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ORIGINAL ARTICLE

Genetic variants in *ADIPOQ* gene and the risk of type 2 diabetes: a case–control study of Chinese Han population

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Abstract This study was to evaluate the association between *ADIPOQ* gene variants and type 2 diabetes mellitus (T2DM). TaqMan® assay was performed to test the genotypes in T2DM patients ($n = 1{,}105$) and normal control subjects ($n = 1{,}107$). Serum adiponectin concentration was measured by ELISA kit. The variant genotypes rs7649121AT and rs7649121AT/TT, compared with the AA genotype, were associated with a significantly decreased risk of T2DM [Adjusted OR (95% CI) = 0.79(0.66–0.95), 0.80(0.67–0.96), respectively]. In stratified analysis, rs2241767AG genotype increased the risk of T2DM in obesity group [Adjusted OR (95% CI) = 1.32(1.03–1.69)]. Patients with genotype AG/GG of rs2241767 had lower levels of serum adiponectin than those with the genotype AA ($P = 0.044$). Haplotype analyses were not significant. Crossover analysis of rs7649121 and environmental risk factor (obesity) indicated that the protect effect of rs7649121AT/TT maybe offset by the environmental risk. Those who exposed to environmental risk factor (obesity) had a chance to attack T2DM compared with those who did not expose to the two factors [Adjusted OR (95% CI) = 1.64(1.30–2.06)]. This study suggested that the *ADIPOQ* gene polymorphisms were associated with the risk of T2DM in a Chinese Han population.

Keywords *ADIPOQ* gene · Type 2 diabetes mellitus · Adiponectin · Single-nucleotide polymorphism

Introduction

Type 2 diabetes mellitus (T2DM), which accounts for more than 90% of diabetes worldwide, is a common, complex, chronic disease with rapidly growing global importance [1]. The number of people suffered from diabetes worldwide will reach 300 million by 2025. This disease is considered a major medical burden on society. T2DM is a metabolic disorder characterized by insulin resistance and pancreatic β-cell dysfunction. Insulin resistance, caused by impaired insulin actions in adipose tissue, skeletal muscle, and liver is a major reason of T2DM [2]. There are strong evidences that genetic and environmental factors jointly determine its susceptibility.

Adiponectin is the gene product of *ADIPOQ* which has been mapped on chromosome 3q27 and consisted of three exons and two introns. Adiponectin is a 244 amino-acid protein exclusively secreted by adipose tissue [3] and also an adipocytokine which plays a pivotal role in the regulation of insulin action and the metabolism of glucose [4].

Adiponectin has been reported to be linked with several features of the metabolic syndrome and diabetes [5]. Its plasma concentration plays an important role in modulating

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insulin sensitivity and glucose homeostasis [6, 7] and has been found to be at a decreased level in people with T2DM [8]. Various single-nucleotide polymorphisms (SNPs) of the human adiponectin gene (*ADIPOQ* gene) were reported to be associated with obesity, T2DM, and insulin sensitivity in many ethnic groups [9, 10, and 11]. Thus, *ADIPOQ* gene has emerged as a susceptibility gene for T2DM and obesity [12].

In this study, we provided a systematic investigation, including high-density SNPs of the *ADIPOQ* gene to examine potential association between genetic variants in *ADIPOQ* gene and T2DM risk in a Chinese Han population.

Results

Basic characteristics

Between the cases and controls, significant differences existed in SBP, DBP, BMI, TC, TG, FPG, HDL-C, LDL-C, and adiponectin. There were no significant differences in the distribution of age and gender (Table 1).

Logistic regression analysis for the association between variant genotypes of *ADIPOQ* and T2DM

Among the 2,212 subjects, the successfully genotyped rates of the ten SNPs were all more than 97%. The genotype distributions of the ten SNPs satisfied Hardy–Weinberg equilibrium (all $P > 0.05$ in controls) excepted rs7649121 in controls. The variant genotypes rs7649121AT and rs7649121AT/TT were associated with a significantly decreased risk of T2DM [Adjusted OR (95% CI) = 0.79(0.66–0.95), 0.80(0.67–0.96)] compared with the AA genotype (Table 2).

The stratified analysis showed that significantly higher risk of T2DM was observed in obesity people (BMI ≥ 24) who carried the rs2241767AG genotype [Adjusted OR (95% CI) = 1.32(1.03–1.69)] (Table 3). However, a decreasing risk of T2DM was observed in men, youngsters (≤50-year old), and obesity people (BMI ≥ 24) who carried rs7649121AT genotype [Adjusted OR (95% CI) = 0.61(0.47–0.80), 0.59(0.41–0.85), 0.75(0.58–0.97)] (Table 4).

As presented in Table 5, patients with rs2241767AG/GG genotype had lower levels of serum adiponectin than those with the AA genotype of rs2241767 ($P = 0.044$).

