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Sequence Note

The Evolving Molecular Epidemiology of HIV Type 1 among Injecting Drug Users (IDUs) in Malaysia

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ABSTRACT

Earlier studies in the 1990s indicate that human immunodeficiency virus type 1 (HIV-1) subtype B has been the predominant subtype among injecting drug users (IDUs) in Malaysia. More recent studies performed between 2003 and 2004, however, show a high prevalence of unique CRF01_AE/B intersubtype recombinants among IDUs. To determine the subtype distribution among IDUs in Kuala Lumpur prior to the emergence of CRF01_AE/B intersubtype recombinants, the *gag–pol* or the reverse transcriptase gene was sequenced from IDUs who were diagnosed as HIV positive between 1993 and 2002. Subtype B was present at 50.0% followed by CRF01_AE/B recombinant at 41.7%, with more CRF01_AE/B recombinants detected between 2000 and 2002. All CRF01_AE/B recombinants shared similar recombination patterns. Interestingly, we found that this potential new candidate of circulating recombinant form (CRF) could have emerged as early as the mid-1990s. The results showed evidence of changing HIV-1 molecular epidemiology toward the predominance of CRF01_AE/B intersubtype recombinants among IDUs in Kuala Lumpur.

THE EXTENSIVE DIVERSITY OF HIV-1 has significant impact on disease pathogenesis, long-term antiretroviral treatment, and the development of an effective vaccine. Subtype information is particularly important when clinical investigations on antiretroviral drugs or candidate vaccines are initiated in a select population.

In Malaysia, very little information is available about the predominant HIV-1 subtypes circulating among injecting drug users (IDUs). This is in spite of the fact that of the 64, 439 HIV infections reported between 1986 and December 2004, 76% were among IDUs. HIV-1 subtype B has been the predominant subtype among IDUs since the early phase of the epidemic, followed by CRF01_AE.[1–3] Studies have shown that coexistence of two or more subtypes circulating recombinant forms (CRFs) in any population can lead to coinfection in individuals with high-risk practices and therefore to the generation of an intersubtype recombinant strain.[4–7] In our laboratory, we recently demonstrated that the major circulating HIV-1 strain among IDUs between 2003 and 2004 was CRF01_AE/B intersubtype recombinants, which were also present in high prevalence in other risk groups.[8] These unique recombinant forms (URF) shared similar recombination profiles in the protease and reverse transcriptase (RT) genes and led to evidence of the emergence of a new CRF circulating in Kuala Lumpur, Malaysia. However, recent data on the predominant subtype/CRF among IDUs prior to the detection of CRF01_AE/B recombinants in 2003–2004 are still lacking. To demonstrate the changing molecular epidemiology of HIV-1 among IDUs, we studied the subtype/CRF distribution among former IDU prison inmates diagnosed seropositive for HIV-1 between 1993 and 2002.

Plasma samples were collected from 36 consenting former IDU prison inmates in Kuala Lumpur in 2004. These IDU subjects were diagnosed while still incarcerated where HIV testing upon admission to a prison is mandatory in Malaysia. All inmates were antiretroviral naive at the time of sampling. HIV-1 RNA was extracted and the nucleotide sequences of the partial *gag–pol* gene encoding for protease and reverse transcriptase were determined by nested PCR and direct sequencing using a modified in-house genotypic drug resistance protocol described previously.[8] These nucleotide sequences were aligned

