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Contribution of *HPC1* (*RNASEL*) and *HPCX* variants to prostate cancer in a founder population

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Abstract

Background—Prostate cancer is a genetically complex disease with locus and disease heterogeneity. The *RNASEL* gene and *HPCX* locus have been implicated in hereditary prostate cancer; however, their contributions to sporadic forms of this malignancy remain uncertain.

Methods—Associations of prostate cancer with two variants in the *RNASEL* gene (a founder mutation, 471delAAAG, and a non-synonymous SNP, rs486907), and with five microsatellite markers in the *HPCX* locus, were examined in 979 cases and 1,251 controls of Ashkenazi Jewish descent. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression models.

Results—There was an inverse association between *RNASEL* rs486907 and prostate cancer in younger men (<65 years) and those with a first-degree relative with prostate cancer; men with AA genotype had ORs of 0.64 and 0.47 (both $p<0.05$), respectively, in comparison to men with GG genotype. Within the *HPCX* region, there were positive associations for allele 135 of bG82i1.1 marker (OR=1.77, p=0.01) and allele 188 of DXS1205 (OR=1.65, p=0.02). In addition, allele 248 of marker D33 was inversely associated (OR=0.65, p=0.05) with Gleason score ≥7 tumors.

Conclusions—Results suggest that variants in *RNASEL* contribute to susceptibility to early onset and familial forms of prostate cancer, whereas *HPCX* variants are associated with prostate cancer risk and tumor aggressiveness. The observation that a mutation predicted to completely inactivate RNASEL protein was not associated with prostate cancer, but that a missense variant was associated, suggests that the effect is due to either partial inactivation of the protein, and/or acquisition of a new protein activity.

Introduction

Prostate cancer remains the most commonly diagnosed solid tumor and the second leading cause of cancer deaths among men in the United States [1]. It is a multi-factorial complex disease where both genetic and environmental factors contribute to its incidence [2]. One of the strongest risk factors for prostate cancer is family history; having a first-degree relative

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diagnosed with prostate cancer is associated with a two- to three-fold elevation in the relative risk [3, 4], and both early age at diagnosis and multiple affected family members are strong predictors of risk in relatives. These results suggest an important inherited component to disease risk. However, deciphering the underlying genetic basis of this disease has been enigmatic [5–7].

Linkage analyses studies of hereditary prostate cancer (HPC) families have identified several candidate susceptibility loci for HPC including *HPC1* at 1q24-31 [8], and *HPCX* at Xq27-28 [9]. Further positional cloning of the 1q24-25 region provided evidence that the *RNASEL* gene, located on 1q25, was a strong candidate gene for prostate cancer susceptibility linked to the *HPC1* region [10]. This gene encodes RNase L- a latent endoribonuclease-, which mediates the antiviral and proapoptotic activity of the interferon-inducible 2–5A system, and has been shown to play a role in regulating cell proliferation and apoptosis [11–13]. Since the initial discovery of the prostate cancer association with the *HPC1* region and the *RNASEL* gene, several studies have examined associations between genetic markers in these candidate gene/loci and hereditary, as well as sporadic prostate cancer [10, 14–39]. However, despite the initial promising results that *RNASEL* was an important prostate cancer susceptibility gene, the studies mentioned above examining associations between either mutations or single nucleotide polymorphisms (SNPs) in this gene have provided conflicting evidence. Similarly discordant results were also found for the *HPCX* prostate cancer susceptibility region located at Xq27-28 [9, 40–44]. These inconsistent results could have been due in part to 1) the relatively small sample sizes of some studies, with limited power to detect modest associations, 2) potentially false-positive findings, 3) the genetic heterogeneity of prostate cancer, or 4) classification errors in discriminating between hereditary, familial, and sporadic forms of prostate cancer.

Of the above studies, only a few have examined associations between prostate cancer and genetic variants in the *RNASEL* gene in a founder population such as Ashkenazi Jews [15, 20, 28, 30, 32], and all of these studies had a relatively small sample size (<400 prostate cancer patients). Moreover, no studies have examined associations between prostate cancer risk and genetic markers in the *HPCX* region in this founder population. Thus, the goal of the current study was to examine associations between prostate cancer risk and the founder frameshift mutation 471delAAAG and a non-synonymous SNP (rs486907, Arg462Gln) in *RNASEL,* as well as five polymorphic microsatellite (STR) markers in the *HPCX* region, in a large case-control study of 2,230 Ashkenazi Jewish men. Furthermore, we were interested in examining whether associations varied by age at diagnosis, family history of prostate cancer, or histopathological characteristics of the tumors.

Materials and Methods

Study Population

Prostate cancer cases (n=979) and controls (n=1,251) were Ashkenazi Jewish men recruited from the general population as previously described [45]. Briefly, from 1998 through 2005, study participants were mailed informed consent forms, questionnaires, and materials to obtain a DNA sample. Informed consent and study materials were received from 1,014 self-reported cases of prostate cancer and 1,270 men without a history of prostate cancer (controls). However, the final study sample consisted of 979 prostate cancer cases and 1,251 controls, who satisfied the criteria of having both parents of Ashkenazim descent, completing a self-administered questionnaire, and providing a DNA sample.

