1 **Characterisation of the HIV-1 Molecular Epidemiology in Nigeria: Origin, Diversity,**
2 **Demography and Geographic Spread**

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13 Running head: HIV-1 Molecular epidemiology in Nigeria

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1 **ABSTRACT**

2 Nigeria has been reported to have the highest number of AIDS-related deaths in the world.

3 In this study, we aimed to determine the HIV-1 genetic diversity and phylodynamics in

4 Nigeria. We analysed 1442 HIV-1 *pol* sequences collected 1999-2014 from four

5 geopolitical zones in Nigeria. Phylogenetic analysis showed that the main circulating

6 strains was the circulating recombinant strain (CRF) 02_AG (44% of the analysed

7 sequences), subtype G (8%), and CRF43_02G (16%); and that these were introduced in

8 Nigeria in the 1960s, 1970s and 1980s, respectively. The number of effective infections

9 decreased in Nigeria after the introduction of free antiretroviral treatment in 2006. We also

10 found a significant number of unique recombinant forms (22.7%). The majority of those

11 were recombinants between two or three of the main circulating strains. Seven of those

12 recombinants may represent novel CRFs. Finally, phylogeographic analysis suggested

13 multiple occasions of HIV-1 transmissions between Lagos and Abuja (two of the main

14 cities in Nigeria), that HIV-1 epidemic started in these cities, and then dispersed into rural

15 areas.

1 **IMPORTANCE**

2 Nigeria has the second largest HIV-1 epidemic in the world with the highest number of
 3 AIDS-related deaths. The few previous reports have focused on local HIV-1 subtype/CRF
 4 distributions in different Nigerian regions, and the molecular epidemiology of HIV-1 in
 5 Nigeria as a whole is less well characterized. In this study, we describe the HIV-1
 6 spatiotemporal dynamics of the five dominating transmission clusters representing the
 7 main characteristics of the epidemiology. Our results may contribute to inform prevention
 8 strategies against further spread of HIV-1 in Nigeria.

1 INTRODUCTION

2 Thirty-seven years after the first AIDS cases were described^{1,2}, HIV-1 is still a major public
3 health problem that affects approximately 36.7 (30.8–42.9) million people globally³. Sub-
4 Saharan Africa accounts for approximately 70% of all those infections. Nigeria, the most
5 populous country in this region, has been ranked as the country with the highest number of
6 AIDS-related deaths and the second highest number of HIV-1 infected cases in the world^{4,5}.
7 HIV-1 serological surveys in Nigeria were initiated in 1991, and an adult prevalence of
8 1.8% (760,000) was reported⁶. This figure gradually increased to 5.8% in 2001 (2.6
9 million), before declining to 2.9% in 2016 (3.1 million)^{3,6}. Previous reports on circulating
10 HIV-1 strains in Nigeria have identified subtype G and the circulating recombinant form
11 (CRF) 02_AG as the most common⁷⁻⁹. In addition, CRF43_02G was recently shown to be
12 highly prevalent in the capital Abuja¹⁰. However, estimates on the contribution of each
13 strain to the Nigerian HIV-1 epidemic have varied considerably, with estimates of
14 frequency varying between 22% and 50% for subtype G, and between 19% and 60% for
15 CRF02_AG⁸⁻¹⁵. These variations may be due to differences between geographic areas and
16 transmission groups. A clearer picture of the HIV-1 subtype/CRF distributions in Nigeria
17 is therefore warranted. In addition, it has been suggested that the genetic composition of
18 the infecting HIV-1 strain may influence disease progression rate, probability and
19 efficiency of transmission, interaction with the host, response to viral treatment and vaccine
20 development¹⁶⁻²³.

21

22 Advances in molecular biology techniques, databases, bioinformatics tools and expanded
23 disease surveillance programs have provided an opportunity for scientists to conduct HIV-

1 epidemiological studies to understand the diversity, origin and transmission dynamics of
 2 HIV-1 by phylodynamic approaches²⁴. Applying established epidemiological models on
 3 HIV-1 gene sequences in combination with data on time and location of sampling,
 4 epidemic growth rate, number of effective infections, timing, origin and dispersal of
 5 different HIV-1 lineages can be obtained²⁴⁻²⁶.

6

7 The objective of the current study was to characterize the molecular epidemiology of HIV-
 8 1 in Nigeria using a large dataset of *pol* sequences collected from 1999-2014.
 9 Phylodynamic approaches were employed to determine the HIV-1 diversity and to uncover
 10 the demographic history and the HIV-1 dissemination routes of the main circulating strains
 11 within Nigeria. This study increase the understanding of the Nigerian HIV-1 epidemic and
 12 may inform HIV-1 intervention strategies aiming at reducing the spread of HIV-1 in
 13 Nigeria.

