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Sequence variants of *elaC homolog 2 (Escherichia coli)* (*ELAC2*) gene and susceptibility to prostate cancer in the Health Professionals Follow-Up Study

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Two non-synonymous single-nucleotide polymorphisms (SNPs), Ser217Leu and Ala541Thr, in the *elaC homolog 2 (Escherichia coli)* (*ELAC2*) gene have been related to prostate cancer risk in previous studies, though with inconsistent results. The association of *ELAC2* haplotypes with prostate cancer risk has not yet been explored. We assessed whether sequence variants in *ELAC2* were associated with the risk of total or aggressive prostate cancer. In a nested case–control design within the Health Professionals Follow-Up Study, we identified 659 participants with prostate cancer diagnosed after they provided a blood specimen in 1993 and before January 2000. Controls were 656 age-matched men without prostate cancer who had had a prostate-specific antigen test after providing a blood specimen. We genotyped eight tagging SNPs in *ELAC2* to test for the association between sequence variances in *ELAC2* and prostate cancer. No individual SNP (including Ser217Leu) was associated with the risk of prostate cancer. Ala541Thr is a rare SNP in this population. One common haplotype (hap4) was statistically significantly associated with an increased risk of prostate cancer [odds ratio (OR) = 1.39, 95% confidence interval = 1.05–1.85]. Two common promoter SNPs and three common haplotypes were statistically significantly associated with aggressive prostate cancer (carriers versus non-carriers—snp2: OR = 1.43, snp3: OR = 0.69, hap1: OR = 1.47, hap2: OR = 0.72, hap4: OR = 1.51; global *P*-value for all common haplotypes = 0.11). Common SNPs and haplotypes of *ELAC2* were associated with risk of aggressive prostate cancer.

Introduction

elaC homolog 2 (Escherichia coli) (*ELAC2*) is located on chromosome 17p11, spans 27 kb and includes 24 exons. Identified as a prostate cancer susceptibility gene (1), it is expressed at high levels in rat testis and some mouse tissues (2). Although ELAC2 is expressed at low levels in mouse prostate (2), experimental studies showed that *ELAC2* plays a role in germline proliferation, which is related to cell cycle and sterility (3), and thus may relate to prostate carcinogenesis. The amino acid sequence of ELAC2 is similar to that of PSO2 DNA interstrand cross-link repair proteins and the 73 kD subunit of messenger RNA 3′ end cleavage and polyadenylation specificity factor (CPSF73), which may in turn be related to blocking transcription, replication and segregation of DNA and regulation of messenger RNA modifications (1,4).

Abbreviations: BMI, body mass index; CI, confidence interval; ELAC2, elaC homolog 2 *(Escherichia coli)*; OR, odds ratio; QC, quality control; SNP, single-nucleotide polymorphism.

Two non-synonymous polymorphisms in *ELAC2*, Ala541Thr and Ser217Leu, have been well studied. The allele frequency of Thr541 was lower in Japanese subjects (0–1.1%) (5–7) than in African-Americans (21%) (8) or Caucasians (2.7–10.6%) (8–20). However, comparing Thr541 carriers with non-carriers, the risk of sporadic prostate cancer was higher among Japanese subjects [odds ratio (OR) = 3.4–5.1] (6,7) than among Caucasians (OR = 1.0–2.2) (8–20). Furthermore, Thr541 was not associated with hereditary prostate cancer (10,12).

In contrast, the Leu217 allele is more prevalent in Caucasians (27–46%) than in African Americans (23%) (8) or Japanese (0–3.3%) (5,6). Three (5,10,17) out of 14 studies found that the Leu217 allele was statistically significantly associated with prostate cancer [Leu217 carriers versus non-carriers—all cases versus controls: OR = 0.78 (10); non-aggressive cases versus controls: OR = 1.34 (17); sporadic cases versus controls: OR = 3.11 (5)], whereas the remaining studies found a null relationship. A meta-analysis showed that the Leu217 variant was statistically significantly associated only with familial prostate cancer (OR = 1.37) (6). Taken together, 80% of studies showed no evidence of association between Leu217 and prostate cancer risk. Some studies indicated that the joint effect of Ser217Leu and Ala541Thr was associated with a higher risk of prostate cancer than Leu217 alone (1,9,17), whereas others failed to observe this joint effect (6,10–12,15,16).

