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Genetic diversity of the human immunodeficiency virus (HIV-1) in the Kaliningrad region

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Introduction. As is currently known, the epidemic process in the Kaliningrad Region was mainly associated with the spread of the recombinant form of HIV-1 (CRF03_AB); however, regular HIV importations from other countries and continents has created favorable conditions for emergence and spread of various recombinant forms of the virus.
The most complete information on the diversity of recombinant forms in the region is also necessary to understand the structure of drug resistance (DR).
The aim of the study was to explore the HIV-1 genetic diversity in the Kaliningrad Region.
Materials and methods. We studied 162 blood plasma samples obtained from patients from the Kaliningrad Region, both with confirmed virological failure of antiretroviral therapy (ART) and with newly diagnosed HIV infection. For reverse transcription and amplification of HIV genome fragments, diagnostic «AmpliSense HIVResist-Seq».
Results and discussion. The various recombinants between subtypes A and B (74%) were predominant in study group: recombinant was between CRF03_AB and subtype A (33.95%) and CRF03_AB-like (13.58%) were the most common. Among the «pure» subtypes of the virus, subtype A6 (16.67%). The circulation of subtypes B (3.70%) and G (1.23%) was also noted.
Ninety-six patients (59.26%) were identified with at least one mutation associated with antiretroviral (ARV) drug resistance.
Conclusion. The observed diversity of subtypes and recombinant forms of the virus implies that the new recombinants are actively emerging in the studied region, both between existing recombinant forms and "pure" subtypes, as well as between "pure" subtypes.

Keywords: *human immunodeficiency virus; HIV; recombinant forms of HIV; HIV drug resistance; laboratory diagnostics*

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Генетическое разнообразие вируса иммунодефицита человека (ВИЧ-1) в Калининградской области

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Введение. Как известно на сегодняшний день, эпидемия ВИЧ-инфекции в Калининградской области преимущественно была связана с распространением рекомбинантной формы вируса (CRF03_AB), однако регулярные заносы ВИЧ из других стран и частей света создали благоприятные условия для формирования и распространения его разнообразных рекомбинантных форм.
Наиболее полная информация о разнообразии рекомбинантных форм в регионе необходима для понимания структуры лекарственной устойчивости (ЛУ), так как влияние ассоциированных с ней мутаций на приспособленность вируса может быть неодинаковым для разных субтипов, причём рекомбинантные формы могут сочетать в своём геноме наиболее удачные паттерны мутаций, что позволит ВИЧ с большей эффективностью противостоять антиретровирусной терапии.
Цель работы. Изучение генетического разнообразия ВИЧ-1 в Калининградской области.
Материалы и методы. Исследованы 162 образца плазмы крови, полученные от пациентов из Калининградской области как с подтверждённой вирусологической неэффективностью антиретровирусной терапии, так и с впервые выявленной ВИЧ-инфекцией. Для обратной транскрипции и амплификации ВИЧ использовали диагностический набор «АмплиСенс HIVResist-Seq» (ЦНИИЭ, Россия).
Результаты и обсуждение. Доминирующими в группе являлись различные рекомбинанты между субтипами A и B (74%), в том числе CRF03_AB и субтипом A (33,95%) и рекомбинантная форма, схожая с CRF03_AB (CRF03_AB-like (13,58%). Среди «чистых» субтипов вируса доминирует характерный для территории Российской Федерации суб-субтип – A6 (16,67%), одновременно с ним циркулируют субтипы B (3,70%) и G (1,23%).
Были выявлены 96 пациентов (59,26%) хотя бы с одной мутацией, ассоциированной с ЛУ к антиретровирусным препаратам.
Заключение. Выявленное разнообразие субтипов и рекомбинантных форм вируса указывает на то, что в исследуемом регионе продолжается активный процесс формирования новых рекомбинантов, причём между как уже существующими рекомбинантными формами и «чистыми» субтипами, так и между «чистыми» субтипами.

Ключевые слова: *вирус иммунодефицита человека; ВИЧ; рекомбинантные формы ВИЧ; лекарственная устойчивость ВИЧ; лабораторная диагностика*

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Introduction

The human immunodeficiency virus type 1 (HIV-1) was first isolated in the 1980s and has been circulating in the human population for almost 100 years. The stable phylogenetic structure that has been developed over that time is represented by three main groups: N, O, M; the latter, in its turn, is divided into 12 distinct subtypes comprising viral isolates that are more closely related to each other than to isolates from other subtypes [1, 2]. Classification of HIV-1 was originally based on sequences of subgenomic regions or individual genes; however, recent improvements in sequencing methods made it possible to classify HIV-1 based on full-length genomes or sequences from multiple subgenomic regions. As a result, isolates could be identified with distinctive parts of their genomes corresponding to different subtypes: These isolates are products of recombination between parental strains belonging to different subtypes. When a particular recombinant form is identified in three or more individuals with no direct epidemiological linkage, it is classified as a circulating recombinant form (CRF). At present, there are more than one hundred known CRFs [3] being responsible for at least 20% of global HIV epidemics [4], with recombinant forms prevailing in several regions such as Western and Central Africa (CRF02_AG) [5, 6] and Southeast Asia (CRF01_AE) [7, 8].

Recombinant forms of the virus are the product of recombination during reverse transcription. During the minus-strand deoxyribonucleic acid (DNA) synthesis, the reverse transcriptase (RT) shifts from one ribonucleic acid (RNA) strand to the other with a high frequency, suggesting that at least both genomic RNA copies are used alternatively as templates. The copy choice switching rate in HIV-1 for highly similar template RNAs is estimated at $3 \times 10^{-4} – 1.4 \times 10^{-3}$ events per nucleotide, i.e. 3–12 template switches per genome replication [9–11]. Importantly, the formation of virions containing two different genomic RNAs depends on fulfilment of certain prerequisites: First, two or more viruses with different genotypes must infect the same cell; second, genomic RNAs of different origin must be subsequently co-packaged. Such situation can be associated either with coinfection or superinfection of a patient with different virus subtypes.

The fact that the Nef and Vpu proteins downregulate the expression of CD4 and co-receptors during HIV-1 infection implies that superinfections are of extremely rare occurrence [12]. Nevertheless, the *in-situ* hybridization of cells from patients showed that single cells could contain more than four different proviruses [13, 14]. In addition, the high recombination rates and spread of recombination forms demonstrated by recent studies imply high rates of coinfection *in vivo* [4, 15]. Thus, different HIV subtypes must co-circulate in a region to activate the generation of recombinant forms of the virus.

In the Russian Federation, the most prevalent sub-subtype of the virus is A6 also known as IDU-A (Injecting Drug Users) or A-FSU (former Soviet Union countries). This sub-subtype was previously classified as A1; however, being significantly different from other HIV-1 variants of sub-subtype A1 by its structure and transmission, it was set apart as an individual, relatively uniform group [16, 17]. In the meantime, some regions have become favorable for co-circulation of several subtypes, including the Kaliningrad Region and its central city, which is a major transportation hub with railroads and highways, sea and river ports, and the international airport.

As is currently known, the epidemic process the Kaliningrad Region was initially associated with the spread of the recombinant form of the virus (CRF03_AB) among injecting drug users. Later, the HIV infection expanded beyond the vulnerable groups of population. In addition, regular importation of HIV from other countries and continents has created favorable conditions for

emergence of various new recombinant forms of the virus in the region [18, 19].

Notably, the spread of subtypes and recombinant forms in the HIV-1 epidemic is very dynamic: the contemporary virus genetic diversity is represented by a mixture of recombinants, which emerged during earlier stages of the global epidemic, and recombinants, which emerged later; all of them are contributing to creation of more complex recombinant forms, which subsequently will make their contribution to the dynamics of the HIV-1 global population. It can be assumed that the process of generation of increasingly complex recombinant forms will constitute the core of the virus evolution in the Kaliningrad Region.

The aim of the study was to explore the HIV-1 genetic diversity in the Kaliningrad Region.

