Increased Risk of Prostate Cancer and Benign Prostatic Hyperplasia Associated with a CYP17 Gene Polymorphism with a Gene Dosage Effect

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ABSTRACT

The CYP17 gene (CYP17) codes for the cytochrome P450c17α enzyme, which mediates two key steps in the sex steroid synthesis. There is a polymorphism (a T-to-C substitution) in the 5′-untranslated region, which may influence the transcription level of CYP17 mRNA. There is a continuing controversy as to whether the variant allele is associated with a subset of breast cancer or polycystic ovary syndrome. In prostate cancer research, there are contradictory data concerning the CYP17 risk allele. We explored the association between CYP17 polymorphism and a risk of prostate cancer or benign prostatic hyperplasia (BPH) in a Japanese population. This study included 252 prostate cancer patients, 202 BPH patients, and 131 male controls. A 451-bp fragment encompassing the polymorphic site was amplified by PCR, treated with restriction enzyme MspI, and electrophoresed on an agarose gel. The MspI-un digested allele with the published sequence and the MspI-digested variant allele were designated as A1 and A2, respectively. There was a significant difference (P < 0.05) in the genotypes between prostate cancer patients and male controls, and between BPH patients and male controls. Men with the A1/A1 CYP17 genotype had an increased risk of prostate cancer [odds ratio (OR), 2.57; 95% confidence interval (CI) = 1.39–4.78] and BPH (OR, 2.44; 95% CI = 1.26–4.72) compared with those with the A2/A2 genotype. Men with the A1/A2 genotype had an intermediate risk of prostate cancer (OR, 1.45; 95% CI = 0.84–2.54) and BPH (OR, 1.60; 95% CI = 0.89–2.87) compared with those with the A2/A2 genotype. The trend of an increasing risk of prostate cancer and BPH with an increasing number of the A1 allele was statistically significant (prostate cancer versus male control, P = 0.003; OR, 1.57; 95% CI = 1.16–2.12; BPH versus male control, P = 0.008; OR, 1.55; 95% CI = 1.12–2.13). There was no significant association between the CYP17 genotype and the tumor status (grade and stage) of prostate cancer. Our results suggest that the A1 allele of the CYP17 polymorphism is associated with an increased risk of prostate cancer and BPH, with a gene dosage effect. However, the CYP17 genotype does not seem to influence the disease status in prostate cancer.

INTRODUCTION

Ethnic and geographic differences in clinical prostate cancer incidence have been well documented (1–3). The incidence in African-Americans and that in American Whites is at least 10 times and 5 times higher, respectively, than in the Japanese (2, 3). However, African-Americans and that in American Whites is at least 10 times and 5 times higher, respectively, than in the Japanese (2, 3). However, the presence of the A2 allele may increase the risk of prostate cancer (18, 20), whereas from the United States and Austria, reported that the presence of the A2 allele may increase the risk of prostate cancer (18, 20), whereas another study from Sweden claimed that the A1/A1 genotype may significantly increase the risk (19).

In this study, we analyzed the CYP17 genotype in a native Japanese male population who are considered to be less influenced by environmental factors for prostate cancer than those in Western countries (3).

MATERIALS AND METHODS

Subjects. A total of 585 subjects consisting of 252 prostate cancer patients, 202 BPH patients, and 131 male controls treated at Akita University Medical Center and related community hospitals were enrolled in this study. Most subjects were previously enrolled in a study of vitamin D receptor gene polymorphisms and prostate cancer risk (21). All of the prostate cancer patients were diagnosed histologically with specimens obtained from transrectal needle
biopsy or transurethral resection of the prostate for voiding symptoms. All of the BPH patients had various degrees of lower urinary tract symptoms and showed an apparent prostatic enlargement by digital rectal examination. The PSA levels were measured in all of the BPH patients, and men with elevated PSA levels (≥4.0 ng/ml; the Tandem-R assay; Hybritech Inc., San Diego, CA) were proved not to have prostate cancer by transrectal biopsies. Serum PSA was measured using the Tandem-R assay in most cases. When serum PSA was measured by kits other than the Tandem-R, the measured PSA level was adjusted to that of the Tandem-R assay using a formula published elsewhere (22). The male control group consisted 131 volunteers >60 years old who were selected mainly from among the patients admitted because of nonurological diseases and showed no signs of prostate cancer and no prostatic enlargement by digital rectal examination. They all were tested for serum PSA levels (the Tandem-R assay), and men with abnormal PSA levels were omitted from the normal controls or received further examination, including prostate biopsy, to rule out any prostatic disease conditions.

