Title

Genetic diversity and drug resistance mutations in reverse transcriptase and protease genes of HIV-1 isolates from Southwestern Siberia

Nadezhda B. Rudometova[1], Nadezhda S. Shcherbakova[1], Dmitry N. Shcherbakov[1], Elena V. Mishenova[3], Elena Delgado[2], Alexandr A. Ilyichev[1], Larisa I. Karpenko[1], Michael M. Thomson[2]

[1] Department of Bioengineering, State Research Center of Virology and Biotechnology «Vector», Koltsovo, Novosibirsk, Russia.

[2] HIV Biology and Variability Unit, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain.

[3] Budgetary healthcare institution of the Republic of Altai «Center for the Prevention and Control of AIDS», Gorno-Altaysk, Russia

Corresponding authors: Nadezhda B. Rudometova, State Research Center of Virology and Biotechnology Vector, Koltsovo, Russia (e-mail: andreeva_nb@vector.nsc.ru); Larisa I. Karpenko, State Research Center of Virology and Biotechnology Vector, Koltsovo, Russia (e-mail: lkarpenko@ngs.ru); Michael M. Thomson, HIV Biology and Variability Unit, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain (e-mail: mthomson@isciii.es).

Abstract

The analysis of a pol gene fragment encoding protease and part of reverse transcriptase was carried out for 55 sera collected in 2016 and 2018 from HIV-1-infected patients diagnosed in 2014-2018 living in the south of Western Siberia, Rusia: Altai Territory (n=11), Republic of Altai (n=15), Kemerovo region (n=18) and Novosibirsk region (n=11). CRF63_02A was the dominant genetic form (>70%) in the Altai Territory and Kemerovo and Novosibirsk regions, with subsubtype A6 comprising <30% of samples. In the Altai Republic, subsubtype A6 was predominant (53%), with 33% of viruses belonging to CRF63_02A. Four CRF63_02A/A6 unique recombinant forms were identified in the Altai Territory, Kemerovo Region, and the Altai Republic. A majority (11 of 15) CRF63_02A viruses from Kemerovo grouped in a cluster. Antiretroviral drug resistance mutations were found in 6 (14%) of 43 drug-naïve patients. This study provides new insights in HIV-1 molecular epidemiology and prevalence of transmitted antiretroviral drug resistnace mutations in Southwestern Siberia.

Keywords

HIV-1; CRF63_02A; A6; resistance mutations; highly active antiretroviral therapy; drug resistance

Introduction

Currently, about 37 million people in the world are living with HIV-1, which makes this disease one of the leading global public health problems (UNAIDS, 2018). Despite decades of intensive research, due to the high ability of the virus to elude the immune response, an effective strategy to cure HIV-1 has not been developed yet.

Russia is one of the leading countries in HIV-1 incidence in the world, surpassing some African countries. According to the molecular epidemiological analysis of HIV-1 isolates circulating in Russia, the A6 subsubtype continues to dominate in most of the Russian Federation, with the exception of some areas of Siberia, where the recombinant variant CRF063_02A prevails, with lower prevalences of CRF02_AG and unique recombinant forms (URF)[1, 2, 3]. In the distribution of HIV-infected people according to the main risk factors for infection, sexual transmission through heterosexual contacts is predominant [4, 5].

In the Russian Federation at present, 81% of HIV-infected people know their status, but only 36% of them are on antiretroviral therapy (ART) and 75% have suppression of viral load[6].

