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Genetic Contributions to Childhood Obesity: Association of Candidate Gene Polymorphisms and Overweight/Obesity in Korean Preschool Children

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This study was aimed to investigate the association of candidate gene polymorphisms and obesity or overweight in young Korean children. A total of 190 Korean preschool children (96 control, 48 overweight, and 46 obese children) were genotyped for the angiotensin converting enzyme (ACE) insertion (I)/deletion (D), angiotensin II type 2 receptor (AT2) C3123A, transforming growth factor (TGF)-β1 T869C, vascular endothelial growth factor (VEGF) T460C, and tumor necrosis factor (TNF)-α G308A polymorphisms. No differences were found among the groups with respect to age, sex, birth weight, blood pressure levels, and serum concentrations of glucose and total cholesterol. Obese children showed a higher incidence of ACE DD genotype and D allelic frequency compared to the controls (odds ratio [OR], 2.7, 95% confidence interval [CI], 1.01–7.21; OR, 2.5, 95% CI, 1.49–4.19; all $P < 0.05$). The frequency of TC genotype and C allele in the TGF-β1 T869C polymorphism (OR, 2.08, 95% CI, 1.01–4.27; OR, 1.93, 95% CI, 1.15–3.21) and that in the VEGF T460C polymorphism (OR, 2.5, 95% CI, 1.19–5.28; OR, 2.15, 95% CI, 1.26–3.68) was also higher in obese children than in control subjects (all $P < 0.05$). Overweight children exhibited a higher frequency of the A allele in the AT2 C3123A polymorphism compared to the controls (OR, 1.72, 95% CI, 1.03–2.88, $P < 0.05$). There were no differences in the TNF-α G308A polymorphism among the groups. The ACE I/D, AT2 C3123A, TGF-β1 T869C, and VEGF T460C polymorphisms can affect susceptibility to obesity or overweight in Korean children.

Keywords: Angiogenic Proteins; Child Obesity; Genetic Variation; Renin Angiotensin System

INTRODUCTION

Childhood obesity has become a major health concern and is occurring at progressively younger ages (1). The rate of severe obesity in children continues to increase, particularly among young children and is expected to continue (1). Moreover, some of the complications of obesity such as type 2 diabetes, hypertension, and hyperlipidemia were formerly observed only in adults but are now frequently detected in obese children in some populations (1,2). Recently, the genetic predisposition to the development of obesity has been extensively studied and polymorphisms in candidate obesity-related genes have become the target of intensive investigation (3,4).

The renin angiotensin system (RAS) has been focused on its potential roles in obesity and several co-morbidities. In addition to its traditional role in the blood pressure (BP) control, the RAS has been implicated in the metabolic regulation (5). Adipose mass has a positive correlation with RAS activity, and RAS gene expression shows a close relationship with the severity of obesity (5,6). The angiotensin converting enzyme (ACE) insertion (I)/deletion (D) polymorphism has been widely studied among RAS polymorphisms, and associations with diabetic nephropathy and obesity have been shown in adults (7,8). The angiotensin II type 2 receptor (AT2) gene is also implicated in hypertension or other obesity-related metabolic parameters (8). Several studies have shown the association of the AT2 C3123A polymorphism with cardiometabolic diseases (9,10).

Growing adipocytes produce numerous angiogenic factors, which provoke neovascularization either alone or collectively during fat tissue expansion (11). Among them, transforming growth factor (TGF)-β1 is a multifunctional growth factor that controls proliferation, differentiation and other pleiotropic functions in various cells (12). In humans, increased TGF-β1 activity is related to body mass index (BMI) and abdominal adipose tissue in morbid obesity (13,14). It has been reported that the C allele of the T869C polymorphism is highly associated with serum TGF-β1 level and that carriers of this allele show elevated risk for obesity and type 2 diabetes (15,16). Vascular endothelial growth factor (VEGF) is also the major angiogenic factor in adipose tissue. It stimulates proliferation and migration of endo-

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thelial cells and has an impact on the reciprocal interaction between endothelial cells and adipocytes (11). Serum VEGF levels are increased in obese subjects, and the manipulation of VEGF has been proposed to be a pharmacological target in the treatment of obesity (11,17). Genetic variations in the VEGF gene were associated with differences in VEGF expression levels and susceptibility to the obesity phenotype (18,19). Tumor necrosis factor (TNF)-α is a multifunctional pro-inflammatory cytokine produced mainly by macrophages and adipocytes (20). The TNF-α G308A polymorphism has been associated with obesity, hyperleptinemia, insulin resistance, and elevated transcriptional activity of TNF-α (21).

