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Emerging trends of HIV epidemiology in Asia

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Abstract

The main molecular trait of HIV-1 is the inherent capacity to vary, recombine, and diversify, which gives it a clear edge to evade the human immune system and survive through the generation of complex molecular forms, termed recombinants. In a setting of coinfection, molecular and biological interactions between diverse HIV-1 subtypes may promote the emergence of circulating recombinant forms through the shuffling of viral genomes, which results in increased intra- and inter- host viral variation and altered biological properties. The focus of this review is on Asia, which has the highest proportion of HIV-1 recombinants circulating worldwide, with the top in South and Southeast Asia, amounting to 89% of its total HIV-1 infection. The HIV-1 strains which are spreading in this geographic area are CRF01_AE, subtypes B and C. Given the rapid spread and active establishment of some of the recombinant forms in Asia, it is essential to understand how they differ from their parental strains, the acquisition of certain molecular traits, and their biological attributes upon recombination, which give these strains an epidemiologic edge. The current epidemic provides strong evidence that the parental subtypes are being replaced via competition with possibly more versatile HIV-1 recombinant forms. This appears to be an ongoing phenomenon and has resulted in an HIV-1 epidemic shift, with the expansion and dissemination of a wide variety of HIV-1 forms within this geographic region.

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Introduction

The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimated that 2.5 million new HIV infections and 2.1 million AIDS death occurred in 2007, and almost 33.2 million adults and children worldwide are currently living with HIV or AIDS, the great majority of them in the developing world[1]. Among the major regions around the world, sub-Saharan Africa remains the region with the highest number of people living with HIV (estimated 22.5 million in 2007), while Oceania remains the region with the lowest number of people infected with HIV. This review focuses on the Asian region, which consists of Bangladesh, DPR Korea, Republic of Korea, Laos, Malaysia, the Maldives Bhutan, Brunei, Cambodia, China, India, Indonesia, Hong Kong, Japan, Nepal, the Philippines, Singapore, Mongolia, Myanmar, Sri Lanka, Thailand, and Vietnam. As Asia is home to more than half of the world's population[2], without a doubt the HIV epidemic in this region is bound to have an enormous impact on the global HIV epidemic in addition to being a huge future economic burden to these countries. The focuses of this review are (i) the epidemiologic trends of HIV-1 in Asia, (ii) the distribution of major HIV-1 subtypes and intersubtype recombinants in different parts of Asia, as well as (iii) the impact of HIV-1 recombination in drug resistance and vaccine development in the region.

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Global HIV subtypes and their distribution

Through phylogenetic analysis, HIV-1 strains can be classified into three groups: the "major" group M, the "outlier" group O and the "new" group N (also described as non-M non-O). Group M, being the predominant HIV-1 strain, has dominated more than 90% of HIV-1 infections. Within group M there are known to be at least nine genetically distinct subtypes (or clades) of HIV-1, which are distributed globally. These are subtypes A, B, C, D, F, G, H, J and K, as well as circulating recombinant forms (CRF) with certain subtypes predominating in different geographic regions[3]. Furthermore, there are other strains of HIV-1 group M sub-subtypes (A1, A2, A3, F1 and F2), which are frequently regarded as independent subtypes by some scientists[3]. The HIV-1 subtype classification applies to all genomic regions, although it was initially based on the *gag* and/or *env* sequences. The intrasubtype genetic variation ranges from 15-20%, whereas the variation between subtypes is approximately 25-35%, depending on the subtypes and genome regions examined[4]. Even as the global availability of sequencing techniques has increased, the classification of HIV-1 strains into subtypes and CRF remains a complex issue and the definitions are subject to change as new discoveries are made. Apart from the intricate HIV-1 subtype classification, the different subtypes have distinct global distribution patterns. "Pure" subtype C or HIV-1 recombinant forms containing at least the *env* gene of subtype C accounts for 50% or more of all HIV-1 infections worldwide[5,6], partly as the result of the recent pandemics in Southern Africa, South America and Asia[6-9]. Other main subtypes such as A, B, G, and D were responsible for 12, 10, 6, and 3% of HIV-1 infections, respectively[5]. The remaining subtypes (F, H, J, K) together caused as little as 0.94% of infections[5]. In terms of the worldwide distribution of the recombinant forms, two most important CRF (CRF01_AE and CRF02_AG) have each contributed to 5% of HIV-1 infections, whereas CRF03_AB is only responsible for 0.1% globally and other recombinants accounted for the remaining 8% of infections[5].

HIV epidemiologic trends in Asia

There are an estimated 4.9 million people living with HIV in Asia, with 440,000 new infections in Asia in 2007[2]. In Asia, particularly in East Asia, there was a 90% increase in the number of people living with HIV, as well as a leap of approximately 20% in the number of people who acquired HIV, in 2007 compared to the year 2001[1]. On the contrary, the number of new infections in South and Southeast Asia has only increased by 14% between 2001 and 2007. The predominance of high-risk behaviors, mainly involving injecting drug use, unprotected paid sex, and sex between men are especially evident in the HIV epidemic in Asia. In 2005, sex workers and their clients accounted for about 49% of the epidemic in South and Southeast Asia (excluding India), while 22% of infections were in injecting drug users (IDU). Meanwhile, men who have sex with men have a small and yet significant amount (5%) of HIV infections[10].

Although the current HIV-1 genetic variability in Asia is only a fraction in terms of the global diversity of HIV-1[11], the epidemic is dynamic, with a constant influx of new HIV-1 strains/subtypes leading to the emergence of complex new generations of recombinant forms[12,13]. A few countries, including the Philippines, Laos, Korea, and Japan[14], have escaped the serious effects of the HIV epidemic and its spread. While the underlying reasons for this remain unclear, it is crucial for these countries to be heedful in order to avoid the fate of some other countries that once experienced a low rate of HIV, for instance Indonesia. An extreme diversity in the HIV-1 infection pattern has been observed in several Asian countries, with the lowest HIV prevalence rates seen in Mongolia and DPR Korea, while Cambodia, Thailand, and Myanmar remained the worst affected geographic areas. Notably, the incidence of HIV-1 infection has gradually declined in Cambodia and Thailand, yet increased in China, Indonesia, and Vietnam. Particularly in China, the increase is caused by the extensive introductions of HIV-1 and its multiple subtypes as a consequence of the economic boom and the incessant influx of diverse nationalities[8,15]. Home to nearly half of world's population, India, Indonesia, and China raise more concerns in terms of their HIV-1 prevalence rates. Although the overall prevalence rates in Asia are still lower among adults, HIV transmission rates could be higher than the initial estimation and are expected to rise as human efflux and influx continue, due to the burgeoning economies in this region[16]. In addition, in many Asian countries, the proportion of women living with HIV is continually and gradually on the rise[10]. Overall, the HIV-1 molecular epidemiology and its rapid spread in Asia are interesting. It has provided clues on the HIV-1 risk groups, focal routes of HIV-1 dissemination and its correlation with the newly emerging trends in HIV-1 infections, as well as the possible nature of infecting strains.

HIV-1 subtype displacement and its recombinants: the distribution of HIV-1 strains in Asia

In many regions around the world, an apparent displacement of the existing HIV-1 subtype (particularly subtype C)

by another new strain has been significant; for instance, subtype B and CRF01_AE in southern China[7,8], subtype B in southern Brazil[9] and a number of subtypes in Kinshasa, Democratic Republic of Congo[17]. The initial predominance of a certain single subtype within a specific transmission group in these regions is possible[18]. However, there is no substantial evidence to suggest that HIV-1 strains such as CRF01_AE and subtypes (e.g. A, B, C, D) are any more or less transmissible by a specific route, in a specific ethnic group, or in a specific cell type[19-25]. Furthermore, the initial predominance could possibly be lost during an epidemic, as observed in Thailand; before the late 1980s, there was primary segregation of subtype B among the IDU and CRF01_AE among the heterosexuals, which later became uniformly mixed in the Thai population and continues in the present day epidemic[11].

