Genome Sequence of a Novel HIV-1 Circulating Recombinant Form (CRF57_BC) Identified from Yunnan, China

Huamian Wei,^{1,*} Yongjian Liu,^{2,*} Yi Feng,¹ Jenny Hsi,¹ Hui Xing,¹ Xiang He,^{1,3}
Lingjie Liao,¹ Takebe Yutaka,^{1,4} Jingyun Li,² and Yiming Shao¹

Abstract

We identified a novel HIV-1 circulating recombinant form (designated CRF57_BC) from a total of four patients with no obvious epidemiologic linkage in western Yunnan (Dehong prefecture) in China. Two strains (09CN.YNFL37 and 10CN.DHFL17) were identified in this study. An additional two strains (341 and 1439) were found among strains reported in a previous study. CRF57_BC was composed of subtype B and subtype C, with one subtype B segment inserted into the *gag* region of the subtype C backbone. Subregion tree analysis showed that the B regions originated from a Thai B lineage and the C regions were from an India C lineage. The emergence of CRF57_BC may reflect the continual generation of various forms of intersubtype recombinants in western Yunnan.

HIV-1 CAN BE DIVIDED into four major phylogenetic groups: the M group, N group, O group, and the newly defined P group, of which the M group is the most widely epidemic in the world. In recent years, many cases of viral recombination between M group subtypes have been reported, and the circulation of recombinant strains is salient for HIV-1 prevention and control. In China, the main circulating strains are CRF01_AE, CRF07_BC, CRF08_BC, and subtype B, according to the third national HIV-1 molecular epidemiology survey.¹ As the putative place of origin and introduction for these strains within China, the government of Yunnan province has devoted much effort to HIV prevention and control. However, the ongoing generation of multiple forms of HIV-1 intersubtype recombinants is still occurring; new recombinant form strains have continued to be reported in this area.^{2,3} Here we identify another circulating recombinant form (CRF) strain (CRF57_BC) found in high-risk populations in western Yunnan and characterized its genomic features.

Blood plasma from three HIV-positive patients (YNFL37, DHFL17) in Dehong prefecture, Yunnan was collected (Table 1). The patients were diagnosed as HIV-1 positive in 2009 and recruited as part of a national HIV-1 drug resistance

survey in Yunnan. The study was approved by the institutional review boards of the National Center for AIDS/STD Control and Prevention of China. Near full-length genome (NFLG) amplification and sequencing were performed as previously reported.⁴ Recombination breakpoints were determined using RIP and jpHMM (www.hiv.lanl.gov) and SimPlot.⁴ Breakpoint confirmation and the origin of each segment were then analyzed by phylogenetic trees. The standard subtype reference alignment file including all HIV-1 group M, CRF01_AE, CRF07_BC, CRF08_BC, and CRF31_BC was downloaded from the Los Alamos National Laboratory HIV Database (www.hiv.lanl.gov). Nucleotide sequences were first aligned by using Clustal W, and then adjusted manually using BioEdit.⁵ Phylogenetic trees were constructed with MEGA 5.0 by using the neighbor-joining method with 1,000 bootstrap replications to confirm the subtype of mosaic fragments.⁶

An extensive database search revealed that two recombinant strains, 341 and 1439, show high-level of homology with the two strains in this study^{3,7} (Table 1). The four strains formed a distinct monophyletic cluster, distantly related to all known HIV-1 subtypes/CRFs (Fig. 1). Recombination analysis shows that the sequences were

¹State Key Laboratory for Infectious Disease Prevention and Control, National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Beijing, China.

²Department of AIDS Research, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, China.

³Guangdong Provincial Institute of Public Health, Guangzhou, China.

⁴AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan.

*These authors contributed equally to this work.

TABLE 1. DEMOGRAPHIC AND EPIDEMIOLOGIC CHARACTERISTICS OF STUDY SUBJECTS HARBORING CRF57_BC

Strain name	Year of sampling	Geographic origin (prefecture, province)	Sex	Age	Risk factor	Accession number	Reference/remark
341	2007	Dehong, Yunnan	Male	35	Injecting drug user	HM776939	Liu <i>et al.</i> ⁷
1439	2009	Dehong, Yunnan	Male	30	IDU	JX679207	Li <i>et al.</i> ³
09CN.YNFL37	2009	Dehong, Yunnan	Female	18	Heterosexual	KC870044	This study
10CN.DHFL17	2010	Dehong, Yunnan	Male	27	Heterosexual	KF250400	This study

NA, not available.

FIG. 1. Phylogenetic tree analysis of the near full-length genome (NFLG) sequences of the four isolates. All HIV-1 group M reference sequences were used to construct the neighbor-joining phylogenetic tree. Node stability was assessed by bootstrap analysis with 1,000 replications, and bootstrap values above 0.7 are shown at the corresponding nodes. All CRF57_BC isolates in this study are marked gray. The scale bar represents 2% genetic distance.

composed of subtypes B and C, with one region (II) of subtype B inserted into the subtype C backbone (I, III) (Fig. 2). The breakpoint positions refer to HXB2 coordinates, and were located by HIV Sequence Locator (www.hiv.lanl.gov/content/sequence/LOCATE/locate.html). Therefore, the map of the recombinant genome is as follows: C (790–1,200 nt), B (1,225–1,808 nt), and C (1,863–9,417 nt). Subregion segments were verified by phylogenetic analysis, which was well confirmed by bootstrap values above 0.7. The analysis also indicated that the parental origin of subtype B regions is probably from a Thai B lineage and the subtype C regions originate from an India C lineage (Fig. 3). The recombinant structure is distinct from any known CRFs to date. Therefore, these new recombinants are now designated CRF57_BC.

