Analysis of the *RNASEL* Gene in Familial and Sporadic Prostate Cancer

Liang Wang,[1] Shannon K. McDonnell,[2] David A. Elkins,[1] Susan L. Slager,[2] Eric Christensen,[1] Angela F. Marks,[1] Julie M. Cunningham,[1] Brett J. Peterson,[2] Steven J. Jacobsen,[2] James R. Cerhan,[2] Michael L. Blute,[3] Daniel J. Schaid,[2] and Stephen N. Thibodeau[1]

Departments of [1]Laboratory Medicine and Pathology, [2]Health Sciences Research, and [3]Urology, Mayo Clinic and Foundation, Rochester, MN

The *RNASEL* gene on chromosome 1q25 was recently identified as a candidate gene for hereditary prostate cancer (PC). To confirm these findings, we screened 326 patients from 163 families with familial PC for potential germline mutations, by use of conformation-sensitive gel electrophoresis, followed by direct sequence analysis. A total of six variants were identified, including one intronic and five exonic changes (three missense and two silent alterations). There were no unequivocal pathogenic changes. To further test for potential associations between genes and increased risk for disease, the three missense polymorphisms (Ile97Leu, Arg462Gln, and Glu541Asp) were genotyped in 438 patients with familial PC and in 510 population-based control subjects. Association testing revealed no significant differences between patients and control subjects for either the Leu97 variant (χ^2 trend test = 1.42; $P = .23$) or the Asp541 variant ($\chi^2 = 1.52$; $P = .22$). However, significant differences were detected for the Arg462Gln genotypes ($\chi^2 = 5.20$; $P = .02$; odds ratio [OR] = 0.54; 95% confidence interval [CI] 0.32–0.91) when the genotype Gln/Gln was compared with Arg/Arg. In subset analyses, associations were also observed in the younger group (age at diagnosis ≤64 years) ($P = .0008$; OR = 0.29; 95% CI = 0.13–0.66), in node-negative patients ($P = .01$; OR = 0.48; 95% CI 0.27–0.84), patients with stage T_1/T_2 disease ($P = .008$; OR = 0.39; 95% CI 0.2–0.75), and patients with low-grade disease ($P = .01$; OR = 0.40; 95% CI 0.20–0.78). To evaluate whether this variant was also associated with sporadic PC, we genotyped an additional 499 patients with sporadic PC. Differences in frequency were not detected between patients with sporadic disease and control subjects. However, the same association was observed between patients with familial disease and patients with sporadic disease for the entire group ($\chi^2 = 4.82$; $P = .03$), as well as in the subset analyses. These results suggest that polymorphic changes within the *RNASEL* gene may be associated with increased risk of familial but not sporadic PC.

Introduction

It has been known for some time that prostate cancer (PC) tends to cluster in some families (Cannon et al. 1982; Meikle and Stanish 1982; Steinberg et al. 1990; Spitz et al. 1991; Carter et al. 1992; Goldgar et al. 1994; Whittemore et al. 1995). Segregation analysis suggests that this familial clustering can best be explained by at least one rare dominant susceptibility gene (Carter et al. 1992; Schaid et al. 1998). However, evidence also points to a complex genetic basis, involving multiple susceptibility genes and variable phenotypic expression. On the basis of linkage studies of families with high risk of PC, six PC-susceptibility loci have been postulated to exist: *HPC1* (MIM 601518) localized to chromosome 1q24-25 (Smith et al. 1996); *PCAP* (MIM 602759) to 1q42.2-43 (Berthon et al. 1998); *CAPB* (MIM 603688) to 1p36 (Gibbs et al. 1999); *HPCX* (MIM 300147) to Xq27-28 (Xu et al. 1998); HPC20 to 20q13 (Berry et al. 2000); and *HPC2* (MIM 605367) to17p (Tavtigian et al. 2001). Among the six loci, two candidate genes have been proposed: *HPC2/ELAC2* on 17p (Tavtigian et al. 2001) and *RNASEL* on 1q25 (Carpten et al. 2002). Variations of the *HPC2/ELAC2* were initially reported to be associated with PC risk (Rebbeck et al. 2000). However, recent studies suggest that it likely plays a more limited role in sporadic and hereditary PC (Rokman et al. 2001; Suarez et al. 2001; Wang et al. 2001; Xu et al. 2001).

