Association Study of Retinoic Acid Related Orphan Receptor A (RORA) Gene and Risk of Prostate Disorders

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Purpose: Prostate cancer (PCa) and benign prostate hyperplasia (BPH) are two prevalent disorders among men with considerable mortality and morbidity. Several association studies have been conducted in different populations to find genetic loci linked with these disorders. Retinoic acid-receptor-related orphan receptor alpha (RORA) codes for a transcription factor which regulates expression of several cancer-related genes. Besides, RORA has been shown to be down-regulated in PCa tissues and cell lines.

Materials and Methods: In the present study we evaluated genotype and allele frequencies of rs11639084 and rs4774388 variants within RORA gene in PCa and BPH patients compared with healthy subjects.

Result: The rs11639084 and rs4774388 alleles were not different between PCa and normal groups 95% CI: 0.52-1.24, OR = 1.04, *P* = .34; 95% CI: 0.48-1.33, OR = .79, *P* = .39 respectively. Moreover, we did not detect any significant difference in allele, genotype or haplotype frequencies of these SNPs between the other study groups.

Conclusion: The mentioned RORA variants are possibly not involved in the pathogenesis of PCa and BPH. Future studies are needed to assess the associations between other variant within this gene and PCa risk to suggest a putative mechanism for involvement of RORA in PCa.

Keywords: benign prostatic hyperplasia; prostate cancer; retinoid-related orphan receptor alpha; RORA; single nucleotide polymorphism

INTRODUCTION

Prostate cancer (PCa) and benign prostate hyperplasia (BPH) are two prevalent disorders among men with considerable mortality and morbidity[(1)]. Several researches have focused on evaluation of risk factors of these disorders at genomic [(2,3)] and transcriptomic levels [(4)]. Among candidate genes whose involvement in PCa pathogenesis has been demonstrated is retinoic acid-receptor-related orphan receptor alpha (RORA). This gene encodes a transcription factor which is categorized as one of the orphan nuclear receptors. The existence of response elements for RORA in the promoter region of cell cycle-related genes implies its involvement in the regulation of cell cycle. Moreover, expression of RORA in androgen-independent PCa cells has suppressed cell growth as demonstrated by in vitro and in vivo studies [(5)]. More evidences for tumor suppressor role of RORA have been provided by observation of its down-regulation in various cancer tissues [(6)]. Certain single nucleotide polymorphisms (SNPs) within this gene have been associated with risk of breast cancer in different ethnic groups [(7,8)]. Despite the prominent role of RORA in PCa pathogenesis, there is no study for assessment of the association between RORA variants and risk of PCa or BPH. Consequently, in the present study we evaluated genotype and allele frequencies of rs11639084 and rs4774388 variants in PCa and BPH patients compared with healthy subjects to find if these variants are involved in the pathogenesis of these disorders or can be used as genetic risk factors for PCa or BPH.

MATERIALS AND METHODS

Study participants

Subjects

In the present case-control study a total of 144 PCa cases, 177 BPH cases and 112 normal males participated. PCa and BPH patients were selected from newly diagnosed patients in whom the histological examination of samples obtained from transrectal needle biopsy or transurethral resection of the prostate confirmed the diagnosis of disorder. Control subjects were normal age-matched males selected from a routine hospital-based health survey during 2016. The thee study groups have also been matched in age and body mass index (BMI). The study protocol has been approved by ethical committee of Shahid Beheshti University of Medical Sciences. All study participants were selected from hospitals affiliated with Shahid Beheshti University of Medical Sciences after assessment of their compli-

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Table 1. Demographic and clinical data of study participants.

