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Transforming growth factor-beta1 (509 C/T, 915 G/C, 869 T/C) polymorphisms are not related to obesity in Turkish children

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SUMMARY: Kanra AR, Tulgar-Kınık S, Verdi H, Ataç FB, Yazıcı AC, Özbek N. Transforming growth factor-beta1 (509 C/T, 915 G/C, 869 T/C) polymorphisms are not related to obesity in Turkish children. Turk J Pediatr 2011; 53: 645-650.

Increasing expression of transforming growth factor-beta 1 (TGF-β1) from fatty tissue affects the serum level and hence may stimulate expression of the other cytokines. The studies concerning the relation between TGF-β1 polymorphisms and obesity have been performed in adults, and diverse results have been reported. In this study, we aimed to investigate the association of TGF-β1 509 C/T, 915 G/C, 869 T/C polymorphisms in childhood obesity and related pathologies.

Two hundred and seventy-one children and adolescents were included in the study. One hundred and twenty-one of these cases were in the Obese Group and 150 were in the Control Group. In the Obesity Group, we searched the carbohydrate and lipid metabolism disorders such as insulin resistance, dyslipidemia and hepatosteatosis.

The results of this study revealed the lack of an association between TGF-β1 509 C/T, 915 G/C and 869 T/C polymorphisms and obesity. There were no relations between the polymorphism genotypes and obesity-related metabolic disturbances.

Key words: obesity, children, transforming growth factor-β1 gene polymorphism.

Obesity is a result of an imbalance between nutrient intake and energy expenditure. Increased positive energy balance causes fat storage. With increasing epidemics of obesity all over the world, further research has been focused on both the genetic and environmental factors affecting energy balance in children and adolescents. Family food choices, diet composition (in particular fat intake), low physical activity, and lifestyle changes are responsible for fat gain in predisposed individuals. Although these factors are responsible for obesity, studies in twins and adopted children suggest a genetic factor in the etiology of obesity[1-3].

Transforming growth factor-beta (TGF-β) is a multifunctional cytokine that is produced by a variety of cells. It is capable of regulating the growth and differentiation of many cell types. Elevated expression of TGF-β has been shown in adipose tissue of obese mice[4,5].

Tumor necrosis factor alpha (TNF-α) and TGF-β have been shown to be potent inducers of plasminogen activator inhibitor-1 (PAI-1) synthesis in a number of cell systems[4,6-11]. In obesity, PAI-1 and TGF-β levels are increased in visceral and subcutaneous fat tissues[8]. Although some reports suggest increased TGF-β level in adipose tissue in human and mice[12-14], there is limited data in the literature concerning TGF-β concentration in obese humans. Studies in obese adults showed decreased levels of TGF-β[15,16]. Byrne et al[17]. found increased PAI-1 and decreased TGF-β activity during a fat tolerance test in healthy men. In a study by Yener et al.[18], serum TGF-β levels were positively correlated with postprandial glucose and age and inversely correlated with body

mass index (BMI) and waist circumference. In a previous study[19], we determined that obese children had lower TGF-β levels compared to leans. However, lower TGF-β levels were not correlated with lipids, insulin resistance (IR) and BMI in this study[34]. On the contrary, Romano et al.[20] showed higher serum TGF-β levels in obese women who had impaired insulin sensitivity.

Transforming growth factor (TGF)-β gene regulation and expression levels are affected by the presence of single nucleotide polymorphisms (SNPs) in certain loci. Among these, 509 C/T, 915 G/C (Arg25 Pro, codon 25) and 869 T/C (Leu10Pro, codon 10) are the most frequently studied polymorphisms[21-25]. Since SNPs can affect TGF-β expression, numerous studies have examined the association between SNPs and diabetes, obesity and inflammatory diseases[23,24,26,27]. However, phenotypic differences resulting from altered expression due to these SNPs is sometimes inconsistent[22]. We could find no data in the literature concerning TGF-β gene polymorphisms and childhood obesity.

Herein, we studied TGF-β1 509 C/T, 915 G/C and 869 T/C polymorphisms in the TGF-β gene in obese children. We also aimed to investigate the relationships between TGF-β genotype and obesity-related metabolic disorders.

Material and Methods

Two hundred and seventy-one unrelated children and adolescents were enrolled in the study. One hundred and twenty-one of these cases comprised the Obese Group. The Control Group was comprised of 150 children who were healthy and non-obese. All cases included in the study (obese and control) were clinically free of symptoms and were not on any medication.