RNASEL (National Center for Biotechnology Information nucleotide accession number NM_021133; MIM 180435) was recently identified by a positional-cloning/candidate method, and germline mutations were reported to cosegregate within families with PC linked to the *HPC1* region, at 1q24-31 (Carpten et al. 2002). This gene has elsewhere been shown to play a role in regulating cell proliferation and apoptosis through the 2–5A pathway and has been suggested as a candidate tumor-suppressor gene (Hassel et al. 1993; Lengyel 1993).

To confirm whether alterations of *RNASEL* are associated with familial PC risk, we screened 326 patients

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Address for correspondence and reprints: Dr. Stephen N. Thibodeau, Laboratory Genetics/Hilton 970, Mayo Clinic Rochester, 200 First Street SW, Rochester, MN 55905. E-mail: sthibodeau@mayo.edu

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with PC (two affected members per family) from 163 families that were defined as having familial PC (Berry et al. 2000), for potential germline mutation. We also examined the frequency of three polymorphisms (Ile97Leu, Arg462Gln, and Glu541Asp) among 438 patients with familial PC, 499 patients with sporadic PC, and 510 control subjects, for potential associations with the presence of PC.

Subjects and Methods

Patients with Familial PC

Ascertainment of families with PC has been described elsewhere (Berry et al. 2000). In brief, on the basis of surveys of 12,675 men performed at Mayo Clinic, ~200 high-risk families were identified; families having a minimum of 3 men with PC were enrolled for further study. Blood was collected from as many family members as possible, resulting in a total of 473 affected men from 181 families. For 163 of these families, DNA information was available on multiple living affected men. For the remaining 18 families, DNA information was available on only a single affected individual. All men with PC who contributed a blood specimen had their cancers verified by review of medical records and pathologic confirmation. One family has Hispanic ancestry; the remainder consist of non-Hispanic white men.

In an effort to identify potential gene alterations that segregate with disease, two affected members (the proband and the youngest available affected man) from each of 163 families were selected for further analysis (total 326 patients). For the association study, all affected men from the same generation (i.e., siblings and cousins) were included. The decision to study men of the same generation was made to avoid large differences in ages and to avoid secular trends based on year of diagnosis. Thus, 438 patients (consisting of singletons, siblings, and cousins) were used for the association study. The research protocol and informed consent forms were approved by the Mayo Clinic institutional review board.

Patients with Sporadic PC

Patients with sporadic PC were selected from respondents to our family history survey who reported no family history of PC. To ensure that the sporadic group was similar to the familial group, except for family history, eligible patients with sporadic disease were selected by frequency matching them to the familial index patients (i.e., matched to have equal frequencies of categories, rather than one-to-one matching), according to year of diagnosis, age at diagnosis, and number of brothers. Multiple patients with sporadic disease were identified for each familial case, and these case sets were randomly sampled for recruitment, with a goal of recruiting ~500 men with sporadic PC. A total of 1,001 invitations were sent to men who initially reported no family history of PC. Our second survey determined that only 740 of these men were eligible (i.e., still no family history of PC), and, of these men, 501 agreed to contribute a blood sample. At the time when assays were performed, blood samples were available for 499 men. All but 11 of these men were treated surgically for their PC.

