УДК 6616.2-002.5:575.22](571.56)
https://doi.org/10.20538/1682-0363-2020-4-102-109

Comparative analysis of N-acetyltransferase 2 genotyping results among patients with newly diagnosed pulmonary tuberculosis residing in the Sakha Republic (Yakutia)

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ABSTRACT

Aim. To assess the variability of the *NAT2* gene and to comparatively analyze the prevalence of *NAT2* polymorphisms and acetylation types among Yakut and Russian patients newly diagnosed with pulmonary tuberculosis (TB), permanently residing in the Sakha Republic (Yakutia).

Materials and methods. The study included 197 patients with newly diagnosed pulmonary TB (132 Yakuts and 65 Russians) aged (43.3 ± 14.4). The following single-nucleotide polymorphisms were analyzed, using real-time polymerase chain reaction (PCR): *NAT2*5* (rs1801280, T341C), *NAT2*6* (rs1799930, G590A), *NAT2*7* (rs1799931, G857A), *NAT2*11* (rs1799929, C481T), *NAT2*12* (rs1208, A803G), and *NAT2*13* (rs1041983, C282T). Genetically determined basal metabolic rates were calculated using the NATpred online tool.

Results. 75% of residents, both of Yakut and Russian ethnicity, were identified as carriers of *NAT2* polymorphic variants known to be related to isoniazid biotransformation. *NAT2*6* and **13* allelic variants were more frequent in Yakuts (occurring in 40.9% and 64.4%, respectively); variants *NAT2*5, *6, *11, *12*, and **13* were more common in Russians (69.2; 55.4; 67.7; 69.2, and 64.6%, respectively). The *NAT2*5, *7, *11*, and **12* polymorphisms were found to be significantly ethnicity-dependent. The study established substantial prevalence of medium acetylation type (58.3%) in Yakuts and slow acetylation type in Russians (61.5%). Correlations were shown between ethnicity and different prevalence rates of rapid, medium, or slow acetylation types among patients with TB.

Conclusion. The observed *NAT2* polymorphism distribution patterns and isoniazid acetylation types among Yakut and Russian patients with newly diagnosed pulmonary TB demonstrated that pharmacologic responses can be significantly different between ethnic groups. Findings of pharmacogenetic studies in Yakut and Russian populations should be incorporated in clinical practice for personalized administration of isoniazid.

Key words: Yakut, Russian, tuberculosis, isoniazid, pharmacogenetics, polymorphism, *NAT2*, acetylation, isoniazid acetyltransferase.

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Conflict of interest. The authors declare no obvious or potential conflict of interest related to the publication of this article.

Source of financing. The study was financially supported by the Republican clinical hospital no. 3 of the Ministry of Health of the Sakha Republic (Yakutia).

Conformity to the principles of ethics. All patients signed an informed consent to take part in the study. The study was approved by the Ethics Committee of the Phthisiatry Research-Practice Center (Protocol No. 3 of 26.09.2018).

For citation: Krasnova N.M., Efremova E.N., Egorova A.A., Filippova O.I., Chertovskikh Y.V., Rudykh Z.A., Alekseeva E.A., Tatarinova T.E., Sokorutov D.A., Val N.S., Vinokurova M.K., Karvchenko A.F., Vengerovskii A.I., Sychev D.A. Comparative analysis of N-acetyltransferase 2 genotyping results among patients with newly diagnosed pulmonary tuberculosis residing in the Sakha Republic (Yakutia). *Bulletin of Siberian Medicine.* 2020; 19 (4): 102–109. https://doi.org/10.20538/1682-0363-2020-4-102-109.

Сравнительный анализ результатов генотипирования гена N-ацетилтрансферазы 2 у пациентов с впервые выявленным туберкулезом органов дыхания, проживающих в Республике Саха (Якутия)

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РЕЗЮМЕ

Цель. Оценить вариабельность гена *N*-ацетилтрансферазы 2 (*NAT2*), провести сравнительный анализ распространенности его полиморфизмов гена *NAT2* и типов ацетилирования среди якутов и русских с впервые выявленным туберкулезом органов дыхания, проживающих в Республике Саха (Якутия).

