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Transmitted Drug Resistance in Newly Diagnosed, Treatment-Naïve, HIV Type 1-Infected Patients in Hungary

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Highlights

- HIV-1 TDR among newly diagnosed individuals is 10.7% in Hungary.
- The prevalence of TDR in Hungary is 9.5% for NRTIs, 1.2% for NNRTIs and 0.6% for PIs.
- Ongoing spread of resistant strains may increase the prevalence of TDR in Hungary.

Abstract

Objectives: Transmitted HIV-1 drug resistance (TDR) may affect the success of first-line antiretroviral treatment. The aim of this study was to monitor the presence of HIV-1 strains carrying transmitted drug resistance-associated mutations (TDRMs) in newly diagnosed, treatment-naïve patients in Hungary.

Methods: 168 HIV-infected individuals diagnosed between 2013 and 2017 were included in the study; most of them (93.5%) belonged to the homo/bisexual population. HIV-1 subtypes and TDRMs were determined by analysing the protease and reverse transcriptase coding regions of the *pol* gene by the Stanford HIV Drug Resistance Database. Transmission clusters among patients were identified using phylogenetic analysis.

Results: Although subtype B HIV-1 strains were predominant (87.5%), non-B subtypes including F, A, CRF01_AE, CRF02_AG, D and G were also recorded, especially in young adults. The overall prevalence of TDR was 10.7% (18/168; 95% CI: 6.9-16.3%). Subtype B HIV-1 strains carried most of the TDRMs (94.4%). Nucleoside reverse transcriptase inhibitor (NRTI)-associated mutations were the most prevalent indicators of TDR (16/168; 9.5%; 95% CI: 5.9-14.9%), followed by mutations conferring resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) (2/168; 1.2%; 95% CI: 0.3-4.2%) and protease inhibitors (PIs) (1/168, 0.6%; 95% CI: 0.1-3.3%). Phylogenetic analysis revealed that most NRTI-associated resistance mutations were associated with a single monophyletic clade, suggesting early single source introduction and ongoing spread of this drug resistant HIV-1 strain.

Conclusions: Onward transmission of drug resistant subtype B HIV-1 strains accounted for the majority of TDRs observed among treatment-naïve HIV-infected individuals in Hungary.

Keywords: HIV-1, transmitted drug resistance, Hungary, transmission cluster, ongoing transmission

1. Introduction

Human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), represents a serious global health

challenge: it is estimated that in 2017, 940 000 people died of AIDS-related diseases globally, and AIDS is still the 4th most common cause of death in low-income countries [1,2]. Although antiretroviral therapy (ART) has significantly reduced morbidity and mortality in HIV-infected patients, the development of drug resistance poses a threat to the long-term success of ART [3]. Transmission of drug resistant strains to therapy-naïve individuals could cause first-line regimen treatment failure. Thus, international guidelines recommend testing for transmitted drug resistance associated mutations (TDRMs) before initializing ART of treatment-naïve patients either with acute or chronic HIV-1 infection [4,5].

Hungary is a country of low HIV prevalence, even though the annual number of newly diagnosed HIV infections increased from 0.62 in 2003 to 2.75 in 2015, per 100 000 population. Between 1985 and 2017, altogether 3567 HIV-infected persons were diagnosed in Hungary, a country of 10 million, and the cumulative number of registered AIDS cases was 944. The main route of HIV infection is homo/bisexual contact and the proportion of males is 88% among HIV-positive patients with known gender [6].

In the era of combination antiretroviral therapy (cART), HIV-associated death decreased significantly in Hungary, similarly to other European countries. Treatment standards of HIV-positive patients are according to Hungarian guidelines which are in accordance with the recommendations of European AIDS Clinical Society [7]. cART is offered to each HIV-positive patient irrespective of

their $CD4^+$ T cell count and it is estimated that at present more than 1900 patients receive cART in Hungary.

Because only limited information was available about the molecular epidemiology of HIV-1 in Hungary, we aimed to monitor circulating HIV-1 subtypes, assess the prevalence of transmitted drug resistance (TDR), and reveal potential transmission clusters among treatment-naïve, HIV-1-infected individuals in Hungary.

2. Materials and Methods

2.1. Study population

Peripheral blood samples were collected between 2013 and 2017, within 1 year (median: 0.7 month; interquartile range, IQR: 0.36-1.13 months) of HIV diagnosis from 168 patients attending the Dermatology, Venereology and Dermatooncology Clinic of Semmelweis University, Budapest and the Center for HIV, South-Pest Central Hospital, Budapest. South-Pest Central Hospital hosts the only Center for HIV in the country, where 98% of HIV-infected patients are treated. All patients were newly diagnosed and antiretroviral therapy-naïve, and all of their data were anonymized. This study has been approved by the Institutional Bioethics Committee of South-Pest Central Hospital and by Medical Research Council, Scientific and Research Ethics Committee, Budapest, Hungary.

