Featured Article

Genetic Analysis of the RNASEL Gene in Hereditary, Familial, and Sporadic Prostate Cancer

Fredrik Wiklund,1 Björn-Anders Jonsson,2 Anthony J. Brookes,3 Linda Strömqvist,3 Jan Adolfsson,4 Monica Emanuelsson,1 Hans-Olov Adami,5 Katarina Augustsson-Bälter,5 and Henrik Grönb erg1

Department of 1Radiation Sciences, Oncology, and 2Medical Biosciences, Pathology, University of Umeå, Umeå, Sweden; and 3Center for Genomics and Bioinformatics, 4Oncologic Center, Department of Surgical Sciences, and 5Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden

ABSTRACT

Purpose: The RNASEL gene has been proposed as a candidate gene for the HPC1 locus through a positional cloning and candidate gene approach. Cosegregation between the truncating mutation E265X and disease in a hereditary prostate cancer (HPC) family and association between prostate cancer risk and the common missense variant R462Q has been reported. To additionally evaluate the possible role of RNASEL in susceptibility to prostate cancer risk, we performed a comprehensive genetic analysis of sequence variants in RNASEL in the Swedish population.

Experimental Design: Using 1624 prostate cancer cases and 801 unaffected controls, the truncating mutation E265X and five common sequence variants, including the two missense mutations R462Q and D541E, were evaluated for association between genotypes/haplotypes and prostate cancer risk.

Results: The prevalence of E265X carriers among unaffected controls and prostate cancer patients was almost identical (1.9 and 1.8% in controls and cases, respectively), and evidence for segregation of E265X with disease was not observed within any HPC family. Overall, the analyses of common sequence variants provided limited evidence for association with prostate cancer risk. We found a marginally significant inverse association between the missense mutation D541E and sporadic prostate cancer risk (odds ratio, 0.77; 95% confidence interval, 0.59–1.00) and reduced risk of prostate cancer in carriers of two different haplotypes being completely discordant.

Conclusions: Considering the high quality in genotyping and the size of this study, these results provide solid evidence against a major role of RNASEL in prostate cancer etiology in Sweden.

INTRODUCTION

Accumulating evidence from epidemiologic and genetic studies indicates that hereditary predisposition has a considerable impact on the development of prostate cancer. Linkage analyses suggest that a number of chromosomal regions harbor prostate cancer susceptibility genes. However, lack of consistency between studies additionally indicates that prostate cancer is a genetically heterogeneous disorder, with multiple genetic and environmental factors involved in its etiology (1). In 1996, the first prostate cancer susceptibility locus, HPC1, was mapped to chromosome 1q24-25 (2). Subsequent studies of linkage to HPC1 have proven inconclusive; some confirmed linkage (3–6), although others did not (7–11). However, in a pooled analysis of 772 families from nine international groups confirmatory linkage evidence was found with a maximum heterogeneity logarithm of odds score of 1.4 (12).

RNASEL maps to HPC1 and is involved in the IFN-regulated 2′-5′-linked oligoadenylates system that mediates cell proliferation and apoptosis (13, 14) and has been suggested as a candidate tumor suppressor gene (15). RNASEL was recently identified as a candidate gene for HPC1 through a positional cloning and candidate gene approach. A truncating mutation (E265X) and an initiation codon mutation (M1I) was reported to cosegregate within two hereditary prostate cancer (HPC; ref. 2) families linked to HPC1 (16). Furthermore, loss of the wild-type allele and reduced RNASEL activity was observed in microdissected tumors carrying a germ-line mutation. In Finnish HPC families, RNASEL mutations did not explain disease segregation; however, the truncating mutation E265X was associated with prostate cancer risk (17).

In addition to rare mutations, several polymorphisms in RNASEL have been reported. A common missense variant of RNASEL, R462Q, has been associated both with increased (17, 18) and decreased (19, 20) risk of HPC. Recently, Xiang et al. (21) reported that the R462Q variant reduces the ability of RNase L to cause apoptosis in response to activation by 2-5A. Hence, this polymorphism might have a functional role in prostate cancer development. Another missense variant, D541E, was associated with an increased risk for prostate cancer in a study from Japan (19), although several other studies found no association (17, 18, 20).

