Association of *HPC2/ELAC2* Polymorphisms with Risk of Prostate Cancer in a Population-based Study[1]

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Abstract

Genetic polymorphism in *HPC2/ELAC2* was recently associated with risk of sporadic prostate cancer. To determine the contribution of two *HPC2/ELAC2* missense variants (*Ser*^{217}*Leu* and *Ala*^{541}*Thr*) to the risk of developing prostate cancer, we conducted a population-based case-control study of middle-aged men (40–64 years). Cases ($n = 591$) were ascertained from the Seattle-Puget Sound Surveillance, Epidemiology, and End Results Cancer Registry and Controls ($n = 538$) from the same general population were identified through random-digit dialing. Subjects were residents of King County, Washington, and were frequency matched on age. Cases (32%) had a slightly higher frequency of the *Leu*^{217} variant compared with controls (29%), but there were no differences in the frequency of the *Thr*^{541} allele (4%). When considering joint genotypes, white men homozygous for the *Leu*^{217} variant on an *Ala*^{541}/*Ala*^{541} background had an increased risk of prostate cancer [odds ratio (OR) = 1.84; 95% confidence interval (CI), 1.11–3.06]. Different risk profiles were also observed when cases were stratified by disease aggressiveness. Men with at least one *Leu*^{217} allele had an elevated risk (OR = 1.34; 95% CI, 1.02–1.76) of less aggressive prostate cancer (localized stage and Gleason score ≤7), with a stronger association among men with two *Leu*^{217} alleles (OR = 1.73; 95% CI, 1.08–2.77). The *Ala*^{541}*Thr* polymorphism was not associated with risk, and neither variant was associated with more aggressive prostate cancer phenotypes. We estimate that the *Ser*^{217}*Leu* genotype may account for ~14% of less aggressive prostate cancer cases and 9% of all sporadic cases in the general United States population of white men <age 65 years.

Introduction

Prostate cancer is the most frequently diagnosed solid tumor among United States males, with >220,000 cases diagnosed each year (1). Hospital-based (2, 3) and population-based case-control studies (4–7), as well as cohort studies (8–10), suggest the existence of susceptibility genes. Relative risk estimates associated with a history of prostate cancer in a first-degree relative range from 1.7 to 3.7 (11), with younger ages at diagnosis and multiple relatives with prostate cancer conveying even higher risks (2). Segregation analyses indicate that rare autosomal dominant highly penetrant genes may account for ~10% of the disease in the general population of men up to age 85 years and an excess of up to 43% of the disease in men diagnosed before age 55 years (12–14). Putative prostate cancer susceptibility loci have been mapped to several genomic locations in studies of high-risk families defined by multiple affected men (15–21).

Of the loci described from high-risk family studies, to date, the first successful cloning was reported for the putative susceptibility locus, *HPC2/ELAC2* (20). Although protein-truncating mutations in *HPC2/ELAC2* appear to be extremely rare in families with hereditary prostate cancer, it has been hypothesized that the combination of a rare *Ala*^{541}*Thr* missense change in linkage disequilibrium with a more common *Ser*^{217}*Leu* variant is associated with risk of prostate cancer (20). A few recent investigations found a positive association between one or both of the *HPC2/ELAC2* variants and prostate cancer (20, 22, 23), but most studies reported no relationship between *Ser*^{217}*Leu* or *Ala*^{541}*Thr* alleles and risk of prostate cancer (24–28). However, results of these negative studies are difficult to interpret because of potential selection biases resulting from the use of selected case series and/or controls from different underlying populations than those from which the prostate cancer patients were ascertained. For example, some studies compared cases from high-risk prostate cancer families with control men who participated in prostate cancer screening programs (23, 25, 27) or who were blood donors (26). We have therefore conducted a population-based case-control study in a geographically defined community to examine the role of genetic polymorphism in the *HPC2/ELAC2* gene and risk of prostate cancer.