Материалы и методы. В исследование включены 197 пациентов (132 якута и 65 русских) в возрасте (43,3 ± 14,4) года с впервые выявленным туберкулезом органов дыхания. Методом полимеразной цепной реакции в режиме реального времени исследованы однонуклеотидные полиморфизмы *NAT2*5* (rs1801280, T341C), *NAT2*6* (rs1799930, G590A), *NAT2*7* (rs1799931, G857A), *NAT2*11* (rs1799929, C481T), *NAT2*12* (rs1208, A803G), *NAT2*13* (rs1041983, C282T). Генетически детерминированную скорость метаболизма рассчитывали с помощью онлайн-калькулятора NATpred.

Результаты. Полиморфные варианты гена *NAT2*, ассоциированные со скоростью биотрансформации изониазида, встречаются у 75% якутов и всех русских, проживающих в Якутии. Якуты являются частыми носителями аллельных вариантов *NAT2*6* и **13* (с частотой встречаемости 40,9 и 64,4% соответственно), русские – носителями *NAT2*5, *6, *11, *12 и *13* (с частотой встречаемости 69,2; 55,4; 67,7; 69,2 и 64,6% соответственно). Распределение полиморфизмов *NAT2*5, *7, *11, *12* значимо зависит от национальности. Установлена большая распространенность промежуточного типа ацетилирования (58,3%) среди якутов, медленного типа – среди русских (61,5%). Различия распространенности быстрого, промежуточного и медленного типов ацетилирования у пациентов с туберкулезом зависят от национальности.

Заключение. Особенности распределения полиморфизмов гена *NAT2* и типов ацетилирования изониазида среди пациентов якутской и русской национальности с впервые выявленным туберкулезом органов дыхания свидетельствуют о том, что фармакологический ответ может значительно различаться среди пациентов национальных групп. Данные фармакогенетического исследования у якутов и русских необходимо учитывать в клинической практике для персонализированного применения изониазида.

Ключевые слова: якуты, русские, туберкулез, изониазид, фармакогенетика, полиморфизм, NAT2, медленный, быстрый, промежуточный, тип ацетилирования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Исследование выполнено при финансовой поддержке ГАУ РС(Я) «Республиканская клиническая больница № 3» Министерства здравоохранения Республики Саха (Якутия).

Соответствие принципам этики. Все пациенты подписали информированное согласие на проведение исследования. Исследование одобрено локальным этическим комитетом ГБУ РС(Я) «Научно-практический центр «Фтизиатрия» (протокол № 3 от 26.09.2018).

Для цитирования: Краснова Н.М., Ефремова Е.Н., Егорова А.А., Филиппова О.И., Чертовских Я.В., Рудых З.А., Алексеева Е.А., Татаринова Т.Е., Сокорутов Д.А., Валь Н.С., Винокурова М.К., Кравченко А.Ф., Венгеровский А.И., Сычёв Д.А. Сравнительный анализ результатов генотипирования гена N-ацетилтрансферазы 2 у пациентов с впервые выявленным туберкулезом органов дыхания, проживающих в Республике Саха (Якутия). *Бюллетень сибирской медицины.* 2020; 19 (4): 102–109. https://doi.org/10.20538/1682-0363-2020-4-102-109.

INTRODUCTION

Conventionally recommended treatment for newly identified drug-sensitive pulmonary tuberculosis consists of a combination of 4 most effective anti-TB drugs, such as isoniazid, rifampicin, pyrazinamide, and ethambutol, administered in standard doses (http://cr.rosminzdrav.ru/#!/schema/943). In reality, individual differences in pharmacologic responses to these drugs, developing quite often, include poor chemotherapy outcomes in some patients, possible development of *M. tuberculosis* drug resistance followed by disease relapse, and adverse drug reactions [1]. In particular, isoniazid is a drug with a known hepatotoxic effect, which can cause liver damage with clinical manifestations ranging from asymptomatic hyperenzymemia (10–20% of patients) to severe hepatitis or acute hepatic failure (0.5–1%) [2]. Toxic liver effect is produced by highly active isoniazid metabolites, hydrazine and acetylhydrazine [3, 4].

