UGT1A1 GENE VARIANTS AND TOTAL BILIRUBIN LEVELS IN HEALTHY SUBJECTS AND IN GILBERT SYNDROME PATIENTS

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1. INTRODUTION

The Gilbert syndrome (GS) is a benign form of unconjugated hyperbilirubinemia, mainly associated with variants in *UTGT1A1* gene [1]. In Caucasians, behind the most common genetic polymorphirsm, the seven repeat observed at the promoter *A(TA)nTAA* motif, another polymorphism a *T>G* transition at position -3279, at the phenobarbital responsive enhancer module, has also been associated with GS [2]. The aim of this study was the analysis of the impact of both promoter and/or coding region of *UGT1A1* variants on bilirubin levels, in GS patients and in healthy controls. Additionally, bioinformatics tools were used to study the new identified variants in order to evaluate their clinical significance.

2. MATERIAL AND METHODS

Subjects

This study included 45 GS Caucasian patients (36 males and 9 females, of the gastroenterology department of Vila Nova de Gaia/Espinho Hospital Center, EPE, with clinical diagnosis of GS, based on standard criteria [3]. Hyperbilirubinemia was defined for concentrations of total bilirubin levels (TBL) above 17.1 µmol/L. Data from GS patients were obtained from medical records. At the time of study they all presented normal physical examination, liver function tests and blood cell counts. Healthy control participants (n=161; 45 males and 116 females; were included in this work. All participants, GS patients and healthy controls, gave their informed consent to participate in this study.

Assays

Blood samples of controls were collected in the morning after 8 hours of fasting in order to obtain plasma and buffy coat for DNA extraction. TBL concentration was determined by using a colorimetric method. Genomic DNA was extracted from blood samples using standard salting out method. All patients and controls were screened for the presence of the *TA* insertion in the *TATA* box region of *UGT1A1* gene by PCR amplification, with the introduction of a fluorochrome label (NED™) in the reverse primer as previously described [1] and the length of fragments were determined by capillary electrophoresis. Direct sequencing of the five exons, and the c.-3576 to c.-3209 region of *UGT1A1* gene was done in independent sequencing reactions, using the respective forward and reverse primers, and by the Dye Terminator Cycle Sequencing Kit (Perkin Elmer, Boston, USA) [1].

Bioinformatics Tools and Data Analysis

The potential pathogenicity of the new variants and the correspondent altered sequences were analyzed by 5 web available tools: Polyphen-2, SIFT, A-GVGD, Grantham Distance and BLOSUM62.
For statistical analysis appropriate tests were applied using Statistical Package for Social Sciences (version 18, SPSS Inc., Chicago, USA).

3. RESULTS

Data from genotyping revealed the presence of two promoter polymorphisms, c.-41_-40dupTA and c.-3279T>G, in both groups (GS patients and controls) with significant different allelic and, consequently, diplotype distribution (table 1).

Table 1. Diplotypes frequencies of the two promoter *UGT1A1*polymorphisms and total bilirubin levels (TBL) in GS patients and in controls.

Diplotypes c.-41_-40dupTA / c.-3279T>G	GS Patients % (n)	GS Patients TBL (µmol/L)	Controls % (n)	Controls TBL (µmol/L)	p*
$[TA]_6$ / $[TA]_6$ -TT	0% (0)	-	33.5% (54)	7.7 ± 3.1	<0.0001
$[TA]_6$ / $TA]_6$ -TG	0% (0)	-	11.2% (18)	6.9 ± 3.4	
$[TA]_6$ / $[TA]_7$-TG	8.9% (4)	27.8 ± 7.4	32.9% (53)	9.6 ± 4.9	
$[TA]_7$ / $[TA]_7$-TG	4.4% (2)	29.1 ± 0.5	0% (0)	-	
$[TA]_6$ / $[TA]_6$-GG	0% (0)	-	1.9% (3)	8.4 ± 3.8	
$[TA]_6$ / $[TA]_7$-GG	8.9% (4)	34.8 ± 7.6	10.6% (17)	9.5 ± 4.3	
$[TA]_7$/ $[TA]_7$-GG	77.8% (35)	37.9 ± 13.3	9.9% (16)	19.5 ± 9.9b	

TBL: total bilirubin levels that are presented as mean ± standard deviation (SD); [a] p-value for difference in total bilirubin levels between the several diplotypes; [b] Multiple comparison analysis reveals that only $[TA]_7$/$[TA]_7$-G/G TBL were significant different from other diplotypes.

