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Role of *HPC2/ELAC2* in Hereditary Prostate Cancer[1]

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

Departments of Laboratory Medicine and Pathology [L. W., D. A. E., E. C., A. F. M., J. M. C., S. N. T.], Health Sciences Research [S. K. M., S. L. S., B. J. P., S. J. J., J. R. C., D. J. S.], and Urology [D. A. E., M. L. B.], Mayo Clinic and Foundation, Rochester, Minnesota 55905

ABSTRACT

The *HPC2/ELAC2* gene on chromosome 17p was recently identified as a candidate gene for hereditary prostate cancer (HPC). To confirm these findings, we screened 300 prostate cancer patients (2 affected members/family) from 150 families with HPC for potential germ-line mutations using conformation-sensitive gel electrophoresis, followed by direct sequence analysis. The minimum criteria for our families with HPC was the presence of 3 affected men with prostate cancer. A total of 23 variants were identified, including 13 intronic and 10 exonic changes. Of the 10 exonic changes, 1 truncating mutation was identified, a Glu216Stop nonsense mutation. This nonsense variant was found in 2 of 3 affected men in a single family. The remaining nine alterations included five missense, three silent, and one variant in the 3′ untranslated region. To additionally test for potential associations of polymorphic variants and increased risk for disease, we genotyped two common polymorphisms, Ser217Leu and Ala541Thr, in 446 prostate cancer patients from 164 families with HPC and 502 population-based controls. The frequency of the Leu217 variant was similar for patients (32.3%) and controls (31.8%), as was the frequency of the Thr541 variant (5.4% among patients *versus* 5.2% among controls). In contrast to previous reports, we found no association of the joint effects of Leu271 and Thr541 (odds ratio, 1.04; 95% confidence interval, 0.57–1.89). Overall, our results did not reveal any association between these two common polymorphisms and the risk for HPC. The finding of a nonsense mutation in the *HPC2/ELAC2* gene confirms its potential role in genetic susceptibility to prostate cancer. However, our data also suggest that germ-line mutations of the *HPC2/ELAC2* are rare in HPC and that the variants Leu217 and Thr541 do not appear to influence the risk for HPC. Cumulatively, these results suggest that alterations within the H*PC2/ELAC2* gene play a limited role in genetic susceptibility to HPC.

INTRODUCTION

PC[3] is one of the most common human cancers, occurring in as many as 15% of men in the United States. It has been known for some time that PC tends to cluster in some families (1–7). Segregation analysis suggests that this familial clustering can best be explained by at least one rare dominant susceptibility gene (8, 9). However, evidence also points to a complex genetic basis, involving multiple susceptibility genes and variable phenotypic expression. On the basis of linkage studies, five PC susceptibility loci have been postulated to exist for HPC: *HPC1* localized to chromosome 1q24–25 (10), *PCAP* to 1q42.2–43 (11), *CAPB* to 1p36 (12), *HPCX* to Xq27–28 (13), and *HPC20* to 20q13 (14). However, none of the putative susceptibility genes have thus far been identified. Recently, Tavtigian *et al.* (15) demonstrated linkage to another site on chromosome 17p. Positional cloning and mutational screening within the refined interval identified a candidate PC predisposition gene, *HPC2/ELAC2*. This gene was reported to harbor mutations that cosegregated with PC in two kindreds. The function of this gene has yet to be elucidated.

In addition to possible germ-line mutations, two common polymorphisms (Ser217Leu and Ala541Thr) in *HPC2/ELAC2* have been reported to increase the risk for PC (15, 16). These variants have been estimated to be responsible for ≤5% of PC in the general population.

To confirm whether alterations of *HPC2/ELAC2* are associated with HPC, we screened 300 PC patients (2 affected members/family) from 150 families with HPC (14) for potential germ-line mutation. We also examined the frequency of two common polymorphisms (Ser217Leu and Ala541Thr) in a sample set consisting of 446 HPC patients and 502 controls.

