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Genetic diversity and drug resistance mutations of HIV-1 in Leningrad Region

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Abstract

Introduction. The spread of the human immunodeficiency virus type 1 (HIV-1) has become a global concern and has approached the pandemic status. St. Petersburg, a major transportation, tourist, cultural, industrial center, and a border city, is characterized by high migration of the population. The growing number of migrants can contribute to importation and spread of new genetic variants of the virus and trigger recombination processes in the virus population in St. Petersburg and the Leningrad Region.

The **aim** is to characterize the present-day HIV-1 subtype-specific profile and drug-resistance mutations among patients with virological failure on antiretroviral therapy (ART) in the Leningrad Region.

Materials and methods. The study performed in 2016–2018 was based on clinical material from HIV-infected individuals living in the Leningrad Region and having confirmed virological failure on ART. The genetic diversity and distribution of drug-resistance mutations of the HIV-1 isolates were assessed through analysis of nucleotide sequences of the virus *pol* gene fragment that included regions encoding protease and the reverse transcriptase region.

Results. In the group (n = 138), most of the patients had sub-subtype A6 (97.4%) common in Russia, though a few patients had subtype B and a recombinant containing circulating recombinant form CRF_03AB and sub-subtype A1. The tests showed that 95.79% of patients had at least one significant drug-resistance mutation; in most cases (73%) the virus was resistant to 2 classes of antiretroviral drugs and in some cases (8%) — to 3 classes. A total of 105 different drug-resistance mutations were found at 35 positions of the virus genome.

Conclusions. The high prevalence of HIV-1 drug-resistance mutations among ART patients with virological failure calls attention to surveillance of drug resistance of the virus both among ART-experienced patients and ART-naïve individuals.

Keywords: *HIV-1, HIV-1 drug resistance, HIV-1 genotyping, antiretroviral therapy, molecular epidemiology*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the St. Petersburg Pasteur Institute (Protocols No. 3, April 7, 2010, and No. 47, December 25, 2018).

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Научная статья
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Генетическое разнообразие и мутации лекарственной устойчивости ВИЧ-1 в Ленинградской области

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Аннотация

Введение. Проблема распространения вируса иммунодефицита человека первого типа (ВИЧ-1) к настоящему времени обрела общемировое значение и фактический статус пандемии. В Санкт-Петербурге — крупном транспортном, туристическом, культурном, промышленном и приграничном центре — наблюдается высокая миграционная активность населения. Это может способствовать заносу и распространению новых генетических вариантов вируса, а также рекомбинационным процессам в вирусной популяции Санкт-Петербурга и Ленинградской области.

Цель — охарактеризовать современный субтипический профиль и мутации лекарственной устойчивости ВИЧ-1 среди пациентов с вирусологической неэффективностью антиретровирусной терапии (АРТ) в Ленинградской области

Материалы и методы. В ходе работы в 2016–2018 гг. был исследован клинический материал от ВИЧ-инфицированных лиц из Ленинградской области с подтверждённой вирусологической неэффективностью АРТ. Для оценки генетического разнообразия и структуры мутаций лекарственной устойчивости полученных изолятов ВИЧ-1 проводили анализ нуклеотидных последовательностей фрагмента гена *pol* вируса, включающего области, кодирующие протеазу и участок обратной транскриптазы.

Результаты. В обследованной группе (*n* = 138) преобладал характерный для России субсубтип A6 (97,4%), однако в единичных случаях были обнаружены субтип B и рекомбинант, образованный циркулирующей рекомбинантной формой CRF_03AB и субсубтипом A1. По результатам анализа у 95,79% пациентов выявлена хотя бы одна значимая мутация лекарственной устойчивости, чаще всего (73% случаев) вирус был устойчив к 2 классам антиретровирусных препаратов, но иногда (8% случаев) — к 3 классам. Всего были определены 105 различных мутаций фармакорезистентности в 35 позициях генома вируса.

Выводы. Учитывая высокую встречаемость мутаций фармакорезистентности ВИЧ-1 у пациентов с вирусологически неэффективной АРТ, представляется необходимым надзор за лекарственной устойчивостью вируса как у получающих АРТ, так и у АРТ-наивных лиц.