According to the Central Scientific Research Institute of Epidemiology of of The Federal Service on Customers' Rights Protection and Human Well-being Surveillance, in 2015, the primary HIV-1 antiretroviral (ARV) drug resistance rate in the Russian Federation was around 6% in 2015, and in 2017 in some regions of the country increased to 9.7%[7]. If the ARV drug resistance in the population reaches 10%, this means that it is necessary to review the treatment strategy[8]. At the same time, according to the results of Kirichenko et al., the prevalence of transmitted drug resistance associated with mutations from the Surveillance Drug Resistance Mutation (SDRM) list in nine federal districts of the Russian Federation amounted to 5.3%, which is classified by WHO as moderate. The highest prevalence of drug resistance was found for drugs of the class of nonnucleoside reverse transcriptase inhibitors (NNRTI) (rilpivirin, nevirapine, and efavirenz)[9]. In the neighboring country of Kazakhstan, a low prevalence (3%) of drug resistance mutations to ARV drugs in the group of untreated patients was noted[10]. Overall, ARV drug resistance in Former Soviet Union countries has been reported to range from 3.4% in Latvia to 8.3% in Georgia[11].

The main reason for the pharmacologic resistance of HIV-1 is the inaccuracy of reverse transcription in combination with a high virus reproduction rate, with such concomitant factors as low patient compliance with ART in combination with poor adherence to the treatment regimen[12]. At the same time, drug resistance appears not only in patients with ARV drug experience due to the occurrence of mutations (acquired), but

also in patients without ARV drug experience (drug-naive patients) as a result of the transmission of HIV-1 variants that already contain resistance mutations (primary or transmitted) [13-14].

This study aims to determine the genetic diversity and drug resistance mutations in reverse transcriptase and protease genes of HIV-1 isolates from Southwestern Siberia, the region most affected by HIV-1 in the Russian Federation.

Materials and methods

Patients

The study used 55 sera from HIV-1-infected patients collected in 2016 and 2018: 11 from the Novosibirsk Region, provided by the Center for the Prevention and Control of AIDS and Infectious Diseases of Novosibirsk; 18 from the Kemerovo region, provided by the Kemerovo Regional Center for the Prevention and Control of AIDS and Infectious Diseases; 11 from the Altai Territory, provided by the Altai Regional Center for the Prevention and Control of AIDS and Infectious Diseases (Barnaul); and 15 from the Altai Republic, provided by the Center for the Prevention and Control of AIDS of the Republic of Altai. The study was approved by the ethical committees of these centers.

RNA extraction

The total RNA pool was isolated from the sera of HIV-1-infected patients using the QIAamp Viral RNA Mini Kit reagent kit (QIAGEN, Germany) and the RNA / DNA reagent kit from the MAGNO-sorb clinical material (AmpliSens, Russia) according to the manufacturer's recommendations.

PCR and sequencing

Amplification of a fragment of the HIV-1 pol gene encoding protease and reverse transcriptase was performed using a method described previously[2, 15]. The nucleotide sequence of the amplified fragments was determined by the Sanger method using the Big Dye Terminator v 1.1 Cycle Sequencing Kit (Applied Biosystems, USA) on the ABI Prizm Genetic Analyzer 310 instrument at the Genomika Research Center, Novosibirsk, and ABI 3730 XL DNA Analyzer at Genomic Unit, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain.

Sequence analysis

Electropherograms were assembled, analyzed, and edited using the SeqMan v6.1 (DNASTAR, Inc, Madison, WI, USA) and BioEdit v7.2.5 programs. Multiple alignment of the nucleotide sequences was performed with MAFFT v7. Phylogenetic analysis was performed via maximum likelihood with IQ-TREE v1.6.12 using the general time reversible with FreeRate evolutionary model (GTR+R). Node support was assessed by ultrafast bootstrapping with 1000 replicates. To construct the phylogenetic trees, reference sequences from the Los Alamos HIV Sequence Database (http://www.hiv.lanl.gov/) were included. Recombination was analyzed by bootscanning using the SimPlot 3.5 program[16]. This analysis was done using a window of 180 nt moving in 20 nt increments, with tree construction using the neighbor-joining method and the Kimura 2-parameter substitution model.

ARV drug resistance mutations were determined using the HIVdb program at the Stanford University Database[17]. Levels of resistance to antiretroviral drugs were determined according to the HIVdb program: 15–29, low level of resistance; 30–59, intermediate level of resistance; more than 60, high level of resistance.

