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Association of common missense changes in *ELAC2* (*HPC2*) with prostate cancer in a Japanese case–control series

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Abstract The recently identified prostate cancer susceptibility gene *ELAC2* (*HPC2*) harbors two common missense variants, a serine to leucine substitution at residue 217 (Leu217) and an alanine to threonine substitution at residue 541 (Thr541). We genotyped the two variants in a Japanese cohort consisting of 350 prostate cancer patients 242 male

population controls, and 114 male low-risk controls. Both missense alleles, Leu217 and Thr541, were carried at higher frequency in Japanese patients than in the controls (Leu217, $P = 0.0012$; Thr541, $P = 0.0145$), and the odds ratios associated with carrying these sequence variants were higher in Japanese than in Caucasians. Although the Leu217 and Thr541 variants of *ELAC2* are less common in Japanese than in Caucasians, both variants confer significantly increased risk of prostate cancer in Japanese. Carriage of these variants was not associated with age at diagnosis, tumor stage, or tumor grade in these Japanese prostate cancer patients. The allele-specific pattern of risk observed in Japanese and familial Caucasian patients was qualitatively similar; however, the magnitude of that risk was considerably greater in Japanese than in Caucasians.

Key words *ELAC2* · *HPC2* · Modest risk variant · Prostate cancer · Genetics

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Introduction

ELAC2 (*HPC2*), the first prostate cancer susceptibility gene identified through genetic linkage and positional cloning, was described in late 2000 (Tavtigian et al. 2000). Orthologs of *ELAC2* are readily identifiable among all eukaryotes for which complete genome sequences are available. In addition, the carboxy half of *ELAC2* is very similar to a gene, typified by *Escherichia coli* *elaC*, present in all prokaryotes. Sequence analysis suggests that *ELAC2* encodes a metal-dependent hydrolase. However, the function of the gene product remains unknown, as does the mechanism by which the altered function influences the risk of prostate cancer (CaP).

Initial mutation screening of *ELAC2* in prostate cancer patients from Utah (Tavtigian et al. 2000, 2001) revealed four nucleotide sequence variants that altered the predicted amino acid sequence of the protein. Two of these variants, the frameshift mutation 164insG and the missense variant Arg781His, are rare or pedigree-specific changes associated

with a high risk of CaP. The other two variants are common missense variants: Ser217Leu (S217L), with a Leu217 allele frequency of about 30% in Caucasians of northern European ancestry, and Ala541Thr (A541T), with a Thr541 allele frequency of about 5% in the same population. Two primary hypotheses emerged from the initial case-control association study to examine these common missense changes (Tavtigian et al. 2001): (1) Thr541 is in very strong linkage disequilibrium with Leu217 such that virtually all Thr541 carriers also carry Leu217. If confirmed, the two missense variants could be considered to define a three-allele system: Ser217/Ala541 (SA allele), Leu217/Ala541 (LA allele), and Leu217/Thr541 (LT allele). (2) Both Leu217 and Thr541 confer a modestly increased risk of CaP, with the risk of prostate cancer conferred by the individual alleles decreasing in the order $LT > LA > SA$.

Subsequent studies have both screened familial prostate cancer patients for germline mutations in *ELAC2* (Xu et al. 2001; Rokman et al. 2001; Wang et al. 2001) and looked for associations between common sequence variants in this gene and CaP-related phenotypes (Xu et al. 2001; Rokman et al. 2001; Wang et al. 2001; Rebbeck et al. 2000; Vesprini et al. 2001; Suarez et al. 2001). Rare novel sequence variants that alter the protein coding sequence of *ELAC2* have been found in familial CaP patients (Rokman et al. 2001; Wang et al. 2001). However, as these resequencing studies have focused on variant discovery in CaP patients without measuring the summed frequency of such variants in controls, it is not known whether such variants are actually more common in patients than in controls. In addition, the variants that have been found are less than 100% penetrant and do not account for all of the cases in the pedigrees in which they are present, making it difficult to link them with risk of disease. While association studies have confirmed strong linkage disequilibrium between the common missense variants Thr541 and Leu217, they have left the relationship between carriage of these common *ELAC2* missense variants and risk of CaP ambiguous. For instance, Rebbeck et al. (2000) observed an association between carriage of the LT allele of *ELAC2* and risk of CaP in a hospital-based CaP case-control series, and Suarez et al. observed an association between carriage of the LT allele of *ELAC2* and risk of CaP in a series of familial CaP cases vs. low-risk controls (Suarez et al. 2001). In contrast, other studies have seen no significant evidence for an association between the common missense variants in *ELAC2* and risk of CaP (Xu et al. 2001; Rokman et al. 2001; Wang et al. 2001; Vesprini et al. 2001).

