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HIV Drug Resistance at Patients on HAART and Transmitted HIV Drug Resistance (tHIVDR) in Treatment Naive Patients in Belarus

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Abstract

Of the 59 patients (adults) who have been identified to have a virus with a high level resistance to HAART drugs, in 38 (64.4%) patients mutation M184V/I was identified. In 27 (45.8%) patients (13 female and 14 male) K103N mutation was detected. In 20 (33.9%) patients (16 male and 4 female) we found G190G/S/A mutation. Of the 18 children-patients, born to HIV-infected mothers, in 15 (83.3%) cases (9 girls and 6 boys) we detected HIV resistance mutation M184V. In 10 (55.6%) and 2 (11.1%) cases mutations in position G190S and K103S were found, respectively. Of 82 samples collected from newly diagnosed HIV-infected antiretroviral naïve patients only 6 samples (7.3%) had other resistance mutations which can be classified as 'minor' or 'other' according to HIVDR database of Stanford University: L10V - PI minor mutation associated with resistance to most PIs when present with other mutations; L33F - PI minor mutation selected by FPV/r, DRV/r, LPV/r, ATV/r, and TPV/r, and contributes decreased susceptibility to these PIs; V118I - accessory mutation usually occurred with multiple TAMs and contributes some resistance to each of the NRTIs including 3TC and FTC; T74S is 'other' mutation associated with reduced NFV susceptibility; and V108I - accessory mutation, causes low-level resistance to NVP and EFV.

Keywords: HIV; IDUs; Resistance; Mutations; Sequencing; Subtypes

Introduction

One of the main factors influencing the level of efficiency of highly active antiretroviral therapy (HAART) is the high degree of variability of certain regions of the genome of human immunodeficiency virus (HIV) that leads to the emergence of mutations associated with resistance to antiretroviral therapy (ART). ART treatment regimen use more than 30 drugs of 5 different classes, but, unfortunately, we already identified some mutations in the genome of the virus which cause developing HIV resistance to these drugs [1,2]. At present day there is no alternative to HAART, although it cannot be considered 100% effective treatment, but HAART regimen help and significantly improve the living status of HIV-infected patients. Early diagnoses of HIV resistance mutations development and consequent changing of treatment regime are the bases of HAART efficiency.

Belarus witnesses consistently high growth of HIV/AIDS cases. Over the last 5 years more than 1,000 new HIV-infection cases were revealed annually. By August 1st 2013 - 15,038 (158 infected people per 100 thousand population) patients with HIV/AIDS were registered, during 7 months period in the year 2013 - 860 new HIV infection cases were officially registered (Figure 1). The largest number of HIV/AIDS cases were reported in the age group from 15 to 29 years (8613 cases), but at present day the majority of new HIV cases are being recorded in the age group from 30 up to 45 years, the primary mechanism of HIV transmission is sexual. For instance, if in year 2012 the proportion of sexual HIV transmission mechanism amounted to 77.2% of all new cases; in 2013 already 83% of the patients were infected through sexual rout.

Currently, in Belarus - 4,500 patients are on HAART, while in year 2008 only 1,200 patients were receiving this therapy, in year 2004 - only 15 patients. It is planned, that by the year 2015 - 7,000 patients will be on ART. In this report we present the results of studies on detecting the resistance mutations in HIV/AIDS patients on HAART as well as at HIV/AIDS patients which are not receiving antiretroviral therapy in Belarus.

Materials and Methods

The article presents data on the identification of high-level resistance mutations developed by 77 patients (59 adults and 18 children) who have been on HAART during the period from year 2008 to year 2013. Information on these patients is shown in Tables 1 (adult) and 2 (children).Blood plasma from 96 patients initially identified with HIV/AIDS, who were not receiving antiretroviral therapy, has been investigated for the presence of primary resistance mutations.

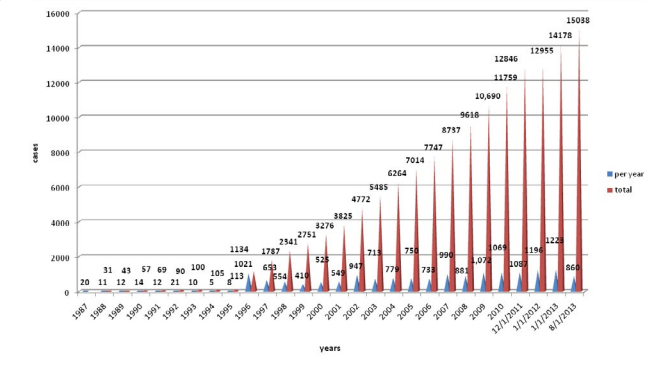

Figure 1: HIV/AIDS dynamics in Belarus (1987 – 01.08.2013)