Results

Epidemiological data

The study analyzed a total of 55 sera of HIV-positive patients living in the south of Western Siberia: Altai Territory (capital is Barnaul), the Republic of Altai (capital is Gorno-Altaisk), Kemerovo region (capital is Kemerovo) and Novosibirsk region (capital is Novosibirsk city) (Figure 1a).

Data from HIV-1-infected patients (Table 1) indicate that there were more men included in the study than women: 51.8% (29/55) men vs 48.2% (25/55) women. The average age of the patients was 38 ± 8.9 years (range, 25 to 62 years). In 58.2% (32 of 55) of cases with known transmission routes, infection occurred through heterosexual contact; in 34.5% (19 of 55), it was transmitted through intravenous drug use; and in four (7.3%) cases the transmission route could not be established. The proportion of patients on ART was 21.8% (12/55). Most of them (10 patients) were from the Altai Republic and two were from the Altai Territory.

Molecular genetic analysis of a gene pol

To molecularly characterize HIV infection, an amplification of a portion of the pol gene encoding protease and reverse transcriptase was performed. Good quality informative sequences for further analysis were obtained from 100% of samples. The phylogenetic analysis of the amplified fragment showed that the recombinant form CRF63_02A prevails in the examined samples from three regions and the A6 subtype also circulates in all four regions, reaching 53.4% in the Republic of Altai (Figure 1b). Eleven of 15 (73.3%) CRF63_02A sequences from Kemerovo Region grouped in a strongly supported cluster.

Recombination analysis by bootscanning showed that among the 55 studied HIV-1 variants, 4 were CRF63_02A/A6 recombinants in PR-RT fragment: two (18RU04GA and 18RU14GA) from the Republic of Altai, one (16RU38) from the Altai Territory, and one (16RU39) from the Kemerovo Region (Figure 3).

Drug resistance mutations to protease inhibitors, NRTIs, and NNRTI

Major mutations associated with drug resistance to protease inhibitors (PIs) were not detected in the analyzed nucleotide sequences. Most of the studied samples contained polymorphic mutations K20I (70%), L10I/V (22%) and V11I (2%).

Regarding the reverse transcriptase segment, NRTI and NNRTI drug resistance mutations were detected (Table 2). Among the NRTI drug resistance mutations, mutations M41L (in two drug-naïve patients) and K65R (in two drug-naïve patients) were found. The M41L mutation causes low levels of azidothymidine (AZT) resistance; the K65R mutation results in a moderate level of resistance to ABC, FTC, 3TC and a high level of resistance to TDF. This represents a NRTI SDRM prevalence of 9.3% (4 out of 43 drug-naïve patients analyzed). Among the mutations that confer resistance to NNRTI drugs, the mutation K103N (3 drug-naïve patients) was identified, which leads to a high level of resistance to EFV and NVP. This represents a NNRTI SDRM prevalence of 7% (3 out of 43 drug-naïve patients analyzed). In one drug-naïve patient, a total of two mutations were found: K65R and K103N. This represents a NRTI+NNRTI SDRM prevalence of 2.3% (1 out of 43 drug-naïve patients analyzed). Thus, among the 43 treatment-naïve HIV-1-infected participants, 6 (14%) were infected with HIV-1 variants with SDRMs.

Discussion

The total number of HIV-infected people registered in the Russian Federation has reached 1,376,907[18]. The Siberian Federal District is one of the most affected by the HIV epidemic - 279,760 HIV-infected. The leading regions in Siberia in terms of infection are Kemerovo, Novosibirsk, Tomsk, and Omsk regions, and

Krasnoyarsk and Altai Territories[19, 20, 21, 22, 23]. In these regions, epidemiological surveillance of circulating strains is being carried out, and the dominant variants of HIV-1 have been identified in most of them. At the same time, information on the subtype of HIV-1 isolates in some regions is completely absent. An example would be the Altai Republic. While infection rates are currently low in the region, there has been a significant increase in new infections in recent years. In total, for all the years of observation (from 1999 to 2019), 1,050 HIV-infected people were registered in the Altai Republic.

