An Emerging HIV-1 Recombinant (CRF58_01B) Disseminating Among People Who Inject Drugs in Malaysia

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INTRODUCTION

HIV pandemic is depicted primarily by the circulation of HIV-1 group M comprising of 11 subtypes and sub-subtypes (A1, A2, B-D, F1, F2, G, H, J and K) and to date 61 circulating recombinant forms (CRFs). In Southeast Asia, active inter-subtype recombination involving three main circulating genotypes, subtype B (including subtype B'), CRF01_AE and CRF33_01B[1], have contributed to the emergence of novel unique recombinant forms. In view of the increasing epidemiological impact of HIV-1 recombinants, for example CRF33_01B[1,2] in Southeast Asian countries, it is very likely that novel recombinants could arise especially among the high risk injecting drug population.

We report the emergence of a newly emerging novel HIV-1 CRF, designated as CRF58_01B by HIV Los Alamos National Library and characterised by the near full length recombinant genomes sequenced from six epidemiologically-unlinked people who inject drugs (PWID).

METHODOLOGY

Molecular epidemiology study of 258 HIV-1-infected PWID between year 2009 and 2011 identified six individuals with identical recombinant structures based on *gag*-RT sequencing

HIV-1 RNA extraction from plasma samples, amplification and sequencing of near full length genomes (~9kb) using primer sets that produced ten overlapping segments

Alignment of near full length HIV-1 sequences, phylogenetic, recombination analyses (similarity plot, bootscan and informative sites) and sub-region tree reconstructions

Maximum clade credibility (MCC) trees were constructed using coalescent-based Bayesian Markov chain Monte Carlo (MCMC) sampling (BEAST v1.7) for the shared sub-regions

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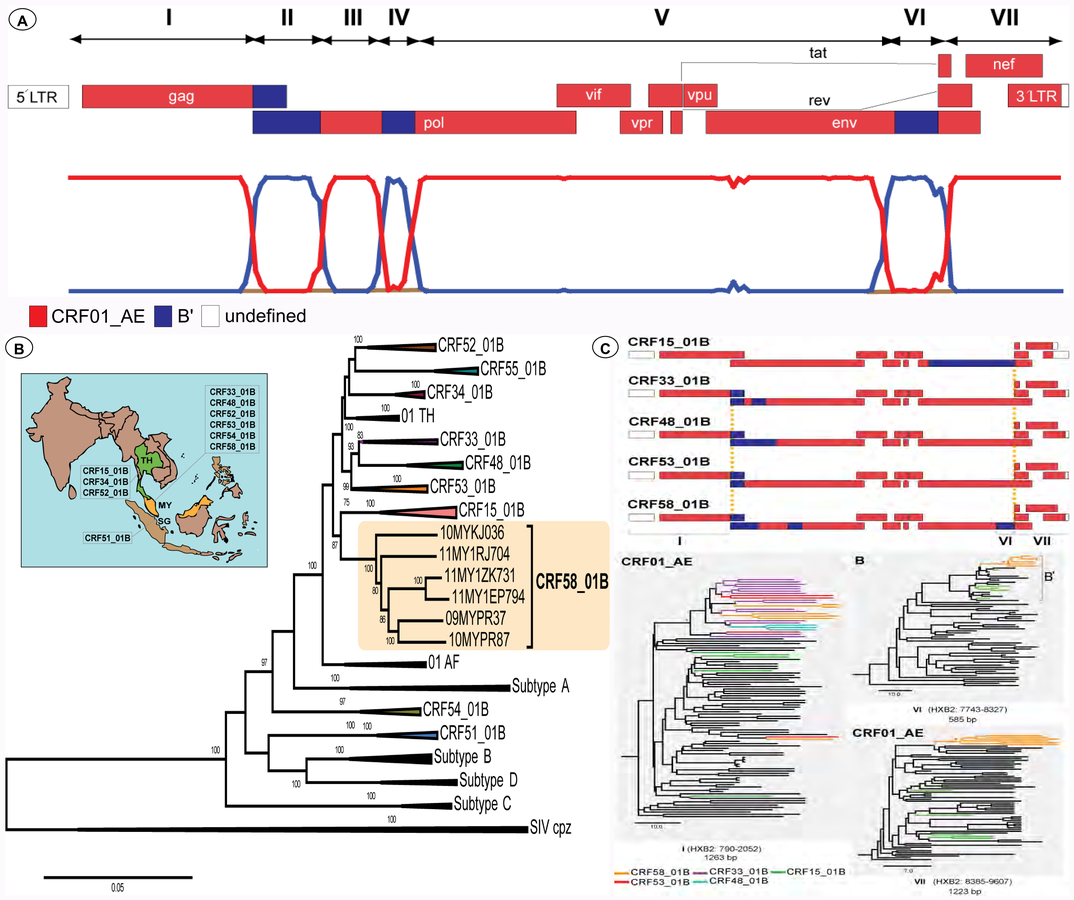

