

# Evaluation of a 3-base pair indel polymorphism within pre-microRNA-3131 in patients with prostate cancer using mismatch polymerase chain reaction-restriction fragment length polymorphism

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**Abstract.** The present study aimed to examine the impact of a 3-bp indel (rs57408770) polymorphism within the pre-microRNA (miR)-3131 polymorphism on prostate cancer (PCa) risk in a sample of an Iranian population. In total, 340 subjects, including 177 patients with PCa and 170 patients with benign prostatic hyperplasia, were enrolled in the present case-control study. A mismatch polymerase chain reaction-restriction fragment length polymorphism method was designed for genotyping the 3-bp indel (rs57408770) polymorphism. The present findings demonstrated that the indel variant significantly increased the risk of PCa in codominant [odds ratio (OR)=2.23, 95% confidence interval (CI)=1.13-4.37; P=0.021, insertion (ins)/ins vs. deletion (del)/del] and recessive (OR=2.33, 95% CI=1.25-4.36; P=0.009, ins/ins vs. del/del + del/ins). In conclusion, to the best of our knowledge, the present findings for the first time proposed that a 3-bp indel variant of miR-3131 may be a risk factor for susceptibility to PCa in a sample of an Iranian population. Further studies with different ethnicities and larger sample sizes are required to validate the present findings.

## Introduction

Prostate cancer (PCa), the second most common malignancy in men, is the fifth leading cause of cancer-related mortality among men globally (1). The incidence rate of PCa in Iran

is lower than that in the rest of the world (2-4). Despite the high prevalence of PCa, little is known about the mechanisms underlying the development and progression of PCa. It has been proposed that genomic and environmental factors contribute to the development and progression of PCa (5-8). Twin studies have indicated that 42% of the variation in PCa risk may be attributed to genetics (9). Single nucleotide polymorphisms (SNPs), the most common type of genetic variation in the human genome, have been demonstrated to be associated with the risk of developing PCa (10-12).

MicroRNA (miR) are small, non-coding, endogenous, single-stranded RNA molecules that are ~22 nucleotides in length (13,14). They regulate gene expression by directing sequence-specific degradation or inhibiting translation of target mRNA (13,14). Mounting evidence has suggested that mutation or SNPs in miR genes may affect target-binding activity, expression, or processes of mature miR, thus affecting the expression of their target genes (15,16). Polymorphisms in mature and/or pre-miR sequences may affect miR biogenesis and be associated with the development of various types of cancer (17-21). Small insertions and deletion (indels) polymorphisms are one of the most common genetic alterations in the human genome that influence human traits and diseases (22,23). There is limited information regarding the association between pre-miR-3131 polymorphisms and cancer risk. Recently, Wang *et al* (20) investigated the impact of a 3-bp indel polymorphism (rs57408770) in pre-miR-3131 on hepatocellular carcinoma (HCC) and observed that the insertion (ins) allele significantly increased the risk of HCC in a Chinese population. To the best of our knowledge, for the first time, the present study aimed to determine the impact of a 3-bp indel polymorphism (rs57408770) within pre-miR-3131 on PCa susceptibility in a sample of an Iranian population.

## Patients and methods

**Patients.** The present case-control study involved 177 patients with PCa (mean age, 61.45±6.78 years) and 177 individuals with benign prostatic hyperplasia (BPH) as controls (mean age,

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62.43±7.68 years) admitted to hospital between February 2014 and March 2015. All cases and controls were elected from the Department of Urology, Shahid Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences (Tehran, Iran). The study design and enrolment procedure were described previously (17,18,24). The project was approved by the local Ethics Committee of Zahedan University of Medical Sciences (Zahedan, Iran) and written informed consent was taken from all participants. Peripheral blood samples were collected in tubes containing EDTA and genomic DNA was extracted using the salting out method, as described previously (25).

**Genotyping.** Mismatch polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods for genotyping were designed. Mismatched C was introduced into the forward primers of rs57408770 at -2 bp from the polymorphic site to create an *AluI* (New England BioLabs, Inc., Ipswich, MA, USA) restriction site. The forward and reverse primers were 5'-CTGTGCAGCTGACTCTGAGAA GACG-3' and 5'-TATTGGCTCCTAGGAAGGCTGAGT-3', respectively.

