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Original Article

The impact of BMI, Smoking, Family History and Ala 119 Ser (rs1056827) Polymorphism of *CYP1B1*2* Genes with Susceptibility to Prostate Cancer among Iranian Men

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Background and Aims: The genes involved in detoxification and the elimination of toxic metabolites have a vital role in cancer pathogenesis. Also, there is evidence that higher amounts of body fat are associated with increased risks of several cancers. The current study aims to identify the relationship of age, body mass index (BMI), smoking, family history, and polymorphism rs1056827 of CYP1B1 with prostate cancer.

Materials and Methods: A total of 103 patients and 103 healthy men as control groups were enrolled in the current study. The DNAs were extracted using the salting-out method after venesection, and the genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism. The obtained data were analyzed by SPSS version 23 using X^2 statistical tests and logistic regression.

Results: The results showed a significant association between the study groups regarding age, BMI, and family history of the disease ($p = 0.024$, $p = 4.08\times10^{-4}$, and $p = 3.58\times10^{-19}$, respectively). Investigation of genetic models except to additive model showed a significant relationship between TT (Ser/Ser) genotype and prostate cancer. It also showed a strong association between this genotype with BMI and family history.

Conclusion: The current study results showed that the carriers of the TT genotype with a high BMI have a higher chance of developing prostate cancer. Further studies in this area will provide stronger results.

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Introduction

Prostate cancer is the fifth leading cause of death worldwide [1]. The studies indicated that age, race/ethnicity, and family history are the predominant risk factors for prostate cancer. Also, a small proportion of cases are related to high-penetrance genes, another critical risk factor for prostate cancer [2]. Several studies suggested that higher body mass index (BMI) values can increase the risk of cancer incidence. The biological mechanisms that link BMI and cancer are still poorly understood. However, insulin-like growth factors, sex-related hormones, and adipokines are the factors that might explain this association [3].

The prostate is an androgen-dependent organ [4, 5] that genetic changes and exposure to chemical compounds play essential roles in susceptibility to prostate cancer [6, 7].

According to the studies, the big family of cytochrome P450 and enzymes associated with them are significant in susceptibility to different cancers such as prostate cancer due to their key role in the metabolism of internal compounds and external chemicals. On the other hand, the enzymes mentioned earlier are the key factors in the detoxification and metabolism of drugs and environmental chemicals [8].

Cytochrome P450 family or the CYP enzymes metabolize steroid and lipid hormones and xenobiotic materials such as medicines [9].

The *CYP1B1* gene encodes an extrahepatic cytochrome P450 enzyme activating many procarcinogens, including polycyclic aromatic hydrocarbons, heterocyclic, and arylamines, and nitroaromatic hydrocarbons, which can cause DNA damage to cells. Also, CYP1B1 is involved in the oxidative metabolism of estrogens and catalyzes the hydroxylation of estrogens at the C-4 position to 4-hydroxy CEs, which can form depurinated DNA adducts that showed carcinogenic effects in several animal models [10, 11]. CYP1B1 is one of the main enzymes of the cytochrome P450 family situated on the short arm of chromosome 2 in the 2P 21-22 chromosome region [12]. Rs1056827 polymorphism, also known as Alal119Ser polymorphism, is one of the four principal polymorphisms of the *CYP1B1* gene. This polymorphism is located in exon 2 of *CYP1B1*, and guanine alkane is replaced with thymine alkane or the replacement of serine and alanine amino acids in the polypeptide chain [10]. Rs1056827 is associated with the increased risk of different cancers such as breast, endometrial, prostate [13], colorectal cancers [14], and laryngeal cancer [15], as well as renal cell carcinoma in males [16]. The current study investigates the relationship between age, BMI, family history, smoking, and rs1056827 polymorphism with susceptibility to prostate cancer among Iranian males.

Materials and Methods

The current case-control study was conducted on 103 Iranian men with primary adeno-carcinoma of the prostate, referring to the Reproductive Health Research Center, Department of Gynecology and Obstetrics at Imam Khomeini Hospital in Tehran, using

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Digital Rectal Exam (DRE), serum prostate-specific antigen (PSA) levels, and prostate acupuncture biopsy. Histopathological reports of prostate cancer were confirmed and underwent radical prostatectomy as the control group. One hundred three individuals were selected who did not have any specific disease such as diabetes or hypertension, did not take any particular medication, and had no cancer, including prostate cancer, from September 2014 to August 2016. Since family history is an undeniably strong predictor of hereditary cancers, we collected information about prostate cancer in first-degree relatives, including parents, brothers, or children, from all participants. This study was conducted in accordance with all Ethical Committee criteria and obtained written consent from patients (IR.IAU.TMU.REC.1395.092).