DNA was obtained from the peripheric blood for TGF-β1 509 C/T, 915 G/C and 869 T/C genotyping of the children.

Each subject's height was measured using a standard wall-mounted stadiometer. Weight was measured with a calibrated electronic scale. BMI was calculated using the weight/height^2 (kg/m^2) formula. Children with a BMI above the 95th percentile for age and sex were defined as obese (as defined by the National Center for Health Statistics, www.cdc.gov). Relative BMI (relBMI) was calculated using the following formula: subject's BMI × 100/50th percentile BMI for the subject's age and sex. Children with a relBMI <110 were defined as normal, $110 \leq$ relBMI < 120 as overweight, and relBMI ≥ 120 as obese[29].

In the Obese Group, we searched for IR, dyslipidemia and hepatosteatosis.

The levels of glucose, lipid and insulin were assessed in the venous blood following an overnight fast (10–12 hours). Serum glucose levels were measured using the glucose hexokinase method. Serum total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, and triglyceride (TG) levels were studied using Roche diagnostics methods (GbmH, Germany). Serum very low density lipoprotein (VLDL) cholesterol levels were measured by the Friedewald formula. Serum insulin levels were measured using the chemiluminescence method (DPC, Los Angeles, CA, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) score was used to determine IR. This score was calculated with the following formula: HOMA-IR=fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5[30,31].

After prepubertal children in the Obese Group were excluded, HOMA-IR >3 was defined as IR in the pubertal obese children[32]. Children were considered to have excessive TG and LDL cholesterol levels if blood concentrations were ≥ 130 mg/dl. HDL was considered low at a level of ≤ 40 mg/dl. The age- and sex-specific 90th percentile (P_{90}) for TG as well as for LDL cholesterol values was set as the upper limit and the age- and sex-specific 10th percentile (P_{10}) for HDL cholesterol was defined as the lower limit[33].

In obese patients, appearance of hyperechogenic (bright) liver in ultrasonography implicated steatosis (Ellegra Siemens (German) with 3.5 MHz convex probe)[34].

Ethics: The study protocol was approved by the ethics committee of Başkent University, and informed consent was obtained from all participants' parents.

Genotyping

Genomic DNA was prepared from leukocyte pellets by sodium dodecyl sulfate lysis, ammonium acetate extraction and ethanol

Table I. The Clinical Characteristics of the Obese and Control Groups

Mean ±SD (median) Min-Max	Control (n=150)	Obese (n=121)	P
Age (year)	10.7 ±2.4 (10.0) 8.0-17.0	12.5 ±3.1 (12.8) 5.5-17.8	<0.001
Relative BMI	88.2 ±10.2 (88.0) 66.0-115.0	151.7 ±20.5 (149.0) 125.0-228.0	<0.001
Female/Male	85 / 65	60 / 61	0.270
HOMA-IR		3.4±2.2 (0.5-17.0)	
HDL (mg/dl)		43.9±11.1 (11.0-78.0)	
LDL (mg/dl)		96.4±23.6 (45.0-167.0)	
Triglyceride (mg/dl)		112.3±62.6 (27.0-460.0)	

BMI: Body mass index. LDL: Low density lipoprotein. HDL: High density lipoprotein. HOMA-IR: Homeostasis model assessment of insulin resistance.

precipitation. The primers used and the conditions for polymerase chain reaction (PCR) analysis were as described previously[35].

-509 T/C: A 153 base pair (bp) PCR product was cut with Eco 81 I for TGF-β -509 T/C. The uncut product (153 bp) showed the presence of the T allele. If the PCR product was cut into two fragments of 117 and 36 bp, it revealed the C allele.

896 T/C (codon 10, - Leu10Pro): 869 T/C (codon 10, - Leu10Pro) was determined after digestion with MspA1I , which yielded 161, 67, 40, and 26 bp bands in the presence of the T allele and 209- and 149, 67, 40, 26 and 12 bp bands in the presence of the C allele.

915 G/C (codon 25 Arg25Pro): The PCR product was digested with BglI for TGF-β 915G/C (codon 25 Arg/Pro). Detection of 131, 103 and 60 bp products yields the G allele; 131 and 163 bp indicated the C allele.

