Genetic characterization of a novel HIV-1 CRF01_AE/ CRF07_BC recombinant form among men who have sex with men in Baoding City, Hebei Province, China

Xuegang Yang
the People's Hospital of Baoding https://orcid.org/0000-0002-1145-3465
Huiying Zhu
The People's Hospital of Baoding
Weina An
The People's Hospital of Baoding
Jing Zhao
The People's Hospital of Baoding
Xinli Lu
Hebei Provincial Centre for Disease Control
Wenlong Sun
The People's Hospital of Baoding
Yongqin Li (yongqlee@163.com)
The People's Hospital of Baoding https://orcid.org/0000-0001-6255-9334

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Abstract

Nowadays, a large number of unique recombinant forms have been found among sexual transmission populations in China. This study reported a novel HIV-1 second-generation unique recombinant form (URF), which was isolated from an HIV-positive man named BD201AJ who was infected through men who have sex with men(MSM) in Baoding City, Hebei province, China. This is different from a CRF01_AE/CRF07_BC recombinant strain from Hebei province, in China. The analysis result of the near full length genomic characterization showed that the genome comprises at least twelve interlaced segments, including six CRF07_BC and six CRF01_AE segments, with CRF07_BC as the main framework. No similar breakpoints between our strain and the other strains in the Los Alamos HIV database were observed. This highlights the importance of monitoring HIV-1 molecular epidemiological characteristics and the ongoing generation of recombinant viruses may provide an important insight for future analysis the recombination mechanism of HIV-1.

Full Text

As we all known, Human Immunodeficiency Virus (HIV) is one of the deadliest pathogens. HIV/AIDS has been a major public health issue threatening human health since the first patient was reported in 1981[1]. The extremely high genetic variability and high-speed replication capacity of HIV leads to the continuous formation of new circulating recombinant forms (CRFs) and unique recombinant forms (URFs). Hebei Province is located in North China, Baoding city is located in central Hebei Province, and is the most populous city in Hebei Province, and shared a border with Beijing and Tianjin, and was also the second area severely affected by HIV-1 in Hebei Province. Lu *et al* reported that in 2019, 77.5% HIV-1 infected individuals were MSM in Hebei province, 49.6% were infected with CRF01_AE strain and 29.7% were CRF07_BC strain[2]. Among the HIV cases reported in 2020, 98.3% of them were infected with HIV through sexual transmission in Baoding city, Hebei Province. Moreover, homosexual transmission accounted for 91.7%, which indicates the high prevalence of HIV transmission among MSM in Baoding city, Hebei Province[3]. Our previous study showed that CRF01_AE (53.0%) and the CRF07_BC(26.5%) were the two major HIV circulating recombinant forms among MSM in Baoding city, Hebei Province[4]. The co-circulation and dual infections of the CRF01_AE and CRF07_BC will undoubtedly contribute to the emergence of the second-generation recombinant strains. In this study, we detected and characterized a second generation HIV-1 recombinant strain (BD201AQ) composed of CRF01_AE and CRF07_BC subtype isolated from a MSM infected with HIV-1. BD201AQ is a Chinese citizen of Han ethnicity, residing in Baoding, 28-year-old man, unmarried, primary school education, diagnosed by Baoding CDC in November 2020, and had been infected through homosexual by self-report. $CD4^+$T cell count of the man was 333 cells/μl by the first time of blood collection. This study was approved by the institutional review boards of the people's Hospital of Baoding. Written informed consent was obtained from the subject prior to sample collection.

NFLG sequence of BD201AQ was amplified due to the discordant subtypes of *gag* and *pol*. RNA was extracted from 200μL of plasma samples, then was reversely transcribed into cDNA using the superscript

 First-strand synthesis system (Invitrogen). And the NFLG was obtained in two halves by nested PCR amplification using TaKaRa reagent (TaKaRa). The PCR conditions of two rounds were consistent: 94℃ 5 minutes followed by 30 cycles of 94℃ 30 seconds, 60℃ 30 seconds and 72℃ 6 minutes, and an extension of 72℃ 10 minutes. The positive products were detected by 1% agarose gel electrophoresis, then purified and sequenced by SinoGenoMax (China) with a series of special primers. What's more, the chromatogram data were assembled by ContigExpress software (a component of Vector NTI version11.5.1, Invitrogen).

