

Association of two Common Single Nucleotide Polymorphisms (+45T/G and +276G/T) of *ADIPOQ* Gene with Coronary Artery Disease in Type 2 Diabetic Patients

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ABSTRACT

Background: Adiponectin, an adipocyte-secreted hormone, is known to have anti-atherogenic, anti-inflammatory, and anti-diabetic properties. In the present study, the association between two common single nucleotide polymorphisms (SNPs) (+45T/G and +276G/T) of *ADIPOQ* gene and coronary artery disease (CAD) was assessed in the subjects with type 2 diabetes (T2DM). **Methods:** Genotypes of two SNPs were determined by polymerase chain reaction-restriction fragment length polymorphism in 200 subjects with T2DM (100 subjects with CAD and 100 without CAD). **Results:** The frequency of TT genotype of +276G/T was significantly elevated in CAD compared to controls ($\chi^2=7.967$, $P=0.019$). A similar difference was found in the allele frequency of +276G/T between two groups ($\chi^2=3.895$, $P=0.048$). The increased risk of CAD was associated with +276 TT genotype when compared to reference GG genotype (OR=5.158; 95% CI=1.016-26.182, $P=0.048$). However, no similar difference was found in genotype and allele frequencies of SNP +45T/G between two groups. There was a CAD protective haplotype combination of +276 wild-type and +45 mutant-type allele (276G-45G) (OR=0.37, 95% CI=0.16-0.86, $P=0.022$) in the subject population. **Conclusion:** Our findings indicated that T allele of SNP +276G/T is more associated with the increased risk of CAD in subjects with T2DM. Also, a haplotype combination of +45G/+276G of these two SNPs has a protective effect on the risk of CAD. **DOI: 10.7508/ibj.2016.03.004**

Keywords: Adiponectin, Type 2 diabetes, Coronary artery disease, Single nucleotide polymorphisms

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INTRODUCTION

The incidence of coronary artery disease (CAD) is two to four times more frequent in diabetic patients than to non-diabetic subjects, which indicates the major cause of mortality and morbidity in this population^[1]. The circulating levels of adiponectin, an adipocyte-derived hormone, have been

demonstrated to be decreased in patients with the phenotypes of metabolic syndrome^[2], including obesity^[3], type 2 diabetes (T2DM)^[4], and insulin resistance^[5]. Furthermore, it has been shown that low plasma levels of adiponectin are also correlated with CAD^[6,7].

The adiponectin-coding gene, *ADIPOQ*, is located on chromosome 3q27, a genomic region identified as a

susceptibility locus for the metabolic syndrome, T2DM and CAD through genome-wide scans^[8,9]. The effects of two common single nucleotide polymorphisms (SNPs) of *ADIPOQ* gene, T/G substitution in exon 2 (+45T/G) and G/T substitution in intron 2 (+276G/T), on cardiovascular disease have been investigated in Caucasian and Korean populations^[9,10]. The G allele at the +45T/G polymorphism has been shown to be associated with higher serum adiponectin concentrations^[11,12] and to improve insulin sensitivity^[13].

A study has reported the protective effect of the G allele at the +45T/G polymorphism on the risk of CAD in European populations^[14]. On the other hand, results from another study conducted on Italian population^[15] demonstrated that +276G/T polymorphism is associated with CAD risk in diabetic patients. In Asia, +276G/T polymorphism is not only correlated with T2DM^[16] and cardiovascular disease in Japanese patients^[17] but also with the metabolic syndrome in Korean subjects^[18]. However, this variant was not correlated with T2DM or insulin resistance in another Korean population^[19]. Therefore, it is likely that due to differences in clinical or ethnic backgrounds of the studied populations, the results of previous *ADIPOQ* genetic association studies are contradictory^[14-19]. In addition, little is known about the association between two common SNPs of the *ADIPOQ* gene and the risk of CAD in Iranian subjects with T2DM. The aim of the current study was to investigate the association between two common SNPs (+45T/G and +276G/T) of the *ADIPOQ* gene and CAD in Iranian subjects with T2DM.

MATERIALS AND METHODS

Patients

In this study, 200 subjects with T2DM were selected based on American Diabetes Association (ADA) and divided into two groups: 100 patients with CAD (CAD cases) and 100 patients without CAD (controls). All patients enrolled in the study were Iranians with ancestry in the Khuzestan Province, Iran. The carotid angiography of all patients was carried out at the Department of Cardiology, Ahvaz Golestan Hospital (Ahvaz, Iran). All the recorded data of T2DM patients, who agreed to participate in the study, were reviewed. Criteria for the selection of T2DM and CAD patients have been described elsewhere^[20]. The selected criterion for CAD, confirmed by coronary angiography, was $\geq 50\%$ stenosis of at least one segment of a major coronary artery or its main branches. The control group consisted of T2DM patients aged over 35 years old, who had normal

