T.V. Borisova, V.G. Pshennikova, F.M. Teryutin, A.V. Solovyov, G.P. Romanov, S.A. Fedorova, N.A. Barashkov

ESTIMATION OF THE MUTATION AGE C.1621C>T P.(GLN541*) IN THE *FYCO1* GENE RESPONSIBLE FOR THE DEVELOPMENT OF AUTOSOMAL RECESSIVE CONGENITAL CATARACT IN THE YAKUT POPULATION

DOI 10.25789/YMJ.2022.80.02

УДК 575.1

The main cause of congenital or juvenile cataract with autosomal recessive inheritance in Yakutia is the c.1621C>T p.(Gln541*) nonsense mutation in the exon 8 of the FYCO1 gene. Previous studies have shown that the c.1621C>T p.(Gln541*) mutation has spread to the territory of Yakutia as a result of the founder effect. The initial assessment of the average "age" of the mutation using the data of linkage disequilibrium for three STR markers: D3S3685, D3S3582 and D3S3561 showed a result of ~10.4 ± 2.6 generations (260 ± 65.0 years). In the present study, we used a different approach to determine the "age" of the c.1621C>T p.(Gln541*) mutation using the DMLE+ 2.3 software based on the analysis of 25 SNP markers. The calculated DMLE+ 2.3 "age" of the mutation, taking into account the 95% confidence interval, varies from 25 to 67 generations (from 625 to 1675 years). Comprehensive data show that the c.1621C>T p.(Gln541*) mutation could have occurred between the 4th and 18th centuries with the most likely time of expansion from 11th century.

Keywords: congenital autosomal recessive cataract, CTRCT18, c.1621C>T (p.Gln541*), FYCO1, Yakuts, founder effect, Eastern Siberia.

Introduction. Congenital cataract is one of the main causes of childhood blindness [20]. It is known that from 8.3 to 25% of congenital or juvenile cataracts are inherited [11; 7; 16] by autosomal dominant type [20], less often by autosomal recessive and X-linked type [20; 13]. In the Yakut population, congenital or juvenile cataract with autosomal recessive inheritance is one of the most common orphan diseases, occurring at a frequency of 1 in 8257 people [22]. In this regard, we have previously conducted studies to find the main genetic cause of autosomal recessive cataract in Yakutia. Whole exome analysis revealed a new c.1621C>T p.(Gln541*) nonsense mutation exon 8 of the *FYCO1* gene (NM_024513.3) [9]. This substitution leads to the formation of a premature stop codon p.(Gln541*) in the functionally significant Coiled-coil domain and truncates the polypeptide chain of the FYCO1 protein [9]. Among the studied patients with congenital cataract in Yakutia, in 86% of patients (25 out of 29), the c.1621C>T p.(Gln541*) mutation was found in the homozygous state. Taking into account the significant contribution (86%) of the c.1621C>T p.(Gln541*) mutation to the etiology of the disease in the territory of Yakutia, haplotypes of mutant chromosomes were analyzed using 6 STR markers. The results of the study showed the unity of origin of all the studied mutant chromosomes, which indicates the spread of the c.1621C>T p.(Gln541*) mutation in the territory of Yakutia as a result of the founder effect. Phylogenetic analysis revealed the highest diversity of haplotypes in the central subpopulation of the Yakuts, which indicates the beginning of the spread of mutant chromosomes in the territory of Yakutia from the Lena-Amga interfluve [9]. The carriage frequency for this mutation in the population of Yakuts was 7.9%, in Evens - 2%, Evenks - 1.7%, in the populations of Russians, Yukagir, Dolgan and Chukchi this variant was absent.

Previously, the estimation of the "age" of the mutation was carried out using the method described in Risch et al. [12]. To determine the "age" of the mutation, the data of linkage disequilibrium for three STR markers were used: D3S3685, D3S3582, D3S3561 (~6.3 Mb). This method gives an estimate of the "age" separately for each marker under study and is based on the "genetic clock" approach [14]. The average "age" of the c.1621C>T p.(Gln541*) mutation in the Yakut population was ~10.4 ± 2.6 generations (260 ± 65.0 years), which indicated the start of the expansion of mutant chromosomes in the 18th century [9] and corresponded to the time when the first Russian explorers appeared in Yakutia. However, the results of screening indicated the absence of this mutation in the Russian population. In order to clarify the data obtained earlier, in this work, we applied a different approach to estimating the "age" of the c.1621C>T p.(Gln541*) mutation using the DMLE+ 2.3 software [19], which makes it possible to carry out calculations based on the linkage disequilibrium of several genetic markers.

