Common Polymorphisms in the Adiponectin and Its Receptor Genes, Adiponectin Levels and the Risk of Prostate Cancer

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Abstract

Background: Adiponectin, an insulin-sensitizing adipokine, is inversely associated with adiposity and prostate cancer risk and progression. However, the role of genetic variation in the adiponectin (*ADIPOQ*) and receptor genes (*ADIPOR1/R2*) in prostate cancer is largely unknown.

Methods: In a nested case–control study of 1,286 cases and 1,267 controls within the Physicians' Health Study, we evaluated 29 common single-nucleotide polymorphisms (SNP) in *ADIPOQ* ($n = 13$), *ADIPOR1* ($n = 5$), and *ADIPOR2* ($n = 11$) in relation to the risk of prostate cancer. In subgroups, we also evaluated the association of genotype and circulating adiponectin levels ($n = 951$) and prostate tumor expression of insulin receptor (IR) and insulin-like growth factor 1 (IGF-IR) receptor ($n = 181$).

Results: Among the 12 tagging polymorphisms in *ADIPOQ*, four (rs266729, rs182052, rs822391, and rs2082940) were significantly associated ($P < 0.05$) with overall prostate cancer risk, with no significant difference by tumor grade or clinical stage. Two of the risk SNPs (rs266729 and rs182052) plus four other SNPs (rs16861209, rs17366568, rs3774261, and rs7639352) were also associated with plasma adiponectin levels, and three of these (rs1686109, rs17366568, and rs3774261) were also significantly associated with IR expression in prostate tumor tissue. One additional SNP was associated with IGFI-R tumor tissue expression (rs16861205). None of the 16 variants in *ADIPOR1/R2* were related to cancer risk or circulating adiponectin levels.

Conclusions: Common variants in the adiponectin gene were associated with prostate cancer risk, plasma adiponectin levels, and IR or IGF-IR expression in the prostate tumor.

Introduction

Emerging evidence suggests that high body mass index (BMI) and adiposity are linked to increased prostate cancer mortality (1, 2). Higher circulating levels of adiponectin, a protein secreted by adipose tissue and inversely correlated with BMI, may be associated with a reduced risk of prostate cancer (3, 4), lower Gleason score (3–6), and lower tumor stage (3–5). We had previously reported that low prediagnostic levels of circulating adiponectin were associated with increased risk of high-grade and lethal prostate cancer in the Physicians' Health Study (PHS; ref. 7), although another smaller prospective study did not yield similar results (8).

It remains unclear whether adiponectin acts directly on tumorigenesis or indirectly through its effects on insulin resistance (9), which is also highly regulated by the insulin-like growth factor 1 (IGF-I) signaling system. Adiponectin activates the AMP-activated protein kinase (AMPK), stimulates fatty acid oxidation, acts as a direct endogenous inhibitor of inflammation (10–12), and activates anti-inflammatory cytokines through the NF-κB pathway (13–15). It also reduces angiogenesis (10) and inhibits prostate cancer cell proliferation *in vitro* (16). Adiponectin receptor expression (*ADIPOR1/R2*) has been shown in androgen-dependent and androgen-

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independent prostate cancer cell lines (16–18), and lower expression has been observed in prostate tissue of prostate cancer patients than in healthy men or men with benign prostatic hyperplasia (5).

Several polymorphisms in the adiponectin and its receptor genes modulate levels and function and have been linked to obesity, insulin resistance (19–22), and prostate cancer risk (23). Three studies have evaluated single-nucleotide polymorphisms (SNP) in the adiponectin gene with respect to prostate cancer risk (23) of which one recently reported significant associations (rs266729, rs822395, rs822396, and rs1510299 in *ADIPOQ* and rs12733285 and rs7539452 in *ADIPOR1*; ref. 23). The other 2 studies—a case–control study of African Americans and a cohort of Finnish smokers—yielded no associations in candidate SNPs selected for potential functionality and prostate cancer (24, 31). None of these studies evaluated concurrent associations between genotypes and circulating adiponectin levels. In the current case–control study nested within a long-term prospective cohort of U.S. male physicians, we comprehensively evaluated common haplotype tagging SNPs throughout genes encoding adiponectin (*ADIPOQ*) and its 2 receptors (*ADIPOR1/R2*). We examined these SNPs in relation to the risk of total prostate cancer as well as high-grade and aggressive disease. In subgroups with available data, we also assessed whether plasma adiponectin levels, and expression of tumor biomarkers involved in insulin sensitivity [insulin receptor (IR) and IGF-IR], were correlated with these genotypes as potential mediators of risk.

Methods

Study population

We conducted a nested case–control study within the PHS, a randomized trial of aspirin and β-carotene for the primary prevention of cancer and cardiovascular disease among U.S. male physicians (25, 26). The study began in 1982 among 22,071 physicians aged 40 to 84 years free of baseline cardiovascular disease or cancer (except for nonmelanoma skin cancer). Follow-up information and mortality data are 97% complete on all participants. The research protocol was approved by the Human Subjects Committee at Brigham and Women's Hospital, and all participants provided written informed consent.

