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Predominance of Subtype A and G HIV Type 1 in Nigeria, with Geographical Differences in Their Distribution

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ABSTRACT

The purpose of this study was to generate data on the relative prevalences of the HIV-1 subtypes circulating in Nigeria. A total of 252 HIV-1-positive samples collected during an epidemiologic survey conducted in April 1996 were genetically characterized by HMA (heteroduplex mobility assay) and/or sequencing. Samples were collected in Lagos, Calabar, Kano, and Maiduguri. Overall, the predominant *env* subtypes were A (61.3%) and G (37.5%). Subtype A is more prevalent in the south ($p < 0.001$), about 70% in Lagos and Calabar, whereas a quarter of the samples was classified as subtype G in these states. In contrast, subtype G is predominant in the north ($p < 0.001$), representing 58% of the samples in Kano. In the northeastern region, Maiduguri, almost similar proportions of subtype A and G were seen, 49 and 47.4%, respectively. A total of 37 samples was also sequenced in the p24 region from the *gag* gene; 13 (35%) had discordant subtype designations between *env* and *gag*. The majority of the *gag* (12 of 17) and *env* (14 of 22) subtype A sequences clustered with the A/G-IBNG strain. Within subtype G, three different subclusters were seen among the envelope sequences. These different subclusters are observed among samples obtained from asymptomatic individuals and AIDS patients from the four Nigerian states studied. In conclusion, we observed a limited number of HIV-1 subtypes circulating in Nigeria, with subtypes A and G being the major *env* subtypes responsible for the HIV-1 epidemic. Nevertheless, the high rate of recombinant viruses (A/G) and the different A/G recombinant structures indicate a complex pattern of HIV-1 viruses circulating in this country.

INTRODUCTION

With the characterization of many isolates of HIV from Africa and from other regions of the world, numerous genetic subtypes of the virus have been identified. Phylogenetic analysis revealed three groups of HIV-1 isolates: group M, the major group with at least 10 different genetic subtypes in the envelope (A–J); group N; and group O.[1,2] Preliminary data indicate a heterogeneous distribution and dominance of different genetic subtypes depending on the country analyzed.[3,4] In Africa all the known HIV-1 genetic subtypes, including groups N and O, are present.[4,5] Recombination events among sequences of different genetic subtypes of HIV-1 group M have been frequently identified. Since the first report on recombinant HIV-1 viruses, increasing numbers of recombinant HIV-1 genomes have been recognized and recombinant forms of epidemiologic importance have been identified.[5–10] The implications of this genetic diversity for vaccine development, pathogenesis, disease progression, and virus transmission still remain to be elucidated.[3] A relationship between genetic subtype and natural resistance against antiretroviral drugs has been observed. In particular, group O viruses are resistant to nonnucleoside reverse transcriptase (RT) inhibitor (TIBO)[11] and within group M, some subtype F samples are less susceptible to TIBO and some subtype G samples are less susceptible to protease inhibitors.[12,13] But one of the most important conse-

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quences of the genetic variability is the implication for the efficiency of diagnostic assays. Group O antibodies are not always detected by some commercial HIV antibody assays,[14,15] non-B seroconversions are detected after B seroconversions,[16] and some viral load assays are not able to detect and correctly quantify non-B HIV-1 viruses.[17–19] For all of these reasons, it is important to study the geographic distribution of the different HIV-1 genetic subtypes.

To date, there have been relatively few systematic large-scale attempts to characterize HIV isolates from different parts of the world, and especially from Africa. As part of an assessment of the geographic distribution of HIV-1 subtypes in West Africa, we studied the prevalence of different genetic subtypes of HIV-1 in Nigeria.

Nigeria is Africa's most populous country, with more than 100 million inhabitants representing nearly 25% of the total population of sub-Saharan Africa. Thirty-five percent of the people in Nigeria live in urban areas. Between 1986 and 1989 the HIV prevalences were low, so that Nigeria was for a long time regarded as a low-prevalence country. Since then different studies have shown a growing increase in HIV prevalence in the general population and in populations at risk of infection.[20–22] The HIV epidemic is rapidly spreading in all population groups in Nigeria, but the fastest growing rate continues to be seen in female sex workers.[23] There is also evidence to suggest that a rural HIV epidemic may be emerging in parts of Nigeria.[23] Nigeria is located between the well-established HIV-1 epidemic of Central Africa and the epicenter of the HIV-2 infection, found mainly on the extreme west coast of Africa. HIV-1 is predominant but HIV-2 and dual infections have been documented.[21–25] A limited number of strains from Nigeria have been genetically characterized, and almost all were recombinant viruses, with different mosaic genomes involving subtype A and G sequences.[26–28] Also, a few cases of HIV-1 group O infections have been described.[23,29]

Between March and April 1996, we conducted a large serosurvey of selected population groups from different geographic locations in Nigeria (southwest, southeast, north, and northeast), which has been published.[23] The purpose of this study was to generate data on HIV infection, especially on the relative prevalences of the HIV-1 subtypes circulating in Nigeria.

MATERIALS AND METHODS

Specimen and DNA isolation

A total of 330 HIV-1-positive samples was collected during an epidemiologic survey conducted in April 1996 in 4 of the 30 states from Nigeria: Lagos State (southwest) in Lagos, Cross River State (southeast) in Calabar and in Ikom, Borno State (northeast) in Maiduguri, and Kano State (north) in Kano.[23] Participants were mainly recruited among female sex workers (FSWs), tuberculosis patients and patients clinically suspect for HIV infection, pregnant women, and blood donors.

Peripheral blood mononuclear cells (PBMCs) were collected by Ficoll gradient centrifigation from all HIV-positive samples. Plasma and cell pellets were stored at −20°C and shipped on dry ice for further genetic characterization.

DNA was extracted from the dry cell pellets with an IsoQuick isolation kit (Microprobe, Garden Cove, CA) or a Qiagen (Courtabeauf, France) DNA isolation kit.

Heteroduplex mobility assay

The V3–V5 region from the envelope gene was amplified by a nested polymerase chain reaction (PCR) as previously described[30] with ED5 and ED12 as outer primers, and with ES7 and ES8 as inner primers. The PCR conditions were as follows: a first denaturation step for 5 min at 94°C followed by 30 cycles of 94°C for 15 sec, 55 or 50°C for 30 sec, and 72°C for 2 min, with a final extension for 7 min at 72°C for the first round. Five microliters from this amplification was used for the second round with the inner primers, using the following cycling conditions for 40 cycles; 94°C for 15 sec, 55 or 50°C for 30 sec, and 72°C for 1 min. The reaction mixture consists of 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.1% Triton X-100, 1.25 mM $MgCl_2$ for the first round or 1.8 mM $MgCl_2$ for the second round, 10 pmol of each primer, a 0.2 mM concentration of each dNTP, and 2.5 U of *Taq* polymerase.