exercise tolerance test and negative history of CAD. Smokers, alcoholism, pregnant individuals, subjects under insulin therapy, and those with chronic liver and renal disease as well as acute infectious diseases, such as active diabetic foot, were not included. Demographic parameters of the subjects, including age, gender, duration of diabetes, height, weight, BMI, systolic and diastolic blood pressures were measured as described previously^[20]. Serum levels of glucose, total cholesterol, HDL cholesterol and triglyceride were measured by enzymatic methods. Friedewald's formula^[21] was used to calculate the level of LDL cholesterol. Homeostasis model assessment of insulin resistance was used to determine insulin resistance as well as fasting serum insulin, and adiponectin was measured by ELISA as previously described elsewhere^[20]. The study was approved by the Clinical Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, and all subjects gave a written informed consent. Venous blood samples from all patients were collected in EDTA-containing tubes following an overnight fasting of at least 12 h.

Genotyping the +45T/G and +276G/T polymorphisms of *ADIPOQ* gene

Genomic DNA was extracted from the whole blood using a DNA purification kit (SinaClon BioScience Co., Tehran, Iran). *ADIPOQ* +45T/G and +276G/T polymorphisms were determined using polymerase chain reaction-restriction fragment-length polymorphism analysis. The DNA fragment containing each SNP was amplified using the following primers:

+45T/G (rs 2241766):

5'-GCA GCT CCT AGA AGT AGA CTC TG-3' (forward) and 5'-TCT GTG ATG AAA GAG GCC AG-3' (reverse)

+276G/T (rs 1501299):

5'-GTC TCT CCA TGG CTG ACA GT-3' (forward) and 5'-GGT GAA GAT GGG AAA GGG GA-3' (reverse)

The amplification reaction was performed in a 20- μ l volume containing 12.5 μ l commercially available PCR premix (AccuPower PCR PremiX; Bioneer, Daejeon, Korea), 2.0 μ l (10 pmol/ μ l) forward and reverse primers, 2.0 μ l (50 ng/ μ l) genomic DNA and 6.5 μ l sterile nuclease free water. Thermocycling conditions for +45T/G consisted of an initial denaturation at 94°C for 5 min, followed by 30 cycles of 30 s at 94°C, 30 s at 60°C and 30 s at 72°C with a final extension at 72°C for 5 min. The resulting 367-bp PCR product for +45T/G was digested with *Sma*I (New England Biolabs, Beverly, MA) at 37°C for 12 hours

according to the manufacturer's protocol. The genotype was defined by the absence (T) or presence (G) of the *SmaI* restriction site. Thermocycling conditions for +276G/T consisted of an initial denaturation at 94°C for 5 min, followed by 30 cycles of 30 s at 94°C, 30 s at 58°C, and 30 s at 72°C with a final extension at 72°C for 5 min. The resulting 504-bp PCR product for +276G/T was digested with *BsmI* (New England Biolabs, Beverly, MA) at 37°C for 12 hours according to the manufacturer's protocol. The genotype was defined by the presence (G) or absence (T) of the *BsmI* restriction site. For genotyping, digested fragments were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining.

Statistical analysis

All statistical analyses were performed with the SPSS version 15.0 software (SPSS, Inc., Chicago IL, USA). Continuous variables were expressed as mean±SD and compared using student's *t*-test. All frequencies were estimated using the gene counting method, and both polymorphisms were tested for Hardy-Weinberg's equilibrium using the chi-squared test. Categorical variables were presented as total number (percentage) and compared by χ^2 -test. OR and 95% CI were estimated for CAD by logistic regression to assess the relative risk conferred by a particular allele and genotype. The haplotypes distribution in CAD and control groups were estimated according to the two-stage iterative method, named expectation maximization algorithm, using the software SNPstats (<http://www.bioinfo.iconcologia.net/SNPstats>). To determine the association between haplotypes and the

risk of CAD, a logistic regression model was used, and the most common haplotype was considered as the reference group. The statistical significance level was $P<0.05$.

RESULTS

Clinical characteristics of studied participants

Demographic and clinical characteristics of the studied participants are shown in Table 1. Patients with CAD were older than those without CAD and also had higher systolic blood pressure, diastolic blood pressure, fasting plasma glucose, homeostasis model assessment of insulin resistance and insulin levels, and the lower levels of total cholesterol, triglyceride, adiponectin, and HDL-cholesterol.