Population Controls for Association Study

From a sampling frame of the local population provided by the Rochester Epidemiology Project (Melton 1996), 475 men were randomly selected for a clinical urologic examination (Oesterling et al. 1993). This examination included digital rectal examination (DRE) and transrectal ultrasound (TRUS) of the prostate, abdominal ultrasound for post void residual urine volume, measurement of serum levels of prostate-specific antigen (PSA) and creatinine, focused urologic physical examination, and cryopreservation of serum for subsequent sex hormone assays. Any patient with an abnormal DRE, elevated serum PSA level, or suspicious lesion on TRUS was evaluated for prostatic malignancy. If the DRE and TRUS were unremarkable but the serum PSA level was elevated (>4.0 ng/ml), then a sextant biopsy (three cores from each side) of the prostate was performed. An abnormal DRE or TRUS result, regardless of the serum PSA level, prompted a biopsy of the area in question. In addition, a sextant biopsy of the remaining prostate was performed. Those men who were found to be without PC on the basis of this extensive workup at baseline or at any of the follow-up examinations through 1994 were used for the control population ($n = 372$). To make up for study attrition, the sample was augmented with men who were randomly selected from the population to undergo an identical workup ($n = 138$), resulting in a sample of 510 men without evidence of PC (Roberts et al. 2000).

Conformation-Sensitive Gel Electrophoresis (CSGE) and Direct Sequencing

The *RNASEL* gene has eight exons (Carpten et al. 2002). The sequences for the PCR primers covering the coding sequence for six exons were kindly provided by Dr. Carpten, of the National Human Genome Research Institute, National Institutes of Health. CSGE has been successfully used for mutation screening (Ganguly et al. 1993; Couch et al. 1996; Korkko et al. 1998). When compared with sequencing, the CSGE detection rates in our laboratory were 85%–100% (Park et al. 2000). Because the technique is dependent on formation of heteroduplexes, we mixed two samples from different families to maximize this possibility and to allow for more efficient screening. PCR was performed for 30 cycles,

with initial denaturation at 94°C for 12 min, followed by 94°C for 20 s, 58°C for 30 s, and 72°C for 1 min. The reaction was processed in a total volume of 12.5 μl that consisted of dATP, dGTP, and dTTP (200 μM each); 50 μM of dCTP; and 0.1 μl of ^{33}P-dCTP, 2 mM of $MgCl_2$, 50 ng of template DNA, 1 × Ampli*Taq* Gold buffer II, and 0.5 units of *Taq*AmpliGold DNA polymerase (Perkin-Elmer). The PCR product was then denatured at 96°C for 5 min and was cooled to 65°C during the course of 30 min. The reannealed product (5 μl) was then mixed with 1 μl of loading dye (30% glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanol FF). This mixture (0.5 μl) was loaded on the conformation-sensitive gel, which consisted of 15% of acrylamide and 1,4-bis (acrolyl) piperazine (ratio 19:1), 0.5 × TTE buffer (44.4 mM Tris, 14.25 mM taurine, and 0.1 mM EDTA, pH 9.0), 15% of formamide, and 10% of ethylene glycol. The gel was run at 30 W for 5 h. When altered bands were detected, the patient samples were reamplified separately, and 200 ng of purified PCR product and 3.8 pmol of sequencing primer were mixed and sequenced using an ABI 377 automated sequencer.

Genotyping by Pyrosequencing

Three polymorphisms (Ile97Leu, Arg462Gln, and Glu541Asp) in the *RNASEL* gene were genotyped in 438 patients with familial PC, 499 patients with sporadic PC, and 510 control subjects. The PCR primers and pyrosequencing primers are as follows:

1. 97F/97R: biotin-5′-TCTGCTTCGTCATGGTGCT/CACACTCATTGACATCTGCTCC-3′;
2. 97-pyrosequencing: 5′-CAATCGCTGCGAGGA-3′;
3. 462F/462R: biotin-5′-TGGAAGCGTGTTTGGATGT/TGTGGTTGCAGATCCTGGT-3′;
4. 462-pyrosequencing: 5′-TAGATGACAGGACATTT-3′;
5. 541F/541R: biotin-5′-GGCTGGTCCTCTATGTGGTA/GGTCCTTAGTTTCCTCATCTGG-3′;
6. 541-pyrosequencing: 5′-TCATTACTTTGAGCTTTC-3′.