Cases were diagnosed with adenoma-carcinoma of the prostate between 1978 and 2005 (mean and median year of diagnosis was 1996). Clinical information on Gleason score, as well as the extent of disease based on presence of tumor invasiveness, tumor present at

resection margins, prostate capsule invasion, seminal vesicle involvement, and lymph node involvement, was obtained from pathology reports of prostate biopsies or from surgery of men who underwent radical prostatectomy; records were available on 902 (92%) of the 979 cases. All participants completed a 40-page questionnaire and donated a DNA sample (i.e., buccal cells and/or a blood sample). The 40-page questionnaire included detailed questions regarding personal, demographic, and lifestyle habits, detailed medical history, family history of prostate cancer and other cancers, information on prostate cancer screening, and information about prostate cancer diagnosis. The study protocols and materials were approved by the Institutional Review Board of the Albert Einstein College of Medicine, and written informed consent was obtained from all study participants. Men were informed and consented that results of genetic testing would not be available to them at the end of the study.

DNA Isolation

Participants were sent self-contained kits of blood collection materials for obtaining a blood sample at their next health care appointment, a buccal cell collection kit consisting of four to six Dacron swabs, and/or a mouthwash collection kit consisting of a 44 ml bottle of Scope brand mouthwash and two 25 ml screw top collection tubes. Genomic DNA was isolated as previously described (45).

Genotyping Assays

PCR and genotyping of *RNASEL* variants—DNA samples were screened by Pyrosequencing (Pyrosequencing AB, Uppsala, Sweden, www.pyrosequencing.com) for the presence of two RNASEL variants, SNP rs486907 (Arg462Gln) and the deletion 471delAAAG (primer sequences are available from R. D. Burk).

The presence of each PCR product was confirmed by ethidium bromide-stained agarose gel electrophoresis, and the products were submitted to the Albert Einstein College of Medicine Sequencing Facility (Bronx, NY) for pyrosequencing. For quality control, each 96-well reaction plate contained a sequence-verified positive control for each of the expected alleles and a negative (water) control. In addition, 5–10% of all of the samples were genotyped in duplicate.

***HPCX* Simple Tandem Repeat (STR) Marker Analysis—**Genotyping was performed on five STR markers in the HPCX region (Xq27-28) of the X chromosome as previously described [46]. Microsatellite STR marker bG82i1.0 (B270) is an ATTT repeat whose alleles range in size from 210 to 226 bp. Microsatellite STR marker bG82i1.1 (B97) is a CA repeat whose alleles range in size from 131 to 153 bp. Microsatellite DXS1205 is a CA repeat whose alleles range in size from 160 to 198 bp. Microsatellite STR marker, D3S2390 (D33) is an ATCT repeat whose alleles range in size from 244 to 272 bp. Microsatellite STR marker, DXS984 (D60) is a CA repeat whose alleles range in size from 165 to 189 bp. All forward primers (Integrated DNA Technologies) were ordered in duplicate, one modified with a Cy5 fluorescent label on the 5′ end and one without. For each reaction, a mixture of 30% Cy5-labeled forward primer and 70% non-labeled forward primer was used for optimal detection (primer sequences are available from R. D. Burk).

Microsatellite STR markers B270, B97, D33, and D60 were amplified simultaneously in a multiplex PCR with a final volume of 15 μl consisting of DNA (10 ng), dNTP (0.2 mM each; Invitrogen), $MgCl_2$ (2.5 mM), AmplitaqGold polymerase (0.02 U; Perkin-Elmer), 1× reaction buffer, and 1.0 μM, 0.75 μM, 0.04 μM, and 0.1 μM of each primer set, respectively. The DXS1205 microsatellite was amplified individually in a final volume of 15

μl consisting of DNA (10 ng), dNTP (0.2 mM each; Invitrogen), $MgCl_2$ (2.5 mM), 1.0 μM each primer, AmplitaqGold polymerase (0.02 U; Perkin-Elmer), and 1× reaction buffer.

Following amplification, the samples were concentrated by heating uncovered at 95 °C for 3 min. Thereafter, 1.5 μl of the PCR product was then combined with 1.5 μl of a loading dye containing three Cy5.5-labeled size standards (100, 200, and 300 bp; Visible Genetics, Inc.) to serve as internal size standards for each lane of the gel. The PCR products were then resolved on a 6% denaturing polyacrylamide gel in a 1× TBE buffer using a Micro-Gene Clipper sequencer (Visible Genetics, Inc.). The areas under all of the resulting microsatellite marker peaks were measured, and subsequent calculations to determine the allele number were performed using Gene Objects 3.1 software (Visible Genetics, Inc.). To ensure consistency, each gel (16 lanes per gel) was run with a sequence-verified positive control.

