The relationship between adiponectin gene polymorphism and occurrence of obesity at Zagazig University Hospitals

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Received 25 March 2019
Accepted 14 April 2019

Egyptian Journal of Obesity, Diabetes and Endocrinology 2018, 4:90–99

Background
Obesity is considered a severe rapidly growing health problem all over the world. Adiponectin is an adipokine produced and secreted by adipose tissues and widely known as antidiabetic, anti-inflammatory, antiatherogenic, and cardioprotective factor. The adiponectin gene has various single nucleotide polymorphisms (SNPs).
Objective
Our aim of this study was to determine the genotype frequency of SNPs (276 G→T) in adiponectin gene and its relationship with the occurrence of obesity and to detect the association between adiponectin gene polymorphism and different degrees of obesity.
Patients and methods
A total of 96 volunteers were included and divided into the following: group I had 48 healthy nonobese control volunteers, and group II included 48 obese patients not having any disease.
Anthropometric parameters were measured by standard procedures. Random blood sample was collected for routine and research investigations. PCR assay with restriction fragment length polymorphism was used to examine the adiponectin gene SNP276G>T polymorphism.
Results
Genotypes distributions of 276G>T polymorphisms were significantly different between obese and nonobese cases. T allele was significantly associated with obese individuals ($P<0.05$) with odds ratio (OR) of 2.13 and found to be significantly associated ($P<0.05$) and risky for BMI more than or equal to 30, with OR 3.86. Both of the genotype TT and allele T were significantly associated and risky for abdominal obesity ($P<0.05$) with OR of 3.57 for TT genotype and 3.61 for T allele.
Conclusion
The T allele and TT genotypes at the 276 locus of the ADIPOQ gene were associated with higher risk of obesity.

Keywords:
adiponectin gene polymorphism, anthropometric parameters, obesity

Egypt J Obes Diabetes Endocrinol 4:90–99

2356-8062

Introduction

Obesity is considered a severe rapidly growing health problem all over the world [1] being described nowadays as the New World Syndrome [2]. It is expected to become the most dangerous common health problem of the 21st century [3].

It is a medical condition in which excess abnormal body fat accumulates to the extent owing to an imbalance between energy intake and output, so it may be hazardous to human health [4]. Obesity is considered a leading but preventable cause of death worldwide, with increasing prevalence in adults and children [5]. It is one of the most common silent killers around the world that reduces the expectancy of life by 6–7 years on average [6].

According to WHO most recent reports, approximately 600 million adults (13%) and 42 million children under the age of 5 years were obese, but it is more common in women than men [7].

Unfortunately, the Middle East and North Africa regions have the highest rates of overweight and obesity of the developing world, with its effects on local health service and prevalence of severe comorbidities [8].

In Egypt, very apparent paradox is that obesity prevalence is very high compared with the economic development level of the country where urban Egyptian

women have a higher prevalence of obesity compared with most other developing countries [9].

Adipose tissue has been recognized to be an active endocrine organ, producing some important molecules involved in fatty acid and glucose metabolism [10].

Adiponectin is an adipokine produced and secreted by adipose tissues and is widely known as a antidiabetic, anti-inflammatory, antiatherogenic, and cardioprotective factor [11,12].

The adiponectin gene has various single nucleotide polymorphisms (SNPs). One of these SNPs is the 45T/G polymorphism, encountered in exon 2 of the adiponectin gene, which has been frequently found to be associated with obesity and breast cancer, and the second rs1501299 (G276T) is in intron 2 of the ADIPOQ gene [13].

Patients and methods

This study was carried out in the Department of Internal Medicine, Outpatient Clinic of Endocrinology and Medical Biochemistry Department Laboratory, Faculty of Medicine, Zagazig University, and the Outpatient Clinic of the Department of Internal Medicine, Abu Hammad General Hospital. The study was approved by the ethical committee of Zagazig University, and informed consent was taken from each participant before starting the study.

Patients

A total of 96 Egyptian patients participated in the study. The study participants were recruited among the patients attending the outpatient clinic of internal medicine at Zagazig hospitals. All patients provided a written informed consent to participate in this study. They were further divided into the following:

Group I: this group included 48 control patients who had been matched for BMI, sex, age, and socioeconomic background. Their BMI was less than or equal to 25 kg/m^2. It comprised 22 males and 26 females. Their ages were between 19 and 55 years, with a mean±SD value of 28.28±7.54 years.

Group II: this group included 48 patients, comprising 23 males and 25 females. Their BMI was more than or equal to 25 kg/m^2.

