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Research article

ASSESSMENT OF HEALTH RISKS CAUSED BY OVERWEIGHT IN CHILDREN DEPENDING ON THE FTO GENE RS9939609 POLYMORPHISM

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In this study, we aimed to estimate the association between the rs9939609 FTO (fat mass and obesity associated) polymorphism and a risk of overweight in children living in the Baikal region. We performed a case – control study that included 113 schoolchildren living in industrial centers of the Baikal region (Irkutsk, Angarsk, and Ulan-Ude). Anthropometric parameters were measured and body mass index was calculated with its values being ranked in accordance with the WHO BMI curves depending on a sex and age. Genotyping of the rs9939609 FTO polymorphism was performed by allele-specific amplification with real-time results detection. To assess likelihood of an association between the FTO gene allele and overweight and obesity, relative risk (RR) and 95 % confidence interval (CI) were calculated.

The assessment revealed the A allele of the rs9939609 FTO polymorphism to be by 1.29 times more frequent in the examined children with overweight and obesity (48.44 %) than in the children from the reference group (37.65 %). The FTO rs9939609 polymorphism was authentically associated with likelihood of elevated risks of overweight and obesity in children with the homozygous AA genotype (RR = 2.806, 95 % CI: 1.650–4.772; STD = 0.271). Our study confirms that the rs9939609 polymorphism of the FTO gene is a risk factor of overweight and obesity for children from the Baikal region who have the A allele of the homozygous AA genotype. Prevailing frequency of the TT genotype (29.2 %) as compared with the AA genotype (10.62) is likely due to influence of assimilation processes on urbanized territories in the Baikal region.

Keywords: *children, FTO gene, rs9939609, polymorphism, risk, overweight, obesity, Baikal region.*

The Roadmap on prevention of noncommunicable diseases up to 2025 developed by the WHO includes nine global targets; control of obesity and overweight is one of them [1–3]. Preservation of human potential makes this issue truly vital for healthcare experts, scientists and the society as a whole [4–6]. The WHO Regional Office for Europe reported the results of the studies accomplished within Childhood Obesity Surveillance Initiative (COSI) in 2018–2020. According to it, 29 % of children aged 7–9 years in 33 participating countries had overweight, obesity included [7]. The study that was accomplished in Moscow in 2017–2018 within this Initiative and included 2166 7-year old children established overweight in 27 % of boys and 22 % of girls and obesity in 10 % and 6 % of children accordingly [6].

Genetic background is estimated to account for more than 50 % in common obesity etiology [8]. Child obesity has exogenous rea-

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sons in vast majority of cases and primary factors causing it include absence of physical activity or its low levels, quantities of consumed foods and energy [9–11], and provision of vitamins [12]. A small share of obesity cases can occur due to endogenous reasons where common risk factors include sex, age, ethnic group, and genetic polymorphisms [2, 11]. The fat mass and obesity associated FTO gene encodes an alpha-ketoglutarate-dependent dioxygenase that regulates transcription and translation through the methylation of DNA/RNA [8]. Although the molecular mechanism by which the gene participates in obesity still remains unclear, its polymorphisms are closely connected with a risk of overweight and obesity [8, 13]. The most unambiguous association with a risk of obesity has been identified for rs9939609 polymorphism located in 16q12.2. According to A.K. Baturin with colleagues (2019), the essence and intensity of the association between FTO gene polymorphisms as well as the A allele frequency are largely determined by respondents' race and ethnic group [14]. The aforementioned association between the rs9939609 FTO polymorphism and a risk of obesity has been evidenced by data obtained on various populations including the West [15] and East Asia [11, 13], South America [3, 16], as well as in different regions in Russia [5, 10, 17–19]. Still, S. Doaei with colleagues (2019) [20] combined adjusted odds ratios (*OR*) from 8 eligible case-control studies in their review and established the association between rs9939506 and obesity to be significant in the European subgroup (OR=1.68 [1.2–2.36]), but not in the Asian subgroup (*OR* = 0.94 [0.81–1.10]; *OR* = 0.95 [0.80–1.14]; *OR* = 2.31 [0.96–5.58]).

