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Polymorphic locus rs555621 of the *FSHB* gene is association with the obesity in women

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Introduction. Obesity is one of the most common chronic diseases in the world. Genetic factors are involved in the formation of obesity and one of the candidate genes for obesity are genes associated with the age of menarche. It is believed that women with early menarche have a higher risk of developing obesity and polymorphisms associated with early menarche may also be risky for the obesity development.

The aim of the study: To study the association of the menarche-correlated rs555621 *FSHB* gene with the obesity in women. **Materials and Methods.** The DNA samples of 171 women served as material for the study. Genotyping of the rs555621 *FSHB* gene was carried out. For each woman, the body mass index was calculated and the age of menarche was estimated. **Results.** Among women with obesity the frequencies of the A allele and AA genotype of the rs555621 were 1,32 times (OR=2.04 95% CI 1.19-3.51 p=0.009) and 2.01 times (OR=3.03 95% CI 1.41-6.53 p=0.004) respectively higher than in women without obesity. The G allele rs555621 was associated with the obesity in the additive (OR=0.51 95%CI 0.29-0.89, p_{perm}=0.02) and dominant (OR=0.38 95%CI 0.18-0.79, p_{perm}=0.009) models. It was found that in women with obesity, menarche occurs 0.7 years earlier than in women without obesity (p<0.001). The rs555621 *FSHB* was associated with the age of menarche: earlier menarche was observed in women with the AA genotype (12.57 years), and later menarche was detected in women with the GG genotype (13.00 years, p<0,001). **Conclusions.** Thus, it was found that the AA genotype of the rs555621 *FSHB* was associated with early menarche and was associated with an increased risk of obesity.

Keywords. obesity; rs555621; *FSHB;* association; age of menarche

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Introduction

One of the most important medical and social problems in the world at present is obesity [1]. Obesity is considered an abnormal or excessive accumulation of fat, which can negatively affect health [1]. According to the WHO, in 2016, more than 1.9 billion adults over the age of 18 were overweight, of which over 650 million were obese [2]. Among children and adolescents aged 5 to 19, over 340 million people are obese or overweight [2]. Over the past 40 years, the prevalence of obesity has increased 2-fold in more than 70 countries around the world [1]. Obesity is more common among women than among men (15% of women and 11% of men) [2]. Obesity and overweight are known major risk factors for cardiovascular (heart disease, hypertension, stroke), endocrine (diabetes), musculoskeletal (osteoarthritis), oncology (cancers of breast, endometrial, kidney, prostate, etc.), etc. diseases [2,3,4]. High body mass index (BMI) is the cause of death of 4 million people in the world annually, with more than 2/3 of them due to cardio-vascular disorder [1].

Genetic factors are involved in the formation of obesity [5] and one of the candidate genes for obesity are genes associated with the age of menarche [6,7]. It is believed that women with early menarche have a higher risk of developing obesity and polymorphisms associated with early menarche may also be risky for the obesity development [6,7]. A

number of genes are known to be associated simultaneously with both menarche age and obesity/BMI/height such as us *LIN28B,FTO,TNNI3K,MAP2K5,FANCL,GPRC5B*, etc. [6,7]. However, these data are not always unambiguous, often the revealed connections are multidirectional and contradictory (for example, loci associated with early menarche are associated with low height (rs7846385 *PXMP3*, rs4549631 *C6orf173*)[6], etc.) and further research on this issue is needed.

The aim of the study: to study the association of the menarche-correlated rs555621 *FSHB* gene with the obesity in women.

Materials and Methods

Study objects

The material for the study was DNA samples of 171 women aged 20-30 years, of Russian nationality, living in the Belgorod region, born in the Central Chernozem region of Russia [8] for the period 1985-1995, without severe somatic diseases, having a normal BMI (BMI=18.5-24.99, n=125) or obesity (BMI>30, n=46). For each woman, we analyzed the following medical and biological indicators: year of birth, age, height, weight, age of menarche. The age of menarche was considered to be the age (full years) of the first menstrual spotting from the date of birth. BMI calculation was made for each woman: BMI = Body weight (kg) / Height $(m)^2$. The biomedical characteristics of the formed sample of women are presented in Table 1. In this sample of women, associations of the rs555621 *FSHB* with the age of menarche were studied. The biomedical characteristics of women, depending on the presence/absence of obesity, are presented in Table 2. The association of the rs555621 *FSHB* with obesity was studied on these samples of women.