Their ages were between 19 and 55 years with a mean value±SD of 39.25±7.6 years.

There were eight individuals with BMI 25–30 kg/m^2, four males and four females. Also, forty individuals (19 males and 21 females) have BMI≥30 kg/m^2.

For all patients, a complete medical history was obtained by questionnaire. History taking included questions about smoking habits, history of hypertension and type 2 diabetes, and current medication used.

Methods

All patients and control groups were subjected to the following:

(1) History taking, including personal, present, and past medical and surgical, family, and socioeconomic history.
(2) General examination of vital signs, blood pressure, and heart and abdominal examination.
(3) Anthropometric assessment (Quetelet,1830–1850).
Anthropometric measurements were carried out according to Jelliffe (1966), where weight, height, and waist circumference were measured.
The weight of each participant was obtained using electronic digital LCD weighing scale, wearing light cloths and without shoes to the nearest 0.5 kg. Height was measured to the nearest 1 cm while the participants stood straight and without shoes using a plastic tape.
Waist circumference was measured while the individuals were breathing normally to the nearest (cm) in standing position, midpoint between margin of lower rib and the iliac crest using a plastic tape.
(4) BMI calculation:
BMI is calculated according to the following formula (Quetelet, 1830–1850):

BMI=weight (kg)/height (m)^2. Obesity was considered if BMI is 30 kg/m^2 and more as proposed by WHO (2004) (Table 1)
(5) Determination of adiponectin gene.

Table 1 Classification of obesity (WHO, 2000)

BMI (kg/m^2)	Classification
<18.5	Underweight
18.5–24.9	Normal weight
25.0–29.9	Overweight
30.0–34.9	Class I obesity
35.0–39.9	Class II obesity
≥40.0	Class III obesity

276 G>T SNP:

It is done by PCR-based restriction fragment length polymorphism (RFLP).

Three milliliters of venous blood samples was taken from every participant under complete aseptic condition in sterile EDTA-containing tubes using standardized protocol and equipment for DNA extraction. Extracted DNA was stored at −20°C till time of assay.

Adiponectin gene polymorphism (G276T) analysis

The DNA analysis was done using PCR to identify the polymorphism of adiponectin gene at position G276T, and then RFLP technique followed it.

The test was done in five main steps:

(1) DNA extraction from peripheral blood leukocytes collected in EDTA sample.
(2) The extracted DNA was amplified.
(3) The amplified PCR products were detected by agarose gel electrophoresis and ultraviolet light transillumination.
(4) The amplified PCR product length of 196 bp was obtained by restriction enzyme Mva 1269I, at 37°C for 15 min.
(5) Electrophoresis for digested amplified PCR products on agarose gel with ethidium bromide, and then visualized by ultraviolet transilluminator for identification of polymorphism.

Extraction of DNA and genotyping

DNA has been isolated using the DNA extraction kits in which standard DNA isolation from 500 µl of whole blood in approximately 45 min, including treatment of RNase.

The average DNA concentration (127.49±5.05 µg/ml) was obtained from absorbance at 260 nm. All samples of DNA were examined at a 260/280 nm absorbance ratio.

The DNA was checked by electrophoresis on 1.5% agarose gel with an ethidium bromide. Isolated DNA was used for detection of SNP in the adiponectin gene (276 G>T). The SNP was detected by PCR.

T",5,0,2,0,280pt,240pt,0mm,0mm>Detection of single nucleotide polymorphisms adiponectin 276G>T

Forward primer* 5? – GGC CTC TTT CAT CAC AGA CC – 3?.

Reverse primer* 5? – AGA TGC AGC AAA GCC AAA GT –3?.

PCR product length of 196 bp was obtained by restriction enzyme Mva 1269I°. RFLP conditions were 37°C/24 h.

Gel electrophoresis for PCR-digested products was done using 1.5% agarose gel and 50× tris-acetate-EDTA buffer. Ethidium bromide 5 mg/ml was added to the agarose before pouring into the tray.

Sample preparation and loading

Each digested product sample was prepared and electrophoresed on 1.5% agarose gel and visualized under ultraviolet transilluminator with 100 base pair ladder and photographed.

Statistical analysis

Data collected throughout history, basic clinical examination, laboratory investigations, and outcome measures were coded, entered, and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences, version 20.0 (Armonk, New York, US), software for analysis. According to the type of data, qualitative data were represented as number and percentage, and quantitative data were represented by mean±SD. The following tests were used to test differences for significance: difference and association of qualitative variable by χ^2, differences between quantitative independent groups by t test, and risk by odds ratio (OR). P value was set at less than 0.05 for significant results and less than 0.001 for high significant result.