The NFLG sequence was 8,971 nucleotides (nt) (relative position to reference HXB2 672-9613). The sequence spanned the gag, pol, env, tat, rev, vif, vpr, vpu, and nef genes as well as the 5' portion of the 3' long terminal repeat (LTR). The NFLG phylogenetic tree (Fig.1) analysis suggested that BD201AQ was closely relatd to CRF01_AE references, but formed a distinct monophyletic cluster separately from them. SimPlot analysis determined that the BD201AQ was a recombinant form composed of CRF01_AE and CRF07_BC, with eleven obvious breakpoints (Fig. 2A). Bootscanning analysis was subsequently carried out to position the exact recombination breakpoints by using CRF01_AE (Genbank accession number KC596062 and KC596061) and CRF07_BC (BJ070032 and GZ070087) as putative parental reference sequences, and subtype FI (AF077336 and AF005494) as an outgroup (Fig. 2B). Bootscanning analysis revealed eleven unique recombination breakpoints at nt 1170, 2463, 2941, 3209, 3375, 3890,4142, 4619, 5570, 8693, 8829 relative to the HXB2 genome. The BD201AQ NFLG was consequently divided into twelve regions: CRF01_AE(HXB2, 711-1170nt); CRF07_BC (HXB2, 1171- 2463nt); CRF01_AE(HXB2, 2464-2941nt); CRF07_BC (HXB2, 2942- 3209nt); CRF01_AE(HXB2,3210-3375nt),CRF07_BC(HXB2,3376-3890nt);CRF01_AE(HXB2,3891-4142nt);CRF07_BC(HXB2,4143-4619nt);CRF01_AE(HXB2,4620-5570nt),CRF07_BC(HXB2:5571-8693nt), CRF01_AE (HXB2: 8694-8829nt), CRF07_BC(HXB2:8830-9513nt)A mosaic map of the analyzed NFLG is shown in Fig. 2C. Subregion phylogenetic analyses were used to confirm the genetic origins of each segment. It was shown that the gnomic segment I III were all clustered into the CRF01_AE cluster 1cluster 5 and gnomic segment and were all clustered into the CRF01_AE cluster4 (Fig. 3). CRF01_AE cluster 1 was prevalent among heterosexuals and IDUs , CRF01_AE cluster 4 and 5 were related to homosexual transmission[5]. The segment of BD201AQ was too short to construct Neighbor-joining phylogenetic tree. BD201AQ has intra-subtype recombination, which does not rule out the possibility of double infection. Meanwhile, the CRF07_BC segment region IIand were clustered into a specific CRF07_BC_N that prevalent among MSM in northern China (Fig. 3).

Interesting, a lot of novel unique recombination forms (URFs) composed of CRF01_AE and CRF07_BC has been reported in Hebei, Beijing and Tianjin in recent years[6-9]. Among the four CRF01_AE/CRF07_BC recombinants reported in Hebei Province, two cases used CRF01_AE as the backbone to insert four CRF07_BC fragments, and two cases used CRF07_BC as the backbone to insert three and four CRF01_AE fragments, respectively. The CRF01_AE fragment is from the CRF01_AE cluster 4 strain in the Chinese MSM population[6,7]. Jiao *et al*. reported that the Beijing BJ2015EU16 strain includes two CRF07_BC and one CRF01_AE fragment, with CRF07_BC as the backbone and two recombination breakpoints in the vpu and env genes[8]. Zhou *et al*. reported that the two cases in Tianjin consisted of the CRF01_AE backbone

and part of the CRF07_BC sequence, with four breakpoints in the gag, pol, vif, and tat genes[9]. It can be seen that the BD201AQ of this study is different from the above reports, not only there are breakpoints in the gag, pol, vif, env and nef regions, but also more breakpoints, reaching 12.

In conclusion, we reported here a novel HIV-1 recombinant form (BD201AQ) isolated from a MSM in Baoding City, Hebei Province, China. The phylogenetic analyses of BD201AQ showed that the intra-subtype recombinant segments of CRF01_AE were traced back to cluster 1, 4 and 5 of CRF01_AE strains, which were prevalent among HIV-1 infections in China[5,10]. CRF07_BC segments were derived from HIV-1 circulating genotypes among MSM in China. The NFLG sequence feature analysis showed this recombinant form to be distinct from previously reported CRFs and URFs. The emergence of new complex recombinant forms increases the diversity of HIV-1 epidemic in this region of Hebei Province, China, which indicated the very complexity of the local HIV epidemic.