Additional evaluation of the impact that the RNASEL gene have on prostate cancer risk are required. Our aim was to assess...
the possible role of **RNASEL** sequence variants on prostate cancer risk in a Swedish population. We used a large population-based case-control study, composed of 1624 prostate cancer cases and 801 controls. The truncating mutation E265X and several common polymorphisms in **RNASEL** were evaluated, both for individual association and through haplotype analyses.

**MATERIALS AND METHODS**

**Patients with Prostate Cancer and Controls.** This study used men enrolled into the population based case-control study Cancer Prostate in Sweden. A detailed description of the Cancer Prostate in Sweden study has been given elsewhere (22). The Cancer Prostate in Sweden study is composed of all prostate cancer patients, ages \( \leq 79 \) years, diagnosed between July 1, 2001, and September 30, 2002, from the central and northern part of Sweden and all patients, ages \( \leq 65 \) years, diagnosed in the same calendar period from the southeastern part of Sweden and the area of Stockholm. In total, 1961 prostate cancer patients were invited for participation and 1444 (74%) agreed to participate. All these cases donated a blood sample and answered a questionnaire, including questions regarding family history for prostate cancer. For all cases with at least one reported family member with prostate cancer, based on the initial questionnaire, a more detailed family history was obtained through a second questionnaire followed by a telephone interview.

Of the 1444 cases included in the study, 143 were classified as familial prostate cancer cases (two relatives with verified prostate cancer in a nuclear family) and 51 as HPC cases (three or more members with prostate cancer in a nuclear family). Detailed clinical data, including tumor-node-metastasis stage, Gleason score, prostate-specific antigen level at time of diagnosis, means of diagnosis and primary treatment were obtained through linkage to the National Prostate Cancer Registry. Cases were classified as either localized (tumor stage I-II and Gleason sum \( \leq 8/grade \) I-II and prostate-specific antigen \( < 100 \) or advanced (tumor stage III-IV or Gleason sum \( \geq 8/grade \) III or prostate-specific antigen \( \geq 100 \)).

The continuously updated Swedish Population Registry encompasses the individually unique national registration number assigned to all residents. From this register, control subjects were randomly selected and frequency matched according to geographical origin (northern part of Sweden versus southeastern part of Sweden and the area of Stockholm) and to the expected age distribution of the cases (within 5-year age groups). The controls were recruited during the same calendar period as the cases. Of 1697 controls invited, 866 (51%) agreed to participate. Subsequently, eight controls were excluded because linkage to the Swedish Cancer Registry revealed that they were diagnosed with a cancer of the prostate before inclusion. At the time of this study, DNA samples were available for 801 controls and 1427 cases (1247 sporadic prostate cancer cases, 131 familial prostate cancer cases, and 49 HPC cases).

**Patients with Familial Prostate Cancer and HPC.** Recruitment of Swedish families with prostate cancer is an ongoing activity that started at the Department of Oncology, University of Umeå, in 1995. Identification of families has been based mainly on referrals by collaborating urologists and oncologists throughout Sweden but also on self-reported family history of prostate cancer from cases included into the Cancer Prostate in Sweden study, described above. Blood has been collected for as many family members as possible. For the present study, all affected family members with DNA information available (excluding cases from the Cancer Prostate in Sweden cohort already selected for this study) were included, resulting in 184 HPC cases and 25 familial prostate cancer cases. All diagnoses of prostate cancer in the families were confirmed both by reference to the Cancer Registry and by direct examination of medical records.

**Total Study Population.** Our total study population is composed of 1636 prostate cancer patients and 801 unaffected controls. Of the 1636 prostate cancer patients, 156 (9.5%) and 233 (14.2%) were classified as familial prostate cancer and HPC cases, respectively. Pertinent characteristics of the study population are given in Table 1. Written informed consent was obtained from each subject. The ethical committee at the Karolinska Institutet and University of Umeå approved the study.

