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The Frequency of UGT2B7*2 (802C>T) Allele among Healthy Unrelated Jordanian Volunteers

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Abstract

The UDP-Glucuronesyl Transferase (UGT) 2B7*2 802C>T is the most common functional genetic variant on UGT2B7 gene with reported influence on drug response.

Objective: To determine the frequency of UGT2B7*2 (802C>T) genetic variant among Jordanian population.

Methods: The DNA samples from 90 healthy Jordanian volunteers were analyzed by polymerase chain reaction followed by restriction fragment length polymorphism assays (PCR-RFLP) to determine the UGT2B7*2 allele.

Results: The frequency (95% confidence interval) of UGT2B7*2 802C allele was 0.59 (0.52 to 0.66) and for UGT2B7*2 802T was 0.41 (0.34 to 0.48), which was similar to the Caucasian but different than African ethnic populations. The UGT2B7 genotype frequency among Jordanians was 0.31 (0.22 to 0.4) for wild, 0.57(0.47 to 0.67) for heterozygote and 0.12 (0.06 to 0.18) for homozygote UGT2B7*2 genotype.

Conclusion: This study reported for the first time the UGT2B7*2 frequency and genotype profile among Jordanians. The findings of this study will increase our understanding in explaining the inter-individual variation in drug response among Jordanian population.

Keywords: UGT2B7; Genetic Variant; Jordanians; UDP-Glucuronosyltransferase

Introduction

UDP-Glucuronosyltransferase 2B7 (UGT2B7) is phase II drug metabolizing enzyme. It has a major role in the glucuronidation of endogenous compounds such as estrogen, aldosterone, bile acids, retinoid and fatty acids [1]. In addition, this UGT isoform metabolizes around 10% of the total prescribed drugs. It metabolizes opioids, the Nonsteroidal Anti-Inflammatory Drug (NSAIDs), the antiviral zidovudine and the anticonvulsant valproic acid [1-3]. The glucuronidation of these drugs mainly inactivates and enhances their elimination through converting them to more hydrophilic chemicals. However, it is reported that glucuroronitaed morphine is 100 time more effective than the morphine itself [4,5].

The UGT2B7 is predominantly expressed in the liver, but tissue distribution analysis has also demonstrated UGT2B7 expression in the gastrointestinal tract, kidney, pancreas and brain [6].

There is an inter-individual variation in the glucuronidation activity of UGT2B7. The activity of UGT2B7 is influenced by the age, gender, disease and the genetic polymorphism. The UGT2B7 is encoded by UGT2B7 gene. This gene is localized at chromosomal position 4q13 and contains six exons spanning nearly 16 kb [1].

The UGT2B7*2 (802C>T) allele is the most common UGT2B7 genetic allele with significant effect on UGT2B7 glucuronidation capacity and drug response [7]. The 802C>T variant on UGT2B7 gene is a non-synonymous exonic genetic variant leads to the substitution of histidine to tyrosine in codon 268 [7]. Although *in vitro* study showed that UGT2B7*2 802C>T genetic variant increased the UGT2B7 activity, multiple *in vivo* clinical studies reported that UGT2B7*2 genotyped volunteers had less UGT2B7 activity than the wild type [8]. In comparison with the wild type UGT2B7 genotype, it has been shown that UGT2B7*2 genotype altered clinically the metabolism and drug response of epirubicin, morphine and diclofenac [9-12]. The UGT2B7*2 allele was also found to affect the conjugation of the endogenous arachidonic acid metabolite; 20-hydroxyeicosatetraeonic acid which may contribute the variation in the cardiovascular homeostasis [8].

There is a variation in the frequency of UGT2B7*2 genetic variant among different ethnic groups. The frequency of UGT2B7*2 is higher among Caucasians (50%) than Asian Japanese (29%) and Africans (21%). This inter-ethnic variation may contribute to different drug response among different ethnic groups [7].

Although the UGT2B7*2 genetic variant was investigated in many ethnic populations, there is lack of information regarding the UGT2B7*2 genetic allele among Jordanian population. Therefore, the present study aimed to find the frequency of UGT2B7*2 genetic variant among unrelated healthy Jordanian volunteers.

Method

Sample Collection

A total of 90 healthy unrelated Jordanian volunteers (30 males and 60 females), with an average age ± standard deviation of (23 years ± 3 years) agreed to participate in the study and signed an informed consent. From each volunteer, 2 ml venous blood was collected in EDTA tubes. Individuals were unrelated Jordanian, healthy with no serious or chronic disease. Most of the volunteers were Al-Zaytoonah University students.

DNA Extraction

DNA was extracted using Wizard® Genomic DNA purification kit (Promega, Madison, WI, USA) according to the manufacture protocol.

