The Role of Kallikrein10 (KLK10) Polymorphism in Prostate Cancer Susceptibility

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Purpose: The present study aims to investigate the potential role of Kallikrein 10 (KLK10) genotype and allele frequencies in predisposition to prostate cancer.

Materials and Methods: KLK10 (rs7259451) gene polymorphisms were determined by real-time polymerase chain reaction analysis in patients with prostate cancer (n = 69) and controls (n = 76).

Results: KLK10 gene frequencies were significantly different in the case and control groups (P = .028). GG carriers were significantly higher in the control group (P = .034), whereas TT carriers were higher in the prostate cancer group (P = .033). Furthermore, The patients with GG genotype had the lowest PSA levels while TT carriers had the highest (P = .005).

Conclusion: According to the results, we suggested that carrying variant T allele and also carrying homozygote TT genotype could be a potential risk, while ancestral homozygote GG genotype and G allele are risk reducing factors for prostate cancer.

Keywords: KLK10 gene; polymorphism; prostate cancer; PSA; rs7259451

INTRODUCTION

Prostate cancer is the most common cancer among men aged over 40 years. Moreover, after lung cancer, it is the second most common mortality factor in cancer-related deaths. Incidence rates of prostate cancer have more than doubled in the last decades, because of great improvements in diagnostic assets and high-tech screening methods[(1)]. Although prostate cancer is a multifactorial disease with several potential risk factors, such as smoking, obesity, age, diabetes and environmental changes, recent advances in genetics have contributed in understanding its pathological metabolism. However, prostate cancer etiology is still vague and investigations about genetic polymorphisms enable to display individual differences and predispositions [(2)]. Thanks to recent improvements in genetic analysis, many genes and their variations have been demonstrated in cancer research. There are many polymorphisms associated with prostate cancer tendency[(3,4)]. These genetic relations help to understand the molecular basis of the disease as well as provide a clinical diagnostic utility. The protein function and effectiveness could be altered by genomic variations, called polymorphism. These variations have different impacts on cancer and cancer prognosis. They also give rise to predispositions via initiating a physiological process indirectly[(5,6)].

Human kallikrein-related peptidases, catalyse peptide bond hydrolysis, are a member of the serine peptiases family. Kallikrein 3 (KLK3), the most well-known family member, named as Prostate-Specific-Antigen (PSA), is a well-known kallikrein (KLK) and has great importance in prostate cancer prognosis and high PSA levels could lead the biochemical failure which predisposes the patients to metastasis[(7)]. Another family member, called Kallikrein10 (KLK10), is a protein that is involved in steroid hormone stimulation via affecting hormone-receptor complexes. The human tissue KLK is located on chromosome 19q13.4 and encoded by steroid hormone-regulated genes. It has been demonstrated that dysregulation of KLK expression is associated with multiple diseases, such as cancer[(8)].
Human KLK was determined as eligible biomarker for diagnosis and prognosis for many cancer types, such as ovarian, breast and prostate. Investigations have focused on understanding the relationship between prostate cancer and the polymorphisms of several KLK genes. Therefore, this study is aimed to investigate the potential role of KLK10 genotype and allelic frequencies in the tendency to prostate cancer (PC).

MATERIALS AND METHODS

The study population consisted of 69 patients who re-

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Table 1. The comprehensive comparison of sensitivity