From the nine possible diplotypes combinations, four were found in GS patients and six in controls. Sequencing analysis of the coding regions of the *UGT1A1* gene allowed the identification of nine (Fig. 1) additional single-nucleotide polymorphisms (SNPs), all of them in a heterozygous state. Out of these, five were identified in GS patients and four in controls (table 2).

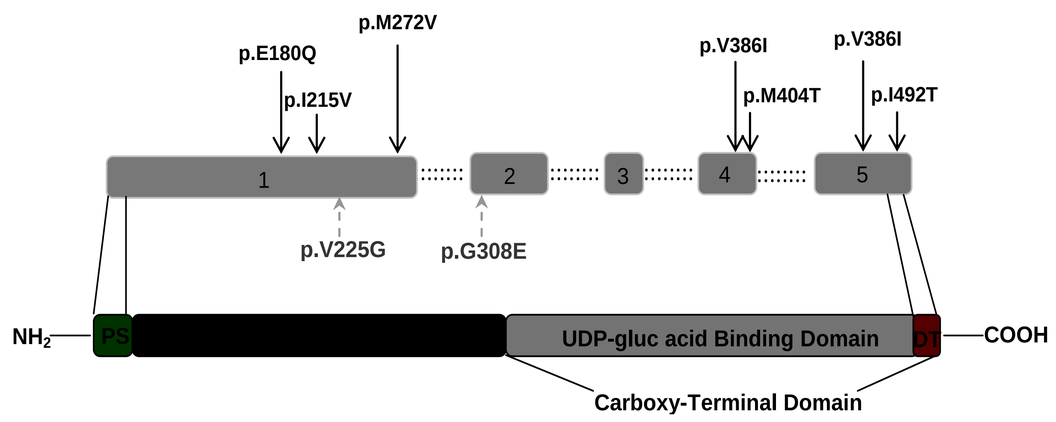

Fig. 1 - Representation of *UGT1A1* gene and UGT1A1 protein (adapted from Servedio *et al*. 2005). Arrows indicate the position of the altered residue in the new identified variants. Variants p.V225G and p.G308E were already described [4,5] .

Table 2. Coding region variants and bilirubin levels in GS patients and controls.

Samples	TBL (µmol/L)	cDNA variation	Effect on protein	Functional Prediction(Score)*	Polymorphism c.-41_-40dupTA	Polymorphism c.-3279T>G	References
Patients							
1/GS	29.4	c.538G>C	p.E180Q	Tolerated (4)	$[TA]_7$/$[TA]_7$	T/G	This study
2/GS	27.7	c.674T>G	p.V225G	Deleterious (3)	$[TA]_6$/$[TA]_7$	T/G	[4]
3/GS	50.1	c.674T>G	p.V225G	Deleterious (4)	$[TA]_7$/$[TA]_7$	G/G	[4]
4/GS	54.2	c.923G>A	p.G308E	Deleterious (4)	$[TA]_6$/$[TA]_7$	G/G	[5]
5/GS	36.6	c.1211T>C	p.M404T	Deleterious (4)	$[TA]_7$/$[TA]_7$	G/G	This study
6/GS	51.3	c.1423C>T	p.R475C	Deleterious (4)	$[TA]_6$/$[TA]_7$	G/G	This study
Controls							
1/C	5.1	c.643A>G	p.I215V	Tolerated (5)	$[TA]_6$/$[TA]_7$	T/G	This study
2/C	10.1	c.814A>G	p.M272V	Tolerated (4)	$[TA]_7$/$[TA]_7$	G/G	This study
3/C	4.3	c.1156G>A	p.V386I	Tolerated (4)	$[TA]_6$/$[TA]_6$	T/T	This study
4/C	5.8	c.1475T>C	p.I492T	Deleterious (3)	$[TA]_6$/$[TA]_7$	T/G	This study

The annotation of the mutations was done according to the recommendation of the HGVS. TBL: total bilirubin levels. * Scores are given empirically, taken in account the number of algorithms in agreement.

4. CONCLUSIONS

- **Bilirubin levels are mainly determined by the presence of the *TA* duplication in the repetitive *TATA*-box sequence of the gene promoter;**
- **The other promoter polymorphism, c.-3279T>G, is an additional factor for the development of hyperbilirubinemia;**
- **The presence of variants in the coding region of the gene seems to be associated with increased bilirubin levels and, therefore, associated with GS;**
- **We describe, for the first time, seven new variants in the coding region of the gene, expanding the spectrum of known *UGT1A*1 variants.**

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