However, few studies have investigated the genetic polymorphisms and the prevalence of metabolic abnormalities among young children with obesity or overweight (22). In the present study, we aimed to examine whether the ACE I/D, AT2 C3123A, TGF-β1 T869C, VEGF T460C, and TNF-α G308A polymorphisms could be indicators of genetic susceptibility for obesity or overweight in young Korean children. Given the association between these modulators and obesity, we hypothesized that polymorphisms of these genes might affect the risk of developing childhood obesity or overweight.

MATERIALS AND METHODS

Study population

Preschool children aged 4 months to 6 years who visited our institutions for acute infections were included in this study for evaluation of the association between genotypes and overweight or obese status. Body weight and height were measured for all children according to standard procedures at the time of admission to the hospital (23). The weight was measured to the nearest 0.1 kg, using a digital scale, with children barefoot and with hospital gowns only. Height was measured to the nearest 0.1 cm, using a wall-measured unit size. In infants unable to stand, length was measured in cm while lying on a solid surface with a rigid headboard. Overweight or obese status in infants < 24 months was defined by using weight for length (WFL) measurements corresponding to age and gender. The same status in children aged between 2 to 6 years was determined using BMI (kg/m^2) matched according to the age and gender. Based on a report defining obesity in young children (24), all subjects were subcategorized into three groups as follows: control (WFL or BMI < 85th percentile), overweight (85th ≤ WFL or BMI < 95th percentile), and obese (WFL or BMI ≥ 95th percentile). BP measurements were taken thrice for the subjects using a standard mercury sphygmomanometer equipped with cuff bladder of –40% width of the arm circumference of the child. To strengthen the accuracy of BP measurements, the average of 3 readings was used for data analysis. Subjects with a perinatal history of premature birth, intrauterine growth retardation, small or large birth weight (≤ 2,500 or ≥ 4,000 g) and chronic diseases were excluded from the study. After the parental informed consent of all the subjects, non-fasting blood samples were obtained for DNA analysis and for measurements of the serum levels of glucose and total cholesterol.

Genetic analysis

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood using a salting-out method. Specific oligonucleotide primers were designed for ACE I/D, AT2 C3123A, TGF-β1 T869C, VEGF T460C, and TNF-α G308A polymorphisms by using previously published genomic sequences (7,9,19,25). Polymerase chain reaction (PCR) amplification for each polymorphism was performed in a total reaction volume of 20 μL with 2 μL of genomic DNA, 5 μL of each of the primer, 250 μM dNTP, 1.5 mM $MgCl_2$, 40 mM KCl, 10 mM Tris-HCl, and 1 U Taq DNA polymerase (Bioneer, Daejeon, Korea). Thermal conditions included denaturation at 94°C for 5 minutes, followed by 30 cycles at 94°C for for 30–40 seconds, annealing at 52°C–65°C for 30–35 seconds, extension at 72°C for 1 minutes, and a final extension at 72°C for 5 minutes. All PCR reactions