Table 1 Baseline characteristics of individuals between the case and control groups

Variables	Case (n = 1,105)	Control (n = 1,107)	P-value
Gender (male:female)	536:569	516:591	0.372
Age (years)	57.07 ± 11.11	57.02 ± 11.39	0.920
SBP (mmHg)	137.99 ± 20.58	127.93 ± 18.13	**<0.001**
DBP (mmHg)	85.02 ± 11.80	79.35 ± 10.42	**<0.001**
BMI (kg/m^2)	24.88 ± 3.55	24.15 ± 3.26	**<0.001**
TC (mmol/l)	5.15 ± 1.36	5.04 ± 0.95	**0.033**
TG (mmol/l)	2.61 ± 2.75	1.59 ± 1.03	**<0.001**
FG (mmol/l)	10.96 ± 4.05	5.07 ± 0.53	**<0.001**
HDL-C (mmol/l)	1.13 ± 0.46	1.42 ± 0.37	**<0.001**
LDL-C (mmol/l)	2.82 ± 0.96	2.57 ± 0.88	**<0.001**
Adiponectin (mg/l)	6.28 ± 1.87	7.14 ± 2.62	**<0.001**

Data were presented as means ± SD. *SBP* systolic blood pressure; *DBP* diastolic blood pressure; *TC* total cholesterol; *TG* triglyceride; *FG* fasting plasma glucose; *HDL-C* high-density lipoprotein cholesterol; *LDL-C* low-density lipoprotein cholesterol

Bold values indicate statistical differences

Haplotype analysis

PHASE (Ver 2.1) was used to determine the haplotypes. The total successfully genotyped subjects were 2,195 in two potential functional SNPs and 2,054 in eight tagging SNPs. Compared with the most common haplotype, there were no halotypes which may increase or decrease risk of T2DM after adjusting for age, gender, and BMI (Tables 6, 7).

Crossover analysis in assessing gene–environmental interaction

Rs7649121 which had significant difference between case and control groups in the single-locus analysis was assessed the gene–environmental interaction by Crossover analysis. Rs7649121AT/TT was defined as the genotype factor (G+), and obesity (BMI ≥ 24) was defined as the environmental factor (E+). The result indicated that those who exposed to environmental risk factor (obesity) had chances to attack T2DM compared with those who did not expose to the two factors [Adjusted OR (95% CI) = 1.64(1.30–2.06)] (Table 8).

Discussion

Currently, there are many studies on the association between *ADIPOQ* gene and T2DM. However, most of the studies are fragmented and lacked of a comprehensive analysis about Chinese population, and only focused on rs1501299 and rs2241766 [13, 14, and 15]. In order to investigate the association of *ADIPOQ* gene and diabetes completely, we studied both potential functional SNPs and tagging SNPs to find gene polymorphisms. Ultimately, two potentially functional SNPs and eight tagging SNPs of *ADIPOQ* were selected to investigate this association in a Chinese Han population.

