



## Genetic Predictors of Liver Diseases in Residents of the Far North of Russia

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### KEYWORDS

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### ABSTRACT:

We studied the possible association between genetic variants of the enhancer of the *FADS1* and *FADS2* genes with a predisposition to obesity using a sample of 450 DNA samples from Yakut volunteers without chronic diseases living in Yakutia. Three groups were formed from them: with normal BMI (n=162), with obesity (n=184) and with pre-obesity (n=104). Analysis of the distribution of alleles of the rs174537 polymorphism of the *FADS1* and *FADS2* gene enhancer showed a higher frequency of the T allele - from 73.1% to 79.1% in all studied groups. As a result of a comparative analysis of the average body mass index depending on the genotype, it is noted that in the group of obese people in carriers of the TT genotype (BMI = 33.6 ± 0.09) it was significantly higher (td = 3.93, p = 0.0001) than in carriers of the TG genotype (BMI=33.1±0.09). We believe that, in combination with high frequencies of unfavorable alleles of the *PNPLA3* and *FABP2* genes in the Yakut population, there may be a high risk of diseases associated with lipid metabolism in the liver.

### 1. Introduction

Yakutia is one of the largest and most contrasting regions in the world in terms of temperature; the annual amplitude of temperature indicators is about 102.8 C. The winter period is characterized by temperature fluctuations from -30° to -70 °C and lasts about 7 months a year. The study of lipid metabolism disorders in the population of Yakutia is most relevant, since due to the loss of traditional farming methods and the transition of the indigenous population from the usual protein-lipid type of diet to a carbohydrate-lipid diet, the indigenous inhabitants of the North have seen an increase in the frequency of manifestations of atherosclerosis and diseases caused by metabolic disorders. [1]. Yakutia is one of the disadvantaged regions for liver diseases. The indigenous population of Yakutia is more susceptible to chronic diffuse liver diseases than the newcomer population [2]. Also, according to state medical statistics of the Russian Federation for 2021, the highest incidence of malignant neoplasms of the liver and intrahepatic bile ducts was in the Republic of Sakha (Yakutia) and amounted to 14.16 per 100 thousand population, when in Russia it was 3.4 per 100 thousand population [3].

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases in developed and developing countries, with a prevalence ranging from 2.8% to 24% in different populations. It accounts for 60% of all chronic liver diseases [4]. Also, up to 15% of all cases of liver cancer are detected in patients with NAFLD [5]. The prevalence of NAFLD increases with increasing body mass index (BMI), so according to Fabbrini E et al (2010), the prevalence of steatosis and steatohepatitis is approximately 15% and 3%, respectively, in non-obese individuals, 65% and 20%, respectively, in individuals with obesity degrees I and II (BMI 30.0–39.9 kg/m<sup>2</sup>) and 85% and 40%, respectively, in patients with extreme obesity (BMI ≥40 kg/m<sup>2</sup>) [6]. Also, the most important factor in the development of NAFLD is dysfunction of visceral adipose tissue [7].

The change from a traditional diet to a modern Western one, in which vegetable oil products predominate (soybean, corn, palm and rapeseed oils, as well as margarine and shortening), leads to a noticeable increase in the content of omega-6 polyunsaturated fatty acids (PUFAs) in the diet, which has a significant impact on human health and is associated with clinical outcomes in various metabolic disorders. [8]. Dietary



PUFAs are metabolized primarily in the liver through a series of fatty acid desaturase steps and extension steps by fatty acid desaturase enzymes (FADS) [9]. The fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*) genes play a critical role in PUFA metabolism. The rs174537 polymorphism, located in the enhancer of the *FADS1* and *FADS2* genes, affects the level of arachidonic acid and is closely related to the enzymatic activity of *FADS1* [10]. The allele frequencies of the rs174537 polymorphism vary greatly between populations [11], which is likely due to differences in diet types.

Individuals homozygous for the ancestral allele rs174537 (TT) demonstrate the slowest conversion of dietary PUFAs, almost half the rate compared to homozygous carriers of the mutant alleles (GG). In addition, carriers of the TT genotype are more often deficient in long-chain PUFAs, in contrast to carriers of the GG genotype, who have significantly higher levels of arachidonic acid in the blood and tissues [12]. In studies by Nobili V. et al. (2018) found that the ancestral T allele is associated with increased fat deposition in the liver. In addition, fat accumulation in hepatocytes is closely associated with various metabolic disorders, including non-alcoholic fatty liver disease, insulin resistance, hyperlipidemia, obesity and cardiovascular diseases [13].

The purpose of this study was to investigate the possible association between genetic variants of the enhancer of the *FADS1* and *FADS2* genes with susceptibility to obesity.

