Short Communication

CYP3A4 and CYP3A5 Genotypes, Haplotypes, and Risk of Prostate Cancer

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Abstract

Previous case-only studies have shown that men with the CYP3A4*1B promoter variant are at an increased risk of developing more aggressive forms of prostate cancer. However, no changes in CYP3A4 activity have been found in CYP3A4*1B carriers, suggesting that its association with disease may simply reflect linkage disequilibrium with another functional variant. CYP3A5 is located within 200 kb of CYP3A4, and a variant in CYP3A5 (*1/*3) correlates with function of the CYP3A5 enzyme. In this study, the potential effect of CYP3A4*1B and CYP3A5*1 on prostate cancer risk and aggressiveness were evaluated in a family-based case-control population. The CYP3A4*1B variant was positively associated with prostate cancer among Caucasians with more aggressive disease (odds ratio [OR], 1.91; 95% confidence interval [CI], 1.02–3.57; P = 0.04), and inversely associated with risk among Caucasians with less aggressive disease (OR, 0.08; 95% CI, 0.01–0.49; P = 0.006) and men with an age of diagnosis <63 (OR, 0.51; 95% CI, 0.26–1.00; P = 0.05). The CYP3A5*1 variant was inversely associated with prostate cancer, especially among Caucasians with less aggressive disease (OR, 0.42; 95% CI, 0.22–0.78; P = 0.006). As expected based on these genotype-level results, the CYP3A4*1B/CYP3A5*3 haplotype was positively associated with disease (OR, 2.91; 95% CI, 1.36–6.23; P = 0.006), and the CYP3A4*1B/CYP3A5*1 haplotype was inversely associated with risk among Caucasians with less aggressive disease (OR, 0.07; 95% CI, 0.01–0.51; P = 0.009). These findings suggest that the CYP3A4 and CYP3A5 variants, or other alleles on the haplotypes they help distinguish, are associated with prostate cancer risk and aggressiveness.

Introduction

Prostate cancer is the most common nonskin-related malignancy in men in the United States. In 2002 ~189,000 men in the United States were diagnosed with prostate cancer, and 30,200 men died from this disease (1). Risk factors include age, ethnicity, family history, and diet (2). A strong family history indicative of a highly penetrant gene is believed to account for only 5–10% of prostate cancers, whereas a larger percentage may be because of common polymorphisms that give rise to a low risk of disease (3, 4). A great deal of interest has focused recently on the role of genes involved in the metabolism, biosynthesis, and regulation of androgens in the occurrence and progression of prostate cancer.

The CYP family of enzymes function in a wide variety of metabolic pathways involving both endogenous and exogenous compounds (5). Their involvement in the metabolism of steroids, as well as environmental xenobiotics, suggests that some may affect prostate cancer risk (3–5). Studies on the activity and expression of CYP3A subfamily members in liver extracts have shown a high degree of polymorphic expression (5). The CYP3A locus consists of four genes, CYP3A4, CYP3A5, CYP3A7, and CYP3A43, all of which reside in a 231-kb region of chromosome 7q21.1 (6).

It has been estimated that up to 60% of the variability in CYP3A4 activity may be because of a genetic component (7). A SNP in the nifedipine-specific response element in the promoter of the CYP3A4 gene (alternatively termed g.-392A>G, CYP3A4*V, CYP3A4*1B, see website7 for unified nomenclature) has been reported (8). Case-only studies of Caucasians (8) and of African-Americans (9) have detected associations between CYP3A4*1B and presentation with biologically aggressive disease. It has been postulated that the presence of the CYP3A4*1B allele decreases the amount of CYP3A4 protein, leading to a reduction of testosterone metabolism and, therefore, more availability of testosterone for conversion to dihydrotestosterone, the most potent androgen affecting the growth and differentiation of prostate cells (8). However, several in vivo studies on the functional effect of CYP3A4*1B have failed to reveal any meaningful link between this polymorphism and activity of the CYP3A4 enzyme (10–13).

CYP3A5 is expressed in a polymorphic manner in 10–29% of adult livers (14–16). Several polymorphic variants in CYP3A5 appear to have a functional effect on CYP3A5 activ-
ity, including an intronic SNP that affects splicing of the CYP3A5 transcript. The CYP3A5*1 allele that produces a correctly spliced transcript has a frequency of 0.15 to 0.45 in Caucasians and African-Americans, respectively (17). The non-functional allele (CYP3A5*3, g.6968A>G) occurs in intron 3 of CYP3A5, creating a cryptic splice site leading to the inclusion of a novel exon, and ultimately a premature stop codon (16, 17). Only individuals with at least one CYP3A5*1 allele express CYP3A5 at a high level (16–18). CYP3A5 represents at least half of the CYP3A content in the liver and jejunum of most individuals carrying a CYP3A5*1 allele, and CYP3A4 levels in those individuals appear to correlate with CYP3A5 levels (17, 18).

