Dear Author,

Please, note that changes made to the HTML content will be added to the article before publication, but are not reflected in this PDF.

Note also that this file should not be used for submitting corrections.

Infection, Genetics and Evolution xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Transmission dynamics of HIV-1 subtype B in the Basque Country, Spain

J.A. Patiño-Galindo [a,b], Michael M. Thomson [c], Lucía Pérez-Álvarez [c], Elena Delgado [c], María Teresa Cuevas [c], Aurora Fernández-García [b,c], Rafael Nájera [c], José A. Iribarren [d], Gustavo Cilla [d], Leyre López-Soria [e], María J. Lezaun [f], Ramón Cisterna [g], F. González-Candelas [a,b,*], on behalf of Group of HIV-1 Antiretroviral Resistance Studies in the Basque Country

[a] Joint Research Unit "Infection and Public Health" FISABIO-Universitat de Valencia, Spain
[b] CIBER in Epidemiology and Public Health, Madrid, Spain
[c] Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain
[d] Hospital Universitario Donostia, San Sebastián, Spain
[e] Hospital Universitario Cruces, Bilbao, Spain
[f] Hospital Universitario Araba, Vitoria, Spain
[g] Hospital Universitario Basurto, Bilbao, Spain

ARTICLE INFO

Article history:
Received 9 December 2015
Received in revised form 15 February 2016
Accepted 22 February 2016
Available online xxxx

Keywords:
Transmission cluster
IDUs
MSM
Antiretroviral resistance

ABSTRACT

This work was aimed to study the HIV-1 subtype B epidemics in the Basque Country, Spain. 1727 HIV-1 subtype B sequences comprising protease and reverse transcriptase (PR/RT) coding regions, sampled between 2001 and 2008, were analyzed. 156 transmission clusters were detected by means of phylogenetic analyses. Most of them comprised less than 4 individuals and, in total, they included 441 patients. Six clusters comprised 10 or more patients and were further analyzed in order to study their origin and diversification. Four clusters included men who had unprotected homosexual sex (MSM), one group was formed by intravenous drug users (IDUs), and another included both IDUs and people infected through unprotected heterosexual sex (HTs). Most of these clusters originated from the mid-1980s to the mid-1990s. Only one cluster, formed by MSM, originated after 2000. The time between infections was significantly lower in MSM groups than in those containing IDUs (P-value < 0.0001). Nucleoside RT and non-nucleoside RT inhibitor (NRTI and NNRTI)-resistance mutations to antiretroviral treatment were found in these six clusters except the most recent MSM group, but only the IDU clusters presented protease inhibitor (PI)-resistance mutations. The most prevalent mutations for each inhibitor class were PI L90M, NRTI T215D/Y/F, and NNRTI K103N, which were also among the most prevalent resistant variants in the whole dataset. In conclusion, while most infections occur as isolated introductions into the population, the number of infections found to be epidemiologically related within the Basque Country is significant. Public health control measures should be reinforced to prevent the further expansion of transmission clusters and resistant mutations occurring within them.

1. Introduction

Since the detection of the first cases of acquired immunodeficiency syndrome (AIDS) in the early 1980s, the pandemic caused by its main causal agent, the human immunodeficiency virus type 1 (HIV-1), has become one of the most important global health problems due to its mortality and morbidity. The latest UNAIDS/WHO report (2013) estimates a total of 35.3 (32.2–38.8) million people infected around the world. In 2012, there were 2.3 (1.9–2.7) million new HIV infections globally, which is a 33% decrease with respect to 2001. In Western European countries, such as Spain, subtype B is the most prevalent among the 9 subtypes of HIV-1. The epidemic of this variant started to spread rapidly among specific risk groups, such as men who have sex with men (MSM) and intravenous drug users (IDUs). Although the rate of infections in these groups decreased during the 1990s thanks to the development of adequate prevention campaigns (UNAIDS/WHO, 2013; Zehender et al., 2010), later years have been characterized by a continuous increment of sexually-related infections, mainly among MSM, while parenteral infections have decreased (ECDC/WHO, 2010).

HIV-1 is a retrovirus of the genus *Lentivirus*. Retroviruses present high evolutionary rates that usually lead to a high genetic diversity. These features are caused by three main factors: polymerization errors of the reverse transcriptase (RT) (Roberts et al., 1988), genetic recombination and an explosive proliferation that leads to enormous effective

* Corresponding author at: Joint Research Unit "Infection and Public Health" FISABIO-Universitat de Valencia, Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Parc Científic de la Universitat de València, c/ Catedrático José Beltrán, 2, 46980 Paterna, Valencia, Spain.
E-mail address: fernando.gonzalez@uv.es (F. González-Candelas).

http://dx.doi.org/10.1016/j.meegid.2016.02.028
1567-1348/

population sizes, which promote the action of natural selection, favoring those mutations that increase the biological fitness of the virus and eliminating the disadvantageous alleles (Moya et al., 2004). These factors have important clinical consequences, such as the rise and spread of mutations related to resistance to antiretroviral drugs, but they also allow the reconstruction of the epidemic history of the virus by using phylogenetic tools (Holmes, 2004).

In the field of molecular epidemiology it is considered that epidemiologically related sequences should group together in a phylogenetic tree, forming transmission clusters, because they all share a common, recent ancestor (Hue et al., 2004, 2005). Coalescent methods for estimating phylogenetic trees (Kingman, 1982; Donnelly and Tavaré, 1995) are used to associate the divergence times from the common ancestor of the sampled individuals in a population with their demographic history. Thus, the results obtained offer information about dates of the introduction of viral variants in populations, the growth rate of infections during the epidemic and the most vulnerable groups of people to the virus (Moya et al., 2004).

