



RCSI

UNIVERSITY
OF MEDICINE
AND HEALTH
SCIENCES

Royal College of Surgeons in Ireland

repository@rcsi.com

Associations between single-nucleotide polymorphisms of ADIPOQ, serum adiponectin and increased type 2 diabetes mellitus risk in Bahraini individuals.

AUTHOR(S)

Fatima Al Hannan, F A. O'Farrell, Maria P. Morgan, Orna Tighe, Kevin G. Culligan

CITATION

Hannan, Fatima Al; O'Farrell, F A.; Morgan, Maria P.; Tighe, Orna; Culligan, Kevin G. (2016): Associations between single-nucleotide polymorphisms of ADIPOQ, serum adiponectin and increased type 2 diabetes mellitus risk in Bahraini individuals.. Royal College of Surgeons in Ireland. Journal contribution. <https://hdl.handle.net/10779/rcsi.10782371.v1>

HANDLE

[10779/rcsi.10782371.v1](https://hdl.handle.net/10779/rcsi.10782371.v1)

LICENCE

CC BY-NC-SA 3.0

This work is made available under the above open licence by RCSI and has been printed from <https://repository.rcsi.com>. For more information please contact repository@rcsi.com

URL

https://repository.rcsi.com/articles/journal_contribution/Associations_between_single-nucleotide_polymorphisms_of_ADIPOQ_serum_adiponectin_and_increased_type_2_diabetes_mellitus_risk_in_Bahraini_individuals_/10782371/1

Associations between single-nucleotide polymorphisms of *ADIPOQ*, serum adiponectin and increased type 2 diabetes mellitus risk in Bahraini individuals

F.A. Al Hannan¹, P.A. O'Farrell¹, M.P. Morgan², O. Tighe² and K.G. Culligan¹

الارتباط بين تعدد أشكال النيوكليوتيد المفرد لجين أديبونيكتين ومعدلات مصبل أديبونيكتين وزيادة خطر الإصابة بالسكري من النمط 2 لدى مجموعة من الأشخاص البحرينيين

فاطمة الهنان، بول أوفاريل، ماريا مورغن، أورنا تاي، كيفن كوليجن

الخلاصة: لقد هدفت هذه الدراسة إلى تقدير تواتر النمطين الجينيين لـ SNPs ($T < +276G$ و $G < +45T$) وتقصي العلاقة بين هذين النوعين من SNPs وبين تركيز الأديبونيكتين والمحددات الأيضية وخطر الإصابة بالسكري من النمط 2 لدى السكان البحرينيين. أجرينا تنميماً جينياً لنوعي SNPs *ADIPOQ* عند 140 مريضاً من مرضى السكري من النمط 2 ليس بينهم قرابة و 66 شاهداً غير مصابين بالسكر باستخدام مقايضة تعدد أشكال طول شدة الاقتران بتفاعل البلمرة المتسلسل. وتم قياس بروفيل الشحوم بالطرق الأنزيمية، وقياس المجموع الكلي للمستويات المصلية لأديبونيكتين بواسطة المقايضة المناعية. فكان لدى مرضى السكري من النمط 2 مستويات منخفضة من الأديبونيكتين مقارنة مع الشواهد. وكان $G < +45T$ أكثر انتشاراً عند المرضى منه عند الشواهد. وكان حدوث أليل G النادر لـ $G < +45T$ أكثر تواتراً من حدوث أليل T الشائع لدى مرضى السكري من النمط 2 مقارنة مع الشواهد، وكان مرتبطاً بوجود مستويات مصلية أدنى للأديبونيكتين. ولم يكن هناك اختلاف كبير في تواترات الأليل والنمط الجيني لـ $T < +276G$ بين مرضى السكري من النمط 2 وبين الشواهد. ولم يكن هناك ارتباط بين كلا SNPs وبين المحددات الأيضية.

ABSTRACT This study aimed to estimate the frequency of the SNPs ($+45T > G$ and $+276G > T$) genotypes and investigate the association between the two SNPs and adiponectin concentration, metabolic parameters and risk of T2DM in the Bahraini population. We genotyped the two *ADIPOQ* SNPs in 140 unrelated T2DM patients and 66 nondiabetic controls using the polymerase chain reaction-restriction fragment length polymorphism assay. Lipid profile was measured by enzymatic methods. Total serum adiponectin levels were measured by immunoassay. T2DM patients had reduced adiponectin levels compared with controls. $+45T > G$ was more prevalent in patients than controls. The rare G allele of $+45T > G$ occurred more frequently than the common T allele in T2DM patients compared with controls, and was associated with lower serum adiponectin levels. There was no significant difference in allele and genotype frequencies of $+276G > T$ between type T2DM patients and controls. There was no association between both SNPs and metabolic parameters.

