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Adipokine Genes and Prostate Cancer Risk

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Abstract

Adiposity and adipocyte-derived cytokines have been implicated in prostate carcinogenesis. However, the relationship of adipokine gene variants with prostate cancer risk has not been thoroughly investigated. We therefore examined common variants of the *IL6*, *LEP*, *LEPR*, *TNF*, and *ADIPOQ* genes in relation to prostate cancer in a case-control study nested within a large cohort of Finnish men. The study sample consisted of 1,053 cases of prostate cancer, diagnosed over an average 11 years of follow up, and 1,053 controls matched to the cases on age, intervention group, and date of baseline blood draw. Logistic regression was used to model the relative odds of prostate cancer. We also examined genotypes in relation to serum insulin, IGF-1, and IGF-1:IGFBP-3 among 196 controls. Variant alleles at three loci (−14858A>G, −13973A>C, −13736C>A) in a potential regulatory region of the *LEP* gene conferred a statistically significant 20% reduced risk of prostate cancer. For example, at the −14858A>G locus, heterozygotes and homozygotes for the A allele had an odds ratio (OR) of prostate cancer of 0.76 (95% confidence interval [95% CI]= 0.62, 0.93) and 0.79 (95% CI = 0.60, 1.04), respectively. At 13288G>A, relative to the GG genotype, the AA genotype was associated with a suggestive increased risk of prostate cancer (OR = 1.29; 95% CI, 0.99, 1.67; P-trend = 0.05). Polymorphisms in the *IL6*, *LEPR*, *TNF*, and *ADIPOQ* genes were not associated with prostate cancer. Allelic variants in the *LEP* gene are related to prostate cancer risk, supporting a role for leptin in prostate carcinogenesis.

Keywords

Leptin; adipokines; cytokines; prostate cancer; epidemiology

Introduction

Prostate cancer is a highly heritable condition, with more than 40 percent of the variability in risk attributed to genetic factors (1). The heritability of prostate cancer is believed to be due largely to multiple low penetrance susceptibility loci, as several linkage studies including a large number of case families have failed to identify any high penetrance alleles.

Genome-wide association studies have uncovered several genetic variants in the regions of chromosome 8q24, 17q12, and 17q24.3 (among others) that are associated with increased risk of prostate cancer (2;3). However, these polymorphisms confer only modest increases in prostate cancer susceptibility and, therefore, much of the genetic heritability of prostate cancer remains to be explained.

Recently, there has been substantial interest in genes related to adipokines, which are cell to cell signaling molecules secreted by adipose tissue, and their role in prostate carcinogenesis. Several adipokines, particularly leptin, adiponectin, tumor necrosis factor-alpha, and Interleukin-6, affect processes involved in carcinogenesis, including those in the prostate (4;5). Adipokine levels are correlated with fat mass, and it has been proposed that adipokines are critical molecular intermediates of the relationship between obesity and cancer (6). Evidence for such a link is provided by studies demonstrating that caloric restriction and/or physical activity decreases fat mass, lowers circulating levels of many adipokines, and also inhibits tumor growth (7;8).

Despite biological plausibility, investigations into the relationship of adipokines and adipokine genes with prostate cancer have yielded conflicting results. Leptin, a neuroendocrine hormone essential for the regulation of energy balance (9) and lipid metabolism (10), has been associated with prostate cancer in some (11;12) but not all studies (13–15). Allelic variants in the leptin gene have been associated with prostate cancer, however, these findings were based on a limited number of prostate cancer cases, i.e. 150 cases (16) and 69 cases (12). Adiponectin, a cytokine involved in glucose regulation and fatty acid catabolism, has been associated with reduced risk of prostate cancer in two small studies (17;18) but not in a subsequent larger study (15), and no studies have investigated variants of the adiponectin gene in relation to prostate cancer risk. Tumor necrosis factor-alpha, a key player in the development of inflammation and apoptotic cell death, has not been investigated in relation to prostate cancer although two *TNF* gene variant studies yielded no association with prostate cancer (19;20). Interleukin-6, another inflammation-related cytokine, has not been associated with prostate cancer (15) nor have genetic variants in the *IL6* gene (21).

While evidence for a relationship between adipokine gene variants and prostate cancer is limited, published studies have included relatively few prostate cases, and thus had limited statistical power to detect modest associations. Furthermore, existing studies have examined only a small number of SNPs in these genes. Finally, existing studies have not examined whether allelic variants in these genes may alter levels of adiposity-related biomarkers, such as insulin and IGF-1, and whether such alterations could potentially mediate any observed associations between adipokine gene variants and prostate cancer risk. In an effort to address these limitations, we examined genetic polymorphisms of potential biologic relevance in the interleukin 6 (*IL6*), leptin (*LEP*), leptin receptor (*LEPR*), tumor necrosis factor-alpha (*TNF*), and adiponectin (*ADIPOQ*) genes in relation to prostate cancer in a large case-control study, nested within a cohort of Finnish men.

