IL-6 Genomic Variants and Risk of Prostate Cancer

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Purpose: To evaluate the role of Interleukin-6 (IL-6) single nucleotide polymorphisms in prostate cancer (PCa) and benign prostate hyperplasia (BPH).

Materials and Methods: We genotyped two IL-6 intronic variants (rs1800795 and rs2069845) in PCa cases, BPH cases and healthy men referred to Labbafinejad and Shohadaye Tajrish Medical Centers using tetra ARMS-PCR method.

Results: The study included 130 PCa cases, 200 BPH cases and 200 healthy men. The C allele of rs1800795 was associated with PCa risk in the assessed population (OR (95% CI) = 1.45 (1.06-1.98)). However, the frequency of rs2069845 variants was not significantly different between PCa, BPH and control groups. The A C haplotype (rs2069845 and rs1800795 respectively) was associated with PCa and BPH risk (OR (95% CI) = 1.67 (1.12- 2.48); OR (95% CI)= 1.78 (1.25 – 2.54)). Besides, the A G haplotype (rs2069845 and rs1800795 respectively) has a protective effect against both PCa and BPH in the assessed population (OR (95% CI) = 0.63 (0.46-0.87); OR (95% CI)= 0.6 (0.45-0.79)).

Conclusion: Consequently, the results of the current study provide further evidence for contribution of IL-6 in prostate cancer.

Keywords: IL-6; prostate cancer; benign prostate hyperplasia

INTRODUCTION

Prostate cancer (PCa) and benign prostate hyperplasia (BPH) are two androgen-dependent pathological conditions with shared inflammatory elements as well as common genetic and epigenetic changes[(1)]. Both diseases have been associated with BK virus (BKV) infection[(2)]. Expression of certain matrix nuclear proteins can differentiate these two conditions[(3)]. The high prevalence of these disorders among aged males has surged researchers to find genetic susceptibility loci[(4-6)] with possible application as biomarkers or therapeutic targets[(7)]. A recent meta-analysis of literature has shown the age-standardized rate of prostate cancer was 9.11 in Iran[(8)]. Inflammatory responses have a well-documented role in cancer pathogenesis through modulation of tumor microenvironment, distortion of cytokine balance and production of reactive oxygen species[(9)]. Among cytokine, the role of interleukin (IL)-6 in prostate cancer pathogenesis has been vastly evaluated. Multiple lines of evidence point to its role in this type of malignancy. First, serum IL-6 levels have been correlated with PCa burden as defined by serum prostate specific antigen (PSA) levels or clinically apparent metastases[(10)]. Moreover, its higher levels might be an indicator of irresponsiveness to hormone ablation therapy[(11)]. IL-6 act as a paracrine factor that modulates PCa autophagy and neuroendocrine differentiation[(12)]. The regulatory role of IL-6 on is exerted through the AMPK/mTOR pathway[(13)]. The role of IL-6 in induction of cell proliferation and prevention of apoptosis is exerted through various cancer-associated signal pathways such as the Janus tyrosine family kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, the extracellular signal-regulated kinase 1 and 2 (ERK1/2)-mitogen activated protein kinase (MAPK) pathway and the phosphoinositide 3-kinase (PI3-K) pathway[(9)]. Experimental studies have shown that IL-6 induces and/or augments the conversion of prostate cancer cells from an androgen-dependent to an androgen-independent phenotype[(14)]. Functional variants within IL-6 coding gene including the rs1800795 and rs2069845 single nucleotide polymorphisms (SNPs) have been previously shown to alter circulating IL-6 levels[(15-17)]. Moreover, the C allele of rs1800795 has been associated with increased risk of PCa in an American population but the association did not remain significant after accounting for multiple tests[(18)]. The GG genotype of this SNP has been associated with an increased risk of metastasis of primary breast cancer[(19)]. Considering the role of IL-6 in PCa pathogenesis as well as the presence of com-

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Table 1. The nucleotide sequences of primers used for genotyping (SNP: single nucleotide polymorphism, bp: base pair).

