

Frequency of the UGT1A1*28 Polymorphism in a Romanian Cohort of Gilbert Syndrome Individuals

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ABSTRACT

Background & Aims: Gilbert syndrome (GS) is characterized by unconjugated hyperbilirubinemia without liver disease or overt hemolysis and it is found in 3-10% of the general population. Inherited hyperbilirubinaemia is attributable to a reduced UGT1A1 activity. The UGT1A1 promoter (TA) repeats variants are documented of being involved in abnormally elevated bilirubin levels. The aim of the present study is to analyze the impact of UGT1A1 promoter variants on bilirubin levels in Romanian patients clinically suspected with GS.

Methods: The study group included 897 subjects: 292 GS patients and 605 healthy controls. Genomic DNA was extracted from the peripheral blood leukocytes. All individuals were screened for the presence of the (TA) insertion in the TATA box region of UGT1A1 gene by PCR amplification. This case-control study was conducted at the Department of Medical Genetics, Synevo, Romania.

Results: UGT1A1*28 (7TA) revealed the highest frequency (61.87%) of all individuals, while the UGT1A1*1 (6TA) allele was found in 36.79%. We identified two other variants of the *UGT1A1* gene, depending on the number of TA repeats in the promoter: 5TA (0.61%) and 8TA (0.72%). The (TA)7/7 homozygous genotype was identified in 32.33% of all individuals, while the (TA)6/7 heterozygous genotype was the most prevalent (57.64%). The wild type (TA)6/6 was identified in 7.36% of the whole cohort.

Conclusions: Because other polymorphisms have been associated with GS, the absence of the UGT1A1*28 allele does not rule out this condition. The results suggest that in the Romanian population there is a strong correlation between the UGT1A1*28 polymorphism and hyperbilirubinemia in patients with GS.

Key words: Gilbert syndrome – UGT1A1 gene – variant – hyperbilirubinemia – promoter – (TA) repeats

Abbreviations: DNA: deoxyribonucleic acid; EDTA: ethylene diamine tetraacetic acid; GS: Gilbert syndrome; OMIM: Online Mendelian Inheritance in Men; PCR: polymerase chain reaction; TA: thymine-adenine; UDP: uridine diphosphate; UGT: uridine diphosphate glucuronosyltransferase; UGT1A1: UGT 1A isoform 1.

INTRODUCTION

Gilbert syndrome (GS) (OMIM#143500) is characterized by mild, chronic, unconjugated hyperbilirubinemia in the absence of liver disease or overt hemolysis and is found in approximately 3-10% of the general population.

The diagnosis of this disorder is made by the detection of elevated unconjugated bilirubin levels and normal liver function. Pathogenesis may involve complex defects in the liver uptake of bilirubin. The inherited

hyperbilirubinaemia, characterized by intermittent episodes of jaundice (the most widely recognized signs) is attributable to a reduced UDP-glucuronosyltransferase 1A isoform 1 (UGT1A1) activity [1, 2].

The UGT1A1 is a phase II drug-metabolizing enzyme responsible for converting a wide array of drugs to water-soluble glucuronides, suitable for renal or biliary elimination. It is also the main isozyme able to conjugate bilirubin, the endogenous pigment resulting from the catabolism of the natural haeme [1, 3-5].

The uridine diphosphate glucuronosyltransferase (UDP-glucuronosyltransferase, or UGT) enzymes are a superfamily of enzymes that metabolize a wide range of molecules such as bilirubin, steroids, toxins, and drugs, including irinotecan. The disorder affects approximately 3-7% of individuals in the general population, and affects individuals of all races. Gilbert syndrome is diagnosed more often in males than in females. It

is present at birth, but may remain undiagnosed until the late teens or early twenties. Gilbert syndrome was first described in the medical literature in 1901 [2, 3, 6, 7].

The UGT genes are mapped to chromosome 2q37, are often polymorphic, and genomic processes, such as the copy-number variations, variant splicing, and epigenetic factors are likely to contribute to their diversity. Promoters containing seven thymine adenine (TA) repeats have been found to be less active than the wild-type six repeats, and the serum bilirubin levels of persons homozygous or even heterozygous for seven repeats have been found to be higher than those with the wild-type six repeats [3, 8-10].