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NN	GenBank data, ID,region	sex	age	HIV infection/ HAART started	Rout of infection	HIV subtype	Data of high/level mutations detection	PIs	NRTIs	NNRTIs
1	2	3	4			5		6	7	8
1.	**Vit-2-A HE657489**,	f	1978	2002/2005	heterosex	A1	2008	-	D67N; K70R; M184V; K219Q	V179DV; Y188L
2.	**Gom-28-A** -	m	1970	1998/2004	IDU	A1	2008	D30N; N88D;+ минорные: L10V; T74S	M41L; D67N; K70R; T215F; K219Q	G190S
3.	FR686468,**MnObI-28-cpx;**	f	1982	2008/2008	heterosex	CRF06_cpx	2010	-	-	K103N; K238T
4.	HE657525 **MnObI-24-A**	m	1968	2008/2009	heterosex	A1	2010	-	M184V	K103N; V108IV; P225HP
5.	**Br-18-A**; HE657536	m	1974	2006/2009	heterosex	A1	2010	-	M184V	K101E; E138A; G190A
6.	**MnObI-29**-A HE657524	f	1969	2007/2009	heterosex	A1	2010	-	-	G190S
7.	**MnObI-25-A**; -; HE657522ИНН,	f	1982	2003/2008	IDU	A1	2010/2012	-	V181I; M184V	K103N
8.	**MnObI-27-cpx**; C FR686469	m	1980	2008/2008	heterosex	CRF06_cpx	2010	-	M184V	K103N
9.	**MnObI-30-A1**HE657516	m	1979	1998/2006	IDU	A1	2010	-	-	K103N; E138A
10.	**MnObI-35-A**HE657541	m	1981	2007/2009	heterosex	A1	2010	-	-	K103N; E138A
11.	**MnObI-31-A**HE657526	f	1985	2003/2008	heterosex	A1	2010	-	V118I; M184V	K103N; P225H
12.	**MnObI-34-A;**HE657540	m	1976	2002/2006	IDU	A1	2010	-	Y115F; V118I; M184V; T215F	E138A
13.	**MnObI-01-A**-BY; HE577613.1	m	1959	2008/2008	heterosex	A1	2010	-	M184V	E138A
14.	**MnObI-16-A, HE657523**	f	1978	2003/2006	IDU	A1	2008	-	-	K103N
15.	**MnObI-17-A;HE657529**	f	1987	2004/2008	IDU	A1	2010	L10I	V118I, M184V, T215F	K101H; G190S
16.	**MnObI-06-A;** HE577618,	m	1975	1996/2007	IDU	A1	2010	-	K65R, M184V	K103N; P225H
17.	**MnObI-04-A;** HE577616	m	1971	2002/2007	IDU	A1	2010	L10IL	V118I	K103N
18.	**Vit-3-A;** HE657491	m	1981	2001/2009	IDU	A1	2011	-	T69NT, M184V	Y181CFGV
19.	**Gom-37A1**	m	1977	1998/2006	IDU	A1	2008	-	T215ST	G190GS
20.	**MnObI-40A**HF679227.1	f	1974	2007/2010	heterosex	A1	2011	-	M184V	K103KN
21.	**MnObI-41**	m	1979	2002/2009	IDU	A1	2011	-	M184V	K103N; P225H
22.	**MnObI-42**Рудик HF679229.1	f	1985	2007/2009	heterosex	A1	2011	-	-	K103N
23.	**Br-21-A;**HF679265.1	m	1972	1998/2008	IDU	A1	2011		A62V, T69S, L74LV, M184I	V90I, K101E, G190S
24.	**Br-20-A**HF679264.1	m	1972	1998/2008	IDU	A1	2011	-	D67G, K70EK, M184V	K101Q, Y181CY, G190S
25.	**Vit-5-A;**HF679261.1	m	1976	2001/2010	IDU	A1	2011	-	L74V, Y115F, M184V	V90I, G190S, H221Y
26.	**Br-22-A**; HF679266.1	f	1975	2003/2010	IDU	A1	2012	-	M184V	K103N
27.	**Mn-29-A;**HF679247.1	m	1976	2002/2011	IDU	A1	2012	-	M41L, D67N, K70R, M184V, L210W, T215Y, K219E	-
28.	**Mn-30-A**; HF679248.1	f	1975	1998/2003	heterosex	A1	2012	M46I, I54V, V82A	M184V	-
30.	**MnObI-44-A;**HF679219.1	f	1983	2006/2010	heterosex	A1	2012	-	M184V	K103N, E138Q
31.	**Mn-31A;**HF679249	m	1983	2006/2010	IDU	A1	2012	-	-	K103N
32.	**Br-24-A**HF679271	m	1977	2001/2009	IDU	A1	2012	-	M184V	K103N

