Grade-Specific Prostate Cancer Associations of *IGF1* $(CA)_{19}$ Repeats and *IGFBP3*-202A/C in Blacks and Whites

Cathrine Hoyo, PhD; Janet Grubber, MSPH; Wendy Demark-Wahnefried, PhD; Jeffrey R. Marks, PhD; Stephen J. Freedland, MD; Amy S. Jeffreys, MS; Steven C. Grambow, PhD; Robert M. Wenham, MD; Philip J. Walther, MD, PhD; and Joellen M. Schildkraut, PhD

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Carrying the cytosine-adenosine $(CA)_{19}$ repeat polymorphism in *insulin-like growth factor-1* (*IGF1*) is associated with lower serum proteins and decreased prostate cancer risk. Carrying the -202A/C genotype in *insulin-like growth factor binding protein-3* (*IGFBP3*) also has been associated with lower serum levels of the binding protein. However, the association between this variant and prostate cancer is inconsistent. To test the hypothesis that inconsistencies are partly due to cancer grade-specific differences in strength and direction of associations, we reanalyzed data from our previous Durham Veterans Administration Hospital study of blacks and whites comprising 47 cases (19 African Americans) with Gleason sum ≥7, 50 cases (30 African Americans) with Gleason sum <7 and 93 controls (49 African Americans). Compared to controls, the association between carrying the *IGFBP3* C allele and prostate cancer risk was in $OR_{Low\text{-}Gleason}$=4.0; 95% CI: 1.4–12.3 compared to $OR_{High\text{-}Gleason}$=1.0; 95% CI: 0.4–2.2. Association patterns were similar in African Americans ($OR_{Low\text{-}Gleason}$=3.6; 95% CI: 1.0–13.2 vs. $OR_{High\text{-}Gleason}$=1.4; 95% CI: 0.4–2.3) and whites ($OR_{LowGleason}$=5.6; 95% CI: 0.6–49.0 vs. $OR_{High\text{-}Gleason}$=0.6; 95% CI: 0.2–2.2). The inverse association between carrying the *IGF1* $(CA)_{19}$ repeat variant did not vary by grade or ethnicity. If confirmed in larger studies, these findings support the hypothesis that the association between *IGFBP3* C allele and prostate cancer is grade specific in both ethnic groups.

Key words: Gleason score ■ prostate cancer ■ polymorphisms ■ race/ethnicity

 From the Department of Community and Family Medicine (Hoyo, Schildkraut), School of Nursing (Demark-Wahnefried), Department of Surgery, Division of Urology (Demark-Wahnefried, Freedland, Walther), Department of Obstetrics and Gynecology, Division of Oncology (Wenham), Department of Biostatistics and Bioinformatics (Grambow), Department of Experimental Surgery (Marks), Duke University Medical Center; Cancer Prevention, Detection and Control Research Program, Duke University Comprehensive Cancer Center (Hoyo, Grubber, Schildkraut); Epidemiologic Research and Information Center, Veterans Administration Medical Center (Jeffreys, Grambow); and Department of Surgery, Durham Veterans Affairs Medical Center (Freedland, Walther), Durham, NC. Send correspondence and reprint requests for *J Natl Med Assoc.* 2007;99:718–722 to: Dr. Cathrine Hoyo, Assistant Professor, Department of Community and Family Medicine, Hanes House—Room 347, Trent Drive, Box 2914, Medical Center, Duke University Medical Center, Durham, NC 27710; phone: (919) 681-2441; fax: (919) 684-8675; e-mail: hoyo0001@mc.duke.edu

INTRODUCTION

Prostate cancer incidence and recurrence, as well as important prognostic factors such as tumor grade and volume, differ considerably between African Americans and whites.[1-4] Factors associated with more aggressive disease that may lead to poorer outcomes among African Americans are still unclear. However, familial clusters of prostate cancer, together with higher mortality among African Americans suggest genetic factors play a role.

Recently, our group[5] reported decreased prostate cancer risk in American veterans homozygous for the *insulin like growth factor-1 (IGF1)* cytosine-adenosine $(CA)_{19}$ repeat allele, although others found an increase in prostate cancer risk in Japanese men homozygous for the $(CA)_{19}$ repeat.[6] Poorer survival was also noted in Japanese prostate cancer cases carrying ≥19 *IGF1* CA repeats.[7] However, three U.S. studies mainly comprised of white men[8-10] found no association between carrying the *IGF1* $(CA)_{19}$ repeat and prostate cancer risk. *IGF1* encodes for a potent broad-spectrum mitogen with anti-apoptotic potential that interacts with androgens to enhance prostate cancer cell proliferation in vitro[11] and in vivo.[12] This common polymorphic microsatellite CA repeat locus located 1 kb upstream of the *IGF1* transcription start site has been associated with decreased circulating levels of the protein product in some[13,14] but not all[15-18] studies. Carrying a large number (>18) of these CA dinucleotide repeats is hypothesized to decrease

serum IGF1 levels and thereby decrease prostate cancer risk. However, the handful of epidemiologic studies evaluating this association are conflicting.

