Polymorphisms of ADIPOQ and ADIPOR1 and prostate cancer risk

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ARTICLE INFO

ABSTRACT

Studies have linked prostate cancer risk with insulin resistance and obesity. Circulating levels of adiponectin, a protein involved in insulin resistance and obesity, have been associated with prostate cancer risk. We studied the association of prostate cancer risk with haplotype tagging single nucleotide polymorphisms (SNPs) of the adiponectin (*ADIPOQ*) and adiponectin receptor 1 (*ADIPOR1*) chosen based on their functional relevance or association with other types of cancer. DNA samples from 465 cases and 441 healthy volunteers from New York City were genotyped for *ADIPOQ* rs266729, rs822395, rs822396, rs1501299, and rs2241766 SNPs and *ADIPOR1* rs12733285, rs1342387, rs7539542, rs2232853, and rs10920531 SNPs. We performed both single- and multiple-SNP analyses. We found that rs12733285, rs7539452, rs266729, rs822395, rs822396, and rs1501299 were significantly associated with prostate cancer risk. Haplotype analysis confirmed these results and identified 5 *ADIPOQ* 4-SNP haplotypes and 1 *ADIPOR1* 2-SNP haplotype tightly associated with prostate cancer risk. Importantly, 2 *ADIPOQ* SNPs, rs266729 and rs1501299, have been previously associated with colon and breast cancer risk, respectively, in the same direction as in this study. These findings suggest that variants of the adiponectin pathway may be associated with susceptibility to various forms of common cancers and warrant validation studies.

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1. Introduction

Numerous studies have evaluated the role of obesity in prostate cancer [1-4]. Multiple cohort studies have shown that obesity is associated with increased risk of death from prostate cancer [1,3,5,6], although this association has not been replicated by all studies [2]. The largest prospective trial included a total of 950 000 men and found a 9% excess of prostate cancer in obese individuals [2]. A recent study including 441 men showed that, after adjusting for prostate-specific antigen levels and larger prostate size, obesity was significantly associated with a 98% increase in prostate cancer risk [3].

Studies are also linking consequences of obesity, that is, insulin resistance and the metabolic syndrome, with prostate cancer. Studies of polymorphisms of the genes encoding for insulin and the insulin receptor have shown that IRS G972 GR/RR genotypes were associated with a 2.8-fold risk of prostate cancer (95% confidence interval [CI], 1.5-5.1; P = .0007) [7]. Emerging evidence indicates that one of the cytokines secreted by the adipose tissue, that is, adiponectin, is associated with prostate cancer risk [8-10]. Adiponectin has been found to be an endogenous insulin sensitizer, the circulating levels of which are decreased in obese and diabetic subjects. Moreover, adiponectin has the potential of regulating the secretion of estrogens, tumor necrosis factor–α [11,12], and insulin-like growth factor [13].

Recently, circulating levels of adiponectin have been found to correlate with prostate cancer risk [8-10]. Because its levels are inversely associated with adiposity, it has been suggested that decreased levels of adiponectin may explain the increased risk of prostate cancer in obesity [14].

Several adiponectin polymorphisms have been shown to affect adiponectin levels; and polymorphisms of both the ligand (ADIPOQ) and its type 1 receptor (ADIPOR1) have been associated with risk for insulin resistance, cardiovascular disease, and diabetes mellitus [15-20]. We showed that some of these polymorphisms are associated with breast and colon cancer risk [21,22]. In this study of prostate cancer cases and controls, we genotyped selected haplotype tagging single nucleotide polymorphisms (SNPs) in the genes encoding adiponectin (ADIPOQ) and its type I receptor (ADIPOR1) and determined their association with prostate cancer risk.

2. Materials and methods

2.1. Study participants

Recruitment of prostate cancer cases and healthy controls has been described before [23]. Briefly, DNA was extracted from peripheral blood lymphocytes from 465 consecutive individuals diagnosed with adenocarcinoma of the prostate who received care at the outpatient urology clinic at Memorial Sloan-Kettering Cancer Center from April 2000 to September 2002. The blood samples were collected following completion of diagnostic studies. Patients were unselected for age or family history. Clinical and pathological records were reviewed to confirm the diagnosis of prostate cancer in all

subjects. The study was approved by the Memorial Sloan-Kettering Cancer Center Institutional Review Board (IRB). A population of 441 healthy white male controls aged 20 to 87 years with well-defined ethnic background who had donated blood for various reasons (predominantly prenatal screening for noncancer disease) constituted the control group.

Male controls were matched to cases based on ethnic status and were from the same geographic locations as the cases. None of the controls had any personal history of cancer at the time of blood donation. This was ascertained by a questionnaire completed by each control. Exact age information was not available for 235 controls because it was not collected prospectively, but the age range (20-40) was known. All personal identifiers were permanently removed from both cases and controls in compliance with IRB regulation.