Table 2 The distribution of genotypes in the case and control group

dbSNPs	Genotypes	Cases *N* (%)	Controls *N* (%)	*P*-value	Crude OR (95% CI)	Adjust OR (95% CI)	*P*-value[b]	*P*-value[c]
rs266729		1,095	1,106					
	CC	590 (53.9)	576 (52.1)	N/A	1.00	1.00	N/A	N/A
	CG	408 (37.3)	441 (39.9)	0.259	0.90 (0.76–1.08)	0.90 (0.75–1.08)	0.257	0.258
	GG	97 (8.8)	89 (8.0)	0.695	1.06 (0.78–1.45)	1.08 (0.78–1.49)	0.616	0.649
	CG/GG[a]	505 (46.1)	530 (47.9)	0.397	0.93 (0.79–1.11)	0.93 (0.78–1.06)	0.415	0.407
	C allele	1,588 (72.6)	1,593 (72.1)	0.713	0.98 (0.85–1.11)			
	G allele	602 (27.4)	619 (27.9)					
rs16861194		1,099	1,107					
	AA	743 (67.6)	755 (68.2)	N/A	1.00	1.00	N/A	N/A
	AG	315 (28.7)	312 (28.2)	0.787	1.02 (0.86–1.24)	1.04 (0.86–1.27)	0.636	0.637
	GG	41 (3.7)	40 (3.6)	0.858	1.04 (0.66–1.62)	1.09 (0.69–1.72)	0.695	0.710
	AG/GG[a]	356 (32.4)	352 (31.8)	0.764	1.02 (0.86–1.23)	1.05 (0.88–1.26)	0.583	0.572
	A allele	1,801 (81.9)	1,822 (82.3)	0.757	1.02 (0.88-1.20)			
	G allele	397 (18.1)	392 (17.7)					
rs182052		1,081	1,095					
	GG	354 (32.7)	349 (31.9)	N/A	1.00	1.00	N/A	N/A
	GA	511 (47.3)	514 (46.9)	0.837	0.98 (0.81–1.19)	0.98 (0.81–1.19)	0.868	0.871
	AA	216 (20.0)	232 (21.2)	0.478	0.92 (0.72–1.16)	0.94 (0.74–1.20)	0.641	0.636
	GA/AA[a]	727 (67.3)	746 (68.1)	0.662	0.96 (0.80–1.15)	0.97 (0.81–1.17)	0.757	0.724
	G allele	1,219 (56.4)	1,212 (55.4)	0.489	0.96 (0.85–1.08)			
	A allele	943 (43.6)	978 (44.6)					
rs16861205		1,098	1,104					
	GG	735 (66.9)	756 (68.5)	N/A	1.00	1.00	N/A	N/A
	GA	331 (30.2)	310 (28.1)	0.321	1.09 (0.91–1.32)	1.14 (0.92–1.35)	0.265	0.248
	AA	32 (2.9)	38 (3.4)	0.558	0.87 (0.53–1.40)	0.88 (0.54–1.45)	0.624	0.652
	GA/AA[a]	363 (33.1)	348 (31.5)	0.440	1.07 (0.89–1.28)	1.09 (0.91–1.31)	0.362	0.326
	G allele	1,801 (82.1)	1,822 (82.5)	0.660	1.04 (0.89-1.21)			
	A allele	395 (17.9)	386 (17.5)					
rs822394		1,086	1,099					
	CC	845 (77.8)	866 (78.8)	N/A	1.00	1.00	N/A	N/A
	CA	230 (21.2)	220 (20)	0.515	1.07 (0.87–1.32)	1.06 (0.83–1.27)	0.811	0.820
	AA	11 (1)	13 (1.2)	0.729	0.87 (0.39–1.95)	0.89 (0.39–2.04)	0.774	0.811
	CA/AA[a]	241 (22.2)	233 (21.2)	0.574	1.06 (0.86–1.24)	1.02 (0.83–1.26)	0.855	0.877
	C allele	1,920 (88.4)	1,952 (88.8)	0.669	1.04 (0.86–1.26)			
	A allele	252 (11.6)	246 (11.2)					
rs12495941		1,085	1,092					
	GG	361 (33.2)	391 (35.8)	N/A	1.00	1.00	N/A	N/A
	GT	541 (49.9)	504 (46.2)	0.115	1.16 (0.96–1.40)	1.16 (0.96–1.41)	0.130	0.121
	TT	183 (16.9)	197 (18.0	0.961	1.01 (0.78–1.29)	0.97 (0.75–1.25)	0.809	0.802
	GT/TT[a]	724 (66.8)	701 (64.2)	0.213	1.12 (0.94–1.33)	1.11 (0.92–1.33)	0.274	0.263
	G allele	1263 (58.2)	1,286 (58.9)	0.648	1.03 (0.91–1.16)			
	T allele	907 (41.8)	898 (41.1)					
rs7649121		1,087	1,101					
	AA	657 (60.4)	604 (54.9)	N/A	1.00	1.00	N/A	N/A
	AT	350 (32.2)	411 (37.3)	**0.007**	**0.78 (0.65–0.93)**	**0.79 (0.66–0.95)**	**0.014**	**0.012**
	TT	80 (7.4)	86 (7.8)	0.343	0.85 (0.62–1.18)	0.85 (0.61–1.19)	0.337	0.327
	AT/TT[a]	430 (39.6)	497 (45.1)	**0.008**	**0.79 (0.67–0.94)**	**0.80 (0.67–0.96)**	**0.013**	**0.008**
	A allele	1664 (76.6)	1,619 (73.6)	**0.021**	**0.85 (0.74–0.98)**			
	T allele	510 (23.4)	583 (26.4)					

Table 2 continued

dbSNPs	Genotypes	Cases *N* (%)	Controls *N* (%)	*P*-value	Crude OR (95% CI)	Adjust OR (95% CI)	*P*-value[b]	*P*-value[c]
rs2241767		1,084	1,103					
	AA	514 (47.4)	559 (50.7)	N/A	1.00	1.00	N/A	N/A
	AG	467 (43.1)	452 (41.0)	0.195	1.12 (0.94–1.34)	1.13 (0.94–1.35)	0.197	0.200
	GG	103 (9.5)	92 (8.3)	0.206	1.22 (0.89–1.65)	1.14 (0.83–1.57)	0.404	0.375
	AG/GG[a]	570 (52.6)	544 (49.3)	0.127	1.14 (0.96–1.34)	1.12 (0.95–1.34)	0.172	0.167
	A allele	1,495 (69.1)	1,570 (71.2)	0.110	1.11 (0.98–1.26)			
	G allele	673 (30.9)	636 (28.8)					
rs3821799		1,079	1,101					
	TT	276 (25.5)	301 (27.3)	N/A	1.00	1.00	N/A	N/A
	TC	620 (57.5)	599 (54.4)	0.231	1.13 (0.93–1.38)	1.14 (0.93–1.40)	0.206	0.206
	CC	183 (17)	201 (8.3)	0.957	0.99 (0.77–1.29)	1.04 (0.77–1.33)	0.771	0.738
	TC/CC[a]	803 (74.5)	800 (62.7)	0.352	1.09 (0.91–1.32)	1.12 (0.92–1.36)	0.278	0.227
	T allele	1,172 (54.4)	1,201 (54.6)	0.877	1.01 (0.90–1.14)			
	C allele	986 (45.6)	1,001 (45.4)					
rs1063539		1,085	1,098					
	GG	529 (48.8)	570 (51.9)	N/A	1.00	1.00	N/A	N/A
	GC	460 (42.4)	446 (40.6)	0.239	1.11 (0.93–1.32)	1.11 (0.92–1.32)	0.269	0.273
	CC	96 (8.8)	82 (7.5)	0.151	1.26 (0.92–1.73)	1.16 (0.83–1.61)	0.385	0.379
	GC/CC[a]	556 (51.2)	528 (48.1)	0.140	1.13 (0.96–1.34)	1.11 (0.94–1.32)	0.216	0.207
	G allele	1,518 (70.0)	1,586 (72.3)	0.098	1.12 (0.98–1.27)			
	C allele	652 (30.0)	610 (27.7)					

