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Relationship between Genetic Variants of Glucuronidation Pathway and TNF-A with Increased Risk of Prostate Cancer in Iranian Men

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Abstract

After lung cancer, prostate cancer has the highest mortality rate. Early and accurate diagnosis of this heterogeneous cancer promises more effective treatment. At present biopsy is the only definitive method of diagnosing the disease. Many gene loci are associated with increased susceptibility to this cancer. Here, the relationship between tumor necrosis factor alpha (TNF-α) gene variants and glucuronidation pathway gene variants with increased risk of prostate cancer in a population of Iranian men has been investigated.

Materials and methods: Blood samples were collected from 360 men including 120 healthy, 120 with benign prostate hyperplasia (BPH), and 120 patients with prostate cancer (PCa). DNA was extracted and tested for each variant with specific primers and PCR based methods. Data were analyzed using agarose and polyacrylamide gel electrophoresis, SPSS software, SNP Stats and Student's t-test.

Results: UGT2B17 and UGT2B15 polymorphisms were associated with BPH in comparison to the control group (P value = <0.0001 and P value = 0.007). The only significant association in the cancer group was between G Score and UGT2B15, so that 90% of patients with PCa and G score less than or equal to 6, had GG genotype (0.01). Other variants had no significant relationship with the cause of the disease. Ins Del G haplotype was more common in BPH compared to the control group (P value = 0.011).

Discussion: Observation of the association of UGT2B17 and UGT2B15 with BPH, and the prevalence of Ins Del G haplotype in BPH compared with healthy individuals, increases the likelihood of these genotypes for prostate hyperplasia. Further studies on the genetic composition of pathways associated with prostate function in larger populations is promising to find possible new biomarkers, map the natural state and progression of cellular changes in favor of prostate cancer.

Keywords: Prostate cancer; Prostate hyperplasia; CNV; polymorphism; SNP

Introduction

Prostate cancer is the second most common cancer in Iran, after gastric cancer [1]. In the early stages of PCa, symptoms are rarely appearing. There may be no symptoms at the time of diagnosis even in the advanced states [2]. Blood in the urine and semen [3], recurrent pain in the affected area[4], difficulty in urinating[5], sudden or frequent urination [6], unexplained weight loss [7], pain in the pelvis [8], thighs and back [9], pain or abnormal symptoms in the penis [10], poor urination, and bone pain [11] are signs of cancer progression.

While the underlying causes of prostate cancer remain unknown, the risk of developing prostate cancer is increasing [12]. Having one or more risk factors does not mean that one will definitely develop the disease. The risk of PCa in men under the age of 40 is very low [13]. But, the chance of the disease appearance increases rapidly in people over 50 years old [13], while about 6 out of 10 cases of the patients are found in over 65 years old men [14]. Blacks are more likely to develop PCa than men of other races and are more than twice as likely to die from the cancer as white men [15].

Smoking is one of the most effective risk factors for the disease [16]. This cancer seems to be more common in some families, suggesting that in some cases there may be hereditary or genetic factors [16]. A person with a first degree relative with prostate cancer is two to three times as likely to get the disease as other men [17]. People with a strong family history of breast and ovarian cancer are also more likely to develop prostate cancer than people without a family history [18] . A number of genetic changes increase the risk of prostate cancer, but in general they are likely to account for only a small percentage of cases [19]. Screening tests help in diagnosis and treatment the cancer, early or even before symptoms appear [20]. PCa screening is performed based on PSA level measurement, MRI/ ultrasound and/ or CT scan, and finally the results of pathology, in Iran. Determining the level of PSA in a blood test is not a specific test for cancer [21]. Even if the serum level of prostate-specific antigen rises above normal, the risk of prostate cancer is not very high and a rectal exam is recommended for asymptomatic men [22]. Prostate cancer diagnosis, early and before it spreads to other parts of the body, is one of the most important benefits of screening, which makes the treatment process easier and shorter. Early detection and screening may not improve the health of a patient with advanced prostate cancer or help prolong his life [23, 24]. Some prostate cancers are life-threatening or never cause symptoms, but if they are detected by screening tests, the person may be treated for cancer. Complications of prostate biopsy such as false negative results delay treatment [25] and false positive cases undergo more biopsies[26]. The side effects of biopsy are many and also cause anxiety and worry [27]. Abnormal prostate changes do not always indicate prostate cancer. In some cases, an MRI before a biopsy is recommended [28]. Currently the only reliable way to diagnose this disease is a biopsy that shows a person has prostate cancer. Other measures are taken to find out if the cancerous mass has spread from its original location to other parts of the body [29]. CT scan shows the presence of cancer cells in the bone [5]. Post-diagnosis PET scan is used to identify the stage and degree of disease progression and to diagnose relapse after treatment [30]. For some men, immediate treatment is not necessary or may not be appropriate. The therapist can allow the patient to make treatment based on the stage of the cancer. Surgery, radiotherapy, chemotherapy and palliative care are other current treatments [30].