2.1.1. DNA isolation
DNA from whole blood lymphocytes was extracted using the QIAamp DNA Blood Mini Kit and was stored at –20°C until use for genotyping (Qiagen, CA, USA). All DNA samples underwent whole genome amplification using the Illustra Genomiphi V2 DNA Amplification kit (GE Healthcare, catalog no. 25660032, NJ, USA). The samples were stored at –20°C.

2.1.2. Selection of SNPs
We genotyped the same 10 SNPs within ADIPOQ and ADIPOR1 used to detect the association of these 2 genes with breast as well as colorectal cancer risk [22,24]. Here, we describe our preferences for such selection, First and most importantly, we preferentially chose functionally relevant SNPs; and it has been shown that these 10 SNPs either affect adiponectin levels or are associated with risk for insulin resistance, cardiovascular disease, and diabetes [15-22]. Second, our previous studies also suggest that several of these SNPs are associated with breast and colorectal cancer risk [23,24]. Third, we only selected SNPs with a minimum allele frequency of 10% in white people. Fourth, we selected SNPs capturing variations in the major blocks of each gene identified previously [17,20]. For the ADIPOQ gene, Heid et al [25] genotyped 53 SNPs in 81 unrelated healthy individuals and used 32 of them to identity 2 major haplotype blocks and 18 tag SNPs. We chose to genotype rs266729 (5′ flanking region), rs822395 (intron 1), and rs822396 (intron 1) to tag block 1 and rs1501299 (intron 2) and rs2241766 (exon 2) to tag block 2 because these are the 5 most common SNPs. Furthermore, they have been studied extensively with respect to their functionality and relation to diseases such as diabetes mellitus [15-18]. For the ADIPOR1 gene, Soccio et al [25] identified 2 blocks and 6 tag SNPs from more than 28 SNPs using the Caucasians HapMap panel. One block extends from the 5′ flanking region to intron 4, and the other is located at the 3′ end of the gene. Based on this structure, we selected 5 common SNPs for genotyping. For block 1, we selected the following tagging SNPs: rs2232853 (5′ flanking region), rs12733285 (intron 1), and rs1342387 (intron 4). For block 2, we selected rs7539542 (exon 8) and rs10920531 (3′ flanking region).

2.1.3. Genotyping
Genotyping was performed by Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Forest City, CA). Results were ascertained with the SDS 2.3

Table 1 – Characteristics of cases and controls and genotyping results

	Case, n (%) n = 465	Controls, n (%) n = 441	P value
Age			
<50	22 (4.7)	341 (77.3)	
50-60	90 (19.3)	62 (14.1)	
60-70	209 (44.9)	30 (6.8)	
70-80	130 (27.9)	1 (0.2)	
≥80	14 (3.0)	0 (0)	
Ethnicity			
White	415 (89.2)	441 (100)	
Black	29 (6.2)		
Hispanic	8 (1.7)		
Asian	2 (0.4)		
Other/missing	12 (2.6)		
rs2232853			2.9×10^{-5}
GG	200 (43.0)	142 (32.2)	
AG	192 (41.3)	170 (38.5)	
AA	58 (12.5)	124 (28.1)	
rs12733285			.66
TT	48 (10.3)	71 (16.1)	
TC	221 (47.5)	222 (50.3)	
CC	183 (39.4)	145 (32.9)	
rs1342387			.65
TT	112 (24.1)	122 (27.7)	
CT	218 (46.9)	209 (47.4)	
CC	116 (24.9)	107 (24.3)	
rs7539542			.96
GG	43 (9.2)	45 (10.2)	
GC	226 (48.6)	193 (43.8)	
CC	183 (39.4)	194 (44.0)	
rs10920531			.99
CC	158 (34.0)	148 (33.6)	
AC	204 (43.9)	207 (47.0)	
AA	89 (19.1)	72 (16.3)	
rs266729			.05
GG	36 (7.7)	29 (6.6)	
CG	153 (32.9)	208 (47.1)	
CC	268 (57.6)	204 (46.3)	
rs822395			.97
CC	61 (13.1)	47 (10.7)	
AC	165 (35.5)	196 (44.4)	
AA	229 (49.2)	194 (43.9)	
rs822396			.68
GG	29 (6.2)	9 (2.0)	
AG	90 (19.4)	123 (27.9)	
AA	335 (72.0)	301 (68.3)	
rs2241766			.93
TT	296 (63.7)	280 (63.5)	
TG	136 (29.2)	142 (32.2)	
GG	15 (3.2)	16 (3.6)	
rs1501299			.88
TT	36 (7.7)	37 (8.4)	
TG	160 (34.4)	184 (41.7)	
GG	261 (56.1)	203 (46.0)	

The P value for each SNP is for testing the deviation from HWE among controls.

software (Applied Biosystems). All results were automatically called. A total of 5% of samples were genotyped in duplicate and showed 100% concordance. All but one SNP (rs2232853) were in Hardy-Weinberg equilibrium (HWE). Because rs2232853 significantly deviated from HWE, we did not include it in our analyses.

