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Recombination in HIV-1: Update and Implications

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Abstract

Recombination is a common feature of retroviruses first described in the early 1970s. Although recognized as mutagenic strategy for rapid evolution and adaptation for avian and murine retroviruses, the implications or even possibility of recombination between heterogeneous HIV isolates was unclear until a few years ago. It is obvious that recombination can occur between HIV-1 quasispecies in a host, initially infected with single HIV-1 strain. However, the principal of retroviral interference and HIV-specific host immune response was thought to block any superinfection of a human host by a second HIV-1 isolate. Recent identification of individuals infected with HIV-1 isolates from two subtypes and intersubtype HIV-1 recombinants suggests that superinfections do occur at some low frequency in the population. It is not surprising that HIV-1 recombinants are detected with the greatest frequency in Africa, specifically in regions where many subtypes (or clades) co-circulate. However, a continual introduction of new subtypes (e.g. clade A, C, D, and F) worldwide could increase the occurrence of HIV-1 recombination outside of Africa. For example, intersubtype recombinants have now been identified in Brazil, Argentina, Russia, and India. In contrast to the A/E recombined HIV-1 in Thailand, these chimeric viruses are not related to recombined HIV-1 strains in Africa but are the result of recent recombinations between clades co-circulating in that country. Analysis of a limited set of HIV-1 chimeric genomes reveals no selection for specific recombination sites in the HIV-1 genome. Even though "hot spots" for recombination may occur *in vitro*, it is apparent that viral fitness may be a deciding factor in the selection and transmission of specific recombined viruses in the population. Increases in intersubtype recombination and transmission of recombined isolates can lead to major antigenic shifts and will undoubtedly effect the development of new vaccine and chemotherapeutic strategies.

Key words

HIV. Recombination. Diversity. Evolution.

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Recombination as a source of high genetic variability

Genetic variation inherent to RNA viruses has been characterized in great detail with HIV[1,2]. The extensive heterogeneity observed in the worldwide epidemic originates from the rapid viral turnover

(10^{10} viral particles/day) in an HIV-infected individual[3,4], high rate of incorrect nucleotide substitutions during HIV reverse transcription (10^{-4}/nt) in the absence of proof-reading mechanisms[5], and the pliant conformations/functions of many HIV-1 proteins. In addition to this rapid accumulation of minor genotypic changes, different HIV-1 strains can also recombine at a high rate, generating large genetic alterations and possible antigenic shifts[6-8]. Recombination between two genetically distinct HIV genomes is preceded by the production of heterodiploid virus from a cell co-infected with at least two different viruses. Co-infected cells can produce diploid virus carrying a copy of each distinct RNA genome. During the subsequent round of *de novo* infection both RNA strands are potential templates for proviral DNA synthesis by HIV-1 reverse transcriptase[9]. A copy-choice, rather than break-and-union mechanism is believed to be responsible for retrovirus recombination[10,11]. In a copy-choice model, recombinants result from template-switching by the polymerase during synthesis of a new strand[10,11]. These templates switches are often in addition to the first and second template switch/strand transfer events necessary for completion of minus and plus strand synthesis, respectively[12-14]. Recombination during proviral DNA synthesis generates a chimeric genome containing genetic information from both RNA strands. As the number of crossovers increases, so does the complexity of the resulting hybrid genome. Recombination can produce heterogeneous viral populations, the survivors of which would represent larger evolutionary jumps than that observed with variants generated by nucleotide substitutions[11,15,16].

HIV diversity and worldwide distribution

Like other retroviruses, the genomic structure of HIV is based in three structural genes: Group-specific antigen *(gag)*, polymerase *(pol)*, and the hypervariable envelope (*env*)[17]. Since 1992, *env* sequences have been used to classify and group prevalent viruses observed in the global epidemic. Two types of HIV resulting from distinct zoonotic introductions are recognized: HIV-1, predominant throughout the world, and HIV-2, found primarily in West Africa[18,19]. HIV-1 can be further subdivided into three highly divergent groups (main [M], outlier [O], and a new [N] highly divergent)[19-21]. Overall, group N appears equidistant to groups M and O {734} whereas a larger genetic distance separates the latter (35 to 49%)[22,23]. HIV-1 group M strains responsible for the worldwide epidemic and over 90% of current/new infections can be separated into 10 different subtypes or clades (A-J)[19], whereas as the rare group O now consists of at least two distinct clades[23,24] (Fig. 1A). At the level of nucleotide sequence, HIV-1 intrasubtype divergence can vary from 5% to 15% in *env* nucleotide sequence, whereas viruses in different subtypes (intersubtype) differ by 15 to 30%[19,25].

