Description of HIV-1 Group M Molecular Epidemiology and Drug Resistance Prevalence in Equatorial Guinea from Migrants in Spain

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Abstract

**Background:** The HIV epidemic is increasing in Equatorial Guinea (GQ), West Central Africa, but few studies have reported its HIV molecular epidemiology. We aimed to describe the HIV-1 group M (HIV-1M) variants and drug-resistance mutations in GQ using sequences sampled in this country and in Spain, a frequent destination of Equatoguinean migrants.

**Methods:** We collected 195 HIV-1M pol sequences from Equatoguinean subjects attending Spanish clinics during 1997-2011, and 83 additional sequences sampled in GQ in 1997 and 2008 from GenBank. All (n = 278) were re-classified using phylogeny and tested for drug-resistance mutations. To evaluate the origin of CRF02_AG in GQ, we analyzed 2,562 CRF02_AG sequences and applied Bayesian MCMC inference (BEAST program).

**Results:** Most Equatoguinean patients recruited in Spain were women (61.1%) or heterosexuals (87.7%). In the 278 sequences, the variants found were CRF02_AG (47.8%), A (13.7%), B (7.2%), C (5.8%), G (5.4%) and others (20.1%). We found 6 CRF02_AG clusters emerged from 1983.9 to 2002.5 with origin in GQ (5.5 sequences/cluster). Transmitted drug-resistance (TDR) rate among naïve patients attended in Spain (n = 144) was 4.7%: 3.4% for PI (all with M46IL), 1.8% for NRTI (all with M184V) and 0.9% for NNRTI (Y188L). Among pre-treated patients, 9/31 (29%) presented any resistance, mainly affecting NNRTI (27.8%).

**Conclusions:** We report a low (~5%) TDR rate among naïve, with PI as the most affected class. Pre-treated patients also showed a low drug-resistance prevalence (29%) maybe related to the insufficient treatment coverage in GQ. CRF02_AG was the prevalent HIV-1M variant and entered GQ through independent introductions at least since the early 1980s.

Introduction

The human immunodeficiency virus type 1 group M (HIV-1M), responsible for the HIV pandemic, originated from a zoonotic transmission from chimpanzees into humans in Cameroon [1]. For decades, the virus evolved and diversified into different lineages in the Congo River basin, where the highest viral diversity circulates [2]. Today, HIV-1M is sub-divided into 9 pure subtypes (A-D, F-H, J and K) and recombinants between them (http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html): 58 circulating recombinant forms (CRFs) and multiple unique recombinant forms (URFs).

Equatorial Guinea (GQ), a Spanish colony until 1968, is a small country located in West Central Africa between Cameroon and Gabon. The estimated number of people living with HIV in GQ (20,000 in 2009) increases annually, and antiretroviral treatment (ART) only reached 48% of them in 2011 [3]. Despite its location, close to the epicenter of HIV-1 pandemic, only two local studies [4,5] have so far reported the HIV molecular epidemiology in GQ. Ortiz et al. included 76 HIV-1M env gene (gp11) sequences obtained in 1999 from general HIV-infected population. Djoko et al. evaluated 41 HIV-1M pol sequences sampled in 2008 from military personnel in Malabo – the capital city. Both studies revealed CRF02_AG as the prevalent HIV-1M variant, as happens in the neighboring countries [6].

The latter also reported a rate of transmitted antiretroviral drug resistance (TDR) of 4.2%. This rate represents the percentage of naïve patients infected with HIV variants carrying drug resistance mutations, and threatens the effectiveness of ART. Recent studies report an increasing TDR prevalence in low- and middle-income countries following ART scale-up [7]. This rise especially affects sub-Saharan Africa, driven by the resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI) [8,9]. The TDR rate in Cameroon is around 8-9% [10,11] and lower than 5% in Gabon [12]. HIV molecular epidemiology studies are crucial in these areas to prevent the widespread transmission of drug resistance, especially those lacking systematic surveillance as GQ.
We aimed to describe the circulating HIV-1M variants and the presence of antiretroviral drug resistance mutations in Equatorial Guinea. To achieve this, we applied phylogenetic methods to a combination of viral sequences sampled in Spain (frequent destination of Equatoguinean migration) and publicly available sequences sampled in the sub-Saharan country.