Apart from the subtype displacement by another "pure" HIV-1 subtype, variants of recombinant viruses are beginning to become more prevalent in certain regions around the world. These recombinant forms of HIV-1, designated as CRF, together with numerous unique recombinant forms (URF), are most prevalent in areas where multiple subtypes co-circulate and therefore play a major role in the global AIDS epidemic[26]. To date, 34 CRF have been identified (Los Alamos HIV database: http://www.hiv.lanl.gov/). More other new CRF have recently been characterized and are yet to be available from the Los Alamos HIV database, namely CRF35_AD[27], CRF36_cpx[28] and CRF37_cpx[29]. It is apparent that the recombinant forms are more likely to be widespread and affect the HIV-1 global pandemic. As CRF become increasingly important epidemiologically, they tend to gradually replace the "pure" subtypes, as has been evident from epidemics in some of the geographic regions, such as Thailand, Brazil and West Africa, which may eventually phase out the pure HIV forms previously predominating in different geographic regions of the globe.

Most of the inferences regarding the HIV epidemic in Asia have been derived from more than 13,000 partial nucleotide sequences of HIV-1 in the Los Alamos HIV database. Unfortunately, only 1% of these sequences are available as complete HIV genomes, which has hampered a detailed characterization of complex, new, second and third generation recombinant forms. By referring to the HIV database, a majority of the HIV-1 sequences from Asia are CRF01_AE, subtypes B or C[11,30,31]. Further, several recombinants resulting from these viral strains in different geographic regions have also been documented in many Asian countries[11,32-37] (Table 1).

The global variation in the HIV-1 strains, the dynamic nature of the HIV-1 epidemic, and accidental epidemiologic transmissions can give rise to a highly unequal geographic distribution of viral variants. Although few HIV-1 geographic "recombination hotspots" have been identified around the world, such as central Myanmar[34], Yunnan province of China[38], Argentina[39], Brazil[40], East Africa[41,42] and more recently Cuba[43], it has to be noted that recombinant HIV strains have been reported from almost all geographic regions of the globe where multiple HIV-1 subtypes have been circulating. While the predominant viral forms in the global HIV epidemic are subtypes A and C[44], a different and even more complex HIV genetic diversity has been found in Asia. The HIV spread and its epidemiology in Asia are interesting and closely related to the routes of spread of the epidemic. This is evident from the distribution of subtype C (Fig. 1), which was originally found primarily in India and Africa, and is now spreading to northern India, Myanmar, and Thailand[45]. In India itself, subtype C has dominated the HIV-1 epidemic and accounts for almost 97% of infections, but the biological aspects that explain this high rate of infection remain unclear. Apart from the predominant subtype C, the A/C and B/C intersubtype recombinants have also been recently identified in northeastern India[46]. Emergence of A/C recombinants is also consistent with the epidemic in Bangladesh[47], from where triple recombinants between subtypes A, C and G have been recently reported. Beyrer, et al.[48] also demonstrated the spread of subtypes B and C as well as B/C recombinants through the drug route from eastern Myanmar into Yunnan province of China and moving to north and west into Xinjiang province of China (Fig. 1).

The CRF01_AE, which was originally identified in Thailand[49,50], appears to circulate in major parts of Asia, particularly Southeast Asia (Fig. 1). It is responsible for 84% of all infections, whereas other recombinants account for only 4% of the total HIV-1 infection in the South and Southeast Asia HIV epidemic. Together, CRF01_AE and other recombinants account for nearly 89%, the highest across the world[5]. Since the beginning of HIV pandemic in the last two decades until recently, changes in HIV subtype distribution in Asia have been overwhelming. From the late 1980s to 1990s in Asian countries with a high HIV prevalence, subtype B' (known as the Thai variant of subtype B) was the predominant strain and was most frequently observed among IDU[51,52], while concurrently in Thailand and other areas, CRF01_AE was introduced independently in commercial sex workers[50]. Interestingly in the last decade, a gradual yet evident spread of the Thai variant of CRF01_AE was witnessed in many countries of Asia[53]. The change with CRF01_AE taking over, even among IDU, was later observed in countries of Southeast Asia, including Thailand, Cambodia and Vietnam[53]. Also, prior to the year 2000, countries around Southeast Asia, such as Indonesia

Table 1. The geographic distribution of HIV-1 subtypes and circulating recombinant forms (CRFs), based on the information available from the Los Alamos HIV sequence database

Country	HIV-1 Subtype/CRF	Subtype distribution* (%)
Bangladesh	C	62.9
	A	11.4
	01_AE	8.6
	U	5.7
	G	5.7
	B	5.7
Cambodia	01_AE	97.2
	B	1.7
	Other	1.1
China	B	54.2
	08_BC	18.3
	01_AE	13.0
	07_BC	5.5
	C	3.3
	BC	3.0
	Other	2.8
Hong Kong	01_AE	100.0
India	C	91.7
	B	5.5
	A	1.8
	Others	0.9
Indonesia	01_AE	75.0
	B	25.0
Japan	B	78.9
	01_AE	20.1
	Other	1.0
Malaysia	01_AE	60.7
	B	18.0
	01B	17.4
	U	1.2
	33_01B	1.2
	Other	1.5
Myanmar	B	46.9
	01_AE	19.6
	01B	10.2
	01C	8.6
Myanmar (cont.)	C	6.5
	BC	5.7
	01BC	2.0
	Other	0.4
Nepal	C	100.0
North Korea	G	100.0
Philippines	B	75.4
	01_AE	12.3
	A	7.0
	C	3.5
Philippines	B	75.4
	01_AE	12.3
	A	7.0
	C	3.5
	D	1.8
Singapore	01_AE	66.8
	B	31.3
	C	1.5
	Other	0.4
South Korea	B	90.4
	02_AG	2.5
	A	1.4
	D	1.3
	01_AE	1.3
	Other	3.1
Taiwan	B	35.6
	07_BC	34.7
	01_AE	15.2
	G	6.6
	C	3.5
	A	2.1
	Other	2.3
Thailand	01_AE	89.7
	B	8.2
	01B	1.0
	Other	1.1

*Subtype distribution presented is based on the data from Los Alamos HIV Sequence database (http://hiv-web.lanl.gov/) and does not conclude the epidemiology of HIV-1 in Asia. The data (consist of a listing of the available sequences and subtypes) stored in the database are taken from the publications in the literature and are essential to reflect the intensity of study done at particular region as well as to give an approximate indication of the subtype distribution.

and Malaysia, demonstrated predominantly CRF01_AE and subtype B.

Although the HIV/AIDS epidemic was recognized later in Southeast Asia than elsewhere, local risk behaviors have allowed the epidemic to rapidly expand. Today, IDU account for up to 70% of HIV-1 transmissions in several Asian countries, including China, Indonesia, Malaysia, Myanmar, eastern India and Vietnam[15,54]. Also, there is ample evidence that heterosexual transmission through sex workers has increased over the last few years[15]. Following this trend, a dramatic shift was observed with CRF01_AE, now disseminating faster in all risk groups[55,56], together with a continued emergence and spread of CRF and URF involving the CRF01_AE and B subtypes[35,36,57]. The emergence of CRF01_AE/B recombinants in the Southeast Asia region, particularly in Thailand and Malaysia, is best explained by the CRF01_AE and B subtype co-circulation and dual infections[57,58], which in turn provide

