Short Communication

CYP3A4 and *CYP3A5* Genotypes, Haplotypes, and Risk of Prostate Cancer[1]

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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[6] 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 website[7] 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-

Received 9/30/02; revised 3/10/03; accepted 5/27/03.
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[1] This work was supported in part by NIH Grants RO1 CA88164 and RO1 CA94211, Department of Defense Prostate Cancer Research Program DAMD17-98-1-8589, the General Motors Foundation, and The Cleveland Clinic Foundation Taussig Cancer Center. Part of the work of J. S. W. reported in this paper was undertaken during the tenure of a Visiting Scientist Award by the IARC.
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[6] The abbreviations used are: CYP, cytochrome P450; OR, odds ratio; CI, confidence interval; PSA, prostate-specific antigen; SNP, single nucleotide polymorphism.
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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 nonfunctional allele (*CYP3A5*3*, g.6986A>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 of PSA levels whenever possible (93% of controls). Participants 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 reclassified as cases. Keeping them in the study is important, because automatically excluding men with elevated PSA levels regardless 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′-TCTGTGTGAGGAGTTTGGTGAGGAAAG-3′, and 5′-CTGTGCTCTGCCTGCAGTTGGAAG-3′. The SNuPe reaction primer was 5′-GCCATAGAGACAAGGGCA-3′, and products were analyzed on a MegaBACE 1000 DNA Analysis Workstation (Amersham Biosciences). Genotyping of *CYP3A5* was performed with an allele-specific PCR assay (amplification refractory mutation system; Ref. 25). The common forward primer 5′-GAGAGTGGCATAGGAGATACCCACGTATG-3′ was used with either the "G" allele primer: 5′-GGTAATGTGGTCCAAACAGGGAAGAGATTC-3′ or the "A" allele primer: 5′-GGTAATGTGGTCCAAACAGGGAAGAGATTT-3′. A control primer set was included to verify amplification. Complete *CYP3A5* genotype information was obtained, whereas *CYP3A4* genotype information was obtained for 433 cases and 469 controls.

Using the genotype 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 cancer. In addition to an independent analysis of genotypes comparing 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 characteristics of the cases 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 (<63 *versus* ≥63). The regression models adjusted for potential confounding by age, all of the *P*s 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

Table 1 Genotype and haplotype frequencies of *CYP3A4* and *CYP3A5* variants in prostate cancer cases and sibling controls[a]

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

[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 individuals.
[d] Counts are for the number of haplotypes.

Table 2 Family-based associations between *CYP3A4* and *CYP3A5* genotypes and risk of prostate cancer among cases and sibling controls

Genotype	OR (95% CIs), P[a]		
	No stratification	Stratified by disease aggressiveness[b]	
		Low	High
CYP3A4			
*1A/*1A	1.0 (referent)	1.0	1.0
All Subjects *1A/*1B or *1B/*1B	0.76 (0.48–1.20), 0.24	0.20 (0.07–0.60), 0.004	1.32 (0.77–2.27), 0.31
Caucasians *1A/*1B or *1B/*1B	0.83 (0.48–1.42), 0.49	0.08 (0.01–0.49), 0.006	1.91 (1.02–3.57), 0.04
African-Americans *1A/*1B or *1B/*1B	0.61 (0.27–1.36), 0.23	0.66 (0.16–2.65), 0.56	0.49 (0.17–1.43), 0.19
CYP3A5			
*3/*3	1.0 (referent)	1.0	1.0
All Subjects *3/*1 or *1/*1	0.73 (0.51–1.05), 0.09	0.45 (0.25–0.82), 0.009	1.10 (0.68–1.77), 0.69
Caucasians *3/*1 or *1/*1	0.71 (0.49–1.04), 0.08	0.42 (0.22–0.78), 0.006	1.16 (0.70–1.91), 0.56
African-Americans *3/*1 or *1/*1	1.00 (0.29–3.52), 1.00	0.92 (0.10–8.69), 0.94	0.70 (0.08–5.75), 0.74

[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 $\geq$T2c.

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 *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; $P = 0.006$; 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 *CYP3A5*1* than *CYP3A4*1B* (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-

Table 3 Family-based associations between *CYP3A4* and *CYP3A5* haplotypes and risk of prostate cancer among cases and sibling controls

Haplotype (*CYP3A4/CYP3A5*)	ORs, 95% CIs, P^a		
	No Stratification	Stratified by disease aggressiveness[b]	
		Low	High
All Subjects			
*1A/*3	1.0 (referent)	1.0	1.0
*1A/*1	0.90 (0.60–1.34), 0.60	0.62 (0.33–1.16), 0.13	1.17 (0.70–1.98), 0.55
*1B/*1	0.65 (0.41–1.02), 0.06	0.22 (0.09–0.53), 0.0007	1.05 (0.60–1.82), 0.87
*1B/*3	2.91 (1.36–6.23), 0.006	4.51 (0.64–31.74), 0.13	3.15 (1.18–8.37), 0.02
Caucasians			
*1A/*3	1.0	1.0	1.0
*1A/*1	0.76 (0.48–1.21), 0.25	0.62 (0.31–1.25), 0.18	0.92 (0.51–1.65), 0.77
*1B/*1	0.76 (0.44–1.33), 0.34	0.07 (0.01–0.51), 0.009	1.80 (0.95–3.43), 0.07
*1B/*3	2.36 (0.85–6.54), 0.10	3.93 (0.47–32.82), 0.21	4.18 (0.71–24.79), 0.11
African-Americans			
*1A/*3	1.0	1.0	1.0
*1A/*1	1.24 (0.49–3.18), 0.65	2.49 (0.29–21.1), 0.40	0.83 (0.16–4.19), 0.82
*1B/*1	0.63 (0.26–1.56), 0.32	1.80 (0.12–28.0), 0.68	0.38 (0.09–1.54), 0.17
*1B/*3	4.08 (1.35–12.4), 0.01	[c]	3.05 (1.22–7.61), 0.02

[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.
[c] Extremely unstable results due to small sample size.

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 highest 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 CYP3A5 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.

Acknowledgments

We thank Anu Loukola for assistance with setting up the SNuPe assay.

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Cancer Epidemiol Biomarkers Prev 2003;12:928-932.