Advances in Brief

Polymorphisms in the Prostate Cancer Susceptibility Gene *HPC2/ELAC2* in Multiplex Families and Healthy Controls[1]

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Abstract

Two polymorphisms in the newly cloned prostate cancer susceptibility gene, *HPC2/ELAC2*, are suspected to be associated with an increased risk of developing the disease. These missense variants result in a serine (S) to leucine (L) substitution at amino acid residue 217 and an alanine (A) to threonine (T) substitution at residue 541. We genotyped these polymorphisms in 257 multiplex prostate cancer sibships and in 355 race-matched healthy unrelated controls. A significant increase in the frequency of the *T* allele is seen in the prostate cancer subjects compared with controls. There is, however, little evidence for excess clustering of the *T* allele within the multiplex families known to be segregating this allele, and there is no evidence for linkage of prostate cancer to the *HPC2/ELAC2* region of chromosome 17p11.2 in these families. The *T* allele shows no association with either Gleason score or age-of-onset in segregating families.

Introduction

Recently, Tavtigian *et al.* (1) announced the positional cloning of the first CaP[3] susceptibility gene (*HPC2/ELAC2*) to be identified as the result of a whole genome scan of high risk CaP pedigrees. Two-point linkage analysis performed on a subset of the families yielded a heterogeneity lod score of 4.43 at *D17S1289*. Analysis of recombinants in two Utah kindreds allowed narrowing of the interval to about 1 Mb. Subsequent analysis of the genomic sequence across this region revealed two independent multiexon genes, *04CG09* and *HPC2/ELAC2*. Mutation screening of genomic DNAs from an early age-of-onset multiplex CaP family revealed a frameshift mutation in *HPC2/ELAC2* that throws translation out of frame after amino acid 547 and that cosegregated with the CaP phenotype (1). In addition, two missense variants, a serine (S) to leucine (L) substitution at amino acid 217 and an alanine (A) to threonine (T) substitution at amino acid 541, were found to be associated with the diagnosis of CaP. Rebbeck *et al.* (2) confirmed this association in a sample of 359 incident CaP cases and 266 matched controls. The association was attributable to a significant increase of the *T* allele at amino acid residue 541 in the CaP cases compared with controls.

Materials and Methods

We genotyped these polymorphisms in 257 multiplex CaP sibships and 355 unrelated controls. All of the multiplex sibships were ascertained from patients seen at Washington University School of Medicine by staff urologists, or were

referred by other area urologists, or were participating in CaP support groups, or responded to our published solicitations. Two hundred and thirteen of these families were included in our initial genome scan (3), 22 were added to our follow-up linkage study of chromosomes 1 and 16 (4), and 22 are new to this study.

The control subjects were ascertained from a large pool of men who have been followed for many years as part of a long-term CaP study in which men are screened at 6-to-12 month intervals with PSA blood tests and DRE of the prostate (5). The size of this pool allowed us the luxury of identifying especially healthy men. To be enrolled in the control series, the subjects were required to meet the following four criteria: (*a*) be at least 65 years old; (*b*) never have registered a PSA level in excess of 2.5 ng/ml; (*c*) never have had a DRE suspicious of CaP; and (*d*) have no known family history of CaP. This last criterion was assessed by inquiring about the subject's brothers, father, grandfathers, and maternal and paternal uncles. As a consequence of the first criterion, the control subjects were significantly older than the case subjects (71.7 *versus* 65.5 years; $P < 0.0001$). All of the subjects in this study were of European ancestry. The protocol of this study was approved by the Human Studies Committee of Washington University, and informed written consent was obtained from all of the participants.

The S217L variation was detected as follows: 37 ng of genomic DNA was amplified in 8 μl of total volume using standard 1.5 mM $MgCl_2$ polymerase buffer and 1.25 μM each of 217*HPC2/ELAC2*-5′ (CAGCTCACCTTGTGCAGTGT) and 217*HPC2/ELAC2*-3′ (GCCCAGGAAGAAGGATCTGT) primers, and 0.1 unit of *Taq* polymerase. The DNA was first denatured at 94°C for 2 min and then amplified in 35 cycles of 92°C for 30 s, 63°C for 1 min, and 72°C for 1 min. Each PCR product of 294 bp was digested with 2 units of *Taq*I (New England Biolabs, Inc.) at 65°C for 2.5–3 h. One-half of the DNA was electrophoresed at 130 V for 1.5 h on 3% agarose 3:1 (Amresco) to separate the fragments, 294 (L) *versus* 157 and 137 (S).