MATERIALS AND METHODS

HPC Cases. Ascertainment of PC families was described previously (14). In brief, a total of 12,675 surveys were sent to men who received a radical prostatectomy or radiation therapy at Mayo Clinic from 1967 to 1997. From these surveys, ~200 high-risk families were identified. More detailed family histories were obtained over the telephone, and three to four generation pedigrees were constructed. Families having a minimum of 3 affected men with PC were enrolled for additional study. For the purposes of this study, we have defined HPC as those families having a minimum of 3 affected men with PC. Blood was collected by a number of methods from as many family members as possible, resulting in a total of 473 affected men from 181 families. For 164 of these families, DNA was available on multiple living affected men. For the remaining 17 families, DNA was available on only a single affected individual. All men who contributed a blood specimen and who had PC had their cancers verified by review of medical records and pathological confirmation. One family has Hispanic ancestry; the remainder are Caucasian.

For our mutation study, 2 affected members (the proband and 1 randomly selected affected male) from each of 150 HPC families were selected for additional analysis (total 300 patients). For our association study, we used all affected men from the same generation (*i.e.*, siblings and cousins) to avoid large differences in ages and secular trends according to year of diagnosis. Thus, 446 HPC cases, consisting of singletons, siblings, and cousins, were used for our association study. The research protocol and informed consent forms were approved by the Mayo Clinic Institutional Review Board.

Population Controls for Association Study. The Olmsted County Study of Urinary Symptoms and Health Status among men cohort was initiated in 1989–1990 and has been established and maintained by our research team over the past 10 years (17, 18). The initial cohort was drawn from the population of Olmsted County, which serves as the laboratory for the Rochester Epidemiology Project (19). The initial cohort was randomly selected from an age- and residence (City of Rochester *versus* balance of Olmsted County)-stratified sampling frame constructed from the Rochester Epidemiology Project. Of the 2115 men from the initial cohort, 475 were selected for a clinical urological examination (in-clinic cohort; Ref. 20). This examination included: DRE and TRUS of the prostate, abdominal ultrasound for postvoid residual urine volume, serum PSA and creatinine measurement, focused urological 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

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[2] To whom requests for reprints should be addressed, at Laboratory Genetics/HI 970, Mayo Clinic Rochester, 200 First Street SW, Rochester, MN 55905. Phone: (507) 284-4696; Fax: (507) 284-0043; E-mail: sthibodeau@mayo.edu.

[3] The abbreviations used are: PC, prostate cancer; HPC, hereditary prostate cancer; DRE, digital rectal examination; TRUS, transrectal ultrasound; PSA, prostate-specific antigen; CSGE, conformation-sensitive gel electrophoresis; BMI, body mass index; OR, odds ratio; CI, confidence interval.

Table 1 *DNA sequence of primers used for PCR amplification and sequencing*

Primers	Primer sequence (5′ to 3′)	Sizes (bp)
Exon 2 + 3 forward/reverse	GGCTCGGTAGTCCAACGTG/TCCCAGAGACTTCCCACCA	445
Exon 4 forward/reverse	GCTGGAAGGTGGAGATGTAGA/AAGCGTTTCAACGACCAGGTA	295
Exon 5 forward/reverse	GTCTCCTCCACTGGCCTGAT/CTGCACTCCAGCCTGGTAAG	471
Exon 6 forward/reverse	ATTCAGTTGCTCGTGGTTGG/TAGGCATAAGTCAGACATCCGT	355
Exon 7 forward/reverse	TTGGCTGTCAGCTCACCTTG/AGCCCAGGAAGAAGGATCTGT	303
Exon 8 forward/reverse	TCTGTCTTCTCCAGTCCTAGGTG/TTGTCTCAGGTAACAGGAGTGC	458
Exon 9 + 10 forward/reverse	CGTTGGTCAGGCTGGTCTC/TCGAATGCAGCCGTGTTG	624
Exon 9 + 10 forward/reverse (nested)	CTGGAATTACAGGCGTGAGC/GCCTGAAGAAGACAGACTCTGC	447
Exon 11 forward/reverse	AACCTTAATGCCAAGCCTCC/CACAGCTCTTGCCACAAGATG	445
Exon 12 forward/reverse	GTCTTGGTGTGTCCTGAGTCTG/TGGAATCGTGTGGACACTGC	368
Exon 13 + 14 forward/reverse	CCTCCTAACAGACGCTGCAA/TGGAGAGCCTCCTGGAACAT	443
Exon 15 forward/reverse	GGAACTTCCTGCAGATTGTCC/CCACCAGCACTCCACTTAATG	313
Exon 16 forward/reverse	CAGTCTCCTGAGAGCCAGTACTT/GACTGGTGAGTACAGCAGGACTT	418
Exon 17 forward/reverse	TCAGCCTCTGAACCATCAGC/GGCGCAGAAGGACTATGTTG	372
Exon 18 + 19 forward/reverse	GCTGACGCATGTCTCCTGTT/CCAGTGTCCACCTTGAGCTT	382
Exon 20 + 21 forward/reverse	GTTCCAGATGTCCAAGAAACG/CCTGCATTCACCTCCAGCTA	423
Exon 22 forward/reverse	AACGTCAGCAGAGGCAGGA/TGCCACAGGTCAAGGAAGC	398
Exon 23 forward/reverse	GTTGAGTTCAGCTTCAGTCTGC/GTTCGACCTGGTTAGTGATGG	332
Exon 24 forward/reverse	TGACTGCATCCCTTCCAGC/GCACCAGTCCTAAGAGGCATC	500
Exon 7-TaqI/BfaI-forward/reverse	GCACCAACCATGGCAGAGT/AGCCCAGGAAGAAGGATCTGT	227
Exon 7-pyrosequencing	GCGATCTTCAGACTCC	
Exon 17-Fnu4HI-forward/reverse	GCAGCTGTGCCGTCATTAC/GGCAAGTTTGGAAGCGGAG	197
Exon 17-pyrosequencing	CAGGTGGGACACAAAC	