The PCR amplification products were detected by electrophoresis on a 1% agarose gel and visualized by ethidium bromide staining. To avoid PCR product cross-contamination, pre-PCR and post-PCR manipulations were performed in separate rooms.

Heteroduplex molecules were obtained by mixing 5 μl of two divergent PCR-amplified DNA fragments (the unknown patients strain with a plasmid from typed reference strains) denatured at 94°C for 2 min and renatured by rapid cooling on wet ice. The reference plasmids used in this study were the following: A1 (RW20, Rwanda), A2 (IC144, Ivory Coast), A3 (SF170, Rwanda), B1 (BR20, Brazil), B2 (TH14, Thailand), B3 (SF162, United States), C1 (MA959, Malawi), C2 (ZM18, Zambia), C3 (IN868, India), C4 (BR25, Brazil), D1 (UG21, Uganda), D2 (UG38, Uganda), D3 (UG46, Uganda), E1 (TH22, Thailand), E2 (TH06, Thailand), E3 (CAR7, Central African Republic), F1 (BZ162, Brazil), F2 (BZ163, Brazil), G1 (RU131, Russia), G2 (LBV21-7, Gabon), G3 (VI525, Gabon), H1 (CA13, Cameroon), H2 (VI557, Democratic Republic of Congo), H3 (VI997, Belgium). The reaction was performed in 100 mM NaCl, 10 mM Tris-HCl (pH 7.8) and 2 mM EDTA in a final volume of 8 μl. The heteroduplex formation was resolved by electrophoresis analysis at 250 V for 3 hr on a nondenaturing 5% polyacrylamide gel in TBE buffer (88 mM Tris-borate, 89 mM boric acid, 2 mM EDTA) and was detected after being stained with ethidium bromide. The electrophoretic mobility of the heteroduplexes was inversely proportional to the sequence divergence of the two annealed strands.

Sequencing of part of the envelope

The genetic subtype in the envelope determined by heteroduplex mobility assay (HMA) of the V3–V5 region of the envelope (700 bp) was confirmed by direct sequencing of the corresponding region for some samples. For the samples that could not be amplified with the HMA primers, a fragment of approximately 1100 bp was amplified by a nested PCR, using new designed primers S1 (CTATTTGTGCATCWGATGCTAAAGC) and S2 (CCTGTACCGTCAGCGTTATTGAC) as outer primers and ED5nig (TTATGGGATGAAAGYCTAA-

AGCCAT) and ES8nig (ACTTCTCCAGTTGTCCCTCATATCT) as inner primers. This 1100-bp fragment was subsequently sequenced. Nucleotide sequences were obtained by direct sequencing of the PCR products. For some samples, because of the significant presence of quasispecies, PCR products were purified with a QIAquick PCR purification kit (Qiagen) and were cloned in the pGEM-T easy vector (Promega, Madison, WI) before sequencing.

Genetic subtyping in the gag *region*

A 700-bp fragment, corresponding to the p24 region from the *gag* gene, was amplified with previously described primers G00–G01 and G60–G25.[31] The PCR conditions were as follows: a first denaturation step for 3 min at 92°C, followed by 30 cycles of 92°C for 10 sec, 55°C for 30 sec, and 1 min at 72°C, with a final extension for 7 min at 72°C, in a final volume of 50 μl. The reaction mixture consists of 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.1% Triton X-100, 1.4 mM $MgCl_2$, 10 pmol of each primer, a 0.2 mM concentration of each dNTP, and 2.5 U of *Taq* polymerase. One microliter from this amplified product was used for the second round, using the same reaction mixture and PCR conditions for 40 cycles, in a final volume of 100 μl.

The PCR amplification products were detected by electrophoresis on a 1% agarose gel and visualized by ethidium bromide staining.

Nucleotide sequences were obtained by direct sequencing of the PCR products. The amplified DNA was purified with a QIAquik gel extraction kit (Qiagen). Cycle sequencing was performed by fluorescent dye terminator technology (dye terminator cycle sequencing with AmpliTaq DNA polymerase FS; Perkin-Elmer, Roissy, France) according to the instructions of the manufacturer. Electrophoresis and data collection were done on an Applied Biosystems (Foster City, CA) 373A automatic DNA sequencer (Stretch model).

Phylogenetic analysis

Phylogenetic relationships of the new viruses were estimated from sequence comparisons with previously reported representatives of group M. Nucleotide sequences were aligned by CLUSTAL W[32] with minor manual adjustments, bearing in mind the protein sequences. Sites where there was a gap in any of the sequences, as well as areas of uncertain alignment, were excluded from all sequence comparisons. Phylogenetic trees were constructed by the neighbor-joining method and reliability of the branching patterns assessed using the bootstrap approach implemented by CLUSTAL W. Genetic distances were calculated by the Kimura two-parameter method.[33]

RESULTS

Study population

A total of 330 HIV-1-positive samples was collected during an epidemiologic survey conducted in April 1996 and previously published.[23] Among the 330 HIV-1-positive samples, only 1 had antibodies to HIV-1 group O and the genetic characterization of this sample has been previously described.[34] Only 252 samples were sufficiently well preserved for efficient PCR amplification to identify the genetic subtype.

Overall, 160 HIV-1-positive samples obtained from female sex workers (FSWs) were genetically characterized: 64 from Lagos, 66 from Maiduguri, and 30 from Calabar/Ikom. All women were full-time, low-income FSWs, with a mean number of clients per week of 3.5 (2–7), 5.7 (2–20), and 2.9 (2–20) for FSWs from Lagos, Calabar/Ikom, and Borno, respectively. The mean age of these women was 28.3 (18–60) years in Lagos, 25 (18–40) years in Calabar/Ikom, and 25.7 (18–43) years in Maiduguri. Forty-one outpatients, suspected to have AIDS, were studied: 15 from Lagos, 15 from Maiduguri, 10 from Kano, and 1 from Calabar. One-third of this population group were female patients and the mean age of all the patients together was 30.3 years old and ranged from 12 to 55 years. A total of 27 tuberculosis patients, mainly male patients with a mean age of 31.7 years, ranging from 15 to 62 years, was included from Lagos ($n = 15$), Maiduguri ($n = 7$), and Calabar ($n = 5$). An additional 25 samples obtained from miscellaneous population groups were also genetically characterized: 6 STD patients from Lagos ($n = 5$) and Calabar ($n = 1$), 7 blood donors from Lagos, 6 pregnant women (1 from Lagos, 4 from Kano, and 1 from Calabar), 1 truck driver from Calabar, and 7 individuals living in a rural community (Gadar Tumburuwa in Kano State).