Since data on genetic factors and their associations with obesity in children and adolescents are still rather controversial [21, 22], it seems interesting to analyze associations between the rs9939506 polymorphism (the FTO gene) in children living in the Baikal region. It is located in two geographical areas, Central Asia and Eastern Siberia and therefore has many ethnical groups such as Slavic, Mongolian, Tungus and Turkic ones with certain assimilation between them.

In this study, our aim was to estimate the association between the rs9939609 FTO polymorphism and a risk of overweight in children living in the Baikal region.

Materials and methods. The study was a case-control one, cross-sectional, observational. Genetic testing was accomplished on 113 schoolchildren aged 7–17 years, 60 boys and 53 girls. They lived in industrial centers in the Baikal region (Angarsk, Irkutsk, and Ulan-Ude). Investigation and sampling of biological material (buccal epithelium) was accomplished only after a parent or a legal representative of each participating child gave their informed voluntary consent to it and after the approval by the Local Committee on Ethics of the East-Siberian Institute of Medical and Ecological Research (the meeting report No. 32 issued on September 10, 2019). The study was accomplished in conformity with the Declaration of Helsinki (1964) Medical Research Involving Human Subjects with all the amendments. We used several criteria for including children in the research: informed consent provided by parents or legal representatives; a child was born after a full-term pregnancy and was breast-fed for longer than 3 months after birth; a child did not have any acute or decompensated chronic diseases at the moment the study was being accomplished[1]; a child attended common physical training classes at school without any limitations and did not do any sports beyond school; an information form was filled in correctly. Anthropometric parameters were estimated considering age and sex; sigma Z-scores of body mass index (BMI) were cal-

[1] Bogdanova O.G., Efimova N.V., Mylnikova I.V. Comparative nutritional characteristics in schoolchildren with different nutritional status. *Gigiena i sanitariya*, 2022, vol. 101, no. 9, pp. 1072–1079. DOI: 10.47470/0016-9900-2022-101-9-1072-1079 (in Russian).

culated according to the respondents' age and then compared with the WHO standards[2]. The aforementioned standards were applied due to the examined respondents belonging to different ethnic groups[1], a situation quite typical for the Baikal region.

Samples of deoxyribonucleic acid (DNA) were obtained from the participants' biological material (buccal epithelium) using conventional procedures. We applied a multicomponent lytic solution able to destroy DNA-protein complexes and then sorbed it on magnetic particles covered with silica gel and washed with ethanol. The final stage involved elution in a buffer solution. DNA was extracted by using a RealBest DNA-extraction3 reagent kit (Vector-Best closed JSC, Russia) and automated pipetting system epMotion 5075 (Eppendorf, Germany). Genotypes were identified by allele-specific PCR and real-time detection of the results. We used TaqMan probes complimentary to DNA polymorphisms and relevant reagents (Syntol, Russia). Amplification was accomplished with a CFX96 Real Time System (Bio-Rad, USA) in real time [5, 10, 14].

To estimate associations between genetic polymorphisms and overweight and obesity (OV and OB), all the examined respondents were divided into Group One ('case', $n = 32$) made of children with OV and OB, and Group Two ('control' or the reference group, $n = 81$) made of children with normal body weight.

The data were tested for normality of distribution by asymmetry and excess and then analyzed with non-parametric statistics. We calculated frequencies of genotypes, alleles, and median percentile BMI trends (*Me* (P25–P75)) by using PASW Statistics 20 [5]. We identified a relative risk (*RR*) and its 95 % confidence interval (CI) and then tested authenticity of the results by Mann – Whitney U-test using DeFinetti software available on the website of Institute of Human Genetics (Munich, Germany)[3] [24]. The critical statistical significance was taken at $p < 0.05$ and $p < 0.01$.

Results and discussion. Overweight (OV) was identified in 23.89 % of the examined respondents and 4.42 % of them had obesity; 25.0 % and 5.0 % of the boys and 22.64 % and 3.77 % of the girls accordingly. Our results are similar to those obtained by the multi-centered study of children aged 5, 10 and 15 years ($n = 5182$) living in Astrakhan, Ekaterinburg, Krasnoyarsk, Saint Petersburg and Samara [25]. Overweight frequency varied between 18.8 and 22.0 % among them; obesity, between 4.7 and 6.7 %.