The efficacy of highly active antiretroviral therapy (HAART) introduced in the 1990s is hampered by the emergence of resistance mutations in HIV-1 (Costagliola et al., 2007). Genotypic tests of resistance to antiretroviral drugs, after sequencing of the protease and reverse transcriptase (PR/RT) regions, are carried out routinely in many countries, including Spain, both for the design of individualized antiretroviral treatments and for the assessment of the frequency of certain resistances in the population (Costagliola et al., 2007). The widespread use of these tests has led to large, publicly accessible data sets of HIV-1 sequences that, combining analyses of their evolutionary history with epidemiological data, allow depicting the epidemic as well as characterizing its phylodynamics (Hue et al., 2004; Bello et al., 2010; Kouyos et al., 2010).

Genotypic tests of resistance for samples from the Basque country have been performed since 2001. The epidemic in the Basque Country has previously been analyzed using molecular data by Cuevas et al. (2009), who reported the existence of five major HIV-1 subtype B transmission clusters, with sizes ranging between 7 and 18 patients. Here, we have used a larger number of samples and we have also included a representative set of reference sequences to perform a more detailed phylogenetic analysis.

The objective of the present study was to characterize the epidemic of HIV-1 subtype B in this region and analyze the transmission dynamics of some relevant cases. For this, we have used a dataset of 1727 sequences comprising HIV-1 PR/RT genomic regions obtained from 2001 to 2008 in the Basque Country as part of the genotyping program for the search of drug resistance mutations. The largest HIV-1 subtype B transmission clusters detected were subjected to dated phylogenetic analysis (Drummond and Rambaut, 2007). The prevalence of mutations associated with antiretroviral drug resistance was estimated. The results obtained may help in the design of proper HIV prevention campaigns and treatments in this region.

2. Methods

2.1. Dataset

A total of 2497 HIV sequences 1200 nt long and spanning the full PR and partial RT coding regions were obtained from patients attending the main health centers in the Basque Country, Spain (cities of Bilbao, San Sebastián and Vitoria) from 2001 to 2008. In cases of multiple sequences from a single patient, only the earliest one was included. Thus, each viral sequence represented a different patient. Additionally, 8504 worldwide sequences were retrieved from Los Alamos HIV dataset (http://www.hiv.lanl.gov) and were used as reference sequences to ensure the validity of the transmission chains detected in the Basque sample. All the sequences were aligned with MUSCLE v3.5 (Edgar, 2004).

2.2. Phylogenetic reconstruction

In order to identify transmission clusters, defined as viral lineages derived from the same variant in the Basque population, two phylogenetic trees for the dataset of sequences which included both the Basque and the reference sequences (11,001 sequences in total) were obtained using FastTree 2.1 software (Price et al., 2010)) using the GTR + Γ (4 categories) substitution model: (i) a tree obtained from a full codon alignment, in which 40 codons associated with major resistance in PR (30, 32, 46, 47, 48, 50, 54, 58, 74, 76, 82, 83, 84, 88, 90) and RT (41, 62, 65, 67, 69, 70, 74, 75, 77, 100, 101, 103, 106, 108, 115, 116, 151, 181, 184, 188, 190, 210, 215, 219 y 225) (Johnson et al., 2013) were removed, yielding a total length of 1080 nt, and (ii) a tree obtained from only third-codon positions of the original alignment (length of sequences = 400 nt).

We considered as potential transmission clusters those clades formed by at least 2 sequences of Basque origin present in both trees with SH-like local support ≥0.90 (Christin et al., 2012). Furthermore, clusters with support between 0.90 and 0.95, and/or including at least 10 patients, were further validated after their joint analysis with three random datasets of 1000 subtype B reference sequences (full codon alignments without resistance mutations). Basque sequences included in these clusters were incorporated to the three datasets and analyzed by maximum-likelihood with PhyML 3.0 (Guindon et al., 2010). Clusters with Basque Country-only sequences were considered only if they had Chi2-based approximate Likelihood-ratio test (aLRT) support >0.999. Clusters were classified depending on the major transmission route (>50%) for the corresponding patients.

Only HIV-1 subtype B clusters were considered for further analysis. All the sequences were subtyped with the REGA HIV-1 Subtyping Tool — Version 2.0 (http://dbpartners.stanford.edu/RegaSubtyping/; De Oliveira et al., 2005).

2.3. Dated phylogenies

The molecular clock signal of each transmission group equal to or larger than 10 individuals was assessed by performing linear regression analyses between the parameters “root-to-tip divergence” and “sampling date” with the software Path-O-Gen v1.4 (Drummond et al., 2003), using the phylogenetic trees from each transmission cluster, obtained as subtrees from the full-codon FastTree tree, as input.

These transmission groups were further analyzed using the full codon alignments of 1080 nt. Dated phylogenies were obtained using a Bayesian MCMC coalescent method, as implemented in BEAST v1.8.1 (http://beast.bio.ed.ac.uk/; Drummond and Rambaut, 2007). The SRD06 model, which partitions by codon position ($HKY_{112} + \Gamma_{112}$), was used for all the BEAST analyses, as it fits better in most viral protein-coding regions (Shapiro et al., 2006). A log-normal prior (median = 0.002 substitutions per site and year, s/s/y, 95% HPD upper limit = 0.0039 s/s/y) was placed on the ucld.mean parameter (Hue et al., 2005; Zehender et al., 2010). Under a relaxed molecular clock model, the most appropriate demographic model [either constant demographic size, exponential growth, logistic growth or Bayesian Skyline Plot (BSP)] was determined as the one with the lowest Akaike Information Criterion (AIC) value (Baele et al., 2012).

For each transmission cluster we performed at least two independent runs of Bayesian MCMC, with chain lengths ranging between 5 and 10 million states, sampling every 10,000 generations. Subsequently, these runs were combined after discarding a 10% burn-in. All the parameters were estimated from an effective sampling size >200 using the software Tracer 1.5 (http://tree.bio.ed.ac.uk/software/tracer/). Trees generated from the two BEAST runs were combined and summarized after discarding a 10% burn-in using TreeAnnotator (http://beast.bio.ed.ac.uk/).

