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INVESTIGATION OF THE FAT MASS AND OBESITY-ASSOCIATED (*FTO*) GENE IN PRESCHOOL CHILDREN

YAĞ KİTLESİ VE OBEZİTE İLİŞKİLİ (*FTO*) GENİN OKUL ÖNCESİ ÇOCUKLARDA ARAŞTIRILMASI

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ABSTRACT

Objective: Obesity is a complex disease defined as being overweight. Previous studies have highlighted familial, environmental, and genetic factors as effective predictors of high body mass index (BMI) and obesity in the preschool period. These studies particularly emphasized that habits acquired in the preschool years affect BMI and obesity. In this population-based case-control study, it was aimed to reveal for the first time the relationship between fat mass and obesity-related (FTO) gene expression and BMI and obesity in preschool children aged 2-6 living in Turkey.

Materials and Methods: Buccal mucosal swabs were collected from 54 preschool children from 3 kindergartens located in Istanbul, Turkey. In the study, the *FTO* gene expression level in a total of 54 (n=25 girls and n=29 boys) children who were obese (n=14) and non-obese (n=40) according to the International BMI index was determined by the 'Quantitative Polymerase Chain Reaction' (qPCR) technique.

Result: A correlation was found between *FTO* gene expression and BMI and obesity in obese and non-obese children with a Mann-Whitney U test (p=0.005).

Conclusion: Additional research is needed to better understand the function and prevalence of the *FTO* gene mutations by performing sequencing analysis of the gene simultaneously in preschool children in Turkey.

Keywords: *FTO* gene, obesity, overweight, preschool children, BMI

ÖZ

Amaç: Obezite fazla kilolu olma olarak tanımlanan çok faktörlü bir hastalıktır. Önceki çalışmalar ailesel, çevresel ve genetik faktörlerin okul öncesi dönemde vücut kitle indeksi (VKİ) ve obezitenin etkili belirleyicileri olarak bulunmuştur. Özellikle okul öncesi yıllarda kazanılan alışkanlıkların VKİ ve obezite üzerinde etkisi olduğu vurgulanmıştır. Bu nüfusa dayalı vaka kontrol çalışmasında, Türkiye'de yaşayan yaşları 2-6 arasında değişen okul öncesi çocuklarda yağ kütlesi ve obezite ile ilişkili (*FTO*) gen ekspresyonu ile VKİ ve obezite arasındaki ilişkinin ilk defa açığa çıkarılması amaçlanmıştır.

Gereç ve Yöntem: İstanbul, Türkiye'de yaşayan 3 adet anaokuluna kayıtlı, yaşları 2-6 arasında olan toplam 54 okul öncesi çocuktan bukkal mukoza sürüntüleri toplandı. Çalışmada, uluslararası VKİ indeksine göre obez olan (n=14), obez olmayan (n=40) toplam 54 (n=25 kız ve n=29 erkek) çocukta *FTO* gen ekspresyon düzeyi 'Quantitative Polymerase Chain Reaction' (qPCR) tekniği kullanılarak incelendi.

Bulgular: Obez olan ve olmayan çocukların *FTO* gen ekspresyonu ve obezite arasında anlamlı ilişki $2^{-\Delta\Delta CT}$ formülü ile Mann-Whitney U testi kullanılarak bulundu (p=0,005).

Sonuç: Okul öncesi çağında olan çocuklarda *FTO* geninin işlevini daha iyi anlamak için daha fazla bireyle gen dizileme analizi araştırmalarına ihtiyaç olduğu düşünülmektedir.

Anahtar kelimeler: *FTO* geni, obezite, fazla kilo, okul öncesi, VKİ

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INTRODUCTION

The alarming increase in childhood obesity is impacting both developed and developing countries (1). The World Health Organization has reported that around 60% of adults and one in three school-age children in Europe are currently affected by obesity whereas, in the US, obesity affects more than 17% of children (2). Due to childhood obesity, some children are at risk of developing certain diseases that were previously thought to be adult diseases, such as obstructive sleep apnea, hypertension, type 2 diabetes mellitus, dyslipidemia, and other comorbidities (3). Also, these children are more vulnerable to feelings of low self-esteem, despair, and anxiety, and some experience bullying, which has an impact on their psychological and emotional well-being (4). Furthermore, the risk of suffering from an obesity-related disease is linked to an increased risk of early death and increased healthcare spending (5). For all these reasons, research which seeks to detect the factors underlying childhood obesity is very important.

