Investigation of Interleukin-1β Polymorphisms in Prostate Cancer

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Abstract. *Background: Cytokine-mediated immune and inflammatory responses are considered to play an important role in the pathogenesis of prostate cancer. The present study investigated certain interleukin-1β (IL1β) polymorphisms and their association with prostate cancer. Materials and Methods: Genotyping of the IL1B-31(rs 1143627 G>A) and IL1B-511(rs 16944 A<G) polymorphisms were performed using real-time polymerase chain reaction for blood samples from a group of patients with prostate cancer (n=71) and controls (n=76). Results: The frequency of the IL1B-31(rs1143627) AG heterozygote genotype was found to be significantly lower in patients compared to controls (odds ratio=0.648, 95% confidence interval=0.463-0.908; p=0.036). The genotypic frequencies of IL1B-511(rs16944) between prostate cancer patients and controls were statistically significantly different (p=0.001). The frequency of AG genotype for IL1B-511(rs16944) was 0.5-fold lower in patients with prostate cancer than in the controls (odds ratio=0.546; 95% confidence interval=0.377-0.791; p=0.001). Conclusion: Our data show that individuals carrying the IL1B-31(rs1143627) and IL1B-511(rs16944) AG genotypes had a decreased risk for developing prostate cancer. Out of all the possible combinations analyzed, IL1B-31(rs1143627) G with IL1B-511(rs16944) G combination had a protective association with prostate cancer.*

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Key Words: prostate cancer, interleukin-1β, polymorphism.

Prostate cancer is the most common malignancy and the second leading cause of cancer-related death among men in the world (1). Twin studies showed that up to 50% of the risk of prostate cancer may be explained by genetic factors (2). However, common genetic polymorphims are likely to play an important role in sporadic cases of prostate cancer (3).

Early identification of patients at high risk of progression could be useful to anticipate therapy. The most important prognostic markers of prostate cancer are Gleason score, preoperative serum prostate-specific antigen (PSA) and pathological stage (4). Some predictors for biochemical recurrence exist. However, the accurarcy of prediction could be improved by incorporating new prognostic markers into clinical trials. Evidence from epidemiological, histopathological, genetic and molecular research support the existence of a relation between inflammation and prostate cancer (5).

Cytokine-mediated immune and inflammatory responses have been consired to play an important role in the pathogenesis of prostate cancer (6). Interleukin-1β (IL1B) is a 17-kDa cytokine member of the interleukin-1 family, which is involved in inflammation and immunity. It is produced by B-lymphocytes, macrophages, endothelial cells, fibroblast, dendritic cells. The *IL1B* gene is located on chrosome 2q14 and its expression has been found to be associated with various cancer types (7). *IL1B* polymorphisms are associated with an increase in the production of IL1B when compared to the wild-type gene (8). *IL1B* polymorphisms have also been linked to several kinds of malignant tumors, such as gastric cancer (9), hepatocellular cancer (10) and lung cancer (11).

Prostate cancer cases have decreased in the past decade because of practical screening methods for PSA. PSA is a 33-kDa glycoprotein containing a polypeptide of 240 amino acids that is mainly synthesized by the epithelial cells of the prostate gland (12). Serum PSA levels can be increased in benign prostatic cases because of structural disorders of the prostate; it has been shown that higher levels of PSA are

Table I. *Demographic characteristics of the study population.*

Parameter		Prostate cancer (n=71)	Control (n=76)	*p*-Value
Age (years), mean±SD		67.61±7.34	67.53±8.77	0.967
Body mass index (kg/m), mean±SD		27.01±3.71	27.28±3.55	0.773
Smoking (pack year), mean±SD		30.56±18.68	27.75±17.04	0.594
Prostate-specific antigen (ng/ml) mean±SD		25.94±41.14	3.03±2.66	0.006*
Gleason score, mean±SD		7.74±0.88	-	-
Family history of cancer, n (%)‡	Yes	31 (43.7%)	9 (11.8%)	-
	No	38 (53.5%)	10 (13.2%)	-
	NA	2 (2.8%)	57 (75%)	-
Pathological T-stage, n (%)	T2a	9 (12.7%)	-	-
	T2b	10 (14.1%)	-	-
	T2c	29 (40.8%)	-	-
	T3a	11 (15.5%)	-	-
	T3b	10 (14.1%)	-	-
	NA	2 (2.8%)	-	-
Clinical T-stage, n (%)	Early (T1+T2)	61 (85.9%)	-	-
	Late (T3+T4)	8 (11.3%)	-	-
	NA	2 (2.8%)	-	-

n: Number of individuals; NA: not available. *statistically significant difference, ‡first degree.

found in malignancy (13). PSA is a useful diagnostic marker for patients with prostate cancer. Although PSA has been used as tumor marker for a long time, its role in pathologies has not been precisely determinated. Recent evidence suggests that genetic variations may contribute to individual variation in serum PSA levels. However, genetic variations associated with PSA levels have not been identified (14).

