

# UGT1A1 gene variations in individuals with and without clinical diagnosis of Gilbert Syndrome



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## 1 - INTRODUCTION

Bilirubin is a non-polar metabolite, results from catabolism of haemoglobin and is bound to glucuronic acid in the liver by the uridine diphosphate glucuronosyltransferase (UGT1A1). Molecular studies showed that the presence of two extra bases [A(TA)<sub>7</sub>TAA] in the promoter region of the *UGT1A1* gene is responsible for the reduced UGT1A1 glucuronization activity and is the main cause of unconjugated hyperbilirubinemia observed in patients with Gilbert Syndrome (GS). However, individuals with normal bilirubin levels and no clinical symptoms of SG may also present this polymorphism in homozygosity<sup>1,2,3</sup>. This polymorphism is not sufficient to explain the inter-individual variation and the presence of hiperbilirubinemia<sup>4</sup>.

The aim of this work is to determine the presence of other mutations in the *UGT1A1* gene, downstream of the TA duplication, and how they may contribute towards the inter-individual variation of serum bilirubin levels.

## 2 - MATERIAL AND METHODS

### Subjects and assays

To perform this study we groups: one comprising 36 individuals without clinical diagnosis of GS (14 with 6/6TA, 11 with 6/7TA, and 11 with 7/7TA repeats); the other group consisting of 36 patients clinically diagnosed with GS. In both, bilirubin levels were determined and direct sequencing of the *UGT1A1* gene was performed. Exclusion criteria for both groups has the presence of hematologic or hepatic disorder and been under medication. For each individual, after an overnight fasting, venous blood samples were collected in order to determine total and direct-reacting bilirubin and. Genomic DNA was isolated for molecular study of *UGT1A1* gene promoter, followed PCR amplification as previously described<sup>5</sup>. In order to study the pathogenicity of the new mutations, sequences were analyzed in the Biosoftware *Alamut*. For statistical analysis, the Statistical Package for Social Sciences, version 17.0 was used. The variable TA repeat polymorphism was categorize in Normal (6/6), Heterozygous (6/7) and Homozygous (7/7). To compare groups we use Kruskal-Wallis test. Significance was accepted at  $p < 0.05$ .