33.	**Br-26-A;**HF679270.1	m	1984	2002/2010	IDU	A1	2012	-	-	K103N
34.	**Br-27-A;**HF679277.1	m	1955	1997/2011	IDU	A1	2012	-	A62V, M184V	Y181C
35.	**Mn-33-A;**HF679251	m	1983	2004/2011	IDU	A1	2012	-	A62V, K65R, M184IMV	V90I, V179T, Y181C, G190S
36.	**Br-29-A;**HF679273	f	1982	2006/2010	heterosex	A1	2012		M184V	K101E, G190A
37.	**MnObl-47-A;**HF679230	f	1967	2009/2009	heterosex	A1	2012		M184V	K103N
38.	**MnObl-48-A;**HF679235	m	1981	2009/2010	IDU	A1	2012		L74V, Y115F, M184V	K101E, Y181C, G190S
39.	**MnObl-49-A;**HF679232	m	1977	2008/2010	heterosex	A1	2012		D67N, K70R, M184V, T215Y, K219Q	K103N, P225H
40.	**MnObl-50-A1**; HF679216	f	1982	2004/2010	heterosex	A1	2012	-	L74LV, M184V	K103N, Y181C
41.	**Gom-29-A;**HF679272	m	1975	2008/2009	heterosex	A1	2012	-	M41L, K70KN, V75IM, M184V, T215Y	Y181C
42.	**MnObl-53A1;**HF679231	m	1985	2006/2011	IDU	A1	2012	-	-	G190GS
43.	**MnObl-55A1**	m	1984	2006/2011	IDU	A1	2012	-	-	K103KN
44.	**MnObl-56A1;**HF679210	f	1980	2005/2010	heterosex	A1	2012	-	D67DN, M184V, T215NSTY	K101E, G190A
45.	**MnObl-57A1**; HF679212	m	1970	2007/2010	IDU	A1	2012	-	-	K103N
46.	**MnObl-58A1** HF679234	m	1981	2007/2010	IDU	A1	2012		D67DN, K70R, M184V, T215IT, K219EK	Y181CY
47.	**MnObl-61A/B**	m	1975	1998/2008	IDU	A/B	2012	-	-	K103S, G190A
48.	**MnObl-63A1,**HF586690	m	1972	1997/2009	IDU	A1	2012	-	K65KR, K70EK, M184V	K101H, Y181CY, G190S
49.	**Gom-30A1**, HF679275	f	1989	2008/2010	heterosex	A1	2012	-	K65R, Y115F, M184V	-
50.	**Mn-37B**, HF679255	m	1970	1998/2009	heterosex	B	2012	-	M184V	-
51.	**MnObl-68A1**; HF679223	m	1978	2002/2009	IDU	A1	2012	-	M184V	G190S
52.	**Mn-43A1**	f	1982	2004/2011	IDU	A1	2013	-	-	K103KN
53.	**MnObl-72A1**,	m	1973	1998/2011	IDU	A1	2013	-	-	K103N
54.	**MnObl-75A1**	m	1978	2012/2012	IDU	A1	2013	-	A62V, L74LV, M184I	K101E, Y181CY, G190S
55.	**MnObl-80A1;**	m	1979	2010/2011	IDU	A1	2013	-	-	K103N, Y188L
56.	**MnObl-84A1;**	m	1983	2008/2010	IDU	A1	2013	-	-	G190S
57.	**Mn-47A1;**	m	1967	2003/2008	IDU	A1	2013	-	D67G, L74V, M184MV, T215ST, K219E	K101H, Y181C, G190S
58.	**Mn-51A1**	m	1971	2000/2008	IDU	A1	2012		M184V	-
59.	**MnObl-94A1**	m	1974	2006/2006	IDU	A1	2013		M41LM, A62V, M184V, T215Y	K101E, E138A, G190S
60.	**Gom-38A1**	f	1980	1999/2004	heterosex	A1	2008		-	K103N

Table 1: High/level resistance mutation at patients on HAART (adults).

For detecting the resistance mutations we used commercial kit «ViroSeq™ HIV-1 genotyping system v.2.0» (Abbott, USA) with an analytical sensitivity 2×10^3 copies RNA/ml. This kit allows sequencing of HIV-1 gene polregion encoding of proteases and 2/3 of the reverse transcriptase (1800 bp). Electrophoresis of DNA fragments after sequencing PCR was performed on genetic analyzer ABI PRISM 3100-Avant (Applied Biosystems, USA).

Analysis of the obtained fragments and detecting of HIV resistance mutations to antiretroviral drugs was performed using commercial databases «ViroSeq HIV-1 genotyping system software v2.6 analysis» (Abbott, USA) and Stanford University "HIV Drug Resistance Database", and using software «Sequencing Analysis Software v.5.1.1», «BioEdit», «SeqScape v.2.6».

Phylogenetic analysis of the DNA fragments was performed with the help of the program Mega 4.1 (trees constructed by neighbor-joining method).

Results and Discussion

Resistance mutation in patients on HAART

Of the 59 patients (adults) who have been identified to have a virus with a high level resistance to HAART drugs, there were 39 male (median age 37.2 ± 4.8 years) and 20 female (median age 33.9 ± 4.92 years). 20 patients (6 male and 14 female) were infected hetero-homosexually and 39 (33 male and 6 female) were IDUs. 35 patients came from different parts of the Minsk region, 8 patients from the city of Minsk, 8 patients from the Brest region, 5 patients from the Gomel

