atrie.Sep.2015. vol.15. p. 213-217.

14. F.A. Platonov, K. Tyryshkin, D.G.Tickonov, T.S. Neustroyeva, N.V. Yakovleva,T.M. Sivtseva, O.G.Sidorova, V.P. Nikolaev, L.G. Goldfarb, N. M. Renwick. F. A. Genetic fitness and selection intensity in a population affected with high-incidence spinocerebeller ataxia type 1.[]// Neurogenetics, 2016,17: 179-185, DOI 10-1007,s10048-016-0481-5.

15. Olivopontoserebellar atrophy in large Yakut kinship in Eastern Sibiria. L.G. Golfard, M.P. Chumakov, P.A. Petrov [L.G Golfard, M.P. Chumakov., P.A.Petrov, N.I.Fedorova, D.C. Gajdusek] // Nevrology. 1989,vol.39,n.11, p. 1527-1530

16. Rakowicz M., Sulek A., Sobanska A. Prospecive study of motor cortex excitability and conduction of pyramidal tracts in the presymptomatic spinocerebellar ataxia type 1 gene carriers // Journal of the Neurological Sciences. 2015. Oct. Supplement 1, Vol. 357, p. 284-e284.

17. Ware J.E., Kosinski M., Keller S.D. SF-36 Physical and Mental Health Summary Scales: A User`s Manual // The Health Institute, New England Medical Center. Boston, Mass.-1994.

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ANALYSIS OF ASSOCIATIONS OF GENETIC POLYMORPHISMS 481C> T, 590G> AI 857G> A GENE OF THE ENZYME N-ACETYLTRANSFERASE 2 *(NAT2)* WITH THE RISK OF LUNG CANCER IN THE YAKUTS

ABSTRACT

For the first time the search of polymorphic options of a gene of NAT2 associated with the development of lung cancer in Yakutia has been carried out. Genetic markers of the increased and lowered risk of development of lung cancerin the Yakuts have been revealed. It is established that markers of the increased risk of development of lung cancer for the Yakuts are the allele of NAT2*857A and a genotype of NAT2*857G/A, markers of the lowered risk – NAT2*857G allele, NAT2*857G/G genotype.

Keywords: lung cancer, polymorphic options, N-acetyltransferase-2.Keywords: lung cancer, polymorphic options, N-acetyltransferase-2.

INTRODUCTION

In Russia cancer of a lung takes the leading positions in structure of oncological incidence – the incidence of it for the last 20 years has increased more than twice and it is on the first place among malignant neoplasms [5]. Annually in Russia this pathology is diagnosed more, than for 63 thousand patients. The problem of cancer of lung is relevant also for Yakutia where this form of cancer for many years takes the first place in structure of an oncopathology. In Republic Sakha (Yakutia) with the population in 982,1 thousand people annually more than 300 people get cancer of a lung[2].The sharpness of a problem is caused not only by high prevalence of a disease, but also by late diagnostics as at an early stage lung cancer is possible to diagnose no more than in 15% of cases [4]. It is relevant to study all factors involved in carcinogenesis.

Lung cancer, as well as many oncological diseases, is a multiple-factor disease and in its development an important role is played as outside environment (smoking, asbestos, radon, arsenic, etc.), and genetic factors [7, 9, 11, 17]. By some authors, it is shown that polymorphic options of a gene of NAT2 make a contribution to development of oncological diseases including cancer of a lung [8, 13, 18].

The gene of NAT2 is localized on a short chromosome arm 8 (8p23.1), has about 9900 PN extension, contains 2 exons and it is expressed mainly in a liver and intestines [3, 14]. The N enzyme-acetyltransferase-2 coded by this gene represents the protein with a molecular weight of 33 cd consisting of 290 amino-acid residues. This enzyme localized in cytoplasm participates in process of biotransformation of aromatic amines, which are present at the environment. A source of aromatic amines are industrial wastes, pollution of water, air, and a number of medicines [3, 15].