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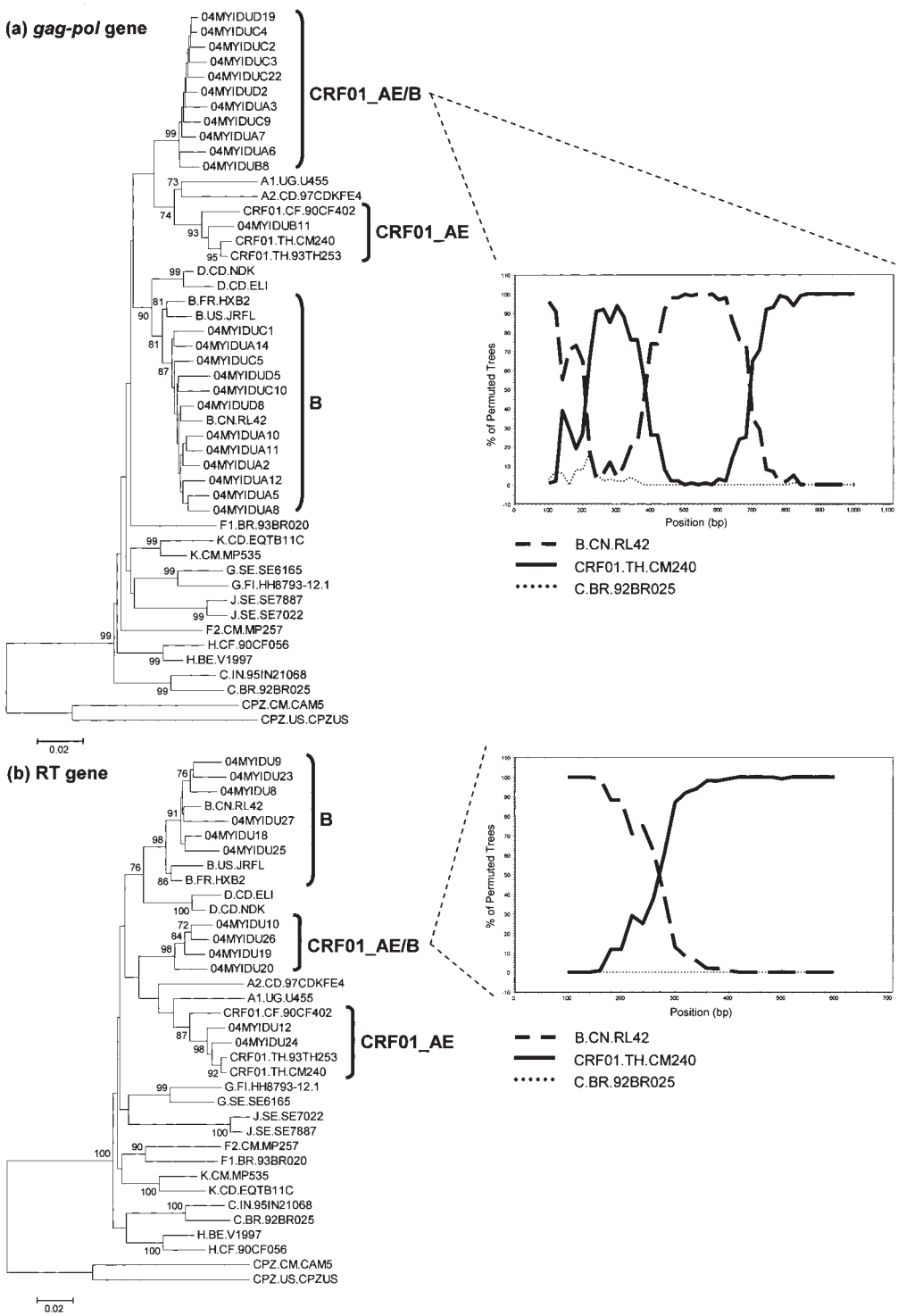

FIG. 1. Neighbor-joining phylogenetic analysis and bootscan plots of **(a)** 24 *gag–pol* gene sequences (1.1 kb) and **(b)** 12 reverse transcriptase gene sequences (696 bp) of HIV-1 isolated from former IDUs in Kuala Lumpur, Malaysia. The Kimura two-parameter method was used for estimating pairwise evolutionary distance and the reliability of the branching orders was assessed by bootstrap analysis with 1000 replicates. Bootstrap values of more than 70% were shown at the nodes of the tree. The reference sequences used for multiple alignments were as follows: subtype A (A1.UG.U455 and A2.CD.97CDKFE4), subtype B (B.FR.HXB2, B.US.JRFL, and B.CN.RL42), subtype C (C.IN.95IN21068 and C.BR.92BR025), subtype D (D.CD.NDK and D.CD.ELI), and CRF01_AE (CRF01.TH.93TH253, CRF01.TH.CM240, and CRF01.CF.90CF402), subtype F (F1.BR.93BR020 and F2.CM.MP257), subtype G (G.SE.SE6165 and G.FI.HH8793-12.1), subtype H (H.CF.90CF056 and H.BE.V1997), subtype J (J.SE.SE7887 and J.SE.SE7022), and subtype K (K.CD.EQTB11C and K.CM.MP535). SIVcpz reference isolates (CPZ.CM.CAM5 and CPZ.US.CPZUS) were used as outgroup. Bootscan analysis was plotted using HIV-1 subtype B′ (RL42) and CRF01_AE (CM240) as the putative parental strains, with subtype C (90BR025) as an outgroup. The bootstrap values were calculated for a window of 200 bp moving in increments of 20 bp along the alignment.

