



Evaluating the association of *APOA2* polymorphism with insulin resistance in adolescents



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ABSTRACT

Background: 265T>C SNP in the *APOA-II* gene promoter may be associated with obesity risk and insulin resistance (IR). This study aims to analyze the association between the *APOA2* – 265T>C SNP and risk for obesity and IR in adolescents.

Material and methods: The study was conducted on 500 adolescents. They were 240 obese and 260 non-obese individuals, aged 16–21 years old. Their mean age was 18.25 ± 2.54 years. Variables examined body weight, height, waist circumference (WC), systolic and diastolic blood pressure (BP), body fat percentage (BF%), and abdominal visceral fat layer. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was used as a biomarker for IR. BF% was assessed by body composition analyzer and abdominal visceral fat thickness was determined by ultrasonography. The *APOA2* – 265T>C polymorphism genotype was analyzed by PCR amplification of a 273-bp fragment.

Results: Genotype frequencies were in Hardy–Weinberg equilibrium. The frequency of the mutant C allele was significantly higher in obese cases than non-obese cases. After multivariate adjustment, waist, BF%, visceral adipose layer and HOMA-IR were significantly higher in homozygous allele CC carriers than TT + TC carriers. Homozygous individuals for the CC allele had statistically higher values of energy intake, total fat (g/day) and saturated fat (SATFAT) than carriers of the T allele.

Conclusions: Homozygous individuals for the C allele had higher obesity risk than carriers of the T allele and had elevated levels of visceral adipose tissue. Moreover, the present study shows that the CC polymorphism is associated with the development of IR [OR 1.89

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(1.35–2.91), $P = .012$] and remains significant after adjusting for gender, age and body mass index.

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Introduction

Insulin resistance, which represents a reduced physiological response of the peripheral tissues to the action of the normal levels of insulin, is a major finding in several metabolic disorders, including type 2 diabetes and metabolic syndrome. Apolipoprotein A-II (APOA2) is the 2nd most abundant protein of HDL particles, but its function remains largely unknown (Blanco-Vaca et al., 2001). Obesity-linked genetic variations in the presence of other routine habits such as smoking, physical inactivity and unhealthy food intake may greatly raise the risk of a person developing heart diseases (cardiovascular diseases, CVD).

Excess body fat, obesity, is one of the most common disorders in clinical practice. The location of the body fat is a major determinant of the degree of excess morbidity and mortality due to obesity (Björntorp, 1997). At least two components of body fat are associated with obesity-related adverse health outcomes. These are the amount of subcutaneous truncal or abdominal fat, and the amount of visceral fat located in the abdominal cavity. Each of these components of body fat is associated with varying degrees of metabolic abnormalities and independently predicts adverse health outcomes. Many complex traits are thought to be inherited since they often run in families. However, these complex traits do not show typical mendelian pedigree patterns. These non-mendelian diseases may depend on several susceptibility loci, with a variable contribution from environmental factors. Discovering the major susceptibility locus may be the key to advances in understanding the pathophysiology of a disease.

There have been several studies using association approaches in order to undertake systematic searches for candidate genes in obesity defined as elevated body mass index (BMI, kg/m^2) (Rankinen et al., 2002).

Apolipoprotein A-II (APOA-II) is the second most common protein in high-density lipoproteins. APOA-II appears to impair the reverse cholesterol transport and antioxidant function of high-density lipoprotein, which is consistent with the observation that increased APOA-II levels promote the development of atherosclerosis (Warden et al., 1993). A functional polymorphism representing a T-to-C substitution at the –265 position of this gene has been associated with waist circumference and lower levels of plasma APOA-II in European men (van't Hooft et al., 2001), suggesting that genetic variation at the *APOA2* may be associated with body fat distribution phenotypes. Lower levels of visceral adipose tissue (VAT), both absolute and relative to their total body fat, have been reported in African-American compared with White women (Albu et al., 1997; Lara-Castro et al., 2002), which may be related to differences in genetic make-up between women of different ethnic backgrounds.

New obesity loci continue to be identified through genome-wide association studies in populations of increasing size and ethnic diversity (Heid et al., 2010; Speliotes et al., 2010) but understanding of the mechanisms by which known genetic variants contribute to obesity remains limited. Several well established obesity candidates encode proteins that appear to modulate obesity risk via energy intake, a key determinant of obesity risk (Cole et al., 2010).

