ELAC2/HPC2 Involvement in HEREDITARY AND SPORADIC PROSTATE CANCER

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Abstract

The ELAC2/HPC2 gene at 17p11 is the first candidate gene identified for human prostate cancer (PRCA) based on linkage analysis and positional cloning (S. V. Tavtigian et al. Nat. Genet., 27:172–180, 2001). A truncating mutation was found in one hereditary prostate cancer (HPC) family, whereas two missense variants, Ser217Leu and Ala541Thr, were reported to be associated with increased PRCA risk in the general population. Here, we screened for mutations of the ELAC2/HPC2 gene in 66 Finnish HPC families. Several sequence variants, including a new exonic variant (Glu622Val), were found, but none of the mutations were truncating. We then analyzed the frequency of the three found missense variants in 1365 individuals, including hereditary (n = 107) and unselected (n = 467) PRCA, benign prostatic hyperplasia (n = 223), and population controls (568 healthy male blood donors). Ser217Leu and Ala541Thr variants carried no significantly elevated risk for HPC or PRCA, although the latter variant was associated with benign prostatic hyperplasia. The previously undescribed Glu622Val variant had a 1.0% population prevalence, but a significantly higher frequency in PRCA cases (3.0% odds ratio, 2.94; 95% confidence interval, 1.05–8.23). We conclude that ELAC2/HPC2 truncating mutations are rare in HPC, but that rare variants of the ELAC2/HPC2 gene may require additional study as risk factors for PRCA in the general population.

Introduction

To explore the genetic causes of PRCA, several groups have applied linkage analysis to localize susceptibility genes for PRCA. Five candidate loci, HPC1 (1q24-q25; MIM 601518), PCAP (1q42.2-q43; MIM 602759), HPCX (Xq27-q28; MIM 300147), CAPB (1p36; MIM 603688), HPC20 (20q13; MIM 176807) have been reported (1). In addition, putative susceptibility loci may also be located at chromosomes 11p and 16 (1). Causative genes have not yet been isolated for any of these chromosomal regions. Tavtigian et al. (2) found suggestive linkage of HPC pedigrees to 17p11 and by positional cloning identified the ELAC2 as a candidate HPC2 gene at this locus. The function of the ELAC2/HPC2 gene is unknown, but it is homologous to PSO2 (SNM1), a DNA interstrand cross-link repair protein, and CPSF73, a subunit of mRNA 3’ end-cleavage and polyadenylation factor. The ELAC2/HPC2 gene displayed several sequence variants: missense mutations Ser217Leu, Ala541Thr, and Arg781His, and a frame-shift mutation 1641 insG (2). Two previous studies (2, 3) found an association between Ser217Leu and Ala541Thr and their combination with PRCA. In addition, the 1641 insG was found to segregate with disease in a Utah PRCA family (2).

Here, we explored the significance of the ELAC2/HPC2 gene in PRCA causation in Finland. The Finnish population is genetically relatively homogeneous (4, 5). Therefore, there may be a limited number of PRCA-causing mutations, and the effect of individual risk genes could be identified more readily than in more heterogeneous populations. Furthermore, allele association studies of Finns may be less problematic than in more admixed populations, where ethnic and other population differences between cases and controls may bias the results. We first screened for mutations across the 24 exons of the ELAC2/HPC2 gene in probands from 66 HPC families. An association analysis was then carried out to explore the role of missense mutations of the ELAC2/HPC2 gene in 1365 DNA specimens, including HPC families, unselected PRCA patients, unselected patients with prostate symptoms attributed to BPH, and an independent series of 568 healthy male blood donors. Unselected consecutive PRCA specimens came from a single hospital, TAUH, which acquires a population-based selection of PRCA patients in the region.