and TRUS were unremarkable and the serum PSA level was elevated (>4 ng/ml), 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 based on this extensive work-up at baseline or at any of the follow-up exams through 1994, with augmentation with random samples from the population accrued over that time, were used as the control population for this study ($n = 502$; Ref. 21).

Control Population for Mutation Screening. DNA was also available from 200 healthy blood bank donors. These specimens were used to determine the frequency of variant alleles identified through mutation screening.

PCR Primers. On the basis of published sequences (GenBank accession no. AF304370 for cDNA and AC005277 for genomic DNA), we designed 21 pairs of primers for amplifying 23 of the 24 exons containing coding sequences. The primers for mutational screening were generally selected to cover ≥50 bp on either side of the coding sequence. The sequences of these primers are listed on Table 1.

CSGE and Direct Sequencing. CSGE has been successfully used for mutation screening (22–24). When compared with DNA sequencing, we have observed the detection rate for CSGE to range between 85 and 100%, depending on the gene analyzed (25). Because this 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, 60°C for 30 s, and 72°C for 1 min. The reaction was processed in total volume of 12.5 μl consisting of 200 μM each dATP, dGTP, and dTTP; 50 μM dCTP and 0.1 μl of ^{33}P-dCTP; 2 mM $MgCl_2$; 50 ng of template DNA; 1 × AmpliTaq Gold buffer II; and 0.5 unit of TaqAmpliGold DNA polymerase (Perkin-Elmer). The PCR product was then denatured at 96°C for 5 min and cooled to 65°C over 30 min. The reannealed product (5 μl) was then mixed with 1 μl of loading dye (30% glycerol/0.25% bromphenol blue/0.25% xylene cyanol FF). This mix (0.5 μl) was loaded on the CSGE gel consisting of 15% of acrylamide/1,4-bis (acrollyl) piperazine (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 ABI377 automated sequencer.

Genotyping. Two polymorphisms (Ser217Leu and Ala541Thr) in the *HPC2/ELAC2* gene were genotyped in 446 cases with HPC and 502 population controls. The primer pair Exon7-TaqI/BfaI (Table 1) was used to amplify a 227-bp region containing the Ser217Leu variant. The primer pair Exon17-Fnu4HI was used to amplify a 197-bp fragment containing the Ala541Thr variant. All PCR reactions were carried out in a 12.5-μl reaction volume consisting of 1 × AmpliGold buffer II, 2 mM $MgCl_2$, 100 μM each deoxynucleotide triphosphate, 6.25 pmol of each primers, 0.5 unit of TaqAmpliGold DNA polymerase, and 50 ng of template DNA. PCR was performed using a Tetrad thermal cycler (MJ Research, Cambridge, MA) with the following conditions: initial denaturation at 94°C for 12 min, followed by 35 cycles at 94°C for 20 s, 60°C for 30 s, and 72°C for 1 min. Five μl of the PCR product was digested with the appropriate restriction enzyme (TaqIa for Exon 7 and Fnu4HI for Exon 17; New England Biolabs), according to the manufacturer's recommendation. Fragments were resolved on a 3% agarose gel and recorded on a Gel Documentation System (Bio-Rad).