Heterogeneity of HIV-1 subtypes is reflective of the global diversity observed in the AIDS pandemic. As of December 1998, an estimated 33 million people were infected with HIV, three-fourths of which reside in developing countries (www.unaids.org). A "founder" effect rather than some host restrictive/prevalence factor appears responsible for the predominance of specific subtypes in geographical regions outside of Africa, e.g. subtype B in the Americas and Europe. Although specific subtypes tend to predominate in specific geographic regions, current patterns of transmission and population migration are increasing the rapid spread of pre-existing and new HIV-1 subtypes in most countries and regions[26,27] (Fig. 1B). For example, subtype E and F infections are now common along with subtype B infections in South East Asia and South America, respectively[28-30]. In Europe, a predominance of subtype B infections has now been clouded by numerous reports of established and sporadic introductions of new subtypes in different countries, e.g. subtype F in Romania[31], G in Russia[32], E in the Czech Republic[33], group O in Spain[34]. In many African countries, increased commerce, trade, travel and wars have lead to established introduction of multiple subtypes in most countries[35]. Extensive seroprevalence and molecular epidemiological studies by UNAIDS, WHO, and others have identified subtypes A, B, C, D, and E in Uganda[36]. Similar subtype diversity is now emerging in India, Thailand, the Philippines, and other countries in SE Asia[28,37-40]. Potential for co-infection (see below)[41-46] and genetic recombination in these populations[6,15,19,47] has complicated the classification of new isolates based on current subtype divisions. Finally, more than 10% of HIV-1 strains described in the HIV database[19] might be mosaic or recombinant forms, bearing interspersed segments of genetic information from two or more subtypes[19,47].

HIV-1 dual infections and recombination. The "superinfection paradox"

A central dogma of retroviral co-infections is the inability of a cell infected with one retrovirus to be superinfected by a retrovirus of the same type. Interference by the primary infecting virus usually involves a block or down-regulation of the host cell receptors for the superinfecting strain[48-51]. Although HIV-1/ HIV-2 mixed infections have been described[52-55], and HIV-1 could superinfect HIV-2-infected cells *in vitro*[56,57], previous studies found that most if not all HIV-1 isolates could interfere or block infection of each other[50,58,59] suggesting an inability of HIV-1 infected individuals to be superinfected with another HIV-1 strain. Moreover, it was thought that in rare incidences of an HIV-1 dual infection[51] detection would be difficult since one isolate would likely out-replicate and predominate over the other[60,61]. Independent of retroviral interference, it had been reported that host immune response might interfere with HIV superinfection of humans. HIV-specific CD8+ cytotoxic T cell response, found to be less strain-specific than humoral responses and ac-

