Molecular Epidemiology of HIV Type 1 in Ukraine:
Birthplace of an Epidemic

MAGDI DARWISH SAAD,1 ALLA M. SHCHERBINSKAYA,2 YUKA NADAI,3 YURI V. KRUGLOV,2
SVIETLANA V.ANTONENKO,2 MARIYA G. LYULLCHUK,2 OLGA N. KRAVCHENKO,2
KENNETH C. EARHART,1 JOSE L. SANCHEZ,3 DEBORA L. BIRX,4 and JEAN KIRKLAND CARR4

ABSTRACT

During the 1990s, HIV-1 spread rapidly through drug networks in Ukraine and from there throughout the
former Soviet Union. To examine the origins of this epidemic, the genetics of HIV-1 in Ukraine were studied.
Proviral DNA from PBMC was extracted and PCR amplified. Part of pol and nearly full genomes of HIV-1
were sequenced and characterized. The predominant genetic form in 163 strains was subtype A (66%), fol-
lowed by subtypes B (30%), C (2%), D (1%), and a new AB recombinant form (1%). HIV strains from Kiev
were diverse having subtypes A, B, C, and D. In Crimea, Donetsk, Poltava, and Odessa, however, the strains
were overwhelmingly subtype A, while in Nikolaev subtype B predominated. After the near simultaneous in-
troduction of subtypes A and B in Ukraine, subtype B remained where it was introduced while subtype A
spread widely, creating the fastest growing epidemic in the world.

INTRODUCTION

According to the United Nations Program on HIV/AIDS (UNAIDS), the HIV/AIDS epidemic in the countries of
Eastern Europe and Central Asia was growing rapidly, the most rapid increase documented in the global pandemic to date.1 It was estimated that 1.3 million people in the region were living with HIV infection by the end of 2003, and about 30% of those had become infected in the past 2 years.1

Ukraine, where the estimated prevalence of HIV among the adult population was 1.4% in 2003, is one of the most affected countries in Europe.2 The spread of HIV in Ukraine before 1995 had a sporadic character; single isolated instances of HIV in-
festation (around 50 per year) were recorded. The ratio of HIV-infected men and women was approximately one. For 8 years between 1987 and 1994, only 398 Ukrainians were registered as HIV infected.2 Transmission was predominantly heterosexual, though men with men sexual behavior was clearly underre-
ported.2

Between 1994 and 1996, the number of HIV cases reported in Ukraine rose from 50 per year to 12,000 per year. The in-
crease was associated with a steep rise in HIV prevalence in injecting drug users (IDU), coupled with a markedly increased use of illegal drugs that followed the dissolution of the Soviet Union.3 Since 1996, the number of HIV-positive IDU has con-
tinued to grow dramatically. UNAIDS estimated there were 360,000 people in Ukraine with HIV infection in 2003.1

Geographically, the IDU epidemic in the countries of the former Soviet Union originated in Ukraine with near simultane-
ous outbreaks in two southern cities: Odessa and Nikolaev.4 From molecular data, it is now known that the epidemic in

Odessa was caused by subtype A while the epidemic in Niko-
layev was caused by subtype B.5 During this period, the two epidemics must have intermingled because the subtypes re-
combined to form a new circulating recombinant form, CRF03_AB, which caused an explosive IDU epidemic in Kalin-
ingrad in 1996.6 Since that time, the subtype A strain from Odessa has spread throughout the countries of the former So-
viet Union and beyond.7,8

The geographic distribution of HIV infection in Ukraine, however, is non-uniform. The most affected regions of the
country are Odessa, Nikolaev, Donetsk, and the capital of the

1U. S. Naval Medical Research Unit No. 3, Cairo, Egypt.
2Institute of Epidemiology and Infectious Diseases, Academy of Medical Sciences of Ukraine, Kiev, Ukraine.
3HIV Research Program, Henry M. Jackson Foundation, Rockville, Maryland.
4Walter Reed Army Institute of Research, Rockville, Maryland.
Crimea, Sevastopol (regions in the east and the south of the country). Southern Ukraine, bordering the Black Sea, is considered one of the main ports for heroin trafficking.

In the late 1990s, the epidemic began to spread into the general population; the number of people infected by unprotected heterosexual exposure has been steadily increasing. In 2003, about one-third of HIV-infected adults were women and an increasing number of children were born with HIV infection. In Ukraine in 2003, the majority of new infections were still found in IDU but some 30% were now attributable to heterosexual exposure.

Over the past 2 decades, the HIV epidemic in Ukraine has proceeded steadily through the three WHO defined phases: 1987–1994, initial phase; 1995–1998, concentrated phase in IDU; and 1998–2003, generalized phase. With each phase the challenge of containment increased. Ukraine is currently facing the difficult challenge of preventing HIV transmission in an environment where heterosexual sex is becoming the primary mode of transmission.

