Gene 634 (2017) 47–52

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

# Genetic variations in UGT2B28, UGT2B17, UGT2B15 genes and the risk of prostate cancer: A case-control study

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ARTICLE INFO

*Keywords:*
Prostate cancer
Benign prostatic hyperplasia
UGT2B family
Single-nucleotide polymorphism
Copy number variation
Androgen

ABSTRACT

Glucuronidation is a major pathway for elimination of exogenous and endogenous compounds such as environmental carcinogens and androgens from the body. This biochemical pathway is mediated by enzymes called uridine diphosphoglucuronosyltransferases (UGTs). Null (del/del) genes polymorphisms in *UGT2B17*, and *UGT2B28* and D85Y single-nucleotide polymorphism (SNP) of *UGT2B15* have been reported to increase the risk of prostate cancer. The goal of this study was to determine the association of mentioned genetic variants with the risk of prostate cancer.

We investigated the copy number variations (CNVs) of *UGT2B17* and *UGT2B28* loci and the association between rs1902023 polymorphism of *UGT2B15* gene in 360 subjects consisted of 120 healthy controls, 120 prostate cancer (PC) patients and 120 benign prostatic hyperplasia (BPH) patients. No association was detected for the mentioned polymorphisms and the risk of PC. However, a significant association was detected between *UGT2B17* copy number variation and BPH risk (OR = 2.189; 95% CI, 1.303–3.675; p = 0.003). Furthermore, we observed that the D85Y polymorphism increases the risk of BPH when analyzed in combination with the copy number variation of *UGT2B17* gene (OR = 0.135; 95% CI, 0.036–0.512; p = 0.003).

Our findings suggest that the D85Y polymorphism of *UGT2B15* and CNVs in *UGT2B28* and *UGT2B17* genes is not associated with prostate cancer risk in Iranian patients. To our knowledge, this is the first report that implicates the role of CNV of *UGT2B17* gene in BPH.

## 1. Introduction

Mainly, prostate diseases can be divided into three groups: 1) prostate cancer, 2) benign prostatic hyperplasia, and 3) prostatitis. Prostate cancer, which is the third most common cancer among Iranian males (Pakzad et al., 2016), is considered as the second most common malignancies in men all around the world and is recognized as a major public health problem (Cimino et al., 2016). BPH is the most prevalent age-related pathophysiological conditions of prostate gland, occurring approximately 80% of men over age 70 years (Roehrborn, 2005; Taheri et al., 2017b).

It has been recognized for many years that androgenic steroid hormones, including testosterone and dihydrotestosterone (DHT), are important for prostate growth and function (Wilding, 1991; Khvostova et al., 2015). Hence, androgenic hormone imbalance is considered to be an important risk factor for both mentioned diseases (Bauman et al., 2006; Cuzick et al., 2014; Kpoghomou et al., 2013). Levels of these hormones are influenced by individual genetic predisposition and environmental factors (Kral et al., 2011). So far, many studies have been done on genetic variations related to steroid hormone metabolism to clear their role in prostate cancer. In recent years, a growing body of evidences proposed that uridine UGTs genes are involved in the androgenic hormone metabolism (BELANGER et al., 2003; Labrie et al., 2006; Belledant et al., 2016; Gauthier-Landry et al., 2015). These genes encode enzymes that are responsible for the process of glucuronidation, a major androgen metabolic pathway (SCHäNZER, W., 1996;

*Abbreviations:* UGT, uridine diphosphoglucuronosyltransferases; 3α-DIOL, androstane -3α-DIOL; ADT, androsterone; DHT, dihydrotestosterone; BPH, benign prostatic hyperplasia; PC, prostate cancer; CNV, copy number variation; SNP, single-nucleotide polymorphism; Vmax, maximal velocity

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http://dx.doi.org/10.1016/j.gene.2017.08.038
Received 16 February 2017; Received in revised form 27 July 2017; Accepted 30 August 2017
Available online 04 September 2017
0378-1119/ 

BELANGER et al., 2003). The glucuronidation reaction consists of transfer of the glucuronosyl group from UDP-glucuronic acid to active androgen (DHT) and inactive metabolites of DHT, androsterone (ADT) and androstane -3α-DIOL (3α-DIOL) (Gauthier-Landry et al., 2015).

The UGT2B subfamily has seven functional enzymes (Mackenzie et al., 2005). According to previous investigations, three UGT2B classes of enzymes (UGT2B15, UGT2B17, and UGT2B28) (Turgeon et al., 2001) are primarily involved in the metabolism of steroids and wide variety of endobiotic and xenobiotic substrates (Nagar and Remmel, 2006; Miners and Mackenzie, 1991). Recently, a series of case-control studies have investigated the association of these gene polymorphisms with the risk of PC (Grant et al., 2013; Vidal et al., 2013).

A common genetic polymorphism in *UGT2B15*, D85Y, that includes G-to-T point mutation, resulting in an aspartate to tyrosine substitution in codon 85 (D85Y) (Levesque et al., 1997) has shown altered androgen clearance in prostate (Chouinard et al., 2008; Vidal et al., 2013). In comparison to Y85 variant, D85 variant has a lower Vmax of glucuronidation for its substrates 3α-DIOL and DHT (Hajdinjak and Zagradišnik, 2004; Grant et al., 2013; Jakobsson et al., 2006; Levesque et al., 1997; Chouinard et al., 2008). Some of previous studies (Grant et al., 2013; MacLeod et al., 2000; Park et al., 2004) have shown that DD genotype could increase the risk of prostate cancer by increasing higher exposure of the gland to androgen hormones. Whereas, others did not. Thus, to explain these contradictory results, Xiao Zhong and colleagues (Zhong et al., 2017) performed a meta-analysis and suggested that the *UGT2B15* D85Y gene variant is protective against prostate cancer. Based on this report, the plausible hypothesis that explains the protective mechanism is that the variant may has enhanced ability to metabolize DHT and thus reduce the risk of prostate cancer by decreasing the testosterone levels.