2.2. Statistical analysis

We used logistic models to analyze our data with 3 different methods. The first method was the standard single-SNP analysis that estimates and tests the main effect(s) of one SNP at a time. The second method simultaneously estimates and tests all main effects of the SNPs, referred to as *multiple-SNP nonepistatic analysis*. Our third method was the multiple-SNP epistatic analysis, simultaneously fitting all main effects and epistatic interactions. Multiple-SNP models can relieve multiple-testing problem encountered in single-SNP analysis, can accommodate linkage disequilibrium (LD) among the SNPs, and have the advantages of providing potentially increased power and reduced false positives to detect causal variants, of better separating highly correlated predictors, and of more efficiently detecting epistatic interactions [24,27-29].

We coded the main-effect predictors by using different genetic models (Cockerham, codominant, dominant, and recessive) and constructed all epistatic interactions by multiplying 2 corresponding main-effect variables. We denoted common homozygote (ie, the homozygote with higher frequency), heterozygote, and rare homozygote for each SNP by c, h, and r, respectively. Our first genetic model was the Cockerham model that defines 2 main effects for each SNP, an additive contrast as –1, 0, and 1 for c, h, and r, and a dominance contrast as –0.5 for c and r and 0.5 for h, respectively [28,29]. The additive effect represents the genotypic effect (r – c)/2, and the dominance effect measures h – (c + r)/2 in the logit probability of being cases. A positive additive effect (ie, odds ratio [OR] >1) indicates that the rare homozygote increases cancer risk compared with the common homozygote, and a positive dominance effect indicates that the heterozygote increases risk compared with the mean of 2 homozygotes.

The codominant model also introduced 2 main effects for each SNP, with the 2 main-effect predictors being 2 indicator variables with the common homozygote c chosen as the reference group. Under the codominant model, the 2 main effects, also denoted by additive and dominance, represent the genotypic effect differences between r and c (ie, r – c) and between h and c (ie, h – c) in the logit probability, respectively. A positive additive or dominance effect (ie, OR >1) indicates that the rare homozygote or heterozygote is associated with increased cancer risk compared with the common homozygote. The dominant (or recessive) model defined one main effect for each SNP, using one indicator variable with the rare (or common) homozygote as the reference group and the other 2 genotypes combined into a category. The main effect in the dominant or recessive model represents the genotypic effect difference c/h – r or r/h – c, respectively.

We performed single-SNP and multiple-SNP nonepistatic analyses using all the above 4 genetic models. Our multiple-SNP epistatic analyses used the Cockerham and the codominant models, both introducing 4 epistatic effects for each pair of SNPs. Our multiple-SNP analyses used Bayesian hierarchical logistic models as described previously [26,27].

We used 2 summary measures, the deviance and the Akaike information criterion (AIC), to compare different models. Lower deviance and AIC means better fit to data. To evaluate the risk prediction ability of the multiple-SNP logistic

models, we calculated the true-positive rate (or sensitivity) and the false-positive rate (or 1 – specification) that the model discriminates cases and controls, and plotted the receiver operating characteristic (ROC) curves.

We also performed haplotype analysis based on the results of single- and multiple-SNP analyses. Using the expectation-maximization algorithm implemented in Haplore [30], the haplotype frequencies were estimated separately for cases and controls on SNPs that were found to be significantly associated with prostate cancer risk from the single- and/or multiple-SNP analysis. The posterior probability of each haplotype for each sample was then calculated and used in the logistic regression to test if there was an association between a haplotype and the prostate cancer risk. For each haplotype in the analysis, the other haplotypes were grouped to be used as the reference group. Because the majority of cases and all controls are white, only white samples were used in the haplotype analysis. Haplotypes with a frequency less than 5% were not considered in the analysis. Because *ADIPOQ* and *ADIPOR1* are on chromosome 3 and 1, respectively, the haplotype analysis was conducted for these 2 genes separately. For *ADIPOQ*, 4 SNPs—rs266729, rs822395, rs822396, and rs1501299—were used. For *ADIPOR1*, 2 SNPs—rs12733285 and rs7539542—were used.

