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UGT1A1*28 relationship with abnormal total bilirubin levels in chronic hepatitis C patients

Outcomes from a case–control study

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Abstract

Gilbert syndrome (GS) is a frequent benign clinical condition, marked by intermittent unconjugated hyperbilirubinemia, mostly due to the polymorphism uridine diphosphate-glucuronosyltransferase 1A1*28 (UGT1A1*28). Hyperbilirubinemia has been reported in a GS patient undergoing hepatitis C treatment, and other UGT isoforms polymorphisms have been linked to worse outcomes in viral hepatitis. Yet, little is known to GS contributions' to the liver disease scenario. Our aim was to assess UGT1A1 genotypes' frequency in chronic hepatitis C (CHC) patients and correlate with total bilirubin (TB). This is a case–control study in a large tertiary medical center. Cases were CHC patients confirmed by hepatitis C virus (HCV)–polymerase chain reaction. Exclusion criteria were hepatitis B virus or human immunodeficiency virus (HIV) coinfection. Control were healthy blood donors. UGT1A1 promoter region gene genotyping was performed, and bilirubin serum levels were available for HCV patients. Genotypes and alleles frequencies were similar in case (n=585; $P=0.101$) and control groups (n=313; $P=0.795$). Total bilirubin increase was noticed according to thymine–adenine repeats in genotypes ($P<0.001$), and the TB greater than 1 mg/dL group had more UGT1A1*28 subjects than in the group with TB values <1 mg/dL (18.3 vs 5.3; $P<0.001$). Bilirubin levels are linked to the studied polymorphisms, and this is the first time that these findings are reported in a chronic liver disease sample. Among patients with increased TB levels, the frequency of UGT1A1*28 is higher than those with normal TB. Personalized care should be considered to GS, regarding either abnormal bilirubin levels or drug metabolism.

Abbreviations: CHC = chronic hepatitis C, GS = Gilbert syndrome, HCFMUSP = Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, PCR = polymerase chain reaction, TA = thymine–adenine, TB = total bilirubin, UGT1A1 = uridine diphosphate-glucuronosyltransferase 1A1.

Keywords: chronic, Gilbert syndrome, hepatitis C, hyperbilirubinemia

1. Introduction

Gilbert syndrome (GS) is a frequent clinical condition, marked by intermittent unconjugated hyperbilirubinemia and jaundice.[1] Its frequency varies among different populations, and it mostly relates to a 20% to 30% decrease of the uridine diphosphate-glucuronosyltransferase 1A1 (UGT1A1) enzyme activity due to the insertion of thymine–adenine (TA) groups in the enzyme's promoter region. Wild TATA box have 6 TA groups, while GS alleles have 7 or 8 TA copies. TA 7/7 polymorphism is called UGT1A1*28.[2]

GS is widely considered to be a benign condition as it does not lead to hepatocyte death and chronic hepatic disease.[3] Although, as UGT1A1 is not only a major enzyme in the bilirubin homeostasis, but an important metabolizer for other endogenous substrates and xenobiotics, enzyme function-decreased individuals are at risk for other conditions, such as severe drug effects and cancer. Most notorious, UGT1A1*28 patients treated with irinotecan present an increased risk for drug toxicity, such as diarrhea and neutropenia.[4–7] SN-38, irinotecan's active metabolite, is eliminated via UGT isoforms, among them, UGT1A1 pathway.[8] Also, higher risk for breast[9] and colorectal[10] cancers has been reported in GS, most probably as a result of carcinogens detoxification decrease[11,12] and estrogen-related disease.[13]

In the pharmacogenomics era, 1 key point is how new discoveries could change the clinical practice. Serum bilirubin

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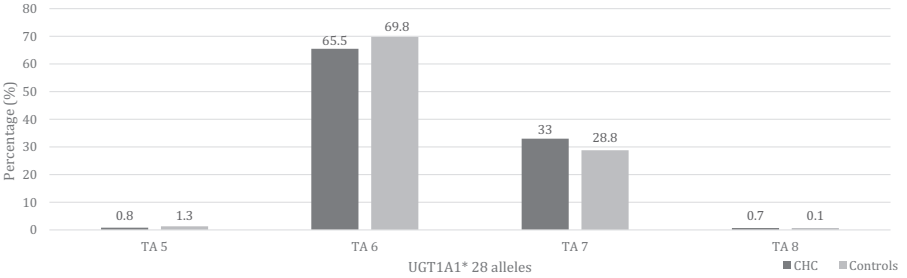

