INS PstI Polymorphism and Prostate Cancer in African-American Men

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BACKGROUND. Both prostate cancer and diabetes mellitus are common diseases in African-American men. High insulin levels and insulin resistance have been implicated in prostate cancer development, which has prompted a recent investigation of a possible role for germline variation in the insulin gene (*INS*) and prostate cancer risk.
METHODS. Four hundred sixty-six African-American men with and without prostate cancer from the Flint Men's Health Study were typed for the *INS Pst1* genotype using restriction digest and direct sequencing. An association between the *Pst1* genotype and prostate cancer was examined using crude and age-adjusted logistic regression models.
RESULTS. African-American men who were homozygous for the *INS PstI* CC genotype were 1.59 times more likely to be diagnosed with prostate cancer compared to men with the TT or TC genotypes (95% CI = 0.93–2.72). The association appeared stronger among diabetics compared to non-diabetics; however this observation was not statistically significant.
CONCLUSIONS. Our study, taken together with the report of [Ho et al. Br J Cancer 88:263–269, 2003], suggests that the *INS Pst1* CC genotype is associated with prostate cancer risk in African-American men. Germline variation in the *INS* gene should be more fully explored in multiethnic studies to elucidate the molecular variant(s) associated with prostate carcinogenesis. *Prostate 65: 83–87, 2005*.

KEY WORDS: *INS*; **polymorphism; African-American; prostate cancer**

INTRODUCTION

Prostate cancer is the most common cancer among American men and is the second leading cause of cancer deaths in the United States. Age, African-American race, and family history are well-established risk factors for this disease [1]. African-American men have an approximately 1.5-fold higher incidence of prostate cancer and a nearly 2.5-fold higher incidence of distant disease compared to Caucasian men [2]. The likely causes which explain the racial differences in prostate cancer development and progression have not been fully elucidated, but likely include genetic, environment, and sociological factors.

African-American men are also more commonly diagnosed with type 2 diabetes mellitus compared to Caucasian men. Using data from the Third National Health and Nutrition Examination Survey (NHANES), Robbins et al. [3] showed that African-American race is associated with an approximately 50% increase in the risk of type 2 diabetes mellitus in men (age-adjusted OR = 1.43, 95% CI = 1.03–1.99). In this study, the excess risk of diabetes could not be explained by other factors including socioeconomic variables and body size. The

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proposed pathophysiological mechanism for type 2 diabetes is peripheral insulin resistance in combination with relative insulin deficiency.

Several groups have explored the potential relationship between prostate cancer and various aspects of insulin physiology. Using data from a case-control study of prostate cancer conducted in China, Hsing et al. [4] demonstrated that men in the highest tertile of insulin resistance had a 2.8-fold increased risk or prostate cancer (95% CI = 1.6–4.7). Other reports have shown an increased risk of prostate cancer in men with high insulin levels [5] as well as a relationship between serum insulin levels and advanced tumor stage and risk of prostate cancer recurrence [6,7].

Given the potentially important role of insulin in prostate cancer development, Ho et al. [8] tested genetic variation in the insulin gene (*INS*) to determine its potential association with prostate cancer susceptibility. Tight linkage disequilibrium has previously been reported between 10 SNPs spanning a 4.1 kb genomic segment on chromosome 11p15.5 containing the *INS* gene, therefore these investigators elected to test a single SNP (+1127 *INS* Pst1) in the 3′ untranslated region (UTR) of the gene. Using DNA samples from a hospital-based case-control study, Ho et al. [8] detected an increased risk of prostate cancer in men who had the homozygous CC *INS* Pst1 genotype (OR = 1.74, 95% CI = 0.99–3.05). The strongest association, however, was observed in the subset of men who were over age 54 years, either African-American or Caucasian, and who did not report a personal history of diabetes (OR = 6.33, 95% CI 1.87–21.4).

The Flint Men's Health Study (FMHS) is a community-based case-control study of prostate cancer in African-American men between the ages of 40–79. To further explore the potential association between diabetes, germline variation in the *INS* gene and prostate cancer, we genotyped 466 men with and without prostate cancer for the +1127*INS* Pst1 SNP and herein describe the association of the CC genotype with prostate cancer in our study population.