Please cite this article as: Patiño-Galindo, J.A., et al., Transmission dynamics of HIV-1 subtype B in the Basque Country, Spain, Infection, Genetics and Evolution (2015), http://dx.doi.org/10.1016/j.meegid.2016.02.028

2.4. Detection of intra-subtype recombination

Intra-subtype recombination might introduce spurious long branches in the phylogenies of the transmission clusters considered (Hughes et al., 2009). We grouped all sequences from these clusters and checked for the presence of recombination events by performing five different recombination analyses implemented in RDP3 software: RDP, Geneconv, Bootscan, Maxchi and Chimera (Martin et al., 2010; Martin and Rybicki, 2000; Padidam et al., 1999; Martin et al., 2005; Smith, 1992; Posada and Crandall, 2001). The criterion used to consider the existence of recombination was to obtain significant evidence of recombination in at least two different analyses.

2.5. Estimates of time between infections in transmission clusters

The internal branch lengths of the transmission clusters allow us to estimate the time between infections (Lewis et al., 2008). 95% HDPs for the median time between infections at each transmission cluster were estimated from the tree files produced with BEAST. We obtained the median and the upper and lower 95%HPD limits for the internal branch lengths of each tree (Lewis et al., 2008) using an in-house Perl script combined with an R-script (R Development Core Team, 2011). The distributions of the median internal branch lengths were compared among transmission groups by ANOVA tests. Tukey's tests were performed as post-hoc analyses.

2.6. Estimate of prevalence of drug resistance mutations

Mutations associated with resistance to PR and RT inhibitors (Johnson et al., 2013), both in the total dataset and in each of the transmission groups analyzed, were detected using the Stanford University HIV Drug Resistance Database [http://sierra2.stanford.edu/sierra/servlet/JSierra, (Liu and Shafer, 2006)] and their prevalence was estimated. Only major mutations were taken into account for the protease gene.

3. Results

3.1. Detection of transmission clusters

Of the 2497 HIV-1 sequences analyzed, 2311 belonged to subtype B and had been obtained from a total of 1727 different patients. Of these, 833 corresponded to IDUs, 340 to people infected through unprotected heterosexual sex (HT), 181 to MSM, and 49 to people vertically infected (VERT). 175 sequences belonged to people infected through sexual contact, not specifying whether it was heterosexual or homosexual. No risk factor was known for 149 people.

A total of 156 HIV-1 subtype B transmission clusters were consistent with the two phylogenetic reconstructions obtained with FastTree (from full-codon and third-positions alignments). In total, 441 (25.5%) sequences in the Basque country dataset were included in a transmission cluster. Most of these clusters (93.6%) contained 4 individuals at most (Fig. 1A). Transmission clusters of IDUs were the most abundant (Fig. 1B), followed by groups formed by people infected through unprotected heterosexual sex (HT), MSMs, and clusters containing both IDUs and HTs (IDU/HT). IDU clusters encompassed the largest number of patients (n = 126), followed by MSM (n = 124), HT (n = 75) and IDU/HT (n = 81) (Fig. 1C). MSM were significantly more likely to group in a transmission cluster than patients from other risk groups (Fisher's exact test: P-value = 2.2E-4; odds-ratio = 1.76, 95% confidence interval = 1.30–2.37). Only 6 of the detected clusters comprised at least 10 individuals that, altogether, represented 4.8% of the Basque sample (83 individuals). Four of these clusters were formed by MSM (clusters C, D, E and F), one was classified as an IDU cluster (cluster B) and another was classified as IDU/HT (cluster A). All of them were validated with the maximum likelihood analyses (all had aLRT >0.99 in the reconstructions with PhyML), and all but cluster B had been reported previously by Cuevas et al. (2009): A (cluster M2 in Cuevas et al.), C (M5), D (M1.3), E (M1.1, M1.2), F (M3). MSM were significantly more likely to group in a large transmission cluster than UDIs and HTs (Fisher's exact test: P-value = 2.00E-15; odds-ratio = 8.95, 95% confidence interval = 5.12–15.84).

3.2. Bayesian coalescent analyses

No evidence of intra-subtype recombination events was found in any of the six transmission clusters considered. Fig. 2 shows the dated phylogenies of the transmission clusters reconstructed with BEAST using dated-tips and considering the demographic model that yielded the lowest AIC value in each group. tMRCA estimates differed among groups with the earliest date corresponding to group B (median tMRCA = 1983.8), and the latest to group D (2000.5) (Table 1).

The ANOVA test comparing the median lengths of internal branch concluded that there were significant differences between transmission clusters (F = 37,382.3, df = 5 and 47,463.27, P-value <2.2E-16), being significantly shorter in MSM than in IDU or IDU/HT transmission clusters (all Tukey's test comparisons: P = 0.00; Table 1). The same results (not shown) were obtained with estimates obtained using all the sequences as contemporaneous.

3.3. Resistance mutations in transmission clusters

The prevalence of mutations associated with antiretroviral drug resistance in each transmission cluster and in the complete dataset are shown in Table 2. While mutations associated with resistance to protease inhibitors (PIs) were found only in one transmission group (B, most prevalent mutation L90M: 0.30), all transmission groups except D presented mutations associated with resistance to nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs, respectively). Groups A and B presented the largest number of NNRTI and NRTI resistance mutations, respectively. Among NRTI mutations, T215D/Y/F had the highest prevalence (1.0 in cluster F, 0.50 in cluster B), followed by M184I/V (0.50 in cluster B, 0.27 in cluster A) and M41L (0.40, also in group B). The prevalence of NNRTI-resistance mutations was lower than those causing resistance to NRTI, with K103N being the most prevalent one (0.36 and 0.20 in groups A and B, respectively), followed by G190A/S (0.20 and 0.19 in groups B and E, respectively). In the complete subtype B Basque dataset, mutations associated with resistance to PIs were also present with low prevalence except L90M and M46I/L (0.13 and 0.11, respectively). The most frequent NRTI-resistance mutations were M184I/V (0.36), L215Y/F (0.26), M41L (0.23), D67N (0.17), L210W (0.15), K70E/R (0.13), and K219D/Q/E/R (0.12). The most frequent NNRT-resistance mutation was K103N/S (0.22), the only one with prevalence >0.10.

