Association of polymorphism in adiponectin (+45 T/G) and leptin (–2548 G/A) genes with type 2 diabetes mellitus in male Egyptians

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Abstract

Introduction: Adiponectin is an adipose tissue-specific protein with insulin-sensitizing properties. Many investigators have explored the association between adiponectin single nucleotide polymorphisms (SNPs) and type 2 diabetes mellitus (T2DM) in different ethnic populations from different regions. Leptin is a protein hormone constituting an important signal in the regulation of adipose tissue mass and body weight. The aim of this study was to explore potential associations between SNP +45 T>G of the adiponectin gene and SNP 2548G/A of leptin with T2DM and the effect of SNPs on serum adiponectin and leptin levels.
Material and methods: From the Egyptian population, we enrolled 110 T2DM patients and 90 non-diabetic controls. Serum lipid profile, blood glucose, serum adiponectin, and leptin were measured. Genotyping for two common SNPs of the adiponectin and leptin genes was performed by polymerase chain reaction–restriction fragment length polymorphism.
Results: The G allele and TG/GG genotype of SNP 45 occurred more frequently than the T allele and TT genotype in T2DM patients compares to the controls. Subjects with the GG + TG genotype of SNP 45 were at increased risk for T2DM (OR = 6.476; 95% CI: 3.401–12.33) and associated with a low serum adiponectin level compared with the TT genotype. The serum leptin concentration of GA + AA genotype carriers was not significantly different from that of the GG genotype in the diabetic group.
Conclusions: The G allele carriers who have reduced plasma concentrations of adiponectin may have an association with T2DM, while leptin SNP 2548 G/A is not associated with the risk of development of T2DM in the Egyptian population.

Key words: single nucleotide polymorphism, polymerase chain reaction–restriction fragment length polymorphism.

Introduction

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, accounting for 85% to 95% of cases in developing nations, and the prevalence of T2DM in Egyptians less than 79 years of age is about 11.4% [1]. Obesity leads to an alteration in the profile of hormones secreted by adipose tissue (adipokines) and proinflammatory molecules that contribute to obesity-associated complications. These adipokines

are important determinants of insulin resistance and T2DM [2]. Adipose tissue-derived cytokines, termed adipokines, have been shown to be not only regulators of fibrogenesis, metabolic and inflammatory processes but also potent regulators of angiogenesis. Leptin, visfatin, chemerin and resistin have been found to promote, whereas adiponectin to attenuate, angiogenesis [3]. Adiponectin is a circulating 30-kDa protein located on chromosome 3q27, which has been reported to be linked to T2DM and metabolic syndrome [4]. Adiponectin is an insulin-sensitizing hormone that is negatively regulated by obesity [5]. Hypoadiponectinemia, caused by interactions of genetic factors such as single nucleotide polymorphism (SNPs) in the adiponectin gene and environmental factors causing obesity, appears to play an important causal role in insulin resistance, T2DM, and the metabolic syndrome, which are linked to obesity [6]. Circulating plasma adiponectin concentrations have been reported to be reduced in the obese state. An SNP in the adiponectin gene, in exon 2 (+45T/G), was screened and evaluated for its association with T2DM and serum adiponectin levels [7]. Most adipokines with pro-inflammatory properties are overproduced with increasing adiposity, e.g. leptin, while some adipokines with anti-inflammatory properties, such as adiponectin, are decreased [8]. Leptin is encoded by a gene located in human chromosome 7q31. Leptin resistance may represent an integrated marker for the inextricably linked disease states of obesity, metabolic syndrome, insulin resistance and T2DM [9]. Several sequence variants have been detected within the 5' flanking region of the human leptin gene [10]. Rare obesity syndromes are associated with mutations of the leptin gene in humans [11]. One polymorphism identified in the 5' untranslated region – c.–2548G>A – has been evaluated in different populations. A few association studies have suggested that individuals with polymorphisms of the LEP gene had a predisposition to developing obesity [12], yet there are findings contradicting this [13]. However, there are no previous longitudinal studies on the association of adiponectin SNP +45T/G and leptin SNP 2548G/A polymorphisms of the adiponectin gene with the risk of T2DM in Egyptian subjects with a high prevalence of obesity.

