

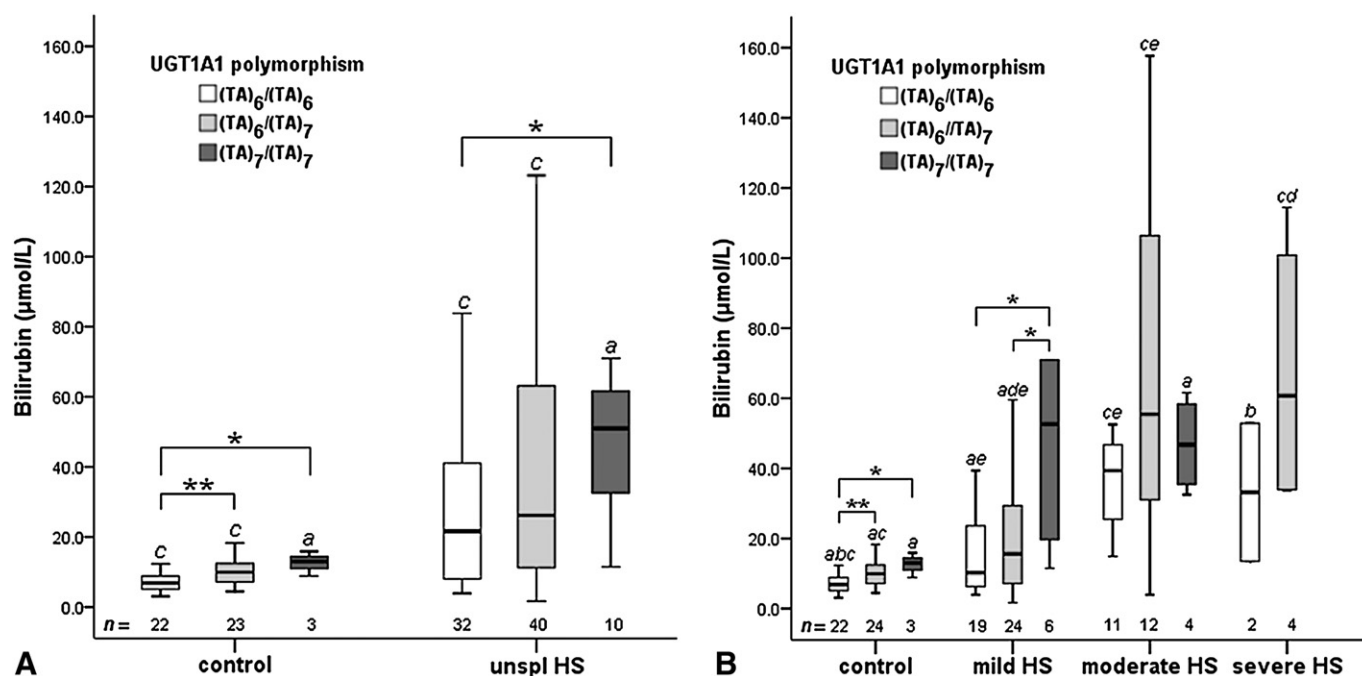
## Letter to the Editor

# Hereditary spherocytosis and the (TA)<sub>n</sub>TAA polymorphism of UGT1A1 gene promoter region—A comparison of the bilirubin plasmatic levels in the different clinical forms

To the editor,

Hereditary spherocytosis (HS) is the most common non-immune hemolytic anemia in individuals of northern European ancestry, affecting 1 in 2000 [1]. HS is classified as mild, moderate or severe according to the severity of the symptoms, family history and analytical presentation—hemoglobin (Hb) concentration, reticulocyte count and serum bilirubin levels [1]. When performing the classification of HS in our patients during the last years, we observed that bilirubin levels were sometimes inconsistent with the other parameters defining the severity of this anemia. This was particularly evident in some mild HS cases that presented unexpectedly high bilirubin plasma concentration, which was not in agreement with the other analytical parameters. A potential cause for this discrepancy could be the co-inheritance of HS and