Associations entre les polymorphismes mononucléotidiques du gène *ADIPOQ*, l'adiponectine sérique et le risque accru de diabète de type 2 dans la population bahreïnienne

RÉSUMÉ La présente étude avait pour objectif de mesurer la fréquence des polymorphismes mononucléotidiques ($+45T > G$ et $+276G > T$) des génotypes et d'évaluer l'association entre ces deux polymorphismes et la concentration d'adiponectine, les paramètres métaboliques et le risque de diabète non insulino-dépendant (DNID) dans la population bahreïnienne. Nous avons génotypé les deux polymorphismes mononucléotidiques du gène *ADIPOQ* chez 140 patients atteints de DNID sans lien de parenté et 66 témoins non diabétiques en recourant à l'analyse du polymorphisme de longueur des fragments de restriction par réaction en chaîne de polymérase. Le profil lipidique a été mesuré au moyen de méthodes enzymatiques. Les concentrations d'adiponectine totale sérique ont été mesurées par immunodosage. Les patients atteints de DNID affichaient des concentrations d'adiponectine réduites par rapport aux témoins. Le polymorphisme $+45T > G$ avait une prévalence plus élevée chez les patients que chez les témoins. L'allèle rare G du polymorphisme $+45T > G$ apparaissait plus fréquemment que l'allèle commun T chez les patients atteints de DNID que chez les témoins, et était associé à des concentrations d'adiponectine sérique plus faibles. Il n'existait pas de différence significative entre les fréquences des allèles et des génotypes du polymorphisme $+276G > T$ entre les patients atteints de DNID et les témoins. Aucune association entre les deux polymorphismes et les paramètres métaboliques n'a été notée.

¹Department of Basic Medical Sciences, Royal College of Surgeons in Ireland – Medical University of Bahrain, Busaiteen, Kingdom of Bahrain (Correspondence to: F.A. Al Hannan: fhannan@rcsi-mub.com). ²Molecular & Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland.

Received: 23/01/14; accepted: 14/06/16

Introduction

T2DM is a major public health problem worldwide. Five GCC countries are among the world's top 10 highest for diabetes prevalence: Kuwait, Oman, Saudi Arabia, United Arab Emirates and Bahrain, which is ranked third (1,2). The International Diabetes Federation estimated that the prevalence of diabetes in the Bahraini population aged 20 years was 20% in 2011 (3). Statistics from the World Health Organization predict that the number of patients with diabetes in Bahrain will triple by the year 2025.

Recently, adipose tissue has become increasingly seen as a metabolically active endocrine and paracrine organ. It secretes several mediators, known as adipokines, which participate in diverse metabolic processes (4). There is great interest in clarifying the role of adipokines as possible mediators between obesity, insulin sensitivity and diabetes. Adiponectin is a protein secreted mainly from adipocytes, which plays an important role in regulation of lipid and glucose metabolism (5). Several studies have demonstrated that serum adiponectin concentrations are decreased in individuals with obesity (2), insulin resistance, dyslipidaemia (6) and T2DM (7). Thus, adiponectin may have a causal role in the pathogenesis of T2DM and insulin resistance. The *ADIPOQ* gene has been identified and located on human chromosome 3q27 (8). Saito *et al.* determined that *ADIPOQ* spans 16kb and contains 3 exons and 2 introns (8). Genome-wide scans in humans have suggested that this chromosomal region is a susceptibility locus for T2DM and metabolic syndrome (9).

Two SNPs at the *ADIPOQ* locus have been extensively studied: a silent T to G substitution in exon 2 (+45T>G, rs2241766) and a G to T substitution in intron 2 (+276G>T, rs1501299). However, association studies of these two SNPs, either independently or as

a haplotype, with T2DM, adiponectin levels and insulin sensitivity have given conflicting results in different populations and sample types (10,11,12,13,14). It has been seen that the association between these SNPs and T2DM differs among populations suggesting that a genetic factor might be involved. Further studies are needed to elucidate the effect of genetic background on the role of adiponectin.

In the present study, we estimated the frequency of SNPs (+45T>G and +276G>T) genotypes and investigated the association between these two SNPs with adiponectin concentration and metabolic syndrome parameters, in a representative sample from Bahrain. To the best of our knowledge, this is the first study to investigate the association between variants of *ADIPOQ* and serum adiponectin level and its relation to T2DM and metabolic syndrome in a Bahraini population.

Methods

Study participants

We enrolled using convenience sampling 140 unrelated patients with T2DM (age 47 years; 74 women and 66 men) attending the Diabetic Clinic at the Bahrain Defence Force (BDF) Hospital for routine follow-up, and 66 unrelated healthy control individuals without diabetes (age 46 years; 6 women and 60 men) during August 2009 to April 2011. The presence or absence of T2DM was established according to the American Diabetes Association criteria (15), in which diabetes is defined as fasting blood glucose (FBG) ≥ 126 mg/dL (7 mmol/L). Patients with T1DM and women with gestational diabetes were excluded. The controls were from the same geographic areas as the patients and were individuals who visited the blood bank of the BDF hospital for blood donations. The following exclusion criteria were applied to the controls: fever (temperature ≥ 38 °C),

hospitalization in the previous 2 weeks, known diabetes, arterial hypertension [systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 85 mmHg], dyslipidemia [total cholesterol (TC) ≥ 6.21 mmol/L (240 mg/dL), triglyceride 1.7 mmol/L (150 mg/dL), high-density lipoprotein-cholesterol (HDL-C) ≤ 1.03 mmol/L (40 mg/dL)], coronary heart disease, congestive heart failure, stroke, chronic renal disease, active hepatic disease. Individuals were also excluded if they were taking any medication known to affect metabolic profile.

