



## Letter to the Editor

### A novel HIV-1 circulating recombinant form (CRF168\_0107) identified from men who have sex with men in Beijing, China



Dear Editor,

Recent correspondence in this journal reported several novel second-generation HIV-1 circulating recombinant forms (CRFs) between CRF01\_AE and CRF07\_BC among men who have sex with men (MSM) in China.<sup>1,2</sup> Recombination occurring during the simultaneous infection of two or more viruses into the same cell has resulted in the emergence of unique recombinant forms (URFs), accounting for 2.8% of infections in China.<sup>3,4</sup> Over the past decade years, CRF07\_BC and CRF01\_AE have become the predominant circulating strains, especially among the MSM population in China,<sup>4</sup> along with the frequent recombination between the two strains resulted in serial novel CRFs\_0107.<sup>1,2</sup> According to the LANL HIV sequence database (<http://www.hiv.lanl.gov>), since CRF79\_0107 was first identified in Shanxi, China in 2017, a total of 18 second-generation CRFs between CRF01\_AE and CRF07\_BC have been reported nationwide. Beijing, as the capital of China, attracted a host of talents and visitors nationwide or worldwide with convenient communications. Approximately seventy percent of newly identified individuals got infected via MSM. The complex genetic diversity prevalent in Beijing, together with the inherent capability of active sexual behavior and multiple sexual partners, contributed to the increasing URFs.<sup>4,5</sup> In the present study, we identified a lineage of novel CRF among four HIV-1-infected MSM in Beijing, China, designated as CRF168\_0107. To trace the origin and viral transmission of this strain, we obtained the near-full length genome amplification (NFLG) sequences and made a further inference by systematical phylogenetic analyses.

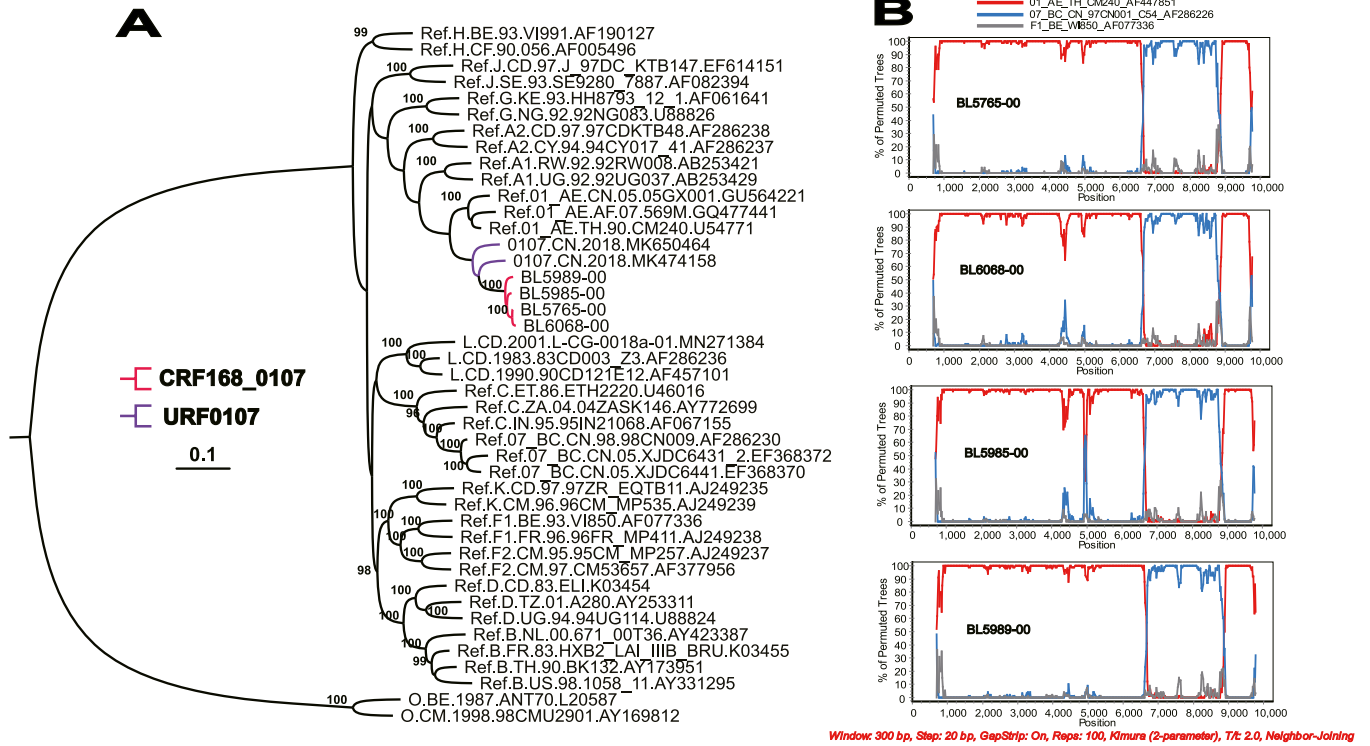
During our consecutive surveillance of HIV-1 molecular epidemiology and pretreatment drug resistance in Beijing, we identified a monophyletic transmission cluster consisting of four MSM individuals by discordant subtyping between partial *pol* and *env* gene sequences. The four individuals (BL5989-00, BL5985-00, BL5765-00 and BL6068-00) were diagnosed in 2022 (n=1) and 2023 (n=3), without obvious epidemiological linkage, except for the close sexual partners between BL5989-00 and BL5985-00. The basic demographic and laboratory information for the four individuals was presented in [Table S1](#). Written informed consent was obtained from all participants before sample and epidemiology data collection. The study was approved by the Ethics Committee of the Beijing Center for Disease Prevention and Control (approval number: 2022 No. [13]).