2.2. Sample preparation and sequencing

Plasma of ART-naïve HIV-1-infected patients was obtained from EDTA-anticoagulated whole blood samples, centrifuged at 10 000 rpm for 2 min and stored at -80°C. Viral RNA was extracted from plasma using the NucliSENS miniMAG nucleic acid purification system and specific magnetic extraction reagents (bioMérieux, Marcy l'Etoile, France). The protease (PR) and reverse transcriptase (RT) coding regions of the *pol* gene, which are potentially affected by drug resistance related mutations were reverse transcribed using RobusT II

(Finnzymes, Espoo, Finland), AccessQuick (Promega, Madison, WI, USA) and SuperScript III (Invitrogen, Carlsbad, CA, USA) one-step RT-PCR kits and further amplified by nested PCR as described earlier [8,9]. Purified PCR products were subjected to direct sequencing using the DYEnamic ET Dye Terminator Cycle Sequencing (Amersham Biosciences, Buckinghamshire, UK) and BigDye Terminator v3.1 Cycle Sequencing (Applied Biosystems, Foster City, CA, USA) kits. Capillary electrophoresis and base calling was implemented by MegaBACE 1000 (Amersham Biosciences) and Applied Biosystems 3500 Genetic analyser systems.

HIV-1 RNA quantification was performed using the NucliSENS nucleic acid purification and amplification system (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions.

2.3. Subtyping and drug resistance interpretation

For HIV-1 subtype determination, the PR and RT coding sequences were analysed using the Stanford HIVDB subtyping program and the COMET tool [10,11]. The presence and clinical relevance of TDRMs in the examined samples were assessed by the Stanford HIV Drug Resistance Database algorithm according to the WHO Surveillance Drug Resistance Mutations list [10,12].

2.4. Phylogenetic and statistical analysis

In addition to the 168 sequences newly reported in this study, in the phylogenetic analyses we included a further 30 partial *pol* sequences that we reported earlier from Hungary [8]. To enable the identification of transmission clusters local to the country, we constructed a set of background control sequences by pooling the 5 most similar NCBI BLASTN search results (excluding our sequences already submitted to GenBank from Hungary) for each of our 198 partial *pol* sequences [13], then removing duplicate sequences. Some samples had been amplified to yield two contigs (covering PR and partial RT, respectively); these were concatenated (inserting indeterminate 'N' nucleotides in the missing positions based on alignment to the HXB2/K03455 reference sequence) before submitting to the BLAST search. The combined set of Hungarian and background sequences was aligned using MAFFT version 7.212 [14], followed by manual inspection and correction of the multiple alignment using Unipro UGENE version 1.31.1 [15], and the removal (gap-stripping) of nucleotide positions that were absent or indeterminate in at least 50% of the Hungarian sequences or of the combined set. This procedure resulted in a multiple alignment covering HXB2 positions 2253-3379 (codons PR1-RT277), with a 63-bp gap between codons RT12-33 where data were missing for samples that had been amplified with two contigs; the total length of the alignment was 1064 base pairs. Steps of the data pipeline were automated with scripts implemented in (Bio)python [16] and R [17].

Maximum likelihood phylogenetic trees were constructed with RAxML version 8.2 [18] under the GTRCATI model; branch support values were computed based on automated bootstrap replicates (-autoMR method of RAxML).

Potential transmission clusters were identified by using Cluster Picker 1.2 [19] with a maximum genetic distance threshold 0.045 nucleotide substitutions per site within the cluster and bootstrap support ≥98%. Clades consisting of at least 3 Hungarian sequences were assigned as transmission clusters. To examine the effect of the genetic distance threshold on the number and size of clusters, two more stringent thresholds, namely 0.03 and 0.02 nucleotide substitutions per site were also investigated.

The 95% confidence interval of the prevalence of TDR was calculated using Wilson score interval [20]. Fisher's exact test was used to compare frequencies between groups; temporal trends and multivariate analyses of the prevalence of HIV-1 subtypes and TDR were tested by logistic regression. All statistical tests were performed with R version 3.5.0 [17]. Statistical significance was defined at p value ≤0.05.

3. Results

3.1. Characteristics of study population

A total of 168 therapy-naïve patients diagnosed as HIV-1 positive between 2013 and 2017 were analysed. The majority of the patients were men who have

sex with men (MSM; 157/168; 93.5%), while heterosexual transmission was recorded in 11 (11/168; 6.5%) cases. The majority of the patients were male (161/168; 95.8%). The median age of the study group was 36 years (IQR: 29-43 years). At the time of resistance testing the median viral load was 63 500 copies/ml (IQR: 14 000-265 000 cps/ml) and the median $CD4^+$ T cell count was 381 cells/µl (IQR: 220-564 cells/µl). The characteristics of the study population are summarized in Table 1.