1 MATERIALS AND METHODS

2 Sequence dataset

3 We analyzed 366 previously unpublished HIV-1 *pol* sequences (positions in HXB2
4 K03455: 2253-3364) collected in Abuja, Nigeria from 2006-2011 together with all
5 Nigerian *pol* sequences from the corresponding genetic region available in the Los Alamos
6 HIV-1 sequence database (N=1076, April 2015, <http://www.hiv.lanl.gov/>, Table 1).
7 Missing information on date and location of sampling was obtained through contact with
8 relevant research centers.

9

10 Subtype determination

11 The Nigerian *pol* sequences were aligned with the 2010 All M group (A-K +
12 Recombinants) reference sequence dataset (<http://www.hiv.lanl.gov/>) using the Clustal
13 algorithm, followed by manual editing in MEGA6^{27,28}. The HIV-1 subtype/CRF
14 assignment was determined with maximum-likelihood (ML) phylogenetic analysis in Garli
15 v0.98²⁹, applying the General Time Reversible (GTR) substitution model to infer the ML
16 phylogeny. Branch support was estimated using the approximate likelihood ratio test with
17 the Shimodaira-Hasegawa-like procedure (aLRT-SH) as implemented in the PhyML 3.0³⁰.
18 Branches with aLRT-SH support >0.90 were considered statistically supported²⁵.

19

20 Recombination analysis

21 Putative unique recombinant forms (URFs) and sequences that were difficult to type were
22 analysed by Bootscan in Simplot³¹. Briefly, *pol* sequences were aligned with the LANL
23 2010 HIV-1 reference subtypes for G, CRF4302G and CRF02AG (parental sequences).

1 Recombination breakpoints were identified using a sliding window size of 300 bp and step
2 size of 50 bp.

3
4 In order to define the structure and distribution of the breakpoints across the alignment, we
5 plotted a line graph of the relative frequency of the breakpoints. The K-means univariate-
6 clustering algorithm as implemented in the ‘Ckmeans.1d.dp’ R package was used to define
7 hotspots for recombination³². The gap statistic method implemented in the ‘factoextra’ R-
8 package³³ was employed to estimate the groups based on similarity in breakpoint positions
9 obtained from the Simplot analysis. The recombination hotspot positions were then used
10 to identify groups of three or more URFs with one or more similar breakpoint positions.
11 Finally, for the URFs to be defined as potential new CRFs, we performed a maximum
12 likelihood phylogenetic analysis to assess the epidemiological relatedness among the
13 sequences³⁴.

14

15 **Cluster analysis**

16 A previously described BLAST approach was used to construct a reference sequence
17 dataset for each subtype/CRF, separately^{25,35}. Briefly, we initially constructed a reference
18 set containing at least eight non-Nigerian sequences from the BLAST search of each
19 sequence belonging to the different CRF/subtype group. Redundant sequences from each
20 reference dataset were removed using an in-house Perl script and the Emboss 6.6.0.0
21 package skip redundancy³⁶. Nigerian transmission clusters were defined as clusters with
22 aLRT-SH support >0.90 and ≥80% Nigerian sequences^{25,37}. Clusters of two sequences
23 were defined as dyads, 3-14 sequences as networks, and >14 sequences as large clusters³⁸.

1

2 **Dating and Phylogeographic analysis**

3 The temporal signal in each dataset were assessed by TempEst using the transition-
 4 transversion versus divergence plots³⁹. To determine informative substitution rate priors
 5 for analysis of the main transmission clusters in Nigeria, we randomly sampled 150
 6 sequences for each subtype/CRF, respectively. The evolutionary rates were estimated in
 7 BEAST v1.8⁴⁰ using the SRD06 model⁴¹ with a relaxed uncorrelated lognormal clock
 8 model^{42,43}. Markov chain Monte Carlo (MCMC) simulations were run for 30×10^7 chain
 9 steps, subsampling parameters every 1000 steps. Convergence was assessed in Tracer.v.1.6
 10 (Effective Sample Sizes (ESS) ≥ 100)⁴⁴. Estimated subtype/CRF-specific evolutionary rates
 11 were then used as priors in the subsequent analyses.

12

13 We used a Bayesian discrete phylogeographic approach with a MCMC length of 300
 14 million steps in BEAST v1.8, sampling every 30,000th step, to reconstruct the spatial
 15 dynamics of HIV-1 for the large clusters identified^{40,45}. BEAGLE was used to improve run
 16 time of likelihood calculations, and Tracer v1.6 was used to assess convergence of the runs
 17 (ESS ≥ 100)^{44,46,47}. The demographic history of the viral population, past growth rates, and
 18 effective population sizes were inferred using the Bayesian Skygrid model⁴⁸. Priors for the
 19 TreeModel Root Height were estimated using the Bayesian Skyline model⁴⁹. If applicable,
 20 detailed growth rates were estimated using the exponential growth rate model.

