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Genome-scale analysis of evolutionary rate and selection in a fast-expanding Spanish cluster of HIV-1 subtype F1

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Abstract

This work is aimed at assessing the presence of positive selection and/or shifts of the evolutionary rate in a fast-expanding HIV-1 subtype F1 transmission cluster affecting men who have sex with men in Spain. We applied Bayesian coalescent phylogenetics and selection analyses to 23 full-coding region sequences from patients belonging to that cluster, along with other 19 F1 epidemiologically-unrelated sequences. A shift in the overall evolutionary rate of the virus, explained by positively selected sites in the cluster, was detected. We also found one substitution in *nef*(H89F) that was specific to the cluster and experienced positive selection. These results suggest that fast transmission could have been facilitated by some inherent genetic properties of this HIV-1 variant.

Keywords

HIV-1; subtype F1; transmission cluster; men who have sex with men; selection

1 Introduction

In Western Europe, the HIV-1 epidemic is dominated by subtype B, especially among men who have sex with men (MSM) (Abecasis et al., 2013). However, a large HIV-1 subtype F1

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Author contributions

FD, MTC, ED, MS, LPA, and MMT participated in the sequencing of some of the analysed samples. JAPG, MMT, RS, FGC and JMC conceived and designed the study. JAPG did the analyses. JAPG and JMC conceived the analyses and drafted the manuscript. MMT, RS, and FGC provided critical review and editing of the manuscript. All authors have seen and approved the paper.

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transmission cluster, affecting more than 100 MSM in Spain and other Western European countries, was recently reported. This cluster was identified in an HIV-1 molecular epidemiological surveillance study in the region of Galicia, Northwest Spain, with the first cases diagnosed in 2009 and subsequent rapid expansion resulting in an increase in the prevalence of subtype F1 infections among new HIV-1 diagnoses in 2012 to 20% in Galicia and 43% in the city of A Coruña. Viruses belonging to this cluster were also identified in several other Spanish regions and in four Western European countries (Delgado et al., 2015; Thomson et al., 2012). This subtype is rare in Europe, displaying a prevalence of less than 2% (Abecasis et al., 2013). The transmission cluster presented no major resistance-associated mutations, but was characterized by a rapid expansion among MSM in Spain, which could be accounted for by the epidemiological scenario of HIV-1 in Europe (Bezemer et al., 2008; ECDC, 2013). Nonetheless, it has also been suggested that its efficient transmission might be linked to some intrinsic genetic properties of the viral lineage, given its unusual large size (Delgado et al., 2015; Thomson et al., 2012) and the fact that patients included in this outbreak presented significantly higher viral loads and poorer response to antiretroviral treatment than other HIV-1 variants (Cid-Silva et al., 2018; Pernas et al., 2014).

The hypothesis of positive selection acting on HIV in fast-expanding transmission clusters contrasts with previous works suggesting that adaptive selection is weaker during early infection, when most infections occur due to the lack of awareness of HIV serological status (Maljkovic Berry et al. 2007). Furthermore, mutations that are adaptive in one individual are possibly maladaptive in other individuals and, consequently, HIV transmitted to a new host undergoes reversions of mutations that adaptively occurred in the donor. Altogether, these facts would slow down the evolutionary rate of a viral lineage involved in a fast-expanding epidemic (Maljkovic Berry et al. 2007; Lythgoe & Fraser 2012). However, fast transmissions have also been associated with higher evolutionary rates (Pybus & Rambaut, 2009; Salemi et al., 1999). These studies argued that, if within-host rates slow down during infection, faster transmissions should result in higher long-term evolutionary rates. Also, faster transmission rates could lead mutations to be more likely to occur, which may increase the evolutionary rate.

For this study, the complete coding sequences of 24 publicly available Spanish isolates from a fast-expanding HIV-1 subtype F1 transmission cluster were retrieved. In addition, sequences of 25 HIV-1 subtype F1, worldwide-distributed isolates were included in the analysis. First, we evaluated the impact of recombination in our samples in order to remove its effect in subsequent analyses. Then, we performed Bayesian coalescent analyses to compare genomic evolutionary rates of the Spanish transmission cluster and the epidemiologically unrelated sequences. Finally, we assessed the effect of selection on the evolution of the transmission cluster.