The findings show that another type of germ line polymorphism, the Copy number variation (CNV), play an important role in human diseases (Zhang et al., 2009; Furuya et al., 2015). Germ line CNV as a functional polymorphism in *UGT2B17* and *UGT2B28* has been proposed to be a possible PC risk factor. (Karypidis et al., 2008; Park et al., 2006) However, little attention has been paid to the potential role of CNV of *UGT2B28* in the risk of prostate cancer. Previous findings revealed that the urine testosterone concentrations is different between subjects stratified by *UGT2B17* CNV genotypes, where lower mean concentration was observed in people with two deleted *UGT2B17* alleles in comparison to those who carry one copy of the gene (Jakobsson et al., 2006; Juul et al., 2009).

In overall, mentioned UGT2B variants have been evaluated in relation to prostate cancer risk, with inconsistent findings (Gsur et al., 2002; Hajdinjak and Zagradišnik, 2004; MacLeod et al., 2000; Park et al., 2004; Park et al., 2007; Setlur et al., 2010; Park et al., 2006; Olsson et al., 2008; Karypidis et al., 2008). For example, some studies have shown that null genotype of *UGT2B17* gene is linked to increased risk of PC in African-American and Caucasian races (Park et al., 2006; Karypidis et al., 2008). Whereas some other investigators have shown no association (Gallagher et al., 2007; Vidal et al., 2013). Discrepancies could have been due to the genetic heterogeneity of the populations studied, as well as variable sample sizes of these populations (Vidal et al., 2013).

Unlike prostate cancer, almost no publication is available in the literatures to discuss for the potential association of *UGT2B* genetic polymorphisms with the risk of BPH. The current case-control study was carried out to determine the potential association of *UGT2B15*, *UGT2B17*, and *UGT2B28* genetic polymorphisms with the risk of PC by comparing the genotypes in the three under study groups of PC patients, BPH and healthy controls.

## 2. Materials and methods

### 2.1. Subjects

In a case-control study, 360 peripheral blood samples including 120 samples from PC patients, 120 samples from BPH patients and 120 samples from healthy individuals were recruited from Shohadai-E-tajrish, Labbafinezhad, and Vali-e-asr hospitals. All participants provided written consent and a personal questionnaire covered smoking consumption, body mass index (BMI), PSA level, family history of cancer (All subjects in this research had not family history of Prostate cancer) and detailed medical history.

Patients with PC were selected by using standard clinical procedure of diagnosis including biopsy of the prostate tissue. The exclusion criteria for BPH patients were prostate volume smaller than 25 $cm^3$. In control subjects, health status was based on normal result of Digital Rectal Examination, PSA levels < 4 ng/ml with no lower urinary tract symptoms (LUTS). All subjects were older than 45 years old and were aged matched in the studied groups. Also, all patients who received hormonal treatment or radiotherapy before radical prostatectomy were excluded from the study. The present study was approved in the ethics committee of Shahid Beheshti University of Medical Sciences (IR.sbmu.msp.REC.1395.171).

### 2.2. Genotyping of the UGT2B15 D85Y polymorphism

Genomic DNA from peripheral blood samples were obtained using salting out method. The presence of D85Y polymorphism which converts aspartic acid (D) to tyrosine (Y) at position 85 was investigated by PCR-RFLP method, using forward primer 5′CTGTGGAAAGGTGCTAGT3′ and reverse primer 5′ GAATTTTCAGAA GAGAATCTTCCAGAT3′ (Bioneer, South Korea) (Hajdinjak and Zagradišnik, 2004). The reactions were carried out using Ampliqon Taq 2 × red master mix (Ampliqon, Denmark). The thermal program consisted of an initial denaturing at 95 °C for 5 min, followed by 30 cycles at 95 °C for 30 s; 52.5 °C for 45 s; and 72 °C for 30 s with a final extension at 72 °C for 7 min. The expected length of PCR products was 215 base pair (bp). 5 μl of each PCR product was digested with 1 U of *Sau*3AI restriction enzyme (Fermentas, Hunover, MD, USA) at 37 °C overnight. Digested products were separated on 12% polyacrylamide gel electrophoresis (PAGE). The expected fragments resulting from the restriction digestion were 215-bp bands that attributed to the *UGT2B15* 85YY. The appearance of three different fragments of 215/187/28-bp bands corresponded to the *UGT2B15* 85DY, and two fragments of 187/28-bp showed to be corresponded to the *UGT2B15* 85DD.

### 2.3. Genotyping of the UGT2B17 copy-number variation

In order to detect the CNV in *UGT2B17* gene, to pairs of primers were used, including *UGT2B17*-forward: 5′-TGAAAATGTTCGA TAGATGGACATATAGTA-3′ and *UGT2B17*-reverse: 5′-GACATCAAATTTTGACTCTTGTAGTTTTC-3′ primers (located in the known deleted region and produce a 173 bp PCR product in the absence of deletion); and *UGT2B17*-del-forward: 5′-TGCACAGAGTTA AGAAATGGAGAGATGTG-3′ and *UGT2B17*-del-reverse: 5′-GATCATCCT ATATCCTGACAGAATTCTTTTG-3′ primers (located outside of the known deleted region and produce a 893 bp PCR product in the presence of deletion) (He et al., 2009). PCR condition for amplification of the *UGT2B17*del allele started with an initial denaturation temperature at 93 °C for 5 min, and 30 extra cycles including: Denaturation at 94 °C for 30secods, annealing at 48.2 °C for 30s, replication at 72 °C 35 s and final extension at 72 °C for 5 min. The presence or absence of PCR products were examined by 2% agarose gel.

