CYP17 Promoter Variant Associated with Prostate Cancer Aggressiveness in African Americans


Abstract
Androgens play an important role in the etiology of prostate cancer. The CYP17 gene encodes the cytochrome P450c17 enzyme, which is the rate-limiting enzyme in androgen biosynthesis. A T to C polymorphism in the 5′ promoter region has recently been associated with prostate cancer. However, contradictory data exists concerning the risk allele. To investigate further the involvement of the CYP17 variant with prostate cancer, we typed the polymorphism in three different populations and evaluated its association with prostate cancer and clinical presentation in African Americans. We genotyped the CYP17 polymorphism in Nigerian (n = 56), European-American (n = 74), and African-American (n = 111) healthy male volunteers, along with African-American men affected with prostate cancer (n = 71), using pyrosequencing. Genotype and allele frequencies did not differ significantly across the different control populations. African-American men with the CC CYP17 genotype had an increased risk of prostate cancer (odds ratio, 2.8; 95% confidence interval, 1.0–7.4) compared with those with the TT genotype. A similar trend was observed between the homozygous variant genotype in African-American prostate cancer patients and clinical presentation. The CC genotype was significantly associated with higher grade and stage of prostate cancer (odds ratio, 7.1; 95% confidence interval, 1.4–36.1). The risk did not differ significantly by family history or age. Our results suggest that the C allele of the CYP17 polymorphism is significantly associated with increased prostate cancer risk and clinically advanced disease in African Americans.

Introduction
The incidence of prostate cancer varies significantly across ethnic groups, with African-American men having the highest rates worldwide (1–3). African Americans also appear to present more commonly at an advanced stage with aggressive histology and increased cancer-related mortality (4). Although the more advanced cancers in African Americans may be confounded by social class and access to health care, there is a critical need to explore the etiological pathways (genetic and environmental factors) that contribute to this disparity.

Because the prostate is an androgen-regulated organ, androgens may play a major role in the etiology of prostate cancer. The CYP17 gene encodes the cytochrome P450c17a enzyme that catalyzes two key steps in the steroid biosynthesis pathway. The first step in the biosynthesis pathway involves the conversion of cholesterol to pregnenolone by CYP11A1. Subsequently, pregnenolone is converted to 17α-hydroxypregnenolone and then to dehydroepiandrosterone, a precursor of testosterone, by the P450c17a enzyme. A T to C polymorphism in the 5′ promoter region of the CYP17 gene has been described (5) which has been associated with increased risk for early-onset familial breast cancer (6–8). Also denoted as the A2 allele, this single nucleotide polymorphism may create an Sp1-type promoter site. However recent electromobility shift assays have not confirmed Sp1 binding (9).

The CYP17 gene is a likely candidate for prostate cancer, which, like breast cancer, is hormone-related. To date, four studies have shown an association of the CYP17 gene and prostate cancer risk, however they have been contradictory in terms of which allele is associated. Two studies, from Sweden and Japan, suggested that the T (A1) allele was associated with increased risk for prostate cancer (10, 11), whereas independent studies from the United States and Austria reported that the C (A2) allele confers greater risk (12, 13). It is interesting to note that the C (A2) allele is more prevalent among Asian populations (8, 11, 12, 14). However, CYP17 allele and genotype frequencies do not seem to differ between African Americans and European Americans (8, 12, 15), unlike several other candidate genes for prostate cancer which exhibit striking allele frequency differences that parallel differences in prostate cancer incidence (16–18). To date, no allele and genotype frequency data exists on clinically evaluated indigenous Africans and African-American prostate cancer patients. The purpose of this study was to determine whether differences exist in CYP17 genotype frequencies between African, African-American, and European-American populations and whether the CYP17 polymorphism was associated with prostate cancer risk in African Americans.
Subjects and Methods
Unrelated men were enrolled from three sites for a population-based study of genetic risk factors for prostate cancer. The Howard University Institutional Review Board approved the study, and written consent was obtained from all subjects. All prostate cancer cases were between 40 and 79 years of age and were diagnosed with prostate cancer within the last 2 years. One hundred and eighty-two African Americans (71 prostate cancer patients and 111 healthy male controls) were enrolled from the Washington, DC area. African-American men with prostate cancer were recruited from the Division of Urology at the Howard University Hospital and/or prostate cancer screening at the Howard University Cancer Center. The response rate among the African-American cases was 92%. Healthy African-American male volunteers were enrolled among individuals undergoing regular physical exams at the Division of Urology at Howard University Hospital and/or men participating in screening programs for prostate cancer at the Howard University Cancer Center. The response rate for the African American controls was 90%. The mean age of prostate cancer patients was 66.3 ± 3.3 years and, among controls, 57.3 ± 0.8 years. Clinical characteristics including Gleason grade, PSA, and family history were obtained from medical records.