Past studies of *ELAC2* and prostate cancer risk have focused on the two common missense variants, Ser217Leu and Ala541Thr, and results have been inconsistent. However, two single-nucleotide polymorphisms (SNPs) provide a relatively small amount of information for predicting prostate cancer risk. The relationship between haplotypes in *ELAC2* and the risk of sporadic prostate cancer has not been explored. Therefore, in addition to these two non-synonymous SNPs in *ELAC2*, we selected additional tagging SNPs and hypothesized that haplotypes of *ELAC2* are associated with susceptibility to prostate cancer in the Health Professionals Follow-Up Study. We also explored the relationship between *ELAC2* genetic polymorphisms and aggressiveness of prostate cancer.

Materials and methods

Study population

In this nested case–control study, incident prostate cancer cases were identified from the ongoing Health Professionals Follow-Up Study with follow-up from 1986 through 2000. A total of 51 529 USA men aged 40–75 years were enrolled in 1986. At baseline, every participant completed a mailed questionnaire on demographics, lifestyle and medical history and a semiquantitative food-frequency questionnaire. Information on exposures and diseases was updated every other year, and diet information was updated every 4 years. Deaths were identified through reports by family members or the postal system upon follow-up questionnaires or a search of the National Death Index (21). This study was approved by the Institutional Review Board at the Harvard School of Public Health.

Blood samples (collected in tubes containing sodium ethylenediaminetetraacetic acid) were obtained from 18 018 of the participants between 1993 and 1995. We obtained informed consent from each subject before blood was drawn. Samples were shipped by overnight courier and centrifuged; the aliquots, including plasma, erythrocytes and buffy coat, were stored in liquid nitrogen freezers. QIAGEN QIAamp blood extraction kit (QIAGEN, Valencia, CA) was used for DNA extraction. All DNA samples were whole-genome amplified, and quality control (QC) samples had 100% genotype concordance. Among the men who provided a blood specimen, 95% responded to the year 2000 questionnaire, and the 18 who died of prostate cancer before the end of follow-up were included in the case series.

We identified 659 incident prostate cancer cases and 656 controls; all are Caucasians. Each case was matched with one control who was alive,

Table I. Characteristics of *ELAC2* SNPs among Caucasians

SNP name	SNP name used in Camp *et al.* (22)	rs #	Nucleotide change (amino acid change)	Location	bp relative to start codon ATG	Controls		Cases	
						MAF (%)	HWE, *P*-value	MAF (%)	HWE, *P*-value
Snp1	S1	rs2322779	T → C	Promoter	−12682	16	0.14	19	0.74
Snp2	S4	Not applicable	G → A	Promoter	−6280	30	0.99	33	0.40
Snp3	S5	rs12600940	C → T	Promoter	−3831	49	0.35	46	0.44
Snp4	S7	rs2051974	G → A	Promoter	−381	24	0.81	24	0.17
Snp5	SL	rs4792311	C → T (Ser217Leu)	Exon	6256	32	0.88	32	0.17
Snp6	S11	rs2302069	A → G	Intron	7241	14	0.45	13	0.44
Snp7	AT	rs5030739	G → A (Ala514Thr)	Exon	21363	0	Not applicable	0	Not applicable
Snp8	S17	rs17552022	A → G	Exon	22970	13	0.36	14	0.21

HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency.