### 2.4. Genotyping of the UGT2B28 copy-number variation

We determined the variations of the *UGT2B28* gene copy number as

previously described. Primers used to detect UGT2B28 gene (according to (Menard et al., 2009)):

Forward: 5′-ATGACGCATTCACTCTTAAACTC-3′
Reverse: 5′-CAATTGTGTAGCCAGGAGTGAAGCA-3′
342 bp

Primers used to detect UGT2B28 deletion (according to (Menard et al., 2009)):

Forward: 5′-ATAAAGCTGGAAACAGTCATCCT-3′
Reverse: 5′-ATTAGGACTAGCAGTAACCATTA-3′
450 bp

PCR reactions were conducted in a Bioer Gene Touch thermal cycler with following conditions: Denaturation at 95 °C for 5 min, the extra 35 cycles at 95 °C for 45 s, 45 s at 56 °C and 45 s at 72 °C and a final extension at 72 °C for 5 min.

## 3. Statistical analysis

Chi-square test and One-Way ANOVA test were applied for categorical and continuous variables, respectively, to assess whether there are any statistically significant differences between subjects. Binary logistic regression analysis was used to compute the odd ratios (ORs) with 95% confidence intervals (CIs). The association of the *UGT2B17*, and *UGT2B28* null genotypes (del/del) and D85Y polymorphism with the disease was calculated separately followed by joint analysis. In joint analysis (Choubey et al., 2013), the low activity genotype, D85Y (DD), and null genotype of *UGT2B17* were added together and compared with those having both the high activity genotype, D85Y (YY), and intact form (ins/ins) of *UGT2B17* gene (Table 4). Similarly, the numbers of individuals having both null genotype of *UGT2B17* and low activity genotype, D85Y (DD), were compared with those having ins/ins or ins/del form of *UGT2B17* and DY or YY genotypes of *UGT2B15* gene (Table 4). Deviation of the genotype frequency from the Hardy-Weinberg equilibrium was tested using Chi-square analysis. Reported p-values were two-sided and p-values of $< 0.05$ were considered as statistically significant. All statistical analysis were done using SPSS 18 windows statistical package.

## 4. Results

### 4.1. Characteristics of subjects

The demographic characteristics of study subjects are summarized in Table 1. The ages mean ( ± SD) of patients with PC or BPH and controls were 67.97 ± 7.93, 67.29 ± 8.37 and 66.10 ± 8.29 years, respectively ($p = 0.202$). There was no statistical difference in BMI between the three groups ($p = 0.094$). BPH subjects prostate volume were significantly larger than PC patients ($p < 0.001$). A significant difference in smoking status was found between patients with cancer and healthy controls ($p = 0.013$). The mean of total serum PSA level was significantly higher in PC (20.54 ng/ml) and BPH (5.92 ng/ml) than healthy individuals (2.09 ng/ml) ($p < 0.001$).

### 4.2. UGT2B17, UGT2B28 and UGT2B15 polymorphisms association with PC risk

Our study investigated the association of polymorphisms in *UGT2B17*, *UGT2B28*, and *UGT2B15* genes with susceptibility to PC. Table 1 shows the frequencies of CNV polymorphisms in the *UGT2B17* and *UGT2B28*, and rs1902023. The odds ratio (OR) and 95% confidence intervals are shown in Table 2 and Table 3. We have not found any association between the null genotype (del/del) of *UGT2B17* and *UGT2B28* genes with prostate cancer. Furthermore, no statistically significant association was observed between DD genotype from D85Y polymorphism with prostate cancer (Tables 2 and 3).

The *UGT2B17* del/del and del/ins genotypes were significantly more frequent in the BPH group (65%) compared to the healthy

**Table 1**
Demographic characteristics and genotype frequencies of study groups.

| | Control (n = 120) | BPH (n = 120) | PC (n = 120) | p Value |
|---|---|---|---|---|
| Characteristics | | | | |
| Age[a] | 66.10 ± 8.29 | 67.29 ± 8.37 | 67.97 ± 7.93 | 0.202 |
| BMI[a] | 24.09 ± 4.22 | 24.95 ± 3.39 | 25.04 ± 3.51 | 0.094 |
| PSA[a](ng/mL) | 2.09 ± 1.08 | 5.92 ± 4.04 | 20.54 ± 27.57 | < 0.001 |
| Smoking status | | | | |
| Non smokers | 80 (%67) | 67 (%55.83) | 61 (%50.83) | 0.04 |
| Current or former smokers | 40 (%33) | 53 (%44.17) | 59 (%49.17) | |
| Prostate volume | not available | 62.65 ± 22.53 | 49.25 ± 23.06 | < 0.001 |
| Gleason score | | | | |
| 6 ≥ | NA[b] | NA[b] | 62 (%51.7) | |
| 6 < | NA[b] | NA[b] | 58 (%48.3) | |
| Genotype n (%) | | | | |
| UGT2B28 | | | | |
| Ins/ins | 88 (%73.3) | 87 (%72.5) | 90 (%75) | |
| Ins/del | 27 (%22.5) | 29 (%24.2) | 27 (%22.5) | |
| Del/del | 5 (%4.2) | 4 (%3.3) | 3 (%2.5) | |
| UGT2B17 | | | | |
| Ins/ins | 66 (%55) | 43 (%35.8) | 53 (%44.2) | |
| Ins/del | 49 (%40.8) | 57 (%47.5) | 59 (%49.2) | |
| Del/del | 5 (%4.2) | 20 (%16.7) | 8 (%6.7) | |
| UGT2B15 | | | | |
| DD | 44 (%36.6) | 30 (%25) | 47 (%39.2) | |
| DY | 65 (%54.2) | 64 (%53.3) | 63 (%52.5) | |
| YY | 11 (%9.2) | 26 (%21.7) | 10 (%8.3) | |

[a] Mean ± SD.
[b] Not applicable.