Results

Basic characteristics of all different groups participated in this study are listed in Tables 2 and 3 and Figs 1–5.

Mean±SD of all biochemical parameters, including sex, age, waist circumference, and BMI, was calculated.

Mean±SD of age was 33.32±9.3 years, with minimum 19 years and maximum 55 years. Regarding sex, there were 45 males and 51 females, with percentages of 46.9 and 53.1%, respectively.

Overall, 48 (50%) individuals were nonobese and 48 (50%) were obese patients.

Mean±SD of BMI (29.54±7.6 kg/m^2) distributed as following:

Table 2 Sociodemographic and body state distribution

	Mean±SD [median (range)]
Age (year) (mean±SD)	33.32±9.3
Median (range)	32.0 (19–55)
Waist circumference (mean±SD)	92.25±13.7
Median (range) (cm)	89.0 (72–121)
BMI (kg/m^2) (mean±SD)	29.54±7.6
Median (range)	25.0 (20–46)
Sex [*n* (%)]	
Male	45 (46.9)
Female	51 (53.1)
Obesity [*n* (%)]	
Not obese	48 (50)
Obese	48 (50)
WC (cm)	
<80 cm in female or <94 in male	32 (33.4)
>80 cm in female or >94 in male	64 (66.6)
BMI (kg/m^2)	
<25	48 (50)
25–30	8 (8.3)
>30	40 (41.7)
Total	96 (100.0)

Age was distributed as 33.32±9.3 years, with minimum 19 years and maximum 55 years. waist circumference and BMI were distributed as 92.25±13.7 cm and 29.54±7.6 kg/m^2, respectively. Males and females were nearly matched, and 45.8% were obese. $^{**}P$ value 0.00 ($P<0.05$).

Table 3 Age and sex distribution between obese and nonobese

	Not obese (*N*=48)	Obese (*N*=48)	*T*	*P*
Age	28.28±7.54	39.25±7.6	7.054	0.00**
Sex [*n* (%)]				
Male	22 (45.8)	23 (47.9)	0.04	0.83
Female	26 (54.2)	25 (52.1)		
Total [*n* (%)]	48 (100.0)	48 (100.0)		

No significant difference or association between sex and obesity but obese were significantly older.

(1) BMI less than or equal to 25 kg/m^2 was present in 48 (50%) patients.
(2) BMI 25–30 kg/m^2 was present in 8 (8.3%) patients.
(3) BMI more than or equal to 30 kg/m^2 was present in 40 (41.7%) patients.

Mean±SD of waist circumference was 92.25±13.7 cm, where 32 (33.4%) patients had normal waist circumference (<80 cm in female or <94 cm in male), whereas 64 (66.6%) patients had abdominal obesity (>80 cm in female or >94 cm in male).

(1) Obese individuals comprised 23 (47.9%) males and 25 (52.1%) females. Mean±SD of age in obese patients was 39.25±7.6 years.
(2) Nonobese individuals comprised 22 (45.8%) males and 26 (54.2%) females. Mean±SD of age in nonobese patients was 28.28±7.54 years.

There was no significant difference or association between sex and obesity, but regarding age, obese patients were significantly older ($P<0.05$)

Genotype and alleles frequencies for adiponectin 276G>T are presented in Tables 4 and 5 and Fig. 6.

Total homogenous genotype GG number in our study was found in 18 (18.8%) patients, total heterogeneous genotype GT number was found in 39 (40.6%) patients, and total homogenous genotype TT number in this study was found in 39 (40.6%) patients.

The G allele frequency was found in 75 (39.0%) patients, but the T allele frequency was found in 117 (61.0%) patients.

In obese patients, homogenous genotype GG number was found in five patients with percentage 12.5%. Heterogeneous genotype GT number was found in 19 patients with percentage 39.5%, whereas homogenous genotype TT number was found in 24 patients with percentage 47.9%. The G allele frequency was found in 29 patients with percentage 30.2%, but the T allele frequency was found in 67 patients with percentage 69.8%.

It was found that obese individuals were significantly associated ($P<0.05$) with T allele with OR 2.13.

