Assessment of the association of the adiponectin gene single-nucleotide polymorphism 45T/G with type 2 diabetes mellitus in Egyptian diabetic patients

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Background
Type 2 diabetes mellitus (T2DM) is a common multifactorial genetic disease. Adiponectin is a hormone produced solely by adipocytes and is a regulator of glucose and energy homeostasis. A number of genes and polymorphisms have been reproducibly associated with T2DM in a variety of studies. The gene ADIPOQ, encoding adiponectin, was found to be the main locus contributing to variations in adiponectin serum levels.

Objective
The aim of the work was to investigate the association between single-nucleotide polymorphism in exon 2 (45T/G) of the adiponectin gene with serum adiponectin level, and the occurrence of T2DM, which could allow proper management and genetic counseling for the high-risk carrier.

Patients and methods
The study included 40 patients with T2DM and 40 normal individuals with no family history of diabetes mellitus. BMI, serum fasting and postprandial glucose, lipid profile, fasting insulin, and adiponectin were measured. Molecular study for adiponectin 45T/G gene polymorphism was carried out.

Results
There was no statistically significant difference found when either genotype or allele frequencies were compared between the two groups.

Conclusion
Single-nucleotide polymorphism 45T/G of adiponectin gene was not associated with T2DM.

Keywords:
adiponectin, diabetes mellitus, polymorphism

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Introduction

Type 2 diabetes mellitus (T2DM) is a common multifactorial genetic disease, which is determined by several different genes and environmental factors [1]. It is a chronic disease that requires long-term medical attention to limit the development of its devastating complications and to manage them when they do occur [2]. Diabetes leads to a reduced life expectancy and quality of life; it has risk for heart disease, stroke, peripheral neuropathy, renal disease, blindness, and amputation [3]. A number of genes and polymorphisms have been reproducibly associated with T2DM in a variety of studies [4–6].

Adiponectin is a hormone produced solely by adipocytes and is a regulator of glucose and energy homeostasis [7]. Low plasma adiponectin concentration is associated with a decrease in whole-body insulin sensitivity in humans [8,9], and has been shown to be predictive of future development of diabetes [10].

Serum adiponectin concentration is highly heritable, and a number of genome-wide association studies have identified ADIPOQ, the gene encoding adiponectin, as the main locus contributing to variations in serum levels in European and Asian populations [11,12]. Adiponectin effects may be partly mediated by stimulatory effects of adiponectin on signaling pathways for 5-AMP-activated protein kinase and peroxisome proliferator-activated receptor α [13,14].

The adiponectin gene consists of three exons and two introns located on chromosome 3q27, where a diabetes susceptibility locus has been mapped [15,16]. Genetic associations of single-nucleotide polymorphism (SNP) in exon 2 (45T/G) of adiponectin gene with T2DM and adiponectin level were reported in Japanese population and with insulin resistance (IR) in some White populations [16].

Adiponectin is among the strongest and most consistent biochemical predictors of T2DM [17], and adiponectin levels may be useful for identifying individuals likely to benefit most from interventions to treat 'dysfunctional adipose tissue' and its metabolic complications [18].

Aim

The aim of this study was to investigate the association between SNP 45T/G of the adiponectin gene with serum adiponectin level, and occurrence of T2DM. This could allow proper management and genetic counseling for the high-risk carrier.

Patients and methods

The study included 40 patients with T2DM and 40 normal individuals with no family history of diabetes. They were recruited from to the Internal Medicine Department, Medical Research Institute, Alexandria University.

All participants were asked to freely volunteer to the study, and informed written consent (appendix) was gathered before their inclusion in the study, according to the ethical guidelines of the Medical Research Institute, Alexandria University.

All participants were subjected to the following:

(1) History and thorough clinical examination.
(2) BMI evaluation.
(3) Laboratory investigations, which included the following:
    (a) Estimation of fasting blood glucose (FBG), 2-h postprandial blood glucose (PPG) concentrations, serum lipid profile [total cholesterol (TCH), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C)], serum creatinine, and blood urea [19,20].
    (b) Determination of fasting serum insulin level with calculation of homeostatic model assessment (HOMA) [21].
    (c) Estimation of serum adiponectin level using the ELISA technique [22].
(4) Molecular study for adiponectin 45T/G polymorphism:
    (a) Collection of peripheral blood samples into sterile EDTA vacutainer tubes.
    (b) DNA extraction from blood samples using the salting out technique [23].
    (c) PCR amplification for adiponectin 45T/G was carried out using the following primers (Sigma, Alexandria, Egypt):

Forward primer: 5′-GAA GTA GAC TCT GCT GAG ATG G-3′, and reverse primer: 5′-TAT CAG TGT AGG AGG TCT GTG ATG-3′ [24].

