Original Article

Genetic polymorphisms of estrogen receptor alpha and catechol-O-methyltransferase genes in Turkish patients with familial prostate carcinoma

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OBJECTIVES: Estrogen is one of the most crucial hormones participating in the proliferation and carcinogenesis of the prostate glands. Genetic polymorphisms in the estrogen metabolism pathway might be involved in the risk of prostate carcinoma development. We evaluated the association between genetic polymorphisms in estrogen receptor alpha (ESR1) and catechol-O-methyltransferase (COMT) genes and the risk of developing familial prostate carcinoma. MATERIALS AND METHODS: In this study, 34 cases with prostate carcinoma whose first-degree relatives had prostate carcinoma and 30 healthy age-matched male controls were enrolled. The genotypes of ESR1 and COMT genes were analyzed employing polymerase chain reaction-restriction fragment length polymorphism method. 34 cases with prostate carcinoma, whose first degree relatives had prostate carcinoma and 14 age-matched male controls were enrolled to analyze the genotype of these two genes. **RESULTS:** Among control patients, the *ESR1 Pvull* genotypes of C/C, C/T and T/T were observed in 37%, 26% and 37%, respectively, whereas the C/C, C/T and T/T genotypes were observed in 18%, 41% and 41% of case patients, respectively. Among controls, the ESR1 Pvull allele frequencies of C and T were equally observed, whereas the C and T allele frequencies were observed in 38% and 62% of patients, respectively. Among ESR1 Pvull genotypes there were not any significant difference in terms of genotype (P = 0.199) and allele (P = 0.181) frequencies. Among controls, the ESR1 Xbal genotypes of G/G, G/A and

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A/A were observed in 33%, 37% and 33%, respectively, whereas the G/G, G/A and A/A genotypes were observed in 12%, 47% and 41% of patients, respectively. Among controls, the ESR1 Xbal allele frequencies of A and G were observed equally, respectively, whereas the A and G frequencies were observed in 65% and 35% of patients, respectively. Among ESR1 × bal, there was not any significant difference in terms of genotype (P = 0.111) and allele (P = 0.093) frequencies. But the C/C genotype of the Pvull site and G/G genotype of the Xbal site in the ESR1 gene were associated significantly with the risk of developing prostate carcinoma. The G/G. G/A and A/A genotypes of the COMT gene were observed in 50%, 29% and 21% of control patients and in 53%, 21% and 26% of case patients, respectively. The A and G allele frequencies of the COMT gene were observed in 36.7%, 63.3% of control patients and in 36.8%, 63.2% of case patients, respectively. In COMT gene, there was not any significant difference in terms of genotype (P = 0.843) and allele (P = 0.991) frequencies. But the G/A genotype of the *COMT* gene had a weak tendency toward increased risk. **CONCLUSION:** Polymorphisms of ESR1 gene in the estrogen metabolism pathway were associated significantly with familial prostate carcinoma risk. Single nucleotide polymorphisms of low-penetrance genes are targets for understanding the genetic susceptibility of familial prostate carcinoma.

Key words: Catechol-*O*-methyltransferase, estrogen receptor alpha, familial prostate carcinoma, genetic polymorphism

Introduction

Estrogen is one of the crucial hormones participating in the proliferation and carcinogenesis of the prostate

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glands. Genetic polymorphisms of estrogen receptor alpha (*ESR1*) gene in the estrogen metabolism pathway might be involved in the risk of prostate carcinoma development.^[1] Estrogens exert their effects via cognate receptors, *ESR1* and ER beta. Both receptors are located in the prostate glands and they have been postulated to have important effects on these glands.^[1,2]

