Frequency of I148M Polymorphism of the PNPLA3 Gene Associated with a Risk of Steatosis and Liver Fibrosis in Residents of the Republic of Sakha (Yakutia)

Snezhana S. Sleptsova 1¶, Aleksandr E. Efimov 1¶*, Nikolai M. Gogolev 2, Palmira G. Petrova 3, Sergey I. Semenov 5, Polina I. Golikova 6, Anastasiya L. Danilova 6, Filipp F. Vasilev 6, Alexandra E. Yakovleva 6, Aitalina L. Sukhomyasova 6, Tatiana E. Burtseva 4, Jon O. Odland7,8

1 Department of Infectious Diseases, Phthisiology and Dermatovenerology of the North-Eastern Federal University, Institute of Medicine, Yakutsk, Russia

2 Department of Surgery of the North-Eastern Federal University, Institute of Medicine, Yakutsk, Russia

3 Department of Normal and Pathological Physiology of the North-Eastern Federal University, Institute of Medicine, Yakutsk, Russia

4 Department of Pediatrics and Pediatric Surgery of the North-Eastern Federal University, Institute of Medicine, Yakutsk, Russia

5 Research Center of the North-Eastern Federal University, Institute of Medicine, Yakutsk, Russia.

6 Research Laboratory "Molecular Medicine and Human Genetics" of the North-Eastern Federal University, Institute of Medicine, Yakutsk, Russia.

7 Department of Public Health and Nursing Norwegian Norwegian university of science and technology, Norway
Abstract

Background

Single nucleotide polymorphism (SNP) are the most common type of genetic polymorphism. SNP can significantly affect the expression activity of genes and the level of protein production. Researching the role of SNP in the occurrence of diseases is an important and urgent task, as it allows to predict the risk of pathology, its severity and outcome. Purpose of the study: study of the frequency of I148M polymorphism of the PNPLA3 gene in residents of the Republic of Sakha (Yakutia), associated with a high risk of steatosis and liver fibrosis.

Methods

A total of 3132 peripheral venous blood samples were used for population studies, studies patients with chronic hepatitis B and C, studies patients with NAFLD. Genotyping of DNA samples was carried out by real time-PCR. Reagent kits were used for genotyping I148M polymorphism of the PNPLA3 gene.

Results
In the present study, it was found that in the Yakut population the carriage of the GG genotype (49%) of the PNPLA3 gene I148M polymorphism predominates. When conducting a comparative frequency analysis, there were no statistically significant differences between the control group and the group with NAFLD patients ($p=0.82$). A comparative frequency analysis of the distribution of genotypes and alleles of I148M polymorphism of the PNPLA3 gene in the control group and the group of patients with chronic hepatitis B and C showed that we did not reveal significantly significant differences ($p = 0.45$).

**Conclusions**

The frequency of homozygotes for the mutant G allele of the I148M polymorphism of the PNPLA3 gene in the Yakut population significantly exceeds the frequency indicator of the G allele in other world populations.

**Keywords:** steatosis; fibrosis; gene; Yakutia

1. **Introduction**

Hereditary diseases and congenital malformations make a significant contribution to the morbidity, disability and mortality of people, being not only a medical, but also a social problem.

The successes of modern genetics are associated with the study of multifactorial diseases. The study of genetic polymorphism was crucial in
determining the causes of hereditary changes. Genetic polymorphism is a change in the genome of a population in two or more variants (alleles) with a frequency of at least 1%. Single nucleotide polymorphism (SNP) are the most common type of genetic polymorphism. SNP can significantly affect the expression activity of genes and the level of protein production (mRNA stability, transcription rate, changes in the structure of transcription factor binding sites). Researching the role of SNP in the occurrence of diseases is an important and urgent task, as it allows to predict the risk of pathology, its severity and outcome.

**Purpose of the study:** study of the frequency of I148M polymorphism of the *PNPLA3* gene in residents of the Republic of Sakha (Yakutia), associated with a high risk of steatosis and liver fibrosis. The study showed that the frequency of the mutant allele of functional polymorphism I148M of the *PNPLA3* gene in the Yakut population was higher than in other known world populations.

2. Materials and methods

2.1 Blood samples

Whole blood samples (N = 1561) were obtained during scientific research expedition of the Medical Institute of North-Eastern Federal University (NEFU, Yakutsk, Russia) to the districts of Republic of Sakha (Yakutia, Russia) in 2016. Republic of Sakha (Yakutia) is located in the northeastern part of Siberia.
Figure 1. Distribution of study samples. Whole blood samples were collected from four districts (ulus) of Republic of Sakha (Yakutia): Central Economic Zone (Tattinskiy and Aldanskiy ulus); Western economic zone: Verkhnevilyuyskiy ulus; Arctic economic zone (Verkhoyanskiy and Eveno-Bytantayskiy ulus).

Inclusion in the study control group (n=1425) required that all individuals belong to the Yakut, Even or Russian ethnic group, were healthy and unrelated. Patients with non-alcoholic fatty liver disease (NAFLD) (n = 97), chronic hepatitis B and chronic hepatitis C (n = 39) were included in the study. All of them are living in the above areas. Verification of NAFLD, chronic hepatitis B and chronic hepatitis C was carried out using clinical data, laboratory studies, ultrasound studies and serological blood tests (ELISA).

