Allelic Variants of Aromatase and the Androgen and Estrogen Receptors: Toward a Multigenic Model of Prostate Cancer Risk

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ABSTRACT

Purpose: The purpose of this study was to determine whether polymorphisms in the CAG repeat in exon 1 of the androgen receptor (AR), two intronic restriction sites in the estrogen receptor (ESR1) XbaI and ESR1 PvuII, and an Arg264Cys substitution in the aromatase gene (CYP19) contribute to prostate cancer risk.

Experimental Design: A case-control study was performed with 88 Caucasian prostate cancer patients and 241 Caucasian male controls. Logistic regression models were used to assess individual and joint contributions of genotypes to prostate cancer risk.

Results: For single polymorphisms, only the AR repeat number was significantly related to increased prostate cancer risk [age- and BMI-adjusted odds ratio (OR), 1.14; 95% confidence interval (CI), 1.04–1.25], suggesting a 14% increase in risk for each missing CAG repeat. When subjects were classified as either long (>23 AR CAG repeats) or short (<23 repeats) carriers, a significant increase in risk was also observed (age- and BMI-adjusted OR, 1.75; 95% CI, 1.05–2.95; P = 0.04). The aromatase C/T was associated with an increase in risk of borderline significance (age- and BMI-adjusted OR, 2.50; 95% CI, 0.99–6.28). When examining the effects of two polymorphisms on prostate cancer risk, homozygosity for the ESR1 XbaI restriction site together with a longer AR was more frequent among controls (32%) than cases (18%; age- and BMI-adjusted OR, 0.39; 95% CI, 0.19–0.78). The aromatase C/C genotype together with a longer AR was also more frequent among controls (55%) than cases (41%; age- and BMI-adjusted OR, 0.51; 95% CI, 0.30–0.89).

Conclusions: Estrogen and aromatase may play a role in prostate cancer. A multigenic model of prostate cancer susceptibility is also supported.

INTRODUCTION

Because normal growth and function of prostatic tissue is under the control of DHT, there has been much interest in the role of sex steroid hormones in the etiology of prostate cancer. However, despite strong biological support, there has been no consistent epidemiological link established between steroid hormones and prostate cancer risk. Recent studies have found an association between prostate cancer risk and polymorphisms in several genes along the sex steroid pathway, including the AR (1) and 5α-reductase [SRD5A2 (2, 3)], which catalyzes the conversion of testosterone to DHT. Thus, it is possible that the discrepancies among epidemiological studies investigating the link between prostate cancer and steroid hormones may be due in part to polymorphisms in genes involved in the metabolism and action of steroid hormones.

The goal of this study was to investigate the association of prostate cancer and polymorphisms in the AR, aromatase, and estrogen receptor (ESR1). To our knowledge, there have been no previous studies of the relationship of the aromatase and ESR1 polymorphisms and prostate cancer, nor have there been any studies of the interactions among these polymorphisms on prostate cancer risk. All three genes are involved in sex steroid metabolism and action (Fig. 1). The effect of androgens is ultimately mediated through the AR. The AR contains an intragenic CAG repeat in exon 1, and the length of this repeat is inversely correlated with the ability of the AR to transactivate other genes (4, 5). It is possible that lifelong enhanced AR activity is related to prostate cancer. Studies (1, 6–9) linking short AR alleles to increased prostate cancer risk support this hypothesis. Aromatase (CYP19) catalyzes the conversion of androgens to estrogens. The effects of the resulting estrogens are mediated through the estrogen receptor. The prostate is influenced by estrogen from peripheral sources as well as through aromatase activity in its stroma (10). Data from a prospective study suggest that low levels of estradiol may be a risk factor for...
prostate cancer (11). There is also evidence of elevated levels of aromatase activity and mRNA expression in stromal cells in prostate cancer (12), as well as increasing evidence of cross-talk between estrogens and androgens in regulating gene expression in the prostate (13, 14). Moreover, circulating estrogens can compete with androgens for binding to sex hormone-binding globulin, and it is generally assumed that sex hormone-binding globulin synthesis is regulated by and is a reflection of the estrogen/androgen balance (15). Thus, it is possible that individual and combined genetic variations in the AR, estrogen receptor, and aromatase genes, which may alter the availability of sex steroid hormones, can alter an individual’s risk of prostate cancer. The work presented here addresses that hypothesis.

