Genome Sequence of a Novel HIV-1 Circulating Recombinant Form 54_01B from Malaysia

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We report here the first novel HIV-1 circulating recombinant form (CRF) 54_01B (CRF54_01B) isolated from three epidemiologically unlinked subjects of different risk groups in Malaysia. These recently sampled recombinants showed a complex genome organization composed of parental subtype B' and CRF01_AE, with identical recombination breakpoints observed in the *gag*, *pol*, and *vif* genes. Such a discovery highlights the ongoing active generation and spread of intersubtype recombinants involving the subtype B' and CRF01_AE lineages and indicates the potential of the new CRF in bridging HIV-1 transmission among different risk groups in Southeast Asia.

Intersubtype homologous recombination is an important mechanism that leads to diversification of the HIV-1 population, thereby potentially assisting the recombinants to evade host immunity (6) or antiretroviral therapy (4). Recent estimates show that the HIV-1 circulating recombinant form (CRF) and other minor recombinants account for approximately 20% of global HIV-1 infections (1). Here, we described the genomes of a novel HIV-1 recombinant, designated CRF54_01B by the Los Alamos National Laboratory, isolated from two recently infected subjects and collected from Kuala Lumpur, Malaysia.

HIV-1 RNA was extracted from plasma of consenting subjects, reverse transcribed, and amplified using sets of primers (8) designed to span the complete genome of HIV-1, including the *gag*, *pol*, *env*, *tat*, *rev*, *vif*, *vpr*, *vpu*, and *nef* genes and the noncoding 5' and 3' long terminal repeats (LTRs). The contiguous nucleotide sequences generated by an ABI Prism 3730XL DNA analyzer (Applied Biosystems) were assembled and codon aligned with a comprehensive list of reference sequences retrieved from the HIV database (www.hiv.lanl.gov). A neighbor-joining tree (5) was reconstructed based on the Kimura two-parameter model implemented in MEGA version 5.05 (7) to deduce the relationships between each isolate. Bootstrap inference of 1,000 replicates was applied to ascertain the reliability of branching orders. To establish the precise recombination structure, the closely related putative parental strains, namely, 90THCM235 (CRF01_AE) and 96TH_NP1538 (subtype B'), determined by similarity plot (3), were used for bootscanning and informative site estimation with appropriate window/step sizes. Subgenomic phylogenetic trees were constructed to confirm the origin of each region.

In the present study, we have observed that recombinants 09MYSB023 (9,069 bp) and 08MYKL044 (8,159 bp), together with a previously reported unique recombinant 07MYKLD49 (8,942 bp) (2), formed a distinct monophyletic cluster distantly related to all known HIV-1 genotypes. Bootscan mapping indicated five identical recombination breakpoints located in the *gag* (2 breakpoints), *pol* (2 breakpoints), and *vif* genes (1 breakpoint), and shared among all three recombinants, forming six genomic regions of different sizes, namely, region I (relative to the HXB2 numbering system, positions 853 to 990), region II (HXB2, 1041 to 1163), region III (HXB2, 1200 to 3164), region IV (HXB2, 3224 to 3500), region V (HXB2, 3542 to 5478), and region VI (HXB2,

5481 to 8765). The ancestral origin of each region has been traced by maximum likelihood analysis to the respective parental lineages; regions I, III and V were grouped with subtype B', and regions II, IV, and VI were grouped with CRF01_AE. The emergence of CRF54_01B indicates a continual, active transmission of unique recombinants of significant epidemiological impact in the region. Interestingly, the isolation of CRF54_01B from various risk groups (heterosexual, homosexual, and injecting drug user populations) highly suggests the wide circulation of this genotype and extensive bridging of HIV-1 transmission across risk groups that may complicate disease prevention.

Nucleotide sequence accession numbers. The genome sequences of HIV-1 CRF54_01B isolates 09MYSB023 and 08MYKL044 have been deposited in GenBank under accession no. [JX390976](https://www.ncbi.nlm.nih.gov/nuccore/JX390976) and [JX390977](https://www.ncbi.nlm.nih.gov/nuccore/JX390977), respectively.

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The authors declare that no competing interests exist for this work.

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