4. Discussion

We have analyzed 1727 HIV-1 subtype B sequences from different patients obtained from health centers in the Basque Country, Spain, between 2001 and 2008 to assess the HIV-1 epidemics in this population. The large size of the dataset and the time-span in which these sequences were obtained provide enough confidence to consider the results obtained in this work as representative of the epidemic scenario of HIV-1 in this region.

The results obtained from this work suggest that the HIV-1 subtype B epidemic in the Basque Country is characterized by a majority of infections occurring as isolated introductions of the virus, although a representative 25.5% of the patients were included in transmission clusters, which ranged in size between 2 and 18 individuals. This proportion is much lower than the 47% sequences grouping in transmission clusters found by Cuevas et al. (2009) among newly diagnosed individuals. The smaller size of their dataset (261 vs 1727 patients), methodological

Please cite this article as: Patiño-Galindo, J.A., et al., Transmission dynamics of HIV-1 subtype B in the Basque Country, Spain, Infection, Genetics and Evolution (2015), http://dx.doi.org/10.1016/j.meegid.2016.02.028

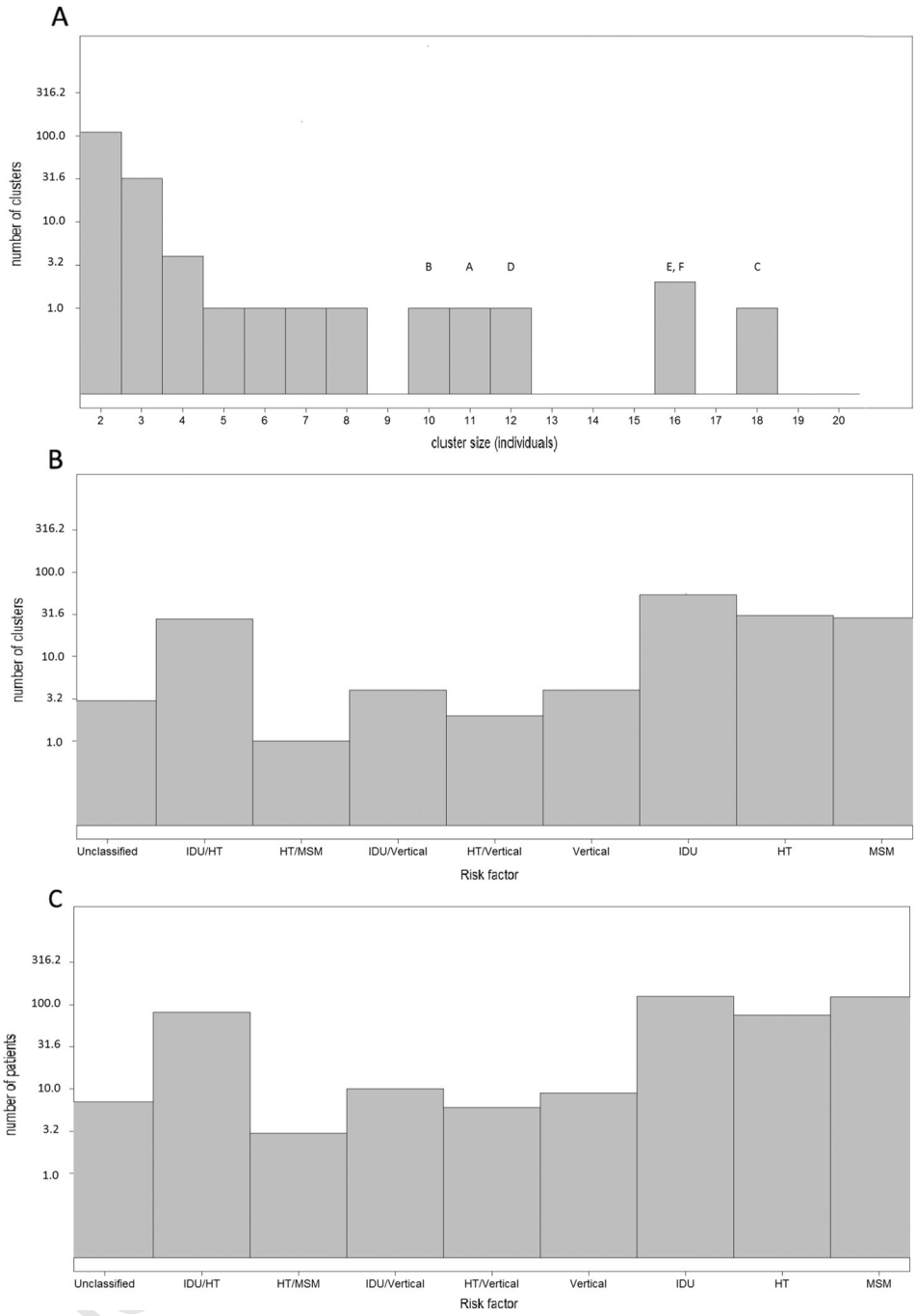

Fig. 1. Distribution of sizes, in log scale, of the 156 transmission groups found in the Basque Country (2001–2008) with the phylogenetic analyses. Block letters on each bar indicate the 6 main transmission clusters that were analyzed using BEAST (panel 1 A), number of transmission clusters depending on the risk group in which their members are included (panel 1B) and total number of patients for each risk group included in transmission clusters (n = 441) (panel 1C).

differences in phylogenetic analyses, and the low number of subtype B reference sequences (n = 4) used in that study can explain the markedly different proportions of individuals included in transmission clusters between both analyses. An additional factor that might explain the differences observed is the inclusion of non-newly infected patients in our analyses, which may cluster with less frequency due to higher number of nucleotide substitutions (longer external branches).