Age-adjusted incidence rates of CaP vary dramatically both by geographic location and by ethnicity (Muir et al. 1987; Parkin et al. 1992; Kolonel 1996). While some incidence-rate differences between ethnic groups may be due to allele-frequency differences for modest risk sequence variants (Shibata et al. 1997; Makridakis et al. 1997; Walker et al. 1998), so little is understood about the genetic component of prostate cancer susceptibility that the magnitude of this effect cannot yet be estimated. One consistent pattern is that Asian men residing in Asia experience considerably lower CaP incidence and mortality rates than Caucasians living in the United States. However, CaP inci-

dence among first- and second-generation Asian Americans is substantially higher than that in men living in Asia, sometimes approaching the rate in Caucasians (Kolonel 1996; Cook et al. 1999). As this immigration effect was well recognized before prostate-specific antigen (PSA) screening came into common use and is only partially accounted for by differences in screening and/or reporting (Cook et al. 1999; Shibata et al. 1997), a large fraction of the difference in prostate cancer incidence between United States Caucasians and Asians residing in Asia is presumably attributable to environmental, lifestyle, and/or dietary differences. Thus, one might expect that the genetic component of susceptibility to CaP is less confounded by these factors, and consequently easier to detect, in Asian men residing in Asia than in Caucasian men residing in the United States. Therefore, even though point estimates of allele frequencies of the missense variants of *ELAC2* are numerically lower in Asians than in Caucasians (Vesprini et al. 2001), we undertook to analyze these variants in a Japanese case-control series.

Subjects and methods

Study subjects

Three groups of male Japanese adults were identified for this study.

Prostate cancer cases. This group consisted of 350 patients over the age of 45 who were diagnosed with prostate cancer in Nippon Medical School Hospital, Yokohama City University Hospital, Akita University Hospital, Kyoto University Hospital, Kouchi Medical school Hospital, Nagoya City University Hospital, or Chiba University Hospital over the period 1995–2001. All of the patients had prostatectomy with postoperative pathological validation of the diagnosis; however, information on age at diagnosis, tumor stage, and tumor grade were available only for subsets of patients, as follows: age at diagnosis, 255 (72.9%). The mean age at diagnosis was 66.2 (range of 45–90). Tumor stage, scored by the Jewett staging system, where stages A and B describe locally confined prostate tumors, stage C describes tumors that have spread across the prostatic capsule or involve the seminal vesicles but without evidence of more distant metastasis, and stage D describes node-positive tumors and tumors that have metastasized to distant sites, 295 (84.3%); tumor grade, by Gleason score (range of 2–10), 257 (73.4%); tumor grade, by a summary description of well differentiated, moderately differentiated, or poorly differentiated, 177 (50.6%). These clinical data are summarized in Table 1.

Low-risk controls. This group consisted off 114 adult male Japanese with a mean age of 64.5 (52–78), who were negative for prostate cancer after urological examination, which included digital rectal examination (DRE) and rectal ultrasound echography, and had normal serum PSA levels (less than 4 ng/ml).

