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ORIGINAL ARTICLE

Prostate Cancer

Genetic variations of the *ADIPOQ* gene and risk of prostate cancer in Chinese Han men

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Adiponectin secreted by adipose tissue has been implicated in prostate carcinogenesis. Genetic variations in *ADIPOQ* are thought to influence the activity of adiponectin, thus relating to cancer occurrence. In this hospital-based case-control study of 917 prostate cancer (PCa) cases and 1036 cancer-free controls, we evaluated the association of single nucleotide polymorphisms in *ADIPOQ* with risk of PCa and adiponectin levels in Chinese Han men. Variants of *ADIPOQ* were genotyped by Taqman polymerase chain reaction method. The plasma adiponectin concentrations were measured by enzyme-linked immunosorbent assay (ELISA) in a subset of cases and controls. We found that the *ADIPOQ* rs3774262 variant AA genotype was associated with both decreased PCa risk [adjusted odds ratio (OR): 0.66, 95% confidence interval (CI) =0.48–0.92] and increased plasma adiponectin levels ($P = 0.036$ and 0.043), with significant difference by tumor grade, clinical stage, and aggressiveness. A significant interaction between *ADIPOQ* rs3774262 and body mass index was observed in modifying the risk of PCa ($P = 6.7 \times 10^{-3}$). *ADIPOQ* rs266729 and rs182052 were not related to PCa risk or plasma adiponectin levels. Our data support that *ADIPOQ* rs3774262 may affect PCa risk in combination with plasma adiponectin levels in Chinese Han men. It may contribute to the molecular basis for the association between obesity and PCa.

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INTRODUCTION

Prostate cancer (PCa) is the second most commonly diagnosed cancer and the sixth most common cause of cancer-related mortality among men worldwide.[1] Large geographical variations in PCa risk suggest that lifestyle and environmental factors may also contribute to its etiology. In particular, it has been reported that overweight and obese individuals in populations across the Asia-Pacific region had a significantly increased risk of mortality from PCa.[2]

Many studies have investigated the roles of adipose tissue-derived factors (adipokines) as putative molecular mediators between obesity and PCa. As the most abundantly circulating adipokine, levels of circulating adiponectin are negatively correlated with central obesity, body mass index (BMI), visceral fat accumulation, and insulin resistance.[3] Because adiponectin levels are inversely correlated with adiposity, it has been suggested that increased levels of adiponectin may explain the decreased risk of PCa,[4,5] lower Gleason score,[4–7] and early tumor stage.[4–6] Several single nucleotide polymorphisms (SNPs) in *ADIPOQ* encoding adiponectin have been shown to be associated with PCa risk in Caucasians,[8] of which rs266729 had been previously shown to be associated with risk of multiple cancers.[9–12] A nested case-control study further confirmed that rs266729 and rs182052 were associated with not only PCa risk, but also plasma adiponectin levels in Caucasians.[13] Recently, another SNP (rs3774262) in *ADIPOQ* was reported to be associated with both reduced cancer risk and obesity measurements in Chinese populations.[14]

In the current case–control study, we investigated the associations between three SNPs in the *ADIPOQ* gene and PCa risk in Chinese Han men. We also assessed whether plasma adiponectin levels were correlated with these genotypes as potential risk mediators between *ADIPOQ* genetic variations and PCa risk in two subgroups of subjects.

MATERIALS AND METHODS

Study population

We identified 1000 Chinese Han eligible patients with newly diagnosed and histopathologically confirmed primary PCa from Fudan University Shanghai Cancer Center (FUSCC, Shanghai, China) between January 2007 and June 2011; 917 of them (92%) agreed to participate in this study. All patients came from Eastern China. The tumors were histopathologically confirmed as primary PCa assessed independently by two pathologists as routine diagnosis at FUSCC. All pathologic diagnoses were performed according to the World Health Organization (WHO) criteria of PCa, and any histologic diagnosis other than adenocarcinoma subtype of PCa was excluded. As previously described, exclusion criteria also included those cases who suffered from malignancies other than PCa, with a family history of PCa and those had radiotherapy or chemotherapy before recruitment.[15] In the