Statistical Analysis

The deviations of genotype frequencies for both *RNASEL* and *HPCX* genetic variants from the Hardy-Weinberg Equilibrium among controls were assessed by chi-square tests. Unconditional logistic regression models were used to examine associations between genetic variants of *RNASEL* and *HPCX* and prostate cancer and to compute odds ratios (ORs) and 95% confidence intervals (CIs) [47]. For the *RNASEL* frameshift mutation 471delAAAG, subjects with this founder mutation were grouped and the risk of prostate cancer was calculated in comparison to non-mutation carriers. For the *RNASEL* SNP rs486907, a non-synonymous SNP resulting in the substitution of arginine for glutamine at position 462 (Arg462Gln), we examined associations between prostate cancer and each genotype separately; the most commonly observed genotype among controls was used as the reference category. In addition, we examined risk of prostate cancer associated with *RNASEL* rs486907 in recessive and dominant modes of inheritance. A test for trend was used to examine the risk of prostate cancer associated with 0, 1, and 2 copies of the *RNASEL* rs486907 minor A allele and p-values for trend were calculated from logistic regression models.

Using chi-square tests, we assessed global associations between prostate cancer and five *HPCX* region micro-satellite polymorphic markers, B270, B97, DXS1205, D33, and D60, which spans a 330-kb region on Xq27-28. These *HPCX* microsatelite markers were selected based on a previous case-control study that showed an association of prostate cancer with these markers [46]. For each *HPCX* STR marker, allele frequencies were computed for cases and controls, and associations between each allele with a frequency of 2% or higher and prostate cancer were assessed by unconditional logistic regression models. In these models, each allele was compared to the grouped other alleles in the same STR marker. For the global association tests, alleles of microsatellite markers with a frequency less than 2% were grouped together; associations between these alleles and prostate cancer were not assessed separately.

Associations between genetic variants in *RNASEL* and *HPCX* and prostate cancer were adjusted for age at diagnosis (cases) and age at study participation (controls). Additional adjustment for first-degree family history of prostate cancer and prostate specific antigen (PSA) test or digital rectal examination (DRE) screening did not change the risk estimates of genotypes by 15% or higher (considered as confounding); thus, the final models were adjusted only for age. In addition, associations between genetic variants in *RNASEL* and *HPCX* and prostate cancer were examined in strata defined by age at diagnosis (<65 vs. ≥65 years; age 65 was used as cutoff point since this was the average age at diagnosis in our study population), and by first-degree family history of prostate cancer (yes vs. no). To test effect modification, interaction terms between variants in the *RNASEL* genes or *HPCX* markers and age (<65 vs. ≥65 years) or first-degree family history of prostate cancer were

included in logistic regression models containing the main effects. The log likelihood of reduced models with main effects only were compared with the log likelihood of fully saturated models that also contained the interaction terms, using a likelihood ratio test to determine statistical significance [48].

Associations between genetic variants in the *RNASEL* gene and the *HPCX* region and prostate cancer were also examined according to histopathological features of prostate cancer, including Gleason score and a composite measure of disease aggressiveness. Gleason scores were obtained from either biopsy or from surgical pathology reports. For these analyses, prostate cancer cases were grouped into two strata: those with Gleason scores of 2–6 and those with Gleason scores of 7–10. Aggressive prostate cancer was characterized as either having a Gleason score ≥7 or at least two of the following characteristics documented on the pathology report: presence of tumor invasiveness, tumor present at resection margins, prostate capsule invasion, seminal vesicle involvement, and lymph node involvement. The frequency of alleles/genotypes in each group of cases (i.e., those with high (7–10) or low (2–6) Gleason score cancers) were compared to the frequency of alleles/genotypes among controls using polytomous logistic regression models [49]. SAS version 9.1 (SAS Institute, Cary NC) and STATA version 9 (STATA Corporation, College Station, TX) were used for statistical analyses.

Results

Table 1 gives characteristics of 979 prostate cancer cases and 1,251 controls genotyped for the study. Cases were slightly older at study participation compared to controls (average age of 69.4 vs. 68.3, respectively, p=0.01) and were more likely to report a first-degree family history of prostate cancer (28% vs. 14%, respectively, p<0.0001). However, there were no differences in educational attainment or lifestyle characteristics between cases and controls. The majority of cases (95.5%) and controls (98.8%) had undergone PSA testing or DRE screening for prostate cancer. The average age at diagnosis for prostate cancer cases was 64.5 years, and the majority of cases were diagnosed with prostate cancer because of an abnormal PSA or DRE test (85.2%). Most cases had a Gleason score of 2–6, 24.8% had a Gleason score of 7, and 11.7% had Gleason score 8–10; approximately 50% of the cases were classified as having aggressive prostate cancer (Table 1).

RNASEL variants and prostate cancer

We examined associations between prostate cancer and two *RNASEL* variants: the founder frameshift mutation 471delAAAG (15) and a non-synonymous SNP, rs486907, at position 462 (Arg462Gln). Although the associations between these two *RNASEL* variants and overall prostate cancer risk was not significant (Table 2), when the data were stratified by age at diagnosis (Table 3A), there was an inverse association between *RNASEL* SNP rs486907 and prostate cancer in men less than 65 years of age (OR = 0.64, 95% CI 0.41 – 1.00) when comparing men with the AA vs. GG genotype. There was also a significant trend of decreasing risk with increasing number of A alleles (p-value = 0.048). By contrast, among men aged 65 or older there was no association between rs486907 and prostate cancer (Table 3A).