Materials and methods. *Patients.* This study consisted of DNA samples from 24 Yakut patients with congenital cataract who had the c.1621C>T p.(Gln541*) mutation in the *FYCO1* gene in the homozygous state. DNA samples from 22 healthy Yakut patients constituted a control sample.

Estimation of the "age" of the c.1621C>T p.(Gln541) mutation in the FYCO1 gene using DMLE+ 2.3*

The DMLE+ 2.3 program allows to make a conclusion about the "age" of a mutation (in generations) based on the observed linkage disequilibrium of several genetic markers, using the Markov chain Monte Carlo algorithm [19]. To cor-

Institute of Natural Sciences M.K. Ammosov NEFU: **BORISOVA Tuyara Valeryevna** – graduate student, borisovatv96@gmail.com, ORCID: 0000-0002-5019-067X; **ROMANOV Georgiy Prokopyevich** – research assosiate, ORCID: 0000-0002-2936-5818; **FEDOROVA SARDANA ARKADYEVNA** – Doctor of Biology, chief researcher, ORCID: 0000-0002-6952-3868;
YSC CMP: **PSHENNIKOVA Vera Gennadiyevna** – PhD in Biology, external researcher, ORCID: 0000-0001-6866-9462; **TERYUTIN Fedor Mikhailovich** – PhD in Medicine, senior researcher; ORCID: 0000-0002-8659-0886; **BARASHKOV Nikolay Alekseevich** – PhD in Biology, Head. lab. ORCID: 0000-0002-6984-7934; **SOLOVYOV Aisen Vasilyevich** – PhD in Biology, research associate, In-t of humanitarian research and problems of the small peoples of the North of the Russian Academy of Sciences, ORCID: 0000-0003-0914-3609.

rectly estimate the “age” of a mutation using the DMLE+ 2.3 software, it is necessary to calculate the proportion of mutant chromosomes in the sample (proportion of population sampled) and the population growth rate (d). The proportion of mutant chromosomes in the sample was calculated based on the frequency of heterozygous carriage of the c.1621C>T p.(Gln541*) mutation. Since the exact number of Yakuts is known only since the time of the First General Population Census of 1897 in the Russian Empire [4], there was not enough data to accurately calculate the population growth rate. In this regard, we used the population growth rate parameter d=0.085 used by Rannala and Reeve [18] to estimate the age of the mutation *SLC26A2* gene in the Finnish population, which, presumably, like the Yakut population, originated from a small ancestral population [14].

Results and Discussion. As a result of comparing the allele frequencies of two samples of individuals using the χ2 test, 25 SNP markers (69,851 kb) were identified for further analysis. We calculated the expected number of homozygotes in the Yakut population (0.3%) from the known frequency of heterozygous carriers of the c.1621C>T p.(Gln541*) mutation, which is 7.9% [9], and determined the proportion of mutant chromosomes in the sample to be 0.05. The average “age” of the c.1621C>T p.(Gln541*) mutation in the *FYCO1* gene with a proportion of mutant chromosomes in the sample of 0.05 was estimated at 38 generations (950 years), taking into account 95% CI, the “age” is within 25–67 generations (from 625 to 1675 years) (Fig. 1, A).

To assess the accuracy of the calculation of the “age” of the mutation and identify possible errors, we used two more values for the proportion of mutant chromosomes in the sample, 0.04 and 0.06 (Fig. 1, B and C). If the estimated proportion of mutant chromosomes is lower than the calculated one and equals 0.04, the average “age” of the mutation is 39 generations (975 years), taking into account 95% CI, is 28–72 generations (from 700 to 1800 years) (Fig. 1, B). If the proportion of mutant chromosomes is higher than calculated and equals 0.06, the “age” of the mutation is 37 generations (925 years), and at 95% CI it is 23–66 generations (from 575 to 1650 years) (Fig. 1, C). Thus, the change in the proportion of mutant chromosomes in the sample had little effect on the final estimate.