**Table 2**
Distribution of UGT2B28 and UGT2B17 copy number variants in BPH, PC and controls.

| groups | No deletion | Deletion[a] | p-value | OR (95% CI) |
|---|---|---|---|---|
| Independent analysis | | | | |
| UGT2B28 | | | | |
| Controls (n = 120) | 88 (% 73.3) | 32 (% 26.7) | Ref. | – |
| BPH (n = 120) | 87 (% 72.5) | 33 (% 27.5) | 0.885 | 1.043 (0.590–1.843) |
| PC (n = 120) | 90 (% 75) | 30 (% 25) | 0.768 | 0.917 (0.514–1.634) |
| PC vs. BPH | – | – | 0.660 | 0.879 (0.494–1.563) |
| PC vs. BPH + controls | – | – | 0.673 | 0.897 (0.543–1.482) |
| UGT2B17 | | | | |
| Controls (n = 120) | 66 (%55) | 54 (%45) | Ref. | – |
| BPH (n = 120) | 43 (%35) | 77 (%65) | 0.003 | 2.189(1.303–3.675) |
| PC (n = 120) | 53 (%44) | 67 (%56) | 0.094 | 1.545 (0.929–2.571) |
| PC vs. BPH | – | – | 0.188 | 0.706 (0.420–1.186) |
| PC vs. BPH + controls | – | – | 0.822 | 1.052 (0.677–1.635) |

[a] Ins/del + del/del.

**Table 3**
Distribution of UGT2B15 D85Y genotype in BPH, PC and controls.

| Groups | DD | DY + YY | p-Value | OR (95% CI) |
|---|---|---|---|---|
| Independent analysis | | | | |
| UGT2B15 | | | | |
| Controls (n = 120) | 44 (%37) | 76 (% 63) | Ref. | – |
| BPH (n = 120) | 30 (%25) | 90 (%75) | 0.051 | 1.737 (0.997–3.027) |
| PC (n = 120) | 47 (%40) | 73 (% 60) | 0.690 | 0.899 (0.534–1.515) |
| PC vs. BPH | – | – | 0.019 | 0.518 (0.298–0.899) |
| PC vs. BPH + controls | – | – | 0.115 | 0.692 (0.438–1.094) |

**Table 4**
Distribution of joint UGT2B17 and UGT2B15 genotypes in BPH and controls.

| Groups | Del/del + DD | Ins/ins + YY | p-Value | OR (95% CI) |
|---|---|---|---|---|
| Joint analysis | | | | |
| Controls | 3 | 40 | Ref. | – |
| BPH | 15 | 27 | 0.003 | 0.135 (0.036–0.512) |

| Groups | Del/del + DD | Ins/del or ins/ins + DY or YY | p-Value | OR (95% CI) |
|---|---|---|---|---|
| Joint analysis | | | | |
| Controls | 3 | 117 | Ref. | – |
| BPH | 15 | 105 | 0.008 | 0.179 (0.051–0.637) |

subjects (45%) (OR = 2.189; 95% CI, 1.303–3.675; p = 0.003) (Table 2). The *UGT2B15* DY and YY genotypes were significantly more common in the BPH group (75%) compared to cancer patients (60%) (OR = 0.518; 95% CI, (0.298–0.899; p = 0.019)). However, the *UGT2B15* DY and YY genotypes were not significantly different between BPH group (75%) and healthy controls (63%) (OR = 1.737; 95% CI, (0.997–3.027; p = 0.051) Table 3). The ORs for the combined effect of *UGT2B15* and *UGT2B17* genotypes are presented in Table 4. We found that the existence of both null genotype (del/del) of *UGT2B17* and DD genotype of *UGT2B15* considerably increase the risk of BPH (Table 4). The level of association was strong when null genotype of *UGT2B17* and DD genotype of D85Y were compared with those having either ins/ins form of *UGT2B17* and YY genotype of *UGT2B15*.

## 5. Discussion

In addition to normal growth and development of prostate gland, development of BPH and PC depends on androgens metabolism and function (Carson and Rittmaster, 2003; Taheri et al., 2017a). In general, have been proposed as a causal mechanism in the BPH formation and in the initiation and progression of prostate cancer (Krieg et al., 1979; Siiteri and Wilson, 1970; Geller et al., 1976; Belis, 1980). Through glucurinidation, three *UGT2B* classes of enzymes (*UGT2B15*, *UGT2B17*, and *UGT2B28*) prevent the accumulation of androgens in the prostate (Nadeau et al., 2011). Due to the importance of androgens in the prostate development and diseases, in the current study, we examined the association of CNV polymorphisms in *UGT2B17*, *UGT2B28*, and rs1902023 of *UGT2B15* and the risk of prostate cancer. However, there are many conflicting data about the association of the two former mentioned genetic variations and the risk of the disease.

Since UGT2B enzymes accelerate the elimination of androgens from the prostate, the lack or reduced amount of enzymes increase the risk of prostate cancer (Rane, and EKSTRöM, L., 2012; Wei et al., 2009). Karypidis et al. (Karypidis et al., 2008) reported an association between *UGT2B17* polymorphism and the risk of prostate cancer in Caucasian population. They showed that individuals carrying ins/ins genotype of *UGT2B17* had a significantly decreased risk of prostate cancer. Additionally, they also found that individuals with deletion of one allele had a dramatically lower mRNA levels in the prostate. Based on Park et al. (Park et al., 2006) report, associations of *UGT2B17* polymorphisms and increased risk of prostate cancer are linked to ethnicity.

