

Prevalence of clinically relevant (TA)*n* UGT1A1 promoter alleles in Indian neonates

UDP-glucuronosyltransferase 1A1 (UGT1A1) is the key enzyme for bilirubin conjugation. It is encoded by the *UGT1A1* gene, which consists of five exons and is a part of the UGT1A locus on chromosome 2q37. Several mutations in the *UGT1A1* gene related to Gilbert's Syndrome (GS) and Crigler Najjar syndrome have been reported and majority of them are point missense mutations, especially in exon 1 and occur in different populations and regions of the world¹. Some of them are highly relevant as they exert a significant effect on enzyme function². Of these, a tandem repeat in the 5'-promoter region of the *UGT1A1* gene [-53 (TA)*n*] has been identified as a main cause of GS³. Usually, six copies of this tandem repeat are present and are associated with normal enzyme activity, while more than six copies are associated with reduced enzyme activity. The frequency distribution of an allele with seven copies [(TA)7] which leads to the GS phenotype is highly variable in different ethnic groups, with an estimated frequency of 0.160 in Asians, 0.387 in Europeans and 0.426 in Africans⁴.

The frequency of the (TA)7 allele was found to be higher among Indians (33.6%)⁵ compared to other Asian populations (10.0–18.8%)⁶. Therefore, it is likely that an increased frequency of the (TA)7 allele would also be found in indigenous Indian newborns and if inherited along with G6PD deficiency, could lead to severe hyperbilirubinemia in these neonates. Although the (TA)7 allele was found to be significantly associated with hyperbilirubinemia in North Indian neonates who were having jaundice⁷, the frequency of this allele has not been determined in indigenous Indian newborns. Hence, the present study was undertaken to determine the frequency of alleles and genotypes of the (TA)*n* repeat promoter polymorphism of the UGT1A1 gene in healthy newborns.

A total of 925 consecutively delivered healthy neonates ≥ 35 weeks gestation with a mean birth weight of 2890 ± 250 g were studied. They had no known risk factors that would affect neonatal hyperbilirubinemia like maternal diabetes, blood group incompatibilities, hemolytic

anaemia, G6PD deficiency, infection, dehydration, hypothyroidism or liver disease. These conditions were evaluated by means of past and family history and clinical and laboratory examinations. These babies were followed up for five days. The study was cleared by the institutional ethics committee. Blood samples were collected from the umbilical cord after obtaining informed consent from the parents. RBC indices were determined using an automated blood cell counter (Sysmex K-1000 Kobe, Japan). Liver function tests were done on an automated biochemistry analyser (Cobas 111, Roche, Germany). Screening for G6PD deficiency was done by the DPIP dye decolorization method.

High molecular weight genomic DNA was extracted from the peripheral blood leukocytes using a commercially available DNA extraction kit (Qiagen GmbH; Hilden, Germany). The 5'-flanking region containing (TA)*n* repeats of the *UGT1A1* gene was amplified by PCR with a FAM-labelled sense primer and an unlabelled antisense primer. The sizes of the PCR products along with 500 LIZ size standards were analysed using a laser-based gene scan application on an ABI PRISM 310 Genetic Analyser (Applied Biosystem). The resultant data were analysed using the Genotyper software. Each repeat number was calculated from the observed PCR product size according to the 5'-flanking region sequence of the *UGT1A1* gene. For confirmation, subsets of samples were randomly selected and subjected to automated DNA sequencing.

The distribution of the number of repeats among our newborns along with previously reported data among ethnic Asians, Nigerian and Sephardic Jewish neonates is summarized in Table 1. Eight of the ten possible UGT1A1 genotypes were observed. The frequencies of (TA)6/(TA)6, (TA)6/(TA)7 and (TA)7/(TA)7 genotypes were found to be 38.90%, 43.02% and 14.90% in our newborns, which is similar to the earlier observations among Indian adults⁵. One hundred and forty-four neonates (15.5%) were found to have the TA promoter genotype (TA)7/(TA)7 or larger [either (TA)7/(TA)8 or (TA)8/(TA)8], which is

almost similar to that seen in ethnic Asian populations⁶ and Jewish neonates, but less than that observed in the combined Nigerian and African ancestry individuals⁸. The frequency of the mutant (TA)7/7 genotype varies widely among different populations, with the highest frequencies reported in populations from Africa, India, Sri Lanka and Bangladesh, and lowest frequencies in populations of Southeast Asia like Hong Kong, Thailand, Indonesia and Vietnam, Melanesia, and the Pacific Islands⁶.

