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High Prevalence of Sequences Included in Transmission Clusters Within Newly Diagnosed HIV-1 Patients in Southern Spain (2004–2015)

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The presence of transmission clusters (TCs) and their epidemiological characteristics in a treatment-naive cohort of HIV-1 patients in southern Spain over a decade (2004–2015) were evaluated. Protease and reverse transcriptase sequences provided by each genotype test were used in the phylogenetic study, performed first by the neighbor-joining method and then confirmed by Bayesian analysis. We collected clinical, immunovirological, and demographic data for all patients included. Our cohort comprised 757 patients, 428 (56.5%) belonging to a TC. Overall, we found 123 TCs, 21 of them comprising five or more individuals and three with ≥10 sequences. Forty-three TCs (35.0%) remained active. The clustered patients were mainly men (92.8%) who had sex with men (MSM) (81.5%), Spanish (80.6%), and young adults (median age at diagnosis of 32.6 years). They had lower percentages of late diagnosis and AIDS cases (42.1% and 13.6%, respectively), whereas the presence of recent seroconverters (31.1%), HIV-1 B subtypes (79.4%), and transmission drug resistance (20.3%) increased within TCs, with regard to not-clustered individuals. Among the TCs of non-B variants, circulating recombinant forms (CRF) were predominant (87.5%), with the highest frequencies for CRF19_cpx (17.0% of non-B subtype sequences in TCs); CRF02_AG (15.9%); and CRF01_AE (9.1%). In conclusion, over half of our cohort was included within a TC. More than a third of TCs found could be considered active transmission events. Belonging to a TC was related to MSM, Spanish origin, recent seroconversion, high prevalence of resistance mutations, and B HIV subtype. Among the non-B genetic forms in TCs, we found a high prevalence of CRF19_cpx, CRF02_AG, and CRF01_AE variants.

Keywords: HIV-1, molecular epidemiology, transmission events, drug resistance mutations, southern Spain

Introduction

The WHO and Spanish clinical guides recommend a genotype resistance test be performed in all new cases of HIV diagnosis. The same recommendation applies to patients already diagnosed with HIV infection starting antiretroviral therapy (ART) or failing the regimen being followed.[1,2] Therefore, protease (PR) and reverse transcriptase (RT) sequences are routinely obtained for all these patients, providing not only information about ART mutation resistances but also making the sequences available for HIV molecular epidemiology and phylodynamic analyses.[3,4] Well-characterized transmission chains traditionally based on surveys and contact studies are often in good agreement with sequence-based phylogenies.[3,5,6]

An HIV-1 transmission cluster (TC) can be defined as a set of HIV-1 sequences linked by direct or indirect epidemiological connection and not by randomness.[7,8] Moreover, the broad meaning of TCs comprises not only persons who are already diagnosed but also undiagnosed HIV-infected individuals. Thus, a TC represents a subset of a wider network, including persons who are not infected with HIV, but who may be at risk for infection. Accordingly, the description of HIV TCs mainly contributing to ongoing transmission in an area should help to improve interventions to prevent possible new infections. From a Public Health perspective, an undiagnosed individual linked to members of a TC can also be identified through partner studies and thus become the target for testing strategies.[9]

The Costa del Sol area (Malaga, southern Spain) has the maximum rate of new diagnosis of HIV in our region (Andalusia), and even one of the highest figures for the whole of Spain.[10,11]

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Objectives

Therefore, to better understand HIV transmission dynamics, we analyzed the relevance of the TCs in the spread of HIV in our area, identifying those in our cohort of newly diagnosed HIV patients during the period from 2004 to 2015 and describing them from different points of view (demographic, clinical, and immunovirological).

Study design

Study population and subtype assignment. This study was carried out at the Virgen de la Victoria Hospital, a reference center for HIV-1 genotypic drug resistance testing on the Costa del Sol (Andalusia, southern Spain) during the period from January 2004 to December 2015. A genotype resistance test is always undertaken at the time of HIV-1 diagnosis and before antiretroviral therapy (ART) initiation. Hence, a partial region of the HIV-1 *pol* gene is routinely available; specifically, the complete PR and partial RT. This analysis included sequences obtained during the study period by RT ± PCR and Sanger sequencing up to the end of 2014, after which we used 454 pyrosequencing (GS Junior Titanium Sequencing Kit®; Roche Diagnostics Gmbh, Mannheim, Germany). The consensus sequence was generated by the modular software Mesquite v 3.02, with a cutoff threshold of 10%.[12] In addition, for Sanger sequencing, we used the Trugene HIV Genotyping Kit® (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY) until 2014, and ViroSeq® HIV-1 Genotyping System v2.0 (Abbott Laboratories, IL) in 2015. Subtyping was performed through REGA v3.0.

Description and statistical analyses of the study population characteristics. We also collected demographic, clinical, and immunovirological data for all the newly diagnosed patients, whose genotype test was available during the study period. Patients within a TC were compared to those outside. Independent predictors of clustering were assessed with logistic regression models. Statistical analyses were carried out by the software SPSS 16.0.

All these patients signed an Informed Consent at their first visit to our hospital and before follow-up, approving routine data use under confidentiality and anonymized, as in this study.