Genetics have a remarkable influence on obesity in an adipogenic environment and childhood obesity is the result of an interaction of genetic factors and environmental effects (3,6). The combination of these factors leads to energy imbalance, i.e. excessive calorie intake compared to the amount of energy consumed, resulting in excessive accumulation of adipose tissue (7). The fat mass and obesity-associated (*FTO*) gene was the first gene that showed consistent associations with overall obesity in adults and children from different ethnic backgrounds (8). In a longitudinal study of twins, researchers found that the impact of a common variant of the *FTO* gene on BMI becomes more pronounced with increasing age in childhood (9). In terms of obesity onset, age-related differences were observed between the studies. The evidence suggests that the relationship between *FTO* variants and obesity-related traits may be age-dependent (10). Lopez-Bermejo et. al. indicated the onset of obesity in the neonatal period (11). In another study, childhood was indicated as the onset period of obesity (12). In addition, Rutters et. al. pointed to adolescence as the period in which the onset of obesity-related traits is seen (13). But these studies were done entirely on individuals of European descent. The pattern and age of onset of obesity, and the association between *FTO* gene expression and childhood BMI/obesity in the Turkish population are not clear. In 2022, a multicenter study in Turkey investigated potential genes and mutations in children with non-syndromic early-onset severe obesity (onset<7 and BMI-SDS>3) and confirmed any pathogenic variants of any mutations in *FTO* intron (14). But another study conducted by Agagunduz et. al. among Turkish adults demonstrated that only *FTO* polymorphism was associated with whole-body fat accumulation, and not abdominal fat accumulation (15).

To our knowledge, no studies have examined the age and sex-specific associations of BMI and *FTO* gene expression patterns in relation to BMI and obesity in preschool children in a population-based in Turkey. Here in this study, we investigate the associations between the *FTO* gene expression and BMI and obesity in preschool children living in Turkey and examine the interactions of this gene expression pattern with gender, food allergy, and familial factors.

MATERIAL and METHOD

Study Population

In this population-based case-control study, preschool children (aged between 2-6 enrolled in 3 kindergartens) living in Istanbul, Turkey, were assessed for body size parameters. A total of 54 (n=25 girls and n=29 boys) children who were obese (n=14) and non-obese (n=40) according to the international BMI index were included in the study. The age and sex-specific BMI cut points recommended by the International Obesity Task Force were used (IOTF)(16). Buccal mucosa samples were collected from 54 preschool children.

The study was approved by the MEF University Ethics Committee (The approval date was 20.02.2019) to collect the swap samples from preschool children. Istanbul University, Istanbul Faculty of Medicine, and the Clinical Research Ethics Committee approved doing the experimental procedure at the Oncology Institute (Date: 03.03.2023, No: 5). Written consent was obtained from the parents prior to the study. A power analysis was used to determine the minimum number of study groups. The result of the power analysis showed that Type I Error=0.09 and Test Power (Confidence Interval) =80% for patient analysis. It was calculated that the minimum number of subjects in our patient group should be 54.

Anthropometric measurements

Anthropometric measurements including weight, height, and fat mass percentage were calculated using a stadiometer; each participant's height was calculated to the nearest centimeter. On common medical scales set to the nearest 0.1 kg, subjects wearing light indoor attire were weighed. To determine BMI, the formula BMI= weight(kg)/height^2 (m) was used. Using age and sex-specific BMI cut-off values suggested by Cole et al., overweight and obesity status were identified (16). Lifestyle habits (eating habits), nationality, food allergy status, breastfeeding duration, and the obesity status of each child were determined using a questionnaire. Oral swabs were used to gather the biological material needed by the study group from the mouth's cheek region. The *FTO* gene's expression in the collected sample was examined.