Materials and methods

The study was performed in 2014–2018 using clinical material from 162 patients from the Kaliningrad Region, both with confirmed virological failure on antiretroviral therapy (ART) and with newly diagnosed HIV infection. To detect HIV resistant strains, the blood plasma was delivered to the North-West District Center for AIDS Prevention and Control (NWD AIDS Center) of the St. Petersburg Pasteur Research Institute of Epidemiology and Microbiology.

The blood plasma was used for measuring the viral load with the AmpliSens HIV-Monitor-FRT reagent kit (the Central Research Institute of Epidemiology, Russia) with the sensitivity threshold of 500 copies/ml. The samples with detectable viral load were further tested through reverse transcription polymerase chain reaction (RT-PCR) and Sanger sequencing. Diagnostic RT-PCR-kit-Pro/Rev and PCR-kit-Pro/Rev kits (the Central Research Institute of Epidemiology, Russia) were used for HIV reverse transcription and amplification; the sequencing reaction was performed in accordance with the manual of the AmpliSens HIVResist-Seq kit (Central Research Institute of Epidemiology, Russia). The genotyping of HIV-1 was based on the analysis of nucleotide sequences of the pol gene fragment of 1,302 nt, encoding protease (PR) and part of reverse transcriptase (RT) in the 2,253–3,554 nt region; the coordinates are given for HIV HXB2 (GenBank accession number K03455.1) in the international GenBank database. The products of the sequencing reaction were analyzed using the ABI Prism 3500 genetic analyzer (Applied Biosystems, United States).

The primary analysis of nucleotide sequences was performed using the NCBI Blast program to compare with nucleotide sequences available in the international GenBank database. The alignment of nucleotide sequences was performed using the MEGA 7.0 software and the ClustalW algorithm [20]. For phylogenetic trees and for the subsequent phylogenetic analysis, we used the Neighbor-Joining algorithm to optimize trees in accordance with the balanced minimum evolution criterion. To assess the significance of phylogenetic relationships, we used the bootstrap method to generate multiple samples for 1,000 independent constructions of each phylogenetic tree.

The genotyping of studied isolates was performed using the REGA HIV-1 Subtyping Tool 3.0 software [21] and the analysis of their phylogenetic relationships with reference sequences from the international GenBank database. To identify and analyze recombinant forms, we used the REGA HIV-1 Subtyping Tool 3.0 software and the parameters preset in the software (window size – 400 bp; step size – 20). The HIV-1 genetic sequences were assessed for drug-resistance (DR) mutations using the Stanford database (Stanford HIV DB) [22]. The mutational profiles were analyzed by building linear diagrams using the Linear Diagram Generator software [23].

The statistical analysis of the data was performed using MS Excel Professional Plus 2013 (Microsoft), Prizm v5.0 (GraphPad Software Inc.) software. The statistical error was measured using the Clopper-Pierson exact interval. The results were presented with a 95% confidence interval (CI). To measure the significance of differences between numerical data obtained in paired comparisons, we used (depending on the characteristics of samples) Fisher's exact test or the χ^2 test with Yates' correction. The probability value as the significance threshold for differences was set at $p < 0.05$.

The study was performed with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the St. Petersburg Pasteur Research Institute of Epidemiology and Microbiology (Minutes No. 3 of 7/4/2010 and Minutes No. 47 of 25/12/2018).

Results and discussion

The sequences of 162 HIV-1 isolates were obtained to be further deposited to GenBank under numbers ON367567–ON367728. The sub-subtype was identified for all of them (**Table 1**). Both the data obtained during the genotyping using the REGA HIV Subtyping Tool 3.0 and jumping profile Hidden Markov Model (jpHMM) (Supplement A) and the results of the phylogenetic analysis conducted using the Mega X software (**Fig. 1**) were taken into consideration.

The dominance of recombinants between subtypes A and B in the region, including CRF03_AB, is supported by the published data on the genetic diversity of the virus in the Kaliningrad Region [16, 18, 19]. Nevertheless, the heterogeneity of recombinants inside the clade in the phylogenetic tree is of interest, being associated with characteristics of epidemiological relationships and new circulating recombinant forms emerging in the region. It requires further studies on full-length genomes of virus isolates from the Kaliningrad Region.

Among recombinants between subtypes A and B, special attention should be given to three isolates marked in the phylogenetic tree (**Fig. 1**). In the dendrogram, recombinants between subtypes A and B form a large heterogenous cluster, while the above isolates are separated from them, although their *pol* gene has fragments related to subtypes A and B, as confirmed by the comparative recombination analysis using different

Table 1. Distribution of isolates by HIV-1 subtypes
Таблица 1. Распределение исследованных штаммов по субтипам ВИЧ-1

Subtype Субтип	Number of isolates Количество штаммов	Sample Share, % Доля в выборке, %	95% CI, % 95% ДИ, %	
			–	+
HIV-1 Subtype A3	2	1,23	0,15	4,39
HIV-1 Subtype A6	27	16,67	11,28	23,31
HIV-1 Subtype B	6	3,70	1,37	7,89
HIV-1 Subtype G	2	1,23	0,15	4,39
HIV-1 CRF03_AB	41	25,31	18,81	32,73
HIV-1 CRF03_AB-like	22	13,58	8,71	19,84
Recombinant of 03_AB, A	55	33,95	26,71	41,79
HIV-1 CRF02_AG	4	2,47	0,68	6,20
Recombinant of A1, B	2	1,23	0,15	4,39
Recombinant of K, J	2	1,23	0,15	4,39

software (**Fig. 2**). At the same time, their subtyping with different online tools (REGA, Geno2Pheno, NCBI, Stanford HIV DB, RIP) precludes from making the conclusion about the genotypic affinity of these isolates. Note that all the three specimens have different location in the dendrogram: isolate HIV1_2014_24_KG is closest to other recombinant forms between subtypes A and B; isolate HIV1_2014_19_KG clusters with subtype B isolates; isolate HIV1_2014_25_KG forms the earliest branch – at the level of divergence of recombinants A and B as well as other HIV-1 subtypes. All the three isolates were collected from patients who were infected relatively recently (less than one year ago) and were not administered ART. The analysis of genetic sequences in chromatograms revealed multiple degenerated fragments, i.e. presence of several different nucleotides at the same positions in the genome. As known, the observed situation can be indicative of the diversity of the viral population in the patient, including coinfection with different HIV subtypes [24]. Thus, it can be assumed that the above isolates are at the beginning of the retroviral recombination process, being primarily represented by virus variants with genomes of A and B isolates co-packaged in the capsid rather than by recombinant forms.

In addition to recombinants between subtypes A and B, the study has revealed CRF02_AG CRFs, which are of rare occurrence in the European part of Russia, and recombinants between subtypes K and J [26].

Among the "pure" subtypes of the virus, the leading place is taken by subtype A common in Russia and represented by two sub-subtypes – A6 (16.67%; 95% CI 11.28–23.31%) and A3 (1.23%; 95% CI 0.15–4.39%); the co-circulating subtypes are subtypes B (3.70%; 95% CI 1.37–7.89%) and G (1.23%; 95% CI 0.15–4.39%).

The studied region demonstrates the distribution of HIV-1 subtypes, which is different from other regions of Russia in general and the North-West Federal District in particular [27–29]. To compare the significance of differences in the genetic diversity among regions of the North-West Federal District, we selected sub-subtype A6, subtype B, and recombinant forms between subtypes A and B, as they have been detected not only in the samples analyzed in this study, but also in isolates from Arkhangelsk [28] and Leningrad Regions [29]. The significance of differences was assessed using the χ^2 test with Yates' correction. No significant differences between the occurrence frequencies of HIV-1 subtypes in Arkhangelsk and Leningrad Regions were found, though statistically significant differences in the genetic diversity were demonstrated by the above regions and the Kaliningrad Region (χ^2 is 254.277; the critical value of χ^2 is 13.277 at the significance level $p = 0.01$).