Population studies of the prevalence of NAFLD among the population of the republic have not been previously conducted. A total of 1006 people were examined from 4 regions of the republic - Verkhnevilyuysky (Western Economic Zone), Tattinsky (Central Economic Zone), Eveno-Bytantaysky and Verkhoyansk (Arctic Economic Zone) regions. The population aged 18 and over was examined.
Among the men examined, there were only 283 (28.1%) people, women - 723 (71.9%). The average age of the examined men was 46.7 ± 15.1 years, women - 47.8 ± 14.4 years. In order to identify various liver pathologies in 994 (98.8%) of the examined, an ultrasound examination (ultrasound) was performed. At the same time, the criteria for steatosis were diffuse hyperechoogenicity of the liver parenchyma, vague vascular pattern. Distribution of patients with signs of NAFLD and changes in the liver and pancreas parenchyma presented in table 1.

Table 1. Distribution of patients with signs of hepatosis / steatosis of the liver and changes in the liver and pancreas parenchyma (n = 629)

| Indicators                        | NAFLD (n=120) | Diffuse changes in the liver parenchyma without NAFLD (n=114) | Diffuse changes in the pancreatic parenchyma (n=395) |
|-----------------------------------|---------------|----------------------------------------------------------------|---------------------------------------------------|
|                                   | n %           | \( \chi^2 \) | p | n % | \( \chi^2 \) | p | n % | \( \chi^2 \) | p |
| men                               | 30 10,6       | 0,8           | 0,37 | 33 11,7 | 0,04 | 0,8 | 112 39,6 | 0,06 | 0,8 |
| 18-59 years, men                  | 26 11,4       |               |     | 27 11,8 |       |     | 91 39,9 |       |     |
| 60 years and older, men           | 4 7,4         |               |     | 6 10,9 |       |     | 21 38,2 |       |     |
| women                             | 90 12,4       | 0,1           | 0,7 | 87 12,0 | 0,3 | 0,6 | 283 39,1 | 0,03 | 0,9 |
| 18-59 лет, women                  | 68 12,2       |               |     | 65 11,7 |       |     | 217 39,0 |       |     |
| 60 years and older, women         | 22 13,3       |               |     | 22 13,3 |       |     | 66 39,8 |       |     |
| Average age men                   | 45,4±14,8     |               |     | 44,4±16,0 |       |     | 46,7±14,6 |       |     |
| Average age women                 | 47,8±14,4     |               |     | 49,2±14,1 |       |     | 47,9±14,6 |       |     |
Note: Group 1 – patients with signs of NAFLD (n = 120); Group 2 – patients with signs of diffuse changes in the liver parenchyma without NAFLD (n = 120); Group 3 – patients with signs of diffuse changes in the pancreatic parenchyma (n = 395).

All individuals provided informed consent before the study was performed. Blood samples were collected in sterile VACUETTE ethylenediaminetetraacetic acid tubes (Greiner Bio-One, Austria).

2.2 Genotyping methods

Genomic DNA was isolated from peripheral blood by phenol-chloroform extraction, ExtractDNA commercial kit (Evrogen, Russia) and kits for isolating genomic DNA from blood using magnetic particles “NucleoMag 96 Blood” (Macherey-Nagel, Germany). The purified DNA was eluted in Milli-Q™ water. The concentration and purity of extracted DNA was determined by measuring the optical density using Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA).

Allele frequencies of single nucleotide polymorphisms were obtained from the SNP database at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/snp). Specific SNP (PNPLA3 p.I148M, rs738409) was chosen for study due to association with pathological states.

Genotyping of p.I148M polymorphism of the PNPLA3 gene was performed by real time-PCR. Real-time PCR was performed using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) and iCycler iQ5 Real-Time PCR Detection System (Bio-Rad, USA). All reagents (except Taq polymerase) were thawed at room temperature before use. After thawing, the contents of the tubes are thoroughly mixed. The 20 uL reaction volume contained 1–2 units Taq DNA polymerase, PCR mixture, Milli-Q™ water and 100 ng of genomic DNA. All components (except DNA samples) were mixed in a separate sterile tube based on the calculation: PCR mixture volume = (component volume for 1 sample) * (number of samples + 2 (negative control). The reaction mixture was mixed by
pipetting followed by vortexing. PCR thermal cycling conditions were as follows: 95°C - 2 min, 94°C - 10 sec, 58°C - 20 sec, 40 cycles.

2.3 Statistics

Statistical analysis was performed using the software STATISTICA 7.0 (StatSoft Inc.). Compliance of genotype frequency distributions with the Hardy-Weinberg equilibrium was established by online calculator provided by Tufts University, Boston, MA, USA (http://www.tufts.edu/mcourt01/Documents/Court%200llab%20%20HW%20calculator.xls). Hardy-Weinberg equation: \( p^2 + 2pq + q^2 = 1 \) (\( p \) - frequency of the dominant allele, \( q \) - frequency of the recessive allele. The significance of differences in the frequencies of genotypes was evaluated using the \( \chi^2 \) criteria (df=1) with Yates correction and Fisher's exact test. All differences were considered significant at \( P < 0.05 \).

3. Results and discussion

Several authors have identified the association of NAFLD with the \( PNPLA3 \) gene and confirmed in different ethnic groups as the cause of the disease [1, 2, 3]. So, in a study by Romeo et al. (2008) [5] identified the association of the \( PNPLA3 \) gene with the development of NAFLD among Americans.