Data Analysis

Normality of distribution of the continuous variables was analyzed using Shapiro-Wilk normality test, and Levene's test was used to assess the homogeneity of variances in the different groups. Parametric test assumptions were not available, and nonparametric tests were used for data analysis. Mann-Whitney U test was used for comparing two independent groups. Differences between more than two independent groups were analyzed by Kruskal-Wallis one way analysis of variance by ranks test, and then multiple comparisons between pairs of groups were carried out according to Dunn test. Friedman test and then Bonferroni-Dunn multiple comparison test were used for comparing dependent groups. The results were expressed as the number of observations (n) and the mean ± the standard deviation ($\overline{X} \pm S_{\chi}$) and median (M). Categorical variables

Table II. The Comparisons of the Genotype Frequencies between Obese and Control Groups

TGFβ	Control n=150 (%)	Obese n=121 (%)	P
509 C/C	12 (8.0)	8 (6.6)	
509 C/T	73 (48.7)	65 (53.7)	0.70
509 T/T	65 (43.3)	48 (39.7)	
Total	150 (100)	121 (100)	
915 C/C	14 (9.3)	13 (10.7)	
915 G/C	116 (77.3)	97 (80.2)	0.45
915 G/G	20 (13.4)	11 (9.1)	
Total	150 (100)	121 (100)	
869 C/C	------	------	
869 C/T	3 (2.0)	2 (1.7)	1.00
869 T/T	147 (98.0)	119 (98.3)	
Total	150 (100)	121 (100)	

Table III. The Comparisons of the Genotypes According to Dyslipidemia

TGF-β	TG ≥150 n=21 n (%)	TG <150 n=99 n (%)	P	LDL ≥130 n=10 n (%)	LDL <130 n=111 n (%)	P	HDL <40 n=46 n (%)	HDL ≥40 n=74 n (%)	p
509 C/C	2 (9.5)	6 (6.1)		0 (0.0)	8 (7.2)		3 (6.5)	5 (6.7)	
509 C/T	13 (61.9)	51 (51.5)	0.47	5 (50.0)	60 (54.1)	0.43	25 (54.3)	40 (54.1)	0,99
509 T/T	6 (28.6)	42 (42.4)		5 (50.0)	43 (38.7)		18 (39.2)	29 (39.2)	
Total	21 (100)	99 (100)		10 (100)	111 (100)		46 (100)	74 (100)	
915 C/C	1 (4.8)	12 (12.1)		1 (10.0)	12 (10.8)		6 (13.3)	8 (10.8)	
915 G/C	18 (85.7)	78 (78.8)	0.54	9 (90.0)	89 (80.2)	0.39	37 (82.2)	58 (78.4)	0.38
915 G/G	2 (9.5)	9 (9.1)		0 (0)	10 (9.0)		3 (4.4)	8 (10.8)	
Total	21 (100)	99 (100)		10 (100)	111 (100)		46 (100)	74 (100)	
869 C/C	—	—		—	—		—	—	
869 C/T	0 (0.0)	2 (2.0)	1,00	1 (10.0)	1 (0.9)	0,15	0 (0.0)	2 (2.7)	0.52
869 T/T	21 (100)	97 (98.0)		9 (90.0)	110 (99.1)		46 (100)	72 (97.3)	
Total	21 (100)	99 (100)		10 (100)	111 (100)		46 (100)	74 (100)	

were analyzed by Pearson χ^2 test and Fisher's exact test when determining the relationships between the variables. Data analyses were performed with SPSS software (Statistical Package for the Social Sciences, version 13.0, SSPS Inc, Chicago, IL, USA). A p value of <0.05 was considered statistically significant.

Results

The clinical characteristics of the Obese and Control Groups together with the laboratory results of the Obese Group are given in Table I.

The TGF-β1 genotypes were not different in the Obese and Control Groups in our study (Table II).