All of China's major prevalent HIV-1 strains are found in Dehong, Yunnan province, commonly considered the epicenter for HIV/AIDS in China. Previous studies have demonstrated that both CRF07_BC and CRF08_BC originated from Dehong prefecture^{2,8} and were spread widely to other parts of the nation through drug trafficking routes. The co-existence of multiple strains along with a sustained active epidemic increases the probability of recombination. However, in contrast to the nationwide transmission of CRF07_BC and CRF08_BC,¹ the newly formed URFs and CRFs were found circulating on only a small scale in Dehong. This may be due to the relatively limited mobility of the local population, compounded by currently strict controls on drug trafficking that further impede high-risk contact and

FIG. 2. Recombination breakpoint analyses of CRF57_BC. **(A)** Bootscan plots of CRF57_BC with subtype B (RL42), subtype C (95IN21068), and subtype J (04CMU11421) as reference genotypes. The parameters of SimPlot bootscan analysis were set as the default except for a window size of 300 and step size of 30. **(B)** Genomic map of CRF57_BC. The mosaic map was generated using the Recombinant HIV-1 Drawing Tool (www.hiv.lanl.gov/content/sequence/DRAW_CRF/recom_mapper.html).

FIG. 3. Phylogenetic analyses of three mosaic segments defined by bootscanning. The phylogenetic trees of the three mosaic segments were constructed with MEGA 5.0 using the neighbor-joining method. The subtype references of the M group are derived from the Los Alamos HIV Sequence Database. Node stability was assessed by bootstrap analysis with 1,000 replications, and only bootstrap values above 0.7 are shown at the corresponding nodes. The sequences of CRF57_BC are marked in bold black.

transmission through other means (e.g., heterosexual transmission).

In this study, we characterized a novel HIV-1 circulating recombinant form CRF57_BC, consisting of different recombinant breakpoints between the subtype B and subtype C strains, from three donors without obvious epidemiologic linkage in Dehong prefecture, Yunnan province, China. The emergence of the new CRF may further complicate the development of ef-

fective vaccines to limit the HIV-1 epidemic in China. In addition, whether recombination may confer selective advantages over parental viruses remains to be investigated.

Sequence Data

The nucleotide sequences of the two isolates 09CN.YNFL37 and 10CN.DHFL17 have been submitted to

GenBank with accession numbers KC870044 and KF250400, respectively.

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Author Disclosure Statement

No competing financial interests exist.

References

1. He X, Xing H, Ruan Y, *et al.*: A comprehensive mapping of HIV-1 genotypes in various risk groups and regions across China based on a nationwide molecular epidemiologic survey. *PLoS One* 2012;7(10):e47289.
2. Yang R, Kusagawa S, Zhang C, Xia X, Ben K, and Takebe Y: Identification and characterization of a new class of human immunodeficiency virus type 1 recombinants comprised of two circulating recombinant forms, CRF07_BC and CRF08_BC, in China. *J Virol* 2003;77(1):685–695.
3. Li L, Chen L, Yang S, *et al.*: Recombination form and epidemiology of HIV-1 unique recombinant strains identified in Yunnan, China. *PLoS One* 2012;7(10):e46777.
4. Wei H, Su L, Feng Y, *et al.*: Near full-length genomic characterization of a novel HIV type 1 CRF07_BC/01_AE recombinant in men who have sex with men from Sichuan, China. *AIDS Res Hum Retroviruses* 2013;29(8):1173–1176.
5. Hall TA: BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Paper presented at the Nucleic Acids Symposium Series 1999.
6. Tamura K, Dudley J, Nei M, and Kumar S: MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007;24(8):1596–1599.
7. Liu Y, Li L, Yang S, *et al.*: Identification and characterization of two new HIV type 1 unique (B/C) recombinant forms in China. *AIDS Res Hum Retroviruses* 2011;27(4):445–451.
8. Tee KK, Pybus OG, Li XJ, *et al.*: Temporal and spatial dynamics of human immunodeficiency virus type 1 circulating recombinant forms 08_BC and 07_BC in Asia. *J Virol* 2008;82(18):9206–9215.

Address correspondence to:

Jingyun Li

Department of HIV/AIDS Research
State Key Laboratory of Pathogen and Biosecurity
Beijing Institute of Microbiology and Epidemiology
20 Dongda Street
Fengtai District
Beijing 100071
China

E-mail: lijy@nic.bmi.ac.cn

Yiming Shao

State Key Laboratory for Infectious
Disease Prevention and Control
National Center for AIDS/STD Control and Prevention
Chinese Center for Disease Control and Prevention
155 Changbai Road
Beijing 102206
China

E-mail: yshao@bnn.cn