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Short Communication: Molecular Epidemiology of HIV Type 1 Infection in Northern Greece (2009–2010): Evidence of a Transmission Cluster of HIV Type 1 Subtype A1 Drug-Resistant Strains Among Men Who Have Sex with Men

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Abstract

A prospective molecular epidemiology study of HIV-1 infection was conducted in newly diagnosed and antiretroviral-naive patients in Northern Greece between 2009 and 2010 using a predefined enrolling strategy. Phylogenetic trees of the *pol* sequences obtained in this study with reference sequences indicated that subtypes B and A1 were the most common subtypes present and accounted for 44.9% and 42.9%, respectively, followed by subtype C (3.1%), CRF02_AG (4.1%), CRF04_cpx (2.0%), and subtypes CRF01_01, F1, and G (1.0%). A high rate of clustered transmission of subtype A1-resistant strains to reverse transcriptase (RT) inhibitors was observed among men having sex with men. Indeed, 15 out of 17 study subjects (88.2%) infected with transmitted drug resistance (TDR) strains were implicated in transmission clusters, 10 of whom (66.7%) were men who have sex with men (MSM), and were also infected with subsubtype A1 strains. The main cluster within subtype A1 (I) included eight men reporting having sex with men from Thessaloniki infected with dual-class RT-resistant strains carrying both T215C and Y181C mutations.

The human immunodeficiency virus type 1 (HIV-1) genome exhibits high genetic heterogeneity, leading to three genetic groups (M, N, and O) and numerous closely related subtypes. HIV-1 group M is further classified in a proposed consensus of nine distinct subtypes (A–D, F–H, J, O, and P) and an increasing number of intersubtype circulating recombinant forms (CRFs).[1,2] In a recent molecular epidemiology study of HIV-1 infection in Europe, the most prevalent subtypes/CRFs were subtype B (66.1%), followed by subsubtype A1 (6.9%), subtype C (6.8%), and CRF02_AG (4.7%) with substantial differences in subtype distribution among European countries, immigrant populations, and patient risk groups.[3]

In this study, we analyzed *pol* sequences of HIV-1 isolates from a prospective molecular epidemiology study, in which we recruited individuals newly diagnosed with HIV-1 infection from 2009 to 2010 in Northern Greece in an effort to characterize the epidemiological and genetic diversity of HIV-1 infection in the region, to determine the prevalence of transmitted drug resistance (TDR), and to gain further insight in the potential risk factors of TDR in relation to the reported risk behavior. The predefined enrollment strategy was for the most part based on the European prospective program (SPREAD) guidelines. Adult, newly diagnosed HIV-1-infected study subjects who had never been exposed to antiretroviral drugs were prospectively recruited. The blood sample was obtained within 3 months of HIV-1 seropositive diagnosis and isolated plasma was used for genotypic resistance analysis. Epidemiological, demographic, and clinical data were collected from each study subject using a standardized questionnaire.

The study subjects were consenting newly diagnosed HIV-1-seropositive patients attending the Division of Infectious Diseases of the AHEPA University Hospital in Thessaloniki, Greece between 2009 and 2010 (Table 1). A table summarizing

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Table 1. Clinical and Epidemiological Information for Study Patients