Figure 1. UDP-glucuronosyltransferase 1A1*28 promoter region frequency distribution among chronic hepatitis C patients and controls (blood donors). Underneath the picture: $P = 0.795$ (Chi-square test). UDP = uridine diphosphate.

level screening is recommended on patients starting irinotecan-based chemotherapy, yet no initial genetic testing is recommended.[14] Concerning the point of care in chronic liver disease and viral hepatitis in the GS scenario, Deterding et al reported 2 patients presenting jaundice and isolated unconjugated hyperbilirubinemia during pegylated interferon and ribavirin treatment for hepatitis C virus (HCV). As no ongoing liver damage was assessed, and genetic testing pointed both patients to be UGT1A*28, treatment was maintained, and sustained virological response was achieved.[15]

Despite its benign condition, GS was associated to delays and increased retention rates in some drug-handling tests used in the past to measure liver activity, such as the menthol and the indocyanine green.[16,17] Also, alleles of other UGT isoforms have been associated with worse prognosis in hepatitis B virus (HBV) and chronic hepatitis C (CHC)—hepatocellular carcinoma and its age of onset.[18] Many questions regarding GS's role in the chronic liver disease scenario can be asked: its incidence in this population, bilirubin levels meaning, prognosis, and even how it affects the decision-making process. Our aim was to assess UGT1A1 genotypes' frequency in a CHC patients group and in healthy blood donors and correlate GS genotypes with total bilirubin levels.

2. Patients and methods

This prospective case–control study was conducted at the University of Sao Paulo School of Medicine, Department of Gastroenterology, Hepatology branch at HCFMUSP (Hospital das Clinicas, University of Sao Paulo School of Medicine). CHC patients were invited as cases to form Group A, and healthy blood donors formed Group B. The present study is reported according to the STROBE Statement: guidelines for reporting observational studies.[19]

2.1. Participants

Consecutive patients from the outpatients clinic at the Gastroenterology Department—Hepatology branch of HCFMUSP (University of Sao Paulo Hospital das Clinicas) and Sao Paulo blood bank Foundation Pro-sangue—Hemocentro de São Paulo HCFMUSP fulfilling criteria for both case and control groups were included in the study. Recruitment was performed between February 24, 2006 and September 14, 2009.

Informed consent was obtained from each patient included in the study and approved by the Institutional Review Board. Study group inclusion criteria were 18 years of age or older and CHC diagnosis confirmed by polymerase chain reaction (PCR). Exclusion criteria were coinfection with HBV and coinfection with HIV. Control group included 313 healthy volunteers from Sao Paulo blood bank Foundation Pro-sangue—Hemocentro de São Paulo HCFMUSP (Fig. 1). Subjects were of both genders and aged between 18 and 86 years. Inclusion criteria were 18 years of age or older and weight more than 50 kg, be well rested and fed. Exclusion criteria were flu-like symptoms, pregnancy, previous tattoo in the past 12 months, general risk factors for sexually transmitted diseases, and other conditions that unable blood donation.

2.2. Variables and data collection

Genetic testing for the UGT1A1 promoter gene was performed in both groups. Wild alleles were considered to be TA5 and TA6 and GS alleles, TA7 (UGT1A1*28) and TA8. Wild phenotype was considered as 5/5, 5/6, and 6/6; GS phenotype as 7/7, 7/8, and 8/8; and heterozygote as 5/7, 5/8, 6/7, and 6/8. Biochemical data were not available for healthy blood donor group according to the data confidentiality policy of the São Paulo blood bank Foundation Pro-Sangue–Hemocentro de São Paulo HCFMUSP. Total bilirubin levels were available for 535 of 585 CHC patients and were analyzed.