Genotype distribution and the association of +45T/G polymorphism in *ADIPOQ* gene with coronary artery disease

Polymorphic fragments of the +45T/G were amplified using specific primers, which resulted in a 504-bp product. PCR products were digested with *SmaI* restriction endonuclease into the fragments of 204 bp and 163 bp (GG homozygote), 367 bp, 204 bp, 163 bp (TG heterozygote) as well as 367 bp (TT homozygote) (Fig. 1). The genotype distributions and allele frequency of +45T/G and their corresponding ORs are shown in Tables 2 and 3, respectively. Genotype frequencies of +45T/G were in Hardy-Weinberg equilibrium in both groups. Between two studied groups with and without CAD, the frequency of genotypes for +45T/G was not different (Table 2,

Table 1. Demographic and clinical characteristics of the studied patients

Variables	Control	CAD	<i>P</i> value
Gender (male/female) (%)	41/59	52/48	0.120
Age (y)	52.20±9.37	57.26±7.42	<0.001
BMI(kg/m ²)	27.97±4.32	26.62±3.90	0.022
SBP (mmHg)	116.44±24.68	129.54±20.72	<0.001
DBP (mmHg)	72.83±15.77	79.65±14.27	0.003
Total cholesterol (mg/dl)	188.92±62.95	183.49±52.03	0.525
Triglyceride (mg/dl)	184.92±72.25	160.50±74.64	0.023
HDL-C (mg/dl)	46.88±10.35	40.13±8.49	<0.001
LDL-C (mg/dl)	105.05±55.09	111.35±48.96	0.413
Fasting glucose (mg/dl)	158.99±57.75	185.51±67.53	0.003
Fasting insulin (μIU/ml)	14.23±14.11	22.26±21.34	0.009
HOMA-IR	5.87±6.11	10.16±9.83	0.002
Duration of diabetes (y)	4.47±3.12	7.83±3.80	<0.001
Adiponectin (μg/ml)	5.35±2.59	3.45±2.52	<0.001

Data are mean±SD; *P* values for continuous variables were calculated by *t*-test. CAD, coronary artery disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance

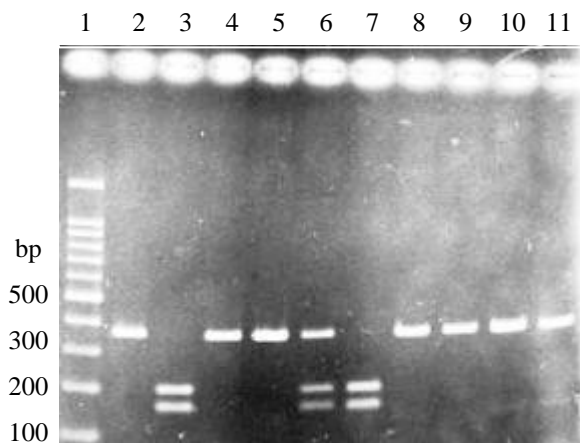


Fig. 1. Representative example of PCR-RFLP product for SNP +45T/G. Lane 1, 100 bp DNA ladder; Lanes 2, 4, 5, 8, 9, 10 and 11, wild type TT genotype (367 bp); Lanes 3 and 7, mutant GG genotype (163 bp and 204 bp); Lane 6, heterozygote TG genotype (367 bp, 204 bp, and 163 bp).

$\chi^2=3.405, P=0.182$). Regarding the allele frequency, in CAD cases (87% for T and 13% for G allele) and in controls (80% for T and 20% for G allele), no significant difference was observed between two groups (Table 2, $\chi^2=1.961, P=0.161$). The ORs for CAD was 0.671 (95% CI=0.358-1.257, $P=0.213$) for GT genotype and 0.217 (95% CI=0.024-1.998, $P=0.176$, Table 3) for GG genotype, which were not significant. There was also no significant difference in the genotypes distribution, including TT and TG+GG genotypes of +45T/G between men and women in two groups ($P>0.05$) (Table 4).

Genotype distribution and association of +276G/T polymorphism in *ADIPOQ* gene with coronary artery disease

A 504-bp DNA fragment was amplified using specific primers of +276G/T. PCR products were digested with *BsmI* restriction endonuclease into the fragments of 321 bp and 183 bp (GG homozygote), 504 bp, 321 bp and 183 bp (GT heterozygote) as well

as 504 bp (TT homozygote) (Fig. 2). The genotype distributions and allele frequency of +276G/T and their corresponding ORs are presented in Tables 2 and 3, respectively. Genotype frequencies of +276G/T were in Hardy-Weinberg equilibrium only in T2DM without CAD. The frequency of TT genotype was significantly increased in CAD cases as compared to controls ($\chi^2=7.967, P=0.019$). A similar significant difference was also found for allele frequency between two groups, which were 66% for G and 34% for T alleles in CAD cases, and 77% for G and 23% for T alleles in controls ($\chi^2=3.895, P=0.048$) (Table 2). The ORs for CAD were 1.930 (95% CI=1.086-3.43, $P=0.025$) for the GT genotype and 5.158 (95% CI=1.016-26.182, $P=0.048$) for the TT genotype (Table 3), which were significant. There was no significant difference in the distribution of GG, and GT+TT genotypes at +276G/T between men of two groups ($\chi^2=2.271, P=0.132$). Also, there was a significant difference in the distribution of GG, and GT+TT genotypes at +276G/T between women of two groups ($\chi^2=4.475, P=0.034$) (Table 4).