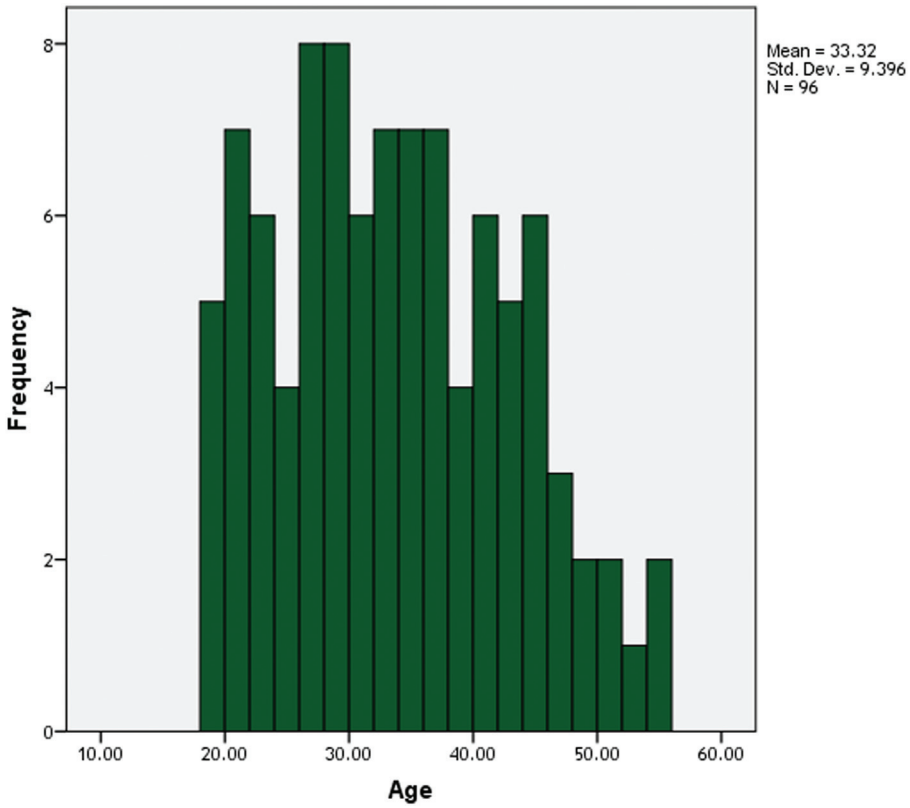
In nonobese patients, homogenous genotype GG number was found in 13 patients with percentage 27.08%. Heterogeneous genotype GT number was found in 20 patients with percentage 41.6%, whereas homogenous genotype TT number was found in 15 patients with percentage 31.2%. The G allele frequency was found in 46 patients with percentage 47.9%, but the T allele frequency was found in 50 patients with percentage 52.1%.

There was no significant difference or association ($P>0.05$).

Comparison among obese between BMI more than or equal to 30 kg/m^2 and 25–30 kg/m^2 is presented in Tables 6 and 7.

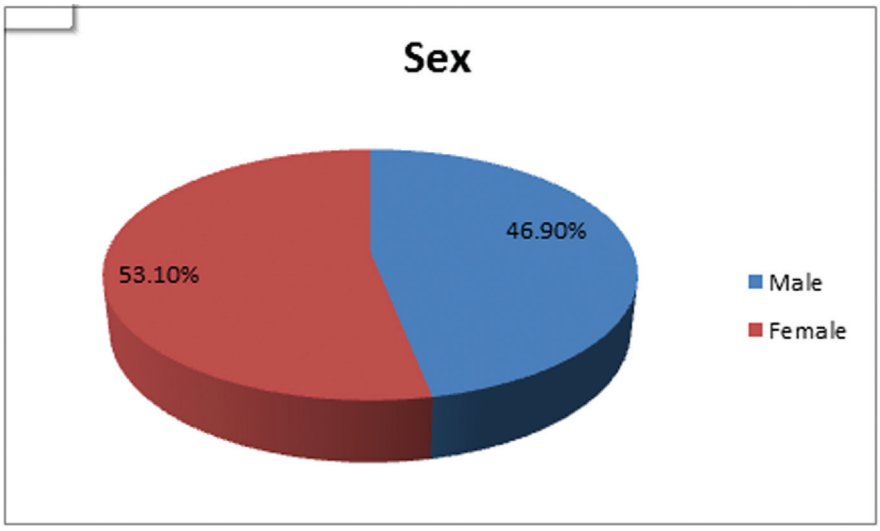
Obese individuals with BMI 25–30 kg/m^2 included four (50%) males and four (50%) females. The mean ±SD of their ages was 42.0±7.34 years. Genotype frequency was GG among two patients, with percentage 25.0%; five patients were GT, with percentage 62.5%; and only one patient was TT, with percentage of 12.5%. The allele frequency was

Figure 1



Age distribution between obese and nonobese patients.