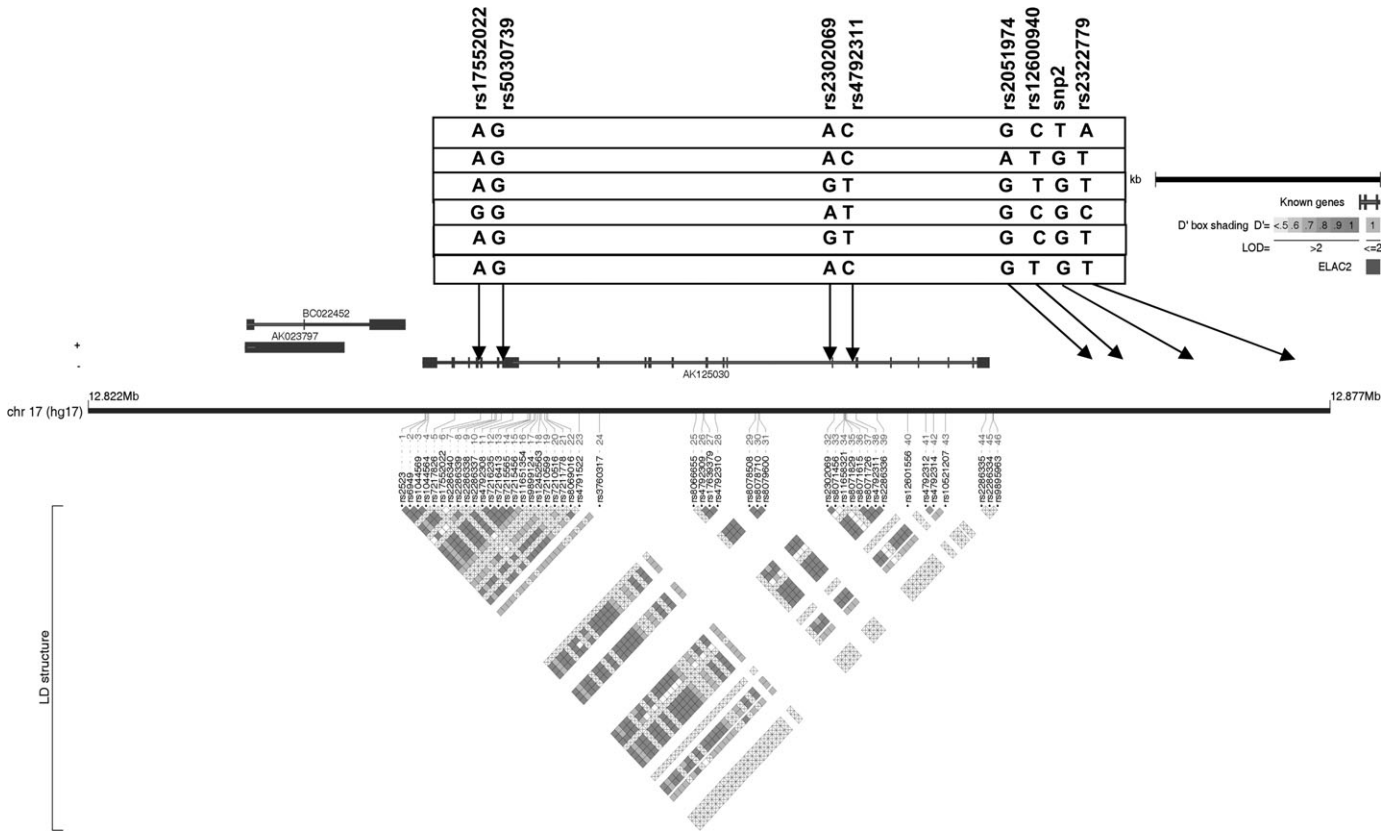

Fig. 1. *ELAC2* linkage disequilibrium plot. This plot was generated by Haploview and Locusview programs. When the approach of Gabriel *et al.* was used, the eight SNPs formed one block. The rs number on the top from left to right corresponds to the SNP name (e.g. snp1, snp2, etc.). The level of pairwise D′, which indicates the degree of linkage disequilibrium between two SNPs, is shown in the linkage disequilibrium structure in red. Six common haplotypes (frequency > 0.05) were identified.

had not been diagnosed with cancer by the date of the case's diagnosis and had a prostate-specific antigen test after the date of blood draw. The latter criterion ensured that controls had the opportunity to have an occult prostate cancer diagnosed. All controls had a prostate-specific antigen test within 2.5 years of the date of diagnosis of their matched case. Because plasma analyses were performed on the same case–control set, cases and controls were matched on year of birth (±1 year), prostate-specific antigen test prior to blood draw (yes/no), time of blood draw (midnight to before 9 A.M., 9 A.M. to before noon, noon to before 4 P.M. and 4 P.M. to before midnight), season (winter, spring, summer and fall) and year of blood draw. To elucidate the effect of sporadic prostate cancer (prostate cancer that occurs occasionally and at random intervals in a population), we also performed analyses excluding subjects with familial prostate cancer cases (two or more family members had prostate cancer cases, $n = 15$ cases and 10 controls).