21

22 Symmetric and asymmetric continuous time Markov chain models were used to model the
 23 location exchange process and the parsimonious description of the location exchange rates

1 was inferred using the Bayesian stochastic search variable selection (BSSVS)
 2 procedure^{24,49,50}. A robust counting approach as implemented in BEAST was used to
 3 estimate the forward and reverse viral movement events between locations along the
 4 branches of the posterior tree distributions⁵¹. Well-supported movements were summarized
 5 using SPREAD v1.0.7 based on a Bayes factor cut-off $>3^{52-54}$. The percentage of viral
 6 movements was summarized using R⁵⁵.

7
 8 All files and scripts are available from the authors upon request.

9 10 **Statistics**

11 Linear by linear association test (LBL) was used to analyze trends over time, using IBM
 12 SPSS V22.0 Armonk, NY: IBM Corporation.

13 14 **Ethics**

15 Approvals were obtained from the local and national institutional review boards
 16 affiliated to the treatment sites and the international collaborating sites including the
 17 University of Maryland, Baltimore, Harvard University, University of Amsterdam and
 18 US center for diseases control.

1 RESULTS

2

3 CRF02_AG, CRF43_02G and subtype G were the major circulating strains in Nigeria

4 We analyzed 1442 HIV-1 *pol* sequences collected from four geopolitical zones in Nigeria
5 between 1999 and 2013 (Table 1). The phylogenetic analysis showed that the CRF02_AG
6 was the most common strain (44% of the analyzed sequences), followed by CRF43_02G
7 (16%), subtype G (8%) and CRF06_cpx (4%). A large proportion of the sequences (23%)
8 were unique recombinant forms (URFs), whereas the remaining sequences were minor
9 variants (each variant accounting for <2%).

10

11 Most sequences were from Abuja (697 sequences, 48%), followed by Lagos (346
12 sequences, 23%) and Jos (216 sequences, 15%). The distribution of different
13 subtypes/CRFs varied within these regions/states with fewer CRF02_AG infections
14 following a North East direction from Lagos (Figure 1). Analysis of trends over time
15 showed an overall decrease in the proportion of CRF02_AG infections in Nigeria (from
16 55% in 2006 to 38% in 2013, $p=0.015$, LBL, Figure 2). Moreover, the analysis also showed
17 an increase in the proportion of unique recombinant forms (URFs) from 16% to 32%, 2005-
18 2009 ($p=0.015$, LBL).

19

20 Four potential recombination hotspots in the *pol* region

21 A detailed recombination analysis was performed on 210 of the 310 sequences classified
22 as URFs. These sequences were initially selected from the maximum likelihood
23 phylogenetic trees if they branched off close to the root between two and had long branches.

There were 655 breakpoint positions recorded among the 210 sequences, with some sequences having more than one breakpoint. These positions were plotted as a frequency plot of breakpoints along the alignment to identify hotspots for recombination (Supplementary Figure 1). Alignment positions around 285-315 (HXB2 K03455 positions: 2538-2568), 503-534 (2756-2787), 720-775 (2973-3028) and 923-956 (3176-3209) were identified as potential recombination hotspots. To define these positions more precisely, we used the IQR of the optimal univariate K-median clustering algorithm with the number of K clusters determined by the gap-statistic method⁵⁶ (Supplementary Figure 2): Recombination hotspot I: 294-312 (HXB2 K03455 positions: 2547-2565); II: 503-533 (2756-2786); III: 729-805 (2982-3058); and IV: 931-957 (3184-3210) (Supplementary Table 1). One-hundred-and-thirty-nine of the 210 (66%) sequences had a recombination breakpoint in the hotspot I region; 104/210 (50%) in hotspot II region; 58/210 (28%) in hotspot III region; and 59/210 (28%) in hotspot IV region. The hotspot positions were unique independent recombination events from the original breakpoint positions as previously identified for the parental sequences of CRF43_02G (HXB2 K03455 positions: 1266, 3325, and 6097) and CRF02_AG (HXB2 K03455 positions: 2391, 3275, and 4175). We identified seven groups of URFs with 3-10 sequences with similar breakpoint positions (Supplementary Figure 6) and that were not epidemiologically linked.