Materials and Methods

Study Population. Study subjects were all residents of King County, Washington, who participated in a previously described population-based case-control study of risk factors for prostate cancer in middle-aged men (29). Briefly, patients included Caucasian and African American men 40–64 years of age with histologically confirmed adenocarcinoma of the pros-

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tate diagnosed between January 1993 and December 1996. Case subjects were identified through the Seattle-Puget Sound SEER[3] Cancer Registry, which covers the 13 northwestern counties of Washington state. All patients <age 60 and a 75% random sample of men ages 60–64 years of age at diagnosis were invited to participate. A total of 971 eligible cases was ascertained for the study, 753 (82.1%) were interviewed, with 591 (78.5%) also providing a blood sample yielding sufficient DNA for genotyping. General population controls of similar age (*i.e.*, 40–64 years, frequency matched to the cases on 5-year age group) were ascertained from King County through random digit telephone dialing (30). We identified a total of 941 eligible controls for the study, interviewed 703 (74.7%) of these men, and DNA samples were available for 538 (76.5%) interviewed controls. There were no differences between interviewed and genotyped case or control subjects with respect to age, race, family history of prostate cancer, indices of body size, income, education, or the clinical characteristics of the patients. The study was approved by the Fred Hutchinson Cancer Research Center's Institutional Review Board.

Genotyping. PCR amplification used primers and conditions as previously described by Rebbeck *et al.* (22). Each reaction used a total of 25 ng of genomic DNA, 0.25 units of Bioloase enzyme (Bioline Systems, Inc., Springfield NJ), 1× NH_4 buffer [16 mM$(NH_4)_2SO_4$, 67mM Tris-HCl, 0.01% Tween 20], and 1.5 mM $MgCl_2$ and was amplified on an Applied Biosystems 9600 GeneAmp PCR System. Samples were digested with *Fnu*4HI and *Taq*I enzymes as previously described (22) and resulting products separated on 3% BMA Metaphor low-melt agarose gels (Fisher Scientific, Pittsburgh, PA). Results were visualized by ethidium bromide staining. There were 10 subjects (8 cases, 2 controls) and 3 subjects (2 cases, 1 control) whose DNA sample did not amplify for the $Ser^{217}Leu$ or $Ala^{541}Thr$ polymorphism, respectively.

For two samples yielding Ser^{217}/Ser^{217} and Ala^{541}/Thr^{541} results, a rare combination that has only been reported in four individuals in the published literature (25, 26), data were checked by direct sequencing in both directions. In brief, DNA was amplified using 1 μl of stock genomic DNA, 0.15 units of Biolase enzyme (Bioline Systems, Inc.), 1× NH_4 buffer [(16 mM $(NH_4)_2$ SO_4, 67 mM Tris-HCl, 0.01% Tween 20)], 0.2 mM deoxynucleoside triphosphate, 2.0 mM $MgCl_2$, and 0.2 μM of primer specific for the region containing the $Ser^{217}Leu$ variant, as well as the region containing the $Ala^{541}Thr$ variant. Amplification was carried out in an ABI Perkin-Elmer 9600 GeneAmp PCR System. Cycling conditions were as follows: 95°C hot-start for 3 min, followed by 25 cycles of 95°C for 30 s, 55°C for 35 s, 72°C for 45 s, and a final extension at 72°C for 2 min. Product size was verified by visualization on a 1.8% agarose gel into which 0.2 mg/ml ethidium bromide were incorporated. Amplified product was cleaned with 10 units of Exonuclease I (Epicenter, Madison, WI) and 1 unit of Shrimp Alkaline Phosphatase (Amersham Biosciences, Piscataway, NJ). Sequencing reactions were performed using the conditions described in the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit manual and run on an ABI 3730 automated sequencer. Data were analyzed using phred, prhap, and consed (31–33). Both samples were homozygous for the amino acids serine and alanine.

Quality control involved genotyping 40 paired samples as blind duplicates that were distributed across all genotyping batches. Laboratory personnel were blinded as to the case-control status of all DNA samples. There was 95% agreement on the $Ser^{217}Leu$ polymorphism (two samples were read as Ser^{217}/Ser^{217} in one assay and Ser^{217}/Leu^{217} in the other assay) and 100% agreement on the $Ala^{541}Thr$ polymorphism in the blind duplicate samples.

