**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 CYP3A4 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) has been reported (5) for unified nomenclature (7). 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*<sup>1</sup> allele that produces a cor-
rectly spliced transcript has a frequency of 0.15 to 0.45 in 
Caucasians and African-Americans, respectively (17). The non-
functional allele (CYP3A5<sup>3</sup>, g.6986A>C) occurs in intron 3 
of *CYP3A5*, creating a cryptic splice site leading to the inclu-
sion of a novel exon, and ultimately a premature stop codon (16, 17). Only individuals with at least one *CYP3A5*<sup>1</sup> 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*<sup>1</sup> 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*<sup>1B</sup> variant allele, an association between *CYP3A4*<sup>1B</sup> 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*<sup>1A</sup>/*1B alleles, *CYP3A5*<sup>1</sup>/<sup>3</sup> alleles, and *CYP3A4*/*CYP3A5* haplo-
types.

**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 
proband 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 of 
PSA levels whenever possible (93% of controls). Particip-
ants with PSA levels >4 ng/ml were informed and advised to 
investigate their disease status with their physician. They were 
retained in the study as controls unless a subsequent diagnosis 
of prostate cancer was made, at which time they were reclass-
cified as cases. Keeping them in the study is important, because 
amatically excluding men with elevated PSA levels regard-
less of their ultimate prostate cancer status can lead to biased 
estimates of association (22, 23). By using a sibling-based study 
design, we are assured that our controls have been ascertained 
from genetic source population of the cases, excluding the 
potential for bias because of population stratification (24).

Genotyping of *CYP3A4* was performed using the SNuPe 
genotyping assay (Amersham Biosciences). A 399-bp PCR 
fragment was generated with the following primers: 5′-TCTT-
GTGTGAGGAGTTTGGTGAGGAAG-3′, and 5′-CTGTG-
GCTCTGCTGGCAGTTGGAAAG-3′. The SNuPe reaction 
primer was 5′-GCCATAGACAGAAGGACA-3′, and products 
were analyzed on a MegaBACE 1000 DNA Analysis Work-
station (Amersham Biosciences). Genotyping of *CYP3A5* was 
performed with an allele-specific PCR assay (amplification 
refractory mutation system; Ref. 25). The common forward 
primer 5′-GAGGTGCGCATAGGGATACCCACGTATG-3′ 
was used with either the “G” allele primer: 5′-GTAATGT-
GGTCCAAACAGGGAAGATTCT-3′ or the “A” allele primer: 
5′-GTAATGTGGTCACGAGAAGATTTT-3′. A control primer set was included to verify amplification. Complete *CYP3A5* genotytye information was obtained, whereas 
*CYP3A4* genotypyte information was obtained for 433 cases and 469 
controls.

Using the genotypyte information, we estimated haplotypes 
with the program PHASE (26), and calculated the linkage 
disequilibrium between *CYP3A4* and *CYP3A5* alleles. We then 
calculated descriptive genotype and haplotype frequencies, 
stratified by case-control status. Finally, conditional logistic 
regression (with family as the matching variable, and a robust 
variance estimator) was used to estimate ORs and 95% CIs for 
the association among genotypes, haplotypes, and prostate can-
cer. In addition to an independent analysis of genotypes com-
paring one or more variants to the nonvariant, both genes were 
simultaneously included in the same regression model to assess 
the potential impact of *CYP3A4* versus *CYP3A5* on prostate 
cancer. Joint genotype and haplotype analysis was performed 
only on individuals who had genotypes for both *CYP3A4* and 
*CYP3A5* (433 cases and 469 controls).

To investigate the potential effect of genotype on disease 
aggressiveness, we stratified the analyses by the clinical char-
acteristics of the cases at diagnosis. Aggressiveness was def-
ined 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*<sup>1</sup> is con-
sidered 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*<sup>1B</sup> = 0.58, *CYP3A5*<sup>1</sup> = 0.66) than Caucasians  
(*CYP3A4*<sup>1B</sup> = 0.04, *CYP3A5*<sup>1</sup> = 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 exam-
ple, the most common haplotype in African-Americans  
(*CYP3A4*<sup>1B</sup>/*CYP3A5*<sup>1</sup>) was present in 53% of control indi-
viduals but only 4% of Caucasian controls. Moreover, although 
the *CYP3A4*<sup>1B</sup>/*CYP3A5*<sup>3</sup> 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 
ethnic specific 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*<sup>1B</sup> 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
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 CYP3A4*1B 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 CYP3A4*1B 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 associations observed for both variants among Caucasians with less aggressive disease (OR, 0.42; 95% CI, 0.22–0.78; P = 0.006). Moreover, when stratifying by the case median age at diagnosis, an inverse association between the CYP3A4*1B 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 CYP3A4*1B/CYP3A5*3 haplotype (i.e., encoding a nonfunctional CYP3A5 protein) was associated positively with 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 CYP3A4*1B/CYP3A5*1 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 CYP3A4 and CYP3A5 variants, or other alleles in linkage disequilibrium with these, in prostate cancer risk. The positive associations we observed for CYP3A4*1B (among Caucasian men) and the CYP3A4*1B/CYP3A5*3 haplotype (among all men) with more aggressive disease agree with the previous findings from case-only studies that the CYP3A4*1B 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 CYP3A4*1B 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 CYP3A4 and CYP3A5 variants in prostate cancer cases and sibling controls$^a$