SNP	Primer sequence	Tm	Annealing temperature	PCR product size (bp)
rs2069845	Forward inner primer (G allele): 5′-GTTTCCCAGTCCTCTTTACACCAACG	66 °C	62 °C	197 bp (G allele)
	Reverse inner primer (A allele): 5′-TTTATGATCTGTTGAAAGACCACTGACCT	66 °C		292 bp (A allele)
	Forward outer primer: 5′-CATCCTGCCTCTGCCATTTCTACTTAA (two outer primers)	66 °C		434 bp
	Reverse outer primer: 5′-ATTCTGACATCTGAGATAATGCCTGG rs1800795	66 °C		
	Forward inner primer (C allele): 5′-CACTTTTCCCCCTAGTTGTGTCTTCCC 206 bp (C allele)	68 °C		61 °C
	Reverse inner primer (G allele): 5′-ATTGAGCAATGTAACGTCCTTTAGCTTC	68 °C		155 bp (G allele)
	Forward outer primer: 5′-CAATACATGCCAATGTGCTGAGTCACTA	68 °C		306 bp (two outer primers)
	Reverse outer primer: 5′-AGAATGATCCTCAGTCATCTCCAGTCCT	68 °C		

mon inflammatory mechanisms in PCa and BPH, we aimed at evaluation of the associations between two functional polymorphisms within this gene (rs1800795 and rs2069845) and risk of PCa and BPH in an Iranian population. The current study is the first association study of IL-6 polymorphisms in Iranian patients with PCa and BPH.

MATERIALS AND METHODS

Patients

The appropriate sample size was calculated for rs2069845 with minor allele frequency of 0.25 assuming the study power of 70% and significance level of 5% to be 120 cases and 120 controls. The current case-control study recruited 130 newly diagnosed PCa cases, 200 newly diagnosed BPH cases and 200 healthy men referred to Labbafinejad and Shohadaye Tajrish Medical Centers. The diagnosis was established based on pathological examination of biopsied samples. The study protocol has been approved by ethical committee of Shahid Beheshti University of Medical Sciences. All study participants signed the informed consent forms. Control subjects were selected from men seeking routine health assessment during 2016 and were matched to patients. Men attributed to control group had no history of lower urinary tract symptoms, inflammatory disease of prostate, prostate enlargement or family history of PCa. Controls had normal PSA levels. PCa or BPH was diagnosed through evaluation of clinical prostate biopsies by an expert pathologist especially in BPH patients with high PSA levels (4.0 ng/ml or more). Exclusion criteria were inadequate pathologic sample, history of former malignancies in other organs and previous chemo-radiotherapy. Blood samples were collected from patients in EDTA tubes before commencement of any therapy such as surgery, radiotherapy, and chemotherapy. Clinicopathological data were collected through filling questionnaires and assessment of medical reports.

Genotyping

Genomic DNA was extracted from blood samples of all study participants using standard salting out method. The rs2069845 and rs1800795 intronic variants within IL-6 gene were genotyped using tetra-primer ARMS-PCR technique and were visualized after staining on 2% agarose gel. The amplification program was started with denaturation step at 95 ºC for 5 minutes followed by 35 cycles of 95 ºC for 45 seconds, specific annealing temperatures for 35 seconds, and 72 ºC for 35 seconds and a final extension step in 72 ºC for 10 minutes. Ten percent of samples were sequenced using ABI 3730xl DNA analyzer (Macrogen, Korea) to confirm the results of tetra-primer ARMS-PCR. The nucleotide sequences of primers used for genotyping are shown in **Table 1**.

Table 2. Demographic and clinical data of study participants (BPH: benign prostate hyperplasia,

Variables	Prostate cancer group	BPH group	Controls
Age (mean ± SD)	66.54 ± 9.5	67.96 ± 3.97	64 ± 5.12
BMI (mean ± SD)	25.06 ± 2.14	24.97 ± 3.47	25.7 ± 1.2
Prostate weight (gr) (mean ± SD)	58 ± 98.31	61.87 ± 29.52	-
PSA (ng/mL) (mean ± SD)	9.13 ± 9.28	8.94 ± 7.2	< 4
<4	25 (19.23%)	39 (19.5%)	200 (100%)
4-10	73 (56.15%)	94(47%)	0
>=10	32 (24.61%)	67 (33.5%)	0
Smoking			
Never smoker (%)	69 (53.1%)	121 (60.5%)	124 (62%)
Current or former smoker (%)	61 (46.9%)	79 (39.5%)	76 (38%)
Gleason score			
<=6	68 (52.3%)	-	-
>6	62 (47.7%)	-	-

Abbreviations: SD: standard deviation, PSA: prostate specific antigen, BMI: body mass index).

Table 3. Exact test for Hardy-Weinberg equilibrium.

