

Features of FTO Gene Polymorphism (rs9939609) in Children with Abdominal Obesity and Metabolic Disorders

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Abstract 76 children with varying degrees of obesity and 40 children with normal body weight were examined. The frequency distribution of the FTO gene polymorphism (rs9939609) was determined. It was revealed that the FTO gene polymorphism (rs9939609) is one of the factors of genetic predisposition to abdominal obesity with a pronounced increase in BMI, while allele A increased the risk of accumulation of excess visceral adipose tissue in abdominal obesity. A significant increase in blood triglycerides and a decrease in high-density lipoprotein cholesterol in children with the AA genotype were determined. Carriers of the A/A and T/A genotypes had a significant disturbance in the level of hunger hormones and saturation of leptin, ghrelin and YY3-36 peptide.

Keywords Abdominal obesity, FTO gene, Leptin, Ghrelin, YY3-36 peptide

1. Introduction

Over the past decades, the prevalence of obesity has increased several times in all countries in the world. At the same time, the most unfavorable dynamics are observed among children and adolescents, since in these age groups, the growth rate of obesity incidence is 8 times faster than its growth rate among the adult population of the planet [1,2]. Obesity has a tendency towards rejuvenation [3]. In the Republic of Uzbekistan, there is also an increase in the primary and general incidence of obesity among children and adolescents, and its growth rate is significant [4].

The need to study risk factors for the development of obesity in children with its complications is determined by the continuing trend of increasing abdominal obesity in the population. An increase in body mass index (BMI) in children and adolescents is recognized as an independent predictor of the development of comorbid conditions that make up the metabolic syndrome [5]. Thus, abdominal obesity in adolescents is recognized as an independent risk factor for stroke at a young age, the development of cardiovascular pathology, diseases of the gastrointestinal tract, and kidney pathology [6].

Genetic factors occupy a leading place in the structure of risk factors for the development of abdominal obesity, amounting to 25 to 70% [7]. The 12th version of the Human Obesity Gene Map includes more than 600 genes, genetic

markers and chromosomal regions directly or indirectly associated with the obesity phenotype. One of these genes is the FTO gene (Fat Mass and Obesity Associated), which is associated with fat mass and obesity [8].

The FTO gene is localized on chromosome 16q12.2 and encodes the synthesis of the FTO protein [3]. The expression product of the FTO Gene is predominantly concentrated in the hypothalamus, namely in the centers of hunger and satiety. The FTO protein is involved in energy metabolism and the metabolism of body cells [7,9]. The existence of a single nucleotide polymorphism (SNP) T/A (rs9939609) in the FTO gene was revealed. The A allele of this gene is associated with the risk of developing primary obesity, while owners of the AA genotype (16% of the population) are more at risk of accumulating fat mass than owners of the TT genotype (37% of the population) [7,8,9].

Currently, special importance is attached to the study of genetic predictors of obesity and its main complications, in connection with which many studies have been conducted. However, studies devoted to the analysis of the influence of genetic factors on the development of obesity and, especially, its comorbid pathology in children of the Uzbek population are incomplete and sporadic, which requires a close study of the problem.

This is especially important since the healthcare system of our country is entrusted with a number of tasks aimed at improving the quality of medical services provided to the population and adapting them to international standards,

including preserving the health of young children, early diagnosis of diseases that arise in them, and reducing complications.

In connection with the above, we were given **a goal:** to study the features of the FTO gene polymorphism (rs9939609) in children with abdominal obesity and find a relationship with metabolic disorders.

2. Material and Methods

Studies were conducted on the basis of family clinics in the city of Samarkand, as well as Samarkand regional branch of the Republican Specialized Endocrinological Scientific and Practical Medical Center named after Academician Y.H. Turakulova (Uzbekistan). The study involved 76 children aged 7 to 18 years with exogenous constitutional obesity, with the average age of the children being 12.02 ± 0.46 years. The control group consisted of 40 children with a normal body weight without the presence of chronic and acute diseases at the time of examination. Children in the control group had a similar age as in the main group, the average of which was 12.14 ± 0.27 years.

Anthropometric studies were carried out using standard measuring instruments (floor stadiometer and medical scales). Anthropometric measurements include: height, body weight, waist and hip circumference. Comparison of the obtained data and assessment of physical development were carried out using cumulative centile tables of WHO age and gender distribution of height and body weight for children 5-19 years old [10]. Body mass index (BMI) was calculated from the measurements.

The results of anthropometric studies were assessed using standard deviations of body mass index (SDS) in accordance with the recommendations of the World Health Organization (WHO) [10]. The basis for the diagnosis of Obesity was the determination of the intersection point of age and BMI, above +2.0 SDS BMI, overweight was diagnosed with indicators ranging from +1.0 to +2.0 SDS BMI, and underweight from -1.0 to -2.0 SDS BMI.

