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Short Communication: Emergence of Novel A/G Recombinant HIV-1 Strains in Argentina

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Abstract

The predominant circulating HIV-1 strains in South America are subtype B and B/F recombinants with different distributions among countries. However, the emergence of other subtypes is a matter of concern and needs continuous monitoring. We identified three different A/G recombinants in Argentina, two of them in vertically infected children from unlinked mothers and one in an adult female. HIV-1 *pol* sequences from the children showed novel A/G recombination patterns and no phylogenetic relationship with previously reported South American A/G sequences. The third A/G recombinant was a CRF06_cpx with African origin. The detection of new or unusual subtypes is important to avoid false-negative PCR HIV-1 early diagnosis due to detection failures and for future vaccine development.

THE HIGH GENETIC VARIABILITY of HIV-1 has allowed the emergence of new genomic structures around the world. HIV-1 is divided into four groups: M, N, O, and P. The outstanding viral diversity of HIV-1 group M is evidenced by its wide classification, which includes nine subtypes (A to D, F to H, J and K) and an infinite number of intersubtype recombinant forms. Most intersubtype recombinants reported worldwide are unique recombinant forms (URFs). However, those identified by full-length sequencing in three or more epidemiologically unlinked individuals are designated as circulating recombinant forms (CRFs). To date 61 CRFs have been characterized, and some of them have attained considerable geographic coverage and importance in the pandemia,[1] for example, CRF01_AE in Thailand, CRF02_AG in West Africa, and CRF12_BF in Argentina.[2,3]

Africa is the origin of the HIV-1 epidemic and therefore harbors almost all the variety of HIV-1 subtypes. Recombinant forms are responsible for almost 10% of all HIV-1 infections in this continent, and the main recombinant forms are mosaic structures containing subtypes A and G, including CRF02_AG and CRF06_cpx. CRF02_AG predominates in West and West Central Africa, accounting for > 50% of the total infections by recombinant HIV-1 strains. CRF06_cpx is second in prevalence, responsible for around 6% of the infections by recombinant strains in Africa, and involves recombination events between subtypes A, G, and at least another two subtypes (J and K).[4] These genetic forms were identified early in the epidemic, and had a limited spread outside the African continent. However, the prevalence of CRF02_AG has been increasing in past years in Africa and Europe, mainly in Italy because of migrations from the sub-Saharan region.[5]

In South America, HIV-1 subtypes are mainly B and BF recombinants. The number of A/G mosaic forms identified is very small, currently reaching a total of 41 GenBank submissions (2 full-length and 39 partial HIV-1 gene fragments). The first A/G recombinants characterized as CRF02_AG by full-length sequencing were found in a heterosexual couple from Ecuador.[6] Between 2003 and 2011, another 18 CRF02_AG strains were found in Ecuador and Brazil based on the analysis of the HIV-1 *pol* genomic fragment.[7,8] Since 2008, HIV-1 gene fragments similar to the CRF06_cpx strain were found in four individuals from Venezuela,[9] four from Colombia, one from Bolivia, and one from Argentina.[10] Parental HIV-1 subtypes A and G in South America are even less frequent than A/G recombinants and up to the present time, no full-length sequence from South American A or G pure HIV-1 strains has been reported.

Our aim was to investigate the recombination patterns and phylogenetic relationship of three different A/G recombinant strains found in two vertically infected children and an adult female from Paraguay but living in Argentina. The study was reviewed and approved by the Ethics Committee of the Garrahan Hospital and the Ethics Committee of the Durand Hospital for children and adults, respectively. An HIV-1 *pol*

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gene fragment of around 1,100 pb encompassing the whole p6gag, protease (PR) *pol*, and codons 1–220 of the reverse transcriptase (RT) *pol* was amplified from plasma samples from two epidemiologically unlinked HIV-1 vertically infected infants (5707 and 4707) born in 2009 and 2013 in the cities of Pergamino and Salto (223 and 183 km from Buenos Aires city, respectively), and from one adult female (FFCA) born in Paraguay.