2 Materials and methods

2.1 Dataset

Twenty four full coding sequences (CDS) from different patients belonging to the Spanish transmission cluster, along with 25 HIV-1 subtype F1 sequences from epidemiologically

unrelated patients, were retrieved from the Los Alamos National Laboratory (LANL) HIV Sequence Database (<http://www.hiv.lanl.gov>). Accession numbers and information on these sequences is provided in Supplementary Table 1). The correct subtype assignment was corroborated with the COMET HIV-1 subtyping tool (<http://comet.retrovirology.lu>). A recombination analysis was performed with RDP4, using five different methods of recombination detection: RDP, Geneconv, Bootscan, Maxchi and Chimaera (Martin et al., 2015). Only those breakpoints detected by at least two of the five methods implemented in RDP4 were accepted. Consequently, recombinant sequences, as well as those lacking information on the collection date, were excluded from subsequent analyses. An alignment consisting of the concatenated non-overlapping regions from all HIV-1 genes (considering the HIV-1 reference sequence HXB2, accession number K03455) was obtained using MAFFT v7 (Katoh & Standley, 2013), and regions with poor homology (“gappy” sites) were trimmed using TrimAl (Capella-Gutiérrez, Silla-Martínez, & Gabaldón, 2009). The final alignment is available upon request.

2.2 Inference and comparison of the genomic evolutionary rates

The selected set of HIV-1 F1 subtype sequences was subjected to a Bayesian coalescent analysis in order to estimate the genomic evolutionary rate of the transmission cluster and to compare it with that of the HIV-1 lineages not included in the Spanish cluster. The presence of molecular clock signal was assessed with a root-to-tip divergence *versus* sample time correlation analysis, performed with Path-O-Gen (now renamed as TempEst; Rambaut et al. 2016), using as input a maximum-likelihood (ML) phylogenetic tree obtained with PhyML (Guindon et al., 2010) under the GTR+ Γ (4 CAT) model. The Bayesian coalescent analysis was performed with BEAST v1.82 (Drummond, Suchard, Xie, & Rambaut, 2012), under a nonparametric demographic model (Bayesian Skyline Plot), combined with the GTR + Γ (4 CAT) nucleotide model and a random local molecular clock model. This clock model has previously been reported to detect evolutionary rate shifts in specific lineages of a phylogeny (Fourment & Holmes, 2014). The comparison of the evolutionary rates estimated from lineages belonging to the transmission cluster and those falling outside was performed by means of a randomization test (Abecasis, Vandamme, & Lemey, 2009).

2.3 Positive selection analysis

The presence of positive selection in the transmission cluster was tested with MEDS, as implemented in HyPhy, a ML method used to detect independent sites under directional selection (adaptive evolution in which mutations to a particular amino acid are selected) (Murrell et al. 2012). A ML phylogenetic tree previously obtained with PhyML (GTR + Γ 4) for the molecular clock signal analysis was used as input, specifying *a priori* that all lineages included in the transmission cluster were susceptible of being under directional selection (foreground branches). Only positively selected sites meeting two conditions were considered. First, in order to minimize the presence of false positives (i.e., sites evolving under neutral evolution or sites in which a deleterious mutation had occurred but had not been removed from the viral population at the time of sampling), those sites in which amino acid variability was caused only by singletons (affecting only one sequence in the phylogeny) and/or only in one step of change along the phylogeny were excluded from the list of positively selected sites. Second, given that the goal of these analyses was to find

evidence of selection specific to the transmission cluster, we only considered those positively selected sites in which the consensus amino acid of the transmission cluster differed from that of the other F1 sequences, as performed with VESPA; (Korber & Myers 1992). Additionally we also required that the amino acid distribution differed significantly between both groups of sequences according to Fisher's exact tests, with P values corrected by using the false discovery rate (Benjamini & Hochberg 1995).