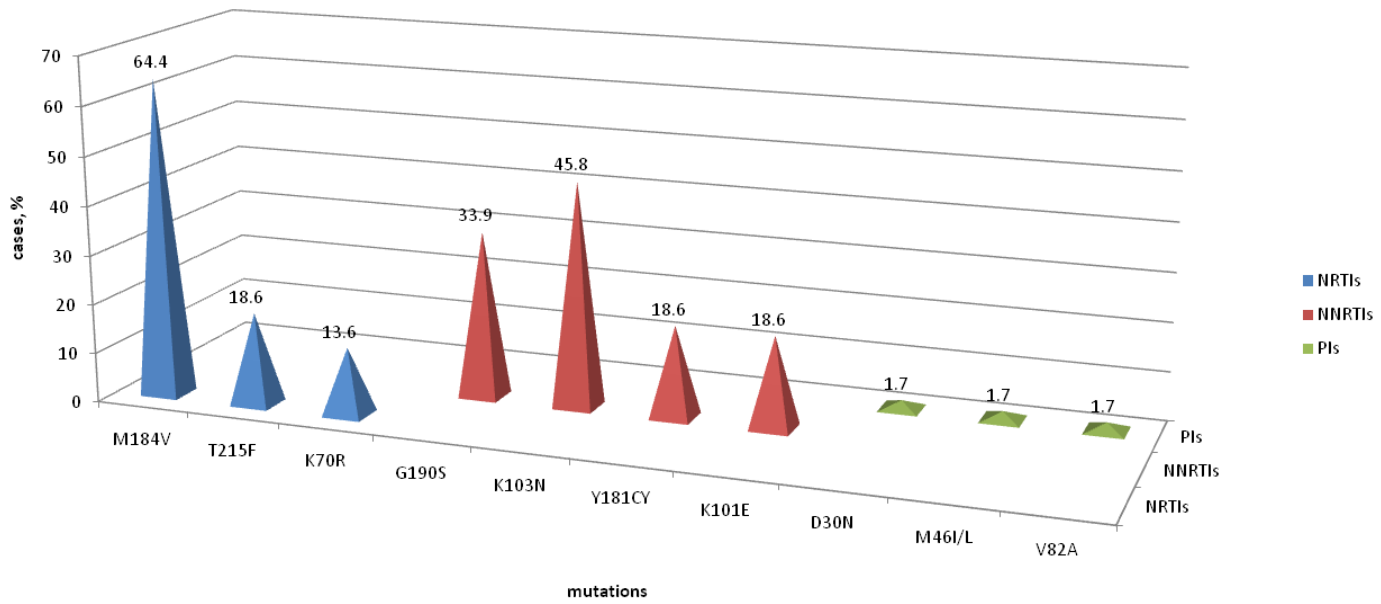

Figure 2: Resistance mutations identified in patients on HAART (adults)

№ п/пv	GenBank data, ID,region	sex	age	HIV infection/HAART started	HIV subtype	Data of high/ level mutations detection	PIs	NRTIs	NNRTIs
1	**2**	**3**	**4**		**5**		**6**	**7**	**8**
1.	**Mg-2-A/ G;**FN995208.	b	2003	2003/2005	CRF02_AG	2009	-	M42L; D67N; T69Xi; K70R; V75M; T215F; K219Q	K101KQ; K103S; G190A
2.	**Gom-26-A;**HE657433	b	1996	1996/2004	A	2008/2012	M46I; I54V; V82F; I84V	A62V; T69Xi; L74I; Y115F; M184V; L210W; T215Y	-
3.	**MnObl-51-A1;** HF679233.1	b	2002	2002/2006	A	2008	-	D67N; K70R; V118I; M184V; T215Y; K219E	V106I; Y188L
4.	**Mg-10-A;** FR686903	b	2003	2003/2008	A	2009	-	M41L; E44DE; D67N; T69Xi; M184V; L210W; T215Y	-
5.	**Mn-24-A,**HF679242	g	2004	2004/2005	A	2009	-	D69N; T69Xi; K70R; V118I; M184V; K219Q	-
6.	**Gom-17-A;**HE657430	g	1996	1996/2004	A	2008	M46I; I54V; V82F+ L10V; T74S; L89V	L74IV, Y115F, V118I, M184V, T215F	K101E, Y181CY, G190S, H221Y
7.	**Gom-27-A;** HE657431	g	2001	2001/2008	A	2010/2012	-	M184V	Y188L
8.	**MnObl-33-A**; HE657542	g	2008	2008/2009	A	2011	-	T69NT, M184V	Y181CFGV
9.	**MnObl-03-A;**HE577615.	g	2004	2004/2006	A	2010	-	D67G, M184V, K219N	G190S
10.	**Gom-32A1**	b	2004	2004/2004	A	2008	M46I; L90M; A71T; T74S	D67N; T69i; K70R; T215FY	
11.	**Vit-4-A; HF67926**	b	2003	2011/2011	A	2012	**M46L**	A62V, D67G	V90I, K101E, G190S
12.	**Br-23-A**; HF679276.1	b	2005	2005/2009	A	2012	**-**	V75MV, M184V	K101E, G190S
13.	**MnObl-46-A;**HF679224	b	2003	2003/2006	A	2012	**-**	D67N, K70R, M184V, K219Q	K101E, G190S
14.	**Mn-35-A;**HF679253	b	2005	2005/2011	A	2012	**-**	A62V, M184V	K103N

15.	**MnObl-54A1**	g	2011	2011/2011	A	2012	-	M184V	K101EQ, G190A
16.	**MnObl-67;** HF679213	g	2004	2004/2011	URF	2012	-	M41L, D67N, T69N, K70R, M184V, T215F, K219E	K101H, G190S
17.	**Gom-23-A;** HE657434	g	1996	2005/2010	A	2011	-	M41L, E44D, V75M, M184V, L210W, T215Y	A98G, K101E, Y181C, G190S
18.	**MnObl-92A1;**	g	2004	2004/2009	A	2012	-	M184V, T215F	K101E, G190S

Table 2: High/level resistance mutation at patients on HAART (children).

