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Association of HPC2/ELAC2 and RNASEL non-synonymous variants with prostate cancer risk in African American familial and sporadic cases

Christiane M. Robbins¹, Wendy Hernandez², Chiledum Ahaghotu³, James Bennett⁴, Gerald Hoke⁵, Terry Mason⁶, Curtis A. Pettaway⁷, Srinivasan Vijayakumar⁸, Sally Weinrich⁹, Paulette Furbert-Harris¹⁰, Georgia Dunston¹⁰, Isaac J. Powell¹¹, John D. Carpten¹, and Rick A. Kittles²

¹Translational Genomics Research Institute, Division of Integrated Cancer Genomics, Phoenix, AZ

²Section of Genetic Medicine, Department of Medicine, Pritzker School of Medicine, The University of Chicago, Chicago, IL

³Division of Urology, Howard University, Washington, D.C.

⁴Midtown Urology, Atlanta, GA

⁵Columbia-Presbyterian Medical Center, New York, NY

⁶Michael Reese Hospital, Chicago, IL

⁷Department of Urology, The University of Texas, M.D. Anderson Cancer Center, Houston, TX

⁸Department of Radiation Oncology, UC Davis Cancer Center, University of California, Davis, Sacramento, CA

⁹Medical College of Georgia, School of Nursing, Augusta, GA

¹⁰National Human Genome Center, Howard University, Washington, D.C.

¹¹Karmanos Cancer Institute, Wayne State University, Detroit, MI

Abstract

Introduction—The *RNASEL* and *HPC2/ELAC2* genes have been implicated in hereditary prostate cancer. Further assessment of the role of these genes in sporadic prostate cancer in African American men is warranted.

Methods—Genotyping of *HPC2/ELAC2* variants (S217L, A541T), along with *RNASEL* variants (R462Q and E541D) was completed in 155 African American sporadic and 88 familial prostate cancer cases, and 296 healthy male controls. Logistic regression analysis was performed

Please send all correspondence and requests for reprints to: John D. Carpten, Ph.D., Senior Investigator/Director, Division of Integrated Cancer Genomics, Translational Genomics Research Institute (TGen), 445 N. 5th Street, Phoenix, AZ 85004, (602) 343-8819 office, (602) 343-8842 fax, jcarpten@tgen.org.
Communicating Author, John D. Carpten, Ph.D., Senior Investigator/Director, Division of Integrated Cancer Genomics, Translational Genomics Research Institute (TGen), 445 N. 5th Street, Phoenix, AZ 85004, (602) 343-8819 office, (602) 343-8842 fax, jcarpten@tgen.org

and Odds Ratios (OR) were calculated, while correcting for both age and population stratification using admixture informative markers.

Results—The HPC2/ELAC2 217L allele was significantly associated with risk of prostate cancer when taking all cases into account (OR=1.6; 1.0–2.6; P=0.03). The RNASEL 541D allele was associated with a decrease in risk of prostate cancer in sporadic cases (OR=0.4; 0.2–0.8; P=0.01). We did not detect an association between prostate cancer risk and the RNASEL R462Q variant. Results from haplotype analyses of the two RNASEL variants revealed highly significant differences in haplotype allele frequencies between cases and controls suggesting a synergistic effect at the *RNASEL* locus. One haplotype in particular (462R-541D) is far more frequent in our control population and shows a strong protective effect against prostate cancer (OR=0.47, $P=8.1 \times 10^{-9}$).

Conclusions—These results suggest that HPC2/ELAC2 and RNASEL may play a role, however minor, in prostate cancer risk among African American men.

Keywords

prostate cancer; African American; genetic susceptibility; *RNASEL*; *HPC2/ELAC2*

Introduction

In 2008, there will be an estimated 186,320 newly diagnosed prostate cancer cases and an estimated 28,660 deaths attributed to this disease (1). It is widely known that the incidence rate of prostate cancer varies widely by ancestry with African American Men (AAM) having among the highest prostate cancer rates in the world. It is estimated that the incidence rate of prostate cancer in the US per 100,000 men will be 255.5 in AAM versus 161.4 in European American Men (EAM) (1). A disparity is further observed when looking at the death rates attributed to prostate cancer amongst these two groups with AAM having a greater than two-fold increase in prostate cancer death rates compared to EAM (1). A number of socio-economic factors have been implicated as contributing to these disparities; however the role of biological factors cannot be ruled out.