**Materials and Methods**

**Study Population**

All the HIV-infected subjects coming from Equatorial Guinea with available HIV-1M pol sequences, and attended in Spanish HIV/AIDS clinics (mainly in Madrid) were collected. They were 195 adult and pediatric patients sampled between 1997 and 2011. Most sequences were previously reported [13–16], while 37 were unpublished. In most cases (136, 69.7%) they included the complete protease (PR, 297nt) and partial reverse transcriptase (RT, 860nt of mean length). In 58 (29.7%) and 1 (0.5%) cases, only the PR or the RT sequence was available, respectively. In addition, all the HIV-1 pol sequences (n = 83) sampled in Equatorial Guinea and available in the GenBank (www.ncbi.nlm.nih.gov/genbank) were retrieved. They belonged to two sequences batches sampled in 1997 (not published) and 2008 [5]. GenBank accession numbers of the sequences included are provided in Accession S1.

**HIV-1M Subtyping**

HIV-1M variants were re-classified by phylogenetic analysis of the 278 pol sequences, including recombinants not available at publishing time. Representative sequences of the HIV-1M 9 subtypes and 49 CRFs downloaded from Los Alamos HIV sequence database (LANL-DB, http://www.hiv.lanl.gov) were used as references. Sequences were aligned manually, and initial neighbor-joining trees were built using MEGAS (http://www.megasoftware.net) under the Kimura two-parameter model with 1,000 bootstrap re-sampling. Subtypes were further confirmed in approximately maximum-likelihood (ML) phylogenetic trees constructed under the general time reversible model of nucleotide substitutions with gamma-distributed heterogeneity rate (GTR + F) using FastTree v2.1.3 (http://www.microbesonline.org/fasttree). The topology robustness was tested by likelihood-based local branch support. In sequences not ascribed to any known subtype or CRF, recombination analyses were performed using SimPlot v3.5.1 and Recombination Detection Program (RDP, v3alpha4).

In the CRF02_AG subset, we searched monophyletic clusters (associations of ≥3 samples) that involved only sequences from Equatoguinean HIV-infected Patients in Spain.

**Results**

**Equatoguinean HIV-infected Patients in Spain**

The main characteristics of these patients are shown in Table 1. Most subjects (61.1%) of this cohort were women, and were infected with HIV due to heterosexual risk practices, in concordance with the general situation in sub-Saharan Africa [20]. Sorting patients according to the sample periods shown in Table 1, we observed a significant increase in the proportion of men: 13.6% in 1997–2001, 44.6% in 2002–2006 and 53.5% in 2007–2011 (P = 0.0002). We found no apparent trends in the remaining analyzed features across time. The epidemiological information was not available in the 83 sequences retrieved from GenBank and sampled in Equatorial Guinea.

**HIV-1M Variants Circulating in Equatorial Guinea**

Table 2 shows the distribution of HIV-1M variants among the 278 pol sequences from Equatoguinean patients, where the recombinant CRF02_AG (47.8%) was predominant. Pure subtypes infected 122 (43.9%) patients, and other CRFs were found in 19 (6.8%) cases. Four sequences were classified as URF (URF_JU, URF_BH, URF_02D and URF_BG) using RDP and SimPlot. In note, the frequency of subtype B was significantly higher in Equatoguinean subjects sampled in Spain than in those sampled in EQ (9.7% vs. 1.2%, P = 0.012).

The new analysis of the sequences permitted to update their classification, especially among recombinants. We re-classified as CRF02_AG 21 sequences initially assigned to subtype G when they were sampled [21,22], 1 CRF11_cpx described as subtype J [22], 1 CRF13_cpx and 1 CRF18_cpx also reported as subtype G [21] and 1 CRF22_01A1 described as subtype A [16]. The recombination analyses revealed that 2 sequences isolated in 1999 and 2000 and reported as pure subtypes G and J [21] were URF_BG and URF_JU, respectively.