Given the heterogeneous nature of prostate tumors, researchers are trying to find more sensitive and specific tumor markers in the blood to replace solid biopsy with liquid biopsy and to create a chance to differentiate between different stages and grades of disease development by providing specific patterns of genetic changes and gene expression [31]. So far, more than 100 gene loci associated with the disease have been identified [32]. Numerous reports have shown an increased risk of prostate cancer in connection with certain single or combined genetic polymorphisms. The aim of this study was to investigate the relationship between rs1800629 and rs361525 TNF-α gene polymorphisms and gene variants of UGT2B28, UGT2B17 and UGT2B15 genes from glucuronidation pathway with increased risk of prostate cancer in Iranian population.

Materials and Methods

Sampling

In this case-control study, 120 men with healthy prostate (control), 120 patients with BPH, and 120 patients with prostate cancer (PCa) were selected from the individuals referred to the urology department of Shohadaye Tajrish Hospital in Tehran between 2019 and 2020. Demographic information was recorded using a conscious questionnaire. The PCa group included 50-year-old men with or without a family history of the disease, high PSA, urinary symptoms, digital rectal examination, CT scan, ultrasound, biopsy with a definitive diagnosis of prostate cancer by a urologist, based on pathological tests and the presence of neoplastic tissue at any stage or degree. Also, body mass index, height and weight, smoking / hookah use or any other drug, place of residence, use of other drugs, PSA level were recorded. The BPH group included men with disturbing urinary symptoms, PSA levels above 4, benign prostate swelling as shown by TR biopsy (rectal examination), pathologically negative results, and a detected prostate volume of more than 30 ml by radiology. The control group included healthy individuals who showed urinary symptoms, abdominal pain, and age 31-41 years. In addition to PSA measurements, ultrasound and urine culture data, their clinical and paraclinical examinations confirmed prostate health. They had no previous surgery, chemotherapy, or radiation therapy for prostate cancer, had no family history of BPH / PCa, and was considered healthy.

Informed consent was received from each patient

DNA extraction from peripheral blood

In this study, 5 ml of peripheral blood of each patient was collected in a tube containing EDTA. Blood samples were taken from the control subjects in the same way and then DNA extraction was performed (DiatomTM DNA prep kit and in some samples using salting out method). The PCR conditions were based on previously works [33, 34]. The forward and reverse primers for examining the studied copy number variations and the gene polymorphisms are shown in Table 1.

Table 1: Genes and the related primers sequences which were used to examine the status of each polymorphism in PCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
UGT2B15 D85Y	CTGTGGAAAGGTGCTAGT	GAATTTTCAGAAGAGAATCTTCCAGAT
UGT2B17: 1. Primer pairs for deleted region 2. Primer set for flanking sequences of the deletion area.	1.TGAAAATGTTCGATAGATGGACATATAGTA 2.TGCACAGAGTTAAGAAATGGAGAGATGTG	1.GACATCAAATTTTGACTCTTGTAGTTTTC 2.GATCATCCTATATCCTGACAGAATTCTTTTG
UGT2B28	1.ATGACGCATTCACTCTTAAACTC 2.ATAAAGCTGGAAACAGTCATCCT	1.CAATTGTGTAGCCAGGAGTGAAGCA 2. ATTAGGACTAGCAGTAACCATTA.
PCR-RFLP rs361525 (-238G/A):	ATCTGGAGGAAGCGGTAGTG	AGAAGACCCCCCTCGGAACC
T-ARMS-PCR rs1800629 (-308G/A):	FOu: AGGACTCAGCTTTCCGAAGCCCCTCCCA; Fin: GGAGGCAATAGGTTTTGAGGCGCAGGG	ROu: TTCTGTCTCGGTTTCTTCTCCATCGCGG Rin: GTAGGACCCTGGAGGCTGAACCCCGTACT.

Enzyme digestion and gel electrophoresis

After the amplification of rs361525 promoter snp and *UGT2B15* D85Y polymorphism, the first variation PCR products were digested by MspI and the second polymorphism products were cut by Sau3AI restriction enzymes (Fermentas, Hanover, MD, USA) and separated by polyacrylamide gel electrophoresis (PAGE, 12%).

Amplification of rs1800629

Tetra-ARMS-PCR technique was used to identify rs1800629 polymorphism. Based on this method, a pair of non-specific primers (Forward outer and Reverse outer) that amplify the entire sequence containing this variant and a pair of specific primers (Forward inner and Reverse inner) that identify and amplify a specific sequence of the interest region were used.

Results

PCR based experiments results were the same as our previous work [33, 34]. The expected fragment of PCR for *UGT2B15* D85Y polymorphism was a 215 bp band, which after restriction digest, could produce two bands of 28 and 187 bp (figure1). The PCR products with YY genotypes showed unique fragments of 215 bp, those with DD genotypes showed two different fragments with lengths of 187 and 28 bp, and DY genotypes demonstrated three bands of 215, 187, and 28 bp on gel electrophoresis (Figure 1).