The HIV-1 *pol* segment was sequenced with the automated sequencer ABI 3130 (Applied Biosystems), and sequences were aligned using the Gene Cutter Tool[11] for phylogenetic and recombination analyses. The phylogenetic trees were inferred by the maximum likelihood (ML) method with the MEGA v6.0 program, under the GTR + G[12] nucleotide substitution model inferred according to the Akaike Information Criterion (AIC) statistics obtained with the jModelTest v0.1 program.[13] Recombination breakpoints were identified with bootscan analysis by SimPlot software v2.5[14] and confirmed with the RIP tool from the Los Alamos Database.[15]

The HIV-1 subtype of the newly characterized HIV-1 strains was investigated by comparison to HIV-1 reference sequences through phylogenetic analysis of HIV-1 *pol* fragments. Figure 1a shows that sequences from the adult woman (FFCA_L691) and her partner (MMaPR_L690) formed a well-defined group with 100% bootstrap support with the CRF06_cpx reference strains. Clustering within the CRF06_cpx group was also observed for a 680-bp V2-C5 *env* fragment (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/aid). Sequences 5707_U864 and 4707_M86 branched between or near the clades defined for subtypes G and A, with low bootstrap support for the nodes. Therefore, they are probably A/G recombinants. The independent branching of the sequences confirms the lack of an epidemiologic relationship between them. In addition, we investigated the phylogenetic relationship of 5707_U864, 4707_M86, and FFCA_L691 with the previously reported A, G, or A/G recombinants from South America (Fig. 1b).

None of the sequences obtained from the vertically infected children clustered with the CRF02_AG or CRF06_cpx

