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Letter to the Editor

Characterization of the first unambiguous HIV-1 CRF07_BC/CRF08_BC circulating recombinant form (CRF160_0708) in Yunnan, China

Dear editor,

Recent correspondence in this journal reported the identification of second and third generation HIV-1 circulating recombinant forms (CRFs) consisting of the previously identified CRFs in China.[1,2] A notable epidemiological feature of HIV-1 genotypes in China is that they are dominated by recombinant viruses, the top four of which include CRF01_AE, CRF07_BC, CRF08_BC and CRF55_01B.[3] In China, more CRFs consisting of any two of CRF01_AE, CRF07_BC and CRF55_01B have been reported recently.[1,2] However, few CRFs were reported from the recombination of CRF07_BC and CRF08_BC. In this study, we identified a novel CRF consisting of CRF07_BC and CRF08_BC in Yunnan Province, designated as CRF160_0708. We further analyzed its recombination structure and investigated its evolutionary history.

From the previous study conducted in Yunnan Province, six *pol* sequences were found not to be classified as known HIV-1 genotypes and formed a unique cluster, indicating a potential novel CRF. Of them, one (21ZT386) was collected in Zhaotong Prefecture in 2021 and the other five (22HH085, 22HH218, 22HH295, 22HH432 and 22HH436) were collected in Honghe Prefecture in 2022. To verify whether these samples represented a novel CRF, the near full-length genome (NFLG) sequences were sequenced and analyzed. No direct epidemiological link was found between the cases. Demographic details are shown in Table S1. Written informed consent was obtained from all participants. The NFLG sequences of subjects 21ZT386, 22HH085, 22HH218, 22HH295, 22HH432 and 22HH436 were 8711 nt (756–9521 in HXB2), 8839 nt (654–9546 in HXB2), 8782 nt (657–9489 in HXB2), 8746 nt (697–9483 in HXB2), 8796 nt (684–9497 in HXB2) and 8793 nt (719–9491 in HXB2)in length, respectively, covering part of the 5′ long terminal repeat (LTR) to part of the 3′ LTR. These sequences have been deposited in GenBank under accession numbers PQ736317 to PQ736322.

The phylogenetic analysis revealed that the six NFLG sequences formed a monophyletic clade with a 100% bootstrap value, clearly distinguishing them from all other known subtypes and CRFs (Fig. 1A). To gain insights into their recombination structure, RIP and Bootscaning analyses were performed. Using Subtype C and Subtype B as reference sequences, we observed the insertion of two Subtype B segments into the backbone of Subtype C, resulting in the formation of five subregions: 1_C (790–2984), 2_B (2985–3264), 3_C (3265–8842), 4_B (8843–8997) and 5_C (8998–9546) (Figs. 1B and 1F). However, when conducting phylogenetic analyses of the individual subregions, it was found that subregions 2B and 4B were related to the corresponding segments of CRF07_BC and CRF08_BC, respectively (Supplementary Figure 1). This suggested that the six NFLG sequences may have originated from recombination events involving CRF07_BC and CRF08_BC.

As both CRF07_BC and CRF08_BC use Subtype C as their backbone (Figs. 1C and 1D), when a bootscanning analysis was performed using CRF07_BC and CRF08_BC as reference sequences, there were some regions where these two CRFs could not be easily distinguished and were replaced by reference sequences that were not candidates. Therefore, phylogenetic analyses were performed on the 15 segments with reference sequence transitions (I to XV) to determine whether the individual segments were derived from CRF07_BC or CRF08_BC (Fig. 1E). As shown in Fig. 2A, subregions I (790–1335), III (1826–2647), V (3645–5369), VII (5812–6868), IX (6977–7315), XII (7728–8053), XIII (8054–8418) and XIV (8419–9215) were clustered with CRF08_BC, whereas subregions II (1336–1825), IV (2648–3644), VI (5370–5811) and XV (9216–9546) were clustered with CRF07_BC. However, it was not clear whether subregions VIII (6869–6976), X (7316–7503) and XI (7504–7727) were derived from CRF07_BC or CRF08_BC. Based on this result, the recombinant structure of the six NFLG sequences was mapped (Fig. 1G). According to the naming rule, they were named CRF160_0708.