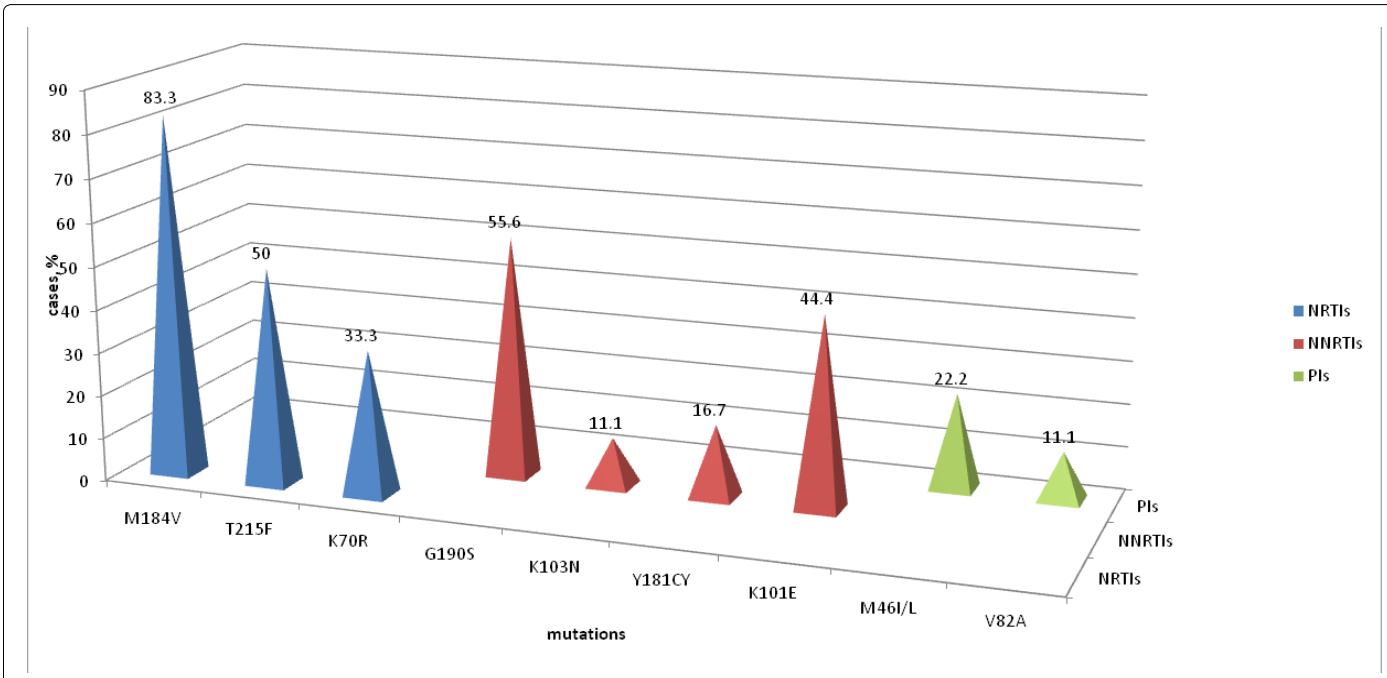

Figure 3: Resistance mutations identified in patients on HAART (children)

region and 3 patients from the Vitebsk region.

55 (93.2%) patients were identified as carriers of human subtype A HIV-1, 3 (6%) were infected with recombinant forms of CRF06_cpx and CRF03_AB and one patient (2%) was infected with subtype B (Table 1).

9 (49.2%) patients have been living with HIV/AIDS for more than 10 years, 25 (42.4%) patients were infected between 5 and 9 years and 5 (8.4%) patients carried the disease for less than 5 years. 40 (25 male and 15 female) during the period of the study received AZT+3TC+NVP (EFV), 7 (5 male and two female) were on the scheme FTC+TDF+NVP, 12 patients were on other schemes and one patient did not get the drugs.

The analysis of the pol gene sequencing results showed that most of the patients in the test group developed resistance mutations to nucleoside (NRTIs) and non-nucleoside (NNRTI) reverse transcriptase inhibitors (Figure 2).

In 38 (64.4%%) patients (24 male and 14 female) mutation M184V/I was identified, which causes high level HIV resistance to NRTIs. In 27 (45.8%) patients (13 female and 14 male) K103N mutation was detected, which determines high level HIV resistance to NNRTIs. In 20 (33.9%) patients (16 male and 4 female) we found G190G/S/A mutation, which is associated with the emergence of high-level resistance mutations to NNRTI.

11 (18.6%) and 8 (13.6%) patients were identified to have T215F and K70R mutations, which determine reduction in susceptibility to AZT, d4T and ADC, as well as AZT, d4T and TDF, respectively. In 15 (25.4%) patients (3 female and 12 male) we detected a combination of mutations M184V+G190S.

In 11 (18.6%) HIV infected patients (6 female and 5 male) was detected combination of resistance mutations K103N+M184V.

More often, in 49.2% of cases (29 people), high-level resistance mutations were detected in patients who have the disease experience of 10 or more years. 25 (42.3%) patients had experience of the disease between 5-9 years. And only 5 (8.5%) patients were infected less than 5 years ago.