Isoniazid is metabolized in the liver through reactions of acetylation and hydrolysis. These reactions are catalyzed by N-acetyltransferase-2 (*NAT2*) and acylamidase, respectively [5]. A *NAT2* isozyme is encoded by a highly polymorphic gene with 106 alleles established to date. *NAT2* activity is determined by single-nucleotide substitution in the backbone region of the encoding gene [6, 7]. Combinations of *NAT2* gene alleles produce a variety of isoniazid acetylation phenotypes: rapid acetylator (presence of 1 or 2 "rapid" alleles); medium acetylator (1 "slow" allele); slow acetylator (2 "slow" alleles) [5, 8].

NAT2 gene polymorphism distribution is known to vary substantially and has been shown to correlate with race, ethnic origin, and place of residence [9–11]. The aim of this study was to assess the variability of *NAT2* gene and to comparatively analyze prevalence of *NAT2* gene polymorphisms and acetylation types among Yakuts and Russians with newly identified pulmonary tuberculosis (PTB).

MATERIALS AND METHODS

Single-center, one stage, observational sampling study was conducted, including 197 patients with newly identified PTB, selected from representatives of 2 ethnic groups living in the Sakha Republic (Yakutia): 132 Yakuts (77 women, 55 men) and 65 Russians (35 women, 30 men). Patients were hospitalized to Phthisiatry Research-Practice Center in Yakutsk during the intensive chemotherapy phase. Patient's average age was 43.3 ± 14.4 years. Inclusion criteria were PTB diagnosed for the first time, age of 18 years or over, informed consent, and Yakut or Russian ethnicity. Ethnicity was established based on self-definition by patients and their parents; family trees were also analyzed to the second generation. In earlier studies, it was shown that ethnic self-definition corresponded

to microsatellite analysis in 99.9% of cases [12]. Descendants from mixed marriages and patients who did not meet any of the inclusion criteria were excluded.

Blood for genetic analysis was obtained from a superficial elbow vein. Using evacuated blood collection systems, whole blood specimens were collected in 4 mL tubes coated with finely dispersed ethylenediaminetetraacetic acid (Zhejiang Gongdong Medical Technology Co., Ltd); then desoxyribonucleic acid (DNA) was isolated using the ExtractDNA Blood reagent kit (Evrogen, Russia). Using Real-Time CFX96 Touch (Bio-Rad, USA) PCR system and GenTest-M *NAT2* (Nomotek, Russia) reagent kit, we identified the presence of the following polymorphic variants: *NAT2*5* (rs1801280, T341C), *NAT2*6* (rs1799930, G590A), *NAT2*7* (rs1799931, G857A), *NAT2*11* (rs1799929, C481T), *NAT2*12* (rs1208, A803G), and *NAT2*13* (rs1041983, C282T). Genetically determined basal metabolic rates were calculated using NATpred online calculator [13].

Statistical analysis was performed using IBM SPSS Statistics ver. 23. Pearson's chi-squared test and its modification with Yates's correction were used for analysis. Compliance of genotype distribution with Hardy – Weinberg equilibrium was checked using 95% Clopper – Pearson confidence intervals. The critical significance level p was 0.05.

RESULTS

Yakut and Russian patients newly diagnosed with TB had the following polymorphic *NAT2* gene variants known to be linked with the isoniazid biotransformation rate: *NAT2*5*, **6, *7, *11, *12,* and **13*. In Yakuts, allele and genotype distributions of *NAT2* polymorphisms were consistent with Hardy – Weinberg equation ($p > 0.05$). In Russians permanently living in Yakutia, allele and genotype distributions of *NAT2*5, *6, *7, *12*, and **13* polymorphisms complied with the Hardy – Weinberg equation, only the *NAT2*11* polymorphism did not correspond to the equilibrium (Table 1).

NAT2 polymorphic variants were found in 75% (99 / 132) of Yakut patients and in all Russian patients (65 / 65). Two most frequent allelic variants found among Yakuts were *NAT2*6* (40.9%) and *NAT2*13* (64.4%). In Russians, the following polymorphic variants were observed with almost the same frequencies: *NAT2*5, *6, *11, *12*, and **13* (69.2%, 55.4%, 67.7%, 69.2%, and 64.6%, respectively) (Table 1).

Statistically significant ethnicity-dependent differences were observed in the prevalence of single-nucleotide polymorphisms (SNPs) *NAT2*5, *7, *11, *12* (Table 1). Polymorphic variants *NAT2*6* and *NAT2*13* were equally frequent among Yakuts and Russians.