Figure A. The near full length mosaic structure of HIV-1 CRF58_01B determined using bootscanning and informative site analysis. CRF58_01B composed of three subtype B' fragments recombined with CRF01_AE in the *gag*-RT, *pol* and *env* regions of HIV-1. HIV-1 reference strains CRF01_CM240 (CRF01_AE) and B'_RL42 (Thai subtype B') were selected as the putative parental genotypes by similarity plotting, and C_95IN21068 (subtype C) as outgroup. The subtype origin of each recombinant segment was confirmed by constructing sub-region trees. **B. Phylogenetic reconstruction of near full length HIV-1 circulating recombinant forms (CRFs) in Southeast Asia.** CRF58_01B forms a strongly supported clade being distinct from other established genotypes. Bootstrap values of greater than 70% were indicated on the branch nodes. The scale bar represents 5% genetic distance (0.05 substitutions per site). **C. Near full length mosaic genomes of CRF58_01B and other established CRFs in Malaysia and Thailand.** CRF58_01B shared two unique recombination breakpoints (indicated in dashed lines) with other established CRFs in the region: CRF33_01B, CRF48_01B and CRF53_01B in the *gag* gene at HXB2: 2053 to 2095nt, and CRF15_01B (from Thailand) in the *env* gene. Maximum clade credibility (MCC) phylogenies were constructed for the shared sub-regions I, VI and VII between CRF58_01B and the established CRFs by including CRF01_AE (sampled from 1990 to 2009 in Malaysia, Thailand, China and Japan) and B/B' reference strains (sampled from 1983 to 2011 in France, United States of America, Japan, Thailand and Myanmar) downloaded from the Los Alamos HIV database (labelled in black) to discern their evolutionary relationship. Monophyletic clusters (with a posterior probability of 1.0) were indicated with an asterisk (*) at the branch nodes. The sub-region trees were scaled in units of time (years).

RESULTS AND DISCUSSION

- All subjects were antiretroviral-naïve males with a mean age of 40.5 ± 6.9 years old from various ethnic groups with a history of unsafe injecting drug use.
- Near full length genomes sequenced from the six epidemiologically unlinked individuals (09MYPR37, 10MYKJ036, 10MYPR87, 11MY1ZK731, 11MY1RJ704 and 11MY1EP794) showed identical mosaic structures consisting of subtype B' and CRF01_AE, with six unique recombination breakpoints in the *gag*-RT, *pol* and *env* regions **(Figure A)**.
- The prevalence of CRF58_01B was relatively low at 2.3% (6/258) compared to approximately 71% of the PWID population predominantly infected with CRF33_01B.
- CRF58_01B shared two unique recombination breakpoints with other established CRFs in the region: CRF33_01B[1], CRF48_01B[3] and CRF53_01B[4] in the *gag* gene, and CRF15_01B[5] in the *env* gene.
- Based on sub-region I, CRF58_01B intermingled with other CRFs isolated from Malaysia (CRF33_01B, CRF48_01B, CRF53_01B) within a robust monophyletic cluster which was distinct from other CRF01_AE and CRF15_01B strains. High rates of recombination in HIV-1 may consequently confound the evolutionary history and classification of sequences[6].
- Extended Bayesian MCMC sampling analysis showed that CRF58_01B and other recently discovered CRFs were most likely to have originated in Malaysia, and that the recent spread of recombinant lineages in the country had little influence from neighbouring countries **(Figure C)**.

CONCLUSION

The isolation, genetic characterization and evolutionary features of CRF58_01B among PWIDs in Malaysia signify the increasingly complex HIV-1 diversity in Southeast Asia that may have an implication on disease treatment, control and prevention.

ACKNOWLEDGEMENTS

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SELECTED REFERENCES

1. Tee et al. *JAIDS* 2006.
2. Chow et al. *PLoS One* 2013.
3. Li et al. *JAIDS* 2010.
4. Chow et al. *JVirol* 2012.
5. Tovanabutra et al. *AIDS* 2001.
6. Abecasis et al. *JVirol* 2007.