Seventy-four European-American healthy male volunteers (mean age, 58.5 ± 2.9 years) were enrolled through various prostate cancer-screening programs in Baltimore, MD, sponsored by the Johns Hopkins Cancer Center. Fifty-six healthy volunteers (mean age, 51.9 ± 1.6 years) belonging to the Edo ethnic group were enrolled in Benin City, Nigeria. Nigerian males were enrolled through a community-based study of risk factors for prostate cancer during the summer of 2000 in collaboration with the University of Benin Teaching Hospital in Benin City, Nigeria. The response rate among the Nigerian controls was 85%. Blood samples were collected from each subject. Ethnicity for all groups was self-reported, and individuals of mixed ancestry were not excluded. All healthy volunteers had PSA levels <4.0 ng/ml and normal digital rectal exams.

Genotyping. The genomic DNA was obtained from isolated lymphocytes using cell lysis, proteinase K-treatment, protein precipitation, and DNA precipitation. Genotyping of the polymorphism in the promoter region of CYP17 gene was performed using Pyrosequencing (19, 20). The primers for the CYP17 polymorphism were designed from the published promoter sequence (National Center for Biotechnology Information accession no. M63871). A 167-bp fragment was amplified in a 50-μl PCR reaction containing 30 ng of genomic DNA, 20 pmol of forward unlabeled 5'-TTC CAC AAG GCA AGA GAT AAC-3' and a reverse biotin-labeled primer (b indicates biotin) 5'-b-GGT CAG CAA GAG ACC CA-3' and 1× PCR buffer II (Perkin-Elmer), 2 mM MgCl2, 0.2 mM dNTP, and AmpliTaq gold DNA polymerase. PCR reactions were performed for 50 cycles; denaturation at 95°C for 30 s, annealing at 54°C for 20 s, and extension at 72°C for 30 s. Biotinylated single-stranded DNA fragments were generated by mixing the PCR product with streptavidin-coated paramagnetic beads (Dynalbeads M280; Dynal, Norway). The PCR products and Dynal beads were mixed with high-salt buffer [0.1% Tween 20, 2 mM NaCl, 0.5 mM EDTA, and 10 mM Tris-HCl (pH 7.6)], incubated for 15 min at 65°C, and spun at 1400 rpm in a thermomixer (Eppendorf). Then the material was resuspended in 0.5 M NaOH and incubated for 2 min to separate DNA stands. Dynal beads containing the immobilized strand were washed in 1× annealing buffer (20 mM Tris-Acetate and 5 mM MgAc2) and resuspended in 45 μl of 1× annealing buffer and 10 pmol of sequencing primer. Then the mixture was incubated at 80°C for 2 min and then cooled to room temperature. Throughout the sample preparation steps, the immobilized fragments coupled to Dynal beads were processed using a manifold device (PSQ 96 Sample Preparation Tool; Pyrosequencing AB, Uppsala, Sweden). An automated pyrosequencing instrument, PSQ96 (Pyrosequencing AB) was used to perform genotyping. The reaction was carried out at 25°C with the sequencing primer 5'-GTC AGG CAA GAT AGA CA-3' added to the reaction. The reaction mixture also contained DNA polymerase (exonuclease-deficient), 40 μM appyrase, 4 μg of purified luciferase/ml, 15 μU of recombinant ATP sulfurylase, 0.1 μM Tris-acetate (pH 7.5), 0.5 mM EDTA, 5 mM magnesium acetate, 0.1% BSA, 1 mM DTT, 10 μM adenosine 5'-phosphosulfate, 0.4 mg of poly(vinylpyrrolidone)/ml, and 100 μg of d-luciferin/ml. The mini-sequencing protocol was carried out by stepwise elongation of the primer strand upon sequential addition of 40 pmol of the different deoxynucleoside triphosphates and the simultaneous degradation of nucleotides by appyrase. As the sequencing reaction continued, the cDNA strand extended and the DNA sequence was determined from the single peaks in the pyrogram using Pyrosequencing software (Pyrosequencing, AB). All samples were genotyped twice directly from genomic DNA. Control DNAs included a known wild-type (TT), a heterozygous mutant (CT), and homozygous mutant (CC) variant samples. The control DNAs were confirmed by direct DNA sequencing using an ABI 377 DNA sequencer (ABI, Foster City, CA). Genotypes from the repeat assay were 100% concordant with initial genotypes.

