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The Relationship between Insulin PSTI Polymorphism and Prostate Cancer in Turkish Population

Türk Popülasyonunda PSTI Polimorfizminin Prostat Kanseri ile İlişkisinin Araştırılması

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ABSTRACT

Purpose: There is positive correlation between insulin and various cancers such as colon, lung and endometrium. Protate cancer is the one of them. The aim of this study, to determine the incidance of INS+1127 *PstI* polymorphism, located in the 3' UTR of insulin gene, between prostate cancer cases and controls and to clarify if this polymorphism have any role in the etiology of prostate cancer.

Methods: We investigated 71 prostate cancer cases and 119 normal controls unrelated to patients. INS+1127 *PstI* polymorphism was investigated with PCR and RFLP method.

Results: At the end of study; INS+1127 *PstI* polymorphism genotypes out to be 67.6 % CC, 28.2 % CT, 4.2 % TT among prostatic cancer individuals and 78.2% CC, 21% CT, 0.8% TT among control group individuals.

Conclusion: As a result, there was no significant difference between patients and control group, in the genotype distribution.

Key Words: INS, polymorphism, prostate cancer, PstI

ÖZET

Giriş: İnsülin ve kolon, akciğer ve endometrium kanserleri gibi bazı kanser türleri arasında pozitif bir korelasyon olduğu bilinmektedir. Prostat kanseri de bunlardan birisidir. Bu çalışmadaki amacımız insülin geni 3' UTR bölgesinde lokalize olan INS+1127 *PstI* polimosfizminin prostat kanseri etiyolojinde rolünü araştırmaktır.

Materyal ve Metod: Çalışmaya 71 prostat kanseri tanısı almış hasta ve 119 normal kontrol bireyi dahil edilmiştir. INS+1127 *PstI* polimorfizm araştırması için PCR ve RFLP tekniği kullanılmıştır.

Bulgular: Çalışmanın sonucunda INS geni *PstI* polimorfizmi genotipleri prostat kanserli hastalarda (n:71) %67,6 CC, %28,2 CT, %4,2 TT; kontrol grubunda (n: 119) %78,2 CC, %21 CT, %0,8 TT olarak belirlenmiştir.

Sonuç: Hasta ve kontroller arasındaki genotip dağılımında istatistiksel olarak anlamlı bir farklılık bulunamamıştır (P=0,078).

Anahtar Kelimeler: INS, polimorfizm, prostat kanseri, PSTI

INTRODUCTION

Prostate cancer is the most common cancer in the male population and a second leading cause of cancer related mortality in men[1]. Although the aetiology of this disease remains largely unclear it is likely caused from complex interactions amongst endocrinological factors such as androgen metabolism, genetic factors, and environmental factors such as alcohol consumption and smoking. In addition of ethnic differences common genetic variants are a significant contributor to the risk of developing prostate cancer; in fact, of all cancer types, prostate cancer is most closely linked to genetic risk factors[2].

The negative effect of insulin in the aetiology of prostate cancer and lead to increased risk of prostate cancer is shown in some studies[3,4,5,6]. Epidemiological studies show a positive correlation between insulin levels and increased risk of developing certain types of cancer, such as colon, lung and endometrium[7,8,9,,10,11,12]. Insulin has a strong mitogenic effect in prostate cells. It mediately influences androgen levels by use of regulation of sex hormone binding globulin (SHBG) levels. And also insulin directly regulates insulin-like growth factor binding proteins (IGFBPs) levels and therefore affects availability of insulin-like growth factor (*IGF-1*) (C16,17). So biological mechanism of insulin also supports its own's tumorigenic effect[13,14,15,16,17]. Thus elevated insulin levels and insulin resistance have been implicated in prostate cancer development[3].

Insulin gene (*INS*) located on chromosome 11p15.5. VNTR region of *INS* is adjacent to the 5 'promoter region (5' UTR) and has a direct effect on the regulation of insulin expression[18]. *INS* VNTR polymorphisms are classified two main groups. First one Class I allele (28-44 repeat) and second one Class III allele (138-159 repeat). Population frequencies are 70% and 30% respectively. The medium-length class II allele is quite rare[19]. The VNTR allelic variants of insulin gene are associated with diabetes mellitus (DM). For example Class I is associated with type I DM, Class III with type II DM[20,21,22,23,24].

While 5' UTR stimulate the pro-insulin translation, 3'UTR has negative affects on protein synthesis. So mutual interference with UTRs significantly affects biosynthesis of proinsulin. In this manner untranslated regions of mRNA has critical role in stabilisation and regulation[25]. The INS+1127 *PstI* polymorphism is located in the 3'UTR *INS*. In the present study, we evaluated the possible association of the INS+1127 *PstI* polymorphism and prostate cancer risk.

