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Evolution and Diversity of HIV-1 in Africa—a Review*

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Abstract. The HIV/AIDS pandemic represents a major development crisis for the African continent, which is the worst affected region in the world. Currently, almost 30 of the 42 million people infected with HIV worldwide live in Africa. AIDS in humans is caused by two lentiviruses, HIV-1 and HIV-2, which entered the human population by zoonotic transmissions from at least two different African primate species. Extensive phylogenetic analyses of partial and full-length genome sequences have helped to gain insights into the evolutionary biology and population dynamics of HIV. One of the major characteristics of HIV is its rapid evolution, which has resulted in substantial genetic diversity amongst different isolates, the majority of which are represented in Africa. Genetic variability of HIV and any consequent phenotypic variation poses a significant challenge to disease control and surveillance in different geographic regions of Africa. This review focuses on the origins and evolution of HIV, current classification and diversity of HIV isolates in Africa and provides an extensive account of the geographic distribution of HIV types, groups, and subtypes in each of the 49 African countries. Numerous epidemiological studies have provided a picture of HIV distribution patterns in most countries in Africa, and these show increasing evidence of the importance of HIV-1 recombinants. In particular, this review highlights that our current understanding of HIV distribution in Africa is incomplete and inadequately represents the diversity of the virus, and underscores the need for ongoing surveillance.

Key words: Africa, evolution, genetic diversity, HIV-1, HIV-2

Introduction

The Joint United Nations Program on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) estimate that since the start of the Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) epidemic over 65 million people worldwide have been infected with HIV (http://www.unaids.org). As of the end of 2002, UNAIDS estimated the number of persons worldwide living with HIV/AIDS as 42 million, and that the AIDS pandemic has claimed the lives of more than 24 million people. The overwhelming majority of these infections (95%) have occurred in developing countries, in particular sub-Saharan Africa, where 29.4 million people are currently living with HIV. The HIV pandemic continues its accelerated spread at a rate of 14,000 new infections each day, generating a total of five million newly infected individuals in 2002 alone. One of the major characteristics of HIV is its rapid evolution that is a consequence of its replication strategy. This permits HIV to escape host immune surveillance, enables the establishment of drug resistant variants, and presents the greatest challenge to the development of an effective HIV vaccine. The ongoing evolution of HIV has resulted in substantial genetic diversity amongst different isolates, the majority of which are represented in Africa. Numerous reviews have looked at the evolution and global diversity of HIV [1–8], however, none have looked at HIV subtype distribution

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throughout Africa in great detail. This review focuses on the diversity of HIV isolates in Africa, in particular it provides an account of the geographic distribution of HIV subtypes in each of the 49 African countries.

Origins and Evolution of HIV

It is generally accepted that AIDS in humans is caused by two lentiviruses of zoonotic origin [9], with SIVsm from the sooty mangabey monkey (*Cercocebus atys*) the most likely source of HIV-2 [10] and SIVcpz from the common chimpanzee (*Pan troglodytes*) the progenitor population for HIV-1 [11]. In the absence of direct epidemiological evidence, molecular evolutionary studies of primate lentiviruses provide the most definitive information about the origins of HIV-1 and HIV-2. Related lentiviruses have been found infecting numerous species of primates in sub-Saharan Africa. The only species naturally infected with viruses closely related to HIV-2 is the sooty mangabey from western Africa, the region where HIV-2 is known to be endemic [12]. Similarly, the only viruses closely related to HIV-1 have been isolated from chimpanzees, and in particular those from western equatorial Africa, again coinciding with the region that appears to be the heart of the HIV-1 pandemic. HIV-1 and HIV-2 have each arisen several times: in the case of HIV-1, the three groups (M, N and O; see classification section below) are the result of at least three independent cross-species transmission events [13], thus placing the origin of the disease to Central Africa (Cameroon, Gabon, Congo, lower Central Africa and Equatorial Guinea). The route of SIV transmission from primates to humans is believed to be blood exposure resulting from bushmeat hunting [14,15]. Evolutionary modelling studies indicate less than a century has passed since the most recent common ancestor of the HIV-1 pandemic strains and, in that time frame, an extraordinarily diverse viral population has developed. The finding of an HIV-1 sequence in an African plasma sample dating from 1959 which clustered near the ancestral node of HIV-1 subtypes B and D (see classification section below) in a phylogenetic tree would certainly imply that the virus was introduced into humans before this time [16]. Attempts to estimate the time of origin of HIV-1 by using phylogenetic analysis are seriously flawed because of the unequal evolutionary rates among different viral lineages, and different selective pressures can lead to dissimilar rates of evolution of the virus in different individuals. However, results from various approaches have concluded that a common ancestor of HIV-1 group M existed in humans around the 1930s [17–19].