Table 1 Selected characteristics of study subjects and ORs and 95% CIs for prostate cancer

	No. of cases (%) (n = 591)	No. of controls (%) (n = 538)	OR (95% CI)[a]
Age (yr)			
40–49	38 (6.4)	48 (8.9)	
50–54	123 (20.8)	106 (19.7)	
55–59	198 (33.5)	199 (37.0)	
60–64	232 (39.2)	185 (34.4)	
Race			
White	559 (94.8)	523 (97.2)	1.00
African American	32 (5.4)	15 (2.8)	2.00 (1.07–3.74)
First-degree family history of prostate cancer			
No	481 (81.4)	481 (89.4)	1.00
Yes	110 (18.6)	57 (10.6)	1.92 (1.36–2.71)
Education			
High school or less	117 (19.8)	101 (18.8)	1.00
Some college	167 (28.3)	148 (27.5)	0.99 (0.70–1.40)
B.A./B.S. degree	160 (27.1)	152 (28.3)	0.92 (0.65–1.31)
Graduate school	147 (24.9)	137 (25.5)	0.93 (0.65–1.33)
Body mass index			
18–23	147 (24.9)	113 (21.0)	1.00
24–26	225 (38.1)	194 (36.1)	0.89 (0.65–1.22)
27–29	125 (21.2)	140 (26.0)	0.68 (0.48–0.96)
≥30	94 (15.9)	91 (16.9)	0.79 (0.54–1.16)
Physical activity, times/week			
None	104 (17.6)	77 (14.3)	1.00
≤1	135 (22.9)	154 (28.6)	0.65 (0.45–0.95)
2–3	198 (33.6)	180 (33.5)	0.82 (0.57–1.18)
≥4	154 (26.1)	127 (23.6)	0.88 (0.60–1.29)

[a] Adjusted for age (categorical).

Statistical Methods. ORs and 95% CIs were calculated according to genotype by unconditional logistic regression (34). In addition to age (categorical), potential confounding factors considered were race, family history of prostate cancer, education, body mass index, PSA screening history, smoking status, and dietary intake (total fat, calories, vegetables, and alcohol). Analyses were also completed according to age (<60, 60–64 years), race (black, white), and first-degree family history (no, yes). In examining the association between genotype and aggressiveness of prostate cancer, clinical characteristics were used to stratify cases into those with less aggressive (localized stage and Gleason scores 2–7) and more aggressive (regional or distant stage or Gleason scores 8–10) disease. Polychotomous logistic regression models (35) were used to compute ORs and 95% CIs by genotype for these two case groups compared with controls.

Results

Selected characteristics of study subjects are provided in Table 1. More cases than controls were African American (OR = 2.00; 95% CI, 1.07–3.74) or had a first-degree relative with prostate cancer (OR = 1.92; 95% CI, 1.36–2.71). The higher incidence of prostate cancer in African-American compared with Caucasian American men (36) suggests the possibility of

[3] The abbreviations used are: SEER, Surveillance, Epidemiology, and End Results; OR, odds ratio; CI, confidence interval; PSA, prostate-specific antigen.

Table 2 Adjusted ORs and 95% CIs for prostate cancer by *HPC2* genotype among whites

Genotype	No. of cases (%)	No. of controls (%)	OR (95% CI)[a]
Ser^{217}*Leu*[b]			
S/S	254 (46.1)	266 (51.1)	1.00
S/L	240 (43.6)	213 (40.9)	1.19 (0.92–1.53)
L/L	57 (10.3)	42 (8.1)	1.42 (0.92–2.20)
Any *Leu*^{217}	297 (53.9)	255 (48.9)	1.23 (0.96–1.56)
Ala^{541}*Thr*[c]			
A/A	514 (92.3)	479 (91.8)	1.00
A/T	42 (7.5)	42 (8.0)	0.91 (0.58–1.42)

[a] Adjusted for age and first-degree family history of prostate cancer.
[b] Analysis excludes 8 cases and 2 controls with missing genotypes; percentages adjusted to account for subjects with missing data.
[c] Analysis excludes 1 case and 1 control with the T/T genotype and 2 cases and 1 control with missing genotype.