Parameters		Prostate cancer (n=69)	Control (n=76)	*p*-Value
Age (years), mean±SD		68.52 ± 7.36	66.49 ± 8.45	0.867
Body mass index (kg/m2), mean±SD		27.01 ± 3.71	27.28 ± 3.55	0.773
Smoking status n (%)		52 (76.5%)	16 (23.5%)	0.415
Diabetes Mellitus n (%)		6 (66.7%)	3 (33.3%)	< 0.001*
CAD n (%)		1 (25%)	3 (75%)	< 0.001*
Hypertension n (%)				
systolic blod pressure >140		17 (81%)	3 (19%)	< 0.001*
diastolic blod pressure >90				
PSA (ng/mL) mean±SD		25.94 ± 41.14	3.0 3± 2.66	0.006*
Gleason score, mean±SD		7.74±0.88	-	-
Pathological T-stagen (%)	T2a	9 (13 %)	-	-
	T2b	10 (14.5 %)	-	-
	T2c	29 (42 %)	-	-
	T3a	11 (15.5 %)	-	-
	T3b	10 (15 %)	-	-
Clinical T-stage n (%)	Early (T1+T2)	61 (88,4 %)	-	-
	Late (T3+T4)	8 (11.6 %)	-	-

*P values less than 0.05 denoted statistical significance. .$\chi 2$: Chi square used for comparison of patients with PC and control group; student t test is used for comparing quantitative data

Abbreviations: CAD:Coronary Artery Disease, PSA: Prostate-spesific antigen, n: number of individuals, SD: standart deviation.

curred from Yeditepe University Urology Department and 76 age-matched healthy controls. Control group consisted of healthy individuals with age 40 -80 years, they were not diagnosed with this disease, following a clinical examination. The patients' group consisted of individuals who had prostate cancer with age range between 40-80 years old. The diagnosis of prostate cancer was demonstrated by the clinical, and pathological examinations. The tumor differentiation status was evaluated using Gleason score criteria. The clinical examinations classified T -stage as early -stage (T1 and T2) and late -stage (T3 and T4). Pathologic T -stage was classified as T2a, T2b, T2c, T3a, and T3b. All patients and control groups gave their informed consent following a detailed explanation of the protocol of the study. Blood samples from all individuals were collected in tubes containing EDTA. Clinical and demographic information's of patients and controls obtained from hospital records. Genomic DNA extraction from 350µl of whole blood was performed by Invitrogen iPrep PureLink gDNA blood isolation kit (Invitrogen, Life Technologies, Carlsbad, California, USA). DNA samples were measured with NanoDrop 2000 (Thermoscientific, Waltham, Massachusetts, USA). Determination of KLK10 gene (rs7259451) polymorphism was performed in Applied Biosystems 7500 Fast Real-Time PCR instrument (Applied Biosystems, Foster City, CA, USA) by using TaqMan Genotyping Assay and TaqMan Genotyping Master Mix (TaqMan Reagents, Applied Biosystems, Foster City, CA, USA). The reactions were carried out with primer sequence as 5'-TAAGGCAAGACTCAGGATAAAACAC[G>T] GTGGTGTGGCCGGGAGCGGTGGCTC-3'. Due to the Minor allele frequency (MAF) analysis, allele frequencies considered as G wild type and T mutant form. Statistical analyses were performed using SPSS Ver. 23 software (SPSS Inc, Chicago, IL, USA). The significant difference between groups was examined by Student's t and one way Anova tests, also demographic and clinic data were compared by Chi square and Fisher's exact tests. Risk estimations were examined with odds ratio (OR) at 95% confidence interval (CI). $P < 0.05$ is denoted as statistically significant.

RESULTS

Demographic and clinical properties are summarized in Table 1. There were no differences between the patients and controls in mean ages. There were also no significant difference regarding BMI and smoking habits between the groups, although diagnosis for diabetes, Coronary Artery Disease (CAD) and hypertension were significantly higher in the PC group ($P < .001$). The patients with PC had significant a statistically higher level of PSA when compared to the control group ($P = .006$). The genotypic and allelic frequencies of KLK10 in PC

Table 2. Kallikrein10 (KLK10) rs7259451 genotypic and allelic frequencies in prostate cancer and control group.