Gene expression analysis

To isolate RNA from the buccal mucosal swap samples, Zymo Research's Quick-RNA MiniPrep Kit (Quick-RNA-TM MiniPrep, USA) was used according to the manufacturer's instructions. Buccal mucosal swabs were fixed in a DNase/RNase-free solution and 300 µL of RNA lysis buffer was added and then centrifuged for 30 s at 10,000 g. The supernatant was transferred into a Spin-Away Filter collection tube and centrifuged for one minute at 10,000 g. 5 µL of DNase I and 75 µL of DNA digestion buffer were added and transferred into a collecting tube and filled with 400 µL of RNA preparation buffer and centrifuged at 10,000 g for 30 seconds. This was washed with 700 µL of RNA

wash buffer twice and centrifuged. The column was placed in a new 1.5 mL micro-centrifuge tube and 700 µL of QIAzole solution was added and left at room temperature for 5 minutes. 140 µL of chloroform was added and centrifuged at +40°C for 15 minutes at 12,000g. The top phase was transferred into a collection tube and 525 µL of 100% ethanol was added and centrifuged at 8000 g for 15 seconds. 700 µL of RWT buffer was added and centrifuged at 8000 g for 15 seconds. Then the columns were filled with 500 µL of RPE buffer and centrifuged at 8000 g for 1 minute and RNA was isolated. The purity and concentration of RNAs were determined by electrophoresis on a 1.5% agarose gel at 150 V. A NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) was used. RNA was determined by measuring absorbance at 260 and 280 nm wavelengths.

A 'Bioline SensiFAST cDNA Synthesis Kit' (Meridian, Bioline, ABD) was used to create the cDNA. After adding 1 ng, 1 µL of template RNA, 4 µL of TransAmp buffer, and 1 µL of Reverse Transcriptase, the total volume was brought to 20 µL by adding RNAse DNAse-free water and a BioRad PCR (BioRas, Singapore) device was used for cDNA synthesis.

qPCR was conducted using the 'SensiFAST SYBR®No-ROXKit-Bioline'(Meridian, Bioline, ABD) kit in Mic PCR Biomolecular Systems (Queensland, Australia) device. 10 µL Sybr Green, 400 nM 1 µL *FTO* Reverse Primer 400 nM, 1 µL *FTO* Forward Primer, 6 µL template cDNA, and 2 µL DNAase/ RNAase Free water were added respectively and the total volume of 20 µL was distributed into Mic PCR tubes. The qPCR procedure was performed at 95 °C for 5 minutes, then at 60°C for 2 minutes, and at 60 °C for 30 seconds. The $2^{-\Delta\Delta CT}$ method was used to analyze the relative changes in obese and non-obese children (17). *GAPDH* gene expression assay was used as the housekeeping gene. *FTO* gene expression was determined.

Statistical Analysis: *FTO* gene expression levels in obese and non-obese preschool children were investigated. The descriptive statistics of the qualitative variables in the study were given as numbers and percentages, and the descriptive statistics of the quantitative variables were given as mean, standard deviation, median, minimum, and maximum. The assumption of normal distribution was examined with the Shapiro-Wilk test. The Mann-Whitney U test was used to compare the mean of two independent groups. The Spearman correlation coefficient was used when examining the relationships between quantitative variables. All statistical analyses were performed using the SPSS program (SPSS version 26; SPSS Science, Chicago, USA), and a p-value=0.005 was considered statistically significant.

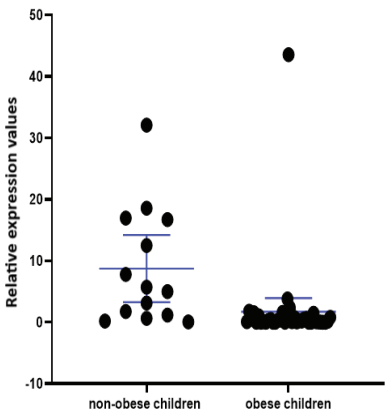
RESULTS

The demographic features of the children are shown in Table 1. In this population-based case-control study 54 preschool children aged between 2-6 were selected for an investigation of the expression level of *FTO* gene expression. Of the children, 51 (94%) were from the Marmara Region of Turkey and 3 (6%) were from Bulgaria (self-reported). We used the $2^{-\Delta\Delta CT}$ method to analyze the *FTO* gene expression in buccal mucosal swabs from obese and non-obese children. To analyze the statistical difference in the expression of the *FTO* gene between obese children and non-obese children, a Mann-Whitney U test was performed.