3.2. HIV-1 subtyping

According to subtyping analysis of the RT and PR coding regions the majority of patients carried subtype B HIV-1 strains (147/168; 87.5%), while subtype F (7/168; 4.2%), A (6/168; 3.6%), CRF01_AE (3/168; 1.8%), CRF02_AG (3/168; 1.8%), D (1/168; 0.6%) and G (1/168; 0.6%) was also detected (Figure 1/A). The occurrence of non-B subtypes was significantly associated with female gender (p=0.043), and with young adults (20-29 years of age, age group II) versus all other adult age groups (age groups III-V; p=0.019; Table 1). The most prevalent non-B subtypes among young adults (20-29 years, age group II) were A (4/49; 8.2%), F (4/49; 8.2%), CRF02_AG (2/49; 4.1%) and G (1/49; 2.0%). The prevalence of HIV-1 subtypes showed no significant association with the route of transmission or $CD4^+$ T cell number at diagnosis (Table 1).

The annual prevalence of non-B subtypes increased significantly among treatment-naïve individuals in the studied period (p=0.008; Figure 1/A). This association remained significant when further co-factors (age or age group; gender) were considered in multivariate tests, or when the test was restricted to the male majority group.

3.3. Prevalence and patterns of transmitted drug resistance

The overall prevalence of transmitted drug resistance was 10.7% (18/168; 95% CI: 6.9-16.3%) in the 168 patients studied. The most frequently observed indicators of TDR were NRTI mutations (16/168; 9.5%; 95% CI: 5.9-14.9%), followed by NNRTI (2/168; 1.2%; 95% CI: 0.3-4.2%) and PI mutations (1/168, 0.6%; 95% CI: 0.1-3.3%; Table 2). The majority of TDRMs were detected in samples carrying subtype B HIV strains (17/18, 94.4%; Table 1), whereas a M230L NNRTI-associated resistance mutation was identified in a CRF02_AG strain. Tests of independence revealed no association between the presence of primary resistance mutations and any of gender, age group, route of transmission or $CD4^+$ T cell count (Table 1). Logistic regression analyses on the year of diagnosis found no temporal trend in the frequency of drug resistance in newly diagnosed infections for either the most frequent NRTI class, or when the occurrence of any drug resistance associated mutation was considered (p=0.73 and 0.70, respectively; Figure 1/B).

The most frequent NRTI mutation was T215E, which occurred in combination with either M41L (11 cases) or L210W (1 case), respectively (Table 2). In 3 sequences T215E occurred alone and the NRTI mutation F77L was also detected in 1 sequence. One patient carried the M230L NNRTI surveillance drug resistance mutation (SDRM). Concomitance of K101E NNRTI SDRM and E138A polymorphic accessory mutation was identified in another patient.

We noticed that one patient carried, in addition to the NRTI resistance mutations M41L and T215E, the PI major mutation M46L, too (Table 2). As far as we know, this is the first observation of a PI major mutation in an ART-naïve patient in Hungary. Triple class resistance was not observed.

3.4. Phylogenetic analysis

To maximize the scope of the comparison, in the phylogenetic analyses we included 30 partial *pol* sequences reported in our earlier study [8], in addition to the 168 sequences reported in this study. Phylogenetic and cluster analysis of the partial *pol* sequences revealed 21 possible transmission clusters containing at least 3 Hungarian sequences with a strong phylogenetic support (bootstrap value ≥98%, maximum genetic distance less than 0.045 nucleotide substitutions per site) (Figure 2), among which 10 clusters included only Hungarian sequences. The size of clusters ranged between 3 and 17. Among the 168 newly diagnosed patients from our present study and 30 further patients from our earlier study, 115 (58.1%) were identified as part of transmission clusters with this definition. The

majority of clustered Hungarian patients were male (99.1%), belonged to the MSM population (95.7%) and carried subtype B HIV-1 strains (94.8%). 56.5% (13/23) of Hungarian samples carrying TDRMs from the present and our earlier study were part of any (in total three) transmission clusters. Cluster II contains 7 Hungarian sequences carrying M41L, T215E NRTI associated resistance mutations (Figure 2/A). Cluster III is composed of two Hungarian sequences carrying M41L, T215E mutations and an additional Hungarian sequence carrying T215E mutation only. The third cluster (Cluster I) carrying only resistant Hungarian sequences consists of two sequences with T215E, and an additional sequence with L210W and T215E NRTI associated resistance mutations. Clade A, a monophyletic group, was formed by all of the Hungarian samples carrying NRTI-associated resistance mutations M41L and T215E derived from our present (11 samples) and earlier (3 samples) study and an additional sample carrying only T215E mutation [8] (Figure 2/A and B). Although bootstrap support was 100% and mean genetic distance was 0.024 within Clade A, the maximum genetic distance was higher than 0.045 substitutions per site therefore this group was not identified as a cluster.

Among Hungarian samples with non-B subtypes, two clusters were identified, three samples with subtype A (Cluster V) and three samples with subtype F (Cluster IV) were classified together (Figure 2/A).