Figure 2



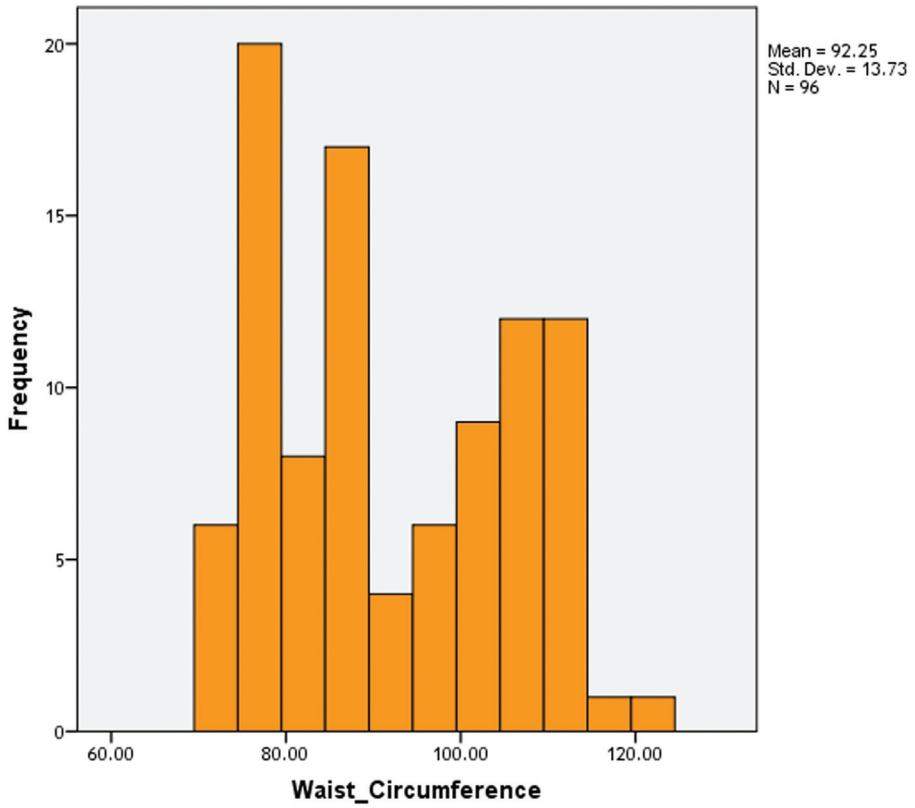
Sex distribution between obese and nonobese patients.

9 G in approximately 56.2% and 7 T in approximately 43.8%.

Obese individuals with BMI more than or equal to 30 kg/m^2 comprised 19 (47.5%) males and 21 (52.5%) females. The mean±SD of their ages was 39.02±7.33.

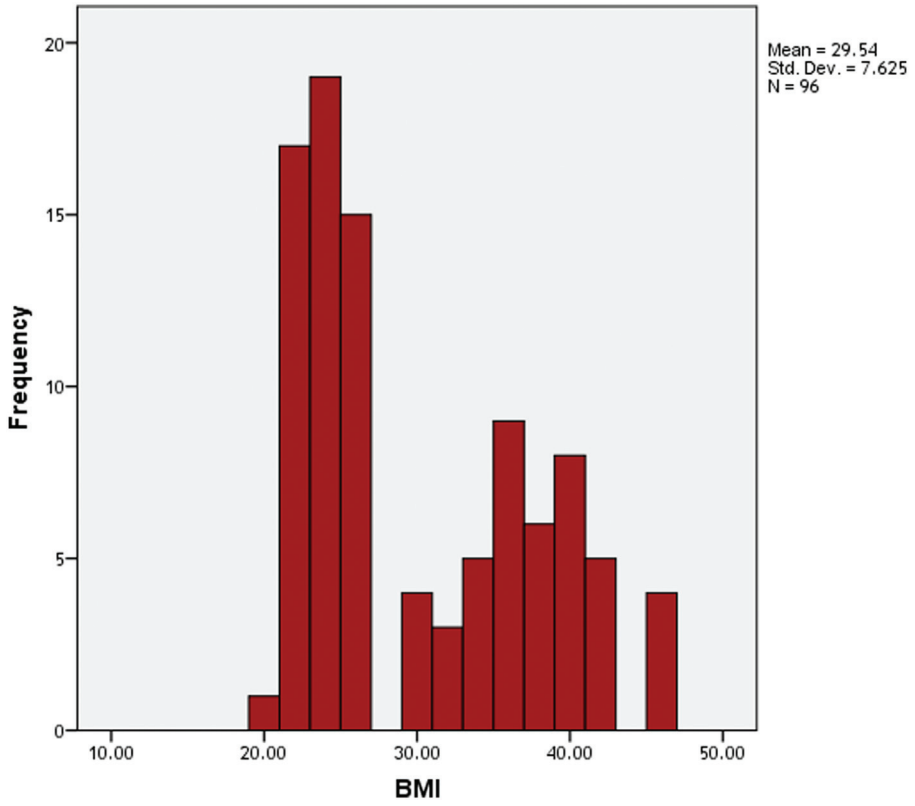
Genotype frequency was GG among three patients with percentage 7.5%, 14 patients were GT with percentage 35.0%, and 23 patients were TT with percentage 57.5%. The allele frequency was 20G in approximately 25.0% and 60T in approximately 75.0%.