At baseline, all study participants completed questionnaires with information on age, height, weight, cigarette smoking, and the presence of diabetes, and 14,916 men (68%) provided a baseline blood sample in 1982. Annual questionnaires updated medical information, including diagnoses of prostate cancer, which were confirmed through medical records and pathology reports reviewed by an End Point Committee of physicians. Deaths were identified through the postal system and next of kin, and cause of death was determined through medical record review and death certificates with nearly 100% mortality follow-up. All participants who provided a baseline blood specimen and who were later diagnosed with a confirmed prostate adenocarcinoma from 1982 to 2004 were eligible cases for this study. For each case, one control was selected at random from those who provided blood, had not had a prostatectomy, and had not reported a diagnosis of prostate cancer at the time of the matched case's diagnosis date. Controls were individually matched to cases by age (within 1 year, if feasible, or 5 years for older men) and cigarette smoking status (never, current, and former).

Clinical and demographic characteristics

Information on Gleason score, tumor-node-metastasis (TNM) stage, and prostate-specific antigen (PSA) at diagnosis was obtained from medical records for all cases. Development of bony metastases was ascertained through annual mailed questionnaires to consenting participants and confirmed by treating physicians. If data on clinical characteristics were not available from medical records, self-reported stage and Gleason score were used from follow-up questionnaires. All clinical covariates were collected while blinded to genotype and plasma level status.

Adiponectin genotyping

We used the web-based tagger application (27) to select linkage disequilibrium (LD) tag SNPs capturing genetic variation in the international HapMap database (HapMap phase II, build 35 data) in the adiponectin (*ADIPOQ*, Chr3q27) and both receptor genes (*ADIPOR1*, Chr1p36.13-q41 and *ADIPOR2*, Chr12p13.31) as well as 5 kb up- and downstream for each gene. An aggressive tagging approach, a maximum combination of 3 SNPs, was used to tag SNPs with a minor allele frequency greater than 0.05 with a minimum r^2 of 0.80 in CEU (27). Evaluation of our tag SNPs showed sufficient genetic coverage using pairwise only tagging allowing for evaluation of single SNP effects. Genotyping was carried out by MassARRAY high-throughput DNA analysis with matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry (Sequenom), and genotypes were called with the automated analysis SpectroTYPER-RT software. We selected 29 SNPs across the 3 genes: 13 in *ADIPOQ*, 5 in *ADIPOR1*, and 12 in *ADIPOR2*. Blind duplicates were run to assess genotyping quality yielding a concordance rate greater than 98% for duplicate samples. All SNPs had >90% genotype passing rates.

Adiponectin plasma levels

Plasma adiponectin concentrations were measured by competitive radioimmunoassay (Linco Research) in the laboratory of N. Rifai (Children's Hospital, Boston, MA) for a subset of 565 men diagnosed with prostate cancer and 440 controls who also had genotype data. Specimens from cases and matched control(s) were analyzed together and placed in random order so the case status remained unknown to laboratory personnel. The median intraindividual coefficient of variation for blinded duplicate quality control samples was 11%. Previously, we showed that 2

adiponectin measurements over a 1-year period had a high BMI-adjusted intraclass correlation [$r = 0.84$, 95% confidence interval (CI), 0.65–0.94; ref. 28], suggesting that a single blood measurement of adiponectin is reasonably accurate and stable, over time and over different transport conditions.

Tissue microarrays

Archival formalin-fixed, paraffin-embedded tissue specimens were available for a subset of surgically treated cases [90.1% radical prostatectomy, $n = 1171$; 8.9% transurethral resection of the prostate (TURP), $n = 115$]. Tissue microarrays (TMA) were created by a manual tissue arrayer (Beecher Instruments). For each patient, a study pathologist circled the dominant nodule or nodule with the highest Gleason score on a hematoxylin and eosin–stained slide, and 3 or more replicate 0.6-mm tissue cores were transferred from the corresponding area on the tissue block into the recipient TMA blocks. The pathologist had no *a priori* knowledge of the clinical or pathologic status of the cases. There were 3 TMAs with 1 to 14 cores per TMA block. We had immunohistochemical data for a subset of the cases for which we had genotyping information for IR and IGF-IR on the TMAs ($n = 169$ for IR and $n = 190$ for IGFI-R).

IR and IGF-IR

Immunohistochemical staining was conducted on 5-μm sections of each TMA by the Anti-Insulin Receptor, β subunit, rabbit immunoaffinity purified IgG (Upstate Cell Signaling Solutions) and the IGF-IR β R rabbit polyclonal antibody (Santa Cruz Biotechnology Inc.) with enzyme-labeled biotin–streptavidin system and solvent-resistant DAB Map kit (Ventana autostainer model Discover XT; Vantana Medical System). Nonspecific reactivity was assessed by omission of the primary antibody. The specificity of staining for IR was confirmed by using placenta as a positive control. The slide was scanned with the BLISS system (Bacus Laboratory) and scored manually by 2 pathologists who were blinded to clinical outcomes. Immunohistochemical analysis of IR showed homogenous cytoplasmic staining in the cancer cells whereas that of IGF-IR yielded mostly membranous and occasional cytoplasmic staining. For both receptors, intensity was scored from 0 to +3—0 (no staining by any tumor cells), 1 (faint or focal staining), 2 (moderate intensity in a convincing number of cells), and 3 (intense staining by a sufficient number of cells). Immunohistochemical staining was completed for 169 men for IR and for 190 men for IGF-IR.