Molecular Basis of Variability in HIV

HIV employs a multitude of schemes to generate variants and/or quasispecies. Mutations, including point mutations, deletions and insertions, can be introduced into the genome during viral cDNA synthesis by the viral reverse transcriptase (RT), owing in part to its lack of DNA proofreading activity [20,21]. Contributing to this is the high level of virus production (10^{10} virions per day) and rate of replication [22]. Sequence diversity can be further obtained via recombination [23]. HIV, like all other retroviruses, is diploid and contains two genomic RNA molecules per virion. Therefore, cells infected with two different strains of HIV-1 might produce heterozygous virions, providing an opportunity for recombination to occur during reverse transcription. The occurrence of HIV-1 recombination in nature is borne out by the identification of genomes that are recombinants between different HIV-1 subtypes. Some of these recombinant viruses have become fixed in the human population and are termed circulating recombinant forms (CRFs) [2]. CRFs represent recombinant HIV-1 genomes that have infected three or more people who are not epidemiologically related, so they are assumed to make an epidemiologically relevant contribution to the HIV-1 M group epidemic.

Classification of HIV Isolates

Since the identification of HIV-1 and HIV-2 as the etiologic agents of AIDS in the early 1980s, both viruses have been intensively characterized by full or partial sequencing of their genomes. The initial HIV isolates characterized were from Europe and North America. As more isolates from different regions of the world were sequenced, it became apparent that they exhibited considerable diversity at the sequence

level. This genetic variation is a hallmark of HIV, and can influence the structure, function and immunogenicity of different strains. Even within an individual, variation exists in the form of quasispecies or microvariants of the infecting strain [24].

The extensive HIV database of nucleotide and protein sequences now available permits a classification of both HIV-1 and HIV-2 into distinct genetic subtypes (Los Alamos National Laboratory HIV sequence database, http://hiv-web.lanl.gov). Subtype designations have been powerful molecular epidemiological markers to track the course of the HIV-1 pandemic. Phylogenetic analyses of numerous strains of HIV-1, isolated from diverse geographic regions, have shown that they can be subdivided into groups, subtypes, sub-subtypes and CRFs. The major group, M, which is responsible for the pandemic worldwide, can currently be subdivided into nine distinct subtypes, namely A, B, C, D, F, G, H, J, and K, including two sub-subtypes (A1, A2 and F1, F2), and into 15 CRFs (http://hiv-web.lanl.gov/CRFs/CRFs.html). The two other lineages of HIV-1 (groups O and N) are highly divergent genetically from M and represent a minority of HIV-1 strains that are endemic in Cameroon and neighbouring countries in West Central Africa. Subtypes within the HIV-1 O (outlier) and N (new or non-M, non-O) group are not yet clearly defined. HIV-2 is very distinct from HIV-1. HIV-2 sequences are nearly as distant from one another as are sequences from the HIV-1 M, N, and O groups, and are classified as HIV-2 groups A, B, C, F or G. Since the identification of HIV-1 recombinants in 1995, several researchers showed that a significant fraction of sequences in the databases, perhaps 10% or more, exhibit a shift in subtype when different genome regions are analysed. Thus extrapolation of the subtype classifications of an HIV strain to genome regions other than those that have actually been sequenced is risky in light of intersubtype recombination. Although there are many ambiguities in the subtyping system, some of these may be resolved as new HIV sequences and better tools for analysis become available. Currently, HIV diversity is studied by various methods, such as serology, heteroduplex mobility assays (HMAs), partial or full length genome sequencing (reviewed in [8]) and more recently a multi-region hybridization assay [25]. The HIV database at Los Alamos National Laboratory in New Mexico is constantly evolving and kept up to date.