The primer pair 97F/97R was used to amplify a 134-bp region containing the Ile97Leu variant. The 462F/462R pair covered a 145-bp region containing the Arg462Gln variant. The 541F/541R pair produced a 108-bp fragment containing the Glu541Asp variant. All PCR assays were performed in a 25-μl reaction volume consisting of 1 × AmpliGold buffer II, 2 mM $MgCl_2$, 100 μM of each dNTP, 10 pmol of each primer, 0.75 U of *Taq*AmpliGold DNA polymerase, and 30 ng of template DNA. PCR was performed using a Tetrad thermal cycler (MJ Research) under the following conditions: initial denaturation at 94°C for 12 min, followed by 40 cycles at 94°C for 20 s, 40 cycles at 58°C for 30 s, and 40 cycles at 72°C for 30 s.

The PCR products were mixed with 25 μl of 2 × binding-washing buffer (10 mM Tris-HCl, 2 M NaCl, 1 mM EDTA, and 0.1% Tween 20, pH 7.6) and 10 μl of Dynabeads (Dynal AS) and were incubated on a thermomixer (Eppendorf) (1,200 rpm) at 65°C for 15 min. To obtain single-strand DNA for subsequent pyrosequencing, Dynabead-immobilized PCR products were denatured in 50 μl of 0.5 M NaOH for 1 min. Immobilized single strands were transferred to 100 μl of 1 × annealing buffer (20 mM Tris acetate and 5 mM $MgAc_2$, pH 7.6) for 1 min and were again transferred to 45 μl of 1 × annealing buffer containing 10 pmol of sequencing primer. After denaturation at 95°C for 3 min, the samples were subjected to DNA sequencing in the PSQ96 system (Pyrosequencing). The genotype of each sample was called automatically by the instrument but was also evaluated manually for potential misclassification.

Statistical Analysis

The association of each of the three polymorphisms (Ile97Leu, Arg462Gln, and Glu541Asp) with familial PC was evaluated by two statistical approaches. The first approach compared genotype frequencies among patients with frequencies among control subjects, using a test for trend in the number of variant alleles that is analogous to the Armitage test for trend in proportions (Sasieni 1997). For the unrelated patients with sporadic disease, the trend test is exactly the Armitage test for trend. For related patients with familial disease, a method that accounts for the correlated family data by correctly computing the variance of the trend test and the variance of the odds ratios (ORs) was used, as described elsewhere (Slager and Schaid 2001).

The second approach was based on unconditional logistic regression, to evaluate the main effects of the variants between each group (patients with familial disease, patients with sporadic disease, and control subjects) while adjusting for age, which is a potential confounding factor. For these analyses, age was defined as age at diagnosis for patients and age at blood draw for control subjects. In addition, age was categorized using quartiles of the combined distribution of patients and control subjects (quartiles were 42–56 years, 57–64 years, 65–69 years, and 70–84 years) or as a continuous variable. To account for genotype correlations among patients from the same family, generalized estimating equations were used (Zeger and Liang 1986), assuming an exchangeable working correlation matrix. All reported *P* values are two sided.

Results

In an effort to identify gene alterations that segregate with disease in our patients with familial disease, two

affected members from each of 163 families were selected for further analysis. Two individuals per family were chosen to take into account the presence of phenocopies, which are presumed to occur frequently in families with hereditary PC.

Among the 326 patients with familial PC who were screened for potential germline mutations, a total of six variants (one intronic and five exonic) were identified and confirmed by DNA sequencing (table 1). Of the five exonic changes, two were common polymorphisms (G1385A and G1623T), whereas the remaining three were rare variants (A289C, A2173G, and C354T). The G→A transition at base pair position 1385 causes an amino acid change from arginine to glutamine at codon 462 (Arg462Gln). The allele frequencies for Arg and Gln are ~63% and 37%, respectively. The G→T transversion at base pair position 1623 results in a substitution of glutamic acid for aspartic acid at codon 541 (Glu541Asp). The allele frequencies for Glu and Asp are ~57% and 43%, respectively. The A→C transversion at base pair position 289 causes a replacement of isoleucine for leucine at codon 97 (Ile97Leu). The allele frequencies for Ile and Leu are ~99% and 1%, respectively. The variants C354T and A2173G are silent. One rare intronic change (T→C) was found in intron 5, 12 nt upstream from exon 5.

