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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

Table 1 UGT allele frequencies with primers' sequences used in the study.

Genotype	Protein	Coding nucleotide change	Amino Acid Change	Allele (wild type) Frequency		Primer	Oligonucleotide sequence
				A	*B*		
UGT1A6*2	UGT1A6.2	A541G	Thr181Ala	0.673	0.673 – Caucasian (American) 0.821 – Mixed population	Forward primer Reverse primer	GGAGAGCAAGTTTGATGCTCTT CAGCTGATGCGAGTTCTTCA
UGT1A7*12	UGT1A7.12	T622C/ C760T	W208R/ R254X	0.606	0.420 – Caucasian (American) 0.380 African (American)	Forward primer Reverse primer	GTGCCCTGCTCCTCTTTCCTAT ACGGGTTTGGGATACTCCAAA
UGT1A8*3	UGT1A8.3	G830A	C277Y	00	0.551 Caucasian (American) 0.760 Caucasian (French Canadian)	Forward primer Reverse primer	GGTATCAACTACCATCAGG CCCTGATGGTAGTTGATACC
UGT1A9*3	UGT1A9.3	T98C	M33T	00	0.063 – Caucasian (American) 0.09 – African (American) 0 – Asian	Forward primer[a] Reverse primer	CCTGCTCTCAGCTGCAGTTCTCT CTTCACTGTGCAATTCAGTGATCTT
UGT2B7*2	UGT2B7.2	C802T	Y268H	0.508	0.460 – Caucasian (American) 0.730 – Asian (Japanese)	Forward primer Reverse primer	GTAAATATCTGTGTCATC GACTATAGAATCATTTCTACTG
UGT2B15*2	UGT1B15.2	G253T	D85Y	0.662	0.448 – Caucasian (American) 0.614 – African (American) 0.630 – Hispanic (American)	Forward primer Reverse primer	GAGCTTGTTCAGAGGGGTCA CAAAACTGCATCTTTACAGAGCTT

A = Saudi Population (Current Study); B = Other Populations.
[a] Also detect G8A mutation.

PCR amplicons were then sequenced by dye termination sequencing using BigDye Terminator Cycle Sequencing V3.1 Kit and 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). DNA sequences were analyzed using the Seqman program of the DNASTAR analysis package (Lasergene, Madison, WI, USA).

2.3. Statistical analysis

Frequencies of the studied polymorphisms are expressed as percentage with 95% confidence interval (95% CI). Data were analyzed using Microsoft Excel® Program (Microsoft Inc., Seattle, WA, USA).

3. Results

Allele and genotype frequencies of UGT1A6*2, 1A7*12, 1A8*3, 1A9*3, 2B7*2, and 2B15*2 are given in Table 2. The most common variant for UGT1A6*2 A/G genotype was the homozygous repeat (AA) with a frequency of 46.5% and the most common allele was (A) with a frequency of 67.3%. For UGT1A6*2 A/C, the most common variant for this genotype was the heterozygous repeat (AC) with a frequency of 45.3% and the most common allele was (A) with a frequency of 65.2%. Similarly, the most common variant for UGT1A7*12 T/C genotype was the repeat (TC) with a frequency of 78.7%, while the most common allele was (C) with a frequency of 60.6%. Both the homozygous (CC) and the heterozygous repeats (CT) of UGT1A7*12 C/T had almost same frequency with the homozygous (CC) repeat having a frequency of 50.3% while the heterozygous repeat (CT) had a frequency of 49.7%. The most common allele was (C) with a frequency of 75.1%. For UGT1A8*3 G/A and UGT1A9*3 T/C, both these genotypes showed only homozygous pattern in all the screened subjects.

For UGT2B7*2 (C802T), the most common variant was the heterozygous repeat (TC) with a frequency of 57.3% and both the alleles (C) and (T) showed a similar frequency of 50.8% and 49.2%, respectively. UGT2B15*2 (G253T) showed the heterozygous repeat variant (TG) with a frequency of 62.3% and the most common allele found was (G) with a frequency of 66.2%.

4. Discussion

There is substantial evidence now which implies that glucuronidation can be attributed to inter-individual and inter-ethnic variations (Burchell et al., 2000). The UGT family of genes is extremely diversified. This can be best illustrated by the UGT1A1 gene, which contains over 50 genetic lesions

Table 2 Allele and genotype frequencies of the studied UGT polymorphisms in a Saudi population.