Drug resistance. The resistance mutations were predicted using Stanford algorithm v7.1.1, available in the Stanford University HIV Drug Resistance Database. Transmitted drug resistance (TDR)-associated mutations were evaluated following the WHO surveillance drug resistance mutation list, updated in 2009.[13] Multiresistance was defined as genotypic resistance to two or three families of antiretroviral drugs.

Cluster identification. All genotypes available from 2004 to 2015 associated with descriptive information from the corresponding patients were submitted to phylogenetic analysis. First, we aligned the PR and RT sequences (numbering positions in HIV relative to HXB2CG: codons 1–99 and 1–335, respectively, with an average overall length of 915 nucleotides) and reconstructed a phylogeny with the maximum-likelihood (ML) method by RAxML software through CIPRES gateway.[14,15] We applied GTR+I+G model, as the best using the Akaike Information Criterion (AIC), determined by FindModel, included in MEGA v6.[16] The reliability of each grouping on the resulting tree was assessed from its bootstrap resampling value, based on 1,000 iterations. In addition, this phylogeny was confirmed by Bayesian analysis if the associated posterior probability (pp) was ≥0.9. The Bayesian approach was carried out by MrBayes v3.2 program,[17] and then visualized by the graphical viewer FigTree. MrBayes analysis was also performed through CIPRES gateway with the evolutionary model GTR+I+G, for 20 million generations, sampled every 1,000 generations and with a 25% default burn-in value for diagnostics.

We relied on Cluster Picker v1.2.1[18] to identify long-lived TCs in our cohort, using as criteria a minimum branch node support of 90% in the phylogeny previously generated by RAxML, as well as a maximum genetic distance within a TC of 4.5%.

TCs of two sequences were defined as pairs, whereas clusters of three to four sequences were considered small clusters, and of four or more as large ones. These latter (≥5 sequences) were analyzed in detail because of their epidemiological relevance. Moreover, the most similar sequences to those comprised in the largest TCs were found by BLASTsearch, and then submitted to a Bayesian analysis similar to that exposed before, to find phylogenetically closeness worldwide.

Finally, clusters containing at least one sequence collected ≤2 years before the end of the study period were defined as active ones.[19]

All the steps mentioned above are summarized in the flow chart depicted in Fig. 1.

Nucleotide sequence accession numbers. Because submission of the whole sequence data set to public databases could enable the identification of transmission networks and thus risk breaching patient confidentiality, we submitted a random sample of 10% to GenBank under accession numbers MK344471–MK344560, KY558407–KY558462, KY766062 for the PR gene, and MK344561–MK344638, KY558463–KY558518, KY766063, KP081425, KP081427, KP081429, KP081432–KP081434, KP081440, KP081443, and KP081447–KP081450 for the RT gene.

Results

Overall description of the cohort

During the period 2004–2015, 784 patients were newly diagnosed with HIV-1 infection in our hospital. Of these, 757 possessed surveillance information (demographic, risk, clinical, and virological data) and were also treatment naive at the time of HIV drug resistance testing. The highest number of new HIV-1 infections was found in 2012, with 92 new diagnoses (12.2%) (Supplementary Fig. S1).

The epidemiological data and distribution of the HIV-1 genetic subtypes are summarized in Table 1. Overall, most patients were male (88.2%), and the median age at diagnosis was 34.7 years (27.7–42.9). Of the 757 patients, 557