SNP	rs2069845			*P*-value	rs1800795			*P*-value
	AA	AG	GG		GG	GC	CC	
PCa	46	69	15	0.15	39	55	36	0.08
BPH	78	91	31	0.60	61	91	48	0.22
Control	82	97	21	0.32	77	87	36	0.19

Abbreviations: SNP: single nucleotide polymorphism, BPH: benign prostate hyperplasia, PCa: prostate cancer

Statistical analysis

The agreement of genotype frequencies with the Hardy–Weinberg equilibrium was assessed using Chi-square test. The associations between genotype frequencies and PCa or BPH were evaluated in three inheritance models including recessive, dominant and co-dominant using Pearson's chi-square test. The P values were corrected through multiplying by the number of SNPs. P values less than 0.05 were regarded as significant. The linkage between rs1800795 and rs2069845 variants were assessed using D' and r values. Haplotype block frequencies and their associations with PCa and BPH were computed using Partition-Ligation–Expectation-Maximization (PL-EM) algorithm[20] (SNPanalyzer 2.0 software) with supposition of 0.01 minimum frequencies for blocks. The results were stated as Odds ratios (OR) and 95% confidence interval of OR (95% CI), *P*-value and Bonferroni adjusted P-values. Patients were matched to control group in variables such as BMI and smoking history.

RESULTS

Demographic and clinical data of study participants

Table 2 shows the demographic and clinical data of PCa, BPH and healthy subjects participated in the study. PCa and BPH patients were age-matched (P = 0.061). The three study groups were not significantly different in smoking (P = 0.39) and BMI (P = 0.79).

Genotyping

The distributions of alleles and genotypes of the assessed SNPs were in accordance with HWE in the three study groups. **Table 3** shows the results of evaluation of HWE.

Figure 1 and 2 show the results of ARMS-PCR for genotyping the mentioned SNPs.

The C allele of rs1800795 was associated with PCa risk in the assessed population (OR (95% CI) = 1.45 (1.06-1.98), Adjusted P = 0.04). However, the frequency of rs2069845 variants was not significantly different between PCa, BPH and control groups. (**Table 4**)

We also assessed the frequencies of IL-6 haplotypes in the three study groups and found significant over-presentation of A C haplotype (rs2069845 and rs1800795 respectively) in both PCa and BPH groups compared with control subjects (OR (95% CI)= 1.67 (1.12- 2.48), Adjusted P = 0.04; OR (95% CI)= 1.78 (1.25 – 2.54), Adjusted P = 0.006 respectively). Besides, the A G haplotype (rs2069845 and rs1800795 respectively) has been shown to exert protective effect against both PCa and BPH in the assessed population (OR (95% CI)= 0.63 (0.46-0.87), Adjusted P = 0.02; OR (95% CI)= 0.6 (0.45-0.79), Adjusted P = 0.001 respectively). **Table 5** shows the detailed information of haplotype analy-

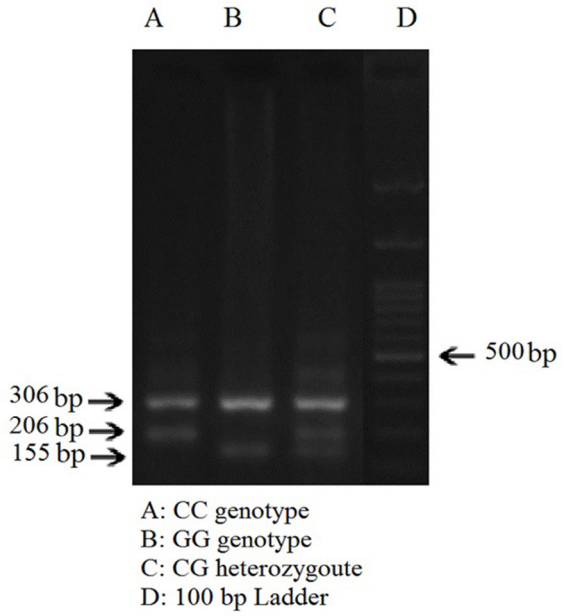

Figure 1. The results of ARMS-PCR for genotyping the rs1800795 and rs2069845 SNPs.