Declarations

Acknowledgments

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Compliance of ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

Written informed consent was signed before sample collection, and the study was approved by Medical Ethics Committee of Baoding People's Hospital.

Sequence data

The nucleotide sequence of BD201AQ have been deposited to GenBank with the accession No OL401523

Contribution Statement

Xuegang Yang: Manuscript writing, data analysis.

Huiying Zhu: Manuscript correction, Sample information collection.

Weina An: Manuscript correction,laboratory procedure,experimental operation.

Wenlong Sun: experimental operation.

Jing Zhao: Sample information collection.

Xinli Lu: Sequence assembly ,data analysis.

Yongqin Li: laboratory procedure,data analysis.

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Figures

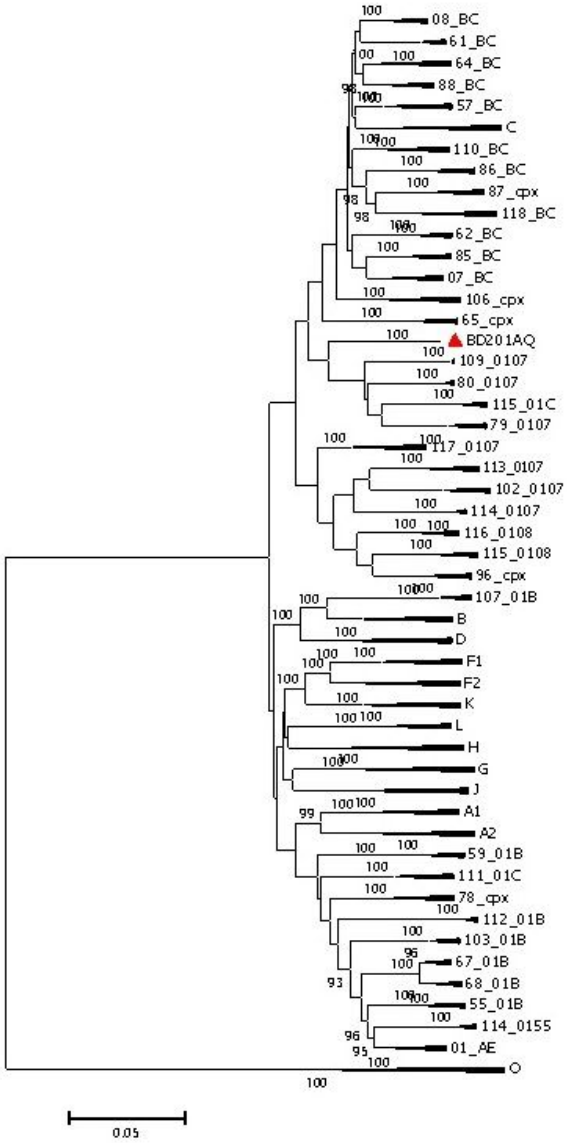

Fig. 1 Phylogenetic tree analyses. The NFLG phylogenetic tree of BD201AQ(8971 bp, ▲) was constructed using the Neighbor-joining method by Mega6.0 software. The stability of each node was assessed by bootstrap tests with 1,000 replicates and only bootstrap values≥90% were shown at the corresponding nodes. The scale bar represents 5% genetic distance.

Figure 1

See image above for figure legend.