To investigate the origin of CRF160_0708, Bayesian evolutionary analyses were performed to estimate the most recent common ancestor (tMRCA) by combining specific subregions: CRF07_BC (including II, IV, VI and XV) and CRF08_BC (including I, III, V, VII, IX, XII, XIII and XIV) (Fig. 2B). Maximum clade credibility (MCC) tree analysis showed that the tMRCAs for the combined CRF07_BC and CRF08_BC subregions were estimated to be 2009.2 (with a 95% highest probability density (HPD) interval of 2006.1–2012.4) and 2008.1 (95% HPD: 2003.9–2012.3), respectively. This suggests that CRF160_0708 probably emerged around 2008 to 2009.

China's HIV-1 epidemic is recognized to have originated in Yunnan Province, a border province in southwestern China.[4] Historically, subtypes C and B were first introduced into Yunnan Province from India and Thailand, respectively, through intravenous drug users (IDUs). [4,5] During the 1990s, the spread of both subtypes among IDUs gave rise to CRF07_BC and CRF08_BC, which spread to other parts of China via different pathways.[6,7] As a result, CRF07_BC became the most widespread strain in China, while CRF08_BC and CRF07_BC remained the dominant strains in Yunnan Province.[8] In the context of a longstanding epidemic, the likelihood of recombination between CRF07_BC and CRF08_BC is extremely high. However, only CRF61_BC has been proposed to be derived from the recombination of CRF07_BC and CRF08_BC, but its recombination structure only distinguished between Subtype B and Subtype C, not between CRF07_BC and CRF08_BC.[9] The reason for this is that 90% of the sequences in these two CRFs are derived from Subtype C, and recombination in these regions is equivalent to intra-subtype recombination, making analysis of recombination breakpoints difficult. Therefore, in this study, the genome was first partitioned based on the transition points of the reference sequences in bootscaning

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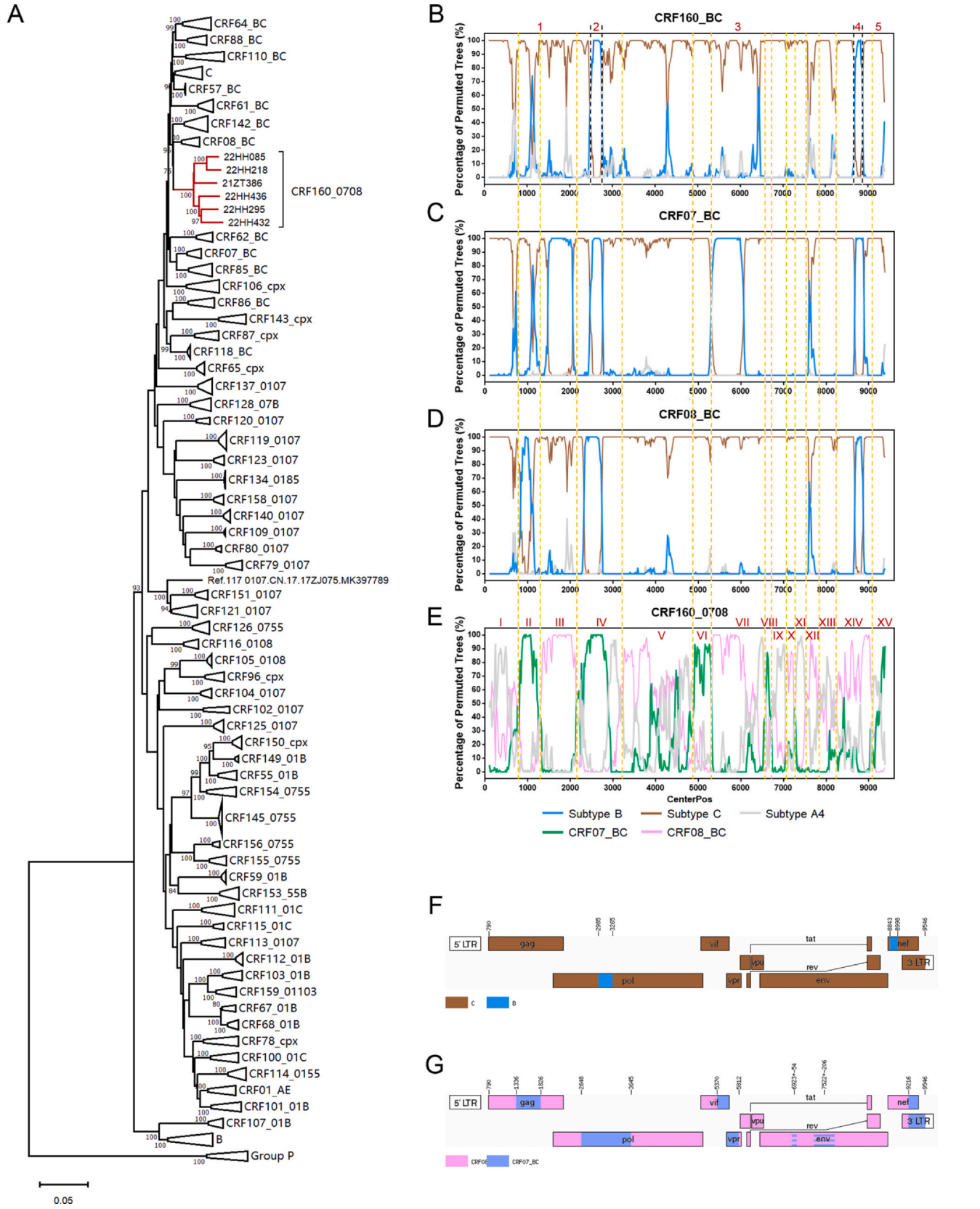