PCR products were then confirmed by means of electrophoresis on 3% agarose gels. Digestion of the PCR products was carried out using the specific restriction enzyme: *Sma*I for 45T/G PCR products [24].

Results

Statistical analysis of the data

Data were fed to the computer and analyzed using SPSS software (version 20.0; SPSS Inc., Chicago, Illinois, USA). Qualitative data were described using number and percentage. Quantitative data were described using range (minimum and maximum), mean, SD, and median. Comparison between different groups with regard to categorical variables was made using the χ^2-test. When more than 20% of the cells have expected count less than 5, correction for χ^2 was conducted using Fisher's exact test or Monte Carlo correction. The distributions of quantitative variables were tested for normality using the Kolmogorov–Smirnov test, the Shapiro–Wilk test, and the D'Agostino test, and histogram and QQ plot were used for vision test. If it revealed normal data distribution, parametric tests were applied. If the data were abnormally distributed, nonparametric tests were used. For normally distributed data, comparisons between the studied groups were made using the *F*-test (ANOVA) and the post-hoc test (Scheffe). For abnormally distributed data, the Kruskal–Wallis test was used to compare the studied groups, and pair-wise comparison was made using the Mann–Whitney test. Significance test results were quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

The study included 40 patients with T2DM and 40 normal controls. They were recruited from the Internal Medicine Department, Medical Research Institute, Alexandria University.

Age, sex, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), serum insulin, FBG, 2-h PPG, homeostatic model assessment for insulin resistance (HOMA-IR), serum adiponectin, and lipid profile data, including TCH, TG, LDL, and HDL, are shown in Table 1.

The diabetic patients had a statistically significantly higher BMI, SBP, DBP, FBG, PPG, HOMA-IR,

TG, TCH, and LDL, but significantly lower HDL compared with controls. Adiponectin level was lower in patients than in controls but the difference was not significant. Insulin level was higher in patients than in controls but the difference was not significant.

Molecular genetics results

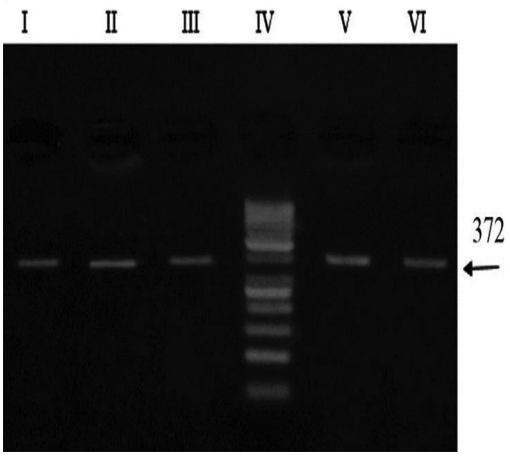
The PCR-RFLP method was used to detect the distribution of genotype and allele frequencies of SNP 45T/G in exon 2 of the adiponectin gene in both patients and controls.

Table 1 Demographic, clinical, and biochemical parameters of studied groups

Characteristics	Cases (N = 40)	Controls (N = 40)	P-value
Age (years)	50.45 ± 9.04	45.15 ± 8.77	0.09
Sex (%)			
Male	42.5	47.5	0.12
Female	57.5	52.5	
BMI (kg/m^2)	32.43 ± 2.0	27.61 ± 2.27	0.001*
SBP (mmHg)	144 ± 19.1	112 ± 12.0	0.001*
DBP (mmHg)	93.0 ± 12.2	74.4 ± 8.60	0.001*
FBG (mg/dl)	208.67 ± 47.46	90.02 ± 11.39	0.001*
PPG (mg/dl)	278.58 ± 52.02	93.87 ± 11.59	0.001*
TCH (mg/dl)	218.85 ± 32.36	185.60 ± 26.70	0.001*
LDL (mg/dl)	132.28 ± 35.48	96.46 ± 30.11	0.001*
HDL (mg/dl)	59.50 ± 11.40	68.93 ± 8.27	0.001*
TG (mg/dl)	134.0 ± 43.98	101.10 ± 27.30	0.001*
Insulin (μIU/ml)	14.21 ± 12.69	9.9 ± 10.66	0.066
HOMA-IR	6.97 ± 5.43	2.1 ± 2.23	0.001*
Adiponectin (ng/ml)	12 669 ± 17 680	16 378 ± 23 213	0.567

DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL, high-density lipoprotein-cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; PPG, postprandial blood glucose; SBP, systolic blood pressure; TCH, total cholesterol; TG, triglyceride; *Statistically significant at $P \leq 0.05$.