In a previous investigation carried out on White Americans participating in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study, the recessive effects for *APOA2* –265T>C polymorphism were observed (Corella et al., 2007). The *APOA2* gene has been associated with obesity and IR in animal and human studies with controversial results. Homozygous individuals for the C allele had higher body mass index (BMI) and obesity risk than did carriers of the T allele, but relationships between *APOA2* –265T>C polymorphism genotype and IR among Egyptian adolescents are unexplored till now.

Therefore, our objectives were to analyze the association between the *APOA2* –265T>C polymorphism SNP and the risk of obesity and IR and study its association with anthropometric measurements, body fat distribution and food consumption in a sample of Egyptian adolescents.

Materials and methods

A descriptive cross-sectional study was conducted on randomly selected 500 Egyptian adolescents. They were 240 obese and 260 non-obese individuals. Their age ranged from 16 to 21 years old and their

mean age was 18.25 ± 2.54 years. Obese cases had BMI greater than 95th percentile for age and gender according to the National Egyptian Growth Curves of Children and Adolescents (Ghalli et al., 2008).

The data were collected from June 2011 to January 2013 and were extracted from a project entitled “Obesity among Youth: Lifestyle and Genetic Factors” funded by the Science and Technology Development Fund (STDF), Egypt. This study protocol was approved by the ethical committee board of the National Research Centre of Egypt (no. 10/223). An informed written consent was obtained from all participants. All individuals were clinically evaluated and anthropometric data were collected.

Anthropometric measurements

Anthropometric variables including height, weight, waist and hip were measured. Body weight was measured with the patients in light clothing and without shoes. Height was measured with the patients standing with their backs leaning against the stadiometer of the same scale. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). WC and hip circumference (HC) were measured in cm using a plastic, non-stretchable tailor's tape. WC was measured with light clothing at a level midway between the lower rib margin and the iliac crest standing and breathing normally. HC was measured at the level at the widest circumference over the buttocks (at the greater trochanter). Subsequently the waist hip ratio (WHR) was calculated as WC divided by HC. Anthropometric measurements were obtained according to standardized equipment and following the recommendations of the International Biological Program (Hiernaux, 1969).

Blood pressure (BP) was measured with the patients sitting with their left arm at heart level using a professional Riester sphygmomanometer manufactured in Japan. Several measurements were made, from which an average BP measurement was obtained. BF% was measured by Tanita Body Composition Analyzer (SC-330).

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the GeneJET™ Genomic DNA Purification Kit (Fermentas, Germany) according to the manufacturer's instructions. Genotyping of $-265\text{T}>\text{C}$ polymorphism in the *APOA2* gene was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis (van't Hooft et al., 2001). Two pairs of primers were used to amplify the promoter region of the *APOA2* gene containing the polymorphism; upstream primer 5' CAT GGG TTG ATA TGT CAG AGC-3' and downstream primer 5' TCA GGT GAC AGG GAC TAT GG 3'.

PCR was carried out in a 25 μL total final volume containing 200 μM dNTPs (Finzyme, Finland), 10 pmol of each primer, 2 U of Taq polymerase (Finzyme, Finland) and 500 ng DNA. Thermal cycling conditions were as follows: denaturation at 95 °C for 10 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 59.5 °C for 30 s, and elongation at 72 °C for 30 s followed by a final elongation of 5 min.

Ten μL of successfully amplified PCR products was digested with a FastDigest BsmI Enzyme (Fermentas, Germany), incubated at 37 °C for 5 min and the fragments were run in 3% agarose gel stained with ethidium bromide, and analyzed under ultraviolet light. The BsmI enzyme cuts the PCR product (273 bp) in two fragments 215 and 58 bp in the presence of the T allele.

We have sequenced 50 cases (25 obese and 25 controls) and compared the genotypes obtained from sequencing and RFLP. No discrepancies in the results between the two methods were observed.

Abdominal ultrasonographic examination

Ultrasonography was carried out by using GE logic α 200-ultrasound machine. Visceral fat layer was measured from the region just above the umbilicus (Hiernaux, 1969). The convex-array probe (3.5 MHz) was used for measuring visceral abdominal fat and anterior wall of the aorta.

Dietary intake

Food intake was carried out using a 24 hour dietary recall. Cases were asked to recall their dietary intakes of the previous 24 h. In particular, we asked about their intake of carbonated beverages, juices, and

Table 1

General characteristics of obese and non-obese adolescents.