In a more sensitive analysis, stricter criteria on the genetic distance were also examined. Cluster analysis with ≥98% bootstrap support and thresholds of

0.03 or 0.02 nucleotide substitutions per site decreased the proportion of clustered Hungarian samples to 41.1% and 29.3%, respectively. The fraction of resistant Hungarian samples in clusters decreased to 21.7% applying either of stricter thresholds.

4. Discussion

Analysis of RT and PR coding regions of 168 newly diagnosed, therapy-naïve HIV-1 positive patients showed that similar to our earlier results, subtype B is still the predominant HIV-1 subtype in Hungary [8,21], although the prevalence and heterogeneity of non-B subtypes and circulating recombinant forms has increased in recent years (Figure 1/A), especially in young adults (Table 1). This observation may reflect the increasing diversity of HIV-1 strains in Europe and in the neighbouring countries [22-29]. Although subtype B strains still predominate in the border countries Slovenia, Austria, Slovakia, Croatia, and Serbia, subtype F and A strains are the most prevalent in Romania and Ukraine, respectively [23-29]. We emphasize, however, that non-B subtypes have not made a significant contribution to TDR in Hungary, because the majority of TDRMs were detected in samples carrying subtype B HIV strains (17/18, 94.4%; Table 1). We also note that in the present study we had no information on the ethnicity or country of origin of the infected patients in the study population, and we therefore cannot reliably deduce the contribution of imported cases to the increasing prevalence of non-B subtypes.

In our current study the overall prevalence of TDR was 10.7% (Table 1), whereas we previously recorded a prevalence of 16.6% among HIV-infected patients diagnosed between 2008 and 2010 [8]. The lower number of cases examined and the different time period may explain the higher TDR prevalence in our earlier study.

In Europe, TDR was fairly stable between 2002 and 2010, and an 8.3% overall prevalence was reported in the SPREAD program between 2008 and 2010 by Hofstra et al. [30]. TDRMs associated with NRTIs were recorded most frequently (4.5%). In the present study we observed a higher prevalence of drug resistant HIV-1 strains carrying NRTI-associated resistance mutations (9.5%). Of note, Hofstra et al. reported a higher prevalence of NRTI-associated resistance mutations in the MSM population than among heterosexuals (5% vs. 3.7%) and a significantly higher prevalence in subtype B HIV-1 strains compared to non-B subtypes (5.6% vs. 2.1%) [30]. Moreover, Frentz et al. reported 11.1% TDRM prevalence in a MSM population compared to 6.6% prevalence in heterosexuals diagnosed between 2002 and 2007 [31]. These observations fit well to our data because in Hungary the main route of HIV infection is homo/bisexual contact and the predominant HIV-1 subtype is subtype B.

In Hungary, similar to data from other European countries, the most frequently observed TDRM was M41L, accompanied with T215E (a T215Y or T215F revertant mutation). This selection of thymidine analogue mutations may have been associated with extensive exposure to the NRTIs zidovudine and stavudine in the pre- and early cART era.

In neighbouring countries, studies on the prevalence of HIV TDRMs have yielded variable results. In Romania, a declining trend of TDRMs was observed among patients infected predominantly with HIV subtype F1, from 26.08% in 1997-2004 to 7.89% in 2005-2011 [28]. In Croatia, between 2006 and 2008,

Grgic et al. detected surveillance drug resistance mutations in 26 of 118 patients (22%) with a pronounced association with NRTIs (19.5%) [26]. The lowest prevalence of TDR in the neighbouring countries was observed in Slovenia: in a six year period (2011-2016) only four out of 168 (2.4%) patients carried viruses with TDRM, followed by 8.0% in Austria from 2003 to 2017 and 8.8% in Serbia between 2002 and 2011 [23,24,27].

Our phylogenetic analysis identified possible transmission clusters with relatively small maximum genetic distance and high support. In addition, our analysis revealed a monophyletic group (designated Clade A; Figure 2/A and B) formed by all of the M41L, T215E carrying strains detected between 2010 and 2017 in our previous and current study, and an additional strain carrying only T215E mutation [8]. Classification of these sequences in a single clade suggests an early single source introduction and ongoing forward spread of this resistant strain in Hungary (Figure 2/A and B). We speculate that the long time interval of onward transmission, from 2010 to 2017, allowed a longer evolutional history of the virus, resulting in a higher maximum genetic distance within this group, while the apparently successful transmission of this strain might indicate either a relatively high fitness (due to potential compensatory mutations), or spreading in a high risk population where environmental factors facilitate HIV transmission. Clade A, together with Cluster I, significantly contributed to the comparatively high overall prevalence of TDR recorded among the untreated, HIV-1-infected individuals of the current study.

The longer time interval (2008-2017) of the studied Hungarian samples in the phylogenetic analysis allowed the use of permissive criteria for transmission clusters. Since the definition of a cluster is not clearly determined in the scientific literature, we applied less stringent as well as stringent genetic distance thresholds**,** similarly to the work of Pineda-Peña et al. [32]). While the number of clustered Hungarian sequences with or without TDRMs decreased with more stringent genetic distance thresholds of 0.03 or 0.02, the classification of the majority of samples carrying TDRMs into a single monophyletic group suggests the forward spread of these resistant HIV-1 strains in Hungary.