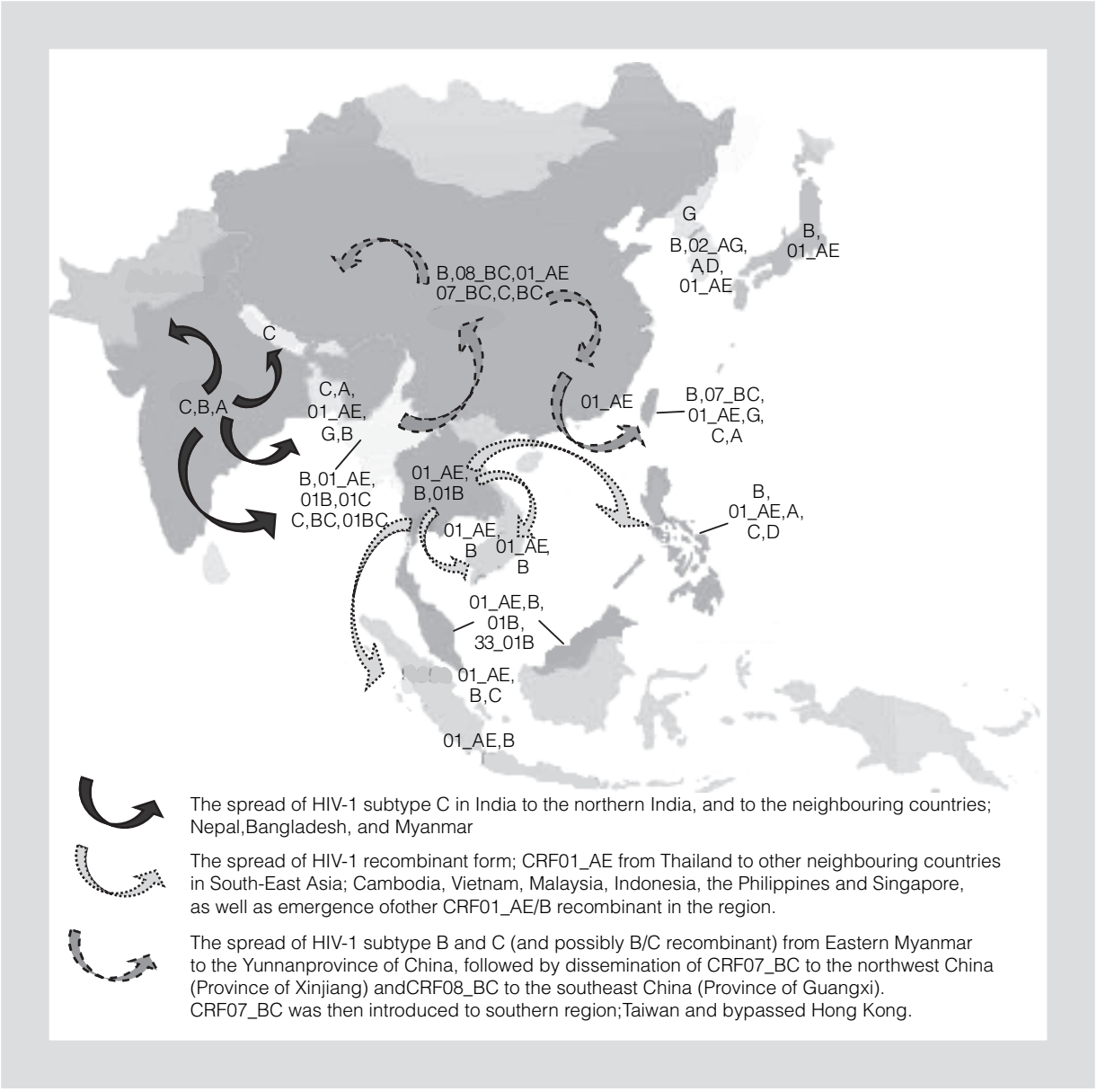

Figure 1. *The complex HIV-1 genetic diversity and the spread of the predominant HIV-1 strains in Asia. Distribution of different HIV-1 subtypes and CRF (demonstrated in descending order, from the most to the least prevalent strain) in major Asian countries, including subtypes A, B, C, D, G, CRF such as 01_AE, CRF01_AE; 02_AG, CRF02_AG; 07_BC, CRF07_BC; 08_BC, CRF08_BC; 33_01B, CRF33_01B and other HIV-1 recombinant forms: BC, B/C intersubtype recombinant; 01B, CRF01_AE/B; 01C, CRF01_AE/C and 01BC, CRF01_AE/B/C. The three different routes of spread for subtype C, CRF01_AE as well as subtype B, C and B/C recombinants are highlighted in green, red, and blue, respectively.*

an ideal environment for recombination[3]. It is certainly possible that these dual or coinfections have somehow created a scenario involving a continuous crossover(s) between CRF01_AE and subtype B in Asia. A collection of data is presented in figure 2 from recently reported full-length or near full-length genome of these CRF01_AE recombinant forms in the Asian region, mostly CRF01_AE/B, although a few are of CRF01_AE/B/C[34] and CRF01_AE/C[59] intersubtype recombinants. Despite the initial predominance of CRF01_AE in Asia, it is believed that this phenomenon started to change as early as in 1991, when the finding of the first case of CRF01_AE/B intersubtype recombinant form was found from an individual who was infected with HIV-1 in Thailand[60]. A few more established HIV-1 CRF were reported; two were from Thailand (CRF15_01B[36] and CRF34_01B[61]), while another one was identified from Malaysia (CRF33_01B)[13]. Most of these CRF01_AE recombinant forms, in particular the CRF01_AE/B intersubtype

recombinants, are more concentrated in Thailand, Myanmar, and Malaysia of Southeast Asia, although there could be more of such in other parts of Asia that are yet fully characterized.

While only a limited number of these newly emerging CRF01_AE/B recombinant forms have been identified, the structure of other recombinant strains remains to be elucidated. Interestingly, the genomic hotspots of recombination tend to reside largely in the *gag* and *env* gene regions. Recently, a new CRF01_AE/B recombinant (CRF33_01B) was identified and was mostly derived from IDU, as well as wide dissemination among various other risk populations in Kuala Lumpur, Malaysia[13]. More HIV-1 recombinant forms are believed to be expanding, and this has been proven by our recent study on clinical samples from Malaysia[12]. We reported some newly identified URF (possible recombinant forms of CRF01_AE and subtype B) based on protease/reverse transcriptase and *env* regions[12] as well as full-length genome characterization of CRF sequenced from an IDU from Kuala Lumpur, Malaysia[62]. Overall, the pattern of the HIV-1 epidemic in Southeast Asia appears to be complex, dynamic, and with a high frequency of intersubtype recombination[15]. There is also evidence suggesting a gradual replacement of the "pure" subtypes with the recombinant HIV-1 strains in this geographic region, as evident from CRF01_AE[11].

In other parts of Asia, for instance in East Asia, the total HIV-1 infections is as little as 3%, but this mostly reflects the distribution in China, since China harbors the vast majority of HIV-1-infected individuals. The HIV epidemic among the IDU in Yunnan province of China is believed to have started in the late 1980s with the initiation of both HIV-1 subtype B (same lineage of subtype B strains isolated in the USA and Europe) and subtype B'[56,63]. Following this, subtype B' increased from 20% of all subtype B strains in 1990 to 90% in 1996, replacing subtype B of U.S./European lineage[50,56,63]. Also in the early 1990s, HIV-1 subtype C strains were identified within this high-risk group in China[7].

In recent years, two closely related CRF (CRF07_BC and CRF08_BC) have been identified among the IDU in China, as a result of the co-circulation of subtypes B' and C. The CRF07_BC is mainly distributed in northwest China (Xinjiang Province), while CRF08_BC is disseminating in southeast China (Guangxi Province) (Fig. 1)[8,64]. Even though both CRF presumably originated from the Yunnan province, their different geographic spread patterns could probably be explained by the variance in the highly prevalent heroin trafficking routes[8]. The CRF07_BC was first introduced into the southern region in 2002 and then spread to other regions in Taiwan in 2004 (Fig. 1). A noticeable spread of CRF07_BC among IDU in Taiwan has recently been documented[65-68]. One of the studies found that in 2004, 44.7% people were infected with subtype B, 53.4% with CRF07_BC, and 1.5% with CRF01_AE, while 98% of IDU were infected with CRF07_BC[68]. Among the Taiwanese CRF07_BC strains, 7 to 11 amino acid deletions were found in both $p6^{gag}$ and $p6^{pol}$ proteins. Overall, the Taiwanese CRF07_BC strains have 97% full-length sequence homology with the prototype counterpart from mainland China. In a recent Chinese study, CRF07_BC from the regions of Ulumuqi and Yili in Xinjiang province was shown to spread continuously[69]. New CRF07_BC isolates showed higher genetic diversity and more potential N-linked glycosylation sites than older isolates, which may possibly give them a biological edge in their transmissibility[69]. While the spread of CRF07_BC in East Asia is more restricted to China and Taiwan, the predominant HIV-1 subtype in other regions within East Asia, for instance Hong Kong, is different. Here, both subtype B (50%) and CRF01_AE (45%) contributed almost equally to the HIV epidemic and are the two major HIV-1 strains circulating in this country. Other countries such as Japan have shown the predominance of subtype B in 81% of the total local HIV-1 infection, followed by subtypes A, C, and CRF01_AE[5]. Closer regions in terms of geographic location, such as central Asia and eastern Europe, would generally have a greater tendency to share a similar HIV-1 dispersal trend, with subtype A being the main circulating HIV-1 strain in both regions.