Gilbert's syndrome (GS). This syndrome, which is estimated to affect 3–10% of the general population [2,3], is a metabolic disorder characterized by a mild and chronic unconjugated hyperbilirubinemia, in the absence of liver and hematologic disease. A polymorphism in the promoter of the bilirubin uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) gene, which expresses the specific isoform for bilirubin conjugation in the liver, has been shown to associate GS with a decrease of enzymatic activity to about 30% [4,5]. The majority of the GS individuals are homozygous for a TA duplication in the (TA)<sub>n</sub>AA motif of the promoter region of UGT1A1 gene [c.-41\_-40dupTA]; this is characteristic of the Caucasian population [2–5], in general, as well as of the Portuguese population [6], in particular. The co-inheritance of HS and GS can exacerbate hyperbilirubinemia, and, therefore, it could be a confounding factor to define HS severity. The aim of our work was to evaluate the prevalence of GS in HS patients and how this co-inheritance may confound the clinical classification of HS and its prognosis. We evaluated bilirubin plasmatic concentration and UGT1A1 gene polymorphisms in 48 healthy individuals and in 125 patients diagnosed with HS by standard screening tests (43 splenectomised and 82 unsplenectomised). The patients were



**Fig. 1.** Bilirubin plasmatic levels according to UGT1A1 polymorphism for (A) control group and total unsplenectomised HS patients (unspl HS) and (B) control group and unsplenectomised HS patients according to their clinical classification (mild, moderate or severe HS). Data presented as median values (inter-quartile range). \* $p < 0.05$  (TA)<sub>7</sub>/(TA)<sub>7</sub> individuals vs. (TA)<sub>6</sub>/(TA)<sub>6</sub> individuals or (TA)<sub>6</sub>/(TA)<sub>7</sub> individuals (Mann–Whitney *U* test); \*\* $p < 0.01$  (TA)<sub>6</sub>/(TA)<sub>6</sub> individuals vs. (TA)<sub>6</sub>/(TA)<sub>7</sub> individuals (Mann–Whitney *U* test); <sup>a</sup> $p < 0.05$  control group vs. unspl HS patients, mild HS patients or moderate HS patients (Mann–Whitney *U* test); <sup>b</sup> $p < 0.01$  control group vs. severe HS patients (Mann–Whitney *U* test); <sup>c</sup> $p < 0.001$  control group vs. unspl HS patients, moderate HS patients or severe HS patients (Mann–Whitney *U* test); <sup>d</sup> $p < 0.05$  mild HS patients vs. severe HS patients (Mann–Whitney *U* test); <sup>e</sup> $p < 0.01$  mild HS patients vs. moderate HS patients or severe HS patients (Mann–Whitney *U* test).

**Table 1**

Bilirubin plasmatic levels for control group and unsplenectomised and splenectomised HS patients according to clinical classification (mild, moderate or severe HS).

	Bilirubin (μmol/L)	
	8.0 (5.6–10.7)	
Control (n = 48)		
HS patients	Unsplenectomised	Splenectomised
Total (n = 82/n = 43)	26.2*** (10.3–52.6)	13.7*** <sup>§§</sup> (10.3–21.0)
Mild HS (n = 49/n = 7)	15.7** (7.0–32.2)	11.3 (7.9–13.8)
Moderate HS (n = 27/n = 22)	42.8*** <sup>EE</sup> (29.8–66.7)	14.6*** <sup>§§</sup> (11.2–24.9)
Severe HS (n = 6/n = 14)	43.5*** <sup>E</sup> (28.6–94.0)	15.4*** <sup>§</sup> (9.8–25.1)

Data presented as median values (inter-quartile range).

\*\*\* $p < 0.01$ , \*\* $p < 0.001$  control group vs. HS patients (Mann–Whitney *U* test).

<sup>§</sup> $p < 0.05$ , <sup>§§</sup> $p < 0.01$ , <sup>§§§</sup> $p < 0.001$  unsplenectomised vs. splenectomised HS patients (Mann–Whitney *U* test).