Patient[a]	*Sex*[b]	*Age (years)*	*Collection date*[c]	*Weeks of infection*[d]	*Country of origin*[e]	*Transmission risk group*[f]	*CD4 (cells/mm^3)*	*Plasma HIV-1 RNA (copies×10^3/ml)*	*Epidemiological information*[f]
GR013	M	29	June, 2009	1	Greece	MSM	844	40.5	Diagnosed with lymphadenopathy
GR015	M	30	June, 2009	4	Greece	MSM	583	120.2	Diagnosed with lymphadenopathy
GR020	M	43	June, 2009	1	Greece	MSM	305	1,340.7	ASM
GR024	M	38	July, 2009	12	Greece	MSM	300	100.1	Diagnosed with lymphadenopathy
GR027	M	22	Aug., 2009	1	Greece	MSM	625	90.5	Diagnosed with Infectious Mononucleosis
GR028	M	27	Aug., 2009	1	Greece	Unknown	946	160.1	ASM
GR029	M	48	Aug., 2009	12	Albania	MSM	160	90.7	ASM
GR031	M	24	Sept., 2009	8	Greece	MSM	265	30.1	ASM
GR033	M	29	Sept., 2009	12	Greece	MSM	378	160.2	ASM
GR035	M	35	Sept., 2009	1	Greece	HSX	805	10.6	ASM
GR036	M	53	Sept., 2009	1	Greece	MSM	134	30.6	ASM
GR041	M	35	Oct., 2009	1	Cameroon	HSX	317	150.0	Diagnosed with lymphadenopathy and VZV
GR043	F	20	Oct., 2009	2	Romania	HSX	241	660.0	Diagnosed with lymphadenopathy
GR044	M	37	Oct., 2009	1	Greece	MSM	656	3.0	ASM
GR046	M	44	Oct., 2009	1	Greece	MSM	234	110.8	Diagnosed with VZV
GR048	M	24	Nov., 2009	2	Greece	MSM/IDU	413	10.8	Diagnosed with lymphadenopathy
GR050	F	52	Nov., 2009	1	Greece	HSX	1,568	20.2	ASM
GR051	M	28	Nov., 2009	1	Greece	MSM	529	230.1	Diagnosed with cervical lymphadenopathy
GR052	F	35	Nov., 2009	2	Nigeria	HSX	593	60.8	ASM
GR054	M	68	Nov., 2009	1	Greece	MSM	817	10.8	ASM
GR055	M	23	Nov., 2009	1	Greece	MSM	405	290.7	ASM
GR056	M	31	Nov., 2009	2	Greece	MSM	436	20.6	ASM
GR057	M	28	Nov., 2009	2	Greece	MSM	684	40.0	Diagnosed with lymphadenopathy, anal warts, and thrombocytopenia
GR058	F	27	Nov., 2009	2	Greece	HSX	309	5,280.5	Diagnosed with lymphadenopathy and high fever
GR060	M	56	Dec., 2009	2	Greece	Unknown	324	10.9	ASM
GR061	M	28	Dec., 2009	2	Greece	MSM	1,086	2.0	ASM
GR062	F	38	Dec., 2009	2	South Africa	HSX	38	160.6	Heterosexual partner of GR063
GR063	M	40	Dec., 2009	2	Greece	HSX	495	3.0	Heterosexual partner of GR062
GR064	M	28	Dec., 2009	2	Greece	IDU	675	30.7	Diagnosed with lymphadenopathy
GR065	M	32	Dec., 2009	12	Greece	MSM	1002	2.0	N/A
GR066	M	44	Jan., 2010	1	Greece	MSM	691	110.4	Flu-like symptoms with high fever
GR068	M	38	Jan., 2010	1	Greece	MSM	396	8.0	ASM
GR070	M	32	Jan., 2010	2	Greece	HSX	732	10.2	Married to seropositive individual
GR073	M	32	Feb., 2010	2	Greece	MSM	584	5.0	Diagnosed with lymphadenopathy and syphilis
GR074	M	32	Feb., 2010	1	Greece	MSM	700	40.5	Diagnosed with lymphadenopathy

(*continued*)

TABLE 1. (CONTINUED)