Haplotype analysis of two *ADIPOQ* single nucleotide polymorphisms in association with coronary artery disease

The haplotype analysis stratified by studied subjects for two loci of *ADIPOQ* gene is shown in Table 5. SNP haplotype frequencies were estimated and compared between diabetic individuals with CAD and without CAD. Based on the four possible *ADIPOQ* haplotypes, the haplotype consisting of wild allele of SNP +276 and mutant allele of SNP +45 was more prevalent in CAD cases compared to the controls (OR=0.37, 95% CI=0.16-0.86, $P=0.022$). The ‘double-mutant’ haplotype (+45G/+276T) was uncommon and was present at very low frequencies in both control and CAD groups. Concerning the association of the haplotype with CAD, our results revealed a significant association between one of the observed common haplotypes (+45G/+276G) and a less risk of CAD in subjects with T2DM (Table 5).

Table 2. Genotype distribution and allele frequencies of two studied single nucleotide polymorphisms in all studied subjects

SNP	Genotype frequency (%)			P ^a	Allele frequency (%)		P	
	TT	TG	GG		T	G		
+45T/G	Control	65 (65.0)	31 (31.0)	4 (4.0)	0.182	80.0	20.0	0.161
	CAD	75 (75.0)	24 (24.0)	1 (1.0)		87.0	13.0	
+276G/T	Control	56 (56.0)	42 (42.0)	2 (2.0)	0.019	77.0	23.0	0.048
	CAD	38 (38.0)	55 (55.0)	7 (2.0)		66.0	34.0	

^aPearson χ^2 -test; number (% of total); SNP, single nucleotide polymorphism; CAD, coronary artery disease

Table 3. The association between genotypes of *ADIPOQ* +45T/G and +276G/T polymorphisms and the risk of coronary artery disease (CAD)

SNP	Genotype	CAD/control	OR (95% CI)	P value
SNP +45T/G	TT	75/65	1.0	
	TG	24/31	0.671 (0.358-1.257)	0.213
	GG	1/4	0.217 (0.024-1.998)	0.176
SNP +276G/T	GG	38/56	1.0	
	GT	55/42	1.930 (1.086-3.43)	0.025
	TT	7/2	5.158 (1.016-26.182)	0.048

Pearson χ^2 -test was used to determine the genotype distribution; OR, odds ratio; CI, confidence interval

DISCUSSION

Identification of the association between genetic susceptibility and CAD, particularly in T2DM, has been the subject of many recent studies and offers the potentials for clinical preventive interventions. Although findings about the association between the two common SNPs of *ADIPOQ* gene and many disorders were documented, reports on their association with CAD are rather inconsistent^[16-20]. The present study showed that SNP +276G/T but not SNP +45T/G of the *ADIPOQ* gene was significantly associated with CAD risk in Iranian subjects with T2DM.

Among more than 10 SNPs of *ADIPOQ* gene reported until now, two common SNPs (+45T/G and +276G/T) were associated with the increased risk of insulin resistance and T2DM in particular^[9-14]. In a case-control study on Italian population, Bacci *et al.*^[15] first reported a significant association between SNP +276G/T and CAD. Recently, a meta-analysis study suggested that the SNP +276G/T of *ADIPOQ* gene is a low risk factor for development of cardiovascular disease in T2DM; however, the association of this

polymorphism with the susceptibility to cardiovascular disease in other populations remains unknown^[22]. Reports from other studies concerning +276 alleles have shown that T allele of this SNP is associated with higher serum levels of adiponectin and can be considered as a protective factor for diabetes, CAD and hypertension or dyslipidemia in American^[23], Finnish^[24], Japanese, and Korean^[25] populations. However, the results in different populations are somehow debating.

In the present studied population, we observed that not only GT and TT genotypes but also T allele of SNP +276G/T were related to an increased risk of CAD. Filippi *et al.*^[26] found similar conclusions and reported that T allele of SNP +276G/T was related to premature coronary heart disease in a case-control study in Italian population. They also observed an association between the SNP +276G/T alleles and the serum levels of adiponectin and found that T allele was associated with lower serum levels of adiponectin, which was in contrast with the present study. Moreover, Gui *et al.*^[27] found similar findings and reported that the adiponectin +276G/T was positively correlated with an increased risk of CAD, and the CAD patients had