<sup>E</sup> $p < 0.05$ , <sup>EE</sup> $p < 0.01$  mild HS patients vs. moderate or severe HS patients (Mann–Whitney *U* test).

classified as having mild, moderate and severe HS, according to Bolton-Maggs et al. [1]; for splenectomised patients, we used the clinical classification of HS established before splenectomy. We evaluated plasmatic total bilirubin levels and the TA duplication in the repetitive TATA box sequence of the UGT1A1 gene promoter, screened by acrylamide gel (15%) analysis of polymerase chain reaction products [6]. The studied subjects were grouped according to the gene polymorphism—(TA)<sub>6</sub>/(TA)<sub>6</sub> for homozygote individuals with normal UGT1A1 transcription, (TA)<sub>6</sub>/(TA)<sub>7</sub> for heterozygote individuals and (TA)<sub>7</sub>/(TA)<sub>7</sub> for individuals with GS.

For the UGT1A1 polymorphism, we found in control group that 45.8%, 47.9% and 6.3% of the individuals were (TA)<sub>6</sub>/(TA)<sub>6</sub>, (TA)<sub>6</sub>/(TA)<sub>7</sub> and (TA)<sub>7</sub>/(TA)<sub>7</sub>, respectively; in the total HS patients (unsplenectomised plus splenectomised), we found similar frequencies—43.2%, 48.0% and 8.8%, respectively; actually, no statistically significant differences were found between control and HS groups ( $p = 0.846$ , Pearson  $\chi^2$ ). The prevalence of GS in both groups was within the estimated range [2,3]. In the control group, unsplenectomised (Fig. 1A) and splenectomised (data not shown) HS patients, bilirubin was higher in (TA)<sub>7</sub>/(TA)<sub>7</sub> individuals, followed by the (TA)<sub>6</sub>/(TA)<sub>7</sub> and (TA)<sub>6</sub>/(TA)<sub>6</sub> subjects. This is likely to result from a decrease in UGT1A1 gene expression, which is reflected in altered enzyme activity, as described elsewhere [7].

Studying the unsplenectomised patients according to HS severity (Fig. 1B), we observed that in mild HS patients, the (TA)<sub>7</sub>/(TA)<sub>7</sub> individuals presented significantly higher values of bilirubin, as compared to the other genotypes. Moreover, the bilirubin levels in these individuals were even higher than in (TA)<sub>6</sub>/(TA)<sub>6</sub> moderate and severe HS patients. In moderate HS, the bilirubin levels of GS individuals were only increased when compared to the (TA)<sub>6</sub>/(TA)<sub>6</sub> subjects. As we had no severe HS patients presenting the (TA)<sub>7</sub>/(TA)<sub>7</sub> genotype, we could not ascertain about their bilirubin levels.

Both splenectomised and unsplenectomised HS patients presented a statistically significant higher bilirubin levels when compared to the control group (Table 1); unsplenectomised patients also presented significantly higher bilirubin values than splenectomised patients, as splenectomy improves the hemolytic features and removes anemia. In both HS groups, the bilirubin concentration increased with HS severity reaching a significant difference in unsplenectomised HS patients.

We must emphasize that, in (TA)<sub>7</sub>/(TA)<sub>7</sub> patients with mild HS, 66% of them presented a bilirubin concentration higher than 34 μM, the highest reference value defined for mild HS cases [1]; in patients with moderate HS, 50% of them also presented a bilirubin concentration higher than 51 μM, the highest value defined for moderate HS cases [1].

In conclusion, our data show that the co-inheritance of GS can be misleading when defining the severity of HS, and, therefore, it would be important to study the UGT1A1 gene polymorphism when the bilirubin values are not in accordance with the other parameters used to define HS severity. Moreover, it is known that the co-inheritance of GS with HS increases the risk for the development of gallstones in HS children [8]; therefore, these studies could also be valuable in future clinical decisions, namely to decide about the performance of splenectomy.

## Acknowledgments

This study was supported by a PhD grant (SFRH/BD/22442/2005) attributed to S. Rocha by Fundação para a Ciência e Tecnologia (FCT) and Fundo Social Europeu (FSE). For blood collection, we thank Serviço de Hematologia Laboratorial do Hospital Santo António, nurses team from Hematologia Clínica do Hospital S. João and laboratory technician Elisabete Machado from Serviço de Hematologia do Hospital Maria Pia.

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18 September 2009