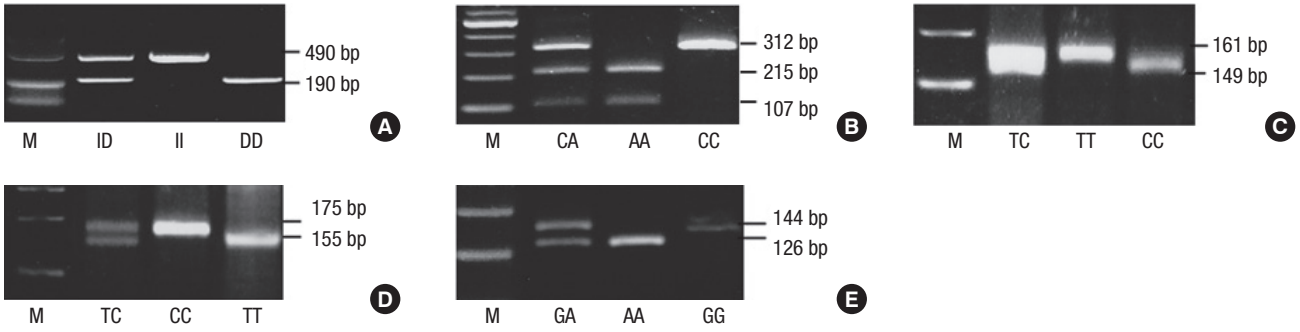

Fig. 1. Determination of genotypes using PCR amplification products. (**A**) ACE I/D polymorphism. (**B**) AT2 C3123A polymorphism. (**C**) TGF-β1 T869C polymorphism. (**D**) VEGF T460C polymorphism. (**E**) TNF-α G308A polymorphism.
PCR = polymerase chain reaction, ACE I/D = angiotensin converting enzyme insertion/deletion, AT2 = angiotensin II type 2 receptor, TGF-β1 = transforming growth factor-β1, VEGF = vascular endothelial growth factor, TNF-α = tumor necrosis factor-α, M = marker, bp = base pairs.

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were performed using a 2720 thermal cycler (Applied Biosystems, Grand Island, NY, USA). Then, restriction fragment length polymorphism analysis was performed by digesting the PCR product with 10 μL of an appropriate restriction enzyme at recommended temperatures. The digested products were electrophoresed on ethidium bromide-stained 2% agarose gels and detected using ultraviolet transillumination (Fig. 1). Specific details of the primer sequences and PCR conditions for each polymorphism are listed in Table 1.

Statistical analysis

Statistical analyses were performed using the SPSS program for Windows (version 18.0; SPSS Inc., Chicago, IL, USA). Continuous variables among the three groups were analyzed using the one-way analysis of the variance test and the Tukey-Kramer post hoc test. Gender distribution and frequencies of the genotypes and alleles among the groups were evaluated by the χ^2 test or Fisher's exact test. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated to identify the association strength. Categorical data are presented as numbers (%) and continuous data as the mean ± standard deviation. Multiple logistic regression analysis was performed to determine the independent effect of each risk allele on the risk of becoming obese/overweight. Statistical significance was set at $P < 0.05$.

Ethics statement

The present study protocol was reviewed and approved by the Institutional Review Board of Korea University Ansan Hospital (IRB No. AS11091). Informed consent was submitted by all subjects when they were enrolled.

RESULTS

Study groups

All subjects were of Korean origin. A total of 96 control, 48 overweight, and 46 obese preschool children were genotyped for ACE, AT2, VEGF, TGF-β1, and TNF-α polymorphisms. No significant differences among the groups were found with respect to age, sex, birth weight, BP levels, and serum concentrations of glucose and total cholesterol (Table 2).

Distribution of ACE I/D polymorphism

The frequencies of the D allele and the DD and ID genotypes were found to be higher in obese children compared to the control (OR, 2.50, 95% CI, 1.49–4.19, $P < 0.001$; OR, 2.70, 95% CI, 1.01–7.21, $P = 0.042$; OR, 2.13, 95% CI, 1.03–4.41, $P = 0.042$, respectively). The frequency of the II genotype was lower in the obese children compared to the controls (OR, 0.21, 95% CI, 0.08–0.52, $P < 0.001$, respectively) (Table 3).

Distribution of AT2 C3123A polymorphism

The frequency of the A allele was higher in overweight children (OR, 1.72, 95% CI, 1.03–2.88, $P = 0.038$) compared to the controls. The frequency of the AA genotype was higher in overweight children than in healthy controls, although the difference was not statistically significant (OR, 2.13, 95% CI, 0.99–4.58, $P = 0.053$). The A allele frequency was also higher in obese children than in the controls, however, it was not significant (OR, 1.60, 95% CI, 0.94–2.72, $P = 0.083$) (Table 3).

Table 2. Clinical characteristics among the three studied groups

Parameters	Controls (n = 96)	Overweight (n = 48)	Obese (n = 46)
Sex, males	62 (64.6)	39 (68.4)	30 (56.6)
Age, mon	22.5 ± 13.3	18.5 ± 16.4	18.8 ± 15.7
Birth weight, g	3,242 ± 354	3,315 ± 390	3,276 ± 408
Systolic BP, mmHg	93.0 ± 9.4	90.8 ± 6.5	90.9 ± 5.3
Diastolic BP, mmHg	53.9 ± 6.4	54.7 ± 6.5	55.2 ± 7.1
Glucose*, mg/dL	98.0 ± 13.9	98.5 ± 15.9	102.7 ± 11.7
Cholesterol*, mg/dL	141.8 ± 21.4	142.6 ± 24.6	137.8 ± 23.9
Body weight, kg	12.1 ± 3.1	12.2 ± 4.1	15.5 ± 7.0†‡
Height, cm	86.3 ± 12.0	79.5 ± 14.0†	84.9 ± 17.4

Values are shown as means ± standard deviation or number (%).
BP = blood pressure, BMI = body mass index.
*These values are non-fasting levels. †$P < 0.05$ vs. controls. ‡$P < 0.05$ vs. overweight.

