ELAC2/HPC2 Polymorphisms, Prostate-Specific Antigen Levels, and Prostate Cancer

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Background: The ELAC2 gene has been proposed to be a prostate cancer susceptibility gene and is being referred to as HPC2, in part because three case–control studies suggested that two common polymorphisms (Ser217Leu and Ala541Thr) are associated with risk. However, four subsequent larger studies have not confirmed this association. In five of the seven total studies, subject selection was influenced by prostate-specific antigen (PSA) levels. We examined the association and possible effect of subject selection in a larger study and a meta-analysis.

Methods: In a population-based study in Australia, 825 case patients and 732 control subjects were genotyped for the Ser217Leu and Ala541Thr polymorphisms of ELAC2. Odds ratios (ORs) for prostate cancer were estimated by unconditional logistic and polytomous regression. A meta-analysis was conducted combining our data with those from seven published studies. The association of genotype with the logarithm of plasma PSA levels in control subjects was analyzed by linear regression.

Results: The ORs for prostate cancer were 0.74 (95% confidence interval [CI] = 0.50 to 1.09) for Leu217 homozygotes and 1.01 (95% CI = 0.68 to 1.50) for Thr541 heterozygotes and homozygotes compared with Ser217 and Ala541 homozygotes, respectively. ORs were not changed by excluding control subjects with elevated PSA levels. Among control subjects, there were no statistically significant associations between genotype frequencies and PSA level for either polymorphism (both P>0.4). The meta-analysis gave pooled OR estimates of 1.04 (95% CI = 0.85 to 1.26) for Leu217 homozygotes and 1.18 (OR = 0.98 to 1.42) for Thr541 homozygotes and heterozygotes. Conclusion: There is no evidence that either ELAC2 polymorphism is associated with prostate cancer or PSA level. [J Natl Cancer Inst 2003;95:818–24]
multiple-case families (7,8) and because two common polymorphisms (Ser217Leu and Ala541Thr) were apparently associated with a modestly increased risk (9). The Ser217Leu polymorphism appeared to act in a recessive fashion and Ala541Thr in a dominant fashion. The apparent association of these polymorphisms with prostate cancer was a major factor in the claim by some investigators that ELAC2 should be renamed HPC2. However, to date, only one study (10) has confirmed this association, and four larger studies (11–14) have found no evidence for the association. A possible reason for the inconsistent findings among the seven studies is that in three studies (9,10,12), the control subjects were excluded if they had an elevated prostate-specific antigen (PSA) level, and in two others (11,14), both case patients and control subjects were selected on the basis of elevated PSA levels. If there was a relationship between PSA levels and the ELAC2 polymorphisms, then excluding control subjects with elevated PSA levels would decrease the prevalence of the genotype(s) associated with higher PSA levels and perhaps provide spurious evidence of an association of the polymorphism with prostate cancer risk. Another issue is statistical power; given their sample sizes, no single study reported to date could exclude odds ratios (ORs) of prostate cancer risk associated with Ser217Leu and Ala541Thr polymorphisms (acting recessively and dominantly) of 1.5 and 2.5, respectively, or less with 80% power at the .05 level of significance.

We sought to resolve the question of whether the Ser217Leu and Ala541Thr polymorphisms in ELAC2 are associated with risk of prostate cancer, possibly through an association with PSA level, by using a population-based case–control study in which the polymorphisms were measured in case patients and control subjects and plasma PSA levels were measured in control subjects. To help resolve the question, we also report a meta-analysis of all published studies of the association between the two ELAC2 polymorphisms and prostate cancer, including the present study.

Subjects and Methods

Subjects

A population-based case–control study of prostate cancer was carried out in Melbourne, Sydney, and Perth, Australia, from 1994 through 1997, with data from population-complete state cancer registers (15); men from Sydney were not included in the current study. Eligible case patients were identified as those men diagnosed before the age of 70 years with histopathologically confirmed carcinoma of the prostate, excluding tumors with Gleason scores of less than 5. Random samples of 100%, 50%, and 25%, respectively, of the case patients diagnosed in the age groups younger than 60 years, 60–64 years, and 65–69 years were asked to participate in this study. Eligible control subjects were identified through government electoral rolls (registration by adults for voting is compulsory in Australia) and were frequency matched to the age distribution of the case patients in a ratio of one control subject to one case patient.