Table 1

Comparison of allele and genotype frequencies of *NAT2* gene polymorphisms in Yakuts and Russians with newly identified pulmonary tuberculosis (PTB)

Polymorphism	Yakuts (n = 132)							Russians (n = 65)						
	Genotype, n (%)			Allele, %		χ^2	P	Genotype, n (%)			Allele, %		χ^2	P
NAT2*5 (T341C)	T/T	T/C	C/C	T	C	1.17	0.558	T/T	T/C	C/C	T	C	1.28	0.527
	91 (68.9)	35 (26.5)	6 (4.6)	0.82	0.18			20 (30.8)*	36 (55.4)*	9 (13.8)*	0.58	0.42		
NAT2*6 (G590A)	G/G	G/A	A/A	G	A	0.64	0.727	G/G	G/A	A/A	G	A	0.004	0.998
	78 (59.1)	49 (37.1)	5 (3.8)	0.78	0.22			29 (44.6)	29 (44.6)	7 (10.8)	0.67	0.33		
NAT2*7 (G857A)	G/G	G/A	A/A	G	A	0.01	0.993	G/G	G/A	A/A	G	A	0.36	0.976
	94 (71.2)	35 (26.5)	3 (2.3)	0.84	0.16			56 (86.2)*	9 (13.8)*	0 (0)*	0.93	0.07		
NAT2*11 (C481T)	C/C	C/T	T/T	C	T	0.00	1.000	C/C	C/T	T/T	C	T	6.70	0.035
	90 (68.2)	38 (28.8)	4 (3.0)	0.83	0.17			21 (32.3)*	40 (61.5)*	4 (6.2)*	0.63	0.37		
NAT2*12 (A803G)	A/A	A/ G	G/G	A	G	0.17	0.916	A/A	A/ G	G/G	A	G	2.07	0.354
	91 (68.9)	38 (28.8)	3 (2.3)	0.83	0.17			20 (30.8)*	37 (56.9)*	8 (12.3)*	0.59	0.41		
NAT2*13 (C282T)	C/C	C/T	T/T	C	T	5.89	0.052	C/C	C/T	T/T	C	T	0.72	0.699
	47 (35.6)	74 (56.1)	11 (8.3)	0.64	0.36			23 (35.4)	34 (52.3)	8 (12.3)	0.62	0.38		

Note. $\chi 2$ – Pearson's chi-square test, p – statistically significant differences (< 0.05). * significant differences, compared with Yakuts, $p < 0.05$.

58.3% (77 / 132) of Yakuts with newly diagnosed TB were characterized by medium acetylators, while in 22.7% (30/132) and 18.9% (25/132) slow and rapid acetylators, respectively, were observed. Among Russians, slow type was detected in 61.5% (40 / 65), medium type – in 35.4% (23 / 65), and rapid type – in 3.1% (2 / 65).

Differences in the prevalence of 3 acetylation types significantly depended on ethnicity ($\chi^2 = 30.977$; $p = 0.000$).

Occurrence of NAT2*6, *7, and *11 genotypes among slow and medium acetylators did not differ much between Yakut and Russian patients, unlike the prevalence of NAT2*5, *12, and *13 polymorphisms, which showed statistically significant differences.

74% (57 / 77; CI [0.62–0.83]) of Yakut medium acetylators were carriers of homozygous T/T genotype of NAT2*5 (T341C). In the group of Russian patients, this carriage was observed in 39.1% of cases (9 / 23; CI [0.19–0.61]) ($p < 0.05$).

Heterozygous T/C NAT2*5 genotype was identified in 24.7% of Yakut (19 / 77; CI [0.15–0.35]) and 60.9% of Russian (14 / 23 CI [0.38–0.80]) ($p < 0.05$) medium acetylators (Table 2).