Parameters	Females (n = 250)		Males (n = 250)	
	Obese (n = 120)	Non-obese (n = 130)	Obese (n = 120)	Non-obese (n = 130)
Age (y)	17.25 ± 3.43	18.25 ± 4.43	17.65 ± 4.33	17.25 ± 3.43
BMI (kg/m ²)	34.40 ± 5.96**	21.99 ± 3.45	32.75 ± 6.075**	19.86 ± 3.33
Waist (cm)	95.90 ± 12.23**	72.57 ± 8.94	98.05 ± 18.90**	71.79 ± 8.54
WHR	.82 ± .06**	.7710 ± .16	.88 ± .129**	.813 ± .06
BF%	33.74 ± 13.05**	25.96 ± 12.93	33.88 ± 13.78**	22.63 ± 11.61
Systolic BP (mm Hg)	110.77 ± 14.81	109.60 ± 13.09	110.67 ± 16.37	108.80 ± 13.23
Diastolic BP (mm Hg)	71.79 ± 10.31	71.80 ± 7.93	71.42 ± 9.746	73.00 ± 10.00

** Statistically significant differences ($P < 0.01$) between obese and non-obese subjects.

other casual intakes. Food frequency method assessed food consumption frequencies per day, week and month bases by using a questionnaire. It was focused on different kinds of food consumption frequencies rather than consumption of specific nutrients. The energy and nutrient contents were computed.

Biochemical analyses

Fasting plasma glucose and serum lipids (total cholesterol, HDL, LDL, triglycerides) were measured by enzymatic colorimetric methods using a Hitachi autoanalyzer 704 (Roche Diagnostics Switzerland). Serum insulin concentration was analyzed by chemiluminescent immunoassay (Immulite 2000, Siemens, Germany). IR (fasting glucose × fasting insulin / 22.5) was calculated using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) method.

Statistical analysis

Quantitative variables were expressed as mean ± S.D., and qualitative variables were expressed as percentages. Differences between groups were tested using an independent two-sample t-test and chi-square test was used to test for differences in the distribution of categorical variables. A multivariate analysis was carried out using various models, which determined the adjusted odds ratio (OR) for the analyzed SNPs (Table 4). The confidence interval was 95%. Gender, age, BMI, waist-to-hip ratio and waist were the confounding variables. To test for possible interactions between genotypes and phenotype parameters, General Linear Model (GLM) ANOVA analysis was preformed.

Statistical analyses were done using SPSS 17.0 software (SPSS). P-values <0.05 were considered statistically significant.

Results

The characteristics of the obese and non-obese cases are given in Table 1, where we compare the anthropometric and clinical variables for the obese and non-obese individuals. There were significant differences in BMI, WC, WHR, and BF% between the two groups. Obese adolescents had higher values than non-obese in both genders. *APOA2* genotype frequencies did not deviate from Hardy–Weinberg equilibrium expectations and did not differ between males and females. Therefore, males and females were analyzed together. The genotype distribution and allele distribution are presented in Table 2. The data indicate that genotype tends to differ significantly between obese and non-obese adolescents in the CC and TT genotypes, remaining significant when the genotypes in CC and TT + TC were grouped; the CC genotype was more common in obese cases than in non-obese cases (CC frequency 32.41% in obese cases and 9.6% in non-obese cases, $P = .001$). The allele frequency of the *APOA2* –265T>C polymorphism was also significantly different between the two groups (Table 2).

APOA2 genotype was evaluated by comparing homozygous minor allele carriers (CC) with combined homozygous major (TT) and heterozygous (TC) subjects (Table 3). After adjusting for age, sex and BMI,

Table 2

Genotype distribution and allele distribution of APOA2 –265T>C polymorphisms.

Polymorphisms	Genotypes			Alleles		Grouped genotypes	
	CC	TC	TT	C	T	CC	TT + TC
Non-obese (n = 260)	25	124	111	174	346	25	235
Percentage (%)	9.6	47.69	42.69	33.46	66.53	9.61	90.38
Obese (n = 240)	73	93	74	239	241	73	167
Percentage (%)	30.41	38.75	30.83	61.04	50.21	30.41	69.58
P value	.001	.04	.001	.001	.001	.001	.001

anthropometric measures showed significant differences between homozygous and heterozygous carriers. BMI, WC, BF% and visceral fat were significantly higher in CC subjects compared with combined heterozygous (TC) and homozygous major (TT) carriers ($P < 0.05$). Moreover, significant elevated level of HOMA-IR was observed in CC subjects compared with the carriers of T allele. Homozygous individuals for the CC allele had statistically higher values of estimated energy intake, total fat intake (g/day) and saturated fat (SATFAT) than carriers of the T allele.