Therefore, we investigated whether adiponectin SNP +45 and leptin SNP 2548G/A polymorphisms and their serum levels are predictors for T2DM.

Table I. Clinical and hemodynamic characteristics of participants and comparison of adiponectin and leptin in the studied groups

Variables	Control	T2DM
n	90	110
Age [years]	50.7 ±1.64	51.0 ±1.26
BMI [kg/m^2]	32.6 ±0.47	33.7 ±0.78
DM duration [years]	–	3.1 ±0.23
FBG [mg/dl]	80.0 ±1.66	209 ±17.7[a]
Serum insulin [µIU/ml]	9.69 ±0.53	14.5 ±0.77[a]
HOMA-IR index	1.92 ±0.13	7.64 ±0.89[a]
Serum TAG [mg/dl]	115 ±5.71	202 ±20.1[a]
Serum TC [mg/dl]	159 ±5.07	204 ±4.07[a]
Serum HDL-C [mg/dl]	50.9 ±1.02	39.5 ±2.17[a]
Serum LDL-C [mg/dl]	87.0 ±3.75	135 ±4.84[a]
TC/HDL-C	3.3 ±0.08	5.53 ±0.39[a]
LDL/HDL	1.72 ±0.08	3.75 ±0.35[a]
Serum adiponectin [µg/ml]	23.3 ±1.35	12.3 ±0.93[a]
Serum leptin [µg/ml]	9.97 ±0.72	50.7 ±4.81[a]

T2DM – Type 2 diabetes mellitus, BMI – body mass index, FBG – fasting blood glucose, TAG – triacylglycerol, TC – total cholesterol, HDL-C – high-density lipoprotein, LDL-C – low-density lipoprotein cholesterol. Data are given as mean + SEM. [a]Significant difference from control group at $p < 0.05$.

Material and methods

The present study was performed in a representative sample of 200 Egyptian male adults who were chosen at random. Ninety non-diabetic subjects with a mean ± SEM age of 50.7 ±1.64 years were randomly recruited from the local population register of Cairo city to act as the control group (I) with normal fasting blood glucose (FBG). Inclusion criteria were as follows: (i) no previous history of hypertension; (ii) no family history of T2DM in first- and second-degree relatives. One hundred ten unrelated Egyptian patients with T2DM (group II) with mean age 51.0 ±1.26 years were recruited from the Endocrinology Department of Kasr Al-Aini Hospitals, Cairo, Egypt.

The study was approved by the Committee on Medical Ethics of Kasr Al-Aini Hospitals. The study was carried out in accordance with the regulations and recommendations of the Declaration of Helsinki. All participants gave their written informed consent prior to participation. A detailed medical history and drug treatments were collected for all participants. The exclusion criteria were as follows: female gender (to avoid the effect of female sex hormones on adipokine serum level), T1DM, insulin treatment, hepatic disease or drug abuse, cancer, any hematologic disorder (assessed by complete blood count for every participant), and smoking. Body mass index (BMI) was calculated as an index of the weight in kg divided by the square of the height in m (Table I).

Biochemical analyses

We collected 12 h fasting venous blood samples from each participant for serum leptin, adiponectin, FBG and lipid determinations. Fasting blood glucose [14], total cholesterol (TC) [15], and triglycerides (TAG) [16] were measured by using standard enzymatic techniques. High-density-lipoprotein (HDL) was determined after precipitation of apolipoprotein B-containing lipoproteins [17] and low-density lipoprotein (LDL-C) was determined according to the method of Hatch and Lees [18]. The reference values for the lipid profile were according to established guidelines [19]. Insulin sensitivity was assessed by indirect HOMA-IR index (Homeostasis Model Assessments) based on FBG and plasma insulin concentration [20]. Adiponectin and leptin were measured in serum aliquots kept frozen at –70°C using ELISA technique. Adiponectin human competitive ELISA Kit was obtained from AdipoGen Inc. (Incheon, Korea) [21]. Leptin was determined by the AssayMax Human Leptin ELISA kit obtained from Assaypro (Missouri, USA) [22].