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present study, PCa were divided into two categories of highly aggressive and less aggressive as defined by the prostate-specific antigen (PSA) levels, pathological staging, and Gleason score. Tumors with PSA serum level >20 ng ml^{-1}, Gleason score of 7 (4 + 3), pathological stages T3 or higher were defined as highly aggressive disease, and the remainder were defined as less aggressive disease.[16] The clinical information, including Gleason score, PSA level at diagnosis, staging and use of medications was determined by reviewing the medical records. During an in-person survey, all cases were interviewed with a questionnaire that collected information about demographic data and potential PCa risk factors including smoking status, occupational exposure, and family history of cancer. Nine hundred and seventeen anthropometric measurements, including weight and height were taken after the interview according to a standardized protocol.

A total of 1151 cancer-free ethnic Han Chinese control subjects were randomly selected from the Taizhou Longitudinal Study during a similar time period[17] and those without response were excluded (n = 104). Frequency matched to cases by age (±5 years) and residence (urban or rural areas). The interviewer-administered questionnaire covered demographic characteristics and environmental exposure factors (e.g. smoking status and use of medications).[17] The Institutional Review Board of FUSCC approved this study, and a written informed consent was obtained from all recruited individuals.

Adiponectin measurement

For laboratory tests, overnight fasting blood samples were provided by the Institutional Tissue Bank at FUSCC (for cases). After the questionnaire interview and physical measurements, 10 ml of overnight fasting blood samples were collected for long-term storage in Taizhou Longitudinal Study (for controls).[17] For a subset of 305 cases and 330 matched controls who had genotype data during a similar time period, plasma adiponectin levels were measured with commercial enzyme-linked immunosorbent assay (ELISA) kit (Abcam Adiponectin Human ELISA Kit, USA) according to the manufacturer's manuals. The minimum detectable dose was 0.7 ng ml^{-1}. The intra- and inter-assay coefficients of variation were 4.5% and 8.7%, respectively.

Single nucleotide polymorphism selection and genotyping

Three SNPs in the *ADIPOQ* gene (rs266729, rs182052, and rs3774262) were selected in an effort to compare results in this investigation with the findings from the published studies of prostate,[8,13] colorectal,[10] and endometrial cancers.[14] These SNPs were originally selected based on their ability to tag the major haplotype blocks in *ADIPOQ* gene, a minor allele frequency of >5% among samples in their study. We chose these significant cancer risk associated SNPs with preferential selection given to SNPs with functional relevance (e.g. plasma adiponectin levels or obesity indicators).

Methods used for DNA isolation and genotyping have been described previously.[18] Briefly, DNA isolation was performed by using the Qiagen Blood DNA Mini KIT (Qiagen Inc., Valencia, CA, USA) with the buffy-coat fraction of the blood samples donated by the participants. The quantification of DNA was determined by a Hybrid Reader (Synergy H4, BioTek Instruments, Inc., USA), and the final concentration of DNA used for genotyping was 2.5 ng ml^{-1}. The TaqMan real-time polymerase chain reaction (PCR) amplification was run with a 7900 HT sequence detector system (Applied Biosystems, Foster City, CA, USA), and the genotypes were determined with the Sequence Detection Software (SDS2.4). The TaqMan genotyping master mix and predesigned primers and probes for each SNP were purchased from ABI (Applied Biosystems). To ensure the accuracy of genotyping results, each 384-well plate included four negative controls (no DNA), and four random-duplicated samples with genotype call rates >98%. Genotyping was randomly repeated in 10% of samples to check for the typing reliability, and the results were 100% in agreement.

Statistical methods

In the present study, we used the WHO cut points for overweight (BMI ≥25 kg m^{-2}) in Asian populations.[19] BMI was calculated as weight (kg) divided by the square of the height (m). Smoking status was divided into smokers and never smokers by whether or not they had smoked for more than 100 cigarettes in their lifetime. Regular use frequency of aspirin or aspirin-containing products was defined as ≥1 pill daily.[20] Men were categorized as statin users if they were taking statins before diagnosis and those who did not have statins use information were considered nonusers.[21]

Pearson's x^2 test was used to evaluate differences in the distributions of the selected demographic characteristics and the *ADIPOQ* genotype frequencies between cases and controls. Hardy-Weinberg equilibrium (HWE) for evaluation of genotype distribution of control subjects was performed by the goodness-of fit x^2 test and the linkage disequilibrium (LD) coefficient r^2 was assessed. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by using univariate and multivariate logistic regression models, respectively. Associations between the genotypes and risk of PCa among subgroups of age, BMI, smoking status, Gleason score, tumor staging, and aggressive grade were further evaluated by stratification analysis. The homogeneity of associations between subgroups was tested by using the Chi-square-based Q test. Haplotype frequencies were estimated using SAS PROC HAPLOTYPE process based on the observed genotypes. P values from the analysis of variance (ANOVA) were used to compare the plasma adiponectin levels by different genotypes.

