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The association between 1349T>G polymorphism of ApE1 gene and the risk of prostate cancer in northern Iran

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Abstract

Prostate cancer (PCa) is the most common malignancy in men and the fourth most common cause of death based on cancer all over the world. Many genes has been shown to be involved in the progress of the prostate cancer. Human apurinic/apyrimidinic endonuclease 1 (*APE1*) is a multifunctional protein that has an important role in the base excision repair (BER) pathway. The aim of this study was to evaluate the association of *ApE1* 1349T>G polymorphism and the susceptibility to prostate cancer in northern Iran population. Samples were collected from 100 patients diagnosed with prostate cancer patients and 100 controls subjects and genotyped by PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism). We observed a significant difference in genotype distributions of *ApE1* 1349T>G polymorphism between patients and controls (P= 0.039). Our findings revealed individuals with the variant TG and GG had a significant increased risk of prostate cancer (GG: OR= 2.50, 95%CI= 1.063-5.874, P= 0.035. TG: OR= 2.40, 95%CI= 1.16-4.95, P= 0.017). Also, more analyses were showed that G allele were associated with increased risk of prostate cancer (OR= 1.493, 95%CI= 1.007-2.21, P= 0.045). The data from this study indicates that the *ApE1* 1349T>G polymorphism is associated with increased risk of prostate cancer. Although more studies should be considered with larger number of patients and control subjects to confirm our results.

Key words: Prostate cancer, gene polymorphism, *ApE1*.

Introduction

Prostate cancer (PCa) is the second most important cause of cancer in men of developing countries and the fourth most common cause of death based on cancer all over the world (1), In Iran, it is the second most commonly diagnosed cancer in men. It has been shown that any damages to our genetic material will be a continuous threat to both our capability to faithfully transmit genetic information to our offspring as well as our own life (2). Genetic factors play an important role in the pathogenesis of PCa. So, there is an expanding interest in the role of genetic factors like single nucleotide polymorphic (SNPs) variants in PCa (3). DNA repair gene system is one of the most important mechanism in human body and have a key role in protecting against gene mutations (4). It has been shown that Direct reversal, mismatch repair, double-strand break repair, nucleotide excision repair (NER) and base excision repair (BER) are five DNA repair systems that support human cells (5). Carcinogens cause damage in DNA like oxidative lesions which removed by Base excision repair (BER) pathway. Any kind of variations in BER genes cause reduce DNA repair capacity and it can causes urological cancers (6).

DNA repair gene systems are one of the most important systems in human body and have a key role in protecting against gene mutations (4). The human apurinic/apyriminic endonuclease 1 (*ApE1*), also known as *ApE*, *APEX* and *Ref-1*, is underlying enzyme in the base excision repair (BER) pathway. BER protects cells against the effect of endogenous and exogenous agents and also is responsible for the repair of DNA brought about oxidation / alkylation damage (5). *ApE1* is a multifunctional protein that located on chromosome 14q11.2-q12, consists of five exons and four introns spanning 2.21 kb. By hydrolyzing 3'-blocking fragments from oxidized and alkylated DNA, *ApE1* comes out with normal 3'- hydroxyl nucleotide termini that are necessary for DNA repair synthesis and ligation at single – or double – strand breaks (7,8).

Recent studies have displayed that single nucleotide polymorphisms (SNP_S) in DNA repair genes may be the critical molecular mechanism of the individual variation of DNA repair capacity (9). Eighteen polymorphisms in *ApE1* have been recognized but transversion of T to G is the most extensively studies polymorphism (10,11). *ApE1* SNP T>G found in exon 5 led to substitution of Asp>Glu at codon 148 which is located within the endonuclease domain of the protein. The *ApE1* Asp148Glu (1349T>G) polymorphism is the only known common non-synonymous *ApE1* coding region variant (12). The aim of this study was to evaluate the association between 1349T>G *ApE1* polymorphisms and the susceptibility to prostate cancer in northern Iran population.

Materials and methods

Subjects

The current study included a total of 100 patients with prostate cancer and 100 disease-free control subjects. Controls and patients were selected from same

population living in the Guilan province, north of Iran, which were recruited between 2013 and 2014. Data on patient characteristics at the study entry for each subject were collected from the oncology clinic of Razi Hospital (Rasht, Iran). Control subjects were selected among individuals getting health examinations at the Health Examination Center of hospital during the same period and any subjects who had a history of cancer were excluded from the study. The control subjects were frequency-matched with the cancer patients based on mean age. Each subject donated 2 ml blood and drawn into EDTA-Coated tubes (Venoject, Belgium), which was used for genomic DNA extraction. This project has been approved by the local licensing committee and informed consent was obtained from all subjects and has been performed according the Helsinki Declaration of 1975, as revised in 1983.