MATERIAL AND METHODS

In the present article it has been done a comparative studying of polymorphism of a gene of NAT2 enzyme arylamine N-acetyltransferase among patients with cancer of a lung and among healthy people, residents of the Sakha (Yakutia) Republic. We have examined 60 patients with cancer of a lung of Yakut ethnic origin from which 43 men, 17 women received treatment in a republican oncological

clinic of the city of Yakutsk. Average age of patients was 58,86±8,72 years. The diagnosis cancer of a lung has been confirmed morphologically, endoscopically and radiologically. During checking has been investigated the group of healthy people which is corresponding to a group of patients on ethnic origin and a gender with no oncological diseases, consisting of 60 people (middle age 49,5±5,75).

For release of DNA the standard method of phenolic and chloroformic extraction was used [12]. The emitted DNA was frozen at a temperature - 400C before carrying out genotyping.

The analysis of polymorphic options 481C>T, 590>T; AU 857>A; was carried out by A of a gene of NAT2 with use of methods of polymerase chain reaction on the thermocycler of "Tertsik" of the "DNK-technology" company (Russia) and T100 of the "Bio-Rad" company (USA).

For amplification used reactionary mix volume 25 мql, which contained 2,5 мql 10 Taq-buffer (67 mMtris-HCl (pH 8,8), 16,6 mM (NH4)2 SO4, 2,5mM MgCl2, 0,01% of Tween-20), 0,1 mkg of genomic DNA, the mix dNTP (dATP, dGTP, dCTP, dTTP on 150 mkM of each), 1 unit of DNA - Thermusaquaticus polymerase (Sintol, Russia) and 5-10 pmoligonucleotidepraymer (F 5' - GCTGGGTCTGGAAGCTCCTC; R 5 '-TTGGGGTGATACATACACAAGGG). The mode of amplification was the following: preliminary denaturation (940C, 5 min.), 28 cycles of amplification: a denaturation – 940C, 45 sec.; annealing – 600C, 45 sec.; synthesis – 720C, 45 sec., the finishing synthesis (720C, 7 min.).

For definition of nucleotide replacements it was carried out hydrolysis of an amplificated fragment by the following restrictions: KpnI (481C > T), BamHI (857G >A), TaqI (590G > A) (fig. 1, 2, 3).

Products of enzymatic hydrolysis were divided by a vertical electrophoresis in 7% poliacrylamide gel with the subsequent processing bromic etidium. Visualization of bands and scanning of gel were carried out in the passing UF-light by means of the "DNAAnalyzer" video system (Moscow).

When comparing frequencies of genotypes the standard criterion $\chi 2$ with Yeats's amendment was used. Statistically significant considered distinctions at $p<0,05$. The relative risk (OR) of development of a disease at a certain genotype was calculated by a standard formula OR=a/bxd/c where a and b – the number of the patients having and not having a mutant genotype respectively and d, c – a number of people in control group, having and not having a mutant genotype. OR is specified with a 95% confidential interval.

RESULTS

The analysis of distribution of frequencies of alleles and genotypes which is carried out by us on three polymorphic locus 481C> T, 590G >A and 857G > NAT2 gene A among sick and healthy people of the Yakut ethnic origin has revealed a number of features of distribution of frequencies of alleles of the studied gene.

Frequency of alleles NAT2*481T in group of healthy individuals of the Yakut ethnic origin was 23,4%. Earlier it has been established that the prevalence of this polymorphic option in various populations varies from 2-43% [16]. In populations of Europe frequency alleles is NAT2*481T 38-43% [1, 6, 16], and in Asian populations of China and Japan with – 6% and 2%, respectively [6], that is there is a gradient of the decrease in frequency given allele from the west to the east. In comparison with literary data on occurrence frequency alleles NAT2*481T in Southeast Asia among Yakuts this value is rather high.

Frequency of alleles NAT2*857A in population of Yakuts - 21,7% that is more, than in other Asian populations. Frequency of given alleles increases from the west to the east, among Europeans it is 2-3%, in populations of Asia – 11-19% [6, 10].