The average “age” of the c.1621C>T p.(Gln541*) mutation obtained in this study for 25 SNP markers (~950 years) significantly exceeds the result of the previous study (260 ± 65.0 years), where the calculation for 3 separate STR markers was used (Table 1). Both of these approaches are used in genetic studies and neither of them can be considered more reliable. However, a study by Clendenning et al [17] showed that the model for estimating linkage disequilibrium used by the DMLE+ 2.3 software [19] could be more effective to determine the origin of relatively "young" mutations in a population with a rapid population growth, in comparison with a method that gives a separate age estimate for each marker [12]. Perhaps the DMLE+ 2.3 approach is more appropriate for the Yakut population.

An earlier estimate of the “age” of the c.1621C>T p.(Gln541*) mutation at 260 years indicated that this mutation had spread during the period of exploration of Eastern Siberia by Russian explorers. However, given the absence of this mutation in the Russian population and the highest carrier rate among the Yakuts (7.9%) [9], it would be more likely to assume that this mutation is related to the ancestors of the Yakuts and to accept an earlier estimate of ~260 years as the lower threshold of “age ». Thus, the estimate of the maximum value of 1675 years obtained in this study can be considered an upper limit for the "age" of the mutation. If we accept both results of the “age” estimate, then the time of the appearance of the mutation is within the range from the 4th to the 18th centuries AD.

According to archaeologist A.I. Gogolev, the physical and linguistic features of the Turkic-speaking ancestors of the Yakuts took shape in the Baikal region from the 6th to the 10th centuries, then in the 13th century Yakuts moved to the valley of the middle reaches of the Lena river [3]. According to A.N. Alekseev, the southern ancestors of the Sakha penetrated the territory of Yakutia much earlier – back in the first half of the 1st millennium AD and by the 10th – 11th century, the ancestors of the Yakuts mastered significant territories of Prilenye [1, 2]. According to genetic data, the time of divergence of N3-lineages of the Y-chromosome of the Yakuts, based on microsatellite diversity, indicates a primary increase in the size of the ancestral population in ~4th century, followed by a secondary expansion starting from the 11th century. [8].