Genetic methods

The polymorphic locus rs555621 of the *FSHB* gene was genotyped in the studied sample of women. The choice of this polymorphic locus for the study is associated with its significant associations with the age of menarche according to previous studies [9], significant regulatory potential [10] (according to HaploReg [11] and GTExportal [12] databases), prevalence in European populations over 5% (according to HaploReg [11]). Genotyping of the rs555621 *FSHB* was carried out on the CFX-96 Real-Time System (Bio-Rad).

Table 1. Characteristics of the studied group of women.

Parameters	$\overline{X}$ ±SD/%(n) (min-max)
n	171
Age, years	25.59±2.53 (20-30)
Height, m	1.64±0.06 (1.50-1.78)
Weight, kg	90.56±11.33 (75-130)
BMI	25.55±5.41 (18.59-44.98)
Proportion of the participants by BMI, % (n): BMI=18.5-24.99 BMI>30	73.10% (125) 26.90% (46)
Age of menarche, years	12.82±1.04 (10-15)
Proportion of the participants by relative age of menarche, % (n): early (<12 лет)	7.01% (12) 89.48% (153) 3.51% (6)

average (12-14 лет) late (>14 лет)	

Table 2

Characteristics of obese and non-obese women

Parameters	Obesity $\overline{X}$ ±SD/%(n) (min-max)	Control $\overline{X}$ ±SD/%(n) (min-max)	p
n	46	125	-
Age, years	25.82±2.19 (21-30)	25.50±2.65 (20-30)	0.51
Height, m	1.64±0.07 (1.50-1.78)	1.64±0.05 (1.50-1.76)	0.88
Weight, kg	90.56±11.33 (75-130)	60.85±5.82 (45-71)	**<0.001**
BMI	33.52±3.53 (30.04-44.98)	22.62±1.84 (18.59-24.97)	**<0.001**
Age of menarche, years	12.30±0.99 (10-15)	13.00±1.00 (10-15)	**<0.001**
Proportion of the participants by relative age of menarche, % (n) early (<12 лет) average (12-14 лет) late(>14 лет)	 13.24% (6) 84.78% (39) 2.17% (1)	 4.80% (6) 91.20% (114) 4.00% (5)	**<0.001**

Statistical methods

To assess the compliance of the empirical distribution of genotypes with the theoretically expected one at the Hardy-Weinberg equilibrium, the criterion χ^2 was used. The associations of the polymorphic locus rs555621 *FSHB* gene with the age of menarche (log-linear regression analysis was used) and obesity (logistic regression analysis was used) in the gPLINK program were studied [13]. Additive, recessive and dominant genetic models were tested with correction for covariates (age, BMI in the analysis of menarche age and age, menarche age in the analysis of BMI) and multiple comparisons (permutation testing was used at a statistically significant level of $p_{perm}<0.05$).

Results and Discussion

Menarche age and obesity

It was found that the average age of menarche in 171 Russian residents of the Central Black Earth of Russia was 12.82± 1.04 years (varied from 10 to 15 years) (Table 1). Menarche at the age of up to 12 years (early menarche) was observed in 7.01% of women, at the age of 12-14 years - in 89.48% and at the age after 14 years (later menarche) – in 3.51% of women. It was revealed that the age of menarche is associated with the development of obesity in women. Women with obesity are characterized by an earlier (by 0.7 years, $p<0.001$) onset of menarche (Table 2). Using logistic regression analysis, it was found that early menarche is a risk factor for obesity (OR= 0.49, 95% CI 0.34-0.71, $p<0.001$).

Associations of rs555621 *FSHB* gene with menarche age

The association of rs555621 *FSHB* with menarche age in Russian women of the Central Chernozem region of the Russian Federation has been established - the G allele of the rs555621 *FSHB* is associated with the menarche in the additive (β = 0.233±0.116, p=0.05, p_{perm}=0.05) and dominant (β = 0.357±0.170, p=0.04, p_{perm}=0.05) of allele interaction models (Table 3). In women who have one allele of the G polymorphic locus rs555621 *FSHB* (genotype A/G) in the genotype, menarche occurs 0.34 years later compared to women whose genotype does not have this allele (genotype A/A), and in women who have two alleles of the G rs555621 *FSHB* in the genotype (genotype G/G), menarche occurs 0.43 years later in comparison with women whose genotype does not have this allele (genotype A/A).