Ключевые слова: *ВИЧ-1, лекарственная устойчивость ВИЧ-1, генотипирование ВИЧ-1, антиретровирусная терапия, молекулярная эпидемиология*

Этическое утверждение. Исследование проводилось при добровольном информированном согласии пациентов. Протокол исследования одобрен Этическим комитетом Санкт-Петербургского научно-исследовательского института эпидемиологии и микробиологии им. Пастера (Протоколы № 3 от 07.04.2010 и № 47 от 25.12.2018).

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

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

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Introduction

According to UNAIDS[1], 31.6-44.5 million people are currently living with human immunodeficiency virus (HIV) worldwide; in 2019, 1.2–2.2 million people became newly infected with HIV [1].

One of the distinctive characteristics of HIV is its high genetic diversity. There are two types of HIV, out of which HIV type 1 (HIV-1) is the most common type. The global distribution of HIV subtypes/sub-subtypes is not homogenous and varies across geographic regions. Subtype B is the dominant HIV-1 clade in Western and Central Europe; however, in Russia and CIS countries, due to specific features of the epidemic process in the region, subtype B is the second most common HIV variant, while the leading place is taken by special sub-subtype A6 also known as IDU-A (from injecting drug users) or A-FSU (from former Soviet Union countries). This sub-subtype was previously classified as A1, but due to significant differences from

[1] UNAIDS data 2020. URL: https://www.unaids.org/en/resources/documents/2020/unaids-data (дата обращения: 05.09.2021).

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other HIV-1 sub-subtype A1 variants in structure and transmission, it was moved into an individual, relatively uniform group [2, 3].

In Russia, there are also HIV-1 recombinant forms combining genome fragments typical of several different subtypes. These virus variants result from the recombination during reverse transcription, when reverse transcriptase (RT) switches the template between two co-packaged genomes, generating a chimeric DNA molecule [1, 3]. If recombinant forms are fully sequenced and detected at least in 3 epidemiologically unrelated patients, they are referred to as circulating recombinant forms (CRF). Complex viruses (*cpx*) represent recombinants of 4 and more subtypes [1]. With the dynamic nature of the HIV-1 pandemic, the number of identifiable CRF is growing steadily, and currently more than 100 CRF[2] have been identified.

Fast evolution of the virus, its molecular and genetic variability resulting from multiple errors made during replication, high mutation and recombination rates of RT enzyme, and non-uniform fixation rate in nucleotide substitutions during molecular evolution lead not only to generation of a large number of subtypes and recombinant forms, but also to accumulation of mutations, some of which can be clinically significant [4]. Although highly active antiretroviral therapy (ART), the primary goal of which is suppression of viral replication, significantly improves the prognosis for HIV-infected patients, reduces mortality and the number of complications associated with HIV-1, improves the survival rate among patients, drug-resistance (DR) mutations of the virus pose a growing threat to the long-term effect of antiretroviral drugs (ARD) [5]. HIV-1 DR to drugs of several groups is becoming of special concern, significantly limiting the capabilities of therapy [6].

HIV infection belongs to the so-called socially significant diseases that pose a threat to the public; it is also declared a threat to national security[3]. During the first six months in 2020, in Russia, there were 38,126 individuals with detected HIV-1 antibodies; by the end of the first half of 2020, there were 1,094,050 Russians with the laboratory confirmed diagnosis of HIV infection[4]. According to the Federal AIDS Center, 5.5% of ARD-naïve patients have drug resistance[5].

The Leningrad Region, a constituent entity of the Russian Federation, is located in the northwest of European Russia. St. Petersburg is the regional center of federal significance; it has an important influence on the region under study.

In St. Petersburg, the HIV infection epidemic started in the late 1980s and was of a relatively small size, as the infection was spread mostly among high-risk groups through sexual contact; the group of the infected was small and was characterized by the dominance of subtype B. In the later years, the number of people in the high-risk group increased significantly when the virus was transmitted into the cohort of injecting drug users. The mid-1990s marked an avalanche-like epidemic spread of HIV-1 in Russia [7, 8]. In the group of injecting drug users, the highest prevalence rates were recorded for sub-subtype A6, and injections became a major route of HIV-1 transmission [9].

