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RESEARCH ARTICLE

Molecular Surveillance of HIV-1 Infection in Krasnoyarsk Region, Russia: Epidemiology, Phylodynamics and Phylogeography

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Abstract: ***Background*:** The information about the dynamics of the viral population and migration events that affect the epidemic in different parts of the Russia is insufficient. Possibly, the huge size of the country and limited transport accessibility to certain territories may determine unique traits of the HIV-1 evolutionary history in different regions.

***Objective*:** The aim of this study was to explore the genetic diversity of HIV-1 in the Krasnoyarsk region and reconstruct spatial-temporal dynamics of the infection in the region.

***Methods*:** The demographic and virologic data from 281 HIV-infected individuals in Krasnoyarsk region collected during 2011-2016 were analyzed. The time to the most recent common ancestor, evolutionary rates, population growth, and ancestral geographic movements was estimated using Bayesian coalescent-based methods.

***Results*:** The study revealed moderate diversity of the HIV-1 subtypes found in the region, which included A6 (92.3%), CRF063_02A (4.3%), B (1.1%), and unique recombinants (2.5%). Phylogenetic reconstruction revealed that the A6 subtype was introduced into Krasnoyarsk region by one viral lineage, which arose around 1996.9 (1994.5-1999.5). The phylogeography analysis pointed to Krasnoyarsk city as the geographical center of the epidemic, which further spread to central neighboring districts of the region. At least two epidemic growth phases of subtype A6 were identified which included exponential growth in early-2000s followed by the decline in the mid/late 2010s.

***Conclusion*:** This study demonstrates a change in the genetic diversity of HIV-1 in the Krasnoyarsk region. At the beginning of the epidemic, subtype A6 prevailed, subtypes B and CRF063_02A appeared in the region later.

Keywords: HIV-1, Russia, Krasnoyarsk region, phylodynamic, phylogeography, molecular epidemiology, Bayesian analysis, epidemiological monitoring.

1. INTRODUCTION

The UNAIDS estimates that Eastern Europe and Central Asia are the only regions in the world where the annual rate of HIV infections continues to rise at an alarming rate [1]. For the last decade, Russia has been reported with the first highest regional HIV prevalence (0.7%), with about 1,000,000 people living with HIV/AIDS [2, 3].

Increasing efforts have been devoted to gathering data on the HIV epidemiology in Russia. A nationwide epidemiological survey indicated that about 70.0% of HIV-positive people were infected with the A6 sub-subtype virus (previously called A_{FSU} [4]), 10.0 % - with subtype B and about 7.0% - with CRF063_02A [5]. The A6 subtype originally came on the scene in the mid-1980s, gradually spreading through heterosexual contact in the city of Odessa, Ukraine [6, 7]. Ten years later, this subtype progressed speedily among Injecting Drug Users (IDU) in Southern Ukraine, marking the beginning of a massive HIV epidemic spread in most countries of the former USSR, including Russia [8-13].

Most studies at this time reported a nearly exponential increase of HIV-1 prevalence by the end of the last millennium [8, 14, 15], with the main driving forces of increased drug usage and socioeconomic crisis at the fall of the Soviet Union [16, 17]. In the Russian Federation alone, a cumulative total of over 89,000 HIV cases were detected by 2000 of which more than 90% were drug-injection related [18, 19]. By the middle of the first decade of 2000, the sexual mode of HIV transmission became actively involved in support of the HIV epidemic

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in Russia [20, 21] with the resultant propagation of both the subtype B and CRF063_02A variants [22-24].

According to available epidemiological information and limited phylodynamic studies [25], subtype B seemed to spread via several independent viral lines long before the beginning of the overwhelming HIV epidemic in Russia and was circulating through sexual contacts (mainly among «male-to-male sex» (MSM) at relatively low transmission rates [26-30]. By contrast, the recent introduction of CRF063_02A to Russia occurred apparently at two-time points, in 2003 and 2007 [31, 32]. During the last few years, this recombinant has quickly overtaken subtype A6 as presented by the number of new HIV infections among heterosexuals in Siberia [33, 34]. Currently, the sexual trend with MSM inclusive is the cause of 54.8% HIV-infection cases in the country [3].

Although the origin and spread of some HIV subtypes in Russia have been previously studied, nonetheless, little is known about the dynamics of the viral population and migration events that affected the HIV-1 epidemic in the separate regions of the country. It is noteworthy that for rapidly evolving pathogens such as HIV-1 the migration is one of the major processes accountable for shaping of the genetic structure of the population, and reconstruction of spread routes, thus, providing the fundamental understanding of the epidemic dynamics [35, 36]. There exists the possibility that the vastness of the country and limited transport accessibility to certain territories may determine the unique traits of the evolutionary history of HIV-1 in different regions.

The Krasnoyarsk region is located in Siberia, Russia and ranks second in size among the subjects of the country with a coverage area of 2.4 million km^2. The population of the region is relatively small with inhabitants of 2.8 million people. However, the present count of people living with HIV/AIDS in the Krasnoyarsk region is 22,228, consequently, making it one of the twenty most HIV affected territories of Russia with high HIV prevalence of 953.5 per 100,000 population.

Since the advent of the HIV epidemic in Russia, information on the spread of HIV-1 in Krasnoyarsk region is based on only one study, which is quite limiting (2008). Rumyantseva *et al.* [37] discovered that the main subtypes of HIV-1 among the risk groups in the region were A6 (92.5%) and B (7.5%), and demonstrated some degree of genetic subdivision in Krasnoyarsk HIV populations. The assumption is that this was the result of the different geographical origin of each sequence (within the region) and was unrelated to the social characteristics of the HIV-infected patients, for example, the transmission mode. However, in this study, the limited number of sequences and geographical locations were analyzed without taking the HIV migration routes into consideration.

