Abstract. The aim of the present study was to determine whether there is an association between the long non-coding RNA (lncRNA) prostate cancer-associated non-coding RNA 1 (PRNCR1) polymorphisms and prostate cancer (PCa) risk in a sample of the Iranian population. This case-control study was performed on 178 patients with PCa and 180 subjects with benign prostatic hyperplasia (BPH). Genotyping assay was performed by polymerase chain reaction-restriction fragment length polymorphism. The findings indicated that the GG genotype of the rs13252298 A>G variant significantly increased the risk of PCa (odds ratio=3.49, 95% confidence interval: 1.79-6.81, P=0.0001) compared with AA+AG. As regards the rs1456315 G>A polymorphism, the AG genotype and G allele significantly increased the risk of PCa. As regards the rs7841060 T>G variant, the findings demonstrated that this TG genotype and the G allele significantly increased the risk of PCa. The rs7007694 T>C variant was not found to be associated with the risk of PCa. Haplotype analysis indicated that GTGA and GTGG significantly increased the risk of PCa compared with rs1456315A/rs7007694T/rs7841060T/rs13252298G (ATTG). The PRNCR1 variants were not found to be significantly associated with the clinicopathological characteristics of PCa patients. In conclusion, our findings support an association between PRNCR1 variants and the risk of PCa in a sample of the Iranian population.

Introduction
Prostate cancer (PCa) is one of the most frequently diagnosed cancers in men and the sixth leading cause of cancer-related mortality among men worldwide (1). The prevalence of PCa differs significantly among populations, indicating that the host's genetic background may play an important role in susceptibility to PCa (2). Single-nucleotide polymorphisms (SNPs) are the most common type of genetic variations in human genome, and they have been found to be associated with the risk of PCa (3-6). Genome tiling arrays have indicated that 1% of the human genome is composed of protein-coding sequences and ~4-9% of the sequences of the human genome are transcribed to non-coding RNAs (ncRNAs) (7).

ncRNAs are documented to be the main regulators of a number of biological processes, such as transcription, splicing, translation, epigenetic gene expression, cell cycle (8-12), stem cell pluripotency and reprogramming (12,13), embryogenesis (14), and regulation of the immune response (15). They are divided into small ncRNAs (<200 nt) and long ncRNAs (lncRNAs; >200 nt) (16,17). LncRNAs are classified according to the correlation between their location and the location of the corresponding protein-coding gene, such as sense, antisense, intergenic, intronic and bidirectional lncRNAs (18,19). Aberrant expression of lncRNAs may contribute to the development and progression of various cancers (20-25).

The lncRNA prostate cancer-associated non-coding RNA 1 (PRNCR1), also referred to as PCAT8 and CARLo3, is mapped to 8q24.21 (26). It has been stated that PRNCR1 is upregulated in PCa and is involved in PCa development by modulating androgen receptor (AR) activity (27). Binding of PRNCR1 to the acetylated AR and its association with DOT1L recruit a second lncRNA, PCGEM1, to the DOT1L-mediated methylated N-terminus of the AR (27). The interactions of these overexpressed lncRNAs may contribute to the development and progression of various cancers (20-25).

To date, certain studies have investigated the impact of PRNCR1 polymorphisms on the risk of various cancers, including prostate (26,28-30), gastric (31,32) and colorectal cancer (33).
PRNCR1 variants that were identified as potential risk factors for cancer were selected (26,28-30,34,35). Genetic risk factors for cancer may vary among diverse populations. Consequently, repeating previously reported genetic associations in other populations is necessary to determine the genetic risk in each population. To the best of our knowledge, there has yet been no study investigating the effect of PRNCR1 variants on cancer risk in the Iranian population. Therefore, the aim of the present study was to determine whether there is an association between the PRNCR1 rs13252298, rs1456315, rs7841060 and rs7007694 polymorphisms and the risk of PCa in a sample of the Iranian population.