The three common missense variants (Ile97Leu, Arg462Gln, and Glu541Asp) were genotyped in 438 patients with familial disease, 499 patients with sporadic disease, and 510 population control subjects to evaluate whether the variant alleles at these loci were associated with an increased risk of PC. Table 2 shows the characteristics of the two PC patient groups and the control group used in this analysis. The age range of all three groups at diagnosis (patients) or at blood draw (control subjects) was ~45–85 years. However, the median age of the control group (55 years) was ~10 years less than that of the familial (66 years) or sporadic (65 years) groups, because a relatively large part of the control sample was <56 years old. Because of this difference, age was included in additional logistic regression models, to statistically adjust for its potentially confounding effects.

Virtually all of the control subjects had a serum PSA level <4 ng/ml, whereas the distribution of PSA values was similar among familial and patients with sporadic disease. Slightly less than half of the patients with familial disease underwent surgery, compared with 98% of the patients with sporadic disease. This difference is likely caused by the method of selection. Probands (from the patients with familial disease) and the patients with sporadic disease were selected primarily from a radical prostatectomy database. The affected relatives of the probands, on the other hand, were not selected on the basis of treatment and therefore represent all possible treatments. Because the sporadic-cancer group is made up almost entirely of men treated with surgery, there are fewer older men in this group. The lower part of table 2 shows that the pathologic variables in the surgical subsets of both groups are similar with respect to nodal status, stage, and grade.

Table 1

Analysis of *RNASEL* Gene in Patients with Familial PC

Variant	Nucleotide Change	Amino Acid Change
Exons:		
2	A289C	Ile97Leu
2	C354T	None
2	G1385A	Arg462Gln
4	G1623T	Glu541Asp
7	A2173G	None
Intron	IVS5−12 T→C	None

We first tested for potential associations of the various genotypes with risk of familial PC. No significant associations between patients and control subjects were found for either the Leu97 variant ($P = .23$) or the Asp541 variant ($\chi^2 = 1.52$; $P = .22$). Analysis of the Arg462Gln variant, on the other hand, showed a significant association between patients with familial disease and control subjects ($P = .02$) (table 3). In subset analyses, the strongest association was seen in the younger patients (age ≤64 years), among whom we observed a significant inverse association of the less common allele for both homozygotes (OR = 0.29; 95% CI 0.13–0.66) and heterozygotes (OR = 0.63; 95% CI 0.41–0.95). We also saw significant associations for node negative patients ($P = .01$), early stage (T_1/T_2; $P = .008$), and low grade (≤6; $P = .01$). However, in each of these subgroups, only the ORs for the homozygotes reached statistical significance, all showing an inverse association with the less common allele. The ORs for the heterozygotes also indicated an inverse association, but the 95% CIs overlapped 1.0 (table 3). Similar results were obtained after adjusting for age (data not shown). In addition to using age quartiles to adjust for the effect of age (see "Subjects and Methods" section), we investigated age as a continuous variable in our regression models, to have more-refined adjustments for age. The results obtained were very similar to the results reported in table 3, further indicating that the imbalance of age is not a major source of bias.

To evaluate whether the Arg462Gln variant is also associated with sporadic PC, we genotyped this allele in 499 patients with sporadic PC. No differences were observed between patients with sporadic disease and control subjects ($P = .92$; data not shown). However, we did detect a significant difference between patients with sporadic versus those with familial disease ($\chi^2 = 4.82$; $P = .03$) (table 4), and this difference remained