The HIV-1 NFLG sequences were amplified and sequenced as described previously.<sup>5</sup> The four sequences were 8998, 8973, 9012, and 8960 nucleotides (nt) in length, respectively, spanning from 5' long terminal repeats (LTR) to 3' LTR corresponding to the location 634–9605 nt of HXB2 strain. The sequences were submitted to GenBank under accession numbers PP975254 to PP975257.

The phylogenetic analysis demonstrated that the four NFLG sequences formed a tightly monophyletic cluster with the Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT) node support value of 100%, loosely clustered with other two URFs 0107 ([Fig. 1A](#)). Notably, our sequences didn't share recombination patterns with the two 0107 URFs from Tianjin ([Fig. S1](#)). To further investigate their recombination structure, recombination analyses were performed using LANL RIP (Recombinant identification program) and Simplot v3.5 software. The genome was divided into three segments, with two recombination breakpoints (I:634–6417 nt, II:6418–8463 nt, and III:8464–9605 nt) and the recombinant gene fragments spanned *env/rev* partial gene regions ([Fig. 1B](#) and [Fig. 2A](#)). The mosaic structure of the recombination was confirmed again by segmental phylogenetic analyses, showing regions I (HXB2:634–6417 nt) and III (HXB2:8464–9605 nt) to cluster with clade CRF01\_AE\_g4 reference sequences, with SH-aLRT support values of 99% and 100%. Segments II (HXB2:6418–8463 nt) of CRF168\_0107 belonged to the CRF07\_BC MSM lineage with an SH-aLRT support value of 100% ([Fig. 2B](#)).

To better trace the origin of CRF168\_0107, we performed Bayesian inference using combined CRF01\_AE regions (I + III) and CRF07\_BC regions (II) to estimate the tMRCA. The estimated tMRCAs for the concatenated CRF01\_AE and CRF07\_BC regions were 2016.9 [95% highest probability density (HPD): 2015.0, 2018.6] and 2018.6 [95% HPD: 2016.3, 2020.7], respectively ([Fig. 2C](#)). Hence, CRF168\_0107 was inferred to originate approximately from November 2016 to July 2018.