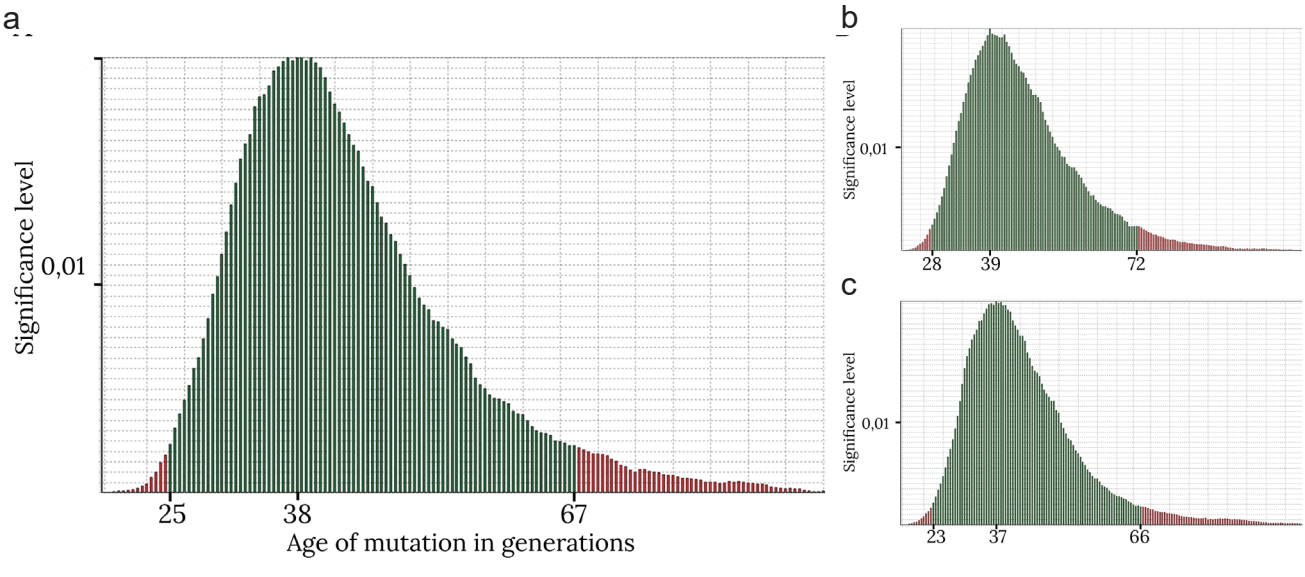

Fig. 1. Plots of estimation of the “age” of the c.1621C>T p.(Gln541*) mutation in the *FYCO1* gene using the DMLE+ 2.3 program [Reeve, 2002]. A - the "age" of the mutation with the value of the proportion of mutant chromosomes in the sample equal to 0.05; B - at 0.04; C - at 0.06.

Comparison of the results of age estimation of the c.1621C>T p.(Gln541*) mutation in the *FYCO1* gene

Method	Markers	Age of mutation (in generations)	Average age (in generations)	Reference
Risch et al., 1995 [12]	D3S3685	185 (~ 7,4)	260 ± 65,0 (10,4±2,6)	[9]
	D3S3582	317,5 (12,7)		
	D3S3561	277,5 (11,1)		
DMLE+ 2.3 [19]	25 SNP markers (69.851 kb)	625 – 1675 (25-67)	950 (38)	[Present study]

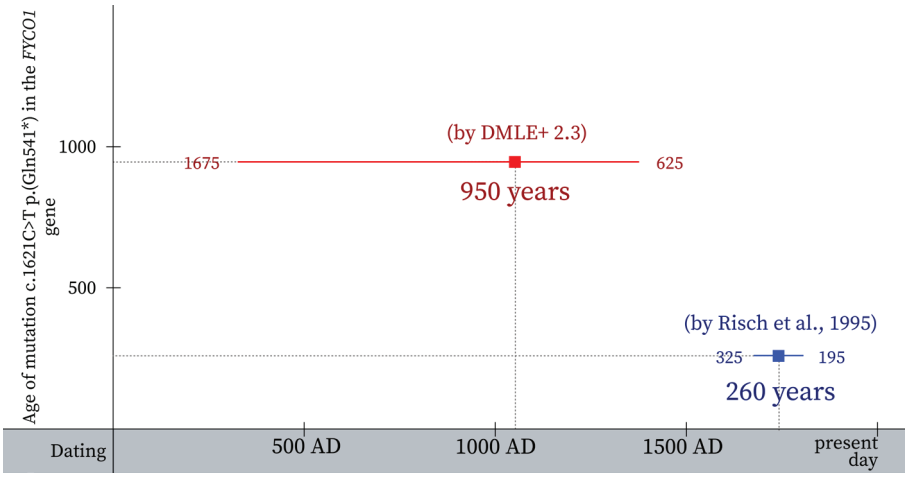

Fig. 2. The "age" of the c.1621C>T p.(Gln541*) mutation in the *FYCO1* gene, estimated using two methods.

Thus, the total period of occurrence of the c.1621C>T p.(Gln541*) mutation is from the 4th to the 18th centuries covers the time of the formation of the Sakha people [1]). At the same time, the average value of the age of the mutation ~950 years coincides with the appearance in the 10-11th centuries on the territory of Yakutia (in Namtsy and Ust-Aldan) sites of the Kulun-Atakh archaeological culture, directly associated with the ancestors of the Yakuts [2].

Conclusions. Thus, the time period from the 4th to the 18th centuries fully corresponds to the time of the formation of the Yakut people according to A.N. Alekseev, starting with the penetration of certain groups of southern pastoral tribes into the territory of Yakutia in the 3rd – 4th centuries. [6]. At the same time, the average mutation age of ~950 years coincides with the appearance in the 10 – 11th centuries. on the territory of Yakutia (in Namtsy and Ust-Aldan) sites of the Kulun-Atakh archaeological culture, directly associated with the ancestors of the Yakuts [2].