Cluster analysis

To determine transmission clusters of the major circulating strains within Nigeria, we analyzed the three dominating forms CRF02_AG, CRF43_02G, and subtype G, respectively. In total, 206 subtype G sequences were analyzed (119 Nigerian and 87 non-

1 Nigerian). The phylogenetic analysis showed four dyads, one network, and one large
 2 Nigerian subtype G cluster (consisting of 81 Nigerian and 11 non-Nigerian sequences,
 3 Table 2 and Supplementary Figure 3). Analyses of the 1161 CRF02_AG sequences (636
 4 Nigerian and 555 non-Nigerian, Supplementary Figure 4) resulted in 12 dyads, 12 networks
 5 and six clusters (Table 2). Finally, we analyzed 295 CRF43_02G sequences (236 Nigerian
 6 and 59 non-Nigerian, Supplementary Figure 5) and all the Nigerian sequences clustered
 7 monophyletically (SH-aLRT=0.99). The majority of the reference sequences obtained by
 8 the BLAST approach did not cluster within the CRF43_02G cluster, suggesting that
 9 CRF43_02G mainly circulates in Nigeria.

10

Dating the origin of five main Nigerian transmission clusters

12 To further dissect the Nigerian HIV-1 epidemic, we focused on the identified large
 13 Nigerian clusters; one subtype G, three CRF02_AG and one CRF43_02G clusters.
 14 Analysis of the temporal signal showed that all clusters had a correlation coefficient above
 15 0.3, indicating a positive correlation between genetic distance and sampling time, and thus
 16 retained sufficient phylogenetic signal to conduct coalescence analyses⁵⁷.

17

18 The median time to most recent common ancestor (tMRCA) of the Nigerian subtype G
 19 cluster was estimated to 1979 (95% HPD: 1967-1980); the CRF02_AG clusters to 1961
 20 (95% HPD: 1946-1974), 1968 (95% HPD: 1958-1978) and 1960 (95% HPD: 1946-1973)
 21 for cluster 1, 2 and 3, respectively; and the CRF43_02G cluster to 1980 (95% HPD: 1975-
 22 1983) (Figure 3). The median HIV-1 evolutionary rates were estimated to
 23 2.1×10^{-3} substitutions/site/year (s/s/y) (95% HPD: $1.7-2.5 \times 10^{-3}$ s/s/y) for the Nigerian

1 subtype G cluster; 1.4×10^{-3} s/s/y (95% HPD: $1.1-1.9 \times 10^{-3}$ s/s/y), 1.3×10^{-3} s/s/y (95%
2 HPD: $1.4-1.8 \times 10^{-3}$ s/s/y), 1.2×10^{-3} s/s/y (95% HPD: $0.9-1.6 \times 10^{-3}$ s/s/y) for the
3 CRF02_AG cluster 1, 2 and 3, respectively; and 4.2×10^{-3} s/s/y (95% HPD: $3.5-$
4 4.8×10^{-3} s/s/y) for the CRF43_02G cluster (Figure 3) .

5

6 To control for bias in the phylogeographic analysis due to oversampling of some locations
7 in the dataset in relation to what is reflected in the epidemic, we randomly selected
8 sequences from different regions based on their HIV-1 prevalence and population growth
9 over time in the different geographic regions. In these analyses, the median tMRCA of the
10 Nigerian subtype G cluster was estimated to 1987 (95% HPD: 1982-1992); and the
11 CRF02_AG clusters to (95% HPD: 1960-1983), 1972 (95% HPD: 1973-1981), 1961 (95%
12 HPD: 1952-1979) for cluster 1, 2 and 3, respectively.

13

14 **Disentangling the demographic history of five main Nigerian transmission clusters**

15 The Bayesian Skygrid analysis indicated that the number of effective infections (i.e. the
16 number of individuals contributing to new HIV-1 infections over time⁵⁸) in the Nigerian
17 subtype G epidemic underwent a fast exponential growth between the 1970s and the mid-
18 1990s with an increase to 10,000 effective infections, followed by a marginal decrease with
19 minor fluctuations from the mid-1990s (Figure 4). The median growth rate was 30% per
20 year (95% HPD: 18%-42%). The three clusters representing the CRF02_AG epidemic
21 displayed a similar pattern with a slow increase in growth rate from the 1980s to 2000s.
22 The median CRF02_AG growth rates were estimated to 22% per year (95% HPD: 13%-
23 32%) for cluster 1, 18% per year (95% HPD: 10%-26%) for cluster 2, and 24% (95% HPD:

1 11%-38%) for cluster 3. Finally, the CRF43_02G cluster also showed an increase in
2 effective HIV-1 infections from 1980 to 2000 (from 100 to 10,000 effective HIV-1
3 infections), followed by a relatively sharp decrease from mid-2000 and forward (Figure 4).
4 The median growth rate was 30% per year (95% HPD: 18%-42%).