Patients and methods

Patients. In total, 358 subjects participated in this hospital-based case-control study, including 178 unrelated men with histopathologically confirmed prostate cancer and 180 age-matched unrelated men with benign prostatic hyperplasia (BPH), with no history of any type of cancer, as the control group (36-39). All cases and controls were selected from a university-affiliated referral center (Shahid Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran). The local Ethics Committee of Zahedan University of Medical Sciences approved the project (IR.ZAUMS.REc.1395.102), and written informed consent was obtained from all the participants. Genomic DNA was extracted by the salting out method and stored at -70°C until use. Peripheral blood samples were collected in tubes containing EDTA and genomic DNA was extracted by the salting out method.

Genotyping. The polymerase chain reaction (PCR)-restriction fragment length polymorphism assay was used for genotyping of the PRNCR1 rs13252298, rs1456315, rs7841060, and rs7007694 polymorphisms. The primer sequences, restriction enzymes and the length of the PCR products are listed in Table I. PCR was performed with the commercially available prime Taq Premix (Genet Bio, Daejeon, Korea) according to the manufacturer’s recommended protocol. Into each 0.20-ml PCR reaction tube, 1 µl of genomic DNA (100 ng/ml), 1 µl of each primer (10 µM), 7 µl of 2X master mix and 6 µl of ddH2O were added. Amplification was performed with an initial denaturation at 95°C for 30 sec, followed by 30 cycles of 30 sec at 95°C, 30 sec at 62°C for rs13252298, 60°C for rs1456315, 56°C for rs7841060, and 64°C for rs7007694, 72°C for 30 sec, with a final extension step at 72°C for 10 min. Subsequently, 10 µl of the PCR products were digested with the appropriate restriction enzymes (Table I). The digested products were separated by agarose gel electrophoresis, visualized by a UV transilluminator and photographed (Fig. 1).

Statistical analysis. All data were analyzed using the statistical package SPSS 22.0 software (IBM Corp., Armonk, NY, USA). The continuous and categorical data were analyzed by the independent samples t-test and χ2 test, respectively. The association among polymorphisms and PCa was calculated by computing the odds ratio (OR) and 95% confidence interval (95% CI) from unconditional logistic regression analyses. Haplotype analysis was performed using SNPStats software (40). The level of statistical significance was set at P<0.05.

Results

Genotypes and allele frequencies of PRNCR1 polymorphisms. The present study included 178 PCA patients with a mean age ± standard deviation of 61.53±6.91 years, and 180 patients with BPH with a mean age of 62.40±7.64 years. No significant difference was found between the groups in terms of age (P=0.258). The genotypes and allele frequencies of PRNCR1 polymorphisms in cases and controls are presented in Table II. As regards the rs13252298 A>G variant, our findings demonstrated that this variant significantly increased the risk of PCa in the recessive (OR=3.49, 95% CI: 1.79-6.81, P=0.0001, GG vs. AA+AG) inheritance model. As regards the rs1456315 A>G polymorphism, the AG as well as the G allele significantly increased the risk of PCa (OR=5.16, 95% CI: 3.16-8.41, P<0.0001 and OR=2.20, 95% CI: 1.60-3.03, P<0.0001, respectively). The TG genotype as well as the G allele of the rs7841060 variant significantly increased the risk of PCa (OR=5.14, 95% CI: 3.15-8.37, P<0.0001 and OR=2.37, 95% CI: 1.71-3.26, P<0.0001, respectively). Our findings demonstrated that the rs7007694 T>C polymorphism was not significantly associated with the risk of PCa. A haplotype analysis was performed, and the findings indicated that GTGA and GTGG significantly increased the risk of PCa compared with rs1456315A/rs7007694T/rs7841060T/rs13252298G (ATTG) (Table III).

Association between clinicopathological characteristics and PRNCR1 polymorphisms. The associations between clinicopathological characteristics, including age, stage, prostate-specific antigen (PSA) levels, Gleason score, perineural invasion and surgical margin, and PRNCR1 polymorphisms are shown in Table IV. The findings did not support an association between PRNCR1 polymorphisms and the clinicopathological characteristics of PCa patients.

The Hardy-Weinberg equilibrium (HWE) was calculated and the findings revealed that the genotype distribution in controls was not in HWE.

