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# Genotypic variability and doping control: effects of the polymorphism of the family of UGT2B genes on a reference population

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## Abstract

The objective of this study was to investigate the use of urine sample for the analysis of the T/E ratio excretion in a database of Caucasian individuals, by the application of sensitive methods for the genotyping of copy number variant (CNV) of the UGT2B17 gene and a common single nucleotide polymorphisms (SNP) of the UGT2B15 gene. Results we achieved show that CNV polymorphisms of UGT2B17 have a main strong impact on T/E ratio while SNP polymorphisms of UGT2B15 seems to impact mainly on A/Etio ratio.

## Introduction

T/E excretion ratio shows high levels of inter-individual variation, and it mainly depends on testosterone glucuronide excretion. Genetic polymorphisms in the uridine diphospho-glucuronosyl transferase (UGT2B) gene family have been associated to this variation. The genotyping of samples can be useful in order to better understand the urinary excreation pattern of steroids. However, genotyping of urine samples requires an additional analysis which is not standardized in the context of antidoping. Human **UGT2B17** CNV polymorphism determines three possible genotypes: ins/ins, ins/del and del/del. The del/del phenotype, mainly expressed within the Asian populations, has been associated to a lower urinary excretion of testosterone glucuronide and consequently, often to lower basal values of the T/E ratio. On the other hand, the steroid profile can be affected also by other factors as human **UGT2B15** gene which presents several SNPs and may involve an altered function of the glucoronidation enzyme.

## Experimental

Genomic DNA (gDNA) from 123 urine or buccal swab samples were extracted by using a PrepFiler DNA extraction kit and quantitated on a standard reference curve with real time quantitative PCR assay. Determination of the UGT2B17 CNV polymorphism was perfomed using a sensitive specific TaqMan assay by a relative quantification method and using a RNAase P as endogenous reference assay. Three control samples with known genotypes were used as external calibrators. Determination of the UGT2B15 SNP polymorphism was performed using a TaqMan SNP genotyping assay. Three control samples with known genotypes were used as external calibrators. All PCR experiments were carried out on a real time qPCR 7500 fast instrument from Life Technologies. Urinary steroid parameters were determined in urine by GC/MS-MS on the total fraction by following the routine laboratory procedures.

## Results and Discussion

As for UGT2B17 gene, significant differences between the three genotyped groups were detected only for T/E ratio (Figure 1). Ins/ins and ins/del groups have similar median T/E values compared to the del/del group (Figure 2). Hence the reduced activity of UGT2B17 enzyme is exclusive of the full homozygous deletion genotype. No gender-dependent statistical differences were detected within any group despite men were observed in general to have slightly higher T/E mean values. As for UGT2B15 SNP polymorphism (rs1902023), 73% of the individuals resulted homozygous wild-type or being heterozygous for the polymorphism (which means normal activity of the enzyme). The remaining 27% of the study population were homozyogous full polymorphic with reduced enzymatic activity (Figure 3). These results for the population are in accordance with

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Hardy-Weinberg equilibrium for bi-allelic systems.

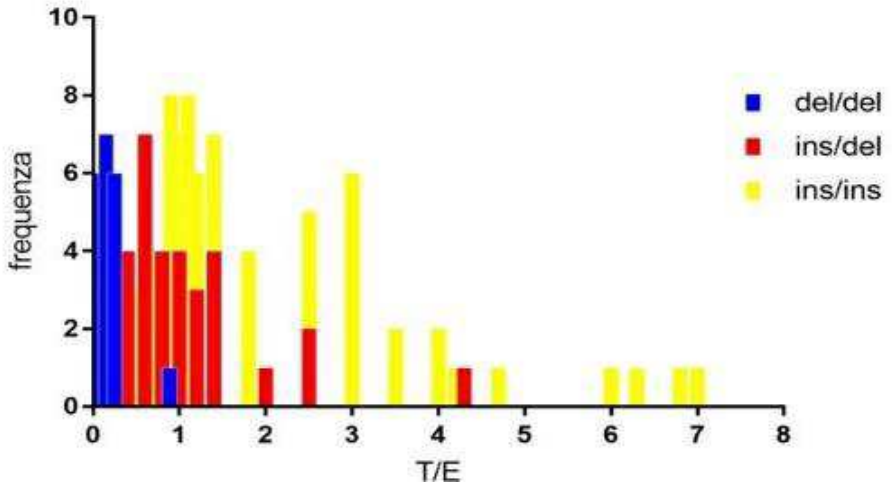


Figure 1: Distribution of T/E excretion ratios (total fraction) according to different UGT2B17 CNV genotypes