Materials and methods

Study Population

The study sample consisted of 1,053 paired cases and controls from the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study. The rationale, design, and objectives of the ATBC trial have been previously described, as well as the main trial findings (22). Briefly, the ATBC trial is a randomized, placebo-controlled prevention trial that was designed to test whether alpha-tocopherol (AT; 50 mg/day), beta-carotene (BC; 20 mg/day), or the combination thereof reduces the incidence of lung cancers. Participants were 29,133

men aged 50–69 years who were living in southwestern Finland and smoked at least 5 cigarettes per day. All study members were eligible and willing to participate, and provided written informed consent prior to the randomization. The study was approved by the institutional review boards of the National Cancer Institute (U.S.A.) and the National Public Health Institute of Finland.

Cases were men diagnosed with incident prostate cancer (International Classification of Diseases 9, code 185) prior to or on April 30, 2003. The cancer cases were identified through the Finnish Cancer Registry, which provides approximately 100% case coverage (23). For cases identified through August 2001, medical records were reviewed by one or two oncologists for stage at a central site. Cases through April 1999 with histology or cytology available were also reviewed and confirmed by pathologists to determine Gleason score. Advanced prostate cancers were defined to include prostate cancers of TNM stage 3 or higher or a Gleason score of 8 or higher. Controls were study participants alive at the time of case diagnosis and were matched to cases on age ($\pm$ 5 years), intervention group, and date of baseline serum blood draw ($\pm$ 30 days).

During up to 18 years of follow-up, 1,347 cases were identified, and whole blood was available for 1,053 cases. We selected 1,053 matched controls with whole blood available. Subsequent to participant selection, we conducted SNPs assays among the samples with DNA available at the time that the assay was initiated. For example, the SNP assays for the *LEP*, *ADIPOQ*, and *TNF* genes were conducted among the 970 cases and 886 controls who had DNA available at that time. After removing participants for whom no DNA was available for any of the assays and/or for whom no assays were successfully completed, our analysis consisted of 1,041 cases and 1,048 controls.

SNP Selection and Genotyping

The single nucleotide polymorphisms (SNPs) were selected through the public databases dbSNP¹, and SNP-500² and a literature review on the *LEP*, *LEPR*, *ADIPOQ*, *TNF*, and *IL6* genes. SNPs were selected for genotyping if they had a minor allele frequency greater than 5% in Caucasian individuals and potential functionality, e.g. SNPs in exons, exon/intron boundaries, putative regulatory regions, or association with adiposity, insulin resistance or related outcomes in previous studies. We considered regulatory regions to be 5' or 3' non-coding regions in, or adjacent to, putative transcription factor binding sites, or in regions previously shown to alter gene expression. The polymorphic loci identified were verified in 102 individuals (SNP-500 population) of self-described Caucasian (n=31), African-American (n=24), Hispanic (n=23) and Pacific Rim (n=24) ethnicity by re-sequencing approximately 300bp of DNA on either side of the putatively polymorphic locus. Genotyping was performed at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics, National Cancer Institute using TaqMan (Applied Biosystems, Foster City, CA). Protocols for each specific assay are available at the SNP 500 web site³. The assays were validated with 100% concordance among the 102 SNP-500 individuals who had had their DNA sequenced.

Quality control was assessed using duplicate masked specimens for 106 control samples. For the majority of SNPs, we observed 100 percent concordance among duplicates. For four SNPs (*LEP* -10928A>C, *ADIPOQ* Ex2+53T>G, *TNF* IVS1+54G>A, and *TNF* 1180bp 3' of STP C>G), we observed 99 percent concordance. For two SNPs (*LEP* -14858A>G and *LEP* -13288A>G), 98 percent concordance was observed. Laboratory personnel were blinded to

¹<http://www.ncbi.nlm.nih.gov/SNP/>

²<http://snp500cancer.nci.nih.gov/>

³<http://snp500cancer.nci.nih.gov>

case-control status. We tested for departures from Hardy-Weinberg equilibrium for each SNP among the control participants.