Table 2

Acetylation types in patients with PTB with different genotypes of *NAT2* gene polymorphisms

Polymorphisms	Genotype	Yakuts (n = 132)			Russians (n = 65)		
		Slow acetylator (n = 30), n (%)	Medium acetylator (n = 77), n (%)	Rapid acetylator (n = 25), n (%)	Slow acetylator (n = 40), n (%)	Medium acetylator (n = 23), n (%)	Rapid acetylator (n = 2), n (%)
NAT2*5 (T341C)	T/T	9 (30.0)	57 (74.0)	25 (100.0)	9 (22.5)	9 (39.1)	2 (100.0)
	T/C	16 (53.3)	19 (24.7)	0	22 (55.0)	14 (60.9)	0
	C/C	5 (16.7)	1 (1.3)	0	9 (22.5)	0	0
NAT2*6 (G590A)	G/G	12 (40.0)	41 (53.2)	25 (100.0)	11 (27.5)	16 (69.6)	2 (100.0)
	G/A	15 (50.0)	34 (44.2)	0	23 (57.5)	6 (26.1)	0
	A/A	3 (10.0)	2 (2.6)	0	6 (15.0)	1(4.3)	0
NAT2*7 (G857A)	G/G	16 (53.3)	53 (68.8)	25 (100.0)	32 (80.0)	22 (95.7)	2 (100.0)
	G/A	12 (40.0)	23 (29.9)	0	8 (20.0)	1 (4.3)	0
	A/A	2 (6.7)	1 (1.3)	0	0	0	0
NAT2*11 (C481T)	C/C	9 (30.0)	56 (72.7)	25 (100.0)	9 (22.5)	10 (43.5)	2 (100.0)
	C/T	18 (60.0)	20 (26.0)	0	27 (67.5)	13 (56.5)	0
	T/T	3 (10.0)	1 (1.3)	0	4 (10.0)	0	0
NAT2*12 (A803G)	A/A	9 (30.0)	57 (74.0)	25 (100.0)	10 (25.0)	8 (34.8)	2 (100.0)
	A/G	19 (63.3)	19 (24.7)	0	23 (57.5)	14 (60.9)	0
	G/G	2 (6.7)	1 (1.3)	0	7 (17.5)	1 (4.3)	0
NAT2*13 (C282T)	C/C	3 (10.0)	19 (24.7)	25 (100.0)	6 (15.0)	15 (65.2)	2 (100.0)
	C/T	18 (60.0)	56 (72.7)	0	27 (67.5)	7 (30.4)	0
	T/T	9 (30.0)	2 (2.6)	0	7 (17.5)	(4.3)	0

Among medium acetylators, 74% of Yakuts (57/77; CI [0.62–0.83]) and 34.8% of Russians (8 / 23; CI [0.16–0.57]) ($p < 0.05$) had A/A genotype of NAT2*12 (A803G). Carriers of heterozygous A/G NAT2*12 genotype were found more frequently among Russian patients (60.9%, 14 / 23; CI [0.38–0.80]), as opposed to rarer occurrence of this genotype among Yakut patients (24.7%, 19 / 77; CI [0.15–0.35]) ($p < 0.05$). C/C NAT2*13 genotype (C282T) was present in 24.7% of Yakuts (19 / 77; CI [0.15–0.35]) and 65.2% of Russians (15 / 23; CI [0.42–0.83]) ($p < 0.05$). In addition, Yakuts were more frequent carriers of T/C NAT2*13 heterozygote (72.7%, 56 / 77; CI [0.61–0.82]) than Russians (30.4%, 7 / 23; CI [0.13–0.52]) ($p < 0.05$).

DISCUSSION

Genetic diversity of the *NAT2* gene and acetylation phenotypes developed as a result of human adaption to living environment. Transition from nomadic to sedentary life profoundly changed food choices, resulting in the body being exposed to novel pathogens and xenobiotics. Further, due to the need for better survival the activity of detoxifying enzymes had been altered, producing a new heritable phenotype of biotransformation [14].

Correlation between ethnicity and the prevalence of *NAT2* gene polymorphisms has been observed across the globe. Based on data from the International Genome Sample Resource (IGSR; https://www.internationalgenome.org/), NAT2*5, *11, and *12 polymorphic variants are more prevalent among the populations of Europe and South Asia (68.4% and 56.5% (first variant); 67.6% and 53.1% (second variant); 67.2% and 58.1% (third variant)). Variants NAT2*5, *11, and *12 have been observed in 7.3%, 7.1%, and 7.7% of the population of East Asia, respectively. NAT2*7 polymorphism is frequent among native population of East Asia (31.8%), but is rare among Europeans (4.6%). NAT2*6 polymorphism has been detected in 58.7% of people living in South

Asia, showing equal rates among populations of Europe (46.9%) and East Asia (43.2%). Proportions of people carrying *NAT2*13* variant are nearly the same among Asian and European races (50.5% of Asians, 69.4% of Europeans).