MATERIALS AND METHODS

Control Subjects

Data collection for the FMHS began in 1996 and concluded in 2002. Informed consent was obtained from all study participants, and protocols were approved by the Institutional Review Board at the University of Michigan Medical School. As described previously, disease-free controls were identified from a probability sample of African-American men in Flint, Michigan or in selected census tracts in neighboring Beecher Township (Genesee County, Michigan) [9]. The source population for controls includes all African-American men aged 40–79 living in Genesee County. Men in older age groups were over-sampled to increase the number of eligible subjects for analyses pertaining to prostate cancer. From the initial sample of 943 men, 732 were willing to participate and determined to be eligible to complete the detailed in-home epidemiologic interview, which covered information on health behaviors such as smoking, drinking and physical activity; occupational exposures, general health condition and medical history of chronic illnesses, family history of prostate cancer and demographic information.

Subjects were then asked to undergo a prostate cancer screening protocol, which included providing a blood sample for a serum total prostate-specific antigen (PSA) measurement, and undergoing a comprehensive urological examination. A total of 379 men completed the interview, blood draw and clinical examination. Men with an abnormal digital rectal examination and/or elevated total PSA concentration ($\geq$4.0 ng/ml) were referred for prostate biopsy. Twenty eight men subsequently diagnosed with biopsy-confirmed prostate cancer were included in the study as cases; the remaining 351 were included in our control sample.

Case Subjects

Prostate cancer case recruitment from the same community began in 1999 and was completed in 2002. Cases were identified using the Genesee County Community-Wide Hospital Oncology Program (CHOP) registry, which includes the three hospitals for the county: Hurley Hospital, Genesys Regional Medical Center, and McLaren Regional Medical Center. Case participation in the study required (1) an in-home interview as in the controls; (2) a review of the hospital and registry records for information on stage, Gleason's grade of differentiation, treatment, and pre-diagnosis PSA value; (3) anthropometric measurements; and (4) a blood sample for DNA and freezer storage of serum and plasma. An expert in genitourinary pathology at the University of Michigan reviewed the pathological material and assigned Gleason grade. One hundred twenty five African-American men aged 40–79 who were residents of Genesee County and who had been diagnosed with prostate cancer completed all aspects of the case protocol. The final case group includes the 28 newly diagnosed cases identified during recruitment of potential controls.

Laboratory Methods

Genomic DNA was extracted from whole blood using a Puregene DNA extraction kit (Gentra Systems, Inc., Research Triangle Park, NC). Of the 351 control and 125 case samples, 9 and 1, respectively, were

removed due to technical reasons. The *INS PstI* genotypes were analyzed for 342 controls without the clinical diagnosis of prostate cancer and 124 cases. Genotyping of the *INS PstI* was performed using minor modifications of the restriction fragment length polymorphism (RFLP) analysis described by Ho et al. [8]. Primers were designed to amplify a 503 bp region of the *INS* gene containing the +1127 *Pst1* SNP. The forward primer sequence was 5′CGGGGGAAGGAGGTGGGACAT, while the reverse primer sequence was 5′ACAACAGTGCCGGGAAGTGGGG. PCR primers were purchased from Invitrogen (Carlsbad, CA). All genotypes were independently interpreted by a second researcher, and 18% of genotypes were confirmed by repeat restriction digest. Direct sequencing was used to independently confirm 7% of all samples, and complete concordance was observed between the genotypes obtained by sequencing and RFLP analysis.

Statistical Analysis

The distribution of the *INS PstI* genotype along with other sociodemographic and clinical characteristics were examined in the overall study population as well as by case status. Differences in genotype frequency between cases and controls were tested using the Mantel–Haenszel Chi-square test. Unconditional logistic regression was performed to obtain multivariable odds ratios with associated 95% confidence intervals for the association between *INS PstI* genotype and prostate cancer, adjusting for potentially important covariates and prostate cancer. The TC and TT genotypes were combined in the final analysis given the rarity of the TT genotype. The final models adjusted simultaneously for *INS PstI* genotype, age, BMI, and diabetes. Differences in the associations between *INS PstI* genotype and prostate cancer by BMI and diabetes status were tested using the Breslow–Day test for homogeneity.