Although, Asian was reported to have a higher prevalence (67–85%) of *UGT2B17* deletion polymorphism rather than Caucasian but the incidence rate of prostate cancer is 2–40 times lower among Asian than Caucasian men (Terakura et al., 2005; Jakobsson et al., 2006; Lee et al., 2016; Haqq et al., 2005). This observation may be explained by the different levels of androgen in the studied subjects. It has been suggested that the androgen level is different among racial/ethnic groups. High levels of androgen may affect the role of *UGT2B17* polymorphisms in developing PC (Vidal et al., 2013). Moreover, the role of other steroid-metabolizing enzymes should be considered in the regulation of androgen levels (Park et al., 2006). Variations in the related coding genes may also play an important role in pathogenesis of PC.

Consistent with previous reports in the literature, we were not able to figure out any association between the studied polymorphisms and the risk of prostate cancer.

To date, studies have shown whole gene deletion of *UGT2B28* is less common among populations than *UGT2B17* gene deletion (Mostaghel et al., 2016). The association between del/del genotype of *UGT2B28* and the risk of cancers was shown in several studies. In Caucasian colorectal cancer patients, no significant association was observed between *UGT2B28* CNV and the disease (Angstadt et al., 2013). In a Chinese study, the aberrant expression of *UGT2B28* gene was reported to play a role in progression of esophageal dysplasia in a chemoprevention trial (Joshi et al., 2006). Therefore, the gene was suggested as a potential candidate in carcinogenesis. Previous studies found no association between prostate cancer risk and null genotype of *UGT2B28* (Setlur et al., 2010; Lee et al., 2016). However, null genotype of *UGT2B28* has been linked to a higher risk of biochemical PC relapse (Nadeau et al., 2011).

There are several studies with controversial results about the association between *UGT2B15* polymorphism (D85Y) and increased risk of prostate cancer. Based on Le'vesque et al. (Levesque et al., 1997) report, *UGT2B15*-Y85 allele has a twofold higher Vmax than *UGTB15*-D85 allele. Although, according to the similar prevalence of the *UGT2B15* alleles and similar kinetic value for DHT, 3*α*-DIOL and ADT, the effect of both alleles on steroid metabolism has a little difference (is similar) (Beaulieu et al., 1996; BELANGER et al., 1998). Our result found no association between the UGT2B15 polymorphism (D85Y) and the risk of prostate cancer consistent with some studies and inconsistent with others. Hajdinjak et al. (Hajdinjak and Zagradišnik, 2004), found significant association between UGT2B15 polymorphism (D85Y) and Gleason grade of prostate cancer, although we were not able to replicate their results. Gsur et al. (Gsur et al., 2002) observed no statistically significant association between D85Y polymorphism of *UGT2B15* and the risk of prostate cancer. However, due to the fact that the BPH basically affected by androgen hormone and the role of the related polymorphism (Hajdinjak and Zagradišnik, 2004), selection of BPH patients as a control group was a key limitation of their study. In order to obstacle this limitation our study was programmed to examine and evaluate three separate groups of PC, BPH and healthy control groups together.

Despite the obvious androgens importance as risk factors for BPH, little is known about the polymorphisms of *UGT2B* genes that contribute to the metabolism of these hormones and their role in BPH creation. There is only a study that assessed the association between polymorphisms of *UGT2B15* D85Y polymorphism and the risk of BPH (Gsur et al., 2002). The frequency distribution of alleles and genotypes of *UGT2B15* D85Y in BPH and PC showed similar pattern and no significant difference was observed in the frequencies of alleles and genotypes between BPH and PC in Gsur et al. report. Although, identifying genetic differences between those subjected to BPH and PC would be very important for elucidating causative factors for cancer development, if hormonal factors were involved, such differences would be expected to modify both entities (Hajdinjak and Zagradišnik, 2004). To date, no studies have investigated the association of the UGT2B17 and UGT2B28 CNVs polymorphism and the risk of BPH.

In our study, a significant association was observed between the polymorphisms of *UGT2B17* and the risk of BPH. We did not find any significant association for *UGT2B2*8 and the disease risk. Additionally, we found no significant increased risk for BPH in men with low activity genotype at D85Y (DD). But rather, we observed significant association between *UGT2B15* D85Y and BPH risk when it was analyzed in combination with the *UGT2B17* copy number variation. Given DHT detrimental role in the adult prostate in that it causes pathologic prostate enlargement (Carson and Rittmaster, 2003), evaluation of variation in

genes involved in DHT metabolism is required for effective therapeutic approaches to BPH. Nowadays, an enzyme such as 5-α-reductase (5AR) with outstanding role in androgen metabolism is used as a therapeutic target in BPH patients (Parsons et al., 2010; Mostaghel et al., 2010; Andriole et al., 2010). Hence, the study of *UGT2B* genes polymorphisms as key enzymes in the androgens metabolism pathways is necessary. Here, for the first time we report an association between UGT2B17 CNV polymorphism and the risk of BPH in Iranian population.

In conclusion, our study provides the first evidence of null genotype involvement in *UGT2B17* as a risk factor for BPH. Also, we observed that *UGT2B15* D85Y polymorphism increased the risk of BPH when analyzed in combination with the null genotype of *UGT2B17*. Replication of our results in future with larger sample sizes may define the *UGT2B15* and *UGT2B17* polymorphisms as new susceptibility markers for BPH and clear the casual link between PC and BPH.

## Acknowledgement

The present article is financially supported by “Research Department of Medicine School, Shahid Beheshti University of Medical Sciences (Grant No.: 7698)”.

## Conflict of interest

The authors declare that they have no conflict of interest.

## References

Andriole, G.L., Bostwick, D.G., Brawley, O.W., Gomella, L.G., Marberger, M., Montorsi, F., Pettaway, C.A., Tammela, T.L., Teloken, C., Tindall, D.J., 2010. Effect of dutasteride on the risk of prostate cancer. N. Engl. J. Med. 362, 1192–1202.