All genotyping results were confirmed by a second technique: pyrosequencing (26). The PCR primers used for pyrosequencing were identical to the those used for the RFLP analysis except that one of the primer was biotin labeled to capture single-stranded molecules for subsequent sequencing (Table1). The PCR products were mixed with magnetic beads (Dynal Biotech, Oslo, Norway) and incubated at 65°C for 15 min. The immobilized strand was then separated in 0.5 M NaOH and transferred to annealing buffer (20 mM Tris-Acetate and 5 mM MgCl2) containing 18 pmol of sequencing primer (Table 1). Pyrosequencing was performed on a PSQ96 instrument (Pyrosequencing AB, Uppsala, Sweden), according to the manufacturer's instructions.

Statistical Analysis. The association of each of the two polymorphisms (Ser217Leu and Ala541Thr) with HPC was evaluated by two statistical approaches. The first was a comparison of the genotype frequencies between cases and controls using a test for trends in the number of variant alleles, analogous to Armitage's test for trends in proportions (27), yet with the appropriate variance to account for the correlated family data (28). The second method was logistic regression, used to evaluate the main effects of the variants (coded as 0,1,2 according to the number of variants in the genotype) but adjusted for the potential confounding factors of age and BMI. For these analyses, age was defined as age at diagnosis for cases and age at blood draw for the controls. BMI (at the time of recruitment for both cases and controls) was calculated as weight in kg divided by height in meters, squared. For the regression analyses, age was categorized using quartiles of the combined distribution of cases and controls (four quartiles: 42–52, 53–62, 63–69, and 70+), and BMI was dichotomized (≤28 *versus* >28). To account for correlations among cases from the same family, generalized estimating equations (29) were used, assuming an exchangeable working correlation matrix. All reported *P*s are two sided.

RESULTS

Mutational Analysis of *HPC2/ELAC2* Gene. Among the 300 HPC patients that were screened for potential germ-line mutations, a total of 23 variants were identified and confirmed by DNA sequencing (Table 2). Among these variants, 13 were intronic, and 10 were

exonic. Of the 10 exonic changes, 9 were located in protein-coding sequence and included 5 missense, 3 silent, and 1 nonsense alteration. The single nonsense mutation in exon 7, Glu216Stop, was identified in pedigree 59. Because this mutation created a restriction site (CGAG > CTAG) recognized by Bfa I, we genotyped all available samples in this family using a Bfa I-based PCR assay (Fig. 1). Sequence analysis confirmed that 2 of the 3 affected men were carriers of this nonsense mutation. This alteration was not present in the one female available for study.

Among the five missense mutations, two (Ser217Leu and Ala541Thr) were reported previously as common polymorphisms (15, 16). The remaining three variants were examined in all available men (affected and unaffected) from carrier families for allele sharing. The Arg211Gln variant in exon 7 was identified in 1 of the 150 families (pedigree 82). Mutational analysis showed that only 1 of 3 affected men carried this mutation. The Gly487Arg variant in exon 16 was found in 2 families, pedigrees 139 and 149. This variant allele was shared by 2 of 2 affected men in family 149 but in only 1 of 2 affected men in family 139. The Gly806Arg variant in exon 24 was found in 1 family (pedigree135). Sequence analysis demonstrated that this variant allele was present in 2 of 2 affected individuals in this family. To additionally evaluate the frequency of these rare alleles, we tested 200 anonymous blood donors. We did not detect the variant alleles Gln211 and Arg806 in any of these normal controls. However, the *Arg487* allele was observed in 2 of the 200 controls.