The authors declare no conflict of interest.

The work was carried out within the framework of the State Assignment of the Ministry of Science and Higher Education of the Russian Federation (FSRG-2020-0016), as well as with the support of RFBR grant № 19-34-60023.

Reference

1. Alekseev A.N. Drevniaia IAkutiia zheleznyi vek i epokha srednevekovia [Ancient Yakutia The Iron Age and the era of the Middle Ages]. Novosibirsk Institute of Archeology and Ethnography of the Siberian Branch of the Russian Academy of Sciences. 1996; 95 (In Russ.).] ISBN 5-7803-0008-9

2. Alekseev A.N., Bravina R.I. Formirovanie iakutskogo naroda [Formation of the Yakut people]. Istoriia IAkutii v 3 t [History of Yakutia in three volumes]. Novosibirsk branch of the Federal State Unitary Enterprise Academic Scientific Publishing and Book Distribution Center Nauka. 2020; V 1: 223-230 (In Russ.).]

3. Gogolev A I Arkheologicheskie pamiatniki IAkutii pozdnego srednevekovia XIV - XVIII vv [Archaeological monuments of Yakutia in the late Middle Ages XIV-XVIII centuries]. Irkutsk Publishing House of the Uni-ty. 1990;188 (In Russ.).] ISBN 5-7430-0077-8

4. Pervaia vseobshchaia perepis naseleniia Rossiiskoi imperii 1897 goda [The first general census of the population of the Russian Empire in 1897]. Ed N.A. Troinitsky. St Petersburg Ed Center Stat Committee of Ministry of Interior. 1897; 1905 (In Russ.).]

5. Fedorova S.A., Khusnutdinov E.K. Geneticheskaia istoriia narodov IAkutii i nasledstvenno-obuslovlennye bolezni [Genetic history of the peoples of Yakutia and hereditary diseases]. Novosibirsk Nauka. 2015; 328 (In Russ.).] ISBN 978-5-02-038663-1

6. Fisher I.E. Sibirskaia istoriia s samogo otkrytiia Sibiri do zavoevaniia sii zemli Rossiiskim oruzhiem [Siberian history from the very discovery of Siberia to the conquest of these lands by Russian weapons]. St Petersburg Imperial Academy of Sciences. 1774; 632 (In Russ.).]

7. A nationwide Danish study of 1027 cases of congenital/infantile cataracts: etiological and clinical classifications. B. Haargaard, J. Wohlfahrt, H.C. Fledelius [et al.] // Ophthalmology. 2004;111(12):2292-8. doi: 10.1016/j.ophtha.2004.06.024.

8. Autosomal and uniparental portraits of the native populations of Sakha (Yakutia): implications for the peopling of Northeast Eurasia. S.A. Fedorova, M. Reidla, E. Metspalu [et al.] // BMC evolutionary biology. 2013. 13:127. doi 10.1186/1471-2148-13-127.

9. Autosomal recessive cataract (CTRCT18) in the Yakut population isolate of Eastern Siberia: a novel founder variant in the FYCO1 gene. N.A Barashkov, F.A. Konovalov, T.V. Borisova [et al.] // Eur J Hum Genet. 2021;29(6):965-976. doi: 10.1038/s41431-021-00833-w.

10. Estimating the age of the p.Cys433Arg variant in the MYOC gene in patients with primary open-angle glaucoma. A.M. Marques, G. Ananina, V.P. Costa [et al.] // PLoS One. 2018;13(11):e0207409. doi: 10.1371/journal.pone.0207409.

11. François J. Genetics of cataract. Ophthalmologica. 1982;184(2):61-71. doi: 10.1159/000309186.

12. Genetic analysis of idiopathic torsion dystonia in Ashkenazi Jews and their recent descent from a small founder population. N. Risch, D. Leon, L. Ozelius [et al.] // Nat Genet. 1995(9):152–159. https://doi.org/10.1038/ng0295-152

13. Hejtmancik J.F. Congenital cataracts and their molecular genetics. Semin Cell Dev Biol. 2008;19(2):134-49. doi: 10.1016/j.semcdb.2007.10.003.

