

## Genomic Characterization of Two Novel HIV-1 Unique (CRF01\_AE/B) Recombinant Forms Among Men Who Have Sex with Men in Beijing, China

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### Abstract

We report here two novel HIV-1 recombinant forms (CRF01\_AE/B) isolated from two HIV-positive male subjects infected through homosexual contact in Beijing, China. Recombination contributes substantially to the genetic diversity of HIV-1, and is likely to occur in populations in which multiple subtypes circulate. Molecular epidemiological studies showed that subtype B, CRF01\_AE, and CRF07\_BC are currently cocirculating in parallel among men who have sex with men (MSM) in China, providing the opportunity for the emergence of new recombinants. Phylogenetic analysis of near full-length genome (NFLG) sequences showed that the unique recombinant forms (URFs) were composed of gene regions from CRF01\_AE and subtype B. The CRF01\_AE region of the recombinants clustered together with a previously described cluster 4 lineage of CRF01\_AE. The B regions of both the recombinants clustered within the B strains. The two recombinants were quite similar with six breakpoints in common. These data highlight the importance of continuous surveillance of the dynamic change of HIV-1 subtypes and new recombinants among the MSM population.

**H**IV-1 IS CHARACTERIZED BY its high degree of genetic diversity. Phylogenetic analyses of HIV-1 isolates derived from different geographic locations have classified HIV-1 strains into four major groups: M, O, N, and group P.<sup>1</sup> HIV-1 group M is predominant in HIV-1 infections worldwide and can be further divided into nine subtypes (A–D, F–H, J, and K), five subsubtypes (A1–A3, F1, and F2), and numerous recombinant forms. To date, 72 circulating recombinant forms (CRFs) have been identified in the Los Alamos HIV database ([www.hiv.lanl.gov](http://www.hiv.lanl.gov)). In addition, many unique recombinant forms (URFs) have developed in populations in which multiple HIV-1 strains cocirculate.<sup>1</sup>

Recombination substantially contributes to the overall genetic diversity of HIV-1. It has been reported that multiple HIV-1 genotypes, including subtype B, CRF01\_AE, and CRF07\_BC, are currently circulating in the men who have sex with men (MSM) population in China.<sup>2</sup> HIV-positive MSM constituted 7.3% of the national total of newly reported HIV cases in 2005. This proportion increased to 32.5% in 2009 and stabilized at around 27% in 2011.<sup>3</sup> The cocircula-

tion and rapid transmission of multiple HIV-1 genotypes provide the opportunity for the generation of new recombination forms. Indeed, we identified and characterized two novel recombinants derived from subtype B and CRF01\_AE, which were highly different from the previously reported CRF01\_AE/B recombinant.<sup>4–8</sup>

Plasma samples were collected from two HIV-positive MSM from an IRB approved study conducted by China CDC. Both of them were infected with HIV-1 through MSM, and were confirmed as HIV-1 seropositive in May 2013 for BJMP3037B and in September 2013 for BJMP3194B, respectively. Participant BJMP3037B had more than 30 MSM sexual partners in his lifetime. Subject BJMP3194B had four male sexual contacts with two in the past 3 months. Subject BJMP3194B was reported to have used nitrite inhalants. The CD4<sup>+</sup> T cell counts were 293 cells/ $\mu$ l and 1,312 cells/ $\mu$ l and the viral loads were 57,300 copies/ml and 29,700 copies/ml for BJMP3037B and BJMP3194B, respectively. They are not sexual partners. More detailed information concerning the two participants is given in Table 1.