**Selection of RNASEL Single Nucleotide Polymorphisms (SNPs).** Located on chromosome 1q22, the **RNASEL** gene with eight exons is \( \sim 13 \) kb. To comprehensively evaluate association between prostate cancer risk and common **RNASEL** sequence variants, we used the following approach. First, we defined the target region for selection of SNPs as 3 kb of the promoter, all exons, introns, and 3'-untranslated region. We then searched the public database National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/SNP) within this target region, resulting in the identification of 34 SNPs. A subset of nine SNPs were then selected, with the criteria of a reported minor allele frequency of at least 5%, a density of one SNP per three kb, and as low as possible proportion of repetitive sequences. Preferentially validated SNPs were selected, and additional attention was paid to SNPs in the promoter region. Besides the two reported missense variants, R462Q and D541E, which were decided to be included in the analyses in advance, this subset consisted of four SNPs in the promoter region, one

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Number</th>
<th>Mean age* (range)</th>
<th>Proportion of advanced tumors†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>801</td>
<td>67.8 (45.5–80.0)</td>
<td>512/1182 (43.3%)</td>
</tr>
<tr>
<td>Sporadic prostate cancer</td>
<td>1247</td>
<td>66.7 (47.3–80.4)</td>
<td>51/124 (41.1%)</td>
</tr>
<tr>
<td>Familial prostate cancer</td>
<td>156</td>
<td>65.8 (47.0–80.0)</td>
<td>15/51 (29.4%)</td>
</tr>
<tr>
<td>Hereditary prostate cancer</td>
<td>233</td>
<td>65.3 (43.0–81.0)</td>
<td></td>
</tr>
</tbody>
</table>

* Age defined as age at diagnosis for cases and age at blood sampling for controls.
† Information of tumor characteristics only available for cases ascertained through the Cancer Prostate in Sweden study.
SNP in intron 4, one SNP in intron 5, and one SNP in 3′-untranslated region (Table 2). These nine SNPs were genotyped in a randomly selected subset of 96 control subjects from the Cancer Prostate in Sweden study. This resulted in the elimination of four SNPs due to failed assay (one SNP), PCR failure (two SNPs), and monomorphic result (one SNP; see Table 2). The remaining five SNPs were all in Hardy-Weinberg equilibrium.

Haplotypes were estimated with a method proposed by Stephens et al. (23), as implemented in the software PHASE. Using a Markov chain Monte Carlo approach, this method incorporates a statistical model for the distribution of unresolved haplotypes based on coalescent theory. Haplotype-tagging SNPs were determined by using the haplotype-tagging SNP2 software. Because removing any one of the five SNPs resulted in a subset of haplotype-tagging SNPs that retained <95% of the diversity observed among the 96 control individuals, all five SNPs were genotyped in the total study population. In addition to the five SNPs, the truncating stop mutation E265X was also genotyped in all individuals.

**Genotyping.** DNA samples were extracted from leukocytes with standard methods. Each DNA plate contained two Centre d’Etude du Polyomorphisme Humain controls, a water blank and blinded internal replicates. Two methods were used for genotyping. The stop mutation (E265X) and the two missense mutations (R462Q and D541E) were genotyped using a mid-kinetic real-time PCRs entailed amplifying short genomic fragments spanning the variant of interest, with one of the primers carrying a 5′-biotin label. Amplifications were performed in 5 μL of volume, containing 1 to 2 ng of genomic DNA, 0.38 μmol/L biotinylated primer, 0.75 μmol/L nonbiotinylated primer, 0.03 units of AmpliTaq Gold (Applied Biosystems, Inc.), 10% dimethylsulphoxide, 1× AmpliTaq Gold Buffer, including 1.5 mmol/L MgCl₂ (Applied Biosystems, Inc.), and 0.2 mmol/L each deoxynucleoside triphosphate. Thermal cycling was conducted on a MBS 384 device (Thermo-Hybaid, Thermo Electron Corporation, Milford, MA) as follows: 1× (10 minutes at 94°C) and 35× (15 seconds at 94°C, 30 seconds at annealing temperature). To verify successful amplification, 0.5 μL of several randomly chosen samples were examined on a 3.0% low-melt agarose gel.