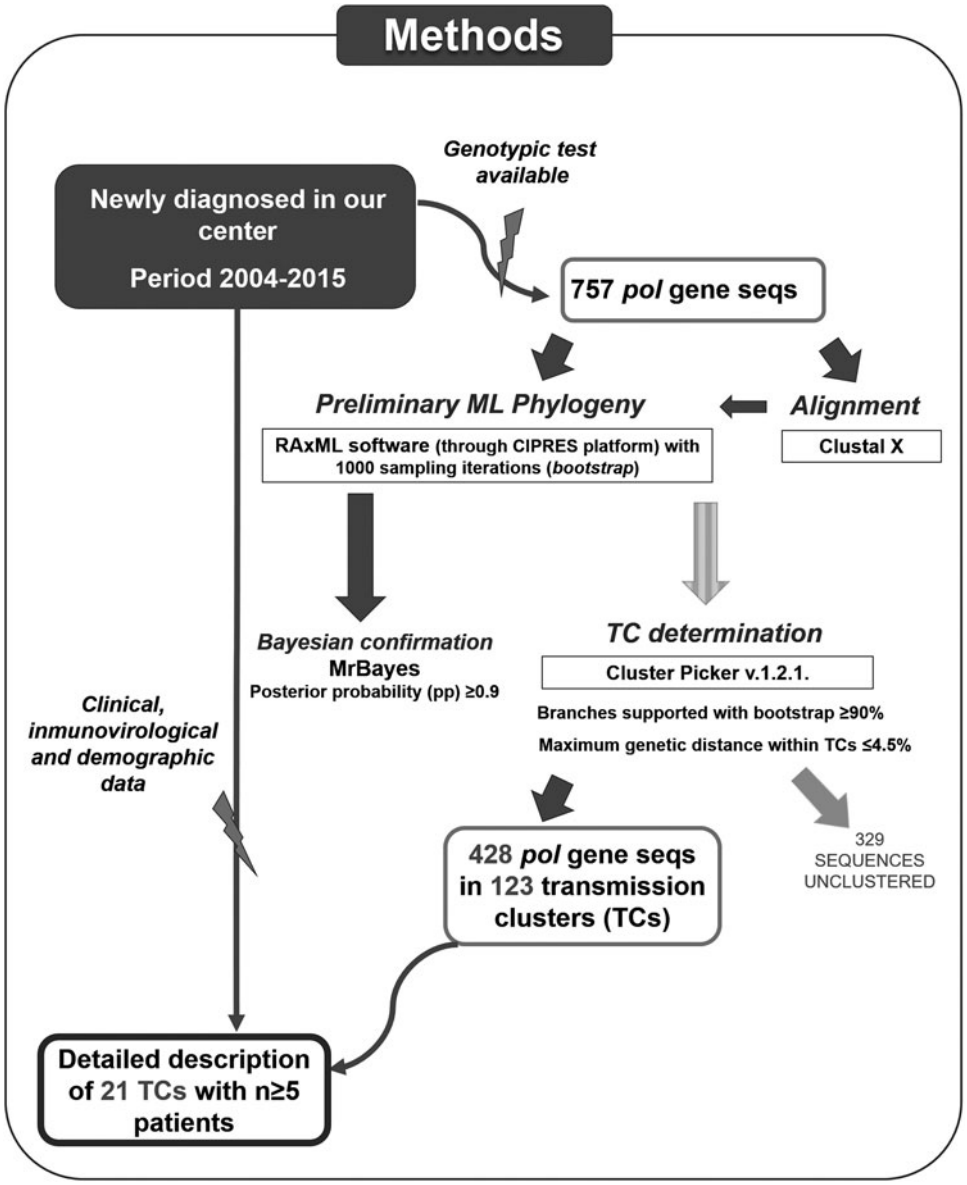

FIG. 1. Flow chart showing the methodology followed in this study.

(73.6%) were Spanish and 535 were men who self-reported having sex with men (MSM) (70.7%). At diagnosis, the initial CD4 count was 372 cells/μL (161–530) and the average first viral load was 4.8 log copies/mL (4.4–5.4). From the whole cohort, 193 patients (25.5%) had a prior negative HIV serology, with a mean time to seroconversion of 14.8 months (7.8–27.5). Almost half the cohort (49.1%) presented a late diagnosis (initial CD4 count <350 cells/mL) and 150 (19.8%) cases of AIDS were recorded during the study period. The overall prevalence of TDR to any drug class among newly diagnosed HIV-1 infections during the study period was 23.3% (176 out of 757). Among these, 35 patients (19.9%) showed TDR to 2 classes of antiretroviral drugs and 2 (1.1%) to the three types considered, but the majority (78.9%) presented TDR involving resistance to only one class. Finally, according to the results provided by REGA v3.0, 572 (75.6%) patients were infected with subtype B viruses and 185 (24.4%) with diverse nonsubtype B genetic forms: 41 of them (22.2%) with 5 different pure non-B subtypes (A1, C, F1, F2, and G) and 77 (41.6%) with 9 circulating recombinant forms (CRFs). The subtype for 67 patients (36.2%) could not be determined precisely by the REGA analysis, but was also suggested to be CRFs other than those stored in its database (beyond CRF47) or unique recombinant forms (URFs) (Table 1).

Prevalence of sequences included in clusters and features

Clusters were identified according to Cluster Picker results, based on the ML phylogeny analysis (subsequently confirmed by Bayesian inference with pp ≥0.9; Supplementary Figs. S2 and S3, respectively) with bootstrap values ≥90% as branch support, and as well as basing on a genetic distance of ≤4.5% divergence between ≥2 individuals. Of the 757 sequences in our cohort, 428 (56.5%) fell into 123 inferred clusters (size ranged 2–39 patients); 71 pairs (57.7%), 31 small clusters containing 3–4 individuals (25.2%), and 21

Table 1. Main Clinical, Demographic, and Virological Characteristics and Distribution of HIV-1 Genetic Forms for the Study Population, According to Whether or Not They Belonged to a Cluster