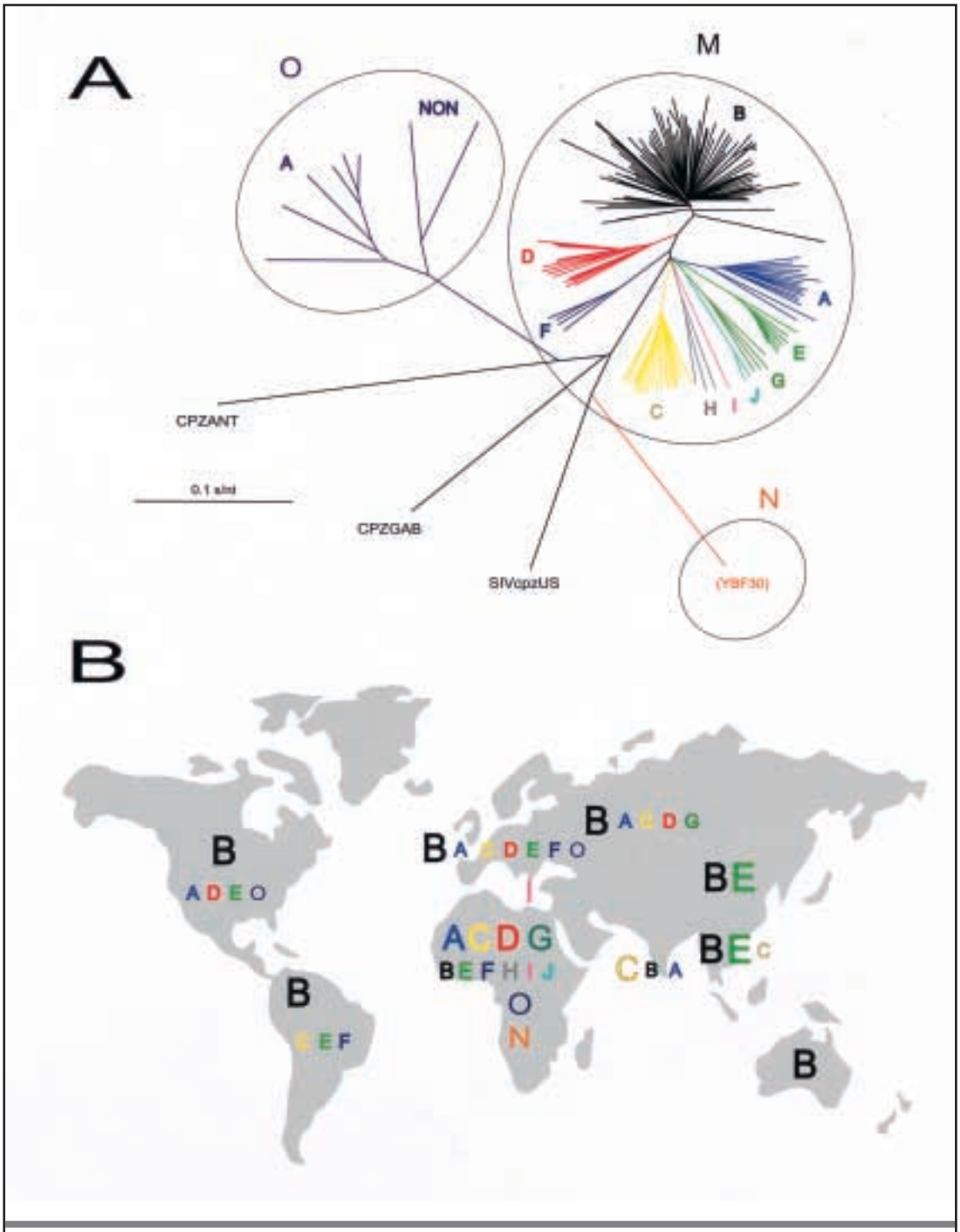

Fig. 1. *(A) Phylogenetic tree analysis of env gene sequences from 187 HIV-1 isolates listed in the HIV Sequence Database (http://hiv-web.lanl.gov)[19]. Full-length envelope sequences were utilized to construct a neighbor-joining tree as previously described[23]. Distance between two sequences could be obtained by summing the length of the connecting branches, using the scale (in nucleotide substitutions per nucleotide [s/nt]). Encircled are the sequences corresponding to different HIV-1 groups: M[19], O[23,24], and N[21] as identified by color. HIV-1 sequences clustering as 'subtype E' and 'subtype I', have been described as HIV-1 A/E[72,73,78,80,81] and AGI[81] intersubtype recombinants, respectively.*
(B) The distribution of HIV-1 isolates throughout the world and in specific geographical regions. Actual distribution of HIV-1 subtypes (A to I in group M) and groups M, N, & O in each continent is indicated; size of the letters defines the prevalence of these subtypes in these continents.

tive in mucosal layers, may be critical in preventing superinfection by other strains of HIV-1[62,63]. Thus, the possibility that recombination between divergent viruses could contribute to the evolution of HIV-1 was not widely considered. However, as the HIV-1 pandemic has continued to spread, simultaneous presence of multiple subtypes in a single geographic region has increased the frequency of mixed infections (Fig. 1B)[42-44,46,64,65]. As discussed below, dual infection of human host with two iso-

lates of different subtypes is the obvious progenitor of intersubtype recombination[66].

Evidence of HIV-1 co-infections were first published in 1995. One report described an acute seroconverter infected with multiple strains of HIV-1 subtype B[43]. Another case in Thailand involved a dual infection with a subtype B and E isolate[44]. Many combinations of dual infections with distinct HIV-1 strains have now been reported in countries where these subtypes co-circulate, e.g. subtype B & E isolate co-infections in Thailand[44,67], B & C, B & D, B & F, and D & F in Brazil[68,69], A & D in Uganda {737}, A & C, C & F, and a triple A, D, & group O infection in Cameroon[70].

Contrary to some earlier theories on HIV-1 evolution, evidence now suggests that intersubtype recombination, as a consequence of dual infection does occur, plays a major role in shaping global genetic diversity of HIV-1[30,41,44,47,71]. Since 1994, numerous HIV-1 isolates were shown to have chimeric genomes in which two different genomic regions, usually *gag* and *env*, cluster with different subtypes[30,47,69,70,72-77]. Increased detection of intersubtype recombinants in the HIV-1 population has placed new emphasis on full-length HIV-1 genome sequencing to identify new recombinants and define specific breakpoints. Consistent with the copy-choice model of recombination[11], sequence analysis of the entire genome from eighteen recombined isolates has revealed complex genome structures with multiple crossovers[19,39,78-86] (Fig. 2A). Surprisingly, 4 of the 18 isolates were intersubtype recombinants of at least 3 different clades. In regions where several subtypes co-circulate, a significant proportion of HIV-1 isolates contain short sub-genomic segments that cluster with subtypes other than that observed throughout the genome. Recombination in many of these viruses may have predated extended evolution due to single substitutions considering many of recombined regions may not be flanked by defined sites of recombination[42,43,47,69-77,85,87-93] (Fig. 2B).

With few exceptions (e.g. B/F and C/B recombinants found in Brazil[30,72,73] and A/B chimera in Russia[74]), more than 80% of HIV-1 intersubtype recombinants have been identified in the 14 African countries (Fig. 3), where multiples HIV-1 subtypes, as well as group O isolates, are known to co-circulate[47,70,72,73,76,77,79,81-83,85-87,90,91,94] (Fig. 1B). The likely epicenter of this disease, e.g. Cameroon, Zaire, Kenya, and Tanzania, has the highest number of reported HIV-1 recombinants and of co-circulating subtypes (7, 5, 3, and 3 different subtypes, respectively). Predominance of subtype A on the continent is reflected in the favored recombination between subtype A and another clade, e.g. A/C, A/D, and A/G (Figs. 2 and 3). By subdividing Africa, it is evident that HIV-1 subtypes A and D have dominated the epidemic in East and Central African countries, whereas the incidence of subtype C in Southern Africa is increasing with the rapid progression of this regional pandemic[35]. Rapid spread of subtype C strains has lead to increased detection of A/C recombinants[47,70,72,73,76,81-83,91]. Interestingly, prevalence of subtype G in Africa is low and yet, A/G recombinants make up the majority (60%) of the reported recombined viruses[95]. A/G recombinants may have achieved a greater fitness in the viral population than the parental G subtype. As described below, inability to isolate a complete subtype E isolate (e.g. not recombined with subtype A) in Africa or Thailand may suggest the "death" of subtype E.