Table 3 Adjusted ORs and 95% CIs for prostate cancer by joint *HPC2* genotypes among whites

Ala^{541}*Thr* genotype	*Ser*^{214}*Leu* genotype		
	S/S	S/L	L/L
A/A	1.00 Ref.[a]	1.19 (0.91–1.55)	1.84 (1.11–3.06)
	254/266	209/184	47/27
A/T		1.17 (0.68–2.01)	0.57 (0.23–1.40)
	0/0	32/28	8/14

[a] Adjusted for age and first-degree family history of prostate cancer; number cases/number controls; analysis excludes 1 case and 1 control with the T/T genotype and 9 cases and 3 controls with missing genotype.

different risk factor profiles. However, because there were too few blacks to analyze separately (32 cases, 15 controls), concerns about population stratification (37) led us to restrict subsequent analyses to white subjects.

Both the *Ser*^{217}*Leu* and *Ala*^{541}*Thr* polymorphisms were in Hardy-Weinberg equilibrium in white cases and controls. As shown in Table 2, there was a modest increase in the relative risk estimate for men homozygous for the *Leu*^{217} allele (OR = 1.42; 95% CI, 0.9–2.2) compared with men with two *Ser*^{217} alleles. The trend test for the possible gene dose effect (*i.e.*, the number of *Leu*^{217} alleles) was not significant (trend $P = 0.07$). The *Thr*^{541} allele was rare in our population and was not associated with prostate cancer risk.

When considering the joint effect of both single nucleotide substitutions (Table 3), there was a significant elevation in risk (OR = 1.84; 95%, CI 1.11–3.06; $P = 0.019$) in men with the *Leu*^{217}/*Leu*^{217} and *Ala*^{541}/*Ala*^{541} genotypes compared with those with the *Ser*^{217}/*Ser*^{217} and *Ala*^{541}/*Ala*^{541} genotypes. Other combined genotypes were not found to be associated with risk. We also examined the joint distribution of genotypes among cases and controls separately. In both prostate cancer cases and controls, the *Thr*^{541} allele was only observed in men who carried at least one *Leu*^{217} allele, confirming that these two polymorphisms are in strong linkage disequilibrium.

Age (<60, 60–64 years) and family history of prostate cancer in a first-degree relative (no, yes) were included in logistic regression analyses to assess possible genotype effect modification. Relative risk estimates were similar in the two age groups for both polymorphisms. In the prostate cancer patients, mean age at diagnosis also did not vary by *Ser*^{217}*Leu* ($P = 0.72$) or *Ala*^{541}*Thr* ($P = 0.42$) genotype. Relative risks differed somewhat by family history. Relative to men with the *Ser*^{217}/*Ser*^{217} genotype, risks associated with the *Ser*^{217}/*Leu*^{217} and *Leu*^{217}/*Leu*^{217} genotypes were OR = 1.25 (95% CI, 0.95–1.64) and OR = 1.50 (95% CI 0.94–2.41) in men without a first-degree family history and OR = 0.93 (95% CI, 0.46–1.88) and OR = 1.07 (0.33–3.47) in men with an affected relative(s). These ORs, however, were not significantly different (P for interaction = 0.62). Men with no family history who had any *Leu*^{217} allele had a 29% (95% CI, 0.99–1.68) increase in risk. Analyses of family history and *Ala*^{541}*Thr* genotype showed that men with any *Thr*^{541} allele were not at elevated risk in either group: OR = 0.91 (95% CI, 0.56–1.47) in men without a family history and OR = 0.64 (95% CI, 0.20–1.99) in men with a family history.