Laboratory assays

Eight common (frequency > 5%) tagging SNPs in *ELAC2* (s1, s4, s5, s7, s11, s13, AT and s17) were selected from the study of Camp *et al.* (22). Laboratory personnel were blinded to case–control status. All case–control matched pairs were analyzed together using the Sequenom system. Multiplex polymerase chain reactions were carried out to generate short polymerase chain reaction products (>100 bp) containing one SNP. The details of polymerase chain reaction and matrix-assisted laser desorption ionization time-of-flight mass spectrometry are available upon request. Six control DNA samples were used for optimization. The SNP s13 in Camp *et al.* (22) failed both Sequenom and Taqman assay designs in our population due to low QC rates. This SNP could be replaced by snp5 (Table I) because they were in the same linkage disequilibrium group according to phylogenetic network analysis from Camp *et al.* (22). Analyses using the Tagger program (http://www.broad.mit.edu/mpg/tagger/) also suggested that the new set of SNPs after replacement can

accurately capture the information within haplotype blocks ($r^2 = 0.984$). Finally, a total of eight SNPs were genotyped in three plexes at the Harvard Partners Center for Genetics and Genomics (Boston, MA). For each SNP, genotyping data were missing for fewer than 5% of the study participants. Sixty-eight QC samples were obtained from 18 external participants who were recruited the same way as the study population, and each of them had two to six duplicates. These QC samples were genotyped together with all other samples in this study. All QC samples passed the QC test with discordance rate = 0.

Ascertainment of prostate cancer

Investigators reviewed the medical and pathology records for men with prostate cancer, reported from the follow-up questionnaire or rarely death certificate, to confirm adenocarcinoma of the prostate and to document clinical presentation, stage and Gleason sum of the tumor. The cases were categorized into regionally invasive or metastatic (stage $\geq$ T3c, N1 or M1), organ-confined or minimal extraprostatic extension (T1b–T3a and N0M0), higher grade (Gleason sum ≥ 7) and lower grade (Gleason sum < 7). Incidental microscopic focal tumors (stage T1a) were excluded because they are generally indolent and susceptible to detection bias due to differential rates of surgery for benign prostatic hyperplasia. In addition, men with a previous cancer (except non-melanoma skin cancer) prior to the date of blood draw were excluded. Confirmed non-T1a tumors between blood draw and 31 January 2000 were included. In the blood subcohort, 92% of cases were confirmed by medical record and 5% by other corroborating information; only 3% were based on self-report (23). We included the self-reported cases in the analyses because the concordance between self-reported and medical record-confirmed cases was high (>90%) in this cohort.

Statistical analysis

The Hardy–Weinberg equilibrium test was performed for each SNP among controls. Haplotype block structure (Figure 1) was determined using Haploview (http://www.broad.mit.edu/mpg/haploview/index.php) and Locusview (http://www.broad.mit.edu/mpg/locusview/) programs. The expectation-maximization algorithm (24,25) was applied to construct haplotypes in each block using the Tagsnp program (26). We estimated haplotype frequencies and their 95% confidence interval (CI) using the progressive ligation algorithm (as implemented in SAS PROC HAPLOTYPE).

Conditional logistic regression models were used to estimate ORs for disease in participants carrying either 1 or 2 versus 0 copies of the minor allele of each SNP and each multilocus haplotype. Haplotype trend regression (27) was used to test global association between *ELAC2* haplotypes and prostate cancer. To assess the risk of sporadic prostate cancer, we performed the same SNP and haplotype association analyses after exclusion of subjects with more than one prostate cancer case in his family. The type I error rate was first controlled by the single multiple-degree-of-freedom test of association between *ELAC2* haplotypes and prostate cancer. Given a statistically significant global test, haplotype-specific tests can provide some guidance as to which variants contribute to the statistically significant global test, although the nominal *P*-values we present do not control the family-wise error rate for these *post hoc* comparisons. Second, we performed Westfall's step-down permutation test (number of permutation tests = 100 000) for SNP and haplotype analyses to correct for multiple comparisons.