DNA Extraction and adiponectin 45 T/G genotyping

SNP 45 T/G polymorphism was screened for nucleotide changes by direct sequencing. The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method was used to determine the distribution of allele and genotype frequencies of the SNP45 T/G polymorphism in exon 2 of the adiponectin gene. Overnight fasting blood samples were obtained from all subjects and genomic DNA was extracted from whole blood using the Qia-amplification extraction kit (Qiagen, USA). The DNA fragments containing SNP45 were amplified by PCR from genomic DNA using the forward primer 5’-GCAGCTCCTAGAAGTAGACTCTGCTG-3’ and the reverse primer 5’-GCAGGT CTG TGATGAAAGAGG-CC-3’ The PCR was performed on 30 ng DNA in 20 ml containing 10 mmol/l Tris HCl, 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, pH 8.3, 0.2 mmol/l dNTP, 0.4 mmol/l forward and reverse primers and 0.035 U/ml Taq polymerase for 35 cycles (30 s at 94°C, 30 s at 60°C, 30 s at 72°C). The PCR fragments (372 bp) were digested using the restriction enzyme SmaI (recognition site: CCC →GGG). Through agarose gel electrophoresis, genotypes were inferred by comparing the length of the restriction fragment. The success rate of all genotyping was > 95%. There were three genotypes identified: (i) wild type TT(372 bp); (ii) heterozygous TG (restriction fragments 372, 209 and 163 bp); and (iii) homozygous mutant GG (restriction fragments 209 and 163 bp) (Figure 1).

DNA Extraction and leptin –2548 G/A genotyping

SNP 2548 G/A polymorphism was screened for nucleotide changes by direct sequencing. LEP –2548 G/A polymorphism was detected by a PCR-RFLP technique using the endonuclease HhaI enzyme. The forward (5’-TTTCTGTAATTTTCCCGTGAG-3’) and reverse (5’-AAAGCAAAGACAGGCATAAAAA-3’) primers were designed based on the LEP promoter sequence. Each of the PCR reactions was performed in a total volume of 25 µl, containing 50 ng of DNA, 5 pmol of each primer, 1.5 µl of 10 x reaction buffer, 2 mM magnesium chloride, 0.2 mM dNTPs, and 1 unit of Taq DNA polymerase. The PCR consisted of an initial denaturation for 5 min (min) at 94°C, followed by 35 cycles of denaturation at 94°C for 45 s (s), annealing at 52°C for 45 s, extension at 72°C for 45 s, and a final extension at 72°C for 7 min. For the restriction fragment length polymorphism (RFLP), these PCR products were digested with restriction enzymes, using CofI Promega (Madison, WI. USA). 1.0 U per 15 µl of the product of PCR was incubated at 37°C for 60 min for the –2548G > A polymorphism in the LEP gene, generating a restriction pattern of 61 bp and 181 bp when the cleavage site is present (Figure 2). The success rate of all genotyping was > 95%.

Statistical analysis

The distribution of the alleles of SNP45 was tested for Hardy-Weinberg equilibrium ($p > 0.05$).