A

LTR	*gag*	*pol*	*Acc*	*env*	*nef*	FRAGMENT	RECOMBINANT	COUNTRY	REF
	A/C	A/C	A/C	C/A	A	Full genome	A / C	India	39
	C/A	A/C	C/A	A/C	C	Full genome	AC	India	39
	C	C	C	C/A	A/C	Full genome	AC	India	39
	A	A	A	A/C		Full genome	A / C	Ethiopia	82
	A/C	A/C	A	C/A	A	Full genome	A / C	Zambia	19,83
	C/A	A/C	C/A	A/C	C	Full genome	A / C	Rwanda	19,81
E	A	A	A/E	E/A	A	Full genome	A / E	Thailand	78
E	A	A	A/E	E/A	A/E	Full genome	A / E	Thailand, C.A.R.	19,80
	G	G	G/A	G	G	Full genome	A / G	Nigeria	19,81
	G	G/A	G/A	G	G	Full genome	A / G	Nigeria	19,81
	A	A/G	G/A	G/A	A/G	Full genome	A / G	Nigeria	19,84
	B/F	B	F/B	F	F/B	Full genome	B / F	Brazil	19,81
G	A	G/A		A	G	Full genome	A / G	Djibouti	79
	A	A	A	H	H	Full genome	A / H	Norway	96
	A/D	A/I	A/D	D/A	A	Full genome	A / D / Y	Zaire	19
	G	G	G/I	G/A		Full genome	A / G / Y	Zaire	19,85
	A/G/I	G/I/A	A/I	A/G/I	Y	Full genome	A / G / Y	Cyprus	19,81
J	A/G	G	J	G	J	Full genome	A / G / J	Burkina Faso	86

Fig. 2. *A summary of all reported HIV-1 recombinant sequences are indicated. Genomic fragments employed for sequencing and recombination analyses for each isolate is listed along with clade classification in various HIV-1 segments/genes, e.g. long terminal repeat (LTR), group-specific antigen (gag), polymerase (pol), rev, tat, vpr, vpu, known as accessory genes (Acc), envelope (env), and nef (nef). Evidence of recombination in a specific gene/fragment is represented by two or more HIV-1 subtypes (e.g. A/C).*
(A) Description of recombination in eighteen full-genome sequences from ten subtypes of group M.

B

LTR	*gag*	*pol*	*Acc*	*env*	*nef*	FRAGMENT	RECOMBINANT	COUNTRY	REF
	A	D		A		gag + pol + env	A / D	Lebanon	75
		A		G		gag + pol + env	A / G	Lebanon	75
	C	C		B		gag + pol + env	C / B	Brazil	73
	B/F	B		[B		gag + pol + env	B / F	Brazil	73
	A	A		E		gag + pol + env	A / E	Thailand	73
	C	C/A		A		gag + pol + env	C / A	Rwanda	73
	A/C	C		A		gag + pol + env	A / C	Rwanda	73
	D/A	D		D		gag + pol + env	D / A	Uganda	73
	A	A		D		gag + pol + env	A / D	Uganda	73
	B	F		B		gag + pol + env	B/F	Brazil	69
	F/B	F		F		gag + pol + env	F / B	Brazil	69
	B/F	F		F		gag + pol + env	B / F	Brazil	69
	B/F	F		B		gag + pol + env	B / F	Brazil	69
	C			B		gag + env	C / B	Brazil	72
	B/F			B		gag + env	F / B	Brazil	72
	C			A		gag + env	C / A	Rwanda	72
	A/D			A		gag + env	D / A	Rwanda	72
	A/C			A		gag + env	C / A	Rwanda	72
	A			E		gag + env	A / E	Thailand	72
	D/A			D		gag + env	A / D	Uganda	72
	A			D		gag + env	A / D	Uganda	72
	A/G			H		gag + env	A /G / H	Zaire	87
	D			A		gag + env	D / A	Sweden	71
	A/D			A/D		gag + env	A / D	Kenya	47
	A/D			D/A		gag + env	A / D	Zaire	47
				A/D		gag + env	A / D	Uganda	47
	A/D					gag + env	A / D	Cote d'Ivoire	47
	D/A					gag + env	A / D	Gabon	47
	A/G					gag + env	A / G	Gabon	47
	A/G					gag + env	A / G	Gabon	47
				C/A		gag + env	C / A	Zambia	47
	B/F					gag + env	B / F	Brazil	47
	G			A		gag + env	G / A	Zaire	85
	A			B		gag + env	A / B	Russia	74
	D			G		gag + env	D / G	Russia	88
	A			G/A		gag + env	A / G	Nigeria	77
		A		E		pol + env	A / E	Camerron	70
		A		G		pol + env	A / G	Cameroon	70
		B		A		pol + env	B / A	Cameroon	70
		A/C		A/C		pol + env	A / C	Cameroon	70
		C/F		C/F		pol + env	C / F	Cameroon	70
		A/2		A		pol + env	A / HIV-2	Cameroon	70
A/D				A		LTR + env	A / D	Tanzania	76
C				A		LTR + env	C / A	Tanzania	76
D				A		LTR + env	D / A	Tanzania	76
C/A				C		LTR + env	C / A	Tanzania	76
C/D				D/C		LTR + env	C / D	Tanzania	76
D/A				D/A		LTR + env	D / A	Tanzania	76
C				D/C		LTR + env	C / D	Tanzania	76
A/C				A		LTR + env	A / C	Tanzania	76
D				C		LTR + env	D / C	Tanzania	76
			B	B		tat + env	B (Intrasubtype)	USA	42
				B		env	B (Intrasubtype)	Australia	43
				B		env	B (Intrasubtype)	Australia	89
				B		env	B (Intrasubype)	USA	64
				B/F		env	B / F	Brazil	30
				B/F		env	B / F	Argentina	92
				A/D		env	A / D	Uganda	90
				C/A		env	C / A	Tanzania	91
				A/D		env	A / D	Tanzania	91
				C/D		env	C / D	Tanzania	91
				C/A		env	C / A	Kenya	94
				G/A		env	G / A	Kenya	94
				D/A		env	D / A	Kenya	94