Figure 3



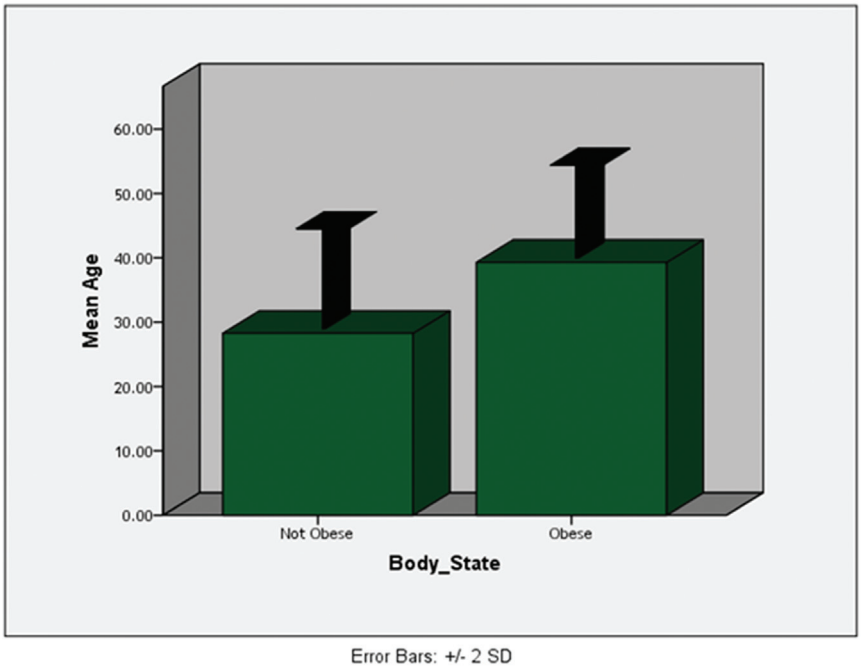
Waist circumference distribution between obese and nonobese patients.

Figure 4



BMI distribution between obese and nonobese patients.

Figure 5



Relationship between BMI and age.

Table 4 Genotypes and allele distribution among the groups

	n (%)
Genotype	
GG	18 (18.8)
GT	39 (40.6)
TT	39 (40.6)
Total	96 (100.0)
Allele	
G	75 (39.0)
T	117 (61.0)

T allele was found significantly associated ($P<0.05$) and risky for BMI more than or equal to 30 kg/m^2, with OR 3.86.

Relationship between abdominal obesity and frequencies of genotype and alleles among groups is present in Table 8.

Waist circumference of less than 80 cm in female or less than 94 cm in male represented 32 patients, with percentage of 33.4%. The genotypes among this group were distributed as follows: genotype GG was found in 12 patients, with percentage 37.5%; genotype GT was found in 13 patients, with percentage 40.6%; and genotype TT was found in seven patients, with percentage 21.8%. The G allele was present in 39, representing 43.9%, whereas the T allele was present in 27, representing 56.1%.

Waist circumference of more than 80 cm in female or more than 94 cm in male represented 64 patients with percentage of 66.6%. The genotypes among this group were distributed as follows: genotype GG was found in six patients, with percentage of 9.3%; genotype GT was found in 26 patients, with percentage of 40.7%; and genotype TT was found in 32 patients, with percentage of 50.0%. The G allele was 36, representing 36.5%, whereas the T allele was 90, representing 63.5%.

It was found that both genotype TT and allele T were significantly associated and risky for abdominal obesity ($P<0.05$) with OR 3.57 for TT genotype and 3.61 for T allele.