Inter-CRF recombination: evidence for second and third generation recombinants and possible epidemiologic implications

Among all the HIV-1 subtypes distributed in Asia, CRF01_AE is reported to play a considerably important role in its epidemic[5]. Besides, it is also apparent that Asia has the highest proportions of recombinants worldwide, with the top in South and Southeast Asia amounting to 89% of its total HIV-1 infection, followed by 61% in East Asia. This epidemiology is bound to become more complex as other recombinant forms are introduced from neighboring geographic regions, for which the previous epidemiologic consequences of these recombinants are known, along with the continuing emergence of novel second and third generation recombinant forms of CRF01_AE in this region. Varied and complex forms of HIV-1 recombinants appear to be emerging continually in geographic areas known as recombination hotspots in Asia, including Myanmar and Yunnan province of China. One study has

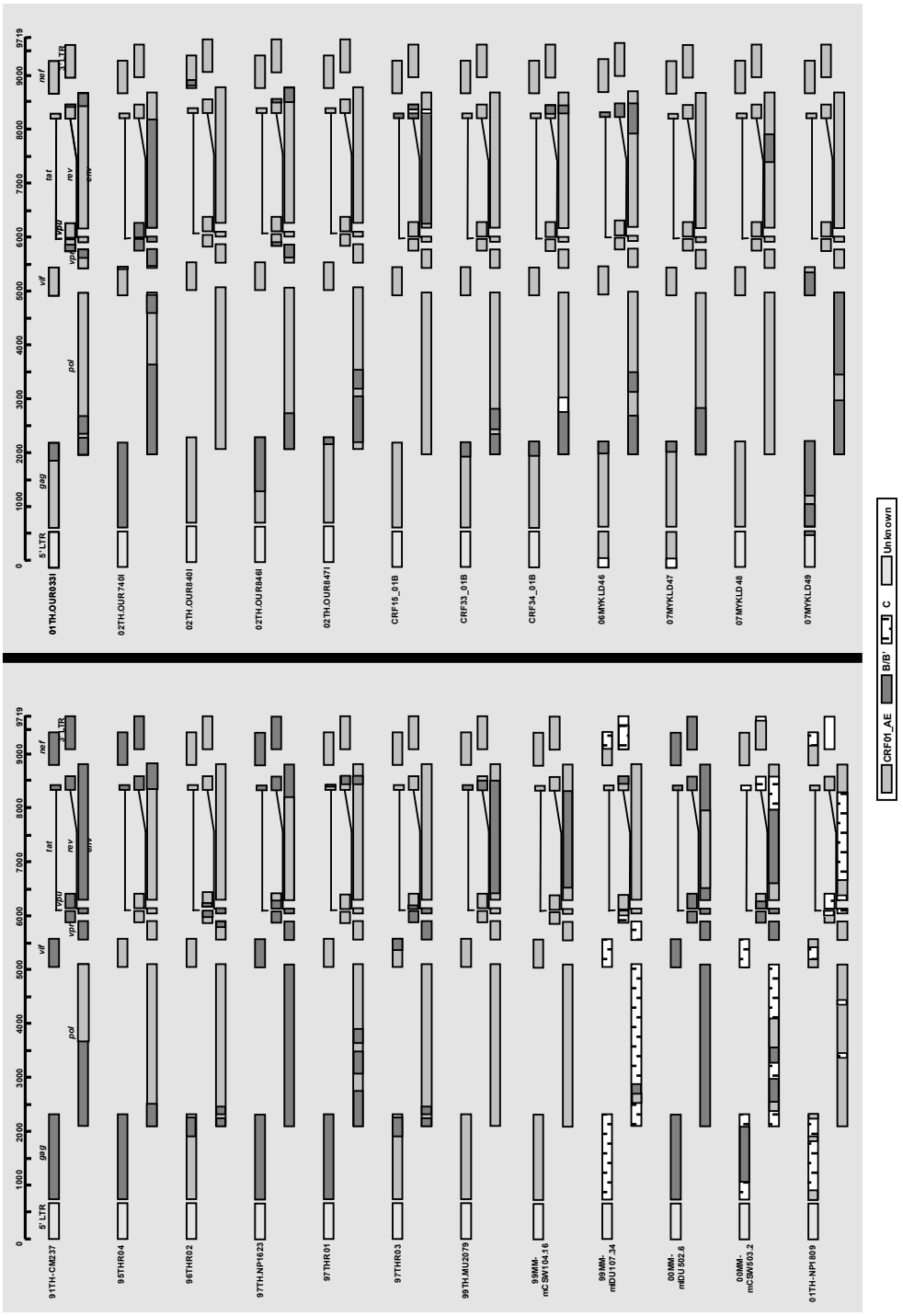

Figure 2. *Schematic representation of subtype structure of recombinant form of HIV-1 CRF01_AE from Asia. The first full-length genome of CRF01_AE recombinant (91TH-CM237) was reported derived from an HIV-1-infected individual from Thailand in 1991*[60]*. Each CRF01_AE recombinants are displayed in ascending order based on the year of the sample; the year is enclosed ahead of the name of the country of origin, followed by the name of the isolate. Country of origin: TH: Thailand; MM: Myanmar; MY: Malaysia. Isolate 91TH-CM237 was the first CRF01_AE recombinant form from Thailand, while 07MYKLD49 (unpublished data) was the latest recombinant of CRF01_AE from Malaysia. CRF15_01B and CRF34_01B are derived from Thailand and CRF33_01B are reported from Malaysia. All are CRF01_AE/B intersubtype recombinant except for isolates 99MM-mIDU107.34 and 00MM-mCSW503.2, which are both CRF01_AE/B/C recombinant from Myanmar and 01TH-NP1809, which is a CRF01_AE/C from Thailand*[11,13,34,57,59,60,61,104,105]*.*

identified a novel inter-CRF recombinant, which is also the second class of HIV-1 inter-CRF recombinants comprised of CRF01_AE and CRF07_BC, in Yangon, Myanmar between 2002 and 2004[70]. Another recombinant (CRF12_BF), which is prevalent in Brazil, was first reported to be circulating among the IDU in Macao where CRF01_AE is the major HIV strain[71]. This suggests the epidemiologically associated transmission of the current HIV infection in the region, and gives clues to the possible initiation of the emergence of novel inter-CRF recombinants between CRF01_AE and CRF12_BF. Also from the same area, a diverse form of HIV recombinant, never previously reported, was recently full-length characterized and comprised of CRF12_BF, CRF14_BG and subtype G[72]. In Yunnan province of China, two CRF (CRF07_BC and CRF08_BC) emerged as a result of the co-circulation of subtypes B and C[8]. As these two CRF continue to co-circulate with the pure subtype B' and C, along with the other URF in Yunnan[38], an ongoing evolution and emergence of novel recombinant forms are anticipated in this region. More new recombinant strains between these two CRF will continue to emerge[73]. In China, a possible emergence of second and third generation recombinant CRF gives rise to more HIV-1 variants with extensive variability in breakpoints and crossover points. A recent study has identified approximately 12% of HIV-1 strains found among the IDU in southeast Yunnan to be the diverse forms of inter-CRF recombinants between CRF07_BC and CRF08_BC[70]. This further provides a good insight into inter-CRF recombinants, and only time will tell regarding the epidemiologic consequences of inter-CRF recombination *in natura*. Overall, there is an emergence of inter-CRF recombinants, together with HIV-1 subtypes, CRF and URF in certain Asian countries. We therefore expect to see a huge impact on the vaccine design, and definitely enormous biological implications at the level of virulence, efficient transmission, and the viral-acquiring fitness of HIV-1 in this region.