Fig. 2. *(B) List of 64 HIV-1 recombinant sequences identified using one or more genomic fragments.*

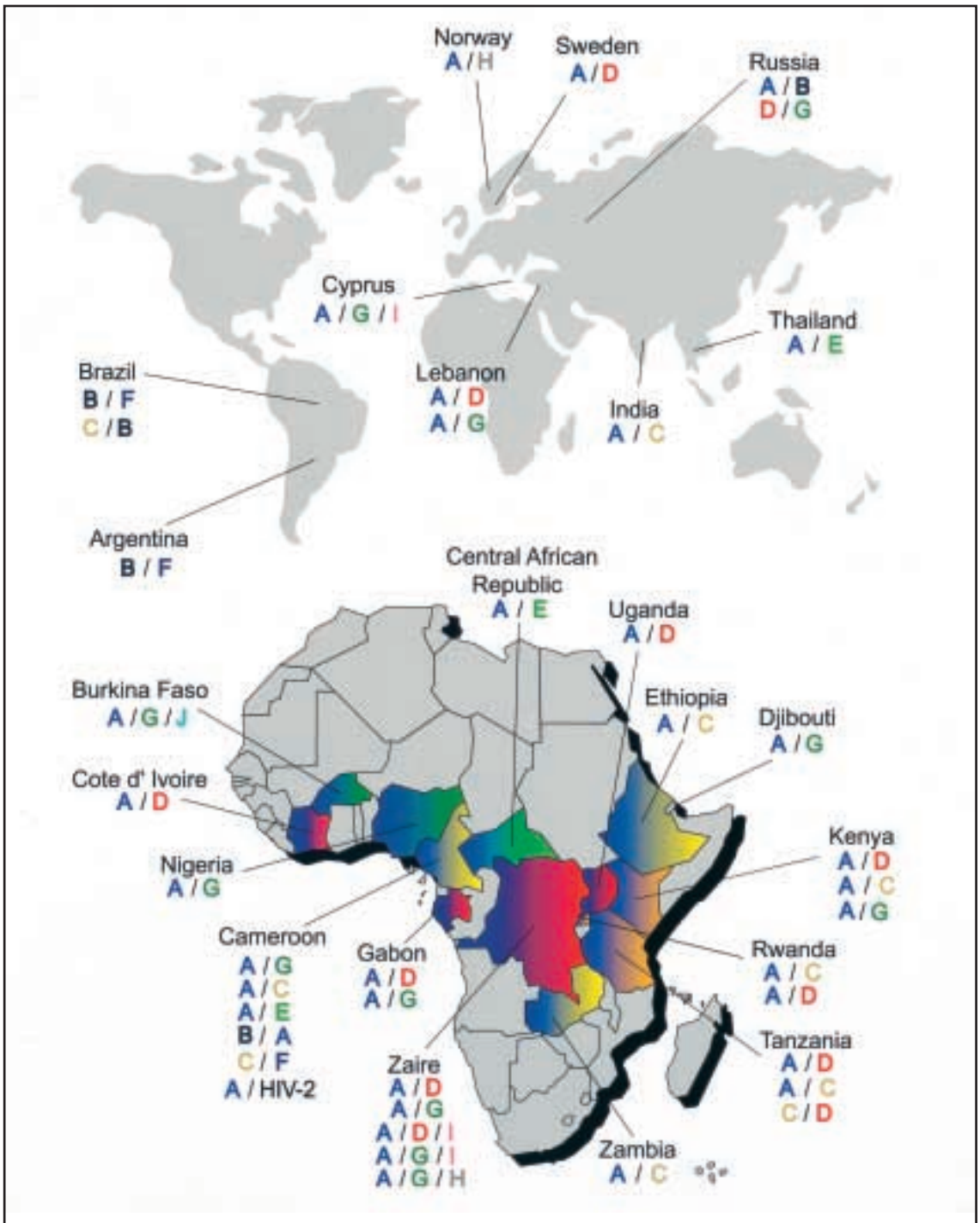

Fig. 3. *Geographic distribution of HIV-1 intersubtype recombinants worldwide. The origin of identification for each hybrid strain from nine countries of America, Europe, and Asia is shown on this map. Numerous HIV-1 mosaic genomes were detected in 14 countries in Africa (see map for origin of identification).*

Rapid spread of HIV-1 through the heterosexual population in Thailand and other countries of Southeast Asia has been caused primarily by HIV-1 strains classified as 'subtype E'[28,29]. However, further phylogenetic analyses based on full-length genomic sequences have identified 'subtype E' viruses as hybrids between clades A and E[72,73,78,80]. In these viruses, *gag* and *pol* genes appear to be of subtype A origin, whereas the accessories and *env* genes are hybrids between subtypes A and E[78,80]. Sequence analysis of A/E recombinant HIV-1 isolates from the Central African Republic revealed an A/E mosaic genome nearly identical to the A/E isolate from Thailand[80]. The true evolutionary origin of "subtype E" is still unknown. However, it is likely that a single recombination between a subtype A and "E" isolate in Africa preceded the introduction of this recombinant virus in Thailand[80].