Two-factor interaction analyses were also carried out by unconditional logistical regression to assess the interactions between the SNPs and environmental factors. The false-positive report probability (FPRP) was calculated based on the previously calculated probability, the power of the current study, and the observed P value, with an effort to detect the false-positive association findings.[22] All statistical tests were two-sided, and a P value of 0.05 was considered significant. All analyses were performed using the SAS Software, version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Characteristics of the study subjects

Among all the subjects, 11 controls failed to be genotyped after repeated assays, likely due to poor quality of DNA. Thus, the final analysis included a total of 917 cases and 1036 controls. The distributions of selected demographic variables between cases and controls were shown in **Table 1**. Approximately 40% of participants were considered overweight, with a significant difference in the BMI between cases and controls (P < 0.001). The two groups were similar with respect to aspirin use and statin use.

Associations between ADIPOQ single nucleotide polymorphisms and prostate cancer risk

The genotype frequencies and their associations with the risk of PCa were summarized in **Table 2**. The genotype frequencies of the three SNPs among the controls were all in agreement with HWE (P = 0.29 for rs266729, 0.83 for rs182052 and 0.09 for rs3774262). In multivariate logistic regression analysis, the rs3774262 was associated with PCa risk

Table 1: Distribution of selected characteristics in PCa cases and controls in Chinese men

Variables	*Cases (n=917)*	*Controls (n=1036)*	*P*
Age (year)	69.1±7.9	68.7±8.9	0.32
BMI (kg m^{-2})	24.4	24.1	<0.001[a]
<25	529 (57.7)	631 (60.9)	0.15
≥25	388 (42.3)	405 (39.2)	
PSA (ng ml^{-1})	27.0	1.01	<0.001[a]
Gleason score, *n* (%)			
≤7 (3+4)	312 (34.0)		
≥7 (4+3)	592 (64.6)		
Stage, *n* (%)			
I	5 (0.5)		
II	423 (46.1)		
III	141 (15.4)		
IV	348 (38.0)		
Adiponectin levels (μg ml^{-1})	7.36±2.11	7.47±2.30	0.54
Smoking status, *n* (%)			
Never	358 (39.0)	397 (38.3)	0.74
Ever	559 (62.0)	639 (61.7)	
Aspirin use, *n* (%)			
Never	670 (73.1)	784 (75.7)	0.95
Ever	215 (23.5)	250 (24.1)	
Statins use, *n* (%)			
Never	745 (81.2)	871 (84.1)	0.52
Ever	149 (16.3)	161 (15.6)	

The numbers of some variables were less than the total number of subjects because some data were unavailable. [a]Medians are presented. *P* value for Mann-Whitney's *U*-test. BMI: body mass index; PSA: prostate-specific antigen; PCa: prostate cancer