The A541T variation was detected by amplification of 37 ng of genomic DNA in 15 μl of final volume using the same conditions as for S 217 L, except that the primers were 541*HPC2/ELAC2*-5′ (CCTGTCCAAAG-CAGACATCA) and 541*HPC2/ELAC2*-3′ (AGGAAAAGACGCAGCC-AAAG), and the annealing temperature was 60°C. Each PCR product of 303 bp was digested with 1 unit of *Fnu 4H*I at 37°C for 3 h. The electrophoretic conditions were the same. All of the alleles had two visible constant bands (the 13-bp product could not be separated from the primers) of 79 and 49 bp. These bands were the positive control for the enzyme function. The 110-bp and 52-bp fragments (the latter comigrated with the 49-bp product) were diagnostic of the *A* allele, and the 162-bp fragment was diagnostic for the *T* allele.

All of the allele calls were independently verified by two of the authors, D. S. G. and either L. N. or N. K. K. Additionally, 15% of the DNA samples were regenotyped, and no discrepancies were observed.

Results and Discussion

Table 1 reports the joint genotype distribution for the two *HPC2/ELAC2* variants in 355 unrelated controls and 257 CaP cases. The genotypic distribution for the cases was obtained by sampling at random a single brother from each of 257 multiplex sibships and repeating the process 1000 times. The mean from these 1000 random samples is reported in Table 1. Similar to the findings of Rebbeck *et al.* (3), we observe complete linkage disequilibrium between the *T*

Table 1 *Joint genotype distribution of the S/L polymorphism at amino acid 217 and the A/T polymorphism at position 541 in a random sample of 257 unrelated prostate cancer cases and 355 healthy unrelated controls*

The joint genotype distribution for the cases was obtained by sampling at random one brother from each sibship. The reported number for each genotype (rounded to the nearest integer) is the mean based, on 1000 realizations of the sampling procedure. Genotypic proportions are given in parentheses.

	Residue 541					
	Cases			Controls		
Residue 217	AA	AT	TT	AA	AT	TT
SS	120 (0.467)			190 (0.535)		
SL	93 (0.362)	21 (0.082)		124 (0.349)	10 (0.028)	
LL	19 (0.074)	4 (0.016)		28 (0.079)	1 (0.003)	2 (0.006)
Allele frequency:	$\bar{L} = 0.3113$			$\bar{L} = 0.2761$		
	$\bar{T} = 0.0486$			$\bar{T} = 0.0211$		

allele and the *L* allele; accordingly, the maximum likelihood estimate of the *L-T* haplotype is the same as the estimate of the *T* allele frequency reported in Table 1. There is no significant difference in the frequency of the *L* and *S* alleles between cases and controls ($X^2 = 1.79$; $P = 0.18$). However, the frequency of the *T* allele is significantly greater in the CaP cases than the control sample ($X^2 = 7.13$; $P = 0.008$).

To determine whether the apparent increase of the *T* allele in our cases is attributable to excess clustering within families, we identified all of the sibships that contained at least one brother with at least one *T* allele. As can be inferred from Table 1, all of the *T*-bearing CaP cases in our sample were *A/T* heterozygotes. Forty families (33 ASPs and 7 affected trios) were identified. To test whether these 40 families provided evidence for excess clustering of the *T* allele, we computed the probability of observing particular genotypic arrays conditional on the observed sibship configurations. For instance, among the ASPs in our sample, only two configurations were observed: *AT,AT* pairs and *AT,AA* pairs. Letting *p* denote the frequency of the *A* allele and $q = 1 - p$, the frequency of the *T* allele, the proportion of ASPs expected to be *AT,AT* was:

$$\frac{1 + pq}{3p^2 + 4pq + q^2} \qquad (A)$$

Similarly, among our affected trios, only two configurations were observed: *AT,AT,AA* trios and *AT,AA,AA* trios. The proportion of affected trios expected to be *AT,AT,AA* was:

$$\frac{\frac{1}{2}p + \frac{1}{4}q}{p + \frac{3}{8}q} \qquad (B)$$

Table 2 reports these expectations for our sample of 33 ASPs and 7 affected trios for selected values of $q < 0.10$. The expected proportion of *AT,AT* ASPs or *AT,AT,AA* trios did not strongly depend on the estimated frequency of the *T* allele.

Among the 33 ASPs in our sample, 10 had configuration *AT,AT*. Three of the 7 trios had configuration *AT,AT,AA*. Because we observed slightly less clustering than expected by chance, these limited data did not support the hypothesis that the *T* allele is overrepresented in segregating CaP families.

The identification and cloning of the *HPC2/ELAC2* gene represents a significant milestone in the quest to understand the genetic architecture of hereditary CaP. The data presented here, however, appear paradoxical. Thus, whereas we were able to demonstrate an increased frequency of the *T* allele in CaP cases compared with controls, we did not find excess clustering in multiplex sibships as would be expected if this allele substantially increases risk. Our approach to detect excess clustering was similar in spirit to the family-based association test

described in Parsian *et al.* (6) or the formal TDT test described by Spielman *et al.* (7), except that, because we did not genotype any parents, ascertainment was through *T*-bearing affected offspring. Thus, although our families did not offer a great deal of power to reject the hypothesis that the *T* allele accounts for a small increase in risk, neither did they provide even a hint of support.