Discussion

LncRNAs are involved in tumorigenesis through their function as proto-oncogenes (41) or tumor-suppressor genes (42). Androgen receptor, a member of the nuclear receptor family, is a ligand-activated transcription factor (43). It has been suggested that lncRNA PRNCR1 promotes prostate carcinogenesis via activating AR (26). SNPs, a class of genetic variations, are commonly used in the prediction of cancer risk (38,44,45), prognosis (46) and clinical outcome (47). Cumulative evidence indicates that non-coding genes may be involved in gene expression complexity in humans (48,49). Abnormal expression of lncRNAs has been found to be associated with the development of numerous cancers (50-52). Genome-wide association studies suggested significant and consistent associations of multiple genetic polymorphisms on chromosome 8q24 with PCa susceptibility (53-58). To
date, several studies investigated the effect of PRNCR1 polymorphisms on the risk of PCa (26,28‑30). However, to the best of our knowledge, no study investigating the impact of PRNCR1 variants on cancer risk in an Iranian population has been conducted to date. The present study aimed to evaluate the possible association between rs13252298, rs1456315, rs7841060 and rs7007694 polymorphisms of PRNCR1 and the risk of PCa in a sample of Iranian population.

Our findings suggested that the PRNCR1 rs13252298, rs1456315 and rs7841060 polymorphisms are significantly associated with increased risk of PCa in our population.

<table>
<thead>
<tr>
<th>PRNCR1 SNPs</th>
<th>PCR primers (5'→3')</th>
<th>Restriction enzyme</th>
<th>Fragment length (bp)</th>
</tr>
</thead>
</table>
| rs13252298 A>G | F: CAGCACCTTGTGCTTCTTCTGATAcAT  
R: TACTCCCCATCTCTGGTCTTACC | EcoRV | AA:196+28  
AG:224+196+28  
GG:224 |
| rs1456315 G>A | F: TTGCATTACCTCAACTAAGCCAAG  
R: GGAATTGAGAACTGAGTGGTTGCTAATAAGTC | AccI | GG:213+30  
GA:243+213+30  
AA:243 |
| rs7841060 T>G | F: CACCAATCCAGGCCATTTTGT  
R: CATTTCAGGTTAGACCACATGAACTCGTA | RsaI | TT:225  
TG:225+197+28  
GG:197+28 |
| rs7007694 T>C | F: GCGAATGCCATTGTGTTGACG  
R: CCTCCAAAGAGAAACGGCT | BstUI | TT:222  
TC:222+200+22  
CC:200+22 |

*PRNCR1*, prostate cancer-associated non-coding RNA 1; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SNP, single-nucleotide polymorphism; F, forward; R, reverse.

![Figure 1](image-url)
In a meta-analysis performed by Chu et al (34), the rs1016343 and rs16901946 variants of PRNCR1 were found to significantly increase the risk of cancer; however, their findings did not support a significant association of the rs13252298, rs7007694 and rs1456315 polymorphisms with cancer risk. Another meta-analysis conducted by Lv et al (35) also revealed that two polymorphisms (rs1016343 and rs16901946) of PRNCR1 were associated with increased cancer risk.

LncRNAs, a new class of functional ncRNAs, are composed of >200 nucleotides and lack protein-coding ability (19). LncRNAs potentially interact with DNA, RNA, as well as protein molecules, to perform diverse regulatory functions, including chromatin remodelling (59), RNA splicing and editing (60), translational inhibition (61), mRNA destruction (62) and epigenetic regulation of gene expression (63-65). The most important function of IncRNAs is involvement in the transcriptional or post-transcriptional regulation of gene expression (66). Abnormal expression of IncRNAs may facilitate tumor cell proliferation, invasion and metastasis (67-70).

There were certain limitations to the present study, including the number of SNPs that were investigated for the PRNCR1 gene, as well as lack of the information regarding survival outcomes and the patients’ response to treatment. The reason for the deviation from HWE in our population was not clear; it may be attributed to genetic drift.

In conclusion, our findings indicated that the PRNCR1 rs13252298, rs1456315 and rs7841060 polymorphisms may be a biomarker for PCa development in a sample of the Iranian population. However, further studies should be performed on PCa and other cancers in different ethnicities with larger sample sizes.
sample sizes to fully elucidate the association between PRNCR1 polymorphisms and cancer risk. In addition, the impact of genetic variants on the expression profile of PRNCR1 should be considered in future studies.

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References