The UGT1A locus contains multiple alternative first coding exons, each of which has its own promoter site, enabling the transcription of nine unique UGT1A enzymes [5]. Every affected person has two copies of the abnormal UGT1A1 gene. Because over half of the people in the general population have at least one abnormal copy of the gene, inheriting two abnormal copies is quite common. People who have only one copy may have slightly higher levels of unconjugated bilirubin but not GS [9-12].

Not all the people who have two copies of the abnormal gene develop elevated serum bilirubin levels high enough for the diagnosis of GS [7], which means that there are other factors involved in this process, too. For example, red blood cells may break down too easily, releasing excess amounts of bilirubin that the impaired enzyme cannot keep up with. Alternatively, movement of bilirubin into the liver, where it would be glucuronidated, may be impaired. These other factors may be due to changes in other genes [9, 13, 14].

The aim of the present study was the analysis of the impact of the promoter of UGT1A1 variants on bilirubin levels in GS patients and in healthy controls, in order to evaluate to what degree the UGT1A1*28 polymorphism is involved in the lower gene expression in Romanian patients clinically suspected of GS.

MATERIAL AND METHODS

The study group consisted of 897 individuals, divided into two groups: group A (GS group) – 292 Caucasian GS patients (214 males and 78 females) with a clinical diagnosis of GS based on standard criteria [8], and group B (non-GS group) – 605 Caucasian healthy controls (506 males and 99 females). Data from the GS patients were obtained from medical records. The controls were selected based on normal liver and/or hematological values, no history of inflammatory or malignant diseases, and no regular medication.

After obtaining the informed consent of each patient, venous blood samples were collected using EDTA as the anticoagulant, in order to obtain the plasma and buffy coat for DNA extraction. The ethical approval of the study was given by the Ethics Committee of the Carol Davila University of Medicine and Pharmacy, Bucharest.

The UGT1A1 gene is located on the long arm of the chromosome 2, cytoband 2q37.1. Genomic DNA was extracted from peripheral blood leukocytes by using standard methods. The DNA extraction was performed with DNeasy Blood & Tissue Kit (Qiagen), followed by PCR and amplicon analysis. All patients and controls were screened for the presence of the (TA) insertion in the TATA box region of UGT1A1 gene by PCR amplification.

Data analysis was performed using the Microsoft Office Excel 2016 software.

RESULTS

Demographic characteristics of the patients

The average age was 28.43 (range 1-73) years. Most of the individuals belonged to the 10-40 year age groups (70% in total: 24% for both 10-20 and 30-40 age groups, and 22% for the 20-30 year age group). The average age was similar: 28.43 (1-73) years for group A and 28.02 (0-73) for group B, respectively. The distribution by age groups is listed in Table I.

In group A, most of the individuals belonged to the 10-40 year old age group (70%): 24% for both 10-20 and 30-40 age groups and 22% for the 20-30 year group. In group B, 58% of the individuals were aged 10-30 at the time of testing: 30% in the 20-30 age group and 23% were aged 30-40 years old.

Regarding the gender distribution, 214 patients (73% of all cases in the group A) and 506 (84% of the controls, group B) were males.

Table I. Age distribution of the study subjects

Age groups (years)	Group A (Cases)	Group B (Controls)
0-10	4	66
10-20	82	143
20-30	86	132
30-40	68	143
40-50	31	66
50-60	14	22
>60	7	33

Clinical manifestations

The signs and symptoms were extremely vague, and included the following: recurrent asymptomatic jaundice, nausea, asthenia, and vague abdominal distension. Eight per cent of the patients were completely asymptomatic (they had first degree relatives previously diagnosed with GS or had high bilirubin levels identified by routine tests) (Table II).