Measures of selected phenotype characteristics

At the pre-randomization baseline visit, the height and weight of participants were measured using standard methods by trained study staff. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Serum samples were collected at the baseline visit after an overnight fast, and stored at -70°C . As part of the parent ATBC study, baseline serum total and HDL cholesterol were assessed for all participants using the CHOD-PAP method (Boehringer-Mannheim) and after precipitation of VLDL and LDL cholesterol with dextran sulfate and magnesium chloride. Serum insulin, glucose, IGF-1, and IGFBP-3 were measured in a subset of 196 controls, using methods previously described for this cohort (24). We calculated the insulin:glucose (I:G) ratio as a surrogate index of insulin resistance and also the IGF-1 to IGFBP-3 molar ratio ($1\text{ ng/ml IGF-1}=0.130\text{ nM}$ and $1\text{ ng/ml IGFBP-3}=0.036\text{ nM IGFBP-3}$) as a measure of bioavailable IGF-1.

Statistical analysis

We used logistic regression to assess the association of genotypes with prostate cancer with the most common genotype serving as the reference category. To assess evidence for a linear trend, we assigned ordinal values of 1, 2, and 3 to genotypes in order of homozygous for the more frequent allele, heterozygous, and homozygous for the less frequent allele. We also examined the odds ratio (OR) of advanced prostate cancer. To assess interactions between variant genotypes and participant characteristics including BMI, baseline serum cholesterol, baseline HDL, and physical activity, we modeled the cross-product terms of genotypes and the characteristic of interest on a continuous scale. Statistical significance was tested by comparing models with and without the cross product terms using the likelihood ratio test. All analyses were adjusted for age at randomization and treatment assignment. Additional adjustment for dietary intake of alpha-tocopherol and beta-carotene did not affect the results. We also analyzed the data using conditional logistic regression models but we did not find any substantial differences in parameter estimates. As the choice of conditional or unconditional models did not materially affect our results or conclusions, we report findings from the unconditional models.

Linkage disequilibrium (LD) between the SNPs was assessed using the r -squared and D' statistics and was visualized using Haploview. Haplotypes were reconstructed within blocks of high LD (25), and haplotype distribution and frequencies were assessed using PHASE software. Phase was estimated with a probability of 99 percent or higher for 99.8 percent of SNPs. We analyzed the relative odds of prostate cancer according to diplotypes (the combination of the two haplotypes for each individual) excluding those diplotypes with a frequency of less than 1%.

The relationship of genotype with serum total cholesterol, HDL, insulin, glucose, insulin:glucose ratio, IGF-1, IGFBP-3, and IGF-1:IGFBP-3 molar ratio were tested among the control participants using ANOVA models adjusted for age at randomization, treatment assignment, and date of blood draw.

Results

The specific genes and SNPs included in our analysis are described in Table 1. The SNP completion percentage was equal to or greater than 97 percent for all SNPs analyzed, except for *LEPR* IVS2+6686G>A (92 percent). Among the control group, all genotypes were distributed in accordance with Hardy-Weinberg equilibrium except for *LEP* -10928A>C

($P=0.04$), and *LEPR* IVS2+6890A>G ($P=0.04$). Among those cases with known stage or Gleason score ($N=843$; 81% of total cases), 575 (68%) cases were localized and 268 (32%) were advanced (Table 2). Cases were slightly older and more likely to have a family history of prostate cancer than controls but were similar with respect to BMI, physical activity, and smoking history (Table 2).

We found that three out of six SNPs in the *LEP* gene were associated (at the $P=0.05$ level) with the risk of prostate cancer (Table 3). For each of the three associated SNPs ($-14858A>G$, $-13973A>C$, $-13736C>A$), carriers of the less frequent allele had an approximately 20% reduced risk of prostate cancer. For example, men with the GA or AA genotype at the $-14858A>G$ locus had relative odds of prostate cancer of 0.76 (95% confidence interval= 0.62, 0.93) and 0.79 (95% confidence interval= 0.60, 1.04), respectively ($P_{\text{trend}} = 0.03$). There was substantial linkage-disequilibrium at each locus among controls (Figure 1), largely explaining the similar results between the three SNPs. Of the remaining SNPs in the *LEP* gene, we also found borderline statistically significant associations at the $-13288A>G$ and $\text{Ex1-11A}>G$ loci ($P_{\text{trend}}=0.05$ and 0.11, respectively). Homozygosity for the A allele at the $13288A>G$ locus was associated with a borderline statistically significant increased risk of prostate cancer ($\text{OR}=1.29$; 95% confidence interval= 0.99, 1.67).

We did not find any evidence of an association between SNPs in the *IL6*, *LEPR*, *TNF*, and *ADIPOQ* genes and prostate cancer, regardless of whether assuming a dominant model (i.e. carrier of either one or two variant alleles versus no variant alleles) or a linear model (i.e. assuming that two copies of the variant allele confer greater risk than one copy) (Table 3).