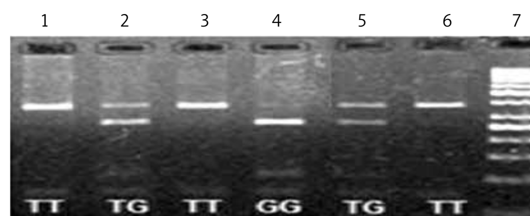

Figure 1. Agarose gel electrophoresis 3% stained with ethidium bromide showing the adiponectin gene amplification after RFLP. Lanes 1, 3, 6 – homozygous TT (372 bp). Lanes 2, 5 – heterozygous TG (372, 209 and 163 bp). Lane 4 – homozygous G (209 and 163 bp). Lane 7 – molecular marker (50 bp)

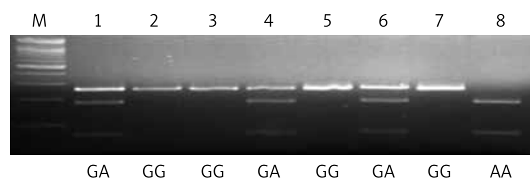

Figure 2. Agarose gel electrophoresis 3% stained with ethidium bromide showing the leptin gene amplification after RFLP. M – Molecular DNA marker. Lanes 1, 4, 6 – heterozygous GA (242, 181, 61 bp). Lanes 2, 3, 5, 7 – homozygous GG (242 bp). Lane 8 – heterozygous AA (181, 61 bp)

Proportions of genotypes of alleles were compared by χ^2 analysis, odds ratios (ORs) and 95% confidence intervals (CI). Descriptive statistics were computed for all variables. The results were expressed as means ± SEM. To determine the statistical significance of laboratory findings, multiple comparisons were achieved using analysis of variance (ANOVA) followed by Bonferroni post hoc analysis. Differences in serum adiponectin and leptin concentrations between individuals with different genotypes were tested by Student's *t*-test. The significance level was set at 0.05 or less. All statistical analyses were performed with SPSS, version 17.0 (SPSS Inc.).

Results

The clinical and demographic data of all participants are presented in Table I. As indicated in Table I, the mean ± SEM of FBG and insulin levels were significantly higher in the diabetic group (II) compared with non-diabetic control (group I) ($p < 0.05$). HOMA-IR (mean ± S.E.) was significantly elevated to 7.64 ±0.89 in the diabetic patients of group II ($p \le 0.05$) in comparison to the control group I (1.92 ±0.13). Regarding lipid profile, TAG and LDL-C in the diabetic group (II) showed a significant increase and HDL-C showed a significant decrease compared to the non-diabetic control (group I) ($p < 0.05$). Moreover, T2DM (group II) showed a significant increase in TC level compared to the non-diabetic control group (204 ±4.07, and 159 ±5.07 mg/dl, respectively) ($p \le 0.05$).

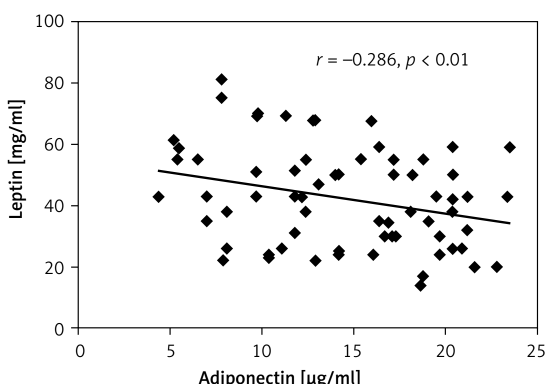

Figure 3. Correlation between adiponectin and leptin concentration in studied diabetic group (n = 68)

In the obese T2DM group (II) serum adiponectin showed a significant decrease in adiponectin level to 12.3 ±0.93 µg/ml compared to the non-diabetic control (group I; 23.3 ±1.35 µg/ml; $p \le 0.05$). Patients with T2DM of group II (50.7 ±4.81 ng/ml) showed a 5-fold increase in leptin level compared to the control group ($p \le 0.05$).

Stepwise multi-regression analysis shows that decreasing adiponectin (< 12.3 µg/ml) is a sensitive discriminator for prediction for a patient with T2DM from the control group (F-ratio = 38.2, $p < 0.001$).