We found 6 large clusters, with sizes ranging between 10 and 18 individuals, that represented almost 5% of the total Basque dataset. Five of these clusters had been reported previously by Cuevas et al. (2009). Cluster B was not detected previously, because none of its sequences was analyzed by these authors for the reasons explained above. Previous studies in different European populations found transmission groups that were mainly formed by MSM (Hue et al., 2005; Kouyos et al., 2010; Lewis et al., 2008). In fact, the four largest transmission clusters found in our analysis (C, D, E, F) were also formed by MSM. Hence, although the MSM population was less frequent than other risk groups in the Basque country sampling, they were the major group associated to transmission clusters. IDUs frequently clustered either as the only risk factor or including also transmissions through unprotected heterosexual sex (IDU/HT), HTs and MSM, thus portraying a more diverse scenario in which IDUs were present in most of the smaller transmission groups. Such clustering of IDUs and HTs has seldom been reported (Kouyos et al., 2010; Holmes et al., 1995).

Please cite this article as: Patiño-Galindo, J.A., et al., Transmission dynamics of HIV-1 subtype B in the Basque Country, Spain, Infection, Genetics and Evolution (2015), http://dx.doi.org/10.1016/j.meegid.2016.02.028

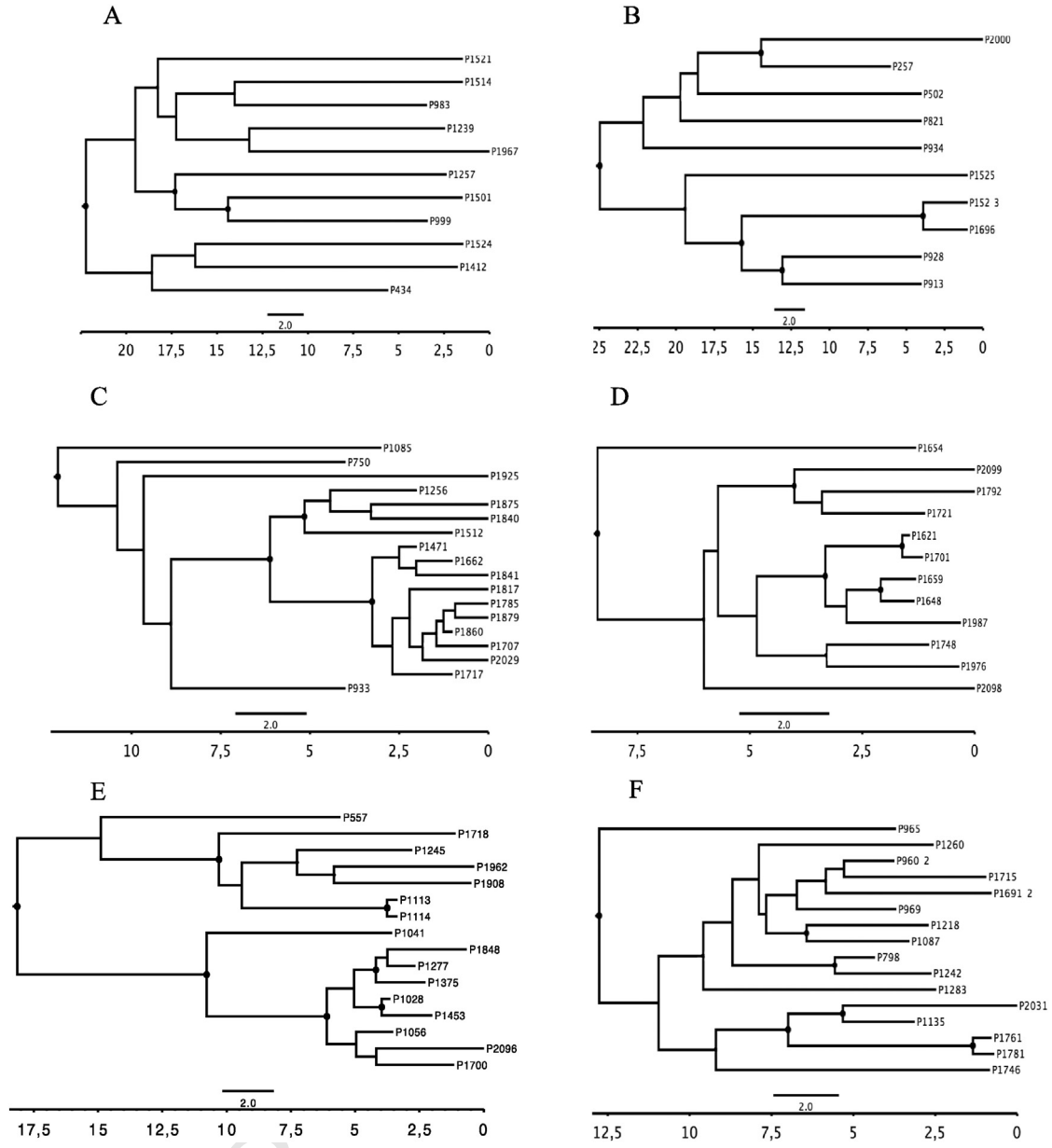

Fig. 2. Dated phylogenies of the six transmission clusters (A to F) analyzed with BEAST, as obtained with tip dating. Branch lengths represent years. Black dots represent nodes with posterior probability ≥0.90.