[a] Analyzed under dominant model

[b] Adjusted for age, gender, and BMI

[c] Thousand times permutation test

Bold values indicate statistical differences

Table 3 Stratified analysis of ADIPOQ gene rs2241767 genotypes with T2DM susceptibility

Stratified characteristics	Cases			Controls			AA	AG		GG	
	AA (%)	AG (%)	GG (%)	AA (%)	AG (%)	GG (%)		*P*-value*	OR (95% CI)*	*P*-value*	OR (95% CI)*
Gender											
Male	265 (50.4)	216 (40.9)	46 (8.7)	257 (49.9)	219 (42.5)	39 (7.6)	1.00	0.900	0.98 (0.76–1.28)	0.464	1.19 (0.74–1.90)
Female	249 (44.7)	251 (45.1)	57 (10.2)	302 (51.4)	233 (39.6)	53 (9)	1.00	0.057	1.28 (0.99–1.66)	0.697	1.09 (0.70–1.69)
Age (year)											
≤50	148 (51)	122 (42.1)	20 (6.9)	144 (51.4)	110 (39.3)	26 (9.3)	1.00	0.721	1.06 (0.74–1.53)	0.408	0.76 (0.39–1.47)
>50	351 (46.1)	332 (43.6)	79 (10.3)	415 (50.5)	340 (41.4)	66 (8.0)	1.00	0.191	1.15 (0.92–1.4)	0.140	1.31 (0.91–1.89)
BMI											
Normal	224 (51.7)	170 (39.3)	39 (9)	278 (49.7)	228 (40.8)	53 (9.5)	1.00	0.669	0.94 (0.72–1.23)	0.581	0.88 (0.55–1.39)
Overweight/ obesity	277 (44.8)	284 (46.0)	57 (9.2)	269 (51.7)	213 (41.0)	38 (7.3)	1.00	**0.027**	**1.32 (1.03–1.69)**	0.112	1.47 (0.93–2.30)

* Adjusted for age, gender, and BMI

Bold values indicate statistical differences

In the single-locus analysis, we observed statistically significant differences between case and control subjects in the genotype distributions of rs7649121. People with rs7649121AT and rs7649121AT/TT genotypes were associated with a significantly decreased risk of T2DM after adjusting for age, gender, and BMI [Adjusted OR (95% CI) = 0.79(0.66–0.95), 0.80(0.67–0.96)] compared with rs7649121 AA genotype. The *P*-value of those

Table 4 Stratified analysis of ADIPOQ gene rs7649121 genotypes with T2DM susceptibility

Stratified characteristics	Cases			Controls			AA	AT		TT	
	AA (%)	AT (%)	TT (%)	AA (%)	AT (%)	TT (%)		*P*-value*	OR (95% CI)*	*P*-value*	OR (95% CI)*
Gender											
Male	318 (60.0)	170 (32.1)	42 (7.9)	257 (50)	217 (42.2)	40 (7.8)	1.00	**0.000**	**0.61 (0.47–0.80)**	0.431	0.83 (0.51–1.32)
Female	339 (60.9)	180 (32.3)	38 (6.8)	347 (59.1)	194 (33)	46 (7.8)	1.00	0.967	0.99 (0.76–1.29)	0.497	0.85 (0.52–1.37)
Age (year)											
≤50	172 (57.7)	103 (34.6)	23 (7.7)	129 (46.6)	125 (45.1)	23 (8.3)	1.00	**0.005**	**0.59 (0.41–0.85)**	0.343	0.73 (0.38–1.40)
>50	469 (61.4)	239 (31.3)	56 (7.3)	473 (57.5)	286 (34.8)	63 (7.7)	1.00	0.194	0.86 (0.69–1.07)	0.515	0.87 (0.59–1.30)
BMI											
Normal	264 (60.3)	141 (32.2)	33 (7.5)	316 (56.6)	205 (36.7)	37 (6.6)	1.00	0.199	0.83 (0.63–1.09)	0.844	1.05 (0.63–1.74)
Overweight/ obesity	371 (60.2)	202 (32.8)	43 (7.0)	278 (53.6)	195 (37.6)	46 (8.8)	1.00	**0.027**	**0.75 (0.58–0.97)**	0.112	0.69 (0.45–1.09)