DNA from whole blood lymphocytes was extracted using the salting-out method. Following the procedure mentioned above, the quality and quantity of the extracted DNA were determined by spectrophotometer and agarose gel then frozen at -20 ˚C. After the DNA isolation, the desired fragment was proliferated by the Eppendorf Mastercycler gradient in Germany. Primers were designed accurately by Macrogen company in South Korea (F:5′: TACGGCGACGTTTTCCAGAT-3′ and R:5′: CGTGAAGAAGTTGCGCAT CA-3′), and the temperature was set to 62.5 ˚C. The collected samples were proliferated to trace the nucleotide polymorphism of G119T in the exon 2 of the *CYP1B1* gene or identify the genotype of the participant. The proliferation program included 95 ˚C for 5 minutes, 95 ˚C for 60 seconds, 62.5 ˚C for 30 seconds, 75 ˚C for 40 seconds at 35 cycles and 72 ˚C for 5 minutes in one cycle for amplification of 231 bp of the gene.

Rs1056827 polymorphism was recognized by restriction enzyme *PdiI* by Thermo Fisher Scientific Inc in Canada. This enzyme has a GCCGGC recognition site, which cuts the site after cytosine. The enzyme carries 5-µL digested products on 2% gel. In the presence of nucleotide G in the desired position, two bands, namely 113bp and 118bp, indicate GG (Ala/Ala) genotype. However, if it changes to T, only one band, namely 231bp, was observed, indicating that the genotype was TT (Ser/Ser). Heterozygous individuals were revealed as 231bp, 118bp, and 113bp fragments, which confirmed GT (Ala/Ser) genotype (Fig. 1).

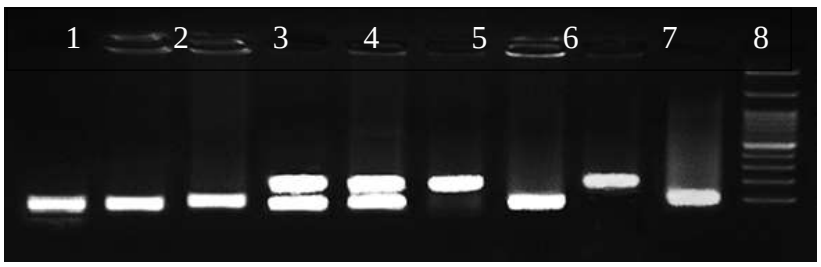

Fig. 1. The results of the enzymatic digestion of PCR product for some samples: wells 1, 2, 3, 7 & 9 are GG genotype, wells 4&5 are GT genotype, wells 6&8 are TT genotype well 10 is DNA marker 100bp.

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Statistical analysis

The allele frequency in both groups was achieved according to Hardy-Weinberg equilibrium using IBM SPSS version 23 software. A chi-square test was also used to evaluate the significant relationships between the genotypes and the risk of susceptibility to prostate cancer in the two groups. Pearson correlation coefficient, chi-square, and Fisher's exact test were employed to compare the distribution of genotypes between the patient and control groups. The association between the genotypes and prostate cancer risk was calculated by odds ratio (OR) and 95% confidence interval. The independent t-test was employed to compare the mean values of the two groups. $p \leq 0.05$ was considered as the significance level.

Results

The case group consisted of 103 males with prostate adenocarcinoma, and the control group included 67 healthy males and 36 males with benign prostatic hyperplasia or enlarged prostate with no sign of cancer in the biopsy. The study participants' demographic and clinical information, including age, BMI, family history, and tobacco smoking, are presented in Table 1.

