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Original article

Screening of Egyptian obese children and adolescents for insertion/deletion (I/D) polymorphism in angiotensin-converting enzyme gene

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ABSTRACT

Background: /aims: The role of angiotensin-converting enzyme (ACE) gene polymorphism in the development of obesity and hypertension in children has not been widely studied. We aimed to screen Egyptian obese children and adolescents for insertion/deletion (I/D) polymorphism in the ACE gene.
Methods: One hundred forty-two children and adolescents were included (70 with simple obesity and 72 controls). Blood pressure was measured, and anthropometric parameters were assessed in all included children and adolescents. Fasting lipid profile, fasting glucose, and insulin were measured. DNA extraction and ACE I/D polymorphism genotyping were also performed.
Results: Obese children had a higher frequency of DD genotype (30% in obese versus 11.1% in controls, $P = .01$) and D alleles (61.8% in obese versus 48.6% in controls, $P = .01$). Obese children with hypertension and prehypertension had higher frequency of DD genotype than II genotype and higher D alleles than I alleles. DD genotype and D allele were independently associated with hypertension (OR: 9.86 and 11.57, respectively, $P < .001$), while dyslipidemia and insulin resistance were not associated with the ACE I/D gene polymorphism.
Conclusion: DD genotype and D-allele of the ACE gene polymorphism were associated with obesity and with hypertension and pre-hypertension in Egyptian children.

1. Introduction

Pediatric obesity has turned into a critical public health problem, as its pervasiveness inside the pediatric population has been extraordinarily expanded in the most recent decades. [1] Morbidities related to obesity, such as hypertension, diabetes mellitus, and hyperlipidemia, were viewed as diseases of adults. Nonetheless, presently, they have progressed toward becoming diseases of children. [2] Obesity is hereditary occurring in approximately 40% of cases, and this happens through numerous genes [3]. Renin-angiotensin system (RAS) mediates controlling blood pressure and the body fluid status [4]. It also controls blood volume and vascular function. The polymorphism in the angiotensin-converting enzyme (ACE) gene takes the form of insertion (I) or deletion (D) of a part in intron 16 that is located at 17q23. Presence of D allele is responsible for the creation of large amounts of ACE. [5,6].

Renin is produced from the juxtaglomerular apparatus of the kidney. It converts angiotensinogen to angiotensin I (Ang-I), and then, it is converted to Ang-II by the ACE [7]. The presence of DD genotype results in 65% higher action of ACE than II genotypes, which, in turn, makes obese children 3.5 times more susceptible to

Abbreviations: ACE, The angiotensin-converting enzyme; BMI, Body mass index; HOMA-IR, homeostasis model assessment for insulin resistance index; HDL, High density lipoproteins; LDL, low density lipoproteins.

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hypertension than normal-weight children [8].

Studies examining the relationship of genes, particulaly angiotensin-converting enzyme insertion/deletion (ACE I/D) on obesity and hypertension in children, are few. The aim of the present study was to screen Egyptian obese children and adolescents for insetion/deletion (I/D) polymorphism in the ACE gene and to assess its relation to hypertension, insulin resistance, and dyslipidemia.

2. Subjects and methods

2.1. Study population

This study was conducted at Ain-Shams University Children Hospital. The local ethical board has approved the study protocol. Informed consent was obtained from guardians of included children and from children over 12 years of age according to the Declaration of Helsinki.

One hundred forty-two children and adolescents aged between 3 and 16 years participated in the study. Among them, 70 had simple obesity and 72 were healthy matched normal controls. Children and adolescents with obesity came for follow-up in the obesity clinic, while controls were healthy children and adolescents who came to the pediatric outpatient clinic with their siblings who had simple illnesses. Cases were diagnosed as having obesity when their body mass index (BMI) was above the 95th percentile or BMI SDS above +2 [9].

Exclusion criteria: Children and adolescents were excluded from the study if they had genetic abnormalities or endocrinal diseases or were receiving drugs that could alter weight, such as antipsychotics, antidepressants, and antiepileptics or if they were taking lipid-lowering drugs.

History was taken from all included children about comorbidities such as diabetes mellitus, hypertension, and heart diseases. Additionally, a thorough clinical examination was performed.