The study protocol was approved by the ethics committees of the Royal College of Surgeons in Ireland – Medical University of Bahrain (RCSI-Bahrain) and the BDF Hospital. Written or verbal informed consent was obtained from the study participants in accordance with the Helsinki Declaration.

Anthropometric and blood pressure measurements

The study participants were asked to complete a self-administered questionnaire on general health. Where patients were unable to complete the questionnaire by themselves, because of illiteracy, assistance was offered. Obesity was defined as body mass index (BMI) ≥ 30 kg/m². For all participants, SBP, DBP, body weight and height were measured, and BMI was calculated as weight divided by height squared.

Blood collection

Venous blood samples (3 mL) were drawn from all participants after an overnight fast for at least 12 hours. The serum and red blood cells were separated immediately by centrifugation at 3,000 g for 10 minutes at room temperature and serum was stored at -80 °C until biochemical analysis. Separate 3 mL venous blood samples were collected in EDTA-coated tubes and stored at -80 °C until DNA extraction for SNP analysis.

Biochemical measurements

Blood samples were analysed for biochemical variables. Haemoglobin A1c was assayed using the latex agglutination reaction on a Roche Cobas c111 automatic analyser (Roche Diagnostics, Indianapolis, IN, USA). FBG, TC, triglyceride, HDL-C and LDL-C were assayed using the Roche Cobas 6000 automatic analyser. FBG was measured using the hexokinase/glucose-6-phosphate dehydrogenase method (16), and serum levels of triglyceride, TC and HDL-C and LDL-C were measured by enzymatic colorimetric test (17).

Total adiponectin levels were measured by quantitative sandwich enzyme immunoassay technique (Quantikine Human Adiponectin ELISA kit; R&D Systems, UK). Adiponectin and biochemical measurements were not performed in 29 samples (11 diabetic and 18 controls) because the participants presented in a nonfasting state.

Genetic analysis

Genomic DNA was isolated from whole blood samples using the phenol-chloroform DNA extraction method (18). Polymerase chain reaction (PCR) amplification was carried out using specific primers for human ADIPOQ, as previously reported by Mackevics et al. (19). For the SNP +45T>G, a 305-bp DNA fragment containing the polymorphic site was amplified by PCR in a DNA Engine Peltier Thermal Cycler (PTC-0200; Bio-Rad, Hercules, CA, USA) using forward primer 5'-TGTGTGTGTGGGGTCTGTCT-3' and reverse primer 5'-TGTGATGAAA-GAGGCCAGAA-3'. For the SNP +276G>T, a 110-bp DNA fragment was amplified by PCR using forward primer 5'-CTACTGATATAAAC-TATATGGAG-3' and reverse primer 5'-CCCCAAATCACTTCAG-GTTG-3'. PCR was carried using 100 ng DNA in 50 µL containing 5 µL 10× Biotherm PCR buffer (Genecraft, Germany) with a final concentration of 1.5 mM magnesium chloride, 0.2 µM

each primer, 200 µM dNTPs and 2.5 U Biotherm Taq Polymerase (Genecraft). Thirty-five cycles of amplification were performed and each cycle consisted of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 30 seconds, with additional steps of initial denaturation at 95 °C for 5 minutes and final extension at 72 °C for 7 minutes (19).

The PCR product (10 µL) of SNP +45T>G (305 bp) was digested with 10 U *Eco88I* (*Aval*) in the recommended 10× Tang Buffer (ER0381; MBI Fermentas, Thermo Fisher Scientific, USA) at 37 °C overnight. The PCR product (10 µL) of SNP +276G>T (110 bp) was digested with 10 U *Hinfl* in the recommended 10× Buffer R (ER0801; MBI Fermentas, Thermo Fisher Scientific) at 37 °C overnight. The fragments were separated on 12% native polyacrylamide gel. The DNA fragments were stained with ethidium bromide and visualized by UV illumination.

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 12.0 (SPSS Inc., Chicago, IL, USA). Genotype and allele frequency distributions were compared using the χ^2 test. Hardy-Weinberg equilibrium (HWE) was calculated to the expected genotype distribution. Comparisons between means were performed using Student's independent *t* test (two-tailed) for normally distributed data or the Mann-Whitney *U* test for parameters presenting with non-normal distribution. Categorical variables were analysed by the chi-squared test. Univariate general linear model analysis was carried out to investigate the effect of SNPs+45T>G and +276G>T on serum adiponectin concentrations and the various metabolic parameters. All analyses were adjusted for gender, age and BMI. $P < 0.05$ was considered significant. Analyses were performed for the whole sample, as well as for men and women separately, to

verify the homogeneity of the genetic effect among men and women.

