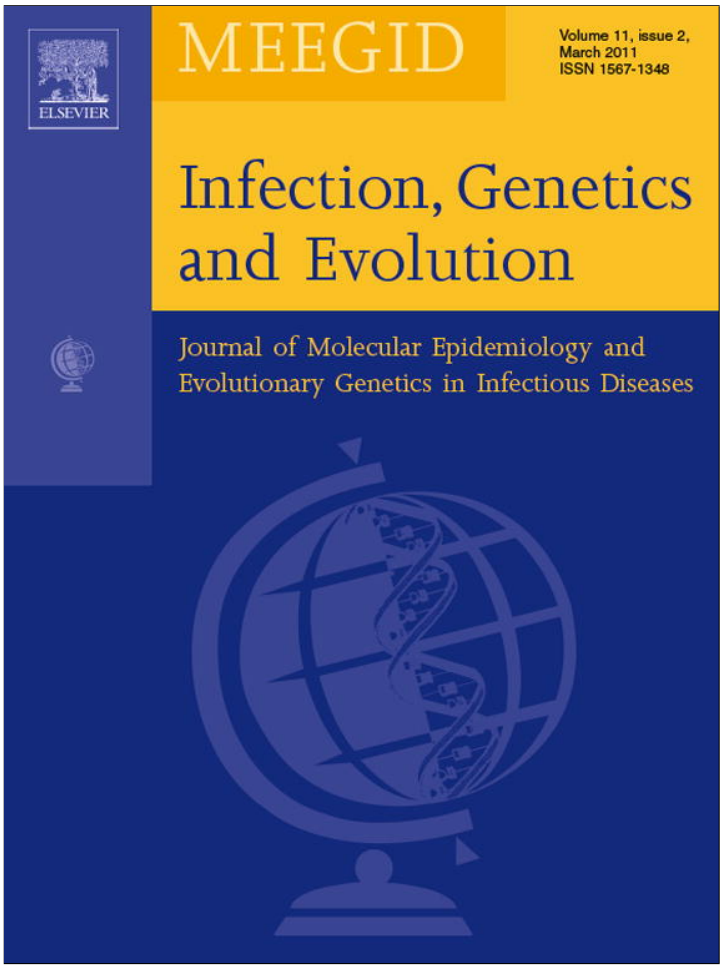

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# Longitudinal analysis of HIV-1 BF1 recombinant strains in vertically infected children from Argentina reveals a decrease in CRF12_BF *pol* gene mosaic patterns and high diversity of BF unique recombinant forms

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ABSTRACT

The HIV-1 epidemic associated to BF1 recombinants in South America is both complex and intriguing, with an underestimated diversity of recombinant structures. Our aim was to explore the characteristics and temporal dynamics of the HIV-1 BF1 epidemic in Argentina, through the study of 172 HIV-1 *pol* BF1 recombinant sequences obtained from HIV-1 vertically infected patients born from 1986 to 2008. Recombination patterns were characterized by bootscanning, subtype signature analysis, and phylogenetic approaches. Proportion of sequences sharing common ancestry and recombination breakpoints with the Circulating Recombinant Form (CRF) CRF12_BF was compared against sequences with a non-CRF12_BF pattern in three study periods, and by fitting the data to a logistic model. Twenty-eight HIV-1 *pol* BF1 mosaic structures were identified, including four of the seven South-American CRF_BF-like patterns. However, common ancestry of these sequences with reference CRF strains only confirmed the presence of CRF12_BF (51.1%) and CRF17_BF (1.2%) among the Argentine BF *pol* sequences. Most non-CRF_BF-like recombinant patterns shared at least one common recombination breakpoint with CRF12_BF. The number of transmissions caused by CRF12_BF viruses decreased in a linear way over time, from 69% in the period 1986–1993 to 46% in 2001–2008. In conclusion, the diversity of HIV-1 *pol* BF1 recombinant structures in Argentina is much more complex than previously described, with at least two CRFs_BF and 26 BF1 unique recombinant forms. For the first time, we provide evidence of a decrease in the proportion of CRF12_BF viruses transmitted from mother-to-child since the start of the epidemic to the present time in Argentina.



## 1. Introduction

Genetic subtyping has been a useful tool to track HIV spread and to study the genesis of the epidemic in specific areas. In Argentina, the HIV-1 epidemic is mostly represented by subtype B, and a wide variety of BF1 recombinant strains. Other HIV-1 subtypes and recombinant forms (A, C, F1, BC, BCF, A2D, and AG) are very atypical, representing less than 1% of the circulating strains (Aulicino et al., 2005a,b, 2007; Carrion et al., 2004; Dilernia et al., 2007; Gomez Carrillo et al., 2004; Pando et al., 2006).

The description of first BF1 Circulating Recombinant Form (CRF) – the CRF12_BF – in Argentina and Uruguay, came along with the initial full-length characterization of local HIV-1 sequences (Carr et al., 2001). Thereafter, several studies have shown that most BF recombinants circulating in Argentina represent either CRF12_BF structures or diverse BF recombinants related to it (Carr et al., 2001; Thomson et al., 2002; Quarleri et al., 2004). However, the prevalence of CRF12_BF varies greatly (from 25% to 75%) depending on the genomic region and population under study (Carr et al., 2001; Dilernia et al., 2007; Quarleri et al., 2004). A second CRF_BF, also described in Argentina in 2001, is the CRF17_BF. Its recombinant structure, based on the single sequence ARMA038 (GenBank accession no. AY037281), is very similar to that of CRF12_BF; but there is virtually no information regarding the origin, prevalence and spread of this CRF. Other CRFs_BF have also been described in South America: CRF28_BF and CRF29_BF in Sao Paulo (Brazil) (De Sa Filho et al., 2006); CRF39_BF and

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CRF40_BF in Rio de Janeiro (Brazil) (Guimaraes et al., 2008); CRF38_BF in Uruguay (Ruchansky et al., 2009); and CRF44_BF in Chile (Delgado et al., 2010); but their presence in Argentina has not yet been investigated.