Table 2: Associations between *ADIPOQ* SNPs and PCa risk in Chinese men

SNP	*Cases (n=917)*	*Controls (n=1036)*	*P*[a]	*OR (95% CI)*	*P*[b]
rs3774262					
GG	461 (50.3)	493 (47.6)	0.03	1.00	
AG	385 (42.0)	426 (41.1)		0.98 (0.81–1.18)	0.82
AA	71 (7.7)	117 (11.3)		0.66 (0.48–0.92)	0.01
AG/AA	456 (49.7)	543 (52.4)	0.24[c]	0.91 (0.76–1.09)	0.31
GG/AG	846 (92.3)	919 (88.7)		1.00	
AA	71 (7.7)	117 (11.3)	0.01[d]	0.67 (0.49–0.92)	0.01
MAF	0.287	0.319	0.04		
rs266729					
CC	470 (51.3)	527 (50.9)	0.88	1.00	
CG	358 (39.0)	414 (40.0)		0.96 (0.79–1.15)	0.63
GG	89 (9.7)	95 (9.1)		1.02 (0.74–1.40)	0.90
CG/GG	447 (48.8)	509 (49.1)	0.87[b]	0.97 (0.81–1.16)	0.71
CC/CG	828 (90.3)	941 (90.8)		1.00	
GG	89 (9.7)	95 (9.1)	0.69[c]	1.04 (0.77–1.42)	0.79
MAF	0.292	0.292	0.96		
rs182052					
GG	264 (28.8)	279 (27.0)	0.63	1.00	
AG	448 (48.9)	514 (49.6)		0.90 (0.73–1.12)	0.35
AA	205 (22.4)	243 (23.5)		0.86 (0.67–1.11)	0.24
AG/AA	653 (71.2)	757 (73.1)	0.36[b]	0.89 (0.73–1.09)	0.25
GG/AG	712 (77.6)	793 (76.5)		1.00	
AA	205 (22.4)	243 (23.5)	0.56[c]	0.92 (0.74–1.14)	0.43
MAF	0.468	0.483	0.36		

BMI: body mass index; SNPs: single nucleotide polymorphisms; PCa: prostate cancer; CI: confidence interval; MAF: minor allele frequency; OR: odds ratio. [a]Two-sided Chi-square tests were used to calculate differences in the frequency distribution of genotypes, combined genotypes, or alleles between cases and controls; [b]Multivariate logistic regression models were used to evaluate associations between the genotypes and risk of PCa with adjustment for age, smoking status and BMI; [c]For dominant genetic models; [d]For recessive genetic models

in the recessive ($P = 0.01$) models with adjustment for age, smoking status, and BMI. Compared with GG genotype, the rs3774262 variant AA genotype was associated with a decreased risk of PCa (OR: 0.66, 95% CI = 0.48–0.92). However, no associations with risk of PCa were observed for other two SNPs. Eight possible haplotypes based on the observed genotypes frequencies were estimated (**Supplementary Table 1**), there were no significant risk associations for these *ADIPOQ* haplotypes.

Associations between ADIPOQ single nucleotide polymorphisms and plasma adiponectin levels

We evaluated differences in adiponectin levels by genotypes in a subset of controls and cases (**Table 3**). rs3774262 was significantly associated with plasma adiponectin levels in ANOVA analysis ($P = 0.036$ and 0.043). Individuals with rs3774262 variant AA genotype had higher levels of plasma adiponectin than those with the AG or GG genotype. Considering that rs3774262 variant AA genotype was inversely associated with PCa risk, these genotype–phenotype and genotype-risk associations were in the expected inverse directions to each other, suggesting a biological causal relationship. There were no significant associations between the other two SNPs and plasma adiponectin levels.

Stratification analysis

We further evaluated the associations between three SNPs and PCa risk by subgroups, assuming a recessive genetic model (**Table 4**). Those who carried rs3774262 variant AA genotype had a significant decreased risk, and this protective effect was more evident in normal weight subjects (OR: 0.47, 95% CI = 0.31–0.71, $P = 3 \times 10^{-4}$), supported by homogeneity test ($P = 9 \times 10^{-3}$). The decreased risk was also observed in men who were younger, never smokers, Gleason score ≥7 (4 + 3), stage III/IV, and high aggressive cancer. Further homogeneity tests suggested, however, that there were no differences in the risk estimates between these strata except for Gleason score, clinical stage, and aggressiveness.

Gene-environment interaction analysis

To explore potential interactions of *ADIPOQ* rs3774262 with age, smoking status, and BMI, we performed gene-environment interaction analyses (**Supplementary Table 2**). Normal weight Individuals harboring the *ADIPOQ* rs3774262 AA genotype had a reduced cancer risk compared with overweight individuals carrying the GG genotype ($P = 6.7 \times 10^{-3}$), which suggesting an interaction effect between BMI and rs3774262. We did not observe any significant interaction of the genotypes with smoking status or age. To account for chance associations from multiple comparisons, the FPR*P* values for significant findings at different prior probability levels were shown in **Supplementary Table 3**.