Dynamic allele-specific hybridization analysis of the PCR product was conducted on membrane macroarrays, with the dynamic allele-specific hybridization-2 protocol (25). Briefly, this entailed transferring samples to the membrane array by centrifugation (28) or robotic gridding. Resulting individual arrays with up to 9600 distinct features were rinsed in 0.1 mol/L NaOH to denature the PCR products. They were then exposed to 2 mL of HE buffer [0.1 mol/L HEPES and 10 mmol/L EDTA (pH 7.9)] containing 4 mmol of the appropriate ROX end-labeled probe. After heating to 85°C and cooling to room temperature, the membrane was briefly rinsed in HE buffer. The array was then soaked in 40 mL of HE buffer containing SYBR Green I dye at 1:20,000 dilution for up to 3 hours. Using a dynamic allele-specific hybridization-2 device (DynaMetrix Ltd.), the membrane was taken through a dynamic allele-specific hybridization heating ramp (heating at 3°C/minute from room temperature to 85°C), whereas fluorescence from the ROX acceptor dye on the probe was monitored. Data were collected at intervals of 0.5°C. We used fluorescence changes with temperature (DNA melting profiles) to distinguish different alleles. This was done by the dynamic allele-specific hybridization-2 device software that uses negative derivatives of fluorescence against temperature to reveal peaks of denaturation rate (target-probe melt-
ing temperatures; Tm) and thereby automatically assign DNA samples into genotype groups.

**Statistical Methods.** We tested Hardy-Weinberg equilibrium for each sequence variant and pairwise linkage disequilibrium between all sequence variants using a replication method (29). For each test, 10,000 permutations were performed, and the Fisher probability test statistic of each replicate was calculated from the new corresponding multilocus table. Empirical P values for each test were estimated as the proportion of replicate data sets found to be as probable as or less probable than the observed data, as implemented in the software package Genetic Data Analysis.

We used unconditional logistic regression to evaluate the association of each of the sequence variants with prostate cancer risk with the statistical software STATA. To adjust for the matching, we included indicator variables, representing each combination of age category (5-year age groups) and geographical region (northern part of Sweden versus southeastern part of Sweden and the area of Stockholm), in the regression model. A likelihood ratio test of a covariate equal to number of rare alleles (0, 1, or 2), corresponding to multiplicative allelic effects on the genotype relative risks, was used to test for association between genotypes and disease. To account for genotype correlations among individuals from the same family (in comparing familial prostate cancer or HPC cases with controls), a modified sandwich variance estimator in clustered logistic regression analysis was used (30). Because the likelihood used for estimation is not a true likelihood, a Wald test was applied to test for association between genotypes and prostate cancer risk.

Association between haplotypes and prostate cancer risk was evaluated with a score test proposed by Schaid et al. (31), as implemented in the software HAPLO.SCORE for the R programming language. On the basis of a generalized linear model, this method allows adjustment for possible confounding variables and provides both global and haplotype-specific tests. In these analyses, age and geographical region was adjusted for (as described above) and haplotypes with frequencies < 0.005 were pooled into a common group. Empirical P values, based on 10,000 simulations, were computed for the global score test and were pooled into a common group. Empirical P values for each test were estimated as the proportion of replicate data sets found to be as probable as or less probable than the observed data, as implemented in the software package Genetic Data Analysis.

No correction was made for multiple comparisons. All reported P values are two sided.

**RESULTS**

In total, 14,466 genotypes were assayed, of which, 13,988 (96.7%) provided a successful result. For the two missense mutations (R462Q and D541E) and the stop mutation (E265X), the success rate was 99.5, 96.7, and 97.4%, respectively. The lowest success rate was observed for the two SNPs located in the promoter and in intron 5, 93.3% in both cases. Quality control of genotyping results based on included Centre d’Etude du Polymorphisme Humain controls (59 samples), repeated study samples (from 115 individuals), and independent genotyping of the R462Q variant in two different laboratories (with two different genotyping methods) provided an estimated error rate of 0.3%. Repeated samples revealed 19 discordant genotyping results of 2,901 samples verified. Repeated samples with discordant genotyping result were blanked in the analysis. All

Centre d’Etude du Polymorphisme Humain samples gave consistent genotypes. No significant deviation of the genotype frequencies from those expected under Hardy-Weinberg equilibrium was observed for any of the six sequence variants, neither among the 801 control individuals nor among the 1504 unrelated prostate cancer cases (all P values > 0.19; range, 0.19 to 1.00). We found strong evidence for pairwise linkage disequilibrium between these variants (all P values < 0.0001) and pairwise D’ estimates between adjacent SNPs were high (mean, 0.94; range, 0.76 to 1.00).