Unlike HIV-1 infection in adults, multiple HIV-1 strains are not expected to co-circulate in children and the precise timing of the transmission event can be traced to the time of birth, providing a unique opportunity to explore the complex and intriguing dynamics of the HIV-1 BF1 epidemic in Argentina, where mother-to-child transmission still accounts for around 6% of all HIV-1 infections (2009 National HIV/AIDS Bulletin: http://www.msal.gov.ar/sida/pdf/Boletin-E-2009.pdf). Only one previous study analyzed HIV-1 diversity in a small group of Argentine children ($n = 23$) born from 1984 to 2000, showing a high genetic diversity in the *vpu-env* segment of BF1 recombinants (Gomez Carrillo et al., 2002). Since then, no other evidence has been provided regarding HIV-1 genetic variability associated to BF1 recombinants in the pediatric population. Therefore, the aim of this study was to investigate the complexity of the HIV-1 epidemic associated to BF1 recombinants in Argentina and the relative prevalence CRFs_BF and BF Unique Recombinant Forms (URFs_BF) over time through the analysis of the HIV-1 *pol* gene in a group of 172 vertically infected children born from 1986 to 2008.

## 2. Materials and methods

### 2.1. Study samples

Samples were obtained from HIV-1 vertically infected patients who attended Hospital de Pediatría "J.P. Garrahan" in Buenos Aires for HIV-1 diagnosis, follow-up, or Genotypic Resistance Testing from 1994 to December 2008. In a group of 300 children, we had previously investigated the HIV-1 subtype to characterize the HIV-1 molecular epidemiology of our population (Aulicino et al., 2006, 2010). In 85% of the children, HIV-1 strains had at least one F1 or BF1 genomic segment, and were recognized as possible BF1 recombinants. Of them, we randomly selected a group of 172 children born from 1986 to 2008 to carry out the present study. Informed consent was obtained from parents or legal guardians in all cases.

### 2.2. Extraction, amplification and sequencing of HIV-1 pol segments

Whole blood samples were obtained by venipuncture into EDTA-containing tubes. After centrifugation, plasma was separated and stored at −70 °C for RNA viral extraction. After Ficoll-Hypaque density gradient centrifugation, peripheral blood mononuclear cells (PBMCs) were treated with a lysis buffer containing Proteinase K and stored at −20 °C.

In a group of 74 patients, HIV-1 DNA was directly amplified from PBMC lysates. In another group of 98 patients, HIV-1 RNA was obtained from 140 μl of stored plasma using the QIAmp viral RNA extraction Kit (Qiagen, Germany) and amplified by RT-PCR followed by a nested PCR as described elsewhere (Aulicino et al., 2010), to obtain a 1168 bp HIV-1 *pol* fragment spanning the last part of *gag* (p6), the complete protease (PR) and the first 230 codons of the reverse transcriptase (RT) (positions 2068–3235 relative to the HXB2 reference strain; GenBank accession number K03455).

The PCR products were purified with QIAquick purification columns (QIAGEN, Germany), and then sequenced using the DYEnamic ET Terminator Cycle sequencing kit (Amersham Biosciences, England). Sequencing reactions were run on an ABI PRISM 3100 automated sequencer and analyzed with the DNA Sequencing Analysis Software v3.3 (Applied Biosystems, USA). Overlapping sequences were assembled into the HIV-1 *pol* consensus sequence using DNASTAR Lasergene version 7.1 and sites with ambiguous bases were changed to the most frequent base at each position.

Sequences used in this study correspond to GenBank accession numbers FJ525802–FJ525872, and HQ158151–HQ158273.

### 2.3. Characterization of HIV-1 pol BF1 recombination profiles

Nucleotide sequences were aligned by CLUSTAL X program integrated in the MEGA 3.1 program (Kumar et al., 2004) and four strategies were used simultaneously to identify recombination breakpoints and characterize the HIV-1 sequences as CRF_BF-like or URF_BF using the HIV-1 *pol* alignment:

(i) Bootscanning was performed with Simplot software version 3.5.1 (Lole et al., 1999). In bootscan analyses, bootstrap values were determined in Neighbor-Joining (NJ) trees constructed using the Kimura two-parameter model, based on 100 re-samplings, supporting branching with the consensus sequences within a 200 bp window moving in steps of 20 bases. Individual query sequences were compared to reference sequences from subtypes B, F1, A, and C. Sequences were considered to have evidence of recombination if SimPlot identified sites of recombination with a maximum bootstrap percentage of at least 70%. Segments not reaching 70% bootstrap value neither for subtype B nor F was considered unresolved by Simplot, and plotted as ambiguous for both subtypes.

(ii) A NJ phylogenetic tree was built only with the CRFs_BF reference sequences, obtaining a high bootstrap support for each CRF_BF node. Query BF1 sequences were added to this tree in groups of 10 sequences to verify the clustering with CRF_BF references. If bootstrap support of CRFs_BF clusters fell below 70%, then conflicting sequences were identified and subject to more detailed analysis. NJ trees were constructed under the Tamura-Nei substitution model in 1000 bootstrapped data sets, as implemented in MEGA 3.1 program (Kumar et al., 2004).