In 76.3% cases (45 people) - resistance mutations were detected in patients who have experience of 2-4 years of therapy. 10 patients had a length of less than 2 years of treatment and 4 patients were treated with drugs for more than 5 years.

Of the 18 children-patients, born to HIV-infected mothers, 9 were boys aged 10.2 ± 1.7 and 9 girls (Table 2). 7 children were from the Minsk region, 5 from the Gomel region, 2 children were from the Mogilev region, 2 from the city of Minsk, 1 child from the Vitebsk region and 1 child from the Brest region. 16 children were infected with HIV-1 subtype A1, one - CRF02_AG and one child - URF (Table 2). In 15 (83.3%) cases (9 girls and 6 boys), we detected HIV resistance mutation

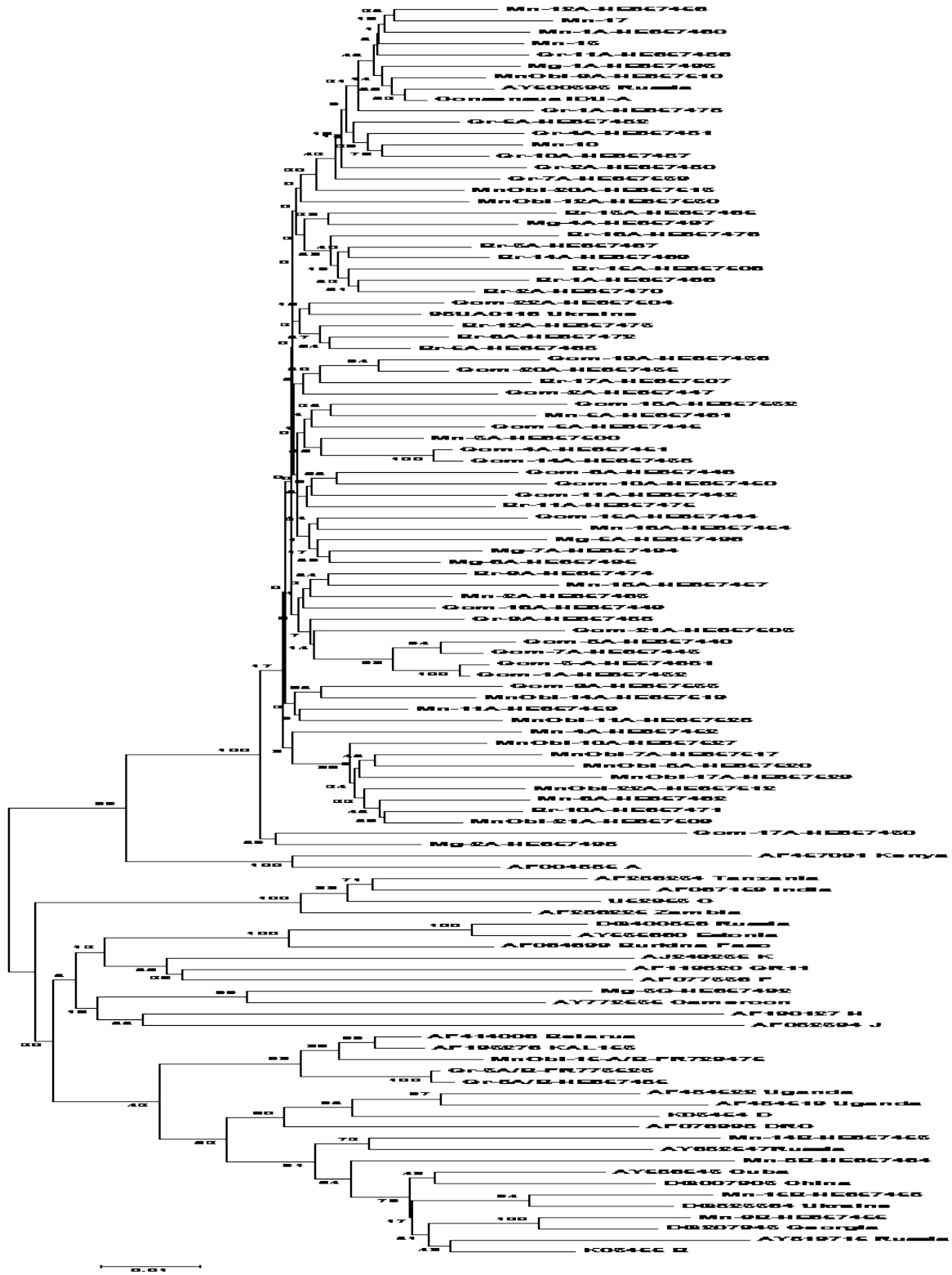

Figure 4: Phylogenetic analysis of HIV-1 gene pol sequences from a newly diagnosed HIV-infected antiretroviral naïve patients isolates. Reference sequences of HIV-1 and viruses specific for the epidemic in the former Soviet Union and Belarus as well as A-K subtypes are included. Reference sequences of HIV-1 subtypes are labeled by their GenBank accession numbers. For IDU-A viruses, the consensus of viruses from Svetlogorsk1 is used as a reference. The neighbor-joining tree were built by using the Kimura two-parameter distance estimation method and pairwise gap deletion. Bootstrap analysis was done with 100 replications, values above 70 are shown.