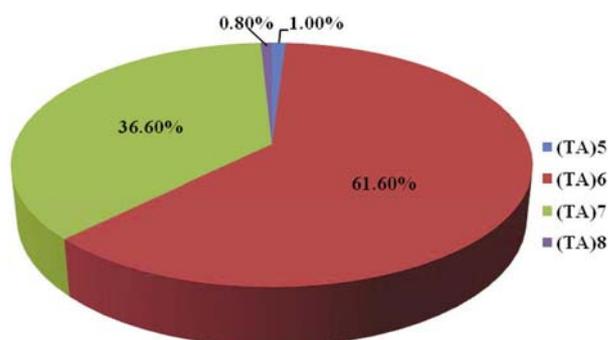
Of the possible 1850 alleles, 19 were (TA)5, 1140 (TA)6, 676 (TA)7 and 15 (TA)8, with a frequency of 0.010, 0.616, 0.366 and 0.008 respectively (Figure 1). An almost equal distribution of (TA)6 and (TA)7 alleles has been reported in the indigenous Nigerian population⁸; however, in the present study the (TA)6 allele was found to be more prevalent than the (TA)7 allele and this finding is similar to earlier reports among healthy Indian adults⁵, ethnic Asian populations⁶ and Sephardic Jews⁸. Neonates with the (TA)5 and (TA)8 alleles were also encountered in a few cases, either in heterozygous or homozygous condition with a frequency of 0.010 and 0.008 respectively, which is lower than that reported from Nigerian infants⁸.

Neonatal hyperbilirubinemia is common in the Asian population, and G6PD deficiency is the main risk factor for the development of severe neonatal hyperbilirubinemia leading to kernicterus⁹. The incidence and severity of neonatal hyperbilirubinemia is significantly higher in Asians, more so in Indians, than in Caucasians⁷. It has been reported that one-third of children with G6PD deficiency develop neonatal jaundice, despite the absence of any biochemical trigger or hematological evidence of hemolysis¹⁰. These findings suggest that genetic factors may be involved in the development of neonatal hyperbilirubinemia.

The present study revealed that a higher frequency of (TA)7/(TA)7 genotype is prevalent in Indian neonates with an allele frequency of 0.366. Previous studies have shown that superimposition of the UGT1A1 promoter variant in G6PD-deficient neonates resulted in a significant increase in the incidence of

Table 1. Distribution of the genotypes for the (TA)*n* promoter polymorphism among the Indian neonates and previously reported ethnic Asian groups (6), indigenous Nigerian and Sephardic Jewish neonates (8)

Genotype	Indian neonates (n = 925)	Ethnic Asian groups (n = 778)	Indigenous Nigerians (n = 88)	Sephardic Jews (n = 262)
(TA)5/(TA)5	1 (0.1%)	–	1 (1.10%)	–
(TA)5/(TA)6	17 (1.83%)	–	5 (5.70%)	–
(TA)5/(TA)7	–	–	5 (5.70%)	–
(TA)6/(TA)8	5 (0.54%)	–	1 (1.00%)	–
(TA)6/(TA)6	360 (38.90%)	388 (49.9%)	19 (21.60%)	116 (44.30%)
(TA)6/(TA)7	398 (43.20%)	292 (37.5%)	38 (43.20%)	110 (42.00%)
(TA)7/(TA)7	138 (14.90%)	98 (12.6%)	16 (18.20%)	36 (13.70%)
(TA)7/(TA)8	2 (0.2%)	–	3 (3.40%)	–
(TA)8/(TA)8	4 (0.43%)	–	–	–

**Figure 1.** Distribution of the alleles for the (TA)*n* promoter polymorphism among the Indian neonates.

hyperbilirubinemia in an allele dose-dependent response¹¹. We have also observed that of the 138 babies with the (TA)7/(TA)7 genotype, 28 (20.1%) developed jaundice (total serum bilirubin levels ≥ 15 mg/dl) as against 10.0% of the babies with the (TA)6/(TA)6 wild genotype. In India, G6PD deficiency has been found to be one of the commonest causes for neonatal hyperbilirubinemia¹². Therefore, a higher frequency of (TA)7 allele along with G6PD deficiency might further increase this inherent imbalance between bilirubin production and conjugation, which will ultimately increase the risk of developing neonatal jaundice in the Indian subcontinent and present as GS in the adults.

GS is a hereditary, non-pathological hyperbilirubinemia condition with varying population frequency. Though this condition is innocuous, the patients of GS keep on moving from one physician to the next due to clinical jaundice. In the absence of a firm diagnostic test, these patients are subjected to various kinds of investigation rarely leading to invasive liver biopsy. However, demonstration of

(TA)7/(TA)7 or more repeats clearly shows that the patient is likely to be a case of GS in the absence of any other liver dysfunction. This simple test can now be done at various centres in the country.

A further implication of the present study is the involvement of more TA repeats in adverse drug reactions of many anticancer drugs (e.g. Taxanes, Irinotecan). Since the toxicity of these drugs can substantially increase in slow metabolisers [(TA)7 or more], prior detection of this polymorphism will help the clinicians in safe and effective drug dosing.

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ACKNOWLEDGEMENT. This study was supported by a grant from the Department of Biotechnology, New Delhi.

Received 24 May 2013; revised accepted 19 July 2013

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