The truncating mutation, E265X, was observed in 15 (1.9%) controls, in 23 (1.9%) sporadic prostate cancer cases, and in five (1.4%) familial prostate cancer or HPC cases (Table 3). There was no significant difference in proportion of mutation carriers between controls and sporadic prostate cancer cases or between controls and familial prostate cancer or HPC cases. Among cases with HPC, four individuals (2.1%) were observed with the stop mutation. This proportion was not significantly different from the proportion observed among controls. The four HPC cases carrying the stop mutations are members of three different families. In one family, including five affected brothers with available DNA samples, two were mutation carriers, whereas the other three did not carry the mutation. In the other two HPC families, including one affected mutation carrier, no DNA samples were available from any other family member. The median age at sampling for the 15 control individuals carrying the mutation was 4 years younger than for the control

<table>
<thead>
<tr>
<th>Variant and genotype</th>
<th>Controls</th>
<th>Sporadic prostate cancer</th>
<th>Familial prostate cancer and HPC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>2575 A&gt;G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>268</td>
<td>35.8</td>
<td>400</td>
</tr>
<tr>
<td>AG</td>
<td>371</td>
<td>49.6</td>
<td>584</td>
</tr>
<tr>
<td>GG</td>
<td>109</td>
<td>14.6</td>
<td>173</td>
</tr>
<tr>
<td>E265X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>780</td>
<td>98.1</td>
<td>1204</td>
</tr>
<tr>
<td>GT</td>
<td>15</td>
<td>1.9</td>
<td>23</td>
</tr>
<tr>
<td>R462Q</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>297</td>
<td>37.3</td>
<td>451</td>
</tr>
<tr>
<td>GA</td>
<td>384</td>
<td>48.2</td>
<td>598</td>
</tr>
<tr>
<td>AA</td>
<td>115</td>
<td>14.4</td>
<td>189</td>
</tr>
<tr>
<td>D541E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>257</td>
<td>32.5</td>
<td>407</td>
</tr>
<tr>
<td>GT</td>
<td>372</td>
<td>47.0</td>
<td>601</td>
</tr>
<tr>
<td>TT</td>
<td>162</td>
<td>20.5</td>
<td>208</td>
</tr>
<tr>
<td>+10721 G&gt;A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>381</td>
<td>50.8</td>
<td>599</td>
</tr>
<tr>
<td>GA</td>
<td>313</td>
<td>41.7</td>
<td>462</td>
</tr>
<tr>
<td>AA</td>
<td>56</td>
<td>7.5</td>
<td>98</td>
</tr>
<tr>
<td>+13861 A&gt;C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>211</td>
<td>27.4</td>
<td>316</td>
</tr>
<tr>
<td>AC</td>
<td>386</td>
<td>50.1</td>
<td>622</td>
</tr>
<tr>
<td>CC</td>
<td>173</td>
<td>22.5</td>
<td>264</td>
</tr>
</tbody>
</table>

* Likelihood ratio test based on a logistic regression model adjusted for age and geographical region.
† Wald test based on clustered logistic regression model with a robust variance estimate adjusted for age and geographical region.
Sequence Variants and Prostate Cancer Risk

individuals who were not carriers ($P = 0.08$, Mann-Whitney test). Stratified analyses based on age (<65 years or >65 years) and tumor aggressiveness (localized or locally advanced) did not reveal any significant differences in proportion of mutation carriers between controls and prostate cancer cases.