The detected clusters of non-B subtypes may indicate that the introduction of subtype A strains (cluster V, Figure 2A) and subtype F strains (cluster IV, Figure 2A) to the Hungarian population was possibly followed by forward spread within the young age group (age group II, Table I), resulting in a higher prevalence of non-B subtypes among young patients.

This study draws attention to the fact that every tenth newly diagnosed, HIV-1-positive patient is expected to carry a partially drug resistant HIV-1 strain in Hungary. Pre-treatment testing of HIV drug resistance contributes to the effective, personalized therapy of patients. With a well-chosen first-line antiretroviral therapy viral suppression can be achieved reliably, reducing the transmission of HIV at the population level, and aiding the completion of the second and third 90 future targets of Joint United Nations' 90-90-90 action plan,

namely 90% of HIV-positive people on antiretroviral therapy and 90% of viral suppression among treated people by 2020 [33].

In conclusion, our data showed that in recent years the overall prevalence of TDR was 10.7% among newly diagnosed, therapy-naïve HIV-1-infected individuals in Hungary. This value is somewhat higher than the corresponding HIV-1 TDR prevalence in most European countries, possibly due to the forward spread of one efficiently transmitted drug resistant strain in at least the last 7 years in this country. The changing patterns of HIV-1 TDR in Hungary, its neighbours, and other countries indicate the necessity of continuous monitoring of TDRMs in newly infected individuals. The appearance of HIV-1 subtypes, circulating recombinant forms and drug resistance mutations not detected in previous studies in Hungary indicates the diversification of the epidemic, and also underlines the importance of a continuous molecular surveillance.

Sequence Data

The nucleotide sequences reported in this study have been submitted to GenBank Nucleotide Sequence Database under accession numbers **KX999940-KX999999**, **KY967234-KY967255**, **MK236491-MK236537** (RT sequences); **KY021932-KY021991**, **KY950415-KY950436**, **MK213272-MK213318** (PR sequences) and **MK250657-MK250695** (PR+RT sequences).

Declarations

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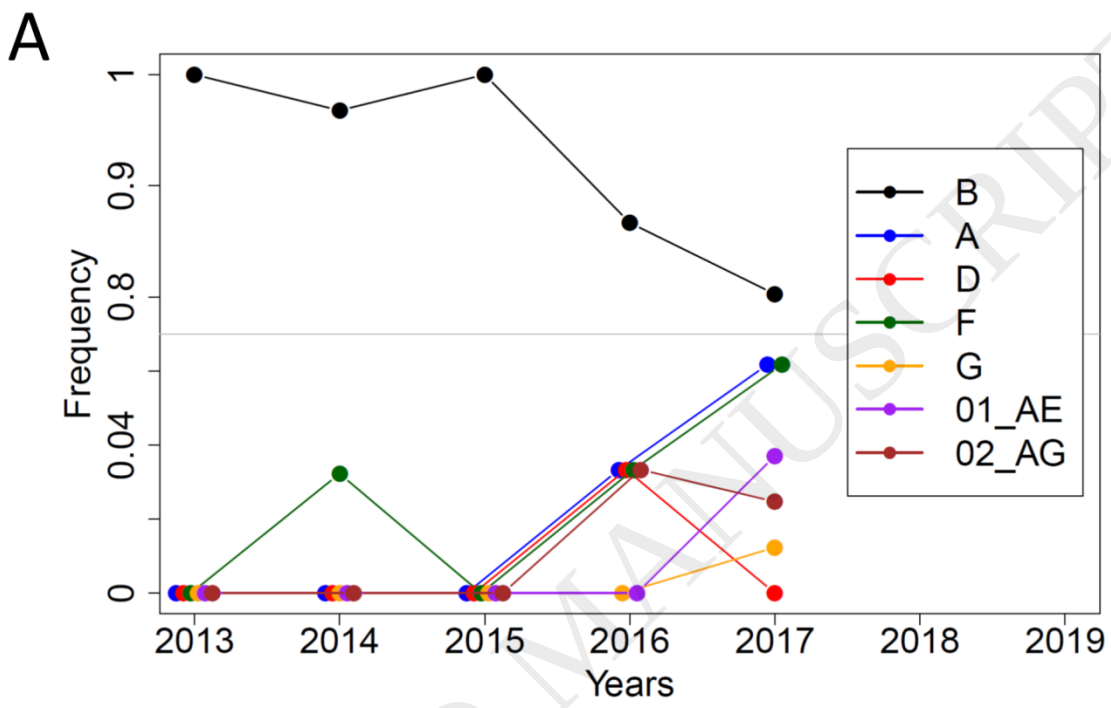
Figure Legends

Figure 1. Temporal trends of HIV-1 subtypes (A) and TDR (B) detected in the studied Hungarian samples.