Table 1. *ELAC2* genotype by clinical characteristics in prostate cancer patients (CaP)

	Count	SA/SA	Any Leu 217	Carry LA	Carry LT
<u>Age at diagnosis</u>		<u>Age</u>			
<55	14	14	0	0	0
55–59	20	18	2	2	0
60–64	60	56	4	3	1
65–69	100	95	5	3	2
70–74	37	34	3	2	1
≥75	24	22	2	2	1
Total	255	239	16	12	5
<u>Age tertiles</u>					
45–63	78	73	5	4	1
64–68	90	86	4	2	2
69–90	87	80	7	6	2
Total	255	239	16	12	5
<u>Jewitt stage</u>		<u>Stage</u>			
A	8	8	0	0	0
B	127	119	8	5	3
C	84	77	7	6	2
D	76	72	4	3	1
Total	295	276	19	14	6
<u>Gleason score</u>		<u>Grade</u>			
2	1	1	0	0	0
3	18	18	0	0	0
4	16	15	1	0	0
5	43	41	2	1	1
6	35	35	0	0	0
7	76	67	9	7	2
8	19	18	1	0	1
9	39	37	2	0	0
10	10	10	0	0	0
Total	257	242	15	8	4
<u>Categories — W/M/P</u>					
Well	35	33	2	1	1
Moderate	99	94	5	4	1
Poor	43	39	4	3	1
Total	177	166	11	8	3

SA, Ser217/Ala541 allele; LA, Leu217/Ala541 allele; LT, Leu217/Thr541 allele; W/M/P, Well/Moderately/Poorly differentiated

Unscreened controls. This group consisted of 242 adult male Japanese volunteers with a mean age of 62.8 (49–73). Serum PSA levels and past or present cancer history were unavailable from this group. The case and control series were recruited from patients in the Nippon Medical School Hospital, Yokohama City University Hospital, Akita University Hospital, Kyoto University Hospital, Kouchi Medical School Hospital, Nagoya City University Hospital, and Chiba University Hospital. This collaborative project was approved by the central institutional review board of each individual institution. Cases, random controls, and low-risk controls of the Japanese panels were all recruited from the same regional populations through visits to participating academic hospitals; thus, the variation of genetic background in the Japanese subjects was negligible

among the sample populations analyzed in the present study.

Genotyping

DNAs were extracted from peripheral blood lymphocytes by conventional methods. Every sample was genotyped for the S217L and A541T molecular variants of the *ELAC2* gene twice, once by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and once by single-strand conformation polymorphism (SSCP) analysis. For genotyping by PCR-RFLP, *ELAC2* exon 7 was amplified as a 296-bp fragment using the forward primer 5'-AGGTGTGCTGATTTAATTGGCG-3' and reverse primer 3'-AGGCAGAGAATTAAGAAAACGCAAG-

5'; the S217L polymorphism was genotyped by restriction digest with *TaqI*. Exon 17 was amplified as a 419-bp PCR product using the forward primer 5'-CCAGCCTTTGTGTAAGTCTAC-3' and reverse primer 3'-GAGACAGAAATTTTCTGCAGTTGCTTC-5' and typed by restriction digest with *Fnu4HI*. After digestion, the PCR products were separated on 3% agarose gels and were visualized by UV light after ethidium bromide staining (Rebbeck et al. 2000).

For genotyping by SSCP, exons 7 and 17 were PCR-amplified using the above mentioned primers (independently from the PCR reactions of the PCR-RFLP genotyping). From each reaction, about 10ng of PCR product was heat-denatured in the presence of 80% deionized formamide and electrophoresed in 8% polyacrylamide gels, with or without 10% glycerol, in 0.5× tris-borate + EDTA (TBE) at 8 V/cm for 10h at room temperature. DNA fragments were visualized by silver staining using the Plus One DNA silver staining kit (Pharmacia Biotech, Tokyo, Japan). Each fragment showing an altered SSCP band pattern was subcloned with an AdvanTAge PCR cloning kit

(Clontech, Palo Alto, CA, USA) and sequenced. There were no discrepancies between genotypes determined by the two methods.