Discussion

Our aim of this study was to determine the genotype frequency of SNPs (276 G>T) in adiponectin gene and its relationship with the occurrence of obesity and to detect the association between adiponectin gene polymorphism and different degrees of obesity.

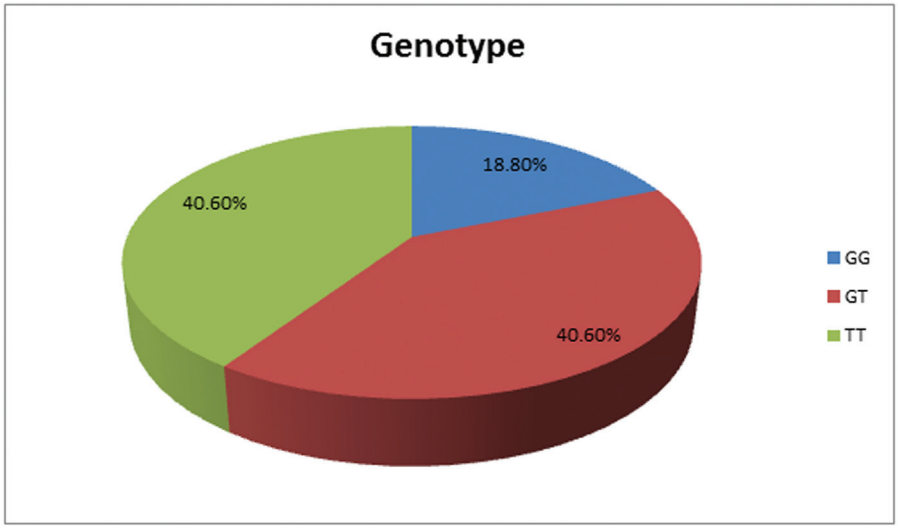
Our data suggest the close association of adiponectin gene with obesity in general and abdominal obesity especially. Our study was on the association of adiponectin 276 G>T SNP with obesity in Zagazig University Hospitals dividing the participated patients into two main groups: control nonobese volunteers and obese patients not having any disease.

Table 5 Genotype and allele distribution between obese and nonobese patients

	Body state [*n* (%)]					
	Not obese	Obese	Total [*n* (%)]	χ^2	*P*	OR (95% CI)
Genotype frequency						
GG	13 (27.08)	5 (12.5)	18 (18.8)	5.6	0.059	
GT	20 (41.6)	19 (39.5)	39 (40.6)			
TT	15 (31.2)	24 (47.9)	39 (40.6)			
Allele frequency						
G	46 (47.9)	29 (30.2)	75 (39.0)	6.32	0.011*	2.13 (1.13–4.2)*
T	50 (52.1)	67 (69.8)	117 (61.0)			

Obese individuals were significantly associated ($P<0.05$) with T allele with OR of 2.13. CI, confidence interval; OR, odds ratio. *P value 0.011.

Figure 6



Genotypes distribution among the groups.

Table 6 Comparison among obese between BMI more than or equal to 30 kg/m^2 and 25–30 kg/m^2

	BMI >30 (*N*=40)	BMI 25–30 (*N*=8)	*T*	*P*
Age	39.02±7.33	42.0±7.34	–0.77	0.44
Sex [*n* (%)]				
Male	19 (47.5)	4 (50.0)	0.017	0.89
Female	21 (52.5)	4 (50.0)		
Total [*n* (%)]	40 (100.0)	8 (100.0)		

No significant difference or association.

Our study confirmed a significant association of 276 T allele frequency in obese patients when compared with control nonobese cases in contrast to those carrying 276 G allele. Moreover, a significant association of 276 T allele frequency has been noticed with the different degrees of obesity, as the higher the BMI, the more significant the association of T allele.

Another significant association was found between 276 T allele and abdominal obesity measured by waist circumference more than 94 cm in males and 80 cm in females.

We could consider that both the T allele and TT genotypes have the higher risk of obesity.

Some studies agree with our finding like those of Salmenniemi *et al.* [14], Yang *et al.* [15,16], and Melistas *et al.* [17] who noticed that patients having the SNP276 T-containing genotypes (TT or GT) experienced central obesity, and they had the greatest risk of being diabetic. They confirmed the correlation between the T allele and obesity. Moreover, those with T allele had great risk of being hyperglycemic than those carrying G allele, which play a protective role against the development of overweight and obesity.

Mackawy *et al.* [18] published data that agree with our study showing that T allele and TT genotypes of 276 G>T SNP associated with higher risk of obesity, lower plasma adiponectin, insulin resistance, and higher parameters of metabolic syndrome and type 2 diabetes mellitus.