In the Obese Group, the frequencies of high TG, high LDL cholesterol and low HDL levels were 18%, 8% and 38%, respectively. After excluding 19 prepubertal children from the Obese Group, 43 of 102 pubertal obese children (42.2%) had HOMA-IR score >3, and 59 of these 102 (57.8%) children had HOMA-IR score ≤3. Fifty-seven of 121 obese children (47%) had hepatosteatosis. There was no relationship between the polymorphism genotypes and metabolic disturbances in obese children (Tables III, IV). Furthermore, the allele frequencies for each polymorphism were not different with respect to high TG and LDL cholesterol levels, low HDL levels,

Table IV. The Relationships between Genotypes and Insulin Resistance Status (HOMA-IR scores) and Between Genotypes and Hepatosteatosis in Obese Patients

TGF-β	HOMA-IR >3 n=43 n (%)	HOMA-IR≤3 n=59 n (%)	P	HS (+) n=57 n (%)	HS (-) n=64 n (%)	P
509 C/C	1 (2.3)	6 (0.10)		3 (5.3)	5 (7.8)	
509 C/T	25 (58.1)	29 (49.2)	0.27	32 (56.1)	33 (51.6)	0.80
509 T/T	17 (39.6)	24 (40.7)		22 (38.6)	26 (40.6)	
Total	43 (100)	59 (100)		57 (100)	64 (100)	
915 C/C	6 (14.0)	6 (10.1)		4 (7.0)	10 (15.6)	
915 G/C	35 (81.4)	48 (81.4)	0.55	48 (84.2)	49 (76.6)	0.19
915 G/G	2 (4.6)	5 (8.5)		5 (8.8)	5 (7.8)	
Total	43 (100)	59 (100)		57 (100)	64 (100)	
869 C/C	—	—		—	—	
869 C/T	1 (2.3)	1 (1.7)	0.67	0 (0)	2 (3.1)	0.50
869 T/T	42 (97.7)	58 (98.3)		57 (100)	62 (96.9)	
Total	43 (100)	59 (100)		57 (100)	64 (100)	

HS: Hepatosteatosis. HOMA-IR: Homeostasis model assessment of insulin resistance score.

hyperinsulinemia, and hepatosteatosis (data not shown).

Discussion

Human adipose tissue has been shown to produce PAI-1, TNF-α, TGF-β, and interleukin-6. Studies in human adipose tissue and mice revealed different results about the relationships between adiposity, cytokines and obesity-related metabolic disorders such as IR and dyslipidemia[26].

Transforming growth factor (TGF)-β gene regulation and expression levels are affected by the presence of SNPs in the gene. The TGF-β gene codes a multifunctional cytokine that controls proliferation, differentiation and some other functions in many cell types. Increased TGF-β expression was associated with BMI and abdominal adipose tissue in morbid obesity[8]. An association has been shown between TGF-β polymorphism (T29C) and both BMI and abdominal obesity in Swedish men[27]. Another study further suggested an association between TGF-β genes and obesity[28]. In our study, we could not find any relation between obesity and the most known TGF-β gene polymorphisms. Similar to our results, Bensen et al.[22] could not find any association between TGF-β (509C/T) genotype, insulin sensitivity and amount of subcutaneous fat tissue.

Dixon et al.[23] found an association between angiotensinogen and TGF-β-producing genotypes (codon 25 Arg/Arg) in obese adults with advanced hepatic fibrosis and non-alcoholic fatty liver disease. On the contrary, we could not show a relationship between TGF-β polymorphisms and hepatosteatosis in obese children.

Park et al.[24] studied 28 polymorphisms in the TGF-β gene in the Korean population. They showed a positive association between TGF-β polymorphisms and insulin levels. They found no significant association between the risk of type 2 diabetes and TGF-β gene polymorphisms except for three SNPs (c.2011+137C>T, c.2589T>G and c.651G>C) that were associated with obesity-related phenotypes. In our study, we failed to show any relationship between TGF-β polymorphisms and obesity in childhood. Further, TGF-β polymorphisms were not related with obesity-associated parameters such as IR, dyslipidemia and hepatosteatosis.

A limitation of our study was the small number of children included in both groups. The mean ages of the groups were statistically different; however, age is not a determining factor for the polymorphism frequencies. We suggest that short duration of increased adipose tissue due to the young age in our study groups and less obvious metabolic impairment in obese children might have affected our study results. Long-term follow-up of these children could help us to understand the interaction between these polymorphisms and metabolic disorders. Further research is needed to identify new genetic or environmental risk factor(s) for childhood obesity that may help in the development of more effective strategies for the prevention and treatment. Moreover, since the cytokines act as a network, and obesity is a multifactorial disease, the molecular pathology cannot be explained on the basis of a single gene. We suggest further studies be undertaken including other cytokines related to fat tissue and obesity.

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