* Adjusted for age, gender, and BMI

Bold values indicate statistical differences

Table 5 Adiponectin level of different genotypes of rs2241767 and rs7649121 in cases and control groups

	Cases (mg/l)	Controls (mg/l)
rs2241767	1,084	1,103
AA	6.39 ± 2.11	7.12 ± 2.65
AG/GG	6.15 ± 1.60[a]	7.15 ± 2.57
P-value	**0.044**[a]	N/A
rs7649121	1,087	1,101
AA	6.24 ± 1.77	7.06 ± 2.62
AT/TT	6.34 ± 2.02	7.25 ± 2.62
P-value	N/A	N/A

Data were means ± SD

[a] $P = 0.044$, versus AA genotype from independent-samples *t* test in cases

Bold values indicate statistical differences

variants were still significant after 1,000 times permutations ($P = 0.012$ and 0.008). Furthermore, we found the association between lower risk of T2DM and rs7649121A>T SNP was more evident among males, younger subjects (≤50-year old), and obesity group (BMI ≥ 24) in the stratified analysis. Sanghera et al. [16] had reported that none of tagSNPs in the *ADIPOQ* gene was associated with T2DM susceptibility in single-site analysis. However, haplotype analysis provided strong evidence of association between the loci with T2DM. In the Sanghera Diabetes Study in Indian, rs182052 and rs7649121 were revealed a significant protective association by the GA haplotype with T2DM ($P = 0.009$, permutation $P = 0.026$). We also found the protective effect of rs7649121 to T2DM in our study. However, we did not find any statistical association between the haplotype of these two SNPs and T2DM risk. Perhaps the difference caused by the ethnic differences or the sample size. Until now, there has been no other analysis of the relationship between this SNP and the T2DM. Therefore, additional studies are needed for confirming these data.

Furthermore, in the stratified analysis, we found rs2241767G variant was associated with an increased risk of T2DM among the obesity subject (BMI ≥ 24), which suggested that rs2241767G variant may be involved in the attack of T2DM with obesity. Research on this SNP has not been reported in other ethnic groups. We only found Lan

Table 6 ORs and 95% CIs for the association between two functional SNPs haplotypes and diabetes in the case–control study

Haplotypes[a]	Case		Control		*P*[b]	Adjust OR (95% CI)
	Chromosome no.	%	Chromosome no.	%		
CA	1192	54.7	1209	54.6	N/A	1.00
GA	593	27.2	611	27.6	0.92	0.99 (0.86–1.14)
CG	387	17.8	384	17.3	0.54	1.05 (0.89–1.24)
GG	6	0.3	8	0.5	0.86	0.91 (0.31–2.64)

[a] Loci of SNPs are written 5′ to 3′ and include the following SNPs: rs266729, rs16861194

[b] Adjusted for age, gender, and BMI

Table 7 ORs and 95% CIs for the association between eight tagging SNPs haplotypes and diabetes in the case–control study

Haplotypes[a]	Case		Control		P[b]	Adjust OR (95% CI)
	Chromosome no.	%	Chromosome no.	%		
CAGTGGCT	343	17.3	334	15.8	N/A	1.00
CTGGAAGC	244	12.3	277	13.2	0.31	0.89 (0.69–1.12)
CAGTGAGC	175	8.7	200	9.5	0.34	0.88 (0.67–1.14)
AAGGGAGC	147	7.3	159	7.5	0.47	0.90 (0.68–1.19)
CAAGAAGT	139	6.9	157	7.4	0.35	0.87 (0.66–1.16)
Others	950	47.5	983	46.6	0.56	0.95 (0.79–1.14)

[a] Loci of SNPs are written 5′ to 3′ and include the following SNPs: rs822394, rs7649121, rs16861205, rs12495941, rs182052, rs2241767, rs1063539, and rs3821799

[b] Adjusted for age, gender, and BMI

Table 8 Crossover analysis in assessing gene–environment (obesity) interaction of rs7649121

Gene G	Environmental E	Case (1,044) *N* (%)	Control (1,076) *N* (%)	P*	OR (95% CI)*
+	+	242 (23.2)	241 (22.4)	0.111	1.22 (0.96–1.56)
+	−	173 (16.6)	242 (22.5)	0.237	0.86 (0.66–1.11)
−	+	366 (35.0)	277 (25.7)	0.000	**1.64 (1.30–2.06)**
−	−	263 (25.2)	316 (29.4)	N/A	1.00

* Adjusted for age and gender

Bold values indicate statistical differences

et al. [17] Chinese Sichuan population study, which included 414 T2DM cases and 405 controls, showed that rs2241767 polymorphism was not associated with T2DM. Whereas, due to the different life and environment backgrounds, this association might be exist in southern Chinese Han population according to our study. Our study demonstrated the probable association between rs2241767, obesity, and T2DM in a larger sample size.