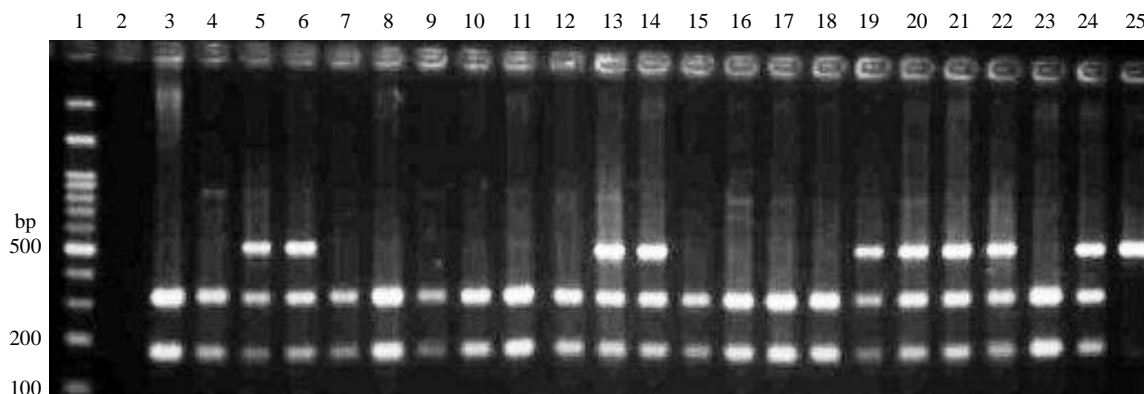


Fig. 2. Representative example of PCR-RFLP product for SNP +276G/T. Lane 1, 100 bp DNA ladder; Lane 2, non-template control; lanes 3, 4, 7, 8, 9, 10, 11, 12, 15, 16, 17, 18, and 23, wild-type GG genotype (183 bp and 321 bp); Lanes 5, 6, 13, 14, 19, 20, 21, 22, and 24, heterozygote GT genotype (504 bp, 321 bp, and 183 bp); Lane 25, mutant TT genotype (504 bp).

Table 4. Genotype distributions of +45T/G and +276G/T in *ADIPOQ* gene based on gender

SNP	Genotype	Males			Females		
		CAD/control	X ²	P	CAD/control	X ²	P
+45T/G	TT	38/28	0.255	0.614	37/38	2.029	0.154
	TG+GG	14/13			11/13		
+276G/T	GG	21/23	2.271	0.132	17/33	4.475	0.034
	GT+TT	31/18			31/26		

Pearson's *t*-test was used to determine the genotype distribution

lower levels of adiponectin, which were not affected by different genotypes of +276G/T polymorphism. Conversely, Esteghamati *et al.*^[28] in a study conducted on an Iranian population revealed that T allele of SNP +276G/T in adiponectin gene is significantly associated with a decreased risk of CAD in T2DM patients, which is inconsistent with our results. This discrepancy may be due to the sample size, statistical analysis methods or ethnical background of the studied population. In addition, their study was performed on a relatively homogeneous Iranian population, whereas our study was carried out in a relatively heterogeneous Iranian population composing of different ethnics, including Arabs, Bakhtiaries, and Persians. Thus, ethnic and regional variations may contribute to the observed differences between these studies. Recently, in a case-control study conducted on a Taiwanese population, a significant association was observed between +276G/T polymorphism and T2DM, and T allele was identified as a risk factor for the prevalence of T2DM. In addition, the genotype frequency of SNP +45T/G revealed no difference between T2DM and controls^[29].

On the other hand, we did not find any significant association between the SNP +45T/G and CAD in T2DM patient, this result is consistent with the reports of several previous studies^[10,15,17,28]. In a study on Iranian population, Esteghamati *et al.*^[28] have shown that +45T/G is not associated with the presence of CAD in T2DM patients, which was in consistent with our finding. In contrast, Sabouri *et al.*^[30] study on other Iranian population with CAD indicated that the

presence of the G allele of +45T/G may be associated with the risk of CAD. The population of their study consisted of only CAD patients, who had several differences in the clinical baseline characteristics, while our studied population included T2DM patients with CAD, which may affect the reported results. Consistent with our findings, Jung *et al.*^[10] have reported that SNP +45T/G in adiponectin gene is not associated with the presence of CAD. They also found no significant association between the severity of CAD and SNP similar to our findings. Conversely, one study on European population has shown that the G allele of the +45T/G polymorphism is associated with the lower risk of CAD^[14]. The protective effect of the G allele was also found in T2DM patients^[23]. However, numerous studies have reported contradictory results in the general population or in T2DM patients^[5,9,10,26-31]. It is speculated that the discrepancy might be due to differences in disease definition and ethnic background.