Diversity of HIV in Africa

In Africa, where the HIV epidemic is believed to be of the longest duration, all the HIV-1 and HIV-2 subtypes have been found. The recognition of distinct types and subtypes of HIV has provided powerful molecular epidemiological markers to follow the course of the HIV global pandemic, and lends definition to strategies for prevention and control. Serologic and genetic studies have combined to provide a striking picture of the areas of highest concentration of the different HIV subtypes on the African continent. Overall, HIV-2 infections are most prevalent in West Africa, whereas HIV-1 group M is broadly distributed throughout Africa. HIV-1 group O infections have been found principally in Cameroon, Equatorial Guinea, and Gabon, but sporadically in other African countries, while HIV-1 group N has only been isolated from Cameroonian patients. There is geographic intermixing of HIV-1 and HIV-2 in West Africa, and of the HIV-1 subtypes in most sub-Saharan regions. Below we document the published data of circulating HIV types and subtypes in Africa. However, it is important to recognize the proportions and distributions of subtypes in many African countries remain unclear as a result of the small numbers of HIV-1 variants that have been subtyped and the potential bias in the way samples were collected. Furthermore, HIV-1 subtyping may have been based on one (*env*) or a few genes, and thus recombinant strains may have been missed. Definitive classification of HIV into groups or subtypes for each country should be based on full length genome sequencing.

Southern Africa

Rampant epidemics are underway in southern Africa and in four countries national adult HIV prevalence has risen higher than 30%. These countries include Botswana (38.8%), Lesotho (31%), Swaziland (33.4%) and Zimbabwe (33.7%) (UNAIDS, 2002). The HIV epidemic in southern Africa is dominated by HIV-1 subtype C, although the presence of other subtypes has been reported. HIV-1 subtype C isolates from Botswana have been described and extensively characterized by several authors [26–28]. A novel recombinant HIV-1 virus has also been isolated in Botswana [29]. The majority of HIV-1 circulating in Zambia is of subtype C origin, although

representatives of subtypes A, D, G and J [30–33], recombinants [34] as well as HIV-1 group O [35] have been identified. Louwagie et al. [36] originally described isolate ZM184 from Zambia, which was later defined as an A2/C recombinant by Gao et al. [37]. Data from three different studies in Harare, Zimbabwe showed the predominant subtype was HIV-1 subtype C [38–40]. McCormack et al. [41] studied the evolution of the HIV-1 epidemic in rural Malawi. In 1982–1984, HIV-1 subtypes A, C and D were all present, though in small numbers. By 1987–1989, 90% of isolates were subtype C and A/C, A/D and D/C recombinants had emerged. Candotti et al. [42] showed a homogeneous circulation of HIV-1 subtype C strains in Ntcheu, Malawi. The predominance of HIV-1 subtype C in South Africa has been well established [26,43–47]. Interestingly, several non-C subtypes and recombinants have been identified [43,48] and a minor HIV-1 subtype B epidemic has been described amongst the homosexual population in South Africa [44,49]. Several groups have sequenced and analysed the full genomes of HIV-1 subtype C circulating in Botswana [27,28], South Africa [43,46,50], and Zambia [50].

There is little published data available on the subtypes circulating in Swaziland and Lesotho, except that reported in a study by Bredell et al. [26] on migrant workers from these two countries working in South African gold mines. Phylogenetic analysis of the gp120 V3–V5 region of three isolates from Swaziland and 13 from Lesotho showed they all clustered with subtype C. Similarly, limited data is available on circulating HIV-1 subtypes in Mozambique. A small number of strains assigned to HIV-1 subtype D [51] and subtype C [26,52,57] on the basis of *env* gene phylogenetic analysis have been reported. No published data of circulating subtypes could be found for Angola and Namibia, although the presence of HIV-1 and HIV-2 in Angola and HIV-1 in Namibia has been established.

