Association of Susceptibility Alleles in *ELAC2/HPC2*, *RNASEL/HPC1*, and *MSR1* with Prostate Cancer Severity in European American and African American Men

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Abstract

Reported associations of *ELAC2/HPC2*, *RNASEL/HPC1*, and *MSR1* with prostate cancer have been inconsistent and understudied in African Americans. We evaluated the role of 16 sequence variants in these genes with prostate cancer using 888 European American and 131 African American cases, and 473 European American and 163 African American, controls. We observed significant differences in *ELAC2*, *RNASEL*, and *MSR1* allele frequencies by race. However, we did not observe significant associations between prostate cancer and any variants examined for both races combined. Associations were observed when stratified by race, family history, or disease severity. European American men homozygous for *MSR1* IVS7delTTA had an elevated risk for localized stage [odds ratio, (OR), 3.5; 95% confidence interval (95% CI), 1.4-6.9], low-grade (OR, 3.2; 95% CI, 1.4-7.3) disease overall, and with low-grade (OR, 2.9; 95% CI, 1.2-7.2) or late-stage disease (OR, 5.2; 95% CI, 1.1-25.7) in family history–negative African Americans. *MSR1* Arg293X was associated with family history–negative high-grade disease (OR, 4.0; 95% CI, 1.1-14.1) in European Americans. *RNASEL* Arg462Gln was associated with low-grade (OR, 1.5; 95% CI, 1.04-2.2) and early-stage (OR, 1.5; 95% CI, 1.02-2.1) disease in family history–negative European Americans. In family history–positive individuals, Arg462Gln was inversely associated with low-grade (OR, 0.43; 95% CI, 0.21-0.88) and low-stage (OR, 0.46; 95% CI, 0.22-0.95) disease. In African Americans, Arg462Gln was associated with positive family history high-stage disease (OR, 14.8; 95% CI, 1.6-135.7). Meta-analyses revealed significant associations of prostate cancer with *MSR1* IVS7delTTA, −14,742 A>G, and Arg293X in European Americans; Asp174Tyr in African Americans; *RNASEL* Arg462Gln in European American's overall and in family history–negative disease; and Glu265X in family history–positive European Americans. Therefore, *MSR1* and *RNASEL* may play a role in prostate cancer progression and severity. (Cancer Epidemiol Biomarkers Prev 2005;14(4):949–57)

Introduction

The incidence of prostate cancer varies widely by geographic location and race. African American men have among the highest prostate cancer rates in the world, with an age-adjusted incidence of 137 per 100,000. This rate is substantially higher than that in European Americans (101 per 100,000; ref. 1). The reasons for this disparity are largely unknown. Risk factors such as diet, lifestyle, and hormones have long been recognized as contributing to the risk of prostate cancer (2). The extent to which these racial differences in incidence are attributed to environmental factors is unclear (3). In addition to age and race, family history of prostate cancer is the only other well-established prostate cancer risk factor (4-7). In part, familial aggregations of prostate cancer may reflect inherited susceptibility. Family-based studies have identified and characterized three genes associated with inherited prostate cancer (hereditary genes): *ELAC2/HPC2* at 17p (MIM 605367; ref. 8), 2′-5′-oligoadenylate-dependent RNase L gene (*RNASEL/HPC1*) at 1q25 (MIM 180435; ref. 9), and macrophage scavenger receptor 1 gene (*MSR1*) at 8p22 (MIM 153622; ref. 10).

ELAC2 was initially predicted to encode an evolutionarily conserved, metal-dependent hydrolase, which could partially explain environmental effects on human prostate epithelial cells by postulating differential interactions with environmental exposures (8). Recently, however, *ELAC2* was shown to encode a 3′ processing endoribonuclease, an enzyme responsible for the removal of a 3′ trailer from precursor RNA (11) and to interact with γ-tubulin, a component of the mitotic apparatus (12), suggesting a possible role for ELAC2 in cell cycle control. Mutations in *ELAC2* are rare. Sequence analyses of *ELAC2* identified two common missense changes, Ser217-Leu and Ala541Thr that have been reported to be associated with prostate cancer (8, 13-15).

RNASEL is a constitutively expressed latent endonuclease that mediates the antiviral and proapoptotic activities of the IFN-inducible 2-5A system (16, 17). Two mutations in *RNASEL* (Met1Ile and Glu265X) were reported to segregate with prostate cancer (9). One of these, Glu265X, was also associated with prostate cancer risk in familial and sporadic prostate cancer in other studies (18, 19). A third deleterious frame-shift mutation, 471delAAAG, was more common among unselected prostate cancer patients compared with controls in Ashkenazi Israeli Jews (20). Several missense variants in *RNASEL* have also been detected (18). One of these variants, Arg462Gln, was implicated in up to 13% of prostate cancer cases using a family-based case-control study (21). Although there is a low frequency of deleterious mutations in *RNASEL*, functional studies strongly implicate a functional role for this gene in prostate cancer. Prostate cancer patients carrying the Glu265X

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or the 471delAAAG mutation showed loss of heterozygosity of the wild-type allele in microdissected prostate tumor DNA (9, 20), whereas Arg462Gln had a significantly lower RNASEL enzymatic activity compared with the wild-type protein (21).

MSR1 is a member of an expanded family of membrane receptors collectively termed scavenger receptors. MSR1 can bind many chemically modified molecules ranging from bacteria to modified lipoproteins (22). *MSR1* maps to the 8p22 chromosome region, which is commonly deleted in prostate cancer (23). Six rare missense variants and one nonsense mutation within *MSR1* were observed to cosegregate with the disease in hereditary prostate cancer families (10). Furthermore, the prevalence of *MSR1* mutations in prostate cancer cases of European and African American descent was substantially higher compared with unaffected men (10). Arg293X and Ser41Tyr were the most common mutations detected among prostate cancer patients of European and African American descent, respectively (24).

Despite reports that these genes are involved in prostate cancer etiology, other studies could not replicate the association between prostate cancer and these genes (25-30). The conflicting results among the different studies may be attributed to several factors, including differences in study design, limited power to replicate some findings, and racial differences in study populations. Prostate cancer screening practices may also influence the results of association studies and the ability to compare studies conducted in countries with different screening practices, particularly if these genes are involved with prostate cancer progression or severity. The aims of the present study were to compare the frequency of 16 alleles in three candidate genes (*ELAC2*, *RNASEL*, and *MSR1*) in African Americans and European Americans, to test for association of a subset of six of these alleles that occur at a frequency of >1% in at least one racial group with prostate cancer by racial group using a case-control sample that was unselected for family history of prostate cancer and to perform a meta-analysis using reports from the literature to provide a comprehensive view of the evidence regarding the role of these genes in prostate cancer etiology.

Materials and Methods

Study Population. Study subjects were all residents of the greater metropolitan Philadelphia area, who were recruited as part of an ongoing case-control study of genetic risk factors for prostate cancer (13). These subjects were recruited through urology clinics of the University of Pennsylvania Health System between 1996 and 2003. Prostate cancer cases included 888 European American and 131 African American men with histologically confirmed adenocarcinoma of the prostate. Case status was confirmed by reviewing medical records using a standardized abstraction form. Clinical characteristics at diagnosis were obtained by medical records abstraction and included clinical and pathologic Gleason grade, prostate-specific antigen (PSA) levels at diagnosis, and tumor-node-metastasis stage. Men were excluded from this study if they reported using finasteride (Proscar or Propecia) at the time of diagnosis. Patients who had a prior diagnosis of cancer at any other site other than nonmelanoma skin cancer were excluded. The median age at diagnosis for the patients was 63 years (range, 55.2-70.8 years) and 61.2 years (range, 52.3-70.1 years) for European Americans and African Americans, respectively ($P = 0.013$). Median Gleason Score at diagnosis was 6.0 in both European Americans and African Americans; 78.4% of cases were T1/T2 and 21.6% were T3/T4 in European Americans and 76.6% of cases were T1/T2 and 23.4% were T3/T4 in African Americans. Median PSA levels were 6.1 and 6.4 ng/mL in European Americans and African Americans, respectively. No statistically significant differences in stage ($P = 0.713$), grade ($P = 0.162$), or PSA levels ($P = 0.194$) were observed by race.