3. Results

3.1. Demographics

DNA was extracted from lymphocytes of blood specimens from 465 consecutive individuals diagnosed with adenocarcinoma of the prostate who received care at the outpatient urology clinic at Memorial Sloan-Kettering Cancer Center from April 2000 to September 2002. The blood samples were collected following completion of diagnostic studies. They were unselected for age or family history. Clinical and pathological records were reviewed to confirm the diagnosis of prostate cancer in all subjects. Once pathological diagnosis of prostate cancer was confirmed, the age of diagnosis was recorded; and all other identifying links were destroyed. The study design and anonymization method were approved by the Memorial Sloan-Kettering Cancer Center IRB. A population of 441 healthy male controls aged 20 to 87 years with well-defined ethnic background who had donated blood for various reasons (predominantly prenatal screening for noncancer disease) constituted the control group. Controls were matched to the cases on ethnicity and were from the same geographic locations as the prostate cancer cases. None of the controls had any personal history of cancer at the time of blood donation. This was ascertained by a questionnaire completed by each control. All personal identifiers were permanently removed from both cases and controls. All healthy controls and the majority of cases (89.2%) were white men. To investigate the potential confounding effects of races, we performed 2 analyses, including only white cases and controls and including all cases and white controls, respectively. These 2 analyses obtained essentially identical results and detected the same significant effects. Before the association analyses, we tested for HWE for each SNP among controls. For

rs2232853, significant deviation from HWE was found. Thus, we did not include rs2232853 in our analyses (Table 1).

3.2. Single-SNP analysis

We performed 4 analyses to assess the relationship of each one of the 9 *ADIPOQ* and *ADIPOR1* SNPs with prostate cancer. Table 2 shows significant main effects with *P* value < .05.

Under the Cockerham model, 4 SNPs were significantly associated with prostate cancer risk (ie, at least one main effect with *P* < .05). For rs822396, both the additive and dominance effects were significant. For rs12733285, only the additive effect was significant. For rs266729 and rs822395, only the dominance effects were significant (Table 2A).

In the codominant model analysis, we found 5 significant SNPs, including the 4 SNPs detected in the Cockerham model and rs1501299. For SNP rs822396, both 2 main effects were significant. For rs12733285, only the first main effects were significant. For rs266729, rs822395, and rs1501299, only the second main effects were significant (Table 2B).

In the dominant model analysis, 2 SNPs, rs12733285 and rs822396, were significantly associated with prostate cancer risk. In the recessive model analysis, however, we detected 3 significant SNPs: rs12733285, rs266729, and rs1501299 (Table 2C).

3.3. Multiple-SNP nonepistatic analysis

We performed 4 multiple-SNP nonepistatic analyses to simultaneously estimate the main effects of all SNPs. Table 3 shows significant main effects with *P* value < .05.

The Cockerham model detected 6 SNPs significantly associated with prostate cancer risk, including the 3 SNPs (rs12733285, rs266729, and rs822396) detected in the single-SNP analysis and 3 additional SNPs (rs753942, rs10920531, and rs1501299). As in the single-SNP analysis, the additive effect of rs12733285 and the dominance effects of rs266729 and rs822396 were significant. Single nucleotide polymorphisms rs753942 and rs10920531 were associated with prostate cancer risk through their dominance effects. The additive effect of rs1501299 was estimated to be significant (Table 3A).

In the multiple-SNP codominant model, 5 SNPs were significantly associated with prostate cancer risk, all detected in the single-SNP and multiple-SNP Cockerham models. As in the multiple-SNP Cockerham model analysis, the multiple-SNP codominant model did not detect SNP rs822395, which was significant in the single-SNP models. For rs12733285, the first main effect was significant. For rs266729 and rs822396, the second main effects were significant. Both the 2 main effects of rs1501299 were estimated to be significant (Table 3B).

The multiple-SNP dominant and recessive models detected the same SNPs as in the corresponding single-SNP models (Table 3C). However, the *P* values for most of the detected effects in the multiple-SNP models were much smaller than those in the single-SNP models. This was also true in the Cockerham and the codominant models.

We could infer the direction of genotypic effects for each SNP on prostate cancer risk from the estimated effects. As shown Table 3, all significant additive effects were negative (ie, OR <1), indicating that the rare homozygotes were all associated with decreased prostate cancer risk. Furthermore,

Table 2 – Single-SNP analyses: ORs, 95% CIs, and P values for significant main effects of SNPs (P < .05), for the analyses including all cases (the top) and only white cases (the bottom)