Table 4 assessed the risk of the possible influence of the APOA2 polymorphism on IR episodes. The study showed that the CC polymorphism is associated with the development of IR [OR 1.89 (1.35–2.91), $P = .012$] and remains significant after adjusting for gender, age and BMI.

Discussion

Obesity is often associated with insulin resistance, and the European Group for the Study of Insulin Resistance suggests that only about 25% of obese patients are insulin sensitive. Increased visceral fat area, increased macrophage infiltration into the omental adipose tissue, enlarged adipocyte size in both omental and subcutaneous fat depots, and omental adipocyte insulin resistance play important roles in the development of insulin resistance in obese patients independent of body mass index (BMI) and total body fat mass.

The present study observed that CC homozygotes had higher BMI, WC, BF%, visceral fat, food consumption and HOMA-IR than carriers of the T allele. These results were consistent with the findings of the previous study of overweight individuals in other populations. Relatively few studies reported the association between APOA2 polymorphisms and phenotypic traits (Armellini et al., 1991; Scott et al., 1985; Ferns et al., 1986; Vohl

Table 3

Associations between APOA2 –265T>C genotype and anthropometric and biochemical parameters and clinical features.

Parameters ^a	CC (n = 98)		TT + TC (n = 402)		V
	Adjusted	Crude	Adjusted	Crude	
BMI (kg/m ²)	32.3 ± 7.4*	35.9 ± 8.8*	23.7 ± 8.3	24.9 ± 8.9	4.5%
Waist (cm)	87.9 ± 15.8*	89.9 ± 15.9*	82.8 ± 17.0	83.9 ± 17.9	3.2%
WHR	.89 ± .11	.99 ± .12	.82 ± .19	.83 ± .29	1.1%
BF%	35.7 ± 11.4*	36.9 ± 12.3*	30.8 ± 12.4	32.9 ± 12.7	3.4%
Systolic BP (mm Hg)	108.7 ± 16.2	109.7 ± 16.7	110.66 ± 13.9	115.6 ± 14.1	1.4/%
Diastolic BP (mm Hg)	71.3 ± 10.4	73.3 ± 10.9	72.1 ± 9.2	75.1 ± 9.9	1.2%
Ultrasound visceral fat (cm)	68.4 ± 13.9*	69.9 ± 13.9*	45.4 ± 12.9	47.4 ± 13.2	2.3%
Energy intake (kcal/day)	1957.1 ± 87.9*	1977.1 ± 89.9*	1499.7 ± 87.1	1500.7 ± 87.9	3.6%
Total fat (g/day)	93.6 ± 31.6*	96.6 ± 31.9*	51.5 ± 29.5	53.5 ± 29.9	3.5%
SATFAT (g/day)	33.9 ± 8.9*	35.9 ± 9.5*	22.2 ± 7.9	23.1 ± 7.8	4.3%
HDL-C (mg/dL)	46.6 ± 7.8	48.6 ± 7.9	48.4 ± 7.8	49.9 ± 7.9	.9%
LDL-C (mg/dL)	115.7 ± 38.9	120.6 ± 38.8	110.64 ± 36.1	116.64 ± 36.9	1.2%
HOMA-IR	8.7 ± 7.5*	9.7 ± 7.6*	2.8 ± 2.5	2.9 ± 2.9	3.6%

V, percentage variance explained in the SNP.

* Statistically significant differences ($P < 0.05$) between CC homozygous subjects and carriers of the T allele for the corresponding variable.

^a Data are adjusted for age, gender and BMI.

Table 4Risk of insulin resistance episode associated with *APOA2* –265C>T polymorphism.

Grouping	OR	95% (CI)	P value
<i>APOA2</i> (CC)	1.89	1.35–2.91	.012
<i>APOA2</i> (CC) + gender + age	1.96	1.18–3.65	.013
<i>APOA2</i> (CC) + gender + age + BMI	2.99	1.11–3.11	.002

et al., 1997). Few genetic variants have been identified in the *APOA2* gene (Martin-Campos et al., 2004). Interestingly, a T–C transition at position –265 (rs no. 5082) affecting element of the *APOA2* promoter has been reported to be functional in 2 independent studies, both demonstrating a –30% drop in basal transcription activity (van't Hooft et al., 2001; Fullerton et al., 2002). In one of these studies, the *APOA2* polymorphism was associated with waist circumference in men (van't Hooft et al., 2001). Another study (Lara-Castro et al., 2005) reported an association between this polymorphism and abdominal fat depots in women.