The impact of HIV-1 recombination

HIV pathogenesis and viral fitness

One of the major characteristics possessed by HIV is its particularly high genetic variability. The HIV-1 tends to diversify in an infected individual where approximately 10^9 new HIV virions are produced each day, with each carrying on average of one mutation, although not all are viable[74,75]. Characterization of the first HIV-1 isolates revealed a variation at the nucleotide sequence level, ranging between 1-8%, with comparable variation at the amino acid level[76,77]. The HIV-1 exhibits an extremely high genetic variability within individual hosts, particularly in the *env* gene hypervariable regions[78], leading to a complex mixture of heterogeneous strains termed "quasispecies" within an infected person[79,80]. These diverse viruses generally have variable antigenic and phenotypic properties and tend to compete among themselves for survival and propagation[81]. Such diversity enhances the inherent capacity of the virus to constantly evade the human immune system and antiretroviral therapy, and poses a challenge in effective drug/vaccine development.

Viral recombination requires the simultaneous infection of a cell with two different proviruses. Upon infection, one RNA transcript from each provirus will encapsidate into a heterozygous virion. In individuals infected by genetically diverse viruses, the low fidelity of highly recombinogenic enzyme reverse transcriptase[82] can facilitate different genomic recombinations. After the subsequent infection of a new cell, a new recombinant that possesses the two parental genomes will be synthesized as a result of reverse transcriptase back and forth jumps between the two RNA templates[82-84]. As a result, the mosaic viruses with discrete breakpoints between the genomic regions of different phylogenetic associations will be produced. Recombination is now recognized as the fundamental component in the generation of HIV diversity and likely a strategy for viral rejuvenation. It is further enhanced by the occurrence of dual or superinfection[85] that is a relatively common event among different strains of HIV-1 (intersubtype recombination)[86]. Initially, intersubtype recombinants were thought to originate from individuals with multiple infections, i.e. simultaneous viral infection of two or more subtypes. This was then made clear through multiple genome region analyses and particularly full-length genome sequencing in order to further identify these recombinant viruses. The same approach was also performed to discover the CRF through full-length genome characterization of the virus isolated from at least three epidemiologically unlinked individuals.

Dual infection (superinfection) and HIV-1 recombination are known to modify viral tropism as well as replication efficiency (fitness), due to the advantages possessed by the recombinant virus over the parental strain. In a separate study, Wang, et al. showed evidence for molecular and biological synergistic and additive interactions *in vitro* between two HIV-1 subtypes in a competition assay[87]. Through molecular interactions and possible recombination, fitter strains can emerge with altered virulence, viral tropism, and entry mechanisms. Previous work has also suggested possible biological differences among the HIV-1 subtypes[88]. It was reported that the long terminal

repeat (LTR) region of CRF01_AE (the predominant HIV-1 strain in Asia) is much more potent *in vitro* than the subtype B LTR. When a recombinant CRF01_AE/B virus was constructed *in vitro,* it exhibited an intense replication advantage compared to the parental subtype B. This indicated that restrained differences in the LTR promoter activity can have a significant impact on viral replication kinetics. A recent profound analysis was done by Kozaczynska, et al.[89] to describe the study over time of HIV-1 isolates in a patient twice superinfected with HIV-1; an initial infection with a subtype B1 strain, followed by first superinfection with a subtype B2 strain and then with CRF01_AE. Again, the LTR of CRF01_AE was found to possess a higher promoter activity, although this was not reflected in the plasma viral load differences. It is remarkable that the later-arriving viruses (strain B2 and CRF01_AE) replicated at much higher levels in blood compared with the first infecting virus B1. Except for the excessive recombination between both subtype B strains, there was only minimal evidence that the different HIV-1 strains found in the patient appeared to influence the evolution of each other.

Fitness is a parameter defining the replicative adaptation of an organism to its environment[90] as a consequence of the interaction of a multitude of viral and host factors[86,91]. Under a certain selective pressure in a defined microenvironment, viral replication will take place to encode virus that replicates at high rates[90]. Thus, one or more strains possessing better viral properties within a given quasispecies will be positively selected, while the unfit variants will be negatively eliminated[90]. The HIV viral factors that affect viral fitness are mainly the biological processes in the virus lifecycle. The survival of the fittest form of HIV recombinant leads to further viral evolution in a complex population, suggesting a continuous evolving of HIV-1 dynamics, mainly attributed to an incessant process of growth, competition, and selection. In an HIV-1 recombinant-related fitness study by Njai, et al.[92], CRF02_AG isolates demonstrated a higher *ex vivo* replicative fitness compared with subtypes A and G from the same geographic region in Cameroon, irrespective of the level of CD4 cell count and coreceptor tropism. A similar study by Konings, et al.[93] showed a 1.4 to 1.9 times higher replication rate increase in the CRF02_AG strains, in contrast to its progenitor subtypes A and G, an adaptation which implies its broader spread and predominance in West Central Africa. In light of these results, it is likely that HIV-1 recombination events in Asia can also contribute to the emergence of viruses with a biological edge in their host, for instance, the widespread CRF01_AE/B intersubtype recombinants.

Challenges to antiretroviral therapy and the future of vaccine development

In Asia, although the number of people receiving treatment has increased more than threefold since 2003, this only represents approximately 16% (one in six people) in need of antiretroviral therapy in this region[10]. Only Thailand is successful in providing the treatment to at least 50% of its people needing it. Effective HIV-1 treatment is always a difficult issue in Asia. With the emergence of new recombinant strains in Asia, this may suggest some advantages over the parental strain and thus may possess important genetic variability for HIV treatment and vaccine development. Studies have been done on HIV-1 strains with different antiretroviral drug sensitivity to determine the potential contribution of recombination to the development of multidrug resistance of HIV-1[94]. A recent mathematical modeling study[95] suggests that treatment targeted at multiple parts of the viral lifecycle may be less prone to drug resistance due to the genetic barrier caused by recombination. However, once selected, mutants resistant to drug regimen(s) may be fitter and able to persist in the population. Independently, coinfected HIV-1 cultures were studied[94], with each harboring resistance to different antiretroviral drugs. Under the drug selective pressure, dually resistant mutants emerged rapidly, suggesting that genetic recombination could contribute to high-level multidrug resistance. Similarly, another separate study[96] was performed on HIV-1 patients who had switched to a new antiretroviral therapy regimen targeting at the reverse transcriptase gene. By week 8, the evolutionary analysis demonstrated a considerably higher viral diversity in the *pol* gene in patients with a decrease in plasma viral load. Also, there was significant evolutionary distance between protease and reverse transcriptase genes in patients who responded well to therapy. This evolutionary distance was unlinked, which may suggest recombination. Hypothetically, it can be explained by the fact that HIV tries to maintain viral fitness in the face of drug pressure by retaining wild-type protease genes, while reverse transcriptase adapts to the new selective pressures. Given the co-circulation of multiple HIV-1 subtypes and CRF in several geographic regions of Asia along with the prevalence of coinfections of HIV-1, it is plausible to believe that the large-scale accessibility of antiretroviral therapy in Asia may lead to multidrug-resistant HIV strains via recombination.

Resistance to antiretroviral drugs is an important issue in the clinical management of HIV-1-related disease, especially when there is an obviously high prevalence of HIV-1 antiretroviral resistance recently in treatment-naive

patients, even higher than those who have been infected longer[97]. Thus, it is becoming more crucial to determine whether HIV-1 subtypes have primary susceptibility to antiretroviral drugs, or if they are in fact capable of developing antiretroviral resistance. An *in vivo* study reported that different group M subtypes were similarly susceptible to the currently used antiretroviral drugs[98]. It has also been reported that patients have shown a difference of drug resistance mutations between subtypes B and non-B viruses after the initiation of therapy[99], while another study reported no effect of viral subtype on the outcomes of antiretroviral therapy[100]. A study done on HIV-1 strains from Asia[101] has shown critical differences at the level of drug resistance mutation patterns between CRF01_AE and subtype B. This study demonstrated that the mutations T69N and V75M in the reverse transcriptase region and L10F, K20I, L33I and N88D in the protease region were more frequently seen in CRF01_AE than in subtype B strains, whereas the mutations D30N, A71V and N88D were exclusive to subtype B. Thus, these analyses clearly show that the genotypic data of drug resistance for one subtype may only be partially applicable or not applicable at all for clinical interpretation of drug resistance. Differential subtype susceptibility to antiretroviral drugs remains an open area for investigation, and little is known about the patterns of drug resistance for each HIV-1 subtype.