As no functional significance has been ascribed to the CYP3A4*1B variant allele, an association between CYP3A4*1B and prostate cancer phenotypes may be because of linkage with a functional polymorphism elsewhere in the CYP3A locus. CYP3A5 is an attractive candidate gene for this association because of evidence that it is expressed in normal and tumor prostate tissue (19, 20), whereas CYP3A4 has been reported as expressed in only 0–14% of normal prostate tissues (19–21). The hypothesis that prostate cancer risk may be associated with CYP3A5 genotypes (17) has been strengthened recently by the report of linkage disequilibrium between the CYP3A4 and CYP3A5 alleles (20). To additionally investigate this possibility, we used a family-based case-control study to investigate the association between prostate cancer and the CYP3A4*1A/*1B alleles, CYP3A5*1/*3 alleles, and CYP3A4/CYP3A5 haplotypes.

Materials and Methods

A study population of siblings (n = 920; 440 cases, 480 controls) was recruited from the major medical institutions in the greater Cleveland area and from the Henry Ford Health System (Detroit, MI). Institutional Review Board approval was obtained from the participating institutions, and all of the study participants gave informed consent. Sibling sets consisted of probands with histologically confirmed prostate cancer and at least one brother without prostate cancer. If unaffected, the brother was either older or no more than 8 years younger than the age of the proband at diagnosis. The disease status of unaffected brothers was additionally confirmed through testing the age of the proband at diagnosis. The disease status of the proband at diagnosis. Aggressiveness was defined as “low” if a case Gleason score was <7 and the tumor category was <T2c, and “high” if the Gleason score was ≥7 or the tumor category was ≥T2c. The tumor category reflects the Tumor-Node-Metastasis System (27). In addition, any possible effect modification by age was evaluated by stratifying by age at diagnosis (<65 versus ≥65). The regression models adjusted for potential confounding by age, all of the Ps are from two-sided tests, and analyses were undertaken with S+ software (version 6.0; Insightful Corp.).

Results

The genotype and haplotype frequencies of the CYP3A4 and CYP3A5 variants by case-control status and ethnicity are shown in Table 1. For the purposes of this study CYP3A5*1 is considered the variant allele because of its lower allele frequency in our population, although biologically it produces the wild-type protein product. Alleles for CYP3A4 and CYP3A5 were in Hardy-Weinberg equilibrium among controls within ethnic groups (P > 0.4). In agreement with previous reports, the frequencies of variant alleles were higher in African-Americans (CYP3A4*1B = 0.58, CYP3A5*1 = 0.66) than Caucasians (CYP3A4*1B = 0.04, CYP3A5*1 = 0.09). The CYP3A4 and CYP3A5 alleles were in relatively strong linkage disequilibrium (D' > 0.7 among controls within ethnic groups). The haplotype frequencies differed greatly between ethnic groups; for example, the most common haplotype in African-Americans (CYP3A4*1B/CYP3A5*1) was present in 53% of control individuals but only 4% of Caucasian controls. Moreover, although the CYP3A4*1B/CYP3A5*3 haplotype was not observed often, it was approximately twice as common among cases than controls, regardless of ethnicity (Table 1).

Initial analysis of CYP3A4 in the entire population and specific ethnic groups indicated no association with prostate cancer (Table 2). However, when the population was stratified by the disease aggressiveness of the case and restricted to Caucasians, the CYP3A4*1B variant was associated positively with disease in the high aggressiveness group (OR, 1.91; 95% CI, 1.02–3.57; P = 0.04) and inversely associated in the low

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CYP3A5

Complete...
aggressiveness group (OR, 0.08; 95% CI, 0.01–0.49; P = 0.006). Moreover, when stratifying by the case median age at diagnosis, an inverse association between the ‐ allele and prostate cancer risk was found in the <63 age stratum (OR, 0.51; 95% CI, 0.26–1.00; P = 0.05; data not shown).