When data were stratified by family history of prostate cancer, among Ashkenazi Jewish men with an affected first-degree relative, there was a significant trend of decreasing risk of prostate cancer with increasing number of A alleles for rs486907 (p-value for trend = 0.046), and men with the AA genotype had an OR of 0.47 (95% CI 0.23 – 0.96) relative to men with the GG genotype (Table 3B). Moreover, there was a stronger reduction in prostate cancer risk (OR = 0.25, 95% CI 0.09 – 0.72; p=0.01) among men with the AA genotype of rs486907 who had both a positive family history of prostate cancer and were <65 years old

at diagnosis relative to men with the GG genotype. There were, however, no effect modifications between the founder mutation 471delAAAG of *RNASEL* and risk of prostate cancer either by age at diagnosis or by first-degree family history of prostate cancer (Table 3).

Finally, we examined associations between these two *RNASEL* variants and Gleason score using polytomous logistic regression models (Table 4). There was a trend (p-value = 0.043) of decreasing risk with increasing number of A alleles for rs486907 among men diagnosed with low-grade (Gleason score 2–6) prostate cancer. Dominant vs. recessive models did not identify any specific pattern of risk inheritance. Results were similar when the analysis was repeated comparing low-grade Gleason score with a Gleason score of 8–10.

HPCX markers and prostate cancer

Associations between prostate cancer and five microsatellite markers (B270, B97, DXS1205, D33, D60) within the *HPCX* region located on Xq27-q28 were examined in only a subset of our study population (717 cases and 1,048 controls), because reagents for the Micro-Gene Clipper sequencer became unavailable within the course of this analysis. The proportion of missing values for all *HPCX* genotypes was higher in prostate cancer cases than in controls, 27% vs. 16%, respectively ($p<0.001$). However, we did not see a significant difference by age, family history of prostate cancer or screening amongst cases and controls with and without missing genotypes. Examinations of each allele with a minor frequency ≥ 2% in relation to prostate cancer risk (Table 2), identified positive associations for allele 135 of STR marker B97 (OR=1.77, 95% CI 1.15–2.71; p=0.01) and allele 188 of STR marker DXS1205 (OR=1.65, 95% CI 1.08–2.54; p=0.02), and a borderline significant inverse association for allele 248 of STR marker D33 (OR = 0.77, 95% CI 0.58–1.01; p=0.06). When we examined the association between prostate cancer and a haplotype constructed by the two risk alleles - i.e., allele 135 of STR marker B97 and allele 188 of STR marker DXS1205—there was an OR of 1.73 (95% CI 1.27–2.40) among men carrying at least one risk allele (only one case, but no controls, carried both these risk alleles).

We also examined whether risk of prostate cancer associated with alleles of these STR markers varied by age at diagnosis or father affected with prostate cancer (Table 3). There was no evidence for effect modifications, although this could also be due to limited power. We had insufficient power to examine maternal family history of prostate cancer (i.e. uncle or grandfather from the mother side affected with prostate cancer) since only 3.1% of all cases and 2.1% of all controls reported a maternal familial history, respectively. In relation to age at diagnosis, risk was slightly higher and statistically significant among older men carrying the risk haplotype- allele 135 of B97 and allele 188 of DXS1205 (OR = 1.88; p = 0.003).

We examined associations between the five *HPCX* markers and Gleason score using polytomous logistic regression models (Table 4). For allele 135 of STR marker B97, risk was similar for low-grade (Gleason score 2–6; OR=1.66, p=0.047) versus high-grade (Gleason score 7–10; OR=1.77, p=0.057) tumors. For allele 188 of DXS1205, there was an increased risk of Gleason score 2–6 (OR=1.84, 95% CI 1.14–2.99), whereas no association was observed for high Gleason score tumors (OR=1.12, 95% CI 0.56–2.21). Finally, for allele 248 of D33 there was no association observed for low-grade tumors, but an OR of 0.65 (95% CI 0.42–0.99) for high-grade tumors. Similar results were observed when analysis was repeated using Gleason score 8–10 to define high-grade tumors.

Discussion

RNASEL and prostate cancer

In this study we examined associations of a frameshift founder mutation (471delAAAG) and of a non-synonymous SNP (rs486907, Arg462Gln) in the *RNASEL* gene to the risk of prostate cancer among Ashkenazi Jewish men. Analyses stratified by age at diagnosis or by family history of prostate cancer indicated that there was evidence for effect modification of prostate cancer with SNP rs486907. Specifically, we observed that men with the AA genotype had 35% to 50% reductions in risk of prostate cancer relative to men with the GG genotype, and this association was significant among men diagnosed at younger ages (<65 years) or among those with a first-degree family history of prostate cancer. Moreover, the inverse association between prostate cancer and this non-synonymous SNP was additive, with an OR of 0.25 (p=0.01) among younger men (age <65 years) who also had a first-degree relative diagnosed with prostate cancer. These characteristics are consistent with an inherited cancer susceptibility gene.

