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ORIGINAL ARTICLE

Prevalence of UDP-glucuronosyltransferase polymorphisms (UGT1A6*2, 1A7*12, 1A8*3, 1A9*3, 2B7*2, and 2B15*2) in a Saudi population

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Abstract Glucuronidation is an important phase II pathway responsible for many endogenous substances and drug metabolism. The present work evaluated allele frequencies of certain UDP-glucuronosyl-transferases (UGT 1A6*2, A7*12, A8*3, A9*3, 2B7*2, and 2B15*2) in Saudi Arabians that could provide essential ethnic information. Blood samples from 192 healthy unrelated Saudi males of various geographic regions were collected. Genomic DNA was isolated and genotyping of various UGTs was carried out using polymerase chain reaction (PCR) followed by direct sequencing. For UGT1A6*2 A/G genotype, the most common variant was the homozygous repeat (AA) and the most common allele was (A) with a frequency of 46.5% and 67.3%, respectively. Similarly, the most common variant for UGT1A7*12 T/C genotype was the heterozygous repeat (TC) with a frequency of 78.7% while the mutant allele (C) was present in 60.6% of the study population. Both UGT1A8*3 (G/A) and UGT1A9*3 (T/C) showed only a wild homozygous pattern in all screened subjects. For UGT2B7*2, the heterozygous repeat (TC) was found with a frequency of 57.3% and the alleles (A) showed a frequency of 50.8%. In contrast, for UGT2B15*2 (G253T), the heterozygous repeat (TG) presented 62.3% of the subjects where the most common allele (G) was with a frequency of 66.2%. In conclusion, our data indicate that Saudis harbor some important

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UGT mutations known to affect enzyme activity. Additional studies are therefore, warranted to assess the clinical implications of these gene polymorphisms in this ethnic group.

1. Introduction

Glucuronidation is an essential metabolic process that is the basis of the detoxification of many drugs and other substances which are mainly taken as various edible forms. There is significant evidence that confirms that the drug elimination and other processes in detoxification display vast inter-individual differences, resulting in variability in both potency and toxicity. For example, during the past few years, multiple drugs used against lung cancer (Slatter et al., 1997; Senderowicz, 2000; Shapiro et al., 2001), colorectal cancer (Iyer et al., 1998; Ando et al., 2000), renal cancer (Innocenti et al., 2000), HIV (Zucker et al., 2001) and B-cell chronic lymphocytic leukemia (Chao and Price, 2001) have led to substantial toxic reactions owing to inter-patient differences in the processes of glucuronidation. This is not surprising, as it has been found that the families of Uridine diphospho-glucuronosyl-tr ansferases (UGTs) are highly polymorphic, and according to the norms of inheritance several monogenic features are predictors of toxicity. Therefore, determining the allelic frequency of these important genes will serve in explaining their role in drug disposition and toxicity.

UGTs are glycoproteins, present in endoplasmatic reticulum (ER) and nuclear membranes that convert many endogenous agents and xenobiotics to less active counterparts that are more water soluble by the conversion of aglycones to D-glucopyronosiduronic acids (glucuronides). In particular, the glucuronidation reactions catalyzed by UGTs are also responsible for clearance of endogenous substrates including thyroid hormones, steroid hormones, bilirubin and bile acids (Tukey and Strassburg, 2000). Consequently, changes in UGTs enzyme function may eventually affect clearance of, and therefore, systemic exposure to those compounds.

Different groups of UGT genes have been identified, each of which includes multiple genes. All of the UGT enzymes produced from these genes have a similar area that identifies UDP-glucuronic acid. UGT1A, on the long arm of chromosome 2 in humans, consists of at least 9 promoters and first exons that can be spliced with four common exons to produce UGT1A1–UGT1A9. The UGT2 family, on the other hand, is divided into 2A (three genes) and 2B (seven genes) subfamilies on chromosome 4. Several functional polymorphisms in UGT 1A and 2B subfamilies are associated with altered glucuronidation activity of important endogenous compounds and clinically used drugs (Tukey and Strassburg, 2001).

UGTs are expressed in a tissue specific fashion in humans, which enables most of the tissues to form glucuronides. Because of the tissue specific regulation of these proteins, each tissue contains a distinct pattern of UGT proteins (Tukey and Strassburg, 2000, 2001). This suggests that the beneficial properties of UGTs in different tissues may have evolved over a period of time to meet the unique challenges essential for glucuronidation. This is best illustrated by the extra-hepatic expression of UGT1A7 (Strassburg et al., 1997), UGT1A8 and UGT1A10 (Strassburg et al., 1998a,b), all of which are present in various tissues of the intestinal tract. Since UGTs are present in a high concentration in the intestinal tract (Tukey and Strassburg, 2001; Strassburg et al., 1998b, 1999), they are thought to play a significant role in the first pass metabolism, and therefore variations in function emerging from pharmacogenetic differences may determine systemic drug levels and therapeutic outcome.

Given the clinical importance of certain UGTs polymorphisms, the focus of this study was to investigate the frequencies of UGT1A6*2, A7*12, A8*3, A9*3, 2B7*2, and 2B15*2 in Saudi Arabians and thus therefore providing essential information on this specific ethnic group. This should also shed some light on the clinical implication of these mutations in relation to disease occurrence and therapeutic efficacy and toxicity of drugs known to be metabolized by these variants.

2. Materials and methods

2.1. Human subjects

A total of 192 apparently healthy unrelated Saudi male volunteers (20–25 year-old) of various geographic regions were recruited to the study from King Saud University, Riyadh, Saudi Arabia. The study's objectives were explained and one time venous blood sample (~20 ml) was obtained in EDTA tubes from each subject after obtaining written informed consent from all participants. The ethical approval of the study was granted by the Institutional Review Board of the College of Medicine, King Saud University, Riyadh, Saudi Arabia.

2.2. Genetic testing

DNA extraction was carried out using Puregene Blood Core Kit C (Qiagen, Germantown, MD, USA) following manufacturer's instructions and quantified using Nanodrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The indicated polymorphic variants were amplified in a Veriti® 96-Well Fast Thermal Cycler (Applied Biosystems, Foster City, CA, USA) in a total volume of 25 µl, containing 20 ng DNA, 0.25 µl (2.5 mM) of dNTPs (Epicentre Biotechnologies, Madison, WI, USA), 2 µl (10 pM) of primers (Metabion, Martinsried, Germany) and 0.3 µl (5 U/µl) of Hotstar Taq DNA polymerase (Qiagen, Germantown, MD, USA). For PCR, an initial denaturation step at 95 °C for 10 min was followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at the indicated temperature for 40 s, and extension at 72 °C for 45 s, followed by a final extension step of 72 °C for 10 min. Primers' sequences are listed in Table 1 with their respective annealing temperature of 56 °C. The PCR amplicons were evaluated by 2% agarose gel electrophoresis and then purified using MCE-membraned Multi-Screen plate (Millipore, Billerica, MA, USA) pre-packed with G-50 superfine cephadex (GE Healthcare, Piscataway, NJ, USA). The purified