The pattern of UGT2B17 PCR fragments on 2.5% agarose gel is shown in Figure 2. Checking for the presence or absence of the gene required two pairs of primers, one pair for exon1 amplification which could create a 173 bp band to confirm the definite presence of the allele and a second pair of primers which could amplify a region of 893 base pairs when the gene was deleted but could produce two fragments of 173 and 893 base pairs in heterozygotes of the deleted allele.

The pattern of UGT2B28 PCR fragments on 2.5% agarose gel is shown in Figure 3. Checking for the presence or absence of the gene required two pairs of primers, one pair for exon1 amplification which could create a 324 bp band to confirm the definite presence of the allele and a second pair of primers which could amplify a region of 450 base pairs in homozygutes of the deletion, but could produce two fragments of 324 and 450 base pairs in heterozygote samples.

The amplification of TNF-α promoter rs361525 polymorphism produced a fragment of 152 bp on 2% agarose gel, while after PCR-RFLP and digestion by MspI restriction enzyme was digested into a 152 bp, a 132bp, and a 20 bp fragments in GA heterozygotes and into two fragments of 132bp and 20 bp in GG homozygotes (Figures 4 and 5).

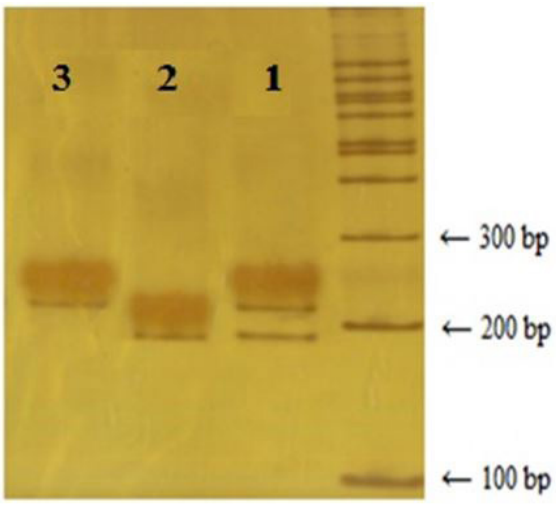

Figure 1: Electrophoresis of PCR fragments of UGT2B15 gene polymorphism after exposure to Sau3AI restriction enzyme on 8% polyacrylamide gel. Lane 1, in which a 187 and a 215 base pair fragments are visible, indicates the DY genotype. The 28bp.fragment is removed from the gel due to its small size, so it is not visible. Lane 2 shows DD genotype containing a cleavage site for the enzyme in both alleles and is cut into two fragments of 187 and 28 bp. Finally, lane 3 represents the YY genotype with 215 bp fragment due to the lack of the enzyme cleavage site

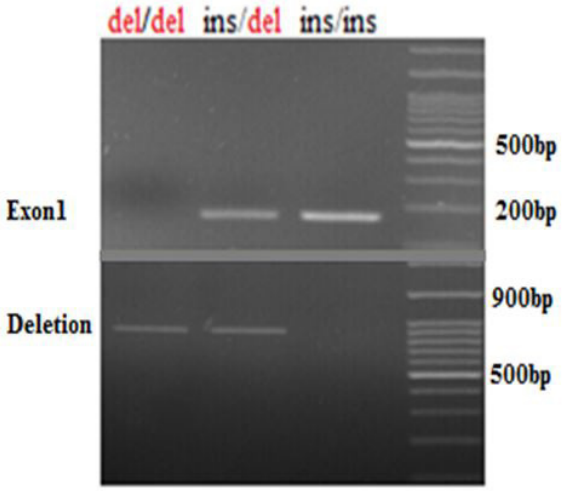

Figure 2: Electrophoresis of UGT2B17 gene PCR fragments on 2.5% agarose gel. The ins/ins genotype indicates that this gene has not been deleted. In this case, the deletion primer will not be able to amplify the region and only one 173 bp fragment will be detected. In the del/ins genotype, one of the alleles of the gene is deleted and the other is present, so two fragments of 173 bp and 893 bp are amplified. In the third line, which shows the del/del genotype, due to the homozygosity for deletion in both alleles of the studied gene, an 893 bp fragment is shown

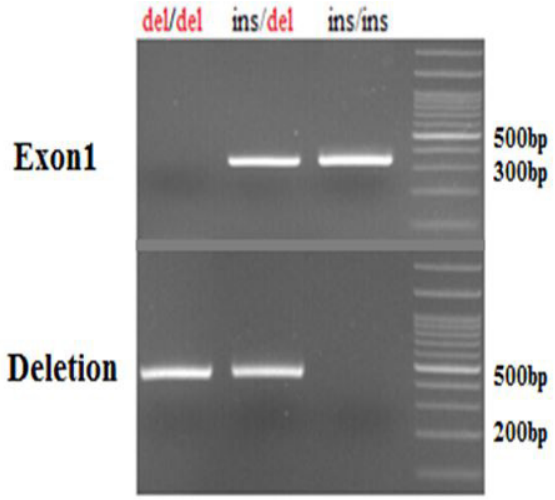

Figure 3: Electrophoresis of UGT2B28 gene PCR fragments on 2.5% agarose gel. The ins/ins genotype indicates that this gene has not been deleted. In this case, the deletion primer will not be able amplify the region and a unique fragment of 324 bp will be detected. In the del/ins genotype, one of the alleles of the gene is deleted and the other is present, so two fragments of 324 and 450 bp are amplified. In the third line, which represents the del/del genotype, due to the deletion of both alleles, a 450 bp fragment is produced