Table 2

Characteristics of Patients with PC and Population-Based Control Subjects

Characteristic[a]	Familial PC (N = 438)[b]	Sporadic PC (N = 499)[b]	Control (N = 510)[b]	P[c]
Median age and range (years)	66 (45–84)	65 (46–79)	55 (42–83)	…
Age quartiles (years):	…	…	…	…
42–56	37 (8.4)	53 (10.6)	273 (53.5)	.001
57–64	133 (30.4)	182 (36.5)	98 (19.2)	…
65–69	137 (31.3)	177 (35.5)	49 (9.6)	…
70–84	131 (29.9)	87 (17.4)	90 (17.7)	…
PSA:	…	…	…	…
<4	41 (12.2)	92 (22.7)	488 (95.7)	.001
4–9.9	155 (46.1)	179 (44.2)	22 (4.3)	…
10–19.9	68 (20.2)	73 (18.0)	0 (.0)	…
⩾20	72 (21.4)	61 (15.1)	0 (.0)	…
Unknown	102	94	0	…
Surgical status:	…	…	…	…
No surgery	232 (53.0)	11 (2.2)	…	…
Surgical patient	206 (47.0)	488 (97.8)	…	…
Pathologic characteristics of all patients:	…	…	…	…
Nodal status:	…	…	…	…
Negative	379 (87.9)	413 (87.7)	…	.91
Positive[d]	52 (12.1)	58 (12.3)	…	…
Unknown	7	28	…	…
Stage:[e]	…	…	…	…
T_1/T_2	272 (71.8)	261 (64.0)	…	.02
T_3/T_4	107 (28.2)	147 (36.0)	…	…
Unknown	7	33	…	…
Grade:	…	…	…	…
<7	251 (57.3)	268 (53.7)	…	.48
⩾7	109 (24.9)	129 (25.9)	…	…
Unknown	78 (17.8)	102 (20.4)		
Pathologic characteristics of surgical patients:	…	…	…	…
Nodal status:	…	…	…	…
Negative	181 (87.9)	411 (89.0)	…	.68
Positive[d]	25 (12.1)	51 (11.0)	…	…
Unknown	0	26	…	…
Stage:[e]	…	…	…	…
T_1/T_2	117 (64.6)	261 (64.0)	…	.88
T_3/T_4	64 (35.4)	147 (36.0)	…	…
Unknown	0	3	…	…
Grade:	…	…	…	…
<7	112 (54.4)	267 (54.7)	…	.66
⩾7	60 (29.1)	129 (26.4)	…	…
Unknown	34 (16.5)	92 (18.9)		

[a] Age is defined as age at diagnosis for patients with familial or sporadic PC and as age at the time of blood draw for control subjects.

[b] Data are no. (%), except as otherwise noted.

[c] Results from Pearson χ^2 test or Mantel-Haenszel test for trend.

[d] Includes patients who had metastatic disease.

[e] Patients whose nodal status was positive are excluded.

significant even after adjustment for age (heterozygous OR = 0.82; 95% CI 0.69–0.97). Moreover, the significant associations from the subgroup analyses between patients with familial disease and patients with sporadic disease were of similar magnitude and in the same direction as those between patients with familial disease and control subjects. In almost all pathologically defined subgroups, the associations were inverse, with the homozygous ORs for the younger, early-stage, node-negative, and lower-grade subgroups reaching statistical significance (table 4).

Discussion

In the present study, gene analysis identified six variants (none of which were pathogenic alterations) within the *RNASEL* gene among our 326 patients with familial PC. Carpten et al. (2002) reported a nonsense variant,

Table 3

Association between PC and the *RNASEL* Arg462Gln Polymorphism: Familial vs. Controls

Subject Characteristic	*N*	No. (%) of Subjects with					OR (95% CI)	
		Arg/Arg	Arg/Gln	Gln/Gln	χ^{2a}	*P*	Gln/Gln vs. Arg/Arg	Arg/Gln vs. Arg/Arg
Control:	493	193 (39.2)	233 (47.3)	67 (13.6)	...	...	...	...
Age ⩽64 years	357	141 (39.5)	164 (45.9)	52 (14.6)	...	...	...	...
Age >64 years	136	52 (38.2)	69 (50.7)	15 (11.0)	...	...	...	...
Familial:	433	198 (45.7)	198 (45.7)	37 (8.6)	5.20	.02	.54 (.32–.91)	.83 (.61–1.13)
Age ⩽64 years	169	92 (54.4)	67 (39.6)	10 (5.9)	11.28	.0008	.29 (.13–.66)	.63 (.41–.95)
Age >64 years	264	106 (40.2)	131 (49.6)	27 (10.2)	.13	.71	.88 (.41–1.91)	.93 (.58–1.49)
Node negative	375	175 (46.7)	171 (45.6)	29 (7.7)	6.46	.01	.48 (.27–.84)	.81 (.59–1.11)
Node positive	52	18 (34.6)	26 (50.0)	8 (15.4)	.40	.53	1.28 (.53–3.11)	1.20 (.64–2.26)
Stage T_1/T_2	268	126 (47.0)	125 (46.6)	17 (6.3)	7.00	.008	.39 (.20–.75)	.82 (.58–1.16)
Stage T_3/T_4	107	49 (45.8)	46 (43.0)	12 (11.2)	1.35	.24	.71 (.34–1.47)	.78 (.49–1.24)
Low grade (⩽6)	248	116 (46.8)	116 (46.8)	16 (6.5)	6.35	.01	.40 (.20–.78)	.83 (.58–1.18)
High grade (>7)	109	46 (42.2)	50 (45.9)	13 (11.9)	.37	.54	.81 (.39–1.68)	.90 (.56–1.44)