<table>
<thead>
<tr>
<th>Genotype/Haplotype</th>
<th>All Subjects$^b$</th>
<th>Caucasians</th>
<th>African-Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Cases</td>
</tr>
<tr>
<td>CYP3A4$^c$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1A/*1A</td>
<td>376 (87%)</td>
<td>402 (86%)</td>
<td>362 (93%)</td>
</tr>
<tr>
<td>*1A/*1B</td>
<td>39 (9%)</td>
<td>52 (11%)</td>
<td>24 (6%)</td>
</tr>
<tr>
<td>*1B/*1B</td>
<td>18 (4%)</td>
<td>15 (3%)</td>
<td>4 (1%)</td>
</tr>
<tr>
<td>CYP3A5$^c$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*3/*3</td>
<td>345 (78%)</td>
<td>366 (76%)</td>
<td>337 (85%)</td>
</tr>
<tr>
<td>*3/*1</td>
<td>78 (18%)</td>
<td>94 (20%)</td>
<td>57 (14%)</td>
</tr>
<tr>
<td>*1/*1</td>
<td>17 (4%)</td>
<td>20 (4%)</td>
<td>3 (0.8%)</td>
</tr>
<tr>
<td>CYP3A4/CYP3A5$^c$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1A/*3</td>
<td>738 (85%)</td>
<td>798 (85%)</td>
<td>710 (91%)</td>
</tr>
<tr>
<td>*1A/*1</td>
<td>53 (6%)</td>
<td>58 (6%)</td>
<td>38 (5%)</td>
</tr>
<tr>
<td>*1B/*1</td>
<td>58 (7%)</td>
<td>73 (8%)</td>
<td>24 (3%)</td>
</tr>
<tr>
<td>*1B/*3</td>
<td>17 (2%)</td>
<td>9 (1%)</td>
<td>8 (1%)</td>
</tr>
</tbody>
</table>

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

$^b$All subjects include 7 Hispanics (4 cases and 4 controls) and 2 Asian-Americans (1 cases and 1 control).

$^c$Counts are for the number of haplotypes.

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**Table 2** Family-based associations between CYP3A4 and CYP3A5 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$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CIs), P$^a$</td>
<td>Low</td>
</tr>
<tr>
<td>CYP3A4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1A/*1A</td>
<td>1.0 (referent)</td>
<td></td>
</tr>
<tr>
<td>All Subjects *1A/*1B or *1B/*1B</td>
<td>0.76 (0.48–1.20), 0.24</td>
<td>0.20 (0.07–0.60), 0.004</td>
</tr>
<tr>
<td>Caucasians *1A/*1B or *1B/*1B</td>
<td>0.83 (0.48–1.42), 0.49</td>
<td>0.08 (0.01–0.49), 0.006</td>
</tr>
<tr>
<td>African-Americans *1A/*1B or *1B/*1B</td>
<td>0.61 (0.27–1.36), 0.23</td>
<td>0.66 (0.16–2.65), 0.56</td>
</tr>
<tr>
<td>CYP3A5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*3/*3</td>
<td>1.0 (referent)</td>
<td></td>
</tr>
<tr>
<td>All Subjects *3/*1 or *1/*1</td>
<td>0.73 (0.51–1.05), 0.09</td>
<td>0.45 (0.25–0.82), 0.009</td>
</tr>
<tr>
<td>Caucasians *3/*1 or *1/*1</td>
<td>0.71 (0.49–1.04), 0.08</td>
<td>0.42 (0.22–0.78), 0.006</td>
</tr>
<tr>
<td>African-Americans *3/*1 or *1/*1</td>
<td>1.00 (0.29–3.52), 1.00</td>
<td>0.92 (0.10–8.69), 0.94</td>
</tr>
</tbody>
</table>

$^a$Adjusted for age.

$^b$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 16 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
catalyzes the 6 hydroxylase activity against dehydroepiandros-


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