Angstadt, A.Y., Berg, A., Zhu, J., Miller, P., Hartman, T.J., Lesko, S.M., Muscat, J.E., Lazarus, P., Gallagher, C.J., 2013. The effect of copy number variation in the phase II detoxification genes UGT2B17 and UGT2B28 on colorectal cancer risk. Cancer 119, 2477–2485.

Bauman, D.R., Steckelbroeck, S., Peehl, D.M., Penning, T.M., 2006. Transcript profiling of the androgen signal in normal prostate, benign prostatic hyperplasia, and prostate cancer. Endocrinology 147, 5806–5816.

Beaulieu, M., Levesque, E., Hum, D.W., Belanger, A., 1996. Isolation and characterization of a novel cDNA encoding a human UDP-glucuronosyltransferase active on C19 steroids. J. Biol. Chem. 271, 22855–22862.

Belanger, A., Hum, D.W., Beaulieu, M., Levesque, É., Guillemette, C., Tchernof, A., Belanger, G., Turgeon, D., Dubois, S., 1998. Characterization and regulation of UDP-glucuronosyltransferases in steroid target tissues. J. Steroid Biochem. Mol. Biol. 65, 301–310.

Belanger, A., Pelletier, G., Labrie, F., Barbier, O., Chouinard, S., 2003. Inactivation of androgens by UDP-glucuronosyltransferase enzymes in humans. Trends Endocrinol. Metab. 14, 473–479.

Belis, J., 1980. Methodologic basis for the radioimmunoassay of endogenous steroids in human prostatic tissue. Investig. Urol. 17, 332–336.

Belledant, A., Hovington, H., Garcia, L., Caron, P., Brisson, H., Villeneuve, L., Simonyan, D., Tetu, B., Fradet, Y., Lacombe, L., 2016. The UGT2B28 sex-steroid inactivation pathway is a regulator of steroidogenesis and modifies the risk of prostate cancer progression. Eur. Urol. 69, 601–609.

Carson, C., Rittmaster, R., 2003. The role of dihydrotestosterone in benign prostatic hyperplasia. Urology 61, 2–7.

Choubey, V.K., Sankhwar, S.N., Tewari, R., Sankhwar, P., Singh, B.P., Rajender, S., 2013. Null genotypes at the GSTM1 and GSTT1 genes and the risk of benign prostatic hyperplasia: a case–control study and a meta-analysis. Prostate 73, 146–152.

Chouinard, S., Yueh, M.-F., Tukey, R.H., Giton, F., Fiet, J., Pelletier, G., Barbier, O., Belanger, A., 2008. Inactivation by UDP-glucuronosyltransferase enzymes: the end of androgen signaling. J. Steroid Biochem. Mol. Biol. 109, 247–253.

Cimino, S., Russo, G.I., Reale, G., Urzì, D., Castelli, T., Favilla, V., Morgia, G., 2016. Pharmacological Role of Dietary Polyphenols in Prostate Cancer Chemoprevention. Springer, Critical Dietary Factors in Cancer Chemoprevention.

Cuzick, J., Thorat, M.A., Andriole, G., Brawley, O.W., Brown, P.H., Culig, Z., Eeles, R.A., Ford, L.G., Hamdy, F.C., Holmberg, L., 2014. Prevention and early detection of prostate cancer. Lancet Oncol. 15, e484–e492.

Furuya, T., Suehiro, Y., Namiki, Y., Sasaki, K., 2015. CNVs associated with susceptibility to cancers: a mini-review. J. Cancer Ther. 6 (413).

Gallagher, C.J., Kadlubar, F.F., Muscat, J.E., Ambrosone, C.B., Lang, N.P., Lazarus, P., 2007. The UGT2B17 gene deletion polymorphism and risk of prostate cancer: a case–control study in Caucasians. Cancer Detect. Prev. 31, 310–315.

Gauthier-Landry, L., Belanger, A., Barbier, O., 2015. Multiple roles for UDP-glucuronosyltransferase (UGT) 2B15 and UGT2B17 enzymes in androgen metabolism and prostate cancer evolution. J. Steroid Biochem. Mol. Biol. 145, 187–192.

Geller, J., Albert, J., Lopez, D., Geller, S., Niwayama, G., 1976. Comparison of androgen metabolites in benign prostatic hypertrophy (BPH) and normal prostate. J. Clin. Endocrinol. Metab. 43, 686–688.

Grant, D.J., Hoyo, C., Oliver, S.D., Gerber, L., Shuler, K., Calloway, E., Gaines, A.R., Mcphail, M., Livingston, J.N., Richardson, R.M., 2013. Association of uridine diphosphate-glucuronosyltransferase 2B gene variants with serum glucuronide levels and prostate cancer risk. Genet. Test. Mol. Biomarkers 17, 3–9.

Gsur, A., Preyer, M., Haidinger, G., Schatzl, G., Madersbacher, S., Marberger, M., Vutuc, C., Micksche, M., 2002. A polymorphism in the UDP-Glucuronosyltransferase 2B15 gene (D85Y) is not associated with prostate cancer risk. Cancer Epidemiology and Prevention Biomarkers 11, 497–498.

Hajdinjak, T., Zagradišnik, B., 2004. Prostate cancer and polymorphism D85Y in gene for dihydrotestosterone degrading enzyme UGT2B15: frequency of DD homozygotes increases with Gleason Score. Prostate 59, 436–439.

HAQQ, C., Li, R., Khodabakhsh, D., Frolov, A., Ginzinger, D., Thompson, T., Wheeler, T., Carroll, P., Ayala, G., 2005. Ethnic and racial differences in prostate stromal estrogen receptor α. Prostate 65, 101–109.