For the intronic variants (Table 2), we identified a 17-bp duplication (CCCACACATCTTCACTA) within intron 5, 44 bp upstream of exon 6, in 13 of 148 mixed HPC samples (mixed samples refer to simultaneous CSGE analysis of 2 patient specimens in a single PCR reaction; see "Materials and Methods"). Subsequent analysis demonstrated this duplication in 9 of 100 mixed normal blood bank controls. We also identified a common 6-bp deletion/insertion polymorphism within intron 10, 182 bp upstream of exon 11. This deletion was found in 113 of 150 mixed cases and 71 of 100 mixed normal blood bank controls. A mononucleotide repeat $(A)_{10-13}$ was found in intron 1, 88 bp upstream of exon 2. The remaining variants were single nucleotide substitution (Table 2).

Table 2 *HPC2/ELAC2 alterations in familial prostate cancer cases*

Exons/ introns	Nucleotide changes	Amino acid changes	Variants in families
Exon 4	G387A	Lys129Lys	
Exon 7	G632A	Arg211Gln	1 of 3 affected men in Family 82
	G646T	Glu216Stop	2 of 3 affected men in Family 59 (see pedigree)
	C650T	Ser217Leu	
Exon 16	G1459C	Gly487Arg	2 of 2 affected men in Family 149, 1 of 2 affected men in Family 139
Exon 17	G1554A	Glu518Glu	
	G1621A	Ala541Thr	
Exon 20	A1893G	Thr631Thr	
Exon 24	G2416C	Gly806Arg	2 of 2 affected men in Family 135
Exon 24 (3′-UTR)	C2632G		
Intron 1	IVS1 −88$(A)_{10}$ > $(A)_{13}$		
Intron 3	IVS3 −4T > A		
Intron 5	IVS5 −14C > T		
	IVS5 −44dup (CCCACACATCTTCACTA)		
Intron 6	IVS6 +132G > A		
	IVS6 +71C > G		
Intron 7	IVS7 −36A > G		
	IVS7 +59T > C		
Intron 10	IVS10 −182del (AGTTAC)		
Intron 12	IVS12 +91C > T		
Intron 14	IVS14 −8C > T		
Intron 17	IVS17 +78G > C		
Intron 19	IVS19 +26C > G		

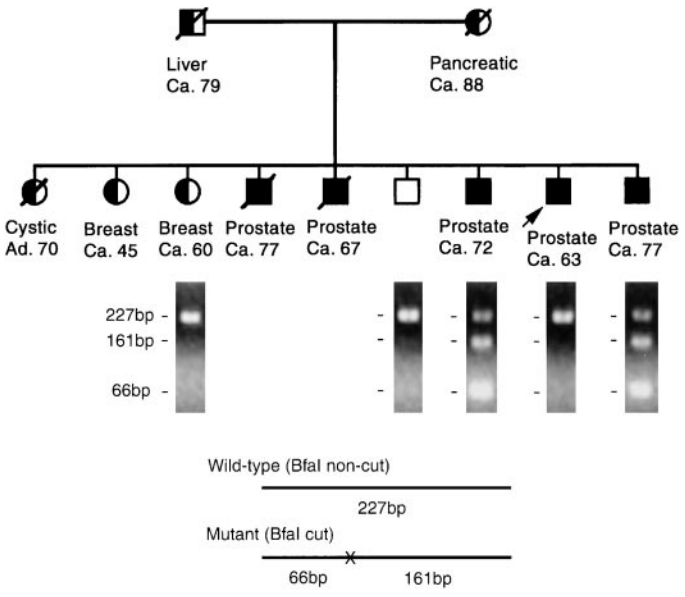

Fig. 1. Segregation of Glu216Stop mutation in Pedigree 59. PCR products were digested with Bfa I and resolved on 3% agarose gel. Two of 3 affected men were found to carry the mutant allele. The *bottom* half of the figure illustrates the fragments generated as a result of the enzyme digest.

Table 3 *Characteristics of cases and controls*

Trait	Cases Median (range)	Controls Median (range)	P^a
Age in years[b]	66.0 (45, 84)	55.0 (42, 83)	<0.0001
BMI	26.6 (17.2, 42.9)	27.8 (18.5, 47.5)	<0.0001
Serum PSA ng/ml	8.4 (0.3, 518.0)	0.9 (0.15, 9.1)	<0.0001

[a] Age is defined as age at diagnosis of PC for cases and age at blood draw for controls.
[b] Wilcoxon's rank-sum test.