An association between *APOA2* –265T>C polymorphism and BMI or obesity only in the presence of high-saturated fat intake in three American populations has been observed (Corella et al., 2009). Moreover, with this gene–diet interaction other studies extend the findings to other geographical areas (Europe and Asia), reporting that when saturated fat intake is low (<22 g/day), this SNP does not have any effect on BMI or obesity. However, when saturated fat intake is high (≥22 g/day), significant differences in anthropometric variables were detected between CC individuals and T-allele carriers. Further adjustment for other macronutrients did not change the significance of these findings, supporting the specificity of saturated fat as a driver of this interaction (Corella et al., 2011). Other studies reported genotype-associated differences in specific intake-related behaviors, which may contribute to obesity risk, identifying the possible role for ghrelin in modulating *APOA2*–nutrient interactions (Smith et al., 2012). Eating behaviors have been identified as related to obesity risk (Mdel et al., 2010; Schlundt et al., 2003) and appear to be associated with *APOA2* genotype in a manner consistent with obesity risk. The relationship between *APOA2*, saturated fat and hormonal regulation of food intake has also been identified, which may be relevant to weight control. The interactions between *APOA2* and saturated fat for obesity may be mediated via modulation of plasma ghrelin and expansion of knowledge of *APOA2* and obesity to include modulation of specific behaviors and hormonal mediators not only broadens our understanding of gene–diet interactions, but also facilitates the pragmatic, future goal of developing dietary guidelines based on genotype (Corella et al., 2011). Lower saturated fat was associated with lower ghrelin in CC carriers, which may theoretically be expected to accompany lower energy intake and smaller body size.

Despite the scarcity of previous data supporting a role of *APOA2* in regulating food intake, numerous experimental evidence demonstrate pivotal roles of another apolipoprotein, *APOA4*, as a satiety signal (Fujimoto et al., 1992; Shen et al., 2005). Fujimoto et al. (1992) were the first to report that *APOA4* is a satiety factor secreted by the intestine after fat absorption and that this function of *APOA4* is not shared by gut *APOA1*. *APOA2* is a member of the apolipoprotein multi-gene superfamily, which includes genes encoding soluble apolipoproteins (e.g., *APOA1* and *APOA4*) that share genomic structure and several functions. Although all these apolipoprotein genes have been found to be related to obesity in at least one epidemiological study (Rankinen et al., 2006), only *APOA4* has been subscribed in regulation of food intake, acting as a satiety signal. The present study shows an association between the *APOA2* polymorphism and food consumption, suggesting a potential new role of *APOA2* in the regulation of human appetite. The mechanisms of this proatherogenic capability of increased human apoA-II could be due to increased concentration of apoB-containing lipoprotein and decreased HDL cholesterol (Chiesa et al., 1998; Zhong et al., 1994).

The relationship between MS and insulin resistance was strong as suggested by previous reports (Sierra-Johnson et al., 2006). Regarding the *APOA2* locus, several associations with obesity, insulin-resistance (IR) and diabetes have been reported in both animal (Castellani et al., 2001, 2004) and human studies (Elbein et al., 1999; Vionnet et al., 2000; Xiang et al., 2004). Type 2 diabetes has been linked to chromosome 1q21–24 in several populations, supporting a role for the *APOA2* gene (located in 1q23) as a potential candidate; however, this association is still controversial. The present study showed that the CC polymorphism is associated with the development of IR [OR 1.89 (1.35–2.91), P = .012] and remains significant after adjusting for gender, age and BMI. Moreover, it was found that the overexpression of *APOA2* in these mice increased IR as demonstrated by 2–3 fold higher fasting insulin levels and a delayed clearance of glucose bolus (Castellani

et al., 2001). These results were consistent with the other observation (Weng and Breslow, 1996) in homozygous APOA2 knockout mice. APOA2 deficiency was associated with lower HDL, free fatty acid, glucose, and insulin levels, suggesting an insulin hypersensitivity state. More targeted mechanistic studies using these experimental models have confirmed the role of APOA2 in IR (Sauvaget et al., 2004).

In summary, the present study emphasized that the homozygous individuals for the APOA2 –265CC allele had higher obesity risk than carriers of the T allele. The functional polymorphism representing a T-to-C substitution at the –265 position of this gene is associated with visceral adipose tissue and food consumption. Moreover, the study showed that the CC polymorphism is associated with the development of IR and remains significant after adjusting for gender, age and BMI.

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