Our study aimed at the exploration of the genetic relationships between the HIV-1 strains circulating in the Krasnoyarsk region, and also the reconstruction of the viral spatial-temporal dynamics in the region. We present the first results of the HIV-1 evolutionary history analysis in the Krasnoyarsk region using the phylogenetic approach based on the Bayesian coalescent-based method.

2. MATERIALS AND METHODS

2.1. Ethics Statement

This study, including the analysis of clinical and laboratory data of HIV-infected patients attending the Krasnoyarsk Regional AIDS Centre, was reviewed and approved by the local ethical committee. Written informed consent was signed by all the subjects involved.

2.2. Study Population

We investigated 281 HIV patients in total who presented between 2011 and 2016 at the Krasnoyarsk regional AIDS Centre. Six patients did not receive the Antiretroviral Therapy (ART). HIV-positive status was determined by the double repeated enzyme immunoassay and confirmed by Western blot. All patients resided permanently in the Krasnoyarsk region and were well-documented from the time of the HIV infection. The demographic, clinical data and treatment information were collected by physicians from the patients' medical charts.

2.3. Genotyping and Dataset

Plasma samples were genotyped using the ViroSeq HIV-1 Genotyping System (Abbott, USA) as per manufacturer's instructions. Sequencing was performed using an automated Genetic Analyzer ABI Prism 3130 (Applied Biosystems, USA). All sequencing electrophoregrams were carefully and manually inspected for sites of ambiguous sequence. Multiple sequence alignments were made using the MAFFT program [38] with the selected reference sequences of various HIV subtypes from the Los Alamos HIV-1 database (https://www.hiv.lanl.gov). The length of the alignment was 1302 nucleotides and covered the entire protease (PR) and partial Reverse Transcriptase (RT) sequences (positions 2253-3554 based on HXB2 numbering). The COMET HIV software [39] and REGA Subtyping Tool v3.0 [40] were used for HIV-1 subtyping. The subtypes of all sequences were confirmed by phylogenetic analysis. New intersubtype or inter-CRFs recombinant sequences were analyzed with the jpHMM program [41]. HIV-1 subtype A6 sequences (N=114) from the Krasnoyarsk region (2007-2008 and 2017-2018 sampling years) from the Los Alamos HIV Sequence Database were additionally included in the analysis (Supplemental Table **S1**). All codons associated with major drug-resistance mutations [42] were removed from the final sequence alignment for phylogenetic and evolutionary (phylodynamics) analysis.

2.4. Phylogenetic and Evolutionary Analysis

The GTR+I+G substitution model was selected as best fitting for each dataset by using jModelTest v2.1.7 [43]. A maximum-likelihood (ML) tree was inferred with IQ-TREE [44] with 1000 replicates for bootstrap support. The cluster reliability was also supported by branch-supports using the Shimodaira-Hasegawa approximate likelihood ratio (SH-aLRT) test [45]. A monophyletic cluster in the phylogeny with an SH-aLRT value over 0.9 containing more than 8 sequences was considered significant.

The Time to the Most Recent Common Ancestor (TMRCA), evolutionary rates and the population growth

were co-estimated by using the Bayesian Markov Chain Monte Carlo (MCMC) in BEAST v1.10.1 [46]. These indicators were estimated in the optimized dataset excluding the following sequences: (i) with more than 1.5% of ambiguous bases; (ii) with particularly high evolutionary rates, and (iii) sequences from epidemiologically linked patients (mother-child, sex partners and hospital-acquired infection). Thus, the reconstruction of the HIV-1 subtype A6 temporal dynamics was based on the set of 293 *pol* sequences isolated at different time frames (2008-2018) in more than 15 different geographic locations (see below). The uncorrelated relaxed log-normal molecular clock model and Bayesian skyline model were selected as the best fitting by using Tracer v1.7.1 [47]. Two independent MCMC chains were run of 500×10^6 steps with 2500 generations logged in, excluding first 25% for each run. Convergence of the chains was estimated based on the Effective Sample Size (ESS) in the Tracer v1.7.1. The parameter estimates with ESS over 200 were accepted. We summarized the Maximum Clade Credibility (MCC) trees from the posterior distribution of trees using a TreeAnnotator. Lastly, the MCC trees were visualized in FigTree v1.4.0 and/or iTOL tool [48].

2.5. Phylogeographic Analysis

The estimates of ancestral geographic movements throughout the phylogenetic history were obtained using the Bayesian discrete phylogeographic approach and Bayesian Stochastic Search Variable Selection (BSSVS) procedure, in which all possible reversible exchange rates between locations were equally likely, were carried in BEAST v1.8.2. We optimized the dataset for this analysis by excluding the part of the Krasnoyarsk city sequences, since their number significantly exceeded the number of sequences from other locations. 189 sequences were finally selected in the dataset. MCMC chains were run for 400×10^6 steps with 2500 generations logged in, excluding first 25% generations for each run. The ESS value for each parameter was over 200. The MCC trees were summarized with TreeAnnotator v1.10.1 and visualized with FigTree v1.4.2. The migration routes and spatial projections were summarized using the SPREAD software [49]. The statistical support for migration routes was determined with Bayes Factor (BF); a route with the BF-support over 3 was considered credible.