Statistical analyses

A χ -squared analysis was used to test the significance of genotype frequency differences between distinctive study groups. As some cells of the contingency tables in Tables 2 and 3 contained an expected value of less than 5, all *P* values in Table 3 were calculated by Fisher's Exact Test (FET). The Mantel-Haenszel χ -squared test with continuity correction was used to summarize data from multiple studies. In the contingency table-based frequency comparisons, *P* values and odds ratios for LT carriers were calculated by two different methods: (1) A full analysis wherein SA/SA, LA/SA, and LA/LA (i.e., non-LT) genotype carriers were grouped into the reference genotype category; and (2) a reference analysis wherein only SA/SA genotype carriers were used as the reference genotype category.

Table 2. Joint genotype distributions of the Ser217Leu and Ala541Thr variants in a Japanese case-control series

	Residue 541								
	Low PSA controls			Unscreened controls			Cases		
Residue 217	AA	AT	TT	AA	AT	TT	AA	AT	TT
SS	114	—	—	238	—	—	331	—	—
SL	—	—	—	4	—	—	13	5	—
LL	—	—	—	—	—	—	—	1	—
	<i>n</i> = 114			<i>n</i> = 242			<i>n</i> = 350		
Allele Freq									
SA	1.0000			0.9917			0.9714		
LA	0.0000			0.0083			0.0200		
LT	0.0000			0.0000			0.0086		

A, Ala541; T, Thr541; S, Ser217; L, Leu217; PSA, prostate-specific antigen

Table 3. Association between prostate cancer and the *ELAC2* genotype

Statistical tests	<i>P</i> values (FET)	Odds ratios	95% CIs
Cases v. combined controls			
any Leu 217	0.0012	5.05	1.9–13.5
carry LA	0.0163	3.72	1.2–11.4
carry LT	0.0145	NA	NA
Cases v. unscreened controls			
any Leu 217	0.0176	3.42	1.2–9.6
carry LA	0.1429	2.52	0.8–7.7
carry LT	0.0863	NA	NA
Cases v. low PSA controls			
any Leu 217	0.0057	NA	NA
carry LA	0.0260	NA	NA
carry LT	0.3436	NA	NA
unscreened controls v. low PSA controls			
any Leu 217	0.3104	NA	NA
carry LA	0.3104	NA	NA
carry LT	NA	NA	NA

NA, not applicable; FET, Fisher's Exact Test; CIs, confidence intervals; V, versus

In order to compare the degree of disequilibrium between different pairwise combinations of loci, a frequency-independent measure of disequilibrium (D' , the proportion of maximum possible disequilibrium (D_{\max}) in the given direction) was calculated, where $D' = \hat{D}/|D_{\max}|$. \hat{D} was calculated using the maximum likelihood technique implemented in the program EH (Xie and Ott 1993), and $\hat{D} = p(1 - q)$ since A541T and S217L are in positive linkage disequilibrium, where p and q are the frequencies of the less common allele at each respective variant, and $p < q$.

Finally, a trend statistic was calculated by grouping the cases and controls into three genotype classes [SA/SA, (LA/SA and LA/LA), and (LT/SA & LT/LA & LT/LT)] and applying the Cochran–Armitage trend test (Cochran 1954; Armitage 1955) (implemented as a summary test analogous to the Mantel-Haenszel χ -squared test) using a simple linear trend (0,1,2) for the row scores.

Results

Comparison of Ser217Leu and Ala541Thr carrier frequencies between Japanese and Caucasians

Our sample set consisted of 706 adult Japanese men: 350 CaP patients, 114 low-risk controls, and 242 men from the general population. All patients and controls were genotyped at S217L and A541T by both PCR-RFLP and SSCP. Genotypes were recorded at both markers for all samples. Genotyping revealed that both Leu217 and Thr541 are present in the Japanese population, but at dramatically lower frequencies than in the Caucasian population samples studied to date. Comparison of all the Japanese men (cases and controls) with a series of Caucasian (European ancestry) low-risk controls (Suarez et al. 2001) is illustrative (Table 4). Carrier frequencies for the Leu217 variant were 3.3% in Japanese versus 46.5% in Caucasian controls ($P < 1 \times 10^{-5}$). Carrier frequencies for the Thr541 variant were 0.8% in Japanese versus 3.7% in the Caucasian controls ($P = 0.001$). As carrier frequencies in the Caucasian controls used for this comparison are among the lowest for any

Caucasian series so far examined, it is clear that the frequencies of these two variants are much lower in the Japanese than in the Caucasian population.