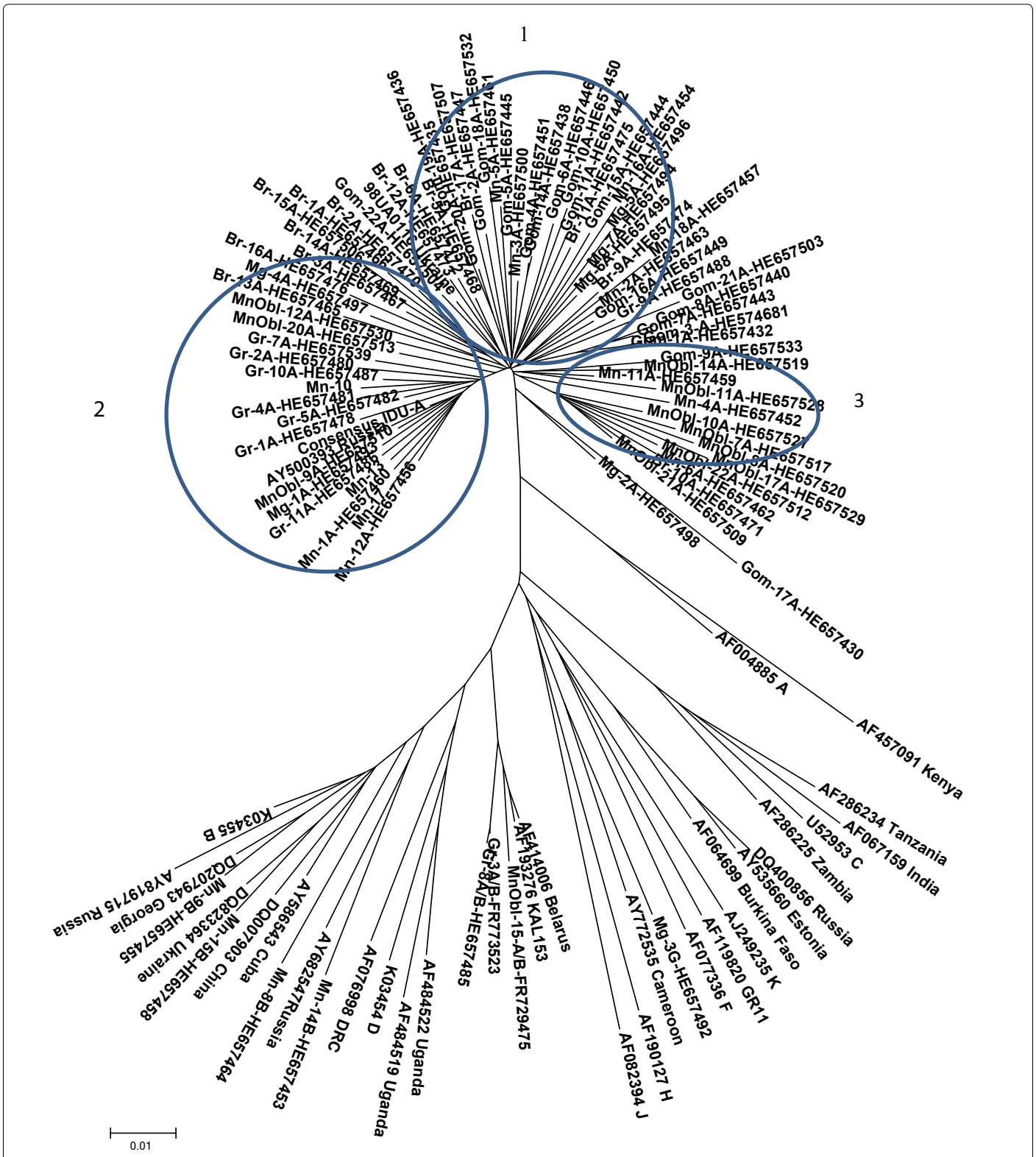

Figure 5: Phylogenetic analysis of HIV-1 gene pol sequences from a newly diagnosed HIV-infected antiretroviral naïve patients isolates. Reference sequences of HIV-1 and viruses specific for the epidemic in the former Soviet Union and Belarus as well as A-K subtypes are included. Reference sequences of HIV-1 subtypes are labeled by their GenBank accession numbers. For IDU-A viruses, the consensus of viruses from Svetlogorsk1 is used as a reference. The neighbor-joining tree were built by using the Kimura two-parameter distance estimation method and pairwise gap deletion. Bootstrap analysis was done with 100 replications, values above 70 are shown.

M184V, which determines, as it was mentioned above, a high level of resistance to NRTIs. In 10 (55.6%) and 2 (11.1%) cases mutations in position G190S and K103S were found, respectively defining, a high level of resistance to NNRTIs (Figure 3). In two children mutation Y188L was identified, which leads to the emergence of high-level HIV resistance to NNRTI. In 4 cases resistance mutation at position M46I/L was identified, which, in presence of other mutations, leads to a reduced sensitivity to HIV protease inhibitors: indinavir (IDV/r), nelfinavir (NFV), fosamprenavir (FPV/r), lopinavir (LPV/r) and atazanavir (ATV/r).