The *RNASEL* gene has been one of the major candidate genes for prostate cancer susceptibility, since mutations in this gene were linked to prostate cancer in high-risk hereditary prostate cancer families [10]. This gene encodes for Rnase L, a latent endoribonuclease which mediates the antiviral and proapoptotic activity of the interferon-inducible 2-5A system (11–13). There is a strong biological plausibility for the involvement of the *RNASEL* gene in prostate cancer, since mutation carriers in this gene exhibited loss of heterozygosity (LOH) and as a consequence were deficient in functional RNase L activity [10].

Several studies have also examined association of the founder mutation 471delAAAG and of the non-synonymous SNP rs486907 in *RNASEL* with sporadic and familial/hereditary prostate cancer in different populations [14–39]. The 471delAAAG is a frameshift, truncating, 4-bp deletion at codon 157, which leads to a premature stop codon at position 164 of the RNASEL protein and is anticipated to inactivate the protein. This founder mutation was initially reported by Rennert et al. to be more frequent among Ashkenazi Jewish prostate cancer patients (6.9%) in comparison to unaffected controls (2.4%) [15]. However, this initial study was based on mutation screening of 87 prostate cancer patients and 83 controls and thus the findings could be due to chance. Following this report, two other studies [20, 32] with larger numbers of prostate cancer patients, reported that the prevalence of this founder mutation was similar in prostate cancer cases and controls:- a finding that did not support an association between the 471delAAAG mutation and prostate cancer risk. In our study, we also found a similar frequency of this mutation among prostate cancer patients (1.8%) and controls (2.0%), indicating the lack of association.

With respect to the non-synonymous SNP rs486907 in *RNASEL*, resulting in the amino acid substitution Arg462Gln and leading to approximately three times lower enzymatic activity, the initial report suggested that this variant could be implicated in 13% of prostate cancer cases [14]. However, other studies reported a lack of overall association between this variant and sporadic prostate cancer in Caucasians [16–18, 27–29, 32, 34, 36], similar to our analysis between *RNASEL* rs486907 and all prostate cancer cases. Nevertheless, we observed that men with the AA genotype of *RNASEL* rs486907 had 35% to 50% reductions in risk of prostate cancer in comparison to men with the GG genotype, which was significant in men diagnosed at younger ages (<65 years) and among those with a first-degree family history of prostate cancer. These results are not completely surprising since the *HPC1* region and *RNASEL* gene were identified in studies of highly aggregated families with early-onset hereditary forms of prostate cancer [10, 50]. Since only 1% of cases and controls were <50 years old in our study, we had limited power to examine associations with very early ages at

prostate cancer diagnosis. The lack of association with an *RNASEL* mutation that inactivates the protein, but a consistent association with a missense mutation, suggests that the effect is due to either partial inactivation of the protein, and/or acquisition of a new protein activity.

HPCX and prostate cancer

In a subset of our study population, we evaluated associations between prostate cancer risk and five micro-satellite STR polymorphic markers (B270, B97, DXS1205, D33, and D60) spanning a region of approximately 330 kb on Xq27-28. Although, associations between these five markers and overall prostate cancer was not statistically significant (all global P-values >0.05), two alleles in two of the STR markers showed a positive association: allele 135 of B97 (OR=1.77, 95% CI 1.15–2.71; p=0.01), and allele 188 of DXS1205 (OR=1.65, 95% CI 1.08–2.54; p=0.02), while allele 248 of D33 marker showed an inverse association (OR = 0.77, 95% CI 0.58 – 1.01; p=0.06). Men carrying at least one of these high risk alleles (allele 135 of B97 or allele 188 of DXS1205) had also an OR of 1.73 (p=0.001) of prostate cancer in comparison to non-carriers. However, both allele 135 of B97 and allele 188 of DXS1205 were positively associated with risk of low Gleason score (2 to 6) tumors (ORs of 1.66 and 1.84, respectively), but not of high Gleason score ≥7. Similar results were observed when analysis was repeated using cases with a Gleason score 8–10.

The *HPCX* region located on Xq27-28 was first identified as a susceptibility region for prostate cancer in a linkage analysis of 360 hereditary prostate cancer families collected in the United States, Finland, and Sweden [9], where a maximum LOD score of 4.6 ($\Phi = 0.26$) was achieved at marker DXS1113. Although there was a large heterogeneity across combined families from different groups, Xu *et al.* [9] concluded that the *HPCX* region may account for 16% of all HPC. Despite this initial evidence, several other linkage studies conducted in independent sets of HPC families in the U.S. [40, 41, 44] and Germany [42] did not find strong linkage in this region, although subset analysis of families with no male-to-male transmission or early age at disease onset (<65 years) showed some evidence for linkage. All of the above studies were, however, conducted in hereditary forms of prostate cancer and thus it is unclear what role the *HPCX* region variants may contribute to sporadic forms of prostate cancer that constitute the majority (85%) of cases.

