

Original Article

ASSOCIATION OF ADIPONECTIN GENE POLYMORPHISMS 276G>T WITH OBESITY AND BIOCHEMICAL PARAMETERS IN ADOLESCENTS

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ABSTRACT

Objective: Several studies have reported the associations between adiponectin (ADIPOQ) gene polymorphisms with obesity and insulin resistance, but the results are inconclusive. The aim of this work was to study the relationship between adiponectin gene polymorphisms 276G>T with obesity and biochemical parameters in Egyptian adolescents.

Methods: Genotyping of 276G>T single nucleotide polymorphisms of adiponectin gene was carried out by PCR-RFLP analysis in 204 adolescents (104 obese and 100 non-obese). Anthropometric and biochemical parameters were measured by standard procedures.

Results: Genotypes distributions of 276G>T polymorphisms were significantly different between obese and non-obese cases. Obese adolescents had a higher distribution of GT/TT genotype compared with non obese subjects. Homozygous carriers (TT) and heterozygous carriers (GT) had higher body mass index, mid upper arm circumference, waist to hip ratio, body fat percentage, fasting glucose and triglyceride levels than GG carriers. The risk of obesity was associated with presence of TT genotype, whereas with T allele.

Conclusions: Presence of the TT allele at the 276 locus of the ADIPOQ gene is associated with higher triglyceride and glucose levels. The study suggests that the ADIPOQ 276G>T polymorphism may be a useful biomarker for obesity and its related complications in adolescents.

Keywords: Adiponectin gene polymorphism, Biomarker, obesity, Adolescents, Biochemical parameters.

INTRODUCTION

Adiponectin (ADIPOQ) is the most abundant gene product in adipose tissue [1, 2]. Adiponectin is encoded by adipocyte, C1q, and collagen domain containing (ACDC), which is located on chromosome 3 at q27, where genome-wide scans have revealed a susceptibility locus for type 2 diabetes (T2D), coronary artery disease (CAD), obesity, and metabolic syndrome [34]. Adiponectin is specifically and abundantly expressed in adipocytes and circulates in the blood at high concentrations [5]. Several adiponectin gene (ADIPOQ) single nucleotide polymorphisms (SNPs) have been shown to influence adiponectin levels and have been associated with risk for obesity, insulin resistance (IR), T2DM, and CVD [6-8]. Two of the most commonly studied SNPs at the ADIPOQ locus are a silent T to G substitution in exon 2 (+45T>G, rs2241766) and G to T substitution in intron 2 (+276G>T, rs1501299). However, association studies of these two SNPs, either independently or as a haplotype, have resulted in conflicting evidence in different populations. The 276G>T has been associated either with increased or decreased levels of plasma total adiponectin in different Caucasian populations [9, 10], while this allele has been positively associated with obesity in Sweden [11], but not in Finland [12]. Reverse associations with insulin resistance (IR) were also observed for SNP 276 G>T in Italian [13] versus Japanese [14] and Polish populations [15]. The association between ADIPOQ locus 276G>T among Egyptian adolescents are unexplored till now.

The aim of the present study was to examine the association of SNP 276G>T in the ADIPOQ gene with obesity and biochemical parameters in Egyptian adolescents.

MATERIALS AND METHODS

This cross sectional study included 204 Egyptian adolescents. The mean age of the patients was 14.47 ± 1.54 years, ranged from 15-17 years. They were 104 obese adolescents (50 males and 54 females) and 100 age and sex matched normal healthy controls. Obese cases had BMI greater than 95th percentile for age and gender according to the National Egyptian Growth Curves of Children and Adolescents.

This study protocol was approved by the ethical committee board of the National Research Centre of Egypt (No.10/223).

Anthropometric and body composition measurements were performed with the subject wearing light clothing and without shoes. For all subjects, body weight and height were measured using a scale and a wall-mounted stadiometer to the nearest 0.5 kg and 0.5 cm respectively. Body mass index (BMI) was computed as weight (in kilograms) divided by height (in meters) squared. Waist circumference (cm) was measured in the middle between the 12th rib and the iliac crest, and hip circumference (cm) was measured around the buttocks, at the level of the maximum extension. Mid upper arm circumference measurement was taken in centimeters with non-elastic tape to the nearest 0.1 mm on the upper left arm (halfway between the acromion process and the olecranon process). The children/adolescent stood relaxed with his/her side to the trained technician and the arm hanging freely at the side; the tape was passed around the arm at the level of the mid-point of the upper arm. The waist-to-hip ratio was then calculated. Body fat % was assessed by Tanita Body Composition Analyzer (SC-330). Blood pressure was measured three times and was averaged for analysis. Fasting plasma glucose and serum lipids (total cholesterol, HDL-C, LDL-C, triglycerides) were measured by enzymatic colorimetric methods using a Hitachi autoanalyzer 704 (Roche Diagnostics Switzerland).