	Total	*In TC*	*Nonclustered*	p[a]	*OR (95% CI)*	p[b]
No. of patients	757	428 (56.5)	329 (43.5)			
Age at diagnosis (years)	34.7 (27.7–42.9)	32.6 (27.0–41.0)	37.6 (29.6–45.5)	**<0.001**		
Age range (years)						
<35	219 (28.9)	143 (33.4)	76 (23.1)	**<0.001**	Ref.	
35–60	494 (65.3)	273 (63.8)	221 (67.2)		0.878 (0.608–1.268)	0.487
>60	44 (5.8)	12 (2.8)	32 (9.7)		0.264 (0.122–0.573)	**0.001**
Sex						
Male	668 (88.2)	397 (92.8)	271 (82.4)	**<0.001**	1.111 (0.621–1.987)	0.724
Female	89 (11.2)	31 (7.2)	58 (17.6)		Ref.	
Origin						
Spanish	557 (73.6)	345 (80.6)	212 (64.4)	**0.001**	1.791 (1.246–2.576)	**0.002**
Immigrants						
African	46 (6.1)	12 (2.8)	34 (10.3)		Ref. (rest of categories)	
South American	86 (11.4)	42 (9.8)	44 (13.4)			
European countries*	65 (8.6)	29 (6.8)	36 (10.9)			
Asian	3 (0.4)	0 (0.0)	3 (0.9)			
Risk behavior						
MSM	535 (70.7)	349 (81.5)	186 (56.5)	**<0.001**	2.467 (1.626–3.741)	**<0.001**
HTX	192 (25.4)	69 (16.1)	123 (37.4)		Ref. (rest of categories)	
IDU	18 (2.4)	3 (0.7)	15 (4.6)			
Others	12 (1.6)	7 (1.6)	5 (1.5)			
Initial viral load (log copies/mL)	4.8 (4.3–5.3)	4.8 (4.4–5.3)	4.9 (4.3–5.4)	0.858		
Initial lymphocyte CD4 count (cells/μL)	353 (161–530)	395 (219–553)	287 (96–491)	**<0.001**		
AIDS cases[c]	150 (19.8)	58 (13.6)	92 (28.0)	**<0.001**	0.680 (0.436–1.061)	0.089
Primary resistance mutations[c]	176 (23.3)	119 (27.8)	57 (17.3)	**<0.001**	2.381 (1.486–3.812)	**<0.001**
Late diagnosis (initial CD4 count <350 cells/μL)[c]	372 (49.1)	180 (42.1)	192 (58.4)	**<0.001**	0.826 (0.583–1.169)	0.280
Recent seroconverters	193 (25.5)	133 (31.1)	60 (18.2)	**<0.001**	1.231 (0.840–1.806)	0.287
Seroconversion time (months)	14.8 (7.7–27.5)	14.9 (7.7–30.1)	13.8 (7.7–23.7)	0.962		
No. of patients	757	428 (56.5)	329 (43.5)			
Subtype						
B	572 (75.6)	340 (79.4)	232 (70.5)	**0.005**	1.207 (0.823–1.770)	0.336
Non-B subtypes	185 (24.4)	88 (20.6)	97 (29.5)		Ref.	
A1	8	0	8			
C	10	5	5			
F1	7	2	5			
F2	2	0	2			
G	14	4	10			
CRF01_AE	11	8	3			
CRF02_AG	30	14	16			
CRF06_cpx	3	0	3			
CRF12_BF	6	2	4			
CRF14_BG	2	1	1			
CRF19_cpx	21	15	6			
CRF24_BG	1	0	1			
CRF29_BF	2	2	0			
CRF47_BF	1	0	1			
Other CRFs/URF**	67	35	32			

The quantitative variables are expressed as average or median and IQR, and the qualitative variables as *n* (%).
[a]*p* Value for the chi-square test.
[b]*p* Value for the univariate logistic regression.
[c]The reference category was the absence of AIDS events, the no presence of TDR, or late diagnosis.
*Other than Spain; **beyond CRF47_BF, REGA v3.0 subtyping results were considered globally with URFs.
In bold, *p* values ≤0.05.
CRF, circulating recombinant forms; HTX, heterosexual transmission; IDU, injecting drug user; MSM, men who had sex with men; URF, unique recombinant forms.

Table 2. Number of Transmission Clusters Found in Our Cohort According to Cluster Picker Results

	No. of clusters by ML (% out of total TCs)	*TC size*	*No. of sequences (% out of total sequences in TCs)*
	71 (57.7)[a]	2	142 (33.2)
	18 (14.6)[a]	3	54 (12.6)
	13 (10.6)[a]	4	52 (12.2)
	7 (5.7)	5	35 (8.2)
	3 (2.4)	6	18 (4.2)
	4 (3.2)	7	28 (6.5)
	3 (2.4)[a]	8	24 (5.6)
	1 (0.8)	9	9 (2.1)
	1 (0.8)	11	11 (2.6)
	1 (0.8)	16	16 (3.7)
	1 (0.8)	39	39 (9.1)
Total TCs	123 (100.0)		
Total seqs.	428		

Size of each TC is indicated by the number of patients associated.
[a]Four TCs with 2, 1 TC with 3, 3 TCs with 4, and 1 TC with 8 individuals, respectively, found by Cluster Picker program, are well supported (bootstrap ≥90%) in RAxML phylogenetic tree with a distance genetic within them ≤4.5%, but they are not confirmed by Bayesian analysis with a posterior probability >0.9.
ML, maximum likelihood; seqs, sequences; TCs, transmission clusters.

Table 3. Prevalence of Transmitted Drug Resistance According to Each Antiretroviral Type

	Total (n = 757)	*In TC (n = 428)*	*Nonclustered (n = 329)*	p
NRTIs	79 (10.4)[a]	46 (10.7)	33 (10.0)	0.422
NNRTIs	117 (15.4)[b]	85(19.9)	32 (9.7)	**<0.001**
PIs	19 (2.5)[c]	11 (2.6)	8 (2.4)	0.549

[a]Forty-three of the 79 patients only show TDR to NRTIs.
[b]Eighty-two of the 117 patients only show TDR to NNRTIs.
[c]Fourteen of the 19 patients only show TDR to PIs.
In bold, p values ≤0.05.
NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; TDR, transmitted drug resistance.

large TCs with ≥5 members (17.0%) (Table 2). Moreover, 180 (23.8%) patients were grouped in 1 of the 21 TCs composed of 5 or more individuals, 3 (14.3%) of these (TCs 30, 70, and 93) with $n \geq 10$. Finally, 43 (35.0%) clusters were defined as active TCs, since they contained at least one sequence collected between January 2014 and the end of the study in December 2015.