## 3 - RESULTS

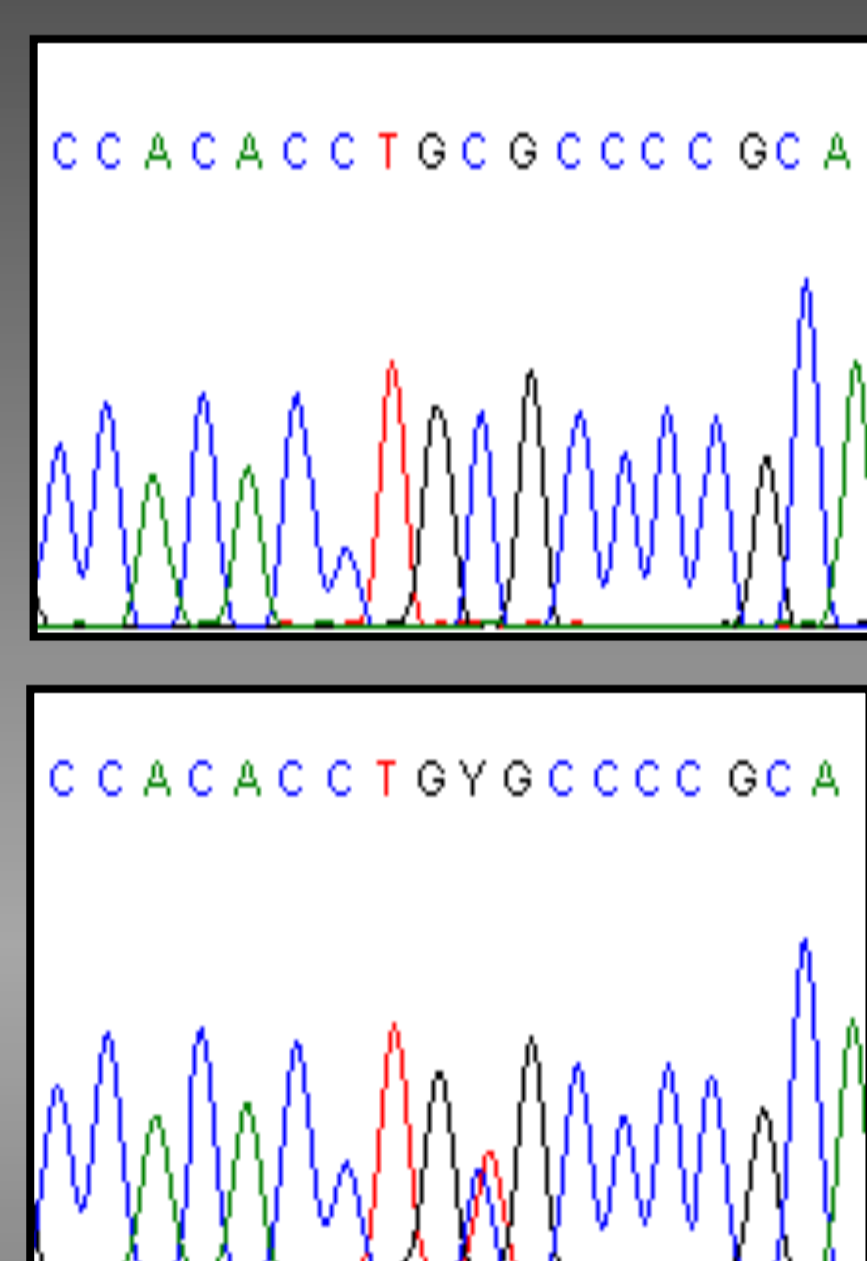


Fig. 1. Partial sequence around the c.1423C>T mutation site, exon 5 of the *UGT1A1* gene. (A) Normal sequence. (B) Heterozygous patient.

Patients (n=36)	Allele 1	Allele 2	Protein Level
28	[TA] <sub>7</sub>	[TA] <sub>7</sub>	-
5	[TA] <sub>7</sub>	[TA] <sub>6</sub>	-
1	[TA] <sub>6</sub>	[TA] <sub>6</sub>	-
1	[TA] <sub>7</sub>	[TA] <sub>6</sub> / c.674T>G	V225G
1	[TA] <sub>7</sub>	[TA] <sub>7</sub> / c.674T>G	V225G
1	[TA] <sub>7</sub>	[TA] <sub>6</sub> / c.923G>A	G308E
1	[TA] <sub>7</sub>	[TA] <sub>6</sub> / c.1423C>T	R475C
Controls (n=36)			
12	[TA] <sub>6</sub>	[TA] <sub>6</sub>	-
11	[TA] <sub>6</sub>	[TA] <sub>7</sub>	-
11	[TA] <sub>7</sub>	[TA] <sub>7</sub>	-
1	[TA] <sub>6</sub>	[TA] <sub>6</sub> / c.1156G>A	V386I
1	[TA] <sub>6</sub>	[TA] <sub>6</sub> / c.643A>G	I215V

Table 1. *UGT1A1* TA polymorphism and other sequence variations of coding regions, in GS patients and in Controls.