Figure 1



PCR products. Lanes I, II, III, V, and VI: 372 bp PCR products. Lane IV: molecular weight marker (50 bp DNA ladder).

The PCR fragments (372 bp) were digested using the restriction enzyme Fast Digest (*Sma*I), where the G allele creates restriction site. Thus, after digestion, homozygous for the wild type T allele presents as a 372 bp fragment, and homozygous for G allele has two fragments, 216 and 156 bp. The heterozygous presents all three fragments (Figs. 1–4).

In T2DM (group I), the TT genotype was found in 55% of patients, whereas the GG genotype was found in 5%, and the heterozygous TG was found in 40%. The T allele frequency was 75%, and the G allele frequency was 25%.

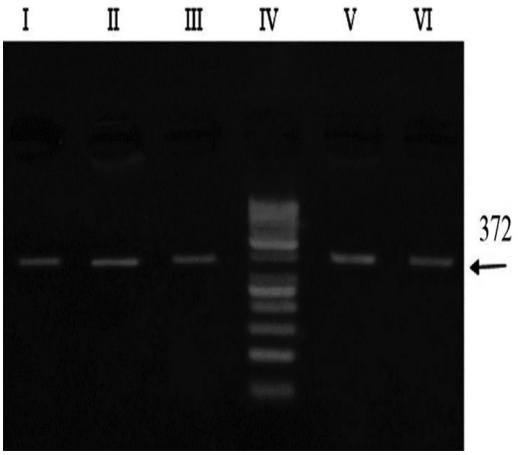
In controls (group II), the TT genotype was found in 57.5% of controls, whereas the heterozygous TG was found in 42.5%, but GG genotype was not found. The T allele frequency was 78.8%, and the G allele frequency was 21.2%.

The G allele was more frequent in patients than in controls, and T allele was more frequent in controls compared with patients, but there was no significant association when compared with the genotype and allele frequencies in patients and in controls ($P > 0.05$) (Table 2).

There were no statistically significant differences with regard to FBG, lipid profile, serum fasting insulin, HOMA-IR, and serum adiponectin between different genotypes in both groups.

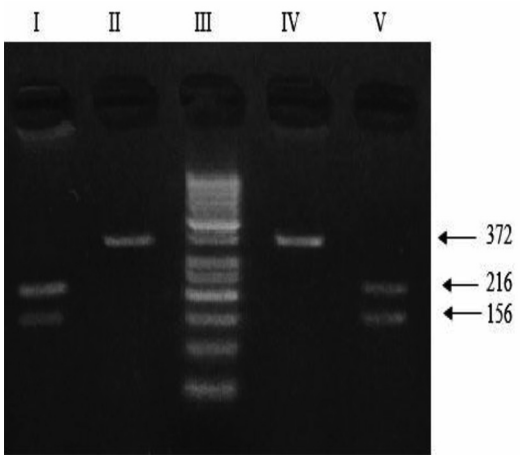
There were significant differences in terms of BMI in the patient group between genotypes ($P = 0.049$), and so the G carriers had significantly higher BMI compared with T carriers. However, in the control

Figure 2



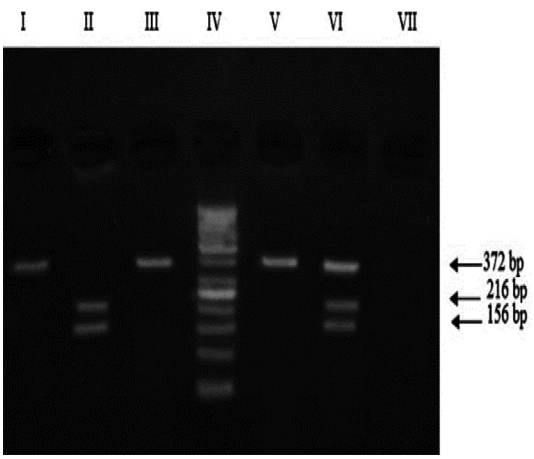
Wild type TT. Lanes I, II, and III: digest products for a homozygous allele TT showed one band at 372 bp. Lane IV: molecular weight marker (50 bp DNA ladder). Lane V and VI: 372 bp PCR product.