2.3. Genetic analysis

Blood samples were collected with standard methods. Peripheral blood leukocytes were obtained, and deoxyribonucleic acid (DNA) isolation was done by commercially available kit QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany) and stored at −20 °C. A 404-bp PCR fragment was amplified using the primers forward UGT1A1F 5′ gaggttctggaagtactttgc 3′ and reverse UGT1A1R 5′ ccaagcatgctcagccag 3′.[2] PCR was performed with 1-U platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA), 1X PCR buffer, 1.5 mM of $MgCl_2$, 0.5 μM of dNTP, and 0.5 μM of forward and reverse primers. Thermal cycle conditions were as follows: preheating at 95 °C by 5 minutes, followed by 30 cycles of 95 °C for 1 minute, 58 °C for 30 seconds, and 72 °C for 30 seconds.

PCR purification was done with commercially kit QIAquick PCR Purification kit (Qiagen, Hilden, Germany). For the sequencing reaction, Big Dye Terminator Cycle Sequencing Standard version 3.1 reagents (Applied Biosystems, Foster City, CA) were used with forward and reverse primers. The samples were applied in a Long Ranger polyacrylamide gel packs (Cambrex, Valais, Switzerland) in ABI377 Prism 377 automated

sequencer (Applied Biosystems). Sequences obtained were transferred into a computer, and the analyses of promoter region of *UGT1A1* gene were done using BioEdit 7.1 and CodonCode (CodonCode corporation, Dedham, MA). Sequences were compared with GenBank database.

For large-scale UGT1A1*28 screening, our group developed a new fragment analysis technique.[20] A standard PCR technique, as described above, was performed. This time, a fluorescent probe was attached to the forward primer UGT1A1F 5′ gaggttctggaagtactttgc-FAM 3′. Megabace 1000 (GE Healthcare, Buckinghamshire, UK) capillary electrophoresis was used to all fragment analysis in order to assess fragments' molecular mass. ET-Rox550 was used as a marker, and fragment profiler software (GE Healthcare) was used to analyze data and compare each genotype's fragment molecular mass to its photon emission. Therefore, a base pair range was described for each UGT1A1 promoter's region genotype, and further fragments' genotype could be predicted by its capillary electrophoresis analysis.

For methodology validation purposes, 76 patients had their samples coded and genotyped by both PCR sequencing and fragment analysis by 2 independent researchers. Sample analysis was blinded, and all results were concordant.

2.4. Statistical analysis

Serum bilirubin values were compared by Mann–Whitney (2 groups) or Kruskal–Wallis followed by Tamhane as post hoc test (3 groups). Chi-square test was used to assess statistical significance between groups in frequency comparisons. Statistical analysis was performed using SPSS v.13 (SPSS Inc., Chicago, IL). Significance was set at 0.05, and all the tests were 2-tailed.

3. Results

Descriptive statistics of both the study (585 patients with chronic hepatitis C) and control (313 blood bank subjects) groups are given in Table 1, which shows significant differences regarding sex and age between the 2 populations, with the control group including more men and young volunteers (57.5%; mean age 33.6 years) compared with the study group, in which more women and older patients are represented (53.6%; mean age 53.1 years).

The distribution of the observed number of (TA) repeats in the study and control groups by sequencing were TA5/6, TA6/6, TA6/7, TA7/7, TA5/7, TA7/8, and TA8/8. There is no significant difference regarding the frequency of the genotypes (Table 2, $P = 0.001$) and allele frequency (Fig. 1, $P = 0.795$) observed between study and control groups.

Table 3 describes total bilirubin (TB) median and interquartile (Q1 and Q3) values according to genotype. TA8/8 genotype

Table 1

Sociodemographic profile of hepatitis C virus patients and controls.

	Controls (n = 313)	CHC patients (n = 585)	
Variables	**n, %**	**n, %**	***P***
Sex			0.002
Male	180 (57.5)	271 (46.4)	
Female	133 (42.5)	314 (53.6)	
Race			0.222
Caucasian	194 (62.0)	362 (62.9)	
Black	45 (14.4)	65 (11.1)	
Yellow	3 (1.0)	13 (2.2)	
Brown	69 (22.0)	121 (20.7)	
Not declared	2 (0.6)	14 (2.8)	
Age			
Mean ± SD	33.6 ± 10.8	53.1 ± 13.0	<0.001

CHC = chronic hepatitis C.

Table 2

Genotypes frequencies in controls and CHC patients.

