CASE SERIES

The discovery of two novel near-full-length HIV-1 recombinant forms in Hebei, China

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Abstract

In the recent years, homosexual contact has been the most common route of transmission of human immunodeficiency virus type 1 (HIV-1) in Hebei, China. In this study, we have identified two novel HIV-1 unique recombinant forms (URFs) that originated from CRF01_AE/C (BDL120) and subtype B/C (BDX016) among men who have sex with men (MSM) in Hebei. A phylogenetic tree and recombination analysis of the near-full-length genome sequences showed that BDL120 was a recombinant form originating from CRF01_AE and subtype C, whereas BDX016 consisted of subtypes B and C, containing eight and nine fragments of each, respectively. In BDL120, four C fragments were inserted into the CRF01_AE backbone, whereas in BDX016, four B fragments were inserted into the C backbone. The findings indicate that the frequent occurrence of HIV-1 recombination events has enriched HIV Databases, reflecting HIV-1 diversity in Hebei.

Keywords: HIV-1; Unique recombination form; Men who have sex with men; Near-full-length genome; Hebei.

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Introduction

In China, the dominant subtypes of HIV-1 are CRF01_ AE (42.5%), CRF07_ BC (28.9%), subtype B'/B (10.9%), and CRF08_BC (10.0%).[1] It is feared that with the co-circulation of different subtypes in some areas, the prevalence and complexity of URFs will increase rapidly. In the recent years, a large number of second-generation recombinant forms, such as CRF01_AE/CRF07_BC, CRF01_AE/B, CRF01_AE/C, and CRF68_01B/CRF01_AE, have been reported.[2-6] The Hebei province of China has a low HIV prevalence.[7] By the end of 2020, the total number of HIV and acquired immune deficiency syndrome (AIDS) cases was 17,891.[6] The prevalence of HIV-1 infections through sexual transmission was 98.9%, while men who have sex with men (MSM) accounted for 77.5% in the sexual contact population. Three main subtypes were: CRF01_AE (49.6%), CRF07_BC (29.7%), and subtype B (13.0%).[8] In this study, the molecular epidemiological characteristics of HIV-1 in the Hebei Province were monitored in order to identify the complex recombinant forms of HIV-1 as soon as possible and provide a reference for the effective control of HIV-1 epidemic.

Case Series

This study included two patients, designated BDL120 and BDX016, respectively. They were confirmed as HIV-1 positive by the Centre for Disease Control and Prevention of Baoding City, Hebei province, China, in February 2023 and May 2023, respectively. They had been infected with HIV-1 through the MSM pathway, aged 28 and 36 years, respectively. Their baseline CD4+ T cell counts were 7 cells/μl and 128 cells/μl, respectively. The study was conducted at Baoding People's Hospital in Baoding City, Hebei Province, and approved by the Medical Ethics Committee of Baoding People's Hospital (protocol number: 2019-03). Written informed consent of patients with BDL120 and BDX016 were obtained before the samples collection.

The extraction, amplification, and sequencing procedures for the viral RNA were performed as described previously.[9] Two near-full-length genome sequences (NFLGs) were submitted to the online tool HIV BLAST (https://www.hiv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html) in order to search for similar sequences. The two sequences were then aligned with subtype reference sequences and Chinese CRFs (https://hiv.lanl.gov/components/sequence/HIV/search/search.html) using MAFFT v7.0,[10] and adjusted manually with BioEdit (v7.2.5.0). The neighbouring-joining (N-J) phylogenetic trees were constructed with MEGA 6.0. Recombination breakpoints were identified with SimPlot (v3.5.1) and Bootscan analyses.

The 8942-bp (HXB2: 758–9700) and 8917-bp (HXB2: 783–9700) NFLG sequences from BDL120 and BDX016, respectively, were obtained. As listed in the NFLG phylogenetic tree, BDL120 could not cluster with the reference sequences, and BDX016 formed a monophyletic branch, indicating that both BDL120 and BDX016 were URFs (Figure 1A).