SNP	Additive effect		Dominance effect	
	OR (95% CI)	P	OR (95% CI)	P
A. Cockerham model				
rs12733285	0.638 (0.469-0.868)	.0034		
	0.759 (0.608-0.948)	.0131		
rs266729			0.573 (0.408-0.806)	.0011
			0.582 (0.412-0.822)	.0017
rs822395			0.676 (0.499-0.915)	.0098
			0.728 (0.533-0.996)	.0425
rs822396	2.166 (1.242-3.778)	.0054	0.377 (0.233-0.611)	5.1×10^{-5}
	1.487 (0.985-2.244)	.0500	0.443 (0.269-0.731)	.0011
B. Codominant model				
rs12733285	0.530 (0.343-0.819)	.0034		
	0.576 (0.369-0.899)	.0131		
rs266729			0.557 (0.420-0.739)	3.5×10^{-5}
			0.593 (0.444-0.792)	.0002
rs822395			0.709 (0.532-0.946)	.0170
			0.722 (0.539-0.968)	.0263
rs822396	2.984 (1.359-6.553)	.0054	0.652 (0.473-0.897)	.0074
	2.210 (0.970-5.036)	.0540	0.659 (0.476-0.914)	.0106
rs1501299			0.669 (0.502-0.890)	.0049
			0.652 (0.486-0.875)	.0035

SNP	Dominant model		Recessive model	
	OR (95% CI)	P	OR (95% CI)	P
C. Dominant and recessive models				
rs12733285	1.641 (1.100-2.449)	.0132	0.723 (0.547-0.956)	.0204
	1.586 (1.054-2.385)	.0238	0.789 (0.592-1.051)	.0584
rs266729			0.604 (0.462-0.791)	1.8×10^{-4}
			0.648 (0.492-0.853)	.0015
rs822396	0.301 (0.138-0.659)	.0021		
	0.407 (0.180-0.925)	.0285		
rs1501299			0.683 (0.520-0.895)	.0048
			0.673 (0.509-0.888)	.0042

all the second main effects except for rs7539542 in the codominant model were negative; and thus, the heterozygotes were associated with decreased risk compared with the common homozygotes. However, the dominance effect of rs7539542 was positive (ie, OR >1). Thus, heterozygotes for this SNP had increased prostate cancer risk compared with the mean of the 2 homozygotes.

3.4. Haplotype analysis

The results from the haplotype analysis confirmed the findings from the single-SNP and/or multiple-SNP analysis (Table 4). For ADIPOQ and ADIPOR1, several haplotypes had different frequencies in cases and controls and were found to be significantly associated with prostate cancer risk. Five 4-SNP ADIPOQ haplotypes and one 2-SNP ADIPOR1 haplotypes were tightly associated with prostate cancer risk (4 haplotypes with $P < 10^{-5}$ and 2 haplotypes with P < .005) (Table 4).

3.5. Multiple-SNP epistatic analysis

In the multiple-SNP epistatic model, significant main effects were detected in the same SNPs as found in our multiple-SNP

nonepistatic Cockerham model; and their estimated ORs were similar to the previous ones. We found that the additive effect for rs12733285 significantly affected prostate cancer risk. Single nucleotide polymorphisms rs753942, rs266729, and rs822396 showed significant dominance effects. Among 180 epistatic interactions, 4 were significant; and others were estimated to be close to zero. The additive-additive interaction between rs1501299 and rs1342387 was significantly associated with prostate cancer risk. The additive-dominant interaction between rs1342387 and rs266729 and the dominance-additive interactions between rs10920531 and rs266729 and rs2241766 and rs7539542 were significantly associated with prostate cancer risk (Table 5A). The epistatic codominant model detected all the significant main effects as in our multiple-SNP nonepistatic model analyses. In addition, we found 2 significant epistatic interactions. The interaction between rs10920531 and rs266729 was also detected in the epistatic Cockerham model analysis (Table 5B).

3.6. Model comparison

Table S3 shows the lowest deviance and AIC in the single-SNP analyses, and the deviance and AIC in the multiple-SNP

Table 3 – Multiple-SNP nonepistatic analyses: ORs, 95% CIs, and P values for significant main effects of SNPs (P < .05), for the analyses including all cases (the top) and only white cases (the bottom)

SNP	Additive effect		Dominance effect	
	OR (95% CI)	P	OR (95% CI)	P
A. Cockerham model				
rs12733285	0.654 (0.492-0.868)	.0028		
	0.685 (0.512-0.916)	.0091		
rs7539542			1.640 (1.139-2.361)	.0066
			1.764 (1.210-2.572)	.0025
rs10920531			0.722 (0.518-1.000)	.0488
			0.717 (0.511-1.006)	.0491
rs266729			0.566 (0.398-0.802)	.0011
			0.575 (0.404-0.818)	.0016
rs822396			0.407 (0.243-0.682)	.0005
			0.486 (0.284-0.832)	.0072
rs1501299	0.758 (0.755-0.996)	.0431		
	0.769 (0.583-1.015)	.0501		
B. Codominant model				
rs12733285	0.436 (0.249-0.764)	.0031		
	0.478 (0.269-0.847)	.0098		
rs7539542			1.675 (1.158-2.426)	.0052
			1.656 (1.137-2.411)	.0072
rs266729			0.492 (0.365-0.663)	2.0×10^{-6}
			0.519 (0.383-0.703)	1.6×10^{-5}
rs822396			0.567 (0.383-0.841)	.0039
			0.583 (0.391-0.868)	.0066
rs1501299	0.584 (0.340-1.000)	.0471	0.606 (0.445-0.827)	.0013
	0.601 (0.347-1.041)	.0536	0.592 (0.431-0.814)	.0009