The results of counting the genotypes showed that the frequency of GG (Ala/Ala) homozygote genotype was 53.40% (n = 55) and 66.99% (n = 69) in the case and control groups, respectively (P = 0.046, OR = 1.771, CI: 1.007- 3.114). Also, TT (Ser/Ser) homozygote genotype was 33.98% (n = 35) and 18.45% (n = 19) in the case and control groups, respectively (p = 0.011, OR: 0.439, CI: 0.231-0.836). TG/GT (Ala/Ser) genotype was 12.62% (n = 13) and 14.56% (n = 15) in the case and the control groups, respectively (P= 0.684, OR= 1.180, CI: 0.531-2.623). The frequency of T-allele was 0.40 and 0.26 in the case and control groups, respectively. However, the abundance of G-allele was 0.60 and 0.74 in case and control groups, respectively (p = 0.002, OR = 0.513, CI: 0.338- 0.780). Also, the logistic regression showed a significant relationship between TT (Ser/Ser) genotype in both groups (p = 0.013, OR = 0.433, CI: 0.223-0.838). Investigation of other genetic models showed a significant relationship between TT (Ser/Ser) genotype and prostate cancer except to additive model. The results are shown in Table 2. Concerning the variable of age in the two subgroups (< 50 and ≥ 50 years), only the GG (Ala/Ala) genotype with an odds ratio of 0.392 was associated with prostate cancer (p = 0.015, OR = 0.392, CI: 0.183- 0.841). The homozygous TT (Ser/Ser) genotype showed a strong association with the BMI of the two subgroups 25 ≥ and 25 < ($p = 3.26 \times 10^{-10}$, OR = 8.75, CI: 3.479- 22.007). In cigarette smoking, only the heterozygous GT genotype showed a statistically significant association with prostate cancer (p = 0.016, OR = 12, CI: 1.199- 120.077). Family history was strongly correlated with all the three genotypes, but the GG (Ala/Ala) and TT (Ser/Ser) genotypes had a decreasing effect, and the heterozygous TG (Ala/Ser) genotype showed a 2.6-fold increase in the incidence of prostate cancer. The relationship between the genotypes with age, body mass index, smoking, and family history are presented in Table 3.

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Table 1. The demographic and clinical information of the study participants

Variable	Range	Mean± Std Error Difference		P-value
		Case	Control	
Age (year)	<50	39	26	0.024
	≥50	64	77	
		54.55±10.92	57.71±8.88	
Body mass index (kg/m^2)	25≥	38	66	4.08×10^{-4}
	25<	65	37	
		25.05±1.91	24.06±2.02	
Family history	Positive	73	10	3.58×10^{-19}
	Negative	30	93	
Smoking	Current	80	89	0.102
	Never	23	14	

Table 2. The results of relationship between genotypes and prostate cancer using logistic regression and genetic models

Genotypes frequency		Case	Control	p-value	Odds ratio (CI 95%)
Rs 1056827	GG(Ala/Ala)	55	69	1 (Reference)	
	TT(Ser/Ser)	35	19	0.013	0.433 (0.223-0.838)
	GT(Ala/Ser)	13	15	0.842	0.920 (0.404-2.094)
Allele wise comparison	G	123	153	1 (Reference)	
	T	83	53	0.002	0.513(0.338-0.780)
			Genetic models		
Dominant	GG	55	69	1 (Reference)	
	TT+GT	48	34	0.046	1.771 (1.007-3.114)
Recessive	GG+GT	68	84	1 (Reference)	
	TT	35	19	0.011	0.439 (0.231-0.836)
Additive	GT			1 (Reference)	
	GG+TT			0.684	1.180 (0.531-2.623)
Codominant	GG	55	69	1 (Reference)	
	TT	35	19	0.012	0.439 (0.231-8.36)

Table 3. The distribution of rs1056827 in subgroups of age, BMI, smoking and family history among the cancer patients and controls

Genotypes RS1056827		Age		P-value	Adjusted OR(CI95%)	BMI		P-value	Adjusted OR (CI 95%)
		<50	≥50			25≥	25<		
GG	Case	25	30	**0.015**	0.392(0.183-0.841)	27	28	0.508	1.271(0.625-2.586)
	Control	17	52			38	31		
TT	Case	13	22	0.224	0.451(0.123-1.654)	4	31	3.26×10^{-10}	8.75 (3.479-22.007)
	Control	4	15			19	0		
GT	Case	1	12	0.099	6(0.598-60.158)	5	8	0.431	1.829(0.404-8.270)
	Control	5	10			8	7		

