Debrisoquine Hydroxylase (CYP2D6) and Prostate Cancer

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Abstract
The p450 hepatic microsomal enzyme system metabolizes exogenous drugs and carcinogens. Debrisoquine hydroxylase (CYP2D6), one member of the p450 hemoproteins, has polymorphic expression leading to poor metabolism of debrisoquine and similar compounds in approximately 7% of Caucasians. The genetic locus for this enzyme has been characterized, and the mutations responsible for the slowed metabolism have been identified. Epidemiological studies of the CYP2D6 phenotype suggest an association between the normal or rapid metabolism phenotype and increased risk of lung and bladder cancer. Preliminary data have also suggested an association with prostate cancer (CaP). We used a PCR-based assay to investigate possible associations between the CYP2D6 B allele, the most common genetic mutation responsible for the poor metabolism phenotype, and CaP. Using genomic DNA isolated from peripheral blood, we genetically typed 571 men with CaP and 767 matched controls, all participants in the Physician’s Health Study. Relative to men homozygous for the wild-type allele, a statistically significant increased risk of CaP of borderline statistical significance (P = 0.07). Introduction
The cytochrome p450 enzymes metabolize a wide range of exogenous and endogenous organic compounds (1). Many pharmacological agents used in medical practice are also substrates for p450 enzymes (2). Approximately 7% of Caucasians have a specific p450 enzyme defect that leads to slow metabolism of debrisoquine as well as more than 30 other pharmacological agents (Table 1; Refs. 3 and 4). Individuals who are PMs are very sensitive to the drugs hydroxylated by this enzyme and can have life-threatening side effects from them (5). Normal individuals are known as RMs. There is a third group of patients recently identified as ultrarapid metabolizers who have multiplication of the active CYP2D6 gene (6).

The variation in individual susceptibility to carcinogens, such as those found in tobacco, stimulated interest in associations between polymorphic p450 expression and carcinogenesis (7). Initial studies, using metabolic ratios of excreted unchanged debrisoquine and its 4-hydroxy metabolite to identify the PM phenotype, demonstrated that PM individuals are less likely to develop lung cancer (7, 8). Similar phenotypic analysis of the debrisoquine polymorphism suggested that they also have a decreased risk of aggressive bladder cancer (9). It has been proposed that this association is the result of decreased activation of procarcinogens by the CYP2D6 enzyme (1).

The CYP2D6 locus has been mapped to 22q13.1 (10, 11) and includes the intact, active gene referred to as CYP2D6 as well as two pseudogenes CYP2D7P and CYP2D8P, which contain inactivating mutations (12). The most common mutations responsible for the PM phenotype have been identified within the CYP2D6 locus (4, 11, 13). A characteristic G to A transition mutation at the junction of exon 3 and intron 4, which leads to an early termination codon and defective mRNA, accounts for approximately 80% of mutant alleles (11). This has been designated as the B allele of CYP2D6. Deletion of the entire CYP2D6 gene (the D allele) accounts for another 15% of PM alleles, and most of the remaining PM alleles are accounted for by a combination of three specific mutant alleles (14).

Individuals who have the PM phenotype are most commonly homozygous for inactivating mutations. However, there is a spectrum of debrisoquine metabolism ranging from individuals with two wild-type alleles and rapid metabolism, to heterozygotes with a single mutant allele and an intermediate metabolic rate, to individuals homozygous for mutant alleles who are PMs (15). Although there is considerable overlap in phenotype between individuals homozygous and those heterozygous for the wild-type allele, a statistically significant “gene dose” effect has been observed (16).

Assays using PCR and RFLPs have supported earlier phenotypic studies associating the PM phenotype with a decreased risk of lung cancer (17, 18) and suggest a decreased risk of liver cancer (19).

Only sparse data link CYP2D6 and CaP. In a preliminary report of patients with a variety of malignancies, 9.3% of patients with CaP (n = 54) were homozygous for a mutant allele compared with 4.3% of individuals without cancer (n = 720; Refs. 20 and 21), for a relative risk of 2.3. However,
perhaps due to the small number of cases, this was not statistically significant.

We used PCR to identify the presence of the CYP2D6 B allele in men with CaP, and matched controls within the Physician’s Health Study to explore the association between the PM phenotype and the risk of CaP.

**Materials and Methods**

**Study Population.** The Physician’s Health Study is a randomized, double-blind, placebo-controlled trial investigating long-term use of aspirin and β-carotene among 22,071 American, predominantly white (97% Caucasian), male physicians, ages 40–84 years, in 1982. Men were excluded if they reported a prior history of myocardial infarction, stroke, transient ischemic attacks, unstable angina, cancer (except for nonmelanoma skin cancer), present renal or liver disease, peptic ulcer disease or gout, contraindications to use of aspirin, or present use of aspirin or other platelet-active agents or vitamin A supplements. The aspirin arm was terminated in January 1988 due to a statistically extreme reduction in the risk of a first myocardial infarction among those in the aspirin group (22).

