatal hyperbilirubinemia in Indonesia, whereas UGT1A1*6 may not affect the incidence of hyperbilirubinemia in neonates in Indonesia. The occurrence of mutations or gene polymorphisms may be related to ethnicity.

One limitation in our study is that we only included samples from one center and in limited number, therefore our sample is only a rough representation of the whole population in Indonesia. In addition, we did not include healthy neonates in our study as a control group, which limits the conclusion that we can take from our study. Discrepancies between ethnicities could also confound both the polymorphism and the bilirubin level. The method of

SNP analyses was also done through RFLP, whereas sequencing would have been superior in accuracy. We suggest conducting further study using a more accurate methods and a larger sample size with a more diverse population.

There is a high incidence of *UGT1A1**60 in Indonesian neonatal patients in Cipto Mangunkusumo Hospital, whereas the *UGT1A1**6 mutational incidence was very low. Despite all patients being of Indonesian descent, intra-ethnic differences in certain ethnicity groups may influence the genetic variation of *UGT1A1**6, in which the Betawi ethnicity showed a small tendency of contributing more heterozygous SNP subjects. On the other hand, the *UGT1A1**60 mutation in neonates with unconjugated hyperbilirubinemia at Cipto Mangunkusumo Hospital is not affected by any particular ethnicity or racial diversity in Indonesia, as it occurred in all subjects. Further study is suggested to explore and confirm the genetic makeup of *UGT1A1* and other related genes which could potentially contribute to a better understanding and treatment of neonatal jaundice of Indonesian neonates.

Conflict of Interest

None declared.

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