The CRF01\_AE was the first CRF observed in China around 1994, and nowadays, it is the top two strain.<sup>3,4,6</sup> Currently, the strains of CRF01\_AE have been further divided into 11 clusters, with great diversity and fast transmission,<sup>7</sup> and clusters 4 and 5 were highly prevalent among MSM in northern China, including Beijing.<sup>8</sup> Two phylogenetic subclusters of CRF07\_BC were circulating in China (CRF07\_BC\_N and CRF07\_BC\_O). CRF07\_BC\_O initially emerged among injecting drug users, subsequently evolving to CRF07\_BC\_N after transmission among the MSM population.<sup>9</sup> The co-circulation of diverse strains in the same geographic region and among the population at high risk contributed to co-infection or super-infection, generating novel recombinants. The above observations happened among the MSM population in Beijing, providing sufficient background for the emergence of inter-subtype recombinants be-



**Fig. 1.** (A) Maximum likelihood (ML) tree of CRF168\_0107 NFLG sequences. The ML phylogenetic tree was constructed using the four NFLGs sequences, two URFs 0107 (ML560464 and MK474158 were from Tianjin, China that showed about 92% similarity to CRF168\_0107 by BLAST), and HIV-1 subtyping references (subtypes A-D, F-H, and J-L, CRF01\_AE, CRF07\_BC, and outgroup O). Database sequences were labeled with subtype, two-letter ISO code of the country of collection, virus names, and GenBank accession. Sequences obtained in our laboratory and similar sequences retrieved from the databases were in red and purple, respectively. Only the SH-aLRT node support values  $\geq 90\%$  were shown; (B) Bootscan analyses of CRF168\_0107. The horizontal axis represented the position in the HXB2 genome of the midpoint of a 300 nt window size with steps in 20 nt increments, and the vertical axis represented the bootstrap value supporting clustering of the query sequence with subtyping references.

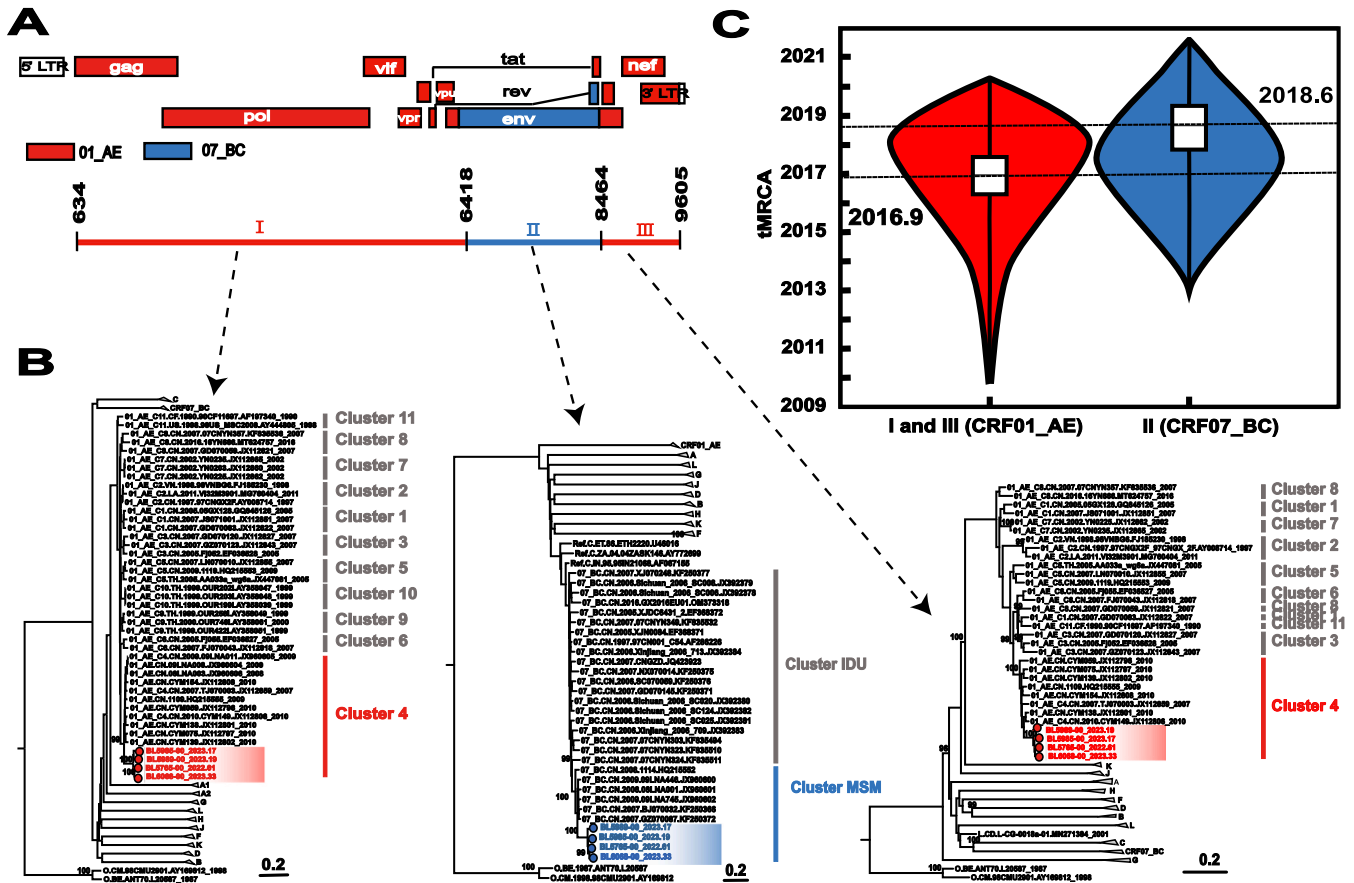
tween the two CRFs. In addition to CRF168\_0107, two documented strains of CRF80\_0107<sup>10</sup> and CRF113\_0107 have been previously identified among the MSM population in Beijing.