Many risk factors such as diet, lifestyle, hormones, age, and race have been recognized as contributing to the risk of prostate cancer; however, family history is the single most significant and reproducible risk factor known, where men with two or three first degree relatives with prostate cancer had a five and 11-fold increased risk of developing prostate cancer, respectively (2). To date three genes have been implicated as hereditary prostate cancer tumor suppressor genes through genetic linkage studies: *HPC2/ELAC2* at 17p11 (MIM 605367; ref.1), 2'-5'-oligoadenylate-dependent *RNASEL* at 1q25 (MIM 180435; ref. 2) and *MSR1* at 8p22 (MIM 153622) (3–5). In addition to harboring rare highly penetrant alleles segregating in families, it is of interest to investigate whether common low penetrant alleles within these genes can increase prostate cancer risk among sporadic cases.

We have set out to assess the role of several known common non-synonymous missense alleles within *RNASEL* and *HPC2/ELAC2* in influencing prostate cancer risk among a cohort of African American prostate cancer cases and age-matched controls. As population

substructuring can confound genetic risk models in admixed populations, we have incorporated data from a number of admixture informative markers (AIMs) to minimize type I errors due to substructuring. Here we present our data on the role of common alleles within *HPC2/ELAC2* and *RNASEL* on prostate cancer predisposition in AAM.

Subjects and Methods

African American sporadic prostate cancer cases and controls

Unrelated men self-described as African American were enrolled for case-control studies of risk factors for prostate cancer. The subjects consisted of 451 African Americans (155 prostate cancer patients and 296 healthy male controls) recruited from the Howard University Hospital (HUH) in Washington, DC (6,7). Incident cases were identified through the Division of Urology at HUH and confirmed by review of medical records. Healthy control subjects unrelated to the cases and matched for age (± 5 years) were ascertained from the Division of Urology at HUH and also from men participating in screening programs for prostate cancer at the Howard University Hospital. The demographic characteristics of participants in the screening program were similar to the patient population seen in the Division of Urology clinics. Recruitment of sporadic prostate cancer cases and healthy controls occurred concurrently, and they each donated a blood sample for research purposes. All prostate cancer cases were between 40 to 85 years of age and were diagnosed with the disease within the last 4 years. Clinical characteristics including Gleason grade, prostatic specific antigen (PSA), tumor-node-metastasis (TNM) stage, age at diagnosis and family history were obtained for all cases from medical records. Disease aggressiveness was defined as “Low” (T category $<T2c$ and/or Gleason grade < 7) or “High” (T category $>T2c$ and/or Gleason grade ≥ 7). All healthy controls had PSA levels $<4.0\text{ng/ml}$ and normal digital rectal examination (DRE). The Howard University IRB approved the study and written consent was obtained from all subjects.

African American hereditary prostate cancer cases

Ascertainment of the African American Hereditary Prostate Cancer Study (AAHPC) multiplex prostate cancer families and corresponding clinical descriptions have been previously described (8,9). Due to barriers in recruiting African American men into hereditary prostate cancer studies, the AAHPC Study Network developed a nation-wide effort to establish Collaborative Recruitment Centers (CRCs) in regions of the US with large African American populations including Atlanta, GA; Chicago, IL; Detroit, MI; Harlem, NY; Houston, TX; rural South Carolina, and Washington, DC (8). Inclusion criteria were, 1) four or more prostate cancer cases, preferably first degree relatives, 2) at least three cases available for sampling, and 3) an average age at diagnosis of <65 years of age for the family. These families all self identified as African American and was verified by the recruitment staff. The proband from 88 AAHPC families was selected for this study. The average age at diagnosis for these probands was 64.9 years of age. All participants gave informed consent and the appropriate Institutional Review Boards (IRB) approved recruitment.

