

# Genetic Subtypes of HIV Type 1 Circulating in Slovakia

M. Habekova,<sup>1</sup> M. Takacova,<sup>1</sup> J. Lysy,<sup>1</sup> M. Mokras,<sup>2</sup> R. Camacho,<sup>3</sup> P. Truska,<sup>4</sup> and D. Stanekova<sup>1</sup>

## Abstract

Slovakia belongs to the group of European countries with a low prevalence of HIV infection. The major proportion of HIV-positive cases in Slovakia is still represented by MSM, followed by heterosexuals infected through unprotected sexual intercourse. This study was conducted to update the description of HIV subtypes circulating in Slovakia. HIV-1 partial *pol* gene sequences from 143 individuals were prospectively collected from 2004 to 2008 and analyzed. Phylogenetic analysis based on HIV-1 partial *pol* gene sequences revealed the highest prevalence of HIV-1 B subtype (93.0 %), predominantly associated with the MSM group. Ten (7.0%) individuals were infected with HIV-1 non-B subtypes. The pure subtypes were more frequent (7; 4.9%) than CRFs (3; 2.1%) and their occurrence was as follows: subtype C (3; 2, 1%), subtype A (2; 1.4%), subtype F (2; 1.4%), CRF\_01AE (1; 0.7%), CRF\_02AG (1; 0.7%), and CRF08\_BC (1; 0.7%). Data show slightly increasing HIV-1 subtype diversity, with HIV-1 subtype B still having the highest prevalence in the Slovak-infected population.

## Introduction

A MAJOR OBSTACLE IN THE CONTROL OF THE SPREAD OF AIDS lies in the diversity of HIV and its enormous evolutionary potential. Numerous HIV variants contribute to the pandemic worldwide and have potential consequences for diagnostics, monitoring, treatment, and the development of an effective vaccine.

HIV genetic diversity could be caused by several factors, such as the rapid turnover of HIV-1 *in vivo*,<sup>1</sup> host selective immune pressure,<sup>2</sup> and recombination events during replication.<sup>3</sup> Due to this variability, HIV-1 variants are classified into three major phylogenetic groups: group M (main), group O (outlier), and group N (non-M/non-O).<sup>4</sup> Group M, which is responsible for the global HIV-1 epidemic, can be subdivided into nine recognized phylogenetic subtypes, or clades (A–D, F–H, J, and K), and within subtypes A and F further into subsubtypes (A1 to A4 and F1 to F2, respectively).<sup>5–8</sup> By January 2009; 43 circulating recombinant forms (CRFs) had been reported.<sup>9</sup> According to recent studies, the most prevalent HIV-1 genetic form worldwide is subtype C, followed by A, CRF02\_AG, and B.

The emergence of CRFs in the global HIV-1 pandemic is important, representing 18% of the infections<sup>10</sup> and appearing predominantly in Southeast Asia (CRF01-AE) and in West and West Central Africa (CRF02-AG).<sup>11</sup> In Europe, in partic-

ular, subtype B is the main circulating genetic form.<sup>12</sup> Trend analysis (1996–2001) for Central and Eastern Europe was based on the supposition that the HIV epidemic reflected the dimension of the so-called bridging population, which corresponds to high-risk groups linked with the general population.<sup>13</sup> Phylogenetic analysis in the Czech Republic,<sup>14</sup> Slovenia,<sup>15</sup> and Austria<sup>16</sup> revealed that HIV-1 subtype B is still dominant, while other HIV subtypes such as A, C, F, and recombinant circulating forms CRF01\_AE and CRF02\_AG are less frequent. Subtype A viruses are predominant in Eastern European countries formerly constituting the Soviet Union, where their spread is driven mainly by injecting drug users. The study accomplished among intravenous drug users (IV-DUs) in Ukraine<sup>17</sup> supported the belief that subtype A was more frequent (66%), followed by subtypes B (30%), C (2%), D (1%), and a CRF03\_AB recombinant form (1%). Unlike the majority of European countries, Romania is characterized by a subtype F epidemic.<sup>18</sup>

