

Genetic and acquired factors that modulate serum bilirubin levels



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1. INTRODUCTION

UDP-glucuronosyltransferase 1A1 (*UGT1A1*) gene is considered a *major* determinant in serum bilirubin levels¹. Patients with Gilbert's syndrome (GS) are often homozygous for a *TA* insertion in the *TATA*-box region of the *UGT1A1* gene. The presence of this *TA*₇ allele decreases *UGT1A1* transcription, and therefore, reduces glucuronidation, resulting in increased unconjugated bilirubin serum levels.

It is well established that other non-genetic factors like gender², age³, or smoking status⁴ influence the inter-individual variation of bilirubin levels. However, there are few studies that relate the influence of genetic and non genetic variables in serum bilirubin concentration.

To clarify this issue, our aim was to determine the influence of the *TA* polymorphism and other non-genetic factors that could contribute to the bilirubin variation in Portuguese population.

2. MATERIAL AND METHODS

Subjects and assays

To perform this study we recruited 81 young adults (62 females and 19 males aged 20,2 ±1,7 years). All volunteers gave their written informed consent. Standardized interviewer-administered questionnaire was performed in all subjects, which included questions about smoking habits, oral contraceptive therapy, caloric intake, fasting time, and physical activity. Exclusion criteria included the presence of liver and/or hematological disorders.

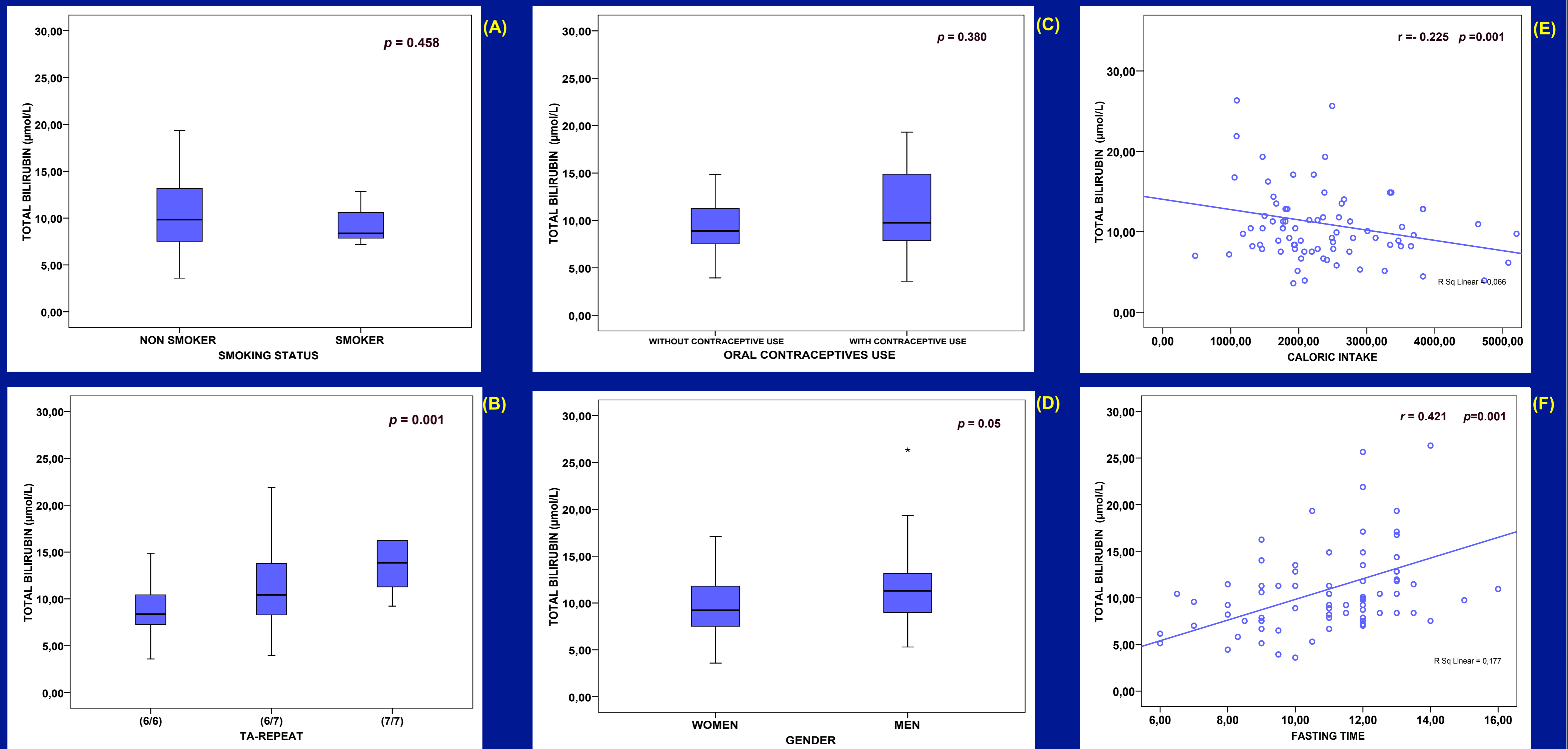
For each individual, after an overnight fasting, venous blood samples were collected in order to determine total and direct-reacting bilirubin. Genomic DNA was isolated for molecular study of *UGT1A1* gene promoter, followed PCR amplification as previously described⁵.

Data Analysis

For statistical analysis, the Statistical Package for Social Sciences, version 16.0 was used. Kolmogorov Smirnov statistics were used to assess sample normality distribution. Multiple comparisons between groups were performed by one-way ANOVA supplemented with Tukey's HSD *post-hoc* test. Spearman correlation coefficient was used to evaluate relationships between sets of data. Logistic regression analysis and adjusted general linear regression model was used to estimate the proportion of the variation of bilirubin concentration explained by different variables. Significance was accepted at $p < 0.05$. Hardy-Weinberg equilibrium was evaluated with the software available at <http://www.changbioscience.com/genetics/hardy.html/>.

3. RESULTS

Fig.1 - Differences between: smokers and no-smokers (A); genotypes 6/6, 6/7 and 7/7 (B); women under contraceptive therapy and without contraceptive therapy (C); gender (D). Correlations between total serum bilirubin levels ($\mu\text{mol/L}$) and: caloric intake (E); fasting time (F).



4. DISCUSSION

Our study showed:

- A clear association between the *TA* repeat polymorphism and bilirubin concentration. Multiple comparison analysis demonstrated that significant statistical differences occur within genotype 6/6 and 6/7 ($p=0.008$) and is more pronounced between 6/6 and 7/7 genotype ($p=0.004$).
- Higher serum bilirubin levels in males.
- No statically significant differences were found in smoking status, oral contraceptive therapy and physical activity.
- Statistically significant correlations were found between bilirubin serum level and fasting time and caloric intake.
- Regression analysis strongly suggest that genetic background plays a *major* role in serum bilirubin levels variation (23%), in this population.
- Moreover, gender, caloric intake, fasting time, could also contribute to the inter-individual variation.

In conclusion, the establishment of factors that might contribute to the observed inter-individual differences will be crucial for many clinical conditions which diagnosis, prognosis and treatment are associated with bilirubin levels.