The differences in the genetic diversity result from the dominance of HIV-1 recombinant forms in the Kaliningrad Region, while they were of rare occurrence in Arkhangelsk and Leningrad Regions. At the same time, the diversity of "pure" subtypes of HIV-1 matches the diversity described in published studies [27–29], with dominating subtype A, primarily sub-subtype A6.

The observed dominance of virus variants, which are recombinants between CRF03_AB and subtype A, as well as the dominance of the recombinant form similar to CRF03_AB, though having a number of differences (CRF03_AB-like), support the assumption stating that the long-lasting co-circulation of recombinant forms and "pure" subtypes of the virus results in emergence of new, more complex recombinant forms and new fragments included in the genome [4].

In addition to the genotypic analysis, we analyzed the frequency of DR associated mutations in this region. The analysis included isolates collected from patients with ART failure (n = 107) and from patients with newly diagnosed infection (n = 55). The primary DR was detected only in two

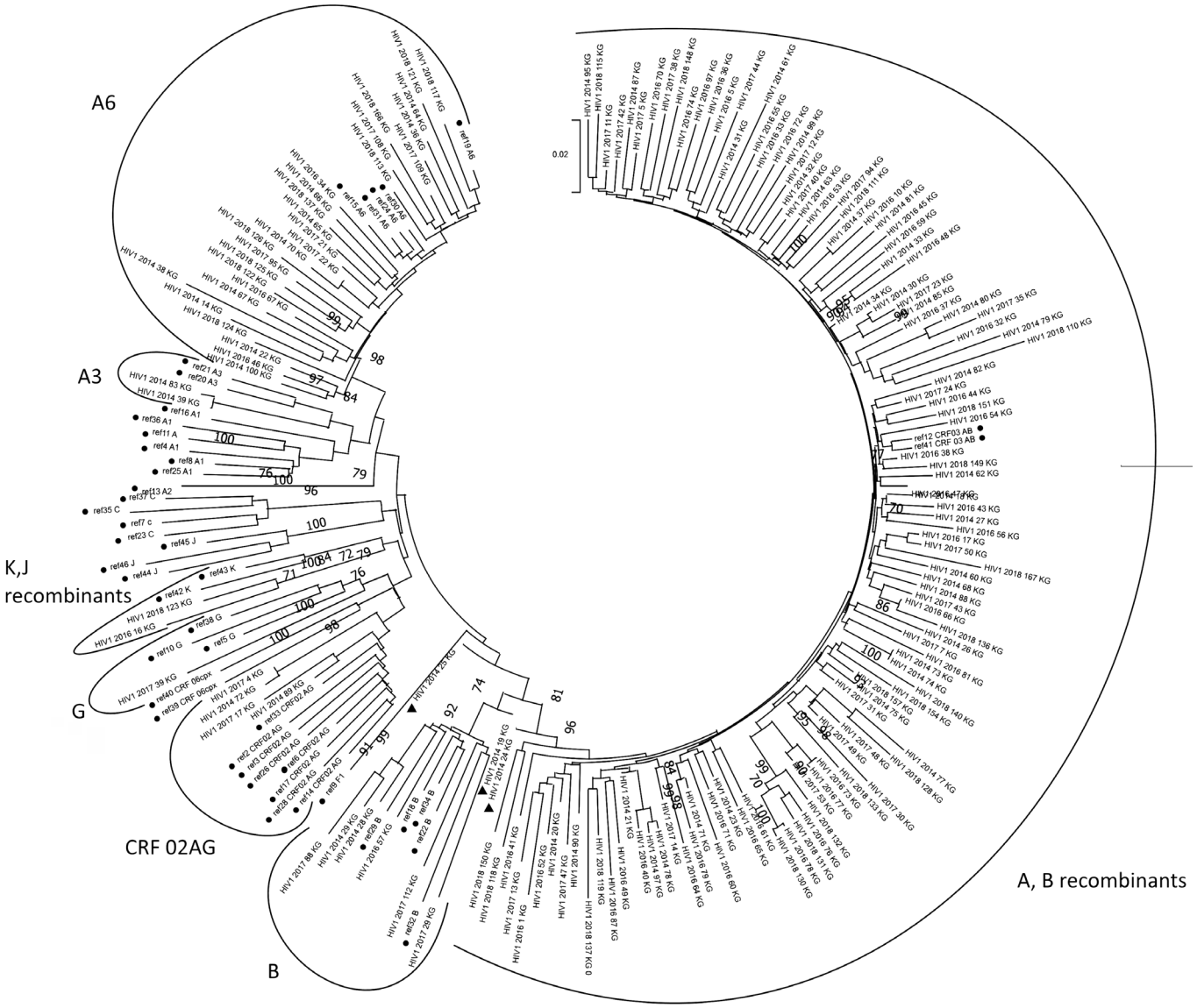

Fig. 1. Results of phylogenetic analysis using the Neighbor Joining algorithm.

• – reference sequences (**table 2**);

▲ – recombinant forms between subtypes A and B, not clustered with other recombinants of this group.

Рис. 1. Результаты филогенетического анализа при помощи алгоритма Neighbor Joining.

• – референсные последовательности (**табл. 2**);

▲ – рекомбинантный формы между субтипами A и B, не кластеризующиеся с другими рекомбинантами этой группы.

cases (3.64%; 95% CI 0.44–12.53%); therefore, the analysis includes all the patients with detected DR mutations.

A total of 80 different DR associated mutations were detected. Most of them were DR mutations to RT inhibitors, including nucleoside reverse transcriptase inhibitors (NRTIs) – 31 substitutions (38.75%; 95% CI 28.06–50.30%) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) – 35 mutations (43.75%; 95% CI 32.68–55.30%); substitutions associated with DR to protease inhibitors (PIs) – 14 (17.50%; 95% CI 9.91–27.62%) account for a lesser proportion of mutational diversity.

96 patients (59.26%; 95% CI 51.27–66.90%) had HIV-1 isolates with at least one mutation associated with DR to antiretroviral agents. The most frequently detected mutations were DR mutations to RT inhibitors. In 13 cases, we detected DR mutations to NRTIs; 4 patients had DR mutations to NNRTIs and 66 had DR mutations to NRTIs + NNRTIs. In addition, 13 patients had DR mutations to PIs: 10 patients – to PIs + NRTIs, and 3 patients – to PIs + NRTIs + NNRTIs.

Among DR mutations to NRTIs, the highest frequency rates were demonstrated by M184V mutations [30] (65.63%; 95% CI 55.23–75.02%), L74V mutations [31] (19.79%; 95% CI 12.36–29.17%), Y115F mutations [32] (14.58%; 95% CI 8.21–23.26%); the other substitutions were detected in 10% of the cases and more rarely.

Table 2. Names of reference sequences from GenBank used in phylogenetic analysis
Таблица 2. Наименование референсных последовательностей из GenBank, использованных в филогенетическом анализе