East Africa

The most information of circulating HIV subtypes from East Africa comes from Kenya, Uganda, and Tanzania, largely as a result of the US Military HIV Program that has conducted extensive epidemiological surveys of potential HIV vaccine sites. The predominance of subtype A and a high proportion of intersubtype recombinants has been well established in Kenya [30,32,36]. Dowling et al. [53] did a comprehensive study of 41 near full length genomes from HIV-1 isolates from blood banks in six locations across southern Kenya. Twenty five of 41 (61%) were non-recombinant, and 16 (39%) were recombinant viruses. Of the 25 pure subtypes, 23 were subtype A, one was subtype C and one was subtype D. Most recombinants consisted of subtype A and either subtype C or subtype D, and some contained sub-subtype A2. HIV-1 group O has also been identified in Kenya [35]. A full length HIV-1 subtype G genome from Kenya has also been characterized [54]. Characterization of the genetic diversity of HIV-1 in Uganda has relied primarily on partial genome sequences, and has identified subtypes A, B, C, D and G [36,55–58]. Harris et al. [59] described full genome sequencing of HIV-1 isolates from Rakai district, Uganda, and showed that among 46 sequences 54% were subtype D, 15% were subtype A and 30% were individually unique recombinants. HIV-1 subtypes A, C and D have been co-circulating in Tanzania, and a large number of unrelated intersubtype recombinants have been reported from this region [60,61]. Koulinska et al. [62] identified CRF10_CD from perinatally infected infants from Dar es Salaam, Tanzania. Rodenburg et al. [50] have sequenced the full genomes of two subtype C viruses from Dar es Salaam. Analysis of nine full genome HIV-1 sequences from Mwanjelwa, Tanzania identified two subtype A, two subtype C, four unique A/C recombinants (mainly C) and one C/D recombinant (not CRF10_CD) [63]. HIV-1 subtype C has been identified as the major circulating subtype in Ethiopia [64–66].

The presence of HIV-1 and HIV-2 in Djibouti has been established [67,68]. In a study by Louwagie et al. [36] five isolates were identified as HIV-1 subtype C [2] and subtype A [3], two of which were later identified as CRF02_AG by full genome sequencing [54]. In another study of HIV-1 strains from 33 individuals infected after overseas deployment to Djibouti, five were *env* subtype A, 11 subtype B, 16 subtype C and 1 subtype E [69]. Little data exists on circulating HIV types and subtypes in Eritrea and Somalia. Ghebrekidan et al. [70] describe the prevalence of infection with HIV-1 in Eritrea in selected high risk cohorts, and no HIV-2 was detected. Somalia has suffered from a long civil war during the last decade so data on HIV infection and subtypes is limited. Louwagie et al. [36] reported

on 1 *env* HIV-1 subtype C sequence from Somalia. Interestingly, Nur et al. [71] report no detection of HIV-1 and HIV-2 antibodies in 256 serum samples collected in the summer of 1995 from blood donors in Somalia.

West Africa

The presence of HIV-1 group M and O as well as HIV-2 has been well established in many countries of West Africa, and the predominance of HIV-1 subtype A, G and CRF02_AG is well recorded. HIV-1 and HIV-2 cocirculate in Benin [72,73], Burkina Faso [74], Ivory Coast [75], Ghana [76], Gambia [77], Senegal [78], Guinea-Bissau [79], Guinea [80], Mali [81], Mauritania [82] and Sierra Leone [83]. HIV-1 group O infection occurs in Benin [84], Senegal and Togo [35].

Strains assigned to HIV-1 subtype G on the basis of *env* gene phylogenetic analysis were reported from Benin [85]. Heyndrickx et al. [30] described the use of *gag* HMA to identify a variety of intersubtype recombinants in Cotonou, Benin at 41% with A/G at 38.5%. Over 70% of HIV-1 infections in Cotonou were subtype A (by *env*) and around one-half of the subtype A infections were CRF02_AG when *gag* sequences were examined [32]. A complex HIV-1 recombinant from Benin, containing parts of subtype A, G and J as well as CRF06_cpx has also been described [86]. Ongoing studies of HIV-1 genotyping in Burkina Faso show a predominance of A and G subtypes [87]. An A/G/J recombinant originally identified by Oelrichs et al. [88], described as CRF06_cpx [89] circulates in Burkina Faso, although the exact prevalence remains to be determined. Evans et al. [75] described the simultaneous isolation of HIV-1 and HIV-2 from an AIDS patient in Ivory Coast, indicating the potential for dual infection. Pieniazek et al. [90] describe the predominance of HIV-2 subtype B in Abidjan, although subtype A is cocirculating. *Env* HIV-1 subtype B [69] and subtype G [91] have been reported from Cote d'Ivoire. Nkengasong et al. [92] described the distribution of genetic subtypes of HIV-1 strains in six regions of Cote d'Ivoire and found that 163 out of 172 samples were subtype A (95%), which are very likely CRF02_AG, three subtype D, four subtype G, one A/D and one A/G recombinant. CRF06_cpx also circulates in Cote d'Ivoire [89]. HIV-1 infection is now dominant in Ghana in contrast to previous surveys during 1986 which showed the dominance of HIV-2 [93]. Circulating HIV-1 subtypes include A, D, G and CRF02_AG [94–96] and HIV-2 subtypes A and B [97].