Recently, another three novel CRFs of CRF125\_0107, CRF158\_0107, and CRF170\_0107 were reported in Yunnan, which were recombined by CRF01\_AE\_g4 and CRF07\_BC\_N.<sup>1,2,11</sup> The high frequencies of recombination between CRF01\_AE and CRF07\_BC suggested that it is imperative to closely monitor virus transmission and evolution across the regions and populations at high risk. Otherwise, the frequent recombination undermined the prevalence of the parental strains.

The epidemiological investigation revealed that case BL6068-00 was at the acute phase of HIV-1 infection, for the last negative testing of HIV antibody on October 11, 2022, and the positive confirmatory WB testing on January 24, 2023. Additionally, the BL6068-00 got infected in Huanghua, Hebei. BL5989-00 was the

sexual partner of BL5985-00. According to the lower CD4 cell counts at diagnosis (Table S1) combined with the topology structure (Fig. 1A), BL5989-00 was the source of the subcluster. All these observations suggest that this novel strain has spread actively among the population.

In conclusion, a novel HIV-1 CRF, designated CRF168\_0107, was identified from the MSM population in Beijing, China. And the backbone genome of CRF01\_AE\_g4 was substituted by CRF07\_BC\_N gene fragment spanning the *env-rev* gene. It is inferred that CRF168\_0107 was estimated to originate around Nov 2016 to Jul 2018. The emergence of CRF168\_0107 introduces further complexity of HIV-1 diversity in China. Furthermore, our findings emphasize the necessity for continuous surveillance of HIV-1 diversity and tracing the transmission, to better control the HIV epidemic with effective measures.



**Fig. 2.** (A) Genome mosaic structure of HIV-1 CRF1168\_0107. Breakpoint positions according to the HXB2 location were indicated; (B) Subregion phylogenetic trees of CRF168\_0107. All the subregion ML trees were reconstructed using the same references, including outgroup O, subtype A-D, F-H, and J-L, CRF01\_AE C1-C11, and Chinese CRF07\_BC. Only the SH-aLRT node support values  $\geq 90\%$  were shown. CRF168\_0107 sequences were colored in red and blue; (C) times to the most recent common ancestor (tMRCA) were shown by the Violin Plot, with the horizontal axis representing the subregion and the vertical axis representing tMRCA. The analysis was conducted using Markov chain Monte Carlo (MCMC) chains with 200 million steps, with sampling from the posterior distribution occurring every 5000 steps, and the best-fit model was GTR +G+I.

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**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Appendix A. Supporting information**

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jinf.2024.106368](https://doi.org/10.1016/j.jinf.2024.106368).

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