Table 7 Genotype and allele distribution among obese patients

	BMI					
	25–30	>30	Total	χ^2	*P*	OR (95% CI)
Genotype frequency [*n* (%)]						
GG	2 (25.0)	3 (7.5)	5 (12.5)	5.93	0.052	
GT	5 (62.5)	14 (35.0)	19 (39.5)			
TT	1 (12.5)	23 (57.5)	24 (47.9)			
Allele frequency [*n* (%)]						
G	9 (56.2)	20 (25.0)	29 (30.2)	6.17	0.012*	3.86 (1.12–3.4)*
T	7 (43.8)	60 (75.0)	67 (69.8)			

T allele was found to be significantly associated ($P<0.05$) and risky for BMI more than or equal to 30 with OR 3.86. CI, confidence interval; OR, odds ratio. **P* value 0.012.

Table 8 Abdominal obesity relationship with genotype and allele distribution

	WC					
	<80 cm in female or <94 cm in male	>80 cm in female or >94 cm in male	Total	χ^2	*P*	OR (95% CI)
Genotype frequency [*n* (%)]						
GG	12 (37.5)	6 (9.3)	18 (18.8)	13.1	0.001**	For TT 3.57 (1.24–10.64)*
GT	13 (40.6)	26 (40.7)	39 (40.6)			
TT	7 (21.8)	32 (50.0)	39 (40.6)			
Allele Frequency [*n* (%)]						
G	39 (43.9)	36 (36.5)	75 (39.0)	16.94	0.00**	3.61 (1.85–7.09)*
T	27 (56.1)	90 (63.5)	117 (61.0)			

Both genotype TT and allele T were significantly associated and risky for abdominal obesity ($P<0.05$), with OR of 3.57 for TT genotype and 3.61 for T allele. CI, confidence interval; OR, odds ratio. ***P* value 0.001 for TT genotype. ***P* value 0.00 for T allele.

Another study revealed data like our results, which was published by Zaki *et al.* [19], who showed that the risk of obesity was associated with presence of T allele and TT genotype.

Our findings are in harmony with previously published study of Jang *et al.* [20,21] where the phenotypic expression of the T allele was observed only among obese patients with elevated BMI only. They stated that there is significant contribution of ADIPOQ_276 SNPs to obese patients where the risk of diabetes is greater in contrast to lean patients with normal BMI.

The present data are in accordance with previously published findings by Filippi *et al.* [22] who reported that the T allele is associated with high risk of obesity and insulin resistance.

We noticed no significant association of the different genotypes GT or GG was found except the genotype TT which associated significantly with abdominal obesity.

Interestingly, regarding the association of obesity with age, it was noticed that obese patients were significantly older, but regarding sex, there was no significant difference or association. This may be owing to the small sample size.

In contrast to our findings, Mohammad *et al.* [23] could not find any significant difference or association in genotype and allele frequencies of SNP276 comparing control group with obese group.

In contrast to our results, other studies have revealed that the 276 T allele did not have any association to obesity and found to be protective for insulin resistance, and the G/G genotype of SNP276 had a higher risk of insulin resistance, such as Hara *et al.* [24], Stumvoll *et al.* [25], Gonzalez-Sanchez *et al.* [26], Xita *et al.* [27], and Fredriksson *et al.* [28].

Moreover, Boumaiza *et al.* [29] observed a protective role among Tunisians of the 276 G>T SNP in obesity risk. Menzaghi *et al.* [30] and Stumvoll *et?A3B2 show $146#?>al.* [25] reported the same.

Possible explanation for these conflicting association findings in various populations can be referred to differences in family history, anthropometric factors, ethnic factors, and environmental factors that may interfere with the results and causing different

findings. Moreover, they suggest the complex relationship between ADIPOQ gene variation and both obesity and insulin resistance [31].

In the current study, the observed T allele frequency in GT and TT genotypes carriers of adiponectin 276 G>T in obese patients, which were significantly higher than in control nonobese patients, suggests the close association of adiponectin 276 T allele to obesity and obesity-related diseases.

Conclusion

The results of the current study represent the association of SNP276 G>T genotypes of the adiponectin gene with general and central obesity in obese patients at Zagazig University Hospitals. Additionally, T allele was significantly associated with obese individuals and found significantly associated and risky for BMI more than or equal to 30 kg/m^2. Moreover, both of the genotype TT and allele T were significantly associated and risky for abdominal obesity. We concluded that the T allele and TT genotypes at the 276 locus of the ADIPOQ gene were associated with higher risk of obesity.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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