HIV-1 Transmission Among Injection Drug Users Leads To Novel Recombinants Circulating In Southern China

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Abstract

Injection drug use has been responsible for most HIV-1 transmission in Asia and has led to the generation and/or spreading of circulating recombinant CRF01_AE, CRF07_BC, and CRF08_BC, which are currently the predominant HIV-1 strains in China. Monitoring HIV-1 transmissions among injection drug users (IDUs) still provides critical information on HIV-1 prevalence and control. In the present study, we detected three HIV-1 sequences, BH048/057/066, from IDUs in Beihai city, Guangxi province, all of which share an extremely complex mosaic recombination pattern. CRF01_AE and CRF08_BC contributed to the rise of new recombinants, which contain CRF08_BC-derived *env* fragments with evidence of further evolution. Our data, combined with those of other studies, suggest that the BH048/057/066 recombinants represent early strains of a circulating recombinant form of HIV-1 in southern China. Information concerning such newly appearing variants is critical for both HIV-1 control and ongoing HIV-1 vaccine trials in Guangxi, China.

THE ROUTES OF TRANSMISSION OF HUMAN immunodeficiency virus type 1 (HIV-1) in China have been expanding since it was first discovered there.¹ Several early outbreaks of HIV-1 infection occurred in southern China, which is located on the trafficking routes for heroin and other drugs from southern/southeastern Asia into China.^{2,3} Therefore, HIV-1 infections in the early outbreaks occurred mainly among injection drug users (IDUs).^{2–5} Although sexual activity has recently become the major pathway for HIV-1 transmission, transmission via IDUs still requires attention. New HIV-1 infections continue to be reported among IDUs, especially in southern China,⁶ which may lead to the generation of novel circulating recombinant forms (CRFs) of HIV-1. These CRFs should be beneficial to the virus, because they combine different gene fragments from different subtypes, making it easier for the virus to adapt to local cell tropisms, counteract host defense mechanisms, and/or spread under evolutionary selection pressure.^{6,7} Here, we report that we have identified a novel recombination form among three HIV-1 sequences retrieved from IDUs in Beihai city in Guangxi province, China.

Previously, in Guangxi province, we successfully retrieved a total of 65 HIV-1 sequences from patients who were infected with HIV-1 through injection drug use.⁶ Analysis of partial *gag/pol* and *env* fragments with the help of MEGA5⁸ suggested that these strains belonged to existing subtypes such as CRF01_AE, CRF07_BC, and CRF08_BC,⁶ with

CRF08_BC and CRF01_AE being the predominant forms in southern China as seen in previous studies.^{9–11} However, three additional strains, termed BH048, BH057, and BH066, presented inconsistent subtyping results in the *gag/pol* and *env* regions (Fig. 1), suggesting the possible existence of HIV-1 recombination forms of CRF01_AE and CRF08_BC among these samples from IDUs in Beihai.

To further confirm our recombination events, we performed single nested polymerase chain reaction (PCR) with the help of ExTaq DNA polymerase (TaKaRa, Japan) and successfully retrieved the 4-kb fragments between known *gag/pol* and *env* sequences of these HIV-1 strains. SimPlot (version 3.5.1)¹² was used to reveal the existence of recombination, and similarity tests (data not shown) were later done to confirm the recombination. The combined sequence of BH048 (covering HXB2 1850–7642) revealed a complex recombination pattern. Recombination between the parental subtypes (i.e., CRF01_AE and CRF08_BC) occurred several times along the sequence (Fig. 2A). Indeed, a mosaic pattern was observed in both *gag* and *pol*, which is different from bootscanning results of BH045 and BH046 that were sampled at the same time as BH048 but were previously determined to be CRF01_AE and CRF08_BC, respectively.⁶ CRF08_BC contributed to *vif* and *vpr*, while CRF01_AE was the source of *vpu* and at least part of *tat* and *rev* (Fig. 2B). Interestingly, although it was genetically related to CRF08_BC, the *env* fragment 6392–7642 of BH048 still contained on

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FIG. 1. Phylogenetic analyses of HIV-1 sequences retrieved from Beihai city in China reveal possible recombination events. **(A)** Phylogenetic analysis performed with retrieved *gag/pol* sequences from Beihai city suggests that BH048/057/066 belong to CRF01_AE. **(B)** Phylogenetic analysis performed with retrieved *env* sequences from Beihai city suggests BH048/057/066 likely evolved from subtype CRF08_BC, based on the locally predominant HIV-1 subtypes. The reference sequences were retrieved from the HIV database at the Los Alamos National Laboratory (www.hiv.lanl.gov). The sequences used correspond to positions 1,850–2,961 in HXB2 for *gag/pol* and 6883–7642 for *env*. Nucleotide sequence alignment was performed with the help of MEGA5 software. Phylogenetic trees were constructed with the neighbor-joining statistical method and Kimura two-parameter as the model. The phylogenetic tree was determined for 1,000 replicates with random seeds. Only strong bootstrap values (>70%) are shown. The scale bar shows nucleotide substitutions per site.

average 167 nucleotide (or 54 amino acids among Env fragment 58–473) differences compared to the CRF08_BC strains circulating in China; the magnitude of the differences was similar to what appeared between subtypes C, CRF07_BC, and CRF08_BC (Table 1), suggesting that the BH048 *env* sequence might represent a significant variant of the CRF08_BC *env* fragment. Surprisingly, despite the evolved *env* fragments, bootscanning results for BH048/057/066 demonstrated an extremely similar recombination pattern among all three HIV-1 sequences (Fig. 2A).