Disequilibrium between Thr541 and Leu217

We observed six Thr541 carriers, all heterozygotes, in our set of 706 Japanese men. Each of the Thr541 carriers also carried Leu217 (Table 4); thus, we observed strong linkage disequilibrium between the two variants, and the maximum likelihood frequency estimate for the LT allele was the same as that for Thr541. As previous studies have also observed near-complete linkage disequilibrium between these two variants, the two missense changes can indeed be considered to define a three-allele (SA, LA, LT) system.

Case–control comparison

The study format allowed for three case–control comparisons (with the controls pooled or with the controls divided into low-risk and population series), as well as a comparison between unscreened controls and low-risk controls (Tables 2, 3). In order to distinguish the effect of carrying Leu217 from that of carrying Leu217 and Thr541, Leu217 carriers were analyzed both as a group and broken into the subsets LA carriers and LT carriers. One patient had the genotype LA/LT and was therefore analyzed in both subsets. Prostate cancer patients were significantly more likely to carry Leu217 than controls ($P = 0.001$). This was true whether they were compared with the low-risk controls ($P = 0.006$) or the unscreened controls ($P = 0.018$). The odds ratio (OR) in the overall case–control comparison, 5.05 [95% confidence interval (CI) 1.9–13.5], was much higher than those reported in previous case–control analyses of the Leu217 variant from primarily Caucasian populations, with even the lower bound of the 95% confidence interval higher than the OR reported in previous studies. When Leu217 carriers were broken down into the subsets of LA carriers and LT carriers, the frequency of LA carriers was higher in the patients than in the controls ($P = 0.016$; OR = 3.72;

Table 4. Joint genotype distributions of the Ser217Leu and Ala541Thr variants in a sample of Japanese prostate cancer patients and controls compared with a Caucasian prostate cancer–control series

	Residue 541					
	Japanese series			Caucasian series		
Residue 217	AA	AT	TT	AA	AT	TT
SS	683	—	—	190	—	—
SL	17	5	—	124	10	—
LL	—	1	—	28	1	2
		$n = 706$			$n = 355$	
Allele Freq						
SA	0.9830			0.7239		
LA	0.0127			0.2549		
LT	0.0042			0.0211		

data series from Suarez et al. (2001)

95% CI, 1.2–11.4). Even when the one LA/LT heterozygote was excluded from the analysis, the frequency of LA carriers was higher in patients than in controls ($P = 0.027$; OR = 3.46; 1.12–10.71), providing strong evidence that, in this population, carriage of Leu217 conferred increased risk of prostate cancer independent of genotype at Thr541.

Interestingly, none of the 356 controls carried Thr541, compared with 6 of the 350 cases. Cases were significantly more likely to carry Thr541, and therefore the LT allele, than controls ($P = 0.015$). In order to estimate an odds ratio for carriage of LT, we added a count of 0.5 to each cell in the contingency table. The estimated odds ratio of 9.7 was higher than that calculated for Leu217 carriers, as expected from the observation that the OR for Leu217 carriers was higher than that for LA carriers. This OR is also higher than that reported in any case–control analysis of the Thr541 variant in primarily Caucasian populations. None of the 114 low risk controls carried either Leu217 or Thr541. However, the carrier frequency difference between the low-risk controls and the unscreened controls was not statistically significant ($P = 0.310$).

Stratified analysis

Cases were stratified by age at diagnosis (<55, 55–59, 60–64, 65–69, 70–74, and ≥ 75 years), age at diagnosis tertiles (45–63, 64–68, and 69–90 years), tumor stage (Jewitt stages A, B, C, or D), tumor grade (Gleason scores 2–10), and tumor grade categories (Gleason scores 2–4, 5–7, or 8–10). Table 1 shows the distribution of the *ELAC2* genotype by each clinical characteristic. There were no statistically significant or even borderline carrier frequency differences between any of the case strata and *ELAC2* genotype.