In the obese T2DM groups (II) (n = 68), adiponectin level was significantly negatively correlated with leptin (r = –0.286, p = 0.01) (Figure 3), in addition to a negative and significant correlation between adiponectin and HOMA-IR (r = –0.272, p = 0.01). Adiponectin was negatively correlated with TC (r = –0.409, p = 0.0001) and LDL-C (r = –0.387, p = 0.001).

Association between SNP45 T>G in the adiponectin gene and T2DM

Table II shows that T2DM (group II) had a statistically significantly lower distribution of the TT genotype than the control (group I) (38% and 80% respectively) and a statistically higher distribution of the TG/GG genotype than the control group (62% and 20% respectively) (χ^2 = 35.32, p = 0.0001). Moreover, the results showed that the diabetic group (II) had a statistically higher distribution of G allele frequency than the control (group I) (35% and 13.5% respectively) and a lower distribution of the T allele frequency than the control group (65% and 86.5% respectively) (χ^2 = 24.62, p = 0.0001).

Subjects with the G/G genotype were at increased risk for T2DM (OR = 6.476, 95% CI: 3.401–12.33) compared with those having the T/T genotype. This finding is consistent with data showing that the G allele of SNP45 is significantly associated with T2DM.

Relationship between SNP +45 T>G and serum adiponectin levels

Results given in Table III revealed that the serum adiponectin concentration of TG + GG geno-

Table II. Differences in allele distribution and genotype frequency of adiponectin gene single nucleotide polymorphism (SNP) +45 T>G between control and T2DM group

Groups	n	Genotype frequency		Allele frequency	
		TT	TG + GG	T	G
Control (group I)	90	72 (80%)	18 (20%)	156 (86.5%)	24 (13.5%)
T2DM (group II)	110	42 (38%)	68 (62%)	143 (65%)	77 (35%)
		χ^2 = 35.32	p = 0.0001	χ^2 = 24.62	p = 0.0001

OR = 6.476, 95% CI: 3.401–12.33.

type carriers was not significantly different from that of the TT genotype in the control group (I). However, in the T2DM group (II) serum levels of adiponectin of the TG + GG genotype were significantly lower than those of the TT genotype.

Association between SNP –2548 G/A in the leptin gene and type 2 diabetes mellitus

The results obtained in this study (Table IV) showed that the obese T2DM group (II) had a lower distribution of the GG genotype than the control (group I) (41%, and 53% respectively) and a higher distribution of the GA/AA genotype than the control group but with no significant difference (59% and 47% respectively) (χ^2 = 2.55, p = 0.1103, OR = 1.579, 95% CI: 0.9002–2.769).

Relationship between SNP –2548 G>A and serum leptin levels

Results given in Table V revealed that the serum leptin concentration of GA + AA genotype carriers was not significantly different from that of the GG genotype in the control group (I). In the T2DM group (II) serum levels of leptin of the GA + AA genotype were higher, but not significantly, than those of the GG genotype (Table V).

Discussion

Type 2 diabetes mellitus is one of the significant comorbidities of obesity [23]. Obesity is associated with an increased risk of developing insulin resistance and T2DM. In obese individuals, adipose tissue releases increased amounts of pro-inflammatory cytokines and other factors that are involved in the development of insulin resistance (IR) and T2DM [24].

Diabetes mellitus often co-exists with obesity and dyslipidemia [25]. This study indicated significant high cholesterol, TAG, and LDL levels in the diabetic group (II) compared to the control group (I). Metabolic syndrome factors (increased BMI, elevated blood glucose, low HDL, and elevated TAG) tend to double the risk of vascular diseases and lead to a six-fold increase in risk for T2DM [26].