Controls were ascertained concurrently with prostate cancer cases. Controls were seen by University of Pennsylvania Health System clinicians for routine checkups, or for the diagnosis or treatment of acute illnesses. Control subjects were excluded from the study if they ever had an elevated PSA test ($\geq$4 ng/mL), abnormal digital rectal examination, previous cancer diagnosis at any site except nonmelanoma skin cancer, or if they reported exposure to finasteride (Proscar or Propecia) at the time of the study ascertainment. After exclusion criteria were applied, a total of 473 and 163 European American and African American controls, respectively, were available for the study. The median age at the time of study ascertainment for the controls was 58 years (range, 47.3-68.7 years) and 59.0 years (range, 48.7-69.3 years) for European Americans and African Americans, respectively. Median PSA levels for European Americans and African Americans at ascertainment were 1.0 and 1.1 ng/mL, respectively.

A standardized questionnaire was used to collect family history of prostate cancer, demographic information, other risk factors, and prostate cancer screening history. A positive family history of prostate cancer was defined as having at least one first-degree relative affected with cancer. Positive family history was reported by 33.7% and 29.0% of the cases compared with 16.1% and 18.4% of the controls in European Americans and African American men, respectively. All study subjects provided informed consent for participation in this research under a protocol approved by the Institutional Review Board of the University of Pennsylvania.

Laboratory Methods. Extraction of genomic DNA from buccal swab samples was done using either an NaOH-based lysis protocol (31) or the QIAamp 96 DNA Buccal Swab Biorobot Kit (Qiagen, Valencia, CA). Sixteen previously reported sequence variants in these genes were selected for study based on their reported putative association with prostate cancer. These included two common single nucleotide polymorphisms (SNPs; Ser217Leu and Ala541Thr) and one rare SNP (Glu622Val) in *ELAC2*; three common variants (−14,742 A>G, IVS-59 C>A, and IVS7delinsTTA) and five rare SNPs (Asp174Tyr, Arg293X, Ser41Tyr, His441Arg, and Val113Ala) in *MSR1*; and one common (Arg462Gln) and four rare variants (Glu265X, Ile97Leu, 471delAAAG frame-shift mutation, and 354 C > T) in *RNASEL*.

Taqman 5′ nuclease PCR primers and probes for alleles of the variant of interest were designed by Applied Biosystems (Foster City, CA) Assay-By-Design^{SM} custom oligonucleotide reagent service. Each probe consisted of an oligonucleotide with a fluorescent reporter dye, a nonfluorescent quencher and minor groove binder. Allele-specific cleavage of probes was detected using different reporter dyes for each probe (6FAM and VIC fluorophores for the each allele) with separate wavelength maxima. PCR amplifications were set up in a 384-well plate format in total volume of 5 μL, containing 50 to 900 nmol/L of each primer, 50 to 250 nmol/L of each probe, Taqman Universal Master Mix (Applied Biosystems), and constant dilutions of 1 to 5 ng of DNA sample. Controls representing each genotype for each variant and a no template (water) control were included in each 384-well plate. PCR was done in MJ Research tetrad thermal cyclers (MJ Research, Inc., Waltham, MA). After an enzyme activation step for 10 minutes at 95°C, 40 two-step cycles were done; a 15-second denaturation at 92°C followed by a 1-minute annealing/extension at 60°C for all variants. After PCR, microtiter plates were transferred to the ABI 7900 HT Sequence Detection System (Applied Biosystems). End-point fluorescence levels of 6FAM and VIC were measured automatically in each well using the SDS 2.1 manufacturer's custom software (Applied Biosystems). Allelic discrimination results were then graphed on a scatter

plot contrasting reporter dye florescence (i.e., allele X versus allele Y). Clustering boundaries for each variant-specific genotype were assigned manually based on the position of the positive control within each cluster, spectral-specific plots specific to each genotype, and sequence results of samples that scattered at the low end of the cluster boundary. Genotypes were called based on their corresponding location within each cluster. Sequence variants were called ''undetermined'' if they clustered in proximity to the negative (water) controls (<1 fluorescence units), showed an abnormal spectral plot (probe-specific fluorophore signal to internal control signal <1), or did not cluster tightly (outliers). Undetermined genotypes that were not confirmed by sequencing were excluded from the present analyses. Percentage of undetermined data for each genotype varied between the different sequence variants with a median genotyping failure rate of 6% and a range of failure rates between 4% and 19%.

To validate the assay performance before testing case and control DNAs, the primers and probes were first tested on a panel of DNA samples composed of 40 European Americans, 40 African American, and 16 CEPH family members (Family 1331, XC01331) obtained from Coriell Human Variation Collection (Coriell Institute, Camden, NJ). Callable genotypes and appropriate allele frequencies were obtained on all variants. The validity of the genotypes for the CEPH family was checked by validation of Mendelian inheritance using the software PedCheck (32). To ensure the reproducibility of the Taqman 5′ nuclease PCR method, ~2.5% of the samples were analyzed in duplicate for all 16 variants.

All results for sequence variants with an uncommon rare allele frequency (<1%) were confirmed by direct sequencing. To confirm inconsistent genotype results, DNA samples were reamplified separately in a 20-μL reaction volume using different PCR primers than those used for the original variant genotyping (PCR primers are available upon request). PCR products were sequenced using Big Dye Terminator v. 1.1 Cycle Sequencing Kit (Applied Biosystems) and an automated ABI 310 Genetic Analyzer instrument (Applied Biosystems).

Statistical Analysis. Allele and genotype frequencies were computed using gene-counting methods. Hardy-Weinberg equilibrium was evaluated assuming random mating (33). Differences in allele and genotype frequencies were evaluated across racial groups by using Fisher's exact tests. Association of allele frequencies with age was done using the Wilcoxon rank sums test.

To further assess the potential functional significance of nine missense variants in *ELAC2*, *RNASEL*, and *MSR1*, we used the software program SIFT, which uses an evolutionary approach to sort intolerant from tolerant amino acid substitutions (34, 35). This analysis is based on the assumption that amino acids with important function tend to be conserved across species. SIFT assigns a substitution probability (P_i) from 0 to 1 for each of the 20 amino acids at each examined position of the protein. The higher a substitution probability, the less likely a particular amino acid is to have a functional effect. We used the output from SIFT to infer substitutions with normalized probabilities of $P_i < 0.05$ as deleterious, whereas those with $P_i \geq 0.05$ were inferred to be tolerated substitutions.