Treatment with thiazolidinediones will increase the adiponectin mRNA levels in adipocytes and adipose tissues. Because rs7649121 and rs2241767 had shown the association with T2DM in the single-locus analysis and stratified analysis, we calculated the adiponectin level in different genotypes of the two SNPs. As presented, patients with the AG/GG genotype of rs2241767 had lower levels of serum adiponectin than those with the AA genotype. It could be tentatively concluded that this SNP may be a genetic marker of T2DM by its phenotype, the levels of adiponectin secretion. We did not find any association between adiponectin level and different genotypes of rs7649121. Woo et al. [18], who studied the white African-American people, stated that rs16861194A, rs182052G, and rs1501299T were associated with higher adiponectin level. In all African-Americans, rs16861194A and rs1501299T were also marginally or significantly associated with higher adiponectin level. We failed to find the association in Chinese Han people, which might be due to differences in type 2 diabetes susceptibility variants. Indeed, some *ADIPOQ* mutations were associated with decreased adiponectin levels in T2DM patients. Adiponectin can regulate glucose levels, insulin action, and lipid metabolism, but how *ADIPOQ* variations decreased the adiponectin level is still unknown.

Haplotypes of *ADIPOQ* gene in the two potential functional SNPs and eight tagging SNPs were analyzed between case and control subjects. With a pity, there was no statistic difference even after adjusting for age, gender, and BMI. The effect of rs7649121 and rs2241767 may be counteracted by age, gender, and obesity. The Haplotypes result of the two potential functional SNPs was in accordance with Wang et al. [19] Chinese ShangHai population. In addition, Wang found rs16861194 (−11426 A>G) in the putative promoter of *ADIPOQ* was associated with T2DM [$P = 0.007$; OR (95% CI) = 1.29(1.08–1.55)] in a Han Chinese. We failed to find this association. The difference maybe caused by statistic bias and genotyping method difference. The age and gender differences in constituent maybe also led to this different result. Up to now, there were no reports about the eight tagging SNPs investigating the association between the haplotypes of SNPs and the risk of T2DM. The exact mechanism of this haplotype effect was not completely clarified. Therefore, potential

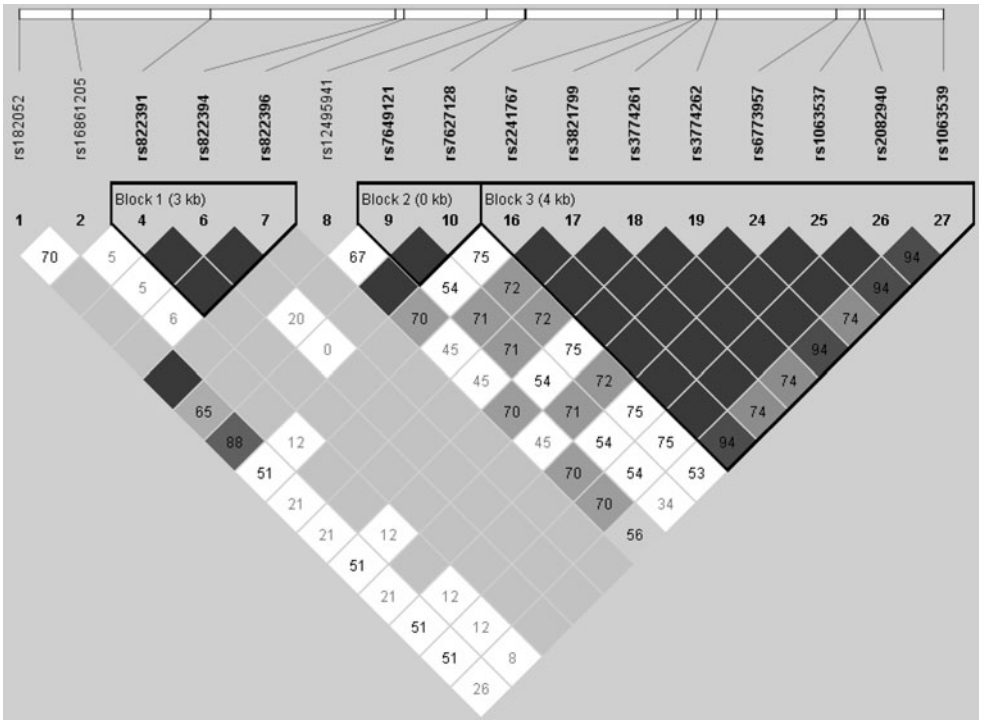

Fig. 1 Linkage disequilibrium (LD) plot of *ADIPOQ* gene region using eight common SNPs with minor allele frequency ≥ 0.05 from HapMap SNP database CHB population. The plot was generated by Haploview 3.2 with the r^2 Color Scheme ($r^2 = 0$, $0 < r^2 < 1$, and $r^2 = 1$ were shown by *white*, shades of *gray*, and *black* color, respectively) and pairwise r^2 values were showed in *diamonds*

locus–locus interactions of SNPs of *ADIPOQ* gene are needed to be further elucidated in future studies.