The approaches of preventive vaccines are mainly focused on safety, high efficiency, affordability, and simplicity, in order to best control the HIV pandemic. However, the development of globally effective HIV vaccines may be challenged by the HIV-1 global genetic and antigenic variability. Importantly, recombination events between diverse HIV strains will impact on vaccine development, especially those vaccine design strategies which are based on live-attenuated viruses. If live-attenuated viruses are used as vaccine, it could probably combine with infecting strains, even if the two are quite divergent. One of the main biological considerations as part of the HIV vaccine efficacy trials is the importance of HIV genetic subtypes in compromising the extent of vaccine-elicited protective immunity and thus the overall effectiveness of a HIV vaccine[102]. In order to ensure the total efficacy, the candidate vaccine should be tested and employed, possibly at the initial stage, on the immunogens representative of the HIV-1 subtypes prevalent in the potential trial population in the region. The best strategy is to enhance the chances of cross-protection and decrease the possibilities of viral immune escape mutants[103], as the candidate vaccines aim to induce broad immune responses against conserved regions of the virus.

Conclusion

The number of people living with HIV in Asia continues to increase almost in tandem with the fast growing economies in the continent. What is more worrisome is the fact that HIV has a tremendous ability to diversify and recombine due to the prevalence and co-circulation of multiple HIV subtypes and CRF in Asia. Gradually, the pure HIV subtypes are phasing out and being replaced by the recombinant HIV strains. It remains to be determined if these strains have some biological edge over their parental subtypes, or if there are other underlying reasons for their facile spread throughout this region. The newer recombinant strains may have a serious impact on the future outcomes of antiretroviral therapy due to the emergence of multidrug resistance via recombination. Further, the possible emergence of a second or third generation of HIV-1 recombinants will not only make the HIV-1 epidemic more complex, it could also severely affect future HIV-1 vaccine design strategy. Therefore, excellent cooperation between scientific and clinical laboratories as well as public health departments is greatly required in the continuous monitoring of the regional distribution of HIV-1 subtypes and recombinants, to improve regional surveillance and epidemiologic data collection. Now, since the transmission routes of several recombinants have been traced between several Asian countries, an active coordination is needed between these countries in order to identify the risk groups and geographic locales where new recombinants or multidrug-resistant HIV-1 strains may emerge. Apart from the generation of reliable and up-to-date estimates of the HIV-1 diversity in Asia, this also ensures more practical moves to improve the control of the wider spread of the infection and possibly more virulent strains of HIV-1.

References

1. UNAIDS/WHO. AIDS Epidemic Update. http://www.unaids.org/en/KnowledgeCentre/HIVData/EpiUpdate/EpiUpdArchive/2007default.asp. Accessed Jan 5, 2008.
2. UNAIDS. 2006 Report on the global AIDS epidemic.
3. Robertson D, Bradac J, Carr J, et al. HIV-1 Nomenclature Proposal: A Reference Guide to HIV-1 Classification Los Alamos, NM: Theoretical Biology and Biophysics Group, Los Alamos National Laboratory; 1999.
4. Korber B, Gaschen B, Yusim K, Thakallapally R, Kesmir C, Detours V. Evolutionary and immunological implications of contemporary HIV-1 variation. Br Med Bull. 2001;58:19-42.
5. Hemelaar J, Gouws E, Ghys P, Osmanov S. Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. AIDS. 2006;20(16): W13-W23.
6. Essex M. Human immunodeficiency viruses in the developing world. Adv Virus Res. 1999;53:71-88.
7. Luo C, Tian C, Hu D, Kai M, Dondero T, Zheng X. HIV-1 subtype C in China. Lancet. 1995;345:1051-2.
8. Piyasirisilp S, McCutchan F, Carr J, et al. A recent outbreak of human immunodeficiency virus type 1 infection in southern China was initiated by two highly homogeneous, geographically separated strains, circulating recombinant form AE and a novel BC recombinant. J Virol. 2000;74:11286-95.