6 **Phylogeographic dispersal of HIV-1 in five main Nigerian transmission clusters**

7 Next, we sought to investigate the spatio-temporal process of the HIV-1 spread in Nigeria
8 using symmetric and asymmetric continuous time markov chain (CTMC) phylogeographic
9 models with the BSSVS procedure in BEAST. The two models gave similar estimations.
10 Results from the asymmetric analysis for subtype G showed a high statistical support for
11 epidemiological linkage between Kaduna and other regions: Abuja (Bayes Factor
12 [BF]=11302), Ibadan (BF=6), Yobe (BF=1609), and Jos (BF=11302). For CRF02_AG
13 clusters, Abuja was connected to Ibadan, Jos, Kaduna, Lagos, Oyo and Maiduguri (all BFs
14 >4). Based on the posterior distribution of the location of origin, the most probable origin
15 of Subtype G, CRF43_02G and CRF02_AG was Abuja (Posterior probability: 0.98).
16 However, these results should be interpreted with caution due to the low number of
17 sequences from some locations.

18
19 Since the dataset contained unbalanced distribution of samples in terms of location, we
20 conducted a control analysis to assess the robustness of our results to over-representations
21 of samples from particular regions in Nigeria (Table 1). We selected samples based on
22 population size and HIV-1 prevalence and run the CTMC analysis on the subsample. This
23 analysis indicated that the most probable root state for all the strains where outside Nigeria.

1

2 **Rates of viral lineage migration**

3 The rates of viral lineage migration within Nigeria were estimated using a robust counting
 4 approach to infer the history of viral movements and epidemiological links between
 5 different locations and the locations that contributed most to the dispersal of HIV-1
 6 subtypes within Nigeria. For subtype G, HIV-1 dispersal from Abuja was estimated at 42%
 7 (95% HPD: 35-48%) and the highest viral migrations from Abuja were to Jos (17%, 95%
 8 HPD: 12-22%), outside Nigeria (10%, 95% HPD: 8-13%), and Lagos (7%, 95% HPD: 4-
 9 10%). Similar results were found for the CRF02_AG clusters, except for slightly higher
 10 migration rates from Abuja to Lagos for cluster 1 (30%, 95% HPD: 25-34%, Figure 5).
 11 The CRF02_AG dispersal from Abuja was estimated to 61%, 52% and 21% from cluster
 12 1, 2 and 3, respectively.

1 DISCUSSION

2 In this study, we aimed to provide a better understanding of the history and spread of the
3 HIV-1 subtypes/CRFs circulating in Nigeria. We applied state of the art phylogenetic
4 approaches on a large set of HIV-1 sequences from individuals of the five most populated
5 geopolitical zones in Nigeria to characterize the Nigerian HIV-1 epidemic and to obtain
6 new insights about its origin and disseminations patterns. In line with previous studies, we
7 identified CRF02_AG, CRF43_02G and subtype G as the most prevalent strains (previous
8 estimates ranging from 19-60% for CRF02_AG^{8,9,11-13} and 22-50% for subtype G^{9-11,14,15}).
9 The prevalence of CRF43_02G has only been reported in one previous study from Abuja
10 (estimated to 19%)¹⁰. These large variations and discrepancies are likely explained by
11 analysis of local geographic regions, and that different subtyping tools differ in accuracy
12 in assigning the correct subtype/CRF (CRFs are often particularly challenging)⁵⁹. It could
13 also be the result of a sampling not reflecting the real prevalence regions and/or over
14 periods.

15

16 For both subtype G and CRF43_02G, we found one well-supported monophyletic clade,
17 respectively. Each clusters harbored the vast majority of the Nigerian sequences from those
18 strains suggesting introductions by a single strain or a limited number of strains that grew
19 out to dominate the epidemic. In contrast, three large Nigerian clusters were found for the
20 CRF02_AG strain indicating multiple introductions that grew out to separate sub-
21 epidemics.

22

1 We observed one large transmission cluster with 68% of all Nigerian subtype G sequences
2 and a coalescent estimate on the time of emergence at 1979 (1967-1980). This estimate is
3 consistent with that subtype G cluster (GWA_{II}) described by Delatorre *et al.* in 2014 and it
4 is approximately six years before the first AIDS cases was identified Nigeria⁶⁰. This
5 indicates that HIV-1 had spread in Nigeria already before the first AIDS case was identified
6 in the country. The three independent transmission clusters for CRF02_AG that originated
7 from within Nigeria had coalescent estimates on the time of emergence from 1960-1980.
8 Our analysis indicated that the one of the CRF02_AG cluster had an earlier origin than the
9 estimated tMRCA for subtype G (i.e. the putative parental strain). This supports a previous
10 study that CRF02_AG is in fact the parental strain of subtype G⁶¹. Interestingly, the
11 CRF43_02G was first fully described and isolated in Saudi Arabia in 2008⁶². Our analysis
12 indicated that it emerged in Abuja already in the early 1980's. In addition, the majority of
13 the CRF43_02G strains in Genbank was of Nigerian origin. Considering the large
14 prevalence of both the putative parental strains CRF02_AG and subtype G, it is therefore
15 plausible that CRF43_02G first emerged in Nigeria. The molecular clock estimates on the
16 date of introduction, from the control analysis accounting for the sampling bias,
17 corresponded well with all the five clusters. This implies that the sampling location had
18 limited effect on these estimations.

