Molecular analysis of HIV-1 subtype A1 and B dispersal patterns of persons with late presentation and advanced disease in Greece

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Introduction

Late presentation of human immunodeficiency virus (HIV) infection is a serious challenge for the management and prevention of HIV infection in Europe.

Too many people throughout the European Region are diagnosed late (51%), increasing the risk of ill health, death and onward HIV transmission. The proportion of those diagnosed late (CD4 count < 350 cells/μL) was 58% in Greece in 2016 (ECDC).

Aim

We aimed to investigate the patterns of HIV transmission among late presenters in Greece using molecular epidemiology, in order to identify risk factors and gaps that need to be addressed at a national level.

Materials and Methods

Study samples included HIV-1 sequences isolated from 6,268 people living with HIV (PLHIV) diagnosed between 1999 and 2015 in Greece. Sequences were available in the PR/RT. We analysed 1,777 (28.4%) and 2,589 (41.3%) sequences of the subtype A1 and B, respectively, which are the most prevalent subtypes in Greece (*Table 1*).

Table 1. *Characteristics of the study population*

		HIV-1 subtype	
		A1 (N=1,777)	B (N=2,589)
		Number (%)	Number (%)
Gender			
	Male	1,198 (67.4)	2,024 (78.2)
	Female	193 (10.9)	240 (9.3)
	Unknown	386 (21.7)	325 (12.5)
Nationality			
	Greek	699 (39.3)	1,442 (55.7)
	Non-Greek	126 (7.1)	129 (5.0)
	Unknown	952 (53.6)	1,018 (39.3)
Risk group			
	MSW	308 (17.4)	376 (14.6)
	MSM	898 (50.5)	1,587 (61.3)
	PWID	145 (8.2)	208 (8.0)
	Other	38 (2.1)	96 (3.7)
	Unknown	388 (21.8)	322 (12.4)
HIV presentation status			
	Non-Late presenter	467 (26.2)	634 (24.5)
	Advanced disease	232 (13.1)	404 (15.6)
	Late presenter	207 (11.7)	240 (9.3)
	Unknown	871 (49.0)	1,311 (50.6)
Period of sampling			
	[1999-2007)	334 (18.8)	959 (37.0)
	[2007-2015]	917 (51.6)	1,185 (45.8)
	Unknown	526 (29.6)	445 (17.2)
		Mean (SD)	**Mean (SD)**
Age (in years)		38.6 (12.6)	37.8 (10.5)

***MSM**: Men who have Sex with Men* ***MSW**: Men who have Sex with Women* ***PWID**: People Who Inject Drugs*

Phylogenetic analysis was performed on subtypes A1 and B sequences from Greece along with a randomly selected global dataset of sequences (subtype A: N=1,500; subtype B: N=2,000; http://hiv.lanl.gov), used as references. Phylogenetic trees were inferred by maximum likelihood method under the GTR model of nucleotide substitution including a gamma (Γ) distributed rates heterogeneity among sites as implemented in RAxML v8.2.10 program. Phylogenetic analysis was repeated in 5 replicates using a different set of randomly selected references.

Local transmission networks (LTNs) were considered as phylogenetic clusters including at least 2 sequences from the same geographic area (Greece) at a proportion higher than 70%. Only sequences belonged to clusters in the repeated replicates were considered as LTNs.

Multivariable logistic regression models were applied for the statistical analysis in Stata 12. Late presenters were defined as persons with initial CD4 count between 200 and 350 cells/μL; those with advanced disease had an initial CD4 count < 200 cells/μL or clinical AIDS regardless of CD4 count.

Results

Phylogenetic analyses revealed that:

- 93.8% (1,667 out of 1,777) of A1 sequences belonged to 38 LTNs, and specifically the largest one included 1,543 (86.8%) of the total subtype A1 sample (*Figure 1 A*).
- 77.1% (1,996 out of 2,589) of B sequences belonged to 166 LTNs (*Figure 1 B*).
- For subtype A1, the percentage of PLHIV within LTNs was 95.2% (N=197) for late presenters, 96.1% (N=223) for those with advanced disease and 95.5% (N=446) for non-late presenters.
- For subtype B, the corresponding figures were 85.8% (N=206) for late presenters, 71.8% (N=290) for those with advanced disease and 89.8% (N=569) for non-late presenters.

Multivariable logistic regression analysis showed that:

- Risk group (MSM vs heterosexuals; OR=6.07; p<0.001) and nationality (Greek vs non-Greek; OR=7.23; p<0.001) were associated with regional clustering of subtype A1 (*Table 2*).
- Year of sampling (later sampling year; OR=1.17 per year; p<0.001) was associated with regional clustering of subtype B (*Table 2*).
- Late presentation or advanced disease status was not associated with regional clustering of subtype A1 (*Table 2*).
- PLHIV with advanced disease had a lower probability (OR=0.48 vs non-late presenters; p<0.001) of belonging to regional clusters of subtype B (*Table 2*).