Previous studies in other European regions estimated that HIV-1 subtype B clusters initiated between the late 1960s and the early 1980s (Hue et al., 2005) or between the early 1990s and the beginning of 21st century (Lewis et al., 2008; Zehender et al., 2010). Dated phylogenies showed the MRCAs from most clades to have diversified from the mid-1980s to mid-1990s. The most recent clusters were D and F, both formed by MSM. Their dates of origin (tMRCAs) are coincident with the increase of infections among MSM after the

Table 1
Transmission routes, size (number of patients), range of sampling dates and root-to-tip divergence vs sampling date correlation coefficient for each clade and estimates of tree heights, internal branch lengths and substitution rates as obtained with BEAST under the best fitting demographic model, using a relaxed molecular clock model, with tip dating.

Cluster	Transmission	Taxa	Range*	Range (dates)	R (root-to-tip divergence vs sampling date correlation)	Best fitting demographic model	Median tree height (95% HPD)*	Median internal branch lengths (95% HPD)*	Median substitution rate x10e-3 (95% HPD)†
A	IDU/HT	11	5.57	Nov 02–Jun. 08	0.03	Exponential	1986.3 (1980.6–1996.9)	2.07 (0.88–4.71)	1.19 (0.54–2.09)
B	IDU	10	4.30	May 04–Oct. 08	0.46	Logistic	1983.8 (1964.8–1996.1)	3.08 (1.36–7.18)	1.54 (0.67–2.73)
C	Homosexual	18	3.98	Nov 04–Nov. 08	0.61	Exponential	1996.6 (1989.9–2001.6)	0.61 (0.30–1.31)	2.20 (1.17–3.38)
D	Homosexual	12	1.44	Jun 07–Nov. 08	(−0.16)	Constant	2000.5 (1992.8–2004.9)	1.00 (0.42–2.44)	1.93 (0.81–3.36)
E	Homosexual	16	5.57	Apr 03–Nov. 08	0.25	Logistic	1990.8 (1979.3–1998.9)	1.39 (0.61–3.09)	1.66 (0.80–2.73)
F	Homosexual	16	4.34	Apr 04–Sept. 08	0.43	Exponential	1996.1 (1988.1–2001.2)	1.22 (0.56–2.65)	1.57 (0.74–2.58)

* Time measured in years.
† Substitution per site and year.

Please cite this article as: Patiño-Galindo, J.A., et al., Transmission dynamics of HIV-1 subtype B in the Basque Country, Spain, Infection, Genetics and Evolution (2015), http://dx.doi.org/10.1016/j.meegid.2016.02.028

Table 2
Prevalence (proportion) of PI-NRTI- and NNRTI-resistance mutations in the HIV-1 subtype B Basque dataset (n = 1727 sequences) and the six largest transmission clusters (A to F).

PI

Cluster	D30N	V32I	M46I/L	I47V/A	G48V	I54 M/L	L76V	V82 A/F/T/S/L	I84V	N88S	L90M
Full dataset	0.05	0.02	0.11	0.01	0.01	0.01	0.01	0.09	0.03	0.01	0.13
A	0	0	0	0	0	0	0	0	0	0	0
B	0	0.10	0	0.10	0.10	0.10	0	0	0	0	0.30
C	0	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0	0

NRTI

	M41L	A62V	K65R	D67N	T69A/D/N/T	K70 E/R	L74I/V	V75I	F77L	Y115F	F116Y	Q151M	M184I/V	L210W	T215Y/F	K219 D/Q/E/R
Full dataset	0.23	0.03	0.02	0.17	0.05	0.13	0.07	0.01	0.01	0.02	0.01	0.02	0.36	0.15	0.26	0.12
A	0	0.09	0.18	0	0	0	0	0	0	0	0	0	0.27	0	0	0
B	0.4	0	0	0.30	0.10	0.10	0.10	0	0	0	0	0	0.50	0.30	0.50	0
C	0	0	0	0	0	0	0	0	0	0	0	0	0.06	0	0	0
D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E	0.19	0	0	0.19	0	0.13	0	0	0	0	0	0	0	0.13	0.13	0
F	0	0	0.06	0	0	0.06	0.06	0	0	0.06	0	0	0	0	1.00	0.06

NNRTI

	V90I	A98G	L100I	K101 E/H/P	K103N/S	V106 A/I/M	V108I	R138 A/G/K/Q/R	V179D/E/F/T/L	Y181C/I/V	Y188 C/L/H	G190A/S	H221Y	P225H
Full dataset	0.05	0.02	0.04	0.06	0.22	0.03	0.06	0.04	0.02	0.08	0.02	0.08	0.03	0.03
A	0.09	0	0.09	0	0.36	0	0.09	0	0.09	0.09	0.09	0	0.09	0.09
B	0	0	0	0.1	0.2	0	0	0	0.10	0.10	0	0.20	0	0.10
C	0.06	0	0	0	0.06	0	0.06	0	0.06	0	0	0	0	0.06
D	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0.13	0	0	0	0	0	0.13	0	0.19	0	0
F	0	0	0	0	0	0	0	0	0	0.06	0	0.06	0	0

commercialization of antiretroviral treatments and subsequent relaxation of prevention measures in this transmission group, especially a decrease of consistent condom usage (ECDC, 2013).

The estimated time between transmissions was significantly lower in MSM groups than in those including IDUs. Hughes et al. (2009) also found that HTs infected with HIV-1 subtypes A and C presented longer times between infections than MSM infected with HIV-1 subtype B. These results may be explained by the known high transmission risk of unprotected anal sex (Baggaley et al., 2010). In MSM who practice unprotected sex, the risk of HIV-1 infection is also increased due to role reversal during sexual intercourse: many individuals practice both insertive and receptive anal sex. This would increase HIV-1 spread by overcoming the low infection rates from receptive to insertive sexual partners (Beyrer et al., 2012).