This report examines the subtype distribution in Ukraine between 2001 and 2002, about 6 years after the beginning of the concentrated phase of the epidemic. By this time, IDU networks from Georgia to Siberia were infected with the strain that originated in Odessa, Ukraine.

**MATERIALS AND METHODS**

**Study population**

Blood samples and questionnaire data were collected from 163 HIV-1-infected patients attending AIDS Centers in six different administrative locations, or Oblasts (Kiev, Odessa, Crimea, Poltava, Donetsk, and Nikolaev) following informed consent. Peripheral blood mononuclear cells (PBMC) were purified from other blood components using the CPT vacutainer procedure (Becton Dickenson, Inc). DNA was extracted from purified lymphocytes using the Qiagen Blood DNA Mini assay (Qiagen Inc., CA) following the manufacturer’s instructions.

**PCR amplification**

The HIV-1 pol gene was amplified from PBMC DNA using a nested PCR method resulting in an amplicon spanning the protease and part of the reverse transcriptase (RT) gene (1.1 kb). In addition, the nearly complete genome of HIV-1 was amplified for selected strains. The PCR primers were positioned to amplify all but 73 nt of the HIV-1 long terminal repeat (LTR).

**DNA sequencing**

Template DNA for automatic sequencing was prepared as described previously. PCR products of the pol gene and the nearly full-length strains were fully sequenced on both strands by using fluorescent dye terminators and an Applied BioSystems (Applied Biosystems Inc., Foster City, CA) Model 3100 DNA sequencer. DNA sequences were assembled using Sequencher software (GeneCodes Inc., Ann Arbor, MI) on Macintosh computers.

**Analysis**

A multiple alignment of the 163 Ukrainian pol gene sequences with selected available HIV-1 reference sequences was generated in MacGDE. Gaps that were introduced to create the alignment were not incorporated in the final analysis. Reference isolates of subtype A (Q2317 from Kenya and UG037 from Uganda, 1990–1992), subtype B (MN and WR27 from the United States, 1986–1988), subtype C (C2220 from Ethiopia, 1986), subtype D (ELI, NDK, from Zaire, 1986–1989), subtype J (SE9173 from Congo, 1993), CRF03_AB (KAL153 from Russia, 1997), and a subtype A strain 97BL006 (accession no. AF193275) from Belarus were used. Briefly, phylogenetic trees were constructed using the neighbor-joining method and the consistency of branching order was evaluated using bootstrap analysis (500 replicates) by MEGA3 software. Subtype J strain SE9173 was used as an outlier to root the tree. Recombinant analysis was done with Bootscan using SimPlot version 3.0. The nucleotide positions of recombinant breakpoints were designated relative to HXB-2 (GenBank Accession No: K03455).

**RESULTS**

Of the 163 patients whose samples were successfully sequenced and phylogenetically analyzed, 44.2% were males and 55.2% were females. The majority of patients were aged between 18 and 45 years, with a mean age of 31 years. One hundred and ten (67.5%) were Ukrainian nationals and 49 (30.1%) were Russians nationals, but all were residents of Ukraine. One hundred and forty-seven (91.4%) reported no contact with foreigners. Ninety-five (55.2%) were enrolled in 2001 and the remaining 73 (44.8%) were enrolled in 2002.

A phylogenetic analysis of the Ukrainian partial pol sequences with reference strains showed that 107 strains (65.6%) were subtype A, 49 (30.1%) were B, 3 (1.8%) were C, 2 (1.2%) were D, and 2 (1.2%) were a unique AB recombinant (Fig. 1). Most of the subtype A strains clustered with strain 97BL006 from Belarus with a bootstrap support value of 99%. The genetic diversity of these strains was extremely low, averaging about 1.1% in this pol region.