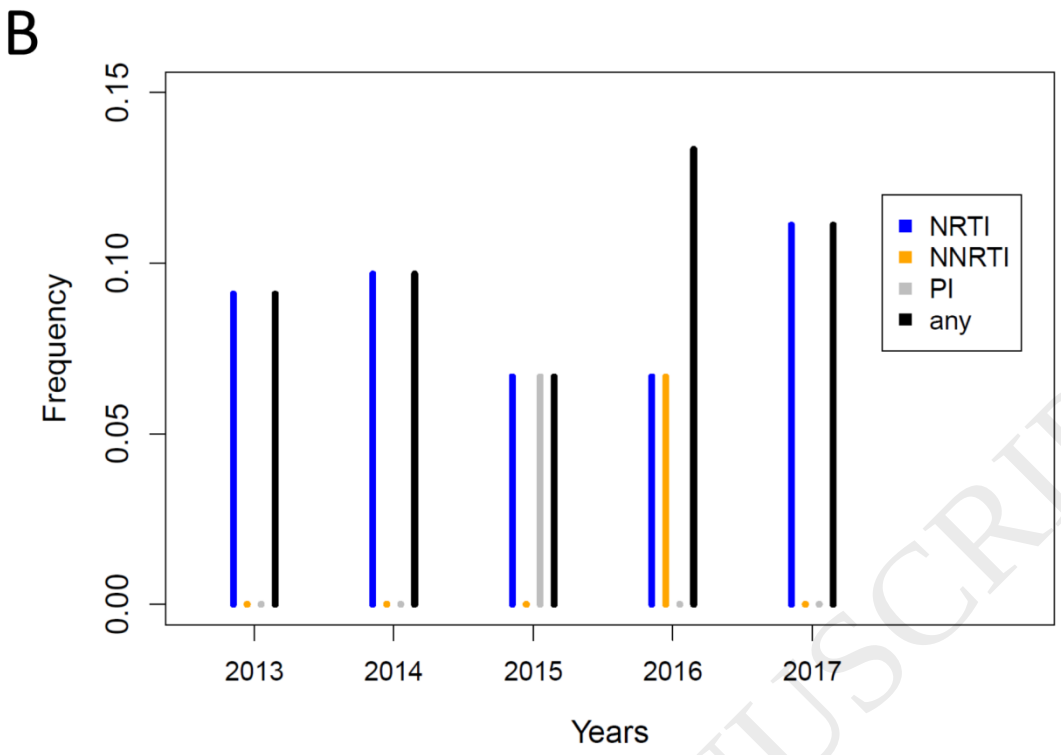
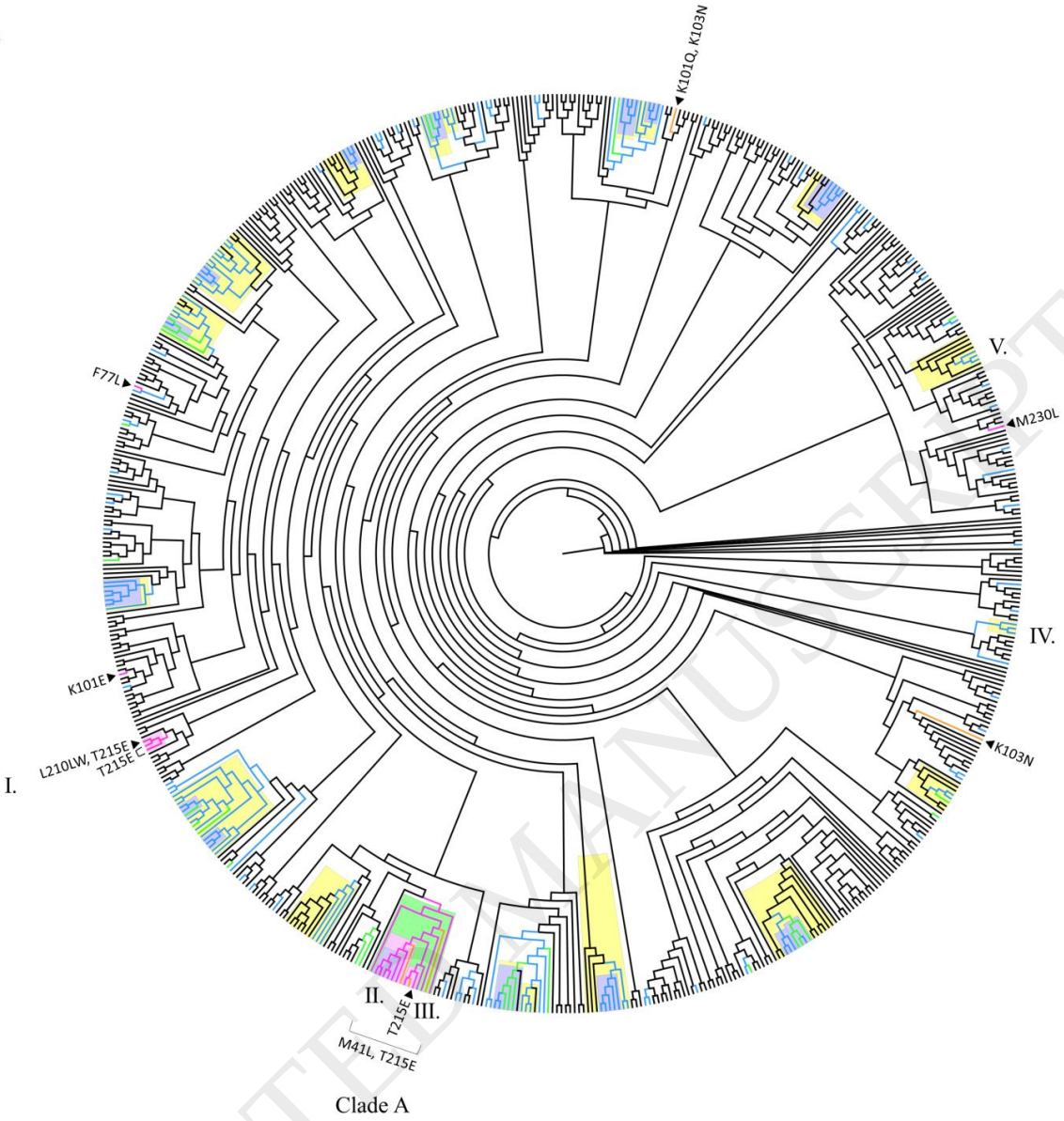