Exclusion of participants with a history of diabetes ($N = 59$) or adjusting for history of diabetes did not materially affect results. Adjustment for body mass index also had little impact on estimated associations. The associations between SNPs and prostate cancer were not modified by BMI, baseline serum cholesterol, baseline HDL, or physical activity (all P for interaction >0.05 ; data not shown).

In order to further investigate our findings in the *LEP* gene, we reconstructed the individual haplotypes and diplotypes based on the six SNPs ($-14858A>G$, $-13973A>C$, $-13736C>A$, $-13288A>G$, $-10928A>C$, $\text{Ex1-11A}>G$) in our study. We found no evidence of phase ambiguity in reconstruction of haplotypes. In analyses of diplotypes, carriers for the three variants at the $-14858A>G$, $-13973A>C$, $-13736C>A$ loci had a reduced risk of prostate cancer ($\text{OR}=0.78$; 95% confidence interval= 0.62, 0.98; $P\text{-value}=0.03$) relative to individuals carrying the common allele at each locus.

In separate analyses using advanced prostate cancer as an outcome, polymorphisms at the *LEPR* IVS1+6808A>G locus were associated with a modest increase in advanced prostate cancer risk. Heterozygosity and homozygosity for the variant allele conferred a relative odds of advanced prostate cancer of 1.50 (95% CI= 0.99, 2.28) and 3.67 (95% CI= 0.23, 59.19), respectively ($P_{\text{trend}}=0.04$). None of the other SNPs were associated with advanced prostate cancer (data not shown). We also examined, in case only analyses, whether genotypes were related to extraprostatic extension (supplementary table 1) or to high grade cancers (supplementary table 2) and found no significant associations.

There was no evidence that the genetic variants in our study were related to BMI, serum levels of insulin, glucose, insulin: glucose ratio, IGF-1, IGFBP-3 or the IGF-1:IGFBP-3 molar ratio (data not shown). However, for all of the above except for BMI, our sample size was modest (196 controls) and thus we cannot exclude the possibility of a moderate or weak association. Polymorphisms at the *TNF* $-487A>G$ locus were associated with increased serum cholesterol ($P=0.03$). The mean mmol/l values for the three *TNF* $-487A>G$

genotypes were 6.3 for GG, 6.3 for GA, and 7.2 for AA. Variants at the *LEP* -13288A>G loci were modestly associated with HDL levels ($P=0.03$), with mean mmol/l values of 1.15 for GG, 1.19 for GA, and 1.23 for AA genotypes.

Discussion

In this nested case-control study of more than 1,000 prostate cancer cases and matched controls, we found that prostate cancer was associated with three out of the six polymorphisms that we investigated in the leptin gene (*LEP*). We observed that allelic variants at any of the -14858A>G, -13973A>C, -13736C>A loci were associated with an approximate 20% reduction in prostate cancer risk. In addition, at the -13288A>G locus, the AA genotype was associated with a suggestive increased risk of prostate cancer. These data may point towards the *LEP* gene as a susceptibility locus for prostate cancer and a role for leptin in prostate tumorigenesis. Polymorphic loci of the *IL6*, *TNF*, *LEPR* and *ADIPOQ* genes were not associated with prostate cancer risk, consistent with previous null studies (19–21;26).

Leptin is a paracrine and autocrine hormone that suppresses appetite and plays a critical role in maintenance of energy balance (27–29). Leptin is also proinflammatory cytokine (30), an angiogenic factor (4;31), and a stimulant of pubertal prostate growth in humans and animals (32–34) and therefore has been implicated as a potential risk factor for prostate tumorigenesis. Additionally, leptin promotes production of IL-6 and IGF-1, factors that have been implicated in tumor growth (35). Leptin has also been hypothesized to influence prostate cancer risk, and particularly prostate cancer progression, by interacting with cytokines, growth factors, sex steroids, and environmental factors to disrupt important homeostatic mechanisms (36). *In vitro* data demonstrates that leptin causes proliferation selectively in androgen-independent prostate cancer cells (37) and that serum leptin levels are associated with advanced progression among men with prostate cancer (37). For this reason, leptin is believed to relate more closely to risk of androgen-independent and/or advanced prostate cancers as opposed to total prostate cancer (36). Elevated leptin levels may also increase prostate cancer risk by triggering the early onset of puberty (34), which has been previously associated with increased prostate cancer risk (38;39).