DISCUSSION

In this hospital-based case–control study of 917 PCa cases and 1036 cancer-free controls, we evaluated the associations between *ADIPOQ* SNPs (rs3774262, rs266729, and rs182052) with PCa risk and adiponectin levels in Chinese men. We found that *ADIPOQ* rs3774262 variant AA genotype was associated with both decreased PCa risk compared with GG or GG/AG genotypes and increased adiponectin levels in a subset of cases and controls. In addition, we observed a significant interaction between rs3774262 and BMI in modifying the risk of PCa.

There is an increasing interest in the correlation between genetic variations in the *ADIPOQ* gene and carcinogenesis. Kaklamani *et al*.[8] evaluated the associations between five potential functional SNPs in *ADIPOQ* and PCa risk in 465 PCa patients and 411 healthy controls in a Caucasian population and reported that four of the five were risk-associated SNPs, which includes rs266729 that was evaluated in the present study. A nested case–control study from the Physicians' Health Study found two risk-associated SNPs (rs266729 and rs182052) that were also overlapped with plasma adiponectin levels among US male physicians.[13] However, we did not have the statistical evidence to support the associations of these two SNPs with PCa risk or adiponectin levels in the current study. It seems the inconsistent findings for the *ADIPOQ* rs266729 and rs182052 SNPs associated with PCa risk between previous and ours studies may be caused by considerably genetic differences in ethnicities.

In the Physicians' Health Study of Caucasians origin,[13] the minor allele frequency of *ADIPOQ* rs182052 in controls was 0.32, which is different from the observed in ours (0.47). The modest number of advanced cases in their study may also limit the statistical power to examine the genetic variants in relation to advanced PCa. In addition, the smaller study size and younger participants in Kaklamani's study may contribute to the observed differences,[8] compared to ours. In other two studies, five SNPs in *ADIPOQ* including rs266729 were evaluated in 131 African American PCa cases,[23] and four potential functional *ADIPOQ* SNPs including rs182052 in a cohort of Finnish men,[24] but neither study yielded any associations between these two SNPs and PCa risk, a finding similar to our results. Moreover, a genome-wide association study[25] and subsequent validation study[11] demonstrated

Table 3: Associations between *ADIPOQ* SNPs and adiponectin levels in Chinese men

SNP	*Cases (n=305)*		*P*[a]	*Controls (n=330)*		*P*[b]
	n	*Mean±s.d.*		*n*	*Mean±s.d.*	
rs3774262						
GG	152	7.08±2.09	0.036	148	7.13±2.23	0.043
AG	125	7.57±2.10		141	7.71±2.30	
AA	28	7.99±2.06		41	7.90±2.42	
rs266729						
CC	148	7.31±2.10	0.902	159	7.54±2.35	0.812
CG	127	7.42±2.16		140	7.45±2.26	
GG	30	7.43±2.00		31	7.25±2.29	
rs182052						
GG	83	7.61±2.31	0.254	94	7.31±2.36	0.563
AG	151	7.17±2.02		161	7.46±2.11	
AA	71	7.50±2.11		75	7.47±2.30	

[a]*P* value for analysis of variance by different genotypes in cases; [b]*P* value for analysis of variance by different genotypes in controls. SNPs: single nucleotide polymorphisms; SD: standard deviation

Table 4: Stratification analysis for associations between *ADIPOQ* SNPs and PCa risk by recessive genetic model in Chinese men