2.6. Statistical Analyses

The temporal signal sufficiency in the Krasnoyarsk HIV-1 A6 datasets was estimated using TempEst v1.5 [50]. Regression of root-to-tip genetic distance against the sampling time was inferred from ML-phylogenies. The correlation coefficient in every dataset was over 0.50 (Supplemental Fig. **S1**). The analysis of discrete categorical (demographic characteristics) was performed with the Pearson chi-square test (χ^2) or Fisher's two-tailed exact test (where necessary) by using STATISTICA v.10.0 software (StatSoft, USA). The differences were considered significant at P less than 0.05.

3. RESULTS

3.1. Demographic Characteristics

There were 281 HIV-1 infected patients in this study, with the median age of 34 years (range 1-85 years). The percentage of men and women was approximately the same (50.9% and 49.1%, respectively). The children under fourteen years old made up 7.1% of all HIV infection cases; the share of persons of working age was 53.0%. The dominant transmission route was intravenous drug usage (IDUs, 44.1%) and heterosexual contacts (HSX, 36.3%). The hospital-acquired HIV-infection accounts for about 2.1%. However, such high numbers could be attributable to the fact that our study included all hospital cases of HIV infection over the entire observation period; in the overall modes of HIV transmission, this route accounts for less than 0.05% (13/37900).

3.2. HIV Subtype Diversity in the Study Population

We analyzed HIV-1 *pol* sequences in all 281 HIV-1 infected patients involved in the study. Among them, 265 (92.3%) were classified as subtype A6, 12 (4.3%) as CRF063_02A, 3 (1.1 %) as subtypes B, and 7 (2.5 %) as unique AB-recombinant forms, labeled as "other" subtypes (Table **1**). The sub-subtype A6 and CRF063_02A were found mainly in injecting drug users (44.9% and 41.7%) and heterosexuals (36.6% and 16.7%), correspondingly. Subtype B infected individuals were males only (100.0%), while all of them reported HSX exposure.

All subtype A6 and CRF063_02A sequences were identified by BLAST searches (and phylogenetic analyses) as related to the strains circulating in the Former Soviet Union (FSU) countries, while subtype B sequences belonged to the Pandemic (Western-B) variant (Fig. **S1**).

3.3. Epidemic Clusters and Phylodynamics of the HIV-1 A6 Subtype

Since the HIV-1 subtype A6 is the most epidemiologically significant strain in the Krasnoyarsk region, our main focus for further analysis was only on this viral population. The maximum-likelihood analysis identified only one HIV-1 A6-cluster in the region, which nevertheless included at least 3 subclusters embracing 345 (92.5 %) sequences (Fig. **1** & Table **2**).

Although, these subclusters revealed no certain genetic structure according to transmission routes and/or geographic locations, we however, noted that most of the sequences from territories and cities of the Krasnoyarsk region, unlike the city of Krasnoyarsk belonged to subcluster 3.

The smallest part of the Krasnoyarsk city sequences prevailing in the total set was observed in the smallest subcluster 2, with 37.5% sequences obtained from the Uyarsky district. One single A6 cluster in the Krasnoyarsk region was dated back to 1996.9. The TMRCA for subclusters 1-3 was estimated from 1999.5 to 2002.1 (Table **2**).

According to the Bayesian evolutionary analysis by sampling trees, the mean evolutionary rate [95% HPD] of the HIV-1 subtype A6 was $3.19\text{x}10^{-3}$ [2.63^{-3}-$3.76\text{x}10^{-3}$] substitutions/site/year. The Bayesian skyline plot indicated at least two growth phases of the HIV-1 subtype A6 effective population size in the Krasnoyarsk region: the exponential growth recorded approximately between 1999 and 2004, and a very slowly increase (stable phase) after 2008, up to the present time. The overall total number of reported HIV cases and

Table 1. General characteristics of HIV-infected patients from Krasnoyarsk region classified by subtype.

-	-	HIV-1 Subtypes				*P* [d]
-	**Total (*N*=281, 100.0%)**	**A6 (*N* =259, 92.3%)**	**CRF063 (*N* =12, 4.3%)**	**B (*N* =3, 1.1%)**	**Others [a] (*N* =7, 2.5%)**	
HIV diagnosis	-	-	-	-	-	0.31
1996-2002	76 (27.0)	74 (97.4)	–	–	2 (2.6)	-
2003-2009	105 (37.4)	102 (97.2)	–	–	3 (2.8)	-
2010-2016	100 (35.6)	83 (83.0)	12 (12.0)	3 (3.0)	2 (2.0)	-
Age (years)	-	-	-	-	-	0.67
< 20	22 (7.8)	20 (90.9)	2 (9.1)	–	–	-
20 - 30	55 (19.6)	47 (85.4)	2 (3.6)	3 (5.5)	3 (5.5)	-
31 - 40	147 (52.3)	141 (70.9)	4 (2.7)	–	2 (1.4)	-
41 - 50	37 (13.2)	34 (91.9)	2 (5.4)	–	1 (2.7)	-
51 - 60	12 (4.3)	10 (83.3)	2 (16.7)	–	–	-
> 60	8 (2.8)	7 (87.5)	–	–	1 (12.5)	-
Gender	-	-	-	-	-	0.39
Male	143 (50.9)	133 (93.0)	5 (3.5)	3 (2.1)	2 (1.4)	-
Female	138 (49.1)	126 (91.3)	7 (5.1)	–	5 (3.6)	-
Group risk	-	-	-	-	-	0.21
HSX [b]	102 (36.3)	94 (92.2)	2 (2.0)	3 (2.9)	3 (2.9)	-
MSM	4 (1.4)	4 (100.0)	–	–	–	-
IDU	124 (44.1)	116 (93.5)	5 (4.0)	–	3 (2.5)	-
MTCT	19 (6.8)	17 (89.5)	2 (10.5)	–	–	-
Others [c]	32 (11.4)	28 (87.5)	3 (9.4)	–	1 (3.1)	-
Sampling year	-	-	-	-	-	0.26
2011	12 (4.3)	12 (100.0)	–	–	–	-
2012	86 (30.6)	84 (97.7)	–	2 (2.3)	–	-
2013	60 (21.4)	55 (91.7)	1 (1.6)	–	4 (6.7)	-
2014	51 (18.1)	41 (80.4)	6 (11.7)	1 (2.0)	3 (5.9)	-
2015	48 (17.1)	45 (93.7)	3 (6.3)	–	–	-
2016	24 (8.5)	22 (91.7)	2 (8.3)	–	–	-