In this work, we investigated the genetic diversity of HIV-1 isolates circulating in the south of Western Siberia, and also studied the profile of mutations that contribute to the emergence of drug resistance of HIV-1 isolates to ARVs.

Phylogenetic and recombination analyses showed that the recombinant form CRF63_02A continues to dominate in the south of Western Siberia, which occurs in more than 70% of cases in the Novosibirsk and Kemerovo regions and the Altai Territory, with the A6 subsubtype accounting for less than 30% of isolates. At the same time, in the Altai Republic (where genetic diversity of circulating HIV-1 strains is first reported here) a different picture is observed in the distribution of HIV-1 subtypes - more than 50% of the studied circulating HIV-1 strains belong to the A6 subsubtype, and the proportion of CRF63_02A is 33% (figure 2b). The observed predominance of CRF63_02A in Novosibirsk and Kemerovo regions is in accordance with previous reports[1, 2]. It is interesting to note that 11 of 15 (73.3%) CRF63_02A viruses from Kemerovo grouped in a local cluster, indicating that most CRF63_02A infections from this region derive from a single introduction. This is similar to the reported A6 subsubtype clusters found in other areas of Russia[24, 25, 26, 27]. With regard to the Altai Territory, we observe a major shift in the genetic composition of the HIV-1 epidemic, with a change from A6 subsubtype predominance (100%) in samples collected in 2008[26] to CRF63_02A predominance (82%) in samples collected in 2016. In addition, it should be noted that among the isolates circulating in the territories of the Kemerovo Region, Altai Territory and the Altai Republic, in isolated cases, CRF63_02A/A6 unique recombinant forms were identified. Similar recombinants are found in Siberia and are described in the literature[1, 18, 19, 23].

Analysis of the protease gene showed no major mutations associated with the resistance to PI resistance. Nevertheless, most of the studied CRF63_02A and A6 strains contained polymorphic mutations K20I, L10I/V, and V11I in the protease gene. Among the mutations causing resistance to NRTI and NNRTI, M41L, K65R and K103N were found. It should be noted that this mutation profile is typical and correlates with previously published data[1, 2, 9, 19]. The overall prevalence of the DR mutations associated with reverse transcriptase inhibitors was 14 %. Comparing with the data obtained earlier, for Russia the level of transmitted drug resistance was at the level of 5.3%[9]. The detection of such mutations in naive patients

indicates infection with a resistant variant of the virus and prevents the use of these drugs in the ART regimen.

Conclusion

The current molecular epidemiological situation of HIV-1 infection in the southwestern Siberian regions of Altai Territory, Republic of Altai, Kemerovo region, and Novosibirsk region is due to the circulation of CRF63_02A and the A6 subsubtype, which have given rise to sporadic cases of CRF63_02A/A6 recombinants. In this study, a relatively small group of people who received ARV therapy and those who did not take ARV drugs were examined.The volume of studies does not make it possible to reliably assess the level of primary resistance in the studied sample, however, the conditional indicator of transmitted drug resistance obtained in this study was 14%. Research in this direction needs to be continued and expanded both for the purpose of monitoring the spread of HIV-1 drug-resistant strains and for studying the characteristics of the spread of HIV-1 variants in the constituent entities of the Russian Federation.

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Sequence data

The HIV-1 protease-reverse transcriptase sequence data were submitted to the GenBank database under the accession numbers MT101799-MT101834 and MT811096-MT811114.