Figure 3



Mutant GG type. Lane I and V: digest products for a mutant homozygous allele GG showed two bands at 216 and 156 bp. Lane II and IV: 372 bp PCR product. Lane III: molecular weight marker (50 bp DNA ladder).

Figure 4



Different genotypes of single-nucleotide polymorphism 45T/G in type 2 diabetes mellitus. Lanes I and III: digest products for a wild homozygous allele TT showed one band at 372 bp. Lane II: digest products for a mutant homozygous allele GG showed two bands at 216 and 156 bp. Lane IV: molecular weight marker (50 bp DNA ladder). Lane V: 372 bp PCR product. Lane VI: digest products for a heterozygous allele TG showed three bands at 372, 216, and 156 bp.

group, there were no significant differences in BMI (P = 0.838).

Discussion

Numerous polymorphisms in the gene coding for adiponectin (ADIPOQ), lying in the 3q27 region, have been described. Some of them seem to influence adiponectin levels or have been associated with IR, T2DM, or its microvascular complications. The most frequent polymorphism in the ADIPOQ gene is the silent T-to-G substitution in exon 2 (45T/G).

Studies with regard to its association with plasma adiponectin, IR, metabolic syndrome, prevalence of diabetes, and diabetic nephropathy report inconsistent results. Some of the differences may be due to ethnic background [25,26].

Some studies have provided evidence that the SNP 45T/G is associated with metabolic syndrome components [27,28]. However, a number of studies have mainly focused on the association of the polymorphism with one or two components of metabolic syndrome, such as obesity and/or T2DM [29,30].

An association of variation within the ADIPOQ gene with diabetic complications is also an ongoing subject for debate.

This study included 40 patients with T2DM and 40 normal controls. There were no significant differences between the two groups with regard to age and sex.

Similar to many previous studies [31,32], the cases had significantly higher BMI, SBP, DBP, FBG, PPG,

Table 2 Genotype and allele frequencies for adiponectin single-nucleotide polymorphism T/G in both groups

Genotype/ allele	T2DM (group I) (n = 40) [N (%)]	Control (group II) (n = 40) [N (%)]	χ^2	P	OR (95% CI)
Genotype					
TT	22 (55.0)	23 (57.5)	0.051	0.882	—
TG	16 (40.0)	17 (42.5)	0.052	0.820	0.98 (0.4–2.4)
GG	2 (5.0)	0 (0.0)	2.051	0.152	—
TG + GG	18 (45.0)	17 (42.5)	0.051	0.822	1.1 (0.5–2.7)
Allele					
T	60 (75.0)	63 (78.8)	0.316	0.574	—
G	20 (25.0)	17 (21.2)			1.24 (0.6–2.6)

CI, confidence interval; OR, odds ratio; T2DM, type 2 diabetes mellitus; Statistically significant at $P \leq 0.05$.

HOMA-IR, TG, TCH, and LDL, but significantly lower HDL compared with controls. Adiponectin level was lower in patients than in controls but the difference was not significant. Insulin level was higher in patients than in controls but the difference was not significant too.

In the current study, the G allele was more frequent in patients than in controls, and T allele was more frequent in controls than in patients, but there was no statistically significant association compared with the genotype and allele frequencies in patients and in controls.

The results obtained in the study in Korea by Lee *et al.* [33] were in accordance with our results, in which there were no statistically significant differences

in allele frequencies of SNP 45T/G on comparing controls with T2DM patients, and the genotype distributions of these SNPs had no association with the risk for T2DM.

Moreover, a study in Polish White populations by Szopa *et al*. [34] and the study in Italian population by Chiodini *et al.* [35] reported that there was no significant association between SNP 45 and the risk for T2DM.

In contrast to our study, Hara *et al.* [36] reported in their study that the SNP 45 was significantly associated with risk for T2DM in Japanese population. Mohammadzadeh and Zarghami [37] also found the same results in Iranian population.

Fan *et al.* [38] used 45 publications in the final meta-analysis study with 9986 T2DM patients and 16 222 controls for SNP 45T/G and found that, in Asians, the SNP 45T/G was significantly associated with risk for T2DM, whereas it was not found to be associated with risk for T2DM in Whites. The discrepancy might be caused by differences in the ADIPOQ_45T/G genotype distribution in different ethnic backgrounds.

In Egypt, the study by Khodeer *et al.* [39] comprising 64 individuals divided into 32 diabetic patients and 32 normal glucose tolerance (NGT) individuals revealed that the T2DM group had a statistically significantly lower distribution of the TT genotype and T allele frequency compared with the NGT group as well as a statistically significantly higher distribution of the TG/GG genotype and G allele frequency compared with the NGT group. This study concluded that the SNP 45 may be associated with T2DM and that, in Egyptians, its risk is higher in G allele carriers.