76 children of the main group had a BMI of +2.0 to $\geq +3$ SDS, i.e. children had a BMI characterizing obesity from degrees I-III, with the average BMI being 32.18 ± 0.5 kg/m², the average SDS BMI was in the range of 2.56 ± 0.11 . At the same time, in the control group, BMI was in the range from +1.0 to -1 SDS, with an average BMI of 19.28 ± 0.26 kg/m² and an average SDS BMI of 1.10 ± 0.08 , which was statistically significantly lower compared with the main group ($P < 0.001$).

The anthropometric data of the children in the main group determined the WV, which had an average level of 102.06 ± 1.51 cm, which was significantly higher compared to the control group 66.74 ± 0.81 cm ($p < 0.001$). Wherein, HV at higher rates in the main group (88.14 ± 1.85 cm) did not statistically differ from the indicators of children in the control group (77.21 ± 1.06 cm; $p > 0.05$).

An important indicator was the ratio of WV/HV characterizing the presence of abdominal obesity, which were statistically higher values in children from the main group with exogenous constitutional obesity (0.98 ± 0.00 compared to the control 0.79 ± 0.01 ; $p < 0.001$).

The presented data characterize significant differences in body weight in the study groups, while age, divided by gender, had no statistical differences (41 (53.9%) boys and 35 (46.1%) girls in the main group, and 21 (52.5%) boys and 19 (47.5%) girls in the control group).

The lipid profile was studied on an automatic biochemical analyzer- Cobas Integra 400 Plus (Roche, Germany) using original test systems (Roche, Germany) with determination of the concentrations of total triglycerides, high-density lipoprotein cholesterol using absorption photometry.

Determination of leptin, ghrelin and YY3-36 was carried out by the enzyme immunoassay method on the Human Reader HS apparatus, using the Human LEPTIN ELISA Kit, Human GHRL (Ghrelin) ELISA Kit, ELISA DSL-10-33600 test systems (manufactured by Elabscience USA).

The study of the FTO gene polymorphism (rs9939609) was carried out using a polymerase chain reaction using the method of allelic discrimination. Reverse transcription and PCR reactions were carried out using commercial kits LLC RPC "Litex" (Russian Federation). DNA samples were isolated from the patients' blood using phenol-chloroform extraction.

The polymerase chain reaction was carried out in a 25 μ l volume in two tubes, one of which contained forward primer 1 and reverse primer, the second - forward primer 2 and reverse primer. After amplification and subsequent electrophoresis in a 3% agarose gel in the presence of ethidium bromide, the amplification results were detected in ultraviolet light. The statistical processing of the obtained data was carried out on a personal computer using the Statistica 10 program. Methods of variational parametric and non-parametric statistics were used to determine the arithmetic mean (M), standard deviation (σ), standard error of the mean (m) relative values (frequency, %). The statistical significance of the obtained measurements was determined by Student's test (t) with a calculation of the probability of error (P). During genetic studies, allele frequencies and frequencies of allelic combinations were calculated and their correspondence to the Hardy-Weinberg equilibrium using the χ^2 criterion with the calculated ones, rejecting the null hypothesis at $P < 0.05$.

3. Results and Discussion

The search for genetic risk factors for the development of obesity has great practical importance, since hereditary factors remain unchanged throughout a person's life. Because obesity is polygenic in nature, the search for those genetic markers that are most responsible for the development of excess weight is especially relevant. Literary sources in recent years indicate that the AA genotype of the FTO gene

When analyzing the mutant homozygous genotype AA, which is responsible for the development of obesity, we did not obtain statistically significant differences compared to the control group, 18.4% in the main group and 17.5% in children of the control group ($\chi^2=0,015$, $p=0,903$, $OR=1,065$, $95\%CI=0,391-2,896$). These indicators were confirmation of scientific research of some world studies.

Genotypes	Main group n=76		Control group n=40		χ^2	P	OR	95%CI
	abc	%	abc	%				
T/T	21	27,6	13	32,5	0,300	0,585	0,793	0,345-1,820
T/A	41	54,0	20	50,0	0,164	0,686	1,171	0,544-2,521
A/A	14	18,4	7	17,5	0,015	0,903	1,065	0,391-2,896
Alleles	n=152		n=80					
T	83	54,6	46	57,5	0,178	0,674	1,125	0,651-1,942
A	69	45,3	34	42,5				

Genotypes	Uniform type of obesity n=39		Abdominal obesity n=37		Control group n=40	
	abc	%	abc	%	abc	%
T/T	16	41,0*	5	13,5	13	32,5
T/A	18	46,1	23	62,1	20	50,0
A/A	5	12,8	9	24,3	7	17,5
Alleles	n=78		n=74		n=64	
T	50	64**	33	44,6	46	57,5
A	28	36	41	55,4	34	42,5