MATERIALS AND METHODS

Selection of Patients and Controls. Blood samples were collected from 88 adult Caucasian patients with histologically confirmed prostate cancer seen by a single physician at the UPtC between April 1994 and June 1998. All prostate cancer patients were eligible for this study, and cases were accrued at initial presentation at UPtC. All cases approached by the staff nurse agreed to participate. Fifty-five subjects were recruited more than 1 year after diagnosis. Blood samples were also obtained from 241 cancer-free community-based Caucasian male controls age 50 years and older. Controls were identified through mailings to population-based lists of age-eligible voters residing near Pittsburgh, Pennsylvania and participated in a previous study (16). Demographic and anthropometric data were obtained from a subject questionnaire or from patient records. Height and weight were measured by study personnel and used to form the BMI. All study subjects gave informed consent for participation, and Institutional Review Board approval was obtained for this study.

AR Genotyping. High molecular weight DNA was extracted from peripheral blood leukocytes by the salting out procedure (17). The CAG repeat polymorphism in exon 1 of the AR gene was genotyped as described previously (18). Reaction mixtures containing 20 ng of genomic DNA, 5 pmol of each oligonucleotide primer (5’-ATC-CAG-GGT-TAT-GTG-GCA-ATG-AC-3’; reverse primer, 5’-ACC-CTG-GCG-TCG-ATT-ATC-TGA-3’) in 50-μl reaction volumes containing 2 μl of DNA, 0.30 μM of each primer, 200 μM of deoxynucleotide triphosphates, 1.5 mM MgCl₂, 5 μl of 10× PCR buffer (500 mM KCl and 200 mM Tris-HCl), 1.5 units of Taq DNA polymerase, and water. After an initial 5-min denaturation at 95°C, 30 cycles of denaturation (94°C, 30 s), annealing (57°C, 40 s), and elongation (72°C, 30 s) were followed by a final elongation step at 72°C for 1 min.

Next, 15-μl aliquots of the PCR products were digested overnight with 5 units of restriction enzyme (New England Biolabs, Beverly, MA) and separated on a 2% agarose gel containing ethidium bromide, and the fragments were visualized by UV illumination. Separate reactions were performed for the XbaI and PvuII restriction sites. Fragment sizes were estimated by comparison to a 1-kb ladder on the same gel. The presence of the XbaI and PvuII restriction sites is indicated by a +, whereas the absence of the sites are denoted by a −.

Aromatase Genotyping. The primers (forward primer, 5’-CGC-TAG-ATG-TCT-AAA-CTG-AG-3’; reverse primer, 5’-CAT-ATG-TGG-CAT-GGG-AAT-3’) were used to amplify the coding and flanking sequences of exon 7 of the aromatase gene. Unique sequence oligonucleotide primers were designed from genomic sequences deposited under GenBank accession number J05105. Amplification was performed in a final volume of 50 μl containing 40 ng of genomic DNA, 1.5 units of Taq polymerase (Life Technologies, Inc.), 1.5 mM MgCl₂, 200 μM of each deoxynucleotide triphosphate, 0.3 μM of each primer, and 5 μl of 10× PCR buffer (500 mM KCl and 200 mM Tris-HCl) and water to a total volume of 50 μl. Thermal cycling consisted of an initial 5-min denaturation step (95°C), followed by 35 cycles of denaturation (94°C, 1 min), annealing (51°C, 1 min), and elongation (72°C, 1 min), and a final elongation step (72°C, 5 min).

The C to T substitution in exon 7, resulting in a single amino acid substitution from Arg by Cys at codon 264, creates a recognition site for the SfòNI restriction enzyme. Thus, 15-μl aliquots of the PCR products were digested overnight with 5 units of SfòNI (New England Biolabs) and separated on a 2% agarose gel containing ethidium bromide, and the fragments were visualized by UV illumination. Fragment sizes were estimated by comparison to a 1-kb ladder on the same gel.

Statistical Analyses. Hardy-Weinberg equilibrium was tested by a χ² goodness of fit statistic. Associations between prostate cancer and genotype were assessed using unconditional logistic regression methods to obtain ORs and 95% CIs. In these models, age, BMI, and number of AR CAG repeats were treated as continuous variables; the polychotomous ESR1 XbaI and ESR1 PvuII genotypes were recoded as dummy variables. The
Arg264Cys CYP19 genotype was treated as a dichotomous variable based on the presence of a Cys allele. To evaluate the combined effect of AR CAG repeat length and other genotypes, subjects were categorized as carriers (<23, the median number of repeats in the control group) or noncarriers (≥23 repeats).