9. Soares E, Martínez A, Souza T, et al. HIV-1 subtype C dissemination in southern Brazil. AIDS. 2005;19(Suppl 4):S81-6.
10. UNAIDS/WHO. AIDS Epidemic Update. http://www.unaids.org/en/HIV_data/epi2006/. Accessed 20 April, 2007.
11. Tovanabutra S, Beyrer C, Sakkhachornphop S, et al. The changing molecular epidemiology of HIV type 1 among northern Thai drug users, 1999 to 2002. AIDS Res Hum Retroviruses. 2004;20:465-75.
12. Wang B, Lau K, Ong L, et al. Complex patterns of the HIV-1 epidemic in Kuala Lumpur, Malaysia: Evidence for expansion of circulating recombinant form CRF33_01B and detection of multiple other recombinants. Virology. Jun 28 2007.
13. Tee K, Li X, Nohtomi K, Ng K, Kamarulzaman A, Takebe Y. Identification of a novel circulating recombinant form (CRF33_01B) disseminating widely among various risk populations in Kuala Lumpur, Malaysia. J Acquir Immune Defic Syndr. 2006;43:523-9.
14. Detels R. HIV/AIDS in Asia: introduction. AIDS Educ Prev. 2004;16(3 Suppl A):1-6.
15. Saksena N, Wang B, Steain M, Yang R, Zhang L. Snapshot of HIV pathogenesis in China. Cell Res. 2005;15:953-61.
16. Anonymous. HIV/AIDS: no one epidemic but many. Lancet. 2004;364:1-2.
17. Vidal N, Mulanga C, Bazepeo S, et al. Distribution of HIV-1 variants in the Democratic Republic of Congo suggests increase of subtype C in Kinshasa between 1997 and 2002. J Acquir Immune Defic Syndr. 2005;40:456-62.
18. Walker P, Pybus O, Rambaut A, Holmes E. Comparative population dynamics of HIV-1 subtypes B and C: subtype-specific differences in patterns of epidemic growth. Infect Genet Evol. 2005;5:199-208.
19. Ball S, Abraha A, Collins K, et al. Comparing the ex vivo fitness of CCR5-tropic human immunodeficiency virus type 1 isolates of subtypes B and C. J Virol. 2003;77:1021-38.
20. Renjifo B, Fawzi W, Mwakagile D, et al. Differences in perinatal transmission among human immunodeficiency virus type 1 genotypes. J Hum Virol. 2001;4:16-25.
21. Renjifo B, Gilbert P, Chaplin B, et al. Preferential in-utero transmission of HIV-1 subtype C as compared to HIV-1 subtype A or D. AIDS. 2004;18:1629-36.
22. Eshleman S, Guay L, Mwatha A, et al. Comparison of mother-to-child transmission rates in Ugandan women with subtype A versus D HIV-1 who received single-dose nevirapine prophylaxis: HIV Network For Prevention Trials 012. J Acquir Immune Defic Syndr. 2005;39:593-7.
23. Yang C, Li M, Newman R, et al. Genetic diversity of HIV-1 in western Kenya: subtype-specific differences in mother-to-child transmission. AIDS. 2003;17:1667-74.
24. Pope M, Frankel S, Mascola J, et al. Human immunodeficiency virus type 1 strains of subtypes B and E replicate in cutaneous dendritic cell-T-cell mixtures without displaying subtype-specific tropism. J Virol. 1997;71:8001-7.
25. Dittmar M, Simmons G, Hibbitts S, et al. Langerhans cell tropism of human immunodeficiency virus type 1 subtype A through F isolates derived from different transmission groups. J Virol. 1997;71:8008-13.
26. Peeters M. Recombinant HIV sequences: Their role in the global epidemic. In: Kuiken C, Hahn B, Korber B, et al., ed. HIV sequence compendium 2000: theoretical biology and biophysics group, Los Alamos National Laboratory, Los Alamos, NM; 2000:I-39-54.
27. Sanders-Buell E, Saad M, Abed A, et al. A nascent HIV type 1 epidemic among injecting drug users in Kabul, Afghanistan is dominated by complex AD recombinant strain, CRF35_AD. AIDS Res Hum Retroviruses. 2007;23:834-9.
28. Powell R, Zhao J, Konings F, et al. Identification of a Novel Circulating Recombinant Form (CRF) 36_cpx in Cameroon That Combines Two CRFs (01_AE and 02_AG) with Ancestral Lineages of Subtypes A and G. AIDS Res Hum Retroviruses. 2007;23:1008-19.
29. Powell R, Zhao J, Konings F, et al. Circulating recombinant form (CRF) 37_cpx: an old strain in Cameroon composed of diverse, genetically distant lineages of subtypes A and G. AIDS Res Hum Retroviruses. 2007;23:923-33.
30. Carr J, Salminen M, Koch C, et al. Full-length sequence and mosaic structure of a human immunodeficiency virus type 1 isolate from Thailand. J Virol. 1996;70:5935-43.
31. Gao F, Robertson D, Morrison S, et al. The heterosexual human immunodeficiency virus type 1 epidemic in Thailand is caused by an intersubtype (A/E) recombinant of African origin. J Virol. 1996;70:7013-29.
32. Ramos A, Hu D, Nguyen L, et al. Intersubtype human immunodeficiency virus type 1 superinfection following seroconversion to primary infection in two injection drug users. J Virol. 2002;76:7444-52.
33. Rodenburg C, Li Y, Trask S, et al. Near full-length clones and reference sequences for subtype C isolates of HIV type 1 from three different continents. AIDS Res Hum Retroviruses. 2001;17:161-8.
34. Takebe Y, Motomura K, Tatsumi M, Lwin H, Zaw M, Kusagawa S. High prevalence of diverse forms of HIV-1 intersubtype recombinants in Central Myanmar: geographical hot spot of extensive recombination. AIDS. 2003;17:2077-87.
35. Tee K, Pon C, Kamarulzaman A, Ng K. Emergence of HIV-1 CRF01_AE/B unique recombinant forms in Kuala Lumpur, Malaysia. AIDS. 2005;19:119-26.
36. Tovanabutra S, Watanaveeradej V, Viputtikul K, et al. A New Circulating Recombinant Form, CRF15_01B, Reinforces the Linkage between IDU and Heterosexual Epidemics in Thailand. AIDS Research and Human Retroviruses. 2003;19:561-7.
37. Tovanabutra S, Brodine S, Mascola J, et al. Characterization of complete HIV type 1 genomes from non-B subtype infections in U.S. military personnel. AIDS Res Hum Retroviruses. 2005;21:424-9.
38. Yang R, Xia X, Kusagawa S, Zhang C, Ben K, Takebe Y. On-going generation of multiple forms of HIV-1 intersubtype recombinants in the Yunnan Province of China. AIDS. 2002;16:1401-7.
39. Thomson M, Delgado E, Herrero I, et al. Diversity of mosaic structures and common ancestry of human immunodeficiency virus type 1 BF intersubtype recombinant viruses from Argentina revealed by analysis of near full-length genome sequences. J Gen Virol. 2002;83(Pt 1):107-19.
40. Sierra M, Thomson M, Rios M, et al. The analysis of near full-length genome sequences of human immunodeficiency virus type 1 BF intersubtype recombinant viruses from Chile, Venezuela and Spain reveals their relationship to diverse lineages of recombinant viruses related to CRF12_BF. Infect Genet Evol. 2005;5:209-17.
41. Dowling W, Kim B, Mason C, et al. Forty-one near full-length HIV-1 sequences from Kenya reveal an epidemic of subtype A and A-containing recombinants. AIDS. 2002;16:1809-20.
42. Harris M, Serwadda D, Sewankambo N, et al. Among 46 near full length HIV type 1 genome sequences from Rakai District, Uganda, subtype D and AD recombinants predominate. AIDS Res Hum Retroviruses. 2002;18:1281-90.
43. Casado G, Thomson M, Sierra M, Najera R. Identification of a novel HIV-1 circulating ADG intersubtype recombinant form (CRF19_cpx) in Cuba. J Acquir Immune Defic Syndr. 2005;40:532-7.
44. Peeters M, Sharp P. Genetic diversity of HIV-1: the moving target. AIDS. 2000;14(Suppl 3):S129-40.
45. Panda S, Wang G, Sarkar S, et al. Characterization of V3 loop of HIV type 1 spreading rapidly among injection drug users of Manipur, India: a molecular epidemiological perspective. AIDS Res Hum Retroviruses. 1996;12:1571-3.
46. Tripathy S, Kulkarni S, Jadhav S, et al. Subtype B and subtype C HIV type 1 recombinants in the northeastern state of Manipur, India. AIDS Res Hum Retroviruses. 2005;21:152-7.
47. Ljungberg K, Hassan M, Islam M, et al. Subtypes A, C, G, and recombinant HIV type 1 are circulating in Bangladesh. AIDS Res Hum Retroviruses. 2002;18:667-70.
48. Beyrer C, Razak M, Lisam K, Chen J, Lui W, Yu XF. Overland heroin trafficking routes and HIV-1 spread in south and south-east Asia. AIDS. 2000;14:75-83.
49. McCutchan F, Hegerich P, Brennan T, et al. Genetic variants of HIV-1 in Thailand. AIDS Res Hum Retroviruses. 1992;8:1887-95.
50. Ou C, Takebe Y, Weniger B, et al. Independent introduction of two major HIV-1 genotypes into distinct high-risk populations in Thailand. Lancet. 1993;341:1171-4.
51. Weniger B, Limpakarnjanarat K, Ungchusak K, et al. The epidemiology of HIV infection and AIDS in Thailand. AIDS. 1991;5(Suppl 2):S71-85.
52. Nerurkar V, Nguyen H, Woodward C, et al. Sequence and phylogenetic analyses of HIV-1 infection in Vietnam: subtype E in commercial sex workers (CSW) and injection drug users (IDU). Cell Mol Biol (Noisy-le-grand). 1997;43:959-68.
53. Nerurkar V, Nguyen H, Dashwood W, et al. HIV type 1 subtype E in commercial sex workers and injection drug users in southern Vietnam. AIDS Res Hum Retroviruses. 1996;12:841-3.
54. Cock K, Weiss H. The global epidemiology of HIV/AIDS. Tropical Medicine and International Health. 2000;5:A3-A9.
55. Kalish M, Luo C, Weniger B, et al. Early HIV type 1 strains in Thailand were not responsible for the current epidemic. AIDS Res Hum Retroviruses. 1994;10:1573-5.
56. Weniger B, Takebe Y, Ou C, Yamazaki S. The molecular epidemiology of HIV in Asia. AIDS. 1994;8(Suppl 2):S13-28.