(iii) Maximum Likelihood (ML) and Bayesian phylogenetic trees for the final HIV-1 *pol* alignment including all BF1 sequences that could be associated with a specific CRF-structure were built to confirm the overall topology and clade support. Phylogenetic trees were constructed under the GTR nucleotide substitution model, with a gamma-distribution model of among site rate heterogeneity and a proportion of invariable sites. Bayesian tree was estimated using MrBayes (Ronquist and Huelsenbeck, 2003). Two runs of four chains each were run for $50 \times 10^6$ generations, with a burn-in of $5 \times 10^6$ generations. Convergence of parameters was assessed by calculating the Effective Sample Size using TRACER v1.4 (http://beast.bio.ed.ac.uk/Tracer), after excluding an initial 10% for each run. ML trees were reconstructed with PhyML (Guindon and Gascuel, 2003) using an online web server (Guindon et al., 2005). Heuristic tree searches were performed using the SPR branch-swapping algorithm, and the approximate likelihood-ratio test (aLRT) based on a Shimodaira–Hasegawa-like procedure was used as a statistical test to calculate branch support. Trees were visualized using the FigTree v1.1.2 program (http://tree.bio.ed.ac.uk/software/figtree/).

CRFs_BF reference sequences used in phylogenetic reconstructions were as follows – **CRF12_BF**: A32989, A32879, ARMA159, URTR23, and URTR35; **CRF17_BF**: PCR0155, BOL119, PSP0073, PSP0096, ARG2233, ARMA038, and ARG1139; **CRF28_BF**: BREPM12817, BREPM12609, and BREPM12313; **CRF29_BF**: BREPM16704, BREPM11948, 99UFRJ_1, and BREPM119; **CRF38_BF**: UY05-4752, UY04-

4022, and UY03-3389; **CRF39_BF**: 03BRRJ327, 03BRRJ103, and 04BRRJ179; **CRF40_BF**: 04BRSQ46, 05BRRJ200, 04BRRJ115, and 05BRRJ055; **CRF44_BF**: CH12 and CH80.

(iv) Subtype signature analysis was used in conflicting BF1 sequences. Signature nucleotides that discriminate between subtypes B and F1 were defined as those found in at least 90% of the reference sequences of one subtype and in less than 10% of those of the other. Reference full-length subtype F1 sequences: BZ163, 93BR020, FIN9363, BrRJ034, BrRJ035, BrRJ042, ARE933, X1670, and CH2. Reference full-length subtype B sequences: WR27, HXB2, RL42, 159367, 04AR151263, 02AR114146, ARMA132, and ARCH054.

### 2.4. Identification of trends in the relative frequency of CRF12_BF over time

CRF12_BF frequency variation along time of HIV-1 transmission (i.e. time of birth) was evaluated by two approaches: (i) by comparing the proportion of CRF12_BF vs non-CRF12_BF in 3 study periods taking into account the year of birth of the children carrying each variant. Comparisons between the earliest and most recent periods were performed by Fisher's exact test; and (ii) by fitting the data to a logistic model, where the time of transmission was considered as the exact date of birth of the children carrying each variant to minimize estimation biases. Statistical significance for the estimated slope was evaluated with a likelihood ratio test.

## 3. Results

### 3.1. Detection of HIV-1 pol BF1 recombination profiles

In order to characterize the BF1 recombination patterns among the HIV-1 *pol* sequences in our group of patients, we selected a 912 pb *pol* genomic segment that codes for Protease (PR) and part of reverse transcriptase (RT) (HXB2 coordinates 2274–3186) because it allows the discrimination among the different CRFs_BF circulating in South America based on bootscan analyses, except for CRF28_BF and CRF29_BF, which share a same recombinant structure in the selected *pol* fragment (Fig. 1a and b). Noticeably, even though CRF12_BF and CRF17_BF appear to share identical recombination breakpoints in *pol* according to the Los Alamos Database, we have verified that the F1 to B breakpoint located at position 3026 in CRF12_BF is shifted almost 200 bp to position 2823 in CRF17_BF, allowing their discrimination. This observation was confirmed by phylogenetic analysis of the *pol* fragment, where CRF12_BF and CRF17_BF reference sequences branched in two well supported independent clusters (Fig. 2), and also by subtype signature analysis. A total of 14 subtype signature nucleotides were found in the *pol* region, at HXB2 coordinates (subtype B/subtype F): 2360 (G/A), 2422 (G/A), 2517 (C/A), 2609 (A/G), 2807 (C/T), 2810 (C/T), 2954 (A/C), 2972 (G/A), 2981 (T/C), 3002 (G/A), 3059 (T/C), 3069 (C/A), 3119 (A/G), and 3182 (G/A). While 80% of the CRF12_BF reference sequences presented subtype B signature nucleotides from position 2517 to 2609, and from 3002 to the 3′-end of the *pol* segment; most CRF17_BF reference sequences had additional subtype B signature nucleotides from position 2954 to 3002, confirming the differences observed in bootscan analyses in the length of the 3′ subtype B *pol* segment of both CRFs. Another discrepancy was found between the reported CRF38_BF recombinant structure (Ruchansky et al., 2009) and our bootscan plot that showed additional subtype F1 insertions within the subtype B *pol* segment initially described for this CRF (Fig. 1a and b); although subtype signature analysis was not sensitive enough to confirm the presence of such small subtype F1 insertions.

Analysis of the bootscan plots of the 172 HIV-1 *pol* BF1 sequences from our pediatric population indicates that: 98 sequences (57.0%) displayed a CRF12_BF-like recombination profile, four sequences (2.3%) had a CRF17_BF-like pattern, two (1.2%) had a single F1 to B recombination breakpoint similar to that of the Brazilian CRF28_BF and CRF29_BF, and another two (1.2%) had a CRF44_BF-like recombination pattern. The remaining 66 *pol* sequences (38.3%) included a variety of BF1 recombination patterns that did not match any known South-American CRF_BF structure.

