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Adolescent obesity and the role of the fat mass and obesity-associated gene polymorphism

Abstract

Purpose: The association between fat mass and obesity-associated (FTO) gene and obesity is unclear in both adults and adolescents. The aim of this study was to examine the role of the FTO gene variant rs9939609 as a candidate gene for obesity and the relationship between insulin resistance (IR), metabolic syndrome (MetS), estimated glomerular filtration rate (eGFR) and neutrophil-to-lymphocyte ratio (NLR).

Methods: Obese adolescents (n=100) and healthy controls (n=100) were included. Rs9939609 polymorphism in the FTO gene was genotyped by PCR-SNaPshot.

Results: The prevalence of insulin resistance (IR), metabolic syndrome (MetS) and hyperfiltration were 47%, 60% and 27%, respectively. There were no significant differences in genotype and allele frequencies between obese adolescents and controls; however, prevalence of MetS in female patients with A allele carriers was more frequent and prevalence of hyperfiltration was less frequent with T allele carriers ($P<0.05$). The NLR levels were higher in A allele carrier obese patients with IR/MetS ($P<0.05$).

Conclusions: We could not confirm the FTO rs9939609 variant as an obesity susceptibility gene in adolescents, but we observed an association with MetS, IR, NLR and hyperfiltration. This is a preliminary study and further work is needed in an independent cohort to clarify the possible effects of FTO gene polymorphism.

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The increasing prevalence of childhood obesity is a major public health problem. Obesity is a multifactorial disease and susceptibility to obesity is determined by genetic and environmental interactions [1]. Genome-wide association studies (GWAS) showed many loci associated with obesity risk and traits [2]. Fat mass and obesity-associated (FTO) gene was the first susceptibility locus for obesity identified by GWAS [3,4]. A common variant (rs9939609) in the FTO gene has been shown to be associated with obesity and related traits in children and adolescents [1,2,5-9]. The association between FTO gene polymorphism and obesity has been demonstrated consistently in Caucasian populations [2]; however, the relationship has not been replicated in all ethnicities. The most obvious reason for failure to replicate results can be related to insufficient statistical power due to the small sample size.

Childhood obesity is an important risk factor for the development of metabolic syndrome (MetS), which is characterized by several factors, including central obesity, elevated plasma fasting glucose (PFG) levels, hypertension and dyslipidemia. The prevalence of MetS is rapidly increasing worldwide, especially in adolescents. Obesity is the major component of MetS; therefore, the FTO gene might also be one of the candidate genes for MetS [7,8]. The association between rs9939609 variant in the FTO gene and MetS has been demonstrated in Lithuanian populations; however, several association studies found inconsistent results in different ethnicities [1,7,8].

Recently, more attention has been drawn to the effects of obesity and related complications on the kidney. Obesity, MetS, insulin resistance (IR) and diabetes mellitus (DM) are associated with glomerular hyperperfusion, glomerulopathy and the development of renal dysfunction. Increased estimated glomerular filtration rate (eGFR) (glomerular hyperfiltration) is one of the early signs of renal complications [10,11]. Based on our current information, there are limited data about the association of rs9939609 variant in FTO gene and hyperfiltration in obese adolescents [12-14].

Obesity is a chronic inflammatory disease characterized by increasing infiltration of proinflammatory immune cells into the adipose tissue. The FTO gene has been found to be associated with inflammatory markers, such as plasma C-reactive protein (CRP) and interleukin-6 (IL-6) levels [15,16]. Recently, several studies have shown that biomarkers of inflammation such as neutrophil/lymphocyte (NLR) and platelet/lymphocyte (PLR) ratio are associated with obesity and related complications [17-19]. These biomarkers show scientific and clinical promise as they are cost-effective and readily available. This article is the first to report on the

association of FTO gene and NLR/PLR values in obese adolescents.

The aim of this study was to examine the role of FTO gene rs9939609 polymorphism on the development of obesity and MetS, as the second was to examine the association with FTO gene and obesity-related traits such as MetS, IR, hyperfiltration and NLR/PLR among Caucasian adolescents in south-eastern Turkey.

Materials and Methods

Patients and control subjects

One hundred unrelated obese adolescent and 100 age and sex-matched healthy controls were selected from among those referred to the Well-Child Outpatient Clinic of Gaziantep University Hospital. The minimum sample size was determined as 85 subjects in each group at the 80% power level with an α error of 5% with MedCalc (version 11.5.1) program. The distribution of AA-genotype was accepted as 16% in the populations according to the literature and the error rate was estimated as 20% in the obese adolescents [3].