Table II. Clinical manifestations in the patients with Gilbert syndrome (group A)

Phenotype	Affected subjects no. (%)
Jaundice	216 (74)
Nausea	193 (66)
Asthenia	190 (65)
Vague abdominal distension	152 (52)
Asymptomatic	23 (8)

Genotyping results

UGT1A1 allele frequencies

The analysis revealed four variants of the UGT1A1 gene, depending on the number of (TA) repeats in the promoter TATA box: 5TA, 6TA, 7TA and 8TA (Table III). The normal wild-type allele is 6TA, the remainder being variants.

Table III. Allele frequencies evaluated in all subjects

UGT1A1 allele	Group A (292 cases, 584 alleles) no. (%)	Group B (605 controls, 1210 alleles) no. (%)	TOTAL (897 subjects, 1794 alleles) no. (%)
5TA	0 (0)	11 (0.91)	11 (0.61)
6TA	0 (0)	660 (54.55)	660 (36.79)
7TA	582 (99.66)	528 (43.63)	1110 (61.87)
8TA	2 (0.34)	11 (0.91)	13 (0.72)

The polymorphism with the highest frequency in the studied population has been UGT1A1 7TA (UGT1A1*28); it was found in 61.87% of all individuals belonging to both groups (Table III). The frequency of the UGT1A1 6TA wild type allele was 36.79% for the whole cohort (54.55% in 605 controls).

UGT1A1 genotype frequencies

Table IV shows the presence of UGT1A1 genotypes revealed by the investigation of the (TA)_n polymorphism in the UGT1A1 gene promoter. The 7/7 homozygous genotype of the UGT1A1 gene (UGT1A1*28) was identified in 32.33% of all individuals.

The 6/7 heterozygous genotype was the most prevalent in our cohort (57.64%), having been observed in 85.45% of the individuals belonging to group B (Table IV). It is known that the 7/7 UGT1A1 genotype is pathognomonic for the Gilbert syndrome, while the 6/7 genotype is considered a common, non-pathogenic variant.

The genotypic and allelic comparison between patients with respect to the presence or absence of jaundice showed a statistically significant association between genotype 7TA/7TA and jaundice ($\chi^2=589.48$, $p=0.00001$).

Table IV. UGT1A1 genotype frequency in our subjects

UGT1A1 Genotype	Group A (292 cases) no. (%)	Group B (605 controls) no. (%)	TOTAL (897 subjects) no. (%)
5TA/7TA	0 (0)	11 (1.82)	11 (1.23)
6TA/6TA	0 (0)	66 (10.91)	66 (7.36)
6TA/7TA	0 (0)	517 (85.45)	517 (57.64)
6TA/8TA	0 (0)	11 (1.82)	11 (1.23)
7TA/7TA	290 (99.31)	0 (0)	290 (32.22)
7TA/8TA	2 (0.69)	0 (0)	2 (0.22)

DISCUSSION

Gilbert syndrome is usually diagnosed at puberty (clinically detected jaundice due to hyperbilirubinemia, consecutive to the steroid inhibition). In our study, 28% of the patients in group A (affected subjects) had ages ranging between 10 and 20 years at testing, while another 30% were below 30 years.

The male gender was previously reported as having a higher prevalence of GS (hyperbilirubinemia) than women (a 2-7:1 ratio). In the group A (GS group), we found a 2.74:1 men to women ratio, consistent with other published studies. This may be explained by the known facts that in men the bilirubin production is higher than in women and at the same time, the bilirubin glucuronidation is inhibited by androgens.

The UGT1A1*28 variant. Allele frequencies

UGT1A1*1 (6TA) is the wild-type and most common allele across populations (61% in Caucasians) and is associated with a normal enzyme activity. In our study, the overall 6TA allelic frequency (in both groups) was 36.79%, with a 54.55% distribution in controls. These frequencies are much lower than those reported in literature for the Caucasian population. The reason for these differences is the more frequent presence of the 6TA/7TA genotype (85.45%) compared with the normal 6TA/6TA one in the control group. The 6TA allele was not identified in the GS group (0%), confirming its reported protective effect on bilirubin levels.