Adipose tissues express a variety of secretory proteins of potential importance for metabolic and vascular diseases. Adiponectin is an adipocyte-derived protein which lessened triglyceride accumulation in skeletal muscle and liver by facilitating fatty acid combustion and energy dissipation [27]. Therefore, deficiencies in adiponectin would presumably trigger insulin resistance, leading to the development of T2DM [28].

Adiponectin SNP45 may play a different role in the pathogenesis of T2DM in various ethnic groups (Table VI).

The present study found that among Egyptian subjects, the T2DM group had a higher distribution of the TG + GG genotype and G allele frequency than the control group. Thus, it is reasonable to speculate that the adiponectin SNP45 polymorphism may be correlated with the prevalence of T2DM in the Egyptian patients. The G allele may be the ultimate risk factor for T2DM Egyptian patients. In order to consolidate the relationship between this SNP45 polymorphism and plasma adiponectin concentrations, plasma adiponectin concentrations were measured using ELISA. Not surprisingly, the results showed that the T2DM group displayed a significantly lower plasma adiponectin concentration than the control group. Furthermore, plasma levels of adiponectin in individuals with the TG + GG genotype in the T2DM group were significantly lower than those with the TT genotype. These data indicate a significant role for the TG + GG genotype in plasma adiponectin levels and in the risk of T2DM [29, 30].

In the present study, patients with T2DM carrying the G allele of SNP45, a putative at-risk allele,

Table III. Differences in serum adiponectin concentrations among different genotype carriers (TT or TG + GG) between control subjects and T2DM group

Groups	Genotype	*n*	Adiponectin [μg/ml]
Control (group I)	TT	72	23.8 ±1.63
	TG/GG	18	21.1 ±1.49
T2DM (group II)	TT	42	15.8 ±0.93
	TG/GG	68	9.46 ±0.79[a]

Data are given as mean + SEM. [a]Significant difference from TT genotype at $p < 0.05$.

Table IV. Differences in allele distribution and genotype frequency of leptin gene single nucleotide polymorphism (SNP) –2548 G>A between control and T2DM group

Group	*n*	Genotype frequency		Allele frequency	
		GG	GA + AA	G	A
Control (group I)	90	47 (53%)	43 (47%)	122 (68%)	58 (32%)
T2DM (group II)	110	45 (41%)	65 (59%)	132 (60%)	88 (40%)
		χ^2 = 2.55	p = 0.1103	χ^2 = 2.584	p = 0.1080

OR = 1.579, 95% CI: 0.9002–2.769.

Table V. Differences in serum leptin concentrations among different genotype carriers (GG or GA + AA) between control subjects and T2DM group

Groups	Genotype	*n*	Leptin [ng/ml]
Control (group I)	GG	47	9.27 ±0.86
	GA/AA	43	10.8 ±1.20
T2DM (group II)	GG	45	40.7 ±6.66
	GA/AA	65	56 ±6.14

Data are given as mean ± SEM.

exhibited much lower plasma adiponectin concentrations than T2DM patients carrying the T allele. This suggests that the SNP45 polymorphism may affect IR, possibly through changes in mRNA stability, levels of adiponectin and eventually reduced plasma adiponectin concentrations. Our findings are similar to those reported by Li *et al.* [31]. Furthermore, this suggests that the adiponectin SNP45 polymorphism may be closely correlated with the prevalence of T2DM.

Also in our study we observed a negative correlation between the two main opposing adipokines, adiponectin and leptin, suggesting their negative and positive correlation with BMI respectively.

Leptin is a hormone that consists of 167 amino acids transcribed by the leptin gene (LEP) and secreted primarily by adipocytes. Leptin is the product of the ob gene, secreted mainly by white adipose tissue [32–36]. It is a 14-kDa protein and is strongly correlated with body fat mass, being elevated in obese subjects [37]. Leptin expression and secretion are highly correlated with body fat and adipocyte number and size [38]. Wauters *et al.* [39] reported that leptin concentration in obese and T2DM was significantly elevated compared to non-diabetic controls, which is consistent with our study that revealed a significant increase in serum leptin levels in the diabetic group (II) compared to the control group (I). The results suggest that increased leptin levels, probably reflecting leptin resistance, were strongly related to insulin resistance.