A/E subtype in Thailand is an example of a recombined 'founder' virus establishing a regional

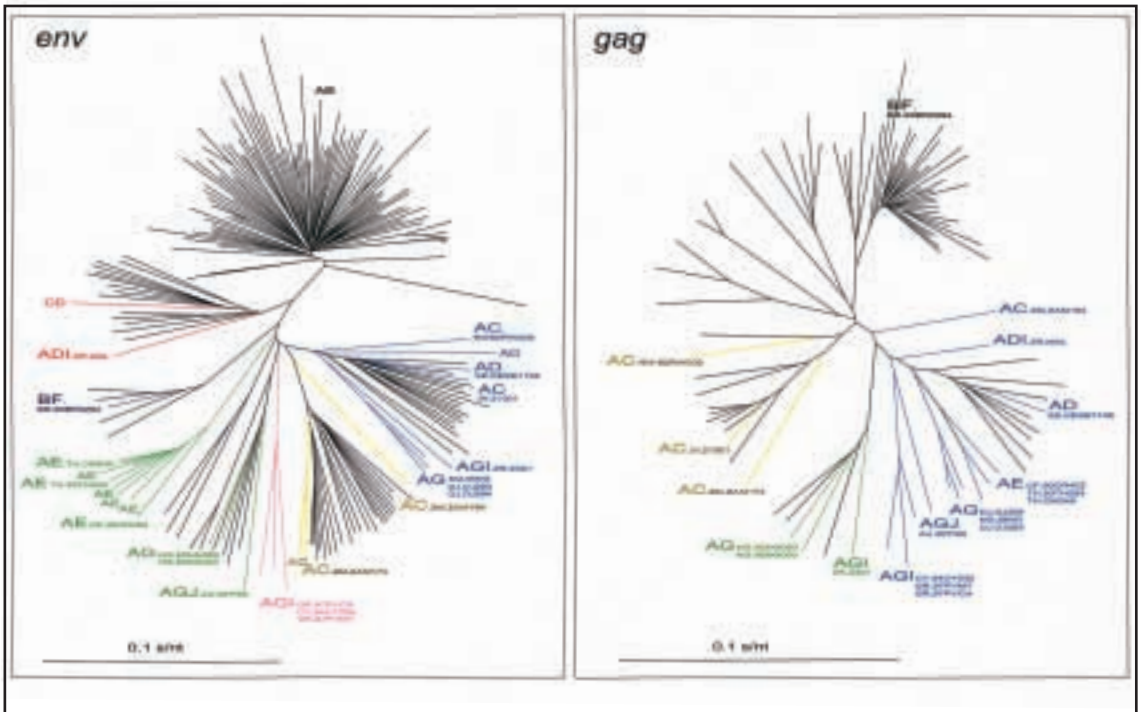

Fig. 4. *Phylogenetic trees showing two segments of several HIV-1 intersubtype recombinant sequences. 195 full-length env and 88 full-length gag sequences were used for this analysis. This tree includes complete names from the Human Retroviruses and AIDS Database[19] for the intersubtype recombinants within or between the env and gag genes. Note that most recombinants have env sequences cluster with one subtype but have gag sequences that cluster with another subtype (e.g., AC.$_{ZM.ZAM184}$ hybrid is subtype C in env, but cluster with subtype A sequences in the gag region).*

pandemic. However, there is evidence of recombination in regions (e.g. the Americas and Europe) where the seroprevalence of HIV is lower than in Africa and where several HIV-1 subtypes were introduced separately[30,47,69,72,96]. In fact, B/F isolates in Brazil were of the first HIV-1 intersubtype recombinants reported[30]. An increasing number of B/F and C/B recombinants, with different genomic breakpoints, have now been described in Brazil and Argentina[69,72,73,81,92]. Only a few cases of HIV-1 recombinant have been identified in Europe, e.g. HIV-1 D/G and A/E in Russia[74,88], HIV-1 A/D recombinant from an African immigrant in Sweden[71], and an A/H/Mal-like hybrid genome in Norway[96]. However, HIV-1 A/B recombinants appear to be circulating among intravenous drug users in Russia[74]. Finally, increased prevalence of subtype C strains in the escalating epidemic in India[37-39] has resulted in detection of A/C recombinant genomes[39]. Thus, appearance of intersubtype recombinants in countries such as Brazil, India, and Russia is likely due to co-infection by co-circulating clades in these regions and not due to the 'founder' effect as with HIV-1 A/E in Thailand.