For genotype-disease associations, we limited our association analyses to alleles with a frequency of at least 1% in either race. Genotype associations at each locus were undertaken by combining putative risk alleles into binary genotype classes. Genotype-disease associations were estimated by computing odds ratios (OR) using unconditional logistic regression. Stratified analyses were undertaken by family history of prostate cancer and race (African American and European American). We further evaluated whether there were differences in the association of genotypes in organ-confined tumors (i.e., stages T1 and T2) compared with tumors diagnosed with extracapsular extension or distant metastasis (i.e., stages T3 and T4). Analyses considered the effect of genotype unadjusted for potential confounders and adjusted for age at time of diagnosis in cases or time of study ascertainment in controls and race. All statistical analyses for associations were undertaken using SAS v. 8.01 (SAS Institute, Inc., Cary, NC) and STATA Statistics Data Analysis version v. 8.0 (Stata Co., College Station, TX). All *P*s were based on two-sided hypothesis tests.

Meta-analyses were undertaken by first performing an extensive literature research for all studies of prostate cancer that considered *ELAC2*, *RNASEL*, or *MSR1*. Analyses were undertaken by generating fixed effects pooled estimates of ORs and 95% confidence intervals (95% CI) using ORs and SEs from individual published studies for specific variants in *RNASEL* and *MSR1*. We did not conduct a meta-analysis for *ELAC2* because several investigators have recently done meta-analyses for *ELAC2* (36-38). We considered every report of variants in *RNASEL* or *MSR1* for which prostate cancer associations had been reported at least twice in the literature for strata defined by race and family history. We included published ORs that were either unadjusted or age adjusted only and did the meta-analyses within strata defined by race (strata defined as African American or Caucasian) and family history (strata defined as family history positive or negative, no family history of prostate cancer, or positive family history including studies ascertained in hereditary prostate cancer families). All computations were undertaken using STATA/SE version 8.0.

Results

Allele Frequency Distribution. As shown in Table 1, the 16 variants were genotyped in 1,019 incident prostate cancer cases (including 131 African Americans) and 636 controls (including 163 African Americans). Allele frequencies were in Hardy-Weinberg proportions within each racial group. Genotyping errors may cause deviations from expected Hardy-Weinberg proportions. However, the deviations from Hardy-Weinberg proportions in our data (Table 1) were random with respect to race and case-control status. Therefore, it is unlikely that these deviations were caused by systematic genotyping errors.

Differences in allele frequency between racial groups were observed for *ELAC2* Ser217Leu and Ala541Thr; *MSR1* −14,742 A>G, IVS7delinsTTA, and IVS5-59 C>A; and *RNASEL* Arg462Gln. A number of other variants were observed in only one racial group, including *MSR1* Asp174Tyr in African Americans; *MSR1* Arg293X and Val113Ala and *RNASEL* Glu265X and 471delAAAG in European Americans. Previously reported variants were not observed in our sample, including *ELAC2* Glu622Val (a relatively common allele in Finnish men) and *MSR1* Ser41Tyr (originally identified in African Americans). One European American control was homozygous for *MSR1* Arg293X, which has not been previously reported. There was no association of allele frequencies with age (data not shown). Therefore, there is no evidence for selection against specific genotypes in the course of an individual's lifetime.

SIFT analysis. The results from the evolutionary SIFT comparison are shown in Table 1. Of the nine variants examined in our sample, only the *RNASEL* Arg462Gln variant was predicted to be an intolerant (i.e., functionally significant) amino acid substitution ($P_i = 0.02$). The reminder eight missense variants were predicted tolerant (low risk) missense changes in this comparison. When we examined the intolerance rate of each of these nine amino acid to any substitution, *RNASEL* Arg^{462} was the least intolerant, with P_i values of <0.05 for 14 of the 20 amino acids. In contrast, *ELAC2* ^{541}Ala tolerated substitutions in all of the 20 amino acids examined.

Table 1. Allele frequencies and SIFT analysis of missense polymorphisms in *ELAC2*, *MSR1*, and *RNASEL* genes

Gene	Nucleotide sequence variant	Amino acid change	SIFT Substitution probability (P_i)	Allele frequency (total number of alleles in sample)			
				African American		European American	
				Controls	Cases	Controls	Cases
ELAC2/HPC2	650 C > T	Ser217Leu	0.15	0.228 (254)*	0.211 (180)	0.296 (656)	0.301 (1,020)
	1621 G > A	Ala541Thr	0.26	0.004 (268)*	0 (190)	0.027 (698)	0.033 (1,072)
	1865 A > T	Glu622Val	0.27	0 (270)	0 (168)	0 (684)	0 (1,048)
MSR1	520 G > T	Asp174Tyr	0.13	0.015 (266)*	0.022 (186)	0 (696)	0 (1,064)
	876 C > T	Arg293X	N/A	0 (262)	0 (166)	0.012 (694)†	0.007 (994)
	121 C > A	Ser41Tyr	0.07	0 (274)	0 (186)	0 (706)	0 (1,070)
	1319 A > T	His441Arg	0.07	0.004 (274)	0 (182)	0.006 (688)	0.001 (1,050)
	−14,742 A > G	None	N/A	0.269 (260)*,†	0.253 (186)†	0.105 (686)	0.105 (1,060)
	IVS5-59 C > A	None	N/A	0.012 (246)*	0.006 (170)	0.059 (628)	0.050 (968)
	338 T > C	Val113Ala	0.13	0 (270)	0 (186)	0.006 (714)	0.002 (1,060)
	IVS7delinsTTA	None	N/A	0.264 (220)*,†	0.218 (156)	0.057 (562)	0.045 (894)
RNASEL/HPC1	793 G > T	Glu265X	N/A	0 (268)	0 (188)	0.001 (714)	0.003 (1,080)
	354 C > T	None	N/A	0.004 (266)	0 (182)	0.003 (682)	0.002 (1,002)
	1385 G > A	Arg462Gln	0.02	0.119 (252)*	0.159 (176)	0.361 (642)	0.370 (1,010)
	289 A > C	Ile97Leu	0.5	0 (254)	0.006 (182)	0.006 (654)	0.004 (1,036)
	471delAAAG	None	N/A	0 (270)	0 (186)	0.001 (700)	0 (1,060)

*Frequency in control groups differs by race (Two-Sided Fisher's Exact Tests: $P < 0.05$).
†Deviations from Hardy-Weinberg proportions based on χ^2 Test at $P < 0.05$.

Association Analysis. For *ELAC2*, we observed no association of ^{217}Leu or ^{541}Thr with prostate cancer overall, by prostate tumor severity, or by family history in European American (Table 2) or African American (Table 3) men. We also did not observe any effect for the homozygous ^{217}Leu genotype.

For *MSR1*, we observed an association of the IVS7delTTA common allele with disease severity in both racial groups (Tables 3 and 4). In European Americans, homozygotes for IVS7delTTA were more common among individuals with no family history of cancer, who also had lower-grade disease (OR, 3.0; 95% CI, 1.3-6.9) and lower-stage disease (OR, 2.2; 95% CI, 1.1-4.4), respectively. When stratified by later age at diagnosis (>60 years), the adjusted OR estimates increased to 3.5 (95% CI, 1.4-8.8), and 3.2 (95% CI, 1.4-7.3) for tumors of higher grade and stage, respectively (results not shown). Among African Americans, IVS7delTTA was more common among individuals with no family history of cancer (OR, 2.0; 95% CI, 1.02-3.9). The ORs increased to 2.9 (95% CI, 1.2-7.2) and to 5.2 (95% CI, 1.1-25.7) for patients with lower-grade disease but with higher stage disease, respectively (Table 2). The effects of this genotype did not differ by age at diagnosis in African Americans (results not shown). No effect was noted for IVS7delTTA genotype in either racial group.

