A POPULATION STUDY ON THE UGT1A1*28 VARIANT ASSOCIATED TO IRINOTECAN TOLERANCE.

Roman R[1], Colomer A[1], Erill N[1], Verdú M[2], Ibáñez R[1], Cordón-Cardó C[1], Puig X[1,2].

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INTRODUCTION

- Irinotecan (CPT-11), a potent inhibitor of topoisomerase I, is currently used as first- or second-line treatment for colorectal cancer, either alone or in combination with 5-fluorouracil based regimens.
- UDP-glucuronosyltransferase 1A1 (UGT1A1) catalyzes the inactivation of SN-38, the active metabolite of irinotecan, through glucuronidation (**Figure 1**).
- UGT1A1*28, a polymorphic variant of this enzyme, displays an additional TA dinucleotide in the promoter TATA box [A(TA)$_7$TAA], instead of the more common form with six repeats [A(TA)$_6$TAA]. The allelic frequency of this variant has been shown to vary between different ethnic groups. Other polymorphic variants presenting five and eight TA repeats have also been found in individuals of african ancestry.
- The UGT1A1*28 variant has been related to up to 70% reduction of the UGT1A1 transcriptional activity. Patients presenting this variant are prone to increased severe toxicities, mainly neutropenia and diarrhea. In fact, a reduction of the starting dose by at least one level of irinotecan has been recommended for individuals presenting the UGT1A1*28 homozygous genotype.

AIM

The objective of our study was to evaluate the presence of the UGT1A1*28 variant in a series of 100 samples from colorectal cancer patients collected from the area of Barcelona, Spain and compare it with the frequency found in a healthy population.

MATERIALS AND METHODS

Patients. This series included a set of 100 normal mucosa samples collected from patients surgically treated for colorectal cancer in the area of Barcelona. There were 58 male and 42 female patients, mean age 65.5 ± 12.4, ranging from 37 to 91. The presence of this variant was also studied in a collection of 102 tonsillectomy samples from otherwise healthy children.

Tissue macrodissection, DNA extraction and quality control. All tissues were routinely formalin fixed and embedded in paraffin. Ten 5-µm thick sections adjacent to a hematoxylin-eosin section were used to perform manual scrapping. DNA was isolated using a proteinase K-phenol/chloroform protocol. The quality of the DNA extracted was checked by amplification of a 268 bp fragment of the human ß-globin gene.

UGT1A1 genotyping. Analysis of the TATA box in the promoter region of the UGT1A1 gene was performed by PCR amplification of 200 ng of extracted genomic DNA, as previously described (Monaghan *et al.*,1996), followed by automated unidirectional sequencing. Genotypes were assigned as follows: 7/7 describes cases homozygous for the UGT1A1*28 variant with seven TA repetitions, 6/6 describes cases homozygous for the common allele with six TA repetitions, and 6/7 represents heterozygous cases (**Figure 2**).

RESULTS

Genotyping could be performed in 91 (91%) colorectal cancer cases. The 7/7 genotype was detected in 11% of cases, while 47% and 42% of the individuals presented 6/6 and 6/7 genotypes, respectively The UGT1A1*28 allelic frequency estimated in this population was 0.32 (**Table**).

Eighty-two (80%) tonsillectomy samples could be assessed. The 7/7 genotype represented the 6% of this population, whereas the 6/6 and 6/7 genotypes were identified in 49% and 44% of cases. There was also one case that presented a 5/6 genotype. The UGT1A1*28 allelic frequency observed in this group was 0.28 (**Table**).

CONCLUSIONS

- The UGT1A1*28 allelic frequency found in the colorectal series is in agreement with those reported for other european populations.
- Based on these data and taking into account the potential severe toxicities and the benefit of a reduced initial dose of irinotecan, it appears plausible to perform a UGT1A1 genotype in colon cancer patients prior to irinotecan treatment.
- Looking at our two different sets of samples separately, a higher allelic frequency was found in the colorectal cancer patients when compared to that found in the tonsils group (0.32 vs. 0.28), though it did not reach statistic significance. This putative increase needs to be further assessed and validated using a larger and well characterized cohort.