Materials and Methods

Human Subjects. Genomic DNA specimens were collected from 467 unselected patients with PRCA and from 223 with BPH, diagnosed at the TAUH between 1996–1999. The mean age of the 467 unselected PRCA cases at diagnosis was 68 years (range, 48–92 years). Information on the tumor grade was available in 94% of the cases, T-stage was available in 94% of the cases, and M-stage (ascertained by bone scan) was available in 82% of the cases. Clinical data were collected from patient records from the hospitals where the patients had been treated. Sixty-six (14.1%) of the unselected PRCA patients had a positive family history of PRCA according to their answer in the questionnaire. Unselected PRCA cases were kept as a single group, but separated in some statistical analyses into those with and without family history. The diagnosis of BPH was based on the PSA-level (>4 or 4–3 when the free/total PSA ratio was <0.16), palpation of the prostate, and transrectal ultrasound. Most of the BPH patients were referred subsequent to a urological examination after seeking help for their lower urinary tract symptoms; and if cancer was not detected, they were diagnosed as having BPH. In addition, we analyzed 568 healthy blood donors from the TAUH and 107 confirmed Finnish HPC families (6). The mean number of those affected in the 107 confirmed HPC families was 2.8 (range, 2–5) and the mean age at diagnosis was 65 years (range, 45–86). Sixty-six families were selected for SSCP. These families had three or more affected members, or two with the proband’s age at diagnosis <65 years. The patients’ diagnoses and family histories were obtained from questionnaires and subsequently confirmed from the medical records or from the Finnish Cancer Registry and from parish records. Written informed consent was obtained from all living patients and, in hereditary cases, also from family members. The research protocols were approved by the Ethical Committee of the TAUH (license 95062 and 93175).

SSCP Analysis for ELAC2/HPC2 Mutation Screening. SSCP analysis of the entire coding sequence of the ELAC2/HPC2 gene was performed using primer sequences designed to include all intron-exon boundaries...
Association Tests. Genotypes of the ELAC2/HPC2 gene were obtained from RFLP analysis and minisequencing as stated above. The association tests were performed with logistic regression analysis, Chi’s square and Fisher’s exact test using the SPSS statistical software package (SPSS 9.0 for Windows; SPSS Inc., 1999).

Results

SSCP Analysis for Sequence Variants of the ELAC2/HPC2 Gene. SSCP revealed 17 variants in the probands of the 66 HPC families (Table 2). None of the variants were truncating mutations. Four of the changes took place in exons, and included the previously characterized missense mutations Ser217Leu (exon 7), Ala541Thr (exon 17). In addition, we found one silent base substitution at codon 520 (exon 17; ACA→ACG, Thr) and a previously undescibed missense mutation, Glu622Val (exon 20). The 622Val variant was found in a family with only two affected members. The proband was a homozygote and diagnosed with PRCA at the exceptionally early age of 45. He had an affected uncle, diagnosed at 72 years, who did not carry the variant.

Analysis of the Frequency of Ser217Leu and Ala541Thr Variants. The frequency of the two previously described missense mutations of the ELAC2/HPC2 gene, Ser217Leu and Ala541Thr, were analyzed by RFLP in four groups of DNA specimens (Table 3). All gene frequencies were found to be in Hardy-Weinberg equilibrium.

Table 1 Primers used for PCR amplification of ELAC2/HPC2 exons

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sequence of sense primer (5’→3’)</th>
<th>Sequence of antisense primer (5’→3’)</th>
<th>Amplicon length (bp)</th>
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Only two Thr541 homozygotes were found, one among the unselected PRCA cases and another among the blood donor controls. The frequencies of Leu217 and Thr541 in HPC patients (42.1% and 7.5%) or in unselected PRCA patients (47.8% and 7.5%, respectively) were not significantly different from those observed in the control group (54.6% and 7.4%). Neither was the combination of these genotypes associated with PRCA. However, we did observe a slightly higher frequency of the Thr541 variant in the group with BPH (OR, 1.73; 95% CI, 1.04–2.87).

Analysis of the Frequency of the Novel Glu622Val Variant. The frequency of the Val622 variant in the blood donor group was 1.0% (5 of 480). A significantly higher frequency was observed for unselected PRCA (14 of 465; 3.0%; OR, 2.94; 95% CI, 1.05–8.23; Table 3). Only 1 of 66 HPC patients (1.5%) and 3 of 223 BPH patients (1.3%) carried the Val622 variant.