To the best of our knowledge, there is a lack of studies reporting a relation between prostate cancer and *IL1B* -31(rs 1143627) and *IL1B* -511(rs 16944) polymorphism. In this study, we investigated the associations between *IL1B* -31 and *IL1B* -511 polymorphisms and prostate cancer in a Turkish population.

Materials and Methods

Patient selection. Our study included 71 patients with prostate cance (mean age=67.61±7.34 years) who were treated at the Department of Urology, Yeditepe University Hospital and Göztepe Research and Education Hospital. The diagnosis of prostate cancer was confirmed by clinical, laboratory and pathological examinations. The tumor differentiation status was evaluated using Gleason score criteria. The clinical T-stage was classified as early-stage (T1 and T2) and late-stage (T3 and T4) by clinical examinations. Pathological T-stage was classified as T2a, T2b, T2c, T3a and T3b. A total of 76 age-matched controls (mean age=67.53±8.77 years) were selected from prostate cancer-negative patients of Urology Clinics of the same hospitals. Clinical parameters (body mass index, serum PSA level, smoking habit) for each participant were collected from the hospital records. Serum samples were taken after obtaining informed consent and the study was conducted prospectively. Local Ethical Committee approval was obtained for the study (protocol no: 63/505). The protocol followed was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects).

DNA extraction. Blood samples from all participants were collected in tubes containing ethylene diamine tetra acetic acid (EDTA). Genomic DNA was extracted from 350 μl peripheral whole blood using Invitrogen iPrep PureLink gDNA blood isolation kit with a iPrep Purification Instrument (Invitrogen, Life Technologies, Carlsbad, CA, USA). The isolation procudure was performed in a closed system and took 45 min; 100 μl of DNA was obtained at the end of the procedure. Consequently, sample DNA concentrations (mean=80±9.98 ng/μl) and optical density ratios (at 260/280 nm) (mean=1.9±0.2) were measured by Nanodrop 2000 (Thermoscientific, Waltham, MA, USA). İsolated DNA samples were preserved at 4˚C until genotyping assessments were conducted.

Genotyping. Analysis of *IL1B-31* (rs 1143627) and *IL1B-511* (rs 16944) were performed by Taqman Allelic Discrimination Assay (Applied Biosystems, Foster City, CA, USA). The TaqMan probes were designed using the Primer Express software (Applied Biosystems). Allelic discrimination analysis with real-time PCR was performed using fluorescein amidite (FAM) for wild-type allele and VIC fluorescent probes for mutant allele. The labeling and sequences of the probes and primers were: IL1B -31: 5-(6FAM)-TTTGAAAGCCATAAAAA-3 (probe 1), 5-VIC-TTTGAAAGCTA TAAAAACAG-3 (probe 2), forward primer: 5-CCCCTTTCCTT TAACTTGATTGTG-3 and reverse primer: 5-AGGTTTGGTATCTG CCAGTTTCTC-3; *L1B-511*: 5-(FAM)-AGAGCTCCCGAGGCA-3 (probe1), 5-(VIC)-AGAGCTCCTGAGGCAG-3 (probe 2), forward primer: 5-CAGCCAAGAAAGGTCAATTTTCTC-3 and reverse primer: 5-CTGTATTGAGGGTGTGGGTCTCTAC-3. The reaction mix included 20 ng genomic DNA, 10 pmol of each primer, 2 pmol of each probe, and 10 μl of 2X master mix (Applied Biosystems) in a final volume of 20 μl. The thermocycling included 40 cycles with 30 s at 95˚C followed by 60 s at 60˚C. The PCR reactions were

Table II. *The distribution of interleukin (IL)1B -31(rs 1143627 G>A) and IL1B -511(rs 16944 A<G) polymorphism and allele frequencies in patient and control groups.*

SNP	Prostate cancer (n=71), n (%)	Control (n=76), n (%)	*p*-Value
IL1B-31 (rs 1143627 G>A)			
Genotype			0.036*
AA	26 (37.7%)	19 (25%)	
GG	16 (23.2%)	11 (14.5%)	
AG	27 (39.1%)	46 (60.5%)	0.008*
Allelle			
A	79 (57.2%)	84 (55.2%)	0.178
G	59 (42.8%)	68 (44.8%)	0.099
IL1B-511 (rs 16944 A<G)			
Genotype			0.001*
AA	21 (29.6%)	10 (13.2%)	
GG	27 (38%)	19 (25%)	
AG	23 (32.4%)	47 (61.8%)	0.001*
Allelle			
A	65 (45.7%)	67 (44%)	0.012*
G	77 (54.3%)	85 (56%)	0.012*

n: Number of individuals; SNP: single nucleotide polymorphism; *statistically significant difference.

performed on a 7500 Fast Real-Time PCR System (Invitrogen, Life Technologies) (11).