All consenting subjects were administered a questionnaire to record family history of cancer and other known or potential risk factors for prostate cancer. We defined a family history of prostate cancer as having at least one first-degree relative diagnosed with the disease. We interviewed 1052 control subjects and 1040 case patients from Melbourne and Perth [50% and 65%, respectively, of those eligible (15)]. Blood samples were collected from 745 control subjects (71%) and 862 case patients (83%). The probability of obtaining a blood sample (data not shown) was independent of age, educational level, and ever being a smoker (all P>.05), but it was greater for case patients than for control subjects (P<.001). It was also greater for men born in Australia than for those born elsewhere (P<.001) and for men with a family history of prostate cancer than for men without such a history (P = .02). The strengths of the effects of country of birth and family history, in terms of ORs of blood sampling, were similar and not statistically different in case patients and control subjects (all P>.4). In case patients, blood sampling was not statistically significantly associated with tumor stage or grade (both P>.06). The great majority of subjects were born in Australia, the British Isles, or Western Europe and were of Caucasian descent; 14 control subjects (2%) and nine case patients (1%) were born in Asia. Statistical analyses were carried out on the 732 control subjects (98% of control subjects with blood samples) and the 825 case patients (96% of case patients with blood samples) for whom both genotypes were measured and complete information on essential variables (i.e., age and family history of prostate cancer) was available.

Genotyping

 Buffy coats were prepared by centrifugation of 10 mL of fresh blood (collected in tubes containing EDTA) at 2000g for 15 minutes. Plasma was removed, and the interphase (buffy coat) layer was removed and stored at –70 °C. Genomic DNA was extracted from the stored buffy coats with a QiAmp Blood Midi kit (Qiagen, Valencia, CA). We used only the internal primers of the nested polymerase chain reaction (PCR) approach described by Rebbeck et al. (9) to amplify the regions of interest in ELAC2. The PCRs and subsequent restriction fragment length polymorphism (RFLP) analyses were performed under standard conditions (9), and products were visualized on 3% agarose gels. RFLP genotype data were confirmed in several ways. PCR products identified to be encoding the Thr541 polymorphism via RFLP analysis were manually sequenced to confirm the genotype. All samples homozygous for Leu217 were redigested to confirm the result (and rule out incomplete digestion). A parallel analysis of a random selection of DNA representing 5% of all DNA in the study was performed via manual sequencing.

Measurement of Plasma PSA Levels

Plasma was separated by centrifugation of 10 mL of fresh blood (collected in tubes containing EDTA) at 2000g for 15 minutes and stored in 0.5-mL aliquots at –70 °C. Plasma PSA levels were measured by a Microparticle Enzyme Immunoassay (AxSYM analyzer; Abbott Laboratories, Abbott Park, IL). The coefficient of variation of the assay at 0.4 ng/mL was 9.5%.

Statistical Analyses

Estimates of allele frequencies and tests of deviation from Hardy–Weinberg equilibrium were carried out using standard procedures based on asymptotic likelihood theory (16). Case–control analyses were conducted with unconditional logistic and polytomous regression (17), adjusting for reference age (age at diagnosis for case patients and age at selection for control subjects), year, study center (Melbourne; Perth), country of birth (Australia; other), and family history (none; any first-degree relative diagnosed with prostate cancer). Study center and calendar year were included because the diagnostic zeal for prostate
cancer screening using PSA varied between centers and over time. Unconditional logistic regression was also used to test for factors associated with blood sampling. Linear regression was used to study the variation of the logarithm of PSA level by genotype, adjusting for age.

We identified the studies to include in the meta-analysis by searching MEDLINE for the keywords “prostate cancer,” “ELAC2,” “HPC2,” and “polymorphisms” for publications listed up to mid-August 2002. Meta-analyses were conducted with standard methods for combining the crude estimates of ORs based on the weighted sum of the log(ORs) with the inverse of the variance as weight \( (18) \). Homogeneity in ORs across studies was tested by calculating the weighted (inverse of variance) sum of the squared differences between the log(OR) and the log-(pooled OR) estimates, assuming that this statistic follows a chi-square distribution with \( n - 1 \) degrees of freedom (where \( n \) = number of studies). Homogeneity in the genotype frequencies across studies was tested by fitting a factor for each study in unconditional logistic regression models separately for case patients and control subjects and by using the likelihood ratio test.

Statistical analyses were carried out with S-plus software \( (19) \). All statistical tests were two-sided.