Genotype	Controls (n = 313)	CHC patients (n = 585)
Wild	162 (51.8)	259 (44.3)
5/5	1 (0.3)	0
5/6	5 (1.6)	9 (1.5)
6/6	156 (49.8)	250 (42.7)
Heterozygote	120 (38.3)	258 (44.1)
5/7	1 (0.3)	0
6/7	119 (38.0)	258 (44.1)
Gilbert syndrome	31 (9.9)	68 (11.6)
7/7	30 (9.6)	61 (10.4)
7/8	1 (0.3)	6 (1.0)
8/8	0	1 (0.2)

$P = 0.101$ for wild, heterozygote, and Gilbert syndrome genotypes. CHC = chronic hepatitis C.

Table 3

Mean and interquartile values according to UGT1A1 polymorphism in chronic hepatitis C patients.

Genotype	TB mean, mg/dL	Q1–Q3, mg/dL	n
TA 5/6	0.49	0.35–0.91	8*
TA 6/6	0.79	0.53–1.26	226†
TA 6/7	0.80	0.59–1.31	239‡
TA 7/7	1.54	1.31–1.92	58
TA 7/8	2.39	1.09–6.67	4§

$P < 0.001$ (Kruskal–Wallis test). TA = thymine–adenine, TB = total bilirubin.
* $P < 0.001$.
† $P = 0.002$.
‡ $P = 0.030$.
§ $P = 0.979$ (Tamhane post hoc test), TA 7/7 genotype as the reference for comparisons.

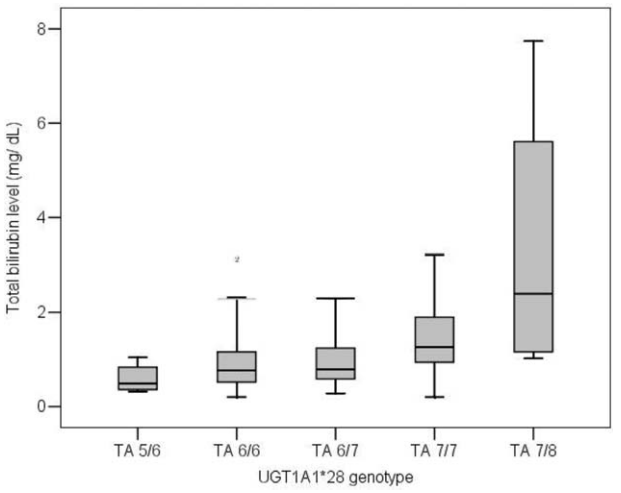

Figure 2. Median and interquartile range of total bilirubin according to number of thymine–adenine (TA) groups in UDP-glucuronosyltransferase 1A1's promoter region found in chronic hepatitis C patients. $P < 0.001$ (Kruskal–Wallis test). TA 5/6 × TA 7/7: $P < 0.001$; TA 6/6 × TA 7/7: $P = 0.002$; TA 6/7 × TA 7/7: $P = 0.030$; TA 7/7 × TA 7/8: $P = 0.979$ (Tamhane test). UDP = uridine diphosphate.

Table 4

Frequency of UGT1A1 alleles according to levels of TB in CHC patients.

	TA 5/6	TA 6/6	TA 6/7	TA 7/7	TA 7/8	TA 8/8	Total
Total bilirubin $\leq$1 mg/dL	7[*] (2.2)	144[†] (45.4)	148[‡] (46.7)	18 (5.3)	0[§]	0[‖]	317
Total bilirubin >1 mg/dL	1 (0.5)	82 (37.4)	91 (41.6)	40 (18.3)	4 (1.8)	1 (0.5)	219

$P<0.001$ Chi-square (all categories). TA = thymine–adenine.

[*] $P=0.004$.

[†] $P<0.001$.

[‡] $P<0.001$.

[§] $P=0.313$.

[‖] $P=1.0$ (2×2 Fisher exact test), TA 7/7 genotype as the reference for comparisons.

group had only 1 subject with 2.52 mg/L. As the number of TA increases, TB levels also increase, as demonstrated in Fig. 2. Frequency of polymorphism TA7/7 (GS) was significantly higher ($P<0.001$) among CHC patients with TB higher than 1 mg/dL (18.3%) when compared to patients with lower level of TB (5.9%), shown in Table 4.