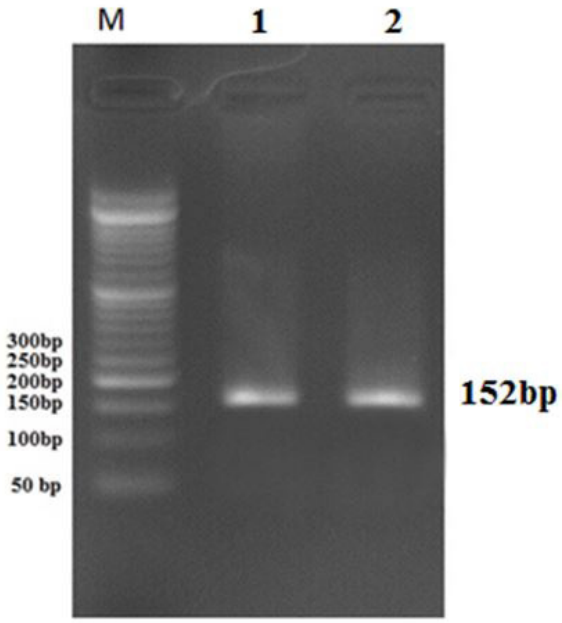

Figure 4: The electrophoresis of TNF-α gene promoter PCR product to show rs361525 polymorphism. Analysis was performed on 2% agarose gel. In this figure, the 152 bp band is the expected amplified fragment. Lanes1 and 2 are samples and M is a 50bp DNA ladder

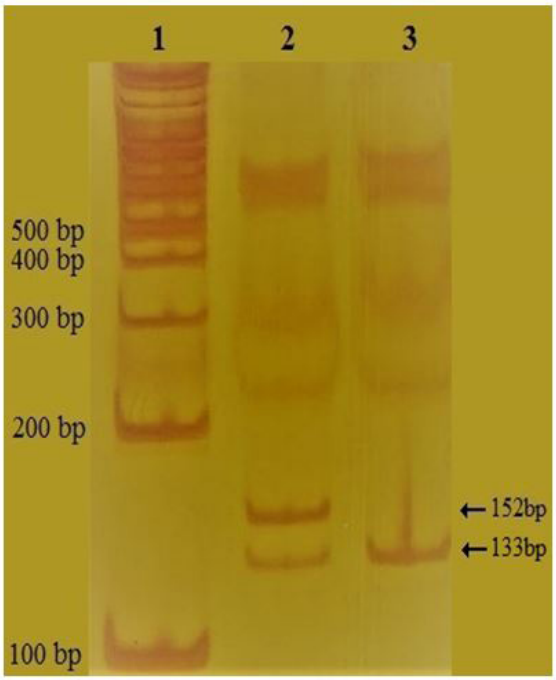

Figure 5: Electrophoresis of TNF-α gene rs361525 polymorphism PCR product after exposure to MspI restriction enzyme on 12% polyacrylamide gel. Lane 1: 100bp DNA ladder. Lane 2: GA heterozygote genotypes with three bands of 152, 133, and 19 bp. Lane 3: GG homozygotes with two fragments of 133 and 19 bp. The 19-pair fragment is not visible in the gel due to its small size. Bp stands for base pairs

For rs1800629 PCR products were electrophoresed directly and without enzymatic digestion on 2% agarose gel. Individuals with the G / G genotype had two fragments of 304 and 197 bp, G/A heterozygotes showed to have three-bands of 304, 197, and 162 bp, and two fragments of 304 and 162 bp. were resulted from A / A homozygotes (Figure 6).

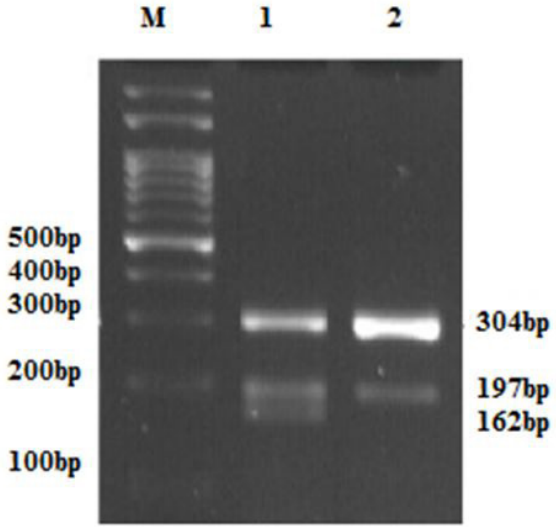

Figure 6: Electrophoresis of PCR fragments related to rs1800629 polymorphism of TNF-α gene on 2% agarose gel. Lane 1, in which three pieces of 304, 197 and 162 base pairs are seen, indicates the GA genotype. Lane 2, which shows 304 and 197 base pairs, represents the GG genotype