### 3.2. Confirmation of common ancestry of CRFs_BF through phylogeny

In order to verify the common ancestry of CRFs_BF-like HIV-1 sequences sharing coincident BF1 recombination breakpoints in *pol*, we performed repetitive phylogenetic analyses to identify and exclude conflicting sequences that severely reduced bootstrap support for CRFs_BF reference clusters and/or branched outside such clusters. Of the 106 *pol* sequences initially classified as a CRFs_BF-like by their recombination profiles in bootscan analyses, 91 were confirmed in the phylogenetic tree as CRF12_BF ($n = 89$) or CRF17_BF ($n = 2$) (Fig. 2). Fifteen sequences (nine CRF12_BF-like, two CRF17_BF-like, two CRF28/29_BF-like, and two CRF44_BF-like) significantly reduced the support of the corresponding CRFs_BF clusters and/or segregated in an independent phylogenetic clade (data not shown), and were therefore considered phylogenetically different from the CRFs_BF despite their coincident recombination breakpoints previously determined by bootscanning. Similar tree topology and clade support were obtained by Bayesian analyses (data not shown).

### 3.3. Recombination patterns present in URFs_BF from Argentina

Among the 66 HIV-1 *pol* sequences with a BF1 recombination pattern not related with any known South-American CRF_BF, 24 different mosaic structures could be distinguished and each one was identified with a roman number (Fig. 3). Seven HIV-1 *pol* sequences could not be completely resolved by Simplot, and ambiguous B/F regions were plotted for patterns X, XIV, XVIII, XXI and XXIII. Pattern I was the most common BF1 structure after the CRF12_BF accounting for 7.6% of the total number of BF1 sequences analyzed. This pattern shared two of the three recombination breakpoints present in CRF12_BF and could represent a second generation of this CRF. Eight other patterns (II–IX) were detected in at least three distinct individuals and could also represent new CRFs_BF, yet unidentified. Full-length characterization of these strains should be carried out to confirm this hypothesis.

Overall, 19 URF_BF1 patterns here detected share at least one common recombination breakpoint with the CRF12_BF. The first F1 to B recombination breakpoint of CRF12_BF (located at position 2473 of the HXB2) was the most conserved, present in 56.1% (37/66) of the sequences. Most patterns showed larger subtype B segments than those present in the CRF12, suggesting that these BF1 recombinants could have originated by successive subtype B insertions into the CRF12_BF background. In contrast, only three sequences (patterns XIII and XIX) showed larger subtype F1 segments than those present in CRF12_BF, indicating the paucity of recombination events between CRF12_BF and F1 strains in harmony with the almost complete absence of subtype F1 in Argentina.

### 3.4. Longitudinal study of HIV-1 pol BF1 recombination patterns in Argentina

The frequency of HIV-1 CRF12_BF and non-CRF12_BF strains were analyzed in children according to the time of birth (i.e. the date of transmission). The proportion of vertically transmitted