The effect of two genes on prostate cancer risk was assessed in three ways. First, we created dummy variables for each possible combination of the two genotypes. We then calculated ORs using the combination of genotypes associated with the lowest risk as determined by univariate analyses as the referent group. Next, we categorized each subject as to whether or not he possessed the lowest risk genotype. We then compared the reduction in risk of this genotype to all other possible genotypes. Finally, we formally tested for interactions between genotypes by including interaction terms in the logistic regression models. All statistical analyses were performed with the STATA statistical software package (Release 5.0; Stata Corp., College Station, TX). All Ps were calculated as two-sided statistics, and P < 0.05 was considered significant.

RESULTS

Cases were slightly younger (68.9 versus 73.6 years; P < 0.05) and had a greater BMI (28.2 versus 27.1 kg/m²; P < 0.05). Table 1 shows the distribution of the variant alleles for the ESR1 and aromatase genes as well as the categorization of cases and controls according to long or short AR (CAG) repeat. A long AR was defined as containing ≥23 CAG repeats, the median number of repeats in the control group. This number is consistent with the repeat lengths among healthy men published by other studies (1, 6). The allele frequencies of the ESR1 PvuII and the ESR1 XbaI polymorphisms were compatible with the control population in Hardy-Weinberg equilibrium.

As shown in Table 1, only the AR CAG repeat length differed significantly between cases and controls. An increase in prostate cancer risk was associated with decreased AR (CAG) repeat number when analyzed as a dichotomous variable (OR, 1.75; 95% CI, 0.1.05–2.94, adjusted for age and BMI) and as a continuous variable (OR, 1.14; 95% CI, 1.04–1.25, adjusted for age and BMI). Thus, there appears to be a 14% increase in prostate cancer risk for each fewer CAG repeat.

There also appears to be an increased risk of prostate cancer associated with the Arg264Cys substitution in the aromatase gene, although this result was of borderline significance (OR, 2.50; 95% CI, 0.99–6.28, adjusted for age and BMI). No other genotype was found to be significantly associated with prostate cancer risk, and no significant interaction between genotypes was found.

AR-CAGs and Estrogen Receptor RFLPs. We further investigated the effect of multiple genotypes on prostate cancer risk. Table 2 shows the distribution of genotypes in cases and controls for the combination AR CAG repeat length and the ESR1 XbaI and ESR1 PvuII genotypes. Using the long AR and the ESR1 XbaI +/+ genotype as the referent group, having either a short AR or an ESR1 XbaI+/- allele (but not both) increased an individual’s risk by about 2-fold, whereas having both a short AR and the absence of the restriction site increased an individual’s risk by about 5-fold. A similar but more moderate finding was evident for the ESR1 PvuII genotype.

In addition, compared with short AR carriage, a long AR together with the ESR1 XbaI +/+ genotype was more frequent among the controls (32%) than the cases (18%; OR, 0.39; 95% CI, 0.19–0.78, adjusted for age and BMI; data not shown). A reduction in risk was also found for the combined long AR and the ESR1 PvuII +/+ genotype, although the result was not significant.

AR-CAGs and Aromatase Arg264Cys Polymorphism. Similarly, we evaluated the effect of the combined AR CAG repeat length and the aromatase genotypes (Table 2). As shown, when compared with men with both a long AR and the aromatase C/C genotype, men with a short AR and the C/T aromatase genotype have over a 3-fold increase in risk. Moreover, compared with short AR carriage, a long AR and the aromatase C/C genotype was more frequent among the controls (55%) than among the cases (41%; OR, 0.51; 95% CI, 0.30–0.89; adjusted for age and BMI; data not shown).