Genetic subtyping of group M HIV-1 isolates in the envelope

The HIV-1 group M strains were genetically typed by HMA in the V3–V5 region of the envelope region. On the 252 samples studied, only 180 samples could be amplified with the primers developed for HMA (ED3–ED14, ED5–ED12, ES7–ES8, and ED31–ED33[30]); the genetic subtype was identified by HMA for 166 samples while 14 were indeterminate by this assay. Almost one-third of the samples, 72 (28.5%), could not be amplified with the HMA primers, and thus a nested PCR with newly designed primers S1 and S2 (outer) and ED5nig and ES8nig (inner) was used. With these primers a 1100-bp fragment, spanning the V2–V5 region, was obtained and subsequently sequenced. The genetic subtype was identified by phylogenetic analysis of the Nigerian samples together with reference strains from each subtype.

By this method, the genetic subtype was identified for an additional 50 samples. A total of 22 (8.7%) samples was still negative with the new primers and the HMA primers, despite the fact that HIV-1 DNA was present in the samples since PCR with the diagnostic Hpol primers[35] was positive for all of them.

Table 1 summarizes the genetic subtypes identified by the different techniques. The predominant *env* subtypes were A followed by G. By HMA 76.6% of the samples were identified as subtype A and 23.4% as subtype G. The majority of samples found indeterminate by HMA or that could not be amplified with the HMA primers were classified as subtype G in the envelope, 57 and 74%, respectively, which shows that the overall prevalence of subtype A in Nigeria has decreased to 61.3% and that the prevalence of subtype G has increased to 37%. Only one subtype C and two subtype D samples were identified, and two of the HMA-indeterminate samples were identified as subtype F, more precisely subclade F2.[36,37]

Geographic distribution of HIV-1 env genetic subtypes in Nigeria

Among the 252 samples analyzed, 107 were from Lagos (southwest), 37 from Calabar (southeast), 20 from Kano (north), and 88 from Maiduguri (northeast). Figure 1 shows the regional distribution of HIV-1 subtypes in Nigeria. From these data it is clear that the genetic subtype distribution differs between the north and the south ($p < 0.001$). Subtype A is more prevalent in the south, about 70% in Lagos and Calabar, whereas a quarter of the samples was classified as subtype G in these states. In contrast, subtype G is predominant in the north, representing 58% of the samples in Kano. In the northeastern region, Maiduguri, almost similar proportions of subtype A and G were seen, 49 and 47.4%, respectively. This geographical difference was not related to different population groups tested in different regions. Female sex workers were tested in Maiduguri ($n = 59$), Calabar ($n = 22$), and Lagos ($n = 55$), and similar subtype A and G prevalences were observed in this particular population group as compared with the overall subtype distribution considering the overall study population. Among female sex workers, the subtype distribution for A and G, respectively, was as follows: 49% for both in Maiduguri, 67.3 and 27.3% in Lagos, and 73 and 23% in Calabar.

Phylogenetic analysis of the HIV-1 group M sequences in the envelope

Figure 2 shows the phylogenetic tree of envelope sequences covering the V2–V5 region, and Table 2 summarizes the demographic characteristics and subtype designations from the viruses represented in the phylogenetic tree. The phylogenetic analysis shows that within the subtype A viruses from Nigeria, two major clusters can be identified, one with strains ($n = 14$) clustering with the A/G-IBNG prototype strain (a complex A/G recombinant strain isolated from a Nigerian patient[26]), and a second group ($n = 6$) of viruses clustering with the nonrecombinant subtype A prototype strains (U-455, 92UG037, SF170, and KENYA). Within subtype A an additional cluster of two strains was seen: MACSW-83 from our study with CA-1 from Cameroon. The CA-1 strain has been described as an A/G recombinant, but different from IBNG.[28] Finally, the CTB30 strain formed a separate single branch. The subclusters do not correspond to strains isolated in a particular geographic region; the two major clusters contain viruses collected in different regions of the country. AG-IBNG viruses were present in FSWs from the northern and southern parts of the country.

Within subtype G, three major clusters can be identified among the Nigerian sequences. One group of viruses clusters with the prototype G/A-92NG003 virus, suggested to be GA-NG003 viruses, supported by 94% of the bootstrap values. A second group of viruses, suggested to be G′, forms a well-defined subcluster, supported by 97 of 100 bootstrap values. This G′ group includes the NG1939, NG1928, and NG1929[28] strains but none of the previously described subtype G prototype strains. Finally, a third group of viruses clustering with the majority of the prototype subtype G strains available in the database is observed. As is observed among the subtype A viruses, the subtype G clusters are not related to the geographic origin of the samples. The G, G′, and GA-NG003 viruses are isolated in the four regions from Nigeria studied. These viruses have also been observed in asymptomatic female sex workers and also in AIDS patients.

None of the GA-92NG003 strains could be amplified with the HMA primers. Among the G′ strains from our study, only one-third of them could be amplified with HMA primers; however, they were indeterminate by HMA and their genetic subtype was identified by sequence analysis. On the other hand, all the subtype G samples from our study clustering with the previously described subtype G prototype strains available in the database were identified by HMA.

Genetic distances were calculated by the Kimura two-parameter method, using the same alignments as used to construct the phylogenetic tree represented in Fig. 2. The overall genetic distance within subtype G was 12.8%, but within the GA-NG003 and G′ subclusters, consisting predominantly of Nigerian strains, the distances were lower. Mean distances of 8.5 and 8.3% were seen, respectively, for the GA-NG003 and G′ subgroups whereas for the subgroup containing the majority of the G references from the database and originating from different African countries the mean distance was 13.4%. Although the mean distance within subtype A was 13.3%, within the AG-IBNG cluster it was 10.5%.