19

20 The demographic analysis indicated an increase in number of effective infections 1970-
21 1995 in all five clusters. This is in line with a rapid population growth during the same
22 period in Nigeria (from 56 to 108 million people, [www.worldometers.info/world-](http://www.worldometers.info/world-population/nigeria-population)
23 [population/nigeria-population](http://www.worldometers.info/world-population/nigeria-population)). Moreover, this increase was followed by a decline in

1 number of effective infections which seem to coincide with when free ART was introduced
2 in 2006⁶, and when Nigeria registered a sharp decrease in HIV-1 prevalence (from
3 approximately 6% to 3%)^{3,6}. However, despite this decrease in HIV-1 prevalence, our
4 analysis indicated a relatively high number of co-circulating strains. Our analysis also
5 indicated that urban areas like Abuja and Lagos might represent major hubs of HIV-1
6 transmissions.

7
8 The recombination analysis indicated several URFs and potentially novel CRFs. Inter-
9 subtype/CRF recombination does not occur randomly on the HIV-1 genome as its
10 frequency varies over the genome with several so-called hotspots for recombination^{63,64}.
11 One main hotspot for recombination in the *pol* region (found in 105 sequences) was close
12 to the *pol PR-RT* border (positions 2547-2565 in the HIV-1 HXB2 reference genome,
13 K03455). This hotspot has previously been reported in a study on HIV-1 subtype B⁶⁵. The
14 hotspot positions II and IV has also been reported elsewhere⁶⁶. The seven identified groups
15 of URFs represents potential novel CRFs circulating in Nigeria or second generation
16 recombinants with different recombination patterns. However, this needs to be confirmed
17 by full-length sequencing. To date, 18 distinct subtype G related CRFs have been described
18 and published worldwide indicating the diversity in Subtype G sequences
19 (<http://www.hiv.lanl.gov/>).

20
21 This is the first study to dissecting the Nigerian molecular epidemiology with a country-
22 wide set of HIV-1 sequences. Understanding the underlying processes and factors that
23 influence HIV-1 transmission, demographic history and migration patterns are pivotal to

- 1 understand the epidemic potential of different HIV-1 strains. Ultimately, this may also
- 2 inform population-based surveillance of infectious diseases.

1 **NOTES**

2 **Acknowledgments**

3 We thank all the study participants and the collaborating health centers in Nigeria.

4

5 **Author contributions**

6 J.N, N.N and J.E. interpreted the data and were responsible for the overall study design.

7 N.N and J.E. were responsible for the overall project coordination. H.R., B.C., P.D, A.A,

8 M.C were medically and organisationally responsible for the clinical sites and collected

9 key epidemiological data of the study participants. J.N, N.F. and J.E analysed the data and

10 contributed in statistical analyses. J.N. and J.E. wrote the manuscript. All authors read and

11 approved the manuscript.

12

13 **Conflicts of interest**

14 The authors or their institutions declare no competing financial interests, and did not at any

15 time receive payment or services from a third party (government, commercial, private

16 foundation, etc.) for any aspect of the submitted work (including data monitoring board,

17 study design, manuscript preparation, statistical analysis, etc.). The authors have no

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19

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3

TABLES

Table 1. Proportion of subtype/CRF in the Nigerian dataset of previously published and new sequences collected in the period 1999-2013

Subtype/CRF	N	%	Geography																		
			Southwest							North Central					North East & North West						
			LAG	IBA	OND	OSU	OYO	ENU	TOTAL	ABV	JOS	NIG	NC	TOTAL	KAD	YOB	MAI	KAN	ADA	BOR	TOTAL
A1	24	1.7	9	2	0	0	0	0	11	7	5	0	0	12	0	0	1	0	0	0	1
B	4	0.3	1	1	0	0	0	0	2	2	0	0	0	2	0	0	0	0	0	0	0
C	14	1.0	2	1	0	0	2	0	5	6	2	0	1	9	0	0	0	0	0	0	0
CRF02_AG	636	44.1	176	27	0	1	18	0	222	284	94	0	10	388	13	0	11	2	0	0	26
CRF06_cpx	64	4.4	18	2	0	0	0	0	20	36	6	0	1	43	0	0	1	0	0	0	1
CRF09_cpx	1	0.1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
CRF11_cpx	4	0.3	1	0	0	0	0	0	1	2	1	0	0	3	0	0	0	0	0	0	0
CRF18_cpx	1	0.1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
CRF19_cpx	1	0.1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
CRF43_02G	236	16.4	33	11	1	0	0	0	45	138	34	0	0	172	8	0	8	3	0	0	19
CRF49_cpx	1	0.1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
D	9	0.6	2	0	0	0	0	0	2	4	2	0	0	6	0	0	1	0	0	0	1
G	119	8.3	24	6	0	0	0	0	30	55	25	1	0	81	4	1	2	0	0	1	8
URF	328	22.7	78	13	0	0	2	1	94	163	46	0	3	212	9	0	11	0	2	0	22
Total	1442	100	346	64	1	1	22	1	435	697	216	1	15	929	34	1	35	5	2	1	78