HIV-1 *gag*/*env* variability showed that the majority of HIV-1 isolates circulating in The Gambia were intersubtype recombinants (68%), and 53% were CRF02_AG variants [98]. In the same study, the authors also identified *gag* subtypes A, B, C, and D as well as *env* B, C, G, and one sequence that was unclassified. Berry et al. [99] found that of 43 HIV-2 clinical samples analysed in The Gambia all were subtype A. HIV-1 subtype A predominates in Senegal, and the majority of HIV-1 subtype A sequences analysed cluster with CRF02-AG [100,101]. Toure-Kane et al. [102] looked at 343 HIV-1 positive individuals from Senegal and found the *env* subtype distribution was 84.6% subtype A, 6.5% subtype B, 4.7% subtype C, 2.5% subtype D, 0.03% CRF01_AE, 0.03% sub-subtype F1, 1.2% subtype G and 0.03% subtype H. Further analysis of *env* and *gag* discordant results (11.6%) showed the predominance of CRF02_AG-like sequences. CRF06_cpx [89] and CRF09_cpx [2] were also identified in Senegal. The presence of HIV-2 subtype A [78] and HIV-1 group O [103] in Senegal has also been documented.

HIV-1 subtype A appears to be dominant in Togo. Molecular genotyping in the *gag* region of 60 HIV isolates in Lome, Togo showed that 80% were subtype A, 16.7% subtype G, 0.02% subtype D and 0.02% subtype H [104]. Andersson et al. [105] analysed *env* and *pol* sequences from 18 individuals from Guinea-Bissau with HIV-1 infection and nine individuals with HIV-1/HIV-2 dual infections. All but one sample (HIV-1 subtype B) clustered among HIV-1 subtype A, and were closely related to CRF02_AG. The HIV-2 isolates were all subtype A. Esteves et al. [79] established that for both HIV-1 and HIV-2 strains circulating in Bissau, Guinea-Bissau, there was dominance of *env* subtype A. Peeters et al. [81] studied HIV-1 and HIV-2 isolates from commercial sex workers in Bamako, Mali, and found that of the HIV-1 isolates, 80.3% were subtype A, 15.1% were subtype G, 3.1% subtype C and 1.5% subtype D. No HIV-1 group O isolates were found. All the HIV-2 isolates belonged to subtype A. CRF06_cpx [89,106] circulates in Mali, although the exact prevalence remains to be determined.

No published references describing the circulating HIV-1 subtypes in Guinea could be found, but Kourouma et al. [80] established the presence of HIV-1 and HIV-2 as well as dual infections in this country. Similarly, seroprevalence rates of HIV-1 and HIV-2 and dual infections in Mauritania have been documented [82]. Gao et al. [107] characterized HIV-2 sequences from two asymptomatic Liberian agricultural workers and analysis of *pol*, *env* and LTR regions revealed the presence of three highly divergent HIV-2 strains, one of which was more closely related to SIVsm and SIVmac than to any virus of human derivation. Exact figures on the extent of the HIV/AIDS problem in Sierra Leone are difficult to obtain, since the country has been devastated by a savage civil war [108], but HIV-1 subtype A and HIV-2 subtypes A and F have been reported [83]. No information is available for Liberia.