Although during the first six months of 2020, the incidence in St. Petersburg and the Leningrad Region did not exceed the average rates across the country, the above regions fall into the category of most affected by HIV infection. Currently, heterosexual transmission is a dominant route of HIV infection. The literature data demonstrate that HIV-1 sub-subtype A6 prevails in the studied region, though there are other concurrently circulating subtypes [10].

The most recent data on prevalence of HIV drug resistance among ART patients with virological failure in St. Petersburg were obtained in 2012 [9], while the information about the current situation pertaining to secondary DR of the virus is almost non-existent. No DR related data about patients in the Leningrad Region can be found in literature.

St. Petersburg, a major transportation, tourist, cultural, industrial center, and a border city, is characterized by high migration of the population. The growing number of migrants can contribute to importation and spread of new genetic variants of the virus and trigger recombination processes in the virus population of the region.

The **aim** of the study was to characterize the present-day HIV-1 subtype-specific profile and drug-resistance mutations among ART patients with virological failure in the Leningrad Region.

Materials and methods

The study performed in 2016-2018 was based on the clinical material collected from 138 patients living

[2] HIV Circulating Recombinant Forms (CRFs) // HIV sequence database (2017). URL: https://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html

[3] Decree of the Government of the Russian Federation of December 1, 2004 No. 715 "On approval of the list of socially significant diseases and the list of diseases that pose a danger to others"; Decree of the President of the Russian Federation of 06.06.2019 No. 254 «On the Strategy for the Development of Healthcare in the Russian Federation for the period up to 2025».

[4] Federal Scientific and Methodological Center for the Prevention and Control of AIDS FBUN of the Central Research Institute of Epidemiology of Rospotrebnadzor. Reference. HIV infection in the Russian Federation as of December 31, 2020. URL: http://www.hivrussia.info/wp-content/uploads/2021/03/VICH-infektsiya-v-Rossijskoj-Federatsii-na-31.12.2020-..pdf.

[5] Federal Scientific and Methodological Center for the Prevention and Control of AIDS FBUN of the Central Research Institute of Epidemiology of Rospotrebnadzor. The level and structure of HIV drug resistance among naive patients in the Russian Federation. URL: http://www.hivrussia.info/wp-content/uploads/2020/12/2020-Rossijskaya-baza-dannyh-LU-VICH-u-naivnyh-patsientov.pdf (дата обращения: 05.09.2021)

in the Leningrad Region and having confirmed virological failure on ART. The material was collected with the voluntary informed consent of the patients. The blood plasma was delivered to the North-West District Center for AIDS Prevention and Control of the St. Petersburg Pasteur Research Institute of Epidemiology and Microbiology to be examined for HIV DR.

The blood plasma was used for viral load tests using the commercial AmpliSens® HIV-Monitor-FRT kit (Central Research Institute of Epidemiology, Russia) with the sensitivity threshold of 500 copies/mL. Samples with detectable viral loads were further tested through RT-PCR and Sanger sequencing. Commercial RT-PCR-kit-Pro/Rev and PCR-kit-Pro/Rev (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 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).

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 MEGAv.7.0 program and the ClustalW algorithm [11]. 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 reliability 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 both using the REGA HIV-1 Subtyping Tool v.3.0 program[6] 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 v.3.0 program and the parameters preset in the program (window size — 400 bp; step size — 20). The HIV-1 genetic sequences were assessed for DR mutations using the Stanford database[7].

[6] REGA HIV-1 Subtyping Tool v.3.0. URL: http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/

[7] Stanford HIV DB. URL: https://hivdb.stanford.edu/hivdb/by-mutations

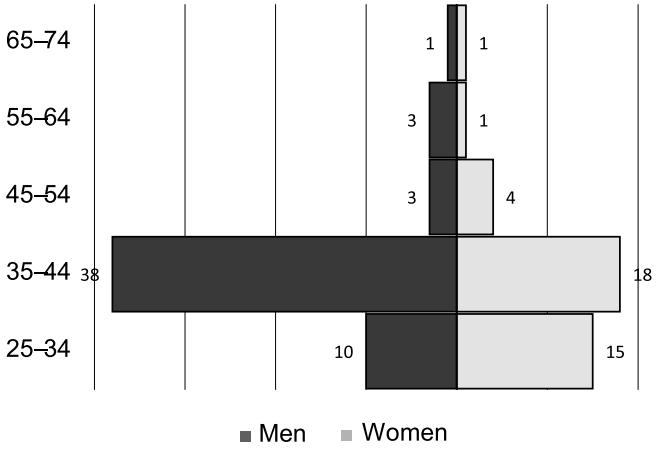

Fig. 1. Age and gender distribution of the examined patients (%).