Results

Association between SNP +45T>G of ADIPOQ and T2DM

To assess whether SNPs +45T>G and +276G>T of *ADIPOQ* contribute to T2DM, we genotyped 140 T2DM patients and 66 control individuals by PCR-restriction fragment length polymorphism (RFLP). After polyacrylamide gel electrophoresis, genotypes were determined by examining the length of the restriction fragments for SNP +45T>G (Figure 1). For SNP +45T>G, fragments of 305 bp for the TT wild-type homozygote (absence of *Aval* cutting site) and 105 and 200 bp for the GG mutant homozygote (presence of *Aval* cutting site) were produced. The heterozygote TG included 3 fragments of 105, 200 and 305 bp.

Genotype frequencies were found to be in agreement with HWE, which is described by the binominal distribution: $p^2+2pq+q^2=1.0$, thus fulfilling this requirement for the present study population. Testing for HWE was done using the χ^2 test ($\chi^2 = 5.36$, $P = 0.02$ in T2DM patients and $\chi^2 = 0.07$, $P = 0.50$ in controls). In the T2DM group, 100 (71.4%) patients were homozygous for the wild-type allele (TT), 32 (22.9%) were heterozygous (TG) and only 8 (5.7%) were homozygous for the mutation (GG) (Table 1). In the non-diabetic control group, 56 (84.8%) individuals were homozygous for the wild-type allele (TT), 10 (15.2%) were heterozygous (TG) and none was homozygous for the mutation (GG). The frequencies of TG and GG genotypes were significantly higher in the T2DM group than the control group. In contrast, the frequency of the TT genotype was significantly higher in the control group. Individuals with the GG genotype were at increased risk for T2DM compared with those having the TT

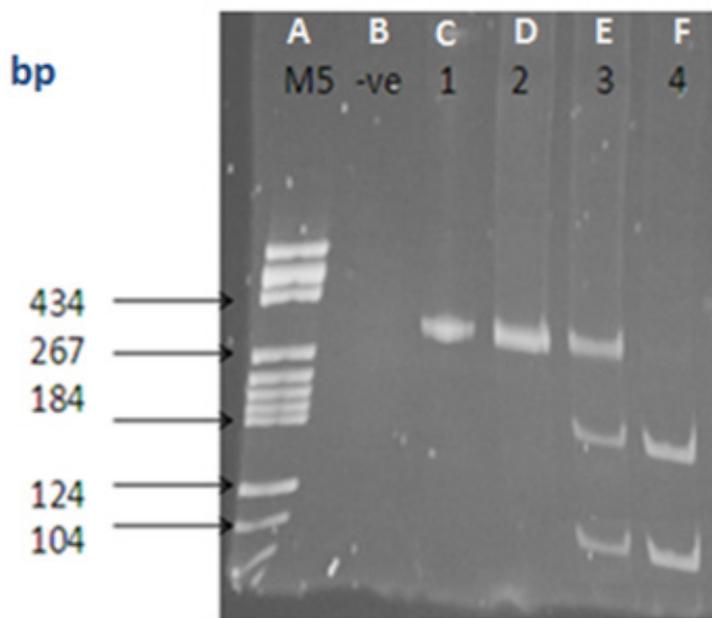


Figure 1 A 12% polyacrylamide gel electrophoresis pattern of polymerase chain reaction-restriction fragment length polymorphism product for single nucleotide polymorphism +45T>G of adiponectin gene. Lane A (M5) represents marker 5 [pBR322 DNA/BsuRI (HaeIII) Marker, SM0271; Fermentas]; Lane B (-ve) shows negative control product with no DNA template; Lane C (sample 1) represents undigested sample (305 bp); Lane D (sample 2) shows wild-type TT (305 bp); Lane E (sample 3) shows heterozygous TG (restriction fragments 305, 200 and 105 bp) digested with 10 U Eco88I (AvaI) in the recommended 10× Tango Buffer (ER 0381; MBI Fermentas); Lane F (sample 4) shows homozygous mutant GG (restriction fragments 200 and 105 bp) digested with 10 U Eco88I (AvaI) in the recommended 10× Tango Buffer.

genotype (relative risk = 2.3, confidence interval = 95%, $P = 0.0137$).

The T allele frequency was significantly higher in the control group (92.4%) than the T2DM group (83%) ($P = 0.009$) (Table 1). In contrast, the G allele frequency was significantly higher in the T2DM group (17%) than the control group (7.6%) ($P = 0.009$).

Association between SNP +276G>T of ADIPOQ and T2DM

After polyacrylamide gel electrophoresis, genotypes were determined by analysing the length of the restriction fragments for SNP +276G>T (Figure 2). For SNP +276G>T, fragments of 110 bp for the GG wild-type

homozygote (absence of *HinfI* cutting site) and 26 and 84 bp for the TT mutant homozygote (presence of *HinfI* cutting site) were produced. The heterozygote GT included 3 fragments of 26, 84 and 110 bp.