**RESULTS**

We first compared the genotype frequencies for each of the two polymorphisms in case patients and control subjects (Table 1). No statistically significant differences in genotype frequencies for either polymorphism between the two groups were observed. The frequency of Leu217 was 0.285 (SE = 0.011) in case patients and 0.315 (SE = 0.012) in control subjects \( (P = .07) \), and the frequency of Thr541 was 0.039 (SE = 0.005) in case patients and 0.040 (SE = 0.005) in control subjects \( (P = .9) \). No deviation from Hardy–Weinberg equilibrium was observed for either polymorphism in case patients, control subjects, or both \( (P \geq .2) \). The data in Table 1 confirm the previously observed strong lack of independence of, and hence presumed linkage disequilibrium between, the two polymorphisms. For example, all 764 Ser217 homozygotes were homozygous for Ala541, whereas if the polymorphisms segregated independently, there would have been an expected 59.4 Ala541Thr heterozygotes and 0.5 Thr541 homozygotes.

For both polymorphisms, there was no statistically significant difference in the genotype distribution by age, city, year of diagnosis or selection, or country of birth (data not shown). There was marginal evidence that the genotype frequencies for the Ser217Leu polymorphism depended on family history of prostate cancer \( (P = .05) \), but the direction of the association was different within case patients and control subjects. Case patients with a family history of prostate cancer \( (n = 152) \) were more likely to be Leu217 homozygotes than case patients without such a family history \( (13\% \text{ versus } 7\%; \ P = .03) \), whereas control subjects with a family history of prostate cancer \( (n = 40) \) were less likely to be Leu217 homozygotes than control subjects without such a family history \( (0\% \text{ versus } 11\%; \ P = .02) \). Among case patients, those with high-grade tumors were less likely to be Leu217 homozygotes than those with moderate-grade tumors \( (4\% \text{ versus } 9\%; \ P = .02) \). We found no statistically significant association between tumor stage and genotype \( (P \geq .3) \).

We next examined the association between the two polymorphisms and prostate cancer risk. Table 2 shows that, when all control subjects were used as a reference group, no statistically significant association of either polymorphism with prostate cancer was observed, either overall or in subgroups with or without a family history of prostate cancer. In addition, no association with genotypes defined by combinations of the two polymorphisms was detected. We did observe a marginally statistically significantly lower risk of prostate cancer in men without a family history of prostate cancer who were homozygous for Leu217 \( (OR = 0.64, 95\% CI = 0.42 \text{ to } 0.97; \ P = .04) \). However, the patterns of risk by genotype were similar in men with or without a family history of prostate cancer \( (all P \geq .06) \) and in men with moderate- or high-grade tumors (data not shown). There was little difference between crude and adjusted risk estimates \( (data \text{ not shown}) \).

After control subjects with elevated PSA levels (defined as more than 2, 2.5, or 4 ng/mL PSA) were excluded (data not shown), the ORs of prostate cancer for men homozygous for Leu217 compared with men homozygous for Ser217 were 0.83 \( (95\% CI = 0.54 \text{ to } 1.26) \), 0.86 \( (95\% CI = 0.56 \text{ to } 1.30) \), and 0.81 \( (95\% CI = 0.54 \text{ to } 1.21) \), respectively. The corresponding OR estimates for men heterozygous or homozygous for Thr541 compared with for men homozygous for Ala541 were 1.10 \( (95\% CI = 0.71 \text{ to } 1.71) \), 1.09 \( (95\% CI = 0.71 \text{ to } 1.67) \), and 1.11 \( (95\% CI = 0.74 \text{ to } 1.68) \). All 95\% CIs included unity. For each PSA cutoff, the number of control subjects excluded was 179, 143, and 68, respectively.

**Meta-Analysis**

We carried out a meta-analysis of all eight \( [\text{the seven previous (8–14) and this one}] \) studies of the association between the Ser217Leu and Ala541Thr polymorphisms and prostate cancer to obtain pooled estimates of association and test for their statistical significance, to test for heterogeneity across studies in terms of ORs and genotype frequencies, and perhaps to provide insight into the reasons for any inconsistencies. The more recent studies have involved substantially larger sample sizes \( [\text{typically about 1000 subjects (11,13,14) compared with 500–600 subjects in earlier studies (8–10,12)]} \) and therefore had more precision in risk estimates and gave point estimates closer to unity and CIs that overlapped unity \( (\text{Figs. 1 and 2}) \). The three studies \( (8–10) \) that found evidence for a nominally statistically significant as-