Statistical Analysis. Genotype and allele frequencies were calculated for each population. Hardy-Weinberg equilibrium analysis of each group was evaluated by contingency table analysis. The SAS Version 6.12 computer program (SAS Institute, Inc., Cary, NC.) was used to compute the two-sided Pearson χ² test. ORs and P values were determined from a comparison of genotypes in Nigerians and European Americans versus African-American healthy controls. Genotypes were also compared between African-American prostate cancer patients and healthy controls. Regression analyses were used to assess whether age at diagnosis and family history modified the relationship between CYP17 and prostate cancer risk. Regression analyses were also performed to compare grade/stage among prostate cancer patients. Grade/stage was defined as low (T1a–T1c and/or Gleason grade ≤7) or high (T2–T4 or N+ or M+ or T1b–T4 and/or Gleason grade ≥8; see Refs. 16 and 17). For the analyses of prostate cancer patients, the regression model controlled for age at diagnosis, PSA (total), and family history (affected first-degree relative).

Results
Fig. 1 shows examples of pyrograms representing the CYP17 genotypes. The C (A2) allele frequency was 30% among the African-American controls. CYP17 genotypes frequencies in the three normal control populations are shown in Table 1.
European Americans and African Americans were consistent population with African-Americans. European Americans (Table 1). Similarities when African Americans were compared with Nigerians or tests revealed no significant differences in genotype frequencies in each population were in Hardy-Weinberg equilibrium. Genotypes in each population were in Hardy-Weinberg equilibrium (P > 0.05; data not shown). Genotype frequencies for European Americans and African Americans were consistent with previous published frequencies (8, 12, 15). Pearson χ² tests revealed no significant differences in genotype frequencies when African Americans were compared with Nigerians or European Americans (Table 1).

The presence of at least one copy of the C (A2) allele was significantly higher among African-American prostate cancer cases (69%), than among controls (50%); P = 0.01 (Table 2). An increased risk for prostate cancer was observed for individuals with at least one copy of the C allele (OR, 2.2; 95% CI, 1.2–4.1). The risk for prostate cancer among heterozygous individuals was intermediate to those who were homozygous for the C allele (ORs of 2.0 and 2.8, respectively; Table 2). This suggests a gene-dosage effect where the risk for prostate cancer increases with number of C allele copies. Additional analyses were performed to examine whether a relationship exists between CYP17 genotype and age at diagnosis (<66 years of age versus >66 years of age) and family history of prostate cancer. No relationship was observed between the CYP17 polymorphism and age of onset in African Americans (P = 0.71). Similarly, no association was observed with family history (P = 0.65; data not shown).

Table 1 CYP17 genotype frequencies in healthy controls from three populations

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>Genotype n (%)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigerians</td>
<td>56</td>
<td>TT 24 (43%)</td>
<td>CC 5 (9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 27 (48%)</td>
<td></td>
</tr>
<tr>
<td>European Americans</td>
<td>74</td>
<td>TT 28 (38%)</td>
<td>CC 8 (11%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 38 (51%)</td>
<td></td>
</tr>
<tr>
<td>African Americans</td>
<td>111</td>
<td>TT 55 (50%)</td>
<td>CC 46 (41%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 46 (41%)</td>
<td></td>
</tr>
</tbody>
</table>

*Two-sided P from Pearson χ² tests comparing genotype frequencies in each population with African-Americans.

Table 2 CYP17 genotype frequencies in African-American prostate cancer cases and healthy controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases</th>
<th>Controls</th>
<th>OR 95% CI</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>22 (31%)</td>
<td>55 (50%)</td>
<td>1.0 (Ref.)</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>38 (54%)</td>
<td>46 (41%)</td>
<td>2.0</td>
<td>1.0–3.9</td>
</tr>
<tr>
<td>CC</td>
<td>11 (15%)</td>
<td>10 (9%)</td>
<td>2.8</td>
<td>1.0–7.4</td>
</tr>
<tr>
<td>TC + CC</td>
<td>49 (69%)</td>
<td>56 (50%)</td>
<td>2.2</td>
<td>1.2–4.1</td>
</tr>
</tbody>
</table>

*Two-sided P from Pearson χ² tests.

stratification of the 71 African-American prostate cancer cases by grade/stage is shown in Table 3. Among men heterozygous for the CYP17 polymorphism, 58% (20 of 34) presented with high grade/stage prostate cancer compared with 49% (18 of 37) with low grade/stage. For the CC genotype, we observed 24% (8 of 37) of men with high grade/stage compared with only 8% (3 of 37) of men with low grade/stage. ORs comparing TC genotype to TT between low and high grade/stage disease suggests an increased risk of presenting with high grade/stage (OR, 2.9; 95% CI, 1.0–9.2). A stronger association was observed when comparing CC genotype to TT (OR of 7.1; 95% CI, 1.4–36.1; P = 0.01). Because of the small number of samples in certain categories, the 95% CIs for the ORs are large.