Table 1. Subtype Distribution among 36 HIV-Infected Former Injecting Drug Users (IDUs) in a Prison in Kuala Lumpur, Malaysia

Isolate	*Ethnicity*[a]	*Date of first positive HIV-1 test (month/year)*	*Subtype/CRF (*gag–pol *gene)*[b]	*GenBank accession number*
04MYIDUA5	I	4/93	B	DQ018345
04MYIDUA12	C	3/94	B	DQ018351
04MYIDUB8	M	8/94	AE/B	DQ018353
04MYIDUC22	M	1/96	AE/B	DQ018362
04MYIDUA2	I	12/96	B	DQ018343
04MYIDUA8	I	6/97	B	DQ018348
04MYIDU26	M	3/98	*AE/B*	AY887057
04MYIDUA10	C	5/98	B	DQ018349
04MYIDU27	M	9/98	*B*	AY887058
04MYIDU19	M	11/98	*AE/B*	AY887043
04MYIDUC10	C	12/98	B	DQ018361
04MYIDU8	M	3/99	*B*	AY887046
04MYIDU9	M	4/99	*B*	AY887047
04MYIDU10	M	4/99	*AE/B*	AY887048
04MYIDUB11	M	5/99	AE	DQ018354
04MYIDU12	C	5/99	*AE*	AY887050
04MYIDUC3	I	2/00	AE/B	DQ018357
04MYIDUC5	M	2/00	B	DQ018359
04MYIDU18	C	2/00	*B*	AY887051
04MYIDU20	I	2/00	*AE/B*	AY887052
04MYIDUC9	I	2/00	AE/B	DQ018360
04MYIDUC1	M	3/00	B	DQ018355
04MYIDUC4	M	3/00	AE/B	DQ018358
04MYIDU23	M	5/00	*B*	AY887054
04MYIDUA7	C	7/00	AE/B	DQ018347
04MYIDU24	I	8/00	*AE*	AY887055
04MYIDUC2	M	11/00	AE/B	DQ018356
04MYIDU25	M	11/00	*B*	AY887056
04MYIDUA11	M	4/01	B	DQ018350
04MYIDUD5	C	10/01	B	DQ018364
04MYIDUD19	M	1/02	AE/B	DQ018366
04MYIDUA14	M	5/02	B	DQ018352
04MYIDUA3	M	8/02	AE/B	DQ018344
04MYIDUD8	M	9/02	B	DQ018365
04MYIDUA6	I	10/02	AE/B	DQ018346
04MYIDUD2	M	12/02	AE/B	DQ018363

[a]I, Indian; C, Chinese; M, Malay.

[b]B, subtype B; AE/B, CRF01_AE/B intersubtype recombinant; AE, CRF01_AE. Subtype/CRF assignment based on reverse transcriptase (RT) gene is listed in italic type.

with selected HIV-1 strains of various reference subtypes obtained from the Los Alamos HIV Database (http://www.hiv.lanl.gov/). Phylogenetic analyses using the neighbor-joining method were performed by *MEGA* version 2.1. Evolutionary distances were assessed using the Kimura two-parameter model with a transition–transversion ratio of 2.0 and the reliability of the branching orders was determined by bootstrap analysis with 1000 replicates.

From 36 subjects (21 Malay, 8 Indian, and 7 Chinese), a 1.1-kb *gag–pol* gene (2195–3301 nt; HXB2 numbering) from 24 samples was successfully amplified and sequenced for analysis. For the remaining 12 samples, a smaller fragment of 696 bp reverse transcriptase gene (2606–3301 nt) was analyzed as an alternative.[8] Phylogenetic analyses of these genes showed that subtype B and CRF01_AE were present at 50.0% (n = 18/36) and 8.3% (n = 3/36), respectively (Fig. 1). Most of the subtype B and CRF01_AE isolates were clustered together with reference strains from Thailand. CRF01_AE/B intersubtype recombinant was present at 41.7% (n = 15/36), with 66.7% (n = 10/15) being present among Malay IDUs.