NRTI resistance mutations were the most prevalent in the Basque dataset, with seven mutations present in more than 10% of the sampled sequences. PI and NNRTI resistance mutations were less frequent, with only two and one cases with a prevalence >0.10, respectively. For the six large transmission clusters, the prevalence of resistance mutations differed both among type of antiviral drug and among the analyzed transmission clusters. While only cluster B presented PI resistance mutations, all the clusters but one presented NRTI and NNRTI resistance mutations. It is important to mention the case of cluster F, in which all patients carry the low-level NRTI resistance mutation T215D. This possible example of drug resistance transmission in the Basque population has been reported previously (Cuevas et al., 2009; Vega et al., 2015). Hence, these results indicate that the dynamics of resistance mutations to antiretroviral drugs may differ among transmission clusters. However, this study lacks sufficient data to perform a detailed analysis of these patterns, and more extensive analyses are necessary to elucidate the factors originating these differences.

In conclusion, our results suggest an epidemic scenario of HIV-1 subtype B in the Basque Country in which most infections appear to correspond to independent introductions in the population, although there exist at least 6 major long-standing and diverse transmission groups. Most of these groups are characterized by a large proportion of MSM, in a disproportionally large frequency with respect to the presence of this risk group in the global sample. Furthermore, a shorter time between infections among MSM relative to other risk groups demonstrates the vulnerability of this collective to HIV-1 infections. Our results reinforce the need to implement prevention campaigns in the MSM population. This study also highlights the relevance and interest of applying Bayesian methods for phylogenetic and coalescent inference in epidemiology.

Acknowledgments

We thank Dr. Daniel Zulaica, coordinator of the Plan for AIDS Prevention and Control and Osakidetza-Basque Health Service for their support in the development of the study.

The Spanish Group of HIV-1 Antiretroviral Resistance Studies in the Basque Country: Álava—Hospital Txagorritxu: Agud J. M. Aldamiz M, Ayensa C, Barroso J, Lezaun M. J, Michaus L, Pérez-Ortolá R, and Portu J. J. H. de Santiago: Andia A and Labora A. Guipúzcoa—Complejo Hospitalario Donostia: Arrizabalaga J, Cilla G, Echevarría J, Iribarren J. A, Rodriguez-Arrondo F, Serrano-Bengoechea E, and Von Wichmann M. A; H. de Zumarraga: Bustillo M.A. Vizcaya — H. de Galdákano: López de Goicoechea M. J and Mayo J; H. de Cruces: Aguirrebengoa K, López Soria L, Goikoetxea J, Montejo M, and Bereciartura E; H. de Basurto: Baraia J. B, Cisterna R, Ezpeleta C, Ferrero O, Ibarra S, Imaz M, Muñoz- Sánchez J, Santamaría J. M, Sota M, and Zubero Z; H. San Eloy: Lizarraga M and Silvariño R.

This work was funded by Convenio de Colaboración entre Osakidetza-Servicio Vasco de Salud y el Instituto de Salud Carlos III (MVI 1158/03) and projects BFU2011-24112 and BFU2014-58656-R from MINECO (Spanish Government).

Please cite this article as: Patiño-Galindo, J.A., et al., Transmission dynamics of HIV-1 subtype B in the Basque Country, Spain, Infection, Genetics and Evolution (2015), http://dx.doi.org/10.1016/j.meegid.2016.02.028

References

Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M.A., Alekseyenko, A.V., 2012. Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. Mol. Biol. Evol. 29, 2157–2167.

Baggaley, R.F., White, R.G., Boily, M.C., 2010. HIV transmission risk through anal intercourse: systematic review, meta-analysis and implications for HIV prevention. Int. J. Epidemiol. 39, 1048–1063.

Bello, G., Aulicino, P., Ruchansky, D., Guimaraes, M., Lopez-Galindez, C., Casado, C., Chiparelli, H., Rocco, C., Mangano, A., Sen, L., Morgado, M., 2010. Phylodynamics of HIV-1 circulating recombinant forms 12_BF and 38_BF in Argentina and Uruguay. Retrovirology 7, 22.

Beyrer, C., Baral, S.D., van Griensven, F., Goodreau, S.M., Chariyalertsak, S., Wirtz, A.L., Brookmeyer, R., 2012. Global epidemiology of HIV infection in men who have sex with men. Lancet 380, 367–377.

Christin, P.A., Besnard, G., Edwards, E.J., Salamin, N., 2012. Effect of genetic convergence on phylogenetic inference. Mol. Phylogenet. Evol. 62, 921–927.

Costagliola, D., Descamps, D., Assoumou, L., Morand-Joubert, L., Marcelin, A.G., Brodard, V., Delaugerre, C., Mackiewicz, V., Ruffault, A., Izopet, J., 2007. Prevalence of HIV-1 drug resistance in treated patients: a French nationwide study. J. Acquir. Immune Defic. Syndr. 46, 12–18.

Cuevas, M.T., Munoz-Nieto, M., Thomson, M.M., Delgado, E., Iribarren, J.A., Cilla, G., Fernandez-Garcia, A., Santamaria, J.M., Lezaun, M.J., Jimenez, L., Lopez-Soria, L.M., Sota, M., Contreras, G., Najera, R., Perez-Alvarez, L., 2009. HIV-1 transmission cluster with T215D revertant mutation among newly diagnosed patients from the Basque Country, Spain. J. Acquir. Immune Defic. Syndr. 51, 99–103.

De Oliveira, T., Deforche, K., Cassol, S., Salminen, M., Paraskevis, D., Seebregts, C., Snoeck, J., van Rensburg, E.J., Wensing, A.M.J., van de Vijver, D.A., Boucher, C.A., Camacho, R., Vandamme, A.M., 2005. An automated genotyping system for analysis of HIV-1 and other microbial sequences. Bioinformatics 21, 3797–3800.

Donnelly, P., Tavaré, S., 1995. Coalescents and genealogical structure under neutrality. Annu. Rev. Genet. 29, 401–421.