Several studies have now described infections by three HIV-1 isolates of different clades (e.g. A/D/O[70]). However, such a 'triple' infection is likely rare and probably not responsible for the relatively high proportion of intersubtype recombinations between three clades[19,85-87] (Fig. 2A and 3). Rather, it is conceivable that two co-infections, the first with two isolates of different subtypes and a second with the recombined isolate and an isolate of a third subtype, were likely responsible for these triple recombinations. Full-length genome sequencing may be necessary to identify triple recombinants. For example, an HIV-1 isolate from Cyprus (94CY032.3) originally identified as subtype I[97] has now been fully sequenced and re-designated as an A/G/I triple recombinant[81] (Figs. 2A and 3). Finally, no group M/group O recombinant strains have been reported in the literature[19] may be attributable to low prevalence of HIV-1 group 0 strains. However, evidence of an intergroup M/O recombinant HIV-1 was recently presented at the 6th Annual International Discussion Meeting on HIV Dynamics and Evolution[98]. Recombination between HIV-1 group O and M strains would represent the largest evolutionary jump between any two HIV-1 strains due to the high genetic distance (>35%) between these groups.

Methods to detect recombined HIV-1 isolates. Are there 'hot spots' for HIV-1 recombination?

Despite detection of a significant number of intersubtype recombinants, chimeric genomes containing segments of two or more isolates are often difficult to isolate and characterize. No distinguishing clinical feature is attributable to infection by a recombined HIV-1 isolate. Homoplasy or 'undirected convergence' (the chance occurrence of identical nucleotides in sequences of different lin-

eages)[99], creates further problems in analyzing potential recombinants. Figure 4 shows several examples of HIV-1 intersubtype mosaic genomes identified as recombinants based on phylogenetic relationships using *gag* and *env* sequences. However, even traditional methods of sequence and phylogenetic analyses are not always sufficient to distinguish mosaic from non-mosaic genomes. Preferential sequencing of the *env* gene for most phylogenetic studies prevents detection of intergenic recombinations, e.g. crossover points between HIV-1 genes. Even cases of intragenic recombination (e.g. within *env*) are often misinterpreted due to a clustering with a specific subtype. After the first reports of HIV-1 recombination[30,47,100], different procedures were developed and adopted for identification of HIV-1 recombinants and mapping of breakpoints. Viral epidemiology signature pattern analysis, or VESPA, is an algorithm designed to reduce the level of homoplasy in sequence data sets, and thereby increase the signal-to-noise ratio[101]. However, VESPA is often difficult to implement and has been replaced with more user-friendly approaches to detect recombinants. One method involves bootscanning for recognition of subtype-specific segments in the HIV-1 recombinant genomes[102]. A sequence of at least 1500 nucleotides from the sample is aligned with reference sequences of different subtypes. Bootstrapped phylogenetic analyses are then applied to segments 200-500 nucleotides in length and with a 50% overlap. Recombinant genomes will cluster with two parental subtypes on either side of a breakpoint in the genome with bootstrap values greater than 70%[102]. Finally, Recombinant Identification Program (RIP) is a computer program developed to recognize recombinant genomes[103]. As with the bootscanning method, query sequences are aligned with isolates of different subtypes for detection of subtype-specific sequences in two distinct genomic regions. RIP can also provide a detailed output of the intersubtype recombinant and possible breakpoints for recombination[103]. Although other methods for identification of recombinants have been applied to HIV-1 (e.g., Likelihood Method[104]), bootscanning and RIP analyses remain the most reliable and utilized techniques for the detection and characterization of intersubtype chimeric genomes.

Many recombined HIV-1 genomes have a complex mosaic appearance due to multiple crossover events[105]. Potential 'hot spots' of recombination in the HIV-1 genome have not been defined and may or may not exist. 'Hot spots' for recombination may be mapped to regions of RNA secondary structures which retard polymerase movement[6,7,106]. A pause opposite a conserved genomic sequence may promote an RNA template switch and generation of a recombined genome[105]. These pause sites are also preferred sites of amino acid substitutions. However, selective sites of mutations or recombination during reverse transcription may not reflect a possible predominance of specific HIV-1 mutants or recombinants in nature. This disparity is likely due to a strong selection for viral fitness in the population and for quasi-species resistant to antiretroviral drugs or host immune response in an infected individual. The information compiled for this review and presented in figure 5 summarizes percentages of recombination sites in the principal genes of HIV-1. From this data set (limited to the reported recombinants), it appears that sites of recombination are distributed randomly along the HIV-1 genome.