Characteristics of TCs

The baseline characteristics of the patients included in a TC in comparison to those not in a TC are also shown in Table 1. Overall, the patients from our cohort who were clustered in a transmission chain were mainly male (92.8%), MSM (81.5%), Spanish (80.6%), and young adults (median age at diagnosis of 32.6 years, IQR: 27.0–41.0). The initial CD4 count for clustered patients was 395 cells/μL (IQR: 219–553), higher than for those who were nonclustered (287, IQR: 96–491) ($p = 0.001$). The viral load at diagnosis was similar for the two groups (4.8 $\log_{10}$ copies/mL vs. 4.9 $\log_{10}$ copies/mL, $p = 0.858$). The percentage of late diagnosis cases was lower within clustered patients than those outside a TC (42.1% vs. 58.4%, $p < 0.001$), as was the case for the AIDS patients (13.6% vs. 28.0%, $p < 0.001$). Patients were more likely to be recent seroconverters when they belonged to a TC than when they were nonclustered (31.1% vs. 18.2%, $p < 0.001$). The prevalence of total TDR was higher in clustered patients (27.8% vs. 17.3%, $p < 0.001$). Moreover, among the 176 patients with TDR in our cohort, 119 (67.6%) were in a TC (Table 1). Twenty-three of these 119 (19.3%) showed TDR to two classes of antiretroviral drugs, while the rest (80.7%) were resistant to a single class. Primary resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) was the most prevalent among the different types of antiretroviral drugs (Table 3), especially in sequences belonging to any TC (19.9 vs. 9.7, $p < 0.001$). The overall prevalence of mutations associated with resistance to each class of antiretroviral drug in the patients who were clustered in any transmission event is shown in Table 4. Thus, considering the 117 sequences with TDR to nucleoside reverse transcriptase inhibitors, the most frequent mutations detected were T69D/N and K219E/Q, present in 36 (34.0%) and 26 (24.5%) clustered individuals, respectively. Regarding NNRTI mutations, V179D/E, K103N, and G190A/S appeared with the highest frequency (37.5%, 29.5%, and 15.9%, respectively) within TCs. Finally, the most prevalent PI-selected mutation was M46I/L, with 10 (90.9%) patients clustered.

We identified more HIV-1 non-B sequences in TCs than outside them (28.2% vs. 19.6%, $p < 0.0001$). Among the 88 nonsubtype B genetic forms clustered in any TC, 11 (12.5% of them) represented pure non-B subtypes (C, F1, F2, and G), whereas the remaining 77 (87.5%) constituted recombinant forms (CRFs or URFs). The most frequent CRFs in our cohort during the study period were (in order of prevalence): CRF19_cpx (17.0% of non-B subtype sequences in TCs); CRF02_AG (15.9%); and CRF01_AE (9.1%) (Table 1). The subtype-specific analysis of the TCs showed that MSM was the major transmission route, independent of the subtype (84.4% and 70.4% for B and non-B genetic forms, respectively). However, heterosexual transmission represented more than a quarter (26.1%) of risk behaviors in patients infected by non-B subtype strains. Injecting drug users were residual in clusters composed of B subtype sequences (0.3%) or very few in the other subtypes (2.2%). Females were more common for the other major subtypes and CRFs than in B subtype clusters (17.0% vs. 4.7%, $p < 0.001$). When B and non-B subtype TCs were analyzed according to origin, we found a higher percentage of Spanish cases within the former (83.8% vs. 68.2%, $p = 0.001$). On the other hand, there was a larger proportion of non-B subtypes among immigrants included in any TC compared with B subtype clusters (31.8% vs. 16.2%, $p = 0.001$). In addition, the ratio of early and late diagnosis was more unbalanced among the subtype B clusters in contrast to non-B TCs (60.6% vs. 39.4% and 47.7% vs. 52.3%, respectively; $p = 0.02$). The same trend is found among B and non-B TCs with regard to AIDS events (11.3%

Table 4. Prevalence of Mutations in the Sequences Grouped in Transmission Clusters

NRTI mutations[a] (n = *106*)		*NNRTI mutations*[b] (n = *88*)		*PI mutations*[c] (n = *11*)	
Mutation	N *(%)*	*Mutation*	N *(%)*	*Mutation*	N *(%)*
M41L	2 (1.8)	K101E	1 (1.1)	M46IL	10 (90.9)
D67N	22 (20.7)	K103N	26 (29.5)	V82L	1 (9.0)
T69D/N	36 (34.0)	V106A	11 (12.5)		
M184I/V	1 (0.9)	V179D/E	33 (37.5)		
T215D/I/S/V	19 (17.9)	Y181I/C	2 (2.2)		
K219E/Q	26 (24.5)	G190A/S	14 (15.9)		
		P225H	1 (1.1)		

[a]Twenty-four of the 46 patients only show TDR to NRTIs.
[b]Sixty-two of the 85 patients only show TDR to NNRTIs.
[c]Ten of the 11 patients only show TDR to PIs.

vs. 20.4%, respectively, $p = 0.03$). We found no significant differences between the two groups, B and non-B clusters, in other clinical or immunovirological parameters, such as initial lymphocyte CD4 count, viral load, or presence of drug resistance.