Central Africa

Numerous studies have assessed the extent of genetic diversity of HIV-1 group M viruses in the Democratic Republic of Congo (DRC; formerly Zaire). The high number of cocirculating HIV-1 subtypes, high intrasubtype diversity, the high numbers of possible recombinant viruses and unclassified strains are all consistent with the presence of an old and mature epidemic in the DRC, suggesting that this region is the epicentre of HIV-1 group M [109]. Several epidemiological surveys in both urban and rural areas of the DRC have confirmed that all known HIV-1 subtypes are cocirculating. Strains assigned to subtype A [36] and subtype G [91] on the basis of *env* gene phylogenetic analysis have been reported. Yang et al. [110] looked at HIV-1 subtype distribution among commercial sex workers from Kinshasa, DRC during the mid 1980s. *Env* analysis showed that of 24 samples, 37.5% were subtype G, 21% subtype A, 12.5% sub–subtype F1, 8% CRF01_AE, 4% subtype D and 4% subtype H, 12.5% were unclassifiable. Analysis of the *gag* region revealed discordant subtypes in many specimens, suggesting a predominance of subtype G (both pure G and CRF02_AG) during the early epidemic. Analysis of 61 HIV-1 isolates from the rural town of Kimpese showed that subtype A was predominant and found in 29 (47.5%) individuals. Other subtypes included subtypes C (1.6%), D (9.8%), F (3.2%), G (6.5%), H (21.3%), J (4.9%), and four sequences were unclassified [111]. Vidal et al. [109] undertook an epidemiologic survey (247 samples) in three regions of DRC, Kinshasa (the capital), Bwamanda (north) and Mbuyi-Maya (south). All known subtypes were found to cocirculate. HIV-1 subtype A was predominant, with prevalences decreasing from north (69%) to south (46%). Subtype prevalences for C, D, G, and H ranged from 7% to 9%, and subtype F, J, K, and CRF01_AE represented 2–4% of the samples. One subtype B strain was identified. The highest prevalence (25%) of subtype C was in the south, while CRF01_AE was seen mainly in the north. Eighteen (29%) of 62 samples had discordant subtype designations between *env* and *gag*. Sequence analysis of the entire envelope from 13 samples confirmed the high degree of diversity and complexity of HIV-1 strains in the DRC; nine had a complex recombinant structure in gp160, involving fragments of known and unknown subtypes. Interestingly, the subtype of 6% of the samples could not be identified, and the unknown fragments from the different strains did not cluster together. Analysis of full length genome sequences from the DRC has revealed the presence of HIV-1 sub-subtype A2 [37], subtype H and subtype H intersubtype recombinants [112], subtype K [113], and subtype J [114]. CRF05_DF [2,115] and CRF11_cpx [116,117] was also isolated from patients in the DRC, although the exact prevalences remain to be established. Of particular interest, Gao et al. [118] characterized the full genome of a highly divergent HIV-1 isolate (83CD003) from Kinshasa, that did not group with any of the known subtypes. Mokili et al. [119] described a new phylogenetic clade of HIV-1 after the analysis of two full length genomes collected from two individuals at a seven year interval, one of which was 83CD003. Pending the identification of at least one partial length sequence of the same lineage from another patient who is epidemiologically unlinked to the previously described patients, this clade has not been named as a subtype yet. This points to the incomplete status of the current HIV database, and the need for continuing surveillance.

Koch et al. [120] established the predominance of HIV-1 subtype C in Bujumbura, Burundi. Genetic analysis of the gp120 of an HIV-1 isolate from Bujumbura showed it grouped with subtype D [121]. Rwanda has a predominantly HIV-1 subtype A epidemic [55,56,122–125], although subtype C [124] and A/C recombinants have been described [122,125]. HIV-1 in Chad has been documented

[126], but very little information on subtypes exists. Lasky et al. [69] identified the genetic subtypes by *env* HMA of HIV-1 strains from two individuals infected after overseas deployment to Chad as subtype B. Full genome sequencing of an isolate from a French patient, either infected in Chad or Yugoslavia showed it was sub-subtype F1 [113]. The presence of HIV-1 group O in Chad has also been documented [35]. Bikandou et al. [127] assessed the molecular epidemiology of HIV-1 *env* from 29 AIDS patients in the Republic of Congo, and found 41% subtype A, 3% subtype D, 21% subtype G, 21% subtype H, 2% subtype J and 2% were unclassified. Results obtained from Taniguchi et al. [128] suggest that the majority of HIV-1 subtypes circulating in the Republic of Congo have mosaic structures, and may have been derived from independent recombinational events. Genetic subtyping based on *vpu*/*env* showed one A/A, 1D/D, 5G/G, 4H/H, two unclassified (U), 9G/A, 2G/H, 1G/J 1H/G, 1U/A, and 1U/J isolates. CRF02_AG was not found in the Congo. One full length HIV-1 subtype G genome from the Congo has been analysed [54]. The circulation of numerous HIV-1 subtypes have been reported from the Central African Republic (CAR), including subtypes A, B, C, D, CRF01_AE, F, G, H and CRF11_cpx [69,129–131]. Studies on CRF01_AE viruses from both Thailand and the Central African Republic suggest that CRF01_AE originated in Africa and then spread through a single introduction into South East Asia [2,132,133]. Several full length CRF01_AE viruses from the CAR have been sequenced [132,133].

