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

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Introduction: Gilbert syndrome (GS, OMIM 606785) is an autosomal recessive condition characterized by unconjugated hiperbilirubinemia in the absence of hemolysis or underlying liver disease due to the reduced activity of the uridine diphosphate-glucuronosyltransferase (UGT1A1). This enzyme is mainly expressed in the liver and has an important role in the glucuronidation of bilirubin, 17β-estradiol, some therapeutic drugs and mutagenic xenobiotics. Absence or severe reductions of UGT1A1 activity are associated with Crigler-Najjar syndrome type I and type II, respectively. Heterozygous carriers of Crigler-Najjar syndrome also present a high incidence of mild hyperbilirubinemia, a feature of GS.

Aim: This work investigated the effect of *UGT1A1* variants on total bilirubin levels in Gilbert patients (n=45) and healthy controls (n=161).

Methods: Total bilirubin levels were determined using a colorimetric method. Molecular analysis of exons 1-5 and two *UGT1A1* promoter regions were performed by direct sequencing and automatic analysis of fragments. Five *in silico* methods predicted the effect of new identified variants.

Results: A significant different allelic distribution, in Gilbert patients and in controls, was found for two promoter polymorphisms. Among patients, 82.2% were homozygous and 17.8% heterozygous for the c.-41_-40dupTA allele; in control group, 9.9% were homozygous and 43.5% heterozygous for this promoter variant, while 46.6% (n=75) presented the [$A(TA)_6TAA$]. For the T>G transition at c.-3279 promoter region, in patients, 86.7% were homozygous and 13.3% heterozygous; in control group, 33.5% were homozygous for the wild type allele, 44.1% were heterozygous and 22.4% homozygous for the mutated allele. The two polymorphisms were in Hardy-Weinberg equilibrium in both groups. Sequencing of *UGT1A1* coding region identified nine novel variants, five in patients and four in controls. *In silico* analysis of these amino acids replacements predicted four of them as benign and three as damaging.

Conclusions: We demonstrated that total bilirubin levels are manly determined by the TA duplication in the TATA-box promoter and by the c.-3279T>G variant. Alterations in the *UGT1A1* coding region seem to be associated with increased bilirubin levels, and therefore with Gilbert Syndrome.