According to literary data in distribution of frequencies of alleles NAT2*590A isn't revealed significant interethnic differences between populations of Asia and Europe. For alleles NAT2*590A at the population of Europe is characteristic occurrence frequency – 26-27%, among inhabitants of Asia 23-30% [6, 10]. Among Yakuts frequency alleles NAT2*590A was equal in control group to 21,7%.

For the purpose of identification of possible associations of polymorphic options of a gene of NAT2 with development of cancer of lung we have carried out the analysis of distribution of allels polymorphic option 481C > T at patients with cancer of a lung and among the people who don't have oncological diseases. We haven't found statistically significant differences in distribution of frequencies of alleles between control group and group of patients with cancer of a lung. In distribution of genotypes between control group and group of sick statistically significant distinctions were also not found (fig. 4).

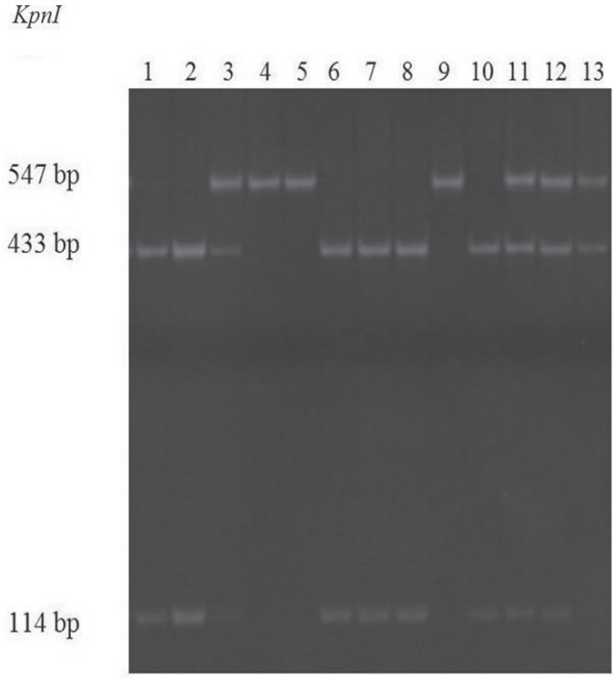

Figure 1. Detection of polymorphism 481C > gene T by NAT2 method of the RFLP-analysis (T/T – a homozygous «wild» genotype (4,5,9); T/C – a heterozygotic genotype (3,11,12,13); C/C – a homozygous mutant genotype (1,2,6,7,8,10)).

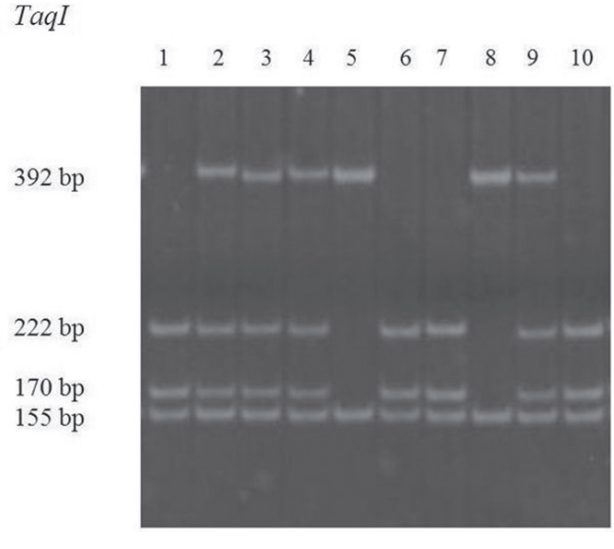

Figure 2. Detection of polymorphism 590G > gene A by NAT2 method of the RFLP-analysis (G/G – a homozygous «wild» genotype (1,6,7,10); A/G – a heterozygotic genotype (2,3,4,9); A/A– a homozygous mutant genotype (5,8)).