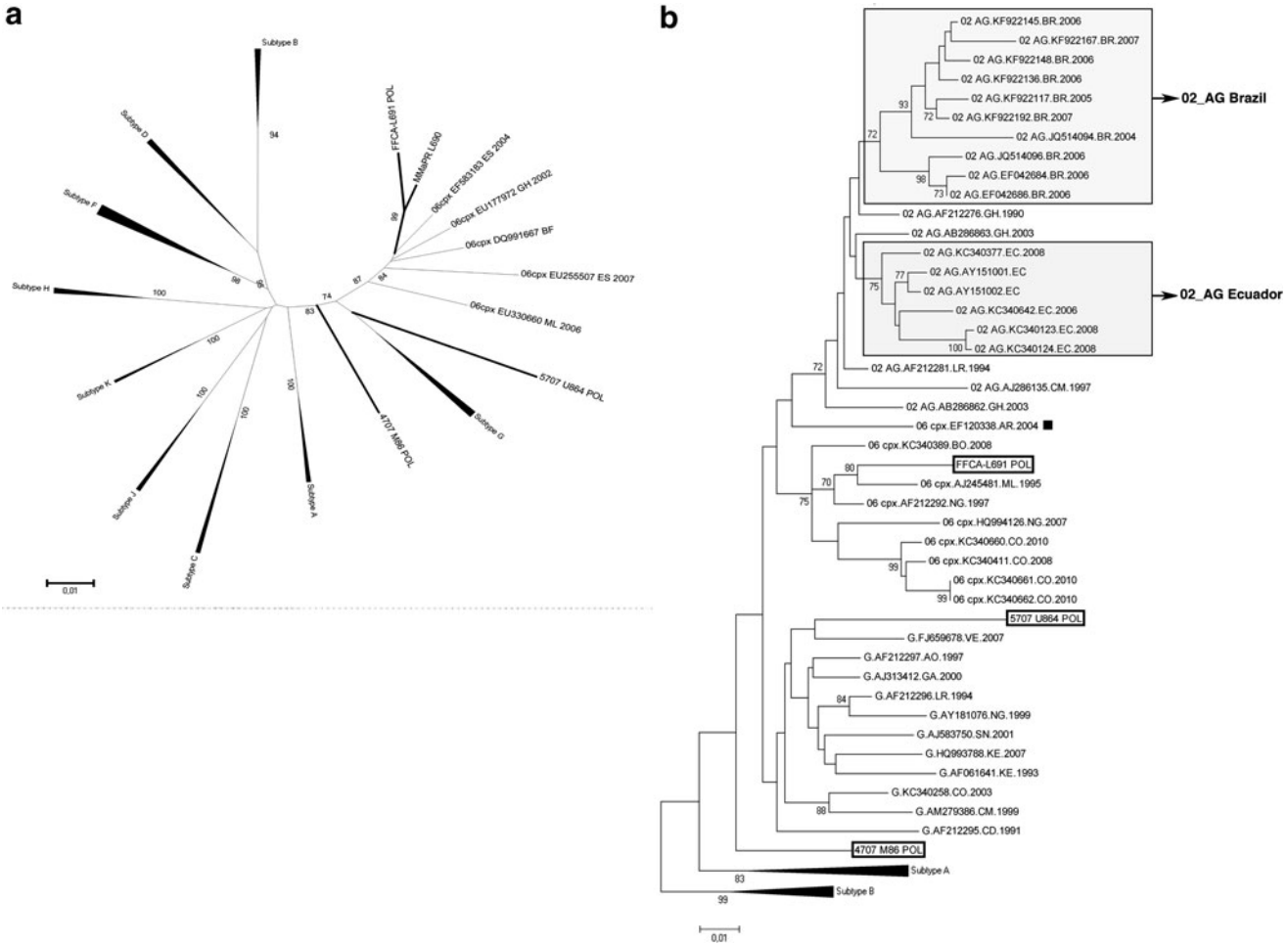

FIG. 1. Maximum likelihood phylogenetic trees of the HIV-1 *pol* region, encompassing the protease and part of the reverse transcriptase of newly identified A/G recombinants from Argentina and subtype reference strains. Bootstrap support values are represented at the nodes for each cluster. **(a)** Analysis of newly identified sequences from Argentina with the reference sequence from the parental HIV-1 subtypes and CRF06_cpx. **(b)** Analysis of newly identified sequences from Argentina with A, G, or A/G recombinants from South America and Africa. Sequences marked with *lined boxes* are the newly identified sequences from Argentina in a woman (FFCA) and two children (5707 and 4707). The sequence marked with a *black square* is the previously identified A/G recombinant from Argentina.[10]

reference strains. 5707_U864 was phylogenetically related with a low bootstrap value (70%) to a subtype G sequence from Venezuela, while 4707_M86 did not show any close phylogenetic relationship with previously reported South American A, G, or A/G recombinant strains.

Within the CRF06_cpx group, sequence FFCA_L691 showed greater similarity to African CRF06_cpx sequences than to CRF06_cpx sequences from South America, in agreement with the fact that FFCA acquired the infection through her partner (MMaPR), an immigrant from Sierra Leone, Africa. In our phylogenetic tree, CRF06_cpx sequences from Colombia and Bolivia form a well-defined group with CRF06_cpx reference strains from Africa, but sequence 06cpx.EF120338.AR.2004 previously identified in an adult female from Argentina[10] branched outside the CRF06_cpx group.

To identify and characterize intersubtype recombination breakpoints in the two newly identified *pol* sequences present in children, sequences were individually analyzed for recombination patterns with consensus references by bootscan analysis with SimPlot 2.5 (Fig. 2a and b). Only A and G subtypes were present in both 5707_U864 and 4707_M86 sequences. However, recombinant breakpoints differed between them. In 4707_M86 the subtype A segment spans approximately 413 nt from position 2114 to 2527 of HXB2 (GenBank Accession Number K03455). In 5707_U864 the subtype A fragment is smaller (approximately 200 nt, positions 2580 to 2778). The small subtype A insertion probably accounts for the low bootstrap support observed for 5707_U864 within the subtype G cluster in the phylogenetic tree (Fig. 1b).

Similar results were obtained with the RIP tool, and subtype assignment was confirmed by phylogenetic analysis of each segment (Fig. 3a and b). None of the sequences shared a recombination breakpoint with CRF02_AG. The HIV-1 *pol* sequence was also available from the mother of 5707, and showed 100% identity to the one found in the child, demonstrating for the first time in Argentina local transmission of novel HIV-1 A/G recombinant strains.

In conclusion, we describe the local circulation of three different A/G recombinants in Argentina: two novel URFs_AG in children and a CRF06_cpx in an adult female and her African partner.

Previously, CRF06_cpx had been identified in four South American countries, including Argentina, where a single CRF06_cpx was reported in an adult woman. However, this sequence was distantly related to the CRF06_cpx strain found in our study, which conformed a well-defined cluster together with other CRF06_cpx strains from Africa, Colombia, and Bolivia. Therefore, it is possible that the previously reported CRF06_cpx strain is in reality a URF_AG that shares no phylogenetic relationship to the novel recombinant A/G strains described in this study.

It has been proposed that recombinants of subtypes A and G possessing breakpoints different from CRF02_AG are commonly generated from dual infections.[16] Due to the almost complete absence of parental A and G subtypes in our country, except for a few scattered cases,[17] the presence of novel recombinant forms in children from Argentina probably represents independent introductions of URFs_AG, rather than local recombination events.