57. Ramos A, Nguyen L, Hu D, et al. New HIV type 1 CRF01_AE/B recombinants displaying unique distribution of breakpoints from incident infections among injecting drug users in Thailand. AIDS Res Hum Retroviruses. 2003;19:667-74.
58. Artenstein A, VanCott T, Mascola J, et al. Dual infection with human immunodeficiency virus type 1 of distinct envelope subtypes in humans. J Infect Dis. 1995;171:805-10.
59. Watanaveeradej V, deSouza M, Benenson M, et al. Subtype C/CRF01_AE recombinant HIV-1 found in Thailand. AIDS. 2003;17:2138-40.
60. Swanson P, Devare S, Hackett J, Jr. Full-length sequence analysis of HIV-1 isolate CM237: a CRF01_AE/B intersubtype recombinant from Thailand. AIDS Res Hum Retroviruses. 2003;19:707-12.
61. Tovanabutra S, Kijak G, Beyrer C, et al. Identification of CRF34_01B, a Second Circulating Recombinant Form Unrelated to and More Complex Than CRF15_01B, among Injecting Drug Users in Northern Thailand. AIDS Research and Human Retroviruses. 2007;23:829-33.
62. Lau K, Wang B, Kamarulzaman A, Ng K, Saksena N. Near-full length sequence analysis of a unique CRF01_AE/B recombinant from Kuala Lumpur, Malaysia. AIDS Res Hum Retroviruses. 2007;in press.
63. Graf M, Shao Y, Zhao Q, et al. Cloning and characterization of a virtually full-length HIV type 1 genome from a subtype B'-Thai strain representing the most prevalent B-clade isolate in China. AIDS Res Hum Retroviruses. 1998;14:285-8.
64. Su L, Graf M, Zhang Y, et al. Characterization of a virtually full-length human immunodeficiency virus type 1 genome of a prevalent intersubtype (C/B') recombinant strain in China. J Virol. 2000;74:11367-76.
65. Chen Y, Lan Y, Lai S, Yang J, Tsai S, Kuo S. HIV-1 CRF07_BC infections, injecting drug users, Taiwan. Emerg Infect Dis. 2006;12:703-5.
66. Lin H, Shih Y, Liu Y, et al. An epidemic of HIV type I CRF07_BC infection among injection drug users in Taiwan. J Acquir Immune Defic Syndr. 2006;42:248-55.
67. Chang S, Sheng W, Lee C, et al. Molecular epidemiology of HIV type 1 subtypes in Taiwan: outbreak of HIV type 1 CRF07_BC infection in intravenous drug users. AIDS Res Hum Retroviruses. 2006;22:1055-66.
68. Lin Y, Lan Y, Chen Y, et al. Molecular epidemiology of HIV-1 infection and full-length genomic analysis of circulating recombinant form 07_BC strains from injection drug users in Taiwan. J Infect Dis. 2007;195:1283-93.
69. Meng Z, Xing H, He X, Ma L, Xu W, Shao Y. Genetic Characterization of Three Newly Isolated CRF07_BC Near Full-Length Genomes in China. AIDS Research and Human Retroviruses. 2007;23:1049-54.
70. Takebe Y. Inter-CRF Recombinants: A New Class of HIV-1 Recombinants and Its Epidemiological Implications. 13th Conference on Retroviruses and Opportunistic Infections. Denver, Colorado; 2006.
71. Chan D, Ip P, Lee S, et al. Identification of a cluster of HIV-1 CRF12_BF in a population of injection drug users in Macao. 4th International AIDS Society conference. Sydney, Australia; 2007.
72. Chan D, Ip P, Lee S, et al. Analysis of the full-length genome sequence of HIV-1 BF recombinant from an injection drug user in Macao. 4th International AIDS Society conference. Sydney, Australia; 2007.
73. Yang R, Kusagawa S, Zhang C, Xia X, Ben K, Takebe Y. Identification and characterization of a new class of human immunodeficiency virus type 1 recombinants comprised of two circulating recombinant forms, CRF07_BC and CRF08_BC, in China. J Virol. 2003;77:685-95.
74. Malim M, Emerman M. HIV-1 sequence variation: drift, shift, and attenuation. Cell. 2001;104:469-72.
75. Burke D. Recombination in HIV: an important viral evolutionary strategy. Emerg Infect Dis. 1997;3:253-9.
76. Haseltine W, Patarca R. AIDS virus and scrapie agent share protein. Nature. 1986;323:115-6.
77. Patarca R, Haseltine W, Webster T, Smith T. Of how great significance? Nature. 1987;326:749.
78. Holmes E, Zhang L, Simmonds P, Ludlam C, Brown A. Convergent and divergent sequence evolution in the surface envelope glycoprotein of human immunodeficiency virus type 1 within a single infected patient. Proc Natl Acad Sci USA. 1992;89:4835-9.
79. Meyerhans A, Cheynier R, Albert J, et al. Temporal fluctuations in HIV quasispecies in vivo are not reflected by sequential HIV isolations. Cell. 1989;58:901-10.
80. Goodenow M, Huet T, Saurin W, Kwok S, Sninsky J, Wain-Hobson S. HIV-1 isolates are rapidly evolving quasispecies: evidence for viral mixtures and preferred nucleotide substitutions. J Acquir Immune Defic Syndr. 1989;2:344-52.
81. Fisher A, Ensoli B, Looney D, et al. Biologically diverse molecular variants within a single HIV-1 isolate. Nature. 1988;334:444-7.
82. Hu W, Temin H. Retroviral recombination and reverse transcription. Science. 1990;250:1227-33.
83. Goodrich D, Duesberg P. Retroviral recombination during reverse transcription. Proc Natl Acad Sci USA. 1990;87:2052-6.
84. Stuhlmann H, Berg P. Homologous recombination of copackaged retrovirus RNAs during reverse transcription. J Virol. 1992;66:2378-88.
85. Jost S, Bernard M, Kaiser L, et al. A patient with HIV-1 superinfection. N Engl J Med. 2002;347:731-6.
86. Quinones-Mateu M, Arts E. Virus fitness: concept, quantification, and application to HIV population dynamics. Curr Top Microbiol Immunol. 2006;299:83-140.
87. Wang B, Lal R, Dwyer D, et al. Molecular and biological interactions between two HIV-1 strains from a coinfected patient reveal the first evidence in favor of viral synergism. Virology. 2000;274:105-19.
88. Jeeninga R, Hoogenkamp M, Armand-Ugon M, de Baar M, Verhoef K, Berkhout B. Functional differences between the long terminal repeat transcriptional promoters of human immunodeficiency virus type 1 subtypes A through G. J Virol. 2000;74:3740-51.
89. Kozaczynska K, Cornelissen M, Reiss P, Zorgdrager F, van der Kuyl A. HIV-1 sequence evolution in vivo after superinfection with three viral strains. Retrovirology. 2007;4:59.
90. Domingo E, Holland J. RNA virus mutations and fitness for survival. Annu Rev Microbiol. 1997;51:151-78.
91. Nijhuis M, Schuurman R, de Jong D, et al. Increased fitness of drug resistant HIV-1 protease as a result of acquisition of compensatory mutations during suboptimal therapy. AIDS. 1999;13:2349-59.
92. Njai H, Gali Y, Vanham G, et al. The predominance of Human Immunodeficiency Virus type 1 (HIV-1) circulating recombinant form 02 (CRF02_AG) in West Central Africa may be related to its replicative fitness. Retrovirology. 2006;3:40.
93. Konings F, Burda S, Urbanski M, Zhong P, Nadas A, Nyambi P. Human immunodeficiency virus type 1 (HIV-1) circulating recombinant form 02_AG (CRF02_AG) has a higher *in vitro* replicative capacity than its parental subtypes A and G. J Med Virol. 2006;78:523-34.
94. Moutouh L, Corbeil J, Richman D. Recombination leads to the rapid emergence of HIV-1 dually resistant mutants under selective drug pressure. Proc Natl Acad Sci USA. 1996;93:6106-11.
95. Fraser C. HIV recombination: what is the impact on antiretroviral therapy? J R Soc Interface. 2005;2:489-503.
96. Gunthard H, Leigh-Brown A, D'Aquila R, et al. Higher selection pressure from antiretroviral drugs in vivo results in increased evolutionary distance in HIV-1 pol. Virology. Jun 20 1999;259(1):154-65.
97. Hanna G, Balaguera H, Freedberg K, et al. Drug-selected resistance mutations and non-B subtypes in antiretroviral-naive adults with established human immunodeficiency virus infection. J Infect Dis. 2003;188:986-91.
98. Palmer S, Alaeus A, Albert J, Cox S. Drug susceptibility of subtypes A,B,C,D, and E human immunodeficiency virus type 1 primary isolates. AIDS Res Hum Retroviruses. 1998;14:157-62.
99. Pieniazek D, Rayfield M, Hu D, et al. Protease sequences from HIV-1 group M subtypes A-H reveal distinct amino acid mutation patterns associated with protease resistance in protease inhibitor-naive individuals worldwide. HIV Variant Working Group. AIDS. 2000;14:1489-95.
100. Frater A, Dunn D, Beardall A, et al. Comparative response of African HIV-1-infected individuals to highly active antiretroviral therapy. AIDS. 2002;16:1139-46.
101. Ariyoshi K, Matsuda M, Miura H, Tateishi S, Yamada K, Sugiura W. Patterns of point mutations associated with antiretroviral drug treatment failure in CRF01_AE (subtype E) infection differ from subtype B infection. J Acquir Immune Defic Syndr. 2003;33:336-42.
102. Garber D, Silvestri G, Feinberg M. Prospects for an AIDS vaccine: three big questions, no easy answers. Lancet Infect Dis. 2004;4:397-413.
103. McMichael A, Hanke T. HIV vaccines 1983-2003. Nat Med. 2003;9:874-80.
104. Viputtijul K, de Souza M, Trichavaroj R, et al. Heterosexually acquired CRF01_AE/B recombinant HIV type 1 found in Thailand. AIDS Res Hum Retroviruses. 2002;18:1235-7.
105. Tovanabutra S, Polonis V, De Souza M, et al. First CRF01_AE/B recombinant of HIV-1 is found in Thailand. AIDS. 2001;15:1063-5.