Exact test showed a Hardy-Weinberg Equilibrium in the studied alleles of the target population (p value > 0.05) (Table 2). The examined groups were compared in terms of genotypes to determine a possible allelic relationship in the genes (Table 3). There was a codominant, dominant, and/ or recessive, or Log-Additive model of allelic relationship between the variants of *UGT2B17* and BPH (p value<0.05). UGT2B17 showed to be associated with BPH in comparison with the control group (P value = <0.0001) (Del versus INS: OR (95% CI) = 2.08 (1.41-3.08). Also, an allelic codominant, dominant, recessive, or Log-Additive model was anticipated for *UGT2B15* and BPH (p value < 0.05).

UGT2B15 was associated with BPH in comparison to the control group (P value = 0.007) (G versus T: OR (95% CI) = 1.64 (1.14-2.37). For other examined variants, there was no significant relationship between allelic models and disease etiology (Table 3).

Since, the understudy genes *UGT2B28, UGT2B17* and *UGT2B15* were located on 4q13.2, different combinations of their alleles were considered as haplotypes. There was a significant relationship between G score and *UGT2B15* in PCa group, so that 90% of patients with GG genotype were found among those with G score ≤ 6 group (Table4).

Table 2: The Exact test results for Hardy-Weinberg Equilibrium. The frequencies of all genotypes in patients and control groups did not significantly deviate from Hardy-Weinberg equilibrium (P>0.05)

	UGT2B28 (frequency)	**UGT2B17 (frequency)**	**UGT2B15 (frequency)**	**rs1800629 (frequency)**	**rs361525 (frequency)**
Control	0.15	0.33	0.077	1	1
BPH	0.48	0.85	0.58	1	1
Prostate cancer	0.46	0.14	0.11	1	1

The decimal values are p values.

The frequency of alleles of each variant in each under study group is indicated by p value. When alleles are in equilibrium, genotypes are also in equilibrium, so the frequency is related to both. Due to the existing balance between the alleles, the selected variants are suitable for study.

Table 3: Results of correlation analysis between UGT2B28, UGT2B17, UGT2B15, rs1800629 and rs361525 polymorphisms and risk of BPH and prostate cancer

Lotus	Model	Geno type	sample size			Prostate cancers vs Controls		BHP vs Controls	
			Prostate cancers	BHP	Controls	Odds Ratio	p value	Odds Ratio	p value
UGT2B28	Allele	Ins	207 (86.2%)	203 (84.6%)	203 (84.6%)	1	0.6	1	1
		Del	33 (13.8%)	37 (15.4%)	37 (15.4%)	0.87 (0.53-1.45)		1 (0.61-1.64)	
	Codominant	Ins/Ins	90 (75%)	87 (72.5%)	88 (73.3%)	1	0.77	1	0.91
		Ins/Del	27 (22.5%)	29 (24.2%)	27 (22.5%)	0.98 (0.53-1.8)		1.09 (0.59-1.98)	
		Del/Del	3 (2.5%)	4 (3.3%)	5 (4.2%)	0.59 (0.14-2.53)		0.81 (0.21-3.11)	
	Dominant	Ins/Ins	90 (75%)	87 (72.5%)	88 (73.3%)	1	0.77	1	0.88
		Ins/Del + Del/Del	30 (25%)	33 (27.5%)	32 (26.7%)	0.92 (0.51-1.63)		1.04 (0.59-1.84)	
	Recessive	Ins/Ins + Ins/Del	117 (97.5%)	116 (96.7%)	115 (95.8%)	1	0.47	1	0.73
		Del/Del	3 (2.5%)	4 (3.3%)	5 (4.2%)	0.59 (0.14-2.53)		0.79 (0.21-3.03)	
	Overdominant	Ins/Ins + Del/Del	93 (77.5%)	91 (75.8%)	93 (77.5%)	1	1	1	0.76
		Ins/Del	27 (22.5%)	29 (24.2%)	27 (22.5%)	1 (0.55-1.83)		1.1 (0.6-2)	
	Log-Additive					0.88 (0.54-1.44)	0.62	1 (0.62-1.6)	1
UGT2B17	Allele	Ins	165 (68.8%)	143 (59.6%)	181 (75.4%)	1	0.1	1	<0.0001
		Del	75 (31.2%)	97 (40.4%)	59 (24.6%)	1.39 (0.93-2.08)		2.08 (1.41-3.08)	
	Codominant	Ins/Ins	53 (44.2%)	43 (35.8%)	66 (55%)	1	0.22	1	5e-04
		Ins/Del	59 (49.1%)	57 (47.5%)	49 (40.8%)	1.5 (0.89-2.53)		1.79 (1.04-3.07)	
		Del/Del	8 (6.7%)	20 (16.7%)	5 (4.2%)	1.99 (0.62-6.45)		6.14 (2.14-17.59)	

