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# Impact of a genetic variant in CYP3A4 on risk and clinical presentation of prostate cancer among white and African-American men☆

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**Abstract**

Genes involved in androgen metabolism are strong candidates for having an important role in the pathogenesis of prostate cancer. CYP3A4, a protein in the cytochrome P-450 supergene family, facilitates the oxidative deactivation of testosterone. In previous studies, patients with the G variant of a genetic polymorphism in CYP3A4 had prostate cancers with clinically aggressive characteristics at diagnosis. The association was strongest among elderly men. We investigated whether the CYP3A4 variant was linked with the diagnosis or clinical presentation of prostate cancer in a case control study of a multiethnic urban population. Biologic specimens were genotyped for CYP3A4, and analyzed for the impact of this genotype on risk and tumor characteristics at presentation, controlling for the effect of several cofactors. The CYP3A4 variant was more common among African-Americans than among white men. Race-stratified analyses revealed little association between the CYP3A4 variant and prostate cancer risk among white men but were limited by the small number of white men with the CYP3A4 variant. Of African-American men, while the variant G allele was not associated with prostate cancer that had less aggressive characteristics, it was associated with risk of aggressive prostate cancer when men with the AG genotype (odds ratio = 9.3, 95% confidence interval 1.3−411) or GG genotype (odds ratio = 11.9 95% confidence interval 1.6−533) were compared with those with the AA genotype. The association between the CYP3A4 genotype and aggressive prostate cancer in African-American men is consistent with findings of other studies. 

*Keywords:* Prostate cancer; Genetic polymorphisms; Racial disparity

## 1. Introduction

CYP3A4, a subgroup of the cytochrome P-450 supergene family, has an important role in metabolic transformation and elimination of many drugs, possibly the most of any cytochrome. Its expression varies by as much as 40-fold in the liver and small intestine, with genetic variation contributing to this heterogeneity [1]. CYP3A4 is involved in the oxidative deactivation of testosterone to biologically less active metabolites [2-4]. Inhibition of this transformation would result in increased bioavailability of testosterone. CYP3A4 is also involved in oxidative metabolism of finasteride [5] and could impact the effectiveness of the chemoprevention of prostate cancer by that agent.

A germ-line genetic variant in the 5′ regulatory region of the CYP3A4 gene (A to G transition, rs2740574) on chromosome 7 has been reported. Alternate names include CYP3A4*1B, –392A>G, and CYP3A4-V [6]. The CYP3A4 variant G allele (referred to as CYP3A4 variant) is much more common among African-American men than white men, Hispanic, or Asian men [7,8].

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Several previous studies found the CYP3A4 G variant was associated with a higher clinical grade and stage, especially among men whose prostate cancer was diagnosed at an older age [7,8]. In other research, the variant was inversely associated with risk among men with less aggressive prostate cancer [9,10]. In this study, we examined the association between the CYP3A4 variant and the diagnosis and clinical presentation of prostate cancer among white men and African-American men. Our analysis controlled for age, body mass index (BMI), a family history of prostate cancer, and history of benign prostatic hypertrophy (BPH), as well as analyses stratified by age at diagnosis and clinical characteristics at presentation. We compared our findings with those of earlier studies to determine the consistency of the effect of this genotype among different studies and study populations.

## 2. Materials and methods

### 2.1. Study population

Methods for the original case-control interview study are fully described by Patel et al. [11]. Cases included men with newly diagnosed prostate cancer identified through the Metropolitan Detroit Surveillance, Epidemiology, and End Results (SEER) cancer registry. A random sample of 50% of all men between 50 and 74 years of age diagnosed with histologically confirmed, invasive prostate cancer between April, 1996, and March, 1998, and living in Wayne County, Michigan, at diagnosis were eligible for the study. Controls 50−74 years old were identified through random digit dialing, supplemented for ages 65−74 years by men randomly selected from a Health Care Financing Administration list of Medicare recipients. Each subject was asked about a previous diagnosis of prostate cancer, but to promote participation and ensure that study subjects were representative of the community, no further screening was performed. Controls were frequency matched to cases by age group (i.e., 50−54, 55−59, 60−64, 65−69, and 70−74 years old) and race. Cases and controls were required to speak English and have telephones. The Wayne State University institutional review board approved each phase of the study, and signed informed consent was obtained from each subject.

In the original interview study [11], a total of 1414 patients and controls were interviewed. Of those subjects interviewed, using the best available information, 64 identified themselves as other than African-American or white men, 46 of the controls reported a previous diagnosis of prostate cancer, 326 were interviewed before amending the consent form to ask if study subjects would be willing to participate in future research (a factor used to determine eligibility for this sub-study), and 100 were not included because of funding limitations, yielding 878 participants from the parent study who were potentially eligible for this study. From these eligible participants, 197 could no longer be located, 13 were now deceased, 14 were medically unable to undergo phlebotomy, and 71 declined. This resulted in 583 participants (66% of those eligible) providing specimens, 13 of which could not be genotyped, leaving 570 participants, including 322 cases and 248 controls. Interview data included age, race (self-identified), height, weight, a history of BPH, and a familial history of prostate cancer. BPH was self-reported and was not further characterized. Family history included the subject's father and male siblings, except for 70 subjects for whom only the paternal history was available.

### 2.2. Deoxyribonucleic acid isolation

Deoxyribonucleic acid (DNA) was isolated from whole blood, buffy coat preparations, or buccal cells. DNA from whole blood was isolated with the Puregene DNA Isolation Kit (Gentra Systems, Inc., Minneapolis, MN), and DNA from buffy coat preparations was isolated with QIAamp DNA Blood Kits (Qiagen, Inc., Valencia CA) using the procedures recommended by the manufacturer. Buccal cells collected in Scope mouthwash (Procter & Gamble Co., Cincinnati, OH) were pelleted and washed. DNA from buccal cells was extracted using Puregene DNA Isolation Kits (Gentra Systems, Inc.) with procedures recommended by their manufacturer. Briefly, the crude DNA extract was deproteinated with 15 $\mu$L of proteinase K and incubated for 1 hour at 55$^{o}$C. Ribonucleic acid (RNA) was digested by adding 15 $\mu$L of RNase A solution to the cell lysate and incubated at 37$^{o}$C for 15 minutes. Protein for the DNA extract was removed with 1.0 mL of protein precipitation solution added to the cell lysate. The tube was then placed in an ice bath for 10 minutes and centrifuged at 2000 X g for 10 minutes. DNA was then precipitated in 3.0 mL 100% isopropanol. The DNA pellet was collected from the supernatant, washed in 70% ethanol after centrifugation at 2000 X g for 3 minutes, briefly air-dried, and resuspended in 200 $\mu$L DNA hydration solution.

### 2.3. Genotyping

Two methods were used: the Amplifluor single nucleotide polymorphism (SNP) genotyping system, based on allele specific hybridization [12]; and primer extension, using high-pressure liquid chromatography (HPLC) [13]. Nested polymerase chain reaction (PCR) was used with both analyses. The first round of PCR amplifications for both used a 25 $\mu$L mix containing 1 $\mu$M each primer (forward 5′-GCTCTGTCTGTCTGGGTTTGG-3′ and reverse 5′-CACACCACTCACTGACCTCCT-3′, described previously [8]), 2 mM deoxynucleoside triphosphate, 10 ng DNA template, 1× PCR buffer, 2.5 mM MgCl2, and 1.25 U of Amplitaq Gold Taq DNA polymerase (Applied Biosystems, Foster City, CA). The reaction was performed in MJ Thermocyclers (MJ Research, Waltham, MA) or Bio-Rad Icy-