Blood bank donors (control group) did not have their TB levels measured, but were considered to be anicteric based on the predonation interview. Although it is known that levels above 2.5 mg are necessary for a clinical diagnosis of jaundice, it is possible that the same phenomena observed in the HCV group patients occurred in the control group; however, these data are not available.

4. Discussion

In this work, we present a UGT1A1 alleles frequency prospective study in a highly multiethnic with complex genetic background population. We address 1 of GS' polymorphisms (UGT1A1*28) in 2 groups, CHC and healthy controls (blood donors). UGT1A1*28 frequencies were 10.4% and 9.6% in CHC patients and in blood donors, respectively (Table 2). Results are similar to those found in other populations. Beutler et al and Premawardhena et al observed a homozygote frequency of 8.6% in Germany, 10% in Iceland, 5% in United Kingdom, 14.8% in Basque, and 6.5% in Catalan. In the African continent, TA7/7 frequency was 17.9%. In Asia, TA7/7 frequencies were 2.6% in Thailand and 1% in China.[21,22]

In studied groups, rare genotypes such us TA 5/5, TA5/6, TA 5/7, TA7/8, and TA8/8 genotypes with low frequencies (0.2%–1.6%) were observed. TA8/8 genotype was found in 1 HCV patient with African ancestry (data not shown). The TA8 allele was first described in an African descendent and is very rare in Caucasians,[21] although it has been observed in Italians, Greeks, and Portuguese.[23–25] Lower TA repeats (TA5 allele) was previously described in Brazilian population, mainly in African descendant subjects.[26]

We observed increasing TB according to the total number of TA copies (Table 3). Also, UGT1A1*28 was more frequent among patients with bilirubin levels greater than 1 mg/dL (Table 4). Even though similar outcomes have been reported in other study populations,[25,27,28] these are key findings in the management of CHC and chronic liver patients, and have not been previously described in such a patient group.

High bilirubin levels are exclusion criteria in many clinical trials,[29–31] which may lead some GS patients to be systematically excluded from these studies. Nonetheless, UGT1A1*28 is associated to a broad spectrum of pharmacogenetics risks.[32] Along with irinotecan, other chemotherapy regimens have been linked with greater toxicity risks, such as sorafenib[33] and belinostat.[34] Atazanavir-associated hyperbilirubinemia has been reported in UGT1A1*28 and other GS patients undergoing highly active antiretroviral therapy for HIV and in chronic myeloid leukemia treatment with nilotinib.[35,36] These drugs may inhibit UGT enzymes' glucoronidation, which is already diminished in GS.[35,37]

Personalized care should be considered in GS. Higher treatment withdrawal has been reported in UGT1A1*28 patients due toatazanavir-associated hyperbilirubinemia and jaundice.[38] Also, atazanavir- and nilotinib-prescribing guidelines have been proposed in this population to reduce side effects.[29,35,39] Considering possible role in the decision-making process in the clinical care, one must ascertain GS chronic liver patients' inclusion in clinical trials, especially with the upcoming drug development for viral hepatitis.

Also, prognostic scores, such as Child–Pugh and Model for End-Stage Liver Disease, which are calculated based on TB levels, might be overestimated by benign increase in TB levels. Further studies should assess this potential source of bias in the clinical practice.

Main study limitation relates to selection bias. Our study had more male subjects in the control group than in the CHC branch, most probably due to minimum weight required for blood donation. Yet, this difference does not affect analysis, as we did not compare bilirubin levels between these 2 groups. Blood donors were younger as well, but again, this does not interfere in the analysis as polymorphisms are genetically inherited and neither relate to age nor sex. On the other hand, bilirubin levels comparison in the hepatitis C group might have being biased by different liver disease stages among UGT1A1 genotypes groups. Other biochemical and clinical features were not available at the time this study was conducted, and adjustment to other variables was not possible. Nonetheless, our results are in accordance with previous studies on bilirubin levels on UGT1A1 genotypes in other specific populations, suggesting that previous literature findings may apply to chronic liver disease in viral hepatitis settings.

In conclusion, there is high genetic variation of the UGT1A1 promoter region gene among CHC patients. Total bilirubin levels correlate to the number of TA group copies in this gene region, and higher frequency of UGT1A1*28 within patients with TB abnormal levels was observed. Personalized care should be considered to GS patients, either regarding their inclusion in clinical trials, prognostic scores, and drug metabolism.

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