

# Characterization of Novel HIV-1 Intersubtype CRF01\_AE/C and A1/C Recombinants from India

Sudhanshu Shekhar Pandey, Madhuri Thakar, Ramesh Paranjape

Department of Immunology, National AIDS Research Institute (ICMR), Pune, India

**We report here three novel HIV-1 intersubtype recombinants from India. One among those is a recombinant between subtype C and CRF01\_AE and another two between A1 and C. A recombinant virus with CRF01\_AE is reported for the first time from India.**

Received 26 June 2015 Accepted 9 July 2015 Published 20 August 2015

**Citation** Pandey SS, Thakar M, Paranjape R. 2015. Characterization of novel HIV-1 intersubtype CRF01\_AE/C and A1/C recombinants from India. *Genome Announc* 3(4):e00849-15. doi:10.1128/genomeA.00849-15.

**Copyright** © 2015 Pandey et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Ramesh Paranjape, rameshsp1952@gmail.com.

Human immunodeficiency virus strains have shown extraordinary genetic diversity, as evidenced by 9 subtypes, >70 circulating recombinant forms (CRFs), and innumerable unique recombinant forms. Of these, subtype C is most prevalent in India, while subtypes A1, B, CRF01\_AE, and CRF02\_AG have occasionally been reported. Apart from these, A1/C (1, 2) and B/C (3) intersubtype recombinants have also occasionally been reported. Here, we report for the first time in India a unique HIV-1 recombinant between subtype C and CRF01\_AE and two novel recombinants between subtype A1 and C. Unique recombinant forms (URFs) between CRF01\_AE and subtype C have been reported from Myanmar, China, and Nepal (Los Alamos HIV Database).

The recombinant strain NARI-FLS\_VB5 was amplified using cocultured proviral DNA using a methodology described previously (4) and sequenced directly from the PCR product using the ABI Prism 3730XL DNA analyzer (Applied Biosystems). The near-full-length genome (NLFG) sequence was initially analyzed for recombination using RIP and for recombination breakpoints using jpHMM at Gobics and SimPlot. Breakpoints were further confirmed using phylogenetic analysis.

Strain NARI-FLS\_VB-5 showed an insertion of CRF01\_AE at three sites, positions 5711 ± 9 to 5901 ± 8, 6025 ± 13 to 8380 ± 7, and 8868 ± 6 to 8978 ± 6 into the backbone of subtype C, whereas at positions 8380 ± 7 to 8593 ± 7, jpHMM showed uncertainty between subtype C and CRF01\_AE. A bootscan analysis of NARI-FLS\_VB-5 showed similar breakpoints to those of jpHMM, with two more small insertions of CRF01\_AE at positions 2871 to 3050 and 4878 to 5013. Combining the results obtained with RIP (5), jpHMM (6), and SimPlot (1) suggests insertions of CRF 01\_AE at five positions in the backbone of subtype C.

Two of the strains, NARI-FLS\_VB27 and NARI-FLS\_IVC3, have been cloned and sequenced using a methodology described previously (4). NARI-FLS\_VB27 and NARI-FLS\_IVC3-1 showed recombination of subtype A1 into the backbone of subtype C. Both clones of NARI-FLS\_IVC3-1 showed an insertion of subtype A1 into the backbone of subtype C in *pol* (positions 3508 ± 17 to 4033 ± 35) and *nef-LTR* (positions 8578 ± 9 to 9632). Of the 11 clones generated for NARI-FLS\_VB27, six clones showed two small insertions of subtype A1 into the backbone of subtype C

int-*vif* (positions 4826 ± 84 to 5162 ± 10 and 5368 ± 10 to 5570 ± 26); one clone showed slightly different breakpoints (positions 4826 ± 84 to 5202 ± 20 and 5455 ± 24 to 5570 ± 26), and four clones showed an insertion of subtype A1 at positions 4826 ± 84 to 5162 ± 10 and uncertainty between subtype A1 and C at positions 5436 ± 5 to 5583 ± 12. Phylogenetic analysis using a recombinant region from positions 5368 to 5583 formed a monophyletic cluster with bootstrap support of 100. The phylogenetic tree suggests that the uncertainty region may be a result of the evolution of the strain after the introduction into the subject. SimPlot analysis also showed breakpoints in concordance with jpHMM (6) for both strains. All the breakpoints used in the study were relative to the HXB2 genome.

A comparison of the URFs characterized in this study with the earlier reported URF 01C and A1C recombinant forms suggest that all three characterized URFs are novel recombinants. The detection of an 01C recombinant also points to the fact that HIV viruses belonging to multiple subtypes are circulating and giving rise to new URFs. This clearly indicates that the HIV epidemic in India is still evolving and needs close monitoring.

**Nucleotide sequence accession numbers.** The near-full-genome shotgun sequences are available in GenBank under the accession numbers [KT175202](https://www.ncbi.nlm.nih.gov/nuccore/KT175202) to [KT175215](https://www.ncbi.nlm.nih.gov/nuccore/KT175215).

## ACKNOWLEDGMENTS

This work was supported in part by a grant from the Department of Biotechnology (DBT), Government of India (BT/PR7054/Med/14/936/2006).

We declare no competing interests.

## REFERENCES

- Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, Ingersoll R, Sheppard HW, Ray SC. 1999. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. *J Virol* 73:152–160.
- Pandey S, Tripathy S, Paranjape R. 2013. Molecular characterization of unique intersubtype HIV type 1 A1/C recombinant strain circulating in Pune, India. *AIDS Res Hum Retroviruses* 29:1245–1253. <http://dx.doi.org/10.1089/AID.2013.0150>.
- Lakhashe S, Tripathy S, Paranjape R, Bhattacharya J. 2008. Evidence of a

- novel B/C recombinant exhibiting unique breakpoints of near full-length HIV type 1 genome from northeastern India. *AIDS Res Hum Retroviruses* 24:229–234. <http://dx.doi.org/10.1089/aid.2007.0229>.
4. Lakhashe S, Tripathy S, Paranjape R, Bhattacharya J. 2008. Characterization of B/C recombinants of near full-length HIV type 1 from northeastern India with mosaics identical to ARE195FL but with a different ancestral origin. *AIDS Res Hum Retroviruses* 24:92–99. <http://dx.doi.org/10.1089/aid.2007.0214>.
  5. Siepel AC, Halpern AL, Macken C, Korber BT. 1995. A computer program designed to screen rapidly for HIV type 1 intersubtype recombinant sequences. *AIDS Res Hum Retroviruses* 11:1413–1416. <http://dx.doi.org/10.1089/aid.1995.11.1413>.
  6. Schultz AK, Bulla I, Abdou-Chekarou M, Gordien E, Morgenstern B, Zoaulim F, Dény P, Stanke M. 2012. jpHMM: recombination analysis in viruses with circular genomes such as the hepatitis B virus. *Nucleic Acids Res* 40:W193–W198. <http://dx.doi.org/10.1093/nar/gks414>.