Single Nucleotide Polymorphism (SNP) genotyping

Genomic DNA was obtained from isolated lymphocytes using cell lysis, proteinase K-treatment, protein precipitation and DNA precipitation. In this study we genotyped two *HPC2/ELAC2* (NM_018127) variants, including rs4792311 (c.650C>T, S217L) and rs34152967 (c.1681G>A, Ala541Thr), along with *RNASEL* (NM_021133) SNPs rs486907 (c.1385G>A, R462Q) and rs627928 (c.1623T>G, D541E). Genotyping was performed using the Sequenom MassARRAY™ genotyping platform with iPLEX™ chemistry according to manufacturer's recommendations. Briefly, iPLEX™ assays were designed utilizing the Sequenom Assay Design software, allowing for single base extension (SBE) designs used for multiplexing. PCR and SBE primer sequences are available upon request. Multiplex assays were performed to amplify 5–10 nanograms of genomic DNA by polymerase chain reaction (PCR). PCR reactions were treated with shrimp alkaline phosphatase (SAP) to neutralize unincorporated dNTPs. Subsequently, a post-PCR single base extension reaction was performed for each multiplex reaction using concentrations of 0.625 microMolar for low mass primers and 1.25 microMolar for high mass primers. Reactions were diluted with 16 microliters of H₂O and fragments were purified with resin, spotted onto Sequenom SpectroCHIP™ microarrays and scanned by MALDI-TOF mass spectrometry. Individual SNP genotype calls were generated using Sequenom TYPER™ software, which automatically calls allele specific peaks according to their expected masses.

To control for possible confounding due to population stratification, a panel of 34 ancestry informative markers (AIMs) was also genotyped for all samples. These markers show large allele frequency differences (δ) between the ancestral populations for African Americans (West Africans and Europeans), and were used to control for the presence of population stratification (PS) due to admixture in our African American samples. Primer sequences, polymorphic sites and other information pertinent to these AIMs have been previously described (10–12).

Statistical analysis

Frequencies for all SNPs were calculated for cases and controls and genotype frequencies were tested for Hardy-Weinberg equilibrium using the χ^2 test. Odds Ratios (OR) and 95% confidence intervals (CI) for the association between prostate cancer risk and genotypes were calculated using binary logistic regression by comparing the genotypes between subjects with prostate cancer and controls using PLINK version 1.00 (13). Analyses were performed on the combined dataset consisting of all PC subjects, and for the hereditary and sporadic cases separately. Haplotype analyses were also performed using PLINK. For all analyses, genetic effects were adjusted for age (at time of diagnosis for case subjects and at time of ascertainment for controls). Linkage disequilibrium (LD) between the SNPs in each gene was obtained using HAPLOVIEW (14), version 3.32. Statistical control for stratification due to population admixture was achieved by introducing individual ancestry (IA) as a covariate in regression analysis. Individual ancestry was estimated from the panel of 34 AIMs using the Bayesian Markov Chain-Monte Carlo (MCMC) method implemented in STRUCTURE 2.1 (15).

Results

Analysis of HPC2/ELAC2 Coding Variants

We performed genotype analysis and genetic association of two common non-synonymous variants in *HPC2/ELAC2*, S217L (650C>T) and A541T (1681G>A), which are 15,107 bases apart. The genotype frequencies for these variants in all cases and controls used in our study can be found in Table 1. Genotype allele frequencies for both variants are in Hardy-Weinberg Equilibrium. The results of logistical regression analyses for *HPC2/ELAC2* genotypes on prostate cancer risk in African Americans, after corrections for age and differences in West African ancestry, are presented in Table 2. Although there was no association with any *HPC2/ELAC2* variant and risk of prostate cancer when assessing all cases, analysis of the *HPC2/ELAC2* S217L (650C>T) variant revealed a slight statistically significant association between heterozygous carriers of the allele and prostate cancer risk among sporadic cases (Odds Ratio (OR) 1.6 (CI, 1.0–2.6), $P=0.03$). We did not detect a statistically significant difference among homozygous carriers of the minor allele. For *HPC2/ELAC2* variant A541T (1621G>A), the minor allele frequency was <1% in both cases and controls; therefore it was not possible to accurately assess risk for this allele or *HPC2/ELAC2* haplotypes in our study population. No association was detected among the familial cases for any of the *HPC2/ELAC2* genotypes.