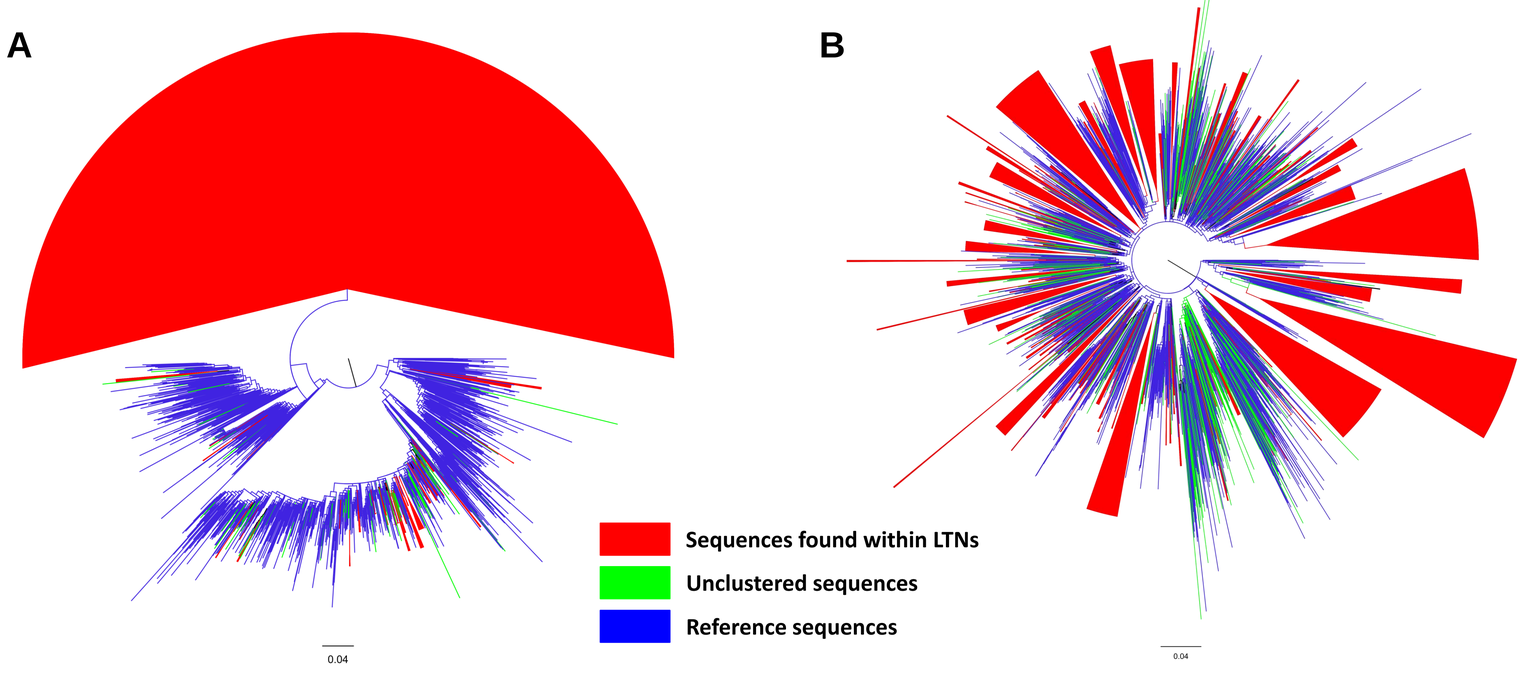

Figure 1. *Unrooted phylogenetic trees estimated by RAxML v8.2.10 of HIV-1 subtype **A.** A1 and **B.** B sequences from Greece and a global reference dataset. Sequences from Greece are marked in light green (unclustered sequences) and red (sequences found within local transmission networks-LTNs) in contrast with sequences from other geographic areas marked in blue. The LTNs are indicated as triangles.*

Table 2. *Multivariate logistic regression estimates using the presence in local transmission networks (LTNs) as the binary outcome variable*

	HIV-1 subtype					
	A1			B		
	Odds ratio	95% Conf. interval	p-value	Odds ratio	95% Conf. interval	p-value
Gender (†Male)						
Female	0.64	(0.35-1.19)	0.158	0.98	(0.63-1.50)	0.915
Unknown	-	-	-	0.42	(0.03-5.28)	0.499
Nationality (†Non-Greek)						
Greek	7.23	(3.67-14.27)	<0.001	1.46	(0.92-2.32)	0.106
Unknown	2.20	(1.09-4.43)	0.028	0.90	(0.54-1.50)	0.698
Risk group (†MSW)						
MSM	6.07	(2.59-14.2)	<0.001	1.26	(0.86-1.85)	0.240
PWID	0.95	(0.41-2.21)	0.909	1.06	(0.63-1.78)	0.837
Other	1.69	(0.36-7.94)	0.506	0.27	(0.15-0.48)	<0.001
Unknown	0.97	(0.39-2.44)	0.951	1.91	(0.81-4.50)	0.138
HIV presentation status (†Non-Late presenter)						
Advanced disease	1.50	(0.56-3.97)	0.419	0.48	(0.33-0.69)	<0.001
Late presenter	0.81	(0.34-1.96)	0.640	0.98	(0.60-1.60)	0.941
Unknown	0.75	(0.39-1.44)	0.384	0.44	(0.32-0.60)	<0.001
Year of sampling	1.01	(0.94-1.08)	0.865	1.17	(1.14-1.21)	<0.001
Age (in years)	0.99	(0.97-1.02)	0.728	0.96	(0.94-0.97)	<0.001

† Reference category ***MSM**: Men who have Sex with Men* ***MSW**: Men who have Sex with Women* ***PWID**: People Who Inject Drugs*

Discussion

- ✓ Our study suggests that most HIV transmissions among PLHIV with late-presentation for subtypes A1 and B and those with advanced disease for subtype A1 occur locally (LTNs), calling for an intensification of testing.
- ✓ This is one of the few studies combining molecular and traditional epidemiology to study HIV dispersal patterns of PLHIV with late diagnosis and advanced disease.

Funding: This study has been supported by Gilead Sciences