Role of HPC2/ELAC2 in Hereditary Prostate Cancer


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 Gln216Stop 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 HPC2/ELAC2 gene play a limited role in genetic susceptibility to HPC.

INTRODUCTION

PC3 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 in 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 services 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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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.
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 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 used for the RFLP analysis except that one of the primer was biotin labeled to capture single-stranded molecules for subsequent sequencing (Table 1). 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) in the context of 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, 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 Ps were 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 nucleotide 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 those used for the RFLP analysis except that one of the primer was biotin labeled to capture single-stranded molecules for subsequent sequencing (Table 1). 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.
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 Gly806Arg variant in exon 24 was shared by 2 of 2 affected men in family 139 but in only 1 of 2 affected men in family 139. The Gly806Arg variant in exon 24 was found in 1 family (pedigree 135). 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 (CCCCACACATCTTCCTCA) 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 (59T/78G) recognized by Bfa I, we genotyped all available samples in this family using a Bfa I-based PCR assay (Fig. 1).

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

<table>
<thead>
<tr>
<th>Exons/ introns</th>
<th>Nucleotide changes</th>
<th>Amino acid changes</th>
<th>Variants in families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 4</td>
<td>G387A</td>
<td>Lys129Lys</td>
<td>Arg211Gln 1 of 3 affected men in Family 82</td>
</tr>
<tr>
<td>Exon 7</td>
<td>G632A</td>
<td>Arg211Gln</td>
<td>Gly216Stop 2 of 3 affected men in Family 59 (see pedigree)</td>
</tr>
<tr>
<td>Exon 16</td>
<td>C650T</td>
<td>Ser217Leu</td>
<td>Gly487Arg 2 of 2 affected men in Family 149, 1 of 2 affected men in Family 139</td>
</tr>
<tr>
<td>Exon 17</td>
<td>G1554A</td>
<td>Gly518Glu</td>
<td>Ala541Thr</td>
</tr>
<tr>
<td>Exon 20</td>
<td>A1893G</td>
<td>Thr631Thr</td>
<td>Gly806Arg</td>
</tr>
<tr>
<td>Exon 24</td>
<td>G2416C</td>
<td></td>
<td>2 of 2 affected men in Family 135</td>
</tr>
<tr>
<td>(3'-UTR)</td>
<td>C2632G</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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).

We also analyzed all variant sequences using a splice site predictor program4 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

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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 6 Association between PC and HPC2–217 and 541 polymorphisms

<table>
<thead>
<tr>
<th>Ser217Leu genotype</th>
<th>Ala541Thr genotype</th>
<th>Population controls (%)</th>
<th>Familial cases (%)</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser/Ser</td>
<td>Ala/Ala</td>
<td>232 (46.2)</td>
<td>198 (44.6)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Ser/Any</td>
<td>Any Thr</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Any Leu</td>
<td>Ala/Ala</td>
<td>218 (43.4)</td>
<td>200 (45.0)</td>
<td>1.05 (0.79, 1.39)</td>
</tr>
<tr>
<td>Any Leu</td>
<td>Any Thr</td>
<td>52 (10.4)</td>
<td>46 (10.4)</td>
<td>1.04 (0.77, 1.39)</td>
</tr>
<tr>
<td>Any</td>
<td>Ala/Ala</td>
<td>450 (89.6)</td>
<td>398 (89.6)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Any</td>
<td>Any Thr</td>
<td>52 (10.4)</td>
<td>46 (10.4)</td>
<td>0.98 (0.61, 1.56)</td>
</tr>
</tbody>
</table>

* OR adjusted for age and BMI.

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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