Statistical analyses

We restricted our analyses to Caucasians to reduce spurious associations by population stratification (29). Using the SAS program package, version 9.1 (SAS Institute), we evaluated Hardy–Weinberg equilibrium and removed one adiponectin SNP (rs7649121) from the analysis because it was severely out of Hardy–Weinberg equilibrium ($P < 0.0001$).

We used unconditional logistic regression to calculate the multivariate OR and 95% CI of prostate cancer associated with each tag SNP genotype, adjusted for the original matching factors of age at randomization (years), cigarette smoking status at baseline (never, former, and current), and time between blood draw and diagnosis (years). We used ANOVA to evaluate the association with each tag SNP genotype and mean plasma adiponectin levels, adjusted for age at baseline and BMI; P_{trend} values were calculated assuming linear changes in risk with additive models.

For total prostate cancer risk and plasma adiponectin levels, we used additive models, and the SNPs were divided into 3 categories, with the most common genotype selected as the reference group, the heterozygous genotype as a second group, and the rare homozygous variant as the third group; P_{trend} values were presented, assuming linear changes in risk. For high-grade and aggressive prostate cancer risk and tumor biomarker expression, we used dominant genetic models due to much smaller numbers, and the SNPs were modeled dichotomously with the same reference group and with heterozygous and rare alleles combined into one category. For calculating the risk of high-grade disease, we restricted cases to those with a Gleason of 4+3, 8, 9, or 10, and for aggressive disease, we considered men who had extra prostatic disease (T_3 or T_4 or N_1 or M_1) or men who developed bony metastases or fatal disease during follow-up (end of follow-up for prostate cancer mortality = Mar 30, 2009). The impact of *ADIPOQ* variation on insulin sensitivity is dependent on adiposity (30). Thus, we examined whether associations between genotype and risk differed according to baseline BMI (kg/m^2) and plasma adiponectin levels ($P_{interaction} < 0.05$).

We used ANOVA for the comparison of continuous variables by genotype, including baseline age (years) and log-transformed adiponectin levels (μg/mL) among cases and controls. To evaluate adiponectin differences, we adjusted for age and BMI. To reduce bias from preclinical disease influencing adiponectin levels at baseline, we excluded cases whose time between blood draw and diagnosis was less than 5 years. To evaluate differences of mean intensity expression levels (scores were averaged across multiple cores per subject) of IGF-IR and IR by genotype, we analyzed cases with available tumor expression levels (IR, $n = 169$; IGFI-R, $n = 190$) and adjusted for age and TMA to account for batch variation by TMA.

We conducted permutation testing to better interpret nominally statistical associations by randomly assigning case–control status 5,000 times for the 29 tagging SNPs. Unadjusted P values were examined in relation to the permuted distribution. We used the SAS program package, version 9.1 (SAS Institute) to carry out statistical analyses with a significance level of 0.05. This study was approved by the Institutional Review Board at Partners Healthcare.

Table 1. Distribution of characteristics at baseline among cases and controls and clinical characteristics at diagnosis (cases) in the PHS, 1982–2009

Characteristics at baseline	Cases ($n = 1{,}286$) *N* (%) or mean (SD)	Controls ($n = 1{,}267$) *N* (%) or mean (SD)	*P*
Age at randomization, y	57.9 (8.4)	57.5 (8.4)	n/a
Cigarette smoking status at baseline			
Never	627 (48.8)	636 (50.2)	n/a
Former	553 (43.0)	532 (42.0)	
Current	106 (8.2)	99 (7.8)	
BMI, kg/m^2, baseline			
<25	746 (58.0)	761 (60.1)	0.56[a]
25–29	499 (38.8)	466 (36.8)	
30+	41 (3.2)	40 (3.2)	
Adiponectin levels, mg/L	7.12 (4.2)	7.18 (4.4)	0.83[b]
Diabetes at baseline	21 (1.6)	17 (1.3)	0.54[a]
Time between blood draw and diagnosis, y	12.2 (5.1)	12.1 (5.0)	0.61[a]
Gleason score at diagnosis			
4–6	750 (58.3)	—	
3 + 4 or 7[c]	234 (18.2)		
4 + 3	103 (8.0)	—	
8–10	173 (13.5)	—	
missing	26 (2.0)	—	
PSA at diagnosis, ng/mL, median (IQR)	7.3 (5.1–12.7)	—	
Missing[d]	350 (27)	—	
Clinical stage[e] at diagnosis			
T_1/T_2, NX/NO	1,079 (83.9)	—	
T_3, NX/NO	63 (4.9)	—	
T_4 or N_1 or M_1	79 (6.1)	—	
Missing	65 (5.1)	—	

Abbreviation: IQR, interquartile range.