TABLE 1. GENETIC SUBTYPES IDENTIFIED BY VARIOUS TECHNIQUES[a]

Technique used to identify subtype	*Genetic subtype*				
	A [n (%)]	*C* [n (%)]	*D* [n (%)]	*F* [n (%)]	*G* [n (%)]
HMA[b] ($N = 166$)	124 (74.6)	1 (0.6)	2 (1.2)	—	39 (23.4)
HMA indeterminate, sequenced[b] ($N = 14$)	4 (28.5)	—	—	2 (14.2)[c]	8 (57.1)
Sequenced, new primers[d] ($N = 50$)	13 (26)	—	—	—	37 (74)
Total ($N = 230$):	141 (61.3)	1 (0.4)	2 (0.8)	2	84 (37.5)

[a]Genetic subtypes were identified by HMA and/or sequencing in the V3–V5 region of the envelope and by sequencing of the V2–V5 region of gp120 for the samples that could not be amplified with the primers previously described for HMA.[30]
[b]Genetic subtype identified in V3–V5 region of gp120.
[c]Strains belong to subclade F2.[36,37]
[d]Genetic subtype identified in V2–V5 region of gp120.

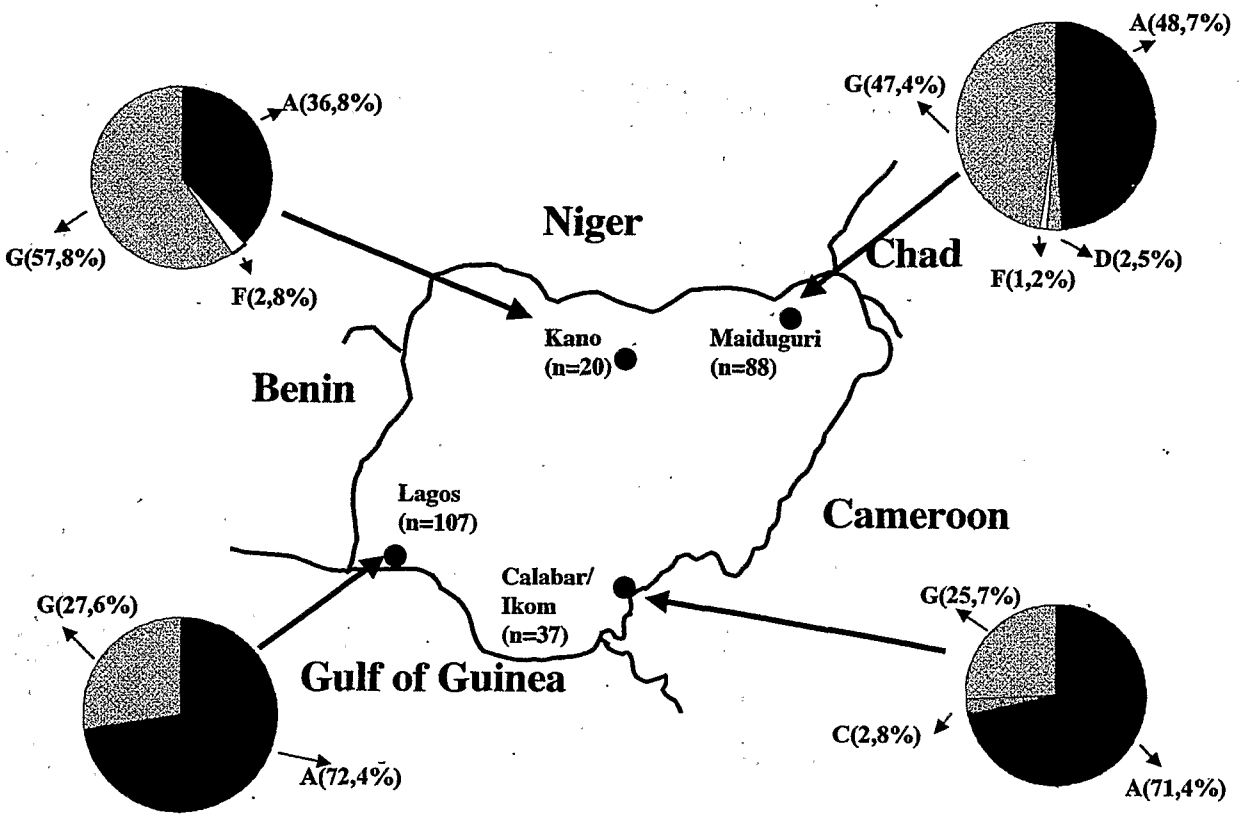

FIG. 1. Distribution of HIV-1 *env* genetic subtypes in various geographic locales from Nigeria.

Genetic subtypes in the gag *and envelope regions*

To determine the proportion of recombinant viruses that circulate in Nigeria, 37 samples were sequenced in the p24 region from the *gag* gene. Among these 37 samples, the genetic subtype in the envelope was characterized either by HMA (n = 10) or by sequencing (n = 27). Table 3 summarizes the demographic characteristics and the detailed subtype designations of the samples simultaneously characterized in *env* and *gag*. Overall, 13 (35%) of the 37 samples had discordant subtype designations between *env* and *gag*. Twenty-three of 37 samples were subtype G in the envelope and 14 were subtype A. However, only 14 of the 23 *env* G samples were also classified as subtype G in *gag*; the remaining samples were either subtype A (n = 7), subtype D (n = 1), or unclassified (n = 1) in the *gag* region. For the 14 *env* subtype A samples, 10 were subtype A in *gag* and 4 were subtype G in *gag*.

Figure 3 shows the phylogenetic tree analysis of the p24 sequences. Similar to the envelope sequences, subclusters were seen among the subtype A p24 sequences. The majority of the *gag* subtype A sequences, 12 of 17, clustered with the A/G-IBNG strain. Only two subclusters were identified among p24 subtype G sequences, whereas in the envelope three distinct subclusters were seen. In *gag* we still have the cluster of Nigerian strains with the prototype subtype G strains from the database, and another group of strains clustering with the GA-NG003 prototype virus. The G′ cluster observed among the envelope sequences is not seen among the p24 sequences; all the G′ envelope sequences cluster with the GA-NG003 strain in *gag*. From the 27 strains for which sequence data are available in *gag* and *env*, we can see that even the recombinant AG-IBNG and GA-NG003 strains are involved in recombination events.

DISCUSSION

The major goal of this study was to determine the prevalence and geographic distribution of the genetic subtypes of HIV-1 in selected populations in Nigeria. Our data show that in Nigeria, the AIDS epidemic is caused mainly by two genetic subtypes, subtypes A and G. However, subtype A was predominant in the southern part of the country, while subtype G was more frequent in the north, a more rural area compared with the south. We previously documented regional differences in relation to HIV prevalences. North–south differences were particularly noticed in FSWs and tuberculosis patients, with significantly higher prevalences in the north than in the south.[23] These findings suggest a geographic variation in the timing and severity of the AIDS epidemic in Nigeria, where an emerging HIV epidemic in the rural areas was described and a growing increase in the HIV prevalence in all population groups was documented.[23]

More than 30% of the samples characterized in *gag* and *env* had discordant subtypes between these two genomic regions.