The results of the genetic tests revealed that the A allele of the rs9939609 FTO polymorphism associated with a risk of obesity was identified in 40.71 % of the examined children. This value is similar to that obtained for European populations where it equals 41.0 % according to the US National Center for Biotechnology Information as of 2022[4]. The A allele of this polymorphism was a bit less frequent among children in Moscow and was identified in 34.4 % of cases [10]. In this study, we were not able to establish any sex-related differences and the share of the A allele was 40.57 % in the examined girls and 40.83 % in the examined boys (Table 1).

Genetic testing in the examined groups detected the A allele of the rs9939609 polymorphism (the FTO gene) in 48.44 % of the children in Group One (OV or OB) and it was 1.29 times higher than the same indicator in the reference group or Group Two (normal

[2] BMI-for-age (5–19 years). *WHO*. Available at: https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/bmi-for-age (February 07, 2023); Tarmaeva I.Yu., Pyrieva E.A., Gmoshinskaya M.V., Bogdanova O.G., Tkachuk E.A., Netunaeva E.A., Safronova A.I., Aleshina I.V. Nutritional features of school children in the Siberian Federal District. *Meditsinskii sovet*, 2021, no. 17, pp. 264–271. DOI: 10.21518/2079-701X-2021-17-264-271 (in Russian).

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[4] dbSNP. Short Genetic Variations. *U.S. National Library of Medicine*. Available at: https://www.ncbi.nlm.nih.gov/snp/rs9939609#frequency_tab (January 20, 2023).

weight) with its value being equal to 37.65 %. Still, likelihood of obesity and overweight for the examined children was not identified when the alleles were compared (A against T, *RR* = 1.46 [0.81–2.61], 0.297); this is in line with the data of the meta-analysis performed by D. Wang with colleagues (2020) in an Asian population where elevated risks of overweight and obesity were established for adults (*OR* = 1.26, 95 % CI: 1.08–1.47, $p = 0.003$) but not for children or adolescents (*OR* = 1.14, 95 % CI: 0.95–1.36, $p = 0.17$) [26].

The AA genotype of the rs9939609 polymorphism (the FTO gene) was established to be 5.06 times more frequent in the children from Group One than in the reference group (Table 2).

Calculation of relative risk (*RR* = 2.81 [1.65–4.77], STD = 0.271) revealed a probable statistically significant association between the homozygous AA genotype of the rs9939609 FTO polymorphism and overweight and obesity in the examined children. Children with such a genotype had overweight and obesity 2.81 times more frequently than children with TT and AT genotypes.

Table 3 provides the results of comparing median percentile BMI trends (*Me*) in the examined children. Obviously, higher BMI was established only in carriers of the homozygous AA genotype of the rs9939609 FTO gene polymorphism in Group One (20.65 (19.58–22.68) kg/m^2). This was 1.16 times higher than in carriers of the same genotype in the reference group or Group Two (17.77 (17.37–18.03) kg/m^2) at the actual U = 0.5 (critical U = 5 for $p < 0.05$, critical U = 2 for $p < 0.01$). Differences were not statistically significant in all the other cases (actual U > critical U).

The results of our retrospective studies on the examined children established that 4.5 % of them had emaciation at birth; the share was 1.3 % at the moment this study was being accomplished. Emaciation detected at birth persisted only in one child up to the moment of the present study (a girl's age was 8 years). One point nine percent of the examined children had overweight at birth and one was obese with this disorder persisting up to the moment of the study (a girl's age was also 8 years). Table 4 reports the results of the retrospective studies; their analysis revealed that the rs9939609 FTO polymorphism had no influence on children's birth weight or length ($p > 0.05$).