To assess the potential relationship between these polymorphisms and severity of disease, we stratified prostate cancer patients into those with clinically less aggressive (localized stage and Gleason scores 2–7) and more aggressive (regional or distant stage or Gleason scores 8–10) tumors (Table 4). Compared with men with only *Ser*^{217} alleles, men with any *Leu*^{217} allele were at increased risk (OR = 1.34; 95% CI, 1.02–1.76; $P = 0.034$) and those with two *Leu*^{217} alleles had an even higher risk estimate (OR = 1.73; 95% CI, 1.08–2.77; $P = 0.023$) for less aggressive prostate cancer phenotypes. The *Ser*^{217}*Leu* genotype was not associated with more aggressive disease. These estimates were similar when stage or grade was examined separately (*i.e.*, elevated ORs associated with the *Leu*^{217} allele were noted for localized stage and for low grade tumors), and results were unchanged after adjustment for PSA screening history. In agreement with the overall results, the *Ala*^{541}*Thr* single nucleotide substitution was not associated with either less aggressive or more aggressive disease.

Discussion

This investigation represents the first population-based association study of these two germ-line *HPC2/ELAC2*-genetic polymorphisms in a group of prostate cancer patients compared with men without the disease who were all ascertained from the same underlying general population and who were frequency matched on age. We found no overall significant associations between prostate cancer risk and either the *Ser*^{217}*Leu* or the *Ala*^{541}*Thr* genotypes examined separately. When considering the joint effect of these single nucleotide polymorphisms, however, there was evidence of an increased relative risk in the subset of men who were homozygous for both the *Leu*^{217} allele and the *Ala*^{541} allele (OR = 1.84; 95% CI, 1.11–3.06; $P = 0.019$). Analyses of genotype by disease aggressiveness showed that the presence of even one *Leu*^{217} allele conferred a borderline significant elevation in risk of less aggressive prostate cancer (OR = 1.34; 95%, CI 1.02–1.76; $P = 0.034$), and the association with less aggressive forms of the disease was strongest in men homozygous for the *Leu*^{217} allele (OR = 1.73; 95% CI, 1.08–2.77; $P = 0.023$). No associations with either *HPC2/ELAC2*-genetic variant and more aggressive prostate cancer phenotypes were apparent.

Our results are consistent with the original report from Tavtigian *et al.* (20) that noted an association with the *Leu*^{217} genotype. Specifically, Tavtigian *et al.* (20) found that *Leu*^{217} homozygotes and individuals with *Thr*^{541} alleles were more frequent in hereditary prostate cancer patients compared with male spouses of women from other nonprostate cancer families ($P = 0.03$ and 0.02, respectively). We also observed a positive association with the *Leu*^{217} variant, particularly in men who were homozygous for the more common *Ala*^{541} allele and in those who were diagnosed with less aggressive disease. Of the

Table 4 Adjusted ORs and 95% CIs for prostate cancer stratified on tumor aggressiveness by *HPC2* genotype among whites

Genotype	No. of controls	No. of less aggressive cases	OR (95% CI)[a]	No. of more aggressive cases	OR (95% CI)[a]
Ser^{217}*Leu*					
S/S	266	160	1.00	94	1.00
S/L	213	162	1.26 (0.95–1.68)	78	1.05 (0.74–1.49)
L/L	42	44	1.73 (1.08–2.77)	13	0.89 (0.46–1.74)
Any *Leu*^{217}	255	206	1.34 (1.02–1.76)	92	1.02 (0.73–1.43)
Ala^{541}*Thr*					
A/A	479	342	1.00	173	1.00
A/T	42	27	0.88 (0.53–1.47)	15	0.96 (0.52–1.78)

[a] Adjusted for age and first-degree family history of prostate cancer by polychotomous logistic regression; the analysis of *Ala*^{541}*Thr* excludes 1 case with less aggressive disease and 1 control with the T/T genotype.

studies published to date, only that of Tavtigian *et al.* (20) found evidence for a role of the *Ser*^{217}*Leu* polymorphism in prostate cancer.