[a] HIV-1 unique A6/B-recombinant form.
[b] Including unprotected heterosexual contacts with IDUs (15/104, 14,4%).
[c] A hospital-acquired HIV-infection (6/32, 18,8%) and unknown-risk HIV-positive samples (25/32, 81,2%).
HSX, heterosexuals; IDU, intravenous drug users; MSM, men who have sex with men; MTCT, mother-to-child transmission.
[d] P-value of the difference between HIV subtypes.

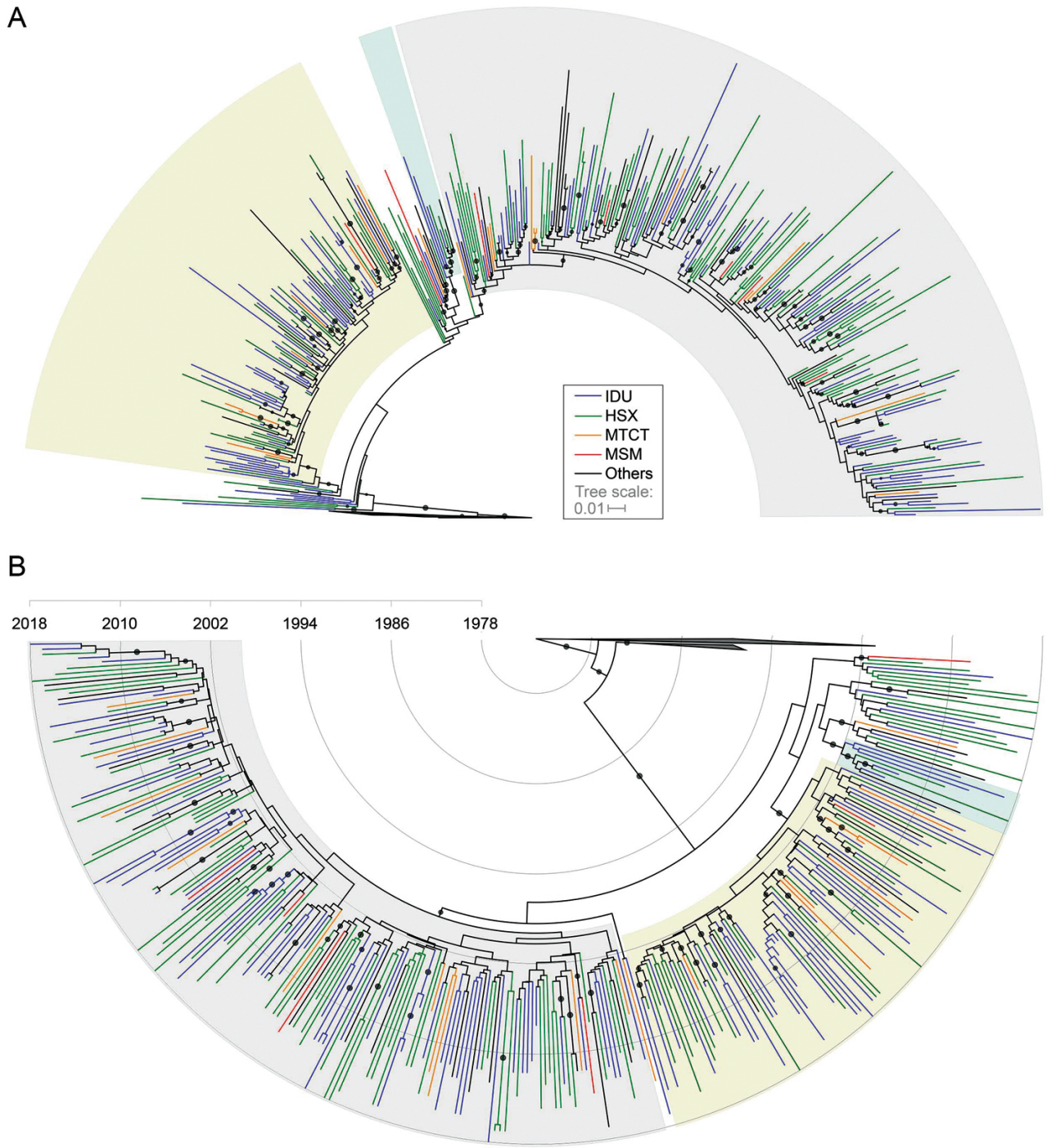

Fig. (1). Phylogenetic trees of the HIV-1 subtype A6 *pol* sequences from HIV-infected persons in the Krasnoyarsk region. (**A**) - Maximum-likelihood tree including 373 subtype A6 sequences (see Material and Methods). Branches are colored according to the risk factors of infection as indicated in the legend. Major significantly supported monophyletic clusters (subclusters, indicated as S1–S3) were identified and colored differently. Circles indicate the key nodes with high state support (SH-aLRT values over 0.9). The tree was rooted using African A1 sequences as outgroup (presented in collapsed form); (**B**) - Bayesian time-scaled maximum clade credibility tree for 293 subtype A6 sequences that were finally selected in the dataset. Branch lengths are drawn to scale with the concentric circles indicating years. Circles indicate the key nodes with high posterior state probability support (PSP over 0.9). The rest designations as on the ML-tree. Abbreviations: HSX, heterosexuals; IDU, Intravenous Drug Users; MSM, men who have sex with men; MTCT, Mother-To-Child Transmission.