**CRF02_AG Cluster Analysis**

In the general ML tree that included the 133 CRF02_AG sequences from the study population and 2,652 worldwide CRF02_AG sequences (Figure 1), we found 7 monophyletic clusters including exclusively Equatoguinean CRF02_AG sequences with ≥95% of statistical support. The geographic origin of the 2,652 CRF02_AG sequences from LANL-DB is described in the legend of Figure 1. The further analysis with BEAST
putative clusters were supported by a posterior probability of 
(0.95) (cluster V) was related to sequences sampled in Central Africa. 
(MRCA) ranged from 1983.9 to 2002.5. Five of the 6 clusters were 
definitive due to their high statistical support. They included 33 
Equatoguinean sequences (mean = 5.5 sequences/cluster). All but 
one (cluster VI) clusters included sequences sampled in both Spain 
and GQ. The dates of their most recent common ancestors 
(dates (n = 38) in order to include at least one sequence 
among the CRF02_AG from LANL-DB according to the ML tree 
putative Equatoguinean clusters (n = 39), their closest relatives 
included a 92 sequence alignment: the CRF02_AG involved in the 
transmitted drug-resistance mutation (TDR) in 7 of the 
patients and 31 (15.9%) to treatment-experienced subjects, with 
naïve subjects, 148 (75.9%) corresponded to antiretroviral-naïve 
patients and 9 (29%) presented any drug-resistance mutation according to the IAS-
USA list [19]. The highest rate (27.8%) was found for NNRTI-
resistance mutations found were M46L (4 cases), and L90M and 
I84V (3 cases each). For NRTI-resistance, the mutation M184V 
resistance was found in all 3 cases. We observed a fluctuation in TDR rates 
in the three periods shown at Table 1:9.7% (3/31) in 1997–2001, 
was found in all 3 cases. We observed a fluctuation in TDR rates 
in the three periods shown at Table 1:9.7% (3/31) in 1997–2001, 
2.5% (2/81) in 2002–2006 and 5.5% (2/36) in 2007–2011, 
probably due to the small sample size in each one. 
HIV-1M Drug Resistance 
Among the 195 sequences collected in Spain from Equatoguinean subjects, 148 (75.9%) corresponded to antiretroviral-naïve patients and 31 (15.9%) to treatment-experienced subjects, with unknown treatment experience in 16 (8.2%) patients. We found at least one transmitted drug-resistance mutation (TDR) in 7 of the 
148 (4.7%) naïve patients following the World Health Organization 
(list [18]. TDR was 6.7% (6/90) in women vs. 1.8% 
(1/56) in men (P = 0.2). Table 4 shows the TDR rate according to 
the antiretroviral class affected. In 5 (3.4%) subjects, the mutations 
affected PR inhibitors (PI), with M46I/L substitution in all cases. 
Another 2 (1.8%) presented the M184V mutation to nucleoside 
reverse transcriptase inhibitors (NRTI). One of the latter also 
presented a drug-resistance mutation to non-nucleoside reverse 
transcriptase inhibitors (NNRTI). No triple-class resistance was 
found. Among the 31 ART-experienced patients, 9 (29%) 
prevalence was significantly higher among patients sampled in GQ 
than in Spain (P = 0.012, chi-square test). 

Table 1. Epidemiologic characteristics at sampling time of 
the 195 Equatoguinean HIV-infected patients followed in 
Spain (1997–2011).

| Characteristic       | n  | % (95% CI)          |
|----------------------|----|---------------------|
| Gender               |    |                     |
| Male                 | 70 | 38.9 (32.1–46.2)    |
| Female               | 110| 61.1 (53.8–67.9)    |
| Unknown              | 15 | –                   |
| Population           |    |                     |
| Adult                | 186| 95.4 (91.5–97.5)    |
| Pediatric            | 9  | 4.6 (2.4–8.5)       |
| Risk practice        |    |                     |
| Heterosexual         | 128| 87.7 (81.3–92.1)    |
| MSM                  | 1  | 0.7 (0.1–3.8)       |
| IDU                  | 2  | 1.4 (0.4–4.9)       |
| Vertical             | 9  | 6.2 (3.3–11.3)      |
| Transfusion/others   | 6  | 4.1 (1.9–8.7)       |
| Unknown              | 49 | –                   |
| ART experience       |    |                     |
| Naïve                | 148| 82.7 (76.5–87.5)    |
| Pre-treated          | 31 | 17.3 (12.5–23.5)    |
| Unknown              | 16 | –                   |
| Sampling period      |    |                     |
| 1997–2001            | 44 | 22.6 (17.3–28.9)    |
| 2002–2006            | 105| 53.8 (46.8–60.7)    |
| 2007–2011            | 46 | 23.6 (18.2–30.0)    |

Mean sampling year (± SD) 2003.96±3.26 –
Mean HIV viral load (log ± SD) 4.3±1.0 –

CI, confidence interval; MSM, men who have sex with men; IDU, injecting drug users; ART, antiretroviral treatment; SD, standard deviation. For naïve and pre-treated patients, the mutations lists from the World Health Organization [18] and from the International AIDS Society-USA [19] were considered, respectively. This information was not available for the 83 HIV-1M sequences retrieved from GenBank and sampled in Equatorial Guinea. 