Pathological grading of the prostate cancer was determined according to the General Rule for Clinical and Pathological Studies on Prostate Cancer by the Japanese Urological Association and the Japanese Society of Pathology, which is based on the WHO criteria and the Gleason pattern (23). Well, moderately, and poorly differentiated carcinoma generally corresponds to Gleason patterns 1–2, 3–4, and 5, respectively (23, 24). In 26 patients, the final pathological grade was not determined because a different or inadequate grading system was used. The clinical or pathological stage was determined by review of the medical records and classified using the Tumor-Node-Metastasis system (25). Prostate cancer was classified into the localized group consisting of T1-4N0M0 (stage A, B, or C by the Whitmore-Jewett system) tumors and the metastatic group consisting of T1–4N+M0–1 or T1–4N0–1M1 (stage D by the Whitmore-Jewett system) tumors. In 11 patients, no definite clinical stage was determined due to inadequate information.

**CYP17 Genotyping Analysis.** DNA was extracted from blood samples collected from each patient using a QIAamp Blood Kit (QiAGEN) or by the standard method with proteinase K digestion followed by phenol-chloroform extraction. The 421-bp fragment encompassing the polymorphic site in the promoter region of CYP17 was amplified by PCR using primers CYP17-F1: 5’-CCATTGCACCTGGAGT and CYP17-R2: 5’-GACAGGAG-GCTCTTGGGGTA. PCR was carried out in a 25-μl aliquot containing 50 ng of genomic DNA, 50 pmol of each primer, 125 μM dNTPs, 1 unit of Taq polymerase (Ampli-Taq Gold DNA polymerase, PE Applied Biosystems), and 1/× reaction buffer supplied by the manufacturer (PE Applied Biosystems). PCR amplification conditions were 10 min of initial denaturation and activation of Ampli-Taq Gold DNA polymerase at 94°C, followed by 35–40 cycles of 30 sec at 94°C, 30 s at 55°C, and 90 s at 72°C, followed by 7 min of a final extension at 72°C. The PCR products were digested overnight with 10 units of MspI (MspI1; New England Biolabs, Inc., Beverly, MA) and electrophoresed on 2.0% agarose gels. When the MspI1 site was present, the 421-bp PCR fragment was divided into 130 and 291 bp by the endonuclease digestion. The genotypes were designated as “A1” when the restriction site was absent, and as “A2” when the restriction site was present, as defined in the other studies (6). Genotyping was performed and checked by laboratory personnel (T. H. and Z. L.) unaware of the case-control status.

**Statistical Methods.** All data were entered into an access database (FileMakerPro, Version 4.0, Claris Co.) and analyzed by Excel 98 and SPSS (Version 6.1, SPSS, Inc.) software. Differences in genotype frequencies and Hardy-Weinberg equilibrium analysis between the three groups and between the subgroups of prostate cancer patients were evaluated by a two-sided 2 × 3 contingency table analysis. Associations between CYP17 genotypes and the development of prostate cancer and BPH were assessed by ORs and 95% CIs. A multivariate logistic regression analysis was performed with the inclusion of a factor of age. In addition, the trend across categories of A2/A2 (A2 homozygote), A1/A2 (heterozygote), and A1/A1 (A1 homozygote) was tested in a logistic regression model by using a variable with the values 0, 1, and 2, respectively. A probability <0.05 was required for statistical significance.

**RESULTS**

The present study included 252 cases of histologically confirmed prostate cancer, 202 cases of BPH with lower urinary tract symptoms, and 131 male controls. The mean ages (±SD) of prostate cancer patients, BPH patients, and male controls were 72.2 ± 8.5, 70.5 ± 9.4, and 75.3 ± 7.2 years, respectively.

Frequencies of the CYP17 genotype in the three groups (prostate cancer, BPH, and male control) are shown in Table 1. The CYP17 allelic distribution in each group was in the Hardy-Weinberg equilibrium (P > 0.05; data not shown). Statistical analyses of the genotype prevalence showed significant differences between prostate cancer patients and the male controls (P = 0.022), and between BPH patients and the male controls (P = 0.018; Table 1). Genotype analysis indicated that the presence of the A1 allele might increase the risk of prostate cancer and BPH, when non-adjusted and age-adjusted ORs were calculated against the A2/A2 genotype (Table 1). Age-adjusted logistic analysis showed that men with the A1/A1 CYP17 genotype had an increased risk of prostate cancer (aOR, 2.57; 95% CI = 1.39–4.78) and BPH (aOR, 2.44; 95% CI = 1.26–4.72) compared with those with the A2/A2 genotype. Although not statistically significant, males heterozygous for the A1 allele also seemed to have an intermediate increased risk of prostate cancer (aOR, 1.45; 95% CI = 0.84–2.54) and BPH (aOR, 1.60; 95% CI = 0.89–2.87) compared with males with the A2/A2 genotype. In addition, using an A2/A2 genotype, an A1/A2 genotype, and an A1/A1 genotype as the values 0, 1, and 2, respectively, we found a statistical significance in a logistic regression model for the risk of prostate cancer and BPH in correlation with an increasing number of the A1 allele (prostate cancer versus male control, P = 0.003; OR, 1.57; 95% CI = 1.16–2.12; BPH versus male control, P = 0.008; OR, 1.55; 95% CI = 1.12–2.13). The results indicate that the presence of the A1 allele may increase the risk of prostate cancer and BPH with a gene dosage effect.