Correlates of clustering

In the multivariable analysis, MSM risk (odds ratio [OR] = 2.467; 95% confidence interval [CI] = 1.626–3.741), Spanish origin (OR = 1.791; 95% CI = 1.246–2.576), and the presence of primary resistance mutations (OR = 2.381; 95% CI = 1.486–3.812) were associated with cluster membership (Table 1). On the contrary, persons with age >60 years were statistically less likely to be in a TC.

Description of TCs of interest

Because of its epidemiological interest, we analyzed in detail the TCs comprising five or more individuals. The main characteristics of these TCs are summarized in the Supplementary Table S1. The TCs were numbered according to Cluster Picker results and as depicted in the RAxML phylogenetic tree (Supplementary Fig. S2). The 100 most similar sequences worldwide identified by BLAST for those comprised in the largest TCs (TCs no. 30, 70, and 93) are shown in the corresponding phylogenetic tree inferred by Bayesian analysis (Supplementary Figs. S4–S6, respectively).

Discussion

More than half the new HIV-1 diagnoses in our cohort were clustered in one of the numerous TCs found. Other similar studies performed in Spain showed a lower percentage of patients associated in clusters.[20,21] Outside Spain, a wide range of clustering results has been reported in European cohorts. A similar percentage was found for Nordic countries (57%),[19] whereas our proportion of TCs was much higher than shown in, for instance, Swiss and Austrian cohorts, both of these around 42–43%.[22,23] Regarding cluster size, the TCs composed of only two individuals (pairs) were the predominant clusters in our population, as also in some of the above-mentioned studies.[19,20,23] Moreover, when we focused on our region and discarded pairs and small TCs ($n = 3$–4 individuals), and only compared the importance of large clusters (≥5 members) among the overall TCs (17.1%), we still found a similar percentage in our cohort than in that for eastern Andalusia (20.0%), despite our more restrictive criteria (*i.e.*, higher bootstrap threshold of clustering).[20] In addition, we found more than one-third of active transmission events at the last enrolment date.

The characteristics of genetically linked individuals correlated reasonably well with those of the overall Spanish HIV epidemic and those of the rest of Western Europe.[19–25] Thus, HIV-1 patients from our cohort included in a TC were mainly males, MSM, and young adults. The contribution of injecting drug users to clustering was residual (0.9%), as expected in Western Europe, but different to that seen in Eastern Europe or Asia.[24] Regarding the origin, Spaniards were predominant, comprising 80.6% of the clustered patients, followed by South American patients (9.8%). The relevance of South American immigration, especially in MSM transmission, as well as the low linkage rate (2.8%) of African immigrants, as presented in this study, have already been highlighted in other series from Spain.[21,26]

HIV-1 surveillance studies in Spain have shown a prevalence of primary resistance mutations to any drug class, ranging from 8.5% to nearly 14%, less than the frequency in our overall cohort (23.3%).[27–29] Nevertheless, the percentage of sequences in clusters harboring drug resistance mutations (27.8%) exceeded the average frequency, so the effectiveness of antiretroviral therapy could be compromised in almost one out of every four patients associated in a TC.

Finally, almost half of non-B infections comprised patients associated in a TC. The high prevalence of sequences of non-B subtypes has typically been linked to migration and heterosexual groups in most Western European countries.[24] However, this pattern is changing, with a sharp increase among MSM transmission local (nonimmigrant) networks.[30] Thus, one of our findings, the presence of 5 clusters of CRF19_cpx subtype, involved 15 MSM patients, 13 of them Spaniards. This subtype has shown an unusual high prevalence in the entire Malaga province, mostly involving Spanish individuals.[31] The predominance of Spanish was also found for TCs subtyped as CRF01_AE (87.5%), but not for CRF02_AG clusters (28.6%). However, one of the limitations of our study was to subtype using REGA v3.0, so we cannot discard the emergence of other interesting non-B clusters in our area belonging to a recombinant form beyond CRF47_BF, the last CRF currently reached

with this automated subtyping tool. Moreover, our study only included sequences from patients with a genotype available, consequently discarding individuals not engaged in clinical care and giving an incomplete view of subtypes landscape and even, of transmission networks.

In conclusion, the analysis of the TCs in our cohort highlights a higher proportion of HIV-1-infected patients linked to any TC, also presenting a higher prevalence of primary drug resistance among them, in comparison with those of other nearby areas. However, the clustered individuals shared similar demographic characteristics to those from other cohorts. Spanish origin and MSM risk behavior were positively associated to belonging to a TC, whereas HIV-1 patients who were older than 60 years showed less likely to be clustered. Nevertheless, the most outstanding features of a significant amount of these TCs were their activity remaining at the end of the study period. These facts are all contributing to the high rate and spread of new HIV-1 infections in our area.