In Slovakia, as in other European countries, the risk of HIV transmission is predominantly (60%) associated with a history of homosexual/bisexual behavior (men having sex with men, MSM).<sup>19</sup> By the end of 2009 less than 30% of HIV-1 infections in Slovakia were transmitted through unprotected heterosexual intercourse and 2.3% through intravenous drug usage. About half of all reported HIV-infected persons in the Slovak Republic live in the capital, Bratislava.<sup>20</sup> The aim of this study

<sup>1</sup>Slovak Medical University, NRC for HIV/AIDS Prevention, Bratislava, Slovakia.

<sup>2</sup>Medical faculty of Comenius University, Derer Hospital, Bratislava, Slovakia.

<sup>3</sup>Laboratório de Biologia Molecular, Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal.

<sup>4</sup>Institute of Public Health of the Capital of Slovakia, Bratislava, Slovakia.

was to assess the prevalence of HIV-1 subtypes and their distribution in epidemiological contexts in the Slovak Republic.

### Materials and Methods

HIV subtyping was obtained from plasma of 143 HIV-infected individuals [117 antiretroviral therapy (ART) naive and 26 ART treated]. Plasma samples were collected for routine genotypic and drug resistance testing between 2004 and 2008 in the National Reference Centre for HIV/AIDS Prevention in Bratislava.

Sequences from 53 plasma samples were generated by population sequencing utilizing the commercial procedure Viroseq (ViroSeq HIV-1 genotyping system, Abbott Molecular, Abbott Laboratories, IL).<sup>21</sup> HIV RNA isolation from plasma of the remaining 90 HIV-infected individuals was performed according to the manufacturer's recommendation using the Macherey-Nagel RNA Isolation Kit (Macherey-Nagel, Duren, Germany) and/or Cobas Amplicor HIV 1 Monitor v.1.5, Isolation procedure,<sup>22,23</sup> followed by nested reverse transcriptase polymerase chain reaction (RT-PCR) of the HIV pol region. The products of nested RT-PCR have been sequenced with the use of in-house procedures according to the ANRS1 sequencing protocol.<sup>24</sup> Sequences had 805 bp and/or 645 bp for RT and 297 bp for protease (PRO) in length, spanning codons (40–240) of RT and the entire (1–99) PRO gene region, respectively.

### Genetic subtyping and phylogenetic analysis

All partial *pol* sequences were first analyzed separately using the REGA HIV-1 Subtyping Tool.<sup>25</sup> HIV-1 subtype determination was carried out by comparing patterns of patient's partial *pol* sequences against consensus sequences representing a variety of HIV-1 subtypes available in the Los Alamos HIV-1 sequence database.<sup>26</sup> The sequences were aligned using CLUSTAL W software<sup>27</sup> and edited with BioEdit Software.<sup>28</sup> The phylogenetic trees were constructed with the use of the *PhyML* tool<sup>29</sup> and methods implemented in this program, using the HKY substitution model. Statistical support for specific clades was computed using Mega 4 software.<sup>30</sup> The bootstrap values (bootstrapping with 1000 replicates) above 80% were considered significant and displayed at the tree nodes.

### Results

The genetic diversity of HIV-1 strains circulating in the Slovak Republic was analyzed. HIV subtyping was done from

plasma of 143 HIV-infected individuals (20 women and 123 men). The median age of women and men at the time of HIV diagnosis was 32 (21;63) and 31 (16;65) years, respectively. All individuals tested were Slovak citizens, with the exception of 3 (2.1 %) who had other nationalities (Ukraine, China, and Russia). The distribution of routes of transmission was as following: homosexual/bisexual (96; 67.1%), heterosexual (34; 23.8%), IVDUs (1; 0.7%), and blood recipients (1; 0.7%). For 11 cases (7.7%) the information about the route of transmission was unknown. Most of the individuals were infected by unprotected sexual intercourse (100% MSM, 51.8% male heterosexuals, 100% females).