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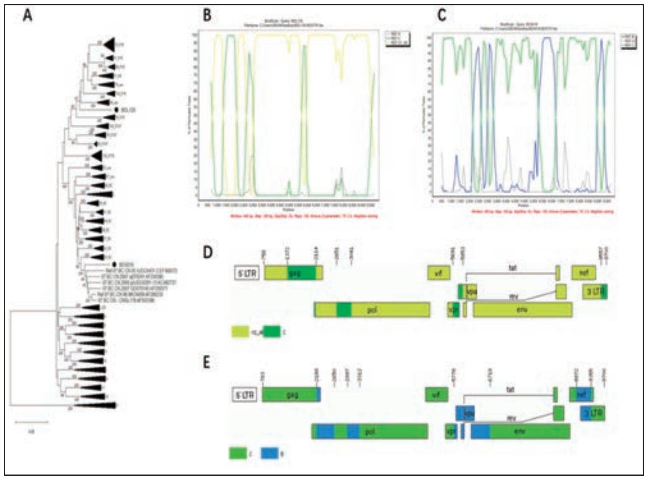

Figure-1: Phylogenetic and recombination breakpoints analysis based on the NFLG sequences of BDL120 and BDX016. *The standard reference sequences of HIV-1 subtypes were downloaded from the HIV Database (www.hiv.lanl.gov/ content/index). (A). The neighbour-joining tree was constructed using MEGA 6.0 with 1,000 bootstrap replicates, and only bootstrap values ≥90% were shown at the corresponding nodes. The scale bar represents 5% genetic distance. Solid black circles represent study subjects BDL120 and BDX016. (B) Bootscanning analysis of BDL120 was performed in SimPlot 3.5.1 software using a window size of 400 bp and a step size of 100 bp. CRF01_AE (yellow line), subtype C (green line), and K subtype (black line) were used as references. (C) Bootscanning analysis of BDX016 was performed in SimPlot 3.5.1 software using a window size of 400 bp and a step size of 100 bp. Subtype B (blue line), subtype C (green line), and subtype H (black line) were used as references. The mosaic maps were obtained using the online Recombinant HIV-1 Drawing Tool (https: //www.hiv.lanl.gov/ content/sequence/ DRAW_CRF/recom_mapper.html). (D) Recombination breakpoints analyses of sequence BDL120. (E) Recombination breakpoints analyses of sequence BDX016. NFLG, near full-length genome.*

The recombination breakpoints showed that BDL120 consisted of four subtype-C fragments inserted into the CRF01_AE backbone, forming eight interlaced chimeric gene fragments (Figures 1B and 1D). Relative to the HXB2 coordinates, the eight sub-regions of BDL120 are: I CRF01_AE (nt 758–1,372); II subtype C (nt 1,373–2,114); III CRF01_AE (nt 2,115–2,651); IV subtype C (nt 2,552– 3,041); V CRF01_AE (nt 3,042–5,691); VI subtype C (nt 5,692–5,953); VII CRF01_AE (nt 5,954–9,557); and VIII subtype C (nt 9,556–9,700).

The recombination breakpoints showed that BDX016 consisted of four subtype-B fragments inserted into the subtype-C backbone, forming nine interlaced chimeric gene fragments (Figures 1C and 1E). The nine sub-regions of BDX016 are as follows: I) subtype C (nt 783–2,189); II) subtype B (nt 2,190–2,650); III) subtype C (nt 2,651–2,997); IV) subtype B (nt 2,998–3,312); V) subtype C (nt 3,313–5,778); VI) subtype B (nt 5,779–6,719); VII) subtype C (nt 6,720–8,972); VIII) subtype B (nt 8,973–9,355); and IX) subtype C (nt 9,356–9,700).

The phylogenetic tree of each sub-region of two NFLGs showed that all the sub-regions clustered with their respective reference sequences (Figures 2A and 2B). Segment VIII of BDL120 was too short to construct a N-J phylogenetic tree.