Briefly, the whole blood sample was incubated with cell lysis solution. After obtaining the nucleus pellet, a nucleus lysis solution was added to the pellet and mixed. Then, a protein precipitation solution was added to the nucleus lysed samples and mixed vigorously. After that, the DNA was precipitated by isopropanol and washed with 70% ethanol and lastly dissolved in nuclease free water. All DNA samples were stored at -20˚C until were used.

Amplification of UGT2B7 Gene

A specific region on UGT2B7 gene was amplified using polymerase chain reaction (PCR) method as described before [13]. Briefly, 100 ng of genomic DNA was amplified in a 50 µl reaction volume containing 1 µl of 10 mMdNTPs, 2 µl of 25 mM $MgCl_2$, 10 µl of green Taq polymerase buffer, 2 µl of 10 pmole/µl from each of the forward (5' TGCCTACACTATTCTAACC 3') and reverse (5' TCTCTGAAAATTCTGCACT 3') primers (Macrogen, South Korea) and 1 unit of Taq DNA polymerase. The thermal cycles were as followings; initial denaturation at 94°C for 5 min, 35 cycles were performed consisting of denaturation at 94°C for 1 min, annealing at 57°C for 1 min and enzyme extension at 72°C for 1 min, followed by final step of elongation at 72°C for 7 min. The PCR product size was 381 bp, as shown on Figure 1.

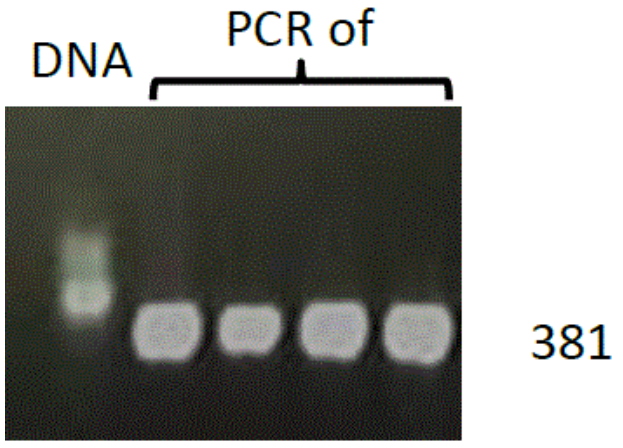

Figure 1: Represented gel-electrophoresis of PCR product amplification of UGT2B7 gene.

Restriction fragment length polymorphism

Ten µl of PCR product was then incubated with 5 units of Fok1 restriction enzyme (Bio labs, England) at 37°C for overnight. The wild-type allele (UGT2B7*1/*1) identified by 2 bands with 312, 69 bp size. Homozygous UGT2B7*2/*2 was identified by 3 bands (201, 111 and 69 bp size) while heterozygous UGT2B7*1/*2 genotype was identified by 4 bands (312, 201, 111 and 69 bp size). As shown on Figure 2, the digested PCR product was separation of on 3% agarose gel for detection of rs2108622 variation after gel staining with ethidium bromide.

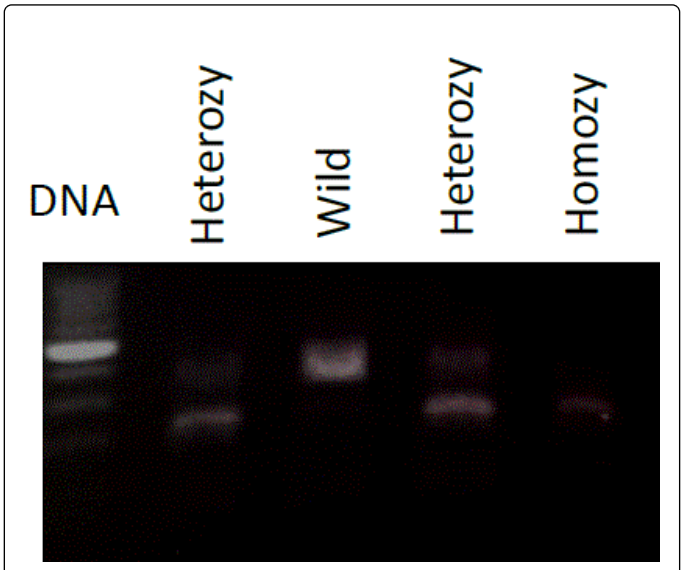

Figure 2: Represented gel-electrophoresis of UGT2B7*2 genotype among sample of healthy Arab Jordanian volunteers.

Statistical Analysis

The deviation from hardy-Weinberg equation was done using χ^2 test. The comparison between the allele frequency among Jordanians with other ethnic groups was done using Z-test, p-value <0.05.