Catechol-*O*-methyltransferase (*COMT*) is an important enzyme involved in estrogen metabolism.^[3] Estrogens are catabolized by hydroxylation reactions and 16-hydroxy, 2-hydroxy and 4-hydroxy compounds are inactivated by *COMT*. These catechol estrogen metabolites induced prostate carcinoma in noble rats.^[4,5] Polymorphisms in the genes *ESR1* and *COMT* are associated with the risk of developing other sex steroid hormone-related carcinomas, such as breast carcinoma.^[1,3,6,7] endometrial carcinoma^[7,8] and ovarian carcinoma.^[4,5,8,9]

Family history is one of the important risk factors for prostate carcinoma.^[1] Several susceptibility loci or candidate genes have been reported to explain the genetic susceptibility of prostate carcinoma.^[1] However, these genetic factors explain the susceptibility of a very small percentage of familial/hereditary prostate carcinoma pedigrees. Another approach to understand the genetic susceptibility is to estimate the odds risk for prostate carcinoma by analyzing the SNPs of low-penetrance genes. In the current study, we considered prostate carcinoma cases with a family history of the disease as high-risk cases. We performed a case-control study to determine the relation of the Pvull and Xbal polymorphisms of ESR1, with the risk of developing familial prostate carcinoma in a Turkish population. We evaluated the association between genetic polymorphisms in estrogen-related enzymes and receptors and the risk of developing familial prostate carcinoma. The aim of this study was to determine whether ESR1 and COMT polymorphisms might be involved in the etiopathogenesis of prostate cancer in a Turkish study population.

Materials and Methods

Patients

In this study, 34 cases with prostate carcinoma, whose first-degree relatives had prostate carcinoma and 30 healthy age-matched male controls were enrolled.

Controls were recruited from out-patient clinics at Cukurova University Hospital. Controls were excluded if they had an abnormal prostate specific antigen level. The genotypes of *ESR1* and *COMT* genes were analyzed.

Genotyping

Cukurova University Hospital Ethics Committee approval and informed consent from patients and controls were obtained before blood samples were drawn. Samples of blood (approximately 7 ml) were collected into Vacutainer tubes containing ethylenediaminetetraacetic acid. Deoxyribonucleic acid (DNA) was extracted from peripheral blood lymphocytes using standard salting out extraction method modified from Miller's method.^[10,11] Samples were diluted to10 ng/L and stored at -20°C. Polymerase chain reactions (PCR) were performed in a total reaction volume of 25 µL containing 20 ng of genomic DNA, primers (10 pmol of each forward and reverse primers), 2.5 ml × 10 PCR reaction buffer, deoxynucleotide triphosphates (0.2 mmol/L) and 1 U/L AmpliTag polymerase. PCR amplification was performed in a GeneAmp 9700 thermal cycler (PE Applied Biosystems). Cycling conditions were 95°C for 10 min for 1 cycle; 96°C for 30 s, 60°C for 30 s and 72°C for 30 s for 35 cycles; and an elongation cycle of 72°C for 10 min.

Genotypes of *ESR1 Pvu*II and *XbaI* in intron 2 were assessed according to the modified method of Hill *et al.*^[12] The sequences of primers for *ESR1* were; 5-AGG CTG GGC TCA AAC TAC AG-3 for the forward primer and 5-CTC TGG GAG ATG CAG CAG AT-3 for the reverse primer. A, T or C allele of the *Pvu*II polymorphism corresponded to the presence or absence of the *Pvu*II restriction site. An A or G allele of the *XbaI* polymorphism corresponded to the presence or absence of the *XbaI* site.

The genotypes of *COMT* were assessed by the method described by Hamajima *et al.*^[13] This method was used to identify a G-to-A polymorphism at codon 158 of *COMT*. A PCR-based restriction fragment length polymorphism assay was performed to detect the presence of the G \rightarrow A transition at position 1947 in COMT (accession no. Z26491). PCR was used to amplify a 185-bp fragment of genomic DNA containing the polymorphism. Briefly, the primer sequences were; 5'-GGAGCTGGGGGCCTACTGTG-3' for the forward primer and 5'-GGCCCTTTTTCCAGGTCTGACA-3' for the reverse primer.