Results

Out of 138 patients, more than a half (59%) were men. In the study group, most of the patients were of 35-44 years old (60%); the median age was 36 years (**Fig. 1**). All the patients had a HIV-1 viral load exceeding 1,000 copies/mL, thus making it possible to obtain virus genome sequences encoding protease and RT region.

Two methods of typing, the phylogenetic analysis and the analysis of sequences using the REGA HIV-1 Subtyping Tool v.3.0 program to improve the accuracy of distribution of HIV-1 subtypes, demonstrated the following distribution of HIV-1 subtypes: sub-subtype A6 common for Russia prevailed (98%); few cases had subtype B (1%) and a recombinant of the circulating recombinant form CRF_03AB and sub-subtype A1 (1%) (**Fig. 2, Table 1**).

The analysis showed that 96% of patients had at least one significant mutation associated with DR for the respective sub-subtype of the virus. We found a total of 105 different DR mutations at 35 positions of the *pol* gene of the HIV-1 virus (**Fig. 3**). Most of the detected mutations (89%) cause DR to nucleoside reverse transcriptase inhibitors (NRTIs) (43%) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) (47%). Among the detected mutations, the smallest proportion (11%) is represented by mutations associated with resistance to protease inhibitors (PIs). The mutations that are most frequently detected in patients are presented in **Tables 2** and **3**.

In most of the detected DR cases (n = 130; 94%) in the analyzed isolates, there were 2 and more mutations. In such patients, the virus is generally resistant to 2 classes of ARDs (n = 101; 73%) and sometimes to 3 classes of ARDs (n = 11; 8%).

Some of the obtained and analyzed nucleotide sequences of the *pol* gene region in the HIV-1 isolates were deposited to the international GeneBank database under numbers OL505461-OL505538.

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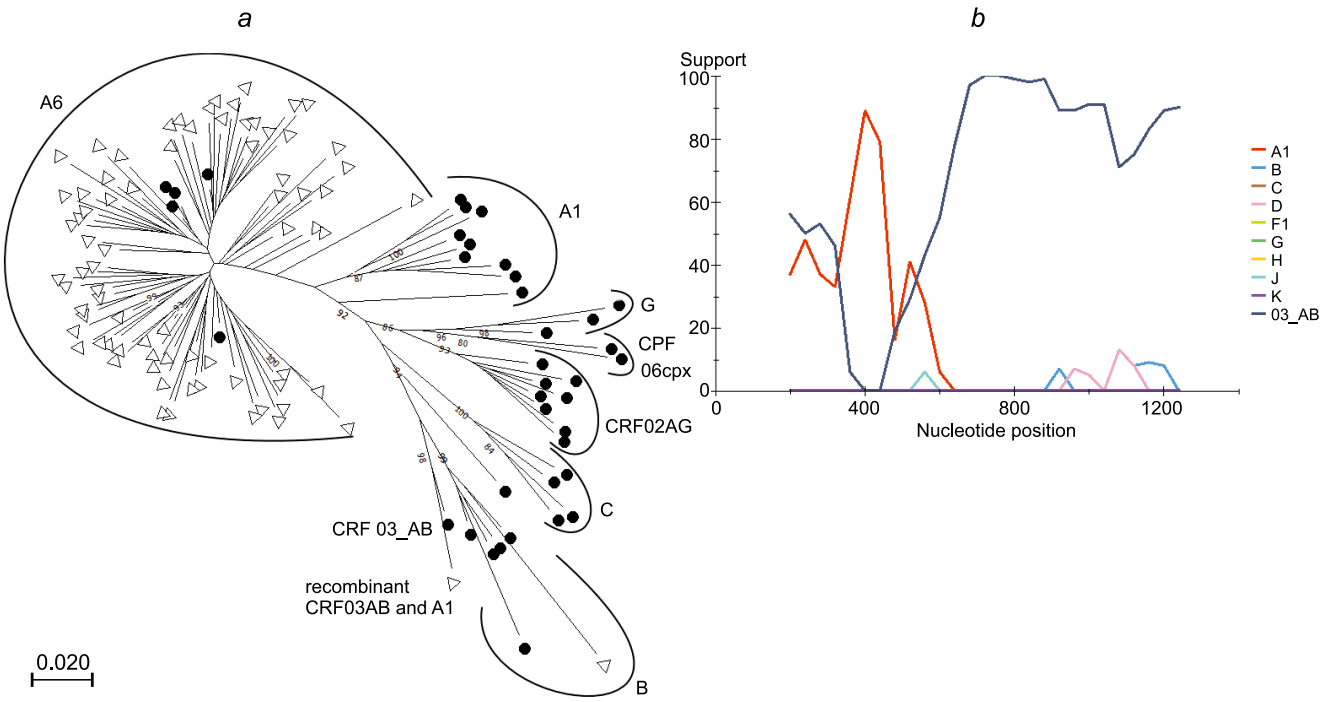