Results

The results showed that the allele frequency of UGT2B7 802C was 0.59 (95% CI of 0.52-0.66) while the UGT2B7 802T allele was 0.41 (95% CI of 0.34-0.48) as shown in Table 1. In comparison with the major ethnic groups, the frequency of UGT2B7 802 C>T among Jordanians was similar to Caucasian (0.52) but significantly higher (z test, p-value <0.05) than African (0.21) and Asian (0.29), as represented on Table 2.

The frequency of wild UGT2B7 802C/C genotype was 0.31, while the heterozygote 802C/T and the homozygote 802T/T were 0.57 and 0.12, respectively (Table 3). The UGT2B7802C>T genotype frequency was within Hardy-Weinberg (χ^2 test, p value >0.05), which indicates that there is no genetic shift in the frequency of UGT2B7802C>T genotype in Jordanians.

Discussion

The UGT2B7 drug metabolizing enzyme plays a major role in endogenous and xenobiotic metabolism [1]. There is a significant inter-individual and ethnic variation in drug glucuronidation [16]. Therefore, in this study investigated for the first time the prevalence of UGT2B7*2 allele among Jordanian population. We found a high frequency of this allele which may effect on drug response and susceptibility to toxicity in Jordanians.

95% confidence interval	Frequency observed	Allele
0.52-0.66	0.59	C
0.34-0.48	0.41	T
	1	Total

Table 1: The allele frequency of UGT2B7*2 802C>T genetic variant among healthy Jordanian population.

Reference	Different than Jordanian (Z test, p-value <0.05)	UGT2B7*2 802T frequency	UGT2B7*2 802C frequency	Ethnic group
[7]	NO	0.52	0.48	Caucasian (White American)
[7]	Yes	0.21	0.79	African (West African)
[14]	Yes	0.28	0.72	Asian (Japanese)
[15]	Yes	0.28	0.72	Hispanic–American

Table 2: The comparison between The UGT2B7*2 802C>T allele frequencies among healthy Jordanians with other ethnic populations.

χ^2 test †	Predicted number (frequency)	Observed number (frequency)	Genotype
>0.05	32 (0.36)	28 (0.31)	Wild (C/C)
	44 (0.49)	51 (0.57)	Heterozygote (C/T)
	14 (0.15)	11 (0.12)	Homozygote (T/T)
	90 (1)	90 (1)	Total

Table 3: The UGT2B7*2 802C>T genotype frequency among healthy Jordanian population. †All of UGT2B7*2 802C>T genotype frequencies were within Hardy-Weinberg equation.

It is found that UGT2B7*2 affected on the inactivation of hydroxyecosterionic acid, a cardiotoxic metabolite of arachidonic acid [8]. The prevalence of the cardiovascular diseases is high among Jordanians [17]. The high frequency of UGT2B7*2 among Jordanians may play a role in the cardiovascular diseases induced by high plasma concentration of hydroxyecosterionic acid. Further investigations are needed to find the association of UGT2B7*2 with Jordanian cardiovascular patients.

However the size of the sample was relatively small, the statistical power of the test (beta=80%) indicates that sample size of 88 volunteers is enough to study the high frequent UGT2B7*2 variant among Caucasian populations. The present study used 90 samples from unrelated volunteers to determine the frequency of UGT2B7*2. Therefore, the sample size of the current study was statistically enough to conclude that UGT2B7*2 variant is high among Jordanian population.

In Jordan, It is found that valproic acid was associated with polycystic ovarian syndrome among epileptic patients [18]. It is reported that UGT2B7*2 affected the glucuronidation of valporic acid [8]. Therefore, it is suggested that UGT2B7*2 may effect on the predisposition of valporic acid on polycystic ovarian syndrome among epileptic patients.

Jordan is an Arabic kingdom in Western Asia. The country's location was part of several kingdoms and empires during the centuries. Because of that middle location, Jordan had served as a connecting between Asia, Africa and Europe and different ethnic groups came into Jordan during the centuries which might produce the variety of the genetic profile in the Jordanian population [19]. The majority of Jordanian population is from Arabic tribes which came from Arabic peninsula. However, the Jordanian population was mixed with other ethnic groups, such as Circassia's, Armenian and Kurds, in addition to the Iraqi and Syrian refugees. In this study, we made sure that the volunteers were originally from the Arabic Jordanian tribes and excluded other mixed groups. Arabs are considered as Caucasians and the results of this study showed that UGT2B7*2 803C>T allele frequency among Jordanians was similar to Caucasians but different than among Africans, Asians and Hispanics.

Conclusion

We reported for the first time the frequency of UGT2B7*2 803C>T genetic variant among Jordanian population which may increase our understanding of the inter-individual variation in drug response and susceptibility to disease in Jordanians.

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