West-Central Africa

The HIV-1 epidemic in Cameroon is characterized by extensive genetic variability in terms of cocirculation of all three HIV-1 groups. Analysis of HIV-1 group M subtypes in Cameroon has revealed the presence of virtually all genetic subtypes, CRF01_AE and CRF02_AG, as well as a variety of other intersubtype recombinants [30,32,69,100,134,135]. Cameroon has a high prevalence of HIV-1 group O viruses [32,35,136–138], and is considered the epicentre of HIV-1 group O. Moreover, two groups reported on intergroup (group M and O) recombinant isolates from Cameroonian patients [139,140]. HIV-1 group N has been identified in Cameroon [141] as well as chimpanzees infected with HIV-1 related viruses sharing group N *env* sequences [142]. Numerous studies describe the HIV-1 group M distribution among samples taken from various cities/towns in Cameroon. All show the prevalence of HIV-1 subtype A and CRF02_AG [32,134,143–148]. Other subtypes found in the abovementioned studies include subtypes B, C, CRF01_AE, F2, D, G, H, J, unique intersubtype recombinants such as A/CR01_AE, A/F2, F2/J and unclassified sequences. Zhong et al. [149] showed that the HIV-1 diversity in rural villages of Cameroon is as broad as has been observed in major cities of Cameroon. They confirmed the predominance of HIV-1 subtype A and CRF02_AG, and the presence of HIV-1 subtypes D, F (F2), G, H, CRF11_cpx, and numerous intersubtype recombinants. Triques et al. [113] analysed the full genome sequences of three isolates from patients in Yaounde, Cameroon and found two were sub-subtype F2, and one was subtype K. Montavon et al. [131] and Wilbe et al. [150] characterized CRF11_cpx from an individual in Cameroon, composed of fragments of subtypes A, G, J and CRF01_AE. Tscherining-Casper et al. [151] initially identified an unusual recombinant isolate with *env* A and protease J. In a later study Wilbe et al. [150] characterized this isolate and designated the new recombinant structure as CRF13-cpx.

Equatorial Guinea (EG) borders to the north with Cameroon, where different subtypes of group M and O are circulating simultaneously. Analysis of 76 plasma samples from HIV-1 seropositive individuals showed 53 were subtype A, with 64% of these sequences clustering with CRF02_AG, 11 were subtype C, four were subtype D, two were subtype F (clustered close to F2), three were subtype G two of them forming a separate cluster with CRF06_cpx, one was subtype H and two were unclassifiable [152]. HIV-1 group O has also been identified in EG [138,153]. HIV-1 group O [35] and HIV-1 subtype B [69], subtype G [36,154,155], CRF02_AG [100] and CRF11_cpx [131] have been reported from Gabon. Phylogenetic analysis of 31 strains from Gabon found two subtype A, four subtype D, one subtype G, one subtype H, eight CRF02_AG, six CRF MAL-like, six unique recombinants and one unclassified [156]. Ousseini et al. [157] described the seroprevalence of HIV-1, HIV-2 and dual infections in Niamey, Niger. Analysis of 110 HIV-1 samples from 1997 ($n = 44$) and 2000 ($n = 66$), showed the predominance of CRF02_AG (54.3%) and CRF06_cpx (18.1%) in Niger [158]. HIV-1 group O is also circulating in

Niger, albeit at a low rate [35]. Esu-Williams et al. [159] provide evidence for HIV-1, HIV-2 and HIV-1 group O [35] in Nigeria. IbNG, the prototype of CRF02_AG was first characterized from a sample collected in Ibidan, Nigeria [160]. It is estimated that, worldwide, at least nine million HIV-1 infections are due to CRF02_AG [2]. McCutchan et al. [161] evaluated the complete *env* from 10 Nigerian patients, and from seven a portion of *gag*. Four were subtype G and six were recombinants, two of which were IbNG-like, and the rest were different. Peeters et al. [162] observed a limited number of HIV-1 subtypes circulating in Nigeria, with subtypes A (61.3%) and subtypes G (37.5%) being the major *env* subtypes responsible for the epidemic. This was confirmed by results of a national molecular epidemiologic survey of HIV-1 strains from 34 out of 36 Nigerian states. Of 230 samples analysed, 44.8% were subtype A, 54.3% subtype G, 0.4% subtype C, 0.4% subtype J and 0.4% unclassifiable [163]. One subtype G and five subtype A strains were sequenced in the full gp160. The subtype G had consistent phylogenies throughout gp160. However all five of the subtype A isolates were recombinants. Two were A/G/J mosaics with common breakpoints, the remaining three had unique breakpoints. None of the five were consistent with CRF02-AG previously reported to be prevalent in West Africa. CRF06_cpx has also been reported in Nigeria [89].