REFERENCES

- Ando M, Hasegawa Y, Ando Y. Pharmacogenetics of irinotecan: a promoter polymorphism of UGT1A1 gene and severe adverse reactions to irinotecan. Invest New Drugs 2005;23:539-45.
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FIGURES

Figure 1. Representation of the metabolic pathway of irinotecan. A: In the presence of the UGT1A1 wild type (6/6) form, and; B: In the presence of the UGT1A1*28 variant.

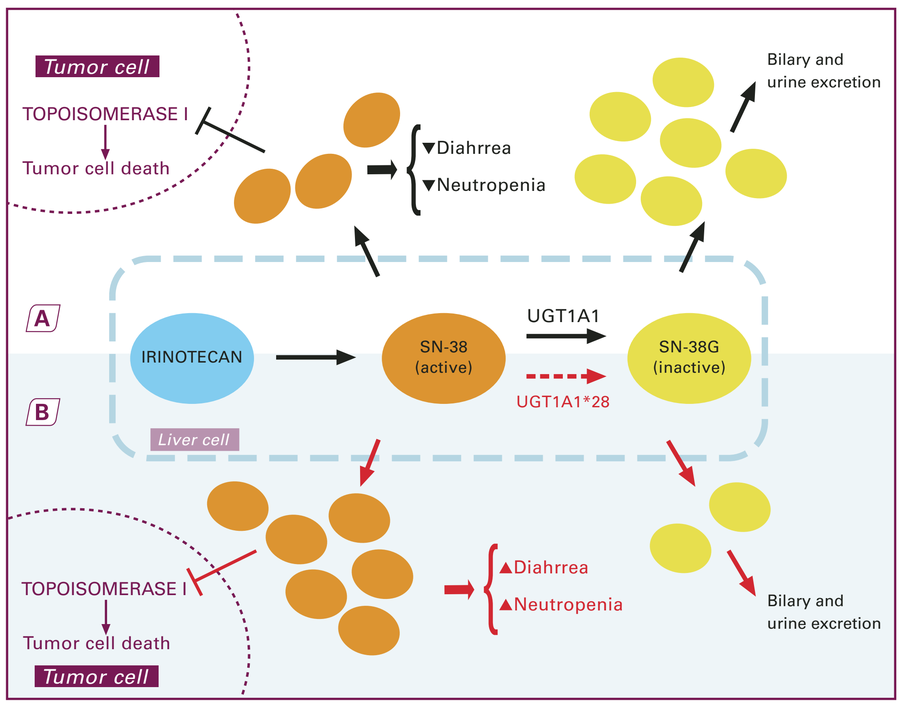
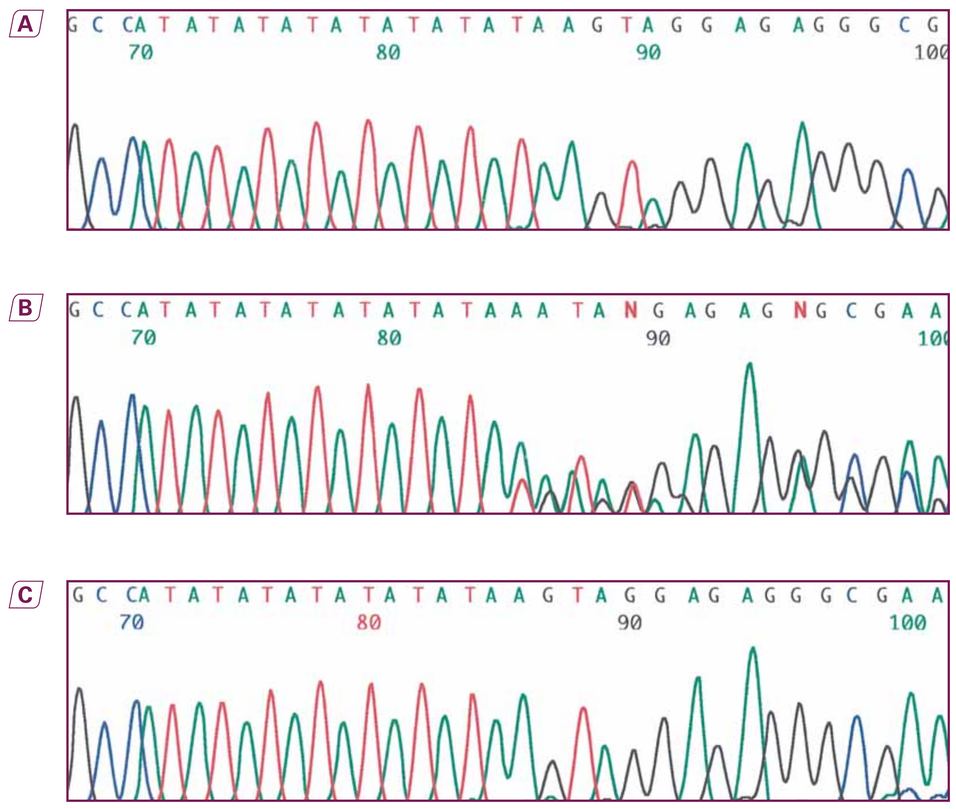