In 9 (50%) and 6 (33.3%) cases mutations T215F and K70R were detected. In 8 and 3 cases mutations K101EQH and Y181CFGV were detected, respectively. K101EQH mutation reduces of HIV-1 sensitivity to NVP, EFV and ETR, and Y181C/I/V mutation leads to a high level of resistance to NVP and DLV.

In 14 children (8 girls and 6 boys) various combinations of mutations leading to a high resistance were found (Table 2).

Most often, high-level resistance mutation were identified in children with the disease experience more than 10 years (9/50%) and 5-9 years (8/44, 4%). 8 children were detected to develop high-level resistance mutations after 2-4 years from the start of treatment, 6 children showed mutations developing after less than 2 years of therapy.

Thus, as it was shown by the undertaken research and studies - adults and children receiving HAART are detected to develop M184V and G190S mutations approximately with the same frequency. At the same time, among children mutation K103N is less often. As research shows, the emergence of resistance mutations is associated with the length of the period of experience of the disease. More than 90% of all patients, 94.4% children and 91.5% adults, who were detected with a high-level resistance mutations, had long period of registered disease experience - more than 5 years (the length of the disease period is considered to start from the day the patient is officially diagnosed to be infected with HIV/AIDS and his/her registration in Western blot, but, obviously, patients can be identified and registered already having an advanced HIV infection, cases of pre-AIDS stage also must be taken into account).

Thus, timely, early-stage identification of HIV/AIDS infection and prompt prescription and provision of the patient with most efficient antiretroviral drug therapy schemes will substantially improve quality of our help to this category of patients. Most frequently mutations were detected in patients after 2-4 years of treatment.For example, in 45 (76.3%) adult patients and 8 (50%) children high-level resistance mutations were detected after 2-4 years of taking ART. We are seriously worried by the fact that in 6 children (33.3%) high-level resistance mutations were identified in less than two years from the start of the therapy. This fact highlights the necessity of strong and careful monitoring of children receiving ART, who live in problem families.

In general, we can talk about a good patient adherence to therapy, only 18.8% of the patients (77 of 313) had resistance mutations of high risk. 39 (66.1%) of 59 adult patients are classified as IDUs, who, according to general experience, do not always express high degree of adherence to therapy [3-5].

96 blood samples were collected from newly diagnosed HIV-infected antiretroviral naïve patients genotype testing was successfully performed on 82 samples. Of 82 samples 43 (51.8%) were from females. Median age – 32-36 years; 81.7% (67) of people were diagnosed as HIV infected 1-3 years ago. Of total of 82 pol sequences analyzed 74 (90%) belong to HIV-1 subtype A; four (4.9%) - subtype B; three (3.7%) to CRF03_AB; and one was subtype G (Figure 4). The viruses carried to subtype A, formed 3 big groups (Figure 5). The Greatest first group including 35 samples, consisted basically of the specimens received from a Svetlogorsk city of the Gomel oblast where, as it is known, in 1996 there was HIV-infection outbreak among IDUs and circulates, a so-called "Svetlogorsk" a HIV-1 subtype A1 variant [6]. Average p-distances in group were 0.039 and fluctuated within 0.012-0.069 that specifies as in circulation before they brought virus, and new cases of an infection. The second group consisting of 24 samples formed patients from the Grodno oblast and a city of Minsk, mainly. There was an outbreak of a HIV-infection among IDUs in the Grodno oblast in the late nineties. Samples were formed around AF413987 (Ukraine), AY500393 (Russia) and consensus–IDU-A. Average p-distances were 0.040 and fluctuated from 0.026 to 0.056 that specifies in virus long circulation in the given population of patients and separate new cases of an HIV-infection. The third group including 12 samples formed patients from the Minsk oblast, basically from the cities of Soligorsk and Slutsk where there was HIV-infection outbreak among IDUs in the beginning of 2000th. Average p-distances between samples in group were 0.039 and fluctuated from 0.023 to 0.056. The HIV-1 from the given group has been designated as «Soligorsk» isolate. Samples AF873832 and FR87227, FR873141 have lain separately from other groups that specifies in independent penetration of a virus on territory of the country.

Apart from resistance mutations included in WHO 2009 list of SDRM, 6 samples (7.3%) had other resistance mutations which can be classified as 'minor' or 'other' according to HIVDR database of Stanford University: L10V – PI minor mutation associated with resistance to most PIs when present with other mutations; L33F – PI minor mutation selected by FPV/r, DRV/r, LPV/r, ATV/r, and TPV/r, and contributes decreased susceptibility to these PIs; V118I – accessory mutation usually occurred with multiple TAMs and contributes some resistance to each of the NRTIs including 3TC and FTC; T74S is 'other' mutation associated with reduced NFV susceptibility; and V108I - accessory mutation, causes low-level resistance to NVP and EFV.

According to the WHO recommendations in case of low prevalence of tHIVDR investigation should be repeated after 2 years, no change of ART regimens is required. However ongoing monitoring of ART programme focusing on risk factors such as continuous access to services, drug quality and supply, prescribing practices, adherence, adverse effects, and treatment failures is absolutely necessary.

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