Genotype frequencies were found to be in agreement with the HWE. Testing for HWE was done using the χ^2 test ($\chi^2 = 12.7$, $P = 0.0003$ in T2DM patients and $\chi^2 = 8.1$, $P = 0.004$ in controls). In the T2DM group, 130 (92.9%) patients were homozygous for the wild-type allele (GG), 8 (5.7%) were heterozygous (TG) and only 2 (1.4%) were homozygous for the mutation (TT) (Table 2). In the nondiabetic control group, 58 (87.9%) individuals were homozygous for the wild-type allele (GG), 6 (9.1%) were heterozygous (TG) and only 2 (3%) were homozygous for the mutation (TT). There were no significant differences in genotype distribution between the T2DM and control groups.

The G allele frequency was 95.7% in the T2DM group and 92.4% in the control group (Table 2). The T allele frequency was 4.3% in the T2DM group and 7.6% in the control group. Neither of these differences was significant.

Relationship between SNPs +45T>G and +276G>T and serum adiponectin levels

Table 3 shows the mean serum adiponectin concentrations according to SNP +45T>G and +276G>T genotypes in the T2DM and control groups. The mean values were divided via a general linear model adjusting for age, gender and BMI. Twenty-nine samples (11 T2DM and 18 controls) without measurement of adiponectin were excluded from the analysis. Serum adiponectin levels were significantly lower in the T2DM than in the control group ($P < 0.05$). In the T2DM group, the mean serum adiponectin levels differed significantly among the three genotype subgroups of SNP +45T>G. There was a clear multiplicative trend of decreasing adiponectin levels per copy of the rare

Table 1 Differences in genotype and allele frequencies of adiponectin gene SNP +45T>G between T2DM patients and controls

		T2DM		Control		P^a
		No.	%	No.	%	
Genotype frequency	T/T	100	71.4	56	84.8	0.046
	T/G	32	22.9	10	15.2	
	G/G	8	5.7	0	0	
Allele frequency	T	232	83	122	92.4	0.009
	G	48	17	10	7.6	

^a χ^2 test. Significant at $P < 0.05$.

SNP = single nucleotide polymorphism; T2DM = type 2 diabetes mellitus.

G allele. The G allele was associated with lower serum adiponectin levels compared with the T allele carriers ($6.5 \pm 4.1 \mu\text{g/mL}$ for T/T genotype, $3.7 \pm 3.4 \mu\text{g/mL}$ for the T/G genotype and $2.1 \pm 2.2 \mu\text{g/mL}$ for the G/G genotype, $P < 0.001$). In the control group, none of the individuals was homozygous for the mutant allele G/G; therefore, the comparison was made between the T/G and T/T genotypes only. Plasma levels of adiponectin in individuals with the T/G genotype were significantly lower than those of individuals with the T/T genotype.

For SNP +276G>T, there was no significant difference in the mean serum adiponectin levels between the three genotype subgroups of SNP +276G>T (Table 3).

Effects of serum adiponectin levels and SNPs+45T>G and +276G>T on features of metabolic syndrome

To assess whether serum adiponectin levels and SNPs+45T>G and +276G>T had any effect on metabolic syndrome, we compared anthropometric and metabolic characteristics among genotype subgroups. A series of metabolic and clinical parameters (age, BMI, SBP and DBP, HbA1c, TC, HDL-C, LDL-C, triglyceride, FBG and insulin resistance estimated by triglyceride/HDL-C index) were analysed with respect to their association with serum adiponectin levels and SNP +45T>G and +276G>T genotypes in T2DM patients. As a result of incomplete clinical data for the control sample, the correlation was only evaluated for T2DM patients. Considering all T2DM patients, serum adiponectin level showed significant inverse correlations with HbA1c and triglycerides ($P = 0.039$ and $P = 0.020$ respectively), and a positive correlation with HDL-C ($P = 0.036$). No association was found with the remaining metabolic parameters ($P > 0.05$). No significant difference in the metabolic parameters among G/G,