The *LEP* gene is the human homologue of the *ob* (obesity) gene in mice that is responsible for hereditary murine obesity (40). The *LEP* SNPs that we examined are located within a putative regulatory region of the gene (i.e. within 10 kilobases upstream of the promoter), where particular variants have been shown to correlate with serum leptin levels (41–44). In particular, the A-allele at the -13288 locus has been associated with increased adipocyte leptin secretion, higher mRNA expression levels in adipocytes, and elevated serum leptin concentrations (41;42;45). In humans, all five of the loci that we examined in the *LEP* gene have been related to overweight and obesity (46–48); excess weight, in turn, is positively correlated with serum leptin levels. Some of the *LEP* gene variants also yield potentially important changes in gene structure. For example, at -13973A>C, the variant A allele results in a novel binding site for growth factor repressor protein and elimination of the original potential binding sites for X-box binding factors and EVI1-myeloid transforming protein (49). This suggests that *LEP* transcription may be altered in carriers of these variants. In addition, the -14858A>G SNP lies in a Krueppel-like C2H2 Zinc-finger factor binding site that is hypermethylated in cancer, albeit the binding site is present for both alleles (49).

Supporting a possible role of leptin gene variants in prostate cancer, previous research into the *LEP* gene promoter by Ribeiro et al. found that carriers of the A allele at the -13288A>G locus had a greater than 2-fold risk of prostate cancer compared to

homozygotes for the G allele (16). In our own data, we also found greater risk of prostate cancer among men who were homozygous for this variant allele, though unlike the findings of Ribeiro et al., we did not observe any stronger associations for advanced stage disease.

More recently, the Cancer Genetics Markers of Susceptibility (CGEMS) project (2;3) published findings further implicating the *LEP* regulatory region in prostate carcinogenesis⁴. In that study, 527,869 SNPs were evaluated in relation to prostate cancer in a case-control study nested within a large cohort (the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial or PLCO) and the most promising 27,326 SNPs were subsequently analyzed in a replication study. In the first stage, the investigators found that markers within *LEP* that were proximal to those investigated herein were suggestively associated with prostate cancer (Figure 2). For example, of the five *LEP* gene markers most proximal to those in our study, the CGEMS statistical tests for association yielded p-values of 0.03, 0.09, 0.22, 0.13, and 0.06.

The CGEMS replication study further investigated several of these SNPs in relation to prostate cancer. This replication effort consisted of more than 10,000 pooled prostate cancer cases from five independent cohorts, including the cases from the current study, which comprise 10 percent of the pooled analysis cases. In the replication study, variants at the rs11763517 locus, a SNP which is in tight linkage disequilibrium with the -13736C>A locus ($r=0.78$ in Caucasians (50)), were related to reduced prostate cancer risk ($P_{\text{trend}}=0.001$; see Figure 2). Heterozygotes and homozygotes for the C allele at this locus had odds ratios of prostate cancer of 0.90 (95% CI= 0.82, 0.99) and 0.83 (95% CI = 0.75, 0.93), respectively, which approximates to our findings for heterozygotes (OR=0.81) and homozygotes (OR=0.78) for the A allele at the linked -13736C>A locus. Thus our findings, taken together with those of CGEMS and those of Ribeiro et al., support the *LEP* gene as a potential susceptibility locus for prostate cancer.

While data from our study, from CGEMS, and from Ribeiro et al. indicate that variants in the *LEP* gene are related to prostate cancer, these results seemingly contrast with epidemiologic studies suggesting no relationship between serum leptin concentrations and prostate cancer (13–15). However, serum-based studies reflect leptin levels at study enrollment, when participants are usually at a relatively advanced age, and these may be only moderately correlated with enhanced leptin levels earlier in life (51;52), such as during adolescence. In addition, the serum leptin and prostate cancer relation may be confounded by complex interrelationships of leptin with androgens, insulin and IGF-1 (53). In this regard, studying *LEP* gene polymorphisms that relate to leptin expression or activity may provide an unbiased estimate of the role of leptin in prostate carcinogenesis, since unlike circulating leptin concentrations, associations with gene variants may be less confounded by lifestyle and/or other physiologic factors (54).

We did not find that polymorphisms in the *LEP* gene or other adipokine-related genes were associated with body mass index. In at least two previous studies, homozygosity for the variant allele at the -13288A>G locus of the *LEP* gene was related to excess weight (46;47). The lack of association in our study between SNPs at the -13288A>G locus and adiposity may reflect characteristics specific to our cohort, namely that all participants were smokers at baseline. Smoking suppresses weight gain and may thereby obscure the relationship between these SNPs and BMI.