Discussion

The strong linkage disequilibrium between the *ELAC2* missense variants Thr541 and Leu217 observed in Caucasian population samples is also maintained in the Japanese population. The variants therefore define three common alleles, SA, LA, and LT. The case–control data are consistent with the hypothesis that both Leu217-containing alleles confer risk of CaP, and the risk conferred decreases in the order $LT > LA > SA$. The potential fourth allele, Ser217/Thr541 (ST allele), is apparently so rare that it may not be possible to address its relationship with CaP risk in a case–control format.

In order to understand how the data reported here fit into the context of the ambiguous results reported from North American and European studies of this gene, we performed a pooled analysis of six of those studies (Table 5). If the case–control series in those studies are classified by two binary variables (familial vs. unselected patients; low-risk vs. unscreened controls) and then analyzed in the four resultant categories, several clear trends emerge. When the analysis is limited to the two studies that compare familial cases to stringently low-risk controls (Xu et al. 2001; Suarez

Table 5. Meta-analysis of case–control association studies between the Ser217Leu and Ala541Thr variants of *ELAC2* and prostate cancer

Study series	Familial cases vs. low-risk controls		All cases vs. low-risk controls		Familial cases vs. unscreened and low-risk controls.		All cases vs. unscreened and low-risk controls.	
	3a, 4 391	537 (1.05–1.80)	1, 3b, 4 978	795 (0.95–1.41)	3a, 4, 5a, 6 942	1,607 (0.91–1.28)	1, 2, 3b, 4, 5b, 6 2,375	2,609 (0.72–1.38)
Leu217 carriers:								
<i>P</i> value (all data)	0.021		0.139		0.396		0.988	
Odds ratio (95% CIs)	1.37		1.16		1.08		1.00	
Thr541 carriers:								
<i>P</i> value (all data)	0.012		0.003		0.135		0.063	
Odds ratio (95% CIs)	1.96	(1.16–3.30)	1.83	(1.23–2.72)	1.27	(0.93–1.72)	1.23	(0.99–1.53)
<i>P</i> value (SA/SA as reference)	0.005		0.002		0.138		0.090	
Odds ratio (95% CIs)	2.15	(1.26–3.69)	1.90	(1.26–2.87)	1.27	(0.92–1.76)	1.22	(0.97–1.53)
Trend test <i>P</i> value	0.003		0.013		0.177		0.442	
Study series definitions:	Controls							
1. Rebbeck et al. (2000)	Age- and race-matched men excluded if history of prostate disease							
2. Vesprini et al. (2001)	Caucasian women							
3a. Xu et al. (2001)	Similar ages as cases, low PSA, normal digital rectal examination (DRE), Caucasian men							
3b. Xu et al. (2001)	Similar ages as cases, low PSA, normal DRE, Caucasian men							
4. Suarez et al. (2001)	Similar ages as cases, low PSA, normal DRE, Caucasian men							
5a. Rokman et al. (2001)	Population [no phenotype info], Caucasian							
5b. Rokman et al. (2001)	Population [no phenotype info], Caucasian							
6. Wang et al. (2001)	Population males. (Mostly low PSA and normal DRE, but some are from the general population. Controls average > 10 years younger than the cases)							