The crossover analysis of rs7649121 and environmental risk factor (obesity) indicated that those who exposed to environmental risk factor had a chance to develop T2DM compared with those who did not expose to the two factors. However, those who with mutation genotypes only or simultaneously exposed to mutation and environmental risk factor had no significant chance to attack diabetes. Therefore, the role of genetic and environmental risk might be offset. In the single-locus analysis, we found rs7649121AT and rs7649121AT/TT can reduce the risk of T2DM, but this protective effect did not show in the crossover analysis. This may be attributed to the role of genetic function in disease development is weak or the sample size is relatively low in the crossover analysis.

Like all other case–control studies, inherent biases existed. The study subjects may not be fully representative of the general population. So this limitation may influence the observed association. Although less than 3% of each loci of the DNA samples failed for genotyping, this may cause some selection bias. The association between *ADIPOQ* variants and T2DM risk were estimated by computing the odds ratios (ORs) and 95% confidence intervals (CIs) from logistic regression analyses with adjustment for age, gender, and BMI. Other limitations relate to the lack of confirmation of our findings in other populations.

In summary, our case–control study provided the evidence that polymorphisms rs7649121A>T and rs2241767A>G in the *ADIPOQ* gene were associated with the risk of T2DM in a Chinese Han population. Our findings are needed to be validated by further functional studies as well as well-designed larger molecular epidemiological studies with diverse ethnic populations.

Materials and methods

Subjects

The study included 2,212 unrelated Han Chinese subjects who agreed to get involved. 1,105 were T2DM patients and another 1,107 were diabetes-free controls. The cases included all eligible patients newly diagnosed with T2DM according to World Health Organization's criteria for diabetes. The subjects were recruited consecutively between March 2008 and August 2009 at in-patient departments from three affiliated hospitals of Nanjing medical university. A diagnosis of T2DM required either a fasting plasma glucose (FPG) $\geq$ 7.0 mmol/l (126 mg/dl) or a 2-h glucose $\geq$ 11.1 mmol/l (200 mg/dl) after an oral glucose tolerance test (OGTT). All the patients were tested by glutamic acid decarboxylase autoantibodies (GAD) and islet-cell antibodies (ICA512) (RSI Company, UK) to get rid of type 1 diabetes. Controls enrolled with routine annual health examinations were non-diabetic determined by an OGTT (75 g of glucose) which was performed according to WHO's criteria (516 male and 591 female; aged 57.02 $\pm$ 11.39 years). These control subjects had no history of T2DM and were frequency-matched to the cases

Table 9 Primers and probe sequences for the amplification of *ADIPOQ* gene SNPs

SNP	Location		Primers and probes
rs266729	NearGene-5′		
		Sense	ATTCTGTTTTGGATGTCTTGTTG
		Antisense	CTTGGACTTTCTTGGCACG
		Probe 1	ATCCTGCCCTTCAA
		Probe 2	TCCTGCGCTTCAA
rs16861194	NearGene-5′		
		Sense	TGTCTTGTTGAAGTTGGTGCTG
		Antisense	GACTTTCTTGGCACGCTCAT
		Probe 1	TGAATTAAATTACGACCCC
		Probe 2	TGAATTAAACTACGACCC
rs182052	Intron		
		Sense	CAGCCCCAAGAGAGAAAGG
		Antisense	GAATTGGACTTCATCTGTGGAC
		Probe 1	CTGAATTTTACCCAGTTC
		Probe 2	CTGAATTTTGCCCAGTT
rs16861205	Intron		
		Sense	GAGAAAAGCCTGGCATATAGTG
		Antisense	TAACCTTCAGCATCCACAGC
		Probe 1	TGTTTCTAAGGCATCC
		Probe 2	TGTTTCTGAGGCATC
rs822394	Intron		
		Sense	TCTTTTACAATCAGAGTCCGTTC
		Antisense	CCACTAATAGGTGCGATCAGC
		Probe 1	TGCTAATCACACTCTT
		Probe 2	TGCTAATCCCACTCTT
rs12495941	Intron		
		Sense	GCAGTGAGGTACCATTATTTCC
		Antisense	CTCCTGATACATATCCCCACAT
		Probe 1	AGGGCATACCTTAACTA
		Probe 2	AAGGGCATAACTTAACTA
rs7649121	Intron		
		Sense	GAGACATTCTTGGAGTTGAGTATTG
		Antisense	CTGGAATCCCACTCCTATGC
		Probe 1	TATACTACCCTCTTCACGTG
		Probe 2	TATACTACCCACTTCACGTG
rs2241767	Intron		
		Sense	TCTTTCATCACAGACCTCCTACA
		Antisense	GCACCATCTACACTCATCCTTG
		Probe 1	CAACCTGAAGTGATT
		Probe 2	CAACCTGAGGTGATT
rs3821799	Intron		
		Sense	CTGCCTTTGGGGAACTCTT
		Antisense	CATCAGGTCCACGGTGAGTAT
		Probe 1	TTTCTTGTGGTAACCAC
		Probe 2	CTTTCTTGTAGTAACCAC
rs1063539	3′UTR		
		Sense	CTCTGGGGCAGGGTTATTC
		Antisense	TCAAAGCATCACAGGACCATT
		Probe 1	ACAGAGACAGTCAACT
		Probe 2	ACAGAGAGAGTCAACT