The most significant polymorphism in the \( PNPLA3 \) gene is I148M (rs738409). The I148M polymorphism is the replacement of the cytosine nucleotide with guanine, which leads to a change in the amino acid isoleucine to methionine at position 148. This replacement leads to a disruption of the mechanism of lipid metabolism in the liver. This polymorphism is associated with susceptibility to NAFLD and affects the histological picture and the development of fibrosis in
children and adolescents with obesity [3]. The frequency of the studied polymorphism in various world populations is presented in Table 2.

Table 2. Frequencies of genotypes and alleles of the polymorphic variant I148M of the *PNPLA3* gene (rs738409) in various populations from the database of the US NCBI (National Center for Biotechnological Information).

| Population                  | Genotype frequency, % | Allele frequency, % |
|-----------------------------|-----------------------|---------------------|
|                             | GG        | GC     | CC     | G      | C      |
| Caucasoid population        |           |        |        |        |        |
| AFD_EUR_PANEL ss24098326    | 0         | 40,9   | 59,1   | 20,5   | 79,5   |
| (n=44)                      |           |        |        |        |        |
| Caucasoid population HapMap-CEU ss76896972 (n=120) | 3,3      | 40     | 56,7   | 23,3   | 76,7   |
| Asian population            |           |        |        |        |        |
| AFD_CHN_PANEL ss24098326    | 0         | 47,8   | 52,2   | 24     | 76     |
| (n=46)                      |           |        |        |        |        |
| Asian population HapMap-HCB ss76896972 (n=90) | 13,3     | 42,2   | 44,5   | 34,4   | 65,6   |
| African population          |           |        |        |        |        |
|                             | 4,3       | 30,4   | 65,3   | 19,6   | 80,4   |
In this study, we genotyping of the polymorphic variant I148M of the \textit{PNPLA3} gene (rs738409). The frequencies of alleles and genotypes of the studied polymorphic variant of the \textit{PNPLA3} gene in residents of the Republic of Sakha (Yakutia) are show in Table 3.

Table 3. Frequencies of genotypes and alleles of the polymorphic variant I148M of the \textit{PNPLA3} gene in residents of the Republic of Sakha (Yakutia), $n = 1464$

| SNP       | Genotype | Genotype frequency | Genotype frequency |
|-----------|----------|--------------------|--------------------|
|           |          | observed           | expected           |                     |
|           |          | %                  | n                  | n                  |
| \textit{PNPLA3} | GG       | 9,8                | 18                 | 15,9               |
| I148M     | GC       | 39,3               | 72                 | 76,1               |
| (G/C), n=175, | CC       | 50,9               | 93                 | 90,9               |
| russian (newcomers) | G       | 30,0               | –                  | –                  |
|           | C        | 70,0               | –                  | –                  |
| \textit{PNPLA3} | GG       | 52,0               | 338                | 346,4              |
| I148M     | GC       | 42,0               | 273                | 256,2              |
| (G/C),    | CC       | 6,0                | 39                 | 47,4               |
|                | G   | 73,0 | –   | –   |
|----------------|-----|------|-----|-----|
| Yakuts         | G   | 26,0 | –   | –   |
| (Verkhnevilyuysky region) | C   |      |     |     |
| **PNPLA3** I148M (G/C), n=22, evens (Eveno-Bytantaysky region) | GG  | 45,5 | 10  | 10,9|
|                | GC  | 50,0 | 11  | 9,2 |
|                | CC  | 4,5  | 1   | 1,9 |
|                | G   | 70,0 | –   | –   |
|                | C   | 30,0 | –   | –   |
| **PNPLA3** I148M (G/C), n=82, yakuts (Eveno-Bytantaysky region) | GG  | 53,7 | 44  | 44,6|
|                | GC  | 40,2 | 33  | 31,7|
|                | CC  | 6,1  | 5   | 5,6 |
|                | G   | 74,0 | –   | –   |
|                | C   | 26,0 | –   | –   |
| **PNPLA3** I148M | GG  | 51,4 | 76  | 75,2|
|                | GC  | 39,9 | 59  | 60,6|
|                |      |      |     |     |
### Notes:

1. G - guanine (guanine), C - cytosine (cytosine);
2. $\chi^2$ - “chi” square

Hardy-Weinberg equilibrium

Determination of the frequency of alleles and genotypes of the polymorphic variant I148M of the *PNPLA3* gene in the studied groups showed

| (G/C), n=148, yakuts (Verkhoyans k region) | CC | 8,8 | 13 | 12,2 |
|--------------------------------------------|----|-----|----|------|
| G                                          | 71 | –   | –  | –    |
| C                                          | 29 | –   | –  | –    |
| **PNPLA3 I148M**                           | GG | 58,8| 10 | 10,7 |
| (G/C), n=17, evens (Verkhoyans k region)   | GC | 41,2| 7  | 5,6  |
| CC                                         | 0  | 0   | 0,7|
| G                                          | 79,0| –  | –  | –    |
| C                                          | 21,0| –  | –  | –    |
| **PNPLA3 I148M**                           | GG | 41,6| 154| 148,6|
| (G/C), n=370, yakuts (Tatta region)         | GC | 43,5| 161| 171,8|
| CC                                         | 14,9| 55 | 49,6|
| G                                          | 63,0| –  | –  | –    |
| C                                          | 37,0| –  | –  | –    |
that this indicator corresponded to Hardy-Weinberg equilibrium, which indicates the representativeness of the sample.

A comparative analysis of the frequencies of the I148M genotypes of the PNPLA3 gene showed no statistically significant differences between groups with individuals of Yakut nationality in the Verkhnevilyuysky, Eveno-Bytantaysky, Verkhoyansk and Tatta regions (Table 4).