SNP	Dominant model		Recessive model	
	OR (95% CI)	P	OR (95% CI)	P
C. Dominant and recessive models				
rs12733285	1.599 (1.061-2.412)	.0221	0.683 (0.503-0.927)	.0358
	1.639 (0.384-7.000)	.0346	0.797 (0.586-1.084)	.0503
rs7539542			1.535 (1.075-2.191)	.0160
			1.482 (1.031-2.130)	.0299
rs266729			0.535 (0.404-0.708)	8.1×10^{-6}
			0.567 (0.426-0.756)	7.6×10^{-5}
rs822396	0.298 (0.129-0.687)	.0037		
	0.395 (0.164-0.950)	.0341		
rs1501299			0.573 (0.427-0.767)	.0001
			0.569 (0.422-0.768)	.0001

nonepistatic and epistatic analyses under the Cockerham and the codominant models. The multiple-SNP epistatic models had better fit to data and prediction power than the multiple-SNP nonepistatic models. This indicates that inclusion of the significant epistatic interactions improves the fit of the model to data and reduces out-of-sample prediction error.

3.7. Prostate cancer risk prediction

The Cockerham and the codominant models yielded almost identical ROC curves and thus the same prediction accuracy (Fig. 1). The epistatic models achieved higher true-positive rate and lower false-positive rate than did the nonepistatic models for any threshold. For the usual threshold of 0.5, the true- and false-positive rates were 70% and 33% for the nonepistatic model and 76% and 27% for the epistatic model, respectively. The areas under the ROC curves (AUCs) were 0.65

(95% CI, 0.62-0.69) and 0.73 (95% CI, 0.70-0.77) for the nonepistatic and the epistatic models, respectively. This indicated that genotypes of the adiponectin pathway are powerful predictors of prostate cancer risk, and a model that includes epistatic interactions further improves prediction accuracy.

4. Discussion

In this clinic-based case-control study, we found that several haplotype tagging SNPs of ADIPOQ and ADIPOR1 are significantly associated with prostate cancer risk. To our knowledge, this is the first report of an association between variants of the adiponectin pathway and risk of prostate cancer.

We performed several analyses taking into account epistatic and nonepistatic genetic models. Both our single- and multiple-SNP analyses in the nonepistatic models showed that rs12733285, rs266729, rs822396, and rs1501299

Table 4 – Haplotype analysis: haplotype frequencies in cases and controls; ORs and 95% CIs

Haplotype analysis for *ADIPOQ* with rs266729, rs822395, rs822396, and rs1501299				
Haplotype	Frequency in cases	Frequency in controls	OR (95% CI)	P value
CAAG	0.361	0.296	1.427 (1.141,1.785)	.0018
CAAT	0.137	0.155	0.838 (0.624,1,126)	.241
CCAG	0.068	0.075	0.873 (0.564, 1.352)	.544
CCAT	0.052	0.110	0.345 (0.222,0.536)	2.17×10^{-6}
CCGG	0.095	0.019	9.403 (4.583, 19.293)	9.89×10^{-10}
CCGT	0.012	0.200	0.012 (0.005, 0.030)	2.62×10^{-21}
GAAG	0.164	0.015	43.486 (17.900,105.638)	8.06×10^{-17}
Haplotype analysis for *ADIPOR1* with rs12733285 and rs7539542				
CC	0.425	0.374	1.310 (1.052,1.625)	9.42×10^{-5}
CG	0.217	0.211	1.048 (0.804,1.365)	.728
TC	0.241	0.300	0.689 (0.540, 0.880)	.0028
TG	0.117	0.117	0.998 (0.699, 1.428)	.994

Only haplotypes with frequency greater than 5% are displayed.