One study in Finland examined allelic associations between 23 markers in the Xq26-28 region and prostate cancer among 108 cases with a family history of prostate cancer and 257 controls [46]. The strongest association was observed between allele ‘180’ of marker DXS1205 and prostate cancer (p=0.0003). We also observed an association with the DXS1205 marker in Ashkenazi Jews, however the strongest allele associated with prostate cancer was 188 (OR=1.65; 95% CI 1.08–2.54; p=0.02) instead of allele 180. Differences in results between the two studies could be related to several reasons including differences in allele detection due to PCR, in study founder populations (Finnish vs. Ashkenazi Jews), in the selection of familial cases in the Finnish study vs. unselected cases in our study, or in sample size.

Our study has strength and limitations that should be carefully considered when evaluating these results. We originally estimated that a sample size of 1,800 Ashkenazi Jewish men would be sufficient to detect a 2-fold increase in prostate cancer risk with an accumulated mutation frequency of 2.0% or higher; however, our study has limited power to detect modest associations. Men were recruited using a novel strategy of recruitment by advertisement and were requested to provide all materials through the mail. As presented in Table 1, over 75% of the study population had at least a college degree that facilitated the completion of the self-administered questionnaire and self-obtained DNA sample. In fact, most cases provided their own pathology reports significantly reducing the labor involved in

obtaining medical records. Nevertheless, such a recruitment strategy has the potential to introduce bias into the study sample. Therefore, these data should be interpreted in light of this recruitment strategy. Despite this fact, the data in Table 1 provide reassurance that not only were our cases comparable to controls with respect to demographic characteristics, but we also demonstrate that the prevalence of the *RNASEL* founder mutations in our study, as well as the other variants in *RNASEL* and *HPCX* are similar to other large studies. Since only 1% of cases and controls were <50 year old, we had limited power to examine associations with very early ages at diagnosis and thus our findings are relevant to men diagnosed with this disease at age ≥ 50 years. Finally, we investigated a limited number of genetic markers in the *RNASEL* gene and *HPCX* region in this study.

Conclusions

In conclusion, results of this study suggest that variants in *RNASEL* contribute to susceptibility in early age at onset and familial forms of prostate cancer, whereas *HPCX* variants are associated with prostate cancer risk and Gleason score. The observation that a mutation predicted to completely inactivate RNASEL protein activity was not associated with prostate cancer, but that a missense variant was associated, suggests that an "activating" or biologically relevant RNASEL protein change does in fact present a risk or protection (depending on the referent allele) for prostate cancer. Our findings are consistent with previous reports suggesting a role of the *RNASEL* gene in familial forms of prostate cancer, as well as in early-onset disease.

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Table 1

Selected characteristics of Ashkenazi Jewish prostate cancer cases and controls

Characteristics	Cases (n = 979)		Controls (n = 1,251)		p*
	n	%	n	%	
Age at participation (years)					0.01
< 50	11	1.1	12	1.0	
50 – 59	146	14.9	284	22.7	
60 – 69	345	35.2	382	30.5	
70 – 79	365	37.3	427	34.1	
≥ 80	112	11.4	146	11.7	
First-degree family history of prostate cancer					<0.0001
Yes	276	28.2	179	14.3	
No	703	71.8	1072	85.7	
Smoking status					0.93
Non smoker	427	43.6	543	43.4	
Former smoker	523	53.4	668	53.4	
Current smoker	24	2.5	33	2.6	
Missing	5	0.5	7	0.6	
Education					0.90
High school or less	63	6.4	86	6.9	
Post secondary/some college	155	15.8	193	15.4	
College degree	259	26.5	320	25.6	
Graduate/professional degree	493	50.4	645	51.5	
Missing	9	0.9	7	0.6	
History of BPH					<0.0001
Yes	323	33.0	538	43.1	
No	616	62.9	682	54.5	
Unknown	40	4.1	31	2.5	

Characteristics	Cases (n = 979)		Controls (n = 1,251)		p*
	n	%	n	%	
Prostate cancer screening					<0.0001
PSA and DRE	933	95.3	1127	90.1	
DRE only	2	0.2	109	8.7	
None	44	4.5	15	1.2	
Age at prostate cancer diagnosis (years)					
< 50	27	2.8			
50 – 59	242	24.7			
60 – 69	443	45.3			
70 – 79	234	23.9			
≥ 80	33	3.4			
Reason for prostate cancer diagnosis					
Abnormal PSA	741	73.1			
Abnormal DRE	123	12.1			
Symptoms	25	2.5			
TURP for BPH	15	1.5			
Other procedures	57	5.6			
Unknown	18	1.8			
Gleason score					
2 – 4	88	9.0			
5 – 6	456	46.6			
7	243	24.8			
8 – 10	115	11.7			
Missing	77	7.9			
Aggressive prostate cancer[ξ]					
Yes	484	49.4			
No	435	44.4			
Missing	60	6.1			

[*] Chi-square p-value

[ξ] Aggressive prostate cancer was characterized as having either a Gleason score of 7–10, or at least two of the following characteristics documented on the pathology report: tumor invasiveness, tumor present at resection margins, prostate capsule invasion, seminal vesicle involvement, or lymph node involvement

Abbreviations: BPH – Benign prostatic hyperplasia; PSA – Prostate specific antigen; DRE – Digital rectal examination; TURP – Transurethral resection of the prostate

