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RESEARCH NOTE

Prevalence of *UGT1A1* $(TA)_n$ promoter polymorphism in Panamanians neonates with G6PD deficiency

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Abstract. A relationship between the polymorphism in promoter region of the *UGT1A1* gene and the development of jaundice has been demonstrated recently. This polymorphism leads to 30% of normal rate transcription initiation of *UGT1A1* gene, thus decreasing the bilirubin glucuronidation. The combination of the G6PD deficiency and polymorphism in neonates and adults may cause pronounced hyperbilirubinaemias. The aim of this study was to analyse the variations in the *UGT1A1* gene promoter in Panamanians neonates with G6PD deficiency and its association with neonatal jaundice (NJ). We identified five different genotypes of TA repeats, in 17 neonates (42.5%) the normal variant TA_6/TA_6 and in the other 57.5% of the subjects: TA_7/TA_7 (12.5%), TA_6/TA_7 (40%), TA_6/TA_8 (2.5%) and TA_6/TA_5 (2.5%). Additionally 75% of the 16 newborns that showed NJ had an abnormal variant in the promoter sequence, although, there was no significant difference ($P = 0.068$). The risk of jaundice in neonates with TA_7 variant was thrice higher in subjects than with other alleles ($P = 0.093$, CI: 0.81–11.67). The TA_7 allele frequency in this study (0.325) was consistent with the global frequency and similar to Caucasians. The results proved that there is no significant relationship between promoter polymorphism in *UGT1A1* and NJ in G6PD deficient Panamanian newborns. Further studies with a greater number of subjects would determine the exact relationship between marked NJ and UGT1A promoter variations.

Keywords. *UGT1A1* gene; polymorphism; G6PD deficiency; neonatal jaundice; Panamanian.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme deficiency in the world affecting about more than 400 million people (Ong *et al.* 2017). This disease is usually asymptomatic, often manifested as acute hemolytic anemia, resulting in hyperbilirubinaemia. Severe cases in the neonatal period may become jaundiced in brain and lead to cerebral palsy with mental retardation (kernicterus) (Fu *et al.* 2018). Severe hyperbilirubinaemia is known to be one of the risk factors associated with G6PD deficiency, such as *UGT1A1* polymorphisms with limited conjugation capacity (Carvalho *et al.* 2011).

The uridine diphosphate-glucuronosyltransferase (UGT) family contains several isoforms but only the A1 isoform (UGT1A1) participates in the conjugation of bilirubin. *UGT1A1* promoter and coding sequence gene variants

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decrease hepatic bilirubin conjugation, hence mutations in the 1A1 exon or its promoter will affect bilirubin glucuronidation and results in Gilbert syndrome (GS) (Zahedpasha *et al.* 2014; Marku *et al.* 2015). A dinucleotide polymorphism in the 5′-promoter region of the *UGT1A1* gene is caused by variability in the number of TA repeats (five to eight in length) in the TATA-box, which normally consists of six repeats (O'Dwyer and Catalano 2006). Functional studies have revealed that increasing the number of repeats in the promoter region leads to a decreased rate of transcription initiation of the *UGT1A1* gene. The wild-type allele (UGT1A1*1) contains six TA repeats, whereas the most common variant allele (UGT1A1*28) contains an extra TA, the addition interferes with binding of the transcription factor IID. This interference leads to 30% of normal rate transcription of UGT1A, hence, homozygous individuals who carry the A $(TA)_7$ TAA allele show significantly higher plasma levels of unconjugated bilirubin and GS (Fertrin *et al.* 2003; Chaouch *et al.* 2012; Marku *et al.* 2015; Liu *et al.* 2017). Interethnic differences have been observed in the frequency of the UGT1A1*28 allele, e.g., the proportion of TA_7/TA_7 genotypes is ∼10% in African population, but less than half of that in Asians (O'Dwyer and Catalano 2006).

Despite the allelic variants of the UGT1A1 promoter cannot cause severe indirect hyperbilirubinaemia by itself, it may have a summative effect on rising bilirubin when coexist with G6PD deficiency (Muslu *et al.* 2006; Saki *et al.* 2011), producing pronounced hyperbilirubinaemias in neonates and adults with these characteristics (Capellini and Fiorelli 2008). The aim of this study was to analyse variations in the promoter area in the *UGT1A1* gene in Panamanians neonates with G6PD deficiency and its association with neonatal jaundice.