Our findings indicated that CAD patients had more lower serum levels of adiponectin than control subjects, which were similar to our previous results^[20]. Also, another investigation reported an association between plasma adiponectin levels and CAD^[32]. We also found that the different genotypes of two common SNPs of *ADIPOQ* gene were not significantly related to the serum levels of adiponectin. These results were obtained and reported in other studies^[19,33]. Nevertheless, the association between *ADIPOQ* SNPs and plasma adiponectin level has been confirmed in some^[23-25] but not all studies^[27,33,34]. Recently, the

Table 5. The estimation of haplotype frequencies and haplotype association with risk of CAD

Haplotype	Control	CAD	OR (95% CI)	P value ^a
Common haplotypes				
+45T/+276G	0.594	0.588	1.00	
+45T/+276T	0.211	0.281	1.54 (0.86-2.74)	0.150
+45G/+276G	0.176	0.066	0.37 (0.16-0.86)	0.022
Uncommon haplotypes				
+45G/+276T	0.019	0.063	4.91 (0.56-42.70)	0.150

^aHaplotype frequency determined by the maximum likelihood method. CAD, coronary artery disease; OR, odds ratio; CI, confidence interval.

results of a population-based study conducted in South India have revealed that SNP +276G/T was significantly associated with lower serum adiponectin level^[35]. Several previous *ADIPOQ* association studies have failed to repeat similar results or have even reported opposite effects of alleles on the adiponectin level. One possible explanation is that *ADIPOQ* gene does not have a key role in the regulation of plasma adiponectin level. On the contrary, several studies have indicated that serum adiponectin levels are highly heritable (30-93%) in different populations^[23,36,37]. A number of genome-wide scans have failed to demonstrate the association between the adiponectin level and *ADIPOQ* poly-morphisms^[10,27,33,34,37]. Also, where the association was observed, there is a possibility of a linkage between polymorphisms and another mutation in the other genes close to the *ADIPOQ* gene^[12,16]. Moreover, it could possibly be understood because the +276G/T is located in intron 2 and might not affect the serum adiponectin level.

In the present study, we used haplotype analysis to determine the association between two common SNPs of *ADIPOQ* locus and the risk of CAD in T2DM subjects. Among four haplotypes, the "double mutant" 45G-276T haplotype was less present in both groups. The haplotype 45G-276G indicated a protective effect against the presence of CAD in T2DM patients as compared to the reference 45T-276G haplotype. Similarly, Esteghamati *et al.*^[28] found that the two haplotypes of adiponectin gene, 45T-276T and 45G-276T, are associated with a decreased risk of CAD in Iranian population. The novel finding of our study is that T2DM patients, who have T allele at position +276 of the *ADIPOQ* gene, probably have a higher risk of CAD than those who carrying G allele.

In conclusion, our study demonstrated that +276G/T, rather than +45T/G of *ADIPOQ* gene, is more associated with the risk of CAD in T2DM patients. Moreover, one haplotype of these two SNPs had protective effect on the risk of CAD in T2DM patients. The frequency of T allele at position +276G/T in female CAD patients was higher than that of control subjects. Furthermore, females carrying the T allele at position +276G/T had a greater risk of CAD than G allele carriers. These findings suggest that genetic factors may exert a greater influence on CAD in women than in men with T2DM.

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CONFLICT OF INTEREST. None declared.

REFERENCES

1. Van Dieren S, Beulens JW, van der Schouw YT, Grobbee DE, Nealbb B. The global burden of diabetes and its complications: an emerging pandemic. *European journal of preventive cardiology* 2010; **17**(1): S3-S8.
2. Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, Nagai M, Matsuzawa Y, Funahashi T. Adiponectin as a biomarker of the metabolic syndrome. *Circulation journal* 2004; **68**(11): 975-981.
3. Buechler C, Wanninger J, Neumeier M. Adiponectin, a key adipokine in obesity related liver diseases. *World journal of gastroenterology* 2011; **17**(23): 2801-2811.
4. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes a systematic review and meta-analysis. *The journal of the American medical association* 2009; **302**(2): 179-188.
5. Almeda-Valdes P, Cuevas-Ramos D, Mehta R, Gomez-Perez FJ, Cruz-Bautista I, Navarrete-Lopez M, Aguilar-Salinas CA. Total and high molecular weight adiponectin have similar utility for the identification of insulin resistance. *Cardio vascular diabetology* 2010; **9**: 26.
6. Kollias A, Tsiotra PC, Ikonomidis I, Maratou E, Mitrou P, Kyriazi E, Boutati E, Lekais J, Economopoulos T, Kremastinos DT, Dimitriadis G, Raptis SA. Adiponectin levels and expression of adiponectin receptors in isolated monocytes from overweight patients with coronary artery disease. *Cardio vascular diabetology* 2011; **10**: 14.
7. Breitfeld J, Stumvoll M, Kovacs P. Genetics of adiponectin. *Biochimie* 2012; **94**(10): 2157-2163.
8. Menzaghi C, Trischitta V, Doria A. Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. *Diabetes* 2007; **56**(5): 1198-1209.
9. Ohashi K, Ouchi N, Kihara S, Funahashi T, Nakamura T, Sumitsuji S, Kawamoto T, Matsumoto S, Nagaretani H, Kumada M, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Hiraka H, Iwashima Y, Ishikawa K, Ohishi M, Katsuya T, Rakugi H, Ogihara T. Adiponectin I164T mutation is associated with the metabolic syndrome and coronary artery disease. *The journal of the American college of cardiology* 2004; **43**(7): 1195-1200.
10. Jung CH, Rhee EJ, Kim SY, Shin HS, Kim BJ, Sung KC, Kim Bs, Lee WY, Kang JH, Oh KW, Kim SW, Park JR. Associations between two single nucleotide polymorphisms of adiponectin gene and coronary artery diseases. *Endocrine journal* 2006; **53**(5): 671-677.
11. Mackevics V, Heid IM, Wagner SA, Cip P, Doppelmayr