Combination of ELAC2/HPC2 Variants and Clinical Features of the Disease. No additional information on disease risks was obtained by combining any two variants (data not shown) in the analysis. We also analyzed the association between the frequency of the three missense variants and disease phenotype, including the age of the patient, tumor grade, Gleason score, T-stage, and M-stage. No significant associations emerged from this analysis (data not shown).

Discussion

Analysis of the ELAC2/HPC2 gene by SSCP in 66 Finnish HPC families failed to identify truncating mutations that would directly implicate a causative role for this gene in HPC. We frequently observed the two missense variants (Leu217 and Thr541) described in the study by Tavtigian et al. (2), and we found one novel missense variant in the coding region (Glu622Val). A silent exonic change and a large number of intronic changes (none of these affecting splice sites) were also observed. To evaluate further the role of the three coding-region missense mutations in PRCA, we determined their frequencies in a population-based study covering 1365 individuals including patients from HPC families, unselected PRCA patients, consecutive patients with BPH, and a group of unselected healthy blood donors.

Our results indicate that the frequencies of the Leu217 and Thr541 variants of ELAC2/HPC2 gene were very similar in the Finnish population as compared with those reported in the studies by Tavtigian et al. (2) and Rebeck et al. (3). We confirmed also the strong linkage disequilibrium between these two missense variants. However, in contrast to the previous studies, we found no evidence of an association between these missense mutations, either alone or in combination, with human PRCA. Interestingly, we did find that the Thr541 variant was slightly more common in the BPH group than in the blood donors (OR, 1.73; 95% CI, 1.04–2.87) or in the two PRCA groups (unselected and HPC).

The previously unreported ELAC2/HPC2 missense mutation, Glu622Val, was found in a small HPC family, where the proband was homozygous for this mutation. Intriguingly, this patient was diagnosed with PRCA at the exceptionally young age of 45 years. His only affected relative, an uncle diagnosed at the age of 72 years, was not a carrier. The Glu622Val changes an amino acid in the carboxyl domain of the protein, about 60 amino acids downstream from the conserved histidine motif (2). The amino acid change is from acidic to neutral and hydrophobic, and the PI of the amino acid changes from 3.22 to 5.97. The glutamate at position 622 is conserved in most species, such as Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, and Mus musculus. These biochemical and evolutionary clues suggest that the Glu622Val variant could affect the protein function. The fact that the observed risk of PRCA in carriers of the Val622 mutation was almost 3-fold in comparison with noncarriers (OR, 2.94; 95% CI, 1.05–8.23) supports this hypothesis. However, these ORs, although being significant, are based on very small numbers of patients. Additional studies in very large scale patient materials are therefore required to unambiguously implicate this or other sequence variants in the relation of the ELAC2/HPC2 gene with PRCA.

Our results on the lack of association between Ser217Leu and Ala541Thr variants of the ELAC2/HPC2 gene and PRCA, agree with two other recent studies. Vesprini et al. (9) analyzed the frequencies of the Leu217 and Thr541 in 431 men with screen-detected PRCA and in 513 controls. No significantly increased risk ratios for PRCA were seen for either of the two missense mutations. Xu et al. (10) analyzed 93 HPC families for mutations across the gene and studied a population-sample of 249 patients with sporadic PRCA and 222 unaffected male controls using association analysis. This study also found no evidence of deleterious ELAC2/HPC2 mutations.

In conclusion, we did not find evidence of truncating mutations of the ELAC2/HPC2 gene in HPC families in Finland. Neither were the Ser217Leu and Ala541Thr variants significantly associated with PRCA in the general population. A novel, rare, disease-associated missense variant, Glu622Val, was observed with an OR of ~3.0. Furthermore, the Ala541Thr variant showed a slight association with BPH. Our results therefore do not support a major role for the ELAC2/HPC2 in the causation of hereditary or unselected PRCA, but they warrant additional studies into the role of rare missense variants of this gene as risk factors for PRCA as well as for BPH.

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References

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