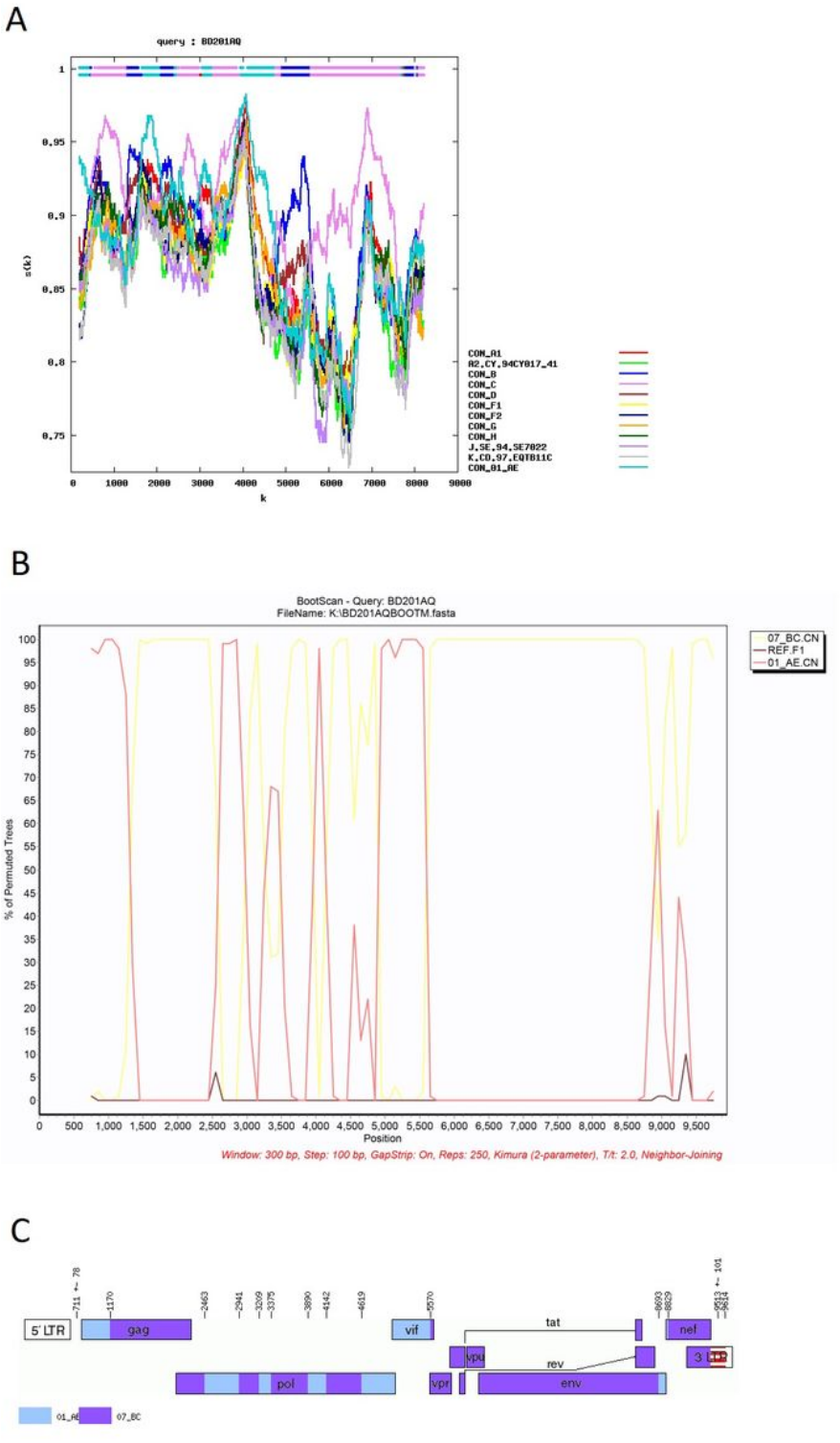

Figure 2

Recombination breakpoint analyses of BD201AQ . (A) RIP analysis of BD201AQ (A). Similarity distance analysis was performed using RIP (version 3.0; Siepel AC, Halpern AL, Macken C, Korber BT, http://hiv-web.lanl.gov) from the Los Alamos National Laboratory HIV Database with default setting except for the window size of 300. (B) CRF01_AE.C and CRF07_BC .C were used as putative parental reference sequences in bootscanning analysis. Subtype F1 was used as an outgroup. The bootscan window was

300 bp with a step size of 90 bp. Recombinant mapping was performed using the Recombinant HIV-1 Drawing Tool (http://www.hiv.lanl.gov/conte nt/seque nce/DRAW_CRF/recom _mappe r.html). (C) The recombinant analysis proved that BD201AQ were composed of 12 interlaced mosaic segments, including CRF01_AE (□ , □ , □ , □ , □ ,□) and CRF07_BC (□ , □ , □ , □ , □and □), with 12 unique breakpoints relative to the HXB2 coordinate .