Currently, over 113 genetic variants of UGT1A1 have been reported at varying frequencies across ethnicities [1, 13]. The frequency of the UGT1A1*28 (7TA) allele varies among ethnicities, being highest in those of African (43%) or European (39%) descent and lowest in those of Asian (16%) descent.

The presence of an additional (TA) repeat in the TATA region of the UGT1A1 promoter region, which controls the production of the bilirubin-UGT enzyme (i.e., 7TA repeats; UGT1A1*28) results in enzyme underexpression, leading to reduced glucuronidation [10, 14, 15]. In many populations, the UGT1A1*28 variant is the most common genetic change that causes GS.

The frequency of the 7TA allele in our cohort was 61.87% in all individuals, with a peak of 99.66% prevalence in the GS group, much higher than that previously reported in Caucasians (due to the high 7TA frequency in controls). The absence of the 7TA allele in the control group (0%) indicates its pathogenic effect on the bilirubin levels (increased).

Variants with 5 or 8 repeats (UGT1A1 *36 / 5TA and *37 / 8TA) occur with much lower frequency in Caucasians (close to 0%), being primarily identified in individuals of African descent. The presence of 8TA repeats has been associated with GS and with decreased glucuronidation *in vitro*.

In concordance with the literature data, the frequency of the 5TA and 8TA alleles in our study group was below 1% (0.61%, 0.72%, respectively) in all individuals. The 5TA variant showed a 0.91% prevalence in controls and was absent in GS patients, being correlated with normal bilirubin levels. The 8TA allele was identified in both groups (0.34% in cases and 0.91% in controls).

The UGT1A1 genotype frequencies

The 6TA/6TA (UGT1A1 *1/*1) and 6TA/7TA (UGT1A1 *1/*28) genotypes are the most common ones in Caucasians, with frequencies of 34% and 55%, respectively. These genotypes are usually non-pathogenic, being associated with normal bilirubin levels [7, 9].

In our study group, the (TA)₆/6 genotype revealed significantly lower frequency (7.36% in all individuals and 10.91% in controls), while the (TA)₆/7 one showed a prevalence comparable to the one reported in literature (57.64% in all 897 individuals and 85.45% in controls).

Almost 10% of the US population is homozygous for UGT1A1*28. The prevalence of (TA)₇ homozygosity in the European populations is 10-15% [4, 14, 15]. The UGT1A1*28 homozygous genotype - (TA)₇/7 - has been associated with increased levels of bilirubin and a high frequency of gallstones

and gallbladder disorders. Individuals with the 7TA/7TA genotype have been reported to undergo cholecystectomy for pigment gallstones much more frequently than those with the 6TA/6TA or 6TA/7TA genotypes [12]. In our cohort, the prevalence of the UGT1A1 *28/*28 genotype was much higher than reported in the literature (32.33%).

The (TA)6/8 genotype (UGT1A1*1/*37) is known to be a less common constellation in the general population and without any proven phenotypical changes. The (TA)5/7 (UGT1A1 *36/*28) and (TA)7/8 (UGT1A1 *28/*37) are very rare genotypes, and only the 7TA/8TA variant is pathogenic [14]. The frequencies for these genotypes in our patients are also low and concordant with these data (Table IV).

CONCLUSIONS

Because other polymorphisms have been associated with Gilbert's syndrome, absence of the UGT1A1*28 allele does not rule out this condition. The results of this study suggest that in the Romanian population there is a strong correlation between the UGT1A1*28 polymorphism and hyperbilirubinemia in patients clinically suspected of having Gilbert syndrome. Further studies should be undertaken to confirm this association. In addition, identification of further mutations in the UGT1A1 gene could contribute to the diagnosis.

Conflicts of interest: None.

Authors' contributions: V.R., R.B. and U.R. designed the study, collected the data and wrote the first draft. V.R., R.U. and C.A. collected the data and created the database. V.R., R.U. and E.P. performed the statistical analysis. C.B., R.B., V.R. and R.U. provided guidance in the design, collection of data and critical revision. All authors approved the final draft of the manuscript.

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