Single Nucleotide Polymorphisms and the Likelihood of Prostate Cancer at a Given Prostate Specific Antigen Level

Stacy Loeb,* H. Ballentine Carter, Patrick C. Walsh, William B. Isaacs, Anna Kettermann, Toshiko Tanaka, Luigi Ferrucci and E. Jeffrey Metter

From the James Buchanan Brady Urological Institute, Johns Hopkins Medical Institutions (SL, HBC, PCW, WBI, AK), and the National Institute on Aging, National Institutes of Health Clinical Research Branch (TT, LF, EJM), Baltimore, Maryland

Purpose: Prostate specific antigen is used for prostate cancer screening but its specificity is limited. Specificity might be increased by considering genotype associated prostate specific antigen levels.

Materials and Methods: We examined associations between single nucleotide polymorphisms on chromosomes 10 and 19 (previously shown to be associated with prostate specific antigen) with prostate specific antigen and prostate cancer in 505 men from the Baltimore Longitudinal Study of Aging.

Results: In a model with age and date the risk ratio for prostate cancer was 1.18 (95% CI 1.13–1.23) per unit increase in prostate specific antigen. Including the interaction between alleles and prostate specific antigen significantly altered the risk ratio for prostate cancer (Cox proportional hazards $p < 0.001$). Specifically prostate cancer risk per unit increase in prostate specific antigen was significantly different in carriers than in noncarriers of a minor allele (1.28 vs 1.10, respectively, Cox proportional hazards $p < 0.001$), whereas men with a minor allele had a significantly higher risk of prostate cancer at prostate specific antigen levels greater than 6 ng/ml.

Conclusions: Our data suggest that genotype influences the risk of prostate cancer per unit increase in prostate specific antigen. Prostate cancer risk stratification using prostate specific antigen and genotype could improve prostate specific antigen test performance.

Key Words: prostate-specific antigen; prostatic neoplasms; polymorphism, single nucleotide; mass screening; genetics

Abbreviations and Acronyms

CaP = prostate cancer

PSA = prostate specific antigen

SNP = single nucleotide polymorphism

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* Correspondence: 600 N. Wolfe St., Marburg 100, Baltimore, Maryland 21287 (telephone/FAX: 410-202-2343; e-mail: stacyloeb@gmail.com).

RECENTLY genome wide association studies have identified sequence variants (single nucleotide polymorphisms) in numerous chromosomal regions that are significantly associated with prostate cancer risk.[1–4] These associations could be causal or indirect as a result of linkage disequilibrium.

Eeles et al reported that SNPs on chromosomes 10 and 19 were associated with serum PSA concentration and CaP risk.[5] Strong associations were found for rs2735839 and rs2659056 (chromosome 19) as well as rs10993994 (chromosome 10). Of note, rs2735839 is in close proximity to *KLK3*, the gene that encodes PSA, whereas rs10993994 is near the transcription start site of the microseminoprotein beta gene that encodes PSP94, a prostatic secretory protein whose expression is decreased in the androgen independent state and may suppress tumor growth.[6]

The notion of a relationship between genotype and PSA is intriguing

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since the majority of CaP is currently detected through PSA based screening and there is no PSA threshold below which CaP can be excluded with certainty.[7] Thus, methods for improving the specificity of PSA are needed. We examined the association of genotype, serum PSA and prostate cancer risk in a longitudinal aging study.

MATERIALS AND METHODS

Our study population consisted of participants in the Baltimore Longitudinal Study of Aging, a previously described prospective cohort study initiated in 1958.[8] All subjects provided written informed consent and the study protocol was approved by the institutional review board. At each evaluation participants underwent a complete medical examination, including CaP screening with PSA and digital rectal examination beginning in 1991. PSA measurements of participants enrolled before 1991 were obtained retrospectively using frozen serum samples when available. After 1991 prostate biopsy was recommended for a PSA greater than 4.0 ng/ml or abnormal digital rectal examination.