As shown in Table 2, the *MSR1* Arg293X nonsense mutation was significantly associated with high-grade disease and the absence of a family history of prostate cancer (OR, 4.0; 95% CI, 1.1-14.1) in European Americans. This effect was higher,

Table 2. Associations of susceptibility alleles in *ELAC2*, *MSR1*, and *RNASEL* and prostate cancer in European Americans

At-risk genotype	Family history of prostate cancer	Age- and race-adjusted ORs (95% CI) compared with controls				
		All cases	Low Gleason cases (Gleason < 7)	High Gleason cases (Gleason ≥ 7)	Encapsulated cases (TNM stages 1 and 2)	Extracapsular cases (TNM stages 3 and 4)
ELAC2, any ^{541}Thr	Total	1.3 (0.70-2.3)	1.1 (0.56-2.1)	1.6 (0.69-3.7)	1.4 (0.73-2.5)	0.64 (0.18-2.2)
	No	1.2 (0.64-2.4)	1.1 (0.52-2.4)	1.4 (0.53-3.8)	1.3 (0.66-2.6)	0.35 (0.05-2.7)
	Yes	1.6 (0.34-7.5)	1.3 (0.25-6.7)	2.4 (0.37-15.6)	1.6 (0.33-8.1)	1.5 (0.20-11.3)
ELAC2, ^{217}Leu/^{217}Leu	Total	1.1 (0.65-1.7)	1.1 (0.67-1.9)	1.3 (0.61-2.5)	1.1 (0.63-1.8)	1.02 (0.45-2.3)
	No	1.04 (0.60-1.8)	1.1 (0.57-2.0)	1.3 (0.60-3.0)	1.1 (0.61-2.0)	0.69 (0.20-2.4)
	Yes	0.98 (0.34-2.8)	1.2 (0.39-3.5)	0.96 (0.21-4.4)	0.96 (0.31-3.0)	1.3 (0.36-5.0)
MSR1, any IVS5-59A	Total	0.83 (0.52-1.1)	0.99 (0.60-1.6)	0.41 (0.16-1.1)	0.99 (0.61-1.6)	0.39 (0.13-1.1)
	No	1.0 (0.59-1.7)	1.2 (0.62-2.0)	0.66 (0.25-1.8)	1.2 (0.69-2.1)	0.38 (0.09-1.7)
	Yes	0.44 (0.17-1.1)	0.62 (0.23-1.6)	—	0.51 (0.19-1.4)	0.30 (0.06-1.5)
MSR1 IVS7deTTA/ IVS7delTTA	Total	1.4 (0.83-2.3)	2.0 (1.1-3.7)	1.1 (0.50-2.4)	2.0 (1.1-3.7)	0.77 (0.37-1.6)
	No	1.5 (0.84-2.7)	3.0 (1.3-6.9)	1.1 (0.45-2.6)	2.2 (1.1-4.4)	0.88 (0.34-2.3)
	Yes	0.93 (0.28-3.0)	0.92 (0.27-3.3)	0.81 (0.13-5.0)	1.2 (0.30-4.4)	0.55 (0.14-2.2)
MSR1, any −14, 742 G	Total	0.90 (0.28-2.9)	1.04 (0.30-3.3)	1.2 (0.23-6.4)	0.86 (0.25-3.0)	1.5 (0.29-8.0)
	No	1.0 (0.26-3.8)	0.98 (0.21-4.5)	1.9 (0.33-10.4)	1.1 (0.26-4.3)	1.4 (0.15-12.9)
	Yes	0.61 (0.05-7.0)	0.93 (0.08-10.7)	—	0.40 (0.02-6.5)	1.2 (0.07-20.0)
MSR1, any 293X	Total	0.80 (0.27-2.4)	0.18 (0.02-1.5)	2.8 (0.84-9.5)	0.93 (0.30-2.9)	—
	No	1.2 (0.37-3.8)	0.28 (0.03-2.4)	4.0 (1.11-14.1)	1.3 (0.40-4.5)	—
	Yes	—	—	—	—	—
RNASEL, any ^{462}Gln	Total	1.1 (0.84-1.5)	1.1 (0.83-1.6)	1.3 (0.83-2.0)	1.2 (0.85-1.6)	1.2 (0.74-2.0)
	No	1.4 (0.99-1.9)	1.5 (1.04-2.2)	1.3 (0.78-2.2)	1.5 (1.02-2.1)	1.4 (0.74-2.6)
	Yes	0.48 (0.24-0.95)	0.43 (0.21-0.88)	0.87 (0.32-2.4)	0.46 (0.22-0.95)	0.58 (0.24-1.4)
RNASEL, ^{462}Gln/^{462}Gln	Total	0.92 (0.62-1.4)	0.95 (0.61-1.5)	1.0 (0.57-1.9)	1.03 (0.68-1.6)	—
	No	0.99 (0.64-1.6)	0.97 (0.58-1.6)	1.2 (0.63-2.4)	1.01 (0.63-1.6)	1.1 (0.48-2.6)
	Yes	0.77 (0.32-1.9)	0.91 (0.36-2.3)	0.56 (0.14-2.3)	1.1 (0.42-2.6)	0.30 (0.06-1.5)

Abbreviation: TNM, tumor-node-metastasis.

although not statistically significant, in patients diagnosed before age 60 (OR, 4. 9; 95% CI, 0.7-36.8; results not shown). *RNASEL* ^{462}Gln was associated with disease severity in European American (Table 2) and African American (Table 3) men, and there were significant differences in this relationship by family history. In European Americans, a significant association of ^{462}Gln with lower-grade (OR, 1.5; 95% CI, = 1.04-2.2) and lower-stage disease (OR, 1.5; 95% CI, 1.02-2.1) was observed in individuals with no family history of cancer. In contrast, ^{462}Gln was less common in men with a family history of prostate cancer (OR, 0.48; 95% CI, 0.24-0.95). In this group, the ORs decreased to 0.43 (95% CI, 0.21-0.88) and 0.46 (95% CI, 0.22-0.95) for patients with lower-grade and earlier-stage disease, respectively (Table 2). In African Americans, however, there was a strong, but only marginally significant, association of ^{462}Gln with positive family history of any cancer (OR, 5.5; 95% CI, 1.0-30.0). This association increased significantly to 14.8 (95% CI, 1.6-136.0) for family history–positive subjects and higher-grade tumors, respectively (Table 3). We did not observe any effect for the homozygous ^{462}Gln genotype in either European American or African American men (Tables 2 and 3). There was also no association of *RNASEL* rare variants with prostate cancer.

Meta-analyses. We identified a number of statistically significant associations after undertaking meta-analyses to evaluate the cumulative relationship of specific variants in *MSR1* and *RNASEL* (Table 4). For *MSR1*, we identified significant associations of any IVS7delTTA, any −14,742 A>G, and any Arg293X with prostate cancer in European Americans with or without a family history of prostate cancer. Because of limited data, we were not able to replicate this result in strata defined by family history, although the association with any −14,742 A>G change was marginally significantly associated with prostate cancer risk in family history–positive disease (OR, 1.1; 95% CI, 0.98-2.4). The only significant association in African Americans was with any Asp174Tyr variant for the group that included both positive and negative family history studies (OR, 2.3; 95% CI, 2.1-3.4).

For *RNASEL*, we observed associations with any Arg462Gln variant in European Americans overall and in the no family history stratum. We also observed a significant association with the homozygous ^{462}Gln/^{462}Gln genotype in European Americans without regard to family history. In European Americans with a positive family history, however, Arg462Gln variant had a moderate opposite effect on prostate cancer risk (OR, 0.74; 95% CI, 0.48-1.0). The strongest association observed from the meta-analysis was among family history–positive European Americans who carried any Glu265X variant (OR, 4.3; 95% CI, 3.1-5.6).