[a]Global χ^2 test (except for "n/a" where age and cigarette smoking status are matching factors).

[b]$P > 0.05$ in F test for ANOVA comparing cases and controls.

[c]Includes $n = 45$ (3.5%) men with Gleason = 7 but no information on major/minor score.

[d]A total of 61% of men with missing PSA at diagnosis ($n = 213$) were diagnosed in the pre-PSA period (<1992).

[e]Pathologic stage available for $n = 465$ cases (27%): $n = 333$ (25.9%) T_2, $n = 116$ (9.0%) T_3/T_4, and $n = 18$ (1.4%) N_1/M_1.

Results

Baseline characteristics are provided in Table 1; men were on average, 57 years at the beginning of follow-up. Table 2 shows the frequency distribution of the 12 *ADIPOQ* SNPs and their associations with prostate cancer risk and plasma levels. Figure 1 summarizes the major findings of the associations of the 12 SNPs with risk of prostate cancer ($P_{trend} < 0.05$), plasma adiponectin levels ($P_{trend} < 0.05$), and tumor tissue expressions of IGF-IR or IR (global $P < 0.05$), with the upwards triangle representing a positive association and downwards triangle an inverse association with the rare genotype. Each of these associations is presented in detail in the following sections.

Genetic variation and prostate cancer risk

Among the 1,286 cases and 1,267 controls, 4 of the 12 tagging SNPs in the adiponectin gene were significantly associated with overall prostate cancer risk using the additive model (rs266729, rs182052, rs822391, and rs2082940) adjusting for age, cigarette smoking status, and time since blood draw (Table 2, Fig. 1). SNP rs266729 is located in the promoter region and is in high LD ($r^2 = 0.73$) with the intronic SNP rs182052. Increased risks were associated with the rare genotype for both SNPs (rs266729, $P_{trend} = 0.049$; rs182052, $P_{trend} = 0.04$). For rs822391, the rare C allele was associated with a decreased cancer risk ($P_{trend} = 0.04$). Rs2082940, located in the 3′untranslated region (UTR) region, showed an allele dosage association with significantly decreasing per-allele ORs [OR (CT) = 0.81, 95% CI, 0.66–0.99; OR (TT) = 0.51, 95% CI, 0.27–0.98; $P_{trend} = 0.006$). Adjustment for multiple comparisons by imputing a new null distribution for the test statistic (based on 50,000 imputations for the 12 SNPs in *ADIPOQ*) yielded an adjusted borderline significant $P = 0.05$ for this SNP. None of these polymorphisms were significantly

Table 2. Multivariate ORs of prostate cancer and adiponectin levels by sequence variants in the adiponectin *(ADIPOQ)* gene in the PHS, 1982–2009

		Cases ($n = 1,286$)	Controls ($n = 1,267$)	Total prostate cancer		Log (Adiponectin), mg/L[b] ($n = 951$)		
	SNP	*N* (%)	*N* (%)	OR[a] 95% CI	P_{trend}	*N*	Mean (SD)	P_{trend}
1	rs266729							
	CC	636 (53)	675 (57)	**1.00 (reference)**	—	483	8.78 (0.61)	0.001
	CG	477 (40)	443 (37)	1.14 (0.97–1.36)		340	8.67 (0.62)	
	GG	79 (7)	65 (5)	1.30 (0.92–1.84)		57	8.58 (0.53)	
					0.049			
2	rs182052							
	GG	545 (45)	564 (47)	**1.00 (reference)**	—	416	8.79 (0.63)	0.0009
	AG	527 (43)	524 (44)	1.03 (0.87–1.22)		406	8.67 (0.62)	
	AA	147 (12)	108 (9)	1.42 (1.08–1.87)		90	8.61 (0.51)	
					0.04			
3	rs168681205							
	GG	1077 (87)	1079 (88)	**1.00 (reference)**		812	8.73 (0.63)	0.14
	AG	161 (13)	138 (11)	1.17 (0.92–1.49)		111	8.62 (0.58)	
	AA	5 (0.4)	7 (0.6)	0.74 (0.23–2.33)		3	8.93 (0.33)	
					0.34			
4	rs168681209							
	CC	1014 (82)	986 (80)	**1.00 (reference)**		744	8.66 (0.63)	<0.0001
	AC	206 (17)	230 (19)	0.87 (0.71–1.07)		163	8.93 (0.52)	
	AA	13 (1)	11 (1)	1.13 (0.51–2.55)		10	9.17 (0.61)	
					0.30			
5	rs822391							
	TT	855 (69)	779 (64)	**1.00 (reference)**	—	622	8.71 (0.62)	0.82
	CT	346 (28)	389 (32)	0.81 (0.68–0.96)		270	8.74 (0.61)	
	CC	47 (4)	52 (4)	0.83 (0.55–1.24)		36	8.66 (0.77)	
					0.02			
6	rs12495941							
	GG	508 (42)	496 (42)	**1.00 (reference)**		358	8.72 (0.61)	0.87
	GT	539 (45)	531 (46)	0.99 (0.84–1.18)		397	8.72 (0.65)	
	TT	162 (13)	148 (13)	1.07 (0.83–1.39)		127	8.74 (0.49)	
					0.70			
7	rs17366568							
	GG	920 (75)	924 (77)	**1.00 (reference)**		689	8.76 (0.60)	0.0003
	AG	282 (23)	256 (21)	1.11 (0.91–1.34)		187	8.61 (0.63)	
	AA	17 (1)	13 (1)	1.31 (0.63–2.71)		12	8.44 (0.61)	
					0.23			
8	rs3821799							
	CC	383 (31)	350 (29)	**1.00 (reference)**	—	282	8.66 (0.66)	0.09
	CT	601 (49)	606 (50)	0.90 (0.75–1.08)		441	8.73 (0.60)	
	TT	249 (20)	259 (21)	0.87 (0.69–1.09)		190	8.76 (0.62)	
					0.20			
9	rs3774261							
	GG	462 (37)	441 (37)	**1.00 (reference)**	—	343	8.63 (0.67)	0.0001
	AG	596 (48)	574 (48)	0.98 (0.83–1.17)		431	8.74 (0.59)	
	AA	183 (15)	193 (16)	0.90 (0.70–1.14)		147	8.86 (0.59)	
					0.43			
10	rs17366743							
	TT	1154 (94)	1136 (94)	**1.00 (reference)**		850	8.72 (0.61)	0.19
	CT/CC	78 (6)	73 (6.1)	1.07 (0.77–1.49)		55	8.83 (0.63)	