Sub-subtype Суб-субтип	Number sequence in phylogenetic tree Номер последовательности на филогенетическом древе	Number sequence in GenBank Номер последовательности из GenBank	Region of origin Регион происхождения
A1	ref4	AF069670	Somali Сомали
A1	ref8	AB287376	Ruanda Руанда
A1	ref16	U51190	Uganda Уганда
A1	ref25	EU110087	Kenia Кения
A1	ref27	AF484509	Uganda Уганда
A1	ref36	AF107771	Sweden Швеция
A2	ref13	AF286237	Cyprus Кипр
A3	ref1	AY521631	Senegal Сенегал
A3	ref20	AY521629	Sweden Швеция
A6	ref15	HQ449397	Russia, Krasnodar Россия, Краснодар
A6	ref19	HQ161930	Russia, Smolensk Россия, Смоленск
A6	ref24	EF589043	Kazakhstan Казахстан
A6	ref30	AY500393	Russia, Moscow Россия, Москва
A6	ref31	AF413987	Ukraine Украина
B	ref18	M17449	USA США
B	ref22	KJ771697	Germany Германия
B	ref29	HM586190	Great Britain Великобритания
B	ref32	AY713409	USA США
B	ref34	AY173951	Thailand Таиланд
C	ref7	AF067155	India Индия
C	ref23	U52953	Brazil Бразилия
C	ref35	U46016	Ethiopia Эфиопия
C	ref37	AY772699	Africa Африка
F1	ref9	AF075703	Finland Финляндия
G	ref5	AF061641	Finland Финляндия
G	ref10	U88826	Nigeria Нигерия
G	ref38	AF084936	Congo Конго
J	ref44	EF614151	Congo Конго
J	ref45	GU237072	Cameron Камерун
J	ref46	AF082394	Sweden Швеция
K	ref42	AJ249235	Cameron Камерун
K	ref43	AJ249239	Cameron Камерун
CRF02_AG	ref2	AF063224	Djibouti Джибути
CRF02_AG	ref3	GU201514	Cameron Камерун
CRF02_AG	ref6	KT124792	Germany Германия
CRF02_AG	ref14	AB231898	Ghana Гана
CRF02_AG	ref17	EU786671	Spain Испания
CRF02_AG	ref26	AB231896	Ghana Гана
CRF02_AG	ref28	AY151001	Ecuador Эквадор
CRF02_AG	ref33	AF377954	Cameron Камерун
CRF06_cpx	ref39	HQ529257.1	Ghana Гана
CRF03_AB	ref12	AF193276	Russia, Kalinigrad Россия, Калининград
CRF03_AB	ref41	AF414006.1	Belarus Беларусь
CRF06_cpx	ref40	MH605500.1	Guinea-Bissau Гвинея-Бисау

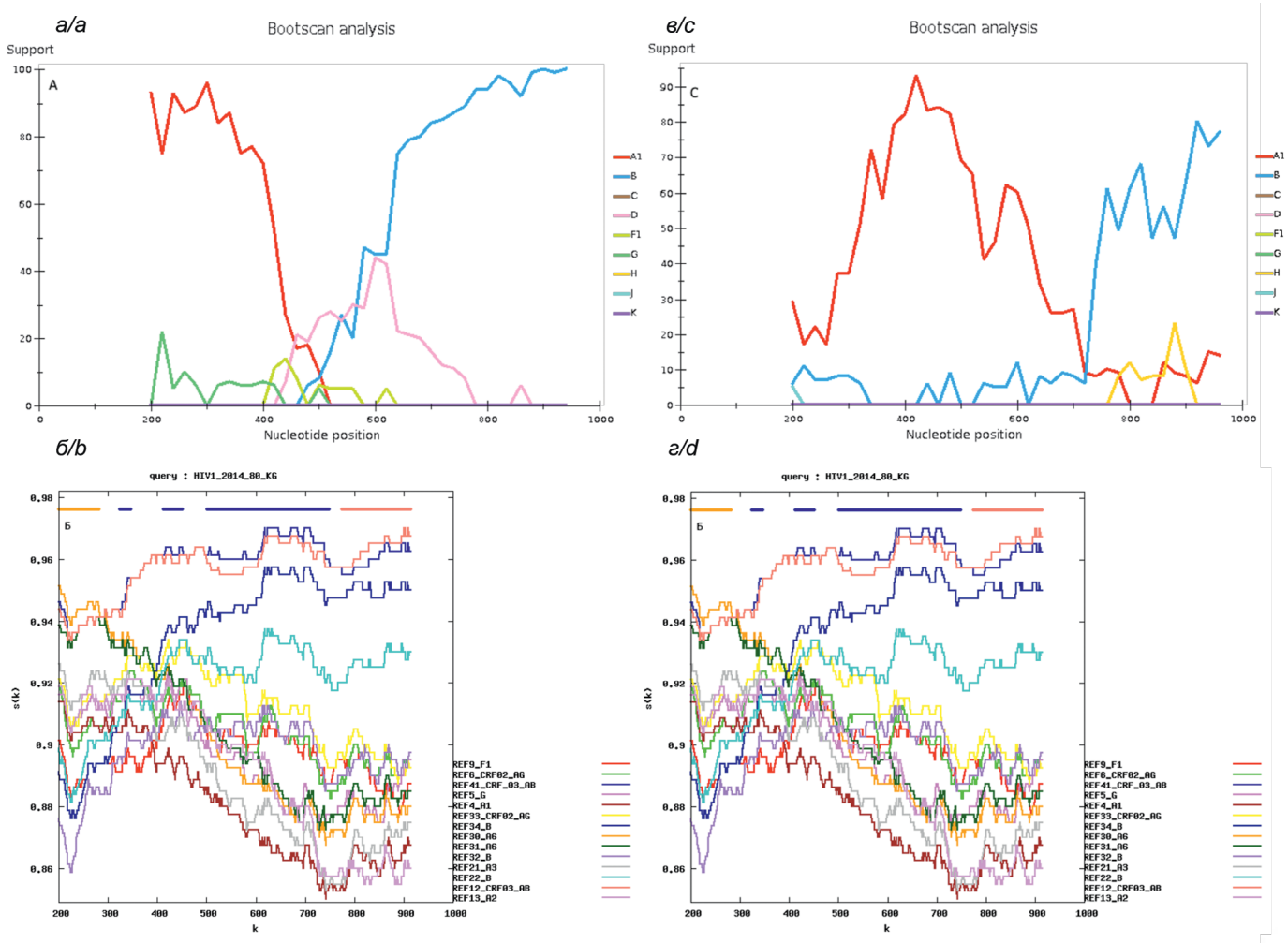

Fig. 2. Comparative recombination analysis of samples 2014_80 (CRF03_AB) and 2014_19 (A + B recombinant) in Rega HIV Subtyping Tool v3.0 [21] and Recombinant Identification Program *(https://www.hiv.lanl.gov/content/sequence/RIP/RIP.html)*

a – sample 2014_80 in Rega HIV Subtyping Tool v3.0; *b* – sample 2014_80 in the Recombinant Identification Program; *c* – sample 2014_19 in Rega HIV Subtyping Tool v3.0; *d* – sample 2014_19 in the Recombinant Identification Program [25].

Рис. 2. Сравнительный рекомбинационный анализ образцов 2014_80 (CRF03_AB) и 2014_19 (A + B recombinant) в Rega HIV Subtyping Tool v3.0 [21] и Recombinant Identification Program [25].

а – образец 2014_80 в Rega HIV Subtyping Tool v3.0; *б* – образец 2014_80 в Recombinant Identification Program; *в* – образец 2014_19 в Rega HIV Subtyping Tool v3.0; *г* – образец 2014_19 в Recombinant Identification Program [25].

The analysis of multiple mutational profiles by building linear diagrams showed stable patterns of DR mutations (**Fig. 3** *a*). The thymidine analog resistance mutations (TAMs) extensively described in the scientific publications were detected in the obtained profiles only in a few cases. There are two pathways of development of TAM patterns: mutations occurring with T215Y (including M41L, L210W and sometimes D67N) comprise the TAM-1 cluster; mutations occurring with K70R (including D67N, T215F, and K219Q) comprise the TAM-2 cluster. Nevertheless, in this case, both mutation clusters are associated with the T215Y substitution, while the development of the pattern along the TAM-2 pathway is known to have the greatest advantage with the T215F substitution, which was also detected in the studied mutational profiles, but not in the TAM patterns [33]. The profiles carrying non-TAM mutations prevailed; they have demonstrated a stable relationship between L74V + Y115F substitutions. These mutations are primarily associated with DR to abacavir and didanosine, though there are data on their association with DR to tenofovir [31, 32], which, in its turn, is included in most of the present-day antiretroviral treatment regimens. Furthermore, in all cases, this combination was detected together with the M184V substitution, which most likely can be explained by the presence of this substitution in most isolates with DR.