We also used a Mann-Whitney U test to analyze the data revealing the difference in expression of the obese group from the non-obese group and to determine whether this was statistically significant. The results showed a difference in *FTO* gene

Table 1: Demographic features of the children

Variables	Children (n=54)
Age (years)	
2 years old	3 (5.5%)
3 years old	7 (13%)
4 years old	12 (22.2%)
5 years old	25 (46.3%)
6 years old	7 (13%)
Gender	
Female	25 (46.3%)
Male	25 (46.3%)
Nationality	
Turkish	51 (94.4%)
Others	3 (5.6%)
Food allergy of children	
Yes	7 (7.4%)
No	47 (92.6%)
Breastfeeding duration	
6 months	9 (16.7%)
6-12 months	18 (33.3%)
12 months and over	27 (50%)
Obesity/Overweight	
Obese	14 (26 %)
Non-obese	40 (74%)



p=0.0001 5.5360 (0.6600-16.97)

Figure 1: Quantitation plots of expression levels of non-obese and obese children. Data are expressed as relative expression units. The bars represent the standard error of the mean (SEM). p value < 0.05 was considered statistically significant using the Mann-Whitney U test.

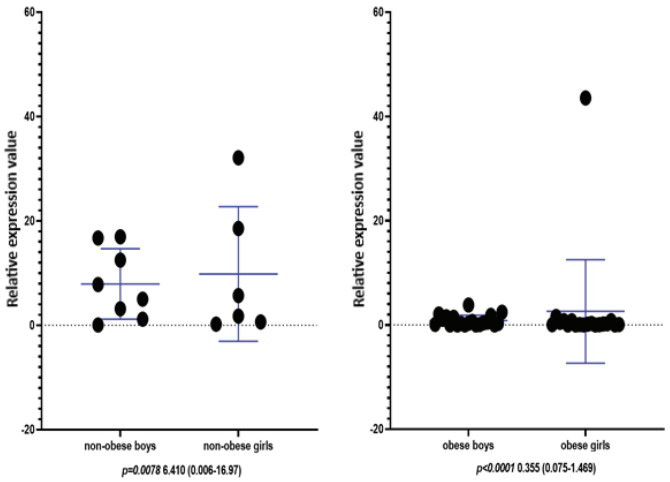


Figure 2: Quantitation plots of expression levels of boys and girls of non-obese (a) and obese (b) children. Data are expressed as relative expression units. The bars represent the standard error of the mean (SEM). p value<0.05 was considered statistically significant using the Mann-Whitney U test.

Table 2: *FTO* gene expression correlation with the parents' weight-height, pregnancy weight of mother, birth weight-height of the children, and breast-feeding duration.

	Mother'sweight	Mother's height	Father's weight	Father's height	Pregnancy weight	Birth weight of the children	Birth height of the children	Breast-feeding duration
FTO gene expression p value	0.919	0.437	0.362	0.745	0.983	0.852	0.565	0.218
r value	0.017	-0.130	0.152	-0.055	0.004	-0.031	-0.096	0.214

Spearman correlation analysis

Table 3: *FTO* gene expression analysis of preschool children with or without food allergy

Food Allergy	N	Mean	Median	Std. Deviation	Minimum	Maximum	p
No	47	12.468	1.1847	43.43699	0.06	255.83	0.085
Yes	7	0.320	0.1686	0.29247	0.13	0.66	

Mann-Whitney U test

Table 4: *FTO* gene expression analysis of obese children between 4-6 months and older than 6 months of age.

Obese children	Mean	Median	Std. Deviation	Minimum	Maximum	p
Between 4-6 months	5.6667	1.0751	9.56681	0.08	32.10	0.788
After 6 months	14.5470	0.8262	51.14744	0.06	255.83	

Mann-Whitney U test

expression levels in obese versus non-obese children shown in Figure 1. The results showed a difference in the gene expression levels in obese versus non-obese, and in boys versus girls in the obese/non-obese groups (Figure 2).

FTO gene expression levels were evaluated in terms of gender, as well as in quantitation plots of expression levels of boys and girls of non-obese (Figure 2a) and obese (Figure 2b) children and there was a difference between the boys versus girls in the obese/non-obese children as shown in Figure 2.