The Bayesian MCC tree (Figure S1) showed that 6 of the 7 putative clusters were supported by a posterior probability of 
with a posterior probability of 
(0.95) (cluster V) was related to sequences sampled in Central Africa (Figure 1). 

HIV-1M Strains and Resistance in Equatorial Guinea

Table 2. HIV-1 group M variants infecting the 278 patients 
from Equatorial Guinea.

| HIV-1M variant | Total (%) | Sampled in Spain (%) | Sampled in GQ (%) |
|----------------|-----------|----------------------|-------------------|
| Pure subtypes  | 122 (43.9)| 84 (43.1)            | 38 (45.8%)        |
| A              | 38 (13.7) | 24 (12.3)            | 14 (16.9%)        |
| B*             | 20 (7.2)  | 19 (9.7)             | 1 (1.2)           |
| C              | 16 (5.7)  | 10 (5.1)             | 6 (7.2)           |
| D              | 14 (5.0)  | 9 (4.6)              | 5 (6.0)           |
| F              | 11 (4.0)  | 7 (3.6)              | 4 (4.8)           |
| G              | 15 (5.4)  | 9 (4.6)              | 6 (7.2)           |
| H              | 8 (2.9)   | 6 (3.1)              | 2 (2.4)           |
| Recombinants   | 156 (56.1)| 111 (56.9)           | 45 (54.2)         |
| CRF02_AG       | 133 (47.8)| 92 (47.2)            | 41 (49.4)         |
| CRF06_cpx      | 4 (1.4)   | 3 (1.5)              | 1 (1.2)           |
| CRF09_cpx      | 1 (0.4)   | 0 (0.0)              | 1 (1.2)           |
| CRF11_cpx      | 7 (2.5)   | 6 (3.1)              | 1 (1.2)           |
| CRF13_cpx      | 3 (1.1)   | 3 (1.5)              | 0 (0.0)           |
| CRF18_cpx      | 1 (0.4)   | 1 (0.5)              | 0 (0.0)           |
| CRF22_01A1     | 3 (1.1)   | 2 (1.0)              | 1 (1.2)           |
| URF b          | 4 (1.4)   | 4 (2.1)              | 0 (0.0)           |
| All            | 278       | 195                  | 83                |

GQ, Equatorial Guinea; CRF, circulating recombinant form; URF, unique recombinant form. Sequences obtained in Spain were sampled between 1997 and 2011, and those obtained in Equatorial Guinea in 1997 (n = 35) (not published) or 2008 (n = 48; [5]).

*Subtype B prevalence was significantly higher among patients sampled in GQ than in Spain (P = 0.012, chi-square test).

bThe four URF were URF_JU, URF_BH, URF_02D and URF_BG. 

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naïve (6/7; 85.7%) and pre-treated (6/9 cases; 66.7%) patients carrying drug-resistant viruses were adult women. They were mainly infected by CRF02_AG (2/7 and 4/9, respectively) and subtype B (2/7 and 3/9 cases, respectively).

Regarding the sequences retrieved from GenBank and obtained in Equatorial Guinea, the antiretroviral status was only available for the 41 samples described in Djoko et al., which were obtained from naïve HIV-infected population in Malabo. Among them, TDR was 4.9% according to the WHO mutations list.

**Discussion**

Despite being located in the River Congo basin, where the HIV epidemics originated [1], only two works [4,5] have provided data about the circulation of different HIV strains and drug resistance in Equatorial Guinea. Here, we expand and complete the information available about the HIV Equatoguinean epidemics combining the use of HIV-1M pol sequences from immigrants in Spain (the main host developed country for Equatoguinean migration) and from patients sampled in Equatorial Guinea. This is the larger study covering transmitted drug resistance in this country and the first one analyzing general HIV-infected population and dating the introduction of CRF02_AG.

**CRF02_AG Recombinant is Prevalent in Equatorial Guinea**

We confirm that recombinant CRF02_AG, the prevalent HIV-1M variant in Western Africa [6], accounts for a half of infections in Equatorial Guinea (Table 2). This is also the most frequent
CRF02_AG Introduction in Equatorial Guinea

The Bayesian MCMC analysis using BEAST revealed 6 monophyletic, highly statistically supported clusters of Equatoguinean CRF02_AG sequences. This could indicate that this variant entered Equatorial Guinea through several independent introductions occurred at least since the early 1980s, and not through a single introduction event. A previous study proposed that CRF02_AG was introduced in Equatorial Guinea from Cameroon [24], but our results showed that only one of the 6 fell within Central African clades, being the rest related to Western African clades. In many cases, sequences from both regions were interspersed in the analysis (Figure 1), which suggests a frequent circulation of CRF02_AG between countries, as others have also indicated [25]. In both Western and Central Africa the presence of this recombinant, originated at least in the early 1970s [24], has increased in the last years [6].