Table 5 – Multiple-SNP epistatic analyses: ORs and 95% CIs, for the analyses including all cases (the top) and only white cases (the bottom)

SNP	Additive	Dominance	Additive:additive	Additive:dominance	Dominance:additive
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
A. Cockerham model					
rs12733285	0.643[b] (0.478-0.864)				
	0.672[b] (0.497-0.908)				
rs7539542		1.657[b] (1.139-2.410)			
		1.543[a] (1.011-2.355)			
rs266729		0.536[c] (0.375-0.767)			
		0.546[c] (0.380-0.784)			
rs822396		0.386[c] (0.228-0.654)			
		0.454[b] (0.262-0.788)			
rs1342387:rs266729				2.091[c] (1.368-3.197)	
				2.038[c] (1.321-3.144)	
rs10920531:rs266729					1.969[b] (1.249-3.105)
					1.971[b] (1.241-3.132)
rs2241766:rs7539542					0.532[a] (0.532-0.876)
					0.542[a] (0.325-0.905)
rs1342387:rs1501299			1.593[b] (1.157-2.191)		
			1.550[b] (1.121-2.143)		
B. Codominant model					
rs7539542		1.732[b] (1.192-2.518)			
		1.702[b] (1.165-2.487)			
rs10920531		0.634[a] (0.424-0.948)			
		0.642[a] (0.427-0.967)			
rs266729	0.435[a] (0.212-0.792)	0.578[b] (0.421-0.792)			
	0.485[a] (0.237-0.995)	0.609[b] (0.442-0.840)			
rs822396		0.561[b] (0.377-0.832)			
		0.577[b] (0.387-0.860)			
rs1501299	0.574[a] (0.333-0.990)	0.597[a] (0.437-0.817)			
	0.591[a] (0.340-1.026)	0.585[a] (0.425-0.806)			
rs10920531:rs26 6729				3.686[a] (1.281-10.608)	
				3.468[a] (1.200-10.020)	
rs12733285:rs26 6729			0.224[a] (0.084-0.597)		
			0.235[b] (0.088-0.627)		

Only effects with P value < .05 are displayed. The term $X_1:X_2$ represents interaction between X_1 and X_2.
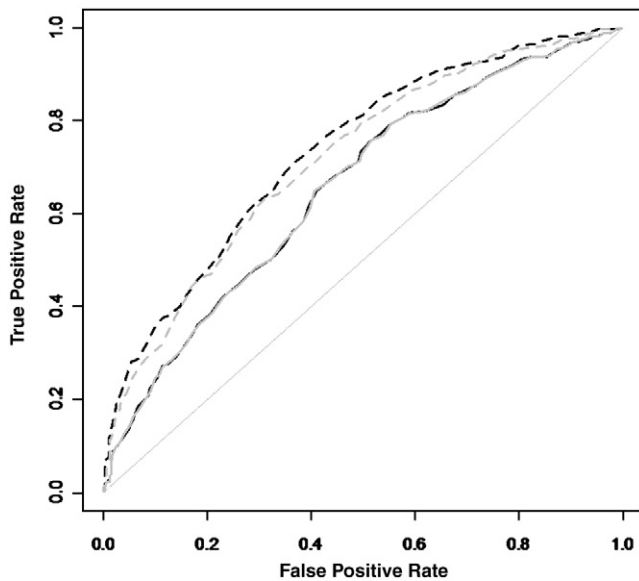[a] ≤.05.
[b] ≤.01.
[c] ≤.001.

Fig. 1 – Receiver operating characteristic curves: ROC curves for risk prediction for multiple-SNP nonepistatic Cockerham (solid black line) and codominant (solid gray line) models and epistatic Cockerham (dotted black line) and codominant (dotted gray line).

are significantly and independently associated with prostate cancer risk. Independent haplotype analysis corroborated these findings. Of all models performed, the epistatic Cockerham model had the best fit. That model showed that several SNPs (most of which were also associated with prostate cancer risk in the other models and in the haplotype analysis) were significantly associated with prostate cancer risk. Rs12733285, rs7539542, rs266729, rs822396, and rs1501299 were significantly associated with prostate cancer risk. Furthermore, the interactions between rs10920531 and rs266729 were significantly associated with prostate cancer risk.

We have previously shown that higher serum adiponectin is associated with a marked reduction in risk of prostate cancer [8] and that lower adiponectin is independently associated with high-grade prostate cancer [9]. Most of the SNPs genotyped are functionally relevant and have been associated with diabetes, insulin resistance, and coronary artery disease. More specifically, rs266729 has been associated with lower adiponectin levels, rs1501299 has been associated with lower adiponectin levels as well as risk for coronary artery disease and insulin resistance, and rs822396 has been associated with ischemic stroke. Furthermore, we previously found that rs266729 is significantly associated with colon cancer [22] and that rs1501299 is significantly associated with breast cancer [21]. Both SNPs were found in multiple models to also be significantly associated with prostate cancer. As in our previous studies, the rare homozygotes of these SNPs increased cancer risk compared with the common homozygotes.

