Spread of CRF33_01B and Emergence of Novel Recombinants Among People Who Inject Drugs in Malaysia

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BACKGROUND

Southeast and East Asia are home to around 4 million people who inject drugs (PWID) with a HIV prevalence rate of approximately 20% in 2010[1]. Malaysia is among the countries facing mega-epidemics from injecting drug use besides China, Vietnam, Russia and Ukraine[2]. Co-circulation of subtype CRF01_AE and B among various risk groups led to an upsurge of unique recombinants (URF) and circulating recombinant forms (CRF), for example CRF33_01B[3] identified in Malaysia had been actively recombining with other subtypes[4] and recently reported in Singapore[5,6], Indonesia[7] and Hong Kong[8]. However the history and magnitude of CRF33_01B transmission among various risk groups including PWID were unknown.

We investigated the rapidly evolving molecular epidemiological profiles of HIV-1 among PWID in Kuala Lumpur through phylogenetic analyses and a high-resolution Bayesian resampling approach.

METHODOLOGY

◊To study the molecular epidemiology and recombination structure of novel URFs among PWID

HIV-1 RNA extraction, amplification and sequencing of *gag-pol* genes (HXB2: 1753-3440nt) → Neighbour-joining phylogenetic and recombination analyses - Simplot, bootscan and informative sites (Simplot v3.5.1)

◊To study the past population dynamics of CRF33_01B

Alignment of CRF33_01B *pol* gene, 1kb (HXB2: 2253-3260nt) compiled from in-house laboratory and LANL databases from 2005 to 2012 →

- ♦Maximum likelihood analysis (PAUP* v4.0 beta)
- ♦Coalescent-based Bayesian Markov chain Monte Carlo (MCMC) sampling (BEAST v1.7)

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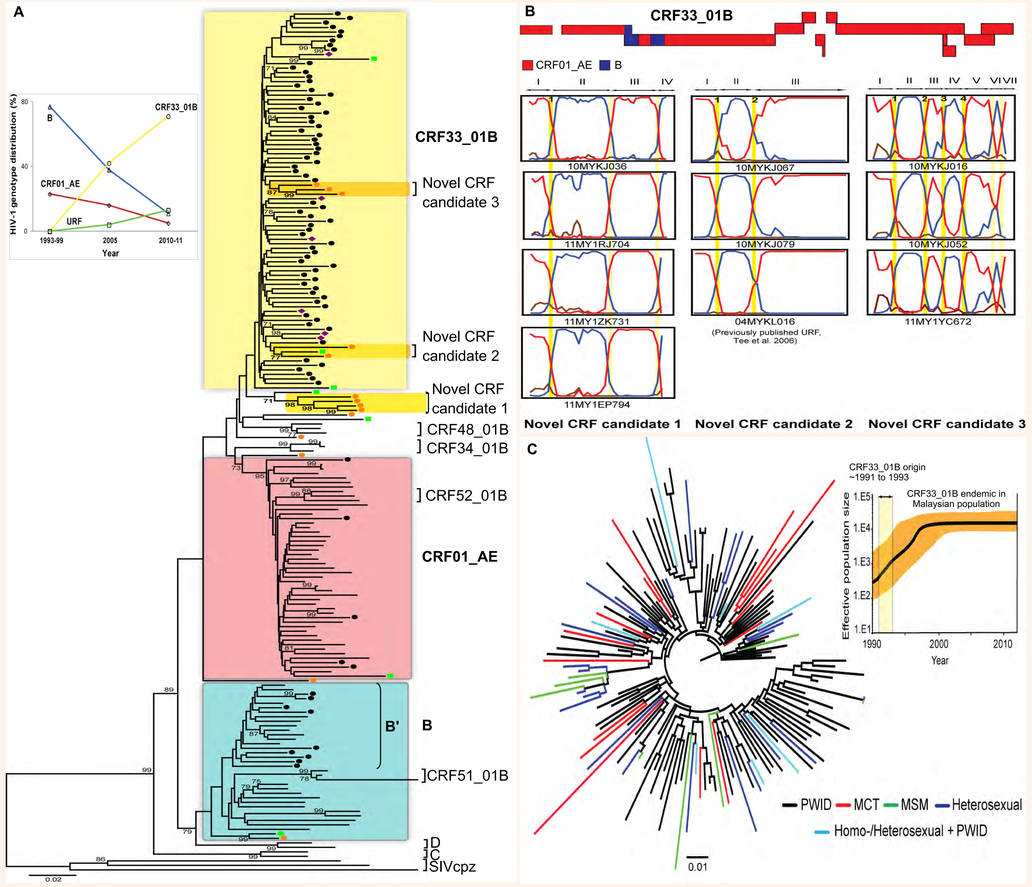