### HIV-1 subtypes B

In the group of 143 analyzed isolates phylogenetic analysis revealed the highest prevalence of HIV-1 subtype B (133; 93.0%). All individuals infected by HIV-1 subtype B were Slovaks, with the exception of one immigrant from Russia (sample coded 4831). A total of 66.4% of all HIV-1 subtypes B were transmitted in the group of MSM. All MSM, except one, were infected by HIV-1 subtype B (98.9%). The relationships between HIV-1 subtypes, risk exposure, and gender are shown in Table 1.

### HIV-1 non-B subtypes

Ten out of 143 patients (7.0 %) were infected with non-B subtypes. The isolates that did not cluster with subtype B originated—with the exception of one MSM—exclusively from patients infected through heterosexual unprotected sexual intercourse. The HIV-1 subtype analysis showed that within the non-B subtypes, pure subtypes were more prevalent (seven isolates, 4.9 %) than CRF(s) (three isolates, 2.1%). Among the pure non-B subtypes, subtype C was the most frequent (three cases, 2.1%), followed by subtype A (two cases, 1.4%) and subtype F (two cases, 1.4%), CRF\_01AE (one case 0.7%), CRF\_02AG (one case 0.7%), and CRF\_08BC (one case 0.7%). With regard to the country of origin of the individuals infected by non-B subtypes, eight were Slovaks (5.6%), one Chinese (0.7%), and one Ukrainian (0.7%). According to the origin of HIV-1 non-B subtypes, these could be transmitted to Slovakia from Africa (five cases, 3.5%), in one case each (0.7 %) from Ukraine, China, and Brazil, and in two cases the areas from which the non-B subtypes originated was unknown (Table 2).

Phylogenetic trees constructed from non-B subtypes are shown in Fig. 1. Non-B isolates revealed in this study were distributed within three monophyletic clades [A (A1), C, F (1)] and three subclades (1 AE, 1 AG, and 1 BC). Two isolates

TABLE 1. DISTRIBUTION OF HIV-1 SUBTYPES ACCORDING TO THE GROUPS AT RISK AND SEX

HIV-1 subtypes vs. groups at risk	B			Non-B			Number total (%)
	Males	Females	Total (%)	Males	Females	Total (%)	
MSM	95	—	95 (66.4)	1	—	1 (0.7)	96 (67.1)
Heterosexuals	11	15	26 (18.2)	3	5	8 (5.6)	34 (23.8)
IVDUs	1	—	1 (0.7)	—	—	—	1 (0.7)
Blood recipients	1	—	1 (0.7)	—	—	—	1 (0.7)
Not known	10	—	10 (7.0)	1	—	1 (0.7)	11 (7.7)
Total	118	15	133 (93.0)	5	5	10 (7.0)	143 (100)

TABLE 2. DISTRIBUTION OF HIV-1 NON-B SUBTYPES ACCORDING TO THE COUNTRY OF ORIGIN, SOURCE OF INFECTION, SEX, MODE OF TRANSMISSION AND COINFECTION WITH HBV, HCV

Patient code	Sex	Country of origin of HIV-infected individuals	Origin of source of HIV infection	Mode of HIV transmission	HIV-1 subtype	Coinfection (HBV, HCV)
5315	F	Ukraine	Ukraine	Heterosexual unprotected intercourse	A	HCV
6143	F	Ukraine	Ukraine	Heterosexual unprotected intercourse	A	HCV
6170	F	Slovakia	Kenya	Heterosexual unprotected intercourse	A	No
5279	M	Slovakia	Africa (?)	Heterosexual unprotected intercourse	C	Not known
6023	F	Slovakia	Zambia	Heterosexual unprotected intercourse	C	No
6577	M	Slovakia	Botswana	Heterosexual unprotected intercourse	C	No
5947	M	Slovakia	Brazil	Heterosexual unprotected intercourse	F1	No
6514	M	Slovakia	Not known	MSM	F1	No
6089	M	China	China	Heterosexual unprotected intercourse	CRF 01_AE	Not known
5007	F	Slovakia	Africa (?)	Heterosexual unprotected intercourse	CRF 02_AG	Not known
4853	F	Slovakia	Not known	Heterosexual unprotected intercourse	CRF 08_BC	Not known