Patient[a]	*Sex*[b]	*Age (years)*	*Collection date*[c]	*Weeks of infection*[d]	*Country of origin*[e]	*Transmission risk group*[f]	*CD4 (cells/mm^3)*	*Plasma HIV-1 RNA (copies × 10^3/ml)*	*Epidemiological information*[f]
GR075	M	38	Feb., 2010	1	Greece	MSM	633	10.3	Diagnosed with lymphadenopathy
GR076	M	22	Feb., 2010	3	Greece	MSM	741	90.5	Diagnosed with high fever and diarrhoic syndrome
GR079	M	30	Feb., 2010	1	Greece	MSM	376	200.5	Diagnosed with high fever
GR080	M	23	Feb., 2010	1	Greece	MSM	737	4.0	Diagnosed with lymphadenopathy and VZV
GR083	M	32	Mar., 2010	3	Greece	MSM	228	100.0	Diagnosed with high fever
GR086	M	33	Mar., 2010	3	Greece	MSM	496	10.9	Diagnosed with lymphadenopathy
GR087	M	22	Mar., 2010	2	Greece	MSM	481	20.8	ASM
GR088	M	47	Apr., 2010	3	Greece	MSM	777	9.0	Diagnosed with lymphadenopathy and ocular HSV
GR090	M	33	Apr., 2010	3	Greece	MSM	564	500.7	Partner of GR114; diagnosed with syphilis
GR091	F	35	Apr., 2010	2	Ukraine	IDU	288	380.0	HCV positive
GR092	M	36	Apr., 2010	3	Greece	MSM	477	410.5	HSV-1
GR093	M	49	Apr., 2010	2	Greece	HSX	389	190.0	ASM
GR094	M	28	Apr., 2010	4	Greece	MSM	737	40.2	Diagnosed with lymphadenopathy
GR096	M	23	May, 2010	1	Greece	MSM	220	130.0	Possible Kaposi sarcoma; seropositive partner
GR097	M	30	May, 2010	2	Greece	MSM	543	420.8	Diagnosed with lymphadenopathy
GR098	M	30	May, 2010	3	Greece	MSM	438	100.2	Diagnosed with lymphadenopathy
GR099	M	37	May, 2010	3	Greece	MSM	260	330.4	Diagnosed with lymphadenopathy and HSV-1
GR100	M	36	May, 2010	1	Greece	MSM	297	290.0	Relapsing aphthous stomatitis
GR101	M	21	May, 2010	1	Russia	MSM	694	9.0	Diagnosed with lymphadenopathy and fever
GR102	M	21	June, 2010	2	Greece	MSM	613	610.0	ASM
GR104	M	34	June, 2010	4	Russia	IDU	250	210.0	ASM
GR105	M	35	June, 2010	2	Albania	MSM	350	330.0	Diagnosed with high fever
GR106	M	26	June, 2010	1	Greece	MSM	324	10.6	ASM
GR107	M	25	June, 2010	1	Greece	HSX	238	10.5	Diagnosed with lymphadenopathy, HSV-1, and syphilis
GR108	M	49	June, 2010	1	Greece	N/A	153	60.9	Diagnosed with neurosyphilis
GR109	M	39	June, 2010	2	Albania	N/A	320	6.0	Diagnosed with syphilis
GR110	M	40	June, 2010	1	Greece	MSM	460	68.0	Diagnosed with infectious mononucleosis
GR111	M	36	June, 2010	1	Greece	MSM	623	10.7	Diagnosed with lymphadenopathy with fever

(*continued*)

Table 1. (Continued)