Discussion

We have comprehensively evaluated the relationship of 16 variants in *ELAC2*, *RNASEL*, and *MSR1* and prostate cancer risk in a sample of European American and African American participants unselected for family history of prostate cancer. Our results do not support the hypothesis that *ELAC2*, *RNASEL*, and *MSR1* are associated with prostate cancer etiology overall. However, our results do suggest that *MSR1* and *RNASEL* may play a role in prostate cancer progression or severity.

Allelic Distributions

Our findings show that there are significant differences in the frequency of putative prostate cancer risk alleles in *ELAC2*, *MSR1*, and *RNASEL* by race. Our results also show that common alleles are more likely to be present in both European Americans and African Americans, whereas rare alleles are more commonly detected in a single racial group. *ELAC2* and *RNASEL* polymorphisms have not been substantially studied in African Americans. Thus, our research is the first to investigate the role of these genes in prostate cancer risk grouping the highest risk racial group for prostate cancer.

Overall, the allele frequencies we observed in our controls for *RNASEL* and *ELAC2* common polymorphisms for European Americans in the United States were similar to previously reported frequencies (Table 1; ref. 13, 14, 21, 25, 26, 39), although a higher frequency of *ELAC2* Ala541Thr has been reported (27). This consistency seems to hold despite the differences in study

Table 3. Associations of susceptibility alleles in *ELAC2*, *MSR1*, and *RNASEL* and prostate cancer in African Americans

At-risk genotype	Family history of prostate cancer	Age- and race-adjusted ORs (95% CI) compared with controls				
		All cases	Low Gleason cases (Gleason < 7)	High Gleason cases (Gleason ≥ 7)	Encapsulated cases (TNM stages 1 and 2)	Extracapsular cases (TNM stages 3 and 4)
ELAC2 ^{217}Leu/^{217}Leu	Total	0.34 (0.07-1.6)	0.34 (0.04-2.8)	0.64 (0.08-5.4)	—	1.2 (0.13-10.0)
	No	0.24 (0.03-2.1)	0.46 (0.05-4.0)	—	—	—
	Yes	0.49 (0.04-5.9)	—	1.1 (0.09-2.9)	—	4.9 (0.28-85.5)
MSR1, any ^{174}Tyr	Total	1.5 (0.36-6.1)	1.5 (0.26-8.3)	1.3 (0.14-12.0)	1.1 (0.19-6.1)	2.2 (0.23-21.1)
	No	1.6 (0.31-7.8)	0.96 (0.10-9.5)	2.4 (0.23-24.2)	1.5 (0.24-9.4)	3.3 (0.31-34.1)
	Yes	1.2 (0.07-20.7)	2.8 (0.15-54.2)	—	—	—
MSR1, any IVS-59A	Total	0.47 (0.05-4.6)	1.1 (0.11-10.7)	—	0.72 (0.07-7.1)	—
	No	1.6 (0.10-25.8)	3.2 (0.19-52.0)	—	2.4 (0.15-39.2)	—
	Yes	—	—	—	—	—
MSR1, IVS7delTTA/ IVS7delTTA	Total	1.7 (0.93-3.5)	2.0 (0.92-4.4)	1.5 (0.57-1.7)	1.8 (0.89-3.5)	3.8 (1.00-14.6)
	No	2.0 (1.02-3.9)	2.9 (1.2-7.2)	1.5 (0.49-4.7)	2.2 (1.00-4.8)	5.2 (1.1-25.7)
	Yes	0.75 (0.20-2.9)	0.38 (0.06-2.4)	0.81 (0.14-4.6)	0.69 (0.15-3.2)	2.5 (0.08-79.2)
MSR1, any −14,742 G	Total	0.95 (0.42-2.2)	0.85 (0.30-2.4)	0.25 (0.03-2.1)	0.84 (0.32-2.3)	1.2 (0.30-5.0)
	No	1.1 (0.45-2.8)	0.72 (0.21-2.4)	0.43 (0.05-3.7)	0.97 (0.33-2.9)	1.9 (0.43-8.5)
	Yes	0.59 (0.08-4.2)	1.3 (0.17-9.5)	—	0.50 (0.04-5.9)	—
RNASEL, any ^{462}Gln	Total	1.5 (0.78-2.8)	1.4 (0.63-3.1)	1.8 (0.70-4.8)	1.8 (0.89-3.6)	0.86 (0.22-3.3)
	No	1.1 (0.55-2.3)	0.99 (0.10-9.8)	0.88 (0.23-3.4)	1.6 (0.75-3.5)	0.68 (0.14-3.4)
	Yes	5.5 (1.00-30.0)	—	14.8 (1.6-136.0)	5.2 (0.67-40.3)	2.42 (0.14-40.7)
RNASEL, ^{462}Gln/^{462}Gln	Total	1.5 (0.29-7.4)	0.96 (0.10-9.4)	3.7 (0.58-23.9)	2.2 (0.43-11.4)	0.73 (0.35-1.5)
	No	1.1 (0.17-6.6)	—	2.7 (0.26-27.7)	1.6 (0.25-9.7)	—
	Yes	—	—	—	—	—

Abbreviation: TNM, tumor-node-metastasis.

Table 4. Meta-analyses of published associations: combined ORs and 95% CIs

Gene	Genotype	Family history positive or negative		Family history negative		Family history positive	
		Caucasian	African American	Caucasian	African American	Caucasian	African American
MSR1	Any IVS7 delTTA	1.4 (1.1-1.7)*,†,‡,§	1.1 (0.74-1.4)*,§	1.3 (0.80-1.7)*,‡	ISD	ISD	ISD
	Any −14,742 A > G	1.5 (1.2-1.8)*,†‡,§	ISD	ISD	ISD	1.7 (0.98-2.4)*,‡	ISD
	Any IVS5-59 C > A	1.2 (0.88-1.6)*,†	2.0 (0.61-3.4)*,†	ISD	ISD	ISD	ISD
	Any Arg293X	1.8 (1.2-2.4)*,†,‡,‖	ISD	0.95 (0.34-1.6)*,¶	ISD	1.2 (0.41-1.9) ‡,‖,¶	ISD
	Any Asp174Tyr	ISD	2.3 (2.1-3.4)*,†,§	ISD	ISD	ISD	ISD
RNASEL	Any Arg462Gln	1.3 (1.1-1.5)*,**,††	ISD	1.5 (1.2-1.8)*,¶	ISD	0.74 (0.48-1.0)*,¶,**	ISD
	^{462}Gln/^{462}Gln	1.4 (1.1-1.7) *,**,††	ISD	0.99 (0.70-1.3)*,¶	ISD	0.98 (0.60-1.4)*,¶,**	ISD
	Any Glu265X	1.2 (0.44-2.0)*,¶,‡‡,§§	ISD	ISD	ISD	4.3 (3.2-5.6)¶,‡‡,§§, NVO	ISD

NOTE: ISD, insufficient data to undertake meta-analysis (i.e., less than two published studies). NVO, no Glu265X variants observed in family history–positive subjects in the present sample.
*Rennert et al. (present study).
†Xu et al. (24).
‡Lindmark et al. (42).
§Miller et al. (40).
‖Seppala et al. (29).
¶Wang et al. (39).
**Rokman et al. (18).
††Casey et al. (21).
‡‡Chen et al. (19).
§§Carpten et al. (9).

design (i.e., hospital based versus population based versus family based) and in the populations of inference. Interestingly, Ala541Thr is very rare (<0.5%) among African Americans compared with European Americans, and therefore is unlikely to explain the higher rate of prostate cancer in the African American racial group unless a significant inverse association with disease were conferred by that allele.