Table 1. Details of primer sequence and reaction conditions used for genotyping of the polymorphisms

Poly-morphisms	Primers	PCR annealing temperature, °C	Restriction enzyme	Alleles	DNA fragment size, bp
ACE I/D	F:5′-CTGGAGACCACTCCCATCCTTTCT-3′ R:5′-GATGTGGCCATCACATTCGTCAGAT-3′	58	-	I D	490 190
AT2 C3123A	F: 5′-GGA TTC AGA TTC TCT TTG AA-3′ R: 5′-GCA TAG GAG TAT GAT TTA ATC-3′	52	AluI	C A	312 215, 107
TGF-β1 T869C	F:5′-TTCCCTCGAGGCCCTCCTA-3′ R:3′-GCCGCAGCTTGGACAGGATC-3′	62	MspA1I	T C	161 149
VEGF T460C	F:5′-TGTGCGTGTGGGGTTGAGCG-3′ R:5′-TACGTGCGGACAGGGCCTGA-3′	65	BstUI	T C	175 155, 20
TNF-α G308A	F:5′-GAGGCAATAGGTTTTGAGGGCCAT-3′ R:3′-GGGACACACAAGCATCAAG-3′	57	NcoI	G A	144 126, 18

ACE I/D = angiotensin converting enzyme insertion/deletion, AT2 = angiotensin type 2 receptor, TGF-β1 = transforming growth factor-β1, VEGF = vascular endothelial growth factor, TNF-α = tumor necrosis factor-α.

Table 3. Genotype and allele frequencies of the ACE, AT2, MMP-9, TGF--β1, VEGF, and TNF-α polymorphisms among the three studied groups

Genes	Geno types	Controls	Overweight	Obese	Overweight vs. controls		Obese vs. controls	
					OR (95% CI)	*P*	OR (95% CI)	*P*
ACE I/D	II	44 (46.8)	25 (52.1)	7 (15.6)	1.24 (0.62–2.48)	0.552	0.21 (0.08–0.52)	< 0.001
	ID	41 (43.6)	19 (39.6)	28 (62.2)	0.85 (0.42–1.72)	0.645	2.13 (1.03–4.41)	0.042
	DD	9 (9.6)	4 (8.3)	10 (22.2)	0.86 (0.25–2.95)	0.242	2.70 (1.01–7.21)	0.042
	D allele	59 (31.4)	27 (28.1)	48 (53.3)*	0.86 (0.50–1.47)	0.572	2.50 (1.49–4.19)	< 0.001
AT2 C3123A	CC	56 (61.5)	25 (52.1)	22 (50.0)	0.68 (0.34–1.38)	0.283	0.63 (0.30–1.29)	0.204
	CA	15 (16.5)	5 (10.4)	8 (18.2)	0.59 (0.20–1.73)	0.337	1.13 (0.44–2.90)	0.806
	AA	20 (22.0)	18 (37.5)	14 (31.8)	2.13 (0.99–4.58)	0.053	1.66 (0.74–3.71)	0.220
	A allele	55 (30.2)	41 (42.7)*	36 (40.9)	1.72 (1.03–2.88)	0.038	1.60 (0.94–2.72)	0.083
TGF-β1 T869C	TT	47 (50.5)	15 (34.9)	12 (26.1)	0.52 (0.25–1.11)	0.090	0.35 (0.16–0.75)	0.007
	TC	32 (34.4)	19 (44.2)	24 (52.2)	1.51 (0.72–3.16)	0.270	2.08 (1.01–4.27)	0.046
	CC	14 (15.1)	9 (20.9)	10 (21.7)	1.49 (0.59–3.78)	0.400	1.57 (0.64–3.86)	0.330
	C allele	60 (32.3)	37 (43.0)	44 (47.8)*	1.59 (0.94–2.68)	0.090	1.93 (1.15–3.21)	0.010
VEGF T460C	TT	44 (51.8)	17 (36.2)	10 (22.7)	0.53 (0.25–1.10)	0.090	0.27 (0.12–0.62)	0.002
	TC	33 (33.8)	24 (51.1)	27 (61.4)	1.64 (0.80–3.38)	0.180	2.50 (1.19–5.28)	0.020
	CC	8 (9.4)	6 (12.7)	7 (15.9)	1.41 (0.46–4.34)	0.550	1.82 (0.61–5.40)	0.280
	C allele	49 (28.8)	36 (38.2)	41 (46.6)*	1.53 (0.90–2.61)	0.120	2.15 (1.26–3.68)	0.005
TNF-α G308A	GG	1 (1.0)	0 (0.0)	0 (0.0)	0.66 (0.03–16.4)	0.800	0.70 (0.03–17.50)	0.830
	GA	23 (24.0)	6 (12.5)	5 (11.1)	0.45 (0.17–1.20)	0.110	0.40 (0.14–1.12)	0.080
	AA	72 (75.0)	42 (87.5)	40 (88.9)	2.33 (0.88–6.17)	0.090	2.67 (0.94–7.53)	0.060
	A allele	167 (87.0)	90 (93.8)	85 (94.4)	2.25 (0.89–5.68)	0.090	2.54 (0.94–6.88)	0.070