MATERIALS and METHODS

This study was performed at the Eskisehir Osmangazi University Medical Faculty Department of Medical Genetics with a study group of 71 prostate cancer patients between the ages of 50 – 86 and a control group of 119 subjects not having prostate cancer determined by the Department of Medical Genetics based on histopathological findings. The cancerous tissue from prostate biopsies was graded according to the Gleason histopathological grading system. Gleason score ≥7 was accepted as high and <7 as low grade tumors. In our study, INS+1127 *PstI* polymorphism ratio was compared among these two groups and analyzed to see if there is a statistically significant difference. Informed consent was obtained prior to participation in the study.

Molecular study

Genomic DNA of patients and control subjects was isolated from peripheral blood using QIAmp DNA Blood Mini Kit. To amplify the gene region containing the INS+1127 *PstI* polymorphism region, 5 µL DNA was added to the mixture containing 5 µL 10x polymerase chain reaction (PCR) buffer (P-2192; Sigma, St. Louis, MO), 5 µL dNTP mixture (Promega, madison, WI) containing 0.2 mM of each nucleotide, 5 µL 1 mM Tris-HCL, 5 µL 5mM KCL, and 4 pmol of F: 5' – GGG TCC CCT GCA GAA GCG TGG CA – 3' and R: 5' – CTC CCT CCA CAG GGA CTC CAT C – 3'

primers each, and 0.2 µL Taq polymerase (D-6677; Sigma) enzyme, and was completed to 50 µL with ddH_2O. For amplification, the reaction mixture was exposed to final extension for 10 min at 72°C after the first denaturation of 3 min at 95°C and 35 cycles of 40 s at 95°C, 1,5 min at 61°C, and 1 min at 72°C. About 15 µL of the obtained 562 bp (base pair) PCR products was added to the reaction mixture composed of 7 µL bidistilled water, 5 units of PstI enzyme, and 2,5 µL buffer, and was left to digestion for 16 h at 37ºC. To separate the fragments that form after digestion, an ultra-pure agarose gel of 2% was added and 20 µL PCR products were subject to 1.5 h of gel electrophoresis. After electrophoresis, 562 bp fragments were determined to be obtained from CC genotype; 562, 470, and 92 bp fragments were determined to be obtained from CT genotype; and 470 and 92 bp fragments were determined to be obtained from TT genotype (Figure 1).

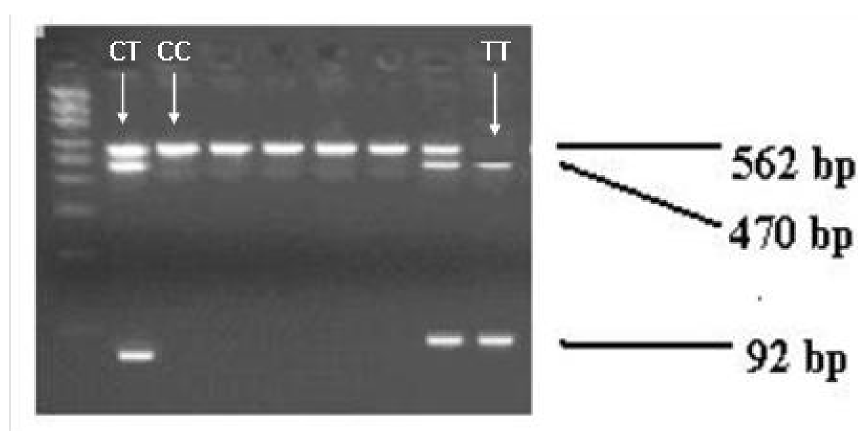

Figure1. Gel electrophoresis view of samples having INS *PSTI* CC, CT and TT alleles.

Table1: Distribution of genotypes among patients and controls

Genotypes	Patients		Controls	
	N	%	N	%
CC	48	67,6	93	78,2
CT	20	28,2	25	21,0
TT	3	4,2	1	0,8

($p>0.05$, patients and controls)

Statistical analysis

SPSS 11.0 software package was used for the statistical analysis of the data. Pearson χ^2 test was used to compare the genotype distributions within the patient and control groups. The association between genotypes and degree of differentiation (high Gleason score versus low Gleason score) was calculated Pearson's χ^2 test as well.

RESULTS

Seventy-one patients found to have prostate cancer were studied for INS+1127 *PstI* polymorphism, and 119 control subjects were studied for INS+1127 *PstI* polymorphism. Among

the patient and control groups, the allele frequencies for the T (mutant) allele were calculated to be 18.3% and 11.3% , respectively, and for the C (wild) allele the allele frequency were calculated to be 81.6% and 88.6%, respectively. When the study and control groups were analyzed for genotype frequencies the TT mutant genotype was determined to be 4.2% and 0.8%, respectively; CT heterozygote genotype was determined to be 28.2% and 21%, respectively; and CC wild-type genotype was determined to be 67.6% and 78.2, respectively. No statistically significant difference was found for the genotype frequency differences among the study and control groups (*p*=0.078, *p*>0.05).