Statistical analyses. Statistical analyses were performed using SPSS version 21 software (SPSS Inc, Chicago, IL, USA). Values are given as the mean±standard deviation (SD). Student's *t*-test was used to examine the significance of differences between the two groups and χ^2 and Fisher's exact tests were used to compare demographic information to expression.

The genotypic distributions in all groups was tested for deviation from Hardy–Weinberg equilibrium (HWE) using the goodness-of-fit test. Linkage disequilibrium (LOD) between variations was assessed using D^0 and r^2 values obtained through the Haploview program (http://www.broad.mit. edu/mpg/haploview/(documentation.php). Multivariate logistic regression analysis was used to study the effect of each genotype on the risk of prostate cancer. The relative risk at a 95% confidence interval (CI) was calculated as the odds ratio (OR). Individuals homozygous for the common genotype were used as the reference to test for any association of genotype with prostate cancer by employing logistic regression model to calculate the OR with 95% CI, *p*-values lower than 0.05 denoted statistical significance.

Results

The demographic characteristic of control and patient groups are given in Table I. The mean age of patients with prostate cancer and healthy controls were 67.61±7.34 and 67.53±8.77 years, respectively. No significant differences were found between patients and controls in terms of median age ($p>0.05$).The patient group had a higher level of PSA ($p=0.006$) when compared to the control group.

Table III. *Multivariate logistic regression analysis relating interleukin (IL)1B-31 rs1143627 and IL1B-511 rs16944 genotype to the risk of prostate cancer.*

Variable	Prostate cancer		
	OR	95% CI	*p*-Value‡
IL1B-31 rs1143627			
GG	Reference		
AG	0.403	0.163-0.995	0.049*
AA	2.478	1.005-6.112	0.029*
IL1B-511 rs16944			
GG	Reference		
AG	0.344	0.159-0.744	0.007*
AA	1.478	0.569-3.839	0.423

OR: Odds ratio, CI: confidence interval. ‡Comparison of patients with prostate cancer with a control group; *statistically significant.

The allelic and genotypic frequencies for *IL1B-31* (rs1143627) and *IL1B-511* (rs16944) polymorphisms in patients with prostate cancer and controls are given Table II. Firstly, we evaluated the association between *IL1B-31* (rs1143627) polymorphism and study groups. The frequency of the AG genotype was found to be significantly lower in patients compared to controls ($\chi^2=6.638$, $p=0.036$; OR=0.648, 95% CI=0.463-0.908, $p=0.008$). At the same time, *IL1B-511* (rs16944) genotypic frequencies in patients with prostate cancer and controls were statistically significantly different ($\chi^2=13.368$, $p=0.001$). The frequency of the *IL1B-511* (rs16944) AG genotype was 0.5-fold lower in patients than in the controls (OR=0.546, 95% CI=0.377-0.791; $p=0.001$).

The genotypic distributions of *IL1B-31* (rs1143627) and *IL1B-511* (rs16944) polymorphisms were consistent with the Hosmer and Lemeshow test. Multivariate logistic regression analysis was performed to correct the OR for the genotype to examine whether the rs1143627 AG and rs16944 AG heterozygote genotypes are independently associated with a reduced risk of prostate cancer (Table III).

We also analyzed frequencies of haplotypes of different combinations to evaluate the synergistic effect on prostate cancer. The results are shown in Table IV. The frequency of the haplotype consisting of the combination of *IL1B-31* (rs1143627) G and *IL1B-511* (rs16944) G alleles was significantly lower in the patients than in the control group (Table IV). Conversely, the frequency of the combination of *IL1B-31* (rs1143627) G and *IL1B-511* (rs16944) A alleles was significantly higher in the patients than in the control group (Table IV).

Distributions of *IL1B-31* (rs1143627) and *IL1B-511* (rs16944) genotypes according to stage of prostate cancer were examined. There were no significant differences in

Table IV. *The frequencies of haplotypes of interleukin 1B (IL1B) gene in patients and controls*

Haplotype	Frequency				
	Overall	Prostate cancer (n=71)	Control (n=76)	Chi square	*p*-Value
rs 1143627 A: rs 16944G	0.384	0.347	0.419	1.604	0.205
rs 1143627 G: rs 16944G	0.249	0.167	0.325	9.738	0.001*
rs 1143627 G: rs 16944A	0.190	0.262	0.123	9.260	0.002*
rs 1143627 A: rs 16944A	0.178	0.224	0.134	4.079	0.043

LOD: 0.87; D': 0.14; r^2: 0.014. *Statistically significant.