2.2. Measurement of anthropometric parameters and blood pressure

All included children were subjected to accurate weight, height, waist circumference, and hip circumference measurment. Body mass index (BMI) was calculated, and BMI SDS was plotted. [9] Further, the waist-to-hip ratio was calculated [10]. Blood pressure was measured accurately at several occasions, and the average was plotted on percentiles [11]. Prehypertension was defined as systolic or diastolic blood pressure ≥90th percentile but less than 95th percentile, and hypertension was defined as systolic blood pressure or diastolic blood pressure ≥95th percentile.

2.3. Measurement of lipid profile and fasting insulin

Fasting cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and fasting blood glucose were measured. Diabetes mellitus was defined as fasting blood glucose more than or equal to 126 mg/dl [12].

Fasting insulin was analysed, and HOMA-IR (homeostasis model assessment for insulin resistance index) was calculated. Insulin resistance was defined as HOMA-IR ≥ 3.16.

2.4. Genetic analysis

DNA was extracted from lymphocytes by the spin column method of GeneJET™ Genomic DNA purification kit (#K072, PureExtreme ®Fermentas Life Sciences, Thermo Fisher Scientific, Vilnius, Lithuania).

Oligonucleotides were synthesized specifically for amplification of a DNA fragment that was 490 bp and located in intron 16 by polymerase chain reaction to determine the ACE I/D genotype (rs4340) [13,14]. The primers used were

Forward: 5′CTGGAGACCACTCCCATCCTTTCT-3′, and reverse: 5′GATGTGGCCATCACATTCGTCAGAT-3′.

PCR analysis (Biometra®) consisted of DNA denaturation at 95 °C followed by 30 cycles of PCR. The produced genotypes were a 190 bp band (D/D); a 490 bp DNA band (I/I); or two bands of 190 bp and 490 bp (I/D). They were separated by electrophoresis on a 2% agarose gel, which was used for separation of bands by electrophoresis in Tris EDTA (ethylenediamine tetraacetic acid)-Borate buffer (TBE). The products were stained and examined under ultraviolet light. This was documented using DNA analysis software (InGenius Syngene™ – UK).

Genotype distribution was tested by Hardy-Weinberg equilibrium (HWE) in obese and controls.

2.5. Statistical analysis

The chi-square test was used to compare categorical data between two groups for categorized data, while the Student t-test was used to compare quantitative data between two groups. The odds ratio (OR) and 95% confidence intervals (95% (CI)) were used to determine liability to disease. The independent effect of different variables on hypertension was determined by logistic regression analysis. A *P value* < .05 was considered to be statistically significant. The statistical analysis was conducted using SPSS version 22.0.

3. Results

The mean age of obese children was 8.9 years, and 57.1% were males. Children in the control group were comparable regarding age and sex ($P > .05$; Table 1). Data of fasting blood glucose, insulin, and fasting lipid profile of all included children are shown in Table 1.

Among the group with obesity, the median BMI SDS was 32.08 (7.05), waist circumference SDS was 4.88, hip circumference SDS was 3.29, and waist-to-hip ratio was 2.23. Of the 70 obese children, 10 (14.3%) were hypertensives and 18 (25.7%) were prehypertensives, while none of the 70 normal children had hypertension, and 5 (6.9%) had prehypertension.

The genotype frequencies among children and adolescents with obesity and controls are presented in Table 2. Of obese children, 30% were DD genotype compared to only 11.1% of controls. Children with DD genotype had a 3.4-fold more risk of obesity (odds ratio [OR] 3.45, 95% confidence interval [CI], 1.26 to 9.48, $P = .005$). In addition, 51.4% of those with obesity were having at least one D allele compared to 38.2% of controls (OR, 1.71, 95% CI, 1.07 to 2.75; $P = .024$).

Children with DD genotype had a higher weight for height SDS than those with II genotype ($P = .001$) and ID genotype ($P = .002$), with no significant difference between ID and II genotypes ($P = .807$). Further, children with DD genotype had higher BMI SDS, waist circumference SDS, and hip circumference SDS than those with II genotype ($P = .003$, .007, and 0.017, respectively). Children with DD genotype have significantly higher BMI SDS than those with ID genotype ($P = .003$), with no significant difference between ID and II genotypes ($P = .408$) as shown in Table 3.

