

Prostate Cancer Risk and ESR1 TA, ESR2 CA Repeat Polymorphisms

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Abstract

Background: Experimental evidence has suggested that estrogen receptor α (coded by the gene *ESR1*) might increase prostate cancer risk, whereas estrogen receptor β (coded by the gene *ESR2*) might reduce prostate cancer risk.

Methods: We investigated the relationship with prostate cancer risk of both a TA repeat polymorphism in the *ESR1* 5' region, *ESR1* (TA)_n, and with a CA repeat polymorphism in intron 5 of *ESR2*, *ESR2* (CA)_n, in a case-control study (545 cases and 674 controls) nested in the Physicians' Health Study.

Results: Prostate cancer risk was highest for carriers of *ESR1* (TA)₂₄ and *ESR1* (TA)₂₅. Replacing one modal *ESR1* (TA)₁₄ allele with one *ESR1* (TA)₂₄ allele yielded an odds ratio of 1.42 (95% confidence interval, 1.00-2.00; $P = 0.05$). Replacing one *ESR1* (TA)₁₄ allele with one *ESR1* (TA)₂₅ allele yielded an odds ratio of 2.10 (95% confidence interval, 1.15-3.84; $P = 0.02$). *ESR2* (CA)_n showed no effects on prostate cancer risk.

Conclusions: The *ESR1* (TA)_n polymorphism might play a role in prostate cancer risk. (Cancer Epidemiol Biomarkers Prev 2007;16(11):2233-6)

Introduction

Effects on risk of prostate cancer of endogenous estrogens and other sex steroid hormones remain controversial. Both estrogen receptors (ER) α and β (coded by the genes *ESR1* and *ESR2*, respectively) are expressed in human and murine prostate tissue and regulate epithelial growth (1-3). Whereas ER α expression has been proposed to increase prostate cancer risk (3), recent evidence suggests that ER β expression might reduce risk by binding with an androstane diol, 5 α -androstane-3 β , 17 β -diol (4-6). Accounting for different estrogen pathways could help to resolve contradictory findings about the effects of endogenous estrogens and androstane diols on prostate cancer risk.

A thymine-adenine (TA)_n dinucleotide repeat polymorphism in the 5' promoter region of the *ESR1* gene might affect ER α activity. In Caucasian populations, the frequency of repeat lengths exhibits a bimodal distribution peaking sharply at (TA)₁₄ followed by very low allele frequency between (TA)₁₆ and (TA)₁₉ and a shallower peak at (TA)₂₃. Most prior epidemiologic research has compared long versus short alleles, defined in various ways (7-13), with some exceptions (14-17).

Longer (TA)_n is associated with increased risk of coronary artery disease (8, 10), lower concentrations of small low-density lipoprotein particles in women (7), blunted endothelial plasminogen activator release in women (18), and reduced adenosine-stimulated myocardial perfusion in men (9). Longer (TA)_n has also been associated with higher osteoarthritis risk (14), reduced bone mineral density and greater history of fractures (11), and reduced risk of endometriosis (15, 16), suggesting reduced ER α expression. However, only a small and nonsignificant reduced risk of endometrial cancer has been associated with longer alleles, defined as (TA)_{≥19}, relative to (TA)_{<19} repeats (12).

A cytosine-adenine (CA)_n dinucleotide repeat polymorphism in intron 5 of the *ESR2* gene has been linked to ER β expression. Longer (CA)_n, defined as (CA)_{≥24}, has been associated with reduced testosterone levels and increased sex hormone binding globulin in women, relative to (CA)_{<24} repeats (13). Longer repeat lengths have also been associated with higher osteoarthritis risk (14), lower bone mineral density in women (19, 20), higher blood pressure (21), and less severe menstrual symptoms (22). However, a large study of endometrial cancer risk found no association with long repeat length, defined as (CA)_{≥22}, relative to (CA)_{<22} (23).

