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Research Article

G1733A (RS6152) polymorphism of the androgen receptor gene in patients with prostate cancer

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ABSTRACT

Aim: The causes of prostate cancer development and molecular mechanism underlying its development and progression are not clearly understood. The aim of this study is to determine the frequency of G1733A (rs6152) polymorphism of the androgen receptor (AR) gene among patients with prostate cancer, and to examine the role of this polymorphism in the development of prostate cancer.

Method: DNA samples isolated from 96 individuals (49 patients with prostate cancer and 47 controls) were analyzed with real time-polymerase chain reaction (real time-PCR) in order to determine G1733A (rs6152) polymorphism genotypes and allele frequencies in the AR gene. The results were evaluated statistically.

Results: Genotype frequency was determined as 91% GG and 9% AG among the controls, and 67% GG and 33% AG among the patients. G allele frequency was 95% in controls and 83% in patients, whereas A allele frequency was 5% in controls and 17% in patients. There was a statistically significant difference between patient and control groups regarding genotype frequency ($p<0.05$).

Conclusion: Based on the results of our study, we can infer that G1733A (rs6152) polymorphism of the AR gene plays a role in development of prostate cancer in the Turkish population.

Keywords: Prostate cancer; androgen receptor gene; AR gene; G1733A (rs6152) polymorphism.

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Introduction

Prostate cancer is the most common cancer in men, and it is the 2nd leading cause of death in the whole world [1]. Despite its clinical significance and high prevalence, the actual causes of development and molecular mechanism underlying its development and progression are not clearly understood [2, 3]. Studies have examined the role of possible factors such as ethnicity, regional variations, dietary habits and androgen levels [4, 5].

Among these factors, although steroid hormones and especially androgens seem to have a major role, the exact effect is not fully clear. Epidemiological studies examining the effects of androgens on prostate cancer have controversial results [6]. Genetic studies on prostate cancer suggest that the genetic polymorphisms of testosterone and other androgens especially might affect androgen metabolism. For example, a polymorphism found in CYP3A4, which has a role in testosterone metabolism, has been shown to create a tendency towards prostate cancer [7]. Although there are a lot of CYP genes and enzymes, only the enzymes belonging to CYP1, CYP2 and CYP3 families have been found to play major roles in drug metabolism; therefore it has been proposed that pharmaco-genetically they may be polymorphisms that might create a tendency towards cancer development. Additionally, other CYP families have essential roles in intermediate metabolisms [8]. In the CYP2C subfamily, CYP2C9 and CYP2C19 enzymes show polymorphism and have significant roles in metabolism [9]. Additionally, CYP3A4 polymorphism causes a tendency towards prostate cancer especially in the Caucasian race. Individuals carrying the polymorphic G allele have 6 times higher risk of developing prostate cancer [10].

The androgen receptor (AR) gene is a transcription factor regulating the physiological actions of androgens. AR gene is localized between Xq11-q12 in the long arm X chromosome, and contains eight exons [11, 12]. AR's belong to the family of steroid hormone receptors, and these hormones include glucocorticoids, mineralocorticoids, thyroid hormone, retinol, estrogen and progesterone. Once the androgen enters the cell, it binds to the AR. Subsequently, this complex is transferred to the nucleus, and binds specific regions in the DNA and initiates mRNA synthesis [13, 14]. Exon 1 of the AR gene includes two polymorphic trinucleotide repeats (CAG and GGC) that encode polyglutamine and polyglycine. In vitro studies have shown that the length of these repeat sequences is associated with AR activity [15, 16]. Additionally, a single nucleotide polymorphism has previously been described, in which G is converted to A (G1733A) in the 3rd nucleotide position of 211th codon between these two repeats [17].

The aim of the present study is to determine the frequency of G1733A (rs6152) polymorphism of the AR gene in patients diagnosed with prostate cancer.

Methods

The study group consisted of 49 cases clinically diagnosed with prostate cancer (Group 2) by the Urology Department of the Faculty of Medicine in Bolu Abant İzzet Baysal University. The control group (Group 1) consisted of a total of 47 healthy men aged over 50 years old, who did not have genetic relationship between them. The control group included 46 individuals who had normal prostate-specific antigen (PSA) levels according to their age, and did not show any abnormal findings during their digital rectal

examination, together with 1 case who had a PSA level of 14 ng/mL, and whose pathology result of the prostate gland biopsy specimen was reported as benign prostate hyperplasia (BPH).

Genomic DNA was manually isolated from 3 ml blood samples using High Pure PCR Template Preparation Kit (Roche Applied Science, Germany). G1733A (rs6152) polymorphism of the AR gene was analyzed with Light Cycler 480 device using LightCycler Hybridization Probe Kit. After PCR amplification with LightCycler 480 device, homozygous wildtype, homozygous polymorphic and heterozygous genotypes were identified based on the degree of Tm peaks in melting curve analysis (*Tm: In melting curve analysis, the temperature of double-stranded DNA sample is gradually increased, and the change in the fluorescence signal is plotted against temperature. The peaks caused by sudden decreases in the fluorescence signal indicate separation of the DNA strands from each other)*. While heterozygous genotype has two peaks at one Tm degree, homozygous polymorphic genotypes have two peaks at two different Tm degrees. These were Tm1: 55.16 °C for Allele A, and Tm2: 63.55 °C for Allele G. Statistical analysis was performed with SPSS ver.15 (Statistical Package for Social Sciences) package software. Results were evaluated using chi-square and Fisher's exact test. P<0.05 was accepted as statistically significant.