No significant difference in genotype frequencies between sporadic prostate cancer cases, familial prostate cancer cases, or HPC cases and controls were observed for any of the five common sequence variants based on a multiplicative genetic model (Table 3). Assuming a dominant or recessive allelic effect on prostate cancer risk did not reveal any significant differences between cases and controls for any of the studied variants, except for the missense variant D541E. With a recessive model, D541E showed a significant inverse association of the less common allele between individuals with sporadic prostate cancer and controls ($\chi^2 = 4.86, P = 0.03$). However, between familial prostate cancer cases or HPC cases and controls, no significant differences were observed under a recessive model. In subgroup analyses based on age and tumor aggressiveness, no significant association between these variants and prostate cancer risk was observed.

Haplotype frequencies of the five common sequence variants were estimated in familial prostate cancer and HPC cases (selecting only the proband among related cases) and sporadic prostate cancer cases and controls (Table 4). A global score test provided no significant difference in haplotype frequency between sporadic prostate cancer cases and controls ($P = 0.46$), nor did a test of the maximum among all score-specific statistics ($P = 0.10$). This latter test should have greater statistical power compared with the global test if only a few haplotypes were associated with prostate cancer risk. Comparing familial prostate cancer and HPC cases with controls did not provide any support for association between the haplotypes and prostate cancer risk (global score test, $P = 0.33$; max-score test, $P = 0.25$). Individual haplotype analyses revealed two haplotypes significantly associated with prostate cancer risk. The frequency of the AAGAA haplotype of SNPs –2575 A>G, R462Q G>A, D541E G>T, 10721 G>A, and +13861 A>C was significantly decreased in sporadic prostate cancer cases compared with controls (2.9 versus 1.5%, $P = 0.010$). This haplotype also occurred at lower frequency among familial prostate cancer and HPC cases (1.6%) compared with controls, yet there was no significant difference ($P = 0.181$). The other haplotype significantly associated with prostate cancer risk was the GTGGC haplotype (containing the opposite allele of each SNP to the above associated haplotype) that also occurred at higher frequencies among controls (3.1%) compared with sporadic prostate cancer cases (2.5%, $P = 0.415$) and familial prostate cancer/HPC cases (0.8%, $P = 0.020$). The stop mutation E265X was in all cases observed on the most frequent haplotype AGTGC. Haplotype analyses, stratified by age and tumor aggressiveness, did not reveal any additional support for association between RNASEL sequence variants and prostate cancer risk (data not shown).

**DISCUSSION**

Linkage analyses of high-risk prostate cancer families provide convincing evidence that the HPC1 locus is likely to harbor a prostate cancer susceptibility gene. In 2002, Carpten et al. (16) proposed RNASEL as a plausible candidate gene for this region through a positional cloning and candidate gene approach. To test the hypothesis that RNASEL sequence variants are associated with prostate cancer risk, we genotyped five common SNPs, including the missense variants R462Q and D541E, and the rare stop mutation E265X in 801 unaffected men and 1636 prostate cancer patients. Overall, our study provided limited support for the hypothesis that RNASEL is a prostate cancer susceptibility gene.

In the initial report of RNASEL as a candidate susceptibility gene for prostate cancer, an index case from each of 26 families at high risk for prostate cancer were screened for mutations in the RNASEL gene. Among these families, eight were linked to the HPC1 region with at least four affected individuals sharing an HPC1 haplotype. In this selected set of families, the stop mutation E265X, detected in one (3.8%) of the index cases, cosegregated with disease status within that family. Rökmans et al. (17) reported the detection of E265X in five (4.3%) index