14. Labuda D., Zietkiewicz E., Labuda M. The genetic clock and the age of the founder effect in growing populations: a lesson from French Canadians and Ashkenazim. Am J Hum Genet. 1997;61(3):768-71.

15. Linkage disequilibrium mapping in isolated founder populations: diastrophic dysplasia in Finland / J. Hästbacka, A. de la Chapelle, I. Kaitila [et al.] // Nat Genet. 1992;2(3):204-211. doi:10.1038/ng1192-204

16. Merin S. Inherited eye diseases. New York:Marcel Dekker Inc.;1991:86–120.

17. Origins and prevalence of the American Founder Mutation of MSH2. M. Clendenning, M.E. Baze, S. Sun [et al.] // Cancer Res. 2008;68(7):2145-53. doi: 10.1158/0008-5472.CAN-07-6599.

18. Rannala B., Reeve J.P. Joint Bayesian estimation of mutation location and age using linkage disequilibrium. Pac Symp Biocomput. 2003;526-534. doi:10.1142/9789812776303_0049.

19. Reeve J.P., Rannala B. DMLE+: Bayesian linkage disequilibrium gene mapping. Bioinformatics. 2002;18(6):894-5. doi: 10.1093/bioinformatics/18.6.894.

20. Robinson G.C., Jan J.E., Kinnis C. Congenital Ocular Blindness in Children, 1945 to 1984. Am J Dis Child. 1987;141(12):1321–1324. doi:10.1001/archpedi.1987.04460120087041

21. Shiels A, Bennett T.M., Hejtmancik J.F. Cat-Map: putting cataract on the map. Mol Vis. 2010;16:2007-15.

22. The structure and diversity of hereditary pathology in Sakha Republic (Yakutia). L. Tarskaia, R. Zinchenko, G. Elchinova [et al.] // Russ J Genet. 2004;40:1264–72. https://doi.org/10.1023/B:RUGE.0000048669.22362.1c.

O.M. Zamorschikova, S.S. Sleptsova, N.R. Maksimova, A.L. Danilova, S.S. Sleptsov, L.I. Petrova

ANALYSIS OF INTERFERON GENE POLYMORPHISM IN PATIENTS WITH HDV INFECTION IN THE REPUBLIC OF SAKHA (YAKUTIA)

DOI 10.25789/YMJ.2022.80.03

УДК 616.36-002.2

The aim of the research: to analyze the frequency of occurrence of polymorphisms rs8105790 of the IFNL3 gene, rs368234815 of the IFNL4 gene, rs1831583 of the IFNA1 gene in healthy people and patients with chronic viral hepatitis D among the ethnic group of Yakuts living on the territory of the Republic of Sakha (Yakutia).

Materials and methods of the study: to study gene polymorphisms in 157 individuals with chronic HDV infection and 160 apparently healthy individuals was used polymerase chain reaction (PCR). Analysis of the results included compliance with the Hardy-Weinberg law, Pearson's chi-squared test ($\chi 2$), odds ratio and its confidence interval.

Results: the people of young working age suffer more from HDV infection, wherein the development of cirrhosis from the moment of infection with the D virus is formed on average over 6.5 years. The high replicative activity of the HDV virus in 74.1% of cases is accompanied by suppression of HBV, but with an increase in the severity of fibrosis and the formation of cirrhosis and liver cancer, there is observed simultaneous replication of hepatitis B and D viruses. According to the data obtained, the risk of developing severe fibrosis in HDV is 1.7 times higher in carriers of the ΔG-allele of the rs368234815 polymorphism of the IFNL4 gene (OR=1.784; 95% CI 0.642–4.959) and 1.8 times higher in the carriers of the C-allele of the rs1831583 polymorphism of the IFNA1 gene (OR= 1.818; 95% CI 0.340–9.713).

Conclusion: the obtained results demonstrate that the C-allele rs1831583 of the IFNA1 gene and the ΔG-allele rs368234815 of the IFNL4 gene predispose to the formation of severe fibrosis in HDV infection, Yakutia.