Failures in HIV-1 detection by nucleic acid-based methods are one of the biggest problems caused by unusual subtypes,[18] as we had previously reported for subtypes A and F.[19] Our test for HIV-1 diagnosis in newborns, an in-house multiplex nested PCR, targets two HIV-1 genomic regions (*gag* and *env*) for increased sensitivity in the detection of unusual subtypes. However, diagnostic problems were also observed in children infected with the A/G recombinants reported in this study. The diagnostic PCR performed at 1 month for child 5707 tested negative due to lack of amplification of both the *gag* and *env* gene fragments, while child 4707 tested positive with amplification of the *gag* fragment alone. In both cases, HIV-1 infection was confirmed with a

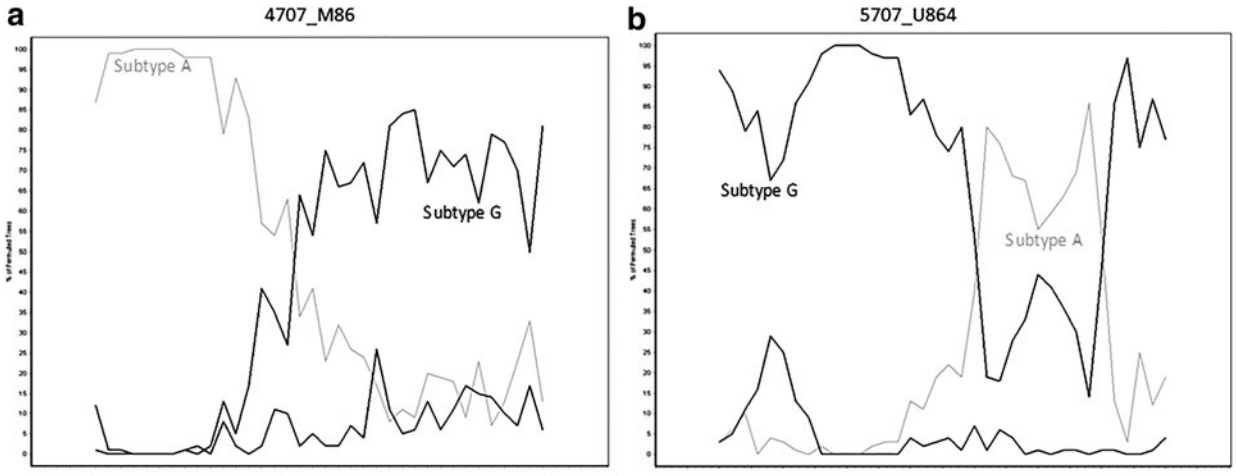

FIG. 2. Bootscanning plot of **(a)** 4707_M86 and **(b)** 5707_U864 sequences from Argentina. *Black curve* represents subtype G and *light gray curve* subtype A. Subtypes C and D were used as outgroups for 4707 and 5707, respectively. In bootscan analyses performed with SimPlot, bootstrap values were determined in neighbor-joining trees constructed using the Kimura two-parameter model, based on 100 resamplings, supporting branching with the consensus sequences within a 200-bp window moving in steps of 20 bases.