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Table 2. Multivariate ORs of prostate cancer and adiponectin levels by sequence variants in the adiponectin *(ADIPOQ)* gene in the PHS, 1982–2009 (Cont'd)

		Cases ($n = 1,286$)	Controls ($n = 1,267$)	Total prostate cancer			Log (Adiponectin), mg/L[b] ($n = 951$)	
	SNP	*N* (%)	*N* (%)	OR[a] 95% CI	P_{trend}	*N*	Mean (SD)	P_{trend}
					0.76			
11	rs2082940							
	CC	969 (80)	907 (76)	**1.00 (reference)**	—	706	8.72 (0.64)	
	CT	226 (19)	260 (22)	0.81 (0.66–0.99)		189	8.67 (0.58)	
	TT	14 (1)	26 (2)	0.51 (0.27–0.98)		17	9.05 (0.50)	0.7
					0.006			
12	rs7639352							
	CC	652 (54)	650 (55)	**1.00 (reference)**	—	514	8.65 (0.65)	0.0001
	CT	453 (38)	428 (37)	1.05 (0.88–1.24)		313	8.78 (0.54)	
	TT	98 (8)	94 (8)	1.04 (0.76–1.40)		70	8.90 (0.61)	
					0.66			

[a]Among Caucasians only, adjusted for age at randomization (y, continuous), cigarette smoking status (never, current, and past), and time between blood draw and event date (y, continuous).
[b]Adjusted for age at randomization and BMI among Caucasians, includes all controls and cases with >5 years between blood draw and diagnosis.

associated with the risk of high-grade (Gleason 4+3, 8–10), regional, or metastatic prostate cancer (T_3–T_4 or N_1 or M_1; Table 3). We found no significant risk associations for any of the 16 SNPs in the *ADIPOR1/R2* regions (Supplementary Tables S1 and S2).

Genetic variation and plasma adiponectin levels

We next evaluated variation in adiponectin levels by genotype in all controls and cases whose prostate cancer was diagnosed at least 5 years after time of blood draw ($n = 951$, Table 2, Supplementary Table S1). Six of the 12 SNPs in *ADIPOQ* were significantly associated ($P_{trend} < 0.05$) with circulating adiponectin levels (rs266729, rs182052, rs168681209, rs17366568, rs3774261, and rs7639352) even after adjusting for baseline age and BMI. Two of these SNPs (rs266729 and rs182052), which were highly linked and significantly inversely associated with plasma adiponectin levels, were also positively associated with the risk of overall prostate cancer ($P_{trend} \leq 0.001$); these genotype–plasma and genotype-risk associations were in the expected inverse directions to each other, suggesting a biological causal link. No variants in *ADIPOR1/R2* were associated with plasma adiponectin levels (Supplementary Table S1).