Statistical analysis were performed using SPSS Inc. Released 2006. SPSS for Windows, Version 15.0. Chicago, SPSS Inc. and the level of statistical significance was accepted as P < 0.05.

Results

Among control patients, the *ESR1 Pvu*II genotypes of C/C, C/T and T/T were observed in 37%, 26% and 37%, respectively, whereas the C/C, C/T and T/T genotypes were observed in 18%, 41% and 41% of case patients, respectively [Table 1]. Among controls, the *ESR1 XbaI* genotypes of G/G, G/A and A/A were observed in 33%, 37% and 33%, respectively, whereas the G/G, G/A and A/A genotypes were observed in 12%, 47% and 41% of case patients, respectively. The C/C genotype of the *Pvu*II site and G/G genotype of the *XbaI* site in the *ESR1* gene were associated significantly with the risk of developing prostate carcinoma.

The G/G, G/A and A/A genotypes of the *COMT* gene were observed in 50%, 29% and 21% of control patients and in 53%, 21% and 26% of case patients, respectively. The G/A genotype of the *COMT* gene had a weak tendency toward increased risk [Table 2].

Discussion

ESR1 is an important mediator of the hormonal response in estrogen-responsive tissue specimens.^[14] The association between bone mineral density and breast carcinoma with genetic polymorphisms of ESR1 has been evaluated extensively. Some studies showed an association between low bone mineral density and the A/A and C/C genotypes of Xbal and Pvull polymorphisms^[15] whereas other studies did not show a significant association between ESR1 genotypes and bone mineral density.^[16] Because high estrogen levels were related to high bone mineral density, the association among bone mineral density, ESR1 polymorphism and breast carcinoma risk were demonstrated.^[17] The relation between ESR1 polymorphism and prostate carcinoma risk was reported by Modugno et al.[18] In their study, the G/G genotype of the Xbal polymorphism and the C/C genotype of the Pvull polymorphism increased the

Table 1: Association of ESR1 genotypes with prostate carcinoma risk

	Number of patients (%)	
	Cases	Controls
Genotype		
ESR1 Pvull		
C/C	6 (18)	10 (37)
C/T	14 (41)	7 (26)
T/T	14 (41)	10 (37)
χ^2	0.199	
ESR1 Xbal		
A/A	14 (41)	9 (33)
A/G	16 (47)	10 (37)
G/G	4 (12)	9 (33)
χ^2	0.111	()
Allele		
ESR1 Pvull		
С	26 (38.2)	30 (50)
т	42 (61.8)	30 (50)
χ^2	0.181	()
ESR1 Xbal		
A	44 (64.7)	30 (50)
G	24 (35.3)	30 (50)
χ^2	0.093	

ESR: Estrogen receptor alpha

Table 2: Association of *COMT* genotypes with prostate carcinoma risk

	Number of patients (%)	
	Cases	Controls
COMT genotype		
G/G	18 (53)	7 (50)
G/A	7 (21)	4 (29)
A/A	9 (26)	3 (21)
χ^2	0.843	
COMT allele frequency		
A	25 (36.8)	22 (36.7)
G	43 (63.2)	38 (63.3)
χ^2	0.991	· · · ·

COMT: Catechol-O-methyltransferase

risk of developing prostate carcinoma, but the difference was not statistically significant. In the current study, the C/C genotype of the *Pvu*II polymorphism and the G/G genotype of the *XbaI* polymorphism were associated with the risk of developing prostate carcinoma.

Conclusion

Polymorphisms of *ESR1* gene in the estrogen metabolism pathway were associated significantly with familial prostate carcinoma risk. The G/A genotype of the *COMT* gene had a weak tendency toward increased risk. Single nucleotide polymorphisms of low-penetrance genes are targets for understanding the genetic susceptibility of familial prostate carcinoma.

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