Genotyping

Genomic DNA was extracted from whole-blood samples using the Gpp solution kit (Gen pajoohan, Iran) according to the manufacturer's instructions. DNA purity and concentration were determined by spectrophotometer at 260 and 280 nm. Each DNA sample was stored in TE buffer (5mμ Tris, HCl, 0.1 mμ EDTA, pH 8.5) at -20˚C until analysis. Polymorphism spanning fragments were amplified by the polymerase chain reaction (PCR) and performed using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The region of *ApE1* including the (1349T>G) SNP site was amplified using primers:
(F: 5' TGAGACCCTATTGATGCCTA 3' and R: 5' TGTTACCAGCACAAACGAG 3'). Polymerase chain reaction products were subsequently digested with restriction enzyme *BtgZI*. The PCR conditions were as follow: initial denaturation at 95˚C for 5 min, 35 cycles of denaturation at 95˚C for 45 s, annealing at 55˚C for 40 s, with a final step at 72˚C for 5 min to allow a complete extension of PCR fragment. A 4-μl from the polymerase chain reaction products was digested with 1.5 U of BtgZ1 restriction enzyme (New England Biolabs), at 55 °C for 12 h and Enzyme digestion products were separated on 2% agarose gel electrophoresis and visualized by ethidium bromide staining. The three possible genotypes were defined by three distinct patterns of bands visualized on the gel: Glu/Glu (197 bp), Glu/Asp (197, 145 and 52 bp), and Asp/Asp (145 and 52 bp). Genotype results were regularly confirmed by randomly selecting 20% of the samples that were regenotyped by another laboratory member to improve the quality of genotyping and its validity and no discrepancy in genotyping was found.

Statistical analysis

Data management and analysis was performed using Medcalc software (version 12.1, Mariakerke, Belgium). Genotype frequencies between cases and controls were compared by the χ^2 test. To estimate the association between the *ApE1* 1349T>G variant and the risk of prostate cancer, Odds ratios and 95% confidence intervals (95%CI) were calculated by logistic regression and differences in demographic variables (including smoking history, body mass index (BMI), and family history of cancer) between patients and control subjects

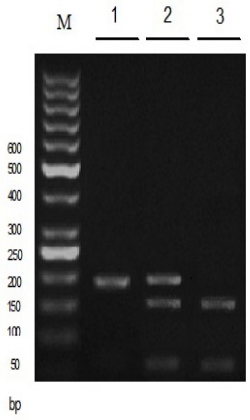

Figure 1. Detection of ApE1 gene polymorphism by PCR-RFLP using *BtgZI* restriction enzyme. Lane 1, fragments presenting the GG genotype for the mutant homozygous patient; lane 2, fragments indicating the TG genotype for heterozygous patients; Lane 3, fragments showing the TT genotype for the wild type homozygous patient; M= 50 bp DNA marker.

were compared by chi-squared (χ2) test. The association between the *ApE1* 1349T>G variant and risk of prostate cancer was investigated by treating the three genotypes (major allele homozygous, heterozygous and variant allele homozygous) as ordinal variables in the analysis. The homozygosity with the more frequent allele among controls was set as the references group. A value of $P < 0.05$ was considered statistically significant.

Results

In the present study 200 subjects including 100 PCa patients and 100 controls were assessed. The mean age of study subjects was (62.6±8.9 years) and for controls (59.1±10.4 years) that was no significantly different between patients and controls, ($P > 0.05$). Genotyping of 1349T>G was done by PCR-RFLP method (Figure 1). The main characteristics of the patients are presented in table 1. Analysis suggested that age, smoking status and family history of prostate cancer were not significantly different between cases and controls. The prevalence of genotype frequencies for TT, TG and GG was 30.0%, 50.0% and 20.0% in controls, and 15.0%, 60.0%, 25.0% in patients, respectively. Statistical analysis showed that there is significant difference between two groups (P= 0.039). The results indicated that the subgroup with GG and TG genotypes were associated with increased risk of prostate cancer (GG: OR= 2.50, 95%CI= 1.063-5.874, P= 0.035. TG: OR= 2.40, 95%CI= 1.16-4.95, P= 0.017). Moreover, analyses were showed that G allele were associated with increased risk of prostate cancer (OR= 1.493, 95%CI= 1.007-2.21, P= 0.045). All information about allele and genotype frequencies and associated ORs (95%CI) for cases and controls are summarized in table 2.