North Africa

Despite the late arrival of HIV/AIDS in North Africa, the visible trend appears to be towards increasing HIV infection rates in several places, though prevalences are still very low (UNAIDS, 2002). Not much information on circulating HIV types and subtypes is available, however, studies conducted in these countries confirm the presence of HIV infected persons. No references describing circulating HIV types and subtypes in Algeria could be found. The only subtype information available from Egypt is an HIV-1 infection outbreak study amongst 13 renal dialysis patients [164]. All 13 outbreak sequences belonged to HIV-1 subtype B, while a non-outbreak sequence was CRF01_AE. In 1999 a cluster of HIV-1 infection was identified amongst 402 children and 19 mothers in Benghazi, Libya. Nosocomial transmission was responsible for the spread of infection, and phylogenetic analysis showed that a monophyletic recombinant HIV-1 form CRF02_AG infected all the patients [165]. V3 serotyping of 200 Moroccan samples indicated that 93.5% were subtype B, 1% were subtype A, 0.5% subtype F and 5% could not be typed [166]. In another study, analysis of the *env* region of 14 Moroccan sequences indicated that 11 were subtype B and three were subtype A [167]. One case of HIV-2 infection has been reported in Morocco [166]. Molecular characterization of 21 *env* sequences from HIV-1 infected Tunisian patients showed that 20 were subtype B and one was CRF02_AG [168]. The analysis of partial *pol* and *env* sequences from 30 strains collected in Khartoum, Sudan showed that 50% were subtype D and 30% were subtype C [169]. Subtype A, B and three unique recombinants were also found. Some strains could not be classified, and one unclassified strain matched another reported previously from the DRC. Sudan borders nine other African countries, and the authors conclude that the large population displacements from East and West Africa allow for the intermixing of HIV-1 subtypes in this region.

The three islands of Comores, Madagascar and Mauritius in the Indian Ocean are also considered part of Africa. The incidence of positive HIV serology in the general population of the Comores [170], Madagascar [171] and Mauritius is low, and no published information on circulating HIV types and subtypes could be found.

Conclusions

Numerous epidemiological studies have provided a picture of the HIV type and subtype distribution in Africa, and these show increasing evidence of the importance of HIV-1 recombinants. The geographic distribution of subtypes is subject to constant change, and our current understanding of HIV distribution is incomplete and inadequately represents the diversity of the virus. More work needs to be done on documenting and monitoring distribution patterns of HIV in Africa, given the presence of numerous cocirculating subtypes and high frequency and wide variety of recombinants.

Genetic variability of HIV and any consequent phenotypic variation poses a significant challenge to disease control and surveillance. The practical implications of HIV variability extend to the efficacy of diagnostic tests, chemotherapeutic agents and

vaccines. For example, *in vitro* data have indicated that HIV-1 group O viruses, as well as HIV-2, are naturally resistant to non-nucleoside RT inhibitors [172,173]. There are also indications of variation in drug susceptibility within group M. For example, some subtype G strains are less susceptible to protease inhibitors [174]. Thus, knowledge of circulating HIV strains can help formulate appropriate antiretroviral treatment programs once drugs are widely available throughout Africa. It remains important to ascertain the molecular diversity at the full genome level of the HIV strains circulating in Africa. This can assist in the selection of genes of appropriate viral strains for inclusion in a suitable vaccine candidate for use in different regions of Africa. Furthermore, knowledge of subtype will minimize the impact of genetic variation as a variable in interpretation of efficacy trials when a vaccine for HIV is tested.

Accumulation of fully sequenced HIV-1 and HIV-2 strains, including those that are more representative of the original patient samples and the geographic diversity of the pandemic, provides a framework for the investigation of antigenic and biological diversity, for the selection of vaccine prototype strains, and for molecular epidemiology. The overview presented here highlights the need for ongoing surveillance of HIV variants in Africa to inform control measures and focus strategies for prevention.

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