Allele frequencies for common variants in *MSR1* (−14,742 A>G, IVS5-59 C>A, and IVS7delinsTTA) varied significantly from the frequencies reported for European American and African American men in other studies in the United States (24, 40). This discrepancy in allele frequencies may be due to heterogeneity between the populations studied, or small sample sizes used to estimate these frequencies in some studies. Differences in allele frequencies in controls among different studies were also noted for the *MSR1* Arg293X and Asp174Tyr polymorphisms which were the most common variants identified among American prostate cancer patients of European and African descent, respectively (10, 30, 40). In the present study, allele frequencies for additional eight very rare alleles in *ELAC2*, *RNASEL*, and *MSR1* fell in the range of 0% to 1%. These frequencies were in general agreement with the findings of other studies of U.S. populations. However, some differences were noted which may reflect differing racial backgrounds of the study populations. For example, *ELAC2* Glu622Val and *RNASEL* 471delAAAG polymorphisms are much more common in the original reports in Finnish and Ashkenazi Jewish men (18, 20) than in the present study sample.

Association Studies. The present study did not identify any association between the common *ELAC2* Ser217Leu and Ala541Thr sequence variants and prostate cancer. These results are in contrast to initial reports (8, 13) that noted an association with the ^{217}Leu and ^{541}Thr genotypes. Three meta-analyses regarding the *ELAC2* polymorphisms and prostate cancer risk have also been published. Severi et al. (38) found no evidence for an association of the two *ELAC2* SNPs and prostate cancer risk in a study compromised of 1,557 individuals in addition to data obtained from seven previously published studies. Meitz et al. (37) using seven published studies and their own results found only a moderate effect of the ^{541}Thr variant on prostate cancer risk with an OR of 1.27. Finally, Camp and Tavtigian (36) constructed a Mantel-Haenszel meta-analysis of six studies. This summary analysis was not consistent with their original findings that ^{217}Leu homozygotes are at increased risk for prostate cancer. Instead, ^{217}Leu showed evidence only for a codominant effect, whereas the effect of ^{541}*Thr* allele on prostate cancer risk depended on the choice of cases and controls. The most significant results were observed in men with familial prostate cancer versus low-risk (family history negative) men. This effect decreased as the case-control comparison broadened to include a wider range of study subjects (36). Because the present sample set was unselected for a family history of prostate cancer and our set included few if any hereditary prostate cancer families, the present finding of no association of *ELAC2* alleles and prostate cancer is consistent with the previous meta-analyses.

The results of this study are also different from the previous findings by our group (13). The cause for this discrepancy is unlikely related to population stratification because the effects were previously seen in a largely Caucasian population in which population stratification is unlikely to result in biased effect estimates (41). New genotype data were generated for the entire sample set, but a careful comparison of the original genotypes published by Rebbeck et al. (13) and the genotype data used in the present analysis showed a very low rate of genotype inconsistency between the two approaches (<1.0% inconsistency among 828 subjects genotyped by both methods). Therefore, it is unlikely that genotype error could explain the difference in the results. Moreover, the present analysis used all of the study subjects previously reported by Rebbeck et al. (13) in addition to new participants. With the larger sample size reported here, *ELAC2* is unlikely to be associated with prostate cancer in our sample of men who are not members of hereditary prostate cancer families. This notion is also supported by recent functional studies that have not observed meaningful differences in the 3′-tRNase activity between the wild-type *ELAC2* and the variant enzymes that harbor the ^{217}Leu or the ^{541}Thr substitutions (11).

For *MSR1*, IVS7delTTA homozygous genotype was significantly associated with family history–negative cancer and lower-grade disease, and this effect was greater in European

Americans with a later age at diagnosis. The association of this variant with disease stage, however, varied by racial group. In contrast, Arg293X nonsense mutation in *MSR1* was associated with high-grade disease in a subset of family history–negative European Americans.

Our study did not find significant association of IVS7-delTTA and prostate cancer. Previous studies in European Americans (10) and African Americans (40) also did not detect any association of IVS7delTTA with prostate cancer. However, no studies were done to investigate the association of *MSR1* genotypes with clinicopathologic characteristics of the disease. Interestingly, all three studies show that IVS7delTTA is more common in cases compared with controls in both racial groups, but this effect was not statistically significant.

Our results were consistent with other studies that have not detected any association of Arg293X with prostate cancer risk (29, 30, 42). We did observe a significant association of Arg293X with high Gleason score in a subset of family history–negative patients, which, although not statistically significant, did tend to be higher in cases with an earlier age at diagnosis (<60 years). Other studies also showed a significantly lower age at diagnosis among Arg293X carriers compared with noncarriers (29, 30). In these studies, however, this effect was primarily associated with a family history of prostate cancer or hereditary prostate cancer. On the other hand, no effect was seen for Asp174Tyr, the most commonly reported rare mutation in *MSR1* among African Americans. In our sample, this variant was more common among prostate cancer patients compared with controls by ~30%. This, however, is in contrast to the 2-fold and over 6-fold differences in mutation frequencies between cases and controls reported in two previous studies (24, 40). Homozygosity for rare *MSR1* sequence variants has been observed for both Arg293X (this study) and Asp174Tyr (40). However, whereas Asp174Tyr homozygous carriers were affected with the disease, the Arg293X homozygous carrier in our study was a 77-year-old control individual. Our results do not support a major role for *MSR1* in the causation of prostate cancer. However, certain *MSR1* sequence variants may influence prostate cancer severity or progression. This notion is also supported by recent immunohistochemical studies that have observed a higher *MSR1* protein expression in antigen presenting cells in inflamed and prostatic intraepithelial neoplasia lesions compared with normal tissue. In contrast, the number of MSR1-positive cells decreased with tumor progression and was significantly associated with higher clinical stage and positive lymph nodes (11).

Interestingly, although deletions of chromosome 8p22-23 are implicated in prostate cancer preferential loss of wild-type allele in prostate tumors of Arg293X carriers has not been reported.

For *RNASEL*, Arg462Gln exhibited a strong association with disease severity, which was significantly affected by family history of prostate cancer and by racial group. In European Americans without a family history, Arg462Gln was associated with an increased risk for early-stage and low-grade disease, whereas in family history–positive individuals, this variant conferred a significant decrease in risk of the less aggressive forms of the disease. In African Americans, however, Arg462Gln showed a very strong association with a positive family history and high-grade prostate tumor.

Inconsistent effects of Arg462Gln in different populations are well documented. Wang et al. (39) clearly showed an inverse association of the more common variant (^{462}Arg) with familial prostate cancer. Subset analysis showed that this association was observed in the younger group with the less aggressive tumors (39). Wang et al., however, did not find any association between Arg462Gln and cases with no family history of prostate cancer (39). A protective effect for the less common genotype was also observed in Japanese men affected with familial prostate cancer (43). In contrast, Rokman et al. reported a positive association of Arg462Gln with hereditary prostate cancer in the Finnish population and no association in patients unselected for family history of prostate cancer (18). A positive effect of this variant was also noted by Casey et al. (21) in a family-based case-control study; however, associations in this study were not stratified by family history of prostate cancer (21). Explanation of these inverse effects is difficult. Wang et al. has suggested that this gene may be a common modifier of other rare susceptibility genes, explaining its effect in familial but not sporadic prostate cancer (39). It also may be that the Arg462Gln variant is in linkage disequilibrium with another undetected polymorphism or unidentified gene close by, and that this effect varies by family history and racial group. Therefore, whereas there is some evidence that *RNASEL* may play a role in prostate cancer etiology, the conflicting literature make it difficult to infer a biologically plausible role of this gene from the association study data.