IL1B-31 (rs1143627) and *IL1B-511* (rs16944) genotypic frequencies by histological tumor stage (p=0.976 and p=0.877, respectively) (Table V).

Table V. *The frequency of interleukin 1B (IL1B) genotypes by tumor stage in patients with prostate cancer.*

SNP	Early stage, n (%)	Late stage, n (%)	Total (n=69), n (%)	*p*-Value
IL1B-31 (rs 1143627)				0.976
AA	24 (34.8%)	2 (2.9%)	26 (37.7%)	
GG	14 (20.3%)	2 (2.9%)	16 (23.2%)	
AG	23 (33.3%)	4 (5.8%)	27 (39.1%)	
IL1B-511 (rs 16944)				0.877
AA	16 (23.2%)	4 (5.8%)	20 (29%)	
GG	24 (34.8%)	2 (2.9%)	26 (37.7%)	
AG	21 (30.4%)	2 (2.9%)	23 (33.3%)	

n: Number of individuals; SNP: single nucleotide polymorphism.

Discussion

Epidemiological, genetic and molecular studies have shown that there is a strong relation between inflammation and prostate cancer (6). However it is reasonable to assume that sequence variants in genes coding pro-inflammatory and anti-inflammatory cytokines effect prostate cancer risk, since cytokine gene polymorphisms may lead to an altered production of cytokines (5, 8). IL1 is a major pro-inflammatory cytokine which has been proposed as a molecular link between inflammation and cancer (5). *IL1B* polymorphisms have been investigated for association with several cancer types. It has been shown that *IL1B* polymorphisms (*IL1B-31* and *IL1B-511*) are associated with increased risk of gastric cancer (9).

In our study, we found the *IL1B-511* (rs16944) G allele was statistically more frequently found in the prostate cancer group (p=0.012). Wang *et al*. showed that *IL1B-511* mutant allele, associated with high IL1B production, in the liver is a genetic marker for the development of hepatocellular carcinoma (10). Our data confirm those of Zienolddiy *et al*. They found that *IL1B-511* (rs16944) mutan allele and *IL1B-31* (rs1143627) mutant allele were statistically more frequent in the lung cancer group (11). In spite of a negative effect of carrying mutant alleles, our study showed that the AG heterozygote genotypes of *IL1B* (rs1143627) and (rs16944) are independently associated with reduced risk for prostate cancer (Table II).

The functional role of *IL1B-31* polymorphisms are far from being clear but Zienolddiy *et al*. implied that the mutant allele is a pro-inflammatory allele (11). Another study also showed that the IL1B-31 mutant allele is associated with breast cancer because of the pro-inflammatory effect (15). The DNA sequence properties of IL1B-31 polymorphisms indicate the importance of IL1B-31 mutant allele. This allelic polymorphism is a TATA-box polymorphism that has been shown to affect DNA–protein interactions *in vitro*, hence modulating *IL1B* gene expression (9). Location of mutant nucleotide at this site will impair the TATA-box, which changes the affinity of regulatory proteins in binding to this sequence. Generally, the presence of a TATA-box in promoter regions is characteristic of an inducible gene. The presence of a wild-type allele at this site may reduce characteristics of the *IL1B* gene, leading to decreased inflammatory reaction (8, 11). Therefore, the mutant allele might be associated with higher transcription of *IL1B*, and inflammation.

The genotypic distributions of *IL1B-31* (rs1143627) and *IL1B-511* (rs16944) were also evaluated by multivariate logistic regression analysis as shown in Table III. A meta-analysis found that *IL1B-31* (rs1143627) polymorphism was weakly significant under a heterozygote model in prostate cancer (16). From our results, the frequency of the *IL1B-31* (rs1143627) AG genotype was found to be significantly lower in patients compared to controls, heterozygosity seemed to be protective aganist prostate cancer for both SNPs.

Among all the possible combinations analyzed here, *IL1B-31* (rs1143627) G and *IL1B-511* (rs16944) G combination

had a protective association with prostate cancer. A number of studies mostly in Caucasian populations support that *IL1B-511* wild-type allele in linkage disequilibrium with *IL1B-31* mutant allele carriers is a risky haplotype for the development of gastric cancer (9) and hepatocellular carcinoma (10).

In conclusion, our present data show that individuals carrying the *IL1B-31* (rs1143627) and *IL1B-511* (rs16944) AG genotypes were at a decreased risk for developing prostate cancer. Among all possible combinations analyzed, *IL1B-31* (rs1143627) G and *IL1B-511* (rs16944) G combination protected against developing prostate cancer. To the best of our knowledge, the data acquired in this study highlight, for the first time, the association of *IL1B* gene variants with prostate cancer in a Turkish population.

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Received July 30, 2015
Revised September 15, 2015
Accepted September 25, 2015