- H, Lejnieks A, Gohlke H, Ladurner G, Illig T, Iglseder B, Kronenberg F, Paulweber B. The adiponectin gene is associated with adiponectin levels but not with characteristics of the insulin resistance syndrome in healthy Caucasians. *European journal of human genetic* 2006; **14**: 349-356.
12. Berthier MT, Houde A, Cote M, Paradis AM, Mauriege P, Bergeron J, Gaudet D, Despres JP, Vohl MC. Impact of adiponectin gene polymorphisms on plasma lipoprotein and adiponectin concentrations of viscerally obese men. *The journal of lipid research* 2005; **46**: 237-244.
 13. Ruchat SM, Loos RJ, Rankinen T, Vohl MC, Weisnagel SJ, Despres JP, Boucard C, Perusse L. Associations between glucose tolerance, insulin sensitivity and insulin secretion phenotypes and polymorphisms in adiponectin and adiponectin receptor genes in the Quebec family study. *Diabetic medicine* 2008; **25**(4): 400-406.
 14. Gable DR, Matin J, Whittall R, Cakmak H, Li KW, Cooper J, Miller GJ, Humphries SE. Common adiponectin gene variants show different effects on risk of cardiovascular disease and type 2 diabetes in European subjects. *Annals of human genetics* 2007; **71**(4): 453-466.
 15. Bacci S, Menzaghi C, Ercolino T, Ma X, Rauseo A, Salvemini L, Vigna C, Fanelli R, Mario U, Doria A, Trischitta V. The +276 G/T single nucleotide polymorphism of the adiponectin gene is associated with coronary artery disease in type 2 diabetic patients. *Diabetes care* 2004; **27**(8): 2015-2020.
 16. Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, Yamauchi T, Otabe S, Okada T, Eto K, Kadowaki H, Hagura R, Akanuma Y, Yazaki Y, Nagai R, Taniyama M, Matsubara K, Yoda M, Nakano Y, Tomita M, Kimura S, Ito C, Froguel P, Kadowaki T. Genetic variation in the gene encoding adiponectin is associated with an increased risk of T2DM in the Japanese population. *Diabetes* 2002; **51**(2): 536-540.
 17. Katakami N, Kaneto H, Matsuoka TA, Takahara M, Maeda N, Shimisu I, Ohno K, Osonoi T, Kawai K, Ishibashi F, Imamura K, Kashiwagi A, Kawamori R, Matsuhisa M, Funahashi T, Yamasaki Y, Shimomura I. Adiponectin G276T gene polymorphism is associated with cardiovascular disease in Japanese patients with type 2 diabetes. *Atherosclerosis* 2012; **220**(2): 437-442.
 18. Hwang JY, Park JE, Choi YJ, Huh KB, Kim WY. SNP276G>T polymorphism in the adiponectin gene is associated with metabolic syndrome in patients with type II diabetes mellitus in Korea. *European journal of clinical nutrition* 2010; **64**(1): 105-107.
 19. Lee YY, Lee NS, Cho YM, Moon MK, Jung HS, Park YJ, Park HJ, Youn BS, Lee HK, Park KS, Shin HD. Genetic association study of adiponectin polymorphisms with risk of T2DM diabetes mellitus in Korean population. *Diabetics medicine* 2005; **22**(5): 569-575.
 20. Mohammadzadeh G, Ghaffari MA. Additional effect of diabetes mellitus type 2 on the risk of coronary artery disease: role of serum adiponectin. *Iranian red crescent medical journal* 2014; **16**(1): e8742.
 21. Friedewald WT, Levi RI, Fredrickson Ds. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the ultracentrifuge. *Clinical chemistry* 1972; **18**(6): 499-502.
 22. Sun K, Li Y, Wei C, Tong Y, Zheng H, Guo Y. Recessive protective effect of ADIPOQ rs1501299 on cardiovascular diseases with type 2 diabetes: a meta-analysis. *Molecular and cellular endocrinology* 2012; **349**(2): 162-169.
 23. Qi L, Li T, Rimm E, Zhang C, Rifai N, Hunter D, Doria A, Hu FB. The +276 polymorphism of the APM1 gene, plasma adiponectin concentration, and cardiovascular risk in diabetic men. *Diabetes* 2005; **54**(5): 1607-1610.
 24. Mousavinasab F, Tahtinen T, Jokelainen J, Koskela P, Vanhala M, Oikarinen J, Keinanen-Kiukkaanniemi S, Laakso M. Common polymorphisms (single-nucleotide polymorphisms SNP+45 and SNP+276) of the adiponectin gene regulate serum adiponectin concentrations and blood pressure in young Finnish men. *Molecular genetics and metabolism* 2006; **87**(2): 147-151.
 25. Jang Y, Lee JH, Chae JS, Kim OY, Koh SJ, Kim JY, Cho H, Jang Y, Lee J, Ordovas J. Association of 276G>T polymorphism of the adiponectin gene with cardiovascular disease risk factors in nondiabetic Koreans. *The American journal of clinical nutrition* 2005; **82**(4): 760-767.
 26. Filippi E, Sentinelli F, Romeo S, Arca M, Berni A, Tiberti C, Verrienti A, Fanelli M, Fallarino M, Sorropago G, Baroni MG. The adiponectin gene SNP+276G>T associates with early-onset coronary artery disease and with lower levels of adiponectin in younger coronary artery disease patients (age < or =50 years). *The journal of molecular medicine* 2005; **83**(9): 711-719.
 27. Gui MH, Li X, Jiang SF, Gao J, Lu DR, Gao X. Association of the adiponectin gene rs1501299 G>T variant, serum adiponectin levels, and the risk of coronary artery disease in a Chinese population. *Diabetes research and clinical practice* 2012; **97** (3): 499-504.
 28. Esteghamati A, Mansournia N, Nakhjavani M, Mansournia MA, Nikzamir A, Abbasi M. Association of +45(T/G) and +276(G/T) polymorphisms in the adiponectin gene with coronary artery disease in a population of Iranian patients with type 2 diabetes. *Molecular biology reports* 2012; **39**(4): 3791-3797.
 29. Tsai MK, Wang HM, Shiang JC, Chen IH, Wang CC, Shiao YF, Liu WS, Lin TJ, Chen TM, Chen YH. Sequence variants of ADIPOQ and association with type 2 diabetes mellitus in Taiwan Chinese Han population. *The scientific world journal* 2014: 650393.
 30. Sabouri S, Ghayour-Mobarhan M, Moohebbati M, Hassani M, Kassaeian J, Tatari F, Mahmoodi-Kordi F, Esmaeili H, Tavakkaie S, Paydar R, Sahebkar A, Tehrani S, Ferns G, Behravan J. Association between 45T/G polymorphism of adiponectin gene and coronary artery disease in an Iranian population. *The scientific world journal* 2011; **11**: 93-101.
 31. Qi L, Doria A, Manson JE, Meigs JB, Hunter D,