UGT1A6*2(R)A/G	AA	GG	AG		Total
N	86	19	77		185
N% (95% CI)	46.5 (39.3, 53.7)	10.3 (5.9, 14.6)	41.6 (34.5, 48.7)		
Allele	A	G			
N	249	115			370
N% (95% CI)	67.3 (62.5, 72.1)	31.1 (26.3, 35.8)			
UGT1A6*2(R)A/C	AA	CC	GG	AC	Total
N	80	22	1	85	188
N% (95% CI)	42.5 (35.45, 49.6)	11.7 (7.1, 16.3)	0.5 (0.01, 2.9)	45.3 (38.1, 52.3)	
Allele	A	C	G		
N	245	129	2		376
N% (95% CI)	65.2 (60.3, 69.9)	34.3 (29.5, 39.1)	0.5 (0.06, 1.9)		
UGT1A7*12(R)T/C	CC	TC			Total
N	40	148			188
N% (95% CI)	21.3 (15.4, 27.1)	78.7 (72.8, 84.6)			
Allele	C	T			
N	228	148			376
N% (95% CI)	60.6 (55.7, 65.6)	39.4 (34.4, 44.3)			
UGT1A7*12(R)C/T	CC	CT	TT		Total
N	94	93	00		187
N% (95% CI)	50.3 (43.1, 57.4)	49.7 (42.5, 56.9)	00		
Allele	C	T			
N	281	93			374
N% (95% CI)	75.1 (70.7, 79.5)	24.9 (20.5, 29.2)			
UGT1A8*3(F)G/A	GG	GA	AA		Total
N	190	00	00		
N% (95% CI)	100	00	00		
Allele	G	A			
N	380	00	00		
N% (95% CI)	100	00	00		
UGT1A9*3(R)T/C	TT	TC	CC		
N	188	00	00		
N% (95% CI)	100	00	00		
Allele	T				
N	376				
N% (95% CI)	100				
UGT2B7*2(C802T)	CC	TC	TT		Total
N	41	106	38		185
N% (95% CI)	22.2 (16.1, 28.1)	57.3 (50.1, 64.4)	20.5 (14.7, 26.4)		100
Allele	C	T			
N	188	182			370
N% (95% CI)	50.8 (45.7, 55.9)	49.2 (44.1, 54.3)			100
UGT2B15*2(G253T)	GG	TG	TT		Total
N	67	119	5		191
N% (95% CI)	35.1 (28.3, 41.8)	62.3 (55.4, 69.2)	2.6 (0.35, 4.8)		100
Allele	G	T			
N	253	129			
N% (95% CI)	66.2 (61.5, 70.9)	33.8 (29.0, 38.3)			100

(Kadakol et al., 2000), most of which impact its functional properties as well as its expression.

UGT1A6*2 mutation influences aspirin metabolism, while UGT1A7*12 may be used to predict mycophenolic acid (MPA) clearance. UGT1A6*2 codes for an enzyme compress its catalytic activity against many UGT1A6 substrates. UGT1A6 also plays a significant role in the metabolism of aspirin and other non-steroidal anti-inflammatory agents (NSAIDs). UGT1A6*2 has also been shown to influence the protective effect of aspirin (Bigler et al., 2001). It has been

found that 87% of individuals, who are homozygous for the UGT1A1*28 allele, which is present in individuals with Gilberts syndrome, are also homozygous for the UGT1A6*2 allele (Wilbert-HM et al., 2003). This suggests that due to the presence of both UGT1A1*28 and UGT1A6*2 genotypes, such individuals may have a reduced capability to glucuronidate bilirubin.

The UGT1A7 allelic polymorphism may change individual vulnerability to cancer by decreasing the capacity to detoxify. It has been shown that UGT1A7*12 causes a 70% decline in the transcriptional performance (Iyer et al., 1998). There have been many SNP association studies which have demonstrated that there is an association of homozygous UGT1A7*12 in 75% individuals with homozygous UGT1A1*28 (Wilbert-HM et al., 2003).

Our data indicate a considerable number of Saudis harbor both these polymorphisms, UGT1A6*2 and UGT1A7*12, which suggest they may be at a risk of developing Gilbert's syndrome. It has also been shown that the amalgam of different variations of UGT1A1 and UGT1A7 can be used to anticipate irinotecan toxicity (Lankisch et al., 2008). Allelic differences in any of the UGT loci can give rise to important biological modifications in drug metabolism ability and in some enzymes that are recognized as glucuronidate carcinogens, an absence of action may play a significant role in the etiology of a carcinogenic incident.