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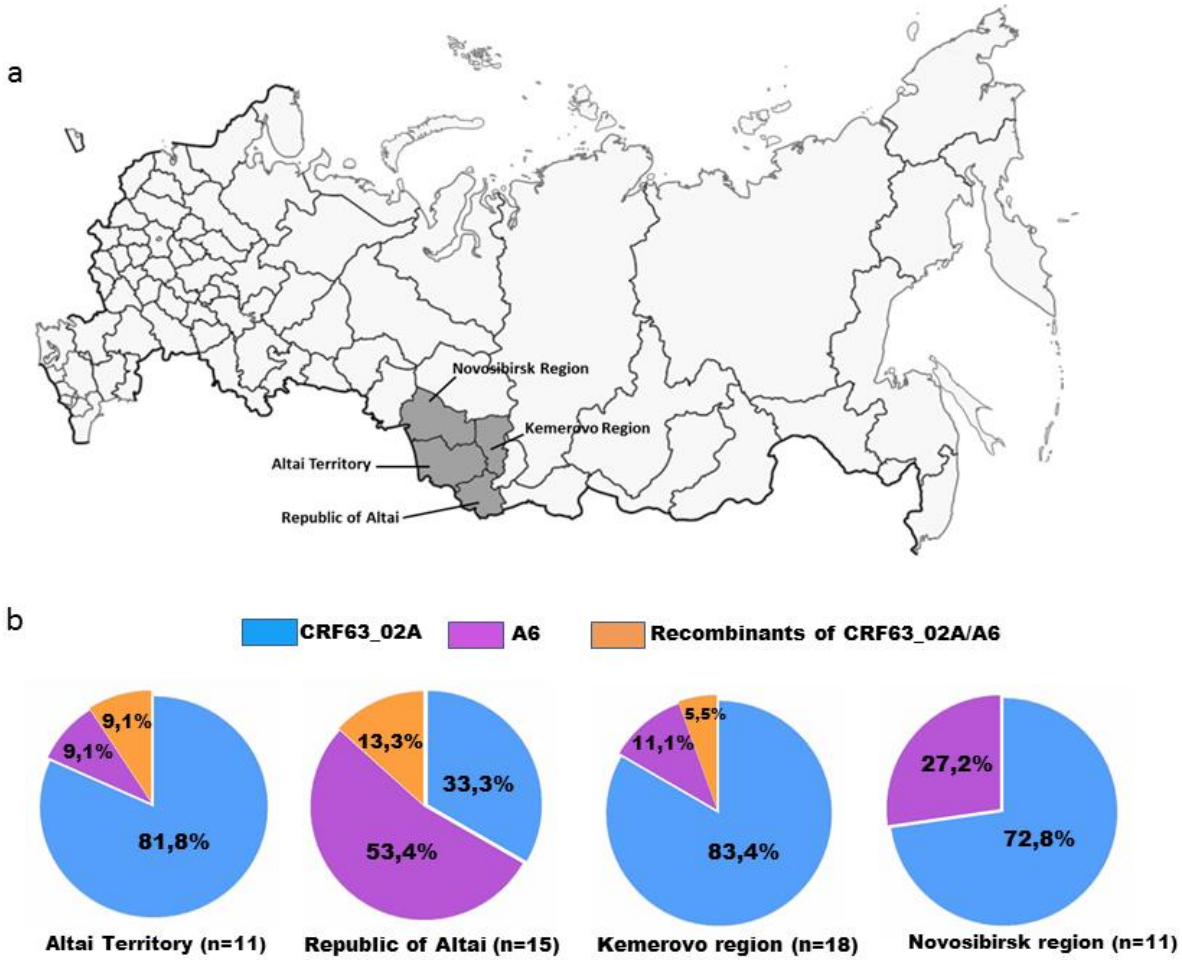

Figure 1. a) Map of the Russian Federation with regions in which the samples were collected marked. b) Distribution of HIV-1 genetic forms identified in samples collected in the four regions of Western Siberia in 2016 and 2018.