Associations of rs555621 *FSHB* with the obesity in women

It was found that among obese women, the frequency of the A/A rs555621 genotype is 2.01 times higher than in non-obese women (p=0.004) (Table 4). Also, the frequency of the rs555621 A allele is 1.32 times higher among obese women compared to non-obese women (p=0.009). These genetic variants are risk factors for obesity (OR=3.03 95%CI 1.41-6.53 for genotype A/A and OR=2.04 95%CI 1.19-3.51 for allele A). The association of the allele G rs555621 with the obesity was revealed in the framework of additive (OR=0.51 95% CI 0.29-0.89, p=0.02, p_{perm}=0.02) and dominant (OR=0.38 95% CI 0.18-0.79, p=0.009, p_{perm}=0.009) allele interaction models (Table 4).

Table 3. The age of menarche in women depending on the genotypes of the polymorphic locus rs555621 of the *FSHB* gene.

Genotypes (genetic model)	n	%	Age of menarche $\overline{X}$ ±SD, years	p
A/A	54	31.58	12.57±1.00	**<0.001**
A/G	88	51.46	12.91±1.07	
G/G	29	16.96	13.00±1.00	
A/A vs. A/G vs. G/G (additive model)	**β = 0.233±0.116, p=0.05**			
A/A vs. A/G + G/G (dominant model)	**β = 0.357±0.170, p=0.04**			
A/A + A/G vs. G/G (recessive model)	β = 0.218±0.213, p=0.30			

Note:β±SE – linear regression coefficient characterizing the change in the age of menarche to the minor allele G and its error, p – significance level.

Table 4

Frequencies of alleles and genotypes of the polymorphic locus rs555621 of the *FSHB* gene in obese women and control group

Alleles, genotypes (genetic model)	Obesity (n=46) abc.(%)	Control (n=125) abc.(%)	OR (95%CI)	p
A	64 (69.56%)	132 (52.80%)	2.04 (1.19-3.51)	**0.009**
G	28 (30.44%)	118 (47.20%)	0.49 (0.28-0.84)	
A/A	23 (50.00%)	31 (24.80%)	3.03 (1.41-6.53)	**0.004**
A/G	18 (39.13%)	70 (56.00%)	0.51 (0.24-1.06)	0.07
G/G	5 (10.87%)	24 (19.20%)	0.51 (0.16-1.55)	0.29
A/A vs. A/G vs. G/G			0.51 (0.29-0.89)	**0.02**

(additive model)				
A/A vs. A/G + G/G (dominant model)			0.38 (0.18-0.79)	**0.009**
A/A + A/G vs. G/G (recessive model)			0.57 (0.20-1.66)	0.30

Note: OR – odds ratio, 95%CI – its 95% confidence interval, p – significance level.

Functional significance of rs555621 *FSHB* in the organism

Using the HaploReg online database, a significant regulatory potential of the rs555621 *FSHB* has been established – it is located in the region of modified histone proteins (H3K27ac) marking "active" enhancers in H9 Cells cell culture, and in the region of modified histones (H3K9ac) marking "active" promoters in primary-peripheral blood mononuclear cells. Using data from the Genotype-Tissue Expression (GTEx) project, associations of the polymorphic locus rs555621 of the *FSHB* gene with the expression level of the *RPL12P30* gene in the thyroid gland and the *ARL14EP* gene in various parts of the brain (cortex, basal ganglia, pituitary gland), thyroid gland, ovaries, subcutaneous adipose tissue were revealed (Table 5). Along with this, the relationship of this polymorphic locus with the level of alternative splicing of the *ARL14EP* gene transcript in the pituitary gland, muscle tissue, thyroid gland, adrenal glands, visceral and subcutaneous adipose tissue is shown (Table 5). It should be noted that the allele G rs555621 *FSHB* ($\beta>0$, $p<0.05$) is associated with an increased level of expression of the *ARL14EP* and *RPL12P30* genes and alternative splicing of the *ARL14EP* gene transcript (Table 5).

