

Original Article

UDP Glucuronosyltransferase 1 (UGT1A1) Gene Promoter Polymorphism among Kuwaitis with Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

Elena Samilchuk¹, Ibrahim Al-Suleiman¹, Esien Usanga², Sadika Al-Awad¹

¹Kuwait Medical Genetics Center, Ministry of Health, Kuwait

²Faculty of Allied Health, Kuwait University, Kuwait

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ABSTRACT

Objectives: The polymorphic (TA)₇ allele of the UGT1A1 gene results in decreased bilirubin conjugation. As a genetic co-factor, it can trigger neonatal jaundice in G6PD deficient individuals. In Kuwait, the incidence of G6PD deficiency is quite high. There are no data, however, on the frequency of the (TA)₇ allele and it is not known how frequently this polymorphism occurs with G6PD deficiency. To answer this question, the UGT1A1 gene promoter polymorphism was studied among G6PD deficient Kuwaitis.

Methods: The UGT1A1 gene promoter polymorphism

was analyzed using polymerase chain reaction followed by polyacrylamide gel electrophoresis.

Results: Among 55 unrelated Kuwaiti blood donors with G6PD deficiency caused by the Mediterranean mutation, nine individuals were found to be homozygous and 26 heterozygous for the (TA)₇. The frequencies of the (TA)₆ and (TA)₇ alleles were 0.6 and 0.4, respectively.

Conclusion: High frequency of the (TA)₇ allele and Mediterranean mutation among Kuwaitis may produce neonatal jaundice in up to 1% of the male and 0.6% of the female newborns.

KEYWORDS: Glucose-6-phosphate dehydrogenase deficiency, Kuwait, UDPglucuronosyltransferase 1

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a result of the mutation in the G6PD gene located on the X chromosome. More than 100 G6PD deficient mutations have been identified^[1]. The most common clinical manifestations of G6PD deficiency are acute hemolytic anemia and neonatal jaundice^[2]. The clinical phenotype depends on the type of the G6PD mutation but G6PD deficiency itself is not sufficient to produce symptoms unless in combination with other factors. Hemolytic anemia is usually provoked by drug administration or infection, while additional genetic co-factors seem to contribute to the development of neonatal jaundice in G6PD deficient individuals. One such co-factor is polymorphism of bilirubin conjugating enzyme, UDP glucurono-syltransferase 1 (UGT1A1), coded by the UGT1A1 gene on chromosome 2. This polymorphism is a result of variation in the number of the TA repeats in the A(TA)_nTAA motif of the UGT1A1 promoter. In addition to the wild-type (TA)₆ allele, the variants with a deletion (TA)₅ and an insertion (TA)₇ and (TA)₈ have been identified. An inverse relationship between the number of TA repeats and the activity of the UGT1A1 promoter were found by using a

reporter gene. The TA insertion leads to reduced expression of UGT1A1 resulting in decreased bilirubin conjugation^[3, 4]. The role of UGT1A1 polymorphism as a triggering co-factor has arisen when it was found that the incidence of hyperbilirubinemia in G6PD-deficient newborns is significantly higher in individuals carrying the (TA)₇ allele^[5]. Moreover, even heterozygotes for G6PD deficient mutation seem to be at increased risk for neonatal hyperbilirubinemia if they carry the (TA)₇ allele^[6]. In both the above-mentioned reports, the G6PD-deficiency in newborns was caused by Mediterranean mutation (563 C→T substitution). This is one of the most common G6PD mutations and can result in both hemolytic anemia and neonatal jaundice. In our previous study, we had shown that Mediterranean mutation is also quite common among Kuwaitis, producing G6PD deficiency in 4.9% males and 2.56% females^[7]. However, there are no data on the frequency of UGT1A1 polymorphism in Kuwaitis (as well as other Arabs) and, therefore, it is not known how frequently this polymorphism occurs with G6PD deficiency. To answer this question, we studied the UGT1A1 polymorphism among G6PD-deficient Kuwaitis with Mediterranean mutation.