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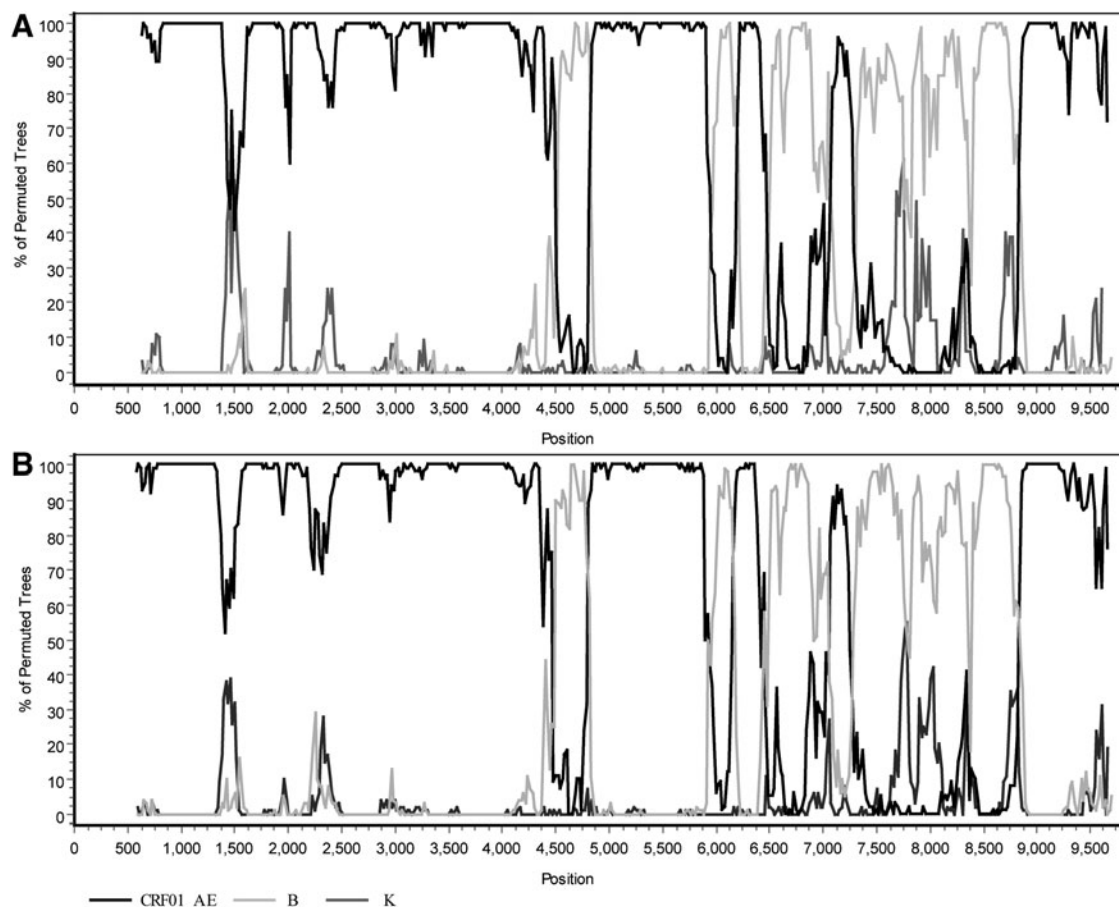
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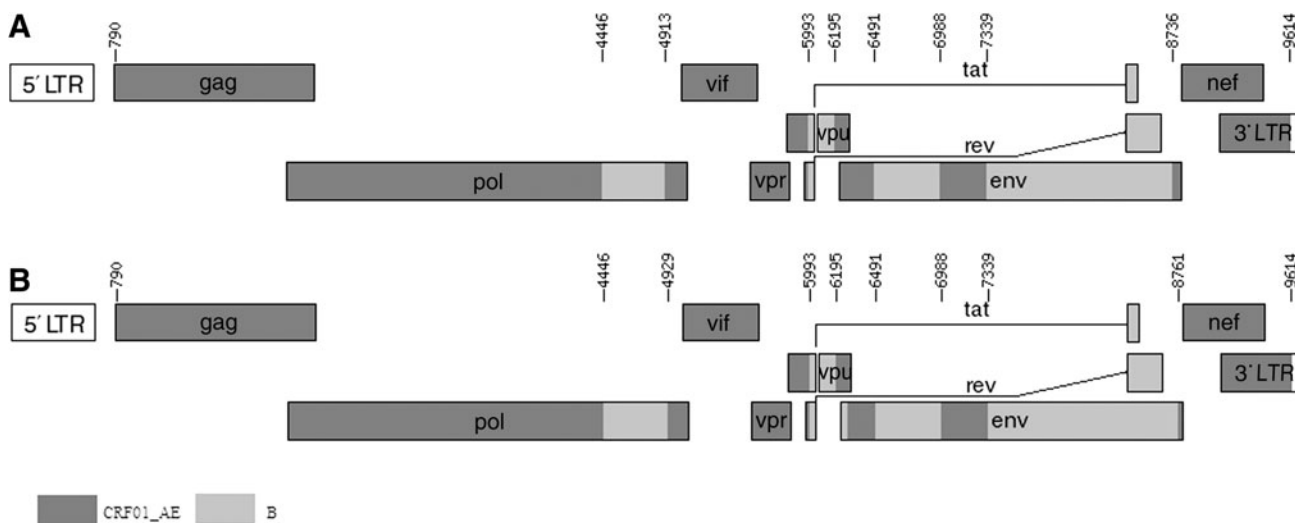