Figure 2. Examples of UGT1A1 genotype sequences. A: Homozygous 7/7; B: Heterozygous 6/7 and C: Homozygous 6/6.



TABLE

Genotype	Colorectal	Tonsils
Homozygous 7/7	10 (11%)	5 (6%)
Heterozygous 6/7	38 (42%)	36 (44%)
Homozygous 6/6	43 (47%)	40 (49%)
Heterozygous 5/6	0 (0%)	1 (1%)
TOTAL	91	82
UGT1A1*28 allelic frequency	**0.32**	**0.28**

UGT1A1 promoter genotypes found in our colorectal and tonsillectomy series, and the resulting UGT1A1*28 allelic frequency.

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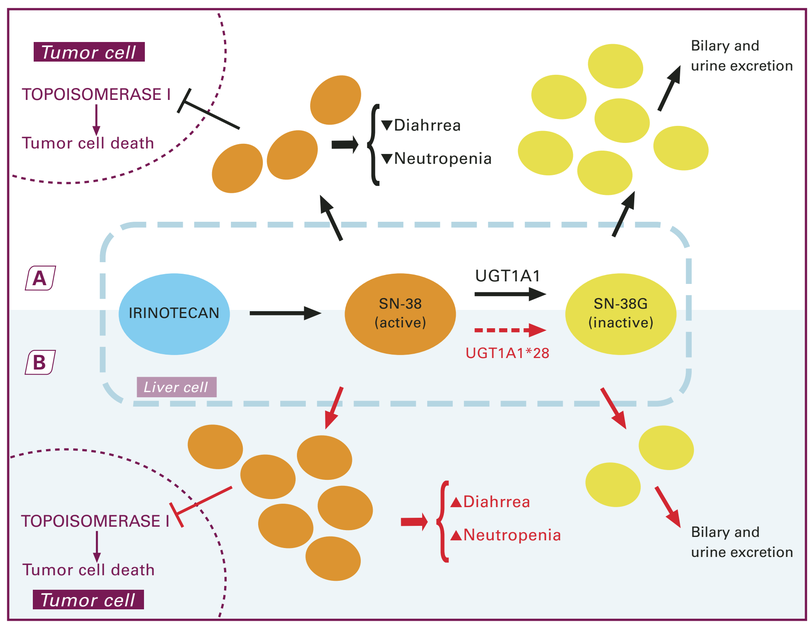

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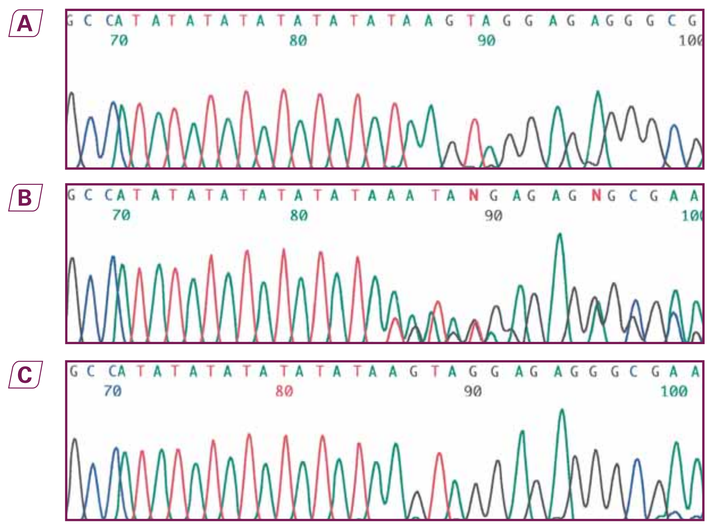

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