Table 2. The size, TMRCA, transmission routes and locations of major HIV-1 subtype A6 subclusters in Krasnoyarsk region.

-	-	Subclusters				
	Total (*N*=373, 100%)	**S1 (*N*=116, 31.1%)**	**S2 (*N*=8, 2.2%)**	**S3 (*N*=221, 59.2%)**	**Un(subcluster) (*N*=28, 7.5%)**	***P***
Transmission route	-	-	-	-	-	0.86
HSX	147 (39.4)	40 (34.5)	3 (37.5)	96 (43.4)	14 (50.0)	
IDU	169 (45.3)	60 (51.7)	4 (50.0)	90 (40.7)	9 (32.1)	
MSM	6 (1.6)	1 (0.9)	-	4 (1.8)	1 (3.6)	
MTCT	22 (5.9)	9 (7.8)	-	12 (5.5)	1 (3.6)	
Others [a]	29 (7.8)	6 (5.1)	1 (12.5)	19 (8.6)	3 (10.7)	
Locations	-	-	-	-	-	0.24
Beresovsky	10 (2.7)	3 (2.5)	1 (12.5)	6 (2.7)	-	
Bogotolsky	4 (1.1)	1 (0.9)	-	2 (0.9)	1 (3.6)	
Divnogorsk	3 (0.8)	-	-	2 (0.9)	1 (3.6)	
Emelyanovsky	4 (1.1)	1 (0.9)	-	2 (0.9)	1 (3.6)	
Eniseysky	6 (1.6)	-	-	6 (2.7)	-	
Kozulsky	2 (0.5)	2 (1.7)	-	-	-	
Kansky	4 (1.1)	1 (0.9)	-	3 (1.4)	-	
Krasnoyarsk	270 (72.3)	96 (82.7)	3 (37.5)	154 (69.7)	17 (60.7)	
Lesosibirsk	10 (2.7)	-	-	9 (4.1)	1 (3.6)	
Norilsk	9 (2.4)	1 (0.9)	-	7 (3.2)	1 (3.6)	
Rybinsky	3 (0.8)	-	1 (12.5)	2 (0.9)	-	
Sukhobuzimsky	4 (1.1)	1 (0.9)	-	3 (1.4)	-	
Sharypovsky	3 (0.8)	-	-	2 (0.9)	1 (3.6)	
Taimyrsky	22 (5.9)	-	-	18 (8.1)	4 (14.2)	
Uyarskiy	4 (1.1)	-	3 (37.5)	1 (0.4)	-	
Others [b]	15 (4.0)	10 (8.6)	-	4 (1.8)	1 (3.5)	
Median TMRCA [95% HPD]	1996.9 [1994.5-1999.5]	2000.7 [1999.4-2001.9]	2002.1 [2000.8-2003.5]	1999.5 [1998.5-2002.2]	*ND*	

[a] A hospital-acquired HIV-infection and unknown-risk HIV-positive samples.
[b] Sequences from other regions of the Krasnoyarsk region (Mansky, Evenkiysky, Partizansky, Achinsky, Boguchanskiy, Bolshemurtinsky, Bolsheuluisky, Sayansky, Kazachinsky, Motyginsky and Turukhansky district, and Sosnovoborsk city)
HSX, heterosexuals; IDU, Intravenous Drug Users; MSM, Men who have Sex with Men; MTCT, Mother-To-Child Transmission; ND, Not-Determined
[c] P-value of the difference between subclusters

new HIV infections per year agreed well with the estimated curves of effective population size for HIV-1 subtype A6 (Fig. **2**).

3.4. Migration Pathway of the A6 Subtype within the Krasnoyarsk Region

Phylogeographic analysis was performed using the Krasnoyarsk region sequences from twelve geographical locations: eight districts and four cities (Table **2**). As expected, the root of the tree was located in Krasnoyarsk city. In addition, the most influential nodes in the network were located in Krasnoyarsk city, suggesting a strong genetic flow in this city. The analysis showed that this city was involved in at least 5 strongly supported migration pathways between 10 different locations in the region (Fig. **3**). According to the inferred MCC genealogy, subtype A6 seemed to have initially appeared in the central part of the Krasnoyarsk region following with simultaneous migrations from Krasnoyarsk city to Eniseysky (BF=3.2), Uyarsky (BF=6.1) and Kozulsky (BF=11.7) districts, and Lesosibirsk city (BF=3.7). We also found the most strongly supported pathways between Sukhobuzimsky and Kansky districts (BF=4.8), Divnogorsk city