Fig. 2. The HIV-1 phylogenetic analysis with the Neighbor-joining algorithm (*a*) and the analysis of the recombination between sub-subtype A1 and CRF_03AB with the REGA HIV-1 Subtyping Tool v.3.0 program (*b*).

a: • — reference sequences (Table 1); Δ — sequences of isolates from the study.

Discussion

The genetic diversity of HIV-1 in the study group correlates with the diversity typical of Russia, demonstrating the absolute dominance of sub-subtype A6. Note that all the isolates were classified as sub-subtype A1 by genotyping with REGA HIV-1 Subtyping Tool v.3.0; however, our own phylogenetic analysis lets us state with confidence that they belong to sub-subtype A6. This discrepancy can be explained by the fact that the latest versions of the software do not include the data confirming that sub-subtype A6 should be separated from sub-subtype A1. In addition, the only case had a complex recombinant of CRF_03AB and subtype A1, the recombination analysis of which we performed within the *pol* gene.

Genotyping is critically important for the further testing of the sample for presence of ARD resistant HIV variants, as genetic variants of the virus can differ in their biological properties, virus evolution rate and disease progression as well as by the contribution of different mutations to development of DR to ART. Therefore, additional studies must be conducted to assess the participation of recombinant forms in the genetic diversity of the virus in the region. The lack of attention to the high diversity of HIV recombinants and unavailability of comprehensive data on most common recombination points can result in misidentification of DR in the virus [21].

Particular attention should be given to the difference in the genetic diversity of subtype-specific pro-

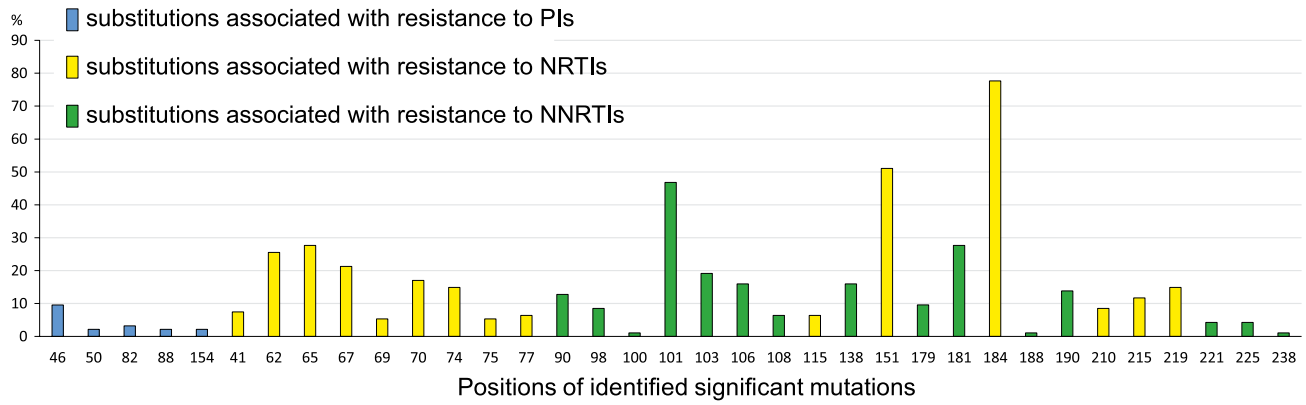

Fig. 3. Frequency of substitutions at positions of the studied *pol* gene region.

