Letters to the Editor

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Meta-Analysis of Associations of the Ser217Leu and Ala541Thr Variants in *ELAC2* (*HPC2*) and Prostate Cancer

To the Editor:

Several factors can contribute to variability in association-study findings. They include, but are not limited to, population-specific linkage disequilibrium between causal variants and marker variants, multilocus interactions, gene-environmental interactions, inadequate statistical power, and different selection criteria for case and control individuals. Meta-analyses afford the opportunity to combine evidence across studies, thereby increasing sample size and power and allowing a more global interpretation of the total data available. We have performed a meta-analysis for data pertaining to the association of alleles of variants Ser217Leu (MIM 605367.0001) and Ala541Thr (MIM 605367.0002) in the *ELAC2* (*HPC2*) gene (MIM 605367) and prostate cancer (MIM 176807) (GenBank accession number AF304370).

The *ELAC2* gene on chromosome 17p11.2 was the first candidate gene for prostate cancer susceptibility to be identified from a linkage analysis and positional cloning project (Tavtigian et al. 2001). Two segregating mutations (1641insG and Arg781His) were found in two extended Utah pedigrees; in addition, the less common alleles of two missense variants (Ser217Leu and Ala541Thr) were observed to be associated with familial prostate cancer (from pedigrees at high risk of prostate cancer [$n = 429$]) when compared with control individuals at low risk (i.e., men who were cancer-free and were not members of pedigrees with a high risk of prostate cancer [$n = 148$]). Initial results indicated that individuals homozygous for Leu217 and individuals carrying the Thr541 allele were at significantly increased risk for prostate cancer and, furthermore, that a combination across both genotypes was the most significant, with an odds ratio (OR) of 2.94 (95% CI 1.52–5.69) (see table 3 of Tavtigian et al. 2001).

Several groups have attempted to confirm these findings, with varying levels of success (only two of the total six studies found significant evidence for the same variants). In brief, Rebbeck et al. (2000) found that carriage of both Leu217 and Thr541 significantly was associated with prostate cancer, in a study of 359 men with newly diagnosed prostate cancer and 266 male age- and race-matched control individuals with an OR of 2.37 (95% CI 1.06–5.29) (see table 2 of Rebbeck et al. 2000). Xu et al. (2001) studied two groups of patients: those with familial disease ($n = 134$) and those with sporadic disease ($n = 228$), in comparison with control individuals at low risk (men who had normal results of a digital rectal examination [DRE] and normal levels of prostate-specific antigen [PSA] [$n = 182$]). No analyses yielded significant results, although several exhibited trends in the expected direction; for example, the OR for carriers of Leu217 compared with control individuals was 1.49 (95% CI 0.94–2.35) (see table 5 of Xu et al. [2001]). Vesprini et al. (2001) studied 431 men with screen-detected prostate cancer, 513 men with elevated PSA but no detectable prostate cancer (the prevalence of benign and neoplastic prostate disease in this group was high), and 922 healthy women. No significant results were found, with the most interesting finding from this study being that there were more double homozygotes among the men with prostate cancer than among either control group ($P = .18$) and, in addition, that there was a modest association between carriage of Thr541 and a family history of prostate cancer ($P = .04$). Suarez et al. (2001) studied 257 men with familial prostate cancer and 355 low-risk control individuals (i.e., those with normal DRE results and normal PSA levels, age >65 years, and no family history of prostate cancer); they found a non-significant trend for carriage of Leu217 and a significant association between carriage of the Thr541 allele and prostate cancer ($P = .008$). Wang et al. (2001) found no evidence for an association of either variant when 446 men with familial prostate cancer were compared with 502 population-based control individuals; however, a novel germline nonsense mutation (Glu216Stop) was found to play an interesting role in a large nuclear family that included multiple individuals with prostate cancer. Rokman et al. (2001) studied 107 men with familial prostate cancer, 467 men with sporadic disease, 223 men with benign prostatic hyperplasia (BPH), and 568 male blood donors. The Leu217 and Thr541 alleles were not found to be elevated significantly in men with prostate cancer (familial or sporadic); however, Thr541 was found