Table 4. Frequencies of genotypes and alleles of the polymorphic variant I148M of the PNPLA3 gene in the Yakut population, N = 1250

| Genotype | Verkhnevilyuysky region (n=650) | Eveno-Bytantaysky region (n=82) | Verkhoyansk region (n=148) | Tatta region (n=370) |
|----------|---------------------------------|---------------------------------|----------------------------|---------------------|
| GG       | 52,0 % 338                      | 53,7 % 44                       | 51,4 % 76                  | 41,6 % 154         |
| GC       | 42,0 % 273                      | 40,2 % 33                       | 39,9 % 59                  | 43,5 % 161        |
| CC       | 6,0 % 39                        | 6,1 % 5                        | 8,8 % 13                   | 14,9 % 55         |

Notes: G - guanine (guanine), C - cytosine (cytosine)

An analysis of the frequency distribution of alleles and genotypes of the I148M polymorphic point of the PNPLA3 gene (rs738409) established similar frequencies in the studied groups of individuals of Yakut and Even nationality.
\( (\chi^2 = 2.07, p = 0.35) \). Statistically significant differences were found between the group with individuals of Russian nationality and the group with individuals of Yakut nationality (\( p < 0.0001 \)) (Table 5).

Table 5. Distribution of alleles and genotypes of I148M polymorphism of the *PNPLA3* gene (rs738409) among residents of the Republic of Sakha (Yakutia), \( n = 1425 \)

| SNP       | Genotype frequency, % | Genotype frequency, % | \( \chi^2 \) | \( p \) | OR | 95% CI |
|-----------|-----------------------|-----------------------|---------------|--------|----|--------|
|           | russian (n=175)       | yakuts (n=1250)       |               |        |    |        |
|           | \( n \) | %   | \( n \) | %   |        |        |
| *PNPLA3*  | GG        | 18  | 9,8  | 612 | 49  | 251,39 | <0,0001 |
|           | GC        | 72  | 39,3 | 526 | 42  | 1,12  | 0,82 – 1,54 |
| I148M (G/C) | CC        | 93  | 50,9 | 112 | 9   | 0,10  | 0,07 – 0,13 |

Notes: ¹ G - guanine (guanine), C - cytosine (cytosine); ² \( \chi^2 \) - “chi” square Hardy-Weinberg equilibrium; ³ OR (odds ratio) - odds ratio; ⁴ CI (confidence interval) - confidence interval.

It is known that the frequency of allelic variants in a population may vary depending on ethnicity. In the present study, it was found that in the Yakut
population the carriage of the GG genotype (49%) of the \textit{PNPLA3} gene I148M polymorphism predominates.

We analyzed the frequency distribution of genotypes and alleles of the studied polymorphic variant of the \textit{PNPLA3} gene according to the available scientific literature. It was found that the frequency of homozygotes for the mutant G allele of the I148M polymorphism of the \textit{PNPLA3} gene in the Yakut population significantly exceeds the rate of the G allele in other world populations. More similar indicators of frequency to the Yakut population according to the GG genotype were established in a study by Romeo et al. (2008) \[4\]. The rate of GG carriage in the Hispanic population was 49.8%. The frequency distribution of the alleles and genotypes of the I148M \textit{PNPLA3} polymorphism in the studied Russian population corresponded to the values found in the world European ethnic populations (Table 6).

Table 6. Distribution of alleles and genotypes of I148M polymorphism of the \textit{PNPLA3} gene (rs738409) in various ethnic populations

| Population    | N  | Genotype frequency, % | A source                |
|---------------|----|----------------------|-------------------------|
|               |    | GG  | GC  | CC    |                           |
| Yakutian      | 1250| 49  | 42  | 9     | Real study               |
| Russian       | 175 | 9.8 | 39.3| 50.9  | Real study               |
| Latin american| 30  | 30  | 40  | 30    | F. Stickel [5]           |
| Uzbekistan    | 50  | 22  | 28  | 50    | Y. Rotman [6]            |
For the first time, the association of the polymorphic variant I148M of the PNPLA3 gene with fatty liver infiltration was established in 2008 [4]. Since then, many studies have been conducted to establish the association of this polymorphism with NAFLD [1, 3, 13, 14, 15, 16]. The association remained significant even despite adjustments for body mass index, diabetic status, alcohol use, and the absence of dyslipidemia [17, 18, 19]. In addition, it was found that this polymorphism is associated with increased serum levels of aminotransferase [4]. In a study by Seko et al. [19] it was found that the GG genotype is a predictor of the development of hepatocellular carcinoma in the Japanese population. In a previous study, it was shown that homozygous carriers

| Language          | Total | M | F | Age | Author |
|------------------|-------|---|---|-----|--------|
| Korean           | 184   | 20| 50| 30  | S.S. Lee[7] |
| Japanese         | 578   | 18| 52| 30  | K. Hotta[8] |
| Chinese (Han)    | 553   | 11| 47| 42  | X.E. Peng[9] |
| Caucasian        | 328   | 9 | 36| 55  | W. Dunn[10] |
| Chinese          | 202   | 8 | 45| 47  | Y. Li [11]  |
| Caucasian        | 326   | 6 | 36| 58  | F. Stickel [5] |
| Italian          | 179   | 3 | 31| 66  | Valenti [1]  |
| German           | 162   | 2 | 34| 64  | M.O. Baclig [12] |
| African          | 38    | 0 | 26| 74  | F. Stickel [5] |

Notes: G - guanine (guanine), C - cytosine (cytosine).
of the GG genotype were characterized by an increased risk of steatohepatitis (3.8 times, CI 95%: 3.03-4.79) and liver fibrosis (2.3 times, CI 95%: 1.77-3.23) [20].