Values are shown as number (%) or OR (95% CI).
ACE I/D = angiotensin converting enzyme insertion/deletion, AT2 = angiotensin type 2 receptor, TGF-β1 = transforming growth factor-β1, VEGF = vascular endothelial growth factor, TNF-α = tumor necrosis factor-α, OR = odds ratio, CI = confidence interval.
*$P < 0.05$ vs. controls.

Table 4. Multiple logistic regression analysis of the risk alleles for prediction of obesity

Dependent variable	Independent variables	B	β	t	*P*	VIF
Obesity	(constant)	–0.024	-	–0.254	0.800	-
	ACE D allele	0.220	0.219	2.393	0.018	1.096
	TGF-β1 869 C allele	0.192	0.196	2.234	0.028	1.005
	VEGF 460 C allele	0.219	0.221	2.414	0.017	1.095

ACE = angiotensin converting enzyme, TGF-β1 = transforming growth factor-β1, VEGF = vascular endothelial growth factor, VIF = variance inflation factor.

Distribution of TGF-β1 T869C polymorphism

The frequency of the C allele was higher in obese children compared to the controls (OR, 1.93, 95% CI, 1.15–3.21, $P = 0.01$). In the obese group the TT genotype was less frequent and the TC genotype was more frequent in the TGF-β1 T869C polymorphism compared to the controls (OR, 0.35, 95% CI, 0.16–0.75, $P = 0.007$; OR, 2.08, 95% CI, 1.01–4.27, $P = 0.046$, respectively) (Table 3).

Distribution of VEGF T460C polymorphism

The C allele frequency was higher in obese children compared to the controls (OR, 2.15, 95% CI, 1.26–3.68, $P = 0.005$). The VEGF T460C polymorphism was associated with a lower frequency of the TT genotype and a higher frequency of the TC genotype in obese children compared to control subjects (OR, 0.27, 95% CI, 0.12–0.62, $P = 0.002$; OR, 2.5, 95% CI, 1.19–5.28, $P = 0.02$, respectively) (Table 3).

Distribution of TNF-α G308A polymorphism

There were no differences in genotypes and alleles of the TNF-α G308A polymorphism among the three groups (Table 3).

Independent effect of the high risk alleles on the obese or overweight phenotype

With multiple logistic regression analysis the D allele of the ACE I/D polymorphism and the C alleles of the TGF-β1 T869C and VEGF T460C polymorphisms showed an independent effect of risk of obesity, respectively (all $P < 0.05$) (Table 4). However, the association between the risk alleles and overweight was not statistically significant in multiple logistic regression analysis (data not shown).

DISCUSSION

The present study investigated genetic susceptibility to obesity in 190 prospectively recruited preschool children. Polymorphi-

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sms in ACE, TGF-β1, and VEGF genes were associated with the presence of obesity and genetic variation in AT2 gene was related with the incidence of overweight in young Korean children. The D allele of ACE gene was more frequently detected in obese children compared to that in the controls. C alleles in both TGF-β1 T869C and VEGF T460C polymorphisms were more frequently found in obese children than those in control subjects. The further multiple logistic regression analysis confirmed this finding by exploring the independent effects of the risk alleles on obesity outcome. Overweight children had a higher frequency of the A allele of AT2 C3123A gene compared to the controls. Our findings indicate that the ACE, AT2, TGF-β1, and VEGF gene polymorphisms might affect the susceptibility of young Korean children to obesity or overweight.