Patient[a]	*Sex*[b]	*Age (years)*	*Collection date*[c]	*Weeks of infection*[d]	*Country of origin*[e]	*Transmission risk group*[f]	*CD4 (cells/mm^3)*	*Plasma HIV-1 RNA (copies × 10^3/ml)*	*Epidemiological information*[f]
GR112	M	24	June, 2010	1	Georgia	MSM	322	210.0	Diagnosed with lymphadenopathy
GR113	M	32	July, 2010	2	Greece	MSM	710	250.1	Diagnosed with syphilis
GR114	M	23	July, 2010	1	Greece	MSM	398	90.8	Partner of GR090, diagnosed with syphilis
GR115	M	27	Aug., 2010	3	Greece	MSM	496	17.0	ASM
GR116	M	51	Aug., 2010	1	Greece	MSM	264	240.0	ASM
GR117	M	48	Aug., 2010	3	Greece	HSX	154	230.0	ASM
GR118	M	38	Sept., 2010	3	Greece	MSM	51	6.0	ASM
GR119	F	41	Sept., 2010	3	Greece	HSX	290	120.4	Oral candidiasis
GR120	M	40	Sept., 2010	1	Greece	MSM	675	340.9	Flu-like symptoms that lasted for 2 months
GR121	M	31	Sept., 2010	2	Greece	MSM	428	10.1	Tonsilitis
GR122	M	25	Sept., 2010	1	Greece	MSM	372	250.5	Diagnosed with cervical lymphadenopathy and rash
GR123	M	25	Sept., 2010	2	Greece	MSM	720	90.6	ASM
GR124	M	57	Sept., 2010	1	Greece	MSM	452	10.9	ASM
GR125	M	26	Sept., 2010	2	Greece	MSM	526	390.3	ASM
GR126	M	33	Sept., 2010	1	Greece	HSX	184	30.2	Diagnosed with cervical lymphadenopathy, fever, and oral candidiasis
GR127	F	22	Sept., 2010	1	Nigeria	HSX	777	3.0	ASM
GR128	M	47	Sept., 2010	1	Greece	MSM	489	10.6	ASM
GR129	M	22	Oct., 2010	3	Greece	MSM	950	9.0	ASM
GR130	M	30	Oct., 2010	1	Russia	IDU	753	10.8	ASM
GR131	M	29	Oct., 2010	1	Greece	MSM	297	4.0	Frequent tonsilitis
GR132	F	51	Oct., 2010	1	Greece	Unknown	963	< 50	ASM
GR133	M	30	Oct,. 2010	1	Greece	MSM	205	110.3	ASM
GR134	M	23	Oct., 2010	2	Greece	MSM	442	50.5	ASM
GR135	M	24	Nov., 2010	3	Greece	MSM	611	210.3	Fever for over 2 weeks with relapsing aphthous stomatitis
GR136	M	26	Nov., 2010	4	Greece	MSM	899	2.0	High fever that lasted 5 days
GR137	M	24	Nov., 2010	1	Greece	MSM	512	10.0	ASM
GR138	M	23	Nov., 2010	1	Greece	MSM	333	70.3	Tonsilitis
GR139	M	33	Nov., 2010	1	Greece	MSM	340	30.4	Diagnosed with VZV
GR140	M	25	Nov., 2010	12	Greece	MSM	154	130.0	Decimal fever
GR141	M	37	Nov., 2010	2	Greece	MSM	746	40.4	ASM
GR142	M	28	Dec., 2010	2	Greece	MSM	379	60.3	ASM
GR143	F	31	Dec., 2010	8	Russia	HSX	382	70	ASM
GR144	M	36	Dec., 2010	2	Greece	MSM	305	1,090.0	ASM
GR145	M	41	Dec., 2010	1	Greece	MSM	656	2.0	ASM
GR146	M	50	Dec., 2010	2	Greece	MSM	496	120.0	ASM

[a]Indicates the laboratory code for each study subject.
[b]F, female; M, male.
[c]Indicates the date of the sample collection.
[d]Indicates the duration from the first known positive HIV antibody test.
[e]Country of birth of the study subjects.
[f]Information provided by the study subjects.

MSM, men who have sex with men; HSX, heterosexual contact; IDU, intravenous drug user, N/A, not available; ASM, asymptomatic; VZV, varicella zoster virus; HSV, herpes simplex virus; HCV, hepatitis C virus.