One study has found an association between a polymorphism in codon 10 of *ESR1* and prostate cancer risk (24). Another recent study detected an effect of a different *ESR1* SNP (rs2234693) on prostate cancer grade at diagnosis, but not total risk of either familial or sporadic prostate cancer (25), and no association with one ER β SNP. They also investigated both *ESR1* (TA)_n and *ESR2* (CA)_n with risk of sporadic and familial prostate cancer (25). They modeled additive effects of all alleles and found no evidence for an overall effect of

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Table 1. Study participants by level of covariates and case-control status

	Cases (n = 545)	Controls (n = 674)
Age at baseline,* mean (SD)	61.09 (7.35)	61.20 (7.64)
Weight (kg), mean (SD)	79.22 (9.40)	77.77 (9.50)
Height (cm), mean (SD)	178.76 (6.14)	177.95 (6.84)
Smoking,* n (%)		
Current smokers	42 (7.71)	57 (8.46)
Former smokers	251 (46.06)	315 (46.74)
Tumor grade, n (%)		
Gleason ≥ 7 or poorly differentiated	190 (34.86)	
Gleason < 7 or well differentiated	296 (54.31)	
Unknown	59 (10.83)	
Stage, n (%)		
Died of prostate cancer	117 (21.47)	
Diagnosed with Metastases	16 (2.94)	
Whitmore-Jewett stage C or D at initial diagnosis	86 (15.78)	
Whitmore-Jewett stage A or B at initial diagnosis	277 (50.83)	
Unknown	49 (8.99)	

*Matching factor.

either polymorphism. We report the results of a study that also investigated the association between prostate cancer risk and ESR1 (TA)_n and ESR2 (CA)_n among participants in the Physicians' Health Study.

Materials and Methods

Participant Selection and Medical Record Review.

Prostate cancer cases were identified from participants in the Physicians' Health Study, which began in 1982 as a randomized trial of aspirin and β -carotene among 22,071 U.S. male physicians (26). Men with a history of myocardial infarction, stroke, transient ischemic attack, unstable angina, cancer (other than nonmelanoma skin cancer), or current renal or liver disease, peptic ulcer, gout, use of platelet-active agents, vitamin A, or β -carotene supplements were excluded from enrollment. Baseline information, including age, smoking history, and race, was collected by self-administered questionnaire, and 93% classified their race as Caucasian. Before randomization, 14,916 men provided a blood sample.

Prostate cancer diagnoses were initially identified by annual follow-up questionnaires, and all cases were confirmed by review of medical records. We follow participants for occurrence of metastases and death. In this article, we defined advanced stage as Whitmore-Jewett stage C or D at diagnosis or having been diagnosed with metastases or having died of prostate cancer during follow-up. We defined high grade as Gleason ≥ 7 or "poorly differentiated" tumors.

For our nested case-control study, prostate cancer cases were matched with one or two controls by age (within 1 year if younger than 55 or within 5 years if older) and smoking status (past, current, or never). Genetic analyses reported in this article include 545 cases and 674 matched controls from diagnosed 1982 to 1995 and who had also provided blood samples.

Genotyping. Genomic DNA was prepared from frozen blood (QIAamp, Qiagen, Inc.). For both polymorphisms, genomic DNA (50 ng) was amplified using 22.5 pmol of each primer in 25 μ L PCR buffer B (Invitrogen Corp.) containing 2 mmol/L MgCl₂ (pH 8.5) plus 1.6 mmol/L dNTP and 1 unit of Platinum Taq Polymerase (Invitrogen). The PCR had an initial denaturing temperature at 95°C (2 min) followed by 35 cycles of denaturing (95°C; 30 s), annealing (60°C; 30 s), and extension (72°C; 30 s). A 7-min extension at 72°C followed the final cycle. Mixed with Gene Scan-500 ROX Size Standard (Applied Biosystems), PCR products were applied to a POP-4 capillary array that was linked to an automated fluorescence detection system, ABI PRISM 3100 Gene Analyzer (Applied Biosystems). The primers for the ESR1 (TA)_n polymorphism were 5'FAM-GTATAAACTATCCAAGATTA-3' and 5'-GCAGAATCAAATATCCAGATG-3' and those for the ESR2 (CA)_n polymorphism were 5'HEX-GGTAAACCATGGTCTGTACC-3' and 5'-AACAAAATGTTGAATGAGTGGG-3'. Using analysis software, GeneScan Analysis 2.1 (Applied Biosystems), ESR1 (TA)_n was calculated as (fragment length - 144 bp) / 2 and ESR2 (CA)_n as (fragment length - 117 bp) / 2. The genetic laboratory was blind to the case-control status of samples.

