

# Genome Sequences of a Novel HIV-1 Circulating Recombinant Form (CRF59\_01B) Identified among Men Who Have Sex with Men in Northeastern China

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**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.**

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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), CRF48\_01B (10), CRF53\_01B (11), and CRF54\_01B (12) in Malaysia, CRF51\_01B in Singapore (13), and CRF52\_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 2718) is B, region III (positions relative to HXB2: 2719 to 6419) 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](https://www.ncbi.nlm.nih.gov/nuccore/KC462190), [KC462191](https://www.ncbi.nlm.nih.gov/nuccore/KC462191), and [JX960635](https://www.ncbi.nlm.nih.gov/nuccore/JX960635).

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## REFERENCES

- Plantier JC, Leoz M, Dickerson JE, De Oliveira F, Cordonnier F, Lemée V, Damond F, Robertson DL, Simon F. 2009. A new human immunodeficiency virus derived from gorillas. *Nat. Med.* 15:871–872.
- Van Heuverswyn F, Li Y, Neel C, Bailes E, Keele BF, Liu W, Loul S, Butel C, Liegeois F, Bienvenue Y, Ngolle EM, Sharp PM, Shaw GM, Delaporte E, Hahn BH, Peeters M. 2006. Human immunodeficiency viruses: SIV infection in wild gorillas. *Nature* 444:164.
- Ou CY, Takebe Y, Weniger BG, Luo CC, Kalish ML, Auwanit W, Yamazaki S, Gayle HD, Young NL, Schochetman G. 1993. Independent introduction of two major HIV-1 genotypes into distinct high-risk populations in Thailand. *Lancet* 341:1171–1174.
- Tovanabutra S, Watanaveeradej V, Viputtikul K, De Souza M, Razak MH, Suriyanon V, Jittiwutikarn J, Sriplienchan S, Nitayaphan S, Benenson MW, Sirisopana N, Renzullo PO, Brown AE, Robb ML, Beyrer C, Celentano DD, McNeil JG, Bix DL, Carr JK, McCutchan FE. 2003. A new circulating recombinant form, CRF15\_01B, reinforces the linkage between IDU and heterosexual epidemics in Thailand. *AIDS Res. Hum. Retroviruses* 19:561–567.
- Tovanabutra S, Kijak GH, Beyrer C, Gammon-Richardson C, Sakthachornphop S, Vongchak T, Jittiwutikarn J, Razak MH, Sanders-Buell E, Robb ML, Suriyanon V, Bix DL, Michael NL, Celentano DD, McCutchan FE. 2007. Identification of CRF34\_01B, a second circulating recombinant form unrelated to and more complex than CRF15\_01B, among injecting drug users in northern Thailand. *AIDS Res. Hum. Retroviruses* 23:829–833.
- Su L, Graf M, Zhang Y, von Briesen H, Xing H, Köstler J, Melzl H, Wolf H, Shao Y, Wagner R. 2000. Characterization of a virtually full-length human immunodeficiency virus type 1 genome of a prevalent intersubtype (C/B') recombinant strain in China. *J. Virol.* 74:11367–11376.
- Piyasirisilp S, McCutchan FE, Carr JK, Sanders-Buell E, Liu W, Chen J, Wagner R, Wolf H, Shao Y, Lai S, Beyrer C, Yu XF. 2000. A recent outbreak of human immunodeficiency virus type 1 infection in southern China was initiated by two highly homogeneous, geographically separated strains, circulating recombinant form AE and a novel BC recombinant. *J. Virol.* 74:11286–11295.
- Han X, An M, Zhang W, Cai W, Chen X, Takebe Y, Shang H. 2013. Genome sequences of a novel HIV-1 circulating recombinant form, CRF55\_01B, identified in China. *Genome Announc.* 1(1):e00050-12. doi: [10.1128/genomeA.00050-12](https://doi.org/10.1128/genomeA.00050-12).
- Tee KK, Li XJ, Nohtomi K, Ng KP, Kamarulzaman A, Takebe Y. 2006. Identification of a novel circulating recombinant form (CRF33\_01B) disseminating widely among various risk populations in Kuala Lumpur, Malaysia. *J. Acquir. Immune Defic. Syndr.* 43:523–529.
- Li Y, Tee KK, Liao H, Hase S, Uenishi R, Li XJ, Tsuchiura T, Yang R, Govindasamy S, Yong YK, Tan HY, Pybus OG, Kamarulzaman A, Takebe Y. 2010. Identification of a novel second-generation circulating recombinant form (CRF48\_01B) in Malaysia: a descendant of the previously identified CRF33\_01B. *J. Acquir. Immune Defic. Syndr.* 54:129–136.
- Chow WZ, Al-Darraj H, Lee YM, Takebe Y, Kamarulzaman A, Tee KK. 2012. Genome sequences of a novel HIV-1 CRF53\_01B identified in Malaysia. *J. Virol.* 86:11398–11399.
- Ng KT, Ong LY, Takebe Y, Kamarulzaman A, Tee KK. 2012. Genome sequence of a novel HIV-1 circulating recombinant form 54\_01B from Malaysia. *J. Virol.* 86:11405–11406.
- Ng OT, Eyzaguirre LM, Carr JK, Chew KK, Lin L, Chua A, Leo YS, Redd AD, Quinn TC, Laeyendecker O. 2012. Identification of new CRF51\_01B in Singapore using full genome analysis of three HIV type 1 isolates. *AIDS Res. Hum. Retroviruses* 28:527–530.
- Liu Y, Li L, Bao Z, Li H, Zhuang D, Liu S, Wang X, Li T, Jia L, Yang S, Li J. 2012. Identification of a novel HIV type 1 circulating recombinant form (CRF52\_01B) in Southeast Asia. *AIDS Res. Hum. Retroviruses* 28:1357–1361.
- An M, Han X, Xu J, Chu Z, Jia M, Wu H, Lu L, Takebe Y, Shang H. 2012. Reconstituting the epidemic history of CRF01\_AE among MSM in Liaoning, northeastern China: implication in expanding MSM epidemic in China. *J. Virol.* 22:12402–12406.
- Salazar-Gonzalez JF, Salazar MG, Keele BF, Learn GH, Giorgi EE, Li H, Decker JM, Wang S, Baalwa J, Kraus MH, Parrish NF, Shaw KS, Guffey MB, Bar KJ, Davis KL, Ochsenaubauer-Jambor C, Kappes JC, Saag MS, Cohen MS, Mulenga J, Derdeyn CA, Allen S, Hunter E, Markowitz M, Hraber P, Perelson AS, Bhattacharya T, Haynes BF, Korber BT, Hahn BH, Shaw GM. 2009. Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. *J. Exp. Med.* 206:1273–1289.
- Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, Ingersoll R, Sheppard HW, Ray SC. 1999. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. *J. Virol.* 73:152–160.
- Kalish ML, Baldwin A, Raktham S, Wasi C, Luo CC, Schochetman G, Mastro TD, Young N, Vanichseni S, Rübsamen-Waigmann H, et al. 1995. The evolving molecular epidemiology of HIV-1 envelope subtypes in injecting drug users in Bangkok, Thailand: implications for HIV vaccine trials. *AIDS* 9:851–857.
- Li Y, Uenishi R, Hase S, Liao H, Li XJ, Tsuchiura T, Tee KK, Pybus OG, Takebe Y. 2010. Explosive HIV-1 subtype B' epidemics in Asia driven by geographic and risk group founder events. *Virology* 402:223–227.