Discussion

In this case-controls study we evaluated the role of *ApE1* 1349T>G polymorphism in 100 PCa patients and 100 controls. Our results suggest that there is a significant association in genotype distribution between cases and controls (*P*= 0.039). The individuals with GG and

Table 1. Characteristics of PCa patients and controls enrolled in the study.

Variable	Controls (n = 100)	Cases (n = 100)	P value
	No. (%)	No. (%)	
Age (mean ± SD)	59.1 ± 10.4	62.6 ± 8.9	0.329
< 50	48 (48.0)	43 (43.0)	
≥ 50	52 (52.0)	57 (57.0)	
Smoking status			0.122
Never	60 (60.0)	50 (50.0)	
Former	13 (13.0)	24 (24.0)	
Current	27 (27.0)	26 (26.0)	
Family history of PCa			0.246
No	80 (80.0)	72 (72.0)	
Yes	20 (20.0)	28 (28.0)	

Table 2. Allele and genotype frequencies of ApE1 1349T>G polymorphism among cases and controls and the associations with risk of prostate cancer.

	controls (n = 100)	patients (n = 100)			
	n (%)	n (%)	OR (95% CI)	P [a]	P [b]
Alleles					
T	110 (55.0)	90 (45.0)	1.00 (reference)	0.05	-
G	90 (45.0)	110 (55.0)	1.49 (1.00- 2.21)		0.045
Genotypes					
TT	30 (30.0)	15 (15.0)	1.00 (reference)	0.039	-
TG	50 (50.0)	60 (60.0)	2.40 (1.16 – 4.95)		0.017
GG	20 (20.0)	25 (25.0)	2.50 (1.06 – 5.87)		0.035

[a] allele and genotype frequencies in cases and controls were compared using χ^2 test.

[b] significance level for allele and genotype frequencies in cases and controls.

TG genotypes were associated with increased risk of prostate cancer (GG: OR= 2.50, 95%CI= 1.063-5.874, P= 0.035. TG: OR= 2.40, 95%CI= 1.16-4.95, P= 0.017). We have also shown that G allele were associated with susceptibility of prostate cancer (OR= 1.493, 95%CI= 1.007-2.21, P= 0.045).

Prostate cancer (PCa) is the second most important cause of cancer in men. The relations between cancer and inter-individual variations in DNA damage and repair process are explained. DNA base excision repair is the main pathway to repair DNA base damages caused by oxidation, radiation, and the loss of bases. There are several enzymes that take part in this pathway, such as DNA polymerase β, DNA ligase III and apurinic/apyrimidinic endonuclease 1 (*APE1*) to protect the genome entirety. The DNA repair activity of *APE1* resides in the C-terminal region, that its endonuclease function makes a nick directly adjoining to 5′ of an apurinic/apyrimidinic (AP) site and produces a hydroxyl group at the 3′ terminus upstream of the nick (13). It is known that 1349T>G polymorphism is the only renowned non-synonymous *ApE1* coding region variant (14). It has been shown that genetic variations can disorder *ApE1* function and play an important role in susceptibility to many kind of cancers. Hao Luo et al. demonstrated that the combined effects of polymorphisms of BER genes may contribute to tumorigenesis in a chinese population (15). Dongying et al. indicated that *ApE1* 1349T>G polymorphism was associated with genetic susceptibility of cancer (16). Moreover, a recent study determined that the G allele of the *ApE1* T1349G variant increased gastric cancer risk (OR = 1.69, P = 0.003) in a Chinese population (17). Ye li et al. detected that *ApE1* 1349T>G polymorphism was associated with the susceptibility to colorectal cancer (18). According to probable effect of this SNP on DNA repair activity of *ApE1*, which in prostate cells may be effective on cancer risk, in present study we investigated the association between 1349T>G *ApE1* SNP and susceptibility to prostate cancer. Previous research findings have been inconsistent and contradictory. Jing et al. showed that no association between Asp148Glu polymorphism and the risk of PCa was found (19). Moreover, findings of the current study are consistent with those of Li X et al who found that the variant genotype of *ApE1* 1349T>G (Glu) was associated with increased risk of prostate cancer (20). The conflicting results of these reports may be due to the differences in sample sizes, and the impact of other genetic and environmental factors. Some limitations of our study including, we only evaluated one SNP in the *ApE1* gene, which was inadequate to assess prostate cancer risk for the gene studies. In addition, our population that were studied was not large enough.

In conclusion, our results indicated that the *ApE1*

1349T>G polymorphism is associated with increased risk of prostate cancer. Further studies with larger numbers of patients and controls are needed to confirm our results.

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