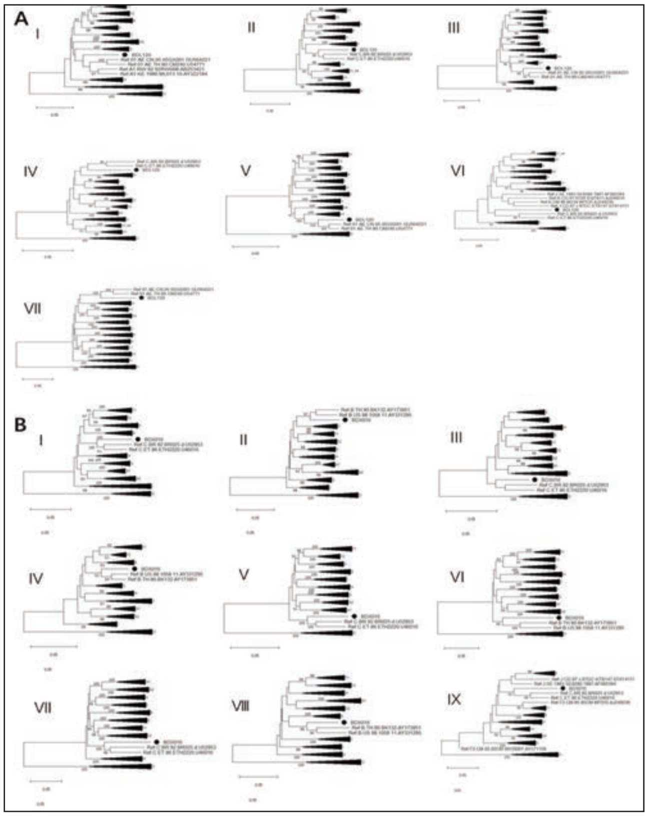

Figure-2: Sub-regional phylogenetic trees of the BDL120 and BDX016. *The phylogenetic trees were constructed using MEGA 6.0 with 1000 bootstrap replicates and bootstrap values ≥ 90% are shown at the corresponding nodes. The scale bar represents a genetic distance of 5%. Solid black circles denote BDL120 and BDX016. (A) Sub-region phylogenetic trees of BDL120. (B) Sub-region phylogenetic trees of BDX016.*

Discussion

Among the 11 prefecture-level cities in Hebei province, Baoding is the most populous and has the second-worst HIV-1 epidemic in the province. In 2020, Shi et al. reported that 91.7% of HIV-1-infected individuals in Baoding city were MSM. Of them, CRF01_AE and subtype B accounted for 49.44% and 17.78%, respectively, whereas subtype C was a minor subtype with a prevalence of 0.8%.[11] Because both patients with BDL120 and BDX016 had been recently infected with HIV-1 via MSM route, and the prevalence of HIV-1 subtype C in the local area is extremely low, it was inferred that they could be infected with the original strains. Literature review indicates that multiple second-generation HIV-1 recombinant strains, including CRF159_01103 and several unique recombinant forms (URFs) such as CRF01_AE/CRF07_BC and CRF01_AE/B have been detected in Baoding city in recent years.[12-15] Notably, the BDX016 strain identified in this study represents the first documented HIV-1 B/C form in Hebei province. Lu Xinli et al.[16] have reported two CRF01_AE/C forms in this region. In this study, a CRF01_AE/C form (BDL120) was observed. These findings collectively highlight an increasing trend in HIV-1 genetic diversity among men who have sex with men

(MSM) in Baoding. This undoubtedly increases the difficulty in prevention and control of HIV/AIDS in Baoding and even in the Hebei province. Therefore, further dynamic surveillance of new URFs, related not only to major strains but also to minor ones, is required.

The limitation of the study is that only 8942 bp of HIV-1 full-length genome was obtained in this study due to the relatively limited technology. In the future, HIV-1 gene structure using next-generation sequencing will be analysed.

Conclusion

In conclusion, two novel HIV-1 recombinant forms have been identified in Baoding, Hebei Province. The frequent recombination of HIV-1 not only increases the adaptability of the virus, but also facilitates its rapid acquisition of drug resistance or its escape from immune surveillance. Further molecular surveillance of HIV-1 diversity in this region should be strengthened to better respond to the HIV-1 epidemic.

Consent for publication: Written informed consent was signed by the participants before sample collection. The use of blood samples for further related studies was also permitted.

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Data availability statement: The data that support the findings of this study is openly available. The nucleotide sequences of BDL120 and BDX016 have been deposited in the National Centre for Biotechnology Information GenBank database under accession numbers PP056114 and PP056115, respectively.

Conflict of interest: None.

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Ethical approval: This study was approved by the Medical Ethics Committee of the Baoding People's Hospital, Baoding, Hebei Province, China (protocol number 2019–03).

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Author Contribution:

JJ & HS: Drafting, writing, review, editing and final approval.

SC: Data curation, resources and final approval.

ZZ & WF: Writing, review, editing and final approval.

PS: Validation and final approval.