Genotype	Prostate cancer (n=69) n (%) $P = .028$*	Control (n=76) n (%) $\chi 2 = 7.178$	*P* value	$\chi 2$	OR	95 % CI
GG	26 (37.7 %)	42 (55.3 %)	.034*	4.489	0.489	0.252-0.951
GT	35 (50.7 %)	32(42.1 %)	.298	1.081	1.415	0.735-2.727
TT	8 (11.6 %)	2 (2.6 %)	.033*	4.524	4.852	0.993-23.703
	Allelic count n (%)					
G Allele	87 (63.1 %)	64 (64%)	0.033*	4.524	0.206	0.042-1.007
T Allele	51 (36.9 %)	36 (36%)	0.034*	4.489	2.043	1.051-3.970

*P values less than 0.05 denoted statistical significance. χ^2: Chi square and Fisher's exact test used for comparison of patients with PC and control group;

Abbreviations: n:number of individuals; χ^2:Chi-Square; OR:odds ratio; CI:Confidence interval

Table 3. KLK10 (rs7259451) genotype variations and prostate volume and PSA levels in patient with prostate cancer

	Prostate Volume (mL) mean±SD		PSA (ng/mL) mean±SD	
GG	45.05 ± 29.79		26.39 ± 7.16	
GT	39.00 ± 13.28	*P* = .236	40.49 ± 13.57	*P* = .005*
TT	47.77 ± 19.11		81.08 ± 70.79	
G allele	42.29 ± 17.00	*P* = .972	33.44 ± 15.80	*P* = .486
T allele	43.07 ± 15.38		40.54 ±3 5.69	

* *P* = values less than 0.05 denoted statistical significance. Student t test and one way ANOVA test used used for comparison of genotypes and alleles

Abbreviations: PSA: Prostate-spesific antigen; SD: standart deviation

and control groups are given in **Table 2**. There were significant differences between the groups in the frequency of KLK10 genotypes (*P* = .028). The frequency of the GG homozygote genotype was significantly higher in the control group than the patient group and those with GG genotype were ~2 fold likely to be healthy control than patients (χ^2 = 4.489, %95 CI= 0.252-0.951, OR= 0.489, *P* = .034). There were no statistically significant correlations between the groups regarding the GT heterozygote genotype (*P* = .298), however, the TT homozygote genotype was significantly higher in the patient group when compared to the control group (P = .033). Ancestral G allele frequency was significantly higher in controls than patients with PC(*P* = .033), while mutant T allele frequency was significantly higher in the patient group (*P* = .034). Sixty-four percent of healthy control participants were carrying the G allele, whereas 36% of them had the T allele.

As it is shown in Table 3, although there was no statistically difference between KLK10 genotypes regarding prostate volume (*P* = .236), the homozygote mutant allele (TT) group displayed the highest prostate volume. According to PSA levels genotype groups are significantly different. The patients with KLK10 GG genotype carriers had the lowest and TT carriers had highest PSA levels (*P* = .005). While there was no statistical difference in allele distributions (*P* = .486), we found that T allele carriers had higher PSA levels than G allele carriers (**Table 3**).

DISCUSSION

In the last decades, polymorphism, expression and genome-wide studies have undergone a great improvement; however, there is no particular evidence to identify prostate cancer susceptibility. On account of this, new studies should be performed in population-based case-control studies. Owing to its tumor suppressing effect, the KLK10 gene accounts for the prediction of cancer prognosis[9]. The human KLK10 gene and its potential role have been investigated in various cancer types in different populations. The present study aims to investigate the associations between KLK10 polymorphism and prostate cancer in a Turkish population. KLK genes and KLK proteins have structural characteristics, such as localization on the same chromosomal domain. They also have analogue translational sites, stop and start codons. KLK genes have five peer exons and four codons with no association with other genes located on the same gene region. Altered KLK gene expressions vary in different cancer types; moreover, it has been asserted that KLK proteins participate in proliferation, angiogenesis and metastasis[8,10]. Several studies indicated that the KLK protein family, the best known of which is KLK3, also known as PSA, is widely used for prostate cancer diagnosis[10]. KLK family members has been investigated as novel serum biomarkers, although there is unclear evidence regarding KLK gene expression and protein levels[11]. Bayani et al. (2008) demonstrated that dysregulated KLK expression levels in breast, ovary and prostate are associated with increased KLK protein levels. They indicated that unbalanced translocations associated with altered protein levels. Furthermore, they showed that there was a relation between cancer progression and KLK protein levels[12].