The analysis of frequency rates for DR mutations to NNRTIs showed that the highest frequency rates were demonstrated by the K103N [34] (36.46%; 95% CI 26.87–46.91%), K101E [35] (12.50%; 95% CI 6.63–20.82%), G190A [36] (11.46%; 95% CI 5.86–19.58%), P225H (15.63%; 95% CI 9.02–24.46%), and Y18C [37] (12.50%; 95% CI 6.63–20.82%) substitutions; the other

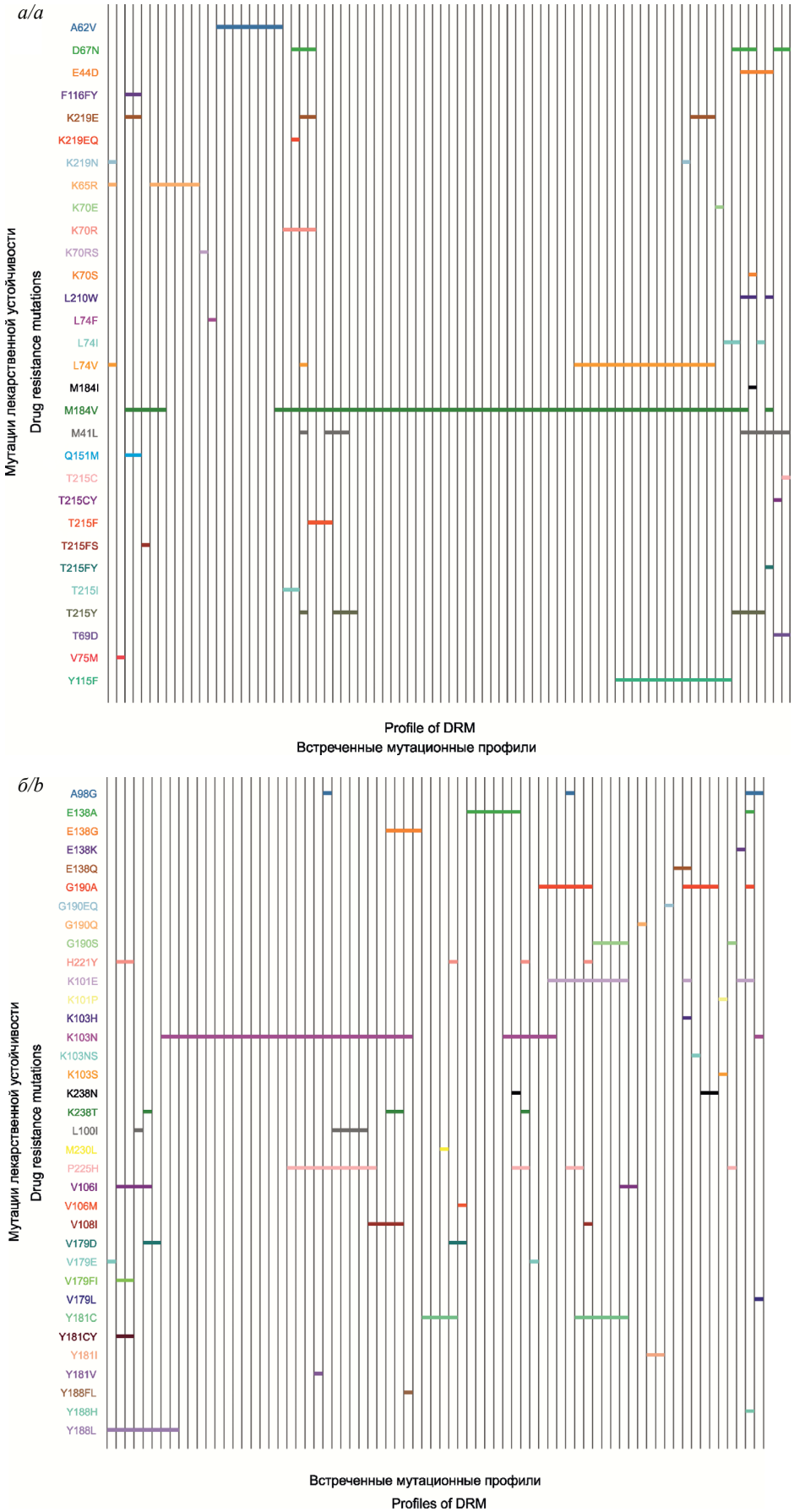

Fig. 3. Results of the study of multiple mutational profiles by constructing line diagrams: *a* – for NRTI resistance mutations; *b* – for NNRTI resistance mutations.

Рис. 3. Результаты исследования множества мутационных профилей путём построения линейных диаграмм: *а* – для мутаций устойчивости к НИОТ; *б* – для мутаций устойчивости к ННИОТ.

mutations were detected in less than 10% of the cases. The analysis of profiles of DR mutations in obtained isolates (**Fig. 3** *b*) revealed a relationship between K101E + G190A/S substitutions; note that this combination was generally detected without the most common K103N mutation. The analysis also revealed a relationship between the substitution for alanine (A) or serine (S) at the 190th position and the subtype of the virus. Substitution 190A was detected only in recombinants between subtypes A and B, while mutation 190S was detected primarily in isolates of sub-subtype A6 (in five of six cases). There are published studies describing the prevalence of the substitution at the 190th position of RT for serine for subtype A [38–40] and alanine for non-A subtypes [38, 41, 42].

Conclusion

The obtained results demonstrate the existence of diverse recombinant forms in the Kaliningrad Region. The dominance of recombinants between CRF03_AB and A suggests that the recombination generally results from the co-circulation of the variant common in the studied region – CRF03_AB, and sub-subtype A6 common in other regions of Russia. The contribution of the co-circulation with subtype B remains unclear.

The observed diversity of subtypes and recombinant forms of the virus implies that new recombinants are actively emerging in the studied region, both between the existing recombinant forms and "pure" subtypes as well as between "pure" subtypes. This activity of the virus adds to the importance of studies on full-length genomes of the isolates obtained in the Kaliningrad Region and of the description of all the recombinant forms currently circulating in the region.