Figure 2. Phylogenetic relations and transmission clusters based on partial *pol* regions of HIV-1 strains from Hungary. (A) A Maximum likelihood phylogenetic tree is shown, composed of 168 HIV-1 sequences derived from our present study, 30 sequences from our earlier study, and corresponding background sequences. Colour code: purple lines indicate Hungarian sequences from the present study carrying transmitted drug resistance-associated mutations (TDRMs); orange lines indicate Hungarian sequences carrying TDRMs from our earlier study (2008 to 2010, [8]); blue lines indicate Hungarian sequences from the present study without drug resistance-associated mutations; green lines indicate Hungarian sequences without drug resistance-associated mutations from our earlier study (2008 to 2010, [8]); black lines indicate background sequences. Yellow-highlighted squares designate possible transmission clusters. Pink-

highlighted squares designate possible transmission clusters of Hungarian sequences carrying TDRMs. Potential transmission clusters were identified with bootstrap support ≥98% and maximum genetic distance less than 0.045 nucleotide substitutions per site. A green-highlighted square designates Clade A, composed of all Hungarian sequences carrying M41L and T215E TDRMs, and a single sequence carrying T215E only. To show the sensitivity of the identification of clusters to the choice of the genetic distance threshold, potential transmission clusters with bootstrap support ≥98% and maximum genetic distance less than 0.02 nucleotide substitutions per site are highlighted with blue squares. Identified TDRMs are indicated next to the corresponding tips of the cladogram. **(B)** An enlarged picture of the subtree corresponding to Clade A is shown. Clade A contains Hungarian sequences from the present study (2013-2017, 12 samples) and our earlier study (2010, 3 samples, [8]). All of the 14 sequences carrying both M41L and T215E TDRMs belong to Clade A, together with a single sequence carrying T215E only. Symbols indicate the year of diagnosis of HIV-1 infection.