Patients with BH048/057/066 (hereafter designated BH048) were all male and were in their 30s when the samples were obtained in 2008, but they shared no other common demographic characteristics, such as marriage status, occupation, or period of IDU. The only apparent connection among these people was their use of injection drugs. Therefore, we believed we might have discovered a new CRF of HIV-1 in Guangxi province, and we therefore checked the circulating status of BH048. Literature screening revealed that HIV-1 strain GXDY1299¹³ and strain 2261¹⁰ share an extremely similar recombination pattern with BH048, yet GXDY1299 and 2261 are transmitted through sexual contact, not injection drug use. Only 11 different nucleotides were detected between BH048 and 2261 in overlapping fragments

1850–5096. Thus, strains of BH048 are now circulating among IDUs and sexual contacts, two high-risk groups for HIV-1 transmission in China.

Recently, new HIV-1 recombination patterns have been reported in China^{10,13}; most of them, however, have been determined to be unique recombinant forms (URFs) that may not actually circulate. URFs are more easily detected among IDUs because of these individuals' high-risk activities, such as sharing needles. Here we report that we have also detected a novel recombinant form of HIV-1 among IDUs in the Guangxi province of China. CRF01_AE and CRF08_BC entered Guangxi province through different drug trafficking routes,² became locally dominant HIV-1 subtypes,⁶ and have now merged again among IDUs to generate recombinant strains of BH048/057/066.

Alerts should be raised in response to the generation of this new recombinant form of BH048, which may be circulating. Evidence from the sequencing of partial or nearly full-length HIV-1 genomes indicates that the 2261 and GXDY1299 strains detected after 2009 share high-level similarity in terms of both nucleotide sequence and recombination pattern with BH048. Thus, our sequences represent an early form of this new HIV-1 CRF. More importantly, both GXDY1299 and 2261 were transmitted through heterosexual activity,

FIG. 2. A novel recombination form of HIV-1 was detected in Beihai city. **(A)** Bootscanning analysis of BH048/057/066. The results indicate that these sequences present a similar, yet mosaic, pattern of CRF01_AE/CRF08_BC recombination. The sequences used correspond to positions 1,850–7,642 in HXB2. BH048/057/066 were first aligned with HIV-1 reference sequences retrieved from the LANL HIV-1 database (www.hiv.lanl.gov). BH048, 057, or 066 was set as the query sequence and the bootscanning was performed with a window size of 200 nt and steps of 20 nt, with the help of SimPlot software. Bootscanning results among the *gag/pol* fragment (HXB2 1,850–2,961) of BH048, BH045 (CRF01_AE), and BH046 (CRF08_BC) were shown on the right for direct comparison of the recombination events. **(B)** A schematic cartoon showing recombination in the BH048 genome. Black indicates CRF08_BC, light gray indicates CRF01_AE, and white indicates unsequenced regions. Dashed lines were used to indicate possible break points determined by bootscanning.

indicating that BH048, which was generated among IDUs, has already started to be transmitted through sexual contact, currently a predominant pathway for HIV-1 transmission. Therefore, although the criteria for a novel CRF designation¹⁴ have not been met, the extremely complex recombination pattern identically observed among all five sequences retrieved by three independent studies suggests that HIV-1 BH048 has continued to spread during the past several years, has moved from one high-risk group to another, and is still responsible for novel infection events.

In addition, with the help of the HIV Drug Resistance Database at Stanford University (<http://hivdb.stanford.edu>),¹⁵ we detected antiviral drug-resistant mutations in two of these three strains (V179D in the reverse transcriptase of strain BH048 and possibly E138K in the integrase of BH057). Therefore, uncontrolled transmission of this new HIV-1 CRF may be coupled with the spread of drug-resistant

mutations. It should also be noted that all the strains of this novel CRF were detected in Guangxi province, where a phase II HIV-1 vaccine trial is currently being conducted. The evolved *env* sequences detected in BH048 suggest an adaptation of this new CRF to local cell tropism, which points to a serious need for further attention and possible adjustments to the process of anti-HIV-1 vaccine development.

Sequence Data

All HIV-1 sequences retrieved from this study have been submitted to GenBank, with accession numbers KF803577 to KF803579.

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TABLE 1. NUMBER OF DIFFERENT NUCLEOTIDES/AMINO ACIDS AMONG *env* FRAGMENTS BETWEEN SUBTYPES C/CRF07_BC/CRF08_BC AND BH048

Subtypes	Nucleotide			
	BH048	C	CRF07_BC	CRF08_BC
BH048 (<i>n</i> = 3)	17 ± 5.2			
C (<i>n</i> = 6)	186 ± 19	131 ± 26		
CRF07_BC (<i>n</i> = 27)	192 ± 13	176 ± 23	110 ± 30	
CRF08_BC (<i>n</i> = 16)	167 ± 18	135 ± 24	179 ± 25	95 ± 35
Subtypes	Amino acid			
	BH048	C	CRF07_BC	CRF08_BC
BH048 (<i>n</i> = 3)	9 ± 1			
C (<i>n</i> = 6)	67 ± 2	61 ± 8		
CRF07_BC (<i>n</i> = 27)	66 ± 13	63 ± 16	56 ± 24	
CRF08_BC (<i>n</i> = 16)	54 ± 8	64 ± 8	62 ± 17	49 ± 17

Sequences corresponding to HXB2 6392–7642 in the RNA genome (or Env 58–473 in protein sequence). The analysis was performed with MEGA5. Data are shown as means ± SD. Subtype C/CRF07_BC/CRF08_BC sequences circulating in China were retrieved from the HIV database at the Los Alamos National Laboratory (www.hiv.lanl.gov).

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Author Disclosure Statement

No competing financial interests exist.

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