PCR was performed using commercially available prime Taq Premix (GeNet Bio, Nonsan, Korea), according to the manufacturer's instructions. Each 0.20 ml PCR reaction tube contained 1 µl genomic DNA (100 ng/ml), 1 µl each primer (10 µM), 7 µl 2X master mix and the appropriate amount of double-distilled H<sub>2</sub>O. Amplification was performed with an initial denaturation at 95°C for 6 min, followed by 30 cycles of 30 sec at 95°C, 30 sec at 65°C and 72°C for 30 sec, with a final extension step of 72°C for 5 min. For genotyping, 10 µl PCR product was digested by *AluI* and the digested products were separated by 2.5% agarose gel electrophoresis. The deletion (del) allele produced a 188-bp fragment, while the ins allele produced 171- and 20-bbp fragments (Fig. 1).

**Statistical analysis.** Statistical analysis was conducted using SPSS v. 22 software (IBM Corp., Armonk, NY, USA). Data were analyzed by independent samples t-tests and  $\chi^2$  tests. Unconditional logistic regression analysis was used to examine the association between the rs57408770 variant and PCa risk.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Patient characteristics.** The present study consisted of 177 patients with PCa (mean age, 61.45±6.78 years) and 177 individuals with BPH (mean age, 62.43±7.68 years). No significant difference was observed between the age of the two groups ( $P = 0.212$ ; data not shown).

**3-bp indel (rs57408770) polymorphism and risk of PCa.** The genotypes and allele frequencies of the 3-bp indel (rs57408770) polymorphism within pre-miR-3131 in patients with PCa and control subjects are demonstrated in Table I. The findings revealed that the indel variant was significantly associated with increased risk of PCa in codominant [odds ratio (OR)=2.23, 95% confidence interval (CI)=1.13-4.37;  $P = 0.021$ , ins/ins vs. reference del/del] and recessive (OR=2.33, 95% CI=1.25-4.36;  $P = 0.009$ , ins/ins vs. reference del/del + del/ins). The ins allele

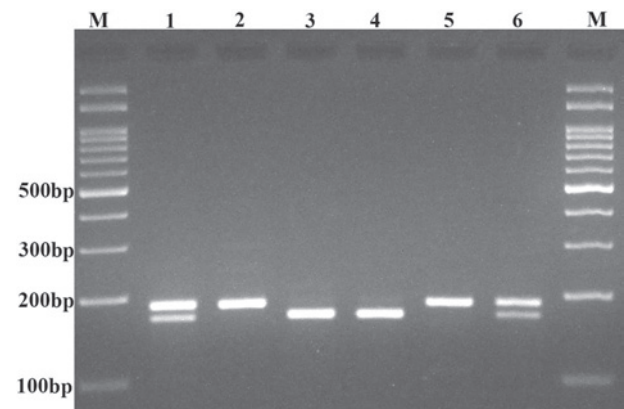


Figure 1. Electrophoresis pattern of the mismatch polymerase chain reaction-restriction fragment length polymorphism method for detection of a 3-bp indel polymorphism within pre-microRNA-3131. M, DNA marker; lanes 1 and 6, insertion/deletion; lanes 2 and 5, deletion/deletion; lanes 3 and 4, insertion/insertion.

was not significantly associated with the risk of PCa (OR=1.33, 95% CI=0.98-1.81;  $P = 0.083$ ).

**Association between 3-bp indel polymorphism of pre-miR-3131 and clinicopathological characteristics.** The association between the 3-bp indel polymorphism of pre-miR-3131 and clinicopathological characteristics, including age, stage, prostate specific antigen levels, Gleason score (18), perineural invasion and surgical margin, are demonstrated in Table II. The findings revealed that the 3-bp indel polymorphism of pre-miR-3131 was only significantly associated with perineural invasion ( $P = 0.015$ ). No significant association was observed between the variant and other clinicopathological characteristics in patients with PCa. The Hardy-Weinberg equilibrium (HWE) was calculated and the results indicated that the genotype distribution in cases and controls was consistent with HWE ( $\chi^2 = 2.41$ ,  $P = 0.121$  and  $\chi^2 = 1.97$ ,  $P = 0.161$ , respectively; data not shown).