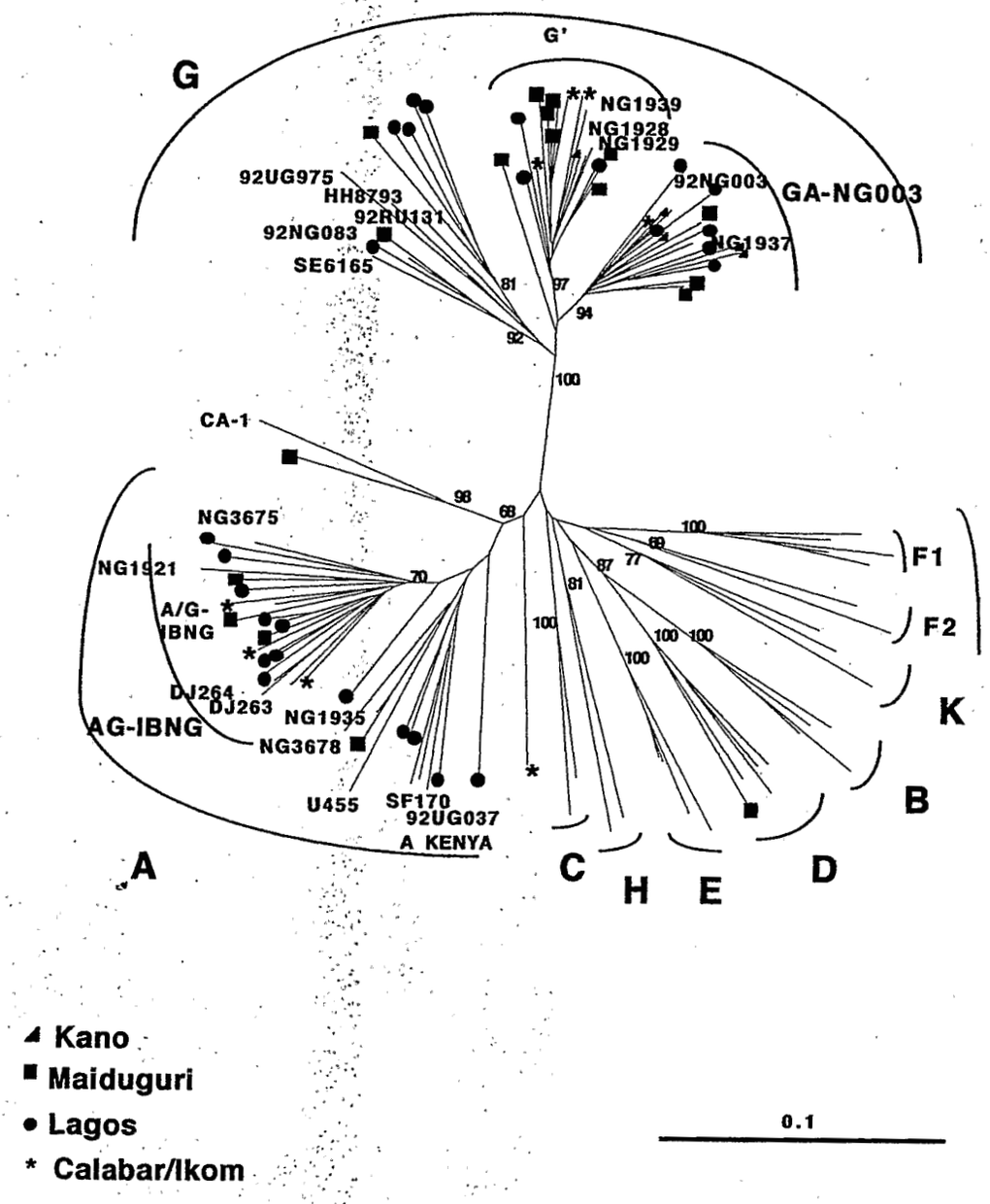

FIG. 2. Phylogenetic tree based on 849 unambiguously aligned nucleotides from the V2–V5 *env* region of the new HIV-1 isolates and reference strains representing the different genetic subtypes: A-U455, A-Kenya, A-92UG037, AG-IBNG, AG-DJ263, AG-DJ264, B-RF, B-OYI, B-JRFL, B-HBX2, C-ETH2220, C-92BR025, D-NDK, D-Z2Z6, D-94UG114, D-ELI, E-90CR402, E-93TH253, E-TN235, E-CM240, F1-93BR020, F1-BZ163, F1-BZ126, F1-95FRMP84, F1-96FRMP411, F2-95CMMP255, F2-95CMMP257, G-92UG975, G-92RU131, G-HH8793, G-SE6165, G/A-92NG083, G/A-92NG003, H-90CF056, H-BCB79, K-96CMMP535, K-97ZREQTB11, and K-ZR36. The analysis was performed as described in Materials and Methods. F1 and F2 correspond to subclades within subtype F,[36] and subtype K has been described.[37] NG1939, NG1929, NG1928, NG1937, NG3675, NG1921, NG1935, and NG3678 are strains from Nigeria.[28]

In addition, an important proportion of the subtype A sequences clustered either in *env* or in *gag* with the recombinant AG-IBNG strain. The IBNG strain was initially isolated from an individual in Ibadan and is a complex mosaic virus with alternating fragments of subtype A and G sequences.[25,38,39] We and others documented that viruses clustering with the AG-IBNG strain have a mosaic A/G genomic structure similar to that of IBNG[26] (Montavon *et al.*[40]). Therefore we can extrapolate that some of the *env* or *gag* subtype A viruses in Nigeria are also complex AG-IBNG recombinants. The possibility that the subtype G strains also contain segments of a different subtype in the unsequenced regions remains also. The two full-length Nigerian subtype G prototype strains also contain small subtype A fragments in some parts of their genome[27] and several of our subtype G strains cluster especially with the GA-92NG003 strain. One study, performed with a limited number of samples from

TABLE 2. DEMOGRAPHIC CHARACTERISTICS, SUBTYPE DESIGNATIONS, AND GENBANK ACCESSION NUMBERS OF THE VIRUSES REPRESENTED IN THE PHYLOGENETIC TREE OF THE ENVELOPE SEQUENCES[a]