A



B

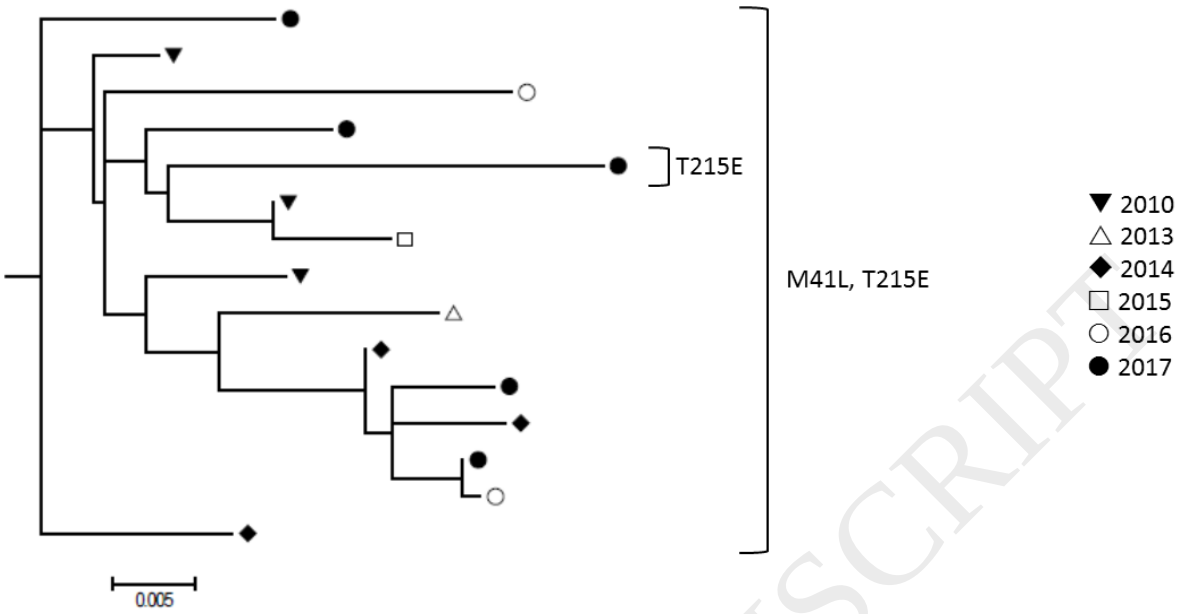


Table 1. Characteristics of the study population

	Total population		**Subtype B**		**Non-B subtypes§**			**Patients with primary resistance**		**Patients without primary resistance**		
	n	%	n	%	n	%	*p*-value	n	%	n	%	*p*-value
Samples	168		147	87.5%	21	12.5 %		18	10.7 %	150	89.3 %	
Sex												
Male	161	95.8 %	**143**	97.3%	**18**	85.7 %	**0.043***	16	88.9 %	145	96.7 %	0.165
Female	7	4.2 %	**4**	2.7%	**3**	14.3 %		2	11.1 %	5	3.3%	
Age group (years)												
I. <20	0		0		0			0		0		
II. 20-29	49	29.2 %	**38**	25.9%	**11**	52.4 %	**0.019***	6	33.3 %	43	28.7 %	0.784
III. 30-39	60	35.7 %	55	37.4%	5	23.8 %	0.330	6	33.3 %	54	36.0 %	1.000
IV. 40-49	39	23.2 %	37	25.2%	2	9.5%	0.166	4	22.2 %	35	23.3 %	1.000

V. >50	20	11.9 %	17	11.6%	3	14.3 %	0.720	2	11.1 %	18	12.0 %	1.000
Route of transmission												
MSM	157	93.5 %	139	94.6%	18	85.7 %	0.143	15	83.3 %	142	94.7 %	0.099
HET	11	6.5 %	8	5.4%	3	14.3 %		3	16.7 %	8	5.3%	
CD4⁺ T cell count (cells/mm³)												
<200	40	23.8 %	34	23.1%	6	28.6 %	0.589	5	27.8 %	35	23.3 %	0.770
≥200	128	76.2 %	113	76.9%	15	71.4 %		13	72.2 %	115	76.7 %	

MSM: men who have sex with men; HET: heterosexual contact

[§]Non-B subtypes detected in this study: F (7/168; 4.2%), A (6/168; 3.6%), CRF01_AE (3/168; 1.8%), CRF02_AG (3/168; 1.8%), D (1/168; 0.6%) and G (1/168; 0.6%).

*Designates statistically significant difference between groups (Fisher's exact test was used to compare frequencies between groups; statistical significance was defined at two-tailed *p* value <0.05). Single age groups were compared with the cumulative data of other age groups.

Table 2. Frequency and resistance profile of transmitted drug resistance mutations (TDRMs) detected in ART-naïve HIV-1-infected individuals in Hungary

NRTI			Resistance profile			
Mutation	Number (n, %)		Potential low	Low	Intermediate	High
M41L, T215E	10 (6.0)			ABC, DDI, TDF	AZT, D4T	
T215E	3 (1.8)		DDI	AZT, D4T		
L210W, T215E	1 (0.6)			ABC, DDI, TDF	AZT, D4T	
F77L	1 (0.6)		AZT, D4T, DDI			
NNRTI						
Mutation	Number (n, %)					
M230L	1 (0.6)				EFV, ETR	DOR, NVP, RPV
K101E, E138A	1 (0.6)			DOR, EFV, ETR	NVP	RPV
Dual class (NRTI+PI)						
Mutation	Number (n, %)					
M41L, T215E, M46L	1 (0.6)		ATV, FPV, IDV, LPV, SQV, TPV	ABC, DDI, TDF, NFV	AZT, D4T	

Bold: transmitted drug resistance mutations (TDRMs)

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; ABC, abacavir; DDI, didanosine; TDF, tenofovir; AZT, zidovudine; D4T, stavudine; EFV, efavirenz; ETR, etravirine; DOR, doravirine; NVP, nevirapine; RPV, rilpivirine; ATV, atazanavir; FPV, fosamprenavir; IDV, indinavir; LPV, lopinavir; SQV, saquinavir; TPV, tipranavir; NFV, nelfinavir.

The presence and the clinical relevance of TDRMs were assessed by the Stanford HIV Drug Resistance Database algorithm

Version 8.7.