[a] Armitage test for trend on allele counts, accounting for related subjects.

E265X, which cosegregated in one of the *HPC1*-linked families. In patients with this alteration, nuclease activity in lymphoblasts was reported to be about half of that seen in noncarriers of the mutation, suggesting that this alteration results in a complete loss of protein. However, the significance of the variant is questionable, since it was also present in 3 of 330 men without PC and in 2 of 258 patients with sporadic PC (Carpten et al. 2002). This mutation was not detected in any of our 326 familial PC patients. Although unequivocal pathogenic mutations were not detected, we cannot exclude the possibility that some might have been missed because of the location of the mutation (e.g., in the promoter region) or because of technical limitations of the CSGE screening method.

In addition to examining the *RNASEL* gene for the presence of specific mutations, we also studied three missense polymorphisms for their association with PC risk. Of the three polymorphisms, only the Arg462Gln variant showed an association with familial PC ($P = .02$), with OR = 0.54 and 0.83 for homozygous and heterozygous carriers of the Gln462 variant, respectively. Furthermore, when subsets of the patients with familial PC were examined, this association was observed with early age at diagnosis (⩽64 years), node-negative status, early-stage (T_1/T_2) disease, and low-

Table 4

Association between PC and the *RNASEL* Arg462Gln Polymorphism: Patients with Familial PC vs. those with Sporadic PC

Patient Characteristic	*N*	No. (%) of Patients with					OR (95% CI)	
		Arg/Arg	Arg/Gln	Gln/Gln	χ^{2a}	*P*	Gln/Gln vs. Arg/Arg	Gln/Arg vs. Arg/Arg
Sporadic PC:	485	191 (39.4)	229 (47.2)	65 (13.4)	...	...	...	...
Age ⩽64 years	228	94 (41.2)	108 (47.4)	26 (11.4)	...	...	...	...
Age >64 years	257	97 (37.7)	121 (47.1)	39 (15.2)	...	...	...	...
Node negative	402	163 (40.6)	184 (45.8)	55 (13.7)	...	...	...	...
Node positive	56	19 (33.9)	28 (50.0)	9 (16.1)	...	...	...	...
Stage T_1/T_2	254	112 (44.1)	106 (41.7)	36 (14.2)	...	...	...	...
Stage T_3/T_4	143	50 (35.0)	75 (52.5)	18 (12.6)	...	...	...	...
Low grade (⩽6)	259	106 (40.9)	123 (47.5)	30 (11.6)	...	...	...	...
High grade (>7)	125	45 (36.0)	63 (50.4)	17 (13.6)	...	...	...	...
Familial PC:	433	198 (45.7)	198 (45.7)	37 (8.6)	4.82	.03	.55 (.33–.93)	.83 (.61–1.14)
Age ⩽64 years	169	92 (54.4)	67 (39.6)	10 (5.9)	6.76	.009	.39 (.17–.93)	.63 (.40–.99)
Age >64 years	264	106 (40.2)	131 (49.6)	27 (10.2)	1.25	.26	.63 (.34–1.20)	.99 (.66–1.48)
Node negative	375	175 (46.7)	171 (45.6)	29 (7.7)	4.91	.03	.49 (.28–.87)	.87 (.62–1.20)
Node positive	52	18 (34.6)	26 (50.0)	8 (15.4)	.01	.92	.94 (.30–2.99)	.98 (.42–2.27)
Stage T_1/T_2	268	126 (47.0)	125 (46.6)	17 (6.3)	2.84	.09	.42 (.21–.85)	1.05 (.71–1.55)
Stage T_3/T_4	107	49 (45.8)	46 (43.0)	12 (11.2)	1.88	.17	.68 (.29–1.62)	.63 (.36–1.09)
Low grade (⩽6)	248	116 (46.8)	116 (46.8)	16 (6.5)	3.04	.08	.49 (.23–1.02)	.86 (.58–1.28)
High grade (>7)	109	46 (42.2)	50 (45.9)	13 (11.9)	.72	.40	.75 (.31–1.79)	.78 (.44–1.38)