We also analyzed all variant sequences using a splice site predictor program[4] but did not find any indication that any of these alterations affected splicing.

Gene Association Studies. Characteristics of the hereditary cases and the population-based controls used for the gene association studies are presented in Table 3. Hereditary cases were significantly older than controls (median 66 years *versus* 55 years respectively, $P < 0.0001$). Nonetheless, there was substantial overlap in the age distribution between cases and controls. When subjects were grouped according to age quartiles, the respective percentage of controls *versus* cases in the four age groups were: 44.4 *versus* 3.8%, age ≤ 53 years; 26.1 *versus* 28.5%, age 53–63 years; 12.0 *versus* 37.8%, age 63–69 years; and 17.5 *versus* 29.9%, age > 69 years. The cases also had a significantly lower BMI than controls (median 26.6 *versus* 27.8 respectively, $P < 0.0001$). Because of these differences, age and BMI were included in all logistic regression models to statistically adjust for potential confounding effects.

The two missense variants, Ser217Leu and Ala541Thr, were genotyped in 446 HPC cases and 502 population controls to evaluate whether alleles at these loci are associated with an increased risk of HPC. The genotype frequencies among the controls of both variants fit Hardy Weinberg proportions (exact test *P*s of 0.76 for Ser217Leu and 0.63 for Ala541Thr). The results of the case-control studies for

[4] Internet address: http://www.fruitfly.org/seq_tools/splice.html.

Table 4 *Association between PC and HPC2–217 polymorphism*

	No.	Genotype frequencies–no. (%)			Trend test (*P*)	Adjusted OR[a] (95% CI)
		Leu/Leu[b]	*Leu/Ser*	*Ser/Ser*		
Population controls	502	49 (9.8)	221 (44.0)	232 (46.2)		1.00 (reference)
Familial PC cases[c]	444	41 (9.2)	205 (46.2)	198 (44.6)	0.06 (0.81)	1.00 (0.82, 1.23)
Node negative	350	37 (10.6)	159 (45.4)	154 (44.0)	0.38 (0.54)	1.05 (0.83, 1.32)
Node positive	37	1 (2.7)	18 (48.7)	18 (48.7)	0.73 (0.39)	0.70 (0.40, 1.22)
Stage T1/T2	257	26 (10.1)	111 (43.2)	120 (46.7)	0.0005 (0.98)	0.98 (0.75, 1.28)
Stage T3/T4	93	11 (11.8)	48 (51.6)	34 (36.6)	2.39 (0.12)	1.26 (0.88, 1.80)
Low grade[d]	332	34 (10.2)	148 (44.6)	150 (45.2)	0.09 (0.76)	1.02 (0.81, 1.28)
High grade	112	7 (6.3)	57 (50.9)	48 (42.9)	0.0005 (0.98)	0.99 (0.68, 1.45)

[a] OR for the effect of allele *Leu* adjusted for age and BMI.
[b] *Leu* denotes the variant allele.
[c] The numbers in the table are slightly smaller than the original total sample (446) because of technical failures (nonamplification) for some specimens.
[d] Low grade defined as Gleason score ≤ 6; high grade as Gleason score > 6.

Ser217Leu are presented in Table 4, and the results for Ala541Thr are presented in Table 5. The *Leu217* and *Thr541* allele frequencies in 446 hereditary cases were 32.3 and 5.4%, respectively. These frequencies did not differ statistically from those found in the unaffected population-based control subjects, 31.8 and 5.2%, respectively (Tables 4 and 5). The Thr541 variant was observed only in the presence of *Leu217* alleles, consistent with the findings by Tavtigian *et al.* and Rebbeck *et al.* (15, 16). Neither of these variants alone was associated with HPC when all cases were compared with the controls or when subsets of cases, stratified on nodal status, stage, and grade, were compared with controls. The upper confidence limits for the ORs suggest that the relative risk associated with the *Ser217Leu* variant allele is <1.23 and that for the variant of Ala541Thr is <1.60. For Tables 4 and 5, the allele covariates in the logistic regression models were included as 0, 1, 2 (*i.e.*, counts of the variant allele in the genotypes), which is appropriate for a log-additive (multiplicative) effect of the variant alleles. To avoid this assumption, we reanalyzed the data using a simple indicator variable for carriers of the variants (*i.e.*, grouping homozygous and heterozygous carriers into a single group); the adjusted ORs and CIs for these analyses were 1.03 (0.80, 1.33) for Ser217Leu and 0.98 (0.61, 1.56) for Ala541Thr, similar to those reported in Tables 4 and 5.