Three (20, 22, 23) of the eight studies to examine these polymorphisms (24–28) found support for an association between the *Ala*^{541}*Thr* missense change and prostate cancer. Rebbeck *et al.* (22) analyzed a case series of 359 radical prostatectomy patients from a large health care system and 266 age- and race-matched medical clinic controls. These investigators observed a 2-fold borderline significant increase in the OR for prostate cancer among men with the rare *Thr*^{541} variant (OR = 2.2; 95%, CI 1.0–4.9). The result was strongest in the subset of men who also had the variant *Leu*^{217} allele (OR = 2.4; 95%, CI 1.1–5.3 for the *Ser*^{217}/*Leu*^{217} or *Leu*^{217}/*Leu*^{217} and *Ala*^{541}/*Thr*^{541} genotypes; there were no *Thr*^{541}/*Thr*^{541} homozygotes in the study). Both Tavtigian *et al.* (20) and Suarez *et al.* (23) reported a significantly higher frequency of the rare *Thr*^{541} allele in prostate cancer patients from high-risk families compared with controls. In all published studies to date, including this study, the frequency of the *Ala*^{541}/*Thr*^{541} and *Thr*^{541}/*Thr*^{541} genotypes combined only accounted for ≤10% of both cases and controls, and the frequency of *Thr*^{541} homozygotes was ≤0.6% in both groups. Given the low frequency of this missense change and the modest relative risk elevation associated with this variant, it is unlikely that the *Ala*^{541}*Thr* polymorphism could account for more than a small proportion of prostate cancer cases in the general population.

An association between germ-line variants in *HPC2/ELAC2* was initially reported as part of a linkage-based study of hereditary prostate cancer families (20). Subsequent reports, however, suggest that protein truncating mutations in *HPC2/ELAC2* are extremely rare, even within hereditary prostate cancer families that feature multiple cases of often early-onset disease and/or prostate cancer in multiple generations. Only 3 of 436 (0.7%) high-risk families screened in four studies reported to date demonstrated even a single individual with a protein-truncating mutation (20, 25–27). Interestingly, in a study of radical prostatectomy patients, Rebbeck *et al.* (22) reported that the overall positive association observed in men with the rare *Thr*^{541} allele, who also had the *Leu*^{217} variant, did not differ according to family history of prostate cancer or race. We also found similar risk estimates associated with *Ser*^{217}*Leu* and *Ala*^{541}*Thr* genotypes in men with and without a first-degree family history of the disease. This suggests that other variants or variants in genes besides *HPC2/ELAC2* may account for the initial linkage results reported at chromosome 17p12 by Tavtigian *et al.* (20).

Several prior association studies examined prostate cancer risk and *HPC2/ELAC2*-genetic polymorphisms in subsets of patients according to clinical features of the disease. Three studies based on patient series treated with radical prostatectomy found no associations between either missense variant and Gleason score or extent of disease defined as whether or not there was extracapsular extension of the tumor (22, 23, 25). We found no associations between risk of less aggressive or more aggressive prostate cancer and the rare *Thr*^{541} variant.

In our population-based study, however, we did observe a significant positive association between the *Leu*^{217} allele and less aggressive phenotypes (localized stage and Gleason scores 2–7) of prostate cancer. If we assume that the prevalence of even one *Leu*^{217} allele is 49% in the general population of United States white men and that this variant confers a relative risk for less aggressive prostate cancer of 1.34 (Table 4), then this genotype may account for 14% of such cases or 9% (0.14 × 0.66) of all sporadic prostate cancer cases in the United States white population of men <65 years (assuming that two-thirds of all patients are diagnosed with less aggressive phenotypes as defined and observed in our study). The latter assumption is consistent with SEER data showing that ~70% of prostate cancer patients have localized stage prostate cancer at diagnosis (36). Furthermore, if 8% of the population is homozygous for the *Leu*^{217} allele, which is associated with a relative risk estimate of 1.73 in men with localized disease and a Gleason score of ≤7, then the *Leu*^{217}/*Leu*^{217} genotype could explain 5.5% of the less aggressive prostate cancer cases in the general United States white population of middle-aged men and 3.6% of all sporadic cases of the disease in such men.