Genetic variation and receptor expressions in the prostate tumors

In a subset of cases whose prostate tumor tissue was available for immunohistochemical analysis, we evaluated tumor expression of IR ($n = 169$) and IGF-IR ($n = 190$) to assess whether alleles associated with risk or circulating levels also yielded differential expression of receptors in tumors. Supplementary Table S3 summarizes tumor expression levels of IGF-IR and IR for each genotype using the dominant model. We observed 3 polymorphisms in *ADIPOQ* (rs168681209, rs3774261, and rs17366568) that were significantly associated with mean intensity levels of IR expression ($P_{trend} \leq 0.02$). One variant in *ADIPOQ* (rs16861205, $P = 0.02$) was associated with IGF-IR expression (Supplementary Table S3). There were no significant associations between *ADIPOR1/R2* polymorphisms and IR or IGF-IR protein expression.

Effect modification

We assessed whether genotype associations with risk varied according to baseline BMI and plasma adiponectin levels. We found that the inverse trend between rs2082940 in *ADIPOQ* and prostate cancer risk (Table 2) was stronger in men with normal/low (<10 μg/mL) baseline plasma adiponectin levels [ORs: OR (CT) = 0.76, 95% CI, 0.53–1.07 and OR (TT) = 0.31, 95% CI, 0.08–1.21, $P_{trend} = 0.03$; data not shown). We also observed a significant interaction for 2 SNPs in *ADIPOR1* (rs10920531 and rs7539542) whereby an increased risk for the rare allele was observed ($P_{interaction} = 0.02$ for rs10920531 and $P_{interaction} = 0.04$ for rs7539542, Supplementary Table S4). We found no significant differences in risk by BMI.

Discussion

In this large prospective analysis, we evaluated the associations of common tagging variants in the genes encoding adiponectin (*ADIPOQ*), adiponectin receptors 1 and 2 (*ADIPOR1/R2*) with prediagnostic plasma levels of

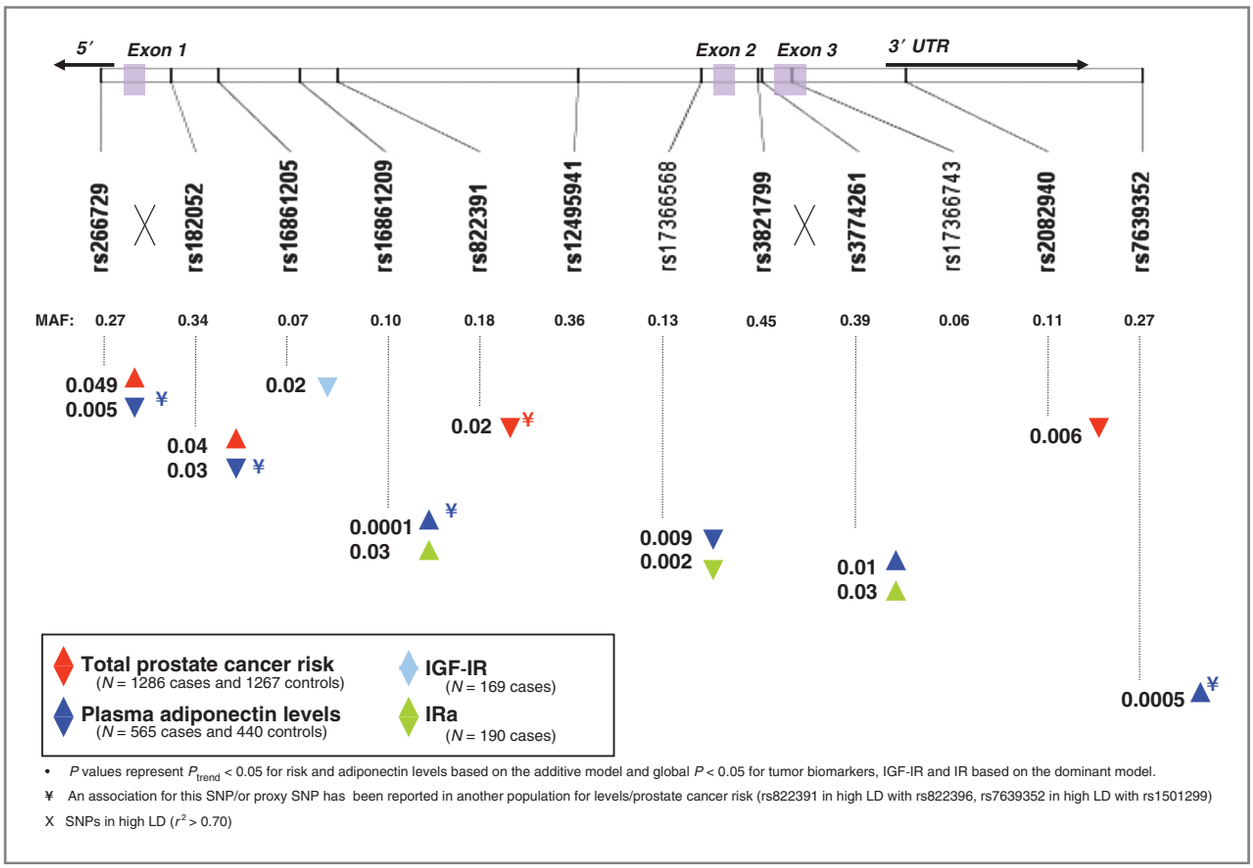

Figure 1. SNPs in *AD/POQ* and associations* with prostate cancer risk, plasma adiponectin levels, and tumor expression of IGF-IR and IR.