Genotypes RS1056827		Smoking		P-value	Adjusted OR(CI95%)	Family history		P-value	Adjusted OR (CI 95%)
		Current	Never			Positive	Negative		
GG	Case	46	9	0.949	1.032(0.394-2.700)	40	15	5.07×10^{-11}	0.56(0.022-0.141)
	Control	58	11			9	60		
TT	Case	27	8	0.265	2.519(0.477-13.30)	25	10	1.3×10^{-5}	0.22(0.003-0.189)
	Control	17	2			1	18		
GT	Case	7	6	0.016	12(1.199-120.077)	8	5	3.25×10^{-4}	2.600(1.307-5.17)
	Control	14	1			0	15		

BMI= Body mass index

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Discussion

In this study, several important factors that influenced prostate cancer, such as age, BMI, smoking, and family history, were studied in two groups of cancerous and healthy individuals. Except for smoking, the results showed that other variables were significantly different between the two groups. Also, further analysis of the genotypes showed that TT (Ser/Ser) and GG (Ala/Ala) genotype had 0.439 fold and 1.771 fold decreasing and increasing effect on the rate of prostate cancer, respectively. The genotypes of rs1056827 were evaluated with different genetic models, and TT (Ser/Ser) genotypes had a significant association with prostate cancer only except for the additive model.

The results were analyzed according to age, BMI, smoking, and family history among the two subgroups for which data were available. The homozygous GG (Ala/Ala) genotype showed a trend to decrease 0.392-fold the risk of prostate cancer with increasing age. There was a strong odds ratio of up to 8.750 fold between BMI and Ala119Ser Polymorphism of *CYP1B1*2* genes. The participants who are habituated to smoking and had the GT (Ala/Ser) genotype significantly increased the risk of prostate cancer by 12 times. The history of prostate cancer in the studied population showed that both homozygous and heterozygous had the decreasing effect of susceptibility to prostate cancer, but the GT (Ala/Ser) genotypes had a 2.6-fold increasing effect.

According to Zhu et al. 2019, this study's results were according to a meta-analysis that revealed a clear association between some polymorphisms of the *CYP1A1* and *CYP1B1* genes and the risk of prostate cancer [17]. Also, Beuten et al. (2008) compared allele, genotype, and haplotype frequencies of six SNPs within *CYP1B1*, such as rs1056827 non-Hispanic Caucasians and Hispanic Caucasians, and found the prevalence of several single-nucleotide polymorphisms and rs1056827 was significantly associated with prostate cancer [18]. In another meta-analysis, the effects of five polymorphisms in *CYP1B1*, such as A119S, in 14 independent studies were investigated by Zhang et al. (2013). Their analysis suggested that A119S was associated with prostate cancer risk under a recessive model in the overall population [19].

The hormone encoding genes play crucial roles in developing prostate cancer; hence, there is a direct relationship between a mutation in such genes and the increased prostate cancer susceptibility [20]. Meanwhile, the mutation in the genes involved in detoxification paths is another key factor in prostate cancer. One of the most important known genes is the cytochrome P450 family [21].

Polymorphisms *CYP1B1*2* or Ala119Ser increase the enzyme activity by two to four times [22]. Also, the study by Yuichiro (2008) showed a variety of genotypes between African-Americans and Caucasians. The TT genotype at codon 119 is highly predominant in African-Americans than Caucasians, or it is 2.71 and 3.55 for the G/T and T/T genotypes, respectively, in blacks compared with whites [23].

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The current study results showed that BMI and family history were associated with an increased risk of prostate cancer, similar to the results of previous studies. Higher BMI may influence prostate cancer prognosis in various ways [24]. Alteration in metabolism in cancer cells permits them to accumulate higher quantities of metabolic intermediates that change the cellular function of cholesterol and fatty acid metabolisms and finally leads to the uncontrolled proliferation of such cells. Also, oxidation of fatty acids is an increase in the production of mitochondrial reactive oxygen species, which at higher levels can be harmful to organelles, including the mitochondria, and predisposition to various pathological states, such as prostate cancer. Older individuals undergo several physiological changes and become vulnerable to various diseases [25].

Conclusion

The current study results suggested that males with a higher BMI were more likely to develop prostate cancer than those with a lower BMI, which was stronger among older subjects. It seems that these results are important in understanding the role of *CYP1B1* polymorphisms in the pathogenesis of prostate cancer.

Conflict of Interest

The authors declared no conflicts of interest.

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