When the Gleason <7 patients and Gleason 7≥ patients were analyzed for the genotype frequencies the TT mutant genotype was determined to be 3% and 5,3%, respectively; CT heterozygote genotype was determined to be 24,2% and 31,6%, respectively; and CC wild-type genotype was determined to be 72,7% and 63,2%, respectively. No statistically significant difference was found for the genotype frequency differences among the two groups (*p*=0.685, *p*>0.05).

Table2: Association of INS *PstI* genotypes with Gleason category

Genotypes	Gleason <7 n=33 (%)	Gleason ≥7 n=38 (%)
CC	24 (72,7)	24 (63,2)
CT	8 (24,2)	12 (31,6)
TT	1 (3)	2 (5,3)

(*p*>0.05, Gleason<7 patients and Gleason≥7 patients)

DISCUSSION

Biological mechanism of insulin supports its own's tumorigenic effect. Pathways of IGF and insulin may play important roles in risk of prostate cancer. The role of insulin in the aetiology of prostate cancer is implicated by the observations from many studies[5]. Already epidemiological studies show a positive correlation between insulin levels and increased risk of developing certain types of cancer, such as colon, lung and endometrium[7,8,9,10,11,12]. In the literature many studies showed association between IGF and insulin pathways genes and prostate cancer risk. Therefore, we evaluated INS+1127 *PstI* polymorphism in *INS* gene in Turkish prostate cancer patients.

The *INS* gene is localized between the insulin-like growth factor 2 (IGF2) gene and the tyrosine hydroxylase (*TH*) gene on chromosome 11p15.5. It has 3 exons. The INS+1127 *PstI* polymorphism is located in the 3'UTR *INS* gene. Untranslated regions of *INS* gene's mRNA has critical role in stabilisation and regulation. The 3'UTR of *INS* gene suppresses translation and also stabilizes the mRNA[26]. In this manner this polymorphism may play a direct role in altering insulin physiology which ultimately contributes to prostate carcinogenesis.

In a study by Ho et al., it was determined that the INS+1127 *PstI* polymorphism increased cancer risk in non-diabetic men who were over 55 years of age. In this study they genotyped 178 cases and 135 controls and they suggested that Black and Caucasian individuals with homozygous CC for INS+1127 *PstI* polymorphism had almost a two fold increased risk of prostate cancer as compared to those with other genotypes. The men who were homozygous CC also were more likely to be older and have a moderately to well-differentiated cancer (Gleason <7). Unlike the Black and Caucasian they observed a negative association between the CC genotype and prostate cancer among the Hispanics[4]. This might cause small sample size or a population stratification bias.

Claeys et al., investigated the potential association between diabetes, germ line variation in the *INS* gene and prostate cancer, they genotyped 466 men wit and without prostate cancer for the INS+1127 *PstI* polymorphism. They also suggested that the *INS PstI* CC genotype is associated with prostate cancer risk in African-American men[3]. We observed that the results of this study were incompatible with the results of Claeys et al. Our results were also incompatible with the results of Ho et al. except the results of their Hispanic population. These changes can be based on ethnic differences.

Neuhausen et al. investigated the association of the *IGF1* CA-repeat, the insulin receptor substrate 1 (*IRS1*) G972R, the *IRS2* G1057D, and the *INS* +1127 INS-PstI polymorphisms and prostate cancer risk in a case-control study. They found that only the IRS1 G972R GR/RR genotypes were associated with a significant increased risk for prostate cancer; other variants were not significantly associated. As a result they suggested that the insulin and/or insulin-like growth factor pathways have a role in the aetiology of prostate cancer. But *INS+1127 PstI* polymorphism was not significantly associated with a significant increased risk for prostate cancer[27]. This study is compatible with our study for CC genotype of the *INS+1127 PstI* polymorphism.

We think the reasons of the detection of differential results to these studies can be inefficient dertermination of groups, only the questionnaire determines whether members of prostate problems for control groups and ethnic differences. The interactions of examined gene products with each other and their structures may be also contributing to differences in the results.

Our study showed that CC genotype of *INS+1127 PstI* polymorphism is not an individual risk factor for prostate cancer in Turkish population. But limitation of our study was the small number of patients included. To better assess the effect of the insulin gene polymorphism in the aetiology of prostate cancer, taking into consideration the other molecules with which they interact large sample groups and different ethnic descent should be studied.

Conflict of interest statement

All the authors declare no conflict of interest.

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