Table 5. Association of the polymorphic locus rs555621 of the *FSHB* gene with the level of expression (eQTL) and alternative splicing (sQTL) of genes in various organs and tissues.

Gene	ref	alt	β	p	Organs
eQTL					
ARL14EP	A	G	0.20	4.5e-10	Thyroid
ARL14EP	A	G	0.19	0.0000024	Brain - Caudate (basal ganglia)
ARL14EP	A	G	0.32	0.0000024	Ovary
ARL14EP	A	G	0.19	0.0000025	Brain - Cortex
ARL14EP	A	G	0.29	0.0000053	Pituitary
ARL14EP	A	G	0.15	0.00001	Adipose - Subcutaneous
RPL12P30	A	G	0.16	0.00035	Thyroid
sQTL					
ARL14EP	A	G	0.35	2.4e-14	Muscle - Skeletal
ARL14EP	A	G	0.38	5.7e-12	Thyroid
ARL14EP	A	G	0.33	1.6e-10	Adipose - Subcutaneous
ARL14EP	A	G	0.34	8.9e-9	Adrenal Gland
ARL14EP	A	G	0.33	2.6e-8	Adipose - Visceral (Omentum)
ARL14EP	A	G	0.38	0.0000093	Pituitary
ARL14EP	A	G	0.35	2.4e-14	Muscle - Skeletal

In our study, it was found that rs555621 *FSHB* polymorphism is associated with the age of menarche and obesity in women in Russian residents of the Central Chernozem region: the allele G rs555621 *FSHB* is associated with late menarche ($\beta = 0.233$- 0.357) and has a protective value in the development of obesity (OR=0.38-0.51), whereas the AA genotype rs555621 is associated with early menarche and is a risk factor for obesity (OR=3.03).The rs555621 *FSHB* is associated with an increased level of expression of the *ARL14EP* and *RPL12P30* genes and a high level of alternative splicing of the *ARL14EP*

gene transcript in various organs important for the formation of menarche and the development of obesity (brain, thyroid gland, adipose tissue, etc.).

The data obtained by us on the relationship of rs555621 *FSHB* with the age of menarche (the G allele is associated with late menarche) are completely consistent with the results of the previously conducted study by He C. et al. [9], in which the G rs555621 allele of the *FSHB* gene was also associated with late menarche. It should be noted that according to the literature, polymorphic loci located in the region of the *FSHB* gene transcription start site (the rs555621 studied by us is located in this region at a distance of 16kb from the 5' end of the *FSHB* gene) play a key role in the development and functioning of the reproductive system in the body [14]. Thus, according to the data of full genomic studies, associations of polymorphic loci located in the region of the 5' end of the *FSHB* gene with the content of follicle-stimulating (rs11031005) and luteinizing (rs11031002) hormones in blood plasma [15] and menopausal age (rs12294104) have been shown [16]. In the population studied by us (Central Chernozem region of Russia), in previous studies, the relationship of polymorphic loci of this region of the genome (near of *FSHB* gene, rs555621/rs1782507/rs11031010/rs11031002/rs11031005) with such BMI-correlated diseases of women as uterine leiomyoma [4], endometriosis [17,18], endometrial hyperplasia [19].

According to Ensembl database (http://www.ensembl.org /) the product of the *FSHB* gene is beta-subunits of follicle-stimulating hormone. Follicle-stimulating hormone stimulates the proliferation of cells of the granulosa layer of follicles and the growth of follicles in the ovaries, induces the synthesis of aromatases converting androgens into estrogens (estradiol), stimulates the synthesis of receptors for luteinizing hormone on granulosa cells of the follicle before ovulation, etc. The *ARL14EP* gene (*ADP ribosylation factor like GTPase 14 effector protein*) encodes a protein with GTPase and advibosylating activity, which interacts with proteins of the cell's actin network (*beta*-actin (ACTB), myosin 1E (MYO1E)) controls the export of molecules of the main histocompatibility complex of class II (http://www.genecards.org /).

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Data Availability Statement: The data generated in the present study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

Conclusions.

It was found that the AA genotype of the rs555621 *FSHB* was associated with early menarche and was associated with an increased risk of obesity.

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