Age and family history are known risk factors for prostate cancer (28,29); previous studies found that, compared with lower body mass index (BMI) (<24.9 kg/m^2), higher BMI ($\geq$30 kg/m^2) was associated with lower risk of all prostate cancer (30), as well as early-onset (<60 years old) (31) and high-grade prostate cancer (32,33), but results were not consistent across studies. Family history of prostate cancer was available in 1990, 1992 and 1996; we checked the consistency of data across these time periods and used the updated information in 1996 for analyses. We used the likelihood ratio test to evaluate how these factors modified the association between *ELAC2* SNPs or haplotypes and the risk of prostate cancer by comparing a model with terms for main effects and interaction terms with the model with terms for main effects only. Because of the possible role of *ELAC2* in germline proliferation and cell cycle (3), aggressiveness of prostate cancer may relate to genetic variations of *ELAC2*. We tested the association between *ELAC2* haplotypes and aggressiveness of prostate cancer by using the definitions for tumor aggressiveness (aggressiveness: stages T3b, T4, N1, M1 or death due to prostate cancer or Gleason sum $\geq$7). All analyses were conducted with SAS release 9.0 (SAS Institute, Cary, NC), and all statistical tests were two sided.

Results

Eight SNPs in *ELAC2* were genotyped. Except for snp7, none of the rest of the SNPs was out of Hardy–Weinberg equilibrium among controls (Table I). Because snp7 (rs5030739, Ala541Thr) was a rare allele with no heterozygote and no homozygote variants in this Caucasian population, it was dropped from the haplotype analyses. The internal blinded QC specimens did not show evidence of genotyping error.

The study population included 659 incident prostate cancer cases and 656 matched controls. Age and BMI distributions were similar for cases and controls (Table II), but family history of prostate cancer was statistically significantly different ($P = 0.02$). The mean age at starting smoking, lifetime average number of cigarettes/day (include non-smokers) and alcohol consumption were similar for cases and controls. The distribution of prostatitis by age group and case–control status did not show statistically significant difference between cases and controls ($P = 0.09$). Among cases, 79% were in tumor stages T1b–T3a, 49% had Gleason grades 5–6 and 36% had aggressive prostate cancer. Eighteen cases died of prostate cancer before 31 January 2000.

No SNP (including the non-synonymous SNP Ser217Leu) was associated with prostate cancer risk (Table III). After dropping the rare SNP (snp7, Ala541Thr), seven SNPs spanning *ELAC2* formed one block using the algorithm of Gabriel *et al.* (34), where blocks identified with the default settings in Haploview were merged if they had multiallelic D′ >0.8, and the cumulative frequency of common (>5% frequency) haplotypes in the merged block was above 80% (35). Six common haplotypes (frequency > 5%) were found with an accumulated

Table II. Characteristics of Caucasians in the Health Professionals Follow-Up Study

Variable	Cases $n = 659$, *N* (%)	Controls $n = 656$, *N* (%)
Age (year) (matching factor)		
≤65	297 (45)	302 (46)
>65	362 (55)	354 (54)
BMI (kg/m^2)		
≤25	405 (61)	489 (59)
25–30	221 (34)	221 (34)
>30	33 (5)	46 (7)
Family history of prostate cancer		
No	528 (80)	557 (85)
Yes	131 (20)	99 (15)
Age started smoking	23.0 ± 5.3	22.9 ± 5.4
Lifetime average cigarettes per day	10.5 ± 6.3	10.9 ± 6.7
Alcohol (g/day)	11.4 ± 14.9	10.5 ± 14.7
Prostate-specific antigen at diagnosis of prostate cancer (ng/ml)	11.3 ± 21.0 ($n = 479$)	Not applicable
Stage		
T1b-T3a	517 (79)	Not applicable
T3b, T4, N1, M1 or death due to prostate cancer	55 (8)	
Missing	87 (13)	
Gleason sum		
2–4	44 (7)	Not applicable
5–6	320 (49)	
7	164 (25)	
8–10	55 (8)	
Missing	76 (12)	
Aggressiveness		
No	419 (64)	Not applicable
Yes	240 (36)	
Missing	0	
Death due to prostate cancer		
No	641 (97)	Not applicable
Yes	18 (3)	
Prostatitis		
≤60	35 (24)	18 (15)
>60	111 (76)	102 (85)

Aggressiveness defined as stages T3b, T4, N1, M1 or death due to prostate cancer or Gleason sum $\geq$7.