Table 2. Characteristics of Patients with Transmitted Drug Resistance Mutations

Patient[a]	Cluster	Sex[b]	Age (years)	Weeks of infection[c]	Country of origin[d]	Transmission risk group[e]	CD4 (cells/mm^3)	Plasma HIV-1 RNA (copies$\times 10^4$/ml)	Subtype	Surveillance drug resistance mutations[e]		
										NRTI	NNRTI	PI
GR013	I	M	29	1	Greece	MSM	844	4.5	A1	T215C	Y181C	—
GR015	I	M	30	4	Greece	MSM	583	12.2	A1	T215C	Y181C	—
GR061	I	M	28	2	Greece	MSM	1086	0.2	A1	T215C	Y181C	—
GR075	I	M	38	1	Greece	MSM	633	1.3	A1	T215C	Y181C	—
GR105	I	M	35	2	Albania	MSM	350	33.0	A1	T215C	Y181C	—
GR117	I	M	48	3	Greece	HSX	154	23.0	A1	T215C	Y181C	—
GR144	I	M	36	2	Greece	MSM	305	109.0	A1	T215C	Y181C	—
GR146	I	M	50	2	Greece	MSM	496	12.0	A1	T215C	Y181C	—
GR083	II	M	32	3	Greece	MSM	228	10.0	A1	T215S	K103N	—
GR087	II	M	22	2	Greece	MSM	481	2.8	A1	T215S	K103N	N88S
GR107	III	M	25	1	Greece	HSX	238	1.5	A1	—	K103N	—
GR136	III	M	26	4	Greece	MSM	899	0.2	A1	—	K103N	—
GR143	III	F	31	8	Russia	HSX	382	0.7	A1	—	K103N	—
GR062	IV	F	38	2	South Africa	HSX	38	16.6	C	—	Y181I	—
GR063	IV	M	40	2	Greece	HSX	495	0.3	C	D67N	Y181I	—
GR033	—	M	29	12	Greece	MSM	378	16.2	B	—	G190A	—
GR098		M	30	3	Greece	MSM	438	10.2	A1	T215C	Y181C	—

[a]Indicates the laboratory code for each study subject.
[b]F, female; M, male.
[c]Indicates the duration from the first known positive HIV antibody test.
[d]Country of birth of the study subjects. N/A, not available.
[e]Defined according to the published list of mutations for surveillance to transmitted drug resistance as recommended by the World Health Organization.[8]
MSM, men who have sex with men; HSX, heterosexual contact; IDU, intravenous drug user, N/A, not available; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

the detailed analyses of the characteristics of the study subjects is presented in a concurrent study utilizing the same study cohort.[4] The study was approved by the Bioethics Committee of the Medical School of the Aristotle University of Thessaloniki. The HIV-1 serostatus of each subject was previously established by commercial enzyme-linked immunoassay and confirmed by Western blotting and blood was drawn within 3 months of HIV-1 diagnosis. An informed consent form was signed by each subject, a questionnaire was filled in with an interviewer, and blood samples were taken by qualified personnel. All samples and questionnaires were coded with a laboratory identifier number so as to ensure patient anonymity. Ninety-eight individuals were included in this study, representing 63.7% of antiretroviral-naive newly diagnosed HIV-1-seropositive patients registered at the database of the AIDS National Reference Laboratory of Northern Greece for the period 2009–2010.