Discussion

Prostate cancer development is influenced by androgens, which are regulated by genetic and environmental factors. Environmental factors such as dietary fat intake play a role in the development of prostate cancer (21). CYP17 is an ideal candidate for prostate cancer because it is directly involved in the production of testosterone. In this study, we examined the role a CYP17 promoter polymorphism plays in prostate cancer among African Americans. The CYP17 polymorphism is in the promoter region and may create an additional Sp1-type site (CCACC) 34 bases upstream of the initiation of translation and downstream from the transcription start site. The presence of this variant may result in increased production of testosterone attributable to an increased rate of transcription (5). This would increase the bioavailability of testosterone for conversion to dihydrotestosterone, ultimately affecting prostate cell growth. Kristensen et al. (9) demonstrated that the CYP17 promoter polymorphism does not create an Sp1 binding site, but suggested that other transcription factors might interact with this polymorphism. However, it is possible that in vivo conditions may favor Sp1 binding to the variant Sp1 site in the prostate, thus bringing about increased transcription of the CYP17 gene.
Two important risk factors for prostate cancer are age and ethnicity. The CYP17 polymorphism was significantly associated with disease and aggressiveness, and its effect did not seem to be modified by age at diagnosis or family history. The CYP17 association with prostate cancer among African Americans may also explain the higher circulating testosterone concentration in African-American men when compared with other ethnic groups (18, 22), because the gene is directly involved in testosterone biosynthesis. It is critical to determine whether differences exist in allele and genotype frequency between populations, because this may help explain some of the differences between populations in prostate cancer prevalence. In this study we showed that the frequency of the CYP17 variant was consistent across control populations consisting of Nigerians, African Americans, and European Americans. This is an important observation, because it suggests that CYP17 may not account for all of the differences in testosterone levels or prostate cancer incidence between populations. Also, allele frequency differences between populations can be a confounder in association studies if not controlled for (23–25), especially in genetic studies on the African-American population, which is highly heterogeneous because of its African ancestry and recent admixture with European Americans.

This is the first study that investigated the relationship between the CYP17 polymorphism and prostate cancer in African Americans. Although the observed association of the CYP17 C (A2) allele with prostate cancer is consistent with previous studies on Austrians (13) and European Americans from South Carolina (12), no association has previously been shown with clinical presentation of the disease. Also, conflicting results have been published as to the associated allele. The T (A1) allele was associated with increased prostate cancer risk in the Japanese (11) and the Swedish populations (10). It has been suggested that the CYP17 genotypes may play either a protective or a promoting role in prostate cancer progression, given different environmental and/or genetic backgrounds (11). Different populations exhibit different environmental factors (diet, lifestyle, etc.), levels of genetic variation, and patterns of genotypic/environment interactions. All of these factors play a role in prostate cancer progression. This may be one of several reasons for the contradictory results. Another reason could be that the T to C promoter polymorphism within the CYP17 gene is in moderate (or incomplete) linkage disequilibrium with the actual disease-related polymorphism. It is likely that the disease allele is older in age than the promoter polymorphism because both promoter alleles (T and C) have been found to be associated with the disease in vastly different populations. Events such as recombination could place the disease allele on different CYP17 haplotypic backgrounds, and so single marker studies would produce conflicting results. This could be evaluated by screening the CYP17 gene for more polymorphisms, estimating the level of linkage disequilibrium, and performing haplotypic (multisite) association analyses on prostate cancer in different populations.

In summary, a common CYP17 variant was associated with increased risk of prostate cancer in African-American men. Comparison of genotypes revealed a significantly higher risk among individuals homozygous for the C allele for developing high grade/stage prostate cancer. In fact, African-American patients with the CC genotype were seven times more likely to present with more aggressive disease. Because the sample sizes were moderate for the African-American samples, the results should be interpreted with caution until larger studies further evaluate the polymorphism. Future research on the role polymorphisms within the CYP17 gene play in prostate cancer and clinical presentation may demonstrate a need for genetic screening, possibly providing better treatment opportunities or prevention strategies. However, other genes have been identified that also are involved in prostate cancer, and CYP17 may play a small but important role in the etiology of prostate cancer.

Acknowledgments

We thank all participants for volunteering in this study. We also thank Norma Foster, Nadege Sylvester, and Dale Young for their assistance in subject enrollment and data and sample processing.

References


Cancer Epidemiology, Biomarkers & Prevention

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