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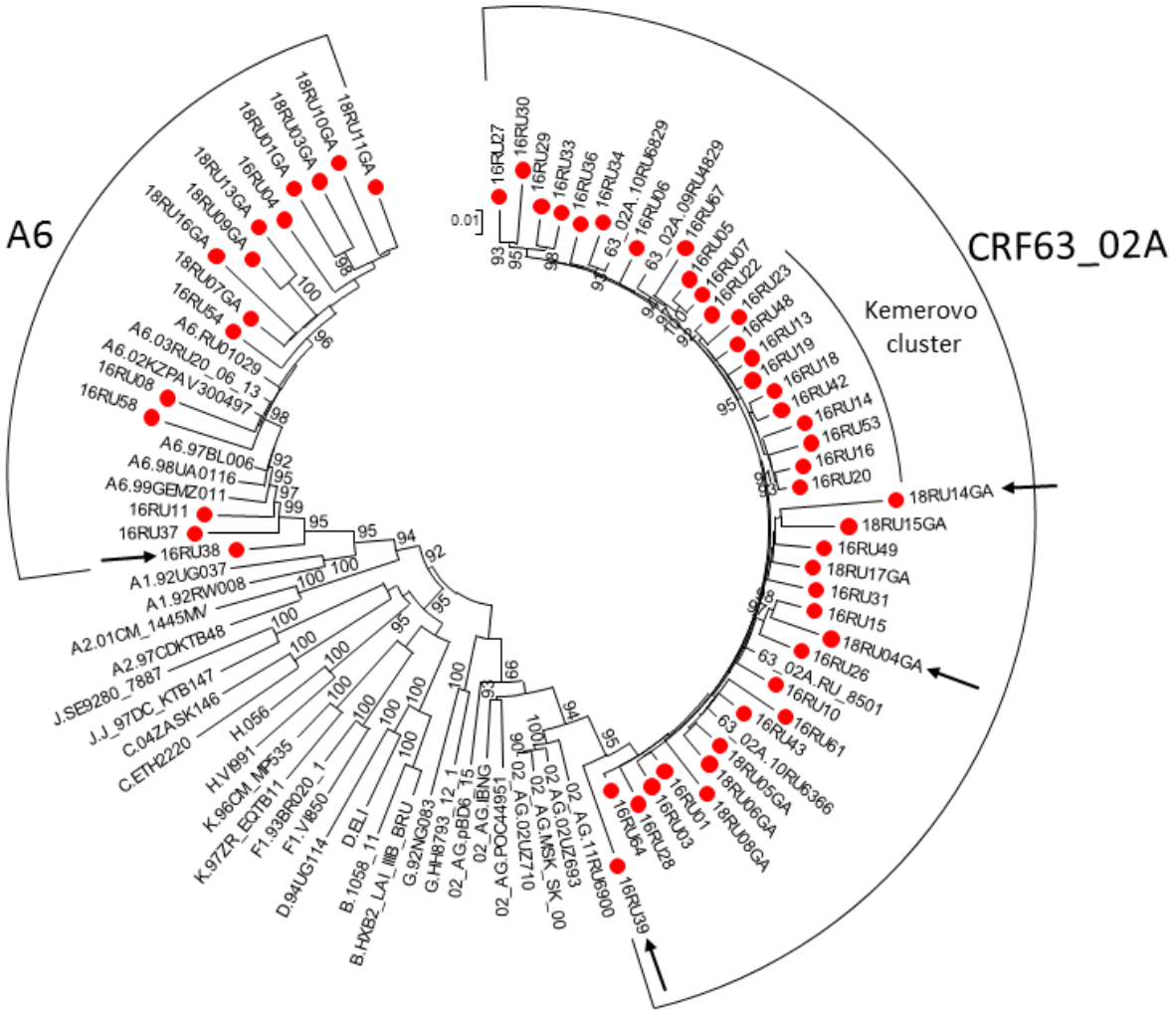

Figure 2. Maximum likelihood phylogenetic trees of HIV-1 pol genes constructed with IQ-TREE. Only bootstrap values ≥90% are shown. Samples from this study are labeled with circles. CRF63/A6 recombinants are signaled with arrows.