All blood samples were processed for population-based nucleotide sequencing of plasma HIV-1 RNA encoding regions of reverse transcriptase (RT) and protease (PR) genes at the National AIDS Reference Laboratory of Northern Greece of the Aristotle University of Thessaloniki by using commercially available kits.[5] The phylogenetic analyses were performed at the Laboratory of Biotechnology and Molecular Virology of the University of Cyprus according to previously published methodologies.[6,7] The GenBank accession numbers for the reference sequences used in the phylogenetic analyses of the *pol* regions are A1-DQ676872, A1-AB253429, A1-AF004885, A1-AB253421; A2-AF286238, A2-AF286237; B-K03455, B-AY331295, B-AY173951, B-AY423387; C-U52953, C-U46016, C-AY772699, C-AF067155; D-AY253311, D-U88824, D-K03454; F1-AF077336, F1-AF075703, F1-AF005494, F1-AJ249238; F2-AJ249236, F2-AJ249237, F2-AY371158, F2-AF377956; G-AF061641, G-AF084936, G-U88826, G-AY612637; H-AF190128, H-AF005496, H-AF190127; J-AF082395, J-AF082394, J-EF614151; K-AJ249235, K-AJ249239; 01AE-U54771, 01AE-AB220944; 02AG-L39106, 02AG-AY271690; 03AB-AF414006, 03AB-AF193276; 04cpx-AF119820, 04cpx-AF049337, 04cpx-AF119819. Transmitted drug resistance mutations (TDRM) were defined according to the mutation list published for surveillance of transmitted drug resistance as recommended by the World Health Organization.[8] Potential drug resistance transmission clusters were define as sequences sharing a most recent common ancestor with $>85\%$ bootstrap support and a mean genetic distance of <0.015 nucleotide substitutions per site.[9]

The study group consisted of 98 HIV-1 newly diagnosed individuals. Eighty-one subjects were Greek citizens living permanently in Northern Greece at the time of the study, although a number of them reported traveling or living abroad in the past, whereas 13 subjects were born in Albania (two subjects), Cameroon, Romania, Nigeria (two subjects), South Africa, Ukraine, Russia (four subjects), and Georgia. Eighty-eight study subjects (89.8%) were male and 10 (10.2%) were female with a median age of 34 years (IQR, 27–41). The most common reported risk factor of HIV-1 transmission was homosexual contact (74.5%), followed by heterosexual contact (15.3%), intravenous drug usage (5.1%), and of unknown reason (5.1%). Investigation for other sexually transmitted diseases showed that seven of the patients were found to be

positive for syphilis and one for HCV. Most of the patients were diagnosed during stage A of their infection according to CDC guidelines and at the time of diagnosis the median CD4 count and the plasma virus load were 468.5 cells/μl (IQR, 307–679.5) and 4.6 log copies/ml (IQR, 4.15–5.31), respectively. Analyses of the HIV-1 *pol* sequences indicated that subtypes B and A1 were the most common subtypes present and accounted for 44.9% and 42.9%, respectively, followed by subtype C (3.1%), CRF02_AG (4.1%), CRF04_cpx (2.0%), and subtypes CRF01_01, F1, and G (1.0%). The GenBank accession numbers obtained in this study for HIV-1 pol sequences are KF671758-KF671855 for protease sequences and KF671856-KF671953 for reverse transcriptase sequences.

A summary of the characteristics of patients with transmitted drug resistance mutations is shown in Table 2. The overall prevalence of transmitted drug resistance mutations (TDRM) to current HIV-1 antiretroviral drugs in the studied patients was 17.4%, of whom 29.4% were infected with viruses carrying a single TDRM. Dual-class and multiclass-resistant mutations were observed in 64.7% and 5.9% of the patients, respectively. The prevalence of nucleoside reverse-transcriptase inhibitor (NRTI) resistance was 12.24% (12 of 98 patients), the prevalence of nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance was 17.35% (17 of 98 patients), and the prevalence of protease inhibitor (PI) was 1.02% (1 of 98 patients). NRTI TDRM were found in 11 patients infected with HIV-1 subtype A1 (T215C, nine patients; T215S, two patients) and in one patient with subtype C (D67N). The highest prevalent mutation was the revertant mutations at position 215 (C/S, 91.7%) followed by D67N (8.3%). NNRTI