Variables	Prostate cancer group	BPH group	Controls
Age (mean ± SD)	68.94 ± 9.89	66.96 ± 10.97	64 ± 9.1
BMI (mean ± SD)	25.07 ± 3.67	24.97 ± 3.47	24.17 ± 4.25
Prostate weight (gr) (mean ± SD)	52.01 ±25.32	62.17 ± 23.63	-
PSA (ng/mL) (mean ± SD)	9.26 ± 9.53	8.54 ± 6.1	<4
< 4	27 (18.75%)	32 (18%)	112 (100%)
4 -10	75 (52.0%)	89 (50.2%)	0
> =10	42 (28.25%)	56 (31.8%)	0
Smoking			
Never smoker (%)	74 (51.3%)	118 (66.6%)	75 (67%)
Current or former smoker (%)	70 (48.7%)	59 (33.4%)	37 (33%)
Gleason score			
< =6	74 (51.3%)	-	-
> 6 7	0 (49.7%)	-	-

Abbreviations: BMI, Body Mass Index, BPH, Benign Prosttae Hyperplasia, PSA, prostate specific antigen, SD, standard deviation.

ance with the study inclusion criteria and signing the informed consent. Control subjects had no history of lower urinary tract symptoms, prostate enlargement or family history of prostate cancer. PCa or BPH was diagnosed through examination of clinical prostate biopsies by an expert pathologist especially in BPH patients with high PSA levels (4.0 ng/ml or more) in whom transrectal biopsies ruled out the presence of PCa. Those with insufficient pathologic sample for evaluation, patients with the history of previous malignancies in other organs and any former chemo-radiotherapy were excluded from the study. Blood samples were collected from patients before initiation of any treatments such as surgery, radiotherapy, and chemotherapy. We also collected clinicopathologic characteristics of study participants including prostate weight, PSA levels and Gleason score through filling questionnaires and evaluation of patients' clinical reports.

Genotyping of rs11639084 and rs4774388

We genotyped the rs11639084 (C/T) and rs4774388 (C/T) variants lcated in the intronic regions of the RORA gene by tetra primer-amplification refractory mutation system-PCR (4P-ARMS-PCR) method using primers designed by PRIMER1 online tool as reported in our previuos study[9]. Briefly, for each reaction we used 100 ng of genomic DNA, 5 pmol/l of outer primers, 10 pmol/l of inner primers and 12.5 µl Taq DNA Polymerase 2 × Master Mix Red (Ampliqon, Denmark). All reactions were performed in a FlexCycler (Analytik Jena, Germany) with a PCR program consisted of an initial denaturation at 95 °C for 5 min and subsequent 35 cycles of 95 °C for 45 seconds, 55 °C for 45 seconds and 72 °C for 45 seconds. The results obtained by 4P-ARMS-PCR method were verified by Sanger sequencing of 10% of total samples in ABI 3730xl DNA analyzer (Macrogen, Korea).

Statistical analysis

SNPStats was used for assessment of allele, genotype and haplotype frequencies in distinct study groups and their accordance with Hardy-Weinberg equilibrium using Chi-square test (two-sided)[10]. The association of rs11639084 and rs4774388 polymorphisms with PCa and BPH risk was evaluated in all assumed inheritance models and described using odds ratios (ORs) and 95% confidence intervals (CIs). Haplotype frequencies for RORA were computed using the SNPAnalyzer program (Istech Ltd, Goyang-si, Korea) based on the expectation-maximization algorithm. The pairwise linkage disequilibrium (LD) between mentioned SNPs was assessed through measurement of D' and r^2 values. D' was described as the ratio of the unstandardized coefficient to its maximal/minimal value. *P* value less than 0.05 were considered statistically significant.

RESULTS

General data of study participants

Clinical and demographic data of study participants including the 144 PCa cases, 177 BPH cases and 112 normal males which were obtained through questionnaires and assessment of clinical reports are summarized in **Table 1**.

Results of SNPs genotyping

The assessed SNPs in the current study are located in intronic regions of RORA gene. Detailed information of these two SNPs is demonstrated in **Table 2**. We assessed the compliance of these SNPs with Hardy-Weinberg equilibrium. As demonstrated in **Table 3**, genotype frequencies of these SNPs in all three study groups are in Hardy-Weinberg equilibrium.

No significant difference has been found in allele and genotype frequencies of rs11639084 and rs4774388 SNPs between three study groups (PCa, BPH and normal controls). **Table 4** shows the allele and genotypes frequencies of these SNPs in distinct study groups.

We further assessed the frequencies of four supposed haplotypes of these two SNPs in distinct study groups but did not detect any difference between PCa, BPH and healthy subjects (**Table 5**).