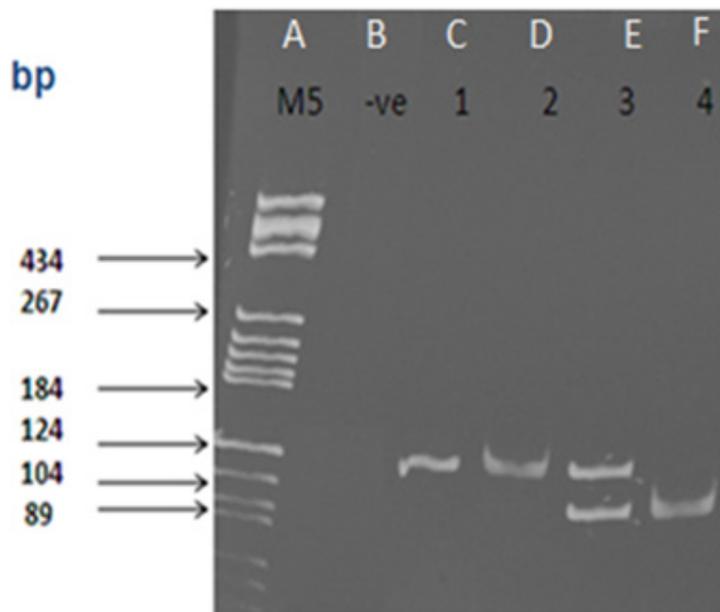


Figure 2 A 12% polyacrylamide gel electrophoresis pattern of polymerase chain reaction-restriction fragment length polymorphism product for single nucleotide polymorphism +276G>T of adiponectin gene. Lane A (M5) represents marker 5 (pBR322 DNA/BsuRI (HaeIII) Marker, SM0271; Fermentas); Lane B (-ve) shows negative control product with no DNA template; Lane C (sample 1) represents undigested sample (110 bp); Lane D (sample 2) shows wild-type GG (110 bp); Lane E (sample 3) shows heterozygous GT (restriction fragments 100, 84 and 26 bp) digested with 10 U *HinfI* in the recommended 10× Buffer R (ER 0801; MBI Fermentas); Lane F (sample 4) shows homozygous mutant TT (restriction fragments 84 and 26 bp) digested with 10 U *HinfI* in the recommended 10× Buffer R. To obtain clear separation between the larger bands of marker 5 and the digested samples, the 26 bp runs off the gel and it was not visualized.

T/G and T/T carriers of SNPs+45T>G and +276G>T was observed in both genders ($P > 0.5$).

Discussion

In the present study, we observed that adiponectin SNP +45T>G was associated with risk of T2DM and adiponectin levels in a Bahraini population, but no significant association was seen with SNP +276G>T. We also observed that the G allele of SNP +45T>G occurred more frequently than the T allele in Bahraini T2DM patients compared to controls. Moreover, the G allele were associated with lower serum adiponectin levels and increased risk for T2DM. However, the data did not show an association of either of these two SNPs with features of metabolic resistance.

Several susceptibility loci for T2DM have been identified by genome-wide linkage scans (20). Two common SNPs in *ADIPOQ*, 45+T>G in exon 2, and +276G>T in intron 2, have been widely studied in several populations including Europeans, Asians and Americans (21,22). Although there is a lack of consistency among studies, the results indicate that variation in *ADIPOQ* is associated with insulin resistance and T2DM (23,24). Ramya *et al.* found that SNP +276G>T of *ADIPOQ* was strongly associated with increased risk of development of T2DM, obesity and hypoadiponectinaemia in 1100 unrelated Indian patients with T2DM (23). Also, in a Spanish population, Gonzalez-Sanchez *et al.* found that both SNPs +45T>G and +276G>T were associated with decreased serum adiponectin levels and risk of impaired

Table 2 Differences in genotype and allele frequencies of adiponectin gene SNP +276G>T between T2DM patients and controls

		T2DM		Control		<i>P</i> ^a
		<i>n</i>	%	<i>n</i>	%	
Genotype frequency	G/G	130	92.9	58	87.9	0.480
	T/G	8	5.7	6	9.1	
	T/T	2	1.4	2	3.0	
Allele frequency	G	268	95.7	122	92.4	0.166
	T	12	4.3	10	7.6	

^a*P* value adjusted for age, body mass index and gender. Calculated from univariate general linear model. Significant at *P* < 0.05.

SD = standard deviation; SNP = single nucleotide polymorphism; T2DM = type 2 diabetes mellitus.

Table 3 Serum adiponectin levels in patients with T2DM and controls with different genotypes for SNPs +45T>G and +276G>T

			Adiponectin Conc (µg/mL)		<i>P</i> ^a
			No.	Mean (SD)	
SNP +45T>G	T2DM	T/T	92	6.5 ± 4.1	0.0005
		T/G	30	3.7 ± 3.4	
		G/G	7	2.1 ± 2.2	
	Controls	T/T	42	7.8 ± 4.3	0.048
		T/G	6	3.8 ± 1.1	
		G/G	0		
SNP +276G>T	T2DM	G/G	119	5.3 ± 3.9	0.101
		T/G	8	9.1 ± 5.8	
		T/T	2	5.8 ± 1.7	
	Controls	G/G	41	7.15 ± 4.9	0.239
		T/G	5	7.1 ± 3.6	
		T/T	2	10.1 ± 0.23	

^a*P* value adjusted for age, body mass index and gender. Calculated from univariate general linear model. Significant at *P* < 0.05.

SD = standard deviation; SNP = single nucleotide polymorphism; T2DM = type 2 diabetes mellitus.

glucose tolerance in 747 unrelated Spanish individuals (12).

However, similar associations were not revealed in a Korean (13) or Chinese (25) population. In the Korean study, plasma levels of adiponectin did not differ significantly according to SNPs +45T>G and +276G>T in either control individuals or T2DM patients. The genotype distribution of these SNPs had no association with the risk of T2DM or metabolic syndrome. In the Chinese study however, only variation at SNP +45T>G was associated with T2DM.

Our results are in agreement with the study of Zacharova et al. who found that the G allele of SNP +45T>G was associated with lower serum adiponectin levels and increased risk for T2DM in the STOP-NIDDM trial (which was conducted in Canada, Germany, Austria, Norway, Denmark, Sweden, Finland, Israel and Spain) (11). Similar results were observed by Mohammadzadeh and Zarghami in an Iranian population (26).