Statistical Methods. Each repeat length allele was scored as 0, 1, or 2 (27, 28) depending on the number of alleles carried by the participant and entered into an unconditional logistic regression following Cunningham et al. (25). Regression models were adjusted for matching factors: age, smoking, and follow-up time. We also conducted further analyses stratifying cases by stage and grade at diagnosis. We report the likelihood ratio test of each full additive model with a model containing only covariates. Given the large number of alleles for each of these polymorphisms, 22 for ESR1 (TA)_n and 14 for ESR2 (CA)_n, the power of these global tests is low. Therefore, we also discuss contrasts with modal alleles (Table 1).

Results

Polymorphisms showed statistically significant global effects neither on total prostate cancer risk [ESR1 (TA)_n

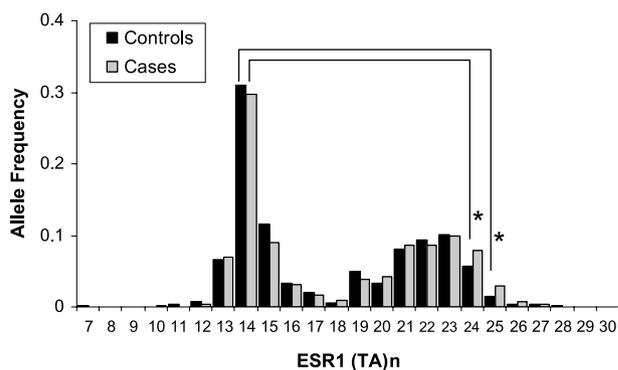


Figure 1. ESR1 (TA)_n distribution by case/control status. Global $P = 0.08$. Contrasts with ESR1 (TA)₁₄ indicated with a "*" $P < 0.05$.

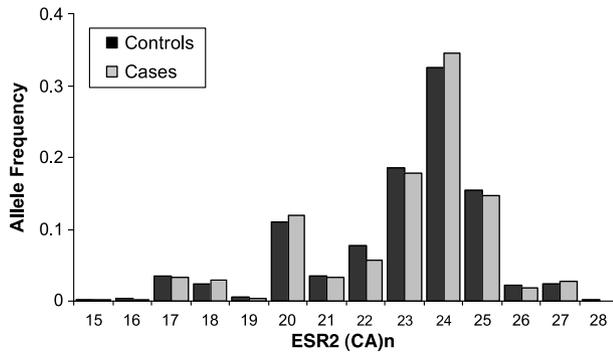


Figure 2. ESR2 (CA)_n distribution by case/control status. Global $P = 0.69$.

global $P = 0.08$, ESR2 (CA)_n global $P = 0.69$] nor on risk of high-grade prostate cancer [ESR1 (TA)_n global $P = 0.31$, ESR2 (CA)_n global $P = 0.38$] nor on risk of advanced prostate cancer [ESR1 (TA)_n global $P = 0.55$, ESR2 (CA)_n global $P = 0.40$]. However, two alleles of ESR1 (TA)_n ($n = 24$ and $n = 25$) were disproportionately carried by cases, relative to modal ESR1 (TA)₁₄ repeats. The effects of these alleles can be expressed as relative odds associated with replacing one ESR1 (TA)₁₄ allele with one ESR1 (TA)₂₄ allele (odds ratio, 1.42; 95% confidence interval, 1.00-2.00; $P = 0.05$) or replacing one ESR1 (TA)₁₄ allele with one ESR1 (TA)₂₅ allele (odds ratio, 2.10; 95% confidence interval, 1.15-3.84; $P = 0.02$; Figs. 1 and 2).