Table 1

Raw Genotypic Counts for Ser217Leu and Ala541Thr

Variant and Genotype	Author (Race of Study Population)													
	Rebbeck (Various)[a]		Suarez (White)[b]		Tavtigian (White)[c]		Vesprini (Various)[d]		Wang (White)		Xu (White)[f]		Xu (White)[g]	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Ser217Leu:														
SS	144	118	120	190	204	76	220	259	198	232	58	97	172	97
SL	139	111	114	134	168	63	169	213	205	221	61	71	156	71
LL	17	21	23	31	57	9	42	41	41	49	15	14	34	14
Total	300	250	257	355	429	148	431	513	444	502	134	182	362	182
Ala541Thr:														
AA	269	232	232	342	387	143	404	478	399	450	111	166	322	166
AT,TT	21	8	25	13	42	5	27	35	46	52	13	16	34	16
Total	290	240	257	355	429	148	431	513	445	502	124	182	356	182

[a] Table 2 of Rebbeck et al. (2000) with additional clarification from the authors to enable Ser217 recessive analysis; >80% of patients were white, and case and control individuals were race and age matched.

[b] Table 1 of Suarez et al. (2001).

[c] From table 3 of Tavtigian et al. (2001), with counts for SS and SL separated.

[d] From table 2 of Vesprini et al. (2001). Only male control individuals were included in the present analysis; >80% of the study population were white.

[e] From tables 4 and 5 of Wang et al. (2001). Case individuals were ascertained from 181 families (180 white non-Hispanic and 1 of Hispanic ancestry).

[f] Patients with familial prostate cancer only; from table 5 of Xu et al. (2001).

[g] Patients with either familial or sporadic cancer; from table 5 of Xu et al. (2001).

to be significantly elevated in men with BPH, and the rare allele of a novel variant (Glu622Val) was found to be significantly increased in both groups with prostate cancer. All studies found Thr541, the less common variant, to be in very strong disequilibrium with Leu217. This disequilibrium makes it essentially impossible to distinguish the effects of Thr541 alone from the joint effect of the two missense changes in the Leu217 + Thr541 allele.

We constructed a Mantel-Haenszel meta-analysis of data from these studies, to consolidate the evidence of association between alleles of Ser217Leu and Ala541Thr and prostate cancer. The Mantel-Haenszel χ^2 test and the Mantel-Haenszel estimate of the OR (e.g., see Kirkwood 1988) were used to provide a summary test and OR, which control for confounding factor(s)—across the various data sources—that may distort results if data are simply pooled. Data from Rokman et al. (2001) could not be incorporated, since their data were presented by allele rather than by genotype. As is evident from the brief descriptions given here, classifications for affected and control individuals varied substantially across studies. For this reason, we analyzed case and control individuals in three groupings: men with familial prostate cancer versus low-risk control individuals (FAM vs. LOW); all men with prostate cancer versus low-risk control individuals (ALL vs. LOW); and all men with prostate cancer versus all control individuals (ALL vs. ALL). Table 1 shows the raw genotypic counts from the studies used in our analyses, and table 2 reports the results for each of the three case/control comparison groups for Leu217 (recessive and dominant), Thr541 (dominant), and a multilocus analysis across both variants (Leu217 dominant and Thr541 dominant).

Our summary analysis of Thr541 data argues strongly in favor of two points. First, there is substantial evidence that carriage of the Thr541 allele, either alone or in combination with carriage of the Leu217 allele, is significantly associated with prostate cancer. Results of the comparisons FAM versus LOW and ALL versus LOW are highly significant for both these tests ($P = .0080$–$.00011$). Second, the results are most significant in the more extreme case/control comparison group (FAM vs. LOW), with effect sizes decreasing as the case/control comparison broadens. For example, for the Thr541 dominant analysis, the ORs decrease from 1.96 (95% CI 1.19–3.22) to 1.81 (95% CI 1.23–2.68) to 1.25 (95% CI 0.97–1.60) (without data of Tavtigian et al. [2001]), or, similarly, from 2.23 (95% CI 1.44–3.46) to 2.01 (95% CI 1.40–2.88) to 1.35 (95% CI 1.07– 1.72) (with data of Tavtigian et al. [2001]) as the comparison groups are expanded from FAM versus LOW to ALL versus LOW to ALL versus ALL, respectively. The dilution of significance and risk-size estimate as the case/control comparison group broadens is to be expected and is consistent with a true genetic risk. Patients with sporadic cancer are more likely to have a larger environmental and smaller genetic component, and population control

individuals are likely to harbor a substantial portion of men with prostate cancer, given the high disease rate.