Anthropometric measurements (height, weight and waist circumference) were performed using standardized protocols. Body mass index (BMI) was calculated as the weight divided by square of height (kg/m^2). International Obesity Task Force's (IOTF) international standards were used to diagnosis of obesity [20]: obesity is defined as a BMI at or above the 95th percentile for children and teens of the same age and sex. The evaluation of BMI was performed by using age-and-sex specific percentile standards of Centers for Disease Control and Prevention (CDC) [21].

The medical records of all obese adolescents were reviewed. The PFG, insulin, serum low-density lipoprotein (LDL)/high-density lipoprotein (HDL) cholesterol, triglyceride, alanine aminotransferase (ALT) and creatinine levels were assessed. The PLR and NLR were calculated from the complete blood count (CBC) parameters. MetS was determined according to the *National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III)* criteria [22]. Insulin resistance was determined by homeostasis model assessment for insulin resistance (HOMA-IR > 3.16) [23]. The eGFR was calculated from serum creatinine (SCr) using the Schwartz formula:

$$[k \cdot \text{height}(\text{cm}) / \text{SCr}(\text{mg/dL}) = \text{mL/min}/1.73 \text{ m}^2$$

For 2-12 years $k=0.55$; for ≥ 12 years: male; $k=0.70$, female; $k=0.55$. The $\text{eGFR} > 165 \text{ mL/min}/1.73 \text{ m}^2$ for 2-12 years and $> 145 \text{ mL/min}/1.73 \text{ m}^2$ for ≥ 12 years were defined as hyperfiltration [24].

TABLE 1. Genotype and allele distribution of the FTO gene polymorphism in obese and control groups and also in adolescent obese group with or without metabolic syndrome

	Obese groups n(%)	Control groups n(%)	Odds Ratio (CI %)	P
Genotype				
T/T	32 (32.0)	41 (41.0)	0.7 (0.38-1.21)	0.1200
T/A	48 (48.0)	36 (36.0)	1.6 (0.93-2.89)	0.0575
A/A	20 (20.0)	23 (23.0)	0.8 (0.43-1.64)	0.3655*
Allele				
T	112 (56.0)	118 (59.0)	0.9 (0.60-1.31)	0.3065
A	88 (44.0)	82 (41.0)	1.1 (0.76-1.68)	0.3065
HWE(p)	0.7921	0.0105		

	Obese adolescent with MetS n(%)	Obese adolescent without MetS n(%)	Odds ratio (CI %)	P
Genotype				
T/T	17 (28.3)	15 (37.5)	0.7 (0.28-1.54)	0.2285
T/A	31 (51.7)	17 (42.5)	1.5 (0.65-3.24)	0.2437
A/A	12 (20.0)	8 (20.0)	1.0 (0.37-2.72)	0.5966*
Allele				
T	65 (54.2)	47 (58.8)	0.8 (0.47-1.47)	0.3105
A	55 (45.8)	33 (41.2)	1.2 (0.68-2.14)	0.3105
HWE (p)	0.7122	0.2109		

*Fisher's exact test

Abbreviations: CI, confidence interval; HWE, Hardy-Weinberg Equilibrium; MetS, metabolic syndrome

The study was approved by the local ethical committee of the University of Gaziantep and the study protocol was carried out in accordance with the Declaration of Helsinki.

Genotyping

Genomic DNA was isolated from EDTA-peripheral blood samples (2 ml) using the salt fractionation procedure. Amplification of rs9939609 in FTO gene was performed using polymerase chain reaction (PCR) technology. The PCR products were checked using agarose (2%) gel electrophoresis and were cleaned up by using NucleoFast96 PCR clean-up plates (Macharey-Nagel). Genotyping of single-nucleotide polymorphism (SNP) were carried out by using SNaPshot multiplex system (Applied Biosystems) on ABI 3130 capillary

electrophoresis instrument (Applied Biosystems) and the electropherograms were analyzed on GeneMapper 4.0 software (Applied Biosystems). The SNaPshot reactions were set according to the manufacturers' protocol.

Statistical analyses

The chi-square test was used to compare genotype and allele frequencies in the case and control groups (Graphpad Instat version 3). The Hardy-Weinberg equilibrium (HWE) was calculated using the de Finetti program. All data were analyzed by using the computer software Statistical Package for the Social Sciences (SPSS) for Windows (version 22.0; SPSS). Results are given as mean±SD, while allele frequencies and the distribution of genotypes are expressed as percentage (%).