The difference between the present study and the study by Khodeer *et al*. [39] may be due to difference in methodology.

In the present study, it was observed that there were no significant differences in FBG for both groups between the genotypes. This result is in agreement with that reported in other studies [33,40].

This study revealed that there were no significant differences in lipid profile (TCH, LDL, TG, and HDL) for both groups between the genotypes. Our findings are in agreement with a recent meta-analysis of available studies, which concluded that the SNP 45 is not significantly associated with lipid profile [41].

However, other studies found an association between this SNP and two components of plasma lipids. In Egypt, the study by Khodeer *et al.* [39] revealed that, compared with SNP 45 T carriers, G carriers showed higher levels of LDL-C and TCH. However, no significant differences were found in TG and HDL between these two genotypes.

Koenig *et al.* [42], in a study on a large cohort from southern Germany comprising 976 diabetic patients, reported that the G carriers of SNP 45T/G show higher levels of LDL and TCH, and lower levels of HDL.

The current study revealed that there was a significant difference in BMI for group I between genotypes ($P = 0.049$), in which the TG/GG genotypes had higher BMI, compared with TT genotypes, and so G carriers had higher BMI compared with T carriers. However, in group II, there were no significant differences between different genotypes in BMI ($P = 0.838$).

Similar results were obtained by previous studies, which confirmed that the G allele of SNP 45 was significantly associated with increased BMI in T2DM patients [27,29].

However, Stumvoll *et al.* [43] reported a positive association between the G allele of SNP 45T/G and obesity in a healthy German population, whereas in Taiwanese nondiabetic individuals the same allele was related to a lower risk for obesity [28].

Our results noted that there were no significant differences in terms of insulin for both groups between the different genotypes, whereas it was observed that the HOMA-IR was higher in the T2DM group; G carriers had somewhat higher (HOMA-IR) compared with T carriers, but the difference was statistically nonsignificant. Moreover, there were no significant differences as regards HOMA-IR in the control group between the genotypes. These results are in accordance with those of Li *et al.* [44], who reported higher HOMA-IR in G carriers versus T carriers in the T2DM group, but the difference was not statistically significant.

The same results were obtained by Nannipieri *et al.* [45], Vasseur *et al.* [46], and Gu *et al.* [47] on Italians, French, and Swedish populations, respectively.

In contrast, in Japanese population, Nakatani *et al.* [48] showed a strong association of this SNP 45T/G with increased IR.

In the current study, patients with TG/GG genotypes had somewhat lower levels of plasma adiponectin as compared with TT patients; however, there were no

significant differences in adiponectin level for both groups between the genotypes.

Many studies are in agreement with our findings. In a study conducted on Finnish men, Mousavinasab *et al.* [49] reported that there was no association between SNP 45T/G and serum adiponectin level. Kim *et al.* [50] also found the same results in Korean population.

Cesari *et al.* [51] and Suriyaprom *et al.* [52] failed to find any association between this SNP and serum adiponectin level.

In the study by Lee *et al.* [33] on Koreans, they genotyped 427 nondiabetic controls and 493 T2DM patients for SNP 45T/G of adiponectin gene and also measured plasma adiponectin concentrations; they revealed that the IR and serum level of adiponectin were not statistically different according to T45G, in both controls and T2DM patients.

Al-Daghri *et al.* [53] experienced two SNPs (45T/G and 276G/T) in Arab population and reported that the relationship between SNP 45T/G and serum adiponectin levels was not observed.

Kacso *et al.* [40] examined 115 T2DM patients and reported that there was no association between SNP 45T/G and serum adiponectin level in a cohort of patients with T2DM from Romania.

Recently, the study performed on Iranian population by Takhshid *et al.* [54] to evaluate the association between SNP 45T/G and serum adiponectin level reported no relation.

In contrast, in the study by Ragab *et al.* [55], who recruited 126 children and 192 adults to investigate the association of adiponectin gene SNPs 45T/G with markers of obesity, circulating total adiponectin concentrations, and IR in nondiabetic obese patients using light cycler real-time PCR, they reported that adiponectin gene SNP 45 is not associated with serum adiponectin, IR, and markers of obesity in nondiabetic obese Egyptians.

In contrast to the present study, the study by Rizk [56] revealed a significant association between the SNP 45T/G in the adiponectin gene and low serum adiponectin levels in Arab gulf populations.