The summary analysis of Leu217 data does not support the original finding by Tavtigian et al. (2001) that homozygotes for Leu217 are at increased risk for prostate cancer. Evidence from the meta-analysis is consistent with a very modest dominant effect. This may indicate that the best model for Leu217 is codominant. Significant results for Leu217 dominant are weaker than those for Thr541, but, as with Thr541, there appears to be a dilution of the effect of Leu217 as the case/control comparison broadens. Leu217 results are more often significant in the most extreme (FAM vs. LOW) case/control comparison and are never significant in the broadest (ALL vs. ALL) comparison. Also supportive of a very modest effect of Leu217 are the ORs for the combined multilocus analysis, which were found to be consistently, although modestly, higher than ORs for the Thr541 dominant analysis.

Our analyses here suggest that the original maximal OR risk estimates of 3.1 (carriage of Thr541 under a FAM vs. LOW comparison [table 3 of Tavtigian et al. 2001) and 2.37 (carriage of Thr541 under an ALL vs. LOW comparison [table 2 of Rebbeck et al. 2000]) for *ELAC2* variants on prostate cancer risk were inflated. Summary results indicate risk ratios as high as 2.4 when highly discordant groups (FAM vs. LOW, in the multilocus analysis including Tavtigian data) are compared, but the results project much lower risks, in the range of 1.3 (ALL vs. ALL, in the Thr541 dominant analyses), for the risk in the general population. If we assume a carrier frequency of 6.6% for risk genotypes (using pooled data), an OR of 1.3 translates to a population-attributable risk of 2% (Lillienfeld and Lillienfeld 1980). This is perhaps a more realistic expectation for common variants in a complex disease, and it suggests that studies may have been underpowered. In conclusion, our summary analyses indicate convincing evidence for the role

Table 2

Results of Mantel-Haenszel Meta-Analysis, Including and Excluding Data of Tavtigian et al. (2001) for the Three Case/Control Comparisons

Comparison, Inclusiveness, and Measure[a]	Analysis			
	Leu217 Dominant	Leu217 Recessive	Thr541 Dominant	Multilocus[b]
FAM vs. LOW:				
Without Tavtigian:				
P	.017	.48	.0080	.0026
OR (95% CI)	1.37 (1.06–1.78)	1.18 (.75–1.85)	1.96 (1.19–3.22)	2.21 (1.32–3.69)
Total sample size	928	928	918	529
With Tavtigian:				
P	.016	.044	.00033	.00011
OR (95% CI)	1.30 (1.05–1.61)	1.48 (1.01–2.16)	2.23 (1.44–3.46)	2.44 (1.55–3.83)
Total sample size	1,505	1,505	1,495	856
ALL vs. LOW:				
Without Tavtigian:				
P	.11	.81	.0029	.0023
OR (95% CI)	1.17 (.96–1.42)	.96 (.67–1.37)	1.81 (1.23–2.68)	1.86 (1.25–2.77)
Total sample size	1,706	1,706	1,680	985
With Tavtigian:				
P	.075	.31	.00014	.00012
OR (95% CI)	1.17 (.98–1.39)	1.18 (.86–1.62)	2.01 (1.40–2.88)	2.05 (1.42–2.96)
Total sample size	2,283	2,283	2,257	1,312
ALL vs. ALL:				
Without Tavtigian:				
P	.21	.86	.081	NA
OR (95% CI)	1.09 (.95–1.24)	1.02 (.81–1.29)	1.25 (.97–1.60)	NA
Total sample size	3,596	3,596	3,571	NA
With Tavtigian:				
P	.15	.29	.013	NA
OR (95% CI)	1.10 (.97–1.24)	1.13 (.90–1.41)	1.35 (1.07–1.72)	NA
Total sample size	4,173	4,173	4,148	NA

[a] FAM vs. LOW comparison includes data from Xu et al. (2001) (familial prostate cancer only) and Suarez et al. (2001). ALL vs. LOW comparison includes data from Xu et al. (2001) (familial and sporadic prostate cancer), Suarez et al. (2001), and Rebbeck et al. (2000). ALL vs. ALL comparison includes data from Xu et al. (2001) (familial and sporadic prostate cancer), Suarez et al. (2001), Rebbeck et al. (2000), Vesprini et al. (2001) (male control individuals only), and Wang et al. (2001).