* differences between groups with uniform and abdominal obesity различия между
- $\chi^2=7,187$, $p=0,008$, $OR=4,452$, $95\%CI=1,426-13,896$;

** differences between groups with uniform and abdominal obesity
- $\chi^2=5,830$, $p=0,016$, $OR=2,219$, $95\%CI=1,157-4,255$

The main goal of our work was to evaluate the contribution of the FTO gene polymorphism (rs9939609) to the development of abdominal obesity and the metabolic syndrome that developed against it in children. In this regard, we divided the children of the main group into two subgroups, depending on the presence or absence of abdominal obesity. The criterion for identifying a child in the group with abdominal obesity was the ratio of waist volume (WV) corresponding to the 90th percentile and above for a certain age and gender recommended in the methodological recommendations of the All-Russian Society of Cardiology (2009) [11]. For children 16 years of age and above, the criterion was the definition of WV ≥ 94 cm in boys and ≥ 80 cm in girls. In accordance with the above criteria, children were divided into a subgroup with a uniform type of obesity (n=39) and an abdominal type of obesity (n=37).

When analyzing the frequency distribution in subgroups, it was revealed that in children with abdominal obesity the frequency of the AA genotype was higher than in other groups - 24.3% (12.85 in children with a uniform type of obesity and 17.5% in controls), but no statistical difference was found compared to other groups. This fact was also observed regarding the heterozygous T/A gene - 62.1% compared to the group with a uniform type of obesity - 46.1% and children in the control group - 50%. In general, the frequency of manifestation of genotypes containing

the mutant allele A (A/A and T/A) was 86.4%, compared with children with a uniform type of obesity 58.9% ($\chi^2=7,187$, $p=0,008$). Thus, the A/A and T/A genotypes were protective for the development of abdominal obesity with the subsequent growth of metabolic complications (Table 2).

The frequency of the homozygous T/T genotype was highest in the group with a uniform type of obesity - 41.0%, which was statistically higher compared to abdominal obesity 13.5% ($\chi^2=7,187$, $p=0,008$, OR=4,452, 95%CI=1,426-13,896), while the chance of encountering this genotype in children with abdominal obesity was 4.452 times greater in children with a uniform type of obesity. Also, the T allele was significantly more common in the group of children with a uniform type of obesity ($\chi^2=5,830$, $p=0,016$, OR=2,219, 95%CI=1,157-4,255); essentially, the chance of encountering this gene in children with a uniform type of obesity was 2.219 times greater compared to children with abdominal obesity in whom the A allele predominated (55.4%).

According to scientific research, the polymorphism of the FTO gene (rs9939609) determines the intensity of the formation of adipose tissue in the body and the regulation of appetite, in this regard, we studied the frequency of distribution of genotypes and alleles according to the degree of obesity based on the distribution of SDS BMI (Peterkova V.A. 2021) [12].

Table 3. Distribution of genotypes and allele frequencies of the FTO gene polymorphism (rs9939609) depending on the degree of obesity in children

Genotypes	I degree of obesity n= 21		II degree of obesity n= 29		III degree of obesity n= 26	
	abc	%	abc	%		
T/T	12	57,2****	7	24,1	2	7,7
T/A	8	38,0	18	62,0	15	57,7
A/A	1	4,8	4	13,8	9	34,6*
Alleles	n=42		n=58		n=52	
T	32	76,1^^^	32	55,1	19	36,5
A	10	23,9	26	44,9	33	63,4

* difference compared to the I degree of obesity - $\chi^2=6,181$, $p=0,013$, OR=11,118, 95%CI=1,279-96,665;
 ** difference compared to the II degree of obesity - $\chi^2=5,632$, $p=0,018$, OR=4,190, 95%CI=1,246—14,089;
 *** difference compared to the III degree of obesity - $\chi^2=5,632$, $p=0,018$, OR=16,000, 95%CI=2,977-85,988;
 ^ difference compared to the II degree of obesity - $\chi^2=4,671$, $p=0,031$, OR=2,600, 95%CI=1,080-6,260
 ^^ difference compared to the III degree of obesity - $\chi^2=14,719$, $p=0,001$, OR=5,558, 95%CI=2,244-13,767

Table 4. Average level of lipid metabolism and adipokines in obese children depending on the distribution of genotypes and allele frequencies of the FTO gene polymorphism (rs9939609)

