Original Article

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

Ayfer Pazarbasi[1], M. Bertan Yilmaz[1], Davut Alptekin[1], Umit Luleyap[1], Zuhtu Tansug[2], Lutfiye Ozpak[1], Muzeyyen Izmirli[3], Dilge Onatoglu-Arikan[1], Sabriye Kocaturk-Sel[1], Mehmet Ali Erkoc[1], Ozgur Turgut[1], Ceyhun Bereketoglu[1], Erdal Tunc[1], Eylul Akbal[1]

[1]Departments of Medical Biology and Genetics, and [2]Urology, Faculty of Medicine, Cukurova University, Adana, [3]Department of Medical Biology, Faculty of Medicine, Bezmi Alem University, Istanbul, Turkey

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 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. Among controls, the *ESR1 Pvu*II 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 Pvu*II genotypes there were not any significant difference in terms of genotype ($P = 0.199$) and allele ($P = 0.181$) frequencies. Among controls, the *ESR1 Xba*I 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 patients, respectively. Among controls, the *ESR1 Xba*I 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 *Pvu*II site and G/G genotype of the *Xba*I 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

Access this article online	
Quick Response Code:	Website: www.ijhg.com
	DOI: 10.4103/0971-6866.124366

Introduction

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

Address for correspondence: Asso. Prof. Ayfer Pazarbasi, Department of Medical Biology and Genetics, Cukurova University, 01330 Adana, Turkey. E-mail: payfer@cu.edu.tr

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 *Pvu*II and *XbaI* 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 AmpliTaq 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 → 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].

Table 1: Association of *ESR1* genotypes with prostate carcinoma risk

	Number of patients (%)	
	Cases	Controls
Genotype		
ESR1 PvuII		
C/C	6 (18)	10 (37)
C/T	14 (41)	7 (26)
T/T	14 (41)	10 (37)
χ^2	0.199	
ESR1 XbaI		
A/A	14 (41)	9 (33)
A/G	16 (47)	10 (37)
G/G	4 (12)	9 (33)
χ^2	0.111	
Allele		
ESR1 PvuII		
C	26 (38.2)	30 (50)
T	42 (61.8)	30 (50)
χ^2	0.181	
ESR1 XbaI		
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

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 *XbaI* and *Pvu*II 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 *XbaI* polymorphism and the C/C genotype of the *Pvu*II polymorphism increased the 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.

Acknowledgments

This work was supported by "Cukurova University Research Projects Funding Unit" project number TF.2007-BAP18.

References

1. Suzuki K, Nakazato H, Matsui H, Koike H, Okugi H, Kashiwagi B, *et al*. Genetic polymorphisms of estrogen receptor alpha, CYP19, catechol-O-methyltransferase are associated with familial prostate carcinoma risk in a Japanese population. Cancer 2003;98:1411-6.
2. Suzuki K, Matsui H, Hasumi M, Ono Y, Nakazato H, Koike H, *et al*. Gene expression profiles in human BPH: Utilization of laser-capture microdissection and quantitative real-time PCR. Anticancer Res 2001;21:3861-4.
3. Mitrunen K, Jourenkova N, Kataja V, Eskelinen M, Kosma VM, Benhamou S, *et al*. Polymorphic catechol-O-methyltransferase gene and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10:635-40.
4. Leav I, Ho SM, Ofner P, Merk FB, Kwan PW, Damassa D. Biochemical alterations in sex hormone-induced hyperplasia and dysplasia of the dorsolateral prostates of Noble rats. J Natl Cancer Inst 1988;80:1045-53.
5. Leav I, Galluzzi CM, Ziar J, Stork PJ, Ho SM, Loda M. Mitogen-activated protein kinase and mitogen-activated kinase phosphatase-1 expression in the Noble rat model of sex hormone-induced prostatic dysplasia and carcinoma. Lab Invest 1996;75:361-70.
6. Andersen TI, Heimdal KR, Skrede M, Tveit K, Berg K, Børresen AL. Oestrogen receptor (ESR) polymorphisms and breast cancer susceptibility. Hum Genet 1994;94:665-70.
7. Weiderpass E, Persson I, Melhus H, Wedrén S, Kindmark A, Baron JA. Estrogen receptor alpha gene polymorphisms and endometrial cancer risk. Carcinogenesis 2000;21:623-7.
8. Berstein LM, Imyanitov EN, Suspitsin EN, Grigoriev MY, Sokolov EP, Togo A, *et al*. CYP19 gene polymorphism in endometrial cancer patients. J Cancer Res Clin Oncol 2001;127:135-8.
9. Garner EI, Stokes EE, Berkowitz RS, Mok SC, Cramer DW. Polymorphisms of the estrogen-metabolizing genes CYP17 and catechol-O-methyltransferase and risk of epithelial ovarian cancer. Cancer Res 2002;62:3058-62.
10. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
11. Pazarbaşi A, Kasap M, Güzel AI, Kasap H, Onbaşioğlu M, Ozbakir B, *et al*. Polymorphisms in the tumor necrosis factor-alpha gene in Turkish women with pre-eclampsia and eclampsia. Acta Med Okayama 2007;61:153-60.
12. Hill SM, Fuqua SA, Chamness GC, Greene GL, McGuire WL. Estrogen receptor expression in human breast cancer associated with an estrogen receptor gene restriction fragment length polymorphism. Cancer Res 1989;49:145-8.
13. Hamajima N, Matsuo K, Tajima K, Mizutani M, Iwata H, Iwase T, *et al*. Limited association between a catechol-O-methyltransferase (*COMT*) polymorphism and breast cancer risk in Japan. Int J Clin Oncol 2001;6:13-8.
14. Cunha GR, Wang YZ, Hayward SW, Risbridger GP. Estrogenic effects on prostatic differentiation and carcinogenesis. Reprod Fertil Dev 2001;13:285-96.
15. Lorentzon M, Lorentzon R, Bäckström T, Nordström P. Estrogen receptor gene polymorphism, but not estradiol levels, is related to bone density in healthy adolescent boys: A cross-sectional and longitudinal study. J Clin Endocrinol Metab 1999;84:4597-601.
16. Bagger YZ, Jørgensen HL, Heegaard AM, Bayer L, Hansen L, Hassager C. No major effect of estrogen receptor gene polymorphisms on bone mineral density or bone loss in postmenopausal Danish women. Bone 2000;26:111-6.
17. Zhang Y, Kiel DP, Kreger BE, Cupples LA, Ellison RC, Dorgan JF, *et al*. Bone mass and the risk of breast cancer among postmenopausal women. N Engl J Med 1997;336:611-7.
18. Modugno F, Weissfeld JL, Trump DL, Zmuda JM, Shea P, Cauley JA, *et al*. Allelic variants of aromatase and the androgen and estrogen receptors: Toward a multigenic model of prostate cancer risk. Clin Cancer Res 2001;7:3092-6.

Cite this article as: Pazarbasi A, Yilmaz MB, Alptekin D, Luleyap U, Tansug Z, Ozpak L, *et al*. Genetic polymorphisms of estrogen receptor alpha and catechol-O-methyltransferase genes in Turkish patients with familial prostate carcinoma. Indian J Hum Genet 2013;19:408-11.

Source of Support: Nil, **Conflict of Interest:** None declared.