Positively selected sites were mapped onto the HXB2 reference genome. A search was performed in LANL database to identify those sites located in human epitopes (antibody, CD8⁺ and/or CD4⁺ T cells), as well as those sites where the transmission cluster presented a consensus amino acid associated with CD8⁺ and/or CD4⁺ T cell immune escape.

3 Results

Recombination analyses performed with RDP4 found evidence of recombination in 3 of the 49 full-genome sequences, including one sequence from the transmission cluster (sequence name VA0043). These sequences were removed from the dataset, as well as those with no collection date information. Consequently, the final HIV-1 subtype F1 dataset consisted of 23 CDS sequences from the Spanish transmission cluster and 19 epidemiologically unrelated HIV-1 F1 sequences (Supplementary Table 1). The resulting alignment of concatenated HIV-1 genes, in their correct coding frame, was 7962 nt long (more than 90% of the full HIV-1 CDS).

The dataset of 42 HIV-1 F1 genomic sequences displayed sufficient clock-like signal as to proceed with the Bayesian coalescent analysis ($R^2 = 0.72$). The resulting dated Bayesian phylogenetic tree displayed evidence for the existence of an acceleration in the evolutionary rate in the transmission cluster, compared with the rest of lineages in the phylogeny (lineages outside the transmission cluster: median = 2.00×10^{-3} substitutions per site and year-s/s/y-, 95%HPD = $1.60 \times 10^{-3} - 2.30 \times 10^{-3}$ s/s/y; lineages within the cluster: median = 3.20×10^{-3} s/s/y, 95%HPD = $2.10 \times 10^{-3} - 3.90 \times 10^{-3}$ s/s/y.; Figure 1a), although the difference was not statistically significant ($P = 0.09$).

MEDS detected 89 sites significantly associated with directional selection, but only 19 sites met the inclusion criteria. Most sites detected to be under selection were located in the *env* gene. Most positively selected sites were included in CD8⁺ or CD4⁺ T cell epitopes (Table 1).

In order to assess whether positive selection could explain the acceleration of the evolutionary rate in the transmission cluster, we repeated the BEAST analyses using an unlinked molecular clock model, in which two different partitions (all positively selected sites detected and the rest of the alignment) were allowed to have different molecular clock models. We found a significant acceleration in the evolutionary rate of the transmission cluster occurring in positively selected sites (lineages outside the transmission cluster: median = 4.90×10^{-3} s/s/y, 95%HPD = $3.90 \times 10^{-3} - 6.0 \times 10^{-3}$ s/s/y; lineages within the cluster: median = 12.70×10^{-3} s/s/y, 95%HPD = $9.60 \times 10^{-3} - 16.20 \times 10^{-3}$ s/s/y; $P < 0.001$. Supplementary Figure 1A) but not in the rest of the alignment (lineages outside the

transmission cluster: median = 1.80×10^{-3} s/s/y, 95%HPD = $1.50 \times 10^{-3} - 2.20 \times 10^{-3}$ s/s/y; lineages within the cluster: median = 1.70×10^{-3} s/s/y, 95%HPD = $1.40 \times 10^{-3} - 2.00 \times 10^{-3}$ s/s/y; $P = 0.18$. Supplementary Figure 1B). In order to validate this finding, we repeated the MEDS positive selection analyses choosing the unrelated F1 sequences as foreground lineages, and performed another BEAST analyses partitioning the selected sites found for this subset and the rest of the genome. No acceleration in the evolutionary rate was found.

Regarding positively selected sites, *nef*H89F substitution (Table 1), which was located in CD8⁺ and CD4⁺ T cell human epitopes (*nef* positions 84-92, wild type sequence AVDLSHFLK), was significantly overrepresented in the transmission cluster (18/23 sequences) compared to the other HIV-1 F1 sequences (3/19; Fisher test: $P < 0.001$). This substitution has been previously associated with a decreased CD8⁺ T cell response for different human HLA alleles (Fukada et al., 2002; Hoof et al., 2010) (Table 1; Figure 1b). It is noteworthy that this epitope has been studied in subtypes A, B, C, D and CRF01_AE but not in subtype F1. However, in our dataset the consensus amino acid of all the positions was wild type with the exception of *nef*89.