**FIG. 2.** Bootscan analysis of the near full-length nucleotide sequences of BJMP3037B (A) and BJMP3194B (B). Bootscan analysis was performed using CRF01\_AE, B, and subtype K reference sequences. The bootscan window was 300 bp with a step size of 10 bp using SimPlot 3.5.1 software. The x-axis is the nucleotide position in the HIV genomic sequence; the y-axis indicates the percentage supporting the clustering with reference sequences.

backbone with four subtype B fragments inserted in the *pol*, *vpu*, and *env* regions (Figs. 2 and 3). There is a very small region derived from CRF01\_AE in the *env* gene; it appears as a “blip” in the bootscanning near position 8,200 with a

300 base window. To demonstrate the genomic graphic structure, the Map-Draw Tool available at the Los Alamos HIV sequence database ([www.hiv.lanl.gov/content/sequence/DRAW\\_CRF/recom\\_mapper.html](http://www.hiv.lanl.gov/content/sequence/DRAW_CRF/recom_mapper.html)) was used to



**FIG. 3.** Genetic map of BJMP3037B (A) and BJMP3194B (B). Map-draw tool is available at the Los Alamos HIV sequence database ([www.hiv.lanl.gov/content/sequence/DRAW\\_CRF/recom\\_mapper.html](http://www.hiv.lanl.gov/content/sequence/DRAW_CRF/recom_mapper.html)).

generate the genomic map of the two new HIV URFs (Fig. 3).

The phylogenetic trees of the individual gene regions confirmed the breakpoints of the two NFLG sequences as follows (Supplementary Fig. S1; Supplementary Data are available online at [www.liebertpub.com/aid](http://www.liebertpub.com/aid)): I (790–4445 nt) CRF01\_AE, II (4446–4912 nt) B, III (4913–5992 nt) CRF01\_AE, IV (5993–6194 nt) B, V (6195–6490 nt) CRF01\_AE, VI (6491–6987 nt) B, VII (6988–7398 nt) CRF01\_AE, VIII (7339–8735 nt) B, IX (8736–9614 nt) CRF01\_AE (BJMP3037B); I (790–4445 nt) CRF01\_AE, II (4446–4928 nt) B, III (4929–5992 nt) CRF01\_AE, IV (5993–6279 nt) B, V (6280–6490 nt) CRF01\_AE, VI (6491–6987 nt) B, VII (6988–7398 nt) CRF01\_AE, VIII (7339–8760 nt) B, IX (8761–9614 nt) CRF01\_AE (BJMP3194B). Bootscan analysis of the two NFLG sequences revealed that they shared six identical breakpoints (nt 4446, nt 4921 ± 8, nt 5993, nt 6491, nt 6988, and nt 7339), using HXB2 as a reference, as shown in the genomic map (Fig. 3). As MSM without any known epidemiological links, it is noteworthy that they are infected with a high degree of consistency in the breakpoints.

We analyzed the parental origin of all the fragments of the two NFLGs. The CRF01\_AE regions for both URFs were from the CRF01\_AE cluster 4 lineage<sup>11</sup> (Supplementary Fig. S1), which is circulating primarily among Beijing MSM. The subtype B regions clustered within the B strains. Subtype B and CRF01\_AE have been circulating in China since the 1990s, predominantly among MSM for subtype B and among heterosexuals for CRF01\_AE.<sup>12,13</sup> Previously, CRF01\_AE was found among MSM in 2005<sup>14</sup> and then experienced a rapid increase, from 3.7% to 30.2% in 2006 and 2008,<sup>2,15</sup> and finally replacing subtype B as the most predominant strain in the MSM population after 2008.<sup>16</sup> Overall, cocirculation of CRF01\_AE and subtype B among MSM is likely to facilitate the generation of different unique recombinant forms of CRF01\_AE and subtype B, as reported here in two novel URFs.

We have previously described two URF NFLG sequences composed of CRF01\_AE/CRF07\_BC second-generation recombinants among Beijing MSM.<sup>17</sup> As the cocirculation of multiple HIV-1 subtypes continues among the MSM population, it is likely that new recombinants of HIV-1 will continue to emerge. Since HIV infections are increasing alarmingly within the MSM risk group in China, monitoring the diversity may provide a means of following the developing epidemic in China.

### Sequence Data

The NFLG sequences of BJMP3037B and BJMP3194B have been deposited in GenBank with accession numbers KP418805 and KP418806 respectively.

### Acknowledgments

This study was supported by the Chinese National Natural Science Foundation of China (81261120393, 81471962), the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award R01AI094562, and the Chinese National Science and Technology Major Projects for Infectious Diseases Control and Prevention (2012ZX1001-002). The funders had no role in the study design, data col-

lection and analysis, decision to publish, or preparation of the manuscript.

### Author Disclosure Statement

No competing financial interests exist.

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