ЛИТЕРАТУРА

1. Korber B., Muldoon M., Theiler J., Gao F., Gupta R., Lapedes A., et al. Timing the ancestor of the HIV-1 pandemic strains. *Science*. 2000; 288(5472): 1789–96. https://doi.org/10.1126/science.288.5472.1789
2. Kuiken C., Foley B., Hahn B., Marx P., McCutchan F., Mellors J.W., et al. A compilation and analysis of nucleic acid and amino acid sequences. In: *Human Retroviruses and AIDS*. Los Alamos; 1999.
3. Los Alamos National Laboratory. HIV Circulating Recombinant Forms (CRFs). Available at: https://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html
4. Simon-Loriere E., Rossolillo P., Negroni M. RNA structures, genomic organization and selection of recombinant HIV. *RNA Biol*. 2011; 8(2): 280–6. https://doi.org/10.4161/rna.8.2.15193
5. McCutchan F.E., Carr J.K., Bajani M., Sanders-Buell E., Harry T.O., Stoeckli T.C., et al. Subtype G and multiple forms of A/G intersubtype recombinant human immunodeficiency virus type 1 in Nigeria. *Virology*. 1999; 254(2): 226–34. https://doi.org/10.1006/viro.1998.9505
6. Montavon C., Toure-Kane C., Liegeois F., Mpoudi E., Bourgeois A., Vergne L., et al. Most env and gag subtype A HIV-1 viruses circulating in West and West Central Africa are similar to the prototype AG recombinant virus IBNG. *J. Acquir. Immune. Defic. Syndr*. 2000; 23(5): 363–74. https://doi.org/10.1097/00126334-200004150-00001
7. Menu E., Truong T.X., Lafon M.E., Nguyen T.H., Müller-Trutwin M.C., Nguyen T.T., et al. HIV type 1 Thai subtype E is predominant in South Vietnam. *AIDS Res. Hum. Retroviruses*. 1996; 12(7): 629–33. https://doi.org/10.1089/aid.1996.12.629
8. Piyasirisilp S., McCutchan F.E., Carr J.K., Sanders-Buell E., Liu W., Chen J., et al. A recent outbreak of human immunodeficiency virus type 1 infection in southern China was initiated by two highly homogeneous, geographically separated strains, circulating recombinant form AE and a novel BC recombinant. *J. Virol*. 2000; 74(23): 11286–95. https://doi.org/10.1128/jvi.74.23.11286-11295.2000
9. Galetto R., Moumen A., Giacomoni V., Veron M., Charneau P., Negroni M. The structure of HIV-1 genomic RNA in the gp120 gene determines a recombination hot spot in vivo. *J. Biol. Chem.* 2004; 279(35): 36625–32. https://doi.org/10.1074/jbc.m405476200
10. Zhuang J., Jetzt A.E., Sun G., Yu H., Klarmann G., Ron Y., et al. Human immunodeficiency virus type 1 recom-bination: rate, fidelity and putative hot spots. *J. Virol.* 2002; 76(22): 11273–82. https://doi.org/10.1128/jvi.76.22.11273-11282.2002
11. Jetzt A.E., Yu H., Klarmann G.J., Ron Y., Preston B.D., Dougherty J.P. High rate of recombination throughout the human immunodeficiency virus type 1 genome. *J. Virol.* 2000; 74(3): 1234–40. https://doi.org/10.1128/jvi.74.3.1234-1240.2000
12. Piantadosi A., Chohan B., Chohan V., McClelland R.S., Overbaugh J. Chronic HIV-1 infection frequently fails to protect against superinfection. *PLoS Pathog* 2007; 3(11): 177. https://doi.org/10.1371/journal.ppat.0030177
13. Gratton S., Cheynier R., Dumaurier M.J., Oksenhendler E., Wain-Hobson S. Highly restricted spread of HIV-1 and multiply infected cells within splenic germinal centers. *Proc. Natl. Acad. Sci. USA*. 2000; 97(26): 14566–71. https://doi.org/10.1073/pnas.97.26.14566
14. Jung A., Maier R., Vartanian J.P., Bocharov G., Jung V., Fischer U., et al. Recombination: Multiply infected spleen cells in HIV patients. *Nature.* 2002; 418(6894): 144. https://doi.org/10.1038/418144a
15. Chen J., Dang Q., Unutmaz D., Pathak V.K., Maldarelli F., Powell D., et al. Mechanisms of nonrandom human immunodeficiency virus type 1 infection and double infection: preference in virus entry is important but is not the sole factor. *J. Virol* 2005; 79(7): 4140–9. https://doi.org/10.1128/jvi.79.7.4140-4149.2005
16. Bobkov A.F., Kazennova E.V., Selimova L.M., Khanina T.A., Ryabov G.S., Bobkova M.R., et al. Temporal trends in the HIV-1 epidemic in Russia: predominance of subtype A. *J. Med. Viro*l. 2004; 74(2): 191–6. https://doi.org/10.1002/jmv.20177
17. Schlösser M., Kartashev V.V., Mikkola V.H., Shemshura A., Saukhat S., Kolpakov D., et al. HIV-1 sub-subtype A6: Settings for normalised identification and molecular epidemiology in the Southern Federal District, Russia. *Viruses*. 2020; 12(4): 475. https://doi.org/10.3390/v12040475
18. Liitsola K., Tashkinova I., Laukkanen T., Korovina G., Smolskaja T., Momot O., et al. HIV-1 genetic subtype A/B recombinant strain causing an explosive epidemic in injecting drug users in Kaliningrad. *AIDS*. 1998; 12(14): 1907–19. https://doi.org/10.1097/00002030-199814000-00023
19. Lebedev A., Pasechnik O., Ozhmegova E., Antonova A., Blokh A., Grezina L., et al. Prevalence and spatiotemporal dynamics of HIV-1 Circulating Recombinant Form 03_AB (CRF03_AB) in the Former Soviet Union countries. *PLoS One*. 2020; 15(10): e0241269. https://doi.org/10.1371/journal.pone.0241269
20. Kumar S., Stecher G., Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol*. 2016; 33(7): 1870–4. https://doi.org/10.1093/molbev/msw054
21. Stanford University. HIV Drug Resistance Database. REGA HIV-1 Subtyping Tool – Version 3.0. Available at: http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/
22. Stanford University. HIV Drug Resistance Database. HIVdb Program: Mutations Analysis. Available at: https://hivdb.stanford.edu/hivdb/by-patterns/
23. Gottfried B. A comparative study on linear and region based diagrams. *J. Spat. Inf. Sci*. 2015; (10): 3–20.
24. Лаповок И.А., Салеева Д.В., Кириченко А.А., Мурзакова А.В., Лопатухин А.Э., Киреев Д.Е. Исследование частоты встречаемости

двойной ВИЧ-инфекции в России. *Инфекционные болезни*. 2020; 18(4): 138–48. https://doi.org/10.20953/1729-9225-2020-4-138-148
25. Los Alamos National Laboratory. RIP: Recombinant Identification Program. Available at: https://www.hiv.lanl.gov/content/sequence/RIP/RIP.html
26. Пасечник О.А., Блох А.И. Распространенность рекомбинантных форм ВИЧ-1 в регионах Российской Федерации и стран СНГ: систематический обзор и метаанализ. *Инфекция и иммунитет*. 2018; 8(2): 127–38. https://doi.org/10.15789/2220-7619-2018-2-127-138
27. Федеральный центр по борьбе со СПИД. Российская база данных. ЛУ ВИЧ у наивных пациентов; 2020. Available at: http://www.hivrussia.info/wp-content/uploads/2020/12/2020-Rossijskaya-baza-dannyh-LU-VICH-u-naivnyh-patsientov.pdf
28. Останкова Ю.В., Щемелев А.Н., Зуева Е.Б., Чурина М.А., Валутите Д.Э., Семенов А.В. Молекулярная эпидемиология и фармакорезистентность ВИЧ у пациентов с вирусологической неэффективностью антиретровирусной терапии в Архангельской области. *ВИЧ инфекция и иммуносупрессии*. 2019; 11(4): 65–72. https://doi.org/10.22328/2077-9828-2019-11-4-79-90
29. Щемелев А.Н., Семенов А.В., Останкова Ю.В., Зуева Е.Б., Валутите Д.Э., Семенова Д.А. и др. Генетическое разнообразие и мутации лекарственной устойчивости ВИЧ-1 в Ленинградской области. *Журнал микробиологии, эпидемиологии и иммунобиологии*. 2022; 99(1): 28–37. https://doi.org/10.36233/0372-9311-216
30. Hung M., Tokarsky E.J., Lagpacan L., Zhang L., Suo Z., Lansdon E.B. Elucidating molecular interactions of L-nucleotides with HIV-1 reverse transcriptase and mechanism of M184V-caused drug resistance. *Commun. Biol*. 2019; 2: 469. https://doi.org/10.1038/s42003-019-0706-x
31. De Luca A., Giambenedetto S.D., Trotta M.P., Colafigli M., Prosperi M., Ruiz L., et al. Improved interpretation of genotypic changes in the HIV-1 reverse transcriptase coding region that determine the virological response to didanosine. *J. Infect. Dis*. 2007; 196(11): 1645–53. https://doi.org/10.1086/522231
32. Lanier E.R., Givens N., Stone C., Griffin P., Gibb D., Walker S., et al. Effect of concurrent zidovudine use on the resistance pathway selected by abacavir-containing regimens. *HIV Med*. 2004; 5(6): 394–9. https://doi.org/10.1111/j.1468-1293.2004.00243.x
33. Hu Z., Giguel F., Hatano H., Reid P., Lu J., Kuritzkes D.R. Fitness comparison of thymidine analog resistance pathways in human immunodeficiency virus type 1. *J. Virol*. 2006; 80(14): 7020–7. https://doi.org/10.1128/jvi.02747-05
34. Ibe S., Sugiura W. Clinical significance of HIV reverse-transcriptase inhibitor-resistance mutations. *Future Microbiol*. 2011; 6(3): 295–315. https://doi.org/10.2217/fmb.11.7
35. Xu H.T., Colby-Germinario S.P., Huang W., Oliveira M., Han Y., Quan Y., et al. Role of the K101E substitution in HIV-1 reverse transcriptase in resistance to rilpivirine and other nonnucleoside reverse transcriptase inhibitors. *Antimicrob. Agents Chemother.* 2013; 57(11): 5649–57. https://doi.org/10.1128/aac.01536-13
36. Madruga J.V., Cahn P., Grinsztejn B., Haubrich R., Lalezari J., Mills A., et al. Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-1: 24-week results from a randomised, double-blind, placebo-controlled trial. *Lancet*. 2007; 370(9581): 29–38. https://doi.org/10.1016/s0140-6736(07)61047-2
37. Archer R.H., Wisniewski M., Bambara R.A., Demeter L.M. The Y181C mutant of HIV-1 reverse transcriptase resistant to nonnucleoside reverse transcriptase inhibitors alters the size distribution of RNase H cleavages. *Biochemistry*. 2001; 40(13): 4087–95. https://doi.org/10.1021/bi002328a
38. Kolomeets A.N., Varghese V., Lemey P., Bobkova M.R., Shafer R.W. A uniquely prevalent nonnucleoside reverse transcriptase inhibitor resistance mutation in Russian subtype A HIV-1 viruses. *AIDS*. 2014; 28(17): F1–8. https://doi.org/10.1097/qad.0000000000000485
39. Останкова Ю.В., Щемелев А.Н., Зуева Е.Б., Чурина М.А., Валутите Д.Э., Семенов А.В. Молекулярная эпидемиология и фармакорезистентность ВИЧ у пациентов с вирусологической неэффективностью антиретровирусной терапии в Архангельской области. *ВИЧ инфекция и иммуносупрессии*. 2019; 11(4): 65–72. https://doi.org/10.22328/2077-9828-2019-11-4-79-9
40. Чурина М.А., Останкова Ю.В., Семенов А.В., Никитина Н.А., Росоловский А.П., Гребенкина Е.В. и др. Молекулярная эпидемиология и фармакорезистентность ВИЧ-1 у пациентов с неэффективностью АРВТ в Великом Новгороде. *ВИЧ-инфекция и иммуносупрессии.* 2017; 9(1): 82–92. https://doi.org/10.22328/2077-9828-2017-9-1-82-92
41. Chen M., Zhu Q., Xing H., Chen H., Jin X., Dong L., et al. The characteristics of pretreatment HIV-1 drug resistance in western Yunnan, China. *Epidemiol. Infect*. 2020; 148: e102. https://doi.org/10.1017/s095026882000093x
42. Cheung K.W., Peng Q., He L., Cai K., Jiang Q., Zhou B., et al. Rapid and simultaneous detection of major drug resistance mutations in reverse transcriptase gene for HIV-1 CRF01_AE, CRF07_BC and subtype B in China using sequenom MassARRAY® system. *PLoS One*. 2016; 11(4): e0153641. https://doi.org/10.1371/journal.pone.0153641