---

**Table 4** Estimated RNASEL haplotype frequencies in controls, patients with SPC and patients with FPC or HPC

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Controls %</th>
<th>Score*</th>
<th>P†</th>
<th>Sporadic prostate cancer %</th>
<th>Score*</th>
<th>P†</th>
<th>Familial prostate cancer and HPC %</th>
<th>Score*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>A G T G C</td>
<td>24.2</td>
<td>23.8</td>
<td>0.60</td>
<td>0.492</td>
<td>21.4</td>
<td>0.77</td>
<td>0.461</td>
<td>13.9</td>
<td>0.495</td>
</tr>
<tr>
<td>G A G A A</td>
<td>19.4</td>
<td>21.2</td>
<td>1.33</td>
<td>0.183</td>
<td>18.7</td>
<td>0.46</td>
<td>0.620</td>
<td>13.9</td>
<td>0.495</td>
</tr>
<tr>
<td>A G T G A</td>
<td>13.9</td>
<td>13.5</td>
<td>0.49</td>
<td>0.625</td>
<td>16.9</td>
<td>0.99</td>
<td>0.319</td>
<td>13.4</td>
<td>0.74</td>
</tr>
<tr>
<td>G A G G C</td>
<td>11.7</td>
<td>12.2</td>
<td>0.24</td>
<td>0.814</td>
<td>13.4</td>
<td>0.74</td>
<td>0.467</td>
<td>8.7</td>
<td>0.62</td>
</tr>
<tr>
<td>A G G G C</td>
<td>8.7</td>
<td>9.0</td>
<td>0.62</td>
<td>0.534</td>
<td>11.3</td>
<td>1.45</td>
<td>0.142</td>
<td>6.1</td>
<td>0.76</td>
</tr>
<tr>
<td>A G G G A</td>
<td>6.1</td>
<td>6.6</td>
<td>0.76</td>
<td>0.445</td>
<td>5.6</td>
<td>0.15</td>
<td>0.885</td>
<td>3.5</td>
<td>2.9</td>
</tr>
<tr>
<td>G A G G A</td>
<td>3.5</td>
<td>2.9</td>
<td>0.68</td>
<td>0.495</td>
<td>3.6</td>
<td>0.36</td>
<td>0.720</td>
<td>3.1</td>
<td>2.5</td>
</tr>
<tr>
<td>G G T G C</td>
<td>3.1</td>
<td>2.5</td>
<td>0.81</td>
<td>0.415</td>
<td>3.8</td>
<td>2.29</td>
<td>0.020</td>
<td>2.9</td>
<td>1.5</td>
</tr>
<tr>
<td>A G G A A</td>
<td>2.9</td>
<td>2.7</td>
<td>0.30</td>
<td>0.763</td>
<td>0.7</td>
<td>0.94</td>
<td>0.363</td>
<td>2.6</td>
<td>2.7</td>
</tr>
<tr>
<td>A G T A A</td>
<td>2.3</td>
<td>2.6</td>
<td>0.07</td>
<td>0.945</td>
<td>4.2</td>
<td>1.27</td>
<td>0.192</td>
<td>2.3</td>
<td>2.6</td>
</tr>
<tr>
<td>G G G A A</td>
<td>0.6</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Score test statistic for association between haplotypes and prostate cancer risk.
† Empirical P values based on 10,000 replications.
patients from 116 Finnish families with HPC. In one of these five families, suggestive evidence of segregation between the mutation and prostate cancer was observed. In that study, they also compared the frequency of E265X mutations in unscreened prostate cancer cases (n = 492) and control individuals (n = 566), without finding any significant association. In a study of 95 men with HPC, originating from 75 unrelated families, Chen et al. (32) found the E265X mutation in one family, with two of three affected brothers carrying the mutation. From these results, it is clear that only a small fraction of prostate cancer families can be explained by segregation of the E265X variant.

We observed virtually identical frequencies of E265X mutation carriers among sporadic prostate cancer cases and control individuals (1.9% in both groups). Among familial prostate cancer and HPC patients, expected to be enriched for genetic variants associated with prostate cancer risk, only 5 (1.4%) of 350 patients carried the E265X mutation; 4 of these had HPC and were members of three unrelated families. None of these families provided evidence for cosegregation between the mutation and disease status; in the only family with DNA samples available from other family members, two of five affected brothers were mutation carriers. Therefore, our results provide no support for the E265X mutation being a high-penetration risk variant for prostate cancer. Furthermore, the complete lack of association between E265X and prostate cancer risk in our study provided no evidence for E265X being a modifier variant for prostate cancer risk.