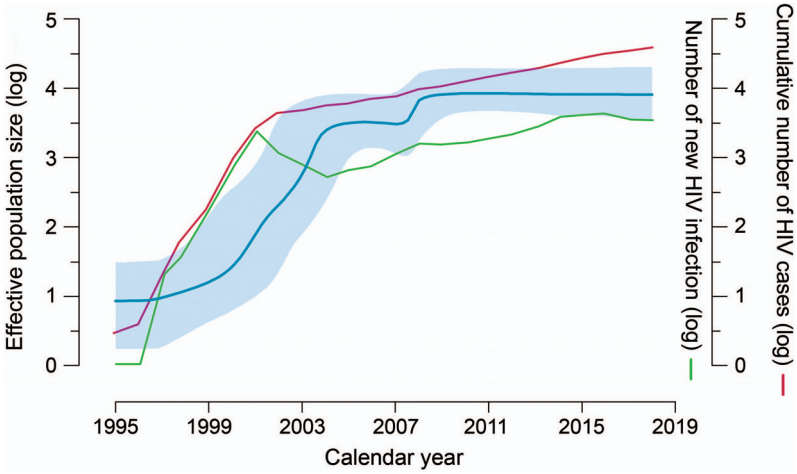

Fig. (2). Demographic history of the HIV-1 subtype A6 in the Krasnoyarsk region. Left y-axis of Bayesian skyline plot represents the median effective number of infection (*Ne*) through time (*t*). Ne(t) estimates are presented as median (solid blue line) and the 95% HPD (blue area). The right y-axis represents the number of reported HIV cases (red line) and new HIV infections (green line). (*The color version of the figure is available in the electronic copy of the article*).

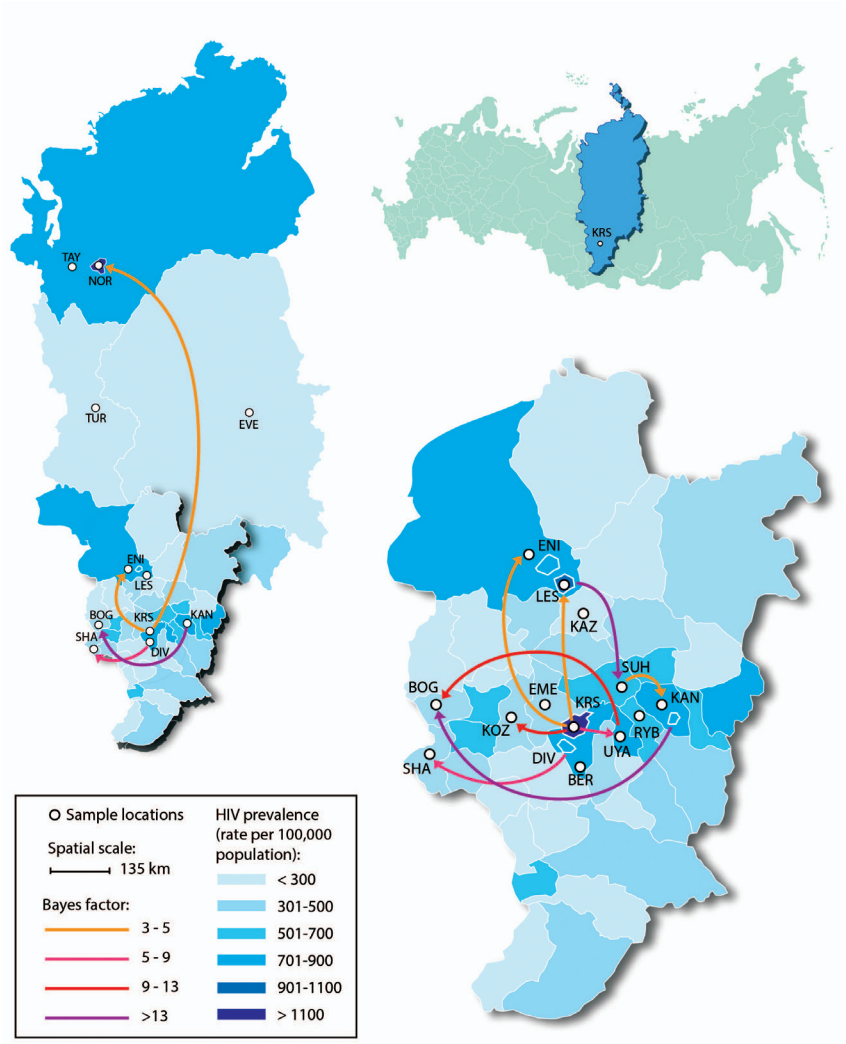

Fig. (3). The migration patterns of the HIV-1 subtype A6 in the Krasnoyarsk region. The geographic locations are colored based on the prevalence of the HIV-1 infection. Arrows between locations indicate migration routes between the HIV-1 A6 sub-populations and corresponds to the locations state transitions along the branches of the Bayesian MCC tree. Arrow colors reflect the Bayes Factor test support for epidemiological linkage between locations. Location: BER-Beresovsky; BMR-Bolshemurtinsky; BOG-Bogotolsky; DIV-Divnogorsk; EME-Emelyanovsky; ENI-Eniseysky; EVE-Evenkiysky; KAN-Kansky; KOZ-Kozulsky; KRS-Krasnoyarsk; LES-Lesosibirsk; NOR-Norilsk; RYB-Rybinsky; SHA-Sharypovsky; SUH-Sukhobuzimsky; TAY-Taymyrsky; TUR-Turukhansky; UYA-Uyarskiy.

and Sharypovsky districts (BF=5.7), Uyarsky and Bogotolsky districts (BF=12.8), Lesosibirsk city and Sukhobuzimsky districts (BF=28.9), and Kansky and Bogotolsky districts (BF=75.0). Other migration pathways in the central area of the Krasnoyarsk region were poorly supported (BF<3). Interestingly, there was no strong evidence of genetic flow from the centre (for example, Krasnoyarsk city or Berezovsky districts) to the north area of the Krasnoyarsk region (Taymyrsky districts) with the exception of Norilsk city; BF for migration pathways from Krasnoyarsk city to Norilsk was 3.0. A phylogeographic analysis, which included the most similar non-Krasnoyarsk sequences from the socially and economically related territories (Murmansk and Arkhangelsk [51]) also did not suggest the strongly supported migration pathways (BF<3) to Taymyrsky districts or Norilsk city from these areas.