adiponectin, and future risk of prostate cancer and receptor (IR and IGF-IR) expressions in prostate tumors. We found that among the 12 genotyped tagging *ADIPOQ* SNPs, 4 (rs266729, rs182052, rs822391, and rs2082940) were significantly associated with the risk of developing prostate cancer. Of these 4 SNPs, 2 highly linked SNPs (rs266729 in the promoter and rs182052 in intron 1, $r^2 = 0.73$, Supplementary Fig. S1) were associated with both lower adiponectin levels and higher prostate cancer risk. In addition, 3 other SNPs (rs168681209, rs17366568, and rs3774261) were significantly associated with IR expression in the prostate and plasma adiponectin levels. There were no associations with the risk of high-grade or aggressive disease, which may suggest that these loci are more relevant for the development rather than progression of prostate carcinomas. We observed no variants in *ADIPOR1/R2* in relation to prostate cancer risk.

To our knowledge, 3 published studies have evaluated genetic variation in the adiponectin gene with respect to prostate cancer risk, selecting SNPs targeted for potential functionality (23, 24, 31). Kaklamani and colleagues (23) recruited Caucasians ($n = 465$ cases and 441 controls in New York city) to evaluate 10 SNPs selected for functionality in *ADIPOQ* and *ADIPOR1* and reported 4 significant SNPs in the adiponectin gene (rs266729, rs822395, rs822396, and rs1501299), of which one was in the same reported direction as our findings (rs266729) and another 2 were in high LD ($r^2 = 0.77$ and 0.92) with our significant findings (rs822396 and rs1501299, respectively; Fig. 1). In the other 2 studies, African Americans were evaluated on 10 SNPs (31), and Finnish smokers were evaluated on 4 SNPs—all targeted for potential functionality (24); neither study yielded associations with prostate cancer risk. The smaller study size and African ancestry of participants in Beebe-Dimmer and colleagues (31) may contribute to the differences in our findings. For the Finnish study (24), there were 2 overlapping SNPs (rs182052 and rs17366743), one of which yielded a significant association in our study (rs182052, OR = 1.41; 95% CI, 1.07–1.86, $P = 0.02$). Compared with all of the previous studies, we used a more comprehensive tagging approach for SNP selections and none of the previous findings linked SNPs to circulating adiponectin levels and receptor expressions in prostate tumors. For the promoter SNP, rs266729, the rare G allele was associated with an increased risk in our population, which is consistent with other studies that have found this allele to be correlated with lower adiponectin levels (19, 20, 32) and higher prostate cancer risk (23). rs266729 may

Table 3. Multivariate ORs[a] of high-grade and aggressive prostate cancer by *ADIPOQ* sequence variants using the dominant model in the PHS, 1982–2009

		High-grade/poorly differentiated PCa (Gleason 4+3 or 8–10, $n = 276$)				Aggressive PCa (T_3, T_4, N_1, M_1 or boney metastases during follow-up, $n = 252$)			
	SNP	N[b] (cases)	OR[a]	95% CI	P	N[b] (cases)	OR[a]	95% CI	P
1	rs266729								
	CC	138	**1.00**	**(reference)**	—	117	**1.00**	**(reference)**	—
	CG/GG	113	1.09	(0.83–1.44)	0.53	104	1.17	(0.87–1.57)	0.31
2	rs182052								
	GG	125	**1.00**	**(reference)**	—	109	**1.00**	**(reference)**	—
	AG/AA	136	0.94	(0.72–1.23)	0.66	128	1.00	(0.75–1.34)	0.98
3	rs168681205								
	GG	234	**1.00**	**(reference)**	—	209	**1.00**	**(reference)**	—
	AG/AA	35	1.08	(0.72–1.61)	0.71	38	1.26	(0.84–1.88)	0.26
4	rs168681209								
	CC	220	**1.00**	**(reference)**	—	205	**1.00**	**(reference)**	—
	AC/AA	46	0.85	(0.6–1.21)	0.36	38	0.81	(0.55–1.19)	0.27
5	rs822391								
	TT	180	**1.00**	**(reference)**	—	166	**1.00**	**(reference)**	—
	CT/CC	86	0.84	(0.64–1.12)	0.24	76	0.79	(0.59–1.07)	0.13
6	rs12495941								
	GG	112	**1.00**	**(reference)**	—	98	**1.00**	**(reference)**	—
	GT/TT	139	0.93	(0.7–1.22)	0.58	126	0.94	(0.70–1.26)	0.66
7	rs17366568								
	GG	202	**1.00**	**(reference)**	—	176	**1.00**	**(reference)**	—
	AG/AA	54	0.9	(0.65–1.26)	0.55	51	1.00	(0.70–1.42)	0.99
8	rs3821799								
	CC	86	**1.00**	**(reference)**	—	79	**1.00**	**(reference)**	—
	CT/TT	180	0.8	(0.6–1.07)	0.13	164	0.83	(0.61–1.12)	0.22
9	rs3774261								
	GG	103	**1.00**	**(reference)**	—	97	**1.00**	**(reference)**	—
	AG/AA	166	0.89	(0.67–1.17)	0.39	147	0.88	(0.66–1.18)	0.39
10	rs17366743								
	TT	245	**1.00**	**(reference)**	—	215	**1.00**	**(reference)**	—
	CT/CC	12	0.76	(0.4–1.43)	0.39	16	1.23	(0.69–2.19)	0.49
11	rs2082940								
	CC	212	**1.00**	**(reference)**	—	198	**1.00**	**(reference)**	—
	CT/TT	52	0.78	(0.56–1.08)	0.13	46	0.76	(0.53–1.08)	0.12
12	rs7639352								
	CC	143	**1.00**	**(reference)**	—	135	**1.00**	**(reference)**	—
	CT/TT	118	0.98	(0.74–1.29)	0.87	100	0.96	(0.72–1.28)	0.77

