We report here a novel HIV-1 circulating recombinant form (CRF) (CRF59_01B) comprised of CRF01_AE and subtype B, with two recombination breakpoints in the pol and vpu-env regions, respectively. CRF59_01B was identified from three epidemiologically unlinked men who have sex with men (MSM) in northeast China. This represents the second CRF identified in the MSM population in China.

Human immunodeficiency virus type 1 (HIV-1) is classified into four groups: M, O, N, and P (1, 2). The group M strains, responsible for the vast majority of HIV infections in the world, comprise eleven subtypes and sub-subtypes, as well as 58 circulating recombinant forms (CRFs) and various types of unique recombinant forms (URFs) (see http://www.hiv.lanl.gov).

In Asia, 12 CRFs have been reported to date: CRF01_AE (3), CRF15_01B (4), and CRF34_01B (5) in Thailand, CRF07_BC (6), CRF08_BC (7), and CRF55_01B in China (8), CRF33_01B (9), CRF46_01B (10), CRF53_01B (11), and CRF54_01B (12) in Malaysia, CRF51_01B in Singapore (13), and CRF53_01B in Thailand and Malaysia (14). CRF51_01B and CRF55_01B are the CRFs identified among men who have sex with men (MSM).

Wide cocirculation and dual infection of CRF01_AE and subtype B in various geographical regions in Asia led to the emergence of various novel CRFs. We describe the genome sequences of a novel CRF (CRF59_01B) isolated from three epidemiologically unlinked MSM in northeastern China.

Near-full-length genome (NFLG) sequences (9.0 kb) were determined from plasma RNA using a single genome amplification method with two sets of primers designed for the determination of the 5' and 3' halves of the HIV-1 genome (15, 16). Amplicons were directly sequenced using the internal walking primers with an ABI 3730XL Sanger-based genetic analyzer. The study was approved by the Institutional Review Board of the First Affiliated Hospital of China Medical University.

The three NFLG sequences of CRF59_01B (accession no. KC462190, KC462191, and JX960635) were 8,804, 8,795, and 8,380 bp in size for strains 11CN.LNSY300392, 10CN.LNSY300533, and 09LNA423, respectively, spanning the non-coding region, the gag, pol, env, tat, rev, vif, vpr, vpu, and nef genes, and part of 3' long terminal repeat (LTR). These three strains formed a distinct monophyletic cluster and did not belong to any known HIV-1 subtype or CRF. Bootscanning and informative site analyses (17) identified four unique recombination breakpoints between CRF01_AE and subtype B at the nucleotide positions (relative to HXB2) 2570 and 2719 in the pol region and 6149 and 8244 in the vpu-env region. These recombination breakpoints were shared among all three strains. Subregion tree analyses further confirmed the parental origin of each region of the recombinant genome as follows: region I (positions relative to HXB2: 790 to 2569) is CRF01_AE, region II (positions relative to HXB2: 2570 to 2719) is B, region III (positions relative to HXB2: 2719 to 6149) is CRF01_AE, region IV (positions relative to HXB2: 6149 to 8243) is B, and region V (positions relative to HXB2: 8244 to 9600) is CRF01_AE. The recombinant structure of CRF59_01B is indeed distinct from any previously reported CRFs. Subregion tree analyses also indicated that subtype B regions were of U.S. or European origin, unlike the subtype B' (Thailand variant of subtype B) (3, 18) lineage associated with bloodborne epidemics in Asia (19), while CRF01_AE regions were of Thailand CRF01_AE origin, not related to the CRF01_AE variants (clusters 1 and 2) that we recently identified among MSM in China (15).

CRF59_01B is the second CRF identified in the MSM population in China. CRF55_01B was identified recently among MSM in southern China, while the new CRF described here was identified among MSM in northeastern China. The emergence of CRF55_01B and CRF59_01B suggests the extensive ongoing generation of new recombinant forms involving the CRF01_AE and subtype B lineages among MSM in various regions in China.

Nucleotide sequence accession numbers. The sequences are available in GenBank under accession no. KC462190, KC462191, and JX960635.

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REFERENCES