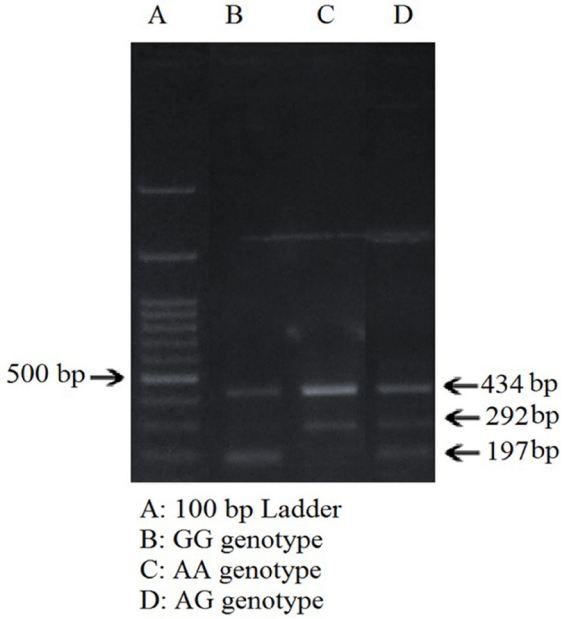

Figure 2. The results of ARMS-PCR for genotyping the rs2069845 SNPs.

Table 4. Association analysis of rs2069845 and rs1800795 polymorphisms and risk of PCa and BPH (*P**: Adjusted *P* value).

SNP	Model		Sample size (%)			PCa vs. Control			BPH vs. Control			PCa vs. BPH		
			PCa (%)	BPH (%)	Control (%)	OR (95% CI)	*P*	*P**	OR (95% CI)	*P*	*P**	OR (95% CI)	*P*	*P**
rs2069845	Allele	G vs. A	99 (38) 161 (62)	153 (38) 247 (62)	139 (35) 261 (65)	1.15 (0.83-1.60)	0.38	0.77	1.16 (0.87-1.55)	0.30	0.61	1.00 (0.0.72-1.37)	0.96	1.00
	Co-dominant	GG vs AA	15 (12)	31 (15.5)	21 (10.5)	1.26 (0.6-2.70)	0.59	1.00	1.59 (0.82-2.94)	0.33	0.66	1.54 (0.85-2.78)	0.35	0.7
		AG vs AA	69 (53)	91 (45.5)	97 (48.5)	1.26 (0.79-2.04)			0.99 (0.64-1.49)			1.11 (0.75-1.64)		
	Dominant	GG+AG vs AA	84 (64.6) 46 (35)	122 (61) 78 (39)	118 (59) 82 (41)	1.27 (0.8-2.00)	0.31	0.61	1.09 (0.73 -1.62)	0.68	1.00	1.17 (0.74-1.85)	0.51	1.00
	Recessive	GG vs AG +AA	15 (12) 115 (88.5)	31 (15.5) 169 (84.5)	21 (10.5) 179 (89.5)	1.11 (0.55-2.24)	0.77	1.00	1.56 (0.86-2.83)	0.14	0.27	0.71 (0.37-1.38)	0.31	0.62
rs1800795	Allele	C vs G	127 (49) 133 (51)	187 (47) 213 (53)	159 (40) 241 (60)	1.45 (1.06-1.98)	0.02	0.04	1.33 (1.00-1.76)	0.05	0.09	1.09 (0.8-1.49)	0.6	1.00
	Co-dominant	CC vs GG	36 (27.7)	48 (24)	36 (18)	1.96 (1.09-3.57)	0.08	0.16	1.69 (0.97.-2.94)	0.16	0.32	2.44 (1.37-4.35)	0.74	1.00
		CG vs	55 (42.3)	91 (45.5)	87 (43.5)	1.25 (0.75-2.08)			1.32 (0.85-2.08)			1.3 (0.87-1.92)		
	Dominant	CG+CC vs GG	91 (70) 39 (30)	139 (69.5) 61 (30.5)	123 (61.5) 77 (38.5)	1.46 (0.91-2.34)	0.11	0.23	1.42 (0.94-2.16)	0.09	0.18	1.02 (0.63-1.66)	0.92	1.00
	Recessive	CC vs CG+GG	36 (27.7) 94 (72.3)	48 (24) 152 (76)	36 (18) 164 (82)	1.74 (1.03-2.95)	0.04	0.07	1.44 (0.89-2.34)	0.14	0.28	1.21 (0.73-2.00)	0.45	0.9

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No strong linkage disequilibrium (LD) has been observed between the analyzed polymorphic sites (LD analysis, $D' = 0.14$; $r = 0.01$; $P = 0.5$).