Our study demonstrated higher frequencies of NAT2*6 and NAT2*13 allelic variants among Yakuts (Table 1), which complies with previously reported prevalence rates among Asians. The frequency of NAT2*5, NAT2*11, and NAT2*12 variants among Yakuts was 31.1%, 31.8%, and 31.1%, respectively, which was inconsistent with previously estimated proportions among Asian people. The allelic variant NAT2*7 had almost the same occurrence among Yakuts (28.8%) and people from East Asia (31.8%); however, its frequency was lower in the population of South Asia (13.5%).

The frequencies of NAT2*5, *6, *11, *12, and *13 polymorphic variants among Russians were 69.2%, 55.4%, 67.7%, 69.2%, and 64.6%, respectively (Table 1). The frequency of NAT2*7 polymorphism in Russians residing in Yakutia was higher than in residents of Europe (13.8% and 4.6%, respectively).

Comparative analysis of NAT2 genotype distribution showed that Russian patients were more frequent carriers of NAT2*5, *11, and *12 than Yakuts (Table 1). To date, evidence is lacking on the contribution of NAT2*5 and *11 genotypes to severity and frequency of isoniazid-induced liver damage in patients with tuberculosis. There is a known correlation between increased risk of isoniazid-induced hepatotoxicity and minor allele homozygous genotypes, compared with the same risk in carriers of major alleles of NAT*5 and *11 [15, 16].

Polymorphic variant NAT2*7 was more frequent in Yakuts (28.8%) than in Russians (13.8%) ($p < 0.05$). Genotype A/A NAT2*7 was observed in a small number of Yakut patients (2.3%) and in none of the Russian patients (Table 1). Few studies have reported inconclusive data on association between minor allele A NAT2*7 and hepatotoxicity risk. Some authors pointed out a higher risk of hepatotoxic reactions to first-line anti-TB drugs in individuals with A/A genotype, in contrast to carriers of G/G genotype [17, 18], while other researchers reported absence of such associations [2, 19].

Major alleles of NAT2*5, *6, and NAT2*7 encode synthesis of NAT2 with altered amino acid sequence and, therefore, lower activity. People with NAT2*5 allele in combination with NAT2*6, or *7 polymorphic variant are slow acetylators [8]. Geographic distribution of slow acetylators has been well studied: this phenotype occurs in 60% of the population of Europe, Middle East, North Africa, and South Asia, and in 10% of the population of East Asia and Native Americans [20].

In Yakut population, the most widespread acetylation type was medium type (58.3%), while Russians mostly were characterized by slow acetylation type (61.5%). The proportion of rapid acetylators was much larger among Yakuts, than among Russians (18.9% versus 3.1%). This is consistent with previous comparative studies among Asians and Caucasians.

In clinical practice, NAT2 polymorphism and genetically determined variability in isoniazid acetylation speed can have a considerable impact on the outcome and safety of tuberculosis pharmacotherapy. A link between liver damage rate and slow acetylation type was confirmed in several meta-analyses [21–24]. Slow acetylators showed high serum concentrations of isoniazid and its toxic metabolites [25]. Rapid acetylators had lower serum isoniazid concentrations, but higher risk of drug resistance to *M. tuberculosis* [25–28].

CONCLUSION

Our study results suggest that NAT2 gene polymorphisms linked to isoniazid acetylation have considerable prevalence rates among Yakuts and Russians. Yakuts mostly tended to be carriers of allelic variants NAT2*6 and *13, while Russians mostly carried variants NAT2*5, *6, *11, *12, and *13. Comparative analysis within the study sample showed the presence of statistically significant differences in frequencies of NAT2*5, *7, *11, and *12 genotypes, depending on ethnicity. As a result of NAT2 genotype combinations, Yakuts tended to develop mostly medium acetylation type, while Russians more often developed slow acetylation type. The observed patterns in distributions of NAT2 gene polymorphisms and acetylation types among Yakuts and Russians with newly identified TB can serve as a confirmation that pharmacologic responses can substantially differ depending on patients' ethnicity.

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Received 19.04.2020
Accepted 29.09.2020