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Supplementary Material

Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
Supplementary Figure S4
Supplementary Figure S5
Supplementary Figure S6
Supplementary Table S1

References

1. AIDS Study Group (GESIDA) of the Spanish Society of Infectious Diseases and Clinical Microbiology and the National AIDS Plan. Executive summary of the GESIDA/National AIDS Plan Consensus Document on Antiretroviral Therapy in Adults Infected by the Human Immunodeficiency Virus (Updated January 2017). Enferm. Infecc. Microbiol. Clin. 2018; 36:435–445.
2. Bennett, D.E., S. Bertagnolio, D. Sutherland, and C.F. Gilks. 2008. The World Health Organization's global strategy for prevention and assessment of HIV drug resistance. Antivir. Ther. 13 Suppl 2:1–13.
3. HueM S., J.P. Clewley, P.A. Cane, and D. Pillay. 2004. HIV-1 pol gene variation is sufficient for reconstruction of transmissions in the era of antiretroviral therapy. AIDS 18: 719–728.
4. Paraskevis, D., G.K. Nikolopoulos, G. Magiorkinis, I. Hodges-Mameletzis, and A. Hatzakis. 2016. The application of HIV molecular epidemiology to public health. Infect. Genet. Evol. 46:159–168.
5. Hue, S., D. Pillay, J.P. Clewley, and O.G. Pybus. 2005. Genetic analysis reveals the complex structure of HIV-1 transmission within defined risk groups. Proc. Natl. Acad. Sci. U.S.A. 102:4425–4429.
6. Vasylyeva, T.I., S.R. Friedman, D. Paraskevis, and G. Magiorkinis. 2016. Integrating molecular epidemiology and social network analysis to study infectious diseases: Towards a socio-molecular era for public health. Infect. Genet. Evol. 46:248–255.
7. Hassan, A.S., O.G. Pybus, E.J. Sanders, J. Albert, and J. Esbjörnsson. 2017. Defining HIV-1 transmission clusters based on sequence data. AIDS 31:1211–1222.
8. Centers for Disease Control and Prevention. Detecting, investigating, and responding to HIV transmission clusters. March 2017. Available at https://www.cdc.gov/hiv/pdf/funding/announcements/ps18-1802/CDC-HIV-PS18-1802-AttachmentE-Detecting-Investigating-and-Responding-to-HIV-Transmission-Clusters.pdf Accessed June 18, 2018.
9. Kimbrough, L.W., H.E. Fisher, K.T. Jones, W. Johnson, S. Thadiparthi, and S. Dooley. 2009. Accessing social networks with high rates of undiagnosed HIV infection: The social networks demonstration project. Am. J. Public Health. 99:1093–1099.
10. Epidemiological Surveillance Service of Andalusia, Health Department of Andalusian Government. HIV/AIDS Infection and Other STI in Andalusia. 2016 Weekly Report. Sevilla: General Section of Public Health and Pharmaceutical Regulation. December 1, 2017, Volume 22 (48). Available at https://www.repositoriosalud.es/bitstream/10668/2782/1/SVEAIS_22_48_2017.pdf Accessed July 12, 2018.
11. General Section of Public Health, Quality and Innovation. Regional Systems of Epidemiological Surveillance. National Centre of Epidemiology. Epidemiological Surveillance for HIV and AIDS in Spain 2017. Update of June 30, 2018. Available at https://www.mscbs.gob.es/ciudadanos/enfLesiones/enfTransmisibles/sida/vigilancia/doc/Informe VIH_SIDA_2018_21112018.pdf Accessed July 17, 2018.
12. Maddison, W.P., and D.R. Maddison. 2006. Mesquite: a modular system for evolutionary analysis. Available at http://mesquiteproject.org Accessed January 31, 2016.
13. Bennett, D.E., R.J. Camacho, D. Otelea, D.R. Kuritzkes, H. Fleury, M. Kiuchi, W. Heneine, R. Kantor, M.R. Jordan, J.M. Schapiro, A.M. Vandamme, P. Sandstrom, C.A. Boucher, D. van de Vijver, S.Y. Rhee, T.F. Liu, D. Pillay, and R.W. Shafer. 2009. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS One 4:e4724.
14. Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313.
15. Miller, M.A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Abstract presented at the Gateway Computing Environments Workshop (GCE), New Orleans, LA, November 14, pp 1–8.
16. Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 30:2725–2729.
17. Ronquist, F., M. Teslenko, P. van der Mark, D.L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M.A. Suchard, and