Results

The control group including healthy individuals had a mean age of 67.93 (51-86) years, and mean PSA level of 2.04 ng/mL (0.1 – 14). The study group including patients diagnosed with prostate cancer had a mean age of 72.38 (49-86) years and a mean PSA level of 37.63 ng/mL (4.5 – 150).

AR gene G1733A (rs6152) genotype frequency was found as 91% GG and 9% AG in the control group, and 67% GG and 33% AG in the patient group. G allele frequency was 95% in Group 1 and 83% in Group 2, whereas A allele frequency was 5% in Group 1 and 17% in Group 2. There were statistically significant differences between the groups regarding genotype frequencies ($p<0.05$) (Table 1).

Table 1. Distribution of androgene receptor gene G1733A (rs6152) genotype and allele between groups.

Groups	PSA level (ng/ml)	Genotypes		Alleles	
		GG n (%)	AG n (%)	G n (%)	A n (%)
Group 1 (n=47)	2.04	43 (91)	4 (9)*	90 (95)	4 (5)**
Group 2 (n=49)	37.63	33 (67)	16 (33)*	80 (83)	16 (17)**

PSA: prostate-specific antigen.
*X^2 test, P =0.004. **Fisher exact test, P =0.005.*

Discussion

Studies that investigate the genetic basis of prostate cancer provide important clues to understand the nature of this cancer type and offer treatment strategies. Significant advances in the fields of molecular biology and epidemiology have widened the horizons in biology and etiology of this cancer. Although the etiopathogenesis of prostate cancer is not fully clear, the evidence suggests the disease is multifactorial [18].

Androgens are the major sex hormones in males, and they are responsible for secondary differentiation. Androgens exert their actions

through AR, which is a ligand-dependent nuclear transcription factor. AR has an oncogenic potential, and plays an important role in development of prostate cancer [19]. The first exon of the AR gene is very polymorphic and contains CAG and GGC repeat sequences. The most important factor in development of prostate cancer is advanced age [20]. Several studies have found associations between prostate cancer and short CAG repeats; on the other hand, some other studies could not find an association between prostate cancer and CAG length [21, 22].

Over 300 somatic and germ line mutations have been described for AR-related diseases. While AR mutations cause androgen insensitivity syndrome, they result in hypersensitivity and increased receptor function in prostate cancer cells [23]. Length of CAG repeats generally vary from 11 to 31 copies; however it was found to be shorter in African Americans [15]. While some studies investigating the relationship between CAG repeats and stage of prostate cancer have found longer repeats in patients with advanced stage disease, others did not detect a difference in CAG repeats [24-27]. Zhai et al. studied the Han population and reported that development and progression of prostate cancer was intensified as the number of CAG repeats in AR gene decreased [28]. Biolchi et al. examined the effect of GGC repeats in the AR gene on development of prostate cancer and BPH in the Brazilian population. They found that individuals with numbers of GGC repeats >19 had higher testosterone levels, BPH frequency and prostate cancer rates [29].

Single point mutations can sometimes lead to shifts, increases or decreases in repeat sequences. These mutations can also increase the cancer risk directly [10]. A polymorphism in the CYP3A4 which functions in testosterone metabolism was shown to create a tendency towards prostate cancer [7]. Additionally, similar to our study, Esteban et al. detected G1733A (rs6152) polymorphism in prostate, breast, colon and esophagus cancers in Mediterranean countries [30]. The same study showed that frequency of polymorphism increased in the normal population as we move from North Mediterranean towards Europe. Francesco et al. examined, the genotype A of the androgen receptor (AR) gene rs6152, which was presented in 45 (18.2%) patients, was the only gene significantly associated with an increased risk of prostate cancer pathologic grade group 4-5 and pT3b/4 disease (all $p<0.05$) [31].

In another study, Fernandes et al examined that Rs743572, rs6162, rs6163, rs1256049 and rs2293275 are promising biomarkers for Prostate Cancer aggression but they did not find any significant association with Prostate cancer for G1733A (rs6152) and rs9332969 [32].

When limitations of our study are considered, first of all control group is not with a previously negative prostate biopsy. Another limitation is that study group seems very heterogeneous in relation with the PSA values (4,5-150). But it is known that the positive predictive value was 24 % when PSA was suspicious but digital rectal examination was not, only 10% when digital rectal examination was suspicious but PSA was not and 49% when both tests were suspicious [33].

Our study is the first study from Turkey to examine the relationship between prostate cancer and G1733A (rs6152) polymorphism of the AR gene. Based on our findings, we can say that G1733A (rs6152) polymorphism of the AR gene might play a role in the development of prostate cancer in the Turkish population. We believe our results will guide future

studies, and studies with larger sample sizes would support our results.

Conclusion

As a result of our study, we can infer that G1733A (rs6152) polymorphism of the AR gene plays a role in development of prostate cancer in the Turkish population. Prospective new researches are needed to assess and support the role of G1733A (rs6152) polymorphism of the AR gene.

Ethics Committee Approval: *The study was approved by the Ethics Committee of Bolu Abant İzzet Baysal University School of Medicine (13.04.2011 No: B.30.2.ABÜ.0.20.05.04.-050.01.04-45).*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

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