Material and methods

The blood samples were obtained from 40 G6PD deficient neonates who were diagnosed by neonatal screening and confirmed with spectrophotometry (WHO 1967) in the Hospital del Niño of Panama. They fulfilled the inclusion criteria of this study and the relatives granted approval for the present study by signing a written consent that was reviewed and approved by an Ethical and Research Committee.

Genomic DNA was isolated from peripheral blood leucocytes using the QIAamp DNA Blood mini kit of Qiagen. The genotyping was performed by fragment analyses using the primers UGT forward labelled with FAM (5′-GAGGGACG ATGGAAACACCTGAC-3′) and UGT reverse (5′-TCCC GCTTGGAGACCGTC-3′). The reaction mix was performed at a final volume of 20 μL, containing buffer 1x, 2.0 mM $MgCl_2$, 0.2 μM dNTP's, 0.2 pM of each primer, 0.5 U of *Taq* polymerase (GoTaq Promega) and 200 ng of DNA. The PCR cycling protocol consisted of an initial step of denaturation at 94°C for 5 min followed by 30 cycles of 60 s at 94°C, 60 s at 55°C and 90 s at 72°C, followed by a final extension of 5 min at 72°C. All PCR assays were performed in the T100 thermal cycler of BioRad. PCR products (PCR-P) were analysed by electrophoresis in 1.5% agarose gel and then stained with GelRed. 1:50 dilutions of PCR-P were performed prior to fragment analysis. The injection mix (1 μL PCR-P; 0.75 μL dH_2O; 0.25 μL size standard GS500 ROX; 12 μL HiDi formamide) for fragment analyses were heated at 93°C for 2 min and immediately placed on ice. The entire 14 μL of each sample was applied to genetic analyser ABI Prism 310 of Applied Biosystem with the following conditions, injection: 5 s, 15 kV; running: 60°C, 15 kV and 30 min. Results were analysed by GeneMapper. The PCR product was expected to be 125-bp long, for alleles with six TA repeats.

To confirm the genotype, we analysed the PCR-P by direct sequencing through an automated capillary electrophoresis DNA sequencer (ABI Prism 310 Applied Biosystem) and the Big Dye Terminator kit on a final volume of 10 μL (Applied Biosystem). For this technique, we analysed the sequence from 5′–3′ using the primer UGT forward (5′-GAGGGACGATGGAAACACCTGAC-3′) at 3.2 pM. Results were analysed by Chromas software. SPSS v. 20.0 software (SPSS, Chicago, USA) with chi-squared test and odds ratio analysis was used to evaluate qualitative data (e.g., the frequency of genotypes and alleles), $P<0.05$ was considered a significant difference.

Results

The 75% (n = 30) of the study population were boys, ratio of male to female is 7:1. The 40% of the newborns had NJ, where 87.5% (n = 14) were boys, while the remaining 12.5% were females (ratio 7:1), nonetheless there was no significant relationship between the sex of neonates and jaundice (P = 0.136). The 10% who were hospitalized due to jaundice were all males.

The fragment analysis method showed a clear distinction between the peaks expected in each form of the promoter gene (figure 1) and the sequence of TA_6/TA_6 and TA_6/TA_7 genotype was confirmed by direct sequencing. Five different genotypes of TA repeats in the TATAA box region of the *UGT1A1* promoter were identified, the allelic and genotypic frequency is shown in table 1; no significant differences were observed between sexes in terms of genotype or allelic frequency (table 1). The 57.5% of the studied newborns presented a variation in the promoter of the *UGT1A1* gene, where the 12.5% were homozygous for the allele TA_7.

According to A $(TA)_n$ TAA variation, the comparison between gender was not significantly associated with an increased risk of jaundice (P = 0.211).