Interestingly, we have not seen any effect for *RNASEL* rare variants, although the allele frequency of the *RNASEL* Glu265X nonsense mutation was 3-fold higher in cases compared with controls in our sample. Thus, this rare variant may be associated with risk but a much larger study will be required to detect this effect. This variant is more common in the Finnish population and was found associated with risk in families with four or more members affected with prostate cancer (18). It was also noted that the median age at disease onset for Glu265X was 11 years less than that for noncarriers in the same families (18). Despite these findings, Glu265X did not completely cosegregate with affected status in several families in two separate studies (18, 19).

Taken together, these data provide only limited support for the hypothesis that *RNASEL* plays a major role in prostate cancer causation, although specific variants, particularly Arg462Gln, may be involved in prostate cancer etiology outside the hereditary prostate cancer setting. This is also supported by functional studies that showed that *RNASEL* Gln^{462} variant had reduced ability to dimerize into its catalytic active form and 3-fold decrease in RNase activity (21, 44). Furthermore, this variant was deficient in causing apoptosis in a mouse RNase $L^{-/-}$ cell line. In contrast, other missense substitutions examined, including Ile97leu, produced similar levels of RNase activity as wild-type enzyme, further supporting the role of Arg462Gln in apoptosis and possibly prostate cancer (44).

Several strengths and limitations should be considered when interpreting the results presented here. Both prostate cancer cases and control study subjects were recruited according to standard selection criteria in a single health system that draws from a wide geographic area in the Philadelphia metropolitan area. Consistent medical records obtained from this health system also allowed consistent diagnostic data from cases and systematic evaluation of prostate cancer absence in controls. Another strength of our approach was the use of high throughput validated genotyping techniques for gathering large amounts of data with a relatively high call rate and accuracy when compared with manual methods. Substantial genotype validation steps, including large-scale duplicate genotyping using multiple assay methods (e.g., for *ELAC2*) were undertaken to ensure genotype fidelity. Replicate studies showed that concordance of genotype calls for the six common variants between the two replicates of the same sample was 1.6%. There was no disconcordance noted for the rare variants. In a recent study done by Applied Biosystems, it was found that the concordance of genotype calls between the SNPlex Genotyping System and the Taqman minor groove binder probe-based assay was 98.8% (45). Therefore, PCR-related genotyping errors may account for ~1% of the total calls. It is unlikely, however, that such a low prevalence of possible miscalls could have substantially affected the results for the

common variants. Thus, whereas genotyping error is low, it was also nondifferential with respect to case status; hence, its likely effect was a small bias in some associations toward the null hypothesis.

A limitation of the present study was low statistical power to detect effects and the number of hypothesis tests conducted here. This problem was particularly evident in African Americans and for some analyses where relatively large OR effects were observed but for which statistical significance was not achieved. Similarly, we were unable to detect significant associations of rare polymorphisms with prostate cancer even in European Americans where the sample size was significantly larger. Type I errors may also have affected our inferences. The present study is confirmatory in that we report only on previously identified genetic variants in three candidate genes. To limit the potential for type I error, we have under taken a study with a relatively large sample size and focused on biologically based hypothesis tests. Supporting the validity of our findings, we have identified associations that were consistent and in the same direction among strata stratified by family history in each population and made our inferences about meaningful associations only when supported by meta-analyses of all associations reported in the literature.

We did meta-analyses of *RNASEL* and *MSR1* to provide a more comprehensive interpretation of the data published to date. The summary analyses of IVS7delTTA, −14,742 A>G, Arg293X, and Asp174Tyr in *MSR1* and Arg462Gln and Glu265X in *RNASEL* support the hypothesis that carriers of these alleles are at increased risk for prostate cancer in one or more age- or family history–specific groups. In some cases, these summary analyses were not consistent with the present results. Most of the meta-analytic summary ORs that provided inconsistent inferences with our data were small. Therefore, it is possible that our individual study may have been inadequately powered to detect these effects. The present analysis and the meta-analysis consistently indicate that *MSR1* IVS5-59 C>A is not associated with prostate cancer risk. For *MSR1*, the meta-analysis results support a significant association of any IVS7delTTA, any −14,742 A>G, and any Arg293X with prostate cancer in European Americans with or without a family history of prostate cancer. *MSR1* Asp174Tyr showed the only significant association with prostate cancer in African Americans overall. The positive effects of these variants were weaker in strata defined by family history. For *RNASEL*, Arg462Gln showed strong positive association in European Americans overall, which increased in family history–negative men and in men homozygous for ^{462}Gln without regard to family history. The meta-analysis, however, was consistent with a marginally significantly negative effect of this variant in the family history–positive stratum, as reported by some groups. Finally, it is important to note that the meta-analyses conducted are limited because there are still relatively few studies in specific family history and racial groups that can be summarized.

Additional support for the association between prostate cancer and Arg462Gln is provided by the SIFT analysis. This analysis showed that Arg at position 462 in *RNASEL* was predicted intolerant not only to Gln but also to the majority of amino acid substitutions, suggesting that most changes in this location would affect the protein function. This is also in agreement with functional studies that showed that Arg462Gln was associated with a major decrease in RNASEL activity (21, 44). Thus, the combination of meta-analysis and SIFT results provides further support for the importance of this variant in prostate cancer. SIFT results do not support the hypothesis that remaining eight *RNASEL*, *MSR1*, and *ELAC2* substitutions examined in this study are functionally important. This result is largely in agreement with our study results. This data is supported by recent analysis results that showed that the degree of cancer risk influenced by a particular SNP in an association study was significantly associated with the degree of evolutionary conservation of the amino acid (46). It is important to note, however, that SIFT analysis results are limited by the sequence information available in public databases. In our analysis the number of homologues sequences used in the SIFT alignment ranged from 6 to 40. As protein databases grow with sequencing results from more organisms, a larger number of homologues sequences will become available, and SIFT prediction may become more accurate.

Summary

We have interpreted the results of our data in the context of the published literature and SIFT analysis. Based on these data, it does not seem that *ELAC2* is associated with prostate cancer outside the context of hereditary prostate cancer families. For *MSR1*, the present data are consistent with the meta-analyses that IVS7delTTA and Arg293X are associated with prostate cancer risk in Caucasians. For *RNASEL*, the present data are consistent with our meta-analysis and SIFT that indicates an association of *RNASEL* Arg462Gln and prostate cancer risk. This association however varies depending on family history of prostate cancer. In addition, the present results suggest that Arg462Gln may influence disease severity, and that this effect possibly varies by family history of cancer and by racial background. Taken together, these results suggest that *MSR1* and *RNASEL* are low-penetrance prostate cancer susceptibility genes that also seem to play a role in disease progression and severity. The present results also warrant further large-scale studies to confirm the role of these genetic variants, particularly to confirm the role of rare variants in these genes in prostate cancer risk.