Fig. 1. Phylogenetic and recombinant analyses based on the near full-length genome sequences. (A) A neighbor-joining phylogenetic tree was constructed using the reference sequences of HIV-1 CRFs identified in China. The sequences of CRF160_0708 are highlighted in red. Values on the branches represent the percentage of 1000 bootstrap replicates, and the scale bar indicates 5% nucleotide sequence divergence. (B) Bootstrap analysis of the candidate CRF. Conditions used for this analysis: Window: 200 bp, step: 20 bp, GapStrip: on, replicates: 100, Kinura (2-parameter), T/t: 2.0. The Subtype B reference group included AY173951, U71182, JF932495, JF932471, JF932490, DQ354118 and DQ354116. The Subtype C reference group included KF835522, KC898995, KC898996, KF250403, AB023804, AF067158 and AF067157. The CRF07_BC reference group included EF368372, EF368370 and AF286230. The CRF08_BC reference group included KC914396, HM067748 and AY008715. The Subtype A4 reference group included AM000053 and AM000054. (C) Genomic structure of CRF160_0708. The mosaic map was generated using the Recombinant HIV-1 Drawing Tool.

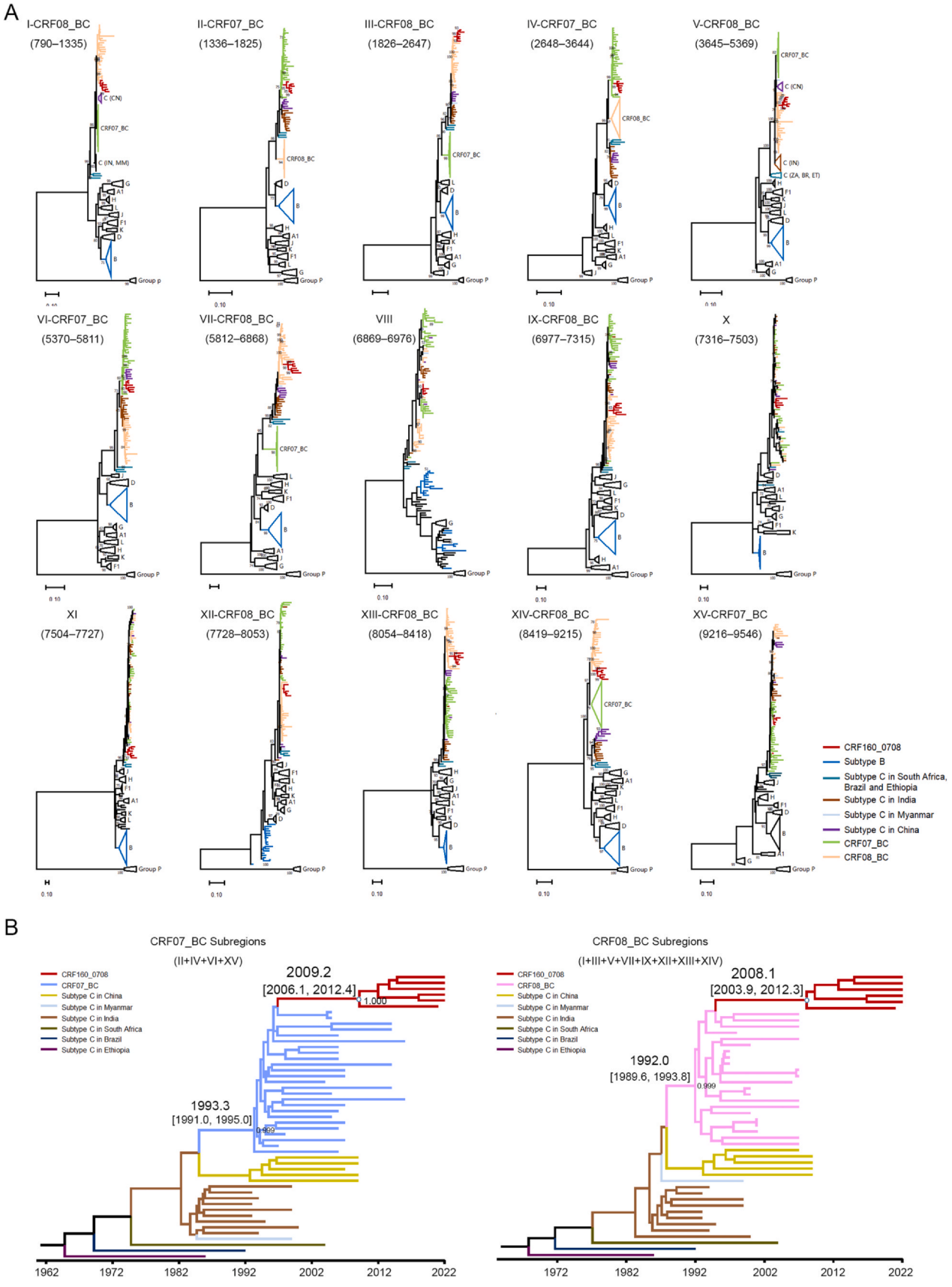

Fig. 2. Phylogenetic and evolutionary analysis of concatenated CRF01_AE and concatenated CRF07_BC subregions from CRF160_0708. (A) The maximum likelihood phylogenetic trees of the 15 mosaic fragments. The reliability of tree branches was assessed by 1000 bootstrap replicates. The scale bar indicates 1% nucleotide sequence divergence. (B) The maximum clade credibility (MCC) trees of combined CRF07_BC segments (II+IV+VI+XV) and combined CRF08_BC segments (I+III+V+VII+IX+XII+XIII+XIV).

analysis, and then each segment was further confirmed by phylogenetic analysis. However, there were still some short regions that cannot be clearly distinguished.

In conclusions, we made the first attempt to resolve the recombination structure of a novel CRF (CRF160_0708), consisting of CRF07_BC and CRF08_BC, which emerged approximately between 2008 and 2009. Our analytical approach may have implications for the analysis of recombination in highly similar HIV-1 genomes. Our findings contribute to the understanding of the evolutionary dynamics and genetic diversity of HIV-1. Future research should focus on elucidating the transmission patterns and potential clinical implications of emerging CRFs.

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Declaration of Competing Interest

We declare that the authors have no conflict of interest in this submission. The funders had no role in the study design, data collation and analysis, interpretation of data, or preparation of the manuscript. We confirm that this manuscript has not been published elsewhere and is not under consideration by any other journal.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106383.

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