It is known that the prevalence of NAFLD varies significantly in different regions of the world. In a study by Wagenknecht et al. (2011) [21] it has been suggested that I148M polymorphism may influence the prevalence of NAFLD in different ethnic groups.

The association of SNP I148M with NAFLD was established in various ethnic groups: Chinese (OR = 1.94, 95% CI: 1.12-3.37), Indian (OR = 3.51, 95% CI: 1.69-7.26), Malay (OR = 2.05, 95% CI: 1.25-3.35), Uyghur (OR = 2.25, 95% CI: 1.23-4.09) [22]. In a study by Lee et al. (2014) [7] it was shown that the frequency of the G allele (31.6%) in the group of patients with NAFLD was significantly higher than in the control group (20.1%). It was also found that the frequency of GC + GG genotypes among NAFLD patients was significantly higher in patients with advanced fibrosis.

In this study, we performed genotyping of patients with NAFLD of Yakut nationality. The results of the comparison of the I148M polymorphism of the PNPLA3 gene in a group of relatively healthy individuals and patients with NAFLD of Yakut nationality are presented in Table 7.
Table 7. Distribution of alleles and genotypes of I148M polymorphism of the *PNPLA3* gene (rs738409) in a group of conditionally healthy individuals and patients with NAFLD of Yakut nationality, n = 1347

| SNP       | Genotype | Control group (n=1250) | Patients with NAFLD (n=97) | χ² | p     | OR Value | 95% CI |
|-----------|----------|------------------------|---------------------------|----|-------|----------|--------|
| PNPLA3    | GG       | 612 49                 | 49 50,5                   | 0,41 | 0,82  | 0,94     | 0,62 – 1,42 |
| I148M (G/C) | GC       | 526 42                 | 38 39,2                   |     |       | 1,13     | 0,74 – 1,72 |
|           | CC       | 112 9                  | 10 10,3                   |     |       | 0,86     | 0,43 – 1,69 |

Notes: ¹ G - guanine (guanine), C - cytosine (cytosine); ² χ² - “chi” – square; ³ OR (odds ratio) - odds ratio; ⁴ CI (confidence interval) - confidence interval

When conducting a comparative frequency analysis, there were no statistically significant differences between the control group and the group with NAFLD patients (p = 0.82).
In a study by Jiang et al. (2014) [23] it was found that the frequency of the G allele in the Chinese population (Qingdao, China) has significant differences between the group with patients with chronic hepatitis B (31.9%) and the group with conditionally healthy individuals (21.9%). Carriers of the GG genotype of PNPLA3 gene polymorphism had a higher risk of hepatitis B in comparison with carriers of alternative genotypes (OR = 1.67, 95% CI: 1.18-2.34). Carriage of the G allele among patients with chronic hepatitis B was a predisposing factor for the development of steatosis, steatohepatitis, lobular inflammation, and accumulation of iron in the liver [24].

The results of a previous meta-analysis showed the effect of the PNPLA3 gene I148M polymorphism on the risk of hepatitis C (OR = 2.20, 95% CI: 1.56-3.11). The frequency of the mutant variant in the group of patients with hepatitis C was 20.4%, the frequency in the control group was 10.23% [25]. A comparative table on the frequency of genotypes of I148M polymorphism in patients with hepatitis C according to the available literature is presented in Table 8.

Table 8. Distribution of alleles and genotypes of the I148M polymorphism of the PNPLA3 gene (rs738409) in a group of relatively healthy individuals and patients with chronic hepatitis C.

| SNP | N | Chronic hepatitis C patients | Control group | A source |
|-----|---|-------------------------------|---------------|----------|

19
In this study, we performed a comparative frequency analysis of the distribution of genotypes and alleles of the I148M polymorphism of the PNPLA3 gene in the control group and the group of patients with chronic hepatitis B and C. In this analysis, we did not reveal significantly significant differences (p = 0.45) (Table 9).
Table 9. Distribution of alleles and genotypes of I148M polymorphism of the *PNPLA3* gene (rs738409) in a group of relatively healthy individuals and patients with chronic hepatitis B and C of Yakut nationality, N = 1289

| SNP   | Genotype frequency, % | OR | 95% CI |
|-------|-----------------------|----|--------|
|       | Control group (n=1250) |     |        |
|       | Patients with chronic hepatitis B and C (n=39) |     |        |
|       | n | % | n | % | \( \chi^2 \) | \( p \) | Value | 95% CI |
| **PNPLA3** | | | | | | | | |
| I148M (G/C) | GG | 612 | 49 | 17 | 39,1 | 1.24 | 0.65 – 2.36 |
| | GC | 526 | 42 | 20 | 54,3 | 1.61 | 0.45 | 0.36 – 1.31 |
| | CC | 112 | 9 | 2 | 13,8 | 1.82 | 0.43 – 7.65 |

Notes: 1 G - guanine (guanine), C - cytosine (cytosine); 2 \( \chi^2 \) - “chi” square; 3 OR (odds ratio) - odds ratio; 4 CI (confidence interval) - confidence interval.