Approximately 50% of FMHS control men were tested for prostate cancer at multiple time points. Therefore, to avoid lead time bias in the multivariable analysis, age was calculated based on the same date for all cases and controls. This date was the most recent follow-up date from the entire sample, with the exception that age at death was used for the 37 controls that died prior this date. Subjects were considered to have a positive family history of prostate cancer if they reported that a father, son, and/or brother had ever been diagnosed with prostate cancer. Subjects were considered to have diabetes based on the subjects' self-report of physician-diagnosed diabetes. BMI was calculated by dividing weight in kilograms by height in meter squared, using measurements of weight and height obtained during the clinical exam and these measures were then categorized based on the World Health Organization (WHO) definitions of overweight and obesity: obese ($\geq$30), overweight ($25 \leq \text{BMI} < 30$), or normal BMI (<25). The Cochran–Armitage trend test was calculated to determine if variables with more than two categories had a linear trend of increasing risk of prostate cancer for each successive category. All analyses were performed using the Statistical Analysis System (SAS v. 8.2, Cary, NC). Two tailed tests were used for all comparisons and *P*-values of less than 0.05 were considered statistically significant.

RESULTS

Overall, the men with prostate cancer that were genotyped in this report were significantly older than men without prostate cancer, 64.3 years versus 56.1 years ($P < 0.0001$). A positive family history for prostate cancer was reported by 21.8% of cases compared to 16.7% of controls ($P = 0.22$). Both BMI and diabetes were positively associated with prostate cancer; however, these associations were not statistically significant (Table I).

TABLE I. Distribution of Clinical Characteristics by Case/Control Status and Associated Odds Ratio for the Diagnosis of Prostate Cancer

	Prostate cancer cases (n = 124)	Disease-free controls (n = 342)	Odds ratio (95% CI)	*P*-value
BMI				0.126
Normal (<25)	25.4%	33.3%	—	
Overweight (25–29.9)	40.2%	36.3%	1.45 (0.87–2.44)	
Obese (≥30)	34.4%	30.4%	1.49 (0.87–2.54)	
Diabetes				0.286
Yes	22.6%	17.8%	1.34 (0.81–2.22)	
No	77.4%	82.2%	—	

The *INS PstI* genotypes obtained from 466 FMHS participants exhibited Hardy–Weinberg equilibrium. The CC genotype was most frequently observed in both cases and controls, 83.9% and 76.6%, respectively (Table II). The TT genotype was observed in only three men or 0.6% of the entire study sample. Our results suggest that men with the *INS PstI* CC genotype had a 1.59-fold greater risk of prostate cancer diagnosis as compared to men with the TT or TC genotype (95% CI = 0.93–2.72). We also pooled the FMHS data with the genotype data from 96 African-American men with prostate cancer and 67 disease-free controls reported by Ho et al. [8]. The pooled estimate of prostate cancer risk associated with the *INS Pst1* CC genotype was 1.53 (95% CI = 1.01–2.32). Among the cases, there was no significant association between the *INS PstI* CC genotype and prostate cancer grade (comparing Gleason score $\leq$6 to Gleason score $\geq$7 cases) or age at diagnosis (data not shown).

No differences were observed in the risk for prostate cancer associated with the *INS PstI* CC genotype in obese compared to non-obese men (Table III). The risk of prostate cancer associated with the CC genotype appeared greater among men who reported the diagnosis of diabetes (OR = 2.35, 95% CI = 0.76–7.26) compared to those who did not (OR = 1.55, 95% CI = 0.82–2.94), although the difference was not significant.

TABLE III. Age-Adjusted Odds Ratios of *INS PstI* CC Genotype by BMI and Diabetes Strata

	Odds ratio (95% CI) CC versus TC/TT
BMI	
Obese ($\geq$30)	1.67 (0.62–4.51)
Non-obese (<30)	1.75 (0.89–3.45)
Diabetes	
Yes	2.35 (0.76–7.26)
No	1.55 (0.82–2.94)

DISCUSSION

This is the first independent confirmation of the association between the *INS PstI* CC genotype and prostate cancer as initially described in a multiethnic, hospital-based case-control study at Albert Einstein College of Medicine in New York City. Since our study population was comprised solely of African-American men, we elected to combine the data from 466 African-American FMHS participants with the subset of 163 African-American men from the New York City study. In this combined sample of 629 men, the *INS PstI* CC genotype was associated with a 1.53-fold increase risk of prostate cancer (95% CI = 1.01–2.32).