The common missense mutation R462Q has been implicated in prostate cancer risk both from epidemiologic and functional studies. Using 423 prostate cancer cases and 454 sibling controls, Casey et al. (18) estimated that heterozygous carriers of this variant have 50% greater risk of prostate cancer than noncarriers, and homozygous carriers have more than double the risk. They also reported a reduction in enzymatic activity of the variant to one-third of normal activity. Additional functional evidence for this variants role in prostate cancer development comes from a recent study observing that the R462Q variant reduced the ability of RNase L to cause apoptosis in response to activation by 2-5A (21). Rökman et al. (17) reported moderate evidence for an increased risk of prostate cancer for homozygous carriers of the R462Q variant in 66 index patients from HPC families. In addition, Wang et al. (20) reported a significant association between this variant and prostate cancer risk among 473 affected men from 181 families; however, they found an opposite trend with odds ratios of 0.8 for heterozygotes and 0.5 for homozygotes.

Despite the functional and epidemiologic findings supporting a role for R462Q in prostate cancer susceptibility, no significant associations between this variant and prostate cancer risk was observed in our study. Nor did we find any significant association with prostate cancer risk for any of the other four common sequence variants studied, except for the missense mutation D541E, which under a recessive genetic model, provided marginally significant evidence for association with sporadic prostate cancer (P = 0.03), with an estimated odds ratio of 0.77 (95% confidence interval, 0.59–1.00) for homozygous carriers compared with homozygous noncarriers. However, because of the large number of tests conducted in our study, this could represent a false positive finding. Moreover, no differences were observed between patients with familial prostate cancer or HPC and controls for this variant, irrespective of choice of genetic model (recessive, multiplicative, or dominant). This missense variant was significantly associated with an increased prostate cancer risk in a study from Japan based on 101 familial prostate cancer cases and 105 controls (19), yet other studies have failed to find an association between this variant and prostate cancer risk (17, 18, 20).

Haplotyping analysis of the five SNPs provided weak support for RNASEL as a risk factor for prostate cancer. Global tests for association between haplotypes and prostate cancer risk were all nonsignificant; however, two specific haplotypes were significantly associated with prostate cancer risk. For both haplotypes, higher frequency was observed among controls compared with prostate cancer cases, indicating protective effects. It is therefore puzzling that these haplotypes were completely discordant for each of the five SNPs, and, as discussed above, due to the many statistical tests performed, the chance of false positive findings has to be considered.

Our study design has a certain limitation. There is the possibility of the existence of other common variants in RNASEL that are not well represented by the five SNPs genotyped in the present study. We used the public database National Center for Biotechnology Information for selection of SNPs, which is suboptimal compared with sequencing an adequate number of individuals from the study population to uncover all existing variants. However, considering the strong linkage disequilibrium between the studied SNPs, we believe that it is most likely that we were able to captured the major part of the genetic variation in the RNASEL gene in our study. Furthermore, because of the large number of individuals in this study, the possibility that our results represent false negative findings is of minimal concern. For example, comparing sporadic prostate cancer patients with controls, using the genotype frequencies observed for the R462Q variant among the controls and assuming a multiplicative genetic model, we have >87% power to detect an odds ratio of 1.5 for homozygotes at a significance level of 5% (two-sided test).

In summary, we failed to support a role of the rare truncating mutation E265X in Swedish prostate cancer. In addition, analyses of common sequence variants, both individually and through haplotypes, provided limited evidence for association with prostate cancer risk. Considering the large and well-characterized study population and the high quality in genotyping, these results provide strong evidence against a role of RNASEL in prostate cancer etiology in Sweden.

ACKNOWLEDGMENTS

We thank all study participants in the Cancer Prostate in Sweden study and all family members who participated in this study, Ulrika Lund for skillfully coordinating the study center at Karolinska Institute, all urologists, including their patients in the Cancer Prostate in Sweden study, and all urologists providing clinical data to the National Registry of Prostate Cancer. We also thank Karin Andersson, Susan Lindh, Gabriella Thörén Berglund, and Margareta Åswärd at the Regional Cancer Registries. In addition, we thank Sören Holmgren and the personnel at the Medical Biobank in Umeå for skilfully handling the blood samples.
REFERENCES


29. Weir BS. Genetic data analysis II. Sunderland, United Kingdom: Sinauer; 1996.


Genetic Analysis of the RNASEL Gene in Hereditary, Familial, and Sporadic Prostate Cancer


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/10/21/7150

Cited articles
This article cites 30 articles, 7 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/10/21/7150.full#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/10/21/7150.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.