Compared to being homozygous for the A allele, being homozygous for the C allele of the single nucleotide polymorphism (A>C) at -202bp of the *IGF-binding protein3* (*IGFBP3*) (*rs2854744*) has been associated with decreased circulating levels of IGFBP3,[16,18] a protein that binds most *IGF1* in circulation, thereby decreasing its bioavailability. An association between carrying the *IGFBP3* C allele and advanced prostate cancer risk in Japanese men has been reported by one study,[19] although our group[5] with equal numbers of African- and European-American participants, and others[10] where European Americans comprised >90% of participants, could not confirm these findings.

Reasons for inconsistent findings are unclear; however, the association between other risk factors such as testosterone[20] and obesity[21] and prostate cancer risk have been shown to vary by grade. It is therefore plausible that grade-specific differences in the association between carrying the *IGF1* $(CA)_{19}$ repeat or the *IGFBP3* C allele at -202bp and prostate cancer risk may explain, in part, inconsistent findings among studies evaluating this association. We present findings of our reanalyses to determine whether carrying these common variants is associated with prostate cancer grade.

MATERIALS AND METHODS

Study Participants

Identification and recruitment of participants, as well as data collection methods, have been detailed elsewhere.[5] Briefly, in a hospital-based, case-control study, newly diagnosed cases of prostate cancer aged 41–75 years were enrolled between January 1999 and July 2001 at the Durham Veterans Administration Medical Center (DVAMC) in North Carolina. Cases confirmed using pathology reports were enrolled on the basis of race (African and European Americans) and age at diagnosis (40–64 and 65–75) to create four age-race strata with ~25% of the cases in each stratum. Controls were DVAMC primary care clinic visitors matched 1:1 to cases based on race (African and

Table 1. Characteristics of cases and controls by Gleason

Characteristics	Controls (n=93) # (%)	Gleason Sum <7 (n=50) # (%)	Gleason Sum ≥7 (n=47) # (%)
Race			
Black	49 (52.7)	30 (60.0)	19 (40.4)
White	44 (47.3)	20 (40.0)	28 (59.6)
Family History of Prostate Cancer in Father or Brother			
Yes	10 (11.0)	9 (18.4)	8 (17.4)
No	81 (89.0)	40 (81.6)	38 (82.6)
Missing	2	1	1
High-School Graduate			
Yes	73 (80.2)	42 (85.7)	40 (87.0)
No	18 (19.8)	7 (14.3)	6 (13.0)
Missing	2	1	1
Ever Had Diabetes			
Yes	39 (42.9)	13 (26.5)	14 (30.4)
No	52 (57.1)	36 (73.5)	32 (69.6)
Missing	2	1	1
Age at Diagnosis			
<60	67 (72.0)	36 (72.0)	33 (70.2)
>60	26 (28.0)	14 (28.0)	14 (29.8)
Body Mass Index			
<25	18 (20.2)	15 (30.0)	11 (24.4)
25–<30	34 (38.2)	21 (42.0)	21 (46.7)
≥30	37 (41.6)	14 (28.0)	13 (28.9)
Missing	4	0	2
Have You Ever Smoked ≥100 Cigarettes			
Yes	72 (79.1)	37 (75.5)	40 (87.0)
No	19 (20.9)	12 (24.5)	6 (13.0)
Missing	2	1	1
Have You Been Screened by PSA in the Last Two Years			
Yes	53 (76.8)	20 (46.5)	20 (47.6)
No	16 (23.2)	23 (53.5)	22 (52.4)
Missing	24	7	5

European American) and age (within five years). The response rate was 71% for cases and 53% in controls. Reasons for nonparticipation were refusal in 40 (27%) cases and 81 (45%) controls, withdrawing from study after consent of two cases (1.3%) and two (1.3%) controls, and our inability to recontact, following consent in two cases (1.1%) and two controls (1.1%).

Data Collection

On scheduled visits to the DVAMC, a trained nurse conducted in-person interviews using a standardized questionnaire, obtained anthropometric measurements, and drew 30 ml of blood with buffy coat stored at -70°C within two hours. Gleason scores were abstracted from pathology reports based on prostate tissue obtained from biopsies.