He, X., Hesse, L.M., Hazarika, S., Masse, G., Harmatz, J.S., Greenblatt, D.J., Court, M.H., 2009. Evidence for oxazepam as an in vivo probe of UGT2B15: oxazepam clearance is reduced by UGT2B15 D85Y polymorphism but unaffected by UGT2B17 deletion. Br. J. Clin. Pharmacol. 68, 721–730.

Jakobsson, J., Ekström, L., Inotsume, N., Garle, M., Lorentzon, M., Ohlsson, C., ROH, H.-K., Carlström, K., RANE, A., 2006. Large differences in testosterone excretion in Korean and Swedish men are strongly associated with a UDP-glucuronosyl transferase 2B17 polymorphism. J. Clin. Endocrinol. Metab. 91, 687–693.

Joshi, N., Johnson, L.L., Wei, W.-Q., Abnet, C.C., Dong, Z.-W., Taylor, P.R., Limburg, P.J., Dawsey, S.M., Hawk, E.T., Qiao, Y.-L., 2006. Gene expression differences in normal esophageal mucosa associated with regression and progression of mild and moderate squamous dysplasia in a high-risk Chinese population. Cancer Res. 66, 6851–6860.

Juul, A., Sørensen, K., Aksglaede, L., GARN, I., Rajpert-de Meyts, E., Hullstein, I., Hemmersbach, P., Ottesen, A.-M., 2009. A common deletion in the uridine diphosphate glucuronyltransferase (UGT) 2B17 gene is a strong determinant of androgen excretion in healthy pubertal boys. J. Clin. Endocrinol. Metab. 94, 1005–1011.

Karypidis, A., Olsson, M., Andersson, S.-O., Rane, A., Ekström, L., 2008. Deletion polymorphism of the UGT2B17 gene is associated with increased risk for prostate cancer and correlated to gene expression in the prostate. Pharm. J. 8, 147–151.

Khvostova, E., Otpuschennikov, A., Pustylnyak, V., Gulyaeva, L., 2015. Gene expression of androgen metabolising enzymes in benign and malignant prostatic tissues. Horm. Metab. Res. 47, 119–124.

Kpoghomou, M.-A., Soatiana, J.E., Kalembo, F.W., Bishwajit, G., Sheng, W., 2013. UGT2B17 Polymorphism and risk of prostate cancer: a meta-analysis. ISRN Oncology 2013, 465916.

Kral, M., Rosinska, V., Student, V., Grepl, M., Hrabec, M., Bouchal, J., 2011. Genetic determinants of prostate cancer: a review. Biomedical Papers 155, 3–9.

Krieg, M., Bartsch, W., Janssen, W., Voigt, K., 1979. A comparative study of binding, metabolism and endogenous levels of androgens in normal, hyperplastic and carcinomatous human prostate. J. Steroid Biochem. 11, 615–624.

Labrie, F., Belanger, A., Belanger, P., Berube, R., Martel, C., Cusan, L., Gomez, J., Candas, B., Castiel, I., Chaussade, V., 2006. Androgen glucuronides, instead of testosterone, as the new markers of androgenic activity in women. J. Steroid Biochem. Mol. Biol. 99, 182–188.

Lee, S.-R., Ahn, M.-H., Choi, Y.H., Leem, S.-H., 2016. Influence of polymorphisms of UDP-glycosyltransferases (UGT) 2B family genes UGT2B15, UGT2B17 and UGT2B28 on the development of prostate cancer in Korean men. Genes & Genomics 38, 225–233.

Levesque, E., Beaulieu, M., Green, M.D., Tephly, T.R., Belanger, A., Hum, D.W., 1997. Isolation and characterization of UGT2B15 (Y85): a UDP glucuronosyltransferase encoded by a polymorphic gene. Pharmacogenet. Genomics 7, 317–325.

Mackenzie, P.I., Bock, K.W., Burchell, B., Guillemette, C., Ikushiro, S.-I., Iyanagi, T., Miners, J.O., Owens, I.S., Nebert, D.W., 2005. Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. Pharmacogenet. Genomics 15, 677–685.

MACLEOD, S.L., Nowell, S., Plaxco, J., Lang, N.P., 2000. An allele-specific polymerase chain reaction method for the determination of the D85Y polymorphism in the human UDPGlucuronosyltransferase 2B15 gene in a case-control study of prostate cancer. Ann. Surg. Oncol. 7, 777–782.

Menard, V., Eap, O., Harvey, M., Guillemette, C., Levesque, É., 2009. Copy-number variations (CNVs) of the human sex steroid metabolizing genes UGT2B17 and UGT2B28 and their associations with a UGT2B15 functional polymorphism. Hum. Mutat. 30, 1310–1319.

Miners, J.O., Mackenzie, P.I., 1991. Drug glucuronidation in humans. Pharmacol. Ther. 51, 347–369.

Mostaghel, E.A., Geng, L., Holcomb, I., Coleman, I.M., Lucas, J., True, L.D., Nelson, P.S., 2010. Variability in the androgen response of prostate epithelium to 5α-reductase inhibition: implications for prostate cancer chemoprevention. Cancer Res. 70, 1286–1295.

Mostaghel, E.A., Zhang, A., Plymate, S., 2016. UDP-glucuronosyltransferase enzymes in prostate cancer progression: is only androgen catabolism involved? Eur. Urol. 69, 610–612.

Nadeau, G., Bellemare, J., Audet-Walsh, E., Flageole, C., Huang, S.-P., Bao, B.-Y., Douville, P., Caron, P., Fradet, Y., Lacombe, L., 2011. Deletions of the androgen-metabolizing UGT2B genes have an effect on circulating steroid levels and biochemical recurrence after radical prostatectomy in localized prostate cancer. J. Clin. Endocrinol. Metab. 96, E1550–E1557.