4 Discussion

This work was aimed at assessing the evolutionary dynamics of an unusually large, fast-expanding HIV-1 F1 subtype transmission cluster, which affected more than 100 MSM in Spain, and has been associated with higher plasma viral loads and poorer virological response to antiretroviral treatment than other HIV-1 variants. Despite the relatively small number of sequences analysed here, a genome-scale analysis has allowed to detect an acceleration of the evolutionary rate of this HIV-1 F1 cluster associated with fast transmissions, which could be explained by the effect of positive selection in this cluster. This is in agreement with previous studies that associated the evolutionary rates with the speed at which transmissions occur in an epidemic (Salemi et al. 1999; Pybus & Rambaut 2009).

The presence of positive selection in transmission clusters has been rarely reported. By performing diversifying selection analyses on three different HIV-1 CRF01_AE clusters affecting MSM occurring in China, Peng et al. (2015) detected that positive selection was present in approximately 5% of the amino acid sites in the *env* gene. In the Spanish F1 transmission cluster, we also found significant evidence of directional positive selection. This result suggests that, although transmission events occurred fast and probably during the first months after infection, this viral lineage had enough time to adapt to the hosts' immune systems. Indeed, most positively selected sites were located in *env*, and a majority of them fell within CD8⁺ and/or CD4⁺ T cell epitopes. One of these positions, *nef*H89F, has been reported to be associated with immune escape from CD8⁺ T cells (Fukada et al., 2002; Hoof et al., 2010).

The rapid expansion of this transmission cluster could be explained by the epidemic scenario characterized by an increased risky sexual behavior among MSM (Bezemer et al., 2008; Diez et al., 2014; González-Domenech et al., 2018; Patiño-Galindo et al., 2017). However, the positively selected positions detected, and particularly *nef*H89F, might be associated

with the already reported high plasma viral loads in the infected patients, that could have facilitated transmission. At this point, it is important to note that variants from this cluster have already been detected in seven Spanish regions, the last cases described in Catalonia (Bes et al., 2017). Also, unpublished results have recently shown the rapid expansion of a new F1 subcluster, closely related with the Spanish variant, presenting 188 reported cases between MSM (Vinken et al., 2017). Altogether, these results remark the increasing prevalence of F1 subtype in geographically distant areas from Europe and supports the hypothesis that the introduction and dissemination of these variants strongly rely on the combination of the epidemic scenario and the viral biological properties.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

a) Dated phylogenetic tree obtained with BEAST (time measured in years) for the HIV-1 subtype F1 analyzed using the random local clock model for the full CDS. "I" labels on nodes represent clades with posterior probability = 1.0, and branches are colored depending on their median evolutionary rate. b) Dendrogram displaying the evolutionary history of the *nef*H89F mutation (blue color) in the transmission cluster.

Table 1

Positively selected positions in the transmission cluster that met the inclusion criteria. Signature amino acid (AA) inside and outside the cluster, location in human antibody (AB), and CD8⁺ and/or CD4⁺ epitopes is specified. For each position, codon is indicated at the corresponding gene (reference sequence HXB2, Genbank accession number K03455).

Position	Signature AA (cluster)	Signature AA (no cluster)	AB epitope	CD8+ epitope (A-list, best defined CTLs)	CD4+ epitope
pol 95	T	P	no	yes	no
vif 50	K	R	no	yes	no
vif 127	Y	H	no	no	no
vpu 14	L	V	no	no	yes
vpu 19	V	A	no	no	yes
env 94	N	D	no	no	yes
env 173	S	H (<50%)	yes	no	yes
env 271	V	I	no	no	yes
env 297	S	T	no	no	yes
env 389	K	G	no	no	yes
env 442	H	N	yes	no	yes
env 467	I	T	yes	no	yes
env 565	M	L	yes	yes	yes
env 621	Q	E	no	no	yes
env 750	S	N	no	no	no
env 797	W	G	no	yes	no
nef 89 *	F	H	no	yes	yes
nef 120	F	Y	no	yes	yes
nef 176	A	E	no	no	yes

* Mutation associated with escape to immune response.