DISCUSSION

Our results suggest that individual polymorphisms as well as combination of genotypes among the AR, ESR1, and aromatase genes contribute to prostate cancer susceptibility. In particular, consistent with several (1, 6, 7) but not all (9, 19)

### Table 1

<table>
<thead>
<tr>
<th>Allele</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR1 XbaI</td>
<td>+/+</td>
<td>34 (41%)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>+/-</td>
<td>38 (46%)</td>
<td>1.39 (0.81–2.39)</td>
<td>1.45 (0.82–2.56)</td>
</tr>
<tr>
<td></td>
<td>-/-</td>
<td>10 (12%)</td>
<td>1.22 (0.54–2.71)</td>
<td>1.65 (0.70–3.92)</td>
</tr>
<tr>
<td>ESR1 PvuII</td>
<td>+/+</td>
<td>26 (32%)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>+/-</td>
<td>34 (42%)</td>
<td>1.01 (0.57–1.83)</td>
<td>1.05 (0.57–1.96)</td>
</tr>
<tr>
<td></td>
<td>-/-</td>
<td>21 (26%)</td>
<td>1.60 (0.81–3.12)</td>
<td>1.85 (0.90–3.81)</td>
</tr>
<tr>
<td>Aromatase</td>
<td>C/C</td>
<td>79 (90%)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>9 (10%)</td>
<td>1.72 (0.72–4.08)</td>
<td>2.50 (0.99–6.28)</td>
</tr>
<tr>
<td>AR CAG</td>
<td>Long</td>
<td>36 (44%)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Short</td>
<td>45 (56%)</td>
<td>1.75 (1.05–2.94)</td>
<td>1.75 (1.02–3.03)</td>
</tr>
</tbody>
</table>

a Missing genotype data due to assay failure are as follows: ESR1 XbaI, 6 cases and 4 controls; ESR1 PvuII, 7 cases and 4 controls; AR CAG, 7 cases and 3 controls.
b Adjusted for age and BMI.
c P = 0.51.
d Bold type indicates P < 0.05.
previous studies, we found a protective effect for the long AR CAG trinucleotide repeat. We found that this protection increased for individuals with a long AR who are homozygous for the ESR1 XbaI RFLP, as indicated by a 30% decrease in the OR for the combined AR-ESR1 genotype compared with men who have the long AR genotype alone (OR = 0.57 for long AR alone compared with OR = 0.39 for long AR and ESR1 XbaI RFLP). According to our control group, approximately 32% of men possess a genotype that reduces their prostate cancer risk by >50%. The protection associated with a long AR was also modestly increased in men with the C/C aromatase genotype, as indicated by a 12% decrease in the OR for the combined AR-aromatase genotype compared with men with the long AR genotype alone (OR = 0.54 for long AR alone versus 0.57 for combined genotype). We also found an increase in risk associated with the aromatase CT genotype, although the result was of borderline significance. Nonetheless, it suggests a possible role for aromatase in prostate cancer. We have reported previously that in older men, bioavailable estrogen was 18.5% lower in carriers (P = 0.03) than in noncarriers of the Arg264Cys polymorphism (P = 0.005; Ref. 20). This observation, together with the observation that low estradiol levels may be predictive of prostate cancer risk (11), suggests that the mutation may exert its effect by reducing circulating estrogen levels throughout a man’s life.

Care must be taken when interpreting these data because the small number of subjects in certain genotype subgroups may make the magnitude of our risk estimates uncertain. Studies with a larger sample size are needed to clarify the complex interactions among the AR, ESR1, and aromatase genes. In addition, more complete demographic and exposure data, including measures of androgen and estrogen exposures, are needed to assess the interaction between these genes and androgen and estrogen exposures.

Another limitation of this study is that we have tested for only a subset of known polymorphisms in the three genes. Except for the androgen repeat, the functional significance of these polymorphisms remains unclear. It is uncertain whether these variants play a causal role in prostate cancer or whether they are merely in linkage disequilibrium with other functional variations within or flanking the genes under study.

We have not included an analysis of other genes involved in steroid metabolism such as 5α-reductase (SRD5A2), which plays a role in the conversion of testosterone to DHT and has been associated with prostate cancer (3). We also have not accounted for endogenous sex hormone levels. In addition, we did not screen controls for prostate cancer using a prostate-specific antigen test. Therefore, we cannot discount the possibility of men with undiagnosed prostate cancer among our controls. However, this would only bias our results toward the null.