TABLE 2. FTO gene T allele and insulin/HOMA-IR levels in obese adolescents without metabolic syndrome and FTO gene A allele and NLR values in obese adolescents with metabolic syndrome or insulin resistance

Obese adolescents without MetS			
	T allele (+) mean±SD (minimum-maximum)	T allele (-) mean±SD (minimum-maximum)	P
Plasma fasting insulin (µIU/mL)	17.0±8.0 (5.9-39.6)	12.4±4.1 (6.8-19.8)	0.034
HOMA-IR	3.6±1.8 (1.3-10.3)	2.7±1.0 (1.4-4.2)	0.050

Obese adolescents with MetS or IR			
	A allele (+) Mean±SD (minimum-maximum)	A allele (-) Mean±SD (minimum-maximum)	P
Patients with IR	1.86±0.65 (0.08-3.04)	1.43±1.38 (1.09-2.25)	0.011
Patients with MetS	1.81±0.66 (0.81-3.56)	1.40±1.37 (1.11-2.20)	0.042

Abbreviations: IR, insulin resistance; MetS, metabolic syndrome

Clinical characteristics and FTO gene polymorphisms were compared using the chi-square test. Multiple comparisons were calculated by the Mann-Whitney U test. Statistical significance was considered as $P < 0.05$.

Results

Clinical characteristics

The mean age of the patients (50 male, 50 female) was 13.6 ± 2.3 years (10-18 years). The prevalence of MetS, IR and hyperfiltration were 60.0% (n=60) 47.0% (n=47) and 27.0% (n=27), respectively.

Genotype and allelic frequencies of FTO gene (rs9939609) polymorphisms

The comparison of genotype and allele frequencies of the FTO rs9939609 variant T/A polymorphisms between obese patients and controls are presented in Table 1. For FTO T/A polymorphism, the distribution of T/T, T/A and A/A genotypes was 32.0%, 48.0% and 20.0%, respectively, in obese children compared with 41.0%, 36.0% and 23.0% in controls ($P > 0.05$). The allele frequencies of T and A were 56.0% and 44.0% in patients compared with 59.0% and 41.0% in controls

($P = 0.306$) (Table 1). There were no significant differences in genotype and allele frequencies between obese patients and controls. No significant deviations from the HWE were observed for the FTO rs9939609 variant T/A polymorphism among adolescent obese patients with or without MetS; however, observed genotype counts were deviated significantly from those expected according to the HWE among cases and controls.

Association between the identified genotypes and clinical/laboratory parameters of patients

The correlations between FTO genotypes and clinical and laboratory parameters of patients were investigated. Serum ALT levels were higher in obese patients with A allele carriers (except for morbidly obese patients) (carriers, 33.71 ± 31.81 U/L (11-155 U/L); non-carriers, 18.00 ± 5.22 U/L (11-31 U/L); $P = 0.050$). No relationship was found between FTO genotypes/alleles and other clinical/laboratory parameters.

The distribution of genotypes and alleles was not different in obese adolescents with or without MetS (Table 1). In females with A allele, the prevalence of MetS was more frequent (carriers, 23/36, 63.9%; non-carriers, 4/14, 28.6%;

$P=0.024$). The e-GFR value was higher in patients with MetS (with MetS, 150.13 ± 20.80 mL/min/ 1.73 m^2 , 119.20 - 211.13 mL/min/ 1.73 m^2 ; without MetS, 141.01 ± 18.82 mL/min/ 1.73 m^2 , 115.57 - 186.66 mL/min/ 1.73 m^2 ; $P=0.035$). In girls, hyperfiltration was less common in T-allele carriers (with T allele, $4/37$, 10.8% ; without T allele, $4/9$, 44.4% ; $P=0.036$). In patients without MetS, plasma fasting glucose (PFG) levels more than 100 mg/dL were more frequent for AA genotype than for the other two genotypes (TT, $0/14$, 0.0% ; TA, $0/17$, 0.0% ; AA, $2/8$, 25.0% ; $P=0.017$). Insulin and HOMA-IR levels were higher in T/wild allele carriers without MetS (Table 2). There were no differences between genotypes and allele frequencies in the same group of MetS.

There was a positive correlation between BMI and NLR values ($r=0.249$, $P=0.018$). The NLR levels were higher in A allele carrier obese patients with IR or MetS (Table 2).

Discussion

The association of the FTO gene (rs9939609) with obesity is confirmed in different populations and age groups [1,3-8]. The AA genotype is associated with obesity and risk has been increased 1.31-1.35 fold in A allele carriers [3,6,8]. Carrying A allele is associated with obesity in Turkish adults [7]. But, no association was found between the rs9939609 variant and obesity in adolescents. The association of the rs9939609 variant with increased obesity risk has been shown previously in Caucasian participants but not in African-Americans [25]. There are other studies with negative results from different ethnicities; including Caucasian, Hispanic, Asian and Oceanic populations [26-28]. These divergent results might be related to different design/methodology of the studies or insufficient statistical power due to the small sample size. It could also be explained by differences in allele frequencies and different environmental factors which may lead to diverse gene effect. Some studies have shown that the FTO gene is associated with early onset obesity in childhood [3,29,30]. The relationship could not be confirmed in this study, as the study was conducted only on adolescent subjects.