Table III. SNP analysis by *ELAC2* genotypes

SNP	0 copies		1 copy		2 copies		*P*-value*
	Case/control	OR	Case/control	OR (95% CI)	Case/control	OR (95% CI)	
All prostate cancer							
Snp1	428/447	1.00	198/186	1.11 (0.87–1.41)	21/12	1.82 (0.89–3.75)	0.20
Snp2	288/314	1.00	293/272	1.18 (0.94–1.49)	64/59	1.19 (0.80–1.75)	0.33
Snp3	179/161	1.00	328/330	0.89 (0.69–1.16)	133/146	0.82 (0.60–1.13)	0.46
Snp4	376/370	1.00	223/237	0.93 (0.73–1.17)	44/36	1.21 (0.76–1.92)	0.52
Snp5	308/296	1.00	263/280	0.90 (0.71–1.13)	72/68	1.01 (0.70–1.46)	0.63
Snp6	480/472	1.00	152/157	0.95 (0.73–1.22)	9/10	0.89 (0.36–2.22)	0.89
Snp8	472/487	1.00	164/149	1.13 (0.88–1.46)	9/8	1.16 (0.44–3.03)	0.63
Sporadic prostate cancer							
Snp1	418/440	1.00	193/183	1.10 (0.87–1.41)	21/12	1.84 (0.89–3.78)	0.20
Snp2	280/312	1.00	287/265	1.21 (0.96–1.53)	63/58	1.21 (0.82–1.79)	0.23
Snp3	178/159	1.00	320/325	0.88 (0.68–1.14)	127/143	0.80 (0.58–1.10)	0.36
Snp4	369/363	1.00	216/234	0.91 (0.72–1.15)	43/36	1.18 (0.74–1.88)	0.49
Snp5	301/293	1.00	258/274	0.91 (0.72–1.15)	69/67	1.00 (0.69–1.45)	0.73
Snp6	471/466	1.00	147/154	0.94 (0.72–1.22)	8/9	0.91 (0.35–2.38)	0.87
Snp8	458/478	1.00	163/148	1.14 (0.88–1.48)	9/8	1.16 (0.44–3.04)	0.59

*P-value was for testing the null hypothesis: $OR_{1\ copy} = OR_{2\ copies} = 1$.

Table IV. ORs between *ELAC2* haplotypes and the risk of prostate cancer

Haplotype	Prevalence among controls, % (95% CI)	0 copies		1 copy		2 copies		*P*-value*
		Case/control	OR	Case/control	OR (95% CI)	Case/control	OR (95% CI)	
All prostate cancer (global test $P = 0.18$)								
Hap1: TACGCAA	26.2 (23.8–28.6)	330/359	1.00	277/250	1.21 (0.96–1.53)	52/47	1.20 (0.79–1.84)	0.24
Hap2: TGTACAA	22.8 (20.5 –25.1)	396/386	1.00	225/239	0.92 (0.73–1.16)	38/31	1.21 (0.73–1.99)	0.53
Hap3: TGTGTGA	12.2 (10.4–14.0)	520/506	1.00	133/142	0.90 (0.69–1.18)	6/8	0.73 (0.25–2.11)	0.65
Hap4: CGCGTAG	8.9 (7.4–10.5)	512/542	1.00	142/109	1.39 (1.05–1.85)	5/5	0.96 (0.26–3.51)	0.07
Hap5: TGCGCAA	8.4 (6.9–9.9)	556/550	1.00	101/101	0.98 (0.72–1.35)	2/5	0.43 (0.08–2.34)	0.59
Hap6: TGTGCAA	5.0 (3.7–6.1)	613/596	1.00	44/55	0.77 (0.50–1.18)	2/5	0.39 (0.08–2.04)	0.25
Others	16.5							
Sporadic prostate cancer (global test $P = 0.03$)								
Hap1: TACGCAA	26.2 (23.8–28.6)	321/354	1.00	271/245	1.25 (0.98–1.58)	52/47	1.23 (0.80–1.89)	0.16
Hap2: TGTACAA	22.9 (20.6–25.2)	388/379	1.00	219/236	0.90 (0.71–1.14)	37/31	1.18 (0.71–1.94)	0.50
Hap3: TGTGTGA	11.9 (10.2–13.7)	510/500	1.00	129/139	0.90 (0.68–1.19)	5/7	0.71 (0.22–2.25)	0.65
Hap4: CGCGTAG	9.0 (7.4–10.5)	498/533	1.00	141/108	1.41 (1.06–1.87)	5/5	0.97 (0.26–3.54)	0.06
Hap5: TGCGCAA	8.5 (7.0–10.0)	544/540	1.00	98/101	0.97 (0.71–1.33)	2/5	0.42 (0.08–2.33)	0.58
Others	21.5							