**Blood Collection.** Study participants completed two mailed questionnaires before randomization in 1982 and additional questionnaires at 6 months, 12 months, and annually thereafter. Before randomization, blood kits were sent to all participants with instructions to have their blood drawn into vacutainer tubes containing EDTA, centrifuged, and to have the plasma and a vial of whole blood returned (by overnight prepaid courier) in polypropylene specimen coolers until receipt the following morning, when they were aliquoted and stored at −82°C. Specimens were received from 500 ml of the thawed whole blood using a commercially available kit (VAH Amp DNA extraction kit; Iqagen, Inc., Chatsworth, CA). DNA concentration and purity were determined by UV absorbance on a Beckman DU640 spectrophotometer. Each sample was diluted to a final concentration of 20 ng/μl and stored at −20°C until analysis.

To identify the polymorphic foci for each case and control, 80 ng of sample DNA was added to the PCR reaction mixture, which included primers 5′-GCTTCCCAACCACTGCCG-3′ and 5′-AATCCCTGCTCCTCCAGGGC-3′ (Gough et al., 1996) at a concentration of 0.25 μM each along with 50 mM KCl, 1.5 mM MgCl2, 250 μM each dNTP, and 1.0 units of AmpliTag (Perkin-Elmer Corp.) in a final volume of 22 μl. All amplifications were performed using MicroAmp tubes (Perkin-Elmer Corp.).

A Perkin-Elmer GeneAmp PCR System 9600 thermocycler was programmed for a two-step PCR. After 4 min at 94°C, samples underwent 35 cycles with a melting step at 94°C for 30 s and an annealing and elongation step at 68°C for 1 min and 30 s. There was a final elongation step for 8 min at 72°C, and samples were then cooled to 4°C.

After amplification, 12 μl of amplified product was digested with 4 units of BstO1 according to the manufacturer’s recommendations (New England Biolabs, Beverly, MA). Digested product was separated using a 1.6% agarose gel containing ethidium bromide. Genotype was based on banding pattern (See Fig. 1).

**Data Analysis.** We conducted analyses to determine whether polymorphisms in CYP2D6 were related to CaP risk. In addition to total CaP, we conducted analyses of tumors that possessed a more aggressive phenotype, as determined by histological grade and tumor stage. In the initial set of matched cases and controls, results were similar using conditional logistic regression and unconditional logistic regression controlling for age and smoking status. Hence, we used unconditional logistic regression, controlling for age and smoking, the matching variables, to compute the relative risk (estimated by the OR) of CaP and 95% CIs. By using unconditional logistic regression, we were able to use the entire set of controls for analyses that used only a subset of the cases (e.g., aggressive cancers), which

![Table 1: Known substrates for CYP2D6](celp.aacrjournals.org)
maximized our power. All reported $P$s are two-sided. The trend across categories of RM (homozygous), RM (heterozygous), and PM was tested by using a variable with the values 1, 2, and 3, respectively, in a logistic regression model.

### Results

Of the 1383 samples, 1338 were successfully amplified. The inability to amplify the 45 samples was attributed to poor quality DNA based on OD260:280 ratios, but we cannot exclude the possibility that some of the samples that failed to amplify were homozygous for large deletions of the CYP2D6 foci. These deletions are uncommon; homozygosity for such an abnormality has an estimated frequency of approximately 0.0015 and probably accounts for very few of the samples failing to amplify.

The overall percentage of poor metabolizers among controls was 5.1% (39 of 767), which is what is expected of a mostly Caucasian population. Among cases, the proportion was slightly higher at 6.5% (37 of 571; Table 1). We found a greater proportion of heterozygotes (OR, 1.19; CI, 0.94–1.51; $P = 0.16$) and homozygotes (OR, 1.37; CI, 0.86–2.20; $P = 0.19$) for the mutant allele among CaP patients (Table 2). We found a trend ($P = 0.07$) of borderline statistical significance for increasing risk with an increasing number of mutant alleles across categories of RM (homozygous), RM (heterozygous), and PM genotypes. The relative risk for aggressive CaP was similar to the overall risk of CaP among patients who were homozygous for the PM allele (OR, 1.32; CI, 0.72–2.44; Table 3).

Smoking history was examined in relation with the CYP2D6 genotype and CaP. Of the 1338 men, 729 had been or were presently smoking tobacco cigarettes. The relative risk for CaP among men homozygous for the PM allele of CYP2D6 who never smoked was 1.29 (CI, 0.66–2.51), similar to the 1.48 (CI, 0.76–2.85) for those with a smoking history.

### Discussion

These data show no overall statistically significant association between the $B$ allele and CaP, but a small increase in the risk of CaP in men with one or two copies of the $B$ allele of CYP2D6, of borderline significance ($P = 0.07$), was observed. This analysis is based on a trend in metabolic phenotype from RM (homozygote, wild type), RM (heterozygote), and PM (homozygote, mutant; Ref. 15).

Although earlier studies of the CYP2D6 phenotype and cancer suggested a protective effect of PMs on the risk of lung, bladder, and liver cancer, our study suggests that the $B$ allele leading to the PM phenotype is associated with an increased risk of CaP. This is in accordance with the preliminary studies by Wolf et al. (20) and Dale Smith et al. (21) that found an increased number of CYP2D6 $B$ alleles in a small number of men with prostate cancer when compared with individuals without cancer. An increased risk of cancer was also associated with the PM phenotype when women with breast cancer were studied (23).