Analysis of RNASEL Coding Variants

Similarly, we assessed the association between two non-synonymous coding variants within the *RNASEL* gene and prostate cancer risk. Two common coding variants separated by 3,220 bases within the *RNASEL* gene were studied, R462Q (1385G>A) and D541E (1623T>G). The results of multivariate logistic regression for these two variants are presented in Table 2. We did not observe an association between *RNASEL* R462Q genotypes and prostate cancer risk for familial or sporadic cases. However, we did detect a marginally significant protective effect for the *RNASEL* 541D allele in all cases combined and sporadic cases specifically. Interestingly the effect was not present when specifically looking at familial cases, but seems to be driven more by sporadic cases (Table 2). This 541D allelic effect seemed to be dominant since it was strongest when individuals carry at least one D allele and there was no increase in risk seen among homozygous E/E carriers.

RNASEL Haplotype analyses

The two common *RNASEL* SNPs were in LD ($D'=88$), prompting the evaluation of the effects of individual haplotypes for all cases combined and separately for sporadic and familial cases. Table 3 reveals that *RNASEL* haplotype frequencies differed significantly when all PC cases combined were compared to controls (Global Test $P = 1.1 \times 10^{-8}$), mainly due to differences in haplotype frequencies between the sporadic prostate cancer cases and controls (Global Test $P = 2.1 \times 10^{-11}$) but not familial cases. Haplotype G -T (462R – 541D) was the most common haplotype and was more frequent in the controls than among the sporadic cases (71% compared to 47%, respectively) and was significantly associated with a decreased risk for prostate cancer (OR = 0.47, $P = 8.1 \times 10^{-9}$). We observed a significantly elevated risk for sporadic prostate cancer among carriers of the second most common haplotype, G-G (462R – 541E) (OR = 2.3, $P = 3.1 \times 10^{-9}$).

Discussion

We have assessed the genetic association between variants in putative susceptibility genes *HPC2/ELAC2* and *RNASEL* and risk of prostate cancer in African American men. Although we did not interrogate the entire genetic diversity within these genes and prostate cancer risk, we focused on a series of non-synonymous coding variants within these two genes that have also been investigated by other research groups. To date, the body of literature surrounding the roles of these genes in prostate cancer is represented by nearly 100 peer reviewed articles since the initial reports of their suspected roles in prostate cancer susceptibility. Among these numerous studies, results have been mixed.

Although *HPC2/ELAC2* was initially discovered as a prostate susceptibility gene through a genetic linkage study for highly penetrant prostate cancer susceptibility genes (3), most subsequent studies have focused on its role as a common low penetrance gene (16–29). An initial case-control study assessing the roles of the S217L and A541T variants as common low penetrant alleles was first reported and the results suggested that individuals who carried a combination of the Leu217/Thr541 minor alleles were at significant risk of developing sporadic prostate cancer (odds ratio = 2.37; 95% CI 1.06–5.29) (16). We were unable to perform this analysis, as the T541 allele was present in <1% of our AA cases and controls. Interestingly, their data were generated on a set of cases and controls from different populations (African Americans and Caucasian) (16). Search results of the NCBI dbSNP database (ref) also suggest that the T541 allele is relatively rare in African Americans (~3%). Multiple groups have investigated the role of *HPC2/ELAC2* variants in sporadic prostate cancer risk, and these largely European case control studies have been met with mixed results (18,21,24–26,30). Interestingly, several groups have reported results of *HPC2/ELAC2* candidate gene case-control studies using Japanese cohorts (20,23,27,28). Interestingly all but one of these studies reported statistically significant associations between *HPC2/ELAC2* coding variants and prostate cancer risk. Importantly, one significant difference between the study that did not report *HPC2/ELAC2* associations, Suzuki et al, (2002), was that this study was based on familial rather than sporadic Japanese prostate cancer cases. Several other studies have also been reported that assessed the role of *HPC2/ELAC2* variants in familial prostate cancer risk (19,31,32). Finally, Camp et al, (2007) performed the first true haplotype-based analysis of the *HPC2/ELAC2* locus by determining the haplotype block structure of the *HPC2/ELAC2* locus and selecting a set of 8 “tagging” SNPs or tSNPs to be interrogated in a set of 99 unrelated hereditary cases and 724 controls from the Utah Caucasian population (33). Using a hapotype approach, they were able to detect significant associations with disease risk (33). This is a very good approach as it is possible that the two commonly investigated coding variants, S217L and A541T, are in weak LD with each other, but in stronger LD with another “shared” variant; therefore the full haplotype would need to be assessed to detect the true association. In any event, one area of *HPC2/ELAC2* investigation remains weak and that is assessment of its role in prostate cancer risk among African American men. Of the 13 studies focusing on *HPC2/ELAC2*, only one report exclusively uses a population of recent African descent. Shea et al, (2002) screened the 5’ and 3’ untranslated regions of *HPC2/ELAC2* and investigated the role of the S217L and A541T coding variants on influencing prostate cancer risk in a cohort of