On the other hand, the observed clustering could also be explained by specific transmission networks, although the available epidemiological information collected in the patients’ clinical records does not point to the presence of any of these networks. In addition, as previously commented, all clusters but one (cluster VI) included both CRF02_AG sequences sampled in Spain and in Equatorial Guinea, which minimizes the chance of a local transmission network. Despite these explanations, this alternative possibility cannot be completely ruled out.

Low Rate of Resistance among HIV-1M-infected Equatoguineans

We observed a TDR rate of 4.7% among the 148 antiretroviral-naïve Equatoguineans attending Spanish HIV/AIDS clinics, a figure close to the limit between low and moderate drug-resistance (5%) according to the World Health Organization [26]. This rate is very similar to that found in the only TDR study performed in Equatorial Guinea (4.9%), which screened military personnel in Malabo [5]. Both are lower than in other sub-Saharan countries, including the neighboring Cameroon, with a TDR around 9% [10,11]. Since ART still reaches only 48% of the HIV-infected population in need of therapy in GQ [3], a lower emergence of resistance than in countries with an older ART scale-up is expected [7].

The highest TDR rate was found for PI (3.4%), led by the presence of the substitution M46I/L in all cases. This was also the most frequent PI-resistance mutation among pre-treated patients, including the neighboring Cameroon, with a TDR around 9% [10,11]. Since ART still reaches only 48% of the HIV-infected population in need of therapy in GQ [3], a lower emergence of resistance than in countries with an older ART scale-up is expected [7].

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transmitted NRTI-resistance mutation [5]. et al., although they only found the substitution D67N as predominance of change M184V) agrees with the study of Djoko personnel living in Equatorial Guinea [5]. This high prevalence contrasts with the rare administration of PI in Equatorial Guinea, where the first line treatment includes the NNRTI efavirenz [3]. For transmitted NRTI-resistance, the low presence found (with the predominance of change M184V) agrees with the study of Djoko et al., although they only found the substitution D67N as transmitted NNRTI-resistance mutation [5].

Studies in Uganda [7,27] have related the expanded use of NNRTI as peripartum prophylaxis among HIV-infected pregnant women to a higher population-wide prevalence of NNRTI-resistance mutations. Thus, the low presence of transmitted NNRTI-resistance mutations in EquatoGuineans compared with other sub-Saharan populations [8] could result from the infrequent administration of this prophylaxis in Equatorial Guinea (only in 19% of HIV-infected pregnant women in 2010) [3].

In treated EquatoGuinean patients attended in Spain, we found a presence of drug-resistance mutations rate of 29%, with NNRTI as the most affected drug class (27.8%). This lower prevalence than in other sub-Saharan antiretroviral-exposed cohorts [28] could reflect a poor adherence as observed among sub-Saharan living in Spain [29], who present great rates of follow-up losses. This has also been reported in Africa [30], caused by the low accessibility of antiretroviral drugs, as happens in Equatorial Guinea, with an antiretroviral coverage of 48% in 2011. In Equatorial Guinea, the use of antiretroviral drugs started in a regular basis in 2005. Thus, previous sporadic treatments and interrupted exposition to drugs could have caused the appearance of resistance mutations that jeopardizes the success of a future systematic antiretroviral programme. Unfortunately, in most cases the information on specific drug exposure for the pre-treated patients was unavailable, which prevent us from a further interpretation of these data.

### HIV Infection Place

The phylogenetic analyses showed that HIV-1M sequences sampled in Spain and/or Equatorial Guinea were interspersed, which suggest that most of the 195 Equatoguinean patients with clinical follow up in Spain were infected in their country of origin. However, we cannot rule out that some of them could have been infected in Spain. This hypothesis would be supported by the overrepresentation of subtype B (the predominant variant in Spain [23] but infrequent in Central Africa) among samples taken in this host country versus those taken in Equatorial Guinea; and by the higher prevalence of transmitted PI-resistance in these subtype B-infected EquatoGuineans sampled in Spain (where treatment is universally available) than in other variants of the study cohort.