**Table 1.** Joint distribution of Ser217Leu and Ala541Thr polymorphisms of ELAC2 in case patients with prostate cancer and population control subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of control subjects (%)</th>
<th>No. of case patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ala/Ala</td>
<td>Ala/Thr</td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>344 (47)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ser/Leu</td>
<td>280 (38)</td>
<td>35 (5)</td>
</tr>
<tr>
<td>Leu/Leu</td>
<td>50 (7)</td>
<td>23 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>674 (92)</td>
<td>58 (8)</td>
</tr>
</tbody>
</table>
association (as indicated by a 95% CI that did not include unity), which included the first two studies to be published (8,9), were among the smaller studies and had the least information (as indicated by having the widest 95% CIs). The pooled OR estimates for prostate cancer risk from the meta-analysis (Fig. 1) were 1.04 (95% CI 0.85 to 1.26) for men homozygous for Leu217 compared with men heterozygous for Leu217 or homozygous for Ser217 [recessive model, data not available from (9) and (13); \(P = .7\)], 1.06 (95% CI = 0.93 to 1.20) for men homozygous or heterozygous for Leu217 compared with men homozygous for Ser217 [dominant model, data not available from (8)]; \(P = .4\), and 1.18 (95% CI = 0.98 to 1.42) for men heterozygous or homozygous for Thr541 \((P = .08)\) compared with men homozygous for Ala541. There was some evidence for at least marginally statistically significant heterogeneity among the studies for the ORs of prostate cancer associated with being homozygous for Leu217 \((P = .1)\) and for being heterozygous or homozygous for Thr541 \((P = .02)\).

We then analyzed the genotype frequencies across all eight studies. Fig. 2 shows that the genotype frequencies were similar in case patients across all studies (Leu217 homozygotes, \(P = .1\); Thr541 heterozygotes and homozygotes, \(P = .3\)). For example, Leu217 homozygotes constituted 8%, 9%, or 10% of case patients in all studies, except for one of the hypothesis-generating studies (8), in which they made up 13% (95% CI = 10% to 17%) of the case patients. The genotype frequencies in control subjects were also similar across all studies for the Leu217 homozygotes, but not for the Thr541 heterozygotes or homozygotes \((P<.001)\). The frequency of the Thr541 heterozygotes or homozygotes in the three studies that reported a

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype</th>
<th>Case patients versus all control subjects, OR (95% CI)</th>
<th>Case patients without family history versus all control subjects, OR (95% CI)</th>
<th>Case patients with a family history versus all control subjects, OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser217Leu</td>
<td>Ser/Ser</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>Ser/Leu</td>
<td>0.94 (0.75 to 1.17)</td>
<td>0.95 (0.75 to 1.19)</td>
<td>0.75 (0.51 to 1.12)</td>
</tr>
<tr>
<td></td>
<td>Leu/Leu</td>
<td>0.74 (0.50 to 1.09)</td>
<td>0.64 (0.42 to 0.97)</td>
<td>1.24 (0.69 to 2.23)</td>
</tr>
<tr>
<td></td>
<td>Ser/Ser</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>Any Leu</td>
<td>0.90 (0.73 to 1.11)</td>
<td>0.89 (0.71 to 1.11)</td>
<td>0.84 (0.58 to 1.21)</td>
</tr>
<tr>
<td>Ala541Thr</td>
<td>Ala/Ala</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>Ala/Thr</td>
<td>0.99 (0.66 to 1.47)</td>
<td>0.97 (0.64 to 1.46)</td>
<td>1.19 (0.61 to 2.30)</td>
</tr>
<tr>
<td></td>
<td>Ala/Ala</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>Any Thr</td>
<td>1.01 (0.68 to 1.50)</td>
<td>1.00 (0.66 to 1.51)</td>
<td>1.19 (0.61 to 2.30)</td>
</tr>
<tr>
<td>Both</td>
<td>Ser/Ser, Ala/Ala</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>Any Leu, Ala/Ala</td>
<td>0.89 (0.71 to 1.11)</td>
<td>0.88 (0.70 to 1.11)</td>
<td>0.80 (0.55 to 1.18)</td>
</tr>
<tr>
<td></td>
<td>Any Leu, Any Thr</td>
<td>0.96 (0.64 to 1.44)</td>
<td>0.94 (0.61 to 1.44)</td>
<td>1.07 (0.54 to 2.12)</td>
</tr>
</tbody>
</table>

*Test for homogeneity of effects between case patients with and without a family history of prostate cancer (defined as a report of at least one first-degree relative with prostate cancer; see text) from polytomous logistic regression models. All statistical tests were two-sided.

Fig. 1. Published estimates of the associations between two ELAC2 polymorphisms and prostate cancer from seven previous publications and this one. The areas of the symbols are proportional to the sample sizes.
nally significant association between genotype and prostate cancer (8–10), two of which excluded control subjects with elevated PSA levels (9,10), were lower than those in the other studies (3%–4% versus 7%–10%; $P<.001$). This difference raises the possibility that lower point estimates of genotype frequencies in these control subjects could be due, in part, to an association between this polymorphism and PSA levels.