Figure A. Phlyogenetic reconstruction of 1.6kb *gag-pol* sequences amplified among people who inject drugs (PWID) in Kuala Lumpur, Malaysia. Bootstrap values of greater than 70% were indicated on the branch nodes. The scale bar represents 2% genetic distance (0.02 substitutions per site) **B. Bootscan analyses of HIV-1 unique recombinant forms (URFs) identified among PWID.** Schematic representation of the full length recombinant structure of CRF33_01B with the four unique recombination breakpoints labelled as 1-4 (HXB2: 2053-2063 nt, 2375-2416 nt, 2538-2540 nt and 2841-2875 nt, respectively) in the gag-pol region. Bootscanning plots of three novel CRF candidates (1 to 3) generated from the 1.6 kb gag-pol gene (HXB2:1753-3440 nt). **C. Maximum likelihood analysis and past population dynamics of CRF33_01B disseminating among various risk groups in Southeast Asia (Malaysia and Indonesia).** The star-like appearance of the phylogenetic tree was characterised by the terminal tree branches (mean length: 0.025 ± 0.014) which were generally longer than the internal branches (mean length: 0.005 ± 0.005) suggested possible high growth rate for CRF33_01B during the early phase of the epidemic in Malaysia. The x-axis represents the time (in units of year) and y-axis represents the effective population size of CRF33_01B. The thick solid line in the plot represents the median estimate and the shaded region represents the 95% highest posterior density (HPD) credible region. The Bayesian skyline plot revealed an exponential increase in effective number of CRF33_01B infections in the host population since its reported emergence around 1991 to 1993[9] until early 2000s and remained stable thereafter at a high prevalence, suggesting persistent viral transmission and endemicity of CRF33_01B among various risk groups in Malaysia.

RESULTS AND DISCUSSION

The HIV-1-infected study population comprised of 336 males (mean age: 31.3 to 44.7 years) and 20 females with a mean CD4+ T-cell count of 438 cells/μl. Among 116 subjects with available serological data, approximately 93% were anti-HCV positive and the majority (89.8%) of subjects were antiretroviral (ARV)-naïve. The *gag-pol* genes were successfully amplified in 75.8% (270/356) of the study subjects.

- ♦Approximately 72% (195/270) of the population was infected by CRF33_01B, followed by CRF01_AE/B' unique recombinants (10%, 27/270), subtype B' (10.7%, 29/270) and CRF01_AE (4.8%, 13/270), indicating a significant shift in genotype replacement among PWID **(Figure A).**
- ♦Three clusters of CRF01_AE/B' recombinants displaying divergent yet phylogenetically-related mosaic genomes to CRF33_01B were identified and characterized, suggestive of an abrupt emergence of multiple novel CRF clades **(Figure B).**
- ♦The founder lineages of CRF33_01B were likely to have first emerged among PWIDs in the early 1990s before spreading exponentially to various high and low-risk populations (including children who acquired infections from their mothers) and later on became endemic around the early 2000s **(Figure C).**

CONCLUSION

We describe the first genomic and epidemiological data demonstrating the massive expansion of HIV-1 CRF33_01B among the high-risk injecting drug use population and its subsequent spread into the general population in Malaysia. The discovery of multiple unique recombinant clusters closely related to CRF33_01B also indicated the emergence of a group of novel second-generation CRFs in Southeast Asia. As a result of the dynamics and extreme genetic variance of HIV-1 in the region, continual effort in designing effective treatment and prevention strategies must be improved.

ACKNOWLEDGEMENTS

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