Variables	*rs3774262 (cases/controls)*		*Adjusted OR*[a] *(95% CI)*	*P*	*P*[b]	*rs266729 (cases/controls)*		*Adjusted OR*[a] *(95% CI)*	*P*	*P*[b]	*rs182052 (cases/controls)*		*Adjusted OR*[a] *(95% CI)*	*P*	*P*[b]
	AA	*GG/AG*				*GG*	*CC/CG*				*AA*	*GG/AG*			
Age (year)															
≤69	35/67	433/478	0.59 (0.38–0.91)	0.016	0.364	44/59	424/486	0.83 (0.55–1.26)	0.384	0.112	88/131	380/414	0.73 (0.54–0.99)	0.045	0.025
>69	36/50	413/441	0.78 (0.49–1.23)	0.281		45/36	404/455	1.36 (0.86–2.16)	0.189		117/112	332/379	1.14 (0.84–1.54)	0.393	
BMI (kg m^{-2})															
<25	34/82	495/549	0.47 (0.31–0.71)	3×10^{-4}	0.009	52/57	477/574	1.11 (0.75–1.65)	0.611	0.811	119/142	410/489	1.00 (0.76–1.32)	0.997	0.483
≥25	37/35	351/370	1.15 (0.70–1.87)	0.585		37/38	351/367	1.03 (0.64–1.66)	0.911		86/101	302/304	0.86 (0.62–1.19)	0.359	
Smoking status															
Never	27/48	331/349	0.58 (0.35–0.96)	0.033	0.591	36/31	322/366	1.25 (0.75–2.07)	0.397	0.294	77/86	281/311	0.97 (0.68–1.37)	0.842	0.709
Ever	44/69	515/570	0.75 (0.50–1.11)	0.151		53/64	506/575	0.94 (0.64–1.38)	0.732		128/157	431/482	0.90 (0.68–1.17)	0.426	
Gleason score															
≤7 (3+4)	37/117	275/919	1.11 (0.75–1.66)	0.598	0.005	28/95	284/941	0.96 (0.61–1.49)	0.842	0.636	75/243	237/793	1.02 (0.76–1.37)	0.904	0.452
≥7 (4+3)	34/117	558/919	0.48 (0.32–0.72)	3×10^{-4}		60/95	532/941	1.09 (0.77–1.53)	0.641		127/243	465/793	0.86 (0.68–1.10)	0.24	
Stage of disease															
I+II	45/117	383/919	0.95 (0.66–1.38)	0.798	0.011	36/95	392/941	0.89 (0.60–1.33)	0.576	0.305	94/243	334/793	0.90 (0.69–1.19)	0.468	0.823
III+IV	26/117	463/919	0.45 (0.29–0.70)	4×10^{-4}		53/95	436/941	1.17 (0.82–1.67)	0.401		111/243	378/793	0.93 (0.72–1.20)	0.56	
Aggressiveness															
Low	23/117	150/919	1.24 (0.76–2.00)	0.388	0.008	18/95	155/941	1.12 (0.66–1.91)	0.677	0.762	40/243	133/793	0.97 (0.66–1.43)	0.889	0.812
High	48/117	696/919	0.55 (0.39–0.78)	9×10^{-4}		71/95	673/941	1.02 (0.74–1.41)	0.901		165/243	579/793	0.90 (0.71–1.23)	0.348	

SNPs: single nucleotide polymorphisms; PCa: prostate cancer; BMI: body mass index; OR: odds ratio; CI: confidence interval. [a]Adjusted for age, smoking status and BMI; [b]*P* value for homogeneity test using the χ^2-based Q-test

that *ADIPOQ* rs266729 and rs182052 SNPs were not associated with colorectal cancer in patients of Ashkenazi Jewish descent or other ethnic groups in Israel, which were consistent with our findings.

The results of the association between *ADIPOQ* rs3774262 and cancer susceptibility have not been consistent for multiple cancers nor for ethnic groups. A case–control study of 648 cases and 659 controls reported no association of *ADIPOQ* rs3774262 with postmenopausal breast cancer risk, BMI, adult weight gain, location of weight gain, or physical activity among US women.[26] While in a study of 1028 endometrial cancer cases and 1003 controls in a Chinese population, Chen *et al*.[14] identified three risk-associated SNPs, of which rs3774262 was in the same reported direction as in our findings, and the other two SNPs (rs1063539 and rs12629945) were in high and moderate LD ($r^2 = 0.84$ and 0.60, respectively) with rs3774262.

ADIPOQ polymorphisms may affect PCa risk through various mechanisms involved in adiponectin. The opposing properties of adiponectin to the majority of other adipokines have resulted in its proposal as an "anticancer" adipokine with respect to PCa.[3] In support of this hypothesis are the findings from case–control studies that found plasma adiponectin levels to be significantly lower in subjects with PCa compared to subjects with benign prostatic hyperplasia or healthy controls.[5,6] For rs3774262, the rare A allele was associated with circulating adiponectin levels and overlapped with PCa risk in the expected opposite direction suggesting potential biological consequences. Genetic studies have previously implicated the *ADIPOQ* locus for a role in influencing variations in adiponectin levels. *ADIPOQ* rs3774261, which is in close proximity of rs3774262 (255 base pair apart) was identified as one of the top hits[27] in a genome-wide association study of plasma adiponectin and replicated in several study populations.[13,28,29] Our results confirmed and extended the evidence implicating adiponectin as a physiological modulator of PCa and point to genetic variations in the *ADIPOQ* gene as a key regulator of this effect.