REFERENCES

1. Korber B., Muldoon M., Theiler J., Gao F., Gupta R., Lapedes A., et al. Timing the ancestor of the HIV-1 pandemic strains. *Science.* 2000; 288(5472): 1789–96. https://doi.org/10.1126/science.288.5472.1789
2. Kuiken C., Foley B., Hahn B., Marx P., McCutchan F., Mellors J.W., et al. A compilation and analysis of nucleic acid and amino acid sequences. In: *Human Retroviruses and AIDS*. Los Alamos; 1999.
3. Los Alamos National Laboratory. HIV Circulating Recombinant Forms (CRFs). Available at: https://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html
4. Simon-Loriere E., Rossolillo P., Negroni M. RNA structures, genomic organization and selection of recombinant HIV. *RNA Biol*. 2011; 8(2): 280–6. https://doi.org/10.4161/rna.8.2.15193
5. McCutchan F.E., Carr J.K., Bajani M., Sanders-Buell E., Harry T.O., Stoeckli T.C., et al. Subtype G and multiple forms of A/G intersubtype recombinant human immunodeficiency virus type 1 in Nigeria. *Virology*. 1999; 254(2): 226–34. https://doi.org/10.1006/viro.1998.9505
6. Montavon C., Toure-Kane C., Liegeois F., Mpoudi E., Bourgeois A., Vergne L., et al. Most env and gag subtype A HIV-1 viruses circulating in West and West Central Africa are similar to the prototype AG recombinant virus IBNG. *J. Acquir. Immune. Defic. Syndr*. 2000; 23(5): 363–74. https://doi.org/10.1097/00126334-200004150-00001
7. Menu E., Truong T.X., Lafon M.E., Nguyen T.H., Müller-Trutwin M.C., Nguyen T.T., et al. HIV type 1 Thai subtype E is predominant in South Vietnam. *AIDS Res. Hum. Retroviruses*. 1996; 12(7): 629–33. https://doi.org/10.1089/aid.1996.12.629
8. Piyasirisilp S., McCutchan F.E., Carr J.K., Sanders-Buell E., Liu W., Chen J., et al. A recent outbreak of human immunodeficiency virus type 1 infection in southern China was initiated by two highly homogeneous, geographically separated strains, circulating recombinant form AE and a novel BC recombinant. *J. Virol*. 2000; 74(23): 11286–95. https://doi.org/10.1128/jvi.74.23.11286-11295.2000
9. Galetto R., Moumen A., Giacomoni V., Veron M., Charneau P., Negroni M. The structure of HIV-1 genomic RNA in the gp120 gene determines a recombination hot spot in vivo. *J. Biol. Chem.* 2004; 279(35): 36625–32. https://doi.org/10.1074/jbc.m405476200
10. Zhuang J., Jetzt A.E., Sun G., Yu H., Klarmann G., Ron Y., et al. Human immunodeficiency virus type 1 recom-bination: rate, fidelity and putative hot spots. *J. Virol.* 2002; 76(22): 11273–82. https://doi.org/10.1128/jvi.76.22.11273-11282.2002
11. Jetzt A.E., Yu H., Klarmann G.J., Ron Y., Preston B.D., Dougherty J.P. High rate of recombination throughout the human immunodeficiency virus type 1 genome. *J. Virol.* 2000; 74(3): 1234–40. https://doi.org/10.1128/jvi.74.3.1234-1240.2000
12. Piantadosi A., Chohan B., Chohan V., McClelland R.S., Overbaugh J. Chronic HIV-1 infection frequently fails to protect