Strengths of our study include a large, well-defined sample of prostate cancer cases and controls, and the use of dense genotyping within a biologically significant region of the *LEP*

⁴<http://cgems.cancer.gov/data/>

gene. In addition, our data on serum insulin, glucose, IGF-1, and IGFBP-3 permitted us to examine if the genotypes under study were related to changes in biologic factors that have been implicated in prostate cancer etiology. However, our study is limited by its use of a population consisting entirely of middle-aged and older-aged smokers. In addition, despite the large sample size, the number of advanced cases was modest. Thus, we had limited statistical power to examine the genetic variants in relation to advanced prostate cancer. We did not comprehensively examine all allelic variants in the *LEPR*, *IL6*, *TNF*, and *ADIPOQ* genes; therefore we cannot rule out the possibility of an association between variants in these genes and prostate cancer risk. Because the indolent period of prostate cancer can span many years, some of the control participants may have had undiagnosed prostate cancer. However, unless the likelihood of indolent disease was related to genotype, this misclassification would have been nondifferential, resulting in odds ratio estimates that are artificially close to the null. This would not explain our positive findings for the *LEP* gene. Furthermore, we also note that the length of follow-up in the study was up to 18 years in length (1985 to 2003) and most of the cases were diagnosed more than five years before the end of follow-up. The controls matched to these cases were followed for greater than five years prior to the end of follow-up and thus indolent disease would, in many instances, have been discovered by the end of follow-up.

In conclusion, this large, nested case-control study found that allelic variants in the *LEP* gene were related to risk of prostate cancer, suggesting that leptin should be further evaluated for its potential role in prostate carcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Linkage disequilibrium of LEP gene polymorphisms among control participants
 Figure 1 depicts the linkage disequilibrium between SNPs in the *LEP* gene in our populations of controls. Figure 1A shows linkage disequilibrium as measured by D' , a measure of agreement between two SNPs given the marginal frequencies of each SNP (values range from 0 to 1). Figure 1B shows linkage disequilibrium as measured by r^2 , a measure of the correlation between SNPs. In each square, the value of D' or r^2 is indicated by a number (e.g. 98 is equal to a D' of 0.98), unless agreement is perfect, in which case no number is listed. At the top of each figure is a bar indicating the relative positions (in base pairs) of each of the six SNPs that we assessed (−14858A>G; −13973A>C; −13736C>A; −13288A>G; −10928A>C; Ex1−11A>G).

Figure 2. Comparison of *LEP* gene results from the current study (ATBC) with publicly available findings from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) and the Cancer Genetics Markers of Susceptibility (CGEMS) replication study

Figure 2 depicts SNPs examined in the ATBC and CGEMS studies within the leptin gene region of 127,466,943 to 127,484,013 bp. The PLCO, as part of the ongoing CGEMS efforts, conducted a genome wide association study where 527,869 SNPs were examined in relation to prostate cancer and published their findings online. The most promising 27,326 SNPs were further examined in a large replication dataset (CGEMS replication study). This figure demonstrates the relative positions of SNPs of interest, the study in which they were examined, and the p-value found in that study. For SNPs in the current study (ATBC), the p-value indicated is for the robust (i.e. linear trend) model (1 degree of freedom) adjusted for age at randomization and treatment group assignment. For SNPs in the PLCO study, the p-value indicated is for a score test with four degrees of freedom adjusted for age at randomization, center of recruitment, an indicator for cases diagnosed within one year of entry to the trial, and three principal components estimated as adjustments for population stratification. In the PLCO, the four degrees of freedom results from analysis of each genotype and of non-aggressive as well as aggressive prostate tumors. For SNPs in the CGEMS replication study, the p-value is for the robust model, adjusted for the same covariates as in the PLCO. Variant alleles (C allele) at rs11763517 are highly correlated with the presence of A alleles at rs12535747 in Caucasians.

Table 1

List of genes and single nucleotide polymorphisms (SNP) evaluated

Gene	Name	Location	SNP/ amino acid change	RS #
<i>IL6</i>	Interleukin 6	7p21	-236C>G	rs1800795
			IVS2+180A>G	rs2069832
			Ex5+132	rs2069849
<i>LEP</i>	Leptin	7q31.3	-14858A>G	rs1349419
			-13973A>G	rs12535708
			-13736C>A	rs12535747
			-13288A>G	rs7799039
			-10928A>C	rs791620
			Ex1-11A>G	rs2167270
<i>LEPR</i>	Leptin receptor	1p31	IVS2+6686G>A	rs1887285
			IVS1+6808A>G	rs7883
			IVS2+6890A>G	rs7602
			Ex4-45A>G	rs1137100
<i>TNF</i>	Tumor necrosis factor-alpha	6p21.3	-487A>G	rs1800629
			IVS1+54G>A	rs3093661
			1180bp 3' of STP C>G	rs3093668
<i>ADIPOQ</i>	Adiponectin	3q27	IVS1+244G>A	rs182052
			IVS1-4514C>T	rs822393
			Ex2+53T>G	rs2241766
			Ex3+117T>C	rs17366743