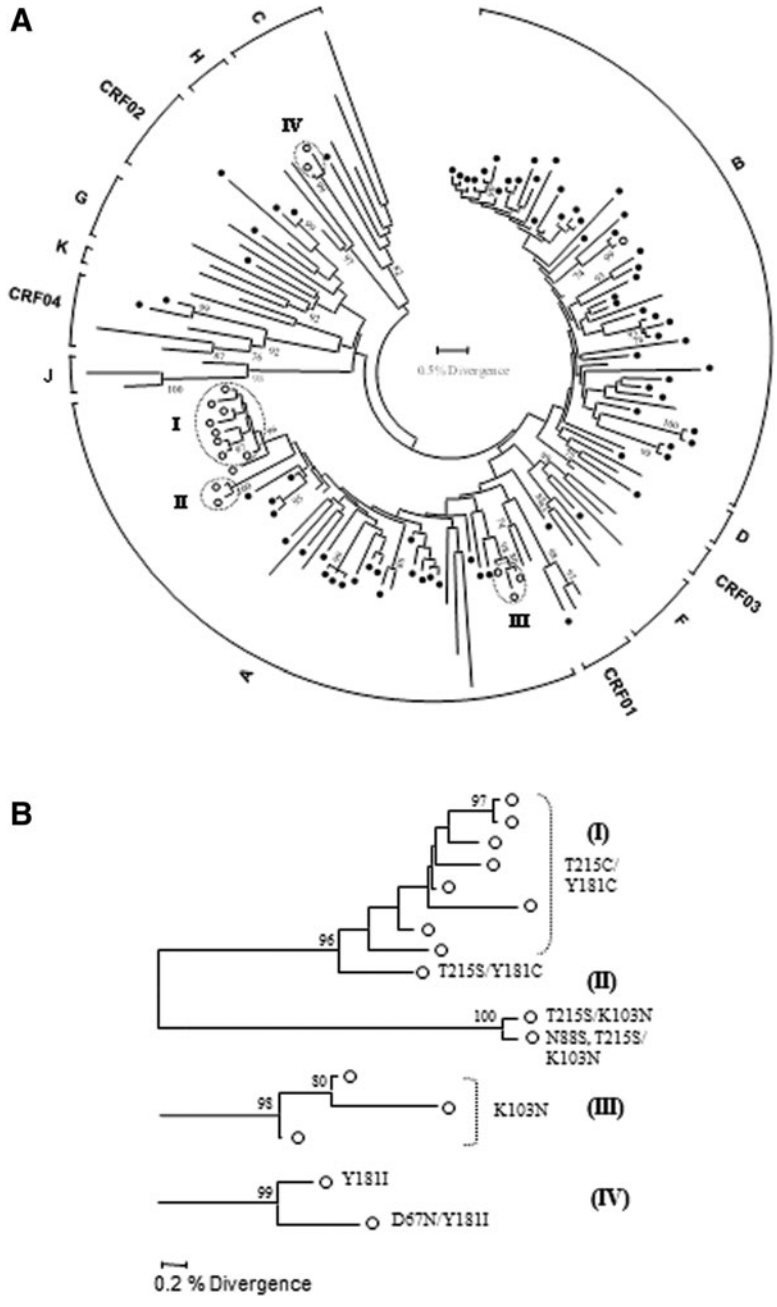

FIG. 1. **(A)** Neighbor-joining tree of *pol* (protease and partial reverse transcriptase) sequences, constructed as described in the text. The circular brackets on the periphery of the tree indicate the determined subtypes and circulating recombinant forms (CRF) as described in the text. The tips of reference sequences are shown with black lines and the patient sequences with circles; those with drug resistance mutations are indicated with open circles. Only consensus bootstrap values greater than 70% out of 1,000 replications are shown at several nodes. The scale at the middle of each tree is used to obtain the percent divergence between any two sequences. Clusters of sequences with transmitted drug resistance mutations (TDRM; indicated with open circles) that have a significant statistical support (>85% bootstrap support) for the branch subtending the cluster and a mean genetic distance of <0.015 nucleotide substitutions per site are indicated by dotted ovals. **(B)** The four observed clusters of drug-resistant strains are magnified for better viewing and the drug resistance mutations associated with each sequences are depicted at the edge of the branches.