The ‐ variant was inversely associated with prostate cancer, especially among Caucasians with less aggressive disease (OR, 0.42; 95% CI, 0.22–0.78; P = 0.006; Table 2). Simultaneously including both of the SNPs in the same regression model did not materially alter the magnitude of the ORs; however, the statistical significance of the inverse associations observed for both variants among Caucasians with less aggressive disease was slightly weakened and more so for than (data not shown).

The haplotype (among all men) with more prostate cancer risk (OR, 2.91; 95% CI, 1.36–6.23; P = 0.006). Although this haplotype is more common in African-Americans than Caucasians, it appears associated with risk in both ethnic groups (Table 3). The haplotype was weakly associated with an inverse risk of prostate cancer (OR, 0.65, 95% CI, 0.41–1.02; P = 0.06), and this association was stronger in Caucasians with less aggressive disease (OR, 0.07, 95% CI, 0.01–0.51; P = 0.009).

**Discussion**

Our findings support the involvement of the and variants, or other alleles in linkage disequilibrium with these, in prostate cancer risk. The positive associations we observed for (among Caucasian men) and the (among all men) with more aggressive disease agree with the previous findings from case‐only studies that the allele is associated with increased prostate cancer aggressiveness among Caucasians (8) and African-Americans (9), where the latter was our previous study on a different population. The lack of a genotype‐level association for among African-Americans agrees with a recent study of African-Americans and Nigerians, which suggested that such an association is because of confounding by population stratification (28). Nevertheless, here we have controlled for population stratification through the use of a sibling case‐control study design, and stratified our analyses to allow for potential effect modification by ethnicity. The inverse as-

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**Table 1** Genotype and haplotype frequencies of and variants in prostate cancer cases and sibling controls

<table>
<thead>
<tr>
<th>Genotype/Haplotype</th>
<th>Alleles</th>
<th>All Subjects</th>
<th>Caucasians</th>
<th>African-Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Cases</td>
</tr>
<tr>
<td>CYP3A4</td>
<td><em>1A</em>/1A</td>
<td>376 (87%) 402 (86%)</td>
<td>362 (93%) 391 (92%)</td>
<td>11 (29%) 8 (21%)</td>
</tr>
<tr>
<td></td>
<td><em>1A</em>/1B</td>
<td>39 (9%) 52 (11%)</td>
<td>24 (6%) 34 (8%)</td>
<td>13 (34%) 16 (42%)</td>
</tr>
<tr>
<td></td>
<td><em>1B</em>/1B</td>
<td>18 (4%) 15 (3%)</td>
<td>4 (1%) 1 (0.2%)</td>
<td>14 (37%) 14 (37%)</td>
</tr>
<tr>
<td>CYP3A5</td>
<td><em>3</em>/3</td>
<td>345 (78%) 366 (76%)</td>
<td>337 (85%) 359 (82%)</td>
<td>6 (16%) 5 (13%)</td>
</tr>
<tr>
<td></td>
<td><em>3</em>/1</td>
<td>78 (18%) 94 (20%)</td>
<td>57 (14%) 75 (17%)</td>
<td>18 (47%) 16 (42%)</td>
</tr>
<tr>
<td></td>
<td><em>1</em>/1</td>
<td>17 (4%) 20 (4%)</td>
<td>3 (0.8%) 3 (0.7%)</td>
<td>14 (37%) 17 (45%)</td>
</tr>
<tr>
<td>CYP3A4/CYP3A5*3</td>
<td><em>1A</em>/3</td>
<td>738 (85%) 798 (85%)</td>
<td>710 (91%) 769 (90%)</td>
<td>21 (28%) 22 (29%)</td>
</tr>
<tr>
<td></td>
<td><em>1A</em>/1</td>
<td>53 (6%) 58 (6%)</td>
<td>38 (5%) 47 (6%)</td>
<td>14 (18%) 10 (13%)</td>
</tr>
<tr>
<td></td>
<td><em>1B</em>/1</td>
<td>58 (7%) 73 (8%)</td>
<td>24 (3%) 31 (4%)</td>
<td>32 (42%) 40 (53%)</td>
</tr>
<tr>
<td></td>
<td><em>1B</em>/3</td>
<td>17 (2%) 9 (1%)</td>
<td>8 (1%) 5 (0.6%)</td>
<td>9 (12%) 4 (5%)</td>
</tr>
</tbody>
</table>

a Frequencies are calculated from the total number of samples successfully genotyped for each variant.

b All subjects include 8 Hispanics (4 cases and 4 controls) and 2 Asian-Americans (1 cases and 1 control).

c Counts are for the number of haplotypes.

d Counts are for the number of individuals.