- Mantzoros CS, Hu FB. Adiponectin genetic variability, plasma adiponectin, and cardiovascular risk in patients with type 2 diabetes. *Diabetes* 2006; **55**(5): 1512-1516.
32. Halvatsiotis I, Tsiotra PC, Ikonomidis I, Kollias A, Mitrou P, Maratou E, Boutati E, Lekakis J, Dimitriadis G, Economopoulos T, Kremastinos D, Raptis S. Genetic variation in the adiponectin receptor 2 (*ADIPOR2*) gene is associated with coronary artery disease and increased *ADIPOR2* expression in peripheral monocytes. *Cardiovascular diabetology* 2010; **9**: 10.
33. Al-Daghri NM, Al-Attas OS, Alokail MS, Alkharfy KM, Hussain T, Yakout S, Vinodson B, Sabico S. Adiponectin gene polymorphisms (T45G and G276T), adiponectin levels and risk for metabolic diseases in an Arab population. *Gene* 2012; **493**(1): 142-147.
34. Heid IM, Wagner SA, Gohlke H, Iglseder B, Mueller JC, Cip P, Ladurner G, Reiter R, Stadlmayr A, Mackevics V, Illig T, Kronenberg F, Paulweber B. Genetic architecture of the *APM1* gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. *Diabetes* 2006; **55**(2): 375-384.
35. Ramya K, Ashok Ayyappa K, Ghosh S, Mohan V, Radha V. Genetic association of *ADIPOQ* gene variants with type 2 diabetes, obesity and serum adiponectin levels in South Indian population. *Gene* 2013; **532**(2): 253-262.
36. Butte NF, Comuzzie AG, Cai G, Cole SA, Mehta NR, Bacino CA. Genetic and environmental factors influencing fasting serum adiponectin in hispanic children. *J Clin endocrinol metab* 2005; **90**(7): 4170-4176.
37. Menzaghi C, Ercolino T, Salvemini L, Coco A, Kim SH, Fini G, Doria A, Trischitta V. Multigenic control of serum adiponectin levels: evidence for a role of the *APM1* gene and a locus on 14q13. *Physiological genomics* 2004; **19**(2): 170-174.