Address correspondence to:

Elena Samilchuk, MD, Ph.D., Kuwait Medical Genetics Center, PO Box: 31121, Sulaibikhat-80901, Kuwait. Tel/fax: (965) 573 5201

E-mail: samilchuk@hotmail.com

MATERIALS AND METHODS

DNA samples from 55 unrelated Kuwaiti blood donors with G6PD deficiency due to the Mediterranean mutation have been analyzed for the UGT1A1 gene promoter polymorphism using PCR followed by polyacrylamide gel electrophoresis (PAGE). DNA was extracted from peripheral blood lymphocytes using the 'salting-out' technique^[8]. The PCR primers (Bili x 5'-ATTAAGTTGGTGTGCGATTGG-3' and Bili z 5'-AGCCATGGCGCCTTTGCTC-3') have been previously reported^[5]. The PCR mixture contained 10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM MgCl₂, 0.001% (w/v) gelatine, 50 μM of each dNTP, 0.4 μM of the primers, 0.75 units of AmpliTaq Gold polymerase (Perkin Elmer) and 200-1000 ng of genomic DNA in a total volume of 25 μl. Thermocycling was performed in the Perkin Elmer 9600 system with initial denaturation at 94 °C followed by 35 cycles at 94 °C; 53 °C, 72 °C (45s each) with a final extension at 72 °C for 7 min. The PCR product was analyzed using electrophoresis in 10% polyacrylamide gel. The gels were stained with ethidium bromide.

Heteroduplex formation was carried out to confirm genotype of homozygous samples where PAGE pattern was ambiguous. PCR product of tested sample was mixed with an equivalent amount of PCR product from control homozygote. The DNA mix was denaturated at 94 °C for 5 min, reannealed at 65 °C for 5 min and examined by 10% PAGE.

RESULTS AND DISCUSSION

Among Kuwaiti samples analyzed only the alleles (TA)6 and (TA)7 were observed (Fig. 1). The (TA)5 and (TA)8 variants, which were previously identified in individuals of African ancestry, were missing in our sample. This is probably due to the low frequencies of these alleles in Kuwait despite the fact that gene flow from Africa has definitely occurred as judged by the presence of A⁻ and A⁺ variants of G6PD in Kuwait. The genotyping by PAGE electrophoresis was very reliable in heterozygotes. In addition to 90 bp and 92 bp fragments produced by (TA)6 and (TA)7 alleles, respectively, there was an extra heteroduplex band. However, because of the small separation of wild-type and mutant fragments, a discrimination between a wild-type homozygote and a homozygote for the TA insertion was problematic in certain cases. Such samples were re-examined using heteroduplex analysis. If the sequence was different from homozygous control, the double band and heteroduplex were observed (Fig. 1). The heteroduplex band, however, was missing if the sequences were identical. Such approach,

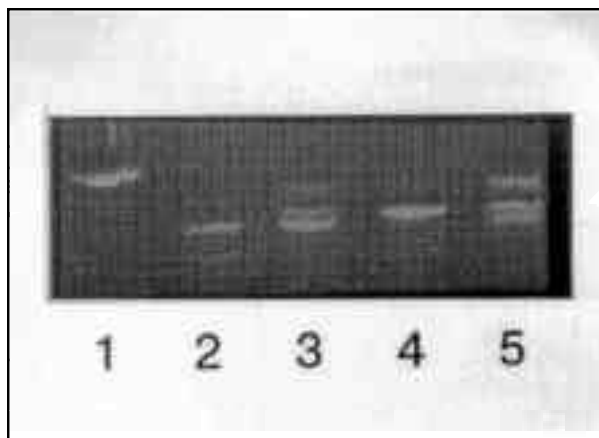


Fig. 1: Detection of UGT1A1 polymorphism by PAGE
1) 100 bp band of DNA ladder, 2) a homozygote (TA)6/(TA)6, 3- a heterozygote (TA)6/(TA)7, 4) a homozygote (TA)7/(TA)7, 5) heteroduplex analysis: a mix of a tested sample with the (TA)6 homozygous control; the observed pattern confirms the (TA)7/(TA)7 genotype of tested sample.

previously used for detection of common deltaF508 mutation in cystic fibrosis^[9] and +TATC1278 in Tay-Sachs disease^[10], seems to be very useful for the analysis of the fragments of the small size difference due to the deletion and insertion of several nucleotides.