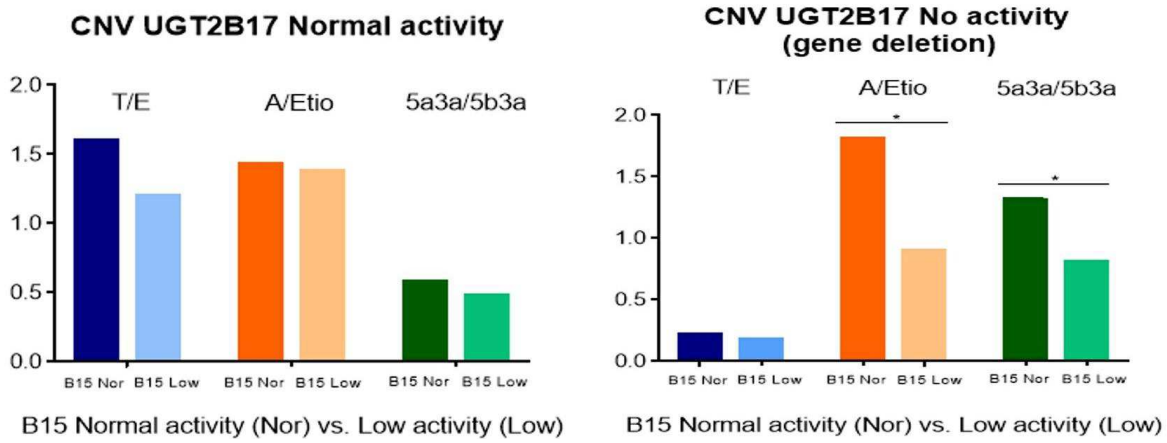


Figure 2: Different excretion patterns of steroids according to different UGT2B17 genotypes

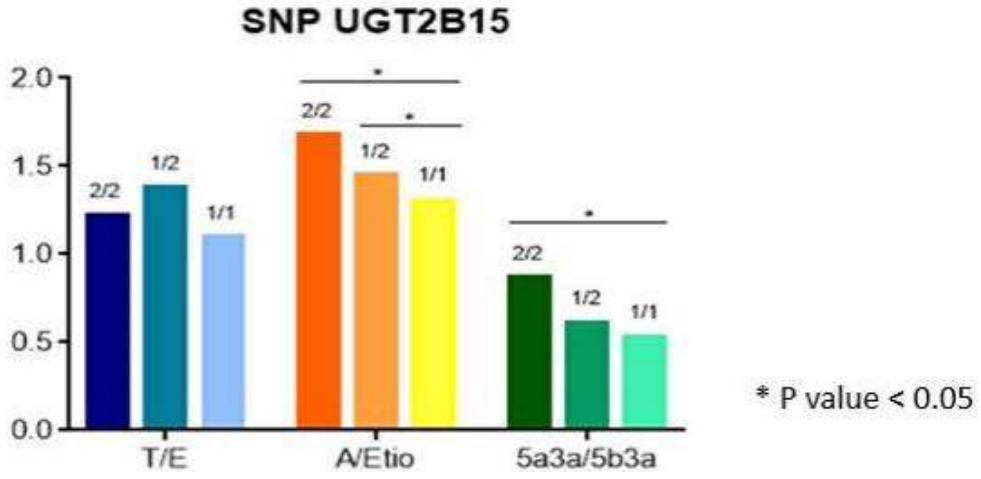


Figure 3: Mean values of T/E, Andro/Etio, 5α3α/5β3α ratios according to different UGT2B15 SNP genotyping

## Conclusions

UGT2B15 polymorphism affects mainly the Andro/Etio and 5α3αdiol/5β3αdiol ratios, but does not seem to have an effect on T/E ratios. T/E ratio is mainly affected by the CNV polymorphism on the UGT2B17 gene regardless of the type of SNP polymorphism that is carried on the UGT2B15 gene. The lowest Andro/Etio ratios are observed on the individuals who are full polymorphic for both UGT2B17 and UGT2B15 gene. Regarding the interpretation of the steroid profile and confounding factors, the reduction of the Andro/Etio ratio due to the full UGT2B15 polymorphism is not as strong as the effect of the 5α-reductase inhibitors.

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