In the evaluation of the relationship between the mutated genotype and the presence of neonatal jaundice (NJ), it was observed that 75% of the 16 newborns that showed NJ had a mutation in at least one of their alleles, these included two

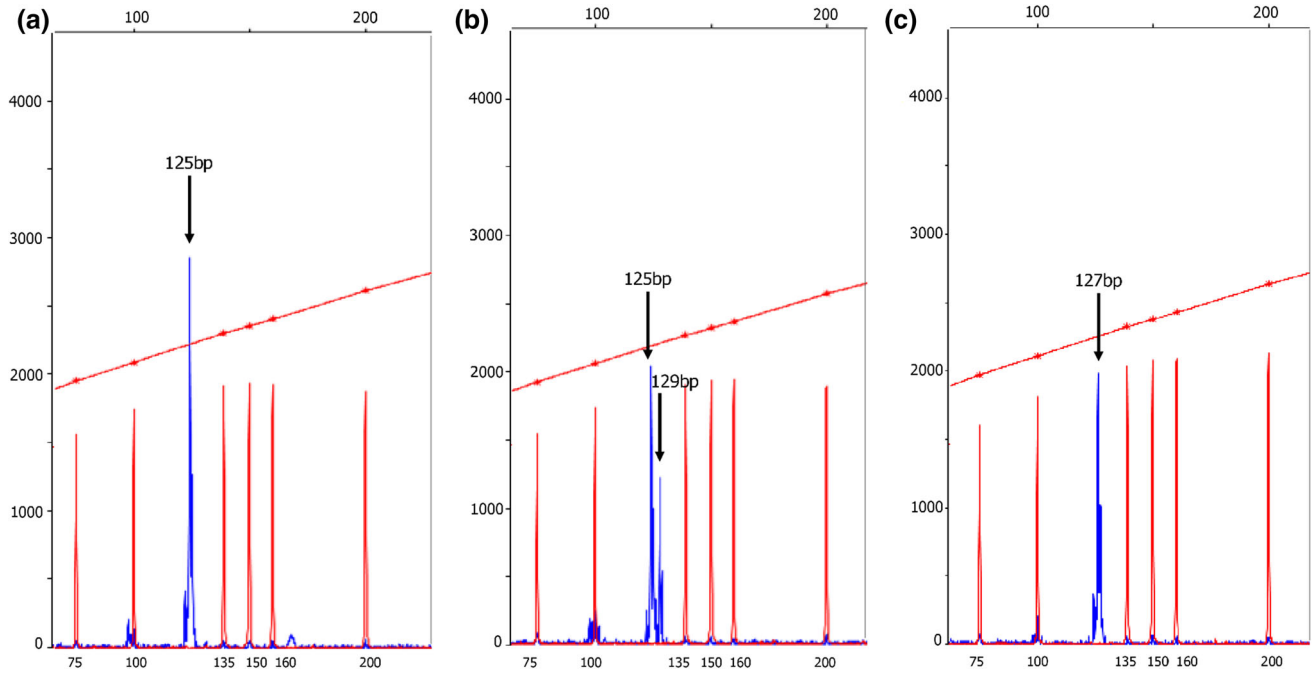

Figure 1. Result of fragment analysis for detection of TA insertion in the TATA box region of the *UGT1A1* gene. The blue line relates to fluorescence of 6-FAM; the two peaks marked with solid arrows indicate: (a) 125-bp product for TA_6/TA_6 genotype alleles (wild type); (b) 125-bp and 129-bp product for TA_6/TA_8 genotype alleles, respectively; (c) 127-bp product for TA_7/TA_7 genotype alleles (GS). The red lines relate to fluorescent dye bound to the molecular weight markers, length of which are marked at the bottom.

Table 1. Genotypic and allelic distribution of the *UGT1A1* gene in the study population.