Table 1

Sex-related distribution of genotypes and frequency of the A and T alleles of rs9939609 polymorphism (the FTO gene) among children in the Baikal region

Sex	Genotypes, abs. (%)			Alleles, %	
	TT	AT	AA	T	A
All the respondents	33 (29.20)	68 (51.13)	12 (10.62)	59.29	40.71
Girls	15 (28.30)	33 (62.26)	5 (9.43)	59.43	40.57
Boys	18 (30.00)	35 (58.33)	7 (11.67)	59.17	40.83

Table 2

Distribution of genotypes and frequency of the A allele of the rs9939609 FTO polymorphism among children in the Baikal region depending on body mass index

Genotypes, abs. (%)	Groups as per body mass index		Relative risk between genotypes (*RR* [CI], STD)		
	Group One	Group Two	AA and TT	AT and TT	AA and AT+TT
AT	15 (46.88)	53 (65.43)	2.44 [1.23–4.85]. 0.350	0.81 [0.40–1.65], 0.364	2.81 [1.65–4.77], 0.271*
TT	9 (28.13)	24 (29.63)			
AA	8 (25.0)	4 (4.94)			
Risk A allele, %	48.44	37.65	1.46 [0.81–2.61], 0.297		

Note: * means the significance of this association is $p < 0.05$ since the 95 % CI does not include one.

Table 3

Distribution of median percentile BMI trends (*Me*) depending on genotypes of the rs9939609 FTO polymorphism among children form the Baikal region

Groups	Body mass index (*Me* (P25–P75), kg/m^2)			Mann – Whitney U-test (U_{actual})		
	TT	AT	AA	TT/AT	AA/TT	AA/AT
All the examined children	17.30 (15.60–19.90)	16.95 (15.50–19.10)	19.45 (18.28–21.68)	1071	270.5	555.5
Group One	23.00 (19.60–23.70)	22.00 (18.75–24.25)	20.65 (19.58–22.68)	73	29	56
Group Two	16.10 (14.48–18.28)	16.20 (15.00–17.90)	17.77 (17.37–18.03)	624	66	153
U_{actual} between Group One and Two	196.5	726	0.5*	447	172.5	402.5

Note: * means a statistically significant difference between actual U < critical U for $p < 0.05$ and $p < 0.01$.

Table 4

Anthropometric parameters of newborns depending on the rs9939609 FTO polymorphism (*Me* (P25–P75))

Parameters	Genotypes		
	TT	AT	AA
Length, cm	52.00 (51.00–53.50)	52.00 (50.00–54.00)	52.00 (51.00–53.00)
Weight, kg	3.40 (3.00–3.60)	3.50 (3.10–3.80)	3.70 (3.55–4.00)
BMI, kg/m^2	12.30 (11.65–13.35)	12.70 (12.00–13.60)	13.40 (12.70–14.40)

We did not establish any associations between the allele A heterozygous AT genotype of the rs9939609 FTO polymorphism in the examined children and a risk of overweight or obesity (95 % CI includes 1, Table 2). Prevalence of the homozygous TT genotype (29.20 %), which was more frequent than the AA genotype (10.62 %) in the examined groups, is likely due to influence of ongoing assimilation processes between incoming and indigenous populations on urbanized territories in the Baikal region [27]. These results are consistent with the data reported by E.A. Bondareva with colleagues (2018) [28]. In their study, detected frequency of the TT genotype varied depending on ethnic peculiarities: it equaled 3.7 % in Altaians; 16.4 % in Russians from Moscow, Arkhangelsk, and Saransk; 49.7 % in Mongolians; and 50.6 % in Kalmyks. The AA genotype, though, was detected in 36.5 % Altaians, 33.2 % Russians, 10.5 % Mongolians, and 24.1 % Kalmyks.

Conclusions. Our study results established emaciation at birth in 4.5 % of the examined children from the Baikal region and it persisted only in one child (8 years old) at the moment the study was being accomplished.

Overweight at birth was identified in 1.9 % of the examined children whereas 23.89 % of them had it at the moment the study was being accomplished and 4.42 % were obese.

We did not establish a statistically authentic association between the rs9939609 (FTO gene) polymorphism and children's anthropometric parameters at birth (birth weight and length and BMI).

Our analysis of the results obtained by genetic tests of children from the Baikal region established a statistically significant association between the A allele AA genotype of the rs9939609 polymorphism (FTO gene) and a risk of overweight and obesity (RR = 2.806, 95 % CI: 1.650–4.772; STD = 0.271). The frequency of the A allele of the

rs9939609 FTO polymorphism that was associated with obesity equaled 40.71 % and this value is not higher than that identified for European populations (41.0 %). Prevailing frequency of the homozygous TT genotype (29.2 %) as compared with the AA genotype (10.62) is likely due to influence of assimilation processes on urbanized territories in the Baikal region.

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