clustered with the HIV-1 A consensus strain. One of these two subtypes (code 6143) originated from a female from Ukraine. The second isolate (code 6170) clustering with subtype A, received from a female having unprotected heterosexual intercourse with her partner, originated from Kenya. Two isolates clustered with consensus strain F (F1). One of them (code 5947) was isolated from a man having unprotected heterosexual intercourse in Brazil. The origin of the second strain (code 6514), similar to F(F1), isolated from MSM is not known.

Three different strains of representative CRFs were used for this analysis and three distinct isolates were similar to them. The strain coded 6089, similar to consensus strain CRF\_01AE, was isolated from a male immigrant from China. The origin of the isolate coded 4853, similar to CRF\_08BC, was not known. The strain (code 5007) clustering with CRF02\_AG and the isolates (codes 5279, 6023, and 6577) clustering with subtype C, isolated from patients having heterosexual intercourse with their partners, originated from Africa.

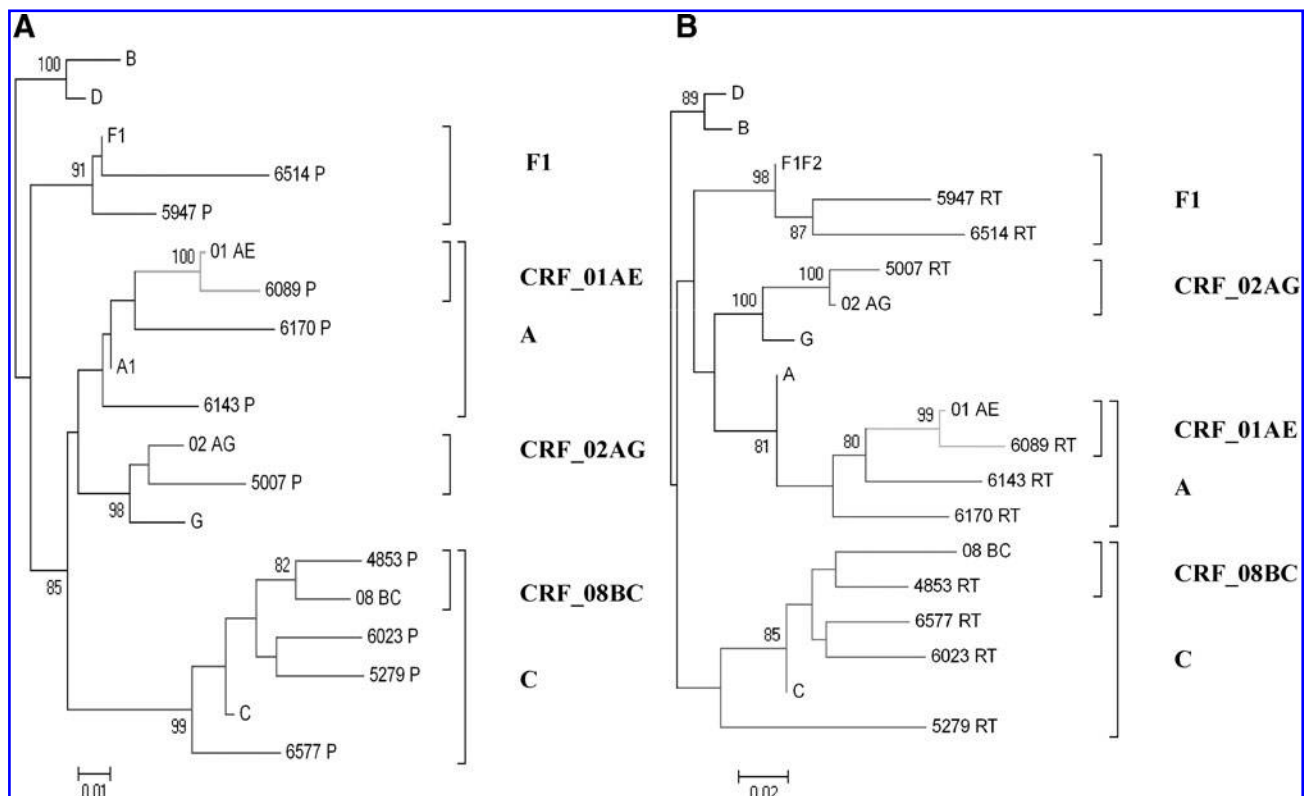


FIG. 1. The phylogenetic trees constructed from non-B subtypes of HIV-1. The sequences of the PRO (A) and RT (B) region of the *pol* gene were analyzed. The phylogenetic trees were constructed with the *PhyML* tool, using the HKY substitution model. Statistical support for specific clades was computed using *Mega 4* software. The bootstrap values (bootstrapping with 1000 replicates) above 80% were considered significant and are displayed at the tree nodes. The bottom scale measures genetic distance in substitutions per nucleotide.

## Discussion

### *HIV-1 subtypes B*

Data of this study showed the high prevalence of HIV-1 subtype B within HIV-infected individuals in Slovakia. HIV-1 subtype B was found in most of the individuals studied (93.0%) and was predominantly associated with the MSM group, with just one exception (subtype F1). Similar results of the highest HIV-1 subtype B prevalence in the Slovak Republic were previously described.<sup>31</sup> Slovakia belongs to the group of countries with the highest HIV prevalence in the MSM group and our study reflects this apparent segregation of HIV-1 subtypes according to the route of transmission. In the Slovak Republic a small number of HIV-infected IVDUs has been reported. In this study one IVDU was tested and found to be a carrier of HIV-1 subtype B.