Isolate	*Age (years)*	*Sex*	*Population group*	*Clinical status*	*Geographic origin*	*Env subtype*	*Accession number*
96NG-IKCSW 013	30	F	FSW	AS	Cross-River	AG-IBNG	AJ 389764
96NG-CCSW 6	22	F	FSW	AS	Cross-River	AG-IBNG	AJ 389758
96NG-IKCSW 015	31	F	FSW	AS	Cross-River	AG-IBNG	AJ 389765
96NG-LCSW 194	21	F	FSW	AS	Lagos	AG-IBNG	AJ 389767
96NG-LCSW 173	24	F	FSW	AS	Lagos	AG-IBNG	AJ 389766
96NG-LCSW 275	20	F	FSW	AS	Lagos	AG-IBNG	AJ 389769
96NG-CSW 099	25	F	FSW	AS	Lagos	AG-IBNG	AJ 389763
96NG-LCSW 203	35	F	FSW	AS	Lagos	AG-IBNG	AJ 389768
96NG-CSW 015*	20	F	FSW	AS	Lagos	AG-IBNG	AJ 389760
96NG-CSW 026	25	F	FSW	AS	Lagos	AG-IBNG	AJ 389760
96NG-CSW 004	25	F	FSW	AS	Lagos	AG-IBNG	AJ 389761
96NG-MACSW 100	19	F	FSW	AS	Maiduguri	AG-IBNG	AJ 389770
96NG-MACSW 130	32	F	FSW	AS	Maiduguri	AG-IBNG	AJ 389771
96NG-MACSW 114	22	F	FSW	AS	Maiduguri	AG-IBNG	AJ 389777
96NG-CSW 008	27	F	FSW	AS	Lagos	A	AJ 389762
96NG-CSW 133	25	F	FSW	AS	Lagos	A	AJ 389772
96NG-LCSW 174	29	F	FSW	AS	Lagos	A	AJ 389774
96NG-LUTOP 080		F	Patient[b]	AIDS	Lagos	A	AJ 389776
96NG-LUTBD 083	28	M	Blood donor	AS	Lagos	A div	AJ 389775
96NG-MACSW 041	20	F	FSW	AS	Maiduguri	A	AJ 389778
96NG-MACSW 061	31	F	FSW	AS	Maiduguri	A	AJ 389779
96NG-MACSW 083	21	F	FSW	AS	Maiduguri	A-CA1	AJ 389780
96NG-CTB30			Tuberculosis	AIDS	Cross-River	A??	AJ 389773
96NG-MAOP	28	M	Patient[b]	AIDS	Maiduguri	D	AJ 389781
96NG-IKCSW 022	19	F	FSW	AS	Cross-River	G′	AJ 389733
96NG-IKCSW 002	30	F	FSW	AS	Cross-River	G′	AJ 389732
96NG-IKCSW 005	20	F	FSW	AS	Cross-River	G′	AJ 389735
96NG-KOP 027	39	M	Patient[b]	AIDSS	Kano	G′	AJ 389738
96NG-CSW 089	25	F	FSW	AS	Lagos	G′	AJ 389731
96NG-CSW 112	18	F	FSW	AS	Lagos	G′	AJ 389726
96NG-CSW 046	19	F	FSW	AS	Lagos	G′	AJ 389730
96NG-MACSW 024	22	F	FSW	AS	Maiduguri	G′	AJ 389750
96NG-MACSW 001	27	F	FSW	AS	Maiduguri	G′	AJ 389745
96NG-MACSW 020	28	F	FSW	AS	Maiduguri	G′	AJ 389749
96NG-MACSW 085	27	F	FSW	AS	Maiduguri	G′	AJ 389754
96NG-MACSW 031	21	F	FSW	AS	Maiduguri	G′	AJ 389751
96NG-MACSW 125	25	F	FSW	AS	Maiduguri	G′	AJ 389746
96NG-IKCSW 021	21	F	FSW	AS	Cross-River	GA-NG003	AJ 389734
96NG-KOP 009	40	M	Patient[b]	AIDS	Kano	GA-NG003	AJ 389723
96NG-KOP 035	25	M	Patient[b]	AIDS	Kano	GA-NG003	AJ 389739
96NG-KGT 021	24	M	Truck driver	AS	Kano rural	GA-NG003	AJ 389737
96NG-KGT 001	22	F	FSW	AS	Kano rural	GA-NG003	AJ 389736
96NG-LCSW 218	26	F	FSW	AS	Lagos	GA-NG003	AJ 389742
96NG-CSW 130	24	F	FSW	AS	Lagos	GA-NG003	AJ 389728
96NG-CSW 127	18	F	FSW	AS	Lagos	GA-NG003	AJ 389727
96NG-CSW 109	18	F	FSW	AS	Lagos	GA-NG003	AJ 389724
96NG-CSW 138	25	F	FSW	AS	Lagos	GA-NG003	AJ 389729
96NG-YOP 069	NA	NA	Patient[b]	AIDS	Lagos	GA-NG003	AJ 389757
96NG-MACSW 018	24	F	FSW	AS	Maiduguri	GA-NG003	AJ 389748
96NG-MACSW 080	22	F	FSW	AS	Maiduguri	GA-NG003	AJ 389753
96NG-MACSW 127	30	F	FSW	AS	Maiduguri	GA-NG003	AJ 389747
96NG-LCSW 189	30		FSW	AS	Lagos	G	AJ 389740
96NG-LUTBD 067	23	M	Blood donor	AS	Lagos	G	AJ 389743
96NG-YOP 013	45	M	Patient[b]	AIDS	Lagos	G	AJ 389744
96NG-LCSW 171	26		FSW	AS	Lagos	G	AJ 389741
96NG-CSW 106	32		FSW	AS	Lagos	G	AJ 389725
96NG-MAOP 005	40	M	Patient[b]	AIDS	Maiduguri	G	AJ 389756
96NG-MAOP 011			Patient[b]	AIDS	Maiduguri	G	AJ 389755
96NG-MACSW 065	25		FSW	AS	Maiduguri	G	AJ 389752

[a]See Fig. 2.
[b]Patient attending hospital with clinical signs of AIDS.