DISCUSSION

The role of IL-6 in the pathogenesis of PCa has been extensively evaluated. However, associations between genomic variants of IL-6 gene and risk of PCa have not been assessed in different populations. In the present study we assessed associations between two functional variants of IL-6 and prostate disorders in a cohort of Iranian patients with prostate disorders. We found significant over-presentation of the rs1800795 C allele in PCa patients compared with healthy subjects. We did not find any difference in allele or genotype frequencies of this SNP between BPH patients and controls which might rule out its contribution in the pathogenesis of BPH despite its putative role in PCa. Although the C allele of rs1800795 has been associated with higher circulating IL-6 levels in human subjects[15,17,21]), the association between this allele and PCa risk in American patients did not remain significant after multiple testing correction. However, authors suggested further evaluation of the association between this genomic variant and PCa risk[18]. Consequently, our results provide additional support for their observation. Moreover, the C allele of rs1800795 has been associated with higher concentrations of circulating C reactive protein (CRP) [16], which has been regarded as a negative predictor of survival in PCa [22]. So this polymorphism might exert its effects in PCa pathogenesis through multiple mechanisms including alterations in IL-6 and CRP levels. However, despite previous studies demonstrated higher IL-6 in carriers of minor allele of rs2069845[17], we did not find significant difference in allele and genotype frequencies of rs2069845 between PCa, BPH and control groups.

Notably, we found significant over-presentation of A C haplotype (rs2069845 and rs1800795 respectively) in both PCa and BPH groups compared with control subjects. On the other hand, the A G haplotype (rs2069845 and rs1800795 respectively) has been shown to exert protective effect against both PCa and BPH in the assessed population. Such data further supports the significance of rs1800795 and unimportance of rs2069845 variants in conferring PCa or BPH risk. However, the implication of other functional variants within these haplotypes cannot be ruled out. As no differences have been found in haplotype frequencies between BPH and PCa groups, assessment of haplotypes cannot differentiate between these two conditions.

Although most of previous studies have demonstrated the usefulness of IL-6 concentrations as predictive biomarkers in PCa patients, some inconsistencies exist. For instance, Nakashima et al. reported serum IL-6 level as a major prognostic factor for prostate cancer and its extent of disease[23]. In line with their study, Alcover et al. highlighted the effectiveness of IL-6 in predicting the biochemical progression of prostate cancer [25]. On the other hand, Pierce et al. failed to detect any association between circulating IL-6 concentration and PCa risk and proposed that rs1800795 may alter PCa risk through other mechanisms[19] among which might be modulation of CRP levels. Alternatively, they suggested that the variability in IL-6 levels or the insufficiency of a single assessment of IL-6 as an indicator of long-standing blood levels might result in failure of

Table 5. The frequencies of haplotype blocks in the three study groups (*P**: Adjusted *P* value).

rs2069845	rs1800795	PCa	BPH	Control	PCa vs. Control			BPH vs. Control			PCa vs. BPH		
					OR (95% CI)	*P*	*P**	OR (95% CI)	*P*	*P**	OR (95% CI)	*P*	*P**
A	G	0.35	0.31	0.47	0.63 (0.46-0.87)	0.005	0.02	0.6 (0.45-0.79)	3.6 E-4	0.001	1.06 (0.77-1.46)	0.73	1.00
G	C	0.22	0.16	0.22	1.07 (0.75 – 1.53)	0.71	1.00	0.89 (0.64 – 1.24)	0.5	1.00	1.2 (0.83– 1.72)	0.33	1.00
A	C	0.27	0.31	0.18	1.67 (1.12- 2.48)	0.01	0.04	1.78 (1.25 – 2.54)	0.001	0.006	0.94 (0.65 – 1.35)	0.73	1.00
G	G	0.16	0.22	0.13	1.23 (0.75- 2.01)	0.41	1.00	1.64 (1.07- 2.49)	0.02	0.08	0.75 (0.47 – 1.19)	0.22	0.87

detecting the expected association[18].
Taken together, the rs1800795, or another variant in LD with it might confer PCa risk possibly through modulation of IL-6 RNA and protein levels or even other independent mechanisms. Considering the short plasma half-life of IL-6[25] and the presence of a circadian rhythm for this cytokine due to the circadian alterations of cortisol[26], we propose assessment of genomic variants within this gene as an alternative to evaluation of its serum concentrations. Such studies would elaborate the role of IL-6 in PCa risk and pave the way for designing personalized therapeutic options.
Our study had some limitations including sample size. Due to relative small sample size, we could not assess associations in subgroups of patients including different grades of PCa. Moreover, we did not have the data about serum level of IL-6 in study participants.

CONCLUSIONS

The rs1800795, or another variant in LD with it is associated with PCa risk possibly through modulation of IL-6 RNA and protein levels or even other independent mechanism.

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CONFLICT ON INTEREST

None declared.

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