Besides the main effect of *ADIPOQ* rs3774262, the present study also provided evidence of gene-environment interactions that were more significant than the modest effect of the single variant with a greater predictive power. In gene-environment interaction analyses and stratified analyses, we found that individuals carrying the *ADIPOQ* rs3774262 AA genotype presented different PCa risk in the normal weight and overweight subgroup. The interaction may have some biological plausibility. Large studies on PCa and overweight, most often measured as high BMI, have generally demonstrated a positive risk association.[30] In addition, obesity could potentially influence the activation of *ADIPOQ* and its receptor genes and subsequent PCa risk.[31,32] A positive association of *ADIPOQ* variants with cancer risk may be limited to persons who are overweight.[33] Our observed interactions further suggest that this SNP may be associated with PCa through their complex association with obesity and might reveal a biological mediation of these factors. Lower levels of adiponectin in obese individuals may result in higher levels of prostatic oxidative stress which may explain the clinical association between obesity, hypoadiponectinemia and PCa.[34]

Previous studies show that low prediagnostic serum adiponectin levels were associated with metastatic and fatal PCa.[3–7,31] Consistently, we found that the protective effect of rs3774262 AA genotype was more obvious in subgroups of Gleason score ≥7, stage III/IV and high aggressive cancer in the stratified analysis. FPRP analyses also supported that all these significant findings were noteworthy. Though some other significant findings in the stratified analysis may be by chance, because of the limited sample size in these subgroups, our data did add further evidence that *ADIPOQ* SNPs may play a role in high-grade or advanced stage PCa.

Our study has some inherent limitations. The present study included limited number of SNPs of the *ADIPOQ* gene and lack of additional rigorous replications. On the other hand, the finding that the risk-associated SNP was also linked with adiponectin levels among cases and controls argues against a chance finding. This hospital-based case–control study may have some selection and information biases, which might be minimized by frequency-matching between cases and controls as well as the adjustment for potential confounding factors in the statistical analyses. We realize that information on dietary factors was not ascertained thus limiting our ability to account for dietary and nutritional differences between two groups. There is emerging evidence that certain nutrients may affect PCa incidence and progression.[35] As diets are made of multiple macro- and micro-nutrients, further prospective studies are warranted, particularly those investigating the relationship between whole foods instead of a single nutritional component.[35] Another potential limitation of this study is that the assessment of body weight status was performed only by BMI. Considering that the relation between BMI and percentage body fat is influenced by ethnicity[36] and previous study suggested that both BMI and waist circumference, also its related waist hip ratio might identically predict the presence of multiple metabolic risk factors in Chinese population.[37] Thus, combining of anthropometric measurements and BMI may be superior to using only one of these parameters. Because chance findings cannot be ruled out due to limited sample sizes, particularly in the subgroup analyses, the interactions we have observed warrant further validation in larger studies.

CONCLUSION

We found that among the three selected SNPs, *ADIPOQ* rs3774262 SNP was associated with risk of PCa and circulating adiponectin levels with suggested biologically plausible directions. Our findings also suggest that a positive association of *ADIPOQ* gene variants with PCa may be more pronounced among persons who are overweight. With these implications, further investigation of SNP-cancer risk associations in a large cohort, in which clinical data will be prospectively collected, as well as more detailed *in vitro* and *in vivo* biological functional studies will be helpful to elucidate how exactly the *ADIPOQ* genetic variations influenced PCa development.

AUTHOR CONTRIBUTIONS

CYG and QXL carried out the laboratory experiments and wrote the manuscript. YZ performed the statistical analysis. DWY and QYW conceived of the study, participated in its design and helped to draft the manuscript. MYW and TYS participated in the statistical analysis. YJY, JCW, and LJ contributed reagents/materials/analysis tools. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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