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References

1. Hsing AW, Tsao L, Devesa SS. International trends and patterns of prostate cancer incidence and mortality. Int J Cancer 2000;85:60–7.
2. Pienta KJ, Esper PS. Is dietary fat a risk factor for prostate cancer? Risk factors for prostate cancer. J Natl Cancer Inst 1993;85:1538–40.
3. Quinn M, Babb P. Patterns and trends in prostate cancer incidence, survival, prevalence and mortality. Part II. Individual countries. BJU Int 2002;90:174–84.
4. Carter BS, Beaty TH, Steinberg GD, et al. Mendelian inheritance of familial prostate cancer. Proc Natl Acad Sci U S A 1992;89:3367–71.
5. Ghadirian P, Howe GR, Hislop TG, et al. Family history of prostate cancer: a multi-center case-control study in Canada. Int J Cancer 1997;70:679–81.
6. Gronberg H, Damber L, Damber JE. Studies of genetic factors in prostate cancer in a twin population. J Urol 1994;152:1484–7; discussion 1487–9.
7. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer: analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 2000;343:78–85.
8. Tavtigian SV, Simard J, Teng DH, et al. A candidate prostate cancer susceptibility gene at chromosome 17p. Nat Genet 2001;27:172–80.
9. Carpten J, Nupponen N, Isaacs S, et al. Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. Nat Genet 2002;30:181–4.
10. Xu J, Zheng SL, Komiya A, et al. Germline mutations and sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. Nat Genet 2002;32:321–5.
11. Takaku H, Minagawa A, Takagi M, et al. A candidate prostate cancer susceptibility gene encodes tRNA 3′ processing endoribonuclease. Nucleic Acids Res 2003;31:2272–8.
12. Korver W, Guevara C, Chen Y, et al. The product of the candidate prostate cancer susceptibility gene ELAC2 interacts with the γ-tubulin complex. Int J Cancer 2003;104:283–8.

13. Rebbeck TR, Walker AH, Zeigler-Johnson C, et al. Association of HPC2/ELAC2 genotypes and prostate cancer. Am J Hum Genet 2000;67:1014–9.
14. Stanford JL, Sabacan LP, Noonan EA, et al. Association of HPC2/ELAC2 polymorphisms with risk of prostate cancer in a population-based study. Cancer Epidemiol Biomarkers Prev 2003;12:876–81.
15. Takahashi H, Lu W, Watanabe M, et al. Ser217Leu polymorphism of the HPC2/ELAC2 gene associated with prostatic cancer risk in Japanese men. Int J Cancer 2003;107:224–8.
16. Zhou A, Hassel BA, Silverman RH. Expression cloning of 2-5A-dependent RNAase: a uniquely regulated mediator of interferon action. Cell 1993;72:753–65.
17. Zhou A, Paranjape J, Brown TL, et al. Interferon action and apoptosis are defective in mice devoid of 2′,5′-oligoadenylate-dependent RNase L. Embo J 1997;16:6355–63.
18. Rokman A, Ikonen T, Seppala EH, et al. Germline alterations of the RNASEL gene, a candidate HPC1 gene at 1q25, in patients and families with prostate cancer. Am J Hum Genet 2002;70:1299–304.
19. Chen H, Griffin AR, Wu YQ, et al. RNASEL mutations in hereditary prostate cancer. J Med Genet 2003;40:e21.
20. Rennert H, Bercovich D, Hubert A, et al. A novel founder mutation in the RNASEL Gene, 471delAAAG, is associated with prostate cancer in Ashkenazi Jews. Am J Hum Genet 2002;71:4.
21. Casey G, Neville PJ, Plummer SJ, et al. RNASEL Arg462Gln variant is implicated in up to 13% of prostate cancer cases. Nat Genet 2002;32:581–3.
22. Platt N, Gordon S. Is the class A macrophage scavenger receptor (SR-A) multifunctional?: the mouse's tale. J Clin Invest 2001;108:649–54.
23. Lieberfarb ME, Lin M, Lechpammer M, et al. Genome-wide loss of heterozygosity analysis from laser capture microdissected prostate cancer using single nucleotide polymorphic allele (SNP) arrays and a novel bioinformatics platform dChipSNP. Cancer Res 2003;63:4781–5.
24. Xu J, Zheng SL, Komiya A, et al. Common sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. Am J Hum Genet 2003;72:208–12.
25. Xu J, Zheng SL, Carpten JD, et al. Evaluation of linkage and association of HPC2/ELAC2 in patients with familial or sporadic prostate cancer. Am J Hum Genet 2001;68:901–11.
26. Wang L, McDonnell SK, Elkins DA, et al. Role of HPC2/ELAC2 in hereditary prostate cancer. Cancer Res 2001;61:6494–9.
27. Vesprini D, Nam RK, Trachtenberg J, et al. HPC2 variants and screen-detected prostate cancer. Am J Hum Genet 2001;68:912–7.
28. Rokman A, Ikonen T, Mononen N, et al. ELAC2/HPC2 involvement in hereditary and sporadic prostate cancer. Cancer Res 2001;61:6038–41.
29. Seppala EH, Ikonen T, Autio V, et al. Germ-Line alterations in MSR1 gene and prostate cancer risk. Clin Cancer Res 2003;9:5252–6.
30. Wang L, McDonnell SK, Cunningham JM, et al. No association of germline alteration of MSR1 with prostate cancer risk. Nat Genet 2003;35:128–9.
31. Walker AH, Najarian D, White DL, et al. Collection of genomic DNA by buccal swabs for polymerase chain reaction-based biomarker assays. Environ Health Perspect 1999;107:517–20.
32. O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 1998;63:259–66.
33. Levene H. On a matching problem arising in genetics. Ann Math Stat 1949;20:91–4.
34. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. Genome Res 2001;11:863–74.
35. Ng PC, Henikoff S. Accounting for human polymorphisms predicted to affect protein function. Genome Res 2002;12:436–46.
36. Camp NJ, Tavtigian SV. Meta-analysis of associations of the Ser217Leu and Ala541Thr variants in ELAC2 (HPC2) and prostate cancer. Am J Hum Genet 2002;71:1475–8.
37. Meitz JC, Edwards SM, Easton DF, et al. HPC2/ELAC2 polymorphisms and prostate cancer risk: analysis by age of onset of disease. Br J Cancer 2002;87:905–8.
38. Severi G, Giles GG, Southey MC, et al. ELAC2/HPC2 polymorphisms, prostate-specific antigen levels, and prostate cancer. J Natl Cancer Inst 2003;95:818–24.
39. Wang L, McDonnell SK, Elkins DA, et al. Analysis of the RNASEL gene in familial and sporadic prostate cancer. Am J Hum Genet 2002;71:116–23.
40. Miller DC, Zheng SL, Dunn RL, et al. Germ-line mutations of the macrophage scavenger receptor 1 gene: association with prostate cancer risk in African-American men. Cancer Res 2003;63:3486–9.
41. Wacholder S, Rothman N, Caporaso N. Population stratification in epidemiologic studies of common genetic variants and cancer: quantification of bias. J Natl Cancer Inst 2000;92:1151–8.
42. Lindmark F, Jonsson BA, Bergh A, et al. Analysis of the macrophage scavenger receptor 1 gene in Swedish hereditary and sporadic prostate cancer. Prostate 2004;59:132–40.
43. Nakazato H, Suzuki K, Matsui H, et al. Role of genetic polymorphisms of the RNASEL gene on familial prostate cancer risk in a Japanese population. Br J Cancer 2003;89:691–6.
44. Xiang Y, Wang Z, Murakami J, et al. Effects of RNase L mutations associated with prostate cancer on apoptosis induced by 2′,5′-oligoadenylates. Cancer Res 2003;63:6795–801.
45. Short S. SNPlex™ system: a novel high-throughput genotyping system utilizing capillary electrophoresis detection platforms. In: SNPs, haplotypes, and cancer: application in molecular epidemiology: abstracts for the American Association for Cancer Research: p. B32, 2003.
46. Zhu Y, Spitz MR, Amos CI, et al. An evolutionary perspective on single-nucleotide polymorphism screening in molecular cancer epidemiology. Cancer Res 2004;64:2251–7.