4. DISCUSSION

In this work, we carried out the analysis of the HIV-1 diversity in the Krasnoyarsk region and the reconstruction of the spatial-temporal dynamics of the most common subtype A6 using sequences from all high-risk populations, as well as sequences from the publicly available database. We focused our analysis on the *pol* gene which has a well-proven record in studies of this nature [52-55]. However, it cannot be excluded that the evolutionary parameters presented here may be overestimated due to the low sample size and the relatively short period of time in which the samples were collected.

Considering the severe situation of HIV infection in the Krasnoyarsk region, it is actually unexpected that only a few publications were discovered to be available on this issue. The only published study dedicated to molecular monitoring [36] was confined mainly to the city of Krasnoyarsk, while the history and spread of HIV-1 in the region was not exhaustively studied. Our results confirmed that the significant proportion of HIV cases in the region is associated with the A6 subtype (92.3% of HIV cases studied) transmitted by heterosexual contacts and injecting drug use mainly (as elsewhere in Russia) [56-60]. In contrast to the previously published results [37], we also observed large numbers of CRF063_02A recombinants in all risk groups whose proportion has increased more than 2.5 times over the past ten years. This recombinant today is the second HIV-1 dominant form (4.3%) in the region, being ahead of the subtype B (1.1%).

Remarkably, all HIV-1 subtype B infected patients were men with reported heterosexual exposure which makes it possible to assume the concealment of the true sexual status because of the stigma and discrimination in the society [61]. There are several arguments in favor of this idea. First, all subtype B sequences were reliably clustered with previously identified sequences from MSM individuals from Russia. Second, this subtype was and still is the most dominant HIV-1 variant in the MSM group in the country [21, 28, 62, 63]. Hence, it could be assumed that in reality, the MSM population is the source of most subtype B cases studied.

In this study at least seven unique recombinant forms (2.5 %) involving the segments of HIV-1 subtypes A6 and B were observed having the recombinant structure similar to the URFs previously identified in the Moscow region [25, 59]. Given the increasing role of recombinants in the regional epidemics [64-66] and the recent rapid spread of the CRF063_02A [31], there is the need to find out if these URFs constitute a new CRF and their role in the epidemic. However, it is quite obvious that the genetic complexity of the HIV-1 epidemic in the region is increasing which suggests the likelihood of future epidemic becoming more heterogenic.

Evaluation of epidemiological and population parameters has become central for the understanding of the dynamics of infectious diseases [67]. By using the coalescent-based approach, we obtained complementary information for the HIV-1 A6 population history in the region, which seems to be characterized by the recent origin and rapid evolutionary dynamics. Our phylogenetic reconstructions and TMRCA estimates indicate that the spread of the HIV-1 subtype A6 in the Krasnoyarsk region involved one viral lineage which arose in 1996.9 (1994.5-1999.5) and subsequently gave rise to the development of the whole epidemic cluster. These data are confirmed by epidemiological observations, which include a large outbreak of HIV infection preceding an exponential increase in the number of HIV infections in the region; and the presence of broad and relatively permanent epidemiological networks as well as low level of external migration in the region at the beginning of the epidemic. The fact that the year in which the first HIV-1 case was reported in the Krasnoyarsk region is in the lower bound of our most conservative estimates, making us believe that the A6 epidemic in the region started around the mid-1990s.

An important factor in the subsequent rapid growth of the epidemic was the large size and prevalence of the network of injecting drug users [68-70]. Some studies have shown that a single penetration of the virus led to an exponential growth in the number of infections [71, 72] in the countries of the former Soviet Union. Considering the geographical location of the Krasnoyarsk region and the migration pathways, it can be assumed that there was a similar epidemic process in this area.

Our estimations of the demographic history of subtype A6 in the region revealed that the fastest growth rate in the effective population size was at the late-1990s/early-2000s with the following stabilization after 2008. These data comply with the HIV incidence increase in this period that was sustained by subtype A6. The saturation of the drug addict population, the inclusion of the slow sexual transmission of the HIV infection, and the introduction of HIV prevention programs and antiretroviral therapy during this period are the probable causes for the reduction of the epidemic in the region by the end of the first decade of the 2000s [72]. The decrease in the number of reported new HIV cases observed during this period may serve as an indirect indication (not evidence) of this conclusion.

In spite of the fact that our findings show the monophyletic origin of subtype A6 epidemic in the region, it is important to draw comments on the epidemiological subclusters identified in this study. In contrast to Rumyantseva *et al.* [37], no clear genetic structure was shown in our analysis based on the geographical locations among the HIV-1 A6

sequences studied, that may adequately describe the clusters' existence. However, no factor was found uniting these sequences. It is possible that the spread of subtype A6 in the region occurred through several independent transmission networks followed by the divergent evolution of the population that led to the observed fact.