cases and controls from the Caribbean island of Trinidad. No significant association was detected between risk of disease and HPC2/ELAC2 genetic variants (22). Ours represents the largest study of HPC2/ELAC2 with participants from a population of recent African descent. Unlike Shea et al, (2002), we do detect an association between the L217 allele and increased prostate cancer risk. Interestingly, there was no variation at the A541T allele within the Trinidad cohort (22), and the T541 allele was also exceedingly rare (<1%) in our cohort.

The true role of RNASEL genetic variation and its influence on prostate cancer risk is similarly controversial. The initial report of *RNASEL* as a prostate cancer susceptibility gene included data on a series of common and rare coding variants that were discovered (4). Independent research groups have extensively studied a number of these common and rare coding variants. Initially, Casey et al, (2002) showed that the R462Q variant resulted in a significant decrease in RNASEL enzymatic activity and that the variant was significantly associated with an increasing risk of prostate cancer in a case control cohort. Several subsequent studies have been reported, which suggest that RNASEL common variants are associated with risk of familial prostate cancer (17,35,36); however these findings are not universally replicable (37–41). Results of a meta-analysis of ten independent RNASEL genotyping studies for the variants E265X, R462Q, and D541E suggested that although there was no overall effect on prostate cancer risk, there was an ethnic associated increase in risk of prostate cancer (<2.0) with the D541E variant in Caucasians regardless of family history (42). In one of the largest single studies to date, Wiklund et al, (2004) reported the results of a genotype analysis of RNASEL variants in a large series of familial and sporadic cases (n=1624) and controls (n=801) from Sweden. Although their results suggested overall that RNASEL variants were not associated with risk, they reported a marginally significant association between decreased risk of prostate cancer and the 541D allele (43). The results of both of these large studies are in alignment with our findings, suggesting that the 541E allele is potentially protective against prostate cancer. In one of the few studies looking at the role of RNASEL variants on prostate cancer risk in underrepresented US populations, Shook et al (2007) genotyped the R462Q and D541E variants in a cohort of non-Hispanic Caucasian, Hispanic Caucasian, and African American prostate cancer cases and controls. They found significant associations between R462Q and increased prostate cancer risk in Hispanic Caucasians and African Americans (44). Furthermore, there was a statistically significant association between the D541E and prostate cancer risk in Hispanic Caucasians. They also reported that the major allele haplotype (G-T) was more common ($P = 0.04$) in controls in the African Americans, but not the other populations that were studied (44). Their study concluded that RNASEL genetic variants have a role in prostate cancer risk in African Americans and Hispanic Caucasians.

Interestingly, the majority of evidence for a role for RNASEL in prostate cancer seems to be in cases with a positive family history, supporting the initial discovery in hereditary patients. And although the jury is still out on a final determination for the role of RNASEL variants in prostate cancer risk, several lines of functional evidence strengthen the case. In what was actually the first replication study for genetic contributions of RNASEL in prostate cancer risk, Casey et al, (2002) reported an association between the R462Q variant and prostate

cancer risk. Furthermore they provided functional data showing that the Q allele resulted in a significant decrease in RNASE enzymatic activity as compared to the R allele (45). More recently, investigators have discovered a novel gammaretrovirus, named XMRV, in the tumors of prostate cancer patients and the virus was 25X more likely to be found in tumors from men who were homozygous for the minor (Q) allele (46). Although there is no evidence that this virus initiates tumorigenesis these clues raise the possibility for a role of RNASEL in prostate cancer.