Next, the prostate cancer group was analyzed according to tumor grade and tumor stage (Table 2). In the tumor grade, no significant difference in genotype frequency was found. Adjusted ORs against the male control group did not show consistent results (Table 2). In the tumor stage, there was a significant difference in the genotype prevalence between metastatic prostate cancer patients and the male controls (P = 0.0224). However, no significant difference was found between the localized prostate cancer patients and the metastatic prostate cancer patients (Table 2). These data suggested that the CYP17 polymorphism had no significant association with the disease status of prostate cancer.

Finally, we investigated whether the CYP17 polymorphism influences the age of onset of prostate cancer. On the basis of median age, the prostate cancer patients were divided into two groups (Table 3). Although an A1/A1 genotype is more frequently observed in prostate cancer patients diagnosed at ≥73 years old, no significant difference in the genotype frequency was found when compared with the prostate cancer patients diagnosed at <73 (P = 0.238; Table 3).

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**Table 1** CYP17 genotype frequencies [%] for all cases and (crude) ORs and aORs against male controls

<table>
<thead>
<tr>
<th>Study group</th>
<th>Genotype</th>
<th>n</th>
<th>A1/A1</th>
<th>A1/A2</th>
<th>A2/A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate cancer</td>
<td></td>
<td>252</td>
<td>95 (38)</td>
<td>111 (44)</td>
<td>46 (18)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>aOR (95% CI)</td>
<td>2.55 (1.25–4.06)</td>
<td>1.40 (0.82–2.39)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>aOR (95% CI)</td>
<td>2.57 (1.39–4.78)</td>
<td>1.45 (0.84–2.51)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPH</td>
<td></td>
<td>202</td>
<td>74 (37)</td>
<td>95 (47)</td>
<td>33 (16)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>aOR (95% CI)</td>
<td>2.45 (1.31–4.57)</td>
<td>1.67 (0.94–2.96)</td>
<td>1.00</td>
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</tr>
<tr>
<td>aOR (95% CI)</td>
<td>2.44 (1.26–4.72)</td>
<td>1.60 (0.89–2.87)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male controls (reference)</td>
<td></td>
<td>131</td>
<td>33 (25)</td>
<td>62 (47)</td>
<td>36 (28)</td>
</tr>
</tbody>
</table>

* Prostate cancer versus male control, P = 0.022.
* BPH versus male control, P = 0.018.
PROSTATE CANCER AND CYP17 GENE POLYMORPHISM

DISCUSSION

Although conflicting results have been documented (7, 14–17), the presence of the CYP17 A2 allele has been described to be an independent risk factor for a subset of breast cancer (8–10). However, the present results indicated that the presence of the A1 allele significantly increases the risk of prostate cancer and BPH with a gene dosage effect. Because cytochrome P450c17 is involved in the production of both androgens and estrogens (4, 5). It has been well accepted that most prostate cancers are androgen-dependent and that an androgen defect prevents normal prostate growth, whereas most breast cancers are estrogen-dependent and estrogens have promoting effects on breast carcinogenesis (4, 26). Consequently, the present results, together with those of the previous documents, could simply suggest that the A1 allele has a more androgenic effect on men and the A2 allele conversely has an estrogenic effect on women. In support of this view, a recent study by Makridakis et al. (27) showed that the CYP17 A1 is significantly associated with higher levels of serum androgen metabolite (androstanediol glucronide) with a gene dosage effect.

On the other hand, three previous studies reported conflicting results on the CYP17 genotype in prostate cancer patients (18–20). One from the United States indicated an increased risk of prostate cancer in the presence of the A2 allele (OR, 1.7; 95% CI = 1.0–3.00; Ref. 18), whereas another from Sweden claimed that men with the A1/A1 genotype had an increased risk (OR, 1.61; 95% CI = 1.02–2.53; Ref. 19). More recently, Gsur et al. (20) reported an increased risk in men with the A2/A2 genotype in a small cohort of prostate cancer patients in Austria. The conclusion in the United States study seems to remain unchanged even when the analysis is restricted to a Caucasian population (18). Although the exact reason for these contradictory results remains unclear, the identical CYP genotype may play either a protective or a promoting role in prostate carcinogenesis given different environmental and/or genetic backgrounds. In support of this view, studies showed that women with an A2/A2 genotype had higher levels of estradiol and estrone (12) and that the A2 allele was associated with significantly higher levels of estradiol (11), whereas the A2 allele was associated with phenotypic modification of a familial form of polycystic ovaries whose sex steroid hormone balance has been shown to be more androgen-dominant than normal (6, 13, 28). These documents suggest that even women with the identical CYP17 genotype have much different phenotypes as far as hormone-dependent diseases are concerned. Because of the multiple enzymatic processes required for steroid hormone syntheses, the one enzymatically hyperactive step may lead to either a hyperestrogenic or a hyperandrogenic hormonal balance according to the difference in activities of the other enzymatic processes which follow.