To test for such an association, we measured plasma PSA levels in control subjects, but we found no evidence for an association between either polymorphism and PSA level. Fig. 3 shows PSA levels by genotypes of the Ser217Leu and Ala541Thr polymorphisms. The geometric mean PSA level was 1.15 ng/mL for Ser217 homozygotes; after adjusting for age, the geometric means were 11% (95% CI $-3\%$ to $28\%$; $P = .2$) higher for Ser217 heterozygotes and 6% (95% CI $-25\%$ to $18\%$; $P = .7$) lower for Leu217 homozygotes. The geometric mean PSA level was 1.19 ng/mL for Ala541 homozygotes; after adjusting for age, the geometric mean was 6% (95% CI $-17\%$ to $36\%$) higher for the Thr541 heterozygotes and homozygotes combined. After adjusting for study center, country of birth, and family history, the estimates changed only marginally, the standard errors and hence CIs were essentially unchanged and, in all instances, the differences in mean PSA levels between genotypes were not statistically significant.

**DISCUSSION**

Our study found no evidence for an association between either of the two ELAC2 polymorphisms and prostate cancer risk...
or plasma PSA level. When we combined our data with that in seven published studies, there was still no evidence to reject the null hypothesis that neither ELAC2 polymorphism was associated with prostate cancer risk. The confidence intervals from the meta-analysis would preclude all but small increases in risks.

When interpreting the eight studies in the meta-analysis, some issues related to the sampling of case patients and control subjects need to be considered. First, some studies used case patients sampled from one or more sources, such as multiple-case families (8–10,12–14), urology clinics (9,12), or PSA screening programs (11), whereas this study and one other (13) used a population register. Consequently, the prostate cancer phenotype is likely to be heterogeneous across studies; if so, this heterogeneity may be masking the existence of a small genetically defined subtype at increased risk. Second, some studies used control subjects sampled through hospitals (9), PSA screening programs (10–12,14), and blood donors (13), and some subjects were even of unstated provenance (8), whereas we used a population register. Third, some of the sampling designs of these studies are potentially problematic because they are prone to several types of selection bias, and their genotype distributions are not necessarily generalizable to the general population. Some designs could also have given different risk estimates, depending on whether or not PSA levels were strongly related to the genotype of one or both of these ELAC2 polymorphisms. However, our data (Fig. 3) indicate that such a relationship between PSA levels and these two ELAC2 polymorphisms is unlikely.

The most plausible reason for the differences between study findings is chance. Despite the different study designs and response rates, the data in Fig. 2 show that the larger studies generally reported similar genotype frequencies for case patients and control subjects. Variations in frequencies between studies were not inconsistent with sampling errors (for studies with 200–800 subjects and genotype frequencies of 10%, the widths of the 95% CIs vary from 8% to 4%). Across all studies, the frequency of Leu217 homozygotes was similar across case patients and control subjects, as was the frequency of Thr541 heterozygotes and homozygotes combined across case patients. In contrast, the frequency of the Thr541 genotype was not homogeneous across control subjects (Fig. 2). Given that the selection criteria for controls differed across studies, this raised the possibility that PSA level might differ by genotype and hence explain discrepancies between study findings. However, our estimates of prostate cancer risk by genotype were not changed by excluding control subjects with elevated plasma PSA levels. Therefore, it is unlikely that increased risks of twofold or more, as originally reported (8,9), pertain to these polymorphisms.

Several criteria have recently been proposed for assessing associations between genetic polymorphisms and disease (20); the claim was that studies “ideally . . . should have large sample sizes, small P values, report associations that make biological sense and alleles that affect the gene product in a physiologically meaningful way,” although the latter condition ignores the possible influence of linkage disequilibrium. The three studies (8–10) that reported a nominally significant association between the two ELAC2 polymorphisms and prostate cancer satisfied the criterion based on a small P value, but those studies now appear to be outliers because of the low frequencies of the putative risk alleles, especially Thr541, in control subjects. Given the lack of compelling evidence from other investigations of multiple-case prostate cancer families for the existence of mutations in ELAC2 that confer a high risk of the disease (11–14) and the lack of evidence from our study and the meta-analysis that the common polymorphisms are associated with any substantial increased risk for prostate cancer, it is premature to refer to ELAC2 as HPC2.

References


NOTES

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