Keywords: chronic hepatitis, liver cirrhosis, gene polymorphism, HDV, IFNL3, IFNL4, IFNA1.

ZAMORSCHIKOVA Olga Mikhailovna – postgraduate student of the Department of Infectious Diseases, Phthisiology and Dermatovenereology of Medical Institute of M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia, olya-botty@mail.ru; **SLEPTSOVA Snezhana Spiridonovna** – M.D., Associate Professor, Head of the Department of Infectious Diseases, Phthisiology and Dermatovenereology, of Medical Institute of M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia, sssleptsova@yandex.ru; **MAKSIMOVA Nadezhda Romanovna** – M.D., chief researcher at Head of Research Laboratory "Molecular Medicine and Human Genetics" of Medical Institute of M. K. Ammosov North-Eastern Federal University, Yakutsk, Russia, nr.maksimova@s-vfu.ru; **DANILOVA Anastasia Lukinichna** – Ph.D. in Biology, senior researcher at Research Laboratory "Molecular Medicine and Human Genetics" of Medical Institute of North-Eastern Federal University named after M.K. Ammosov, Yakutsk, Russia, al.danilova@s-vfu.ru; **SLEPTSOV Spiridon Spiridonovich** – Ph.D. in Biology, associate professor, senior researcher at laboratories of clinical-population and medical-social researches of the Federal State Budgetary Scientific Institution "Yakut Scientific Center for Complex Medical Problems", Yakutsk, Russia, sachaja@yandex.ru; **PETROVA Lyubov Innokentievna** – Ph.D. in Medicine, Associate Professor of the Department of Infectious Diseases, Phthisiology and Dermatovenereology, of Medical Institute of M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia, li.petrova@s-vfu.ru.

Introduction. Chronic viral hepatitis D (CHD) is caused by the hepatitis D virus and is characterized by a predominantly progressive course with the rapid development of liver cirrhosis (LC) than other hepatitis [1,5,4,10].

The Republic of Sakha (Yakutia) is one of the regions with a high prevalence of parenteral viral hepatitis [3,8]. The ongoing annual monitoring of the incidence of chronic viral hepatitis in the Republic of Sakha (Yakutia) shows an excess of the average for the Russian Federation.

Of the 14,975 patients registered in the electronic registry, chronic hepatitis D accounts for 7.8% (1176) of the total number of all chronic viral hepatitis, while HDV infection occurs in 40.8% of people with liver cirrhosis and in 38.5% with hepatocellular carcinoma (HCC). Chronic HDV infection is more often detected in the working-age population with a predominance of indigenous people. The high index of cirrhosis of HDV infection with the development of severe complications leading to early disability and death requires an in-depth study of the causes of liver fibrosis caused by the HD virus.

The mechanisms of genetic predisposition to chronic HDV infection have not yet been elucidated. The role of nucleotide polymorphic variants of interferon I (IFNA1) and type III (IFNL3, IFNL4) genes [2] in the pathogenesis of viral hepatitis is actively studied and is due to binding to cell receptors, as well as participation in the process of viral reproduction inside the cell [14]. Many studies prove the genetic determinism of the development of the chronic course of hepatitis in HBV and HCV infections [11, 16]. Despite the relevance of genetic predictors in the study of the development of chronic HDV infection, so far, no molecular genetic studies have been conducted in the Asian ethnic group in Russia.

Purpose: to analyze the frequency of occurrence of polymorphisms rs8105790 of the IFNL3 gene, rs368234815 of the IFNL4 gene, rs1831583 of the IFNA1 gene in healthy and sick individuals with chronic viral hepatitis D among the ethnic group of Yakuts living in the Republic of Sakha (Yakutia).

Materials and methods: the study was approved by the local ethical committee of the North-Eastern Federal University named after M.K. Ammosov", complies with the ethical principles of the Declaration of Helsinki of the World Medical Association (2013). The selection of the biomaterial was carried out on the basis of the infectious diseases department of the State Budgetary Institution of the Republic of Sakha (Yakutia) "Ya-