Discussion

We did not detect significant global effects of either ESR1 (TA)_n or ESR2 (CA)_n on risk of total, high-grade, or advanced prostate cancer. However, two alleles of ESR1 (TA)_n, $n = 24$ and $n = 25$, were disproportionately carried by cases.

Given that significant overall associations of these polymorphisms with prostate cancer risk or phenotype were not observed in prior large study (25), it is possible that we observed elevation in prostate cancer risk for carriers of ESR1 (TA)₂₄ and ESR1 (TA)₂₅ by chance. Nevertheless, the global tests used both in this study and by Cunningham et al. (25) are similarly underpowered for effects of moderate size. It is therefore worth considering whether our more exploratory findings of effects of individual alleles, in conjunction with other results suggesting reduced estrogenicity of longer repeats, imply a potential role for ER α in prostate cancer carcinogenesis.

Although experimental research shows that ER α expression promotes squamous cell metaplasia (3), human prostate cancer has been associated with a polymorphism at codon 10 of ESR1 (24) that seems to reduce ER α activity, given its opposite association with endometrial cancer (29). Our results also suggest that lower ER α expression increases prostate cancer risk. Substantial prior evidence shows that long repeat length of ESR1 (TA)_n is associated with reduced ER α expression, with the notable exception that no association was found with endometrial cancer.

In summary, we found some preliminary evidence linking a TA repeat polymorphism in the 5' region of ESR1 with both prostate cancer and hormone levels. Our results suggest that ER α activity may reduce prostate cancer risk.

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References

- Cunha GR, Riche W, Thomson A, et al. Hormonal, cellular, and molecular regulation of normal and neoplastic prostatic development. *J Steroid Biochem Mol Biol* 2004;92:221-36.
- King KJ, Nicholson HD, Assinder SJ. Effect of increasing ratio of estrogen: androgen on proliferation of normal human prostate stromal and epithelial cells, and the malignant cell line LNCaP. *Prostate* 2006;66:105-14.
- Risbridger G, Wang H, Young P, et al. Evidence that epithelial and mesenchymal estrogen receptor- α mediates effects of estrogen on prostatic epithelium. *Dev Biol* 2001;229:432-42.
- Guenrini V, Sau D, Scaccianoce E, et al. The androgen derivative 5 α -androstane-3 β , 17 β -diol inhibits prostate cancer cell migration through activation of the estrogen receptor β subtype. *Cancer Res* 2005;65:5445-53.
- Weihua Z, Lathe R, Warner M, Gustafsson J-Å. An endocrine pathway in the prostate, ER β , AR, 5 α androstane 3 β , 17 β diol, and CYP7B1, regulates prostate growth. *Proc Natl Acad Sci U S A* 2002; 99:13589-94.
- Weihua Z, Mäkelä S, Andersson LC, et al. A role for estrogen receptor β in the regulation of growth of the ventral prostate. *Proc Natl Acad Sci U S A* 2001;98:6330-5.
- Demissie S, Cupples LA, Shearman AM, et al. Estrogen receptor- α variants are associated with lipoprotein size, distribution, and particle levels in women: the Framingham Heart Study. *Atherosclerosis* 2006;185:210-8.
- Kunnas TA, Laippala P, Penttilä A, Lehtimäki T, Karhunen PJ. Association of polymorphism of human α oestrogen receptor with coronary artery disease in men: a necropsy study. *BMJ* 2000;321:273-4.
- Kunnas TA, Lehtimäki T, Karhunen PJ, et al. Estrogen receptor genotype modulates myocardial perfusion in young men. *J Mol Med* 2004;82:821-5.
- Lu H, Higashikata T, Inazu A, et al. Association of estrogen receptor- α gene polymorphisms with coronary artery disease in patients with familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 2002; 22:821-7.
- van Meurs JBJ, Schuit SCE, Weel AEAM, et al. Association of 5' estrogen receptor α gene polymorphisms with bone mineral density, vertebral bone area and fracture risk. *Hum Mol Genet* 2003;12: 1745-54.
- Weiderpass E, Persson I, Melhus H, Wedrén S, Kindmark A, Baron JA. Estrogen receptor α gene polymorphism and endometrial cancer risk. *Carcinogenesis* 2000;21:623-7.
- Westberg L, Baghaei F, Rosmond R, et al. Polymorphisms of the androgen receptor gene and the estrogen receptor β gene are associated with androgen levels in women. *J Clin Endocrinol Metab* 2001;86:2562-8.
- Fyliti P, Giannatou E, Papanikolaou V, et al. Association of repeat polymorphisms in the estrogen receptors α , β , and androgen receptor genes with knee osteoarthritis. *Clin Genet* 2005;68:268-77.
- Georgiou I, Syrrou M, Bouba I, et al. Association of estrogen receptor gene polymorphisms with endometriosis. *Fertil Steril* 1999;72:164-6.
- Hsieh Y-Y, Chang C-C, Tsai F-J, Lin C-C, Tsai C-H. Estrogen receptor α dinucleotide repeat and cytochrome P450c17 α gene polymorphisms are associated with susceptibility to endometriosis. *Fertil Steril* 2005;83:567-72.
- Rokach A, Pollack A, Rosen L, et al. Estrogen receptor α gene polymorphisms are associated with the angiographic extent of coronary artery disease. *J Clin Endocrinol Metab* 2005;90:6556-60.
- Hoetzer GL, Irmiger HM, Stauffer BL, DeSouza CA. Estrogen receptor- α thymidine and adenine repeat polymorphism and endothelial fibrinolytic regulation in postmenopausal women. *Am J Obstet Gynecol* 2005;193:366-70.
- Ogawa S, Hosoi T, Shiraki M, et al. Association of estrogen receptor β gene polymorphism with bone mineral density. *Biochem Biophys Res Commun* 2000;269:537-41.
- Scariano JK, Simplicio SG, Montoya GD, Garry PJ, Baumgartner RN.