UGT2B17	Dominant	Ins/Ins	53 (44.2%)	43 (35.8%)	66 (55%)	1	0.09	1	0.002
		Ins/Del + Del/Del	67 (55.8%)	77 (64.2%)	54 (45%)	1.55 (0.93-2.57)		2.19 (1.3-3.67)	
	Recessive	Ins/Ins + Ins/Del	112 (93.3%)	100 (83.3%)	11 (95.8%)	1	0.39	1	0.001
		Del/Del	8 (6.7%)	20 (16.7%)	5 (4.2%)	1.64 (0.52-5.17)		4.6 (1.67-12.71)	
	Overdominant	Ins/Ins + Del/Del	61 (50.9%)	63 (52.5%)	71 (59.2%)	1	0.19	1	0.3
		Ins/Del	59 (49.1%)	57 (47.5%)	49 (40.8%)	1.4 (0.84-2.33)		1.31 (0.79-2.18)	
	Log-Additive					1.46 (0.95-2.25)	0.08	2.14 (1.42-3.22)	2e-04
UGT2B15	Allele	T	157 (65.4%)	124 (51.7%)	153 (63.8%)	1	0.7	1	0.007
		G	83 (34.6%)	116 (48.3%)	87 (36.2%)	0.93 (0.64-1.35)		1.64 (1.14-2.37)	
	Codominant	TT	47 (39.2%)	30 (25%)	44 (36.7%)	1	0.91	1	0.012
		TG	63 (52.5%)	64 (53.3%)	65 (54.1%)	0.91 (0.53-1.55)		1.44 (0.81-2.57)	
		GG	10 (8.3%)	26 (21.7%)	11 (9.2%)	0.85 (0.33-2.2)		3.47 (1.49-8.06)	
	Dominant	TT	47 (39.2%)	30 (25%)	44 (36.7%)	1	0.69	1	0.05
		TG+GG	73 (60.8%)	90 (75%)	76 (63.3%)	0.9 (0.53-1.52)		1.74 (1-3.03)	
	Recessive	TT+TG	110 (91.7%)	94 (78.3%)	109 (90.8%)	1	0.82	1	0.0066
		GG	10 (8.3%)	26 (21.7%)	11 (9.2%)	0.9 (0.37-2.21)		2.74 (1.29-5.84)	
	Overdominant	TT+GG	57 (47.5%)	56 (46.7%)	55 (45.9%)	1	0.8	1	0.9
		TG	63 (52.5%)	64 (53.3%)	65 (54.1%)	0.94 (0.56-1.55)		0.97 (0.58-1.61)	
	Log-Additive					0.92 (0.61-1.38)	0.68	1.76 (1.18-2.62)	0.0045

rs1800629	Allele	G	184 (92%)	204 (92.7%)	206 (93.6%)	1	0.51	1	0.7
		A	16 (8%)	16 (7.3%)	14 (6.4%)	1.28 (0.61-2.69)		1.15 (0.55-2.43)	
	Codominant	GG	84 (84%)	94 (85.5%)	9 6 (87.3%)	1	0.5	1	0.69
		AG	16 (16%)	16 (14.5%)	14 (12.7%)	1.31 (0.6-2.83)		1.7 (0.54-2.52)	
		AA	-	-	-	-		-	
	Dominant	GG	-	-	-	-	-	-	-
		AG+AA	-	-	-	-		-	
	Recessive	GG+AG	-	-	-	-	-	-	-
		AA	-	-	-	-		-	
	Overdominant	GG+AA	-	-	-	-	-	-	-
		AG	-	-	-	-		-	
	Log-Additive					-	-	-	-
rs361525	Allele	G	190 (95%)	213 (96.8%)	215 (97.7%)	1	013	1	0.56
		A	10 (5%)	7 (3.2%)	5 (2.3%)	2.26 (0.76-6.74)		1.41 (0.44-4.52)	
	Codominant	GG	9 0 (90%)	103 (93.6%)	105 (95.5%)	1	0.12	1	0.55
		AG	1 0 (10%)	7 (6.4%)	5 (4.5%)	2.33 (0.77-7.08)		1.43 (0.44-4.64)	
		AA	-	-	-	-		-	
	Dominant	GG	-	-	-	-	-	-	-
		AG+AA	-	-	-	-		-	
	Recessive	GG+AG	-	-	-	-	-	-	-
		AA	-	-	-	-		-	
	Overdominant	GG+AA	-	-	-	-	-	-	-
		AG	-	-	-	-		-	
	Log-Additive					-	-	-	-

*In this table, the allelic and genotypic frequency comparison of the cancer group to control and the BPH group to controls is given in one table. Yellow columns are the frequency of genotypes and alleles in each study group. Gray shows the comparison between PCa and control, and Orange indicates a comparison of BPH with the control group.