Bootscan analysis was performed on all CRF01_AE/B recombinants using SimPlot version 2.5. In the *gag–pol* gene, three breakpoints were identified at 2395, 2555, and 2855 nt in all 11 isolates (Fig. 1a). The small 5′ subgenome of the gene was estimated as subtype B until 2395 nt, followed by CRF01_AE (2396–2555 nt) and subtype B (2556–2855 nt) downstream. The 3′ segment was estimated as CRF01_AE from 2856 nt to 3301 nt. In the shorter RT gene, one breakpoint at 2855 nt was identified in all four isolates with subtype B at the 5′ subregion and CRF01_AE at the 3′ segment after the break-

point (Fig. 1b). These RT segments shared the same breakpoint with the longer *gag–pol* gene at 2855 nt, indicating that these isolates probably had the same recombination patterns if their entire *gag–pol* gene was analyzed. Further analyses by RIP 2.0 (http://www.hiv.lanl.gov/) on all CRF01_AE/B isolates showed that these mosaic viruses shared similar recombination profiles as in the bootscan plots (figures not shown). The subtype/CRF/URF distribution in all subjects is summarized in Table 1. All CRF01_AE/B recombinants identified in this study were similar to those recombinants previously described from the protease and RT genes in our laboratory.[8] However, the recombination breakpoints estimated in the current study may be more accurate since the longer *gag–pol* gene was analyzed and the bootstrap values were plotted with a larger window of 200 bp. Of all the CRF01_AE/B recombinants identified in Kuala Lumpur, none of them shared any recombinant breakpoints with CRF15_01B or other CRF01_AE/B recombinants described elsewhere in the Southeast Asia region.[4–7,9–11]

In previous studies, subtype B was present as the major circulating subtype at 85%, 55%, and 91% between the years 1992–1993, 1992–1996, and 1996–1997, respectively, with CRF01_AE as the second major strain.[1–3] In this study, we demonstrated that subtype B was the major circulating subtype among IDUs followed by CRF01_AE/B recombinant and CRF01_AE. The predominance of subtype B, however, was not high and the prevalence of CRF01_AE/B recombinants was increasing with year of first positive HIV-1 testing, from 31.3% (n = 5/16) between 1993 and 1999 to 50.0% (n = 10/20) between 2000 and 2002. Together with data from 2003–2004 where the prevalence of the CRF01_AE/B recombinant among IDUs was about 83%,[8] the HIV-1 molecular epidemiology among IDUs in Kuala Lumpur has changed toward a predominance of CRF01_AE/B recombinants.[4] Interestingly, the gradual epidemiological shift from subtype B to CRF01_AE among IDUs in Thailand was not observed in this study.[12–15] Instead of becoming "extinct," CRF01_AE has in fact contributed to the appearance of the newly identified CRF01_AE/B recombinant.

The earliest CRF01_AE/B recombinants were detected from IDUs diagnosed in 1994 and later in 1996, suggesting that these recombinants could have emerged in the population as early as the mid-1990s. Since all samples in this study were collected in 2004, the possibility of the subjects being multiply exposed by injecting drug use (or by a different route) leading to intersubtype superinfections cannot be ruled out.[16] The existence of the CRF01_AE/B recombinant in the 1990s might have been overlooked in previous studies, which focused mainly on the *env* or *gag* gene for classifications.[1–3] Molecular study on archival samples of recent seroconverters in the 1990s would therefore determine the actual year of emergence of the CRF01_AE/B intersubtype recombinant in Kuala Lumpur.

This study provides evidence of the epidemiological shift of HIV-1 among IDUs in Kuala Lumpur over the past decade. The reasons for such evolution are not clearly understood. However, the emergence of the CRF01_AE/B intersubtype recombinant at high prevalence suggests that the recombinant strains might have selective advantages in the viral pathogenicity and/or tranmission fitness among IDUs over the two parental strains. The emergence of the new CRF candidate clearly points to the high genetic variability of HIV-1 among IDUs in Kuala Lumpur.

SEQUENCE DATA

Nucleotide sequences were submitted to GenBank under accession numbers as shown in Table 1.

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