ACE is an important regulatory enzyme of the RAS that may have pleiotropic effects. The ACE gene, located on chromosome 17q23, is greatly polymorphic in the promoter and coding regions (26). The most frequently studied ACE gene polymorphism is the 287-bp insertion (allele I) or deletion (allele D) variant in intron 16. This polymorphism is associated with almost half of the variance in circulating activity of the ACE with II, ID, and DD genotypes coupled with lower, intermediate, and higher plasma levels of the enzyme, respectively (27). The subjects with the DD genotype exhibited higher BP, fatness, and increased cardiovascular risks (28). In Japanese obese women, the decrease in body fat after weight loss was considerably less in the ACE DD genotype (3). In this study, the frequencies of the DD and ID genotypes were found to be higher in obese children. The D allele was significantly associated with the obesity in Korean preschool children in an agreement with the previous studies in toddlers, teenagers, and adults (8,22,29). The presence of D allele would increase the serum concentrations of the ACE, and this would increase the production of angiotensin II with its detrimental metabolic effects.

Polymorphism in the AT2 gene was also examined in the present study. Angiotensin II is the most powerful biological product of RAS and exerts its effect via at least two distinct receptor subtypes, the AT1 and the AT2. Generally, AT2 plays a crucial role during fetal development and is highly expressed in fetal tissue (30). Angiotensin II has been shown to regulate adipocyte differentiation and fat tissue growth through AT2, so it might play a role in the pathogenesis of obesity and the regulation of body weight (31). The gene for AT2 is located on the X chromosome (q22–23), and there have been a few studies on the relationship between the AT2 gene C3123A polymorphism and cardiometabolic diseases (9,32). The A allele carriers had significantly lower high-density lipoprotein-cholesterol levels than non-A allele carriers (10). In response to low-energy diets in obese women, subjects with the AA genotype showed less improved levels of systolic BP, cholesterol, carbohydrate, and fat oxidation than those with the CC and CA genotypes (3). In the present study, the A allele was found to be more frequent in overweight children than in healthy controls, although the correlation became non-significant in multiple logistic regression analysis. Obese children also had a higher frequency of the A allele compared to the controls, however, the difference was not significant. Small sample size can contribute to this finding which may become associated with childhood obesity in studies with larger sample sizes. Considering the inconsistent results, further studies are necessary to clearly determine the association between the AT2 C3123A polymorphism and the risk of overweight/obesity.

The TGF-β1 gene codes a multifunctional cytokine that regulates cell growth, differentiation and matrix production in various cell types, including adipocyte precursor cells (12). Increased TGF-β1 expression has been associated with BMI and abdominal adipose tissue in obese subjects (15). The commonly studied T869C polymorphism, located in the promoter region of TGF-β1 gene, may directly influence the expression level. The C allele was highly associated with serum TGF-β1 level, and carriers of this allele showed elevated risk for obesity and type 2 diabetes (15). Rosmond et al. (16) demonstrated that in 284 unrelated, non-diabetic Swedish men, carriers for the C allele had higher BMI, abdominal obesity, insulin and glucose levels. Although we did not measure serum TGF-β1 level in the present study, our findings revealed that the C variant was prevalent in obese children compared to healthy controls. The frequency of the TC genotype was higher in the obese group compared to the controls. The TT genotype and T allele were significantly less frequent in obese children. In contrast, no association between the TGF-β1 T869C polymorphism and obesity was found in Turkish children or adolescents (33). These conflicting results could be attributed to differences in ethnicity and age groups. Although the importance of TGF-β1 signaling in energy homeostasis and pathogenesis of obesity is largely unknown, it is plausible that the presence of the C allele could increase serum concentrations of TGF-β1 and its consequent detrimental metabolic effects.