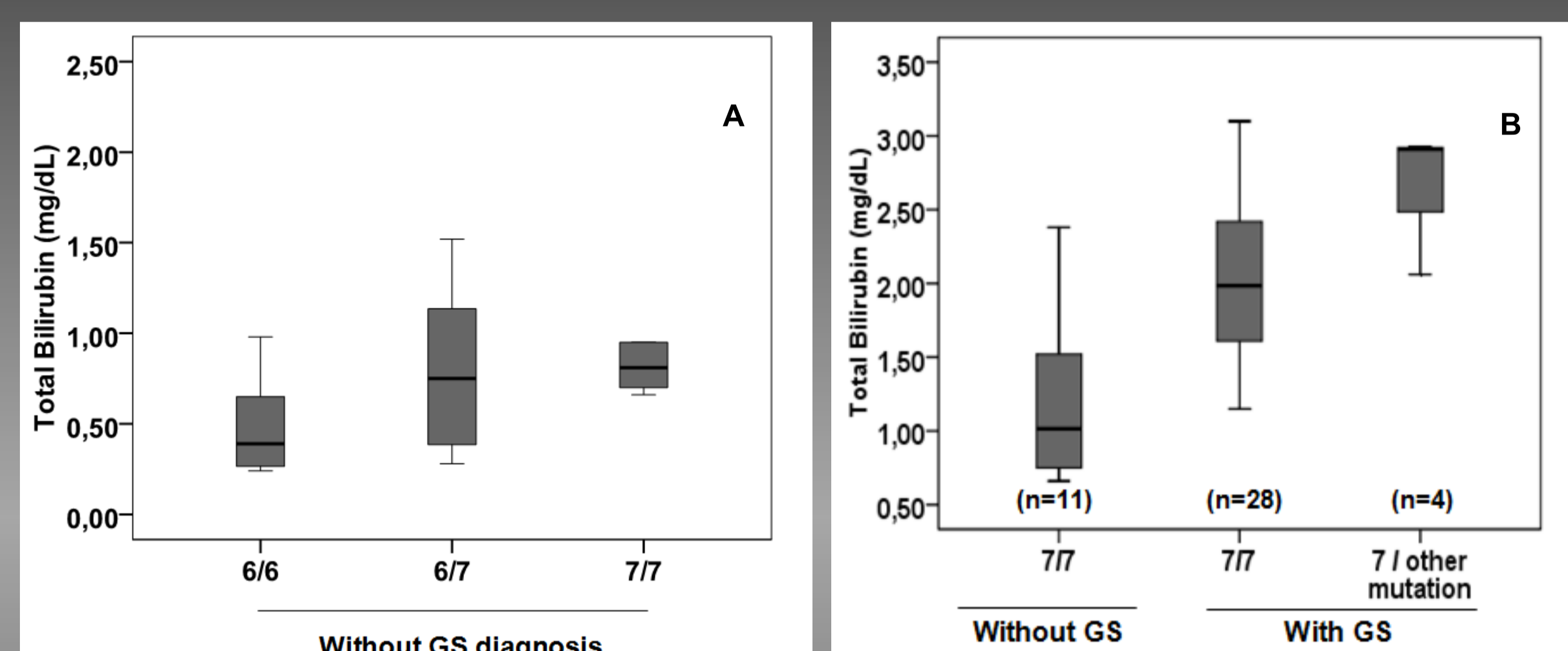


Fig. 2. Differences between total bilirubin concentration (mg/dL) according to: (A) Genotypes 6/6, 6/7 and 7/7 in individuals without GS; (B) between individuals without GS with genotype 7/7 and for the two GS patients groups (with homozygosity for [TA]<sub>7</sub> allele, and with one allele [TA]<sub>7</sub> and the other with a mutation in coding region).

## 3 - DISCUSSION

In the group without GS, TA polymorphism is also responsible for different bilirubin concentrations. In this group no mutations were detected in the 6/7 and 7/7 clusters, but in the 6/6 group two new mutations were found in heterozygosity. These mutations are not associated with increased bilirubin levels. However, they could be associated with GS in the presence of other *UGT1A1* mutations. Most of the patients clinically diagnosed with GS, were homozygous and only one as a normal number of repeats. Molecular analysis showed that one (3,6%) of the 7/7TA patients had another mutation in the *UGT1A1* gene (c.674T>G).. Comparing In the 6/7TA group, one additional mutation was also found in three patients (43%), two of which had been previously described (c.674T>G; c.923G>A) and a new one (c.1423C>T; Fig. 1). As shown in Fig2B mutations in coding regions are associate with increased serum bilirubin levels Additionally, 4 polymorphisms were found (c.864+89C>T; c.997-37T>C; c.997-82A>C; c.997-87A>C) and are not associated with increased serum bilirubin levels.

## 4 - CONCLUSIONS

Our study showed:

- 1) Homozygosity for the TA duplication is associated with GS.
- 2) TA polymorphism is also responsible for the interindividual variation observed among individuals in general population. Statistical differences occur within genotype 6/6 and 6/7 ( $p < 0.0001$ ) and is more pronounced between 6/6 and 7/7 genotype
- 3) We found homozygous for the TA polymorphism, without further mutations in coding regions, that have normal ranges of serum bilirubin concentration. Probably other genetic and non-genetic factors could contribute to the bilirubin variation in Portuguese population
- 4) In the GS group with heterozygosity for the TA duplication, we found variants in 43% of the patients, and mutations in *UGT1A1* coding region are associated with more pronounced hiperbilirubinemia. This two observations emphasize the importance of complete *UGT1A1* sequence analysis.