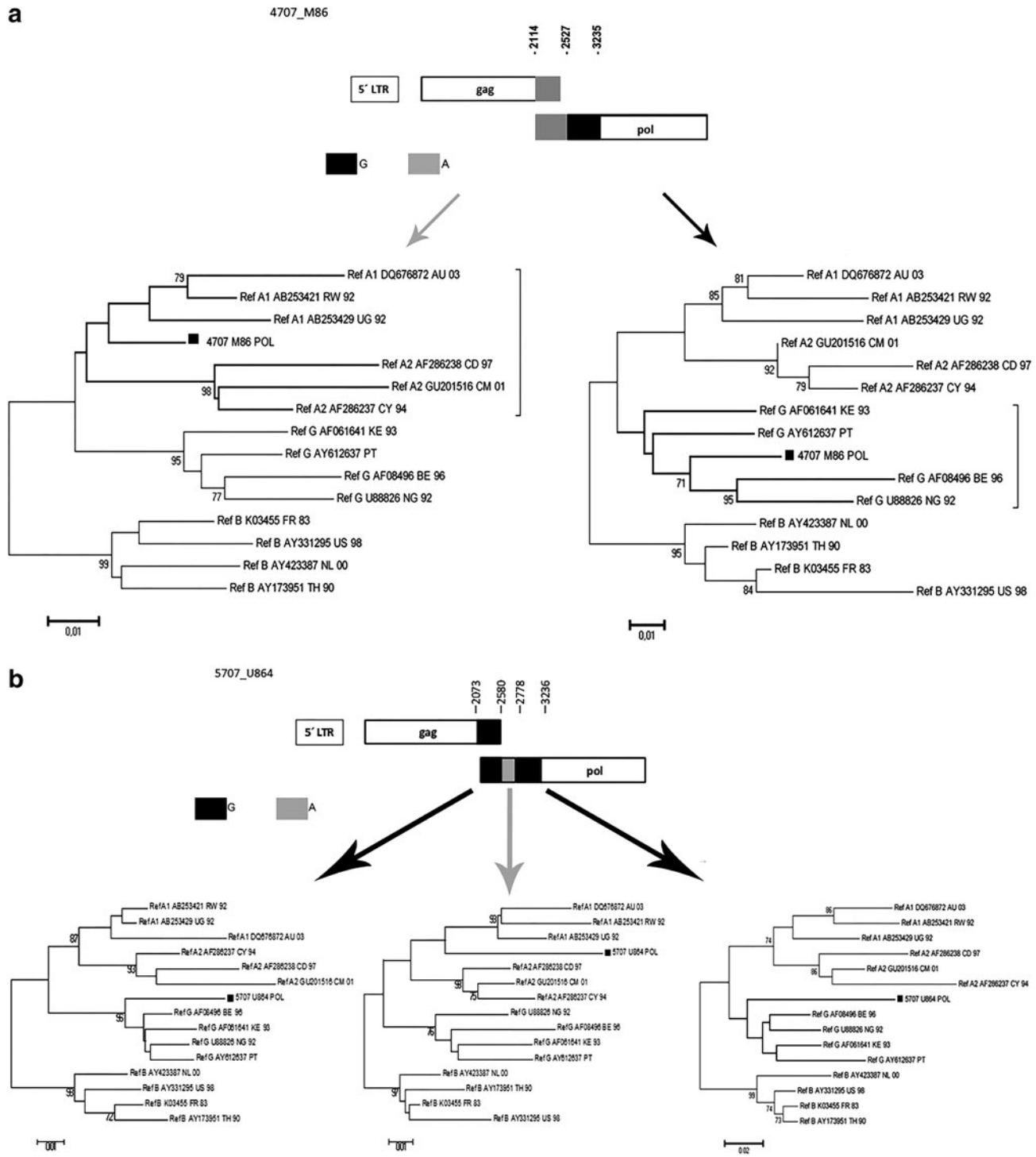

FIG. 3. Schematic representation of the recombination pattern of **(a)** 4707_M86 and **(b)** 5707_U864. Illustrations were obtained from the Recombinant HIV-1 Drawing Tool (www.hiv.lanl.gov/content/sequence/DRAW_CRF/recom_mapper.html). Phylogenetic trees for the nucleotide sequences of regions with different subtypes were constructed using the maximum-likelihood approach. Bootstrap values above 70 are shown at the branch nodes. The clusters containing the query sequences are highlighted and the *black square* represents the newly identified sequences from Argentina 4707 or 5707, respectively.

plasma viral load > 100,000 RNA copies/ml (Roche Cobas TaqMan) and amplification of the HIV-1 *pol* fragment used for the HIV-1 genotypic drug resistance test.

Other implications of uncommon HIV-1 strains include (1) reduction in the sensitivity of the detection of the p24 antigen due to differences in viral epitope recognition by monoclonal antibodies used in the assay,[20,21] (2) potential differences in disease progression,[22] and (3) impairments of vaccine efficacy in clinical trials.

Our results emphasize the importance of continuous surveillance of HIV-1 genetic diversity for the early detection of new emerging viral clades in the population and their spread,

given the unknown impact on transmissibility, pathogenicity, and relevance in future vaccine development.