In our study, we found that in different districts of the Krasnoyarsk region, the HIV-1 prevalence ranged from low in Evenkiysky (0.14%) or Turukhansky district (0.17%) to significant 0.7% in Taymyrsky district (including the city of Norilsk, 1.72%) or 1.14% in Krasnoyarsk city. This large variation in HIV prevalence might be attributable to the differences in the socioeconomic and geographical characteristics, such as the studied populations, the main mode of viral transmission, and limited transport accessibility of individual territories. For example, in the hard-to-reach areas like the Evenkiysky or Turukhansky districts, the main mode of HIV transmission is believed to be injecting drug use, nonetheless, the proportion of IDUs addicts in the total population is on the average. Most HIV cases in the central part of the region are associated with heterosexual contacts; additionally, it was estimated that more than 15,000 injecting drug users live in Krasnoyarsk and Norilsk where the prevalence of HIV among IDUs has been reported to be around 30% [73-75]. Interestingly, 95% of the regional budget is derived from the taxes collected in these cities, therefore, having such powerful economic potentials creates an enabling environment for the advancement in the distribution of HIV.

Although the number of sequences from each location is highly variable and the large numbers of sequences from Krasnoyarsk city may offset the inferred migration routes, the demographic, epidemiological data and phylogeographical reconstruction in aggregate pointed to this city as the geographical origin of the epidemic which further spread to neighboring district of the region.

This is not surprising for several reasons. First, the earliest HIV-cases in the region were reported in Krasnoyarsk and Norilsk cities. Second, this city has the highest HIV infection prevalent in the region. Finally, Krasnoyarsk city is located in the central part of the region, which serves as an important transportation hub. Thus, reported facts match with the hypothesis of Krasnoyarsk city role as a local center of the spread of the epidemic in the region and do not contradict the available epidemiological data. Surprisingly, our data suggested only moderate support for the migration pathway from Krasnoyarsk to Norilsk and did not suggest the genetic flow to the Taymyrsky district (Dudinka city in particular). Both cities are around 1,500 kilometers away from Krasnoyarsk with no road or railway links to it. In contrast to Norilsk, Dudinka is an Arctic port city on the Yenisey River, which is connected by sea to the big ports of Murmansk and Arkhangelsk. It could be hypothesized that if there are only aviation and river communications with the central groups of the districts, then the epidemic process in Taymyrsky district is sustained independently of Krasnoyarsk city. In spite of the geographic accessibility and contrary to our assumption, the results of our analysis, including the most similar non-Krasnoyarsk sequences from these regions, showed that there is no strong evidence backing the movements between the Murmansk/Arkhangelsk and Taymyrskiy districts. Hence, a more geographically extended dataset would be needed to clarify the origin of the subtype A6 population in this area.

The low number of CRF063_02A sequences available from the Krasnoyarsk region prevented us from achieving definite conclusions on the spread routes of this recombinant in the region. Nevertheless, according to (sero) epidemiological data, the most likely scenario is that CRF063-02A initially appeared in the central area of the Krasnoyarsk region and then spread to the rest of the territory as a result of simultaneous independent migrations. Though we have no solid premise to support our claim, however, it is safe to posit that it happened in the early 2010 through the introduction of the virus from the territories to the west from the Krasnoyarsk region where the CRF063_02A has been widely circulated [33, 34, 76].

CONCLUSION

The results of this study provide novel insight into the HIV epidemic in the Krasnoyarsk region, which is becoming increasingly complex due to the introduction of the virus subtypes from other regions of Russia/world and the emergence of viral recombinant forms. Our submission is that the HIV-1 subtype A6, which currently dominates in the epidemic in Krasnoyarsk region was introduced in the region around 1996.

This subtype seemed to have initially appeared in Krasnoyarsk city and then simultaneously spread to the neighboring areas, showing the highest growth in the early 2000s. Despite the confirmed role of Krasnoyarsk city as a local center of the spread of the A6 epidemic in the central area, the epidemic process in the northern territories apparently is sustained independent of Krasnoyarsk city. Subsequently, we would hypothetically state that the spread pattern of the recombinant CRF063_02A may be similar to that of the A6 subtype.

LIST OF ABBREVIATIONS

AIDS	=	Acquired Immunodeficiency Syndrome
ART	=	Antiretroviral Therapy
BF	=	Bayes Factor
BS/SH-aLRT	=	Bayesian Support/ Shimodaira-Hasegawa Approximate Likelihood Ratio Test
BSP-analysis	=	Bayesian Skyline Plot - Analysis
BSSVS	=	Bayesian Stochastic Search Variable Selection
CRF	=	Circulating Recombinant Forms
ESS	=	Effective Sample Size
FSU	=	Former Soviet Union
GTR+I+G	=	General Time Reversible + Invariant Sites + Gamma Distribution
HIV	=	Human Immunodeficiency Virus
HPD	=	Highest Probability Density

HSX	=	Heterosexual Contacts
IDU	=	Injecting Drug Users
MCC	=	Maximum Clade Credibility
MCMC	=	Bayesian Markov Chain Monte Carlo
ML	=	Maximum-Likelihood
MSM	=	Men who have Sex with Men
MTCT	=	Mother-To-Child Transmission
PR	=	Protease
PSP	=	Posterior Probability
RT	=	Reverse Transcriptase
SH-aLRT	=	Shimodaira-Hasegawa Approximate Likelihood Ratio Test
TMRCA	=	Time to the Most Recent Common Ancestor
UNAIDS	=	Joint United Nations Programme on HIV/AIDS
URF	=	Unique Recombinant Forms
USSR	=	Union of Soviet Socialist Republics

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was reviewed and approved by the ethics committee of the I.M. Sechenov First Moscow State Medical University (Sechenov University), Russia, Protocol No. 06-13 on 05.06.2013.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All humans research procedures were in accordance with the standards set forth in the Declaration of Helsinki principles of 1975, as revised in 2008 (http://www.wma.net/en/20activities/10 ethics/10helsinki/).

CONSENT FOR PUBLICATION

The written informed consent was signed by all subjects involved.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this study are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers web site along with the published article.

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