Molecular Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by the salting out method [16].

The genotypes for adiponectin polymorphism 276G>T were determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). PCR products and the digest product were resolved by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. PCR primer sequences were used as following: forward primer, 5'- GGC CTC TTT CAT CAC AGA CC -3' and the reverse primer, 5'- AGA TGC AGC AAA GCC AAA GT -3'. Each reaction contained 25 µL final volumes consisting of,

250 ng genomic DNA, 200 µm dNTPS, 0.5 unit of DNA polymerase (DyNAzyme II, FINZYMES) and 20 pmol of each primer. The thermocycling conditions consisted of initial denaturation at 94°C for 10 minutes, followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and the final extension at 72°C

for 10 minutes. The PCR products were digested with the restriction enzyme, Mva 1269I at 37°C for 15 minutes (Fermentas, Germany) [Fragment size: 148 and 48 bp for wild type allele (GG); 196, 148 and 48 bp for heterozygote allele (GT) and 196 bp for mutant allele (TT)].

Table 1: Distributions of genotype and allele frequencies for 276G>T polymorphisms in non-obese and obese adolescents

	Genotype frequency n (%)			Allele frequency n (%)		Grouped genotypes n (%)
	GG	GT	TT	G	T	GG GT+TT
Non-obese (n=100)	15(15%)	44(44%)	41(41%)	74(37%)	126(63%)	15(15%) 85(85%)
Obese (104)	2(1.9%)	45 (43.3%)	57(54.8)	49(23.55%)	159(76.4%)	2(1.9%) 102(98.1%)
	$\chi^2 = 10.698$ (2df, P=.004)			$\chi^2 = 8.122$ (1df, P=.004)		$\chi^2 = 9.765$ (1df, P=.001)

Table 2: Association of adiponectin 276G>T polymorphism with biochemical and anthropometric measures

Variables	Genotype			P
	GG (n=17)	GT (n=89)	TT (n=98)	
Age (years)	14.6 ± 2.1	13.5 ± 1.9	13.2 ± 2.2	.76
BMI(kg/m ²)	21.9 ± 6.7	33.5 ± 5.7	34.9 ± 5.7	.03
Mid upper arm circumference(cm)	20.9 ± 14.6	34.3 ± 12.5	37.0 ± 13.8	.05
Waist (cm)	81.5 ± 14.6	88.7 ± 15.7	93.9 ± 13.9	.13
Waist to hip ratio	0.71 ± 0.02	0.82 ± 0.01	.91 ± 0.01	.02
Body fat %	21.5 ± 6.5	32.3 ± 8.6	35.7 ± 5.5	.05
SBP (mmHg)	109.7 ± 10.8	114.5 ± 12.6	119.3 ± 14.1	.22
DBP (mmHg)	69.6 ± 11.7	72.2 ± 14.5	70.0 ± 10.6	.56
Glucose (mg/dl)	80.9 ± 10.8	94.0 ± 10.8	96.3 ± 10.8	.03
Cholesterol (mg/dl)	141.7 ± 35.4	145.6 ± 36.1	147.0 ± 38.6	.46
Triglycerides (mg/dl)	89.9 ± 26.7	123.3 ± 25.8	129.3 ± 25.5	.003
HDL-C (mg/dl)	49.3 ± 16.4	45.4 ± 14.7	48.7 ± 19.9	.39
LDL-C (mg/dl)	105.5 ± 26.4	109.5 ± 24.7	116.5 ± 29.8	.34

Significant P is in bold

Statistical analysis

Statistical analyses were performed using SPSS 17.0. The biological parameters values were reported as means– standard deviation (SD) and were compared by the Student t-test. Categorical variables were analyzed by the chi-squared test (χ^2) or by the Fisher exact test for small numbers. For comparison of three or more means of normally distributed continuous variables ANOVA test was used, followed by a Scheffe post-hoc analysis. If distribution of variables was not normal, Kruskal-Wallis followed by Mann-Whitney test was used. For testing normal distribution Kolmogorov-Smirnov test was applied. Odds ratios (ORs), two-tailed P values, and 95% confidence intervals (CI) were calculated as a measure of the association of the SNPs with the presence of obesity. ORs were adjusted to confounders parameters by logistic binary regression. A P value of < 0.05 was considered statistically significant for all tests. With regard to the analysis of the single nucleotide polymorphisms (SNPs), the departure from Hardy-Weinberg equilibrium for all the bi-allelic SNP markers was tested using a chi-square test.