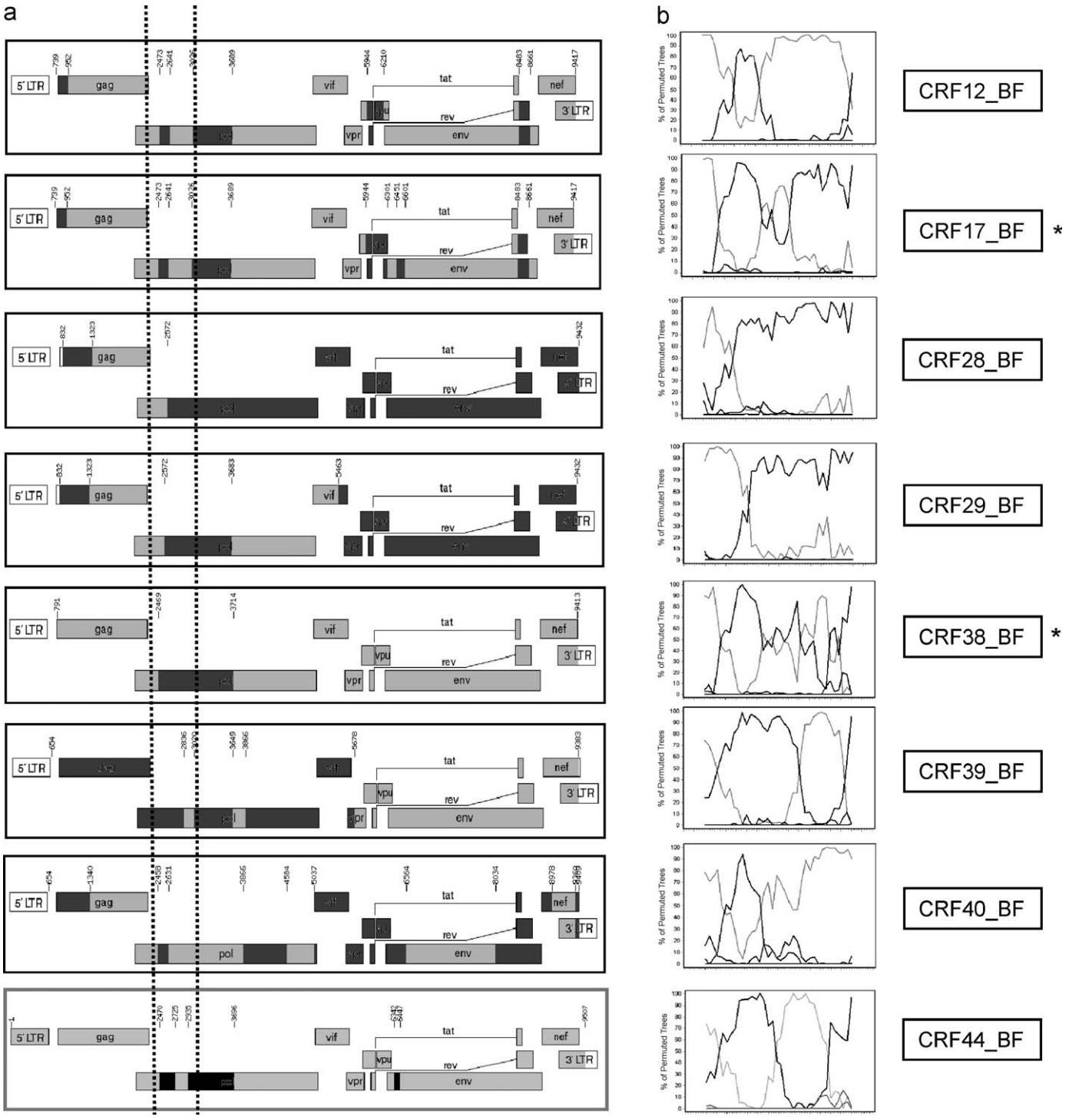


**Fig. 1.** Comparison of BF recombination breakpoints in South-American CRFs_BF. (a) Graphic representation of recombination patterns from South American CRFs_BF. Recombination patterns were obtained from the Alamos National Laboratory database (www.hivdb.lanl.gov/CRFs) except for CRF38_BF, which was reconstructed from Ruchansky et al. (2009) with the Los Alamos Recombinant HIV-1 drawing tool. The vertical dotted lines represent the *pol* segment where almost all CRFs can be distinguished. Subtype B: black; subtype F1: grey. (b) Bootscanning plots of *pol* segment (HXB2 coordinates 2274–3186 bp) from representative CRFs_BF sequences, showing BF recombination breakpoints. Query CRFs_BF sequences: ARMA159 (CRF12_BF), ARMA038 (CRF17_BF), BREPM12817 (CRF28_BF), BREPM16704 (CRF29_BF), UY03_3389 (CRF38_BF), 04BRRJ179 (CRF39_BF), 05BRRJ200 (CRF40_BF), and CH80 (CRF44_BF) were compared to grouped reference sequences from subtype B (MN, WR27, HXB2, RL42) and subtype F1 (RMARE933FL, BZ163, MP411). Subtype A (KE.Q23_17) and C (BR025-d) sequences were used as outgroups. Dark grey lines represent subtype B, light grey subtype F, and black subtypes A or C. *Sequences that differ in their reported BF1 recombination pattern from our bootscanning plots.

strains with a CRF12_BF *pol* genotype (confirmed both by coincident recombination breakpoints and phylogenetic clustering with CRF12_BF references) showed a statistically significant decrease with time from 69% in the period of 1986–1993 to 46% in the period of 2001–2008 (Fisher's exact test, $p = 0.046$) (Fig. 4a). To confirm these results and better characterize the way CRF12_BF frequency decreased over time, we further applied a logistic regression model on the dataset, using the exact date of birth as the time of transmission to minimize estimation biases. Results showed that the decrease in CRF12_BF fitted to a linear function (likelihood ratio test, $p = 0.047$) (Fig. 4b), indicating a relatively constant reduction of the CRF12_BF population over time.