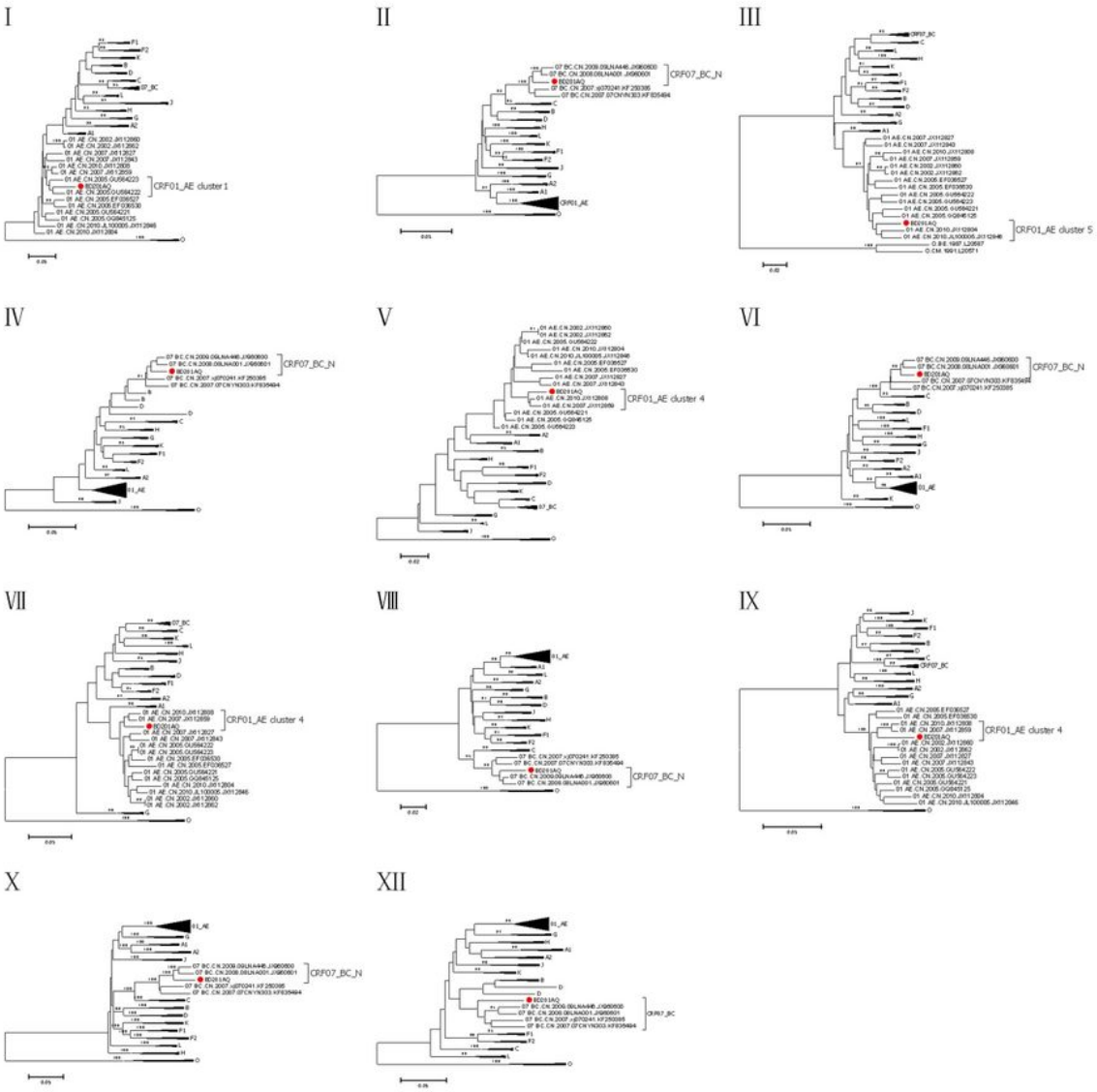

Fig .3 Subregion phylogenetic tree. Subregion phylogenetic analysis of different segments of BD201AQ were constructed by Mega6 using the Neighbor-joining method with 1,000 bootstrap replications .The solid circle (●) marks BD201AQ. Bootstrap values ≥90% were shown at the corresponding nodes. The scale bar represents 5% genetic distance. The segment XI of BD201AQ were too short to construct Neighbor-joining phylogenetic tree.

Figure 3

See image above for figure legend.

Supplementary Files

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- BD201AQ.fasta