Table 2Characteristics of the study populationⁱ

Characteristic	Cases (n= 1041)	Controls (n=1048)	p ⁱⁱ
Age (y)	58.7	58.2	0.07
Family history of prostate cancer (%)	57 (6.3)	30 (3.3)	<.01 ⁱⁱⁱ
Body mass index (kg/m ²)	26.2	26.2	0.86
Vigorous activity during leisure time (%)	208 (20.0)	240 (23.0)	0.10 ⁱⁱ
History of diabetes (%)	33 (3.2)	26 (2.5)	0.34 ⁱⁱ
Smoking status			
Years of smoking	36.5	36.7	0.92
Cigarettes per day	19.3	19.1	0.81
Alpha-tocopherol grp (%)	474 (45.5)	477 (45.5)	0.99 ⁱⁱ
Beta-carotene grp (%)	524 (50.3)	530 (50.6)	0.91 ⁱⁱ
Prostate cancer pathology ^{iv}			
Localized (%)	575 (68.2)		
Advanced (%)	268 (31.8)		
Grade			
Low	462 (72.6)		
High	175 (27.4)		

ⁱ For 12 cases and 5 controls, no DNA was available and/or no assays were successfully completed, thus these participants were excluded from analysis.

ⁱⁱ P values derived from the Wilcoxon signed rank sum test unless otherwise indicated.

ⁱⁱⁱ P derived from the X² test.

^{iv} Due to missing data, numbers may not add to the total of 1,041 cases. Among the 268 advanced cases, tumor size (T) = 3 for 142 cases and T=4 for 36 cases, 18 cases had some degree of confirmed nodal involvement (N>0), and 118 cases were metastatic (M=1)

Table 3

Distribution of adipokine genotypes and the odds ratio of total prostate cancer

Genotype	Cases <i>n</i> (%)	Controls <i>n</i> (%)	OR ⁱ , 95% CI
Leptin gene (<i>LEP</i>)			
-14858A>G; rs1349419			
GG	365 (38.6)	280 (32.7)	1.0 (ref)
GA	419 (44.3)	422 (49.2)	0.76 (0.62, 0.93)
AA	161 (17.0)	155 (18.1)	0.79 (0.60, 1.04)
P for A carrier			0.01
P _{trend} =			0.03
-13973A>C; rs12535708			
CC	443 (46.9)	356 (42.0)	1.0 (ref)
CA	390 (41.3)	381 (44.9)	0.82 (0.67, 0.99)
AA	111 (11.8)	111 (13.1)	0.80 (0.59, 1.08)
P for A carrier			0.03
P _{trend} =			0.04
-13736C>A; rs12535747			
CC	447 (47.3)	362 (42.0)	1.0 (ref)
CA	387 (40.9)	385 (44.7)	0.81 (0.66, 0.98)
AA	112 (11.8)	115 (13.3)	0.78 (0.58, 1.05)
P for A carrier			0.02
P _{trend} =			0.03
-13288A>G; rs7799039			
GG	213 (22.5)	210 (24.3)	1.0 (ref)
GA	453 (47.8)	437 (50.6)	1.02 (0.81, 1.28)
AA	281 (29.7)	216 (25.0)	1.29 (0.99, 1.67)
P for G carrier			0.36
P _{trend} =			0.05
-10928A>C; rs791620			
CC	829 (88.0)	738 (86.9)	1.0 (ref)
CA	113 (12.0)	111 (13.1)	0.91 (0.69, 1.21)
AA	0 (0.0)	0 (0.0)	-
P for A carrier			0.52
P _{trend} =			0.52
Ex1-11A>G; rs2167270			
GG	428 (45.3)	346 (41.2)	1.0 (ref)
GA	404 (42.8)	387 (46.1)	0.84 (0.69, 1.02)
AA	113 (12.0)	107 (12.7)	0.85 (0.63, 1.14)
P for A carrier			0.07
P _{trend} =			0.11
Leptin receptor gene (<i>LEPR</i>)			