et al. 2001), cases are more likely to be Leu217 carriers than controls ($P = 0.021$; OR = 1.37; 1.05–1.80) and are also more likely to be LT carriers than controls ($P = 0.005$; OR = 2.15; 1.26–3.69). Assuming 100% D' linkage disequilibrium between the two missense variants (none of these cohorts contained any Thr541 carriers who were not Leu217 carriers), we find a significant trend ($P = 0.003$) for genotype-conferred risk in the order (LT/SA or LT/LA or LT/LT) > (LA/SA or LA/LA) > SA/SA. Thus, these results are qualitatively identical to those from the Japanese cohort reported here, albeit at considerably lower odds ratios. Two additional case–control series compare unselected cases with low-risk controls (Xu et al. 2001; Rebbeck et al. 2000). When these case and control series are added into the analysis, evidence that Leu217 carriers are at increased risk is no longer significant ($P = 0.139$; OR = 1.16; 0.95–1.41). However, evidence for a higher frequency of Thr541 carriers in cases than in controls is strengthened ($P = 0.002$; OR = 1.9; 1.26–2.87). In contrast, addition of the three case–control series containing unselected controls (Rokman et al. 2001; Wang et al. 2001; Vesprini et al. 2001) to the analysis completely eliminates evidence that Leu217 carriers are at increased risk (OR = 1.00; 0.72–1.38) and leaves only marginal evidence that Thr541 carriers are at increased risk ($P = 0.063$ or 0.090, depending on whether calculated by full or reference method, respectively; OR = 1.2; 1.0–1.5).

The pooled analysis is more powerful than any of the individual studies that it samples because the number of cases and controls subsumed in each category is much larger than in the individual component studies. In particular, when familial CaP cases are analyzed versus low-risk controls, certain marginal results or trends reported in the individual studies become statistically significant. From the pooled analysis, we draw two conclusions: (1) In North American or European Caucasians, the LA and LT alleles of *ELAC2* confer greater risk in the context of familial than sporadic CaP; (2) There is a distinct difference between use of unselected controls and age-matched low-risk controls in these case–control association studies. In support of this latter conclusion, Rokman et al. (2001) reported a higher frequency of LT carriers among men with benign prostate hyperplasia (BPH) than among unselected controls, and Vesprini et al. (2001) noted a trend toward higher LT carrier frequency in men with elevated PSA and BPH than in men with elevated PSA but normal prostate biopsy.

It is not obvious that any simple explanation can entirely resolve the apparent differences between the data sets reported here. While it is reassuring that the Japanese case–control and Caucasian familial case versus low-risk control data sets are qualitatively similar, the basis of the quantitative difference between them remains unclear. One possibility is that diet and lifestyle factors in North America and Europe are responsible for a sufficient fraction of CaP risk in these geographical regions that they confound detection of the risk that is conferred by the LA and LT alleles of *ELAC2*. On the other hand, Japanese men residing in Japan may be subject to gene–environment or gene–gene interactions that magnify risk associated with those alleles.

Analysis of genotyping data from Japanese men residing in North America or Europe could potentially clarify this issue, subject to the limitation that the study would have to be sufficiently powerful to detect an odds ratio of about 1.5 at a carrier frequency of about 1%. The difference in the findings between studies utilizing low-risk controls as opposed to unselected controls is also somewhat puzzling. It may be that the LA and LT alleles of *ELAC2* predispose patients to both malignant and nonmalignant prostate disease in such a way that the use of unselected controls confounds the analysis. Alternatively, these alleles could confer risk of nonmalignant prostatic phenotypes, such as BPH, for which men often seek medical evaluation; coupled with the high rate of occult prostate cancer in older men, this could lead to a true association with BPH and an ascertainment bias-driven association with prostate cancer. Such issues underscore the point that the power of case–control association studies of modest risk sequence variants is very sensitive to case and control category definitions. The optimally designed study would define phenotype categories that are related to or can confound the main disease category and consider such categories separately. Using these phenotypic characteristics, such a study would also define a category of controls at lower risk than the general population.

In summary, we found a significantly higher frequency of both the LA and LT alleles of *ELAC2* in unselected Japanese CaP patients compared with Japanese male controls, with evidence that the CaP risk conferred by the missense-defined alleles of this gene decrease in the order LT > LA > SA. Comparison of these data with a pooled analysis of studies utilizing Caucasian CaP patients and low-risk controls revealed results that are qualitatively very similar and therefore confirmatory, although the odds ratios that we calculated from the Japanese case–control series are considerably higher than those derived from the Caucasian series. While these data provide strong evidence that sequence variants in *ELAC2* can confer increased risk of CaP, we can only speculate as to why the magnitude of this effect differs between ethnic groups.

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