[a] Armitage test for trend on allele counts, accounting for related subjects.

grade (≤6) disease (table 3). Although the Arg462Gln polymorphism exhibited a strong association with familial PC, no such association was observed with sporadic PC. However, significant differences were detected between patients with sporadic versus those with familial disease, including the subgroup analyses (table 4). Overall, these data suggest a role for this polymorphism in familial, but not sporadic, PC. The reported *P* values in the present study are nominal and are not corrected for the statistical testing in multiple subgroups. However, multiple testing will increase the chance of a false-positive result only for the subgroup analyses, not for our overall conclusion that this polymorphism may have a role in familial PC. Independent replication of our findings will be important to support our conclusions.

Interestingly, the increased risk of familial PC was associated with the more common allele (Arg) of the Arg462Gln polymorphism. This polymorphism is located in the protein kinase domain of the conserved region of the *RNASEL* protein. The functional significance of the substitution of the basic amino acid Arg by the neutral amino acid Gln is unclear. Thus, the mechanism by which the Arg462 variant is associated with familial PC is also unclear. Given that Arg462 is the more common (~63%) allele in the population, it may be that this gene is a common modifier of other existing, but rare, susceptibility genes, explaining its effect in familial, but not sporadic, PC. Another possibility is that the Gln462 variant is in linkage disequilibrium with another undetected polymorphism within the *RNASEL* gene or with another unidentified gene nearby. Additional studies are needed to clarify the significance of these findings.

The analysis by subtype also raises some interesting questions. A consistent pattern is seen for the Arg462Gln variant, with no association observed (either direct or indirect) with advanced or aggressive tumors but, rather, an inverse association with less aggressive tumors and with tumors identified in younger men. This suggests the possibility that the variant serves as a marker for more indolent disease. However, since this association is not seen among patients with sporadic disease, additional factors must be present in the familial group. The finding of genetic abnormalities that are associated with early-stage and apparently less malignant tumors has been reported in other malignancies. For example, defective mismatch repair in colon cancer is associated with early-stage disease and an overall improved survival (Thibodeau et al. 1998; Halling et al. 1999). Since there appears to be a correlation between the clinical features and this polymorphism, the ability to detect the effect of this allele may be dependent upon the clinical features within the study population (i.e., the proportion of patients with high versus low grade and high versus low stage). Again, similar subtype analyses should be investigated further in other populations to confirm and extend these findings.

In summary, we failed to detect any pathogenic mutations in the *RNASEL* gene in our 326 patients with familial PC. However, association studies in 438 patients with familial PC, 499 patients with sporadic PC, and 510 population-based control subjects showed that variants within the gene do appear to influence the risk of familial PC, with higher risks observed in subsets of these familial PC patients. The finding of allelic associations within the *RNASEL* gene supports its involvement in familial PC. However, more studies are needed to elucidate the mechanism responsible for this association in familial PC.

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Electronic-Database Information

Accession numbers and the URL for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *RNASEL* [MIM 180435], *HPC1* [MIM 601518], *HPC2/ELAC2* [MIM 605367], *PCAP* [MIM 602759], *CAPB* [MIM 603688], and *HPCX* [MIM 300147])

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