RESULTS

Males and females were analyzed together because homogeneity of the effect was observed by gender. The genotype distributions of the 276G>T SNPs in the obese and non-obese adolescents are presented in Table 1. Genotype distributions did not deviate from Hardy-Weinberg expectations. There were significant differences in the frequency distribution of genotypes between obese and non obese adolescents. As it is seen, there was significant accumulation of homozygous (TT) genotype, grouped GT+TT genotypes and T allele frequency in obese adolescents compared to non obese subjects.

The clinical, biochemical and anthropometric characteristics were analyzed according to different genotypes (Table 2). Significant

genotype effects on biochemical and anthropometric measures were observed in study subjects.

Homozygous (TT) and heterozygous (GT) carriers were associated with higher BMI, mid upper arm circumference, waist to hip ratio, body fat %, fasting glucose concentrations and triglyceride levels compared to the GG carriers.

After adjustment for confounding parameters (age, gender, BMI), the OR of obesity associated with genotype TT carriers OR= **1.74**, P = .04, whereas with T allele carriers OR = **1.91**, P=.01 (Table 3).

Fig. 1: shows the three genotypes (GG, GT and TT) in an obese case, identified by RFLP-PCR digested restriction enzyme.

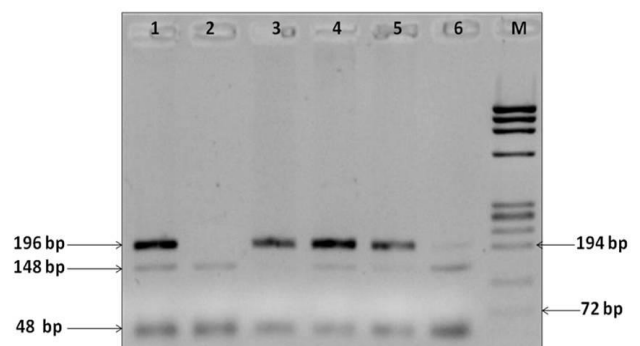


Fig. 1: Represents 2 % agarose gel picture RFLP-PCR digested by the restriction enzyme Mva 1269I, GT genotype: Lanes: 1, 4, 5, 6; GG genotype: Lane 2; TT genotype: Lane 3 and M: ØX174.

Table 3: Associations between of adiponectin 276G>T Genotypes and the Risk of Obesity

Genotype	OR	95% CI	P
TT	1.74	1.19 - 3.98	.040
GT	0.97	0.61 - 1.56	0.91
T (allele)	1.91	2.93 1.23-	0.01

OR, adjusted odds ratio for age, gender and BMI; CI, confidential interval. Significant P is in bold

DISCUSSION

SNPs 45T>G and 76G>T have been studied in several populations including Europeans, Asians, and Americans [17]. Although there is a lack of consistency among studies, the results indicate that genetic variation in the ADIPOQ gene is associated with IR and T2DM [18, 19]. Mediterranean population of Italy 276T allele was associated with higher insulin levels and HOMA-IR [20]. However, other studies have provided opposite results [21, 22]. The present study showed association between 276T genotypes and obesity parameters (BMI, mid upper arm circumference, waist to hip ratio and body fat percentage), whereas with glucose and triglyceride concentrations. SNP 276G>T could influence insulin sensitivity as it has been reported that intronic SNPs may modulate gene expression levels [23]. The present findings are in accordance with previously published data by Jang et al. [24] where the phenotypic expression of the 'risk' genotype for SNP 276G>T was observed only among subjects with elevated BMI.

In an Italian nondiabetic population, the greater IR observed among subjects with the at-risk TT genotype was more pronounced in lean compared with obese individuals of this study [25].

However, a protective role of the 276 G>T SNP in obesity risk has been observed by Boumaiza et al [26] among Tunisians. The same results have been reported by Menzaghi et al. [27] and Stumvoll et al., [28] but Filippi et al. [29] reported that the T allele is associated with IR and obesity risk. This discrepancy might be partly due to the ethnic specificity.

Adiponectin is secreted by adipose tissue and its levels were inversely correlated with Body Mass Index. Significant association between mutated genotypes at G>T, C>T SNPs and metabolic syndrome parameters and weight gain previously reported. SNP analysis did not reveal any significant association of SNPs 45 and 276 with plasma total adiponectin levels [30, 31].

In conclusion, presence of the TT allele at the 276 locus of the ADIPOQ gene is associated with higher triglyceride and glucose levels in Egyptian adolescents. The study suggests that the ADIPOQ 276 G>T polymorphism may be a useful biomarker associated with obesity and its complications. Early diagnosis may help allocate resources for intensive interventions that may benefit children at greatest risk for early obesity-related morbidity.

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