based on age (±5 years) and gender. Each participant was scheduled for an interview; a structured questionnaire was administered by interviewer to collect information on demographic data and environmental exposure history including tobacco smoking and drinking. After interview, an approximately 5 ml venous blood sample was collected from each participant. Genomic DNA was extracted from peripheral blood using proteinase K and phenol/chloroform. Serum adiponectin was detected by ELISA (RapidBio Company, USA). This study was approved by the Research Ethics Committee of Nanjing Medical University.

Polymorphisms selection

Two potentially functional SNPs (rs16861194 and rs266729) of *ADIPOQ* gene located at nearGene-5′ with minor allele frequency (MAF) ≥ 0.05 in the Chinese Han population were identified from NCBI dbSNPs database (http://www.ncbi.nlm.nih.gov/). The selection of tagging SNPs was performed with the tagger programme implemented in Haploview (Ver 3.2). Genotype data were downloaded from Hapmap (http://www.hapmap.org). Haploview was used to assess linkage disequilibrium for all possible SNP pairs by determining r^2 [20]. Eight tagging SNPs were selected based on the pair-wise tagging ($r^2 \geq 0.80$, MAF ≥ 0.05) using genotype data from the unrelated Hapmap CHB individuals [21]. Thus, any marker that was not eventually chosen as a tagging SNP was already strongly correlated with at least one of the tagging markers with $r^2 \geq 0.8$. The 28 SNPs in *ADIPOQ* gene could be tagged by eight tagging SNPs (rs2241767, rs822394, rs1063539, rs12495941, rs16861205, rs182052, rs3821799, and rs7649121), including seven at intron and one at 3′ untranslated region (UTR), and the coverage percentage of the *ADIPOQ* gene by the eight tag SNPs genotyped was 28.57 (Fig. 1).

Genotyping assay

Genomic DNA was extracted from peripheral blood samples of all subjects. 5′-Nuclease TaqMan® assay was used to genotype the polymorphisms in 384-well plates on ABI PRISM 7900HT Sequence Detection system (Applied Biosystems, Foster City, CA). The primers and probes of TaqMan® assays were designed using Primer Express Oligo Design software v2.0 (ABI PRISM) and were available upon request as TaqMan® Pre-Designed SNP Genotyping Assays. The primer sequences used were shown in Table 9. PCR reactions were performed in a 5 μl reaction mixture containing 5 ng DNA, 2.5 μl 2× TaqMan® Universal PCR Master Mix, and 0.083 μl 40× Assay Mix. The PCR conditions were 50°C for 2 min, 95°C for 10 min, 95°C for 15 s, and then 60°C for 1 min, and forty cycles of real-time PCR were performed. Individual genotypes identification was performed by SDS software 2.0 (ABI). Each plate contained two samples from a same individual as positive controls and two blank samples as negative controls for the genotyping quality confirmation. There was 100% consistency in a 5% sample of duplicate testing.

Statistical analysis

The distribution of the general characteristic and genotype frequencies between the T2DM cases and T2DM-free controls were compared using two-sided χ^2-test or Student's *t*-tests. Among controls, genotype frequencies for each SNP were tested for Hardy–Weinberg equilibrium and studied in goodness of fit test. The ORs and 95% CIs of the T2DM associated with the polymorphisms were used by both univariate and multiple logistic regression analysis. Adjustment variables were age, gender, and BMI. Haplotype analyses were based on the PHASE (ver 2.1) Bayesian algorithm. Two-tailed *P*-value less than 0.05 was considered statistically significant. All the statistical analysis was carried out by Statistical Analysis System software (Version 9.1.3; SAS Institute, Cary, NC). Thousand time permutation test was analyzed by Stata 10.0 (StataCorp LP). BMI ≥ 24 kg/m^2 was defined as overweight and obesity [22]. Homozygotes (1/1) and heterozygotes (1/0) for the risk allele and homozygotes for the non-risk allele (0/0) were encoded to an ordered categorical variable for the genotypes (2, 1, and 0). The dominant model was defined as (1/1 + 1/0) versus 0/0.

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