## 2. Materials and Methods

The experimental part of the work on genotyping the rs174537 polymorphism of the *FADS1*, *FADS2* gene enhancer was carried out in the laboratory of hereditary pathology of the department of molecular genetics of the Yakut Scientific Center for Complex Medical Problems (YSC KMP). For the study, DNA samples were used from the biomaterial collection of the YSC KMP using the UNU "Genome of Yakutia" (reg. No. USU\_507512). The study was conducted with the written consent of the participants. The study protocol

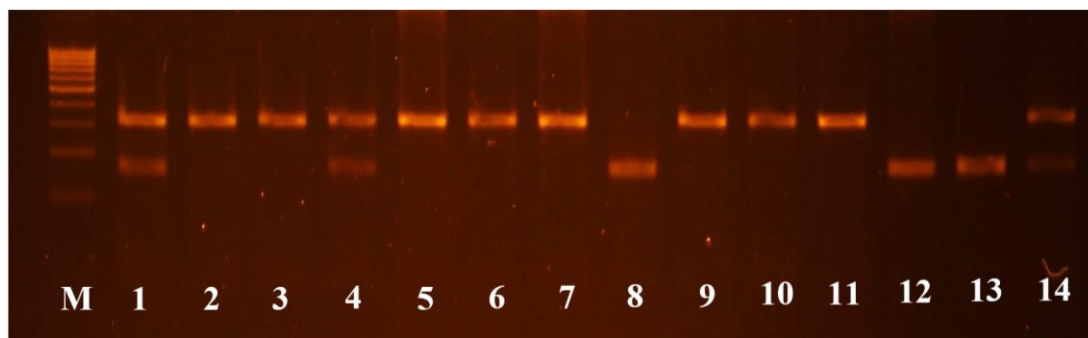
was approved by the local committee on biomedical ethics at the Yaroslavl Scientific Center of Clinical Medicine.

The sample consisted of 450 DNA samples from volunteers without chronic diseases (144 women and 306 men), whose average age was  $49.3 \pm 0.05$  years. All study participants were Yakuts by ethnicity and lived in the territory of the Republic of Sakha (Yakutia). Three groups of subjects were formed: a group with normal BMI ( $n=162$ ), an obese group ( $n=184$ ) and a group with pre-obesity ( $n=104$ ).

A questionnaire was completed for each participant during a personal examination. Height and weight were measured with participants without shoes or outerwear. BMI was calculated by dividing weight (kg) by the square of height ( $m^2$ ).

To carry out molecular genetic analysis, genomic DNA samples were isolated from whole blood using a commercial DNA isolation kit Newteryx (Yakutsk, Russia). Amplification of the gene region containing the rs174537 polymorphic variant was carried out with standard pairs of primers (Forward primer: 5'-CAGGGAGAGAGAGTAAGTA-3' and reverse primer: 5'-AGGTCTGGGAGTATCTCC-3')

The reaction mixture for PCR contained: forward and reverse primers, 10 picomol/ $\mu$ l (1  $\mu$ l); BioMaster HS-Taq PCR-Special (2x) - 12.5  $\mu$ l; deionized water 9.5  $\mu$ l and DNA (100  $\mu$ g/ml) - 1  $\mu$ l. The total volume of the reaction mixture for amplification was 25  $\mu$ l. PCR temperature conditions were as follows: 94 °C for 4 min, followed by 35 cycles at 94 °C for 1 min, 68 °C for 1 min, and 72 °C for 1 min, and a final extension at 72 °C for 5 min. The RFLP mixture with a volume of 20  $\mu$ l consisted of: amplifier - 7  $\mu$ l, deionized water - 10.9  $\mu$ l, restriction buffer - 2  $\mu$ l and restriction endonuclease *AvaI* (2 u.a.). Interpretation of genotyping results was performed based on different band patterns of the region with the rs174537 polymorphism (Figure 1).



**Figure 1.** Electropherogram of the rs174537 polymorphism of the *FADS1*, *FADS2* gene enhancer in a 4% agarose gel after RFLP. M - Step 100 marker; 1, 4 and 14 – GT genotype; 2, 3, 5, 6, 7, 9, 10, 11 – TT genotype; 8, 12 and 13 – genotype GG.

Statistical processing of the obtained data was carried out using the program: “Office Microsoft Excel 2010”. When analyzing the association between the frequency of an unfavorable allele and obesity, a four-field contingency table and the  $\chi$ -square test with Yates' correction were used. To assess the significance of the odds ratio, the boundaries of the 95 % confidence interval (CI 95 %) were calculated.

Mean BMI values by genotype were compared using Student's t test. Results were considered significant at  $p < 0.05$ .