Table 1. GenBank reference sequences used in the phylogenetic analysis

Sub-subtype	GenBank sequence
A1	AF069670_A1_Somalia
A1	AB287376_A1_Ruanda
A1	U51190_A1_Uganda
A1	EU110087_A1_Kenya
A1	AF484509_A1_Uganda
A1	AF107771_A_Sweden
A2	AF286237_A2_Cyprus
A3	AY521631_A3_Senegal
A3	AY521629_A3_Sweden
A6	HQ449397_A6_Krasnodar
A6	HQ161930_A6_Smolensk
A6	EF589043_A6_Kazakhstan
A6	AY500393_A6_Moscow
A6	AF413987_A6_Kiev
B	M17449_B_USA
B	KJ771697_B_Germany
B	HM586190_B_UK
B	AY713409_B_USA
B	AY173951_B_Thailand
C	AF067155_C_India
C	U52953_C_Brazil
C	U46016_C_Ethiopia
C	AY772699_C_Africa
F1	AF075703_F1_Finland
G	AF061641_G_Finland
G	U88826_G_Nigeria
G	AF084936_G_Congo
CRF_02AG	AF063224_CRF02_AG_Djibouti
CRF_02AG	GU201514_CRF02_AG_Cameroon
CRF_06cpx	HQ529257.1_Ghana_crf_06cpx
CRF02_AG	KT124792_CRF02_AG_Germany
CRF02_AG	AB231898_CRF02_AG_Ghana
CRF02_AG	EU786671_CRF02_AG_Spain
CRF02_AG	AB231896_CRF02_AG_Ghana
CRF02_AG	AY151001_CRF02_AG_Ecuador
CRF02_AG	AF377954_CRF02_AG_Cameroon
CRF03_AB	AF193276_CRF03_AB_Kaliningrad
CRF06_cpx	MH605500.1_Guinea-Bissau_crf_06cpx

files obtained in this study and in the study performed in 2012 [9]. The Leningrad Region is characterized by smaller numbers of detected subtypes as compared to St. Petersburg, thus implying significant disconnectedness between the HIV-infected groups of the region and the city despite their geographic proximity.

The frequency of DR mutations was extremely high among ART patients with virological failure. Mutations to RT inhibitors were most frequently detected, while mutations associated with resistance to PIs were detected only in 8 (6%) cases, thus leading to assumption that it can be explained by a higher genetic barrier to DR in PIs and by their less frequent use in treatment regimens administered to the examined patients [22, 23].

Compared with the 2012 data on St. Petersburg, the frequency of detection of HIV-1 DR mutations increased more than 3-fold: from 30% to 95% [9]. The increased number of resistant virus variants can be explained by changes in the HIV-infected population in Russia: during this period, the epidemic went beyond the vulnerable groups of the population, affecting a larger number of HIV-infected people of higher social status. The ARD patients demonstrated increased adherence. There was an increased number of patients with suboptimal treatment adherence, which is a significant contributor to the risk of DR development [24]. At the same time, the region demonstrates an increasing frequency of transmitted primary resistance, which, undoubtedly, contributes to DR prevalence among ARD people, as currently there is no patients' screening for RD mutations prior to their treatment [10].

In addition to the frequency of mutations, there were changes in their distribution (Fig. 3). The most frequently detected mutation is *M184V* (n = 106; 77%). The second most frequent mutation is *Q151M* (n = 70; 51%), which is associated with DR to several NRTIs; note that it was not reported in 2012 [9]. In 2012, the third most frequent mutations were *G190S* (47%) and *K103N* (13%) associated with virus DR to NNRTIs; in this study, the 3rd place is occupied by mutations at the 101st position (n = 65; 47%), and they are also associated with DR to NNRTIs. NRTI and NNRTI-resistance mutations were detected at the same frequency, conjointly in many cases, causing resistance to most of the RT inhibitors.

The major mutation *M46I/L* was detected among PI-resistance mutations in all cases, and the minor mutation *L89T* was detected in 3 cases.

