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

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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) activity. Molecular studies showed that the presence of two extra bases (TA duplication) 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 1,2,3. Consequently, 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. This study was carried out in two 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 was performed. Among the individuals without clinical diagnosis of GS, two new sequence variants were found in heterozygosity (c.643A>G, and c.1156G>A), in the 6/6TA group. No additional mutations were detected in the 6/7 and 7/7 TA groups. In patients clinically diagnosed with GS, 28 were homozygous and 7 heterozygous for the TA duplication, and one with 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). 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 and c.923G>A) and a new one (c.1423C>T). No further mutations were detected in the 6/6TA group. Additionally, 4 polymorphisms were found (c.864+89C>T; c.997-37T>C; c.997-82A>C; c.997-87A>C). In conclusion, we can infer that homozygosity for the TA duplication is associated with GS. In the group without GS, no further 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. Furthermore, in the GS group with heterozygosity for the TA duplication, we found mutations in 43% of the patients, emphasizing the importance of complete UGT1A1 analysis.

References:

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## Is dietary modulation of DNA repair involved in colon cancer prevention?

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Cancer originates from an accumulation of different genetic alterations that include activating mutations in oncogenes, inactivation of tumor suppressor genes, modifications in DNA methylation status and alterations in regulating genome stability. DNA repair enzymes are important to correct mutations and to prevent carcinogenesis initiation. The enzyme methylguanine methyltransferase (MGMT) is responsible for correction of a specific and highly mutagenic type of alkylating DNA damage, MeG, before activating point mutations occur. Also, other enzymes of Base Excision Repair (BER) pathway are relevant for preventing mutagenesis. Diet is an important factor in colon cancer risk. It has been shown that some food-related compounds, such as heterocyclic amines, are potent mutagens for cancer initiation, while other dietary compounds, mostly from fruits and vegetables, can reduce the risk of cancer. Studies have shown that some diet components can modulate DNA repair in different cell types in humans. We investigated the potential of *Salvia officinalis* (SO) herbal tea drinking on colon cancer prevention using the AOM rat model. In F344 rats, SO effects were evaluated at the pre-initiation (SO treatment before azoxymethane (AOM) exposure) and post-initiation (SO treatment after AOM exposure) phases of carcinogenesis. A chemopreventive effect of SO was found in the pre-initiation group that was not seen in the post-initiation group. Immunohistochemistry (Ki67 staining) was used to evaluate the effects of SO tea/AOM treatment on colon epithelial cell proliferation. AOM treatment significantly increased the number of Ki67-positive cells in normal tissue, but this increase was significantly reduced by SO tea treatment. In parallel, an in vitro study using isolated colonocytes from rats that drank SO tea we demonstrated that SO treatment decreased significantly the oxidative H<sub>2</sub>O<sub>2</sub>-induced DNA damage. In Caco 2 cells SO induced BER activity. In view of the ability of SO to modulate DNA repair activity, expression studies of other DNA repair associated genes are in progress, mainly MGMT and N-methylpurine-DNA glycosylase (MPG) involved in alkylation DNA repair, that may provide further mechanisms for prevention of colon carcinogenesis induced by alkylating agents. Acknowledgements: DFNP and AAR supported by the FCT grants SFRH/BD/64817/2009 and SFRH/BD/35672/2007