J.P. Huelsenbeck. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61:539–542.
18. Ragonnet-Cronin, M., E. Hodcroft, S. Hué, E. Fearnhill, V. Delpech, A.J. Brown, and S. Lycett; UK HIV Drug Resistance Database. 2013. Automated analysis of phylogenetic clusters. BMC Bioinformatics 14:317.
19. Esbjörnsson, J., M. Mild, A. Audelin, J. Fonager, H. Skar, L. Bruun Jørgensen, K. Liitsola, P. Björkman, G. Bratt, M. Gisslén, A. Sönnerborg, Nielsen C; SPREAD/ESAR Programme, P. Medstrand, and J. Albert. 2016. HIV-1 transmission between MSM and heterosexuals, and increasing proportions of circulating recombinant forms in the Nordic Countries. Virus Evol. 2:vew010.
20. Pérez-Parra, S., N. Chueca-Porcuna, M. Álvarez-Estevez, J. Pasquau, M. Omar, A. Collado, D. Vinuesa, A.B. Lozano, and F. García-García. 2015. Study of human immunodeficiency virus transmission chains in Andalusia: Analysis from baseline antiretroviral resistance sequences. Enferm. Infecc. Microbiol. Clin. 33:603–608.
21. Yebra, G., A. Holguín, D. Pillay, and S. Hué. 2013. Phylogenetic and demographic characterization of HIV-1 transmission in Madrid, Spain. Infect. Genet. Evol. 14: 232–239.
22. Ambrosioni, J., T. Junier, C. Delhumeau, A. Calmy, B. Hirschel, E. Zdobnov, L. Kaiser, and Yerly S; Swiss HIV Cohort Study. 2012. Impact of highly active antiretroviral therapy on the molecular epidemiology of newly diagnosed HIV infections. AIDS 26:2079–2086.
23. Hoenigl, M., A. Chaillon, H.H. Kessler, B. Haas, E. Stelzl, K. Weninger, S.J. Little, and S.R. Mehta. 2016. Characterization of HIV transmission in South-East Austria. PLoS One 11:e0151478.
24. Beloukas, A., A. Psarris, P. Giannelou, E. Kostaki, A. Hatzakis, and D. Paraskevis. 2016. Molecular epidemiology of HIV-1 infection in Europe: An overview. Infect. Genet. Evol. 46:180–189.
25. Fabeni, L., C. Alteri, G. Berno, R. Scutari, N. Orchi, G. De Carli, A. Bertoli, L. Carioti, C. Gori, F. Forbici, R. Salpini, A. Vergori, R. Gagliardini, S. Cicalini, A. Mondi, C. Pinnetti, L. Mazzuti, O. Turriziani, M. Colafigli, V. Borghi, F. Montella, A. Pennica, M. Lichtner, E. Girardi, M. Andreoni, C. Mussini, A. Antinori, F. Ceccherini-Silberstein, Perno CF, and M.M. Santoro; SENDIH Study Group. 2019. Characterisation of HIV-1 molecular transmission clusters among newly diagnosed individuals infected with non-B subtypes in Italy. Sex Transm. Infect. 95: 619–625.
26. Diaz, A., M.L. Junquera, V. Esteban, B. Martínez, I. Pueyo, J. Suarez, J.M. Ureña, J.A. Varela, M. Vall, J. del Romero, I. Sanz, J. Belda, J. Boronat, P. Gomez, F. Gual, C. Colomo, J. López de Munain, J. Balaguer, M.C. Landa, M.E. Lezaun, M.C. Cámara, E. Fernández, F.J. Bru, I. Alastrue, J.R. Ordoñana, C. de Armas, M.A. Azpiri, L. Gomez, J. Trullén, and M. Diez; STI Study Group and EPI-VIH Group. 2009. HIV/STI co-infection among men who have sex with men in Spain. Euro. Surveill. 14:pii: 19426.
27. Garcia, F., S. Pérez-Cachafeiro, V. Guillot, M. Alvarez, P. Pérez-Romero, M.J. Pérez-Elías, I. Viciana, J.R. Blanco, M. López-Dieguez, and C. de Mendoza; Cohort of the Spanish AIDS Research Network (CoRIS). 2011. Transmission of HIV drug resistance and non-B subtype distribution in the Spanish cohort of antiretroviral treatment naive HIV-infected individuals (CoRIS). Antiviral Res. 91: 150–153.
28. Yebra, G., M. de Mulder, J. del Romero, C. Rodríguez, and A. Holguín. 2010. HIV-1 non-B subtypes: High transmitted NNRTI-resistance in Spain and impaired genotypic resistance interpretation due to variability. Antiviral Res. 85: 409–417.
29. Vega, Y., E. Delgado, A. Fernández-García, M.T. Cuevas, M.M. Thomson, V. Montero, M. Sánchez, A.M. Sánchez, and L. Pérez-Álvarez; Spanish Group for the Study of New HIV-1 Diagnoses in Galicia and Basque Country. 2015. Epidemiological surveillance of HIV-1 transmitted drug resistance in Spain in 2004-2012: Relevance of transmission clusters in the propagation of resistance mutations. PLoS One 10: e0125699.
30. Ragonnet-Cronin, M., S.J. Lycett, E.B. Hodcroft, S. Hué, E. Fearnhill, A.E. Brown, V. Delpech, D. Dunn, and Leigh Brown AJ; United Kingdom HIV Drug Resistance Database. 2016. Transmission of non-B HIV subtypes in the United Kingdom is increasingly driven by large non-heterosexual transmission clusters. J. Infect. Dis. 213: 1410–1418.
31. González-Domenech, C.M., I. Viciana, L. Delaye, M.L. Mayorga, R. Palacios, J. de la Torre, F. Jarilla, M. Castaño, A. Del Arco, E. Clavijo, and J. Santos. 2018. Emergence as an outbreak of the HIV-1 CRF19_cpx variant in treatment-naïve patients in southern Spain. PLoS One 13:e0190544.

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