Because it is possible that the joint effects of the two variants could have a much stronger influence on the risk of PC than either alone, we performed logistic regression analyses similar to those reported by Rebbeck *et al.* (16). However, no combination of genotypes for these two loci were significantly associated with HPC (Table 6).

DISCUSSION

Analysis of the *HPC2/ELAC2* gene revealed a germ-line nonsense mutation (Glu216Stop) in 1 of our 150 PC families. This nuclear family has nine siblings: six brothers, and three sisters (Fig. 1). Remarkably, eight of the nine siblings developed malignancies. Both parents were also diagnosed with cancer. In all, 10 of 11 members of this nuclear family suffered from cancer in their lifetimes. The prevalence of malignancy in this family, and the rarity of the tumor types (other than prostate and breast), suggests a genetic contribution in this lineage.

Mutational analysis demonstrated that 2 of the 3 available affected men in this family carried the nonsense mutation. The 1 unaffected male was not a carrier. Unfortunately, we could not analyze the other 2 affected men because they were deceased, and specimens were not available. Because this mutation is predicted to cause a truncated protein, this alteration is likely to be a causative germ-line change in this family. However, labeling this missense mutation as a causative alteration assumes an inactivating genetic mechanism, for which there is currently no independent evidence. One possible explanation for the lack of a germ-line mutation in the 1 affected male is the presence of a phenocopy. This nonsense alteration was also not detected in the one female available for testing. Because other affected family members were unavailable for testing, it was not possible to additionally explore the role of the Glu216Stop mutation in cancer formation for this family.

In addition to the one nonsense mutation, three novel missense mutations were also identified: *Arg211Gln*, *Gly487Arg*, and *Gly806Arg*. However, only the *Gly487Arg* variant was found in normal blood bank donors, suggesting that this allele may be a rare polymorphism. The *Arg211Gln* alteration was found in only a single individual and in none of the 200 normal blood bank donors. Unfortunately, these data provide no evidence that any of these variants are important in susceptibility to PC. Although germ-line mutation of *HPC2/ELAC2* does not appear to be a common cause of HPC in the present study, identification of a nonsense mutation in 1 family with multiple cancers suggests a limited role of this gene in HPC and, possibly, in other types of cancers as well. Overall, the low frequency of mutations observed in the study is similar to that reported by

Table 5 *Association between PC and HPC2–541 polymorphism*

	No.	Genotype frequencies–no. (%)			Trend test (*P*)	Adjusted OR[a] (95% CI)
		Thr/Thr[b]	*Thr/Ala*	*Ala/Ala*		
Population controls	502	0 (0.0)	52 (10.4)	450 (89.6)		1.00 (reference)
Familial PC cases[c]	445	2 (0.4)	44 (9.9)	399 (89.7)	0.04 (0.85)	1.01 (0.63, 1.60)
Node negative	350	2 (0.6)	37 (10.6)	311 (88.9)	0.32 (0.57)	1.06 (0.64, 1.77)
Node positive	37	0 (0.0)	2 (5.4)	35 (94.6)	0.92 (0.34)	0.57 (0.13, 2.45)
Stage T1/T2	257	0 (0.0)	27 (10.5)	230 (89.5)	0.004 (0.95)	1.07 (0.58, 1.98)
Stage T3/T4	93	2 (2.2)	10 (10.8)	81 (87.1)	1.56 (0.21)	1.14 (0.50, 2.61)
Low grade[d]	333	0 (0.0)	32 (9.6)	301 (90.4)	0.11 (0.74)	0.95 (0.56, 1.61)
High grade	112	2 (1.8)	12 (10.7)	98 (87.5)	1.23 (0.27)	1.41 (0.64, 3.12)

[a] OR for effect of allele *Thr* adjusted for age and BMI.
[b] *Thr* denotes the variant allele.
[c] The numbers in the table are slightly smaller than the original total sample (446) because of technical failures (nonamplification) for some specimens.
[d] Low grade defined as Gleason score ≤ 6; high grade as Gleason score > 6.