Conversely, an evidence for an infection happened in Africa would be the different mutation pattern found in EquatoGuineans sampled in Spain than in a study of general, naive patients [14] (autochthonous and African and non-African foreigners) living in Spain between 1996 and 2010. Thus, the PI-resistance mutation M46L, present in all subtype B viruses with TDR (Table 5), was rare (<1%) among the subtype B-infected population living in Spain, where L90M was predominant. In addition, if the EquatoGuinean patients had been infected in Spain, higher rates of NRTI- and, especially, NNRTI-resistance would have been expected.

In conclusion, the rising HIV drug-resistance transmission in sub-Saharan Africa following antiretroviral rollout highlights the need of periodical surveillance studies to monitor and prevent the resistance emergence, essential information to design or continue the implementation of ART programmes. These studies should be performed among infected people living in the study region. However, in countries lacking surveillance reports (such as Equatorial Guinea), our approach describing the HIV molecular epidemiology of low-income regions from a developed host

### Table 5. Epidemiological and virological characteristics of the 7 antiretroviral-naive and the 9 antiretroviral-experienced patients infected by HIV-1M variants harboring drug resistance mutations.

| # | ART Practice | HIV-1M Strains | Gender | Risk practice | Sampling year | HIV VL (log) | TDR-PI | TDR-NRTI | TDR-NNRTI |
|---|--------------|----------------|--------|---------------|---------------|--------------|---------|----------|----------|
| 1 | Naive Adult B F Htsex 1999 | 2.5 | M46L, L90M | NA | NA |
| 2 | Naive Adult B F NA 1999 | 4.3 | M46L, I84V | NA | NA |
| 3 | Naive Adult C F Htsex 1999 | 1.8 | M46L | NA | NA |
| 4 | Naive Adult CRF02_AG F NA 2005 | 3.6 | – | M184V | – |
| 5 | Naive Adult CRF11_cpx F Htsex 2006 | 2.9 | – | M184V | Y188L |
| 6 | Naive Adult A1 F Htsex 2007 | 2.7 | M46L | – | – |
| 7 | Naive Pediatric CRF02_AG M Vertical 2007 | NA | M46L | – | – |
| 8 | Treated Adult B F Htsex 1999 | 2.3 | M46L | NA | NA |
| 9 | Treated Adult B F Htsex 1999 | 2.3 | M46L, L90M | NA | NA |
| 10 | Treated Adult B F Htsex 1999 | 2.3 | M46L, I84V, L90M | NA | NA |
| 11 | Treated Adult CRF02_AG F Htsex 2003 | 4.0 | F53L, I84V, L90M | M184V | K103N |
| 12 | Treated Adult F2 M Htsex 2006 | NA | – | – | V106I |
| 13 | Treated Adult D M Htsex 2006 | 5.0 | – | – | V90I |
| 14 | Treated Pediatric CRF02_AG F Vertical 2009 | NA | – | M184V | – |
| 15 | Treated Adult CRF02_AG M Htsex 2009 | 6.0 | – | – | K103N |
| 16 | Treated Pediatric CRF02_AG F Vertical 2010 | NA | M46L, I84V | L74V, Y115F, M184V | K103N, G190A |

# number of patient; ART, antiretroviral treatment; VL, viral load; TDR, transmitted drug resistance mutations; PI, protease inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; F, female; M, male; Htsex, heterosexual; NA, data not available; Dash, no mutation found.

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country can provide any knowledge about the HIV epidemic in specific areas.

Supporting Information

Figure S1 Maximum clade credibility tree of the 92 CRF02_AG pol sequences included in the BEAST analysis. The definitive clusters are highlighted in yellow and labeled in accordance to Table 3. The asterisk indicates nodes supported by a posterior probability of ≥0.95. The horizontal axis is expressed in calendar years. Branch colors indicate the origin of the sequences: blue, sequences from Equatoguinean patients (n = 39); green, sequences from Western Africa (n = 21); red, sequences from Central Africa (n = 17); black, other regions (n = 15).

Table S1 Sampling dates of the 92 CRF02_AG pol sequences included in the BEAST analysis. n, number of sequences. (DOCX)

Table S1 GenBank accession numbers of the 278 HIV-1 pol sequences included in this study. (DOCX)

Author Contributions

Conceived and designed the experiments: GY ÅH. Performed the experiments: GY MdM. Analyzed the data: GY MdM ÅH. Wrote the paper: GY ÅH.

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