Nagar, S., Remmel, R., 2006. Uridine diphosphoglucuronosyltransferase pharmacogenetics and cancer. Oncogene 25, 1659–1672.

Olsson, M., Lindström, S., Häggkvist, B., Adami, H.O., Bälter, K., Stattin, P., Ask, B., Rane, A., Ekström, L., Grönberg, H., 2008. The UGT2B17 gene deletion is not associated

with prostate cancer risk. Prostate 68, 571–575.
Pakzad, R., Rafiemanesh, H., Ghoncheh, M., Sarmad, A., Salehiniya, H., Hosseini, S., Sepehri, Z., Afshari-Moghadam, A., 2016. Prostate cancer in Iran: trends in incidence and morphological and epidemiological characteristics. Asian Pac. J. Cancer Prev. 17, 839–843.
Park, J., Chen, L., Shade, K., Lazarus, P., Seigne, J., Patterson, S., Helal, M., Pow-Sang, J., 2004. Asp85tyr polymorphism in the udp-glucuronosyltransferase (UGT) 2B15 gene and the risk of prostate cancer. J. Urol. 171, 2484–2488.
Park, J., Chen, L., Ratnashinge, L., Sellers, T.A., Tanner, J.-P., Lee, J.-H., Dossett, N., Lang, N., Kadlubar, F.F., Ambrosone, C.B., 2006. Deletion polymorphism of UDP-glucuronosyltransferase 2B17 and risk of prostate cancer in African American and Caucasian men. Cancer Epidemiology and Prevention Biomarkers 15, 1473–1478.
Park, J.Y., Tanner, J.-P., Sellers, T.A., Huang, Y., Stevens, C.K., Dossett, N., Shankar, R.A., Zachariah, B., Heysek, R., Pow-Sang, J., 2007. Association between polymorphisms in HSD3B1 and UGT2B17 and prostate cancer risk. Urology 70, 374–379.
Parsons, J.K., Palazzi-Churas, K., Bergstrom, J., Barrett-Connor, E., 2010. Prospective study of serum dihydrotestosterone and subsequent risk of benign prostatic hyperplasia in community dwelling men: the Rancho Bernardo Study. J. Urol. 184, 1040–1044.
Rane, A., EKSTRÖM, L., 2012. Androgens and doping tests: genetic variation and pit-falls. Br. J. Clin. Pharmacol. 74, 3–15.
Roehrborn, C.G., 2005. Benign prostatic hyperplasia: an overview. Reviews in Urology 7, S3–S14.
Schänzer, W., 1996. Metabolism of anabolic androgenic steroids. Clin. Chem. 42, 1001–1020.
Setlur, S.R., Chen, C.X., Hossain, R.R., Ha, J.S., Van Doren, V.E., Stenzel, B., Steiner, E., Oldridge, D., Kitabayashi, N., Banerjee, S., 2010. Genetic variation of genes involved in dihydrotestosterone metabolism and the risk of prostate cancer. Cancer Epidemiology and Prevention Biomarkers 19, 229–239.
Siiteri, P.K., Wilson, J.D., 1970. Dihydrotestosterone in prostatic hypertrophy: I. The formation and content of dihydrotestosterone in the hypertrophic prostate of man. J. Clin. Invest. 49, 1737.
Taheri, M., Habibi, M., Noroozi, R., Rakhshan, A., Sarrafzadeh, S., Sayad, A., Omrani, M.D., Ghafouri-Fard, S., 2017a. HOTAIR genetic variants are associated with prostate cancer and benign prostate hyperplasia in an Iranian population. Gene 613, 20–24.
Taheri, M., Pouresmaeili, F., Omrani, M.D., Habibi, M., Sarrafzadeh, S., NOROOZI, R., Rakhshan, A., Sayad, A., Ghafouri-Fard, S., 2017b. Association of ANRIL gene polymorphisms with prostate cancer and benign prostatic hyperplasia in an Iranian population. Biomark. Med 11, 413–422.
Terakura, S., Murata, M., Nishida, T., Emi, N., Akatsuka, Y., Riddell, S.R., Morishima, Y., Kodera, Y., Naoe, T., 2005. A UGT2B17-positive donor is a risk factor for higher transplant-related mortality and lower survival after bone marrow transplantation. Br. J. Haematol. 129, 221–228.
Turgeon, D., Carrier, J.-S.B., levesque, É., Hum, D.W., Belanger, A., 2001. Relative enzymatic activity, protein stability, and tissue distribution of human steroid-metabolizing UGT2B subfamily members 1. Endocrinology 142, 778–787.
Vidal, A.C., Tucker, C., Schildkraut, J.M., Richardson, R.M., Mcphail, M., Freedland, S.J., Hoyo, C., Grant, D.J., 2013. Novel associations of UDP-glucuronosyltransferase 2B gene variants with prostate cancer risk in a multiethnic study. BMC Cancer 13, 556.
Wei, Q., Galbenus, R., Raza, A., Cerny, R.L., Simpson, M.A., 2009. Androgen-stimulated UDP-glucose dehydrogenase expression limits prostate androgen availability without impacting hyaluronan levels. Cancer Res. 69, 2332–2339.
Wilding, G., 1991. The importance of steroid hormones in prostate cancer. Cancer Surv. 14, 113–130.
Zhang, F., Gu, W., Hurles, M.E., Lupski, J.R., 2009. Copy number variation in human health, disease, and evolution. Annu. Rev. Genomics Hum. Genet. 10, 451–481.
Zhong, X., Feng, J., Xiao, Y., Wang, P., Fan, Q., Wu, R., Hu, W., Huang, C., 2017. Uridine diphosphate-glucuronosyltransferase 2B15 D85Y gene polymorphism is associated with lower prostate cancer risk: a systematic review and meta-analysis. Oncotarget.