Drummond, A., Oliver, G., Rambaut, A., 2003. Inference of viral evolutionary rates from molecular sequences. Adv. Parasitol. 54, 331–358.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

ECDC, 2013. Men who have sex with men. Monitoring implementation of the Dublin Declaration on Partnership to Fight HIV/AIDS in Europe and Central Asia: 2012 progress report (www.ecdc.europa.eu ed).

ECDC/WHO, 2010. HIV/AIDS Surveillance in Europe 2009. European Centre for Disease Prevention and Control, Stockholm.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321.

Holmes, E.C., 2004. The phylogeography of human viruses. Mol. Ecol. 13, 745–756.

Holmes, E.C., Zhang, L.Q., Robertson, P., Cleland, A., Harvey, E., Simmonds, P., Leigh Brown, A.J., 1995. The molecular epidemiology of human immunodeficiency virus type 1 in Edinburgh. J. Infect. Dis. 171, 45–53.

Hue, S., Clewley, J.P., Cane, P.A., Pillay, D., 2004. HIV-1 pol gene variation is sufficient for reconstruction of transmissions in the era of antiretroviral therapy. AIDS 18, 719–728.

Hue, S., Pillay, D., Clewley, J.P., Pybus, O.G., 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.

Hughes, G.J., Fearnhill, E., Dunn, D., Lycett, S.J., Rambaut, A., Leigh Brown, A.J., on behalf of the UK HIV Drug Resistance Collaboration, 2009. Molecular phylodynamics of the heterosexual HIV epidemic in the United Kingdom. PLoS Pathog. 5, e1000590.

Johnson, V.A., Calvez, V., Gunthard, H.F., Paredes, R., Pillay, D., Shafer, R.W., Wensing, A.M., Richman, D.D., 2013. Update of the drug resistance mutations in HIV-1: March 2013. Top. Antivir. Med. 21, 6–14.

Kingman, J.F.C., 1982. The coalescent. Stoch. Process. Appl. 13, 235–248.

Kouyos, R.D., von Wyl, V., Yerly, S., Böni, J., Taffé, P., Shah, C., Bürgisser, P., Klimkait, T., Weber, R., Hirschel, B., Cavassini, M., Furrer, H., Battegay, M., Vernazza, P.L., Bernasconi, E., Rickenbach, M., Ledergerber, B., Bonhoeffer, S., Günthard, H.F., 2010. Molecular epidemiology reveals long-term changes in HIV type 1 subtype B transmission in Switzerland. J. Infect. Dis. 201, 1488–1497.

Lewis, F., Hughes, G.J., Rambaut, A., Pozniak, A., Leigh Brown, A.J., 2008. Episodic sexual transmission of HIV revealed by molecular phylodynamics. PLoS Med. 5, e50.

Liu, T.F., Shafer, R.W., 2006. Web resources for HIV type 1 genotypic-resistance test interpretation. Clin. Infect. Dis. 42, 1608–1618.

Martin, D., Rybicki, E., 2000. RDP: detection of recombination amongst aligned sequences. Bioinformatics 16, 562–563.

Martin, D.P., Lemey, P., Lott, M., Moulton, V., Posada, D., Lefeuvre, P., 2010. RDP3: a flexible and fast computer program for analyzing recombination. Bioinformatics 26, 2462–2463.

Martin, D.P., Posada, D., Crandall, K.A., Williamson, C., 2005. A modified bootscan algorithm for automated identification of recombinant sequences and recombination breakpoints. AIDS Res. Hum. Retrovir. 21, 98–102.

Moya, A., Holmes, E.C., González-Candelas, F., 2004. The population genetics and evolutionary epidemiology of RNA viruses. Nat. Rev. Microbiol. 2, 279–288.

Padidam, M., Sawyer, S., Fauquet, C.M., 1999. Possible emergence of new geminiviruses by frequent recombination. Virology 265, 218–225.

Posada, D., Crandall, K.A., 2001. Evaluation of methods for detecting recombination from DNA sequences: computer simulations. Proc. Natl. Acad. Sci. U. S. A. 98, 13757–13762.

Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 - approximately maximum-likelihood trees for large alignments. PLoS One 5, e9490.

R Development Core Team, 2011. R: A language and environment for statistical computing. (http://www.R-project.org/ed. Vienna, Austria).

Roberts, J.D., Bebenek, K., Kunkel, T.A., 1988. The accuracy of reverse transcriptase from HIV-1. Science 242, 1171–1173.

Shapiro, B., Rambaut, A., Drummond, A.J., 2006. Choosing appropriate substitution models for the phylogenetic analysis of protein-coding sequences. Mol. Biol. Evol. 23, 7–9.

Smith, J.M., 1992. Analyzing the mosaic structure of genes. J. Mol. Evol. 34, 126–129.

UNAIDS/WHO, 2013. Global Report: UNAIDS Report on the Global AIDS Epidemic (Geneva).

Vega, Y., Delgado, E., Fernández-García, A., Cuevas, M.T., Thomson, M.M., Montero, V., Sánchez, M., Sánchez, A.M., Pérez-Álvarez, L., Spanish Group for the Study of New HIV, 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.

Zehender, G., Ebranati, E., Lai, A., Santoro, M.M., Alteri, C., Giuliani, M., Palamara, G., Perno, C.F., Galli, M., Lo, P.A., Ciccozzi, M., 2010. Population dynamics of HIV-1 subtype B in a cohort of men-having-sex-with-men in Rome, Italy. J. Acquir. Immune Defic. Syndr. 55, 156–160.

Please cite this article as: Patiño-Galindo, J.A., et al., Transmission dynamics of HIV-1 subtype B in the Basque Country, Spain, Infection, Genetics and Evolution (2015), http://dx.doi.org/10.1016/j.meegid.2016.02.028