			Sex			Jaundice			Hospitalization		
$N = 40$		Frequency	M	F	*P* value	Yes	No	*P* value	Yes	No	*P* value
Genotype	6/6	0.425	11	6	0.469	4	13	0.211	1	16	0.037
	6/7	0.40	14	2		9	7		1	15	
	7/7	0.125	3	2		2	3		1	4	
	6/8	0.025	1	0		0	1		0	1	
	5/6	0.025	1	0		1	0		1	0	
Alleles	6	0.65	63.3%	70%	0.845	56.3%	70.8%	0.275	50%	66.7%	0.023
	5	0.325	1.7%	0%		3.1%	0%		12.5%	0%	
	7	0.013	33.3%	30%		40.6%	27.1%		37.5%	31.9%	
	8	0.013	1.7%	0%		0%	2.1%		0%	1.4%	

heterozygous combinations: 56.3% TA_6/TA_7, 6.3% TA_5/TA_6 and one mutated homozygous 12.5% TA_7/TA_7. These results contrast with those obtained in the group that did not develop jaundice, in which only 45.9% had some type of variation (12.5% TA_7/TA_7, 29.2% TA_6/TA_7 and 4.2%TA_6/TA_8), this difference showed a tendency towards significance ($P = 0.068$). The risk of NJ with TA_7 variant was thrice higher than subjects with other alleles; although, there was no significant difference ($P = 0.093$, CI: 0.81–11.67). Overall, 25% of the neonates needed hospitalization due to jaundice ($P = 0.010$) distributed uniformly among the genotypes.

Discussion

This study provides unique information about the incidence of NJ, and the frequency of *UGT1A1* promoter polymorphism in Panamanian newborns. G6PD deficiency is one of 10 most important aetiologies of nonhemolytic NJ (Carvalho *et al.* 2011), in our study the incidence of NJ was 40%, which is in accordance with the literature and guidelines where the prevalence is up to 50% of term newborns and 80% of preterm newborns (Brits *et al.* 2018). Also there is clear disparity between genders proportion (7:1) in our population both in G6PD deficiencies and jaundice, although

there is no concrete evidence to support the male vulnerability to jaundice, some theories has been proposed that are based on the higher metabolic rate of male foetuses given that an inverse relationship between lifespan and metabolic rate may reflect significant differences in associated physiological mechanisms such the elimination of serum bilirubin (Tioseco *et al*. 2005).

The relationship between the bilirubin level and the genetic abnormalities that cause GS has aroused growing interest. *UGT1A1* rare and common variants were associated with NJ in different populations. In our study, we identified five different genotypes, the TA_7 allele is the most frequent (32.5%) after the wild type, and this variation in homozygous form is the most common genetic change associated with the unconjugated hyperbilirubinaemia phenotype around the world. The insertion of TA sequence in the TATAA box of the promoter region reduce the expression of *UGT1A1* gene leading to reduced bilirubin glucuronidation activity (Gupta *et al*. 2015; Wisnumurti 2018). Based on the genotype, it is observed that 60% of the subjects with genotype TA_7/TA_7 did not presently have jaundice, Gupta *et al*. 2015 showed that several people with this variant did not have elevated bilirubin levels, demonstrating that the mutation in the promoter region may not be the cause for the appearance of unconjugated hyperbilirubinaemia phenotype. Some individuals with unconjugated hyperbilirubinaemia either entirely lacked the TA_7 allele or have only one. In such individuals, other variations have been identified varying widely between populations. In Gupta *et al*. (2015) also, we detected a subject with rare TA_6/TA_8 genotype. The presence of the TA_8 allele has been previously identified in Africa and is associated even with lower bilirubin glucuronidation activity (Gupta *et al*. 2015; Wisnumurti 2018).

The TA_7 allele frequency for this study was 0.325, consistent with global frequency with regard to populations; Caucasians have been reported between 0.31 and 0.63, and 0.427 in Africans (Muslu *et al*. 2006; NCBI 2018). Our results are similar to Caucasians rather than Africans.

Our study showed that A $(TA)_n$ TAA alleles was not distributed differently in jaundice and nonjaundice neonates with G6PD deficiency. The TA_7/TA_7 genotype is associated with GS, although not all have clinical manifestations due to other factors like male gender may play a role in phenotype (Zahedpasha *et al*. 2014). Some other authors also reported this lack of relationship (Muslu *et al*. 2006; Saki *et al*. 2011).

In the present study, 30% of neonates with jaundice presented a promoter polymorphisms in the *UGT1A1* gene (heterozygous or homozygous), suggesting that there is no significant relationship between variations in *UGT1A1* and NJ in G6PD deficient. Due to the intrinsic limitations, since other physical, biochemical or genetic factors can also contribute to the development of NJ, additional studies are needed to clarify this relationship.

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