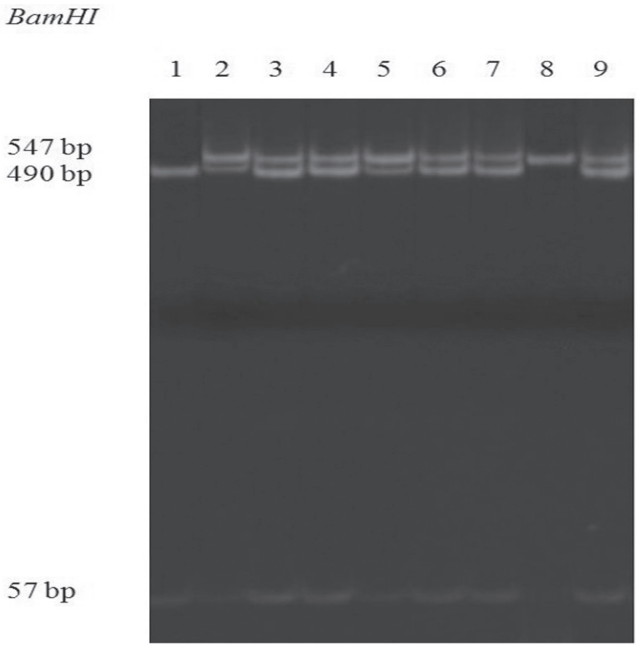

Figure 3. Detection of polymorphism 857 >gene A by NAT2 method of the RFLP-analysis (A/A – a homozygous «wild» genotype (8); A/G – a heterozygotic genotype (2-7,9); G/G – a homozygous mutant genotype (1)).

In group of patients the frequency of occurrence mutant alleles was higher than NAT2*590A in comparison with healthy (34% and 26% respectively), but statistically significant differences when comparing frequencies of alleles (NAT2*590G and NAT2*590A) at sick and healthy isn't found by us (fig. 5). In group of patients we have noted increase in frequency of occurrence of a heterozygote genotype of NAT2*590G/A by 1,7 times in comparison with control group, 40% and 23,3% respectively (χ^2=3,12; p=0,077)

The greatest statistically reliable distinctions of frequencies of alleles and genotypes were observed by us by polymorphic option 857G > A. At patients in comparison with healthy decrease in frequency of occurrence of mutant NAT2*857G alleles - 64,2% and 78,3%, respectively χ^2=42,52 is noted; p =0,000...; OR=2,02; 95% CI=1,10 – 3,78 and increase in frequency of occurrence of wild NAT2*857A alleles 35,8%, 21,7% respectively χ^2=42,52; p=0,000...; OR=6,47; 95% of CI=3,52 - 11,98 (fig. 6).

In group of patients frequency of alleles NAT2*857A increased (35,8%; χ^2=42,52; p=0,000...; OR=6,47; 95% CI=3,52-11,98) and heterozygotic genotype of NAT2*857G/A (71,6%; χ^2=13,43; p=0,000...; OR=0,23; 95% CI=0,10-0,53) and the frequency of a homozygous genotype NAT2*857G/G decreased (28,4%; χ^2=10,95; p=0,000...; OR=3,79; 95% CI=1,66-8,78) in comparison with control of 21,7%, 36,6% and 60,1% respectively.

Thus, in the analysis of associations of polymorphic options 481C > T, 590G >A and 857G >; NAT2 gene A with development by lung cancer in Yakutia have been established the allelic options and genotypes of a gene of NAT2 making a contribution to development of cancer of lung among persons of the Yakut ethnic origin. Markers of the increased risk of development of cancer of lung among Yakuts are the allele of NAT2*857A and a genotype of NAT2*857G/A, markers of the lowered risk - NAT2*857G allele, NAT2*857G/G genotype.

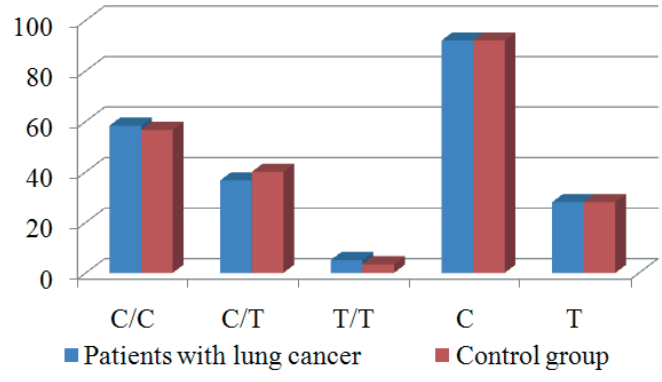

Figure 4. Distribution of frequencies of genotypes and alleles of polymorphic option (481C > T) NAT2 gene at patients with cancer of a lung and control group