Li *et al.* [44] performed a cross-sectional survey in a sample of 151 Chinese adults between 24 and 80 years of age, and they found that the adiponectin SNP 45 is positively correlated with the prevalence of T2DM and low level of plasma adiponectin concentrations in T2DM patients.

Biswas *et al.* [57] included 75 cases of T2DM and 75 apparently healthy controls from South India, and reported a strong association between SNP 45T/G and low adiponectin level in T2DM patients.

In Egypt, Khodeer *et al.* [39] included 64 Egyptians in their case–control study and found an association between SNP 45T/G of adiponectin gene and decreased levels of serum adiponectin in T2DM patients.

Complete sequencing of the ADIPOQ locus has been performed in a large White population where seven SNPs were shown to influence adiponectin levels, but the authors found no evidence of an association between these SNPs and T2DM in a diabetic case–control study [58,59].

These contradictions among studies may be attributed to differences in sample size or family history, environmental factors, anthropometric factors, and ethnic factors that may interfere with the results. Another possible explanation for these contradictory results is the presence of two types of circulating adiponectin, total and high molecular weight (HMW) adiponectin. Assays should differentiate between the types of circulating adiponectin, as not all circulating forms may be functional.

Conclusion

SNP 45T/G of adiponectin gene and serum adiponectin level were not associated with T2DM.

The study suggests that the ADIPOQ 45T/G may be a useful biomarker associated with risk for obesity in diabetic patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus – present and future perspectives. Nat Rev Endocrinol 2011; 8:228–236.

2 Unger RH, Orci L. Paracrinology of islets and the paracrinopathy of diabetes. Proc Natl Acad Sci USA 2010; 107:16009–16012.

3 Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001; 414:813–820.

4 Florez JC, Hirschhorn J, Altshuler D. The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits. Annu Rev Genomics Hum Genet 2003; 4:257–291.

5 Gloyn AL. The search for type 2 diabetes genes. Ageing Res Rev 2003; 2:111–127.

6 McCarthy MI. Progress in defining the molecular basis of type 2 diabetes mellitus through susceptibility-gene identification. Hum Mol Genet 2004; 13:R33–R41.

7 Havel PJ. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. Curr Opin Lipidol 2002; 13:51–59.

8 Zoccali C, Mallamaci F, Tripepi G, Benedetto FA, Cutrupi S, Parlongo S, *et al*. Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. J Am Soc Nephrol 2002; 13:134–141.

9 Tschritter O, Fritsche A, Thamer C, Haap M, Shirkavand F, Rahe S, *et al*. Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. Diabetes 2003; 52:239–243.

10 Daimon M, Oizumi T, Saitoh T, Kameda W, Hirata A, Yamaguchi H, *et al*. Funagata study Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in a Japanese population: the Funagata study. Diabetes Care 2003; 26:2015–2020.

11 Jee SH, Sull JW, Lee JE, Shin C, Park J, Kimm H, *et al*. Adiponectin concentrations: a genome-wide association study. Am J Hum Genet 2010; 87:545–552.

12 Heid IM, Henneman P, Hicks A, Coassin S, Winkler T, Aulchenko YS, *et al*. Clear detection of ADIPOQ locus as the major gene for plasma adiponectin: results of genome-wide association analyses including 4659 European individuals. Atherosclerosis 2010; 208:412–420.

13 Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006; 116:1784–1792.

14 Rabe K, Lehrke M, Parhofer KG, Broedl UC Adipokines and insulin resistance. Mol Med 2008; 14:741–751.

15 Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, *et al*. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proc Natl Acad Sci USA 2000; 97:14478–14483.

16 Kretowski A, Gugała K, Okruszko A, Wawrusiewicz-Kurylonek N, Górska M. Single nucleotide polymorphisms in exon 3 of the adiponectin gene in subjects with type 2 diabetes mellitus. Rocz Akad Med Bialymst 2005; 50:148–150.

17 Sattar N, Wannamethee SG, Forouhi NG. Novel biochemical risk factors for type 2 diabetes: pathogenic insights or prediction possibilities? Diabetologia 2008; 51:926–940.

18 Hajer GR, van Haeften TW, Visseren FL. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J 2008; 29:2959–2971.

19 Burtis CA, Ashwood ER, Bruns DE. Estimation of serum creatinine. In: Burtis CA, Ashwood ER, Bruns DE, editors. *Tietz text book of clinical chemistry and molecular diagnostics*. 4th ed. St Louis: Elsevier Saunders Company; 2006. 870–871.