*P-value was for testing the null hypothesis: $OR_{1\ copy} = OR_{2\ copies} = 1$.

frequency of 84% in controls (Table IV), and the *P*-value for the global test was 0.18. Men carrying one copy of the variant hap4 had a 1.39-fold increased risk of prostate cancer (95% CI = 1.05–1.85) compared with non-carriers. When subjects were restricted to sporadic prostate cancer, results changed very little (the *P*-value for global test became 0.03; hap4: OR = 1.41, 95% CI = 1.06–1.87; data not shown).

We looked at interactions with the following risk factors: age, BMI and family history of prostate cancer. However, none of these risk factors modify the association between sequence variants of *ELAC2* and prostate cancer.

We also explored the main effects for aggressive and non-aggressive prostate cancer separately (Table V). Two SNPs showed a statistically significant association with aggressive prostate cancer (carriers versus non-carriers—snp2: OR = 1.43, 95% CI = 1.06–1.93; snp3: OR = 0.69, 95% CI = 0.50–0.95). For haplotype analysis (global test *P*-value = 0.11), hap1 and hap4 variant carriers had a 1.47- and 1.51-fold increased risk of aggressive prostate cancer (95% CI = 1.08–1.99 and 1.04–2.18, respectively) compared with non-carriers. Hap2 variant carriers showed 0.72-fold risk of prostate cancer (95% CI = 0.52–0.98) compared with non-carriers. Results were not statistically significant for non-aggressive prostate cancer. After correction for multiple comparisons by the permutation test, hap4 is the only haplotype that remained statistically significantly associated with sporadic prostate cancer (*P* for permutation test = 0.0069).

We also assessed whether prostatitis affected the association between *ELAC2* genotypes and the risk of prostate cancer. Prostatitis status did not modify the association between hap1 and prostate cancer (one variant carrier of hap1 versus non-carrier: OR = 5.45, 95% CI = 1.60–18.6, *P* interaction = 0.12) among younger men (age $\leq$ 60) (data not shown). Results were also not statistically significant among older men (age $>$ 60).

Discussion

For main effect, none of the tagging SNPs was associated with the risk of prostate cancer. In contrast, hap4 was associated with an increased risk of prostate cancer. Hap1 and hap4 were related to increased and hap2 to decreased risk of aggressive prostate cancer. This is the first demonstration that common SNPs and haplotypes in *ELAC2* are associated with aggressive prostate cancer. A previous study (22) found that a very rare 8-SNP haplotype (frequency, case: 0.022, controls: 0) was associated with 'familial early-onset' prostate cancer. Since the

Table V. ELAC2 SNP and haplotypes and risk of aggressive and non-aggressive prostate cancer

	Non-carriers		Carriers		*P*-value*
	Cases/controls	OR	Cases/controls	OR (95% CI)	
SNP					
Aggressive prostate cancer					
Snp1	158/447	1.00	78/198	1.10 (0.80–1.52)	0.55
Snp2	94/314	1.00	141/331	1.43 (1.06–1.93)	0.02
Snp3	76/161	1.00	157/476	0.69 (0.50–0.95)	0.03
Snp4	152/370	1.00	83/273	0.74 (0.54–1.01)	0.05
Snp5	112/296	1.00	122/348	0.92 (0.68–1.24)	0.57
Snp6	174/472	1.00	58/167	0.93 (0.66–1.32)	0.69
Snp8	172/487	1.00	63/157	1.13 (0.80–1.58)	0.50
Non-aggressive prostate cancer					
Snp1	270/447	1.00	141/198	1.18 (0.91–1.54)	0.21
Snp2	194/314	1.00	216/331	1.06 (0.83–1.36)	0.65
Snp3	103/161	1.00	304/476	1.00 (0.75–1.34)	0.98
Snp4	224/370	1.00	184/273	1.11 (0.87–1.43)	0.40
Snp5	196/296	1.00	213/348	0.92 (0.72–1.19)	0.53
Snp6	306/472	1.00	103/167	0.95 (0.72–1.26)	0.73
Snp8	300/487	1.00	110/157	1.14 (0.86–1.51)	0.37
Haplotype					
Aggressive prostate cancer (global test $P = 0.11$)					
Hap1: TACGCAA	109/358	1.00	131/298	1.47 (1.08–1.99)	0.01
Hap2: TGTACAA	183/542	1.00	80/269	0.72 (0.52–0.98)	0.04
Hap3: TGTGTGA	160/387	1.00	51/151	0.91 (0.63–1.31)	0.60
Hap4: CGCGTAG	169/383	1.00	57/114	1.51 (1.04–2.18)	0.03
Hap5: TGCGCAA	197/550	1.00	43/106	1.14 (0.76–1.72)	0.52
Hap6: TGTGCAA	189/505	1.00	14/60	0.59 (0.32–1.11)	0.09
Non-aggressive prostate cancer (global test $P = 0.27$)					
Hap1: TACGCAA	221/358	1.00	198/298	1.08 (0.84–1.39)	0.55
Hap2: TGTACAA	329/542	1.00	183/269	1.11 (0.87–1.43)	0.40
Hap3: TGTGTGA	236/387	1.00	88/151	0.89 (0.65–1.20)	0.44
Hap4: CGCGTAG	257/383	1.00	90/114	1.31 (0.96–1.80)	0.09
Hap5: TGCGCAA	359/550	1.00	60/106	0.85 (0.59–1.22)	0.38
Hap6: TGTGCAA	331/505	1.00	32/60	0.82 (0.52–1.30)	0.40