Obese children with hypertension had a significantly higher frequency of DD genotype than those with normal blood pressure as shown in Fig. 1. Logistic regression analysis showed that the DD genotype was independently associated with hypertension (OR, 9.86, 95% CI, 2.98 to 32.65; $P < .001$).

Fasting cholesterol, fasting triglycerides, and fasting LDL did not

Table 1
Clinical and laboratory characteristics of obese children and adolescents with obesity and controls.

Characteristics	Obese children n = 70	Controls n = 72	*P* value
Age (years)	8.91 (3.71)	7.97 (3.15)	.103[a]
Sex (male), *n* (%)	40 (57.1)	45 (62.5)	.515[b]
Positive family history, *n* (%)	31 (44.3)	14 (19.4)	<.001[b]
Fasting blood glucose (mmol/L)	5.41 (1.3)	4.12 (0.39)	<.001[a]
Fasting insulin (μu/ml)	6.09 (2.58)	3.88 (3.31)	<.001[a]
HOMA–IR	3.8 (0.63)	1.01 (0.92)	.001[a]
Total cholesterol (mmol/L)	4.27 (1.56)	3.66 (1.22)	.011[a]
Fasting triglycerides (mmol/L)	1.64 (0.95)	1.08 (0.48)	<.001[a]
Fasting HDL (mmol/L)	1.15 (0.56)	1.37 (0.66)	.040[a]
Fasting LDL (mmol/L)	2.14 (1.28)	2.18 (1.21)	.861[a]

BMI: Body mass index, SDS: Standard deviation score, HOMA-IR: Homeostasis model assessment for insulin resistance. HDL: High-density lipoprotein, LDL: low-density lipoproteins.
Values in parentheses are percentages, or data are presented as mean and standard deviation.
a: Independent *t*-test; b Chi-square test.

Table 2
Distribution of the ACE genotypes and alleles in children and adolescents with obesity and controls.

Genotype	Obese children n = 70		Controls n = 72		OR (95% CI)	*P* value
	n	%	n	%		
Genotype, n (%)						
II	19	27.1	25	34.7	*Referent*	
ID	30	42.9	39	54.2	1.012 (0.472–2.17)	.177
DD	21	30.0	8	11.1	3.45 (1.26–9.48)	.005
Allele, n (%)						
I	68	48.6	89	61.8	*Referent*	
D	72	51.4	55	38.2	1.71 (1.07–2.75)	.024

OR odds ratio, 95% CI confidence interval. P value < .05 indicates a significant difference.

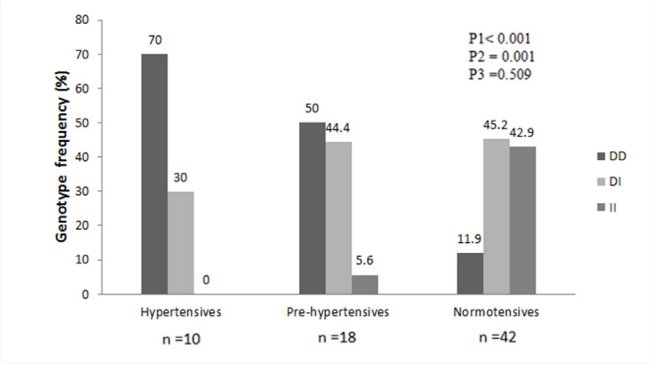

Fig. 1. ACE I/D genotype frequency in relation to blood pressure in subjects with obesity. P1 = hypertensives versus normotensives, P2=Prehypertensives versus normotensives, P3 = hypertensives versus prehypertensives.

show significant difference between DD genotype and II genotype and did not show significant difference between DD genotype and ID genotype. Furthermore, fasting blood glucose, insulin, and HOMA-IR did not show significant difference between DD genotype and II genotype and did not show significant difference between DD genotype and ID genotype as shown in Table 3.

4. Discussion

Many studies in adults have suggested the genetic contribution to obesity. This is the first study to analyze the association of ACE I/D polymorphism with obesity and hypertension in Egyptian children and adolescents.

Thus far, the role of ACE in obesity is not clarified. It is suggested that RAS affects the adipose tissue and the satiety centers [15]. A decrease in renin-angiotensin system activity usually results in weight loss. Further, weight gain could be prevented by providing angiotensin-converting enzyme inhibitors, according to the results of an animal study [16,17].