From 1,806 male Baltimore Longitudinal Study of Aging participants we excluded those who had no PSA measurements (605), men with CaP who had no PSA data before diagnosis (38) or before prostate surgery for prostatic enlargement (80), men who took finasteride (Proscar®) at any time (47), men with an unknown cause of death (54), men with a single outlier PSA value suspected to be laboratory error (2) and men with no genetic information (475). Thus, the final study population included 505 men with PSA measurements and a DNA sample, including 61 with CaP and 444 with no known CaP diagnosis.

All 505 men underwent genome-wide genotyping using the Illumina Infinium HumanHap 550K platform. Several quality control criteria were used to screen the SNPs including minor allele frequency 1% or greater, genotyping completeness 99% or greater and Hardy-Weinberg equilibrium ($p < 0.0001$). We specifically evaluated SNPs previously associated with PSA such as rs10993994 (chromosome 10), rs2659056 (chromosome 19) and rs2735839 (chromosome 19).[5] The outcome of interest in this study was CaP diagnosis (event).

Study group characteristics including age, PSA and followup time were compared using t tests (assuming unequal variance) with a Welch modification to the df. Because of the longitudinal nature of the study with 1 or more PSA measurements for each man during a period up to more than 40 years, mixed effects models were used to examine the relationship of SNPs, age and log (PSA +1) with random effects for intercept and time. A likelihood ratio test was used to evaluate 3 models for each SNP in men without prostate disease. The base model included age at first evaluation, time from first evaluation (time), time squared, date, and random effects for time and subject. The second model then added the SNP, and the third model then added the interaction of SNP, time and time squared. A second PSA analysis included men with prostate cancer, and considered a baseline model including cancer status and interactions of cancer status, time and time squared. A likelihood ratio test was used to compare this baseline model to a second model which added the SNP, and a third model that subsequently included interactions between SNP, time and time squared.

To address whether genotype was associated with CaP risk, time dependent Cox proportional hazards models were examined individually for each genotype by PSA with and without adjustment for age and date. Furthermore, the sum of minor alleles was calculated for each subject. Time dependent proportional hazard models were examined for the total count, as well as comparing the presence of 1 or more minor allele(s) with participants who had no minor alleles. The time dependent models were based on the Anderson-Gill formulation as a counting process using survival functions developed by Therneau,[9] and were evaluated using a likelihood ratio test. Statistical tests were considered significant for $p < 0.05$ and all tests were 2-sided.

RESULTS

In the overall cohort the racial distribution was 75% white, 19% black and 6% other. The mean age (±SD) at initial evaluation was 48.9 (±15.1) and 52.2 (±12.8) years for men with and without CaP, respectively ($p = 0.06$). The mean (±SD) initial PSA was 1.09 (±1.65) ng/ml for men without CaP and 2.07 (±2.70) ng/ml for those in whom CaP developed ($p = 0.007$). The mean (±SD) followup time was similar for men with and without CaP (16.92 ± 10.17 vs 18.11 ± 10.47, $p = 0.39$). The raw genotype data are shown in table 1, stratified by race.

In men without CaP the increment in PSA with age was independent of genotype (rs2735839, $p = 0.22$; rs10993994, $p = 0.15$; rs2659056, $p = 0.28$; likelihood ratio test). However, when comparing PSA increments including men with CaP, an interaction was found between genotype and time ($p < 0.001$). After adjustment for age and date of evaluation there were associations between the genotype by time interaction and the likelihood of CaP ($p = 0.01$).

CaP frequency, PSA and age were similar when comparing men with and those without a minor allele (table 2). Compared to men without a minor allele those with a minor allele had a lower risk of CaP at a PSA of 0 ng/ml (risk ratio 0.39, 95% CI 0.20–0.78).

Table 1. *Allele frequency by race in the study population*

	No. rs10993994			No. rs2735839			No. rs2659056		
	CC	TC	TT	GG	AG	AA	AA	AG	GG
White	144	178	56	285	81	12	211	149	18
Black	21	39	36	43	42	11	84	12	0
Other	7	19	5	17	13	1	11	18	2
Totals	172	236	97	345	136	24	306	179	20