Examination of the geographical distribution of the strains revealed interesting differences. Similar to the molecular genetics in other European capitals, Kiev had subtypes A, B, C, and D (n = 18, 18, 3, and 1, respectively). Subtype B from Kiev separated into two clusters, one with low intersubject diversity and another with higher diversity (Fig. 1A and Fig. 2a). In Crimea, Donetsk, Odessa, and Poltava people were infected almost exclusively with subtype A (n = 17/19, 22/22, 20/22, and 21/21 respectively) (Fig. 2). The subtype A strains from the different oblasts were not different genetically and in fact exhibited strikingly low pairwise distances, with an average of about 1.2%. In contrast, subtype B was the dominant subtype in Nikolaev (22/23). The pairwise genetic distances between strains of subtype B in Nikolaev were also very low, averaging about 1.5%. The few subtype C and D strains were most closely related to strains from Ethiopia and Uganda, respectively (GenBank blast search).
Two strains from Crimea were unique AB recombinants (Fig. 2). The only circulating recombinant form (CRF) consisting of subtypes A and B is CRF03_AB, found in the Kaliningrad epidemic to the north. Bootscan analysis of part of pol revealed that the two strains from Crimea had the same recombinant structure as each other, with most of protease from subtype A but the remainder of the amplified region from subtype B (breakpoint is approximately 2524 and 2531 on HXB2 for 02UACR012 and 02UACR019, respectively) (Fig. 3). Comparison of these recombinants with CRF03 clearly shows that they are not CRF03_AB. Since both of the Crimean recombinants have the same breakpoint in pol they may, or may not, constitute a new CRF; three full genome sequences of the same structure are required to define a new CRF.

Thirteen nearly full genomes were sequenced from Ukraine. Three were subtype B strains from Kiev and the remaining 10 were subtype A strains, consisting of 3 each from Odessa, Donetsk and Poltava and one from Kiev. All strains were non-recombinant. In full genome analysis, all of the subtype A strains clustered with 97BL006 from Belarus (data not shown).

Antiretroviral resistance mutation (ARM) analysis of the 163 strains using the pol gene region indicated 6 (3.7%) strains had mutations corresponding to resistance to nucleoside RT inhibitors (NRTI), nonnucleoside RT inhibitors (NNRTI), or both. Only one of these was from subjects who reported antiretroviral treatment (ART; Combivir and Fortovase). Strain 02UAKV125 was subtype A and had mutations predicting high-level resistance to 3TC and FTC. The remaining five strains were from drug-naive patients and were three subtype B and two subtype A. The proportion of treatment naive subjects demonstrating mutations conferring possible drug resistance is approximately 3.1% (5/163). Observed mutations in RT were A62T, M184I, M184V, M230I, Y188F, and V179D.

**DISCUSSION**

The present HIV epidemic in Ukraine started in the 1990s, with the near simultaneous introductions of subtype B into drug-using networks in Nikolayev and subtype A into drug networks in Odessa.5,19 This study documents that these two subtypes were still contributing to the Ukrainian epidemic in 2001 and 2002, with the majority being subtype A (65.6%) followed by subtype B (30.1%). Although present, subtypes C (1.8%) and
D (1.2%) and a new AB recombinant (1.2%) were common to the region.

The HIV epidemic that began in Nikolayev, with the introduction of subtype B, is still dominated by subtype B (95.6%). The cluster of subtype B in Nikolayev has very low interpatient diversity and thus would have been easy to detect if it had spread. In fact, it was found only as a small component of the epidemic in Kiev and not in any other oblasts. The subtype A strain introduced in Odessa, on the other hand, spread to all of the other oblasts, and from there to Russia, Moldova, Georgia, Uzbekistan, and Kyrgyzstan.\textsuperscript{4,8,20–22} In the history of the HIV pandemic, the transition from a concentrated phase to the generalized phase has often been coincident with the transition from subtype B to non-B. This was true in Thailand, where simultaneous introduction of subtype B in IDU and CRF01\_AE in CSW grew into a generalized epidemic dominated by CRF01\_AE.\textsuperscript{23} In Argentina, the subtype B-dominated, MSM epidemic is in the process of being displaced by heterosexually transmitted BF

**FIG. 3.** Bootscan analysis of Ukraine 02UACR012, 02UACR019, and CRF03\_AB (Kal-153). (A) 02UACR019, (B) 02UACR012, and (C) Kal153. Bootstrap value is plotted of the recombinant with subtype A (thick gray line), subtype B (thick black line), and C (thin light-gray line). The diagram below presents the location of the protease and reverse transcriptase (RT) genes. Dotted lines show the estimated recombinant breakpoints.
recombinants. While it is clear from fitness studies in vitro that subtype B is no less fit than other subtypes, there appears to be selection against subtype B as the virus responsible for generalized, heterosexual epidemics. Understanding the viral elements required for HIV to achieve epidemiologic success is an important challenge.

In 1997, Lukashov and Goudsmit observed a significantly lower rate of nonsynonymous changes over time in the V3 loop of strains infecting IDU than those infecting MSM. On a population level, the observation of low interstrain diversity in IDU epidemics was first reported by O’Neil and colleagues about a subtype C epidemic in Nepal. The phenomenon has since been observed in other IDU epidemics with other subtypes. The subtype A epidemic in the former Soviet Union, however, represents the largest and most long lasting of such epidemics. The subtype A strain has maintained distances in pol of less than 2% between strains from Odessa in 1996, to Irkutsk, Russia, in 1999. The biological mechanism underlying this observation is not understood.