The *INS* gene, comprised of three exons, is located between the insulin-like growth factor 2 (*IGF2*) gene and the tyrosine hydroxylase (*TH*) gene in chromosome 11p15. The *Pst1* polymorphism is located in the 3′ UTR *INS*. Given the likely role of the 3′ UTR in mRNA stability, it is possible that this polymorphism is playing a direct role in altering insulin physiology which ultimately contributes to prostate carcinogenesis. However, tight linkage disequilibrium has also been observed in the region of the *INS* gene [10] and so it is also possible that the *Pst1* C allele is linked to the disease-associated haplotype. In this case, further investigations must be completed to define the specific alteration which leads to prostate cancer in men.

Despite the fact that both our study as well as the Ho et al. [8] observed similar increases in risk of prostate cancer associated with the *Pst1 INS* C allele, there are some differences in our study designs and results that should be noted. The cases and controls in the

TABLE II. Distribution of *INS PstI* Genotypes and Other Characteristics by Case/Control Status and Associated Odds Ratios for Prostate Cancer Diagnosis With 95% Confidence Intervals

Data source	*INS PstI* genotype	Prostate cancer cases	Disease-free controls	Odds ratio (95% CI)
FMHS data				
	CC	104 (83.9%)	262 (76.6%)	1.59 (0.93–2.72)[a]
	TC	19 (15.3%)	78 (22.8%)	—
	TT	1 (0.8%)	2 (0.6%)	—
Ho et al. [8]				
African-American men	CC	78 (81.3%)	48 (71.6%)	1.72 (0.82–3.59)[a]
	TC	17 (17.7%)	18 (26.9%)	—
	TT	1 (1.0%)	1 (1.5%)	—
Pooled data				
	CC	182 (82.7%)	310 (76.8%)	1.53 (1.01–2.32)
	TC or TT	38 (17.3%)	99 (24.2%)	—

[a]Odds ratio compares CC genotype to TC and TT genotypes combined.

New York City study were identified in a clinic setting and were matched by birth year, race and country of origin. The FMHS is a community-based study in which controls were identified using probability sampling minimizing the potential for selection bias. However, one possible limitation of our study was that only approximately 50% of interviewed subjects ultimately completed all aspects of the clinical examination for prostate cancer in the first wave of the study, which may introduce bias due to non-participation. Heeringa et al. [11] examined the potential selection bias due to non-participation that may have occurred in the FMHS and observed that men who completed all aspects of the study protocol were younger, more likely to be experiencing urological symptoms and were more likely to have a family history or prostate cancer compared to those men who did not undergo clinical examination. The high rate of prostatectomy among the FMHS cases (data not shown) suggests that the FMHS cases may have earlier stage disease compared to the target population. Because prostate cancer is a late-onset disease, both our study and that of Ho et al. [8] may have enrolled men as controls who will ultimately be diagnosed with prostate cancer. However, in case-control studies, the selection of controls from the same or similar reference population assumes that these individuals to some unknown degree may later be diagnosed as cases.

The Ho et al. [8] study revealed that the strongest association between *INS* genotype and prostate cancer was in non-diabetic men who were over 55 years of age. The men who were homozygous CC also were more likely to be older and have a moderately to well-differentiated cancer (Gleason score <7). In contrast, no relationship between *INS* genotype and prostate cancer age at diagnosis or Gleason score was detected in the FMHS, and the OR attributed to the CC genotype was higher among diabetics in the FMHS. As the majority of FMHS cases presented with earlier stage disease, it is possible there was not enough variation in our sample for us to detect an association between *INS* genotype and prostate cancer severity. Racial differences is the prevalence of exposure could account for the discrepancies observed by diabetes status. Larger studies of multiethnic cohorts are necessary to further elucidate these potential relationships.

In conclusion, members of the insulin gene family have become intriguing candidates for genetic studies of prostate cancer susceptibility. Although the relationships between diabetes, insulin levels, insulin resistance, and obesity are complex, molecular studies performed in the context of carefully designed epidemiological studies have the opportunity to tease out potential associations. Our study provides some confirmation to the investigation conducted by Ho et al. [8] in that the observed risk associated with the *Pst1 INS* CC genotype in African-American men was similar to the results from their investigation conducted in a multiethnic population. More thorough studies of germline variants in this region will uncover the true causative mutation that may play a role in prostate carcinogenesis directly or indirectly through insulin action.

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