Cross-sectional studies have yielded inconsistent findings concerning the association of SNP +45T>G with T2DM. These conflicting findings could be due to true differences in allelic association

with the disease phenotype in various ethnic populations, which may be the result of different environmental and genetic influences in those populations (27). Our data suggest that this SNP may play a direct role in altering ADIPOQ expression in Bahraini population.

Our observations that the G allele of SNP +45T>G is associated with T2DM and with serum adiponectin levels might be explained by several mechanisms that have yet to be demonstrated conclusively. T2DM is characterized by lower levels of serum adiponectin compared to those in individuals without diabetes. Therefore, the role of the G/G genotype of SNP +45T>G in increasing the risk of T2DM in some populations is consistent with its role in decreasing serum adiponectin levels. This could also be due to the effect of adiponectin on both glucose and lipid metabolism (5). Therefore, deficiencies in adiponectin could presumably trigger development of T2DM.

The mechanism by which these ADIPOQ variants affect adiponectin levels and diabetes in different populations remains unclear. Although SNP +45T>G does not cause an amino acid change (GGT to GGG, Gly15Gly), Yang et al. suggested that the silent +45T>G mutation alters RNA splicing or stability, resulting in allele-specific differential expression of adiponectin (27).

It is also reported that SNP +45T>G is in strong linkage disequilibrium with other SNPs of ADIPOQ or other nearby genes that may affect adiponectin expression, secretion, structure or action, such as SNPs -11377 and -11391 in the 5' promoter region (28).

Although we observed an association between variations in ADIPOQ and adiponectin levels, we did not detect an association between either SNP and the anthropometric and metabolic parameters related to insulin resistance. It is reasonable to infer that the genetic effects of SNP +45T>G on T2DM are

mediated through altered expression of *ADIPOQ* that eventually affects some of the metabolic parameters. Nevertheless, statistical analysis did not reveal any significant association of either SNP with the majority of the clinical and metabolic parameters. This discrepancy has also been shown in other studies in which both SNPs were related to adiponectin concentration but not to the metabolic parameters (19).

Several circumstances might have contributed to this observation. The causal association between metabolic syndrome parameters and T2DM is not entirely clear, and different sets of genes might be involved in the pathogenesis

of the two conditions. The metabolic parameters of insulin resistance might be determined by serum adiponectin levels or *ADIPOQ* expression, as well as other multiple genetic and environmental factors or lifestyle interventions in different populations and sample sizes (29).

In conclusion, this study showed that T2DM is associated with low serum adiponectin concentrations. We also showed that *ADIPOQ* SNP +45T>G was associated with risk of T2DM and low serum adiponectin levels. The current study provides evidence of potential involvement of *ADIPOQ* as a risk factor for T2DM in the Bahraini

population, independent of other known risk factors such as obesity and insulin resistance.

Acknowledgements

We would like to thank Salmaniya Medical Complex and the BDF Hospital for generous support and help. The authors are grateful to Mr. Hassan Al-Basri, Statistician from College of Health Science-Bahrain, for his assistance with the statistical analysis.

Funding: The project was funded by a grant from RCSI-Bahrain.

Competing interests: None declared.