- Estrogen receptor β dinucleotide (CA) repeat polymorphism is significantly associated with bone mineral density in postmenopausal women. *Calcif Tissue Int* 2004;74:501–8.
21. Ogawa S, Emi M, Shiraki M, Hosoi T, Ouchi Y, Inoue S. Association of estrogen receptor β (ESR2) gene polymorphism with blood pressure. *J Hum Genet* 2000;45:327–30.
 22. Takeo C, Negishi E, Nakajima A, et al. Association of cytosine-adenine repeat polymorphism of the estrogen receptor- β gene with menopausal symptoms. *Gend Med* 2005;2:96–105.
 23. Setiawan VW, Hankinson SE, Colditz GA, Hunter DJ, De Vivo I. Estrogen receptor β (ESR2) polymorphisms and endometrial cancer (United States). *Cancer Causes Control* 2004;15:627–33.
 24. Tanaka Y, Sasaki M, Kaneuchi M, Shiina H, Igawa M, Dahiya R. Polymorphisms of estrogen receptor α in prostate cancer. *Mol Carcinog* 2003;37:202–8.
 25. Cunningham JM, Hebring SJ, McDonnell SK, et al. Evaluation of genetic variation in the androgen and estrogen metabolic pathways as risk factors for sporadic familial prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2007;16:969–78.
 26. Steering Committee of the Physicians' Health Study Research Group. Final report on the aspirin component of the ongoing Physicians' Health Study. *N Engl J Med* 1989;321:129–35.
 27. Schaid DJ. Evaluating associations of haplotypes with traits. *Genet Epidemiol* 2004;27:348–64.
 28. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 2002;70:425–34.
 29. Sasaki M, Tanaka Y, Kaneuchi M, Sakuragi N, Dahiya R. Polymorphisms of estrogen receptor α gene in endometrial cancer. *Biochem Biophys Res Commun* 2002;297:558–64.

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