Table 2. Descriptive information of rs11639084 and rs4774388 of RORA gene.

SNP	Position	Minor Allele	MAF	MAC	Type
rs11639084	Chr15:60774317	T	.24	1197	Intron
rs4774388	Chr15:61174799	C	.30	1507	Intron

Abbreviations: MAF, Minor Allele Frequency, MAC, Minor Allele Content (the average amount of minor alleles per subject).

Table 3. Exact test for Hardy-Weinberg equilibrium.

SNP	rs11639084				*P*-value	rs12826786			*P*-value
	All	CC	CT	TT		TT	CT	CC	
Cancer	144	85 (59%)	54 (37.5%)	5 (3.4%)	.47	84 (58.3%)	49 (34%)	11 (7.6%)	.37
BPH	177	119 (67.2%)	52 (29.3%)	6 (3.3%)	1	96 (54.2%)	63 (35.6%)	18 (10.1%)	.13
Control	112	76 (67.8%)	30 (26.7%)	6 (5.3%)	.21	58 (51.7%)	44 (39.2%)	10 (8.9%)	.65

DISCUSSION

In the present study we assessed allele, genotype and haplotype frequencies of two SNPs within RORA gene in Iranian patients with PCa and BPH compared with healthy subjects and demonstrated no significant difference between three study groups. RORA has been shown to participate in the pathogenesis of a wide range of human disorders including attention-deficit hyperactivity disorder [11], bipolar disorder[12], major depression [13], autism[9], multiple sclerosis[14] and breast cancer [7]. Previous studies have provided evidences for RORA contribution in suppression of PCa cell growth. RORA has been shown to down-regulate the expression of 5-lipoxygenase and restrict the mitogenic function of fatty acids on PCa cells. Consequently, RORA has been suggested as a therapeutic target in PCa [15]. In addition, down-regulation of RORA in cancerous tissues might change the expression of its downstream target genes such as CDK inhibitor p21 and N-myc which participate in carcinogenesis process[16]. We have recently demonstrated the association of the rs4774388 within this gene with autism spectrum disorder [9] as well as breast cancer risk[7]. The rs4774388 is supposed to change the affinity of the encoded protein to bind with POU5F1 as predicted by HaploReg v4.1 [17]. The retrogene POU5F1B encodes a homolog of the principal embryonic stem cell transcription factor Oct4. Breyer et al. have demonstrated the correlation between 8q24 risk alleles and decreased expression of POU5F1B gene in prostate tissues. In addition, they suggested the association between harmful POU5F1B missense variants and PCa. Their experiments showed the involvement of POU5F1 in self-renewal capability of embryonic stem cells as well as the pathogenesis of cancer[18]. Besides, participation of RORA in regulation of circadian rhythm[19] and dysregulation of circadian rhythm and melatonin pathways in PCa[20] increase the possibility of contribution of RORA variants in PCa risk. Taken together, we hypothesized that RORA variants might be regarded as risk factor for PCa. The absence of associations between these variants and PCa or BPH risk in Iranian population does not exclude the participation of RORA in the pathogenesis of these disorders.

Our study has some limitations. First, due to the relative small sample size we could not analyze genotype and allele frequencies in subgroups of PCa patients including those with small tumor size, low Gleason grade or low stage separately. In addition, we could not assess the associations between mentioned SNPs and patients' survival or response to treatments. Finally, we did not assess associations between other functional RORA variants and risk of PCa or BPH.

CONCLUSIONS

Although we could not find any association between two variants within RORA gene and prostate disorders, based on the proposed function of this gene, other variants within this gene might alter risk of PCa or BPH. So, future studies are needed to assess the associations between other variant within this gene and PCa risk to suggest a putative mechanism for involvement of RORA in PCa.

Table 4. Allele and genotype frequencies of rs11639084 and rs4774388 SNPs in three study groups (PCa, BPH and normal controls).