against superinfection. *PLoS Pathog* 2007; 3(11): 177. https://doi.org/10.1371/journal.ppat.0030177
13. Gratton S., Cheynier R., Dumaurier M.J., Oksenhendler E., Wain-Hobson S. Highly restricted spread of HIV-1 and multiply infected cells within splenic germinal centers. *Proc. Natl. Acad. Sci. USA*. 2000; 97(26): 14566–71. https://doi.org/10.1073/pnas.97.26.14566
14. Jung A., Maier R., Vartanian J.P., Bocharov G., Jung V., Fischer U., et al. Recombination: Multiply infected spleen cells in HIV patients. *Nature*. 2002; 418(6894): 144. https://doi.org/10.1038/418144a
15. Chen J., Dang Q., Unutmaz D., Pathak V.K., Maldarelli F., Powell D., et al. Mechanisms of nonrandom human immunodeficiency virus type 1 infection and double infection: preference in virus entry is important but is not the sole factor. *J. Virol* 2005; 79(7): 4140–9. https://doi.org/10.1128/jvi.79.7.4140-4149.2005
16. Bobkov A.F., Kazennova E.V., Selimova L.M., Khanina T.A., Ryabov G.S., Bobkova M.R., et al. Temporal trends in the HIV-1 epidemic in Russia: predominance of subtype A. *J. Med. Viro*l. 2004; 74(2): 191–6. https://doi.org/10.1002/jmv.20177
17. Schlösser M., Kartashev V.V., Mikkola V.H., Shemshura A., Saukhat S., Kolpakov D., et al. HIV-1 sub-subtype A6: Settings for normalised identification and molecular epidemiology in the Southern Federal District, Russia. *Viruses*. 2020; 12(4): 475. https://doi.org/10.3390/v12040475
18. Liitsola K., Tashkinova I., Laukkanen T., Korovina G., Smolskaja T., Momot O., et al. HIV-1 genetic subtype A/B recombinant strain causing an explosive epidemic in injecting drug users in Kaliningrad. *AIDS*. 1998; 12(14): 1907–19. https://doi.org/10.1097/00002030-199814000-00023
19. Lebedev A., Pasechnik O., Ozhmegova E., Antonova A., Blokh A., Grezina L., et al. Prevalence and spatiotemporal dynamics of HIV-1 Circulating Recombinant Form 03_AB (CRF03_AB) in the Former Soviet Union countries. *PLoS One*. 2020; 15(10): e0241269. https://doi.org/10.1371/journal.pone.0241269
20. Kumar S., Stecher G., Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol*. 2016; 33(7): 1870–4. https://doi.org/10.1093/molbev/msw054
21. Stanford University. HIV Drug Resistance Database. REGA HIV-1 Subtyping Tool – Version 3.0. Available at: http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/
22. Stanford University. HIV Drug Resistance Database. HIVdb Program: Mutations Analysis. Available at: https://hivdb.stanford.edu/hivdb/by-patterns/
23. Gottfried B. A comparative study on linear and region based diagrams. *J. Spat. Inf. Sci*. 2015; (10): 3–20.
24. Lapovok I.A., Saleeva D.V., Kirichenko A.A., Murzakova A.V., Lopatukhin A.E., Kireev D.E. The study of dual HIV infection prevalence in Russia. *Infektsionnye bolezni*. 2020; 18(4): 138–48. https://doi.org/10.20953/1729-9225-2020-4-138-148 (in Russian)
25. Los Alamos National Laboratory. RIP: Recombinant Identification Program. Available at: https://www.hiv.lanl.gov/content/sequence/RIP/RIP.html
26. Pasechnik O.A., Blokh A.I. The prevalence of HIV recombinant forms in Russia and countries of the CIS: systematic review and metaanalysis. *Infektsiya i immunitet*. 2018; 8(2): 127–38. https://doi.org/10.15789/2220-7619-2018-2-127-138 (in Russian)
27. Federal AIDS Center. Russian database. HIVDR in naive patients; 2020. Available at: http://www.hivrussia.info/wp-content/uploads/2020/12/2020-Rossijskaya-baza-dannyh-LU-VICH-u-naivnyh-patsientov.pdf (in Russian)
28. Ostankova Yu.V., Shchemelev A.N., Zueva E.B., Churina M.A., Valutite D.E., Semenov A.V. HIV molecular epidemiology and pharmaco-resistance in patients with antiretroviral therapy failure in Arkhangelsk district. *VICh infektsiya i immunosupressii*. 2019; 11(4): 65–72. https://doi.org/10.22328/2077-9828-2019-11-4-79-90 (in Russian)
29. Shchemelev A.N., Semenov A.V., Ostankova Yu.V., Zueva E.B., Valutite D.E., Semenova D.A., et al. Genetic diversity and drug resistance mutations of HIV-1 in Leningrad region. *Zhurnal mikrobiologii, epidemiologii i immunobiologii*. 2022; 99(1): 28–37. https://doi.org/10.36233/0372-9311-216 (in Russian)
30. Hung M., Tokarsky E.J., Lagpacan L., Zhang L., Suo Z., Lansdon E.B. Elucidating molecular interactions of L-nucleotides with HIV-1 reverse transcriptase and mechanism of M184V-caused drug resistance. *Commun. Biol*. 2019; 2: 469. https://doi.org/10.1038/s42003-019-0706-x
31. De Luca A., Giambenedetto S.D., Trotta M.P., Colafigli M., Prosperi M., Ruiz L., et al. Improved interpretation of genotypic changes in the HIV-1 reverse transcriptase coding region that determine the virological response to didanosine. *J. Infect. Dis*. 2007; 196(11): 1645–53. https://doi.org/10.1086/522231
32. Lanier E.R., Givens N., Stone C., Griffin P., Gibb D., Walker S., et al. Effect of concurrent zidovudine use on the resistance pathway selected by abacavir-containing regimens. *HIV Med*. 2004; 5(6): 394–9. https://doi.org/10.1111/j.1468-1293.2004.00243.x
33. Hu Z., Giguel F., Hatano H., Reid P., Lu J., Kuritzkes D.R. Fitness comparison of thymidine analog resistance pathways in human immunodeficiency virus type 1. *J. Virol*. 2006; 80(14): 7020–7. https://doi.org/10.1128/jvi.02747-05
34. Ibe S., Sugiura W. Clinical significance of HIV reverse-transcriptase inhibitor-resistance mutations. *Future Microbiol*. 2011; 6(3): 295–315. https://doi.org/10.2217/fmb.11.7
35. Xu H.T., Colby-Germinario S.P., Huang W., Oliveira M., Han Y., Quan Y., et al. Role of the K101E substitution in HIV-1 reverse transcriptase in resistance to rilpivirine and other nonnucleoside reverse transcriptase inhibitors. *Antimicrob. Agents Chemother.* 2013; 57(11): 5649–57. https://doi.org/10.1128/aac.01536-13
36. Madruga J.V., Cahn P., Grinsztejn B., Haubrich R., Lalezari J., Mills A., et al. Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-1: 24-week results from a randomised, double-blind, placebo-controlled trial. *Lancet*. 2007; 370(9581): 29–38. https://doi.org/10.1016/s0140-6736(07)61047-2
37. Archer R.H., Wisniewski M., Bambara R.A., Demeter L.M. The Y181C mutant of HIV-1 reverse transcriptase resistant to nonnucleoside reverse transcriptase inhibitors alters the size distribution of RNase H cleavages. *Biochemistry*. 2001; 40(13): 4087–95. https://doi.org/10.1021/bi002328a
38. Kolomeets A.N., Varghese V., Lemey P., Bobkova M.R., Shafer R.W. A uniquely prevalent nonnucleoside reverse transcriptase inhibitor resistance mutation in Russian subtype A HIV-1 viruses. *AIDS*. 2014; 28(17): F1–8. https://doi.org/10.1097/qad.0000000000000485
39. Ostankova Yu.V., Shchemelev A.N., Zueva E.B., Churina M.A., Valutite D.E., Semenov A.V. HIV molecular epidemiology and pharmaco-resistance in patients with antiretroviral therapy failure in Arkhangelsk district. *VICh infektsiya i immunosupressii*. 2019; 11(4): 65–72. https://doi.org/10.22328/2077-9828-2019-11-4-79-9 (in Russian)
40. Churina M.A., Ostankova Yu.V., Semenov A.V., Nikitina N.A., Rosolovskiy A.P., Grebenkina E.V., et al. HIV-1 drug-resistance and molecular epidemiology in patients with art failure in Veliky Novgorod. *VICh-infektsiya i immunosupressii*. 2017; 9(1): 82–92. https://doi.org/10.22328/2077-9828-2017-9-1-82-92 (in Russian)
41. Chen M., Zhu Q., Xing H., Chen H., Jin X., Dong L., et al. The characteristics of pretreatment HIV-1 drug resistance in western Yunnan, China. *Epidemiol. Infect*. 2020; 148: e102. https://doi.org/10.1017/s095026882000093x
42. Cheung K.W., Peng Q., He L., Cai K., Jiang Q., Zhou B., et al. Rapid and simultaneous detection of major drug resistance mutations in reverse transcriptase gene for HIV-1 CRF01_AE, CRF07_BC and subtype B in China using sequenom MassARRAY® system. *PLoS One*. 2016; 11(4): e0153641. https://doi.org/10.1371/journal.pone.0153641