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GENOME ANNOUNCEMENT

Genome Sequences of a Novel HIV-1 CRF53_01B Identified in Malaysia

Wei Zhen Chow,[a] Haider Al-Darraji,[a] Yeat Mei Lee,[a] Yutaka Takebe,[a,b] Adeeba Kamarulzaman,[a] and Kok Keng Tee[a]

Centre of Excellence for Research in AIDS (CERiA), Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia,[a] and AIDS Research Center, National Institute of Infectious Diseases, Toyama, Shinjuku-ku, Tokyo, Japan[b]

A novel HIV-1 genotype designated CRF53_01B was recently characterized from three epidemiologically unrelated persons in Malaysia. Here we announced three recently isolated full-length genomes of CRF53_01B, which is likely to be phylogenetically linked to CRF33_01B, circulating widely in Southeast Asia. The genome sequences may contribute to HIV-1 molecular surveillance and future vaccine development in the region.

Human immunodeficiency virus (HIV) is a positive-sense, single-stranded RNA virus in the *Retroviridae* family. The virus is characterized by extensive genetic diversity due to its error-prone reverse transcriptase, high viral turnover rates, and genetic recombination (4). HIV-1 group M (main) consists of nine subtypes (A to D, F to H, J, and K), sub-subtypes, and, at present, 52 circulating recombinant forms (CRFs) (www.hiv.lanl.gov), which caused the HIV/AIDS pandemic. In Southeast Asia, the wide cocirculation and dual infection of CRF01_AE and subtype B or B′ (Thai variant of subtype B) led to the emergence of various novel CRFs, specifically CRF15_01B (8) and CRF34_01B (7) in Thailand, CRF33_01B (5) and CRF48_01B (1) in Malaysia, CRF51_01B (3) in Singapore, and CRF52_01B (2) in both Thailand and Malaysia. In this study, we had recently characterized three full-length recombinant genomes of HIV-1 isolated from epidemiologically unrelated persons resembling a novel CRF that is genetically distinct from previously established CRFs reported globally, designated CRF53_01B by the Los Alamos National Laboratory.

Full-length genomes were sequenced from plasma collected from three HIV-1-infected subjects with informed consent from the University Malaya Medical Centre in Kuala Lumpur, Malaysia, during 2010 and 2011. HIV-1 viral RNA was extracted by a column purification method and reverse transcribed using SuperScript III RNase H$^-$ reverse transcriptase (Invitrogen). The genome was amplified by nested PCR using HotStarTaq *Plus* DNA polymerase (Qiagen, Germany) to produce 10 overlapping fragments using primers previously described, sequenced using the ABI Prism 3730XL DNA analyzer (Applied Biosystems), and assembled prior to alignment and phylogenetic and bootscanning analyses (5).

The three full-length genomes of HIV-1 CRF53_01B had sizes of 8,954 bp, 8,599 bp, and 8,174 bp (for isolates 11FIR164, 10MYKJ079, and 10MYKJ067, respectively), spanning the *gag*, *pol*, *env*, *tat*, *rev*, *vif*, *vpr*, *vpu*, and *nef* genes and flanked by the noncoding regions, 5′ and 3′ long terminal repeats (LTRs). Bootscanning and informative site analyses identified unique recombination breakpoints at HXB2 positions 2053 to 2063 and HXB2 2375 to 2422 due to homologous recombination of a short subtype B′ fragment (311 bp) in the *gag*-protease region in a CRF01_AE backbone. Subregion tree analyses further confirmed the parental origin of each region of the recombinant genome: region I (HXB2, positions 649 to 2052), CRF01_AE; region II (HXB2, 2064 to 2374), B′; and region III (HXB2, 2423 to 9617), CRF01_AE. Database search showed another identical mosaic genome previously reported in Malaysia (04MYKL016) (5) that formed a monophyletic cluster with the three full-length genomes sequenced here. Together, these HIV-1 isolates constitute a novel genetic clade within HIV-1 group M. Interestingly, CRF53_01B shares the two recombination breakpoints with CRF33_01B. Maximum likelihood analysis of subgenomic regions revealed that CRF53_01B is closely related to CRF33_01B and is likely to be the ancestor of CRF33_01B, which may have emerged in the early 1990s (6). The emergence of CRF53_01B highlights the alarming increase of HIV-1 complexity in Southeast Asia, which may complicate disease treatment and prevention.

Nucleotide sequence accession numbers. The genome sequences of HIV-1 CRF53_01B isolates 11FIR164, 10MYKJ079, and 10MYKJ067 have been deposited in GenBank under accession no. JX390610, JX390611, and JX390612, respectively.

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The authors have declared they have no competing interests.

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Address correspondence to Kok Keng Tee, k2tee@um.edu.my.

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