The FTO gene variant rs9939609 is associated with PFG/insulin levels or IR/DM independent from BMI [5,31,32]. We found no association between the FTO gene and PFG/insulin/HOMA-IR levels, but PFG levels higher than 100 mg/dL were seen more frequently in AA genotype patients without MetS. Similarly, Liu et al. did not report any association among rs9939609 variant and PFG, insulin levels or IR in adolescents [33]. Another study by Xi et al. showed no association with glucose and lipid levels, despite showing an association of the FTO gene with obesity [34]. The FTO gene

variant rs9939609 is associated with obesity and related phenotypes in severely obese children [1,5-8]. This metabolic diversity may be explained by differences in patients' characteristics.

Although we could not show any association with FTO gene variant and gender, we found that the prevalence of MetS was more frequent in female patients with A allele. Jacobsson et al. found a significant difference between gender and FTO rs9939609 variant in obesity-related traits such as PFG/IR and they concluded that FTO gene may have a gender-specific role for development of IR in children [31,32]. The gender-specific associations between FTO gene variants and glucose homeostasis may be due to higher prevalence of type 2 DM/IR among females and the body fat composition differences between girls and boys [35].

In our study, the distribution of genotypes and alleles was the same in patients with or without MetS. Several other association studies for MetS reported inconsistent results [7,8,34,36]. Carrying of A allele was associated with MetS in adults [7,8]. De Luis et al. found an association between the rs9939609 variant in the FTO gene and MetS in obese females. In accordance with our results, they found that the genotype distribution was not different between patients with or without MetS [37]. Also consistent with our result, they reported higher insulin and HOMA-IR levels in the TT genotype in patients without MetS. These results are different from previously published reports. We cannot explain this diversity, but the same findings in both studies are interestingly and remarkable. This diversity in female gender should be investigated in larger trials.

Recently more attention is drawn to the effect of obesity and related comorbidities on the kidney. Glomerular hyperfiltration (elevated eGFR) due to endothelial damage in obese adolescents is one of the important early-stage renal complications [10,11]. Consistent with the literature, we found that the eGFR value was higher in patients with MetS, but there was no significant difference between genotypes/alleles frequencies of the FTO gene. Coto et al. did not find any relationship between FTO gene rs9930506 polymorphism and reduced eGFR in the elderly patients with MetS [14]. It has been reported a significant association between FTO gene (rs17817449, rs708259 polymorphisms) and chronic kidney disease [12,13]. We also demonstrated that carrying T/wild allele could be a protective role from hyperfiltration in female individuals. It could be speculated that AA genotype of FTO gene (rs9939609) may have a predisposition role development of the renal complications in obese female adolescents.

Obesity is characterized by increasing infiltration of proinflammatory immune cells into the adipose tissue which leads to obesity-related comorbidities. Elevated levels of simple and inexpensive indicator of systemic inflammatory markers, such as NLR/PLR, are also associated with obesity and related complications [17-19]. Higher NLR levels are a significant and independent factor for the development of type 2 DM in adult morbid obese patients [19]. Consistent with other studies, we found a linear correlation between BMI and NLR values. Inflammatory and metabolic marker (sCD40L and visfatin) levels have been found to be higher in postmenopausal women with FTO gene (rs9939609) AT+TT genotype [16]. Surprisingly, we found a significant association between A/variant allele and NLR levels in obese adolescents with IR or MetS. Zimmermann et al. demonstrated that levels of plasma C-reactive protein (CRP) and interleukin-6 (IL-6), which are inflammation markers, were associated with FTO rs9939609 A allele [15]. The A allele of FTO gene may have a possible role in the development of IR and MetS by predisposing adolescent obese patients to inflammation.

The small sample size and the restriction only to the adolescent age group are limitations of our study. This is a preliminary study and the results need to be confirmed in an independent cohort. Nevertheless, we demonstrated the association of the FTO gene with MetS in girls. The main strength of this study is the investigation of the relationship between FTO gene and NLR/ PLR values.

In conclusion, we could not confirm that the FTO rs9939609 variant is a susceptibility gene for the development of obesity in adolescents, but we observed a relationship between MetS, IR, NLR and hyperfiltration. Population studies with larger sample sizes in different age groups are needed determine the exact role of FTO gene for the development obesity and MetS and to clarify gender differences.

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