Considering the striking differences in the age-adjusted incidence of prostate cancer between different racial groups (1–3), we reviewed the frequency of the CYP17 genotype in normal control subjects in the eight previous studies with various clear ethnic backgrounds (Table 4; Refs. 7, 9, 14, 18, 19, 29–31). The CYP17 genotype frequency in the male control group in the present study is comparable to those of Japanese controls in two other studies (A1/A1 = 27%, A1/A2 = 47%, A2/A2 = 26%; P = 0.157. Table 4; Refs. 29, 31). Asians, including Japanese, who have the lowest incidence of clinical prostate cancer, seem to have a higher frequency of the A2/A2 genotype and a lower frequency of the A1/A1 genotype than American Blacks (African-American), American Whites, and Scandinavians. For example, the difference in these genotype frequencies are statistically significant between Japanese and American Blacks (P < 0.0001), and between

<table>
<thead>
<tr>
<th>Study group</th>
<th>n</th>
<th>A1/A1</th>
<th>A1/A2</th>
<th>A2/A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male control</td>
<td>80</td>
<td>21 (26)</td>
<td>37 (46)</td>
<td>22 (28)</td>
</tr>
<tr>
<td>aOR (95% CI)</td>
<td>2.95 (1.32–6.63)</td>
<td>1.66 (0.79–3.49)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>&lt;73 years old</td>
<td>127</td>
<td>42 (33)</td>
<td>58 (46)</td>
<td>27 (21)</td>
</tr>
<tr>
<td>Male control</td>
<td>51</td>
<td>12 (23)</td>
<td>25 (49)</td>
<td>14 (27)</td>
</tr>
<tr>
<td>aOR (95% CI)</td>
<td>2.28 (0.84–5.97)</td>
<td>1.22 (0.52–2.84)</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

* The tumor grade was determined according to the General Rule for Clinical and Pathological Studies on Prostate Cancer by the Japanese Urological Association and the Japanese Society of Pathology (23). Well-, moderately, and poorly differentiated carcinoma generally corresponds to Gleason patterns 1–2, 3–4, and 5, respectively. P = 0.379 by χ² test.

* Localized and metastatic tumor corresponds to stage A–C and stage D (the Whitmore-Jewett system), respectively. P = 0.524 by χ² test.
Japanese and American whites (P < 0.0001). No significant difference was observed between American Blacks and American whites (P = 0.260). On the other hand, the higher frequency of the A1 allele in American Blacks or American Whites than in Asians does not reflect the ethnic difference in breast cancer incidence (1). Presumably, the distinct biological condition caused by the CYP17 genotype will be among various genetic, dietary, and environmental factors regulating hormonal and nonhormonal conditions in the development of prostate cancer and breast cancer.

We did not find any significant association between the CYP17 genotype and disease status in prostate cancer patients. However, the frequency of the A1/A1 genotype seemed to be higher in the patients with metastatic cancer, and the lack of significant association might be due to the relatively small number in each subgroup. As for age at diagnosis, the A1/A1 genotype seemed to be over-represented in the patients diagnosed at the age of ≥73 years. It would be interesting to know whether the CYP17 genotype influences the tissue or serum androgen levels more significantly in an older population than in a younger one.

Our results indicated that the CYP17 genotype is associated with the development of BPH as well as that of prostate cancer to almost the same degree. This connection is in line with the observation that a subset of BPH has a genetic transmission (32). It has been reported that the volume of BPH is positively correlated with serum testosterone (33), therefore indicating a rather small number in each subgroup. For age at diagnosis, the A1/A1 genotype seemed to be over-represented in the patients diagnosed at the age of ≥73 years. It would be interesting to know whether the CYP17 genotype influences the tissue or serum androgen levels more significantly in an older population than in a younger one.

In conclusion, the present study indicated that the CYP17 gene polymorphism may be significantly associated with a risk of prostate cancer, and BPH may be significantly associated with a gene dosage effect. However, the genotype has no significant influence on the disease status in prostate cancer patients.

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