Polymorphisms

PureGene system reagents (Gentra, Minneapolis, MN) were used to extract and resuspend genomic DNA. For *IGF1* repeats, polymerase chain reaction (PCR) was performed using previously published unlabelled primers[5] from Integrated DNA Technologies (Coralville, IA) to amplify the CA repeat. The forward and reverse primers used were 5'-GCTAGCCAGCTGGTGTTATT-3' and 5'-ACCACTCTGGGAGAAGGGTA-3', respectively. The *IGFBP3* single-nucleotide polymorphism located in the promoter region of *IGFBP3* at -202 (A/C) (rs2854744) was analyzed according to Deal (Deal, 2001) with sense primers, 5'-CCACGAGGTACACACGAATG-3' and antisense, 5'-AGCCGCAGTGCTCGCATCTGG-3'.

Statistical Analyses

Statistical analyses are restricted to the 97 local and regional prostate cancer cases with available Gleason scores who were genotyped for either *IGF1* or *IGFBP3* and the 93 controls for whom genotype data for either gene were available. Although age and race matching would suggest the use of conditional logistic regression models, to improve statistical power, both conditional and unconditional logistic regression were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between high- and low-grade prostate cancer, in relation to carrying these common variants. We report results from unconditional logistic regression models, as the associations were similar to those found in conditional logistic regression models. High and low grades were defined as Gleason scores of ≥7 and <7, respectively. Noncarriers of the *IGF1* $(CA)_{19}$ repeat allele, and noncarriers of the *IGFBP3* -202 C allele were referents for the respective analyses and were compared to men who carried ≥1 of these alleles. *IGF1* and *IGFBP3* genotypes did not deviate from Hardy-Weinberg equilibrium. Age at diagnosis (cases) or interview (controls) and race were adjusted for in all models to ensure comparability across studies. In addition, history of prostate cancer in a father or brother, body mass index (BMI) (kg/m^2), height, education (high-school graduate vs. nongraduate), diabetes and cigarette smoking were assessed as potential confounders for the *IGF1* and *IGFBP3* models separately since these factors have been found to be associated with serum IGF1 and IGFBP3 in previous studies (presumably, in part, due to genetic variation). Variables were included in the model one at a time to determine if their inclusion changed the point estimate for the age- and race-adjusted OR for the association between genotype and high- or low-grade prostate cancer by ≥10%. Those found to cause at least a 10% change were retained in the final model. SAS version 9.1 (SAS, Cary, NC) was used for all statistical analyses except the calculation of Hardy-Weinberg equilibrium.

RESULTS

Forty-nine percent of the 97 prostate cancer cases had Gleason scores ≥7. Fifty-eight percent of European-compared to 39% of African-American cases had high Gleason scores (p=0.05). European Americans also were

Table 2. Unconditional adjusted odds ratios and 95% confidence intervals for the association between IGF1 $(CA)_{19}$ repeat allele and IGFBP3 -202 A/C genotypes and high- and low-grade prostate tumors among African and European Americans

Genes/Genotypes	Controls	High-Grade Cases (Gleason ≥7)			Low-Grade Cases (Gleason <7)		
	(n=93)	(n=47)	OR[a]	95% CI	(n=50)	OR[a]	95% CI
IGF1 $(CA)_{19}$ repeat*							
x/x	20	18	1.0[a]	Referent	16	1.0[a]	Referent
$(CA)_{19}$/x or $(CA)_{19}/(CA)_{19}$	63	29	0.4[a]	0.2–1.0	31	0.7[a]	0.3–1.5
Missing	9	0			3		
IGFBP3 -202 A/C							
AA	23	12	1.0[b]	Referent	5	1.0[b]	Referent
AC or CC	69	35	1.0[b]	0.4–2.2	45	4.1[b]	1.4–12.3
Missing	1	0			0		

*x: non-$(CA)_{19}$ allele; a: Adjusted for age at diagnosis/interview, race, height, family history of prostate cancer in father and brother, diabetes, BMI and education; b: Adjusted for age at diagnosis/interview, race, diabetes and BMI

older than African Americans at the time of prostate cancer diagnosis and more likely to be smokers. With the exception of diabetes in which 28% (27% low grade and 30% high grade) and 43% of cases and controls, respectively, reported a history, the distributions of other clinical and demographic characteristics were similar among cases and controls of both ethnic groups. The prevalence of screening for elevated prostate-specific antigen (PSA) in the preceding two years was slightly higher in controls (77%; 95% CI: 67–87%) than in high- (47%; 95% CI: 32–61%) and low- (48%; 95% CI: 33–63%) grade cases. Sixty-two percent of the cases (equally distributed between high and low Gleason scores) were carriers of the *IGF1* $(CA)_{19}$ repeat allele, compared to 63 (68%) controls. Approximately 82% of cases (75% of men with high and 90% with low Gleason sum) and 74% of the controls carried ≥1 C allele at -202bp *IGFBP3*. Neither *IGF1* nor *IGFBP3* genotypes deviated from Hardy-Weinberg equilibrium (p values 0.14 and 0.31, respectively).