We have no clear explanation for why the *Ser*^{217}*Leu* polymorphism is only associated with less aggressive prostate cancer. It is possible that there are different genes and environmental factors that interact in such a way as to limit the aggressiveness of some prostate cancers. It is interesting that adjustment for PSA screening history did not change our results, suggesting that the finding is not explained by screening/early detection. Prostate cancer is known to be a complex and heterogeneous disease, so it seems plausible that different genotypes may alter the aggressive potential of a tumor, particularly in the presence of other genetic variants and/or environmental exposures.

Our data are similar to other published studies that noted strong linkage disequilibrium between the *Ser*^{217}*Leu* and *Ala*^{541}*Thr* variants, making it difficult to separate the effects of the individual variants on risk of prostate cancer. In six (20 22–24 27 28) of eight (25, 26) prior studies, including our study, the *Thr*^{541} allele was only found in men who also carried the *Leu*^{217} allele. A total of only 4 prostate cancer patients has been reported to carry the *Thr*^{541} allele on a *Ser*^{217}/*Ser*^{217} background (25, 26). We initially observed two controls with

this genotype, but upon DNA sequencing, both men were found to be homozygous for both Ser^{217} and Ala^{541}. In our study, the increased risk (OR = 1.8) associated with the Leu^{217}/Leu^{217} genotype was limited to men homozygous for the Ala^{541} allele (Table 3). This differs from the study by Tavtigian *et al.* (20), which reported that Leu^{217} homozygotes were at increased risk (OR = 2.0) only if they also carried a Thr^{541} allele. Differences in study design may have contributed to these divergent findings; Tavtigian *et al.* (20) analyzed prostate cancer cases from high-risk pedigrees and controls who were spouses of cancer patients in other cancer pedigrees. In the study reported here, both the case and control groups were from the same underlying population base. Larger population-based studies will be needed to confirm our results and to assess how well one can disentangle the individual effects of these two genetic variants.

Several strengths and limitations should be considered when interpreting these results. The population-based study design is a major strength that enhances the generalizability of our results, which are less prone to the potential selection biases present in some of the earlier studies. Both patients and comparison men without a history of prostate cancer were ascertained from the same underlying general population. Cases were from a population-based registry and were not selected on the basis of prostate cancer treatment or membership in high-risk prostate cancer pedigrees, and cases and controls were frequency matched on age to assure that both groups had similar age distributions. One limitation of the study, however, was the small number of African-American men, reflecting the population demographics of Northwestern Washington state. There were too few blacks to perform separate analyses of this subgroup. In addition, it is possible that some men in the comparison group had undetected prostate cancer, which would result in misclassification by disease status and attenuate relative risk estimates toward the null. We estimate from prostate cancer screening data for men < age 65 years that no more than 5–10% of these men may have clinically undetected prostate disease and that no more than 5% may have undiagnosed prostate adenocarcinoma. On the basis of self-reported medical history, only 5.8% of controls in our study had never been screened for prostate cancer by either a digital rectal examination and/or a PSA blood test. It is unlikely that such a low prevalence of possibly undetected prostate cancer in the control group could have substantially affected our results.

Another limitation was our inability to examine results with Gleason score 7 tumors included in the more aggressive stratum. According to SEER rules, Gleason scores 5–7 are grouped as one histological grade code for moderately differentiated tumors. Also, our results are based on middle-aged (<65 years) men, who account for 31% of all newly diagnosed patients (38). Thus, associations between prostate cancer and *HPC2* genotypes observed in this study may not reflect the impact of these genetic variants on later onset prostate cancer. It is also possible that participating cases and controls have different genetic profiles than nonparticipants. However, we have no evidence that *HPC2* genotype is associated with study participation.

In summary, our results suggest that *HPC2/ELAC2* polymorphisms may play a role in prostate cancer susceptibility. The Leu^{217} variant was associated with a modest elevation in the relative risk of this disease, particularly less aggressive phenotypes. We estimate that the Leu^{217} allele may account for ~9% of all sporadic prostate cancer cases in the general United States white population of men < 65 years of age. *HPC2/ELAC2* is a member of the *PSO2* gene family that plays a role in DNA interstrand cross-link repair. Functional analysis of the Ser^{217} and Thr^{541} single nucleotide polymorphisms may provide additional insight on the potential role of these variants in prostate cancer.

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