### *HIV-1 non-B subtypes*

In this study 10 HIV-1 non-B subtypes representing 7% of all samples analyzed were revealed. The majority of them (9 of 10 cases) originated from patients infected by unprotected heterosexual contacts. HIV-1 non-B subtypes found in this study arise from areas consistent with the geographic distribution of these subtypes. Two cases of subtype A in this study were observed: one of them (code 6143) was isolated from a woman from Ukraine, an Eastern neighbor country of Slovakia, where this subtype occurs frequently.<sup>32</sup> The next isolates clustering with subtypes A (code 6170), C (codes 5279, 6023 and 6577), and CRF02\_AG (code 5007), respectively, belonged to individuals with sexual partners originating from countries of central and sub-Saharan Africa, where these subtypes circulate.<sup>33</sup> The strain from the sample coded 5947 clustered with consensus strain F (F1) and was isolated from a man having unprotected sexual intercourse in Brazil, where subtype F (F1) is well documented.<sup>34</sup> The strain similar to F (F1) isolated from an MSM (sample coded 6514) and the strain similar to CRF08\_BC isolated from a woman (sample coded 4853) have unknown origins. The strain from the sample coded 6089 isolated from a male immigrant originating from China was similar to consensus strain CRF\_01AE, which represents 12.5% of all sequences from China included in the Los Alamos HIV-1 sequence database.

The molecular epidemiological data of this study are able to confirm multiple entries of HIV-1 infection into the Slovak Republic and show that the genetic patterns of the HIV-1 strains circulating in Slovakia correspond to what is found in other European countries. The findings of this study could help to guide strategies to control the spread of HIV-1 in Slovakia, keeping the local epidemic at a low level.

## Sequence Data

The sequences described in this article have been deposited in the GenBank Nucleotide Sequence Database under accession numbers GQ399633.1, GQ399752, and FN687216 to FN687231.

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## Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:  
Monika Habekova  
Slovak Medical University  
NRC for HIV/AIDS Prevention  
Limbova 12  
833 01 Bratislava, Slovakia  
E-mail: [monika.habekova@szu.sk](mailto:monika.habekova@szu.sk)