Table 2

Associations between *RNASEL* and *HPCX* variants and overall prostate cancer risk [†]

RNASEL		**Cases**		**Controls**		**Statistics**			
		n	**%**	**n**	**%**	**OR***	**95% CI**	**p***	**Global p[‡]**
471delAAAG	No	928	98.2	1212	98.0	1.00	-		0.80
	Yes	17	1.8	25	2.0	0.89	0.74 – 1.68	0.80	
rs486907 (Arg462Gln)	GG	467	48.4	572	46.2	1.00	-		0.63
	GA	414	42.9	556	44.9	0.91	0.76 – 1.09	0.31	
	AA	84	8.7	109	8.8	0.86	0.63 – 1.19	0.36	
p-value for trend							0.23		
GA or AA vs. GG						0.90	0.76 – 1.07	0.24	
AA vs. GG or GA						0.90	0.67 – 1.22	0.51	
HPCX Microsatellite Marker		Cases		Controls		Statistics			
	Allele[ξ]	n	%	n	%	OR*	95% CI	p*	Global p[‡]
B270	210	223	33.4	353	36.0	0.90	0.73 – 1.12	0.35	0.54
	218	206	30.9	275	28.0	1.14	0.92 – 1.43	0.22	
	222	199	29.8	284	28.9	1.06	0.85 – 1.32	0.60	
	226	36	5.4	66	6.7	0.72	0.47 – 1.10	0.13	
B97	131	116	17.2	158	16.0	1.08	0.83 – 1.40	0.59	0.12
	135	49	7.3	42	4.3	1.77	1.15 – 2.71	0.01	
	137	29	4.3	55	5.6	0.78	0.49 – 1.24	0.28	
	143	21	3.1	22	2.2	1.42	0.77 – 2.62	0.26	
	145	35	5.2	51	5.2	1.02	0.65 – 1.60	0.93	
	147	140	20.8	192	19.5	1.07	0.84 – 1.37	0.60	
	149	173	25.7	291	29.5	0.84	0.68 – 1.06	0.14	
	151	93	13.8	153	15.5	0.87	0.66 – 1.15	0.33	
DXS1205	178	242	36.4	378	38.4	0.92	0.75 – 1.13	0.43	0.14

Prostate. Author manuscript; available in PMC 2012 July 24.

RNASEL		Cases		Controls		Statistics			
		n	%	n	%	OR*	95% CI	p*	Global p‡
	180	301	45.3	423	43.0	1.05	0.89 – 1.33	0.43	
	182	21	3.2	46	4.7	0.71	0.42 – 1.21	0.21	
	184	14	2.1	30	3.1	0.65	0.34 – 1.24	0.19	
	188	47	7.1	44	4.5	1.65	1.08 – 2.54	0.02	
	190	21	3.2	36	3.7	0.83	0.48 – 1.45	0.51	
D33	248	93	14.1	168	17.3	0.77	0.58 – 1.01	0.06	0.20
	252	47	7.1	52	5.4	1.41	0.94 – 2.13	0.10	
	256	99	15.0	163	16.8	0.91	0.69 – 1.20	0.52	
	260	174	26.4	228	23.5	1.14	0.91 – 1.44	0.26	
	264	197	29.9	268	27.6	1.11	0.89 – 1.38	0.35	
	268	44	6.7	66	6.8	0.98	0.66 – 1.46	0.93	
D60	171	213	34.3	243	34.6	1.00	0.77 – 1.26	0.99	0.35
	173	164	26.4	158	22.5	1.21	0.94 – 1.56	0.14	
	175	223	35.9	278	39.6	0.86	0.68 – 1.07	0.17	

†Nr of cases and controls that were genotyped for *RNASEL* and *HPCX* vary by genetic marker

*ORs, 95% CI and p-values are adjusted for age, but not for multiple comparisons

ξAlleles represented in Table are those with a minor frequency 2% or higher in controls. The reference category for ORs of each allele of HPCX microsatellite markers are other alleles in the microsatellite marker.

‡Global p-values represent the association of each SNP or marker with prostate cancer

Table 3

Associations between *RNASEL* and *HPCX* variants and prostate cancer risk by age at diagnosis and by first-degree family history of prostate cancer [†]