N, number of sequences; LAG, Lagos; IBA, Ibadan; OND, Ondu; OSU, Osun; ENU, Enugu; ABV, Abuja; NIG, Niger; NC, Other North central areas; KAD, Kaduna; YOB, Yobe; MAI, Maiduguri; KAN, Kanu; ADA, Adamawa; BOR, Borno

1 **Table 2. Number of clusters in the different subtype / CRF groups**

Subtype / CRF	Dyads^a	Networks^b	Large clusters^c	Total
G	8 (4)	8 (2)	94 (1)	104
CRF02_AG	24 (12)	76 (12)	336 (6)	439
CRF43_02G	0 (0)	0 (0)	295 (1)	295
Total	32	84	725	841

^aDyads: Clusters of 2 sequences

^bNetworks: Clusters of 3-14 sequences

^cLarge clusters: Clusters with more than 14 sequences

The brackets () indicates the number of clusters observed for the different groups.

2

1 **FIGURE LEGENDS**

2 **Figure 1. Prevalence of subtypes/CRFs among HIV-1 infected individuals collected** 3 **from different locations in Nigeria.**

4 Molecular diversity of HIV-1 in different zones/regions in Nigeria. The location of the
5 sampled regions are shown by the black dot with a line leading to diversity pie-chart for
6 the city in that particular region. The colors in the pie-chart are defined in the key.
7 Abbreviations for the towns: ABV, Abuja; KAD, Kaduna; MAI, Maiduguri; LAG, Lagos;
8 IBA, Ibadan (Based on the map from <https://d-maps.com/>).

9

10 **Figure 2. Dynamics of subtype/ CRF proportions, ART coverage and overall country** 11 **HIV-1 prevalence over time.**

12 The subtype/ CRF proportion displayed as overall percentage of sequences collected from
13 Nigeria per year. Few sequences (< 20 per year) were collected from 1999-2004 and their
14 proportions had no effect on the LBL association tests. No sequences were collected in
15 2012. The orange bars represent the proportion of HIV-1 infected individuals that were
16 receiving ART in the respective years. The change in proportion of different
17 subtypes/CRFs over time was not significant for subtype G ($p = 0.711$, LBL), CRF43_02G
18 ($p = 0.497$, LBL), CRF02_AG ($p = 0.323$, LBL) and CRF06_cpx ($p = 0.015$, LBL).
19 However the increase of URFs over time was significant ($p = 0.015$, LBL). The y-axis
20 shows the proportions (%) of sequences collected over time for a particular subtype/CRF
21 or the proportion (%) of patients receiving ART. The x-axis represents the time period in
22 years from 1999-2013. The z-axis shows the HIV-1 prevalence in Nigeria over time.

23

1 **Figure 3. Demographic history for the different Nigerian clusters**

2 Pirate plots for the evolutionary rate and time to the most recent common ancestor since
3 2013 for the different 5 clusters in Nigeria. Each bean in the plot has mean indicated as a
4 bold horizontal black line and the Bayesian 95% highest density interval as thin horizontal
5 lines. The raw data from the sampling interval during Bayesian analysis is indicated by
6 black dots inside the beans. The CRF43_02G had a slightly higher evolutionary rate
7 compared to the other clusters.

8

9 **Figure 4. Skygrid plots for the different clusters**

10 Phylodynamic analyses of HIV-1 *pol* gene for subtype G, CRF02_AG, CRF43_02G
11 clusters isolated in Nigeria. Bayesian skygrid plots representing the changes in the effective
12 population size of the virus (N_e)(log) over time. The solid lines represent the estimated
13 median log effective population size and the gray shaded areas represents the 95% HPD
14 intervals.

15

16 **Figure 5. Estimated percentages of CRF02_AG migration events from Abuja to each** 17 **location in the NG, obtained using cluster 1**

18 The density plot for the viral movements from the Abuja (most probable root location) to
19 Lagos, Jos, Kaduna and other towns. Lagos and Jos had the highest percentage of viral
20 movements from Abuja for the CRF02_AG Cluster 1. Infections from outside Nigeria
21 accounted for 10% of the viral movement.