Fat deposition was reported to be mediated by angiotensin II [18]. Moreover, type II diabetes can be prevented by providingg angiotensin-converting enzyme inhibitors [19]. Furthermore, adipose tissue can produce angiotensinogen [20].

RAS can have a major role in control of blood pressure, obesity, and metabolic syndrome through the regulation of vascular tone

Table 3
ACE I/D genotype in relation to anthropometric measures, lipid profile, and glucose homeostasis parameters in children and adolescents with obesity.

	II n = 19	ID n = 30	DD n = 21	II vs. ID P value	ID vs. DD P value	DD vs. II P value
Weight for height SDS, median (IQR)	2.5 (2.28–3.3)	2.51 (1.85–3)	3.64 (2.7–4.44)	.807[a]	.001	.002
BMI SDS, median (IQR)	3.06 (2.65–3.7)	3.26 (2.91–3.72)	3.89 (3.3–5.1)	.408[a]	.003	.001
Waist circumference SDS, median (IQR)	3.91 (3.12–5.08)	4.85 (3.38–5.3)	5.14 (4.08–7.06)	.171[a]	.110	.007
Hip circumference SDS, median (IQR)	2.5 (2.29–3.43)	3.67 (2.94–4.6)	4.27 (2.92–4.91)	.019[a]	.427	.017
Waist/hip ratio SDS, median (IQR)	1.48 (0.6–2.5)	2.1 (1.2–4.25)	2.67 (2–3.75)	.238[a]	.515	.056
Total fasting cholesterol (mmol/L), mean ± SD	4.11 (0.93)	4.62 (1.95)	3.91 (1.31)	.262[b]	.703	.689
Fasting triglycerides (mmol/L), mean ± SD	1.60 (1.16)	1.65 (0.83)	1.67 (0.95)	.857[b]	.952	.824
Fasting HDL (mmol/L), mean ± SD	1.27 (0.42)	1.51 (0.85)	1.24 (0.52)	.227[b]	.200	.974
LDL (mmol/L), mean ± SD	2.12 (1.07)	2.37 (1.41)	1.83 (1.27)	.496[b]	.142	.485
Fasting blood glucose (mmol/L) mean ± SD	5.24 (0.97)	5.71 (1.67)	5.14 (0.82)	.220[b]	.124	.801
Fasting insulin (μu/ml), mean ± SD	6.04 (2.18)	6.46 (2.76)	5.70 (2.63)	.577[b]	.328	.660
HOMA-IR, mean ± SD	3.9 (0.74)	3.6 (0.89)	4.0 (0.89)	.226[b]	.120	.703

BMI: Body mass index; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; HOMA-IR: Homeostasis model assessment for insulin resistance. a: Kruskal–Wallis; b: One-Way ANOVA. Post-hoc analysis was done to compare the genotype subgroups. P value < .05 indicates a significant difference.

and fluid status [21]. ACE gene inhibition leads to a decrease in blood pressure [22].

In the present study, 25.7% of obese children had pre-hypertension and 14.3% had hypertension. This finding was similar to findings from previous studies that concluded that obesity is positively correlated with hypertension [23]. Previous adult studies reported that D allele carriers have a higher BMI than II genotype carriers. They were also more liable to hypertension [24,25]. ACE D alleles were found to have an effect on blood pressure in a study with adolescents. A relation between blood pressure and the ACE I/D genotype was also reported in a previous study [26]. A study on adults has shown that those carrying D/D genotype are 82% more likely to be obese than those carrying other genotypes [27]. In a large previous study on obese children and adolescents, the D allele was associated with hypertension in boys [28].

We compared our results with those of a previous study conducted on adult Egyptian populations due to lack of data in pediatrics, and this study reported an association of hypertension with the DD genotype in Egyptian adult females [29].

In conclusion, we found that the DD genotype of the I/D polymorphism in the ACE gene was significantly higher in obese children and adolescents and in those with hypertension. Lipid profile and glucose homeostasis were not associated with the I/D polymorphism genotypes. Further larger studies should be performed with measurement of ACE level and the ACE gene polymorphism to confirm these results and to search for methods of prevention of obesity and hypertension in children by understanding its genetic etiology.

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Conflict of interest

No conflict of interest to declare.

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