Finally, although numerous studies have been reported on both ELAC2 and RNASEL, data on African Americans is limited. Our study is among the largest in African Americans and suggests a role for RNASEL, but not ELAC2 in prostate cancer susceptibility. We hope to extend these analyses to larger African American datasets and look forward to continued independent evaluation of the role of these two genes in prostate cancer risk among African Americans.

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Table 1Frequency (%) of *ELAC2* and *RNASEL* genotypes in African Americans by case status.

SNP Genotype	All Cases (N=243)	Familial Cases (N=88)	Sporadic Cases (N=155)	Controls (N=296)
<i>ELAC2</i> (S217L)				
CC	56.8	62.5	53.5	61.5
CT	40.7	34.1	44.5	33.4
TT	2.5	3.4	2.0	5.1
<i>ELAC2</i> (A541T)				
GG	99.2	100.0	98.7	98.3
GA	0.8	-	0.3	0.7
AA	-	-	-	-
<i>RNASEL</i> (R462Q)				
GG	75.3	78.8	73.4	75.9
AG	22.5	20.0	24.0	22.3
AA	2.1	1.2	2.6	1.8
<i>RNASEL</i> (D541E)				
GG	15.7	12.1	17.1	8.0
GT	41.9	39.7	42.9	43.7
TT	42.4	48.3	40.0	48.3

Table 2

Genotype associations in African American prostate cancer patients and matched controls.

Gene Genotype	All Cases OR (95%CI) P	Familial PC OR (95%CI) P	Sporadic PC OR (95%CI) P
ELAC2 (S217L)			
CC	reference	reference	reference
1 T allele (CT)	1.5 (0.9–2.2) 0.8	1.1 (0.5–2.3) 0.8	1.6 (1.0–2.6) 0.03
2 T alleles (TT)	0.4 (0.1–1.5) 0.2	0.4 (0.1–3.7) 0.5	0.4 (0.1–1.6) 0.2
1 T allele (CT/TT)	0.2 (0.9–2.0) 0.2	1.0 (0.5–2.1) 0.9	1.5 (0.9–2.3) 0.08
ELAC2 (A541T)			
GG	reference	reference	reference
1 A allele (GA)	1.0 (0.2–5.9) 0.9	-	1.4 (0.2–7.8) 0.7
RNASEL (R462Q)			
GG	reference	reference	reference
1 A allele (GA)	0.9 (0.6–1.5) 0.8	1.6 (0.2–14.3) 0.7	0.7 (0.3–1.6) 0.4
2 A alleles (AA)	1.3 (0.1–15.7) 0.8	-	1.6 (0.1–19.3) 0.7
1 A allele (GA/AA)	0.8 (0.4–1.7) 0.8	1.5 (0.2–13.5) 0.7	0.7 (0.3–1.6) 0.4
RNASEL (D541E)			
GG	reference	reference	reference
1 T allele (GT)	0.4 (0.2–0.8) 0.02	0.4 (0.1–1.5) 0.2	0.4 (0.2–0.8) 0.02
2 T alleles (TT)	0.5 (0.2–0.9) 0.04	0.6 (0.2–2.0) 0.4	0.4 (0.2–0.9) 0.03
1 T allele (GT/TT)	0.4 (0.2–0.9) 0.02	0.5 (0.1–1.6) 0.2	0.4 (0.2–0.8) 0.01

Associations were determined using multivariate logistic regression models, adjusted by age and west African ancestry to estimate OR and 95% CI, using the wildtype genotype as the reference.

Table 3

RNASEL haplotype association analysis

RNASEL Allele		Frequency (P-value)			
R462Q	D541E	All Controls	All Cases	Familial PC	Sporadic PC
A	G	0.14	0.16 (0.29)	0.09 (0.43)	0.17 (0.22)
G	G	0.14	0.32 (6×10^{−9})	0.15 (0.89)	0.34 (2×10^{−11})
A	T	0.01	0.02 (0.59)	0.03 (0.35)	0.02 (0.84)
G	T	0.71	0.50 (5×10^{−9})	0.73 (0.79)	0.47 (6×10^{−11})
Global Test P-value			1.1×10^{−8}	0.706	2.1×10^{−11}