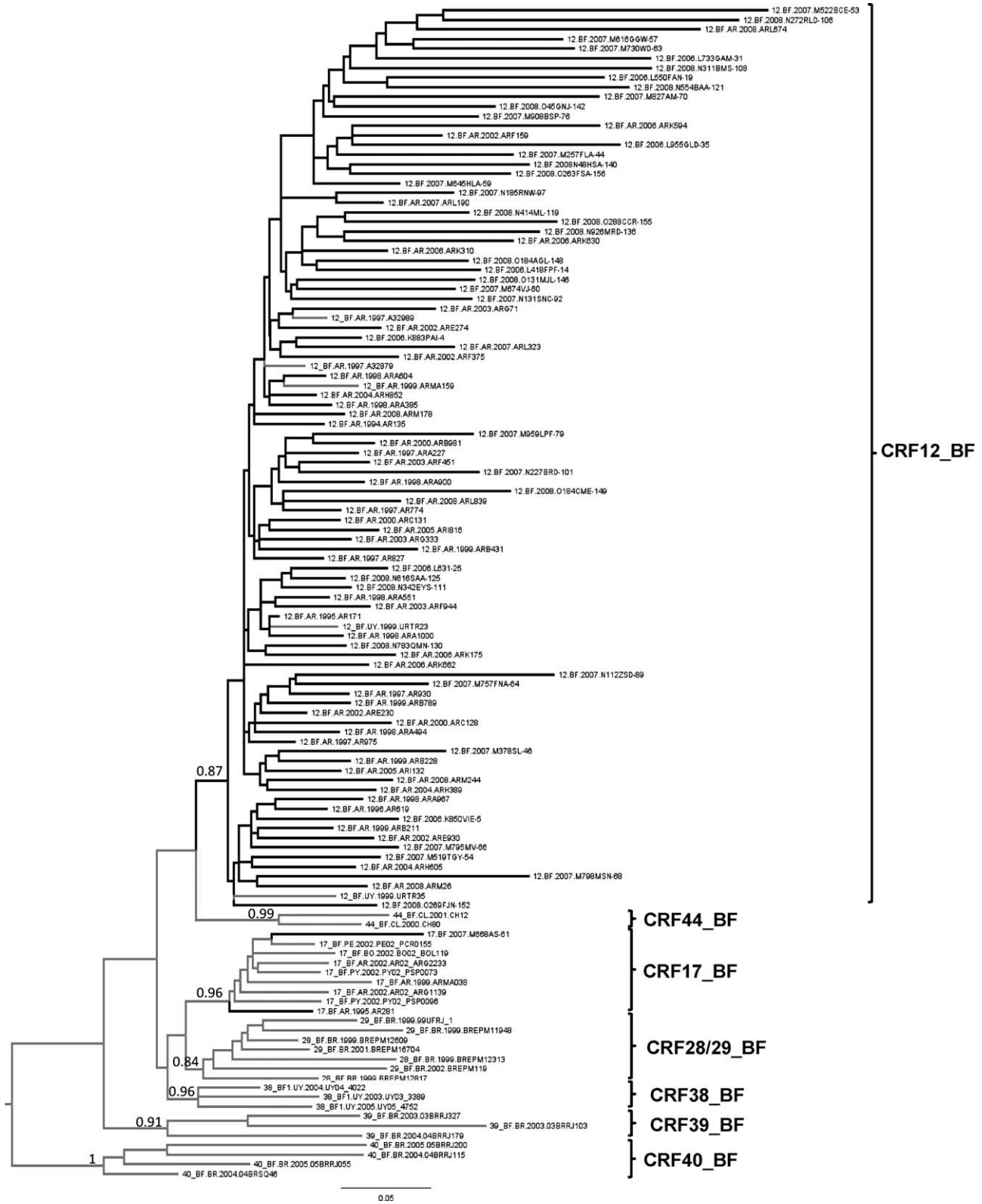


**Fig. 2.** Maximum likelihood phylogenetic tree of HIV-1 *pol* (912 nt) segment from 93 sequences carrying a CRF_BF-like pattern. CRFs_BF reference sequences are identified with grey branches. Support values (aLRT-SH) are represented at the nodes for each CRF_BF cluster. All CRFs_BF show an independent clustering, except for CRF28_BF and CRF29_BF which cluster together. CRFs_BF reference sequences used in phylogenetic reconstructions wereas follows – **CRF12_BF**: A32989, A32879, ARMA159, URTR23, and URTR35; **CRF17_BF**: PCR0155, BOL119, PSP0073, PSP0096, ARG2233, ARMA038, and ARG1139; **CRF28_BF**: BREPM12817, BREPM12609, and BREPM12313; **CRF29_BF**: BREPM16704, BREPM11948, 99UFRJ_1, and BREPM119; **CRF38_BF**: UY05-4752, UY04-4022, and UY03-3389; **CRF39_BF**: 03BRRJ327, 03BRRJ103, and 04BRRJ179; **CRF40_BF**: 04BRSQ46, 05BRRJ200, 04BRRJ115, and 05BRRJ055.