TABLE 3. DEMOGRAPHIC CHARACTERISTICS, SUBTYPE DESIGNATIONS, AND GENBANK ACCESSION NUMBERS OF THE VIRUSES REPRESENTED IN THE PHYLOGENETIC TREE OF THE *gag* SEQUENCES[a]

Isolate	*Age (years)*	*Sex*	*Population group*	*Clinical status*	*Geographic origin*	gag *subtype*	env *subtype*	*Accession number*
96NG-LCSW 193	29	F	FSW	AS	Lagos	A	G[b]	AJ 269994
96NG-LUTBD 067	23	M	Blood donor	AS	Lagos	A	G	AJ 269979
96NG-MAOP 011		F	Patient[c]	AIDS	Maiduguri	A	G	AJ 270013
96NG-MACSW 114	22	F	FSW	AS	Maiduguri	A	AG-IBNG	AJ 270001
96NG-MACSW 127	30	F	FSW	AS	Maiduguri	A	GA-NG003	AJ 270004
96NG-KGT 007	40	M	General population	AS	Kano rural	AG-IBNG	A[b]	AJ 269988
96NG-LCSW 165	30	F	FSW	AS	Lagos	AG-IBNG	A[b]	AJ 269992
96NG-LUTBD 044	31	M	Blood donor	AS	Lagos	AG-IBNG	A[b]	AJ 269998
96NG-MACSW 005	30	F	FSW	AS	Maiduguri	AG-IBNG	A[b]	AJ 270010
96NG-MACSW 115	31	F	FSW	AS	Maiduguri	AG-IBNG	A[b]	AJ 270002
96NG-MHTB 183	33	F	Tuberculosis	AIDS	Lagos	AG-IBNG	A[b]	AJ 270014
96NG-LCSW 275	20	F	FSW	AS	Lagos	AG-IBNG	AG-IBNG	AJ 269997
96NG-MACSW 100	19	F	FSW	AS	Maiduguri	AG-IBNG	AG-IBNG	AJ 270000
96NG-CTB 030	25	M	TB	AIDS	Calabar	AG-IBNG	A-div	AJ 269982
96NG-KGT 001	22	F	General population	AS	Kano rural	AG-IBNG	GA-NG003	AJ 269987
96NG-LCSW 171	26	F	FSW	AS	Lagos	AG-IBNG	G	AJ 269993
96NG-YOP 013	45	M	Patient[c]	AIDS	Lagos	AG-IBNG	G	AJ 269999
96NG-IKCSW 055	20	F	FSW	AS	Calabar	D	G′	AJ 269985
96NG-IKCSW 023	20	F	FSW	AS	Calabar	GA-NG003	G[b]	AJ 269984
96NG-CSW 072	22	F	FSW	AS	Lagos	GA-NG003	G′[d]	AJ 269981
96NG-KAN 094	20	F	Pregnant woman	AS	Kano	GA-NG003	G′[d]	AJ 269986
96NG-MACSW 034	27	F	FSW	AS	Maiduguri	GA-NG003	G′[d]	AJ 270008
96NG-MACSW 138	22	F	FSW	AS	Maiduguri	GA-NG003	G′[d]	AJ 270005
96NG-MACSW 020	28	F	FSW	AS	Maiduguri	GA-NG003	G′	AJ 270006
96NG-MACSW 031	21	F	FSW	AS	Maiduguri	GA-NG003	G′	AJ 270007
96NG-IKCSW 021	21	F	FSW	AS	Calabar	GA-NG003	GA-NG003	AJ 269983
96NG-KOP 035	25	M	Patient[c]	AIDS	Kano	GA-NG003	GA-NG003	AJ 269989
96NG-YOP 069	NA	NA	Patient[c]	AIDS	Lagos	GA-NG003	GA-NG003	AJ 270015
96NG-KOP 052	27	M	Patient[c]	AIDS	Kano	GA-NG003	GA-NG003[d]	AJ 269990
96NG-MACSW 041	20	F	FSW	AS	Maiduguri	GA-NG003	A	AJ 270009
96NG-MACSW 083	21	F	FSW	AS	Maiduguri	GA-NG003	A	AJ 270012
96NG-LCSW 250	35	Male client	FSW	AS	Lagos	G	G[b]	AJ 269996
96NG-MACSW 126	20	F	FSW	AS	Maiduguri	G	G[b]	AJ 270003
96NG-CSW 106	32	F	FSW	AS	Lagos	G	G	AJ 269980
96NG-MACSW 065	25	F	FSW	AS	Maiduguri	G	G	AJ 270011
96NG-KSTD 006	28	M	STD	AS	Kano	G??	GA-NG003[d]	AJ 269991
96NG-LCSW 202	25	F	FSW	AS	Lagos	G	AG-IBNG[d]	AJ 269995

[a]See Fig. 3.
[b]Genetic subtype identified by HMA in the envelope.
[c]Patient attending the hospital with clinical signs of AIDS.
[d]Genetic subtype was identified by phylogenetic tree analysis of a 700-bp fragment in the envelope covering V3–V5.

Nigeria and based on gp160 and partial *gag* sequences, documented that more than half the strains were recombinants, and different A/G recombinant structures were documented.[28]

Compared with other West African countries, lower subtype A prevalences and especially higher subtype G prevalences were seen in Nigeria. It was also interesting to observe that despite the proximity of Nigeria to Cameroon, where all the different HIV-1 groups and subtypes have been documented,[41,42] only sporadic cases of subtype C, D, and F (more specifically F2)[36] *env* subtypes have been detected.

Despite the size of Nigeria and the fact that it is the most populous African country, only two genetic subtypes, A and G, are predominant in Nigeria, but many different A/G recombinant genomic structures have been documented. It can be said that the HIV epidemic is rapidly spreading in all population groups of Nigeria, but the fastest growing rate continues to be seen in female sex workers.[23] We can assume that initially subtypes A and G have been introduced in this latter population group in Nigeria. Their high-risk behavior allows multiple superinfections with other HIV strains and subtypes and can therefore explain the numerous different A/G recombinant genomic structures observed in Nigeria.