20 Burtis CA, Ashwood ER, Bruns DE. Estimation of serum urea. In: Burtis CA, Ashwood ER, Bruns DE, editors. *Tietz text book of clinical chemistry and molecular diagnostics*. 4th ed. St Louis: Elsevier Saunders Company; 2006. 798–799.

21 Calleja AI, García-Bermejo P, Cortijo E, Bustamante R, Rojo Martínez E, González Sarmiento E, *et al*. Insulin resistance is associated with a poor response to intravenous thrombolysis in acute ischemic stroke. Diabetes Care 2011; 34:2413–2417.

22 Fraterrigo G, Fabbrini E, Mittendorfer B, O'Rahilly S, Scherer PE, Patterson BW, Klein S. Relationship between changes in plasma adiponectin concentration and insulin sensitivity after niacin therapy. Cardiorenal Med 2012; 2:211–217.

23 Salazar L. Optimized procedure for DNA isolation from fresh and cryo preserved clotted human blood useful in clinical molecular testing. Clin Chem 1998; 44:1748–1750.

24 Al-Daghri NM, Al-Attas OS, Alokail MS, Alkharfy KM, Hussain T. Adiponectin gene variants and the risk of coronary artery disease in patients with type 2 diabetes. Mol Biol Rep 2011; 38:3703–3708.

25 Schwarz PE, Govindarajalu S, Towers W, Schwanebeck U, Fischer S, Vasseur F, *et al*. Haplotypes in the promoter region of the ADIPOQ gene are associated with increased diabetes risk in a German Caucasian population. Horm Metab Res 2006; 38:447–451.

26 Menzaghi C, Trischitta V, Doria A. Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. Diabetes 2007; 56:1198–1209.

27 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; 51:2306–2312.

28 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; 81:428–434.

29 Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988–1994. Arch Intern Med 2003; 163:427–436.

30 Wang C, Wei D, Wang B, Zhang J, Zhang K, Ma M, *et al*. Effect of lifestyle on the prevalence of the metabolic syndrome among farmers, migrants with Yi ethnicity and Han population in Sichuan province of China. Asia Pac J Clin Nutr 2010; 19:266–273.

31 Li X, Wei D, He H, Zhang J, Wang C, Yu T, *et al*. Association of the adiponectin gene (ADIPOQ) +45T> G polymorphism with the metabolic syndrome among Han Chinese in Sichuan province of China. Asia Pac J Clin Nutr 2012; 21:296–301.

32 Heid IM, Wagner SA, Gohlke H, Iglseder B, Mueller JC, Cip P, *et al*. 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; 55:375–384.

33 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; 22:569–575.

34 Szopa M, Malczewska-Malec M, Kiec-Wilk B, Skupien J, Wolkow P, Malecki MT, Sieradzki J. Variants of the adiponectin gene and type 2 diabetes in a Polish population. Acta Diabetol 2009; 46:317–322.

35 Chiodini BD, Specchia C, Gori F, Barlera S, D'Orazio A, Pietri S, *et al*. GISSI Prevenzione Investigators; SiBioC-GISSI Prevenzione Group Adiponectin gene polymorphisms and their effect on the risk of myocardial infarction and type 2 diabetes: an association study in an Italian population. Ther Adv Cardiovasc Dis 2010; 4:223–230.

36 Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, *et al*. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes 2002; 51:536–540.

37 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; 69:764–771.

38 Fan Y, Wang K, Xu S, Chen G, Di H, Cao M, Liu C. Association between ADIPOQ +45T>G polymorphism and type 2 diabetes: a systematic review and meta-analysis. Int J Mol Sci 2014; 16:704–723.

39 Khodeer SA, Abdu-Allah AM, Abd El-Azeem WF, Mahfouz RG, Khamis S. Adiponectin single nucleotide polymorphism 45T/G and its relationship to adiponectin level in Egyptian patients with type 2 diabetes mellitus. J Pharm Biomed Sci 2011; 1:87–92.

40 Kacso IM, Trifa AP, Popp RA, Bondoe CI, Farcas MF, Lenghel AR, *et al*. Adiponectin gene 45T>G polymorphism is not associated to plasma adiponectin in a cohort of patients with type 2 diabetes from Romania. Rev Română Med Lab 2012; 20:73–79.

41 Zhao T, Zhao J. Genetic effects of adiponectin on blood lipids and blood pressure. Clin Endocrinol (Oxf) 2011; 74:214–222.

42 Koenig W, Khuseyinova N, Baumert J, Meisinger C, Löwel H. Serum concentrations of adiponectin and risk of type 2 diabetes mellitus and coronary heart disease in apparently healthy middle-aged men: results from the 18-year follow-up of a large cohort from southern Germany. J Am Coll Cardiol 2006; 48:1369–1377.