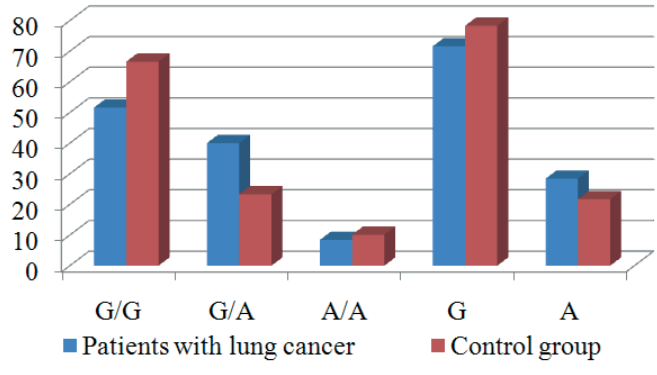

Figure 5. Distribution of frequencies of genotypes and alleles of polymorphic option (590G > A) NAT2 gene at patients with cancer of a lung and control group

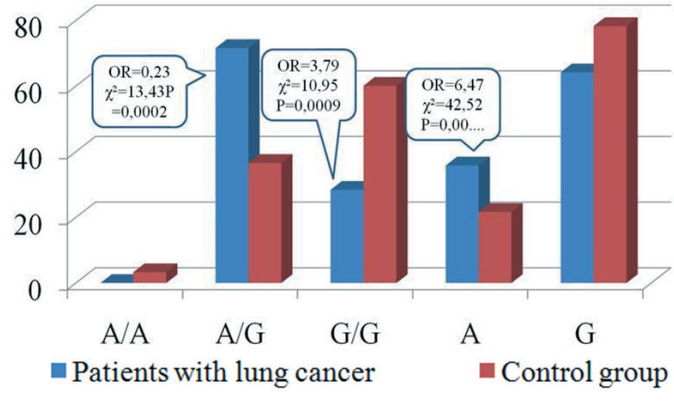

Figure 6. Distribution of frequencies of genotypes and alleles of polymorphic option (857G > A) NAT2 gene at patients with cancer of a lung and control group

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REFERENCES

1. KozhekbaevaZh.M. Gra O.A. FadeevV.S. etal. Assotsiatsiya polimorfizma NAT2 s riskom razvitiya psoriaza v Moskovskoy populyatsii [Association of polymorphism NAT2 with a risk of psoriasis in the Moscow population] Zh. Molekulyarnaya biologiya [J.