A final limitation of this study concerns the different recruitment procedures for cases and controls. All cases were seen by a single physician at UPCL, whereas controls were recruited from the general population. Thus, it is possible that there is some selection bias in our sample. However, because subjects were unaware of their genotypes at enrollment and because it is unlikely that genotypes influenced an individual’s decision to seek care from a particular physician, selection bias should be minimal. In addition, the high percentage of prevalent cases (63%) may bias our results; however, 75% of the cases were enrolled in the study within 3 years of diagnosis; only 19 cases (21%) were enrolled more than 5 years after diagnosis. Because there is no known association of these polymorphisms with survival and because the Surveillance, Epidemiology and End Results 5-year survival rate for prostate cancer is 79% for Caucasians, survival bias introduced by including prevalent cases should not significantly affect our results.

### Table 2: Distribution of combined allelic variants among prostate cancer cases and controls

<table>
<thead>
<tr>
<th></th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)^b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ESR1 XbaI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long AR</td>
<td>+/+</td>
<td>15 (19%)</td>
<td>76 (32%)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>+/−</td>
<td>17 (22%)</td>
<td>45 (19%)</td>
<td>1.52 (0.71–3.26)^c</td>
</tr>
<tr>
<td></td>
<td>−/−</td>
<td>1 (1%)</td>
<td>15 (6%)</td>
<td></td>
</tr>
<tr>
<td>Short AR</td>
<td>+/+</td>
<td>14 (18%)</td>
<td>38 (16%)</td>
<td>1.87 (0.82–4.26)</td>
</tr>
<tr>
<td></td>
<td>+/−</td>
<td>21 (27%)</td>
<td>48 (21%)</td>
<td>2.21 (1.04–4.71)^d</td>
</tr>
<tr>
<td></td>
<td>−/−</td>
<td>9 (12%)</td>
<td>12 (5%)</td>
<td>3.80 (1.36–10.60)</td>
</tr>
<tr>
<td><strong>ESR1 PvuII</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long AR</td>
<td>+/+</td>
<td>13 (17%)</td>
<td>52 (22%)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>+/−</td>
<td>12 (16%)</td>
<td>60 (26%)</td>
<td>0.80 (0.34–1.91)</td>
</tr>
<tr>
<td></td>
<td>−/−</td>
<td>7 (9%)</td>
<td>24 (10%)</td>
<td>1.17 (0.41–3.30)</td>
</tr>
<tr>
<td>Short AR</td>
<td>+/+</td>
<td>11 (14%)</td>
<td>31 (13%)</td>
<td>1.42 (0.57–3.55)</td>
</tr>
<tr>
<td></td>
<td>+/−</td>
<td>21 (28%)</td>
<td>49 (21%)</td>
<td>1.71 (0.77–3.79)</td>
</tr>
<tr>
<td></td>
<td>−/−</td>
<td>12 (16%)</td>
<td>18 (8%)</td>
<td>2.67 (1.03–6.90)</td>
</tr>
<tr>
<td>Aromatase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long AR</td>
<td>C/C</td>
<td>33 (41%)</td>
<td>131 (55%)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>3 (4%)</td>
<td>7 (3%)</td>
<td>1.70 (0.42–6.94)</td>
</tr>
<tr>
<td>Short AR</td>
<td>C/C</td>
<td>40 (49%)</td>
<td>92 (39%)</td>
<td>1.73 (1.01–2.94)</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>5 (6%)</td>
<td>7 (3%)</td>
<td>2.84 (0.85–9.50)</td>
</tr>
</tbody>
</table>

^a Cells do not total 88 cases and 241 controls because of missing genotyping data for some subjects. Only subjects with both analyzed genotypes were included in each analysis.
^b Adjusted for age and BMI.
^c The +/− and −/− genotypes were combined because of the small number of cases in one of the cells.
^d Bold type indicates P < 0.05.
A complete understanding of the role of sex steroid hormones in prostate tumorigenesis requires evaluation of both the genes associated with these hormones as well as the extent to which estrogen and androgen exposure modify the associations of these genes with prostate cancer risk. This understanding can only emerge if research extends beyond single-gene studies to gene-gene and gene-environment interaction studies. To our knowledge, this is among the first studies to explore sex steroid metabolism genes and prostate cancer risk in a multigene model. Such an approach will allow a more precise evaluation of the risks associated with individual genotypes and provide insight into the role of sex steroids in prostate tumorigenesis. Additional studies with larger groups of subjects are needed to confirm these findings and may ultimately help to establish a genetic risk factor profile for prostate cancer.

REFERENCES


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