Thus, in the present study, it was found that the frequency of the mutant allele of functional polymorphism I148M of the *PNPLA3* gene is higher than in other known world populations. A normally functioning *PNPLA3* gene protein regulates
the activity of triglyceride hydrolase and lysophosphatidic acid acyltransferase. I148M polymorphism leads to the replacement of the amino acid isoleucine with methionine at position 148, which does not affect the orientation of the catalytic dyad, but the longer side chain of methionine limits the substrate access to the catalytic serine at position 47 [31]. The size of the access site to the substrate is significantly reduced in the presence of the mutant G allele, which leads to limited access of palmitic acid to the catalytic dyad [32]. In a study by Kumari et al. (2012) [33] it was found that the P PNPLA3 I148M polymorphism induces an increase in lipogenic activity, which leads to an increase in the synthesis of triglycerides in the liver. Pollorphism I148M of the PNPLA3 gene has three effects on the metabolism of triglycerides in the liver: increased synthesis of fatty acids and triglycerides; violation of the hydrolysis of triglycerides; a decrease in the level of polyunsaturated fatty acids [34]. Therefore, it can be assumed that the high frequency of the mutant G allele of the I148M polymorphism of the PNPLA3 gene in the Yakut population may be one of the reasons for the violation of the mechanism of lipid metabolism in the liver.

It is now recognized that the prevalence dynamics of NAFLD is an epidemic. The studies revealed the presence of regional fluctuations in the level of detection of NAFLD. Apparently, this disease has genetic determinism, that is, it depends on ethnic factors. The PNPLA3 gene I148M polymorphism is associated with susceptibility to NAFLD, chronic hepatitis B, chronic hepatitis C. The presence of a
mutant allele affects the histological picture, the development of steatosis, and liver fibrosis. It is likely that due to the high frequency of the mutant G allele of the I148M polymorphism of the \textit{PNPLA3} gene, the Yakut population will be more susceptible to the above pathological conditions. However, in this study, we did not establish an association between the control group and groups with sick liver diseases (NAFLD, chronic hepatitis B, chronic hepatitis C).

In view of the fact that the G14 allele of \textit{PNPLA3} gene polymorphism I148M is associated with an increased risk of developing liver diseases and is a marker of the progression of the pathological process, it is necessary to conduct a thorough and detailed scientific study of this association among the indigenous population of the Republic of Sakha (Yakutia). The establishment of this polymorphism as a marker indicating the risk of adverse development of the disease will allow for effective preventive and therapeutic measures.

4. Conclusion

The study showed that the frequency of the mutant allele of functional polymorphism I148M of the \textit{PNPLA3} gene in the Yakut population was high, as in other world populations.

Therefore, it can be assumed that the high frequency of the mutant G allele of the I148M polymorphism of the \textit{PNPLA3} gene in the Yakut population may be the cause of the hereditary pathology of lipid metabolism in the liver. It is likely that due to the high frequency of the mutant G allele of the I148M
polymorphism of the *PNPLA3* gene, the Yakut population has a high predisposition to the development of steatosis, liver fibrosis, especially in combination with chronic viral hepatitis B, C, D.

**Acknowledgments**

We thank all patients and blood sample donors who have contributed to this study. This study, "A multivariate study of the health status of the indigenous and newcomers of the Republic of Sakha (Yakutia) in order to optimize regional programs to improve the quality of life of the republic’s residents, taking into account territorial, ethnic characteristics in the context of modern socio-economic development", was supported by the Russian Foundation for Basic Research (grant# 18-415-140001 p_a, grant#18-05-60035_Arctica).

**Ethics Statement**

All written informed consent forms signed by the participants or the guardians of the underage participants involved in our study were obtained before beginning the testing procedures. This study was approved by the local Biomedical Ethics Committee of North-Eastern Federal University, Yakutsk, Russia (Yakutsk, Protocol No 11, September 18, 2017).

**Competing interests**

The authors have declared that no competing interests exist.

**Author Contributions**

Conceived and designed the experiments: SS AE NG PP TB. Performed the experiments: SS TB SS ND PG AD FV AY AS. Analyzed the data: SS AE PG AD FV AY AS. Contributed reagents/materials/analysis tools: PG AD FV AY AS. Wrote the paper: SS AE TB AS SS FV JO.
1. Valenti L, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology. 2010 Apr;51(4):1209-17. doi: 10.1002/hep.23622. PubMed PMID: 20373368.

2. Liu YL, Patman GL, Leathart JBS, Piguet AC, Burt AD, Dufour JF et al. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. J Hepatol. 2014. March 07; 61(1):75-81. doi.org/10.1016/j.jhep.2014.02.030.

3. Valenti L1, Alisi A, Galmozzi E, Bartuli A, Del Menico B, Alterio A et al. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. Hepatology. 2010 Oct; 52(4):1274-80. doi: 10.1002/hep.23823. PubMed PMID: 20648474.

4. Romeo S, Kozlitina J, Xing C, Pertsemilidis A, Cox D, Pennacchio LA et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008 Dec; 40(12): 1461-5. doi: 10.1038/ng.257. PubMed PMID: 18820647; PubMed Central PMCID: PMC2597056.

5. Stickel F, Buch S, Lau K, Meyer zu Schwabedissen H, Berg T, Ridinger M et al. Genetic variation in the PNPLA3 gene is associated with alcoholic liver injury in Caucasians. Hepatology. 2011 Jan;53(1):86-95. doi: 10.1002/hep.24017. Epub 2010 Dec 7. PubMed PMID: 21254164

6. Rotman Y, Koh C, Zmuda JM, Kleiner DE, Liang TJ; NASH CRN. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. Hepatology. 2010 Sep; 52(3): 894-903. doi: 10.1002/hep.23759. PubMed PMID: 20684021; PubMed Central PMCID: PMC2932770.