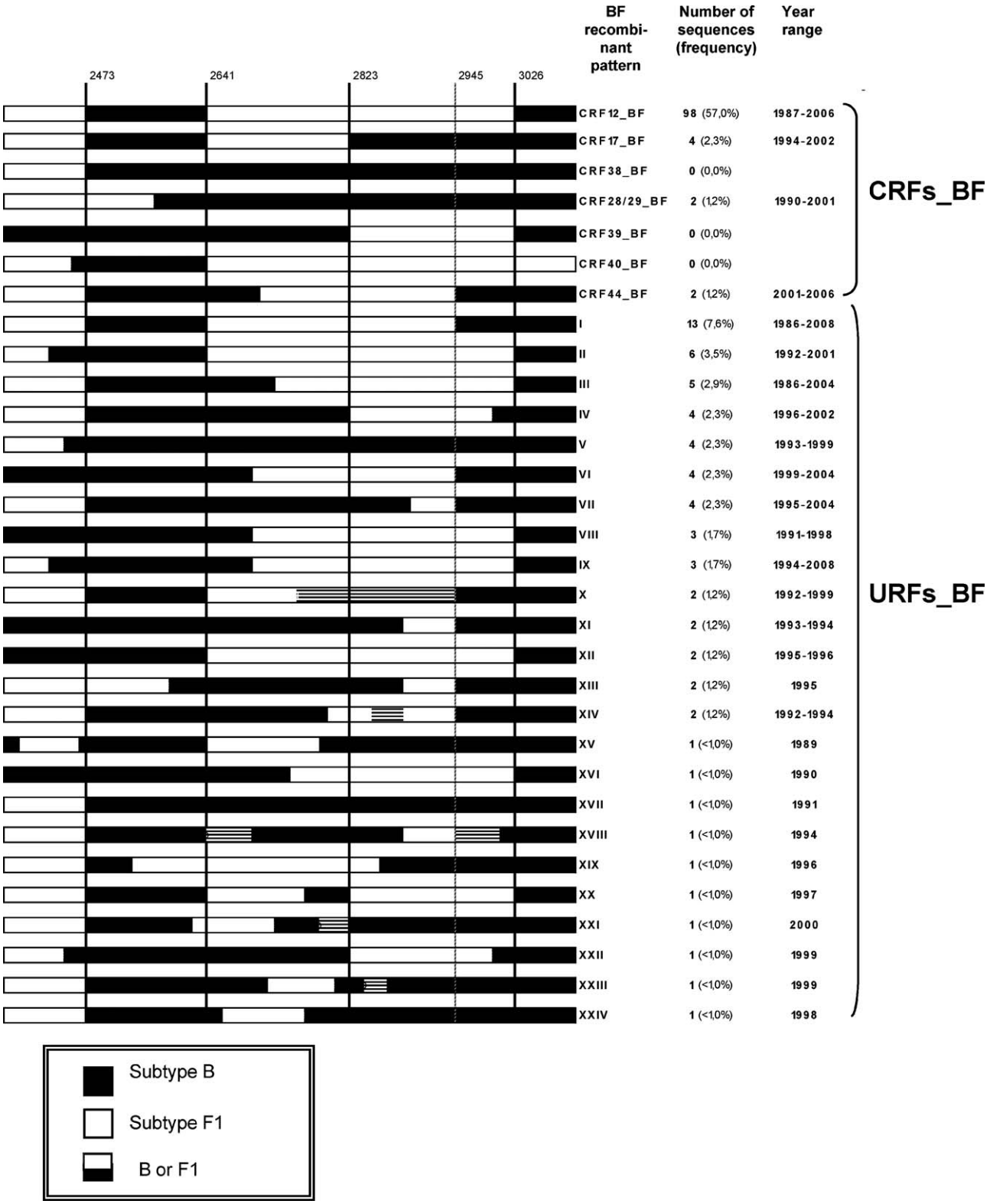


**Fig. 3.** Schematic representation of the BF1 mosaic structures from HIV-1 vertically infected children. Breakpoint patterns were based on the bootscan analysis of the HIV-1 *pol* segment (HXB2 coordinates 2274–3186 bp). Vertical lines indicate coordinates for recombination breakpoints in CRF12_BF or CRF17_BF (straight lines), or in new URFs (dashed line). BF1 mosaic structures were ordered according to their frequency in the dataset, CRFs_BF already described at the top of the list, and URFs_BF below them. The year range includes the earliest and the most recent dates of birth (i.e. time of HIV-1 transmission) of the children carrying BF1 strains with each mosaic pattern.

## 4. Discussion

This study represents the most extensive analysis of the diversity of HIV-1 BF1 recombinants circulating in Argentine vertically infected children born from 1986 to 2008. A total of 28 different BF1 mosaic structures were identified among 172 HIV *pol* sequences analyzed, including four CRF_BF-like mosaic patterns (CRF12_BF, CRF17_BF, CRF28/29_BF, and CRF44_BF). The most common *pol* BF1 recombinant structure in the pediatric population was the CRF12_BF with an overall prevalence of

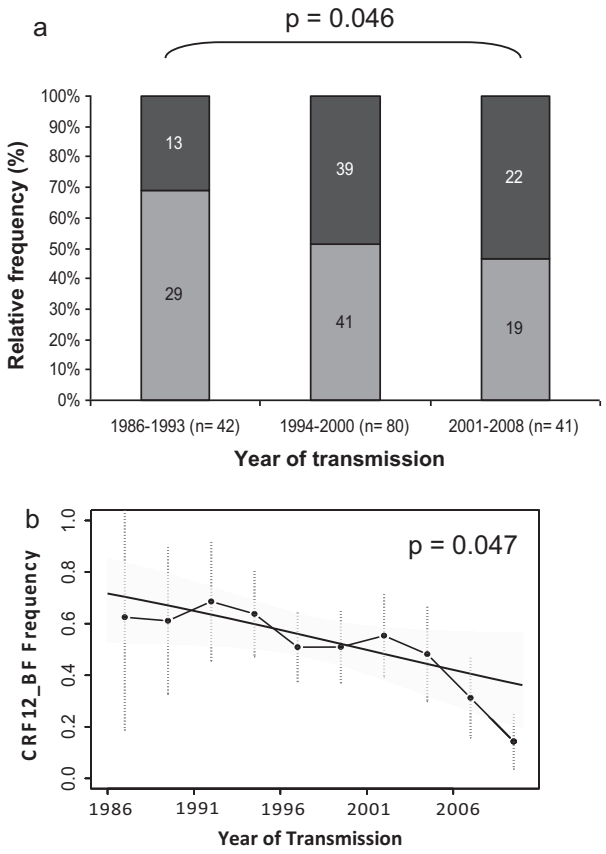


**Fig. 4.** Longitudinal study of HIV-1 *pol* BF1 recombination patterns in children. (a) Relative proportion of CRF12_BF (grey) and non-CRF12_BF (black) transmitted from mother-to-child in three time periods from 1986 to 2008. Statistical significance was assessed between groups using the Fisher's exact test. Nine sequences sharing the CRF12_BF recombination profile but belonging to a differentiated phylogenetic cluster were excluded. (b) Observed frequencies (filled circles) and 95% confidence intervals (dotted lines) for overlapping periods spanning 5 years, every 2.5 years. Straight line and light grey shadow depict fitted values for a logistic model and 95% confidence intervals. Statistical significance was tested using the likelihood ratio test.

~50% over the 22-year period analyzed, consistent with previous studies on adult populations from Argentina (Carr et al., 2001; Dilernia et al., 2007; Quarleri et al., 2004); followed by patterns I (7.6%) and II (3.5%). All other *pol* BF1 recombination patterns, including those similar to other South-American CRFs displayed an overall prevalence below 3%.

HIV-1 *pol* diversity of BF1 recombinants was previously recognized only in adult populations in Argentina, where a few recombination patterns were identified. The largest population analyzed included 284 BF *pol* sequences from patients under treatment failure, where only three BF1 mosaic patterns were described (Quarleri et al., 2004). More recently, however, a larger HIV-1 diversity was recognized among a small group of 16 pregnant women collected in 2005, as seven different BF1 *pol* recombinant structures were described (Gomez Carrillo et al., 2006). In agreement with the last study, we also found a higher diversity of BF1 *pol* mosaic patterns than the initially described by Quarleri et al. (2004). Such contradictory results can be due to a number of factors including the population and period under study, but most probably depends on the methods and criteria used for identification of BF1 recombinant structures in each study.

By bootscan analyses, we found that most BF1 *pol* mosaic patterns displayed a mosaic structure related to CRF12_BF. Nineteen out of the 24 non-CRF_BF-like recombinant patterns shared at least one common recombination breakpoint with CRF12_BF, with larger subtype B segments than those present in the CRF12_BF. In agreement with previous reports (Sierra et al., 2005), our results suggest that most BF1 recombinants in Argentina could be classified as second generation recombinants originated by successive rounds of recombination between the CRF12_BF and the subtype B circulating in Argentina. Recombination of the CRF12_BF with subtype F1 is extremely improbable given the almost complete absence of "pure" subtype F1 in Argentina. Indeed, only three URFs_BF (transmitted around mid 1990s) replaced the CRF12_BF B segment in the *pol* region for a subtype F segment, and probably represent independent introductions of BF1 viruses that did not gain epidemiological relevance in Argentina.