It is of interest that none of the three published whole genome scans of CaP families (3, 8, 9) yield any compelling multipoint evidence of a susceptibility locus on chromosome 17p, although a weak two-point signal was reported by Gibbs *et al.* (9). We have reanalyzed our families for linkage (10, 11) by subdividing them into two groups depending on the presence/absence of the *T* allele. Chromosome 17p genotypes are available on 35 of our 40 *T*-positive sibships and on 200 of the 217 *T*-negative sibships. As can be seen in Fig. 1, the multipoint NPL *Z*-scores for neither subgroup give any evidence of increased allele sharing in the vicinity of *HPC2/ELAC2*.

The frequency of deleterious mutations in *HPC2/ELAC2* is unknown. The 1641 insG insertion reported by Tavtigian *et al.* (1), causes a frameshift in a region of the protein that displays a high degree of amino-acid-sequence conservation among multicellular eukaryotes. In their recent review, Ostrander and Stanford (12) suggested that germ-line changes in *HPC2/ELAC2* are unlikely to be a common cause of CaP because an examination of 45 additional unrelated CaP cases, all with an age-of-onset of 35–55 years, failed to reveal any with a frameshift mutation. Of course it is possible that variation in noncoding regions could alter the transcription of the *HPC2/ELAC2* gene. If such elements are identified and if they are in linkage disequilibrium with the *T* allele, then the association reported here and in the study of Rebbeck *et al.* (2) must be considered secondary and noncausal.

It is also possible that the significant case-control difference that we observed was attributable to having selected an unusually healthy control

Table 2 *Predicted number (of 33) of affected sib pairs with the AT,AT genotype configuration, and the predicted number (of 7) of affected trios to have the AT,AT,AA configuration*

The observed numbers are 10 and 3, respectively.

q^a	ASPs AT,AT	Trios AT,AT,AA
0.03	11.55	3.51
0.05	11.92	3.52
0.07	12.29	3.53
0.09	12.66	3.54

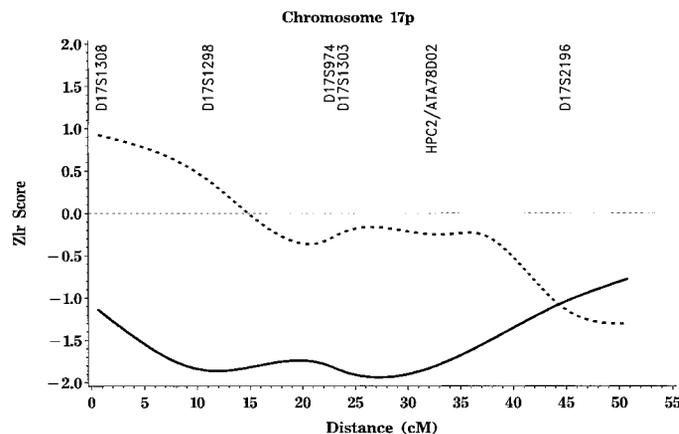*^{a} q*, the allele frequency of the *T* allele.

Fig. 1. Linkage analysis of chromosome 17p in 35 multiplex CaP sibships that contained at least one CaP brother with an *HPC2/ELAC2 T* allele (*solid line*) and in 200 multiplex sibships that contained only brothers who were homozygous for the *A* allele (*broken line*). GENEHUNTER-PLUS (10, 11) was used to compute the Zlr scores.

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sample. Not only did all of our control men have serial PSA measurements that had never exceeded 2.5 ng/ml and consistently negative DRE findings, but none had a first- or second-degree relative with CaP. The frequency of the *T* allele in our controls (0.021) is lower than that reported by Rebbeck *et al.* (2) for their white controls (0.032), albeit the difference is nonsignificant ($X^2 = 1.41$; $P = 0.24$).

Finally, to determine whether the *T* allele affects either Gleason score or age-of-onset, we drew at random 40 unrelated *A/T* heterozygotes (one from each family) and compared them with an unrelated random sample of *A/A* homozygotes drawn from the remaining 217 families. These measurements were unavailable for one of the *A/T* subjects and four of the *A/A* subjects, thereby reducing the sample size slightly. No differences were seen for either mean Gleason score ($P = 0.94$) or mean age-of-onset ($P = 0.30$).

In summary, we found a significant increase in the frequency of the *T* allele in CaP cases drawn from multiplex sibships compared with exceptionally healthy race-matched controls. Analysis of these families, however, failed to reveal any excess clustering of the *T* allele as would be expected if this amino acid substitution substantially increased susceptibility. Furthermore, linkage analysis of families segregating the *T* allele does not suggest that this polymorphism is linked to CaP in this sample.

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