7. Lee SS, Byoun YS, Jeong SH, Woo BH, Jang ES, Kim JW et al. Role of the PNPLA3 I148M polymorphism in nonalcoholic fatty liver disease and fibrosis in Korea. Dig Dis Sci. 2014 Dec;59(12):2967-74. doi: 10.1007/s10620-014-3279-z. Epub 2014 Jul 29. Pub Med PMID: 25069572.

8. Hotta K, Yoneda M, Hyogo H, Ochi H, Mizusawa S, Ueno T et al. Association of the rs738409 polymorphism in PNPLA3 with liver damage and the development of non-alcoholic fatty liver disease. BMC Med Genet. 2010 Dec 22;11:172. doi: 10.1186/1471-2350-11-172. Pub Med PMID: 21176169; Pub Med Central PMCID: PMC3018434.

9. Peng XE, Wu YL, Lin SW, Lu QQ, Hu ZJ, Lin X et al. Genetic variants in PNPLA3 and risk of non-alcoholic fatty liver disease in a Han Chinese population. PLoS One. 2012; 7(11): e50256. doi: 10.1371/journal.pone.0050256. ; Pub Med PMID: 23226254; Pub Med Central PMCID: PMC3511464.

10. Dunn W, Zeng Z, O’Neil M, Zhao J, Whitener M, Yu-Jui Wan Y, rt al. The interaction of rs738409, obesity, and alcohol: a population-based autopsy study. Am J Gastroenterol. 2012 Nov; 107(11):1668-74. doi: 10.1038/ajg.2012.285. PMID: 23032985; PMCID: PMC6677545.

11. Li Y, Xing C, Tian Z, Ku HC. Genetic variant I148M in PNPLA3 is associated with the ultrasonography-determined steatosis degree in a Chinese population. BMC Med Genet. 2012 Nov 23; 13: 113. doi: 10.1186/1471-2350-13-113. PMID: 23176674; PMCID: PMC3523076.

12. Baclig MO, Lozano-Kühne JP, Mapua CA, Gopez-Cervantes J, Natividad FF; St Luke’s Liver Diseases Study Group. Genetic variation I148M in patatin-like phospholipase 3 gene and risk of non-alcoholic fatty liver disease among Filipinos. Int J Clin Exp Med. 2014 Aug 15; 7(8): 2129-36. eCollection 2014. PMID: 25232397; PMCID: PMC4161557.
13. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. Hepatology. 2011 Jun; 53(6): 1883-94. doi: 10.1002/hep.24283. PubMed PMID: 21381068.

14. Krawczyk M, Grünhage F, Zimmer V, Lammert F. Variant adiponutrin (PNPLA3) represents a common fibrosis risk gene: non-invasive elastography-based study in chronic liver disease. J Hepatol. 2011 Aug; 55(2): 299-306. doi: 10.1016/j.jhep.2010.10.042. PubMed PMID: 21168459.

15. Zain SM, Mohamed R, Mahadeva S, Cheah PL, Rampal S, Basu RC et al. A multi-ethnic study of a PNPLA3 gene variant and its association with disease severity in non-alcoholic fatty liver disease. Hum Genet. 2012 Jul;131(7):1145-52. doi: 10.1007/s00439-012-1141-y. PubMed PMID: 22258181; PubMed Central PMCID: PMC3374090.

16. Speliotes EK, Butler JL, Palmer CD, Voight BF; GIANT Consortium; MIGen Consortium. PNPLA3 variants specifically confer increased risk for histologic non-alcoholic fatty liver disease but not metabolic disease. Hepatology. 2010 Sep; 52(3):904-12. doi: 10.1002/hep.23768. PubMed PMID: 20648472; PubMed Central PMCID: PMC3070300.

17. Tian C, Stokowski RP, Kershenobich D, Ballinger DG, Hinds DA. Variant in PNPLA3 is associated with alcoholic liver disease. Nat Genet. 2010 Jan; 42(1):21-3. doi: 10.1038/ng.488. PubMed PMID: 19946271.

18. Takeuchi Y, Ikeda F, Moritou Y, Hroaki H, Yasunaka T, Kuwaki K et al. The impact of patatin-like phospholipase domain-containing protein 3 polymorphism on hepatocellular carcinoma prognosis. J Gastroenterol. 2013 Mar; 48: 405–412. doi.org/10.1007/s00535-012-0647-3.

19. Seko Y, Sumida Y, Tanaka S, Mori K, Taketani H, Ishiba H et al. Development of hepatocellular carcinoma in Japanese patients with biopsy-proven non-alcoholic fatty liver disease: Association between PNPLA3 genotype and hepatocarcinogenesis/fibrosis progression. Hepatol Res. 2017 Oct; 47(11):1083-1092. doi: 10.1111/hepr.12840. PubMed PMID: 27862719.

20. Martínez LA, Larrieta E, Kershenobich D, Torre A. The Expression of PNPLA3 Polymorphism could be the Key for Severe Liver Disease in NAFLD in Hispanic Population. Ann Hepatol. 2017 November-December;16(6): 909-915. doi: 10.5604/01.3001.0010.5282. PubMed PMID: 29055919.