*As is considered, UGT2B17 was associated with the risk of BPH in allelic, Codominant, Recessive, and Log-Additive modes of inheritances. The G allele was more prevalent among BPH cases compared with controls (OR (95% CI) =1.64 (1.14-2.37), P value = <0.0001. For example, in the codominant model, individuals carrying the GG genotype had a significantly higher risk of BPH in comparison with those who carry the TT genotype (GG versus TT: OR (95% CI) = 3.47 (1.49-8.06), P value 0.012). Also, UGT2B15 was associated with the risk of BPH in allelic, Codominant, Recessive, and Log-Additive modes of inheritances (P value= 0.007).

*rs1800629 and rs361525 show no significant association with PCa or BPH in any of the inheritance models. Dash lines: no genotype or allele.

Table 4: Calculations of G score for different allelic composition in each possible genotype

	*G Score		
	≤ 6	> 6	P value
UGT2B28			0.54
Ins/Ins	49 (54.4%)	41 (45.6%)	
Ins/Del	12 (44.4%)	15 (55.6%)	
Del/Del	1 (33.3%)	2 (66.7%)	
UGT2B17			0.26
Ins/Ins	23 (43.4%)	30 (56.6%)	
Ins/Del	34 (57.6%)	25 (42.4%)	
Del/Del	5 (62.5%)	3 (37.5%)	
UGT2B15			**0.01**
TT	19 (40.4%)	28 (59.6%)	
TG	34 (54%)	29 (46%)	
GG	9 (90%)	1 (10%)	
rs1800629			0. 27
GG	40 (47.6%)	44 (52.4%)	
AG	10 (62.5%)	6 (37.5%)	
rs361525			0.5
GG	46 (51.1%)	44 (48.9%)	
AG	4(40%)	6 (60%)	

*Glison score

The only significant difference was observed in comparing the frequency of genotypes of the two groups for UGT2B15. In fact, there is a relationship between GG and G Score, so that 90% of people with GG genotype show a score of G ≤ 6, which confirms the association of this variant with BPH.

The frequency of each haplotype was compared using SNP Stats in three groups. Genotypes of three *UGT2B28- UGT2B17- UGT2B15* genes in BPH group as Ins-Del-G allelic combination was significantly higher than the frequency of this allelic combination in control subjects (p value= 0.011; OR (95%CI) (Table 5). A ***chi-squared*** test was used to derive p-Values.

Table 5: Haplotype frequencies were calculated using the SNP Stats (https://www.snpstats.net/start.htm) based on the expectation maximization algorithm

rs1800629	rs361525	Frequency in Prostate cancer	Frequency in BPH	Frequency in control	Prostate cancer vs Controls		BPH vs Controls		Prostate cancer vs BPH	
					OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value
G	G	0.8763	0.8955	0.9136	1.00	-	1	-	1	-
A	G	0.0737	0.0727	0.0636	1.24 (0.56 – 2.76)	0.59	1.2 (0.55-2.6)	0.65	1.05 (0.48-2.29)	0.9
G	A	0.0437	0.0318	0.0227	2.08 (0.66 – 6.57)	0.22	1.46 (0.45-4.79)	0.53	1.44 (0.5-4.14)	0.5

UGT2B28	UGT2B17	UGT2B15	Frequency in Prostate cancer	Frequency in BPH	Frequency in control	Prostate cancer vs Controls		**BPH vs Controls**		Prostate cancer vs BPH	
						OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value
Ins	**Ins**	**T**	**0.4778**	**0.4133**	**0.4897**	**1.00**	-	**1**	-	**1**	-
Ins	**Del**	**G**	**0.2515**	**0.296**	**0.1827**	**1.41 (0.82-2.44)**	**0.22**	**2.02 (1.18-3.47)**	0.011	**0.72 (0.44-1.19)**	0.2
Ins	**Ins**	**G**	**0.0863**	**0.1012**	**0.1475**	**0.66 (0.35 – 1.24)**	**0.2**	**0.94 (0.5-1.76)**	0.84	**0.75 (0.38-1.49)**	0.41
Del	**Ins**	**T**	**0.1154**	**0.0654**	**0.117**	**1.07 (0.59 – 1.95)**	**0.83**	**0.68 (0.3-1.56)**	0.37	**1.85 (0.79-4.31)**	0.16
Ins	**Del**	**T**	**0.0469**	**0.0353**	**0.026**	**2.02 (0.62 – 6.56)**	**0.24**	**1.48 (0.41-5.29)**	0.55	**1.25 (0.4-3.86)**	0.7
Del	**Del**	**G**	***0**	**0.0701**	**0.0323**	*-	-	**2.97 (0.94-9.37)**	0.065	-	-

Given that the p value is not less than 0.05 in any of the cases, so in terms of frequency, each haplotype of any of the five polymorphisms, shows a similar distribution in cancer and BPH groups compared to the control group. If a particular haplotype, for example, was seen with higher or lower frequently in the BHP samples, it would be possible to discuss it by presenting p value and odds ratio, but none of them showed a significant difference.