43 Stumvoll M, Tschritter O, Fritsche A, Staiger H, Renn W, Weisser M, *et al*. Association of the T-G polymorphism in adiponectin (exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes. Diabetes 2002; 51:37–41.

44 Li LL, Kang XL, Ran XJ, Wang Y, Wang CH, Huang L, *et al*. Associations between 45T/G polymorphism of the adiponectin gene and plasma adiponectin levels with type 2 diabetes. Clin Exp Pharmacol Physiol 2007; 34:1287–1290.

45 Nannipieri M, Posadas R, Bonotti A, Williams K, Gonzalez-Villalpando C, Stern MP, Ferrannini E. Polymorphism of the 3′-untranslated region of the leptin receptor gene, but not the adiponectin SNP45 polymorphism, predicts type 2 diabetes: a population-based study. Diabetes Care 2006; 29:2509–2511.

46 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; 11:2607–2614.

47 Gu HF, Abulaiti A, Ostenson CG, Humphreys K, Wahlestedt C, Brookes AJ, Efendic S. Single nucleotide polymorphisms in the

proximal promoter region of the adiponectin (APM1) gene are associated with type 2 diabetes in Swedish Caucasians. Diabetes 2004; 53(Suppl 1):S31–S35.

48 Nakatani K, Noma K, Nishioka J, Kasai Y, Morioka K, Katsuki A, *et al*. Adiponectin gene variation associates with the increasing risk of type 2 diabetes in non-diabetic Japanese subjects. Int J Mol Med 2005; 15:173–177.

49 Mousavinasab F, Tähtinen T, Jokelainen J, Koskela P, Vanhala M, Oikarinen J, *et al*. 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. Mol Genet Metab 2006; 87:147–151.

50 Kim B, Jang Y, Paik JK, Kim OY, Lee SH, Ordovas JM, Lee JH. Adiponectin gene polymorphisms are associated with long-chain ω3-polyunsaturated fatty acids in serum phospholipids in nondiabetic Koreans. J Clin Endocrinol Metab 2010; 95:E347–E351.

51 Cesari M, Narkiewicz K, De Toni R, Aldighieri E, Williams CJ, Rossi GP. Heritability of plasma adiponectin levels and body mass index in twins. J Clin Endocrinol Metab 2007; 92:3082–3088.

52 Suriyaprom K, Phonrat B, Namjuntra P, Harnroongroj T, Tungtrongchitr R. The −11377C>G adiponectin gene polymorphism alters the adiponectin concentration and the susceptibility to type 2 diabetes in Thais. Int J Vitam Nutr Res 2010; 80:216–224.

53 Al-Daghri NM, Al-Attas OS, Alokail MS, Alkharfy KM, Hussain T, Yakout S, *et al*. Adiponectin gene polymorphisms (T45G and G276T), adiponectin levels and risk for metabolic diseases in an Arab population. Gene 2012; 493:142–147.

54 Takhshid MA, Haem Z, Aboualizadeh F. The association of circulating adiponectin and 45T/G polymorphism of adiponectin gene with gestational diabetes mellitus in Iranian population. J Diabet Metab Disord 2015; 14:30.

55 Ragab S, Ismail NA, Assal H, Salama E, El-Lebedy D, Eman H. Influence of adiponectin gene polymorphisms on adiponectin level and insulin resistance in non-diabetic obese Egyptians. J Appl Sci Res 2013; 9:80–85.

56 Rizk N. Genetic association of adiponectin gene polymorphism in exon 2 with type 2 diabetes and CAD in Qatar. QNRS Repository 2011; 1:12–16.

57 Biswas D, Vettriselvi V, Choudhury J, Jothimalar R. Adiponectin gene polymorphism and its association with type 2 diabetes mellitus. Indian J Clin Biochem 2011; 26:172–177.

58 Demirci H, Yilmaz M, Ergun MA, Yurtcu E, Bukan N, Ayvaz G. Frequency of adiponectin gene polymorphisms in polycystic ovary syndrome and the association with serum adiponectin, androgen levels, insulin resistance and clinical parameters. Gynecol Endocrinol 2010; 26:348–355.

59 Tsuzaki K, Kotani K, Sano Y, Fujiwara S, Gazi IF, Elisaf M, Sakane N. The relationship between adiponectin, an adiponectin gene polymorphism, and high-density lipoprotein particle size: from the Mima study. Metabolism 2012; 61:17–21.