Implications of HIV-1 recombination

a) Diversity and evolution

A high point mutation rate can result in rapid HIV-1 evolution[6,15,16]. However, an efficient mechanism of recombination could generate jumps in HIV-1 evolution and select for viruses with a higher fitness. Recombination could also act as a potential repair/rescue mechanism in instances of deleterious deletions or mutations in one RNA strand of a heterodiploid virus. As described below, recombination of HIV-1 variants with different substitutions has been implicated in emergence of multi-drug resistant viruses. Thus, this mechanism can promote a high rate of sequence exchange among (1) quasi-species during a mono-subtype/strain infection or between (2) different subtypes/strains in a co-infection or superinfection. In addition, the exact site of recombination could generate an amorphous sequence, dissimilar to both subtypes or strains. Selection of these sequences along with flanking regions may alter biological phenotype or promote rapid escape from immune pressure. Regardless of the implications to disease progression or HIV-1 biology, spread of such hybrid viruses poses practical challenges to future diagnostic tests and molecular epidemiological studies. It is quite obvious that full genome sequencing is not feasible as a screen for HIV-1 recombinants. Other techniques such as modified heteroduplex tracking assay[107] or DNA hybridization techniques using overlapping probes from different subtypes allow for rapid detection of recombinants.

b) Drug resistance escape

Several mutations in the HIV-1 pol gene (protease, PR, and reverse transcriptase, RT) confer resistance to antiretroviral drugs[108-110]. Based on a viral turnover of 10^{10} particles per day and a point mutation frequency of 10^{-4}/nt, it is estimated that every single-site and possibly double-site mutant can be generated every day in an HIV-infected individual. However, there is a limited pool of virus containing three mutations, generally required for resistance to three antiretroviral drugs. Several reports have shown that HIV-1 recombination may account for the rapid acquisition of drug resistant substitutions from different quasispecies[111-113]. Multidrug resistant HIV-1 variants have been generated *in vitro* by co-culturing two viruses, resistant to ei-

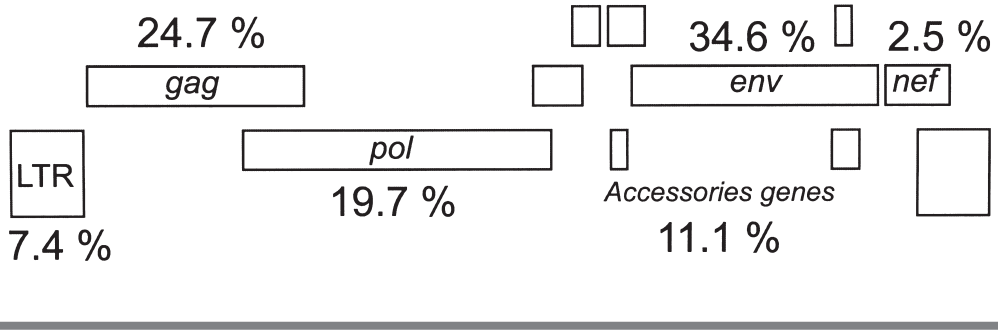

Fig. 5. *A summary of the recombination breakpoints or crossover sites in the HIV-1 genome. This figure was compiled from all the characterized recombinants and is displayed as percentage of breakpoints per genomic region.*

ther a reverse transcriptase or protease inhibitor. Dual resistance of viral progeny is due to linked drug resistant mutations on chimeric genomes with defined breakpoints in the pol gene. Finally, the possibility exists in developing countries where several subtypes co-circulate that treatment of HIV-infected individuals with highly active antiretroviral therapy (HAART) may give rise to both drug resistant and intersubtype recombinants. Considering that many non-subtype B isolates have drug resistant sequences in their wild type genome, intersubtype recombination may pose a significant threat to sustained efficacy of these drugs in these regions.

c) Host immune escape and vaccine development

Evidence of recombination and circulation of these recombined strains suggest that HIV-1-infected individuals can be superinfected with another strain. Interference may be controlled by the divergence of superinfecting HIV-1 strain from established isolate. However, lack of defined clade specificity by humoral or CD8+ T cell-mediated immune response suggests that susceptibility to superinfection by an HIV-1 strain from any subtype may be difficult to predict. Continual spread of HIV-1 recombinant viruses will influence genetic diversity and have a significant impact on vaccine development. Vaccines derived from single isolates or clades may naturally select for recombined HIV-1 strains in the population since these may constitute major antigenic shifts. Although the biological and immunological importance of HIV-1 genetic heterogeneity is not fully understood, it is now clear that any HIV-1 vaccine developed for the global epidemic will have to induce protective immunity against isolates of different monovalent or recombined subtypes.

Conclusions and perspectives

Worldwide spread of different HIV-1 subtypes coupled with intersubtype recombination has serious implications on the efforts to control the AIDS epidemic. Future studies will need to address whether or not recombination influences HIV pathogenesis, e.g. transmission, virulence, and replication. Recombination may be an important mutagenic strategy to increase viral fitness and to evade selective pressure (e.g., antiretroviral therapy, host immune response, and potential vaccines). Finally, it appears that the amount of circulating HIV-1 intersubtype recombinants is underestimated in the population. Improved detection techniques and computer-statistical methods must be developed to identify and characterize both inter- and intra-subtype recombinations. Increased surveillance and molecular epidemiological studies will evaluate the contribution of recombined strains to HIV evolution. Specifically, will new HIV-1 subtypes emerge due to recombination and will HIV-1 subtype classification survive this explosion of genetic diversity? In only a few years, studies on HIV evolution have evolved from tracking a few HIV-1 subtypes in the main HIV-1 group to an inclusion of two new HIV-1 groups (O and N), new subtypes in both groups M and O, and of numerous intersubtype recombinants. The AIDS epidemic is not the result of a single viral isolate but rather a population of HIV-1 strains so diverse that classification as even a single type (e.g. HIV type 1) may be too limiting. Thus, a greater understanding of HIV-1 evolution will definitely impact on the development of any new vaccine or chemotherapeutic strategy.

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