SNP	Model			Sample size (%)		Cancer vs. Control			BPH vs. Control		Cancer vs. BPH	
				Cancer(%)	BPH(%)	Control(%)	OR	*P*-value	OR	*P*-value	OR	*P*-value
		Allele	T vs.	64 (22)	64 (18)	42 (19)	1.04 (.52-1.24)	.34	1.04 (.68-1.61)	.84	.77 (.52-1.14)	.19
			224 (78)	290 (82)	182 (81)							
	Co-dominant	TT vs. CC	5 (3.5)	6 (3.4)	6 (5.4)	1.34 (.39-4.58)		.64	1.56 (.49-5.03)	.45	.86 (.25-2.90)	.80
		CT vs. CC	54 (37.5)	52 (29.4)	30 (26.8)	.62 (.36-1.07)		.08	.90 (.53-1.54)	.71	.69 (.43-1.10)	.12
	Dominant	TT+CT vs.	59 (41)	58 (32.8)	36 (32.1)	.68 (.41-1.14)		.15	.97 (.59-1.61)	.91	.70 (.44-1.12)	.13
		CC	85 (59)	119 (67.2)	76 (67.9)							
rs	Recessive	TT vs.	5 (3.5)	6 (3.4)	6 (5.4)	1.57 (.47-5.30)		.46	1.61 (.51-5.13)	.42	.97 (.29-3.26)	.97
1163		CT+CC	139 (96.5)	171 (96.6)	106 (94.6)							
9084	Over	TT+CC vs.	90 (62.5)	125 (70.6)	82 (73.2)	1.64 (.96-2.81)		.07	1.14 (.67-1.92)	.63	1.44 (.90-2.30)	.12
	dominant	CT	54 (37.5)	52 (29.4)	30 (26.8)							
	Allele	C vs.	71 (25)	99 (28)	64 (29)	1.22 (.82-1.81)		.32	.91 (.62-1.32)	.62	1.34 (.94-1.92)	0.10
		T		217 (75)	255 (72)	160 (71)						
	Co-dominant	CC vs. TT	11 (7.6)	18 (10.2)	10 (8.9)	1.32 (.52-3.30)		.56	.92 (.39-2.13)	.84	1.43 (.64-3.20)	.38
		CT vs. TT	49 (34)	63 (35.6)	44 (39.3)	1.30 (.77-2.20)		.33	1.16 (.69-1.91)	.57	1.12 (.70-1.80)	.62
	Dominant	CC+CT vs.	60 (41.7)	81 (45.8)	54 (48.2)	1.30 (.79-2.14)		.3	1.10 (.69-1.77)	.68	1.18 (.76-1.84)	.64
		TT	84 (58.3)	96 (54.2)	58 (51.8)							
	Recessive	CC vs.	11 (7.6)	18 (10.2)	10 (8.9)	1.18 (.48-2.90)		.71	.87 (.38-1.95)	.73	1.37 (.62-2.99)	.43
rs		TT+CT	133 (92.4)	159 (89.8)	102 (91.1)							
477	Over	TT+CC vs.	95 (66)	114 (64.4)	68 (60.7)	.79 (.48-1.33)		.39	.85 (.52-1.39)	.53	.93 (.59-1.48)	.77
4388	dominant	CT	49 (34)	63 (35.6)	44 (39.3)							

Abbreviations: BPH, Benign Prostate Hyperplasia, OR, Odd Ratio

Table 5. Haplotype frequencies in PCa, BPH and healthy subjects.

rs11639084	rs4774388	PCa	BPH	Control	PCa vs. Control		BPH vs. Control		PCa vs. BPH	
					OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
C	T	.58	.60	.57	1.00	---	1.00	---	1.00	---
C	C	.20	.22	.24	1.20 (.76 - 1.90)	.44	1.12 (.73 - 1.72)	.61	1.04 (.68 - 1.59)	.85
T	T	.17	.12	.14	.81 (.46 - 1.43)	.47	1.21 (.67 - 2.20)	.53	.64 (.38 - 1.10)	.11
T	C	.05	.06	.05	.98 (.32 - 3.04)	.98	.82 (.30 - 2.22)	.69	1.21 (.52 - 2.83)	.66

Abbreviations: BPH, BPH, Benign Prostate Hyperplasia, OR, Odd Ratio, PCa, Prostate Cancer

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CONFLICT ON INTEREST

The authors declare no conflicts of interest.

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