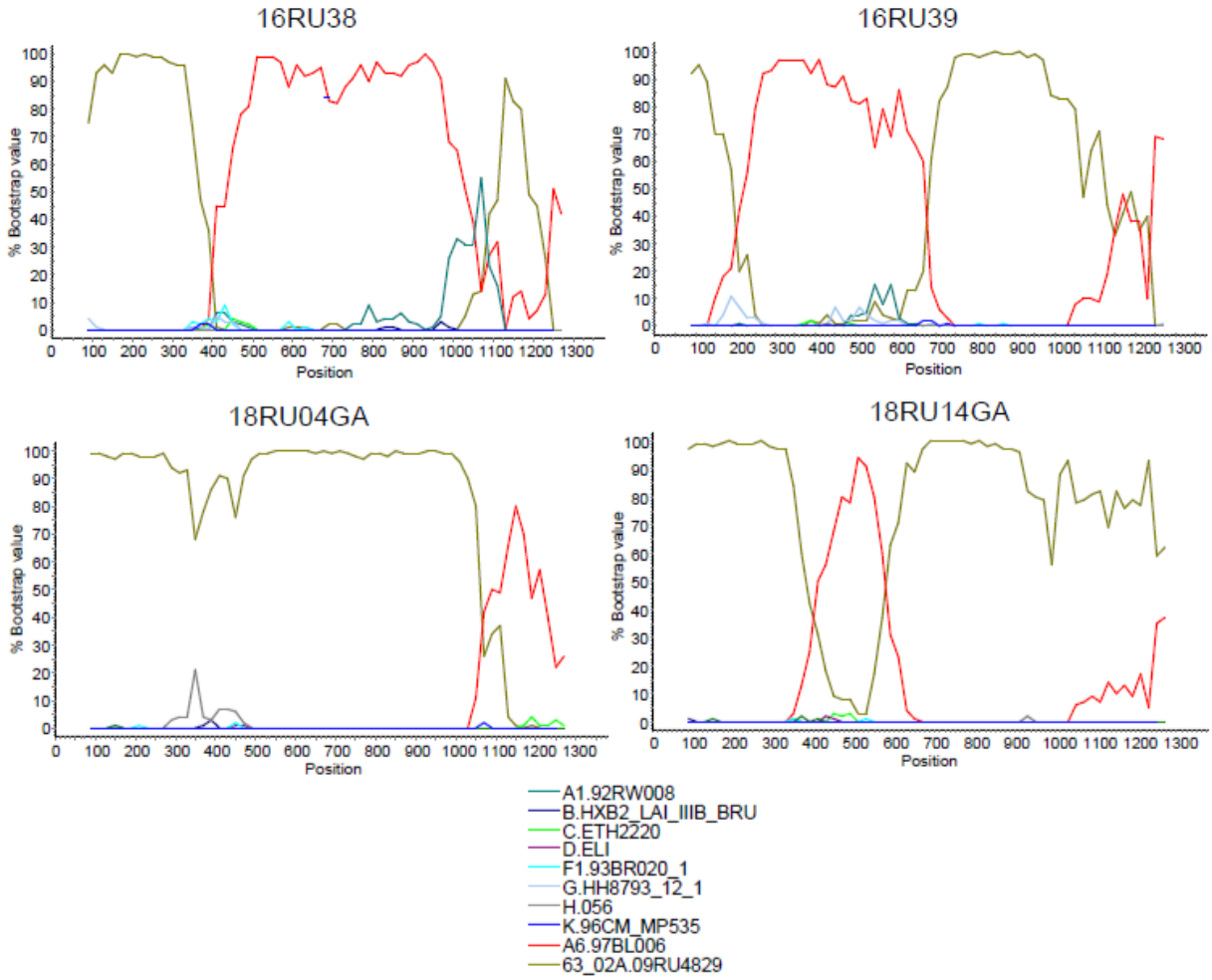

Figure 3. Bootscan analyses of 4 PR-RT CRF63_02A/A6 recombinant sequences from Siberia. Horizontal axis represents nt position from nt 1 of protease. Vertical axis represents bootstrap values supporting clustering with clade references, shown below.