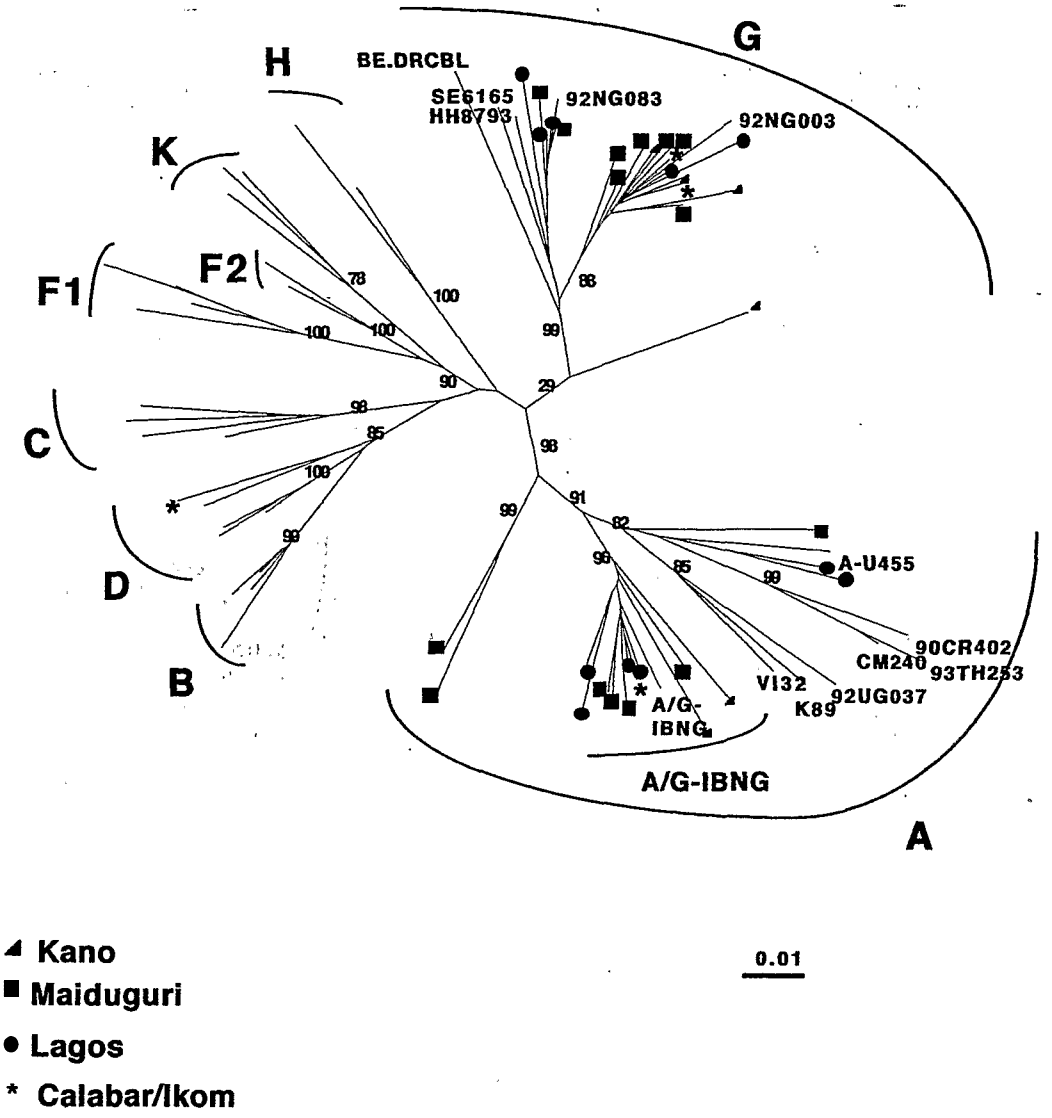

FIG. 3. Phylogenetic tree based on 612 unambiguously aligned nucleotides from the p24 *gag* region of the new HIV-1 isolates and reference strains representing the different genetic subtypes: A-U455, A-K89, A-92UG037, AVI32, AG-IBNG, AG-DJ263, AG-DJ264, B-RF, B-OYI, B-JRFL, C-ETH2220, C-92BR025, C-ZAM18, C-UG268, D-NDK, D-Z2Z6, D-94UG114, D-ELI, E-90CR402, E-93TH253, E-TN235, E-CM240, F1-93BR020, F1-VI174, F1-VI69, F1-BZ126, F1-95FRMP84, F1-96FRMP411, F2-95CMMP255, F2-95CMMP257, G-92UG975, G-92RU131, G-HH8793, G-SE6165, G/A-92NG083, G/A-92NG003, H-90CF056, H-BCB79, K-96CMMP535, K-97ZREQTB11, and K-ZR36. The analysis was performed as described in Materials and Methods. F1 and F2 correspond to subclades within subtype F,[36] and subtype K has been described.[37]

In conclusion, we observed a limited number of HIV-1 subtypes circulating in Nigeria, with subtypes A and G being the major subtypes responsible for the HIV-1 epidemic. However, the high rate of recombinant viruses (A/G) and the different A/G recombinant structures indicate a complex pattern of HIV-1 viruses circulating in this country. Full-length genome sequencing will be necessary to find out to what extent viruses clustering with the GA-92NG003 strain have the same mosaic structure before we can consider that this is a circulating recombinant form. Further genetic analysis is also necessary to determine the precise genomic structure of the G′ viruses in the envelope. Recombinant viruses are already contributing substantially to the global pandemic, and the likelihood of generating recombinant viruses will only continue to increase as the different HIV-1 subtypes spread to all continents.[43–45] In a previous study we documented that even the complex AG-IBNG viruses are implicated in an intergroup (M/O) recombination event[46] and several viruses from Nigeria with discordant *gag* and *env* subtypes had *gag* sequences that clustered with the HIV-1_{IBNG} virus. It becomes more and more evident that the genetic subtype distribution is a dynamic and unpredictable process. More studies are needed in order to understand the role and implications of recombinant viruses in the global evolution of HIV.

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SEQUENCE DATA

The new sequences have been deposited in the GenBank Data Library under the following accession numbers: from AJ 269979 to AJ 270015 for the $p24^{gag}$ sequences and from AJ 389723 to AJ 389781 for the envelope sequences.

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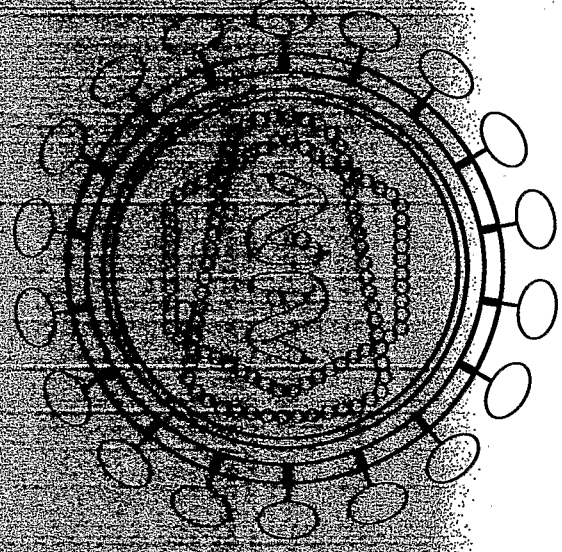

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