We performed a prostate cancer risk prediction model using the ROC curve (Fig. 1). This curve uses all genotypes from cases and controls and assesses the combination of

sensitivity and specificity of different combinations of genotypes. We observed an AUC of 0.73 for the epistatic model. This result shows that the utility of prediction of prostate cancer risk using the adiponectin pathway genotypes is high. It has been suggested by others that an AUC greater than 0.75 should be used for screening individuals with high risk of disease, whereas an AUC greater than 0.5 has some discriminatory ability [31]. If validated in other studies, these SNPs may emerge as a powerful approach to predict prostate cancer risk.

Our study has several strengths and limitations. The age of our controls is significantly lower than the age of our cases. It is possible that age differences in cases and controls affected the allele frequencies observed. Nonetheless, this would be expected to create a bias toward the null hypothesis because it would overestimate the deleterious allele frequency in controls given that a fraction of younger men who would have not yet developed prostate cancer were not removed from the control group. Furthermore, inaccurate information with respect to any variable classification would result in "random misclassification" that would also be expected to result in suppression of effect estimates with a trend toward the null hypothesis. Our study also has several strengths. It includes a relatively large number of sex-matched cases and controls from the same geographic location. The selection of our SNPs was based on previous studies, and the SNPs significantly associated with prostate cancer are functionally significant and have been associated with risk of breast or colon cancer. Although exposure was assessed in the context of a case-control study, it is impossible that prostate cancer would have changed SNP classification, which is already determined at birth. Thus, this study does fulfill the "time sequence" criterion for causality. In summary, our findings together with the available epidemiologic evidence and biologic plausibility support a causal association between these SNPs and risk for prostate cancer.

To our knowledge, this is the first study reporting an association between adiponectin pathway SNPs and prostate cancer risk. A recent study by Beebe-Dimmer et al [32] evaluated the role of adiponectin SNPs in 131 African American prostate cancer cases and 344 controls. In their study, the authors did not find any association between the SNPs they studied and prostate cancer risk. The potential explanations for the differences between these findings and the findings from our analyses are the fact that our population included primarily whites and not African Americans and the fact that the study by Beebe-Dimmer et al included a small number of patients and therefore may have missed a true effect. In addition, it is important to investigate if the 10 SNPs genotyped in our study are in linkage disequilibrium with other SNPs and their coverage. We downloaded HapMap II + III genotypes of CEU samples for ADIPOQ and ADIPOR1 (www.hapmap.org). We used HaploView [33] to calculate the LD measures r^2 and D' between SNPs (Table S2), determine LD blocks ((Figs. S1 and S2), identity tag SNPs, and calculate the coverage. The set of tag SNPs was selected as a minimal set of SNPs such that all SNPs to be captured are correlated at an r^2 greater than 0.80, and the SNPs genotyped in our study were forced to be tag

SNPs [31]. For *ADIPOQ*, there are 24 SNPs with minimum allele frequency (MAF) greater than or equal to 0.05. Three SNPs from our study, rs266729, rs822396, and rs1501299, were also genotyped in the HapMap; and their MAFs are quite similar (Table S1). These SNPs can tag 16.7% (4 of 24) of SNPs using an r^2 threshold of 0.80, and 12 additional SNPs are needed to capture all 24 SNPs. The values of LD measure r^2 based on the HapMap data and our data showed that these SNPs are not in linkage equilibrium with each other and other tag SNPs (Table S2). We found that 2 SNPs, rs822395 and rs2241766, which were genotyped in our study, were not genotyped in the HapMap project. For *ADIPOR1*, there are 17 SNPs with MAF greater than or equal to 0.05 genotyped in the HapMap. All 5 SNPs from our study are also in the HapMap; but the MAFs of 2 SNPs, rs2232853 and rs12733285, are quite different (Table S1). These 5 SNPs can capture 75% (13 of 17) of SNPs using an r^2 threshold of 0.80, and 3 additional SNPs are needed to capture all 17 SNPs. The values of LD measure r^2 based on the HapMap data and our data showed that these SNPs are not in linkage equilibrium with each other and other tagSNPs (Table S2).

The findings from Beebe-Dimmer et al [32] and low coverage of *ADIPOG* gene suggest the need for a confirmatory study with more SNPs within *ADIPOQ* and *ADIPOR1* genes. If confirmed and/or if new findings emerged in subsequent studies, our findings suggest that genetic variants of adiponectin and adiponectin receptor 1 alter prostate cancer risk. The adiponectin axis may emerge as an important modifier of prostate cancer risk. In the future, we may be able to identify a population with a specific combination of adiponectin pathway SNPs who will be identified as high risk for developing prostate cancer and may benefit from adiponectin replacement therapy.

Supplementary materials related to this article can be found online at doi:10.1016/j.metabol.2011.01.005.

Acknowledgment

R E F E R E N C E S

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