The subtype B strain in this Ukrainian population fell into two genetic clusters, one with low interstrain genetic distances and one with distances approaching that of subtype B in Western Europe. Both types of subtype B were present in Kiev, while only the low diversity variant was in Nikolayev. Historically, the epidemic in Nikolayev began with subtype B in IDU; however, we demonstrate that in 2002 a subtype B strain with low genetic diversity was still dominating the epidemic there. The Nikolayev low-diversity B strain, however, recombines with subtype A to form CRF03_AB, which infected drug networks in the Russian city of Kaliningrad. The recombination probably occurred in Kaliningrad itself because no CRF03_AB strains were found in this study of Ukraine.

Two related recombinant strains were detected in Crimea, where the main HIV subtype was A. Both were recombinants of subtypes A and B, and were compared to CRF03_AB, but bootstrap analysis showed that the recombination breakpoint in pol was in a different location (Fig. 3). This suggests the formation of a new recombinant within Ukraine, possibly in Crimea. Future research will determine whether this new recombinant will spread or disappear from the population.

Six strains, two of them A and three B, had ART resistance mutations in the RT gene though only one of them was under ART treatment. This suggests that the rate of primary drug resistance was about 3.1% in this drug-naive, HIV-infected population. Monitoring for resistance mutations in subtype A-infected patients on ART will provide valuable information about the prevalence of these mutations in this strain.

In conclusion, origins of the epidemic spreading through the former Soviet Union could still be clearly seen in the strains present in different parts of Ukraine in 2001 and 2002. Almost all of the subtype A strains collected in most of the cities in Ukraine as well as elsewhere in this widespread epidemic form a monophyletic cluster with the subtype A strains collected in 1996 in Odessa. The descendants of what was probably a single introduction of subtype A have now infected over a million people.

**SEQUENCE DATA**

Nucleotide sequence accession numbers of the pol gene sequences and the full-length sequences from Ukraine are available under GenBank accession nos. DQ823356–DQ823367, respectively.

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**REFERENCES**


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2. Olga A. Rumyantseva, Igor A. Olkhovskiy, Marina A. Malysheva, Ludmila A. Ruzueva, Alexander V. Vasiliev, Elena V. Kazennova, Marina R. Bobkova, Vladimir V. Lukashov. 2009. Epidemiological Networks and Drug Resistance of HIV Type 1 in Krasnoyarsk Region, Russia. Epidemiological Networks and Drug Resistance of HIV Type 1 in Krasnoyarsk Region, Russia. AIDS Research and Human Retroviruses 25:9, 931-936. [Abstract] [Full Text] [PDF] [PDF Plus]


7. Marco Salemi, Maureen M. Goodenow, Stefania Montieri, Tulio de Oliveira, Maria Mercedes Santoro, Danail Beshkov, Ivailo Alexiev, Ivailo Elenkov, Ivan Elenkov, Tsvetana Yakimova, Tonka Varleva, Giovanni Rezza, Massimo Ciccozzi. 2008. The HIV Type 1 Epidemic in Bulgaria Involves Multiple Subtypes and Is Sustained by Continuous Viral Inflow from West and East European Countries. The HIV Type 1 Epidemic in Bulgaria Involves Multiple Subtypes and Is Sustained by Continuous Viral Inflow from West and East European Countries. AIDS Research and Human Retroviruses 24:6, 771-779. [Abstract] [Full Text] [PDF] [PDF Plus]

8. Michael M. Thomson, Elena Vázquez de Parga, Anna Vinogradova, Maria Sierra, Aleksey Yakovlev, Aza Rakhmanova, Elena Delgado, Gema Casado, Mercedes Muñoz, Rocio Carmona, Yolanda Vega, Lucía Pérez-Álvarez, Gerardo Contreras, Leandro Medrano, Saladin Osmanov, Rafael Nájera. 2007. New Insights into the Origin of the HIV Type 1 Subtype A Epidemic in Former Soviet Union’s Countries Derived from Sequence Analyses of Preepidemically Transmitted Viruses. New Insights into the Origin of the HIV Type 1 Subtype A Epidemic in Former Soviet Union’s Countries Derived from Sequence Analyses of Preepidemically Transmitted Viruses. AIDS Research and Human Retroviruses 23:12, 1599-1604. [Abstract] [PDF] [PDF Plus]

9. Bluma G Brenner. 2007. Resistance and viral subtypes: how important are the differences and why do they occur?. Current Opinion in HIV and AIDS 2:2, 94-102. [CrossRef]