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Author Disclosure Statement

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References

1. HIV Sequence Database: Los Alamos National Laboratory. Available at: www.hiv.lanl.gov.
2. Hemelaar J, Gouws E, Ghys PD, and Osmanov S: Global trends in molecular epidemiology of HIV-1 during 2000–2007. AIDS 2011;25:679–689.
3. Aulicino PC, Bello G, Guimaraes ML, *et al.:* 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. Infect Genet Evol 2011;11:349–357
4. Lihana RW, Ssemwaga D, Abimiku A, and Ndembi N: Update on HIV-1 diversity in Africa: A decade in review. AIDS Rev 2012;14:83–100.
5. Mendonca Collaço Véras N, Santoro MM, Gray R, *et al.:* Molecular epidemiology of HIV type 1 CRF02_AG in Cameroon and African patients living in Italy. AIDS Res Hum Retroviruses 2011;27:1173–1182.
6. Carrion G, Hierholzer J, Montano S, *et al.:* Circulating recombinant form CRF02_AG in South America. AIDS Res Hum Retroviruses 2003;19:329–332.
7. Velasco-de-Castro CA, Grinsztejn B, Veloso VG, *et al.:* HIV-1 diversity and drug resistance mutations among people seeking HIV diagnosis in voluntary counseling and testing sites in Rio de Janeiro, Brazil. PLoS One 2014;9:e87622.
8. Delatorre EO, Bello G, Eyer–Silva WA, Chequer-Fernandez SL, Morgado MG, and Couto-Fernandez GC: Evidence of multiple introductions and autochthonous transmission of HIV-1 type 1 CRF02_AG clade in Brazil. AIDS Res Hum Retroviruses 2012;28:1369–1372.
9. Castillo J, Comegna M, Quijada W, *et al.:* Surveillance of HIV type 1 drug resistance among naive patients from Venezuela. AIDS Res Hum Retroviruses 2009;25:1329–1333.
10. Dilernia DA, Gomez AM, Lourtau L, Marone R, Losso MH, Salomón H, and Gómez-Carrillo M: HIV type 1 genetic diversity surveillance among newly diagnosed individuals from 2003 to 2005 in Buenos Aires, Argentina. AIDS Res Hum Retroviruses 2007;23:1201–1207.
11. Gene Cutter: Sequence Alignment and Protein Extraction. Available at www.hiv.lanl.gov/content/sequence/GENE_CUTTER/cutter.html.
12. Lavane C, Preparata G, Sacone C,and Serio G: A new method for calculating evolutionary substitution rates. J Mol Evol 1984;20:86–93.
13. Posada D and Crandall KA: MODELTEST: Testing the model of DNA substitution. Bioinformatics 1998;14:817–818.
14. Lole KS, Bollinger RC, Paranjape RS, *et al.:* Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J Virol 1999;73:152–160.
15. Recombinant Identification Program (RIP 3.0): Available at www.hiv.lanl.gov/content/sequence/RIP/RIP.html.
16. Powell RLR, Konings FAJ, Nanfack, Sherri Burda, *et al.:* Quasispecies analysis of novel HIV-1 recombinants of subtypes A and G reveals no similarity to the mosaic structure of CRF02_AG. J Med Virol 2007;79:1270–1285.
17. Aulicino PC, Kopka J, Mangano AM, *et al.:* Circulation of novel HIV type 1 A, B/C, and F subtypes in Argentina. AIDS Res Hum Retroviruses 2005;21:158–164.
18. Díaz Pernas PL, Riesco Riesco S, Larrú Martínez B, Muñoz-Fernández MA, García-Bujalance S, and de José Gómez MI: False negative diagnosis of HIV-1. An Pediatr (Barc) 2006;65:158–161.
19. Aulicino P, Gómez Carrillo M, Kopka J, Mangano A, Ovejero M, and Sen L: HIV-1 genetic diversity in Argentina and early diagnosis of perinatal infection. Medicina (Buenos Aires) 2006;66:319–326.
20. Kwon JA, Yoon SY, Lee CK, *et al.:* Performance evaluation of three automated human immunodeficiency virus antigen-antibody combination immunoassays. J Virol Methods 2006; 133:20–26.
21. Ly TD, Ebel A, Faucher V, Fihman V, and Laperche S: Could the new HIV combined p24 antigen and antibody assays replace p24 antigen specific assays? J Virol Methods 2007;143:86–94.
22. Nunes CC, Matte MC, Dias CF, *et al.:* The influence of HIV-1 subtypes C, CRF31_BC and B on disease progression and initial virologic response to HAART in a southern Brazilian cohort. Rev Inst Med Trop Sao Paulo 2014;56: 205–211.

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