Table 6 *Association between PC and HPC2–217 and 541 polymorphisms*

Ser217Leu genotype	*Ala541Thr* genotype	Population controls (%)	Familial cases (%)	Adjusted OR (95% CI)[a]
Ser/Ser	*Ala/Ala*	232 (46.2)	198 (44.6)	1.00 (reference)
Ser/Ser	Any *Thr*	0	0	
Any *Leu*	*Ala/Ala*	218 (43.4)	200 (45.0)	1.05 (0.79, 1.39)
Any *Leu*	Any *Thr*	52 (10.4)	46 (10.4)	1.04 (0.57, 1.89)
Any	*Ala/Ala*	450 (89.6)	398 (89.6)	1.00 (reference)
Any	Any *Thr*	52 (10.4)	46 (10.4)	0.98 (0.61, 1.56)

[a] OR adjusted for age and BMI.

Tavtigian *et al.* (15), having found a causative mutation in only 2 of their 42 kindreds with evidence for linkage at 17p. In a study recently published by Xu *et al.* (30), however, there was no evidence of linkage to chromosome 17 in the total sample nor in any of the subgroups tested, and there were no novel mutations in the coding region of *HPC2/ELAC2* in 93 probands with HPC.

In addition to examining the *HPC2/ELAC2* gene for the presence of specific mutations, we also examined two common polymorphisms for their association with PC risk. Despite the recent report by Tavtigian *et al.* and Rebbeck *et al.* (15, 16) suggesting that the Leu217/Thr541 genotypes were significantly more common among hereditary and unselected PC cases compared with control subjects, we failed to detect any statistically significant increased risk of these genotypes in our HPC cases. Both Tavtigian *et al.* and Rebbeck *et al.* (15, 16) found the largest association when considering joint effect of both variants. However a similar analysis for our data resulted in no significant association with an OR of 1.04 (95% CI, 0.57–1.89; Table 6), with an upper confidence limit less than the OR of 2.37 reported by Rebbeck *et al.* (16). For our data, we have ≥98% power to detect an OR of 2.37, with our observed frequency of 10.4% of the controls who carried variants at both sites and an effective sample size of 269 cases (to account for relationships among our 446 HPC cases). A similar result was also reported recently by Xu *et al.* (30). In their association studies, both family-based and population-based tests failed to reveal any statistically significant differences in the allele frequency of these two polymorphisms between patients with PC and control subjects. However, although not statistically significant, Xu *et al.* (30) did note a trend toward higher Leu 217 homozygous carrier rates in patients (9.4%) than in the controls (7.7%; odds radio, 1.3). The analysis of a larger set of samples may be necessary to adequately address this question.

A potential source of bias in our study is that the controls tended to be younger than the cases by ~10 years on average. It is possible that some of our controls will have PC in later years. However, our regression adjustment for age differences failed to suggest that either Leu217 or Thr541 were associated with PC. In addition to using age quartiles to adjust for the effect of age, we added age and the square of age to the regression models to have more refined adjustments for age. All of these analyses were quite close to the results presented in Tables 4–6, suggesting that the imbalance of age is not a major source of bias. Given the similar allele frequencies between our cases and controls, it is very unlikely that our conclusions would differ if some controls developed PC later in life. In fact, we would expect the ORs to be even closer to unity if some of the controls are misclassified. Similarly, the imbalance of BMI levels between cases and controls did not seem to be a source of bias, because the estimated ORs were similar whether or not BMI was included in the regression model.

The genetic complexity of PC, the presence of phenocopies within high-risk pedigrees, and the late age at diagnosis all contribute to the difficulty of identifying PC susceptibility genes. In this study, we detected a nonsense mutation in the *HPC2/ELAC2* gene, confirming its potential role in genetic susceptibility to PC. However, our data also suggests that germ-line mutations of the *HPC2/ELAC2* are rare in HPC and that the variants Leu217 and Thr541 do not appear to influence the risk of HPC. In conclusion, our data suggested that the *HPC2/ELAC2* gene plays a limited role in genetic susceptibility to HPC.

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