Obesity requires neovascularization to supply nutrients and oxygen to increase the size and number of mature adipocytes (11). This process requires the activity of VEGF, which is the most important angiogenic factor involved in fat mass expansion (11). Several studies have shown an increase in serum VEGF in obese patients and have proposed that adipocytes produce angiogenic factors that play central roles in allowing for the expansion of adipose tissue (11,17). The association of VEGF with the development of obesity implies that genetic variation in the VEGF gene may affect susceptibility to this metabolic disorder (18). In the present study, we found that a genetic polymorphism in the VEGF gene could affect susceptibility for obesity in children. The frequency of the C allele and TC genotype in the VEGF T460C polymorphism was higher in the obese group compared

to the controls. Obese children exhibited a lower frequency of the TT genotype and T allele. Our findings cannot be compared to other studies because this is, to the best of our knowledge, the first study investigating the association of the VEGF T460C polymorphism with obesity. However, our findings are plausible because the C allele in the VEGF T460C polymorphism is correlated with a promoter haplotype with greater activity. VEGF promoter haplotypes with the C allele have a 71% higher promoter activity than those that do not carry the allele (19).

TNF-α is also one of the upregulated angiogenic factors in expanding adipose tissues (11). It is a multifunctional cytokine primarily produced by macrophages within adipose tissue (20). Obese mice deficient in either TNF-α or its receptor exhibit protection against developing insulin resistance (20). The G308A polymorphism in the promoter region of the TNF-α gene has been shown to be related with obesity, hyperleptinemia, insulin resistance and elevated transcriptional activity of TNF-α (21). The A allele in the TNF-α G308A polymorphism was associated with a two-fold greater gene expression level (34). Carriers of this allele were more frequently obese than non-carriers (35). In contrast, other studies have revealed no association between this TNF-α mutation and obesity (36). Our present study showed no correlation between genotype and allelic differences of the TNF-α G308A polymorphism in each individual group.

Obesity may have its onset very early in life; accordingly, children comprise a large portion of this disease (1). Experimental and human studies imply the burdening role of early life obesity in long-lasting cardiovascular and renal diseases (37). In the present study, there were no differences of BP levels and serum concentrations of glucose and total cholesterol in preschool children with or without obesity. Although cardio-metabolic changes were not found in this population group, particular attention should be paid to developing strategies for the early prediction and prevention of obesity-associated comorbidities. Our study suggests that the ACE, AT2, TGF-β1, and VEGF genes may be associated with the development of childhood obesity or overweight, although the mechanism of the relationships between these gene polymorphisms and obesity has not yet been elucidated. Several studies have reported a genetic influence on overweight or obesity in Korean children (38,39); however, to the best of our knowledge, this is the first study on the associations between genetic variants and overweight/obesity in Korean preschool children.

This study has some limitations. First, the sample size was somewhat small to gain sufficient statistical power in certain genotypes. Further clarification in a larger study population is needed. Second, other environmental factors (e.g., parental obesity, breastfeeding history, physical activity, etc.) or ethnic predisposition should be also considered. There is no single factor known to cause childhood obesity, but coactions at multiple aspects (e.g., genetic, cellular, physiological, psychological, social, and cultural) contribute to outcomes (40). Finally, functional activity of the studied genes could not be analyzed because we did not measure serum or tissue levels of the gene products. The mechanism of the relationship between the candidate gene polymorphisms and development of obesity should be more clearly determined. Although this was a small-scale study, the present findings suggest the association between the genetic variants of the modulators of adipogenesis and angiogenesis and childhood obesity. Having much knowledge about the genetic contributions to obesity will allow us to get a better understanding for the mechanisms leading to obesity. In the end, this may help predict an individual's risk for obesity at an early age and opens possibilities for introducing the preventive strategies into clinical practice.

Taken together, the ACE I/D, AT2 C3123A, TGF-β1 T869C, and VEGF T460C gene polymorphisms can be related to obesity or overweight in young Korean children. The D allele in the ACE I/D gene and the C alleles in the TGF-β1 T869C and VEGF T460C genes were positively and independently associated with childhood obesity. The A allele in the AT2 C3123A gene showed a possible link with the susceptibility for overweight in children. Further research is needed to identify the roles of these genes in childhood obesity. This may help develop more effective strategies for the prevention and treatment of the obesity epidemic.

DISCLOSURE

The authors have no potential conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Conceptualization: Yoo KH, Yim HE, Hong YS. Data curation: Yim HE, Bae ES. Formal analysis: Yim HE. Funding acquisition: Yoo KH. Investigation: Yoo KH, Yim HE, Bae ES. Writing - original draft: Yim HE, Yoo KH. Writing - review & editing: Yoo KH, Hong YS.

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