High-grade prostate cancer cases were less likely than controls to carry the *IGF1* $(CA)_{19}$ repeat allele ($OR_{High\ Gleason}$=0.4; 95% CI: 0.2–1.0) and the association between carrying this allele and low-grade prostate cancer was in the same direction ($OR_{Low\ Gleason}$ OR=0.7; 95% CI: 0.3–1.5). The direction of this association was similar in African and European Americans, for both high- and low-grade prostate cancer (data not shown). Interestingly, low-grade prostate cancer cases were approximately four times more likely than controls to be *IGFBP3* C allele carriers (adjusted $OR_{Low\ Gleason}$=4.1; 95% CI: 1.4–12.3). However, this association was not found when high-grade cases were compared to controls (adjusted $OR_{High\ Gleason}$=1.0; 95% CI: 0.4–2.2). These associations were similar in African Americans with an $OR_{Low\ Gleason}$=3.6; 95% CI: 1.0–13.2 vs. $OR_{High\ Gleason}$=1.4; 95% CI: 0.4–2.3 and European Americans with an $OR_{Low\ Gleason}$=5.6; 95% CI: 0.6–49.0 vs. $OR_{High\ Gleason}$=0.6; 95% CI: 0.2–2.2).

DISCUSSION

In these analyses, we found a four-fold increase in risk of low-grade prostate cancer among men who carry the *IGFBP3* C allele. To our knowledge, this is the first report of a positive association between low-grade prostate cancer and carrying the *IGFBP3* -202 C allele among African and European Americans. In contrast, this association was not found when high-grade prostate cancer cases were compared to controls. In our previous analyses, when controls were compared to total prostate cancer, carrying this common variant was not significantly associated with risk (OR=2.3; 95% CI: 0.8–6.2).[5] Of the two other studies that have evaluated the association between carrying the *IGFBP3* C allele and prostate cancer risk to date, the study comprising prostate cancer probands aged <73 years with age-matched brothers in Detroit, MI,[10] found no significant association between carrying this allele and prostate cancer risk. Another[19] reported a higher risk of advanced prostate cancer in Japanese men who carried ≥1 C allele.

If carrying the *IGFBP3* C allele is associated with decreased serum concentrations of *IGFBP3*, as suggested in previous studies,[22-24] as well as a higher risk of prostate cancer among individuals with lower circulating levels of *IGFBP3*, as reported by earlier studies from both Europe and the United States,[25-29] our findings suggest that this association is confined to low-grade prostate cancer. Such findings would be consistent with those of ≥1 recent study with a large number of low-grade cases[30] that reported higher prostate cancer risk in men with elevated serum levels of *IGFBP3*. Survival bias is unlikely to explain these findings because men were diagnosed at an early stage, and the cases were enrolled soon after diagnosis. Detection bias is also an unlikely explanation of these findings since screening rates were higher among controls than either case group. Taken together, these findings support the hypothesis that inconsistent results reported previously may be due, in part, to the inherent grade-specific difference in the strength and perhaps direction of the association between serum *IGFBP3* (or carrying the *IGFBP3* C allele) and prostate cancer risk.

Our finding that men with prostate cancer were less likely to carry the *IGF1* $(CA)_{19}$ repeat allele regardless of tumor grade was anticipated since carriers of the *IGF1* $(CA)_{19}$ repeat polymorphism are reported to have decreased *IGF1* circulating levels in some studies[13,14] perhaps via decreased transcriptional efficiency of *IGF1*. However, others[16-18] have found no association between circulating *IGF1* levels and carrying *IGF1* $(CA)_{19}$. In our previous analyses,[5,10,19] being homozygous for the *IGF1* $(CA)_{19}$ repeat allele was significantly associated with lower risk of total prostate cancer (OR=0.3; 95% CI: 0.1–0.7).

A limitation of these analyses is that due to the small number of cases, we were underpowered to find significant race-specific differences. In addition, we did not stratify our analyses by tumor stage because lymph nodes were not always examined, making it difficult to accurately distinguish between local or regional cases. However, due to aggressive PSA-based screening at the VA medical center, most men have clinically localized disease, such that variation by stage is minimal. Nonetheless, our findings suggest that carrying the *IGFBP3* C allele likely predisposes men to low-grade prostate cancer. If confirmed in larger studies, these findings suggest that it is prudent that future studies of prostate cancer account for the possibility that prostate cancer risk factors under evaluation may be grade specific.

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