[a]Among Caucasians only, adjusted for age at randomization (y, continuous), cigarette smoking status (never, current, and past), and time between blood draw and event date (y, continuous).

[b]May not add up to total number of cases ($n = 276$ and $n = 252$) if genotype is missing for some participants.

map to a polymorphic regulatory element in this region with a nucleotide sequence similar to that of an enhancer element, which may explain its influence on serum levels in several study populations (20).

One strength of this study is the comprehensive evaluation of common polymorphisms in 3 genes in the adiponectin pathway in relation to prostate cancer risk, circulating adiponectin levels, and tissue biomarkers expressed in the prostate tumor; these markers are related to the regulation of insulin resistance, which is a critical function of both the adiponectin and IGF-1 signaling systems, and which may play a role in prostate carcinogenesis. We found 3 variants (rs16861209, rs17366568, and rs3774261) that were associated with both circulating adiponectin levels and IR expression in prostate tumor tissue. Variants in the adiponectin gene have been linked

to plasma levels in various studies (19–21, 32, 33). Our findings are consistent with prior evidence for rs16861209 and rs3774261, for which we also found significant differences in IR expression (Fig. 1). We found a novel SNP, rs17366568 in intron 2, related to plasma levels, which was also related to IR expression in prostate tumor tissue.

In vitro data also show that adiponectin can increase the migration activity of prostate cancer cells through upregulation of several pathways including AdipoR1, p38, NF-κB, and AMPK pathways (34). The absence of associations with high-grade or advanced stage disease suggests that potential pathway(s) for risk may be different than those for fatal disease, providing further evidence for heterogeneous etiologies of this disease (35). Our data add to increasing evidence that suggests prostate cancer may be an insulin-responsive disease, although the specific mechanisms are unclear.

One limitation is that our findings are restricted to Caucasians due to the small number of men of other races/ethnicities in our study population ($n = 128$)—the frequency *ADIPOQ* SNP rs266729 is considerably different across populations (minor allele frequency is 47% in Japanese and 35% in persons of European ancestry; International HapMap). Although we cannot rule out chance findings due to limited sample size and multiple comparisons, several lines of evidence suggest the validity of our findings for *ADIPOQ*. First, we have comprehensive coverage (12 tagging SNPs) of *ADIPOQ* as compared with other published data and the Cancer Genetic Markers of Susceptibility (CGEMS). Second, we observed no significant risk associations with *ADIPOR1/R2*, where we examined a greater number of SNPs ($n = 16$), and chance findings are more likely. Third, we identified multiple genotype–phenotype associations (e.g., prostate cancer risk, circulating plasma levels, and prostate tumor expression) with suggested biologically plausible directions. Also, in a multiple-SNP model with 3 variants covering the regions of significance (rs266729, rs822391, and rs2082940), all SNPs remained significant at the $P < 0.05$ level (data not shown). Fourth, one of the risk SNPs identified in our study (rs822391) was in high LD with one of the highest ranking SNPs from CGEMS stage I (rs822396). Finally, our observed interaction between rs2082940 and plasma adiponectin levels is consistent with previous evidence suggesting (30) that *ADIPOQ* variants may have a stronger impact on risk among individuals with a higher adiposity (or lower circulating adiponectin levels).

In conclusion, we observed multiple polymorphic loci in the adiponectin gene associated with prostate cancer risk. Six loci were associated with circulating adiponectin levels, of which 2 overlapped with risk in the expected opposite direction (rs266729 and rs182052) and an additional 3 associated with IR tumor tissue expression (rs16861209, rs17366568, and rs3774261), suggesting potential biological consequences. We did not find any evidence to suggest that variation in the receptor genes, *ADIPOR1/R2*, plays a major role in prostate cancer risk. In our data, several susceptibility loci in *ADIPOQ* were associated with multiple phenotypes and may represent genomic regions with functional variants involved in prostate carcinogenesis that require replication in other studies and a careful evaluation of underlying mechanisms.

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