Although there have been several investigations into the KLK protease family, the association between physiological role and genetic variations is still unclear. Angelopoulou et al. (2009) demonstrated KLK mRNA expressions of cancer tissues. They found that KLK9 and KLK10 have the highest expression levels among cancerous mammary tissues. Thus, it has been suggested that KLK9 and KLK10 participate in proteolytic cascades[13]. KLK proteins are initially translated as preproenzymes that carry signal peptide on N-terminus and mature active enzyme takes place after a short propeptide. The signal cleaves from propeptide domains, and forms a mature enzyme complex. Human KLKs participate in different biological processes, such as regulating neural development, regulating blood pressure, semen liquefaction and also cell proliferation[14]. Therefore, several studies have been conducted on the role of human KLKs in diverse cancer types. Yousef et al. (2005) showed the relation between KLK10 and endocrine related malignancies in silico analyses. Although KLK genes had different expression profiles in various tissues, KLK10 gene expression significantly downregulated in ovarian, breast, testicular and prostate cancer lines. Consequently, the KLK10 gene represented a tumour suppressor function, especially in endocrine-related malignancies and KLK10 could be considered as a cancer biomarker gene[15]. The potential biomarker role of KLK10 gene was documented in human breast, ovary and prostate cancer cell lines by Sidiropoulus et al. (2005). They investigated epigenetic alterations on the tumor suppressor role of KLK10 mRNA expression. However, various mechanisms account for downregulating gene expressions; hypermethylation of CpG islands on the KLK10 gene could explain the specific tumor suppressing mechanism of KLK10 expression profile in cancer cells[16]. Another study demonstrated the tumor biomarker role of altered KLK10 serum level in patients with various malignancies. Although the relation between gene regulation and serum protein level is still unclear, Luo et al. (2001) showed a positive correlation with serum KLK10 level and ovarian cancer severity[17].

The relation between single nucleotide polymorphisms in human KLK10 gene and endocrine-related malignancies, such as prostate, testicular, breast and ovarian cancer, was investigated by Bharaj et al. (2002). Five coding regions of KLK10 gene sequencing analysis performed in different human tumour tissues were obtained from cancer patients and the sequence analysis showed that the mutant variant was significantly higher in prostate tumours than adjacent normal tissues. Thus, they identified human KLK10 gene polymorphisms at codon 50 associated with PC risk[18].

In silico analysis of KLK10 single nucleotide polymorphisms (SNPs) demonstrated that KLK10 expression could be altered by intronic SNPs via regulating transcription factors. Intronic SNPs could change not only gene expressions, but also hormone response element binding domains. Batra et al. (2010) performed KLK10 gene sequencing to analyse possible association between KLK10 and cancer survival. The analysis showed that KLK10 rs 7259451 polymorphism located on the 5'UTR intronic region where upstream of androgen response elements (AREs) clustered. In addition, they asserted that intronic SNPs could regulate translation via epigenetic factors, such as hypermethylation and microRNA alterations[19]. Their results support the present study as regard the importance of KLK10 (rs 7259451) gene polymorphism on PC susceptibility.

CONCLUSIONS

Although the present study has limitations, such as small sample size, to the best of our knowledge, it was the first in vivo study which investigated the association between PC susceptibility and KLK10 intronic SNP. Our results implicated that homozygote ancestral GG genotype and carrying G allele could be protective from PC. Besides not only carrying mutant homozygote genotype TT, but also having T allele, could be a potential risk factor for PC.

CONFLICT ON INTEREST

The authors declare that they have no conflict of interest.

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