The data on the UGT1A1 genotypes in 55 G6PD-deficient Kuwaitis are given in Table 1. Nine individuals were found to be homozygous and 26 heterozygous for the (TA)7. Though our samples were not random but were selected on the basis of G6PD deficiency, the distribution of UGT1A1 genotypes was in agreement with Hardy-Weinberg equilibrium. The frequency of the (TA)6 and (TA)7 alleles among G6PD-deficient Kuwaitis was 0.6 and 0.4, respectively. Taking into consideration this data, as well as the frequency of G6PD deficiency due to the Mediterranean mutation, one can estimate that 1% of male and 0.6% of female newborns in Kuwait can develop jaundice due to genetic interaction of the G6PD and UGT1A1. This approximate figure is based on the assumption that the frequency of hyperbilirubinemia produced by identical genotypes

Table 1

UGT1A1 genotype and allele frequencies among G6PD-deficient Kuwaitis

Genotype	Observed	Expected
(TA)6/(TA)6	20 (36.36%)	19.8
(TA)6/(TA)7	26 (47.27%)	26.4
(TA)7/(TA)7	9 (16.36%)	8.8
Allele	Allele	frequency
(TA)6	0.6	(66/110)
(TA)7	0.4	(44/110)

is similar for different ethnic groups, i.e. among G6PD deficient newborns with the Mediterranean mutation, it is 50% in (TA)7/(TA)7 homozygotes and 32% in (TA)6/(TA)7 heterozygotes^[5].

The interaction between the UGT1A1 and G6PD genes has been shown to influence bilirubin levels not only for Mediterranean mutation, but also for another deficient variant, G6PD Union^[11]. The question whether or not similar interaction would be valid for other G6PD mutations needs to be answered. It is particularly interesting in the case of such a common variant as A-, which is known to produce neonatal jaundice, though rarely^[2]. UGT1A1 polymorphism was also reported as a major determinant of bilirubin level in homozygotes and heterozygotes for beta-thalassaemia^[12,13] and, in this case, the genetic interaction of the abnormal beta-globin gene and UGT1A1 takes place. The question is whether the UGT1A1 polymorphism itself can produce any clinical phenotype. Indeed the (TA)7 allele was reported to be associated with accelerating development of neonatal jaundice^[14]. The most confirmed association, however, is with Gilbert syndrome (a benign condition of decreased bilirubin conjugation due to diminished activity of UGT1A1). Indeed, 92-100% of the chromosomes from patients with Gilbert syndrome carry the (TA)7 allele^[3,15]. In addition, the segregation of the (TA)7 homozygous genotype with the Gilbert phenotype was demonstrated in a family with four affected members^[16].

At the same time, the number of the (TA)7/(TA)7 homozygotes in the population is higher than the incidence of Gilbert syndrome. The (TA)7 homozygosity alone seems, therefore, insufficient for manifestation of this condition^[3].

The frequency of the (TA)7 allele varies in different populations from 0.07 in Japan to 0.4 in Europeans^[3, 4, 17]. However, in Japanese as well as Koreans and Chinese, the other mutation in UGT1A1 gene (Gly71Arg) is more common and contributes to the high incidence of neonatal hyperbilirubinemia in these populations^[17, 18]. The frequency of the (TA)7 allele in Kuwait is similar to that reported in Europeans and, therefore, a similar incidence of Gilbert syndrome in Kuwait can be expected (3-10% of general population). Taking into consideration the high incidence of both G6PD deficiency and thalassaemia in Kuwait,^[19, 20] the UGT1A1 polymorphism screening among the Kuwaiti newborns with neonatal jaundice seems to be warranted.

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