[b] Association test for carriage of both Leu217 and Thr541 versus carriage of neither. "NA" indicates that data were not available from the relevant published papers to perform the multilocus analysis.

of *ELAC2* in prostate cancer, suggest moderate familial risk, and estimate that risk genotypes in *ELAC2* may cause 2% of prostate cancer in the general population.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for prostate cancer [MIM 176807], HPC2/ELAC2 [MIM 605367], Ser-to-Leu change at amino acid 217 [MIM 605367.0001], and Ala-to-Thr change at amino acid 541 [MIM 605367.0002]

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for variant Ser217Leu [AF304370])

References

Lillienfeld AM, Lillienfeld DE (1980) Foundations of epidemiology. 2nd ed. Oxford University Press, New York

Kirkwood BR (1988) Essentials of medical statistics. Blackwell Scientific Publications, Oxford

Rebbeck TR, Walker AH, Zeigler-Johnson C, Weisburg S, Martin AM, Nathanson KL, Wein AJ, Malkowicz SB (2000) Association of HPC2/ELAC2 genotypes and prostate cancer. Am J Hum Genet 67:1014–1019

Rokman A, Ikonen T, Mononen N, Autio V, Matikainen MP, Koivisto PA, Tammela TL, Kallioniemi OP, Schleutker J (2001) ELAC2/HPC2 involvement in hereditary and sporadic prostate cancer. Cancer Res 61:6038–6041

Suarez BK, Gerhard DS, Lin J, Haberer B, Nguyen L, Kesterson NK, Catalona WJ (2001) Polymorphisms in the prostate cancer susceptibility gene HPC2/ELAC2 in multiplex families and healthy controls. Cancer Res 61:4982–4984

Tavtigian SV, Simard J, Teng DH, Abtin V, Baumgard M, Beck A, Camp NJ, et al (2001) A candidate prostate cancer susceptibility gene at chromosome 17p. Nat Genet 27:172–180

Vesprini D, Nam RK, Trachtenberg J, Jewett MA, Tavtigian SV, Emami M, Ho M, Toi A, Narod SA (2001) HPC2 variants and screen-detected prostate cancer. Am J Hum Genet 68:912–917

Wang L, McDonnell SK, Elkins DA, Slager SL, Christensen E, Marks AF, Cunningham JM, Peterson BJ, Jacobsen SJ, Cerhan JR, Blute ML, Schaid DJ, Thibodeau SN (2001) Role of HPC2/ELAC2 in hereditary prostate cancer. Cancer Res 61:6494–6499

Xu J, Zheng SL, Carpten JD, Nupponen NN, Robbins CM, Mestre J, Moses TY, Faith DA, Kelly BD, Isaacs SD, Wiley KE, Ewing CM, Bujnovszky P, Chang B, Bailey–Wilson J, Bleecker ER, Walsh PC, Trent JM, Meyers DA, Isaacs WB (2001) Evaluation of linkage and association of HPC2/ELAC2 in patients with familial or sporadic prostate cancer. Am J Hum Genet 68:901–911

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Regarding "Testing for Population Subdivision and Association in Four Case-Control Studies"

To the Editor:

Ardlie et al. (2002) recently found no evidence for population structure in separate case-control studies of type 2 diabetes and hypertension in U.S. whites and only weak evidence of structure in a case-control study of hypertension in African Americans. These results are consistent with the theoretical results of Wacholder et al. (2000), who found that the magnitude of bias due to unrecognized population stratification is likely to be small under most plausible scenarios. To further evaluate the potential bias due to stratification for these and other conditions, we conducted a series of case-control studies for six common phenotypes in a population-based sample of U.S. adults.

The study population included 444 unrelated adults (231 African Americans and 213 non-Hispanic whites) randomly selected from five U.S. communities as part of the Hypertension Genetic Epidemiology Network (HyperGEN) of the National Heart, Lung, and Blood Institute (NHLBI) Family Blood Pressure Program (Williams et al. 2000). The study was approved by the institutional review boards at each institution, and appropriate informed consent was obtained from human subjects. Phenotypes measured included: (1) obesity (BMI $\geq$ 30), (2) hypercholesterolemia (total plasma cholesterol $\geq$ 240 mg/dl or current use of medications to lower cholesterol), (3) hypertension (systolic blood pressure $\geq$ 140 mmHg, diastolic blood pressure $\geq$ 90 mmHg, or current use of medications to lower blood pressure), (4) diabetes (fasting serum glucose $\geq$ 126 mg/dl, nonfasting glucose $\geq$ 200 mg/dl, self-reported physician diagnosis of diabetes, or current use of hypoglycemic medications), (5) renal dysfunction (serum creatinine $\geq$ sex-specific 90th percentile [1.4 mg/dl in men and 1.1 mg/dl in women]), and (6) cardiovascular disease (self-reported history of heart attack, stroke, or coronary artery bypass surgery). For each