A. Age at Diagnosis

RNASEL	Age at diagnosis < 65 Years						Age at diagnosis ≥ 65 years						
	Cases		Controls				Cases		Controls				
	n	%	n	%	OR[†]	95% CI	n	%	n	%	OR[†]	95% CI	p for interaction
471delAAAG													
No	472	98.1	459	97.9	1.00	-	456	98.3	752	98.0	1.00	-	
Yes	9	1.9	10	2.1	0.87	0.35 – 2.16	8	1.7	15	2.0	1.05	0.42 – 2.59	0.99
rs486907 (Arg462Gln)													
GG	246	49.4	206	43.9	1.00	-	221	47.3	366	47.7	1.00	-	0.08
GA	211	42.4	209	44.6	0.85	0.65 – 1.11	203	43.5	346	45.1	0.99	0.77 – 1.27	
AA	41	8.2	54	11.5	0.64	0.41 – 1.00	43	9.2	55	7.2	1.16	0.74 – 1.82	
p-value for trend					0.048						0.69		
GA or AA vs. GG					0.81	0.63 – 1.05					1.02	0.80 – 1.29	
AA vs. GG or GA					0.69	0.45 – 1.06					1.17	0.76 – 1.80	
HPCX markers													
B97 – 135	22	6.7	18	4.6	1.48	0.78 – 2.81	27	7.9	24	4.1	1.98	1.09 – 3.59	0.47
DXS1205 – 188	19	5.8	15	3.9	1.57	0.78 – 3.14	28	8.3	29	4.9	1.73	0.99 – 3.02	0.74
D33 – 248	56	17.6	71	18.1	0.97	0.66 – 1.42	37	10.8	97	16.8	0.61	0.40 – 0.93	0.09

B. First-degree family history of prostate cancer

RNASEL	Positive first-degree family history of prostate cancer						No first-degree family history of prostate cancer						
	Cases		Controls				Cases		Controls				
	n	%	n	%	OR[†]	95% CI	n	%	n	%	OR[†]	95% CI	P for interaction
471delAAAG													
No	259	98.1	170	96.6	1.00	-	669	98.2	1042	98.2	1.00	-	
Yes	5	1.9	6	3.4	0.52	0.15–1.73	12	1.8	19	1.8	1.09	0.52 – 2.32	0.30

B. First-degree family history of prostate cancer

RNASEL	Positive first-degree family history of prostate cancer						No first-degree family history of prostate cancer						
	Cases		Controls				Cases		Controls				
	n	%	n	%	OR†	95% CI	n	%	n	%	OR†	95% CI	P for interaction
rs486907 (Arg462Gln)													
GG	142	52.2	79	45.1	1.00	-	325	46.9	493	46.4	1.00	-	0.09
GA	113	41.5	77	44.0	0.79	0.53–1.18	301	43.4	479	45.1	0.97	0.79 – 1.19	
AA	17	6.3	19	10.9	0.47	0.23–0.96	67	9.7	90	8.5	1.04	0.73 – 1.48	
p-value for trend						0.046						0.96	
GA or AA vs. GG					0.73	0.50–1.07					0.98	0.81 – 1.20	
AA vs. GG or GA					0.57	0.27–1.11					1.03	0.73 – 1.46	
	Father affected with prostate cancer						No prostate cancer in fathers						
HPCX markers	n	%	n	%	OR†	95% CI	n	%	n	%	OR†	95% CI	
B97 – 135	1	3.6	1	2.1	1.76	0.11–29.3	48	7.4	41	4.3	1.75	1.13 – 2.71	0.57
DXS1205 – 188	2	8.7	1	2.0	4.85	0.42–56.6	45	7.0	43	4.6	1.58	1.02 – 2.45	0.12
D33 – 248	3	10.7	12	24.0	0.40	0.1–1.51	90	14.2	156	17.0	0.80	0.60 – 1.06	0.91

†Nr of cases and controls that were genotyped for *RNASEL* and *HPCX* vary by genetic marker

†ORs and 95% CI are adjusted for age. The reference category for ORs of each allele of HPCX microsatellite markers are other alleles in the microsatellite marker

Table 4

Associations between *RNASEL* and *HPCX* variants and Gleason score [†]

	Controls		Cases with Gleason 2–6 tumors				Cases with Gleason 7–10 tumors			
RNASEL	**n**	**%**	**n**	**%**	**OR[‡]**	**95% CI**	**n**	**%**	**OR[‡]**	**95% CI**
471delAAAG										
No	1212	98.0	522	98.5	1.00	-	333	90.7	1.00	-
Yes	25	2.0	8	1.5	0.75	0.33 – 1.68	9	2.5	1.31	0.60 – 2.86
rs486907 (Arg462Gln)										
GG	572	46.2	271	50.4	1.00	-	154	43.6	1.00	-
GA	556	44.9	227	42.2	0.85	0.68 – 1.06	162	45.9	1.07	0.83 – 1.38
AA	109	8.8	40	7.4	0.70	0.47 – 1.04	37	10.5	1.16	0.77 – 1.77
p-value for trend						0.043				0.42
GA or AA vs. GG					0.83	0.68 – 1.02			1.09	0.86 – 1.39
AA vs. GG or GA					0.75	0.51 – 1.10			1.12	0.75 – 1.67
HPCX markers										
B97 – 135	42	4.3	27	6.9	1.66	1.01 – 2.76	17	7.3	1.77	0.98 – 3.17
DXS1205 – 188	44	4.5	31	7.9	1.84	1.14 – 2.99	11	4.9	1.12	0.56 – 2.21
D33 – 248	168	17.3	62	16.3	0.91	0.66 – 1.26	28	12.1	0.65	0.42 – 0.99

[†] Nr of cases and controls that were genotyped for *RNASEL* and *HPCX* vary by genetic marker

[‡] ORs and 95% CI are adjusted for age. The reference category for ORs of each allele of HPCX microsatellite markers are other alleles in the same microsatellite marker