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**Table 2** Family‐based associations between and genotypes and risk of prostate cancer among cases and sibling controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No stratification</th>
<th>Stratified by disease aggressiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI), P&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Low</td>
</tr>
<tr>
<td>CYP3A4</td>
<td></td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td><em>1A</em>/1A</td>
<td>0.76 (0.48–1.20), 0.24</td>
<td>0.20 (0.07–0.60), 0.004</td>
</tr>
<tr>
<td>All Subjects</td>
<td>0.83 (0.48–1.42), 0.49</td>
<td>0.08 (0.01–0.49), 0.006</td>
</tr>
<tr>
<td>Caucasians</td>
<td>0.61 (0.27–1.36), 0.23</td>
<td>0.66 (0.16–2.65), 0.56</td>
</tr>
<tr>
<td>African-Americans</td>
<td>0.74 (0.44–1.30), 0.09</td>
<td>0.45 (0.25–0.82), 0.009</td>
</tr>
<tr>
<td>CYP3A5</td>
<td></td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td><em>3</em>/3</td>
<td>0.73 (0.51–1.05), 0.09</td>
<td>0.45 (0.25–0.82), 0.009</td>
</tr>
<tr>
<td>All Subjects</td>
<td>0.71 (0.49–1.04), 0.08</td>
<td>0.42 (0.22–0.78), 0.006</td>
</tr>
<tr>
<td>Caucasians</td>
<td>1.00 (0.29–3.52), 1.00</td>
<td>0.92 (0.10–8.69), 0.94</td>
</tr>
</tbody>
</table>

<sup>a</sup> Adjusted for age.
<sup>b</sup> Cases and their brothers stratified by tumor aggressiveness of affected brother. Low aggressiveness: Gleason <7, and T category <T2c. High aggressiveness: Gleason ≥7 or T category ≥T2c.
the promoter region of higher extent than the other CYP3A isoforms, whereas CYP3A7 has
7, or T category event, wherein a portion of the
reported that appear to have arisen from a gene conversion
sociations observed for CYP3A4*1B, CYP3A5*1, and their haplotype among men with less aggressive disease is also consistent with the previous case-only studies; in particular, the CYP3A4*1B variant was essentially protective if one reversed the previous comparison, and contrasted cases with less aggressive disease to those with more aggressive disease (8, 9). Interestingly, the CYP3A4*1B/CYP3A5*3 high-risk haplotype is found more commonly in African-Americans than Caucasians (5.3% versus 0.6%), and the former have the higher incidence and mortality of prostate cancer worldwide.

Other functional variant(s) on the haplotype defined in part by the CYP3A4 and CYP3A5 alleles could be the causal variants that underlie the associations seen here. Two variant alleles predicted to lead to a truncated CYP3A5 protein have been reported to occur at a low frequency in African-Americans (CYP3A5*6, CYP3A5*7; Refs. 16, 17). Seven linked variants in the promoter region of CYP3A7 (CYP3A7*1C) have also been reported that appear to have arisen from a gene conversion event, wherein a portion of the CYP3A4 promoter was substituted for the CYP3A7 promoter sequence (17). CYP3A7 was originally thought to be expressed only during fetal development; however, it appears that this variant promoter (CYP3A7*1C) may allow high expression of CYP3A7 into adulthood (17, 29). Although there is a great deal of overlap in the substrates used by the CYP3A family members, differences in specific substrate affinities exist (5). For example, CYP3A4 catalyzes the 6β-hydroxylation of testosterone to a greater extent than the other CYP3A isoforms, whereas CYP3A7 has higher 16α-hydroxylase activity against dehydroepiandrosterone and dehydroepiandrosterone 3-sulfate (30). Additionally, CYP3A4 effectively inactivates aflatoxin B1, whereas CYP3A7 is more efficient at activating aflatoxin B1 to its carcinogenic form (31). The relative levels of each CYP3A family member may play diverse roles in the metabolism of substrates that may increase or decrease the risk of prostate cancer.

In summary, we have shown that the risk of prostate cancer aggressiveness attributed previously to the CYP3A4 variant is not simply a manifestation of allelic association with the CYP3A5 variant. Moreover, there may exist other functional variants in this region that reside on the haplotypes defined by these variants that affect the risk and aggressiveness of prostate cancer.

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References
CYP3A4/CYP3A5 and Prostate Cancer


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