References

- Klautzer L, Becker J, Mattke S. The curse of wealth - Middle Eastern countries need to address the rapidly rising burden of diabetes. *Int J Health Policy Manag.* 2014 Apr;2(3):109-14. PMID:24757686
- Al-Harbi EM, Farid EM, Gumaa KA, Masuadi EM, Singh J. Angiotensin-converting enzyme gene polymorphisms and T2DM in a case-control association study of the Bahraini population. *Mol Cell Biochem.* 2011 Apr;350(1-2):119-25. PMID:21207118
- International Diabetes Federation. *IDF Diabetes Atlas.* 15th ed. Brussels: International Diabetes Federation; 2011.
- Lau DC, Dhillon B, Yan H, Szmítko PE, Verma S. Adipokines: molecular links between obesity and atherosclerosis. *Am J Physiol Heart Circ Physiol.* 2005 May;288(5):H2031-41. PMID:15653761
- Karbowska J, Kochan Z. Role of adiponectin in the regulation of carbohydrate and lipid metabolism. *J Physiol Pharmacol.* 2006 Nov;57 Suppl 6:103-13. PMID:17228091
- Kitamoto A, Kitamoto T, So R, Matsuo T, Nakata Y, Hyogo H, et al. *ADIPOQ* polymorphisms are associated with insulin resistance in Japanese women. *Endocr J.* 2015;62(6):513-21. PMID:25832963
- Najam SS, Awan FR, Baig SM. Serum adiponectin levels in diabetes, obesity and gender in Punjabi subjects from Faisalabad, Pakistan. *J Pak Med Assoc.* 2014 Oct;64(10):1186-8. PMID:25823162
- Saito K, Tobe T, Minoshima S, Asakawa S, Sumiya J, Yoda M, et al. Organization of the gene for gelatin-binding protein (GBP28). *Gene.* 1999 Mar 18;229(1-2):67-73. PMID:10095105
- Bermúdez VJ, Rojas E, Toledo A, Rodríguez-Molina D, Vega K, Suárez L, et al. Single-nucleotide polymorphisms in adiponectin, *AdipoR1*, and *AdipoR2* genes: insulin resistance and type 2 diabetes mellitus candidate genes. *Am J Ther.* 2013 Jul-Aug;20(4):414-21. PMID:23656997
- Menzaghi C, Ercolino T, Di Paola R, Berg AH, Warram JH, Scherer PE, et al. A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. *Diabetes.* 2002 Jul;51(7):2306-12. PMID:12086965
- Zacharova J, Chiasson JL, Laakso M; STOP-NIDDM Study Group. The common polymorphisms (single nucleotide polymorphism [SNP] +45 and SNP +276) of the adiponectin gene predict the conversion from impaired glucose tolerance to type 2 diabetes: the STOP-NIDDM trial. *Diabetes.* 2005 Mar;54(3):893-9. PMID:15734870
- González-Sánchez JL, Zabena CA, Martínez-Larrad MT, Fernández-Pérez C, Pérez-Barba M, Laakso M, et al. An SNP in the adiponectin gene is associated with decreased serum adiponectin levels and risk for impaired glucose tolerance. *Obes Res.* 2005 May;13(5):807-12. PMID:15919831
- Lee YY, Lee NS, Cho YM, Moon MK, Jung HS, Park YJ, et al. Genetic association study of adiponectin polymorphisms with risk of Type 2 diabetes mellitus in Korean population. *Diabet Med.* 2005 May;22(5):569-75. PMID:15842511
- Vozarova de Courten B, Hanson RL, Funahashi T, Lindsay RS, Matsuzawa Y, Tanaka S, et al. Common polymorphisms in the adiponectin gene ACDC are not associated with diabetes in Pima Indians. *Diabetes.* 2005 Jan;54(1):284-9. PMID:15616040
- American Diabetes Association. *Diagnosis and classification of diabetes mellitus.* *Diabetes Care.* 2009 Jan;32 Suppl 1:S62-7. PMID:19118289
- Neeley WE. Simple automated determination of serum or plasma glucose by a hexokinase-glucose-6-phosphate dehydrogenase method. *Clin Chem.* 1972 Jun;18(6):509-15. PMID:5026763
- Warnick GR, Knopp RH, Fitzpatrick V, Branson L. Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. *Clin Chem.* 1990 Jan;36(1):15-9. PMID:2297909
- Stenesh J. *Dictionary of biochemistry and molecular biology.* New York: John Wiley & Sons; 1989.
- Mackevics V, Heid IM, Wagner SA, Cip P, Doppelmayer H, Lejnieks A, et al. The adiponectin gene is associated with adiponectin levels but not with characteristics of the insulin resistance syndrome in healthy Caucasians. *Eur J Hum Genet.* 2006 Mar;14(3):349-56. PMID:16418740
- McCarthy MI. Growing evidence for diabetes susceptibility genes from genome scan data. *Curr Diab Rep.* 2003 Apr;3(2):159-67. PMID:12728642
- Gable DR, Hurel SJ, Humphries SE. Adiponectin and its gene variants as risk factors for insulin resistance, the metabolic

- syndrome and cardiovascular disease. *Atherosclerosis*. 2006 Oct;188(2):231–44. PMID:16581078
22. Vasseur F, Meyre D, Froguel P. Adiponectin, type 2 diabetes and the metabolic syndrome: lessons from human genetic studies. *Expert Rev Mol Med*. 2006 11 20;8(27):1–12. PMID:17112391
23. Ramya K, Ayyappa KA, 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 Dec 15;532(2):253–62. PMID:24055485
24. Du W, Li Q, Lu Y, Yu X, Ye X, Gao Y, et al. Genetic variants in ADIPOQ gene and the risk of type 2 diabetes: a case-control study of Chinese Han population. *Endocrine*. 2011 Dec;40(3):413–22. PMID:21594755
25. Xia Z, Mo Y, Bian R, Shen J, Ma L. Correlation between single nucleotide polymorphism of adiponectin gene and type 2 diabetes in Chinese. *Chin J Endocrinol Metab*. 2004;20(3):236–7.
26. Mohammadzadeh G, Zarghami N. Associations between single-nucleotide polymorphisms of the adiponectin gene, serum adiponectin levels and increased risk of type 2 diabetes mellitus in Iranian obese individuals. *Scand J Clin Lab Invest*. 2009;69(7):764–71. PMID:19929719
27. Yang WS, Tsou PL, Lee WJ, Tseng DL, Chen CL, Peng CC, et al. Allele-specific differential expression of a common adiponectin gene polymorphism related to obesity. *J Mol Med (Berl)*. 2003 Jul;81(7):428–34. PMID:12750819
28. Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, et al. Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. *Hum Mol Genet*. 2002 Oct 1;11(21):2607–14. PMID:12354786
29. Dedoussis GV, Kaliora AC, Panagiotakos DB. Genes, diet and type 2 diabetes mellitus: a review. *Rev Diabet Stud*. 2007 Spring;4(1):13–24. PMID:17565412