The correlation with the *FTO* gene expression and parents' weight-height, pregnancy weight of mother, birth weight-height of

the children, and breast-feeding duration was also investigated. There was no significant correlation observed between them (p=0.218) as shown in Table 2.

We also evaluated *FTO* gene expression in preschool children with or without food allergy. There was no significant difference between the *FTO* gene expression in children with or without food allergy, and (p=0.085) was detected as shown in Table 3.

We also compared the percentage of obese children aged between 4-6 months and those older than 6 months of age. No significant relationship was detected between obese preschool children who were aged 4-6 months and those older than 6 months of age (p=0.788), as shown in Table 4.

DISCUSSION

This is the first study in Turkey to examine the association between *FTO* gene expression and BMI and obesity in preschool children (aged between 2-6). We found a moderate correlation between *FTO* gene expression and BMI and obesity in prescchool children.

Several studies have shown that the *FTO* gene is known as a susceptibility gene for polygenic obesity. In European populations, the effect of the *FTO* gene on BMI varies at different stages of life (18). However, the results regarding the age of onset of the association have been inconsistent. In this present study, the preschool associations of *FTO* gene expression with BMI and obesity and the risk factors for obesity are investigated for the first time in Turkey. The finding of this study confirms previous research conducted on mice and adult humans which demonstrated an association between *FTO* and the occurrence of obesity. Villalobos et. al. investigated the FTO gene expression level in mestizo Mexican adults and obese individuals who were found to have higher expression of FTO in their subcutaneous adipose tissue compared to healthy individuals (19). In another study, using a mouse model, the researchers discovered that additional copies of the *FTO* gene resulted in increased FTO expression, which in turn promoted the development of obesity. The mice carrying an additional copy of *FTO* exhibited an increase in body weight; furthermore, *FTO* was found to be directly related to the food intake and metabolism of the mice, as an increase in the expression of *FTO* leads to an increase in food intake and body fat (20). Another study indicated that the mRNA level of *FTO* showed a correlation with the BMI of European descent adults, implying that its expression could be influenced by the accumulation of body fat. Notably, the expression of *FTO* in visceral tissues may play a role in the onset of obesity (21). In accordance with the literature findings from different nations, we found a positive association between *FTO* gene expression and obesity in preschool children (22).

Heritable variables are thought to account for 30% to 50% of obese status (23). According to the literature, if both parents are obese, the likelihood of their child being overweight increases by two to three times, and in some cases by as much as fifteen times. In this present study, of the 14 obese preschool children, five of them had obese parents, indicating a possible role of hereditary factors in childhood obesity.

There is growing evidence that these differences in childhood obesity rates are primarily due to inequalities in the physical and social environments in which children grow up (24). For example, the prevalence of obesity among all children in the United States has increased by 10%, while children from households with lower educational attainment, lower income, and higher unemployment have experienced a significant increase in obesity rates, ranging from 23% to 33% from 2003 to 2007 (25). In addition, lower-income families are less likely to recognize their child's obesity or feel the need to intervene in their child's eating and exercise habits (26). Furthermore, low-income communities face several barriers to improving their health conditions (27). As Turkey is a low-income country and obesity is increasingly becoming a major problem, we investigated the expression level of the *FTO* gene expression for the first time in 54 preschool children in the Marmara region (n=51) in Turkey and the Balkans (from Bulgaria, n=3). We analyzed the data taken from obese and non-obese preschool children (2–6 years old) using the Mann-Whitney U test and found it to have a relatively high *FTO* gene expression level (p=0.005).

Childhood obesity has become one of the most serious medical and public health problems of the 21st century. According to statistics, up to 58% of the world's adult population will be obese by 2030 (28). The etiology of obesity is the result of multiple genetic, environmental, and biological effects. Genetic variations also cause the increase in the expression level of specific genes to be one of the main reasons for obesity. This study has both strengths and limitations. One of the limitations has to do with the finding that children from families with obese parents are more likely to be obese. In order to follow this through, however, our study should have continued with an investigation of an additional number of children of different ages, and a mutation analysis performed by sequencing the *FTO* genes should have been conducted.

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