The UGT1A8 is a very rare form of polymorphism and is expressed entirely in the extra-hepatic tissues of the gastrointestinal tract (Tukey and Strassburg, 2000, 2001). It has been found that UGT1A8 plays a role in the metabolism of dietary and environmental carcinogens (Mojarrabi and Mackenzie, 1998; Nowell et al., 1999; Cheng et al., 1998, 1999). UGT1A8*3 has been classified as a low-activity protein on various substrates, including a reduction in MPAG (MPA glucuronide metabolite) formation (Huang et al., 2002). This indicates that it is integral for enzyme function and substrate binding. However, studies have shown that UGTlA8*3 is very rare in humans, which can be considered as an useful marker in evaluating for colorectal cancer. Since our data indicate that Saudis do not harbor any allelic variation for this genotype, it concurs with other studies which report this as a very rare form of polymorphism in other populations around the world as well.

UGT1A9*3 causes decreased glucuronidation of mycophenolic acid (Bernard et al., 2004), 4-hydroxyestrone, 4-hydroxyestradiol (Thibaudeau et al., 2006) and reduced activity of propofol glucuronidation (Girard et al., 2004). Studies have shown the genetic variant in codon 33 of UGT1A9 may help in predicting the individual's vulnerability to the toxicity of drugs, their clearance rate and side effects. Genetic polymorphisms of UGT1A9 gene are very rare among populations (Villeneuve et al., 2003). Our data demonstrate the lack of polymorphism for this genotype in Saudi population as well. Hence, UGT1A9*3 is likely to be a clinically insignificant polymorphism in Saudis. However, additional analysis of functional properties of this polymorphism may help to determine the metabolizer phenotype in populations, which may help in optimizing the drug dose and development of personalized medicine in the future.

UGT2B7 is involved in the glucuronidation of catechol estrogens, bile acids, morphine, MPA, oxazepam and zidovudine with overlapping substrate specificities (Mackenzie et al., 2003). Additionally, UGT2B7*2 allele is affiliated with defective morphine glucuronidation in vitro and hence, homozygous infants with the UGT2B7*2 allele, may be at an enhanced risk of potential life-threatening CNS depression after codeine treatment (Coffman et al., 1997; Ishii et al., 1997). In fact, UGT2B7 exclusively catalyzes the glucuronidation of codeine, morphine and zidovudine (AZT) (Barbier et al., 2000) and non-drug xenobiotic substrates including hydroxylated derivatives of the prototypic carcinogens 2-acetylaminofluorene and benzo[a]-pyrene. Despite being primarily involved in the detoxification of xenobiotic and endogenous substrates, UGT2B7 also plays a vital role in forming bioactive or even toxic compounds like the highly cholestatic D-ring glucuronides of estrogens and the acyl-glucuronides of drugs such as diflunisal that binds to proteins and triggers toxic immunological responses (Worrall and Dickinson, 1995). Various studies have demonstrated that UGT2B7*2 polymorphism nominally impacts enzyme activity and substrate specificity of UGT2B7 (Coffman et al., 1998; Holthe et al., 2002a,b; Bhasker et al., 2000). However, a wide inter-individual variance in the ability to glucuronidate morphine (McQuay et al., 1990; Klepstad et al., 2000; Faura et al., 1998) and AZT (Mentre et al., 1993) suggests that this or other polymorphisms in UGT2B7 may contribute to morphine metabolism variability.

UGT2B15*2 glucuronidates many drugs such as oxazepam, lorazepam and rofecoxib. Our data indicate that heterozygous repeat of UGT2B15*2 accounts for about 62% of the study population. Studies have shown that prostate cancer patients are significantly more likely to be homozygous for the lower activity UGT2B15*2 allele than control individuals (Holthe et al., 2000a,b) Homozygous repeat alleles represent increased risk of prostate cancer associated with this low activity variant. Therefore, our data indicate that Saudis are at a low risk of being afflicted with prostate cancer.

5. Conclusion

This study indicates that Saudi population harbors some important UGT mutations which can affect enzyme activity. Several functional polymorphisms in UGT 1A and 2B subfamilies are associated with altered glucuronidation activity of important endogenous compounds and clinically used drugs. These variations may assist in changing the pharmacokinetic profile of a drug as well as to the etiology of a possible toxic reaction. The current findings also support the need to evaluate UGT polymorphism frequencies within the populations of specific diseases and within patient clinical covariates to understand the contribution in disease pathogenesis and response to drug therapy.

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