### 3. Results and Discussions

According to the results of the study, the distribution of frequencies of alleles and genotypes of the rs174537 polymorphism in all groups was in Hardy-Weinberg equilibrium (Table 1). In all studied groups, a predominance of the TT genotype was revealed, which has a low frequency (~13%) in populations of European origin and is extremely rare in Africans (<1%).

**Table 1.** Frequency distribution of alleles and genotypes of the rs174537 polymorphism of the *FADS1* and *FADS2* gene enhancer with odds ratio (OR).

Groups	Genotype freq, %			Allele freq, %		OR (CI 95%)	p
	GG	TG	TT	G	T		
BMI is normal	4.3	35.2	60.5	21.9	78.1	0.762 (0.51-1.14)	0.22
Pre-obesity	8.7	36.5	54.8	26.9	73.1		
BMI is normal	4.3	35.2	60.5	21.9	78.1	0.830 (0.58-1.18)	0.34
Obesity	7.1	36.4	56.5	25.3	74.7		
Pre-obesity	8.7	36.5	54.8	26.9	73.1	1.089 (0.74-1.60)	0.74
Obesity	7.1	36.4	56.5	25.3	74.7		

Note: BMI – body mass index value, p – novelty, OR – odds ratio

Analysis of the distribution of alleles of the rs174537 polymorphism of the *FADS1* and *FADS2* gene enhancers showed higher chains of the T allele - from 73.1% to 79.1% in all research groups. According to the 1000 Genomes Project, the frequency of the T allele is high, found in Vietnamese (82%), Peruvians (81%), Dai Chinese (78%) and Mexicans (70%). In other populations, the T allele is considered minor and occurs

in 2% in African populations, 16% in South Asian populations, and 35% in European populations [14].

Analysis of the odds ratio of the frequency of genotypes and alleles in patients with normal BMI and abdominal obesity did not reveal statistically significant values.

As a result of a comparative analysis, the average body mass index depending on the genotype showed that in



the group of obese people in carriers of the TT genotype (BMI =  $33.6 \pm 0.09$ ) it was significantly higher ( $t_d = 3.93$ ,  $p = 0.0001$ ) than in carriers of the TG genotype

(BMI =  $33.1 \pm 0.09$ ). In the remaining groups, the differences were not statistically significant. (Table 2).

**Table 2** Average body mass index values depending on the genotype of the rs174537 polymorphism of the *FADS1* and *FADS2* gene enhancer

Выборка	Количество	Генотип	ИМТ (кг/м <sup>2</sup> )	T (сравниваемые генотипы)	p
ИМТ в норме	7	GG	21.6±0.849	0.93 (GG / TG)	0.35
	57	TG	22.4±0.12	1.77 (TG / TT)	0.08
	98	TT	22.7±0.12	1.28 (GG / TT)	0.20
Ожирение	13	GG	32.4±0.7	0.99 (GG / TG)	0.32
	67	TG	33.1±0.09	3.93 (TG / TT)	0.0001
	104	TT	33.6±0.09	1.70 (GG / TT)	0.09
Предожирение	9	GG	27.6±0.43	1.25 (GG / TG)	0.22
	38	TG	27±0.21	0.36 (TG / TT)	0.72
	57	TT	27.1±0.19	1.06 (GG / TT)	0.29

Note: BMI – body mass index values, T – Student’s test, p – significance.

Increased intake of saturated fat, omega-6 fatty acids and trans fatty acids and decreased intake of omega-3 fatty acids leads to various metabolic diseases including obesity. In addition, trans fatty acids interfere with the desaturation and elongation of omega-6 and omega-3 fatty acids, thereby further reducing the amount of arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid available for human metabolism. [15].

Early studies in the Yakut population show a high prevalence of variants of the *PNPLA3* and *FABP2* genes, which are also associated with increased BMI and non-alcoholic fatty liver disease [16, 17]. In studies on three-dimensional liver constructs, Kirk LM et al. (2022) found that hepatocytes with the homozygous TT genotype have the slowest metabolic conversion of linolenic acid to arachidonic acid. They also found that among hepatocytes grown in a medium supplemented with linolenic acid, cells with the TT genotype had the lowest expression of the *FADS1* and *FADS2* genes, while heterozygous cells (GT) had the highest level.

#### 4. Conclusion

As a result of a study of the rs174537 polymorphism of the *FADS1*, *FADS2* gene enhancer, a high frequency of the T allele was established in the Yakuts. We believe that in combination with the high frequencies of unfavorable alleles of the *PNPLA3* and *FABP2* genes in the Yakut population, a high risk of diseases associated with lipid metabolism in the liver is possible.

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