1 FIGURES

2 **Figure 1. Prevalence of subtypes/CRFs among HIV-1 infected individuals collected from different locations in Nigeria.**

3

Figure 2. Dynamics of subtype/ CRF proportions, ART coverage and overall country HIV-1 prevalence over time.

Figure 3. Demographic history for the different Nigerian clusters.

Figure 4. Skygrid plots for the different clusters.

Figure 5. Estimated percentages of CRF02_AG migration events from Abuja to each location in the NG, obtained using cluster 1.

SUPPLEMENTARY DATA

Characterisation of the HIV-1 Molecular Epidemiology in Nigeria: Origin, Diversity, Demography and Geographic Spread

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Files in this Data Supplement

Supplementary Table 1. Summary of the median, mean and interquartile ranges for the 4 hotspot regions in the pol alignment using the univariate K-means clustering Algorithm

Supplementary Figure 1. Potential recombination breakpoint hotspots detected in the pol alignment

Supplementary Figure 2. Optimal cluster determined by the gap statistic method

Supplementary Figure 3. Maximum likelihood phylogenetic tree for Subtype G sequences

Supplementary Figure 4. Maximum likelihood phylogenetic tree for CRF02_AG sequences

Supplementary Figure 5. Maximum likelihood phylogenetic tree for CRF43_02G sequences

Supplementary Figure 6. Groups of sequences with similar recombination breakpoint

Legends for supplementary figures

SUPPLEMENTARY TABLES

Supplementary Table 1. Summary of the median, mean and interquartile ranges for the four hotspot regions in the *pol* alignment using the univariate K-means clustering Algorithm.

	I	II	III	IV
N	221	203	115	116
Min.	147.00	415.00	656.00	864.00
1st Qu.	294.00	503.00	729.00	931.00
Median	305.00	522.00	768.00	948.00
Mean	306.38	519.96	760.44	941.24
3rd Qu.	312.00	533.00	805.00	957.25
Max.	398.00	633.00	858.00	980.00

1 SUPPLEMENTARY FIGURES

2 Supplementary Figure 1. Potential recombination breakpoint hotspots detected in the 3 *pol* alignment.

4

5

1 **Supplementary Figure 2. Optimal cluster determined by the gap statistic method**

1 **Supplementary Figure 3. Maximum likelihood phylogenetic tree of the Subtype G**

2 **sequences (n = 203).**

3

1 **Supplementary Figure 4. Maximum likelihood phylogenetic tree for CRF02_AG**

2 **sequences (n = 1170).**

3

4

1 **Supplementary Figure 5. Maximum likelihood phylogenetic tree for CRF43_02G**
2 **sequences (n = 667).**

3

4

- 1 **Supplementary Figure 6. Groups of sequences with similar recombination breakpoint**
- 2 **patterns that could be potential new CRFs.**

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Potential recombination breakpoint hotspots detected in the

pol alignment. Small vertical lines at the bottom of the graph indicate breakpoint positions along the alignment. Breakpoints detected in each window of 300 nucleotides along the alignment were counted and plotted (solid line).

Supplementary Figure 2. Optimal cluster determined by the gap statistic method. The

gap statistic compares the total intra-cluster variation for different values of k with their expected values under null reference distribution of the data. This plot provides the gap statistic and standard error, identifying k=4 as the optimal cluster with the highest gap statistic.

Supplementary Figure 3. Maximum likelihood phylogenetic tree of the pol region for

Subtype G sequences (n = 203). The blue braches indicate Nigerian sequences while the green indicate an SH-aLRT branch support above 0.9. The black branches indicate the non-Nigerian sequences. We observed one large NG cluster (in blue) that was considered for further analysis

Supplementary Figure 4. Maximum likelihood phylogenetic tree for CRF43_02G

sequences (n = 667). The blue braches indicate Nigerian sequences while the green indicate an SH-aLRT branch support above 0.9. The black branches indicate the non-Nigerian sequences. We observed one large NG cluster (in blue) that was considered for further analysis. The purple sequences were later classified as URFs

- 1 **Supplementary Figure 5. Maximum likelihood phylogenetic tree for CRF02_AG**
- 2 **sequences (n = 1170).** The blue braches indicate Nigerian sequences while the green indicate
- 3 an SH-aLRT branch support above 0.9. The black branches indicate the non-Nigerian
- 4 sequences. We observed 3 large Nigerian clusters (in blue) that were considered for further
- 5 analysis.
- 6
- 7 **Supplementary Figure 6. Groups of sequences with similar recombination breakpoint**
- 8 **patterns that could be potential new CRFs.**