Molecular biology]. 2009, V.43 (1), P. 62-76.
2. Ivanov P.M. Tomskiy M.I. Karataev P.D. Zlokachestvennyie novoobrazovaniya v Yakutii na rubezhe vekov [Malignant neoplasms in Yakutia at the turn of the century] Yakutsk: 2008, 268p.
3. Syichev D.A. et al. Klinicheskaya farmakogenetika [Clinical pharmacogenetics] Moscow: 2007, 248 p.
4. Krivonogov N.G. Demyanenko N.Yu. Dubodelov E.L. Sostoyanie alveolyarno-kapillyarnoy pronitsaemosti u patsientov rakom legkih po dannyim ventilyatsionnoy pulmonostsintigrafii [The condition of alveolar-capillary permeability in patients with lung cancer according to ventilation pulmonoscintigraphy] Sovremennyie naukoemkie tehnologii [Modern high technologies]. 2008, №5, P. 65-66.
5. Trahtenberg A.H., Chistov V.I. Klinicheskaya onkopulmonologiya [Clinical oncology]. Moscow: 2000, 379p.
6. Fraumeni J.F. Jr. Respiratory carcinogenesis: an epidemiologic appraisal, *Journal of the National Cancer Institute.* 1975, Vol. 55(5), pp. 1039-1046.
7. García-Closas M. Malats N. Silverman D. et al. NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses, The Lancet. 2005, Vol. 366(9486), pp. 649–59.
8. Ginsberg R.J. Vokes E.E. Rosenzweig K. Non-small cell lung cancer. Cancer principles and practice of oncology. Ed. V.T. Jr. DeVita, S. Hellman, S.A. Rosenberg. 6th ed. Philadelphia, Lippincott Williams Wilkins, 2001, pp. 925-983.
9. Henry J. L. Chun-Ya H. Bruce K. L. et al. Slow Acetylator Mutations in the Human Polymorphic N-acetyltransferase Gene in 786 Asians, Blacks, Hispanics, and Whites: Application to Metabolic Epidemiology, The *American* Journal of *Human Genetics. 1993, Vol. 52, pp.* 827-834
10. Janerich D.T. Th ompson W.D. Varela L.R. et al. Lung cancer and exposure to tobacco smoke in the household, The New England Journal of Medicine. 1990, Vol. 323(10), pp. 632-636.
11. Johns M. Paulus-Thomas J. Purification of human genomic DNA from whole blood using sodium perchlorate in place of phenol, Analytical Biochemistry. 1989, Vol. 80(2), pp. 276-278.
12. Katoh T.et al.A pilot study testing the association between Nacetyltransferases 1 and 2 and risk of oral squamous cell carcinoma in Japanese people, Carcinogenesis. 1998, Vol. 19, pp. 1803–1807.
13. Kozhekbaeva Zh. M. Glotov A. S. Gra O. A. et al. Analysis of NAT2 Point Mutations with Biological Microchips, Molecular Biology. 2007, Vol. 41(4), pp. 656–664.
14. Pompeo F. Brooke E. Kawamura A. Mushtaq A. Sim E. The pharmacogenetics of NAT: structural Paspects, Pharmacogenomics. 2002, Vol. 3(1), pp. 19-30
15. Rabstein S. Unfried K. Ranft U. et al. Variation of the N-acetyltransferase 2 gene in a Romanian and a Kyrgyz population, Cancer. Epidemiol. Biomarkers. Prev. 2006, Vol.15(1), pp. 138-141.
16. Steinmaus C. Moore L.E. Shipp M. Kalman D. Rey O.A. Biggs M.L. Hopenhayn C. Bates M.N. Zheng S. Wiencke J.K. Smith A.H. Genetic polymorphisms in MTHFR 677 and 1298, GSTM1 and T1, and metabolism of arsenic, Journal Toxicol Environ Health. 2007, Vol. 70(2), pp. 159-70.
17. Wikman H. Thiel S. Jager B. et al. Relevance of N-acetyltransferase 1 and 2 (NAT1, NAT2) genetic polymorphisms in non-small cell lung cancer susceptibility. Pharmacogenetics. 2001, Vol. 11, pp. 157–68.

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GENOME AND GENETIC HEALTH OF THE YAKUT ETHNOS

ABSTRACT

The article presents a survey of the results of studies of the ethnogenesis of the peoples of Siberia in the aspect of the genetic health of the ethnos, adapted to the extreme changes in seasonal changes in the high-latitude zone. The genome inherited from ancestors with a certain protective potential provides metabolic health and stability against stressful environmental influences. The genome of the Yakut ethnos is characterized by three main genetic components (58.5% - Central Siberian, 12.5% - European, 29% - East Asian), as well as a high level of homozygosity of the genome. In the Yakut population, the negative genetic component is manifested by a wide spectrum of age-dependent, genetically predisposed neurodegenerative diseases (multiple sclerosis, Viliuisk encephalitis, Parkinson's disease and diseases with impaired motor functions, including amyotrophic lateral sclerosis and spastic paraplegia and other). Also in recent years, the population is experiencing an increase in the burden of diseases with metabolic disorders. The main reason for the growth of the negative load of health disorders of the modern Yakut population is the consequence of genetic drift and conservatism of the genome.

Keywords: ethnogenesis, Yakut population, genome, metabolic disorders, neurodegenerative diseases