*0: This haplotype (Del Del G) was not present in PCa individuals.

*dash: When there is no haplotype, it is not possible to compare this group with the reference group

Discussion

Prostate cancer is the second most common cancer of men in the world including Iran [35-37]. Numerous studies have examined the effect of functional polymorphisms of *UGT2B28, UGT2B17* and *UGT2B15* enzymes on androgen metabolism [38], including single-nucleotide substitutions (SNPs and variable number of copies (CNV) [33-40]. The copy number variations (CNVs) of *UGT2B17* and *UGT2B28* genes are an important source of variation in their expression which affects the accumulation of dihydrotestosterone [41]. Genetic studies in recent years have shown that in cancer patients where both alleles of the *UGT2B17* gene have been deleted, the amount of 3-alpha-diol glucuronidate in circulation is decreased by 42% [42]. However, Habibi and colleagues did not find any association between the null genotype (del/del) of *UGT2B17* and *UGT2B28* genes with prostate cancer risk in Iranian PCa patients [33]. The present study showed that *UGT2B17* Del allele possibly is associated to the disease development and increase risk of BPH (P value = <0.0001). As we considered, this probable association could be in either mode of codominance, dominance, recessiveness, and/or in log-Additive models which could depend on locus neighboring genes in the form of haplotype.

In vitro studies have identified two enzymes, *UGT2B15* and *UGT2B17*, as the major enzymes involved in glucuronidation of androgens [43]. UGT2B15 protein level decreases in prostate cancer compared to benign hyperplasia and the protein is further reduced in hormone resistant form of prostate cancer and is as low as to be measured in metastasis state [44]. D85Y polymorphism (rs1902023) in *UGT2B15* gene has been shown to be significantly correlated with prostate cancer in some studies [45 ,33]and is a malignant polymorphism at gene codon 85 [33] where thymine replaces guanine base and causes the amino acid aspartic acid to be converted to tyrosine. The occurrence of this substitution in the N-terminal region of the enzyme, affects the protein activity in the second binding domain to the substrate [45]. UGT2B15 D85Y polymorphism does not appear to alter the specificity of substrates, but this polymorphism causes the maximum rate (Vmax) of glucuronidation to almost double and may play a role in individual differences in glucuronidation [46].

Some studies have reported that the TT genotype of *UGT2B15* D85Y is twice as active as the GG homozygotes, so it glucuronates the hormone dihydrotestosterone more rapidly, further protecting the prostate against high levels of androgens, thereby reducing the risk of the disease [47]. Lower activity of the enzyme variant increases the risk of cancer due to the accumulation of dihydrotestosterone in the prostate [48, 49]. Hajdinjak and colleagues showed that the frequency of homozygous G is high in patients with prostate cancer and the frequency of homozygous T is high in controls [45]. A 2013 study by Grant et al. [48], confirmed the association of D85Y polymorphism with prostate cancer, and in this study, as in previous studies by MacLeod et al. 2000 [50]; Hajdinjak et al. In 2004, a homozygous form of G was shown to be associated with an increased risk of prostate cancer [45]. However, some studies, such as a 2002 study by Gsur et al., found no association between this polymorphism and an increased risk of prostate cancer [51].The results of current study showed that *UGT2B15* is associated with BPH in either models of Codominance, Recessive, and Log-Additive (P value = 0.007) and its D85Y, rs1902023 GG genotype is most frequent in individuals with developing PCa (P value = 0.011). This is consistent with the previous reports we pointed here.

Also, the haplotype Ins Del G of the target genes was more common in BPH compared to the control group, one could anticipate that this achievement is an indication of the effect of the association of these three alleles on the etiology of the disease which in turn introduces this genotype as a probably important marker for predisposition a person to prostate hyperplasia.

Recent reports suggest that genetic polymorphisms of cytokines, including tumor necrosis factor-alpha (TNF-α), a proinflammatory molecule, are associated with increased inflammation, cytokine production, and possibly an increased risk of prostate cancer .[52] It is shown that promoter polymorphisms in the TNF-α gene can directly affect TNF-α production, thus causing interpersonal differences in the immune response that may affect susceptibility to prostate cancer [53], and/or malignant tumors like gastric cancer, breast cancer, and hepatocellular carcinoma .[54-56] We revealed that rs361525 TNF-α polymorphism is not associated with prostate cancer, but rs1800629 may increase the risk of PCa. Also, at least in this experimental study, there was no individual with AA genotype which shows that either for this particular polymorphism, the population of Iran is not in Hardy-Weinberg Equation or A allele has a low frequency in Iranian population, and probably has low influence on the incidence of the disease.

According to the results of this study and similar studies, finding functional polymorphisms as probable new biomarkers in the pathogenesis of cancers including prostate cancer encourages us to conduct more extensive research in this area with a larger population. A similar study with a larger population could confirm the results of this work.

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Conflict of interest

Authors have no conflict of interests.

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