In vitro studies have recently demonstrated that a nitrosamine found in tobacco smoke, NNK, is metabolized by CYP2D6 (among other p450 hemoproteins) when the cDNA is cloned into and expressed by a B-lymphoblastoid cell line (24). This nitrosamine is a known rodent pulmonary carcinogen (25), and altered metabolism of NNK may help explain the association between lung and bladder cancer and CYP2D6. However, no definite carcinogen for prostatic epithelium has been identified (26). Thus, the significance of poor metabolism of an exogenous compound such as NNK is unclear in CaP. A personal history of smoking did not seem to influence risk of CaP according to the CYP2D6 genotype.

Testosterone and its 5-hydroxy metabolite, dihydrotestosterone, play a key role in the development of CaP (27). Changes in the metabolism of androgens have the potential to affect the risk of CaP. There is no direct evidence supporting CYP2D6 hydroxylase having androgens as substrates. However, dark DA rats, deficient in CYP2D6 activity, have an increased decrease in hepatic hydroxylation of testosterone and progesterone (28). Alterations in the hydroxylation of testosterone is a potential mechanism for the increased risk of CaP with the PM phenotype. The PM phenotype also is associated with an increased risk of breast cancer (23), another cancer with hormonal dependence. Decreased hydroxylation of androgens and estrogens may lead to delayed clearance and result in higher exposure of the prostate and breast to these compounds. However, in the subset of subjects with available testosterone levels, we found no clear correlation between the CYP2D6 genotype and androgen levels (data not provided).

Another possible explanation for an association between the $B$ allele of CYP2D6 and CaP is in linkage disequilibrium with an unidentified gene that alters the risk of CaP. Recent linkage analysis places the CYP2D6 gene on the 22 chromosome in the region of 22q13.1 (29). This is in close proximity to the platelet-derived growth factor $B$ subunit gene (maximum sex-average lod score, 0.80; $\theta = 0.036$) and to translocation sites for acute lymphocytic leukemia, t(9;22); chronic myelocytic leukemia, t(9;22); and Ewing's sarcoma, t(11;22; Ref. 29). The participation of genes in this region with regard to CaP development is unknown.

The mutation in CYP2D6 analyzed here is the most common, but not the only mechanism leading to the autosomal recessive trait associated with poor metabolism of debrisoquine. The PCR misclassifies approximately 20–30% of alleles leading to the PM phenotype (11, 14). Assuming an incidence of 7% PM phenotype in Caucasian populations, the frequency of alleles leading to PM of debrisoquine would be 0.26. By using an assay that correctly identifies 80% of these alleles, the observed allele frequency would be 20% and the observed homozygotes for the allele is reduced to 4%. This assumes that all mutant alleles not correctly identified are considered normal.

This misclassification of exposure status moderately reduces

### Table 2 Prevalence of CYP2D6 genotypes among CaP patients and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients with CaP ($n = 571$)</th>
<th>Controls ($n = 767$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous for RM allele</td>
<td>Heterozygous for PM allele</td>
<td>Homozygous for PM allele</td>
</tr>
<tr>
<td>62.2% (355)</td>
<td>31.4% (179)</td>
<td>6.5% (37)</td>
</tr>
<tr>
<td>66.2% (511)</td>
<td>28.3% (217)</td>
<td>5.1% (39)</td>
</tr>
</tbody>
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Fig. 1. PCR products of representative genotypes after digestion with BstO1. The size marker used is HaeIII-digested PhilX; Lane 1, homozygous wild type (wt/wt); Lane 2, heterozygous (wt/mt); Lane 3, homozygous mutant (PM; mt/mt).
our statistical power, but should not affect the validity of the association between CaP and CYP2D6. There is no reason to suspect that other mutations not identified would bias the association suggested in this study. Some of the samples that fail to amplify may contain other CYP2D6 mutations, including deletion of the entire gene, but there is no reason to suspect that they would bias the association we observed with the mutations we assessed.

More accurate identification of each individual’s phenotype may strengthen the association between CYP2D6 and CaP. Expanding the genetic analysis to identify the most common mutant alleles leading to the PM phenotype can achieve a 95% concordance between identified genotype and expressed phenotype (13, 19).

Because the polymorphism is relatively uncommon, this study has only modest power to detect relatively small to moderate effects. Although neither relative risk estimate for heterozygotes or homozygotes was statistically significant, the 95% CI (0.94—1.51 for heterozygotes) indicate the range of relative risk estimates that are compatible with the data.

These data are compatible with a possible modest association between the most common mutant allele leading to poor metabolism of debrisoquine and CaP of borderline significance. Although there is no known biological mechanism for such an association, metabolism of exogenous toxins, endogenous androgens, or linkage to an unidentified locus affecting an individual’s risk of CaP are possibilities. Additional studies addressing the association between mutations of the CYP2D6 gene and the PM phenotype and risk for CaP are needed.

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References

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