Genotypes	TG mmol/l	HDL-CS mmol/l	Leptin ng/ml	Ghrelin pg/ml	YY3-36 Peptide pg/ml
T/T n= 21	1,13 \pm 0,03	1,11 \pm 0,02	18,05 \pm 1,29	10,3 \pm 0,52	125,2 \pm 5,1
T/A n= 41	1,24 \pm 0,04 P ₃ <0,03	1,09 \pm 0,01	26,66 \pm 3,26 P ₃ <0,01	9,8 \pm 0,68	134,5 \pm 5,51
A/A n= 14	1,31 \pm 0,03 P ₁ <0,001	1,00 \pm 0,01 P ₁ <0,001 P ₂ <0,01	34,6 \pm 2,27 P ₁ <0,001	5,7 \pm 0,47 P ₁ <0,01 P ₂ <0,001	93,5 \pm 2,1 P ₁ <0,001 P ₂ <0,01

Note: P₁—reliability of differences between A/A and T/T

P₂—reliability of differences between A/A and A/T

P₃—reliability of differences between A/T and T/T

In accordance with this classification, there were 21 children with I degree of obesity, 29 children with II degree of obesity, and 26 children with III degree of obesity and above. The results of the frequency distribution of genotypes of the FTO gene polymorphism (rs9939609) can be seen in Table 3.

As can be seen from the table, there was a significant predominance of the frequency of the AA genotype in children with the III degree of obesity - 34.6% compared to children with the I degree of obesity -4.8% ($\chi^2=6,181$, $p=0,013$, $OR=11,118$, $95\%CI=1,279-96,665$), which was not significantly different from the group with the II degree of obesity (13.8%).

The homozygous T/A genotype predominated in children with the II degree II (62.0%) and the III degree (57.7%) of obesity compared to the distribution frequency in children with the I degree of obesity (38.0%), but the data did not have statistically reliable boundaries. Whereas in children with the I degree of obesity, a predominance of the homozygous T/T gene was observed both in comparison with children with the II degree of obesity ($\chi^2=5,632$, $p=0,018$, $OR=4,190$, $95\%CI=1,246-14,089$), and in compared to children with the III degree of obesity ($\chi^2=5,632$, $p=0,018$, $OR=16,000$, $95\%CI=2,977-85,988$).

When distributing alleles depending on the degree of obesity, it was revealed that if in children with degree 1 the T allele predominated, then in children with degree 3 obesity a mutant gene A was observed, which determines the development of obesity ($\chi^2=14,719$, $p=0,001$, $OR=5,558$, $95\%CI=2,244-13,767$).

Literary sources in recent years indicate that the influence of the mutant allele A contributes to the development of various metabolic disorders, which either contribute to the development or are a consequence of obesity. In this regard, we studied the distribution of the average level of lipid profile and blood adipokines depending on the genotypes of the FTO gene polymorphism (rs9939609) (Table 4).

As can be seen from the presented table, the level of triglycerides was evenly distributed depending on the genotypes, with the lowest level observed in the TT genotype (1.13 ± 0.03 mmol/l) and the highest in the AA genotype (1.31 ± 0.03 mmol/l), while the difference between them was significant ($P<0.001$); also, the level of triglycerides in children with the heterozygous T/A genotype was statistically different from children with a homozygous distribution of alleles (1.24 ± 0.04 ; $p<0.03$). Thus, the level of triglycerides in those who had the mutant allele A was statistically different from those in children with the homozygous TT genotype. The same picture was observed in relation to blood leptin, when the leptin level in children with AA genotypes 34.6 ± 2.27 ng/ml and T/A genotype 26.66 ± 3.26 ng/ml was significantly different from the leptin level in children with genotype TT 18.05 ± 1.29 ng/ml ($P<0.001$ and $P<0.01$, respectively).

There was a significant, reliably distinguishable content of such indicators as HDL cholesterol, ghrelin and peptide YY3-36 in children with the AA genotype from the indicators

of children with the T/A and T/T genotypes, which indicated the implementation of metabolic disorders and pathology of hormones involved in the regulation of feelings hunger specifically in children with the A/A genotype.

4. Conclusions

Polymorphism of the FTO gene (rs9939609) is one of the factors of genetic predisposition to abdominal obesity with a pronounced increase in BMI.

The presence of the A allele increases the risk of accumulation of excess visceral adipose tissue in abdominal obesity. This is explained by the fact that a gene containing nucleotide A is subject to greater expression than a gene containing nucleotide T.

Carriers of the AA genotype are more susceptible to the development of metabolic disorders in obesity, which was reflected in significant pathology of blood triglyceride and HDL cholesterol in the children of this sample.

Expression of the A/A and T/A genotype disrupts the functioning of the hunger center and stimulates an increase in food consumption, which was manifested in a significant, significant increase in leptin, ghrelin and YY3-36 peptide in children with the mutant A allele.

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