Continuous events of recombination between CRF12_BF and subtype B or URFs_BF strains could also explain the observed linear reduction in the number of transmissions caused by CRF12_BF viruses over time, decreasing from 69% in the period 1986–1993 to 46% in 2001–2008. In mother-to-child transmission, multiple HIV-1 infections are not expected to occur, and therefore the increase in URFs_BF structures observed in children reflects the underlying HIV-1 diversity in the female heterosexual adult population at the time of transmission (i.e. near birth). Of note, non-CRF12_BF viruses were detected in children born along the whole study period (1986–2008), indicating that the recombination events leading to URF_BF diversity are not recent and have occurred continuously since the start of the CRF12_BF epidemic, estimated around the early 1980s (Bello et al., 2010).

Phylogenetic analyses of sequences with a CRF_BF-like recombinant pattern showed that CRF12_BF was the most common CRF_BF, representing 51.1% of the BF1 strains. Interestingly, common ancestry with reference CRF12_BF sequences could not be confirmed for 9 of the 98 sequences with a CRF12_BF-like recombinant pattern, as bootstrap support of the CRF12_BF cluster was significantly reduced when these sequences were included in the tree. Previously, Quarleri et al. (2004) named *Pattern I* the sequences sharing the same CRF12_BF mosaic structure at *pol* gene, and showed that these sequences branched into two distinct phylogenetic clusters supported by high bootstrap values without an evident explanation. One of the clusters identified by Quarleri et al. (2004) contained sequences ARCH003, ARCH014, and A32878 that shared the same BF1 recombination breakpoints as the CRF12_BF in the *pol* fragment analyzed, but in a detailed full-length analysis, these sequences displayed a longer subtype B insertion that extends beyond the B/F breakpoint located at position 3689 of CRF12_BF (Carr et al., 2001; Thomson et al., 2002), indicating that the 3′ subtype B *pol* segment has a distinct evolutionary origin from the subtype B present in CRF12_BF recombinants. Of note, the inclusion of these three sequences into our phylogenetic tree also reduced the bootstrap support for the CRF12_BF cluster (data not shown). Therefore, we hypothesize that sequences that did not belong to a CRF_BF cluster despite presenting a CRF_BF-like recombinant pattern probably have a 3′ subtype B segment arising from different subtype B ancestors, and were classified as URFs_BF in our study.

Along with the characterization of CRF12_BF in South-America in 2001 came the description of the first full-length structure of CRF17_BF (Carr et al., 2001). To our knowledge, this is the first time CRF17_BF is included in detailed phylogenetic analyses to study its prevalence and spread in relation to the other South-American CRFs_BF. The mosaic structure of CRF17_BF was reported to resemble that of CRF12_BF in most parts of the genome (Carr et al., 2001; Los Alamos HIV Sequence Database: http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html). However, we found that the BF1 recombination structure of both CRFs could be differentiated at the *pol* gene. Interestingly, the CRF17_BF *pol* BF1 recombination pattern observed in the present study is the same as "*Pattern II*" previously described by Quarleri et al. (2004) who

reported an estimated overall prevalence of 16% in the adult population, suggesting that this CRF could be an epidemiologically important recombinant form in Argentina. However, according to our results, this CRF represented only a minor fraction (<2%) of HIV-1 BF1 recombinants circulating in the Argentine pediatric population, similar to the low prevalence of CRF17_BF in South-America (0.4%) (Laguna-Torres et al., 2005) and Paraguay (2%) (Aguayo et al., 2008).

The CRF28_BF and CRF29_BF have only been described among HIV-1 strains circulating in Brazil (De Sa Filho et al., 2006). Despite the fact that two BF1 *pol* sequences in our pediatric population displayed a CRF28/29_BF-like pattern, their relationship with reference CRF28_BF and CRF29_BF strains could not be confirmed in phylogenetic trees. Noticeably, these two Argentine strains segregated in a sub-cluster within CRF28/29_BF clade with the V62 strain from Venezuela (data not shown), which was described as a URF related to CRF12_BF (Sierra et al., 2005). Likewise, two BF1 *pol* sequences in our pediatric population displayed a recombinant pattern similar to the CRF44_BF recently identified in Chile (Delgado et al., 2010), but they branched in an independent clade in the phylogenetic tree (data not shown). We did not find any BF1 *pol* mosaic structure similar to the CRF38_BF described in Uruguay (Ruchansky et al., 2009), or the CRF39/40_BF described in Brazil (Guimaraes et al., 2008). These results revealed that despite the frequent population exchanges between Argentina and neighboring countries, no evidence of circulation of Brazilian, Chilean or Uruguayan CRFs_BF were found among Argentine children; indicating that factors other than geographic proximity and population flux may limit the dispersion of these HIV-1 BF1 variants in South America.

Recombination plays an important role in HIV-1 evolution and adaptation to the host and its environment. Through the study of a large number of BF1 recombinant sequences from Argentina, we observed a more complex diversity in the recombination pattern of the *pol* region than previously described. Because this study is limited by the analysis of a relatively short fragment of the HIV-1 genome, it would be expected to result in an underestimation of the frequency and diversity of URF as compared to the analysis of full-length genomes. Our results provide evidence of BF1 recombination as a dynamic phenomenon and report for the first time a decrease in the proportion of CRF12_BF viruses transmitted from mother-to-child since the start of the epidemic to the present time in Argentina, and the parallel increase of recombinant viruses with distinct mosaic structures. These findings offers relevant insights to understanding the diversity and temporal dynamics of HIV-1 BF1 strains that spread in Argentina, with future implications for diagnosis, treatment, and vaccine development.

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