G-2548A polymorphism in the leptin gene promoter is close to an SP-1 DNA binding site [40]. Hoffstedt *et al.* [41] reported that nuclear extracts derived from human adiposities were able to bind a DNA fragment spanning the –2548G/A polymorphic site. Interestingly, DNA binding affinity was higher when nucleotides A were present at the –2548 position. Thus it is possible that leptin G-2548A polymorphism could affect lep transcription and therefore leptin synthesis.

Notably, the G-2548A polymorphism in the leptin promoter (Hoffstedt *et al.* [41], Wang *et al.* [42], Duarte *et al.* [43], Hinuy *et al.* [44]) has been associated with obesity and high leptin levels in several populations, although no linkage was found in other groups (Matsuoka *et al.* [45], Wauters *et al.* [46], Heo *et al.* [47], Mergen *et al.* [48]), which clearly highlights the relevance of genetic components of obesity in each population. To date, little is known about the association of lepG-2548A polymorphisms with T2DM in the Egyptians population.

The LEP 2548G >A variant may influence the LEP gene expression and the leptin secretion by adipose tissue [49]. The G2548A polymorphism is located at the 5′ end of the promoter region of LEP, and it has been suggested that this remote region may contain inhibitory elements from transcription in adipocytes [50]. Even though this polymorphism is close to an SP-1 transcription factor binding site, as well as two repetitive sequences, MER11 and Alu, that may regulate LEP

Table VI. SNPs in the adiponectin gene 45 T/G

Parameter association	Population	Reference
GG and TG genotypes were at higher risk for T2DM	Obese Iranians	29
GG associated with T2DM	Japanese	30
G allele associated with T2DM (lower insulin sensitivity), lower adiponectin, higher blood pressure, higher LDL and total cholesterol levels	Chinese (T2DM)	31
GG carriers had higher risk of becoming hyperglycemic/diabetic, associated with increase in BMI. G associated with higher adiponectin levels	French Caucasian	32, 33
No difference in risk for T2DM or IR	Korean (diabetic/non-diabetic)	34
G allele conferred higher risk of developing T2DM than TT genotype	European/Canadian subjects with impaired glucose tolerance	35
GT genotype associated with impaired glucose tolerance	Spanish	36

transcription, the effect of the G to A substitution at nucleotide 2548 in LEP expression remains to be elucidated.

Thus it is possible that lepG-2548A polymorphism could affect lep transcription and therefore leptin synthesis. Although we did not observe a clear association of lepG-2548A polymorphism and obesity status, our study found that among Egyptian subjects, the T2DM group had no statistically significant distribution of the GA + AA genotype and A allele frequency compared to the control group. It is interesting to note that leptin serum levels in individuals with the GA + AA genotype in the diabetic group (II) were higher but not significantly so than those with the GG genotype in the diabetic Egyptian population. This is not in agreement with a previous study that showed an association between AA genotype and leptin resistance in French Caucasians [51].

In conclusion, to our knowledge, this is the first study about the possible relationships between leptin G-2548A and adiponectin 45 T/G polymorphisms and their serum levels and T2DM in an Egyptian population. Altogether, our results evidenced that these associations depend on ethnological factors and characteristics of each population. The adiponectin SNP45 polymorphism is closely correlated with the prevalence of T2DM in Egyptians. The incidence of T2DM is expected to be much higher in G allele carriers, which may be underscored by alterations in adiponectin expression and plasma concentrations in these G allele carriers. SNP leptin 2548 G/A is not associated with the risk of development of T2DM in the Egyptian population, suggesting that this polymorphism does not affect leptin transcription.

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Conflict of interest

The authors declare no conflict of interest.

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