## Discussion

Recent expression profiling studies in PCa suggest that miR may serve as potential biomarkers for PCa risk and disease progression (26-30). Growing evidence has indicated that mutation or polymorphisms in miR genes could affect target-binding activity, expression or processes of mature miR, consequently affecting the expression of their target genes (15,16). Polymorphisms in miR have been demonstrated to be associated with the development of PCa (17,18,31,32). In present study, it was hypothesized that the 3-bp indel polymorphism of pre-miR-3131 may be associated with the development of PCa. The present results indicated that the ins/ins genotype of the pre-miR-313 variant significantly increased the risk of PCa. To the best of our knowledge, there has only been one report regarding the impact of a 3-bp indel polymorphism of pre-miR-3131 on cancer risk (20). Wang *et al* (20) demonstrated that the insertion allele of a 3-bp indel polymorphism of pre-miR-3131 was significantly associated with an increased risk for HCC. Furthermore, their findings revealed that the 3-bp indel polymorphism could affect the expression level of miR-3131 by influencing the binding of

Table I. Genotype and allele frequencies of a 3-bp indel (rs57408770) polymorphism of pre-microRNA-3131 in patients with PCa and controls.

Polymorphism	Group		Odds ratio (95% confidence interval)	P-value
	PCa, n (%)	Controls, n (%)		
Codominant				
del/del <sup>a</sup>	62 (36.5)	67 (39.4)	1.00	-
del/ins	73 (42.9)	86 (50.6)	0.92 (0.58-1.46)	0.723
ins/ins	35 (20.6)	17 (10.0)	2.23 (1.13-4.37)	0.021
Dominant				
del/del <sup>a</sup>	62 (36.5)	67 (39.4)	1.00	-
del/ins + ins/ins	108 (63.5)	103 (60.6)	1.3 (0.73-1.76)	0.654
Recessive				
del/del + del/ins <sup>a</sup>	135 (79.4)	153 (90.0)	1.00	-
ins/ins	35 (20.6)	17 (10.0)	2.33 (1.25-4.36)	0.009
Allele				
del <sup>a</sup>	197 (58.0)	220 (64.7)	1.00	-
ins	143 (42.0)	120 (35.3)	1.33 (0.98-1.81)	0.083

<sup>a</sup>Reference genotype/allele. PCa, prostate cancer; del, deletion; ins, insertion.

Table II. Association of 3-bp indel (rs57408770) polymorphism of pre-microRNA-3131 with clinicopathological characteristics of patients with prostate cancer.

Factors	rs3787016 C>T			P-value
	del/del, n	del/ins, n	ins/ins, n	
Age at diagnosis, years				0.876
≤65	43	52	26	
>65	19	21	9	
Stage				0.578
pT1	2	3	3	
pT2a	9	9	9	
pT2b	6	3	2	
pT2c	26	37	14	
pT3a	5	7	1	
pT3b	14	14	6	
Prostate specific antigen level at diagnosis, ng/ml				0.458
≤4	0	1	0	
4-10	31	32	21	
>10	30	40	14	
Gleason score				0.538
≤7	44	58	28	
>7	17	15	7	
Perineural invasion				0.015
Positive	47	40	19	
Negative	14	33	16	
Surgical margin				0.180
Positive	29	28	10	
Negative	32	45	25	

del, deletion; ins, insertion.

splicing factor SRp20 with pre-miR-3131 (20). Hsa-miR-3131 is located on chromosome 2 in intron 2 of the Indian hedgehog gene and the 3-bp indel variant (rs57408770) is located in the 3' end of miR-3131 (20). A study by Shen *et al* (33) demonstrated that the miR-3131 expression level was upregulated by 92-fold in HepG2 cells treated with *Ganoderma lucidum* polysaccharide, proposing that miR-3131 may be involved in the proliferation and differentiation of HCC cells.

Polymorphisms in miR genes, including pri-miR (17,34,35), pre-miR (36) and mature miR, may affect the processing of miR as well as the regulatory function on their target genes, and consequently may be implicated in the development and prognosis of various types of cancer (37-39). In conclusion, to the best of our knowledge, the present study provided evidence for the first time that the 3-bp indel polymorphism of pre-miR-3131 significantly increased the risk of developing PCa in a sample of an Iranian population. Therefore, the pre-miR-3131 3-bp indel variant may be a potential biomarker for prostate cancer. Further studies with different ethnicities and larger sample sizes are required to certify the present findings.

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