TDRM were found in all 14 patients infected with HIV-1 subtype A1 (Y181C, nine patients; K103N, five patients), two patients with subtype C subtype (Y181I), and one patient with B subtype (G190A). PI TDRM was found in a single patient (GR087) infected with subtype A1 (N88S).

Phylogenetic analyses, presented in Fig. 1, revealed four transmission clusters highly supported by bootstrapping (>85%) and a mean genetic distance of <0.015 nucleotide substitutions per site. Three of the clusters included individuals infected with subtype A1 strains and one cluster with subtype C. The main cluster within subtype A1 (I) included eight men reporting having sex with men infected with dual-class RT-resistant strains carrying both T215C and Y181C mutations. The second subtype A1 cluster (II) involved two men who have sex with men (MSM) individuals, one with a dual-class RT-resistant strain carrying T215S and K103N and one with a triple-class-resistant strain carrying T215S, K103N, and N88S mutations. The third subtype A1 cluster (III) involved three individuals including a woman from Russia and two men from Greece, one of whom is MSM. The only subtype C cluster (IV) involved one heterosexual couple, a woman from South Africa and a man from Greece. Overall, 15 out of 17 study subjects (88.2%) infected with TDR strains were implicated in transmission clusters, 10 of whom (66.7%) were MSM and were also infected with subsubtype A1 strains.

In this study, the molecular epidemiology of HIV-1 infection and TDR in newly diagnosed patients in Northern Greece is presented. Ninety-eight newly diagnosed untreated patients, representing 63.7% of the antiretroviral-naive newly diagnosed HIV-1-seropositive patients at the database of the AIDS National Reference Laboratory of Northern Greece for the period 2009 to 2010, took part in this work, providing demographic and epidemiological characteristics as well as information on risk groups and drug use behavior. The subjects were predominantly young Greek men. The nationalities of the subjects who were not Greek demonstrate the influx of young people from Eastern Europe to the region and possibly its association with neighboring countries in the Balkans.[10]

From retrospective studies carried out in Northern Greece, the main subtypes found were subtype B and subtypes A.[5,11–13] Phylogenetic subtyping of these sequences obtained in the *pol* region of the HIV-1 genome confirmed the frequent presence of subtype B and A strains in this population, but subtypes C, CRF02_AG, CRF04_cpx, CRF01_01, F1, and G were also found in significantly decreased frequencies. Interestingly, 41.1% of MSM were infected with subtype A1 strains, showing that this subtype has a significant prevalence in MSM in Northern Greece, and possibly illustrating spill-over among similar risk groups in the geographic region and neighboring countries.[3,10] This would have to be confirmed by phylogenetic analysis with HIV-1 sequences from comparable risk groups from these countries and other regions in Greece.[10,14,15]

The results of the study showed that within the group tested, the prevalence of TDR to current HIV-1 antiretroviral drugs was 16.32%, which is significantly higher than known prevalence rates of TDR in newly diagnosed patients in other European countries,[16] but is comparable to previously published prevalence rates of TDR in Northern Greece.[13] The phylogenetic analysis of the sequences resulted in four clusters within TDR samples, with small genetic distances (<0.015) and high bootstrap values. Two of the four clusters included MSM infected with subtype A1 TDR strains. The main cluster included eight MSM individuals, seven Greeks and one immigrant from Albania, infected with dual-class RT-resistant strains, whereas the second subtype A1 cluster included two MSM individuals, one with a dual-class RT-resistant strain and one with a triple-class-resistant strain. Interestingly, 88.2% of the patients infected with TDR strains were implicated in transmission clusters, of whom 66.7% were MSM and were also infected with subsubtype A1 strains. Although further investigation would be necessary with a more extended sampling group to obtain a more complete picture of the molecular epidemiology of HIV-1 among MSM in Northern Greece, the results obtained in this prospective study could have an impact on the development of prevention strategies for TDR for the local setting.

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Author Disclosure Statement

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