Aggressive prostate cancer was defined as stages T3b, T4, N1, M1 or death due to prostate cancer or Gleason sum ≥ 7.
*P-value was for testing the null hypothesis: $OR_{carriers} = OR_{non\text{-}carriers} = 1$.

outcomes selected by Camp *et al.* (22) (familial prostate cancer) and our study (>98% were sporadic prostate cancer) were different, our results were not comparable. The results changed very little after we restricted all cases to sporadic prostate cancer.

Two non-synonymous SNPs, Ser217Leu and Ala541Thr, corresponding to snp5 and snp7 in our study, have been widely explored previously. In our study, Ala541Thr was too rare to support statistical analyses. Ala541 of *ELAC2* is located directly beside the histidine motif (1) and is important to 3′-tRNAse catalytic activity for removing a 3′ trailer from precursor tRNA (36). This may explain its association with prostate cancer in some studies (6,7,9,15). A meta-analysis (37) showed that Thr541 carriers accounted for 2% of prostate cancer in the general population. Thr541 is a very rare allele in our study population (Table I), which might be a result of genotyping error. However, Thr541 is in strong linkage disequilibrium with Leu217 (9–12,15,20), which was not associated with the risk of prostate cancer. Therefore, exclusion of Ala541Thr from haplotype analyses had a negligible effect on our results. Fewer than a quarter of previous studies found that either Ser217Leu or Ala541Thr was associated with the risk of prostate cancer. Because of the null findings in the majority of studies on these two SNPs, we included more common tagging SNPs and performed haplotype analyses to capture more genetic information than that provided by individual SNPs.

Our study does have some advantages. We restricted subjects to Caucasians, and thus population stratification is not a concern. Large sample size, high concordance rate in genotyping QC samples, few self-reported cases (3%) and high concordance between self-reported and medical record-confirmed cases (90%) increased the reliability of our findings. However, the low frequency of Thr541 in this population as compared with other Caucasian populations may be a result of sample variation across Caucasian populations. The tagging SNP approach, using a small set of representative SNPs, is a cost-effective approach for haplotype analyses.

A study in *Caenorhabditis elegans* showed that reduction of the gene activity of hoe-1 (the homolog of ELAC2) resulted from activation of mutation in ras and led to subsequent reduction of germline proliferation (3). In addition, the amino acid sequence of ELAC2 is similar to some proteins (1,4) with known functions, as described in the Introduction. This suggests that the sequence variants in *ELAC2* may either indirectly deactivate the mutation in *ras* gene, which leads to inhibition of germline proliferation, or through messenger RNA modification and then the formation of subsequent carcinogenesis in the prostate. We did not observe a statistically significant association between genetic variations in *ELAC2* and the risk of prostate cancer. However, *ELAC2* common SNPs and haplotypes were statistically significantly related to aggressive prostate cancer. More experimental and association studies are warranted to explain the role of *ELAC2* in prostate carcinogenesis.

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