In addition, 2 mutations were detected at the 10th position in the protease region, one of which is *L10LF*, a minor PI-resistance mutation resistance, and the other mutation, *L10I*, detected in 40 (29%) cases, increases replication of viruses with other PI-resistance mutations [25].

Note that the obtained results did not differ significantly from the results obtained by the analysis of HIV-1 subtype-specific and mutation profiles in ART

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Table 2. Most frequent HIV DR mutations in the examined patients

Mutation	Frequency	Description
M184V	106 (77%)	Causes high-level resistance *in vitro* to 3TC and FTC and low-level resistance to ddI and ABC [12]
Q151M	70 (51%)	Causes intermediate/high-level resistance to AZT, ddI, d4T, and ABC and low-level resistance to TDF, 3TC, and FTC [13]
K101E	65 (47%)	Causes intermediate-level resistance to NVP and RPV, low-level resistance to EFV and potentially low-level resistance to ETR [14]
Y181C	37 (27%)	Reduces susceptibility to NVP, ETR, RPV, and EFV more than by 50-fold, 5-fold, 3-fold, and 2-fold, respectively [15]
K65R	37 (27%)	Causes intermediate/high-level resistance to TDF, ddI, ABC, and d4T, and low/intermediate-level resistance to 3TC and FTC. Increases susceptibility to AZT [16]
D67N	28 (20%)	Associated with low-level resistance to AZT and d4T, and contributes to reduced susceptibility to ABC, ddI, and TDF [17]
K103N	26 (19%)	Causes a significant decrease in susceptibility to NVP and EFV [17]
E138A	22 (16%)	Reduces susceptibility to ETR and RPV approximately by 2-fold [18]
G190S	19 (14%)	Causes high-level resistance to NVP and EFV [19]
V90I	18 (13%)	Associated with a minimum decrease in susceptibility to NNRTIs [20]

patients with virological failure from other regions of the North-West Federal District of the Russian Federation. For example, in Arkhangelsk sub-subtype A6 (90%) prevailed as compared to subtype B (9%); the *CRF03_AB* variant was detected in 1 (1%) case. In the group from Arkhangelsk, DR mutations were detected in a smaller number of patients (86.7%) as well as multiple (2 and more) mutations were detected in a smaller number of patients (81.9%), though there were no significant differences. Mutations to RT inhibitors were detected more frequently (84%) than mutations to PIs (16%); isolates with DR only to NRTIs accounted for 17%, isolates with DR only to NNRTIs — 2%, with DR only to PIs — 11%, with DR both to PIs and NRTIs — 12%, with DR to NRTIs and NNRTIs — 47%, with DR to all 3 groups of inhibitors — 12% [26]. Although mutations demonstrate quite similar prevalence rates, their distribution is different. Among patients from Arkhangelsk, mutation *M184V* had the highest prevalence of 72% (67% of all the mutations), similar to the results obtained during this study. At the same time, the prevalence of mutations *K103N* (33%) and *G190S* (a little less than 25%) is more similar to the prevalence reported in the study performed in St. Petersburg in 2012. The similar prevalence of the above mutations was recorded among HIV-infected ART patients with virological failure in Novgorod [27].

Table 3. Frequency of the most common combinations of HIV DR mutations in the examined patients

Mutations	Frequency
M184V + K101E	29 (21%)
M184V + K103N	19 (14%)
M184V + K101E + G190S	17 (12%)
M184V + Q151M + K103N	16 (11%)
M184V + G190S	7 (5%)

Conclusions

HIV-1 A6 (IDUA) remains the dominant genetic HIV-1 variant in the Leningrad Region among patients with virological failure on ART. It has been found that the frequency of HIV-1 DR mutations increased significantly compared to 2012. In all cases, among samples with DR mutations, there were mutations to 2 or 3 groups of drugs. The high prevalence of HIV-1 DR mutations among ART patients with virological failure calls attention to surveillance of HIV-1 DR both among ART-experienced patients and ART-naïve individuals. Lack of surveillance can result in increasing numbers of HIV patients with primary resistance to ART.

Further studies of HIV-1 molecular and epidemic diversity will be essential for monitoring the spread and escalation of the HIV epidemic.

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