Genotype	Cases <i>n</i> (%)	Controls <i>n</i> (%)	OR ⁱ , 95% CI
IVS2+6686G>A; rs1887285			
AA	780 (88.2)	704 (85.5)	1.0 (ref)
AG	95 (10.7)	115 (14.0)	0.74 (0.56, 1.00)
GG	9 (1.0)	4 (0.5)	2.16 (0.66, 7.05)
P for G carrier			0.10
P _{trend} =			0.24
IVS1+6808A>G; rs7883			
GG	835 (87.0)	754 (89.5)	1.0 (ref)
GA	120 (12.5)	87 (10.3)	1.26 (0.94, 1.68)
AA	5 (0.5)	1 (0.1)	4.79 (0.56, 41.16)
P for A carrier			0.08
P _{trend} =			0.05
IVS2+6890A>G; rs7602			
GG	768 (79.2)	650 (77.7)	1.0 (ref)
GA	189 (19.5)	182 (21.7)	0.88 (0.70, 1.11)
AA	13 (1.3)	5 (0.6)	2.17 (0.77, 6.14)
P for A carrier			0.45
P _{trend} =			0.72
Ex4-45A>G; rs1137100			
AA	415 (40.8)	369 (40.2)	1.0 (ref)
AG	462 (45.4)	428 (46.7)	0.96 (0.79, 1.17)
GG	140 (13.8)	120 (13.1)	1.03 (0.78, 1.37)
P for G carrier			0.80
P _{trend} =			0.99
Adiponectin gene (<i>ADIPOQ</i>)			
IVS1+244G>A; rs182052			
AA	266 (28.2)	252 (29.5)	1.0 (ref)
AG	472 (50.1)	400 (46.8)	1.12 (0.90, 1.39)
GG	205 (21.7)	202 (23.7)	0.96 (0.74, 1.24)
P for G carrier			0.55
P _{trend} =			0.82
IVS1-4514C>T; rs822393			
CC	346 (36.8)	307 (36.2)	1.0 (ref)
CT	443 (47.2)	383 (45.1)	1.03 (0.84, 1.27)
TT	150 (16.0)	159 (18.7)	0.84 (0.64, 1.10)
P for T carrier			0.80
P _{trend} =			0.32
Ex2+53T>G; rs2241766			
TT	842 (88.9)	757 (88.1)	1.0 (ref)
TG	105 (11.1)	102 (11.9)	0.92 (0.69, 1.22)

Genotype	Cases <i>n</i> (%)	Controls <i>n</i> (%)	OR ⁱ , 95% CI
GG	0 (0.0)	0 (0.0)	-
P for G carrier			0.55
P _{trend} =			0.55
Ex3+117T>C; rs17366743			
TT	891 (93.7)	806 (94.2)	1.0 (ref)
TC	60 (6.3)	49 (5.7)	1.11 (0.75, 1.63)
CC	0 (0.0)	1 (0.1)	-
P for C carrier			0.68
P _{trend} =			0.76
Tumor necrosis factor gene (TNF)			
-487A>G; rs1800629			
GG	700 (73.8)	641 (74.8)	1.0 (ref)
GA	228 (24.0)	205 (23.9)	1.03 (0.83, 1.28)
AA	21 (2.2)	11 (1.3)	1.72 (0.82, 3.60)
P for A carrier			0.56
P _{trend} =			0.36
IVS1+54G>A; rs3093661			
GG	920 (96.7)	829 (96.6)	1.0 (ref)
GA	31 (3.3)	29 (3.4)	0.96 (0.57, 1.61)
AA	0 (0.0)	0 (0.0)	-
P for A carrier			0.89
P _{trend} =			0.89
1180bp 3' of STP C>G; rs3093668			
GG	914 (96.7)	825 (96.5)	1.0 (ref)
GC	31 (3.3)	30 (3.5)	0.93 (0.56, 1.56)
CC	0 (0.0)	0 (0.0)	-
P for C carrier			0.79
P _{trend} =			0.79
Interleukin-6 gene (IL6)			
-236C>G; rs1800795			
CC	281 (29.4)	250 (29.5)	1.0 (ref)
CG	485 (50.7)	401 (47.3)	1.08 (0.87, 1.34)
GG	191 (20.0)	196 (23.1)	0.87 (0.67, 1.13)
P for G carrier			0.92
P _{trend} =			0.38
IVS2+180A>G; rs2069832			
AA	277 (28.8)	254 (30.2)	1.0 (ref)
AG	492 (51.1)	395 (46.9)	1.15 (0.92, 1.42)
GG	193 (20.1)	193 (22.9)	0.92 (0.71, 1.20)
P for G carrier			0.51

Genotype	Cases <i>n</i> (%)	Controls <i>n</i> (%)	OR ^{<i>i</i>} , 95% CI
P _{trend} =			0.68
Ex5+132; rs2069849			
CC	919 (96.6)	832 (97.2)	1.0 (ref)
CT	31 (3.3)	24 (2.8)	1.17 (0.68, 2.01)
TT	1 (0.1)	0 (0)	-
P for T carrier			0.50
P _{trend} =			0.43

^{*i*} Adjusted for age at randomization and treatment group