21. Wagenknecht LE, Palmer ND, Bowden DW, Rotter JI, Norris JM, Ziegler J et al. Association of PNPLA3 with non-alcoholic fatty liver disease in a minority cohort: the Insulin Resistance Atherosclerosis Family. Liver Int. 2011 Mar; 31(3):412-6. doi: 10.1111/j.1478-3231.2010.02444.x. PubMed PMID: 21281435; PubMed Central PMCID: PMC3703938.

22. Zhang Y, Cai W, Song J, Miao L, Zhang B, Xu Q et al. Association between the PNPLA3 I148M polymorphism and non-alcoholic fatty liver disease in the Uygur and Han ethnic groups of northwestern China. PLoS One. 2014 Oct 7; 9(10):e108381. doi: 10.1371/journal.pone.0108381. Pub Med PMID: 25290313; PubMed Central PMCID: PMC4188522.

23. Jiang M, Xin Y, Wang W, Lin Z, Zhang D, Li C et al. Association between the PNPLA3 I148M polymorphism and chronic hepatitis B in a Qingdao Han Chinese population]. Zhonghua Gan Zang Bing Za Zhi. 2014 May;22(5): 340-3. Chinese. doi: 10.3760/cma.j.issn.1007-3418.2014.05.004. Pub Med PMID: 25180867.

24. Zampino R, Coppola N, Cirillo G, Boemio A, Grandone A, Stanzione M et al. Patatin-Like Phospholipase Domain-Containing 3 I148M Variant Is Associated with Liver Steatosis
and Fat Distribution in Chronic Hepatitis B. Dig Dis Sci. 2015 Oct; 60(10): 3005-10. doi: 10.1007/s10620-015-3716-7. Pub Med PMID: 25986529.

25. Zhang H, Xue L, Chen L, Jiang S, Xin Y, Xuan S. A Meta-Analysis of the Association Between the I148M Variant of Patatin-Like Phospholipase Domain Containing 3 Gene and the Presence of Chronic Hepatitis C. Hepat Mon. 2015 Nov 28; 15(11): e31987. doi: 10.5812/hepatmon.31987. Pub Med PMID: 26834791; Pub Med Central PMCID: PMC4717312.

26. Nischalke HD, Berger C, Luda C, Berg T, Müller T, Grünhage F et al. The PNPLA3 rs738409 148M/M genotype is a risk factor for liver cancer in alcoholic cirrhosis but shows no or weak association in hepatitis C cirrhosis. PLoS One. 2011; 6(11):e27087. doi: 10.1371/journal.pone.0027087. Epub 2011 Nov 7. Pub Med PMID: 22087248; Pub Med Central PMCID: PMC3210131.

27. Valenti L, Rumi M, Galmozzi E, Aghemo A, Del Menico B, De Nicola S et al. Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. Hepatology. 2011 Mar; 53(3): 791-9. doi: 10.1002/hep.24123. Pub Med PMID:21319195.

28. Miyashita M, Ito T, Sakaki M, Kajiwara A, Nozawa H, Hiroishi K et al. Genetic polymorphism in cyclooxygenase-2 promoter affects hepatic inflammation and fibrosis in patients with chronic hepatitis C. J Viral Hepat. 2012 Sep; 19(9): 608-14. doi: 10.1111/j.1365-2893.2011.01580.x. Epub 2012 Jan 28. Pub Med PMID: 22863264

29. Valenti L, Rametta R, Ruscica M, Dongiovanni P, Steffani L, Motta BM et al. The I148M PNPLA3 polymorphism influences serum adiponectin in patients with fatty liver and healthy controls. BMC Gastroenterol. 2012 Aug 16; 12:111. Pub Med PMID: 2289888; Pub Med Central PMCID: PMC3444917.

30. Ezzikouri S, Alaoui R, Tazi S, Nadir S, Elmdaghri N, Pineau P et al. The adiponutrin I148M variant is a risk factor for HCV-associated liver cancer in North-African patients. Infect Genet Evol. 2014 Jan; 21: 179-83. doi: 10.1016/j.meegid.2013.11.005. Pub Med PMID: 24269995.

31-20. Wilson PA, Gardner SD, Lambie NM, Commans SA, Crowther DJ. Characterization of the human patatin-like phospholipase family. J Lipid Res. 2006 Sep; 47(9): 1940-9. Epub 2006 Jun 25. PMID: 16799181.

32. Xin YN, Zhao Y, Lin ZH, Jiang X, Xuan SY, Huang J. Molecular dynamics simulation of PNPLA3 I148M polymorphism reveals reduced substrate access to the catalytic cavity. Proteins. 2013 Mar; 81(3): 406-14. doi: 10.1002/prot.24199. Pub Med PMID: 23042597.

33. Kumari M, Schoiswohl G, Chitraju C, Paar M, Cornaciu I, Rangrez AY et al. Adiponutrin functions as a nutritionally regulated lysophosphatidic acid acyltransferase. Cell Metab. 2012 May 2; 15(5): 691-702. doi: 10.1016/j.cmet.2012.04.008. Pub Med PMID: 22560221; Pub Med Central PMCID: PMC3361708.

34. Li JZ, Huang Y, Karaman R, Ivanova PT, Brown HA, Roddy T et al. Chronic overexpression of PNPLA3I148M in mouse liver causes hepatic steatosis. J Clin Invest. 2012 Nov; 122(11): 4130-44. doi: 10.1172/JCI65179. Pub Med PMID: 23023705; Pub Med Central PMCID: PMC3484461.