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Sequence Note

Identification of All HIV Type 1 Group M Subtypes in Senegal, a Country with Low and Stable Seroprevalence

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ABSTRACT

A total of 343 HIV-1-positive samples obtained between June 1996 and March 1999 was genetically characterized in the envelope region by HMA and/or sequencing. The *env* subtype distribution was as follows: 290 (84.6%) A, 22 (6.5%) B, 16 (4.7%) C, 8 (2.5%) D, 1 (0.03%) E, 1 (0.03%) F1, 4 (1.2%) G, and 1 (0.03%) H. For 77 samples the p24 region from the *gag* gene was also sequenced, and for 9 (11.6%) the subtypes between *env* and *gag* were different. Phylogenetic tree analysis showed the predominance of AG-IBNG-like viruses among *gag* and *env* subtype A sequences. HMA is relatively simple and requires less sophisticated technical facilities compared with sequencing, and in Senegal 323 (94.2%) of the 343 samples could be identified by this technique. However, in the actual configuration of the assay, discrimination between the recombinant AG-IBNG-like recombinant viruses, which are predominant in Senegal, and the nonrecombinant subtype A viruses is not possible.

SENEGAL IS LOCATED at the tip of West Africa and both AIDS viruses, HIV-1 and HIV-2, cocirculate. HIV-2 was initially described from individuals living in Senegal, and in 1985, HIV-1 was nonexistent in the original survey describing HIV-2 in commercial sex workers.[1,2] At present, in Senegal and in other West African countries, HIV-2 prevalences remain low and stable or have even decreased, whereas HIV-1 has become increasingly more common.[3] Compared with neighboring countries in West Africa, HIV seroprevalences are relatively low and stable over time in the general population and in risk groups in Senegal.[4] However, the country has an important trading activity and travel links with many other West and Central African countries, where high seroprevalence rates have been reported in high-risk groups.[5,6] A case of HIV-1 group O infection, with an epidemiological link to Cameroon, has been reported.[7] Since the emergence of the AIDS epidemic, data on the HIV-1 genetic subtypes in Senegal are still limited. We report here prevalences of different genetic subtypes of HIV-1 circulating in Senegal among HIV-1-infected patients seen between June 1996 and March 1999 in the three major hospitals in Dakar, the capital city.

A total of 343 HIV-1-positive samples, obtained from patients attending a hospital for the first time with either clinical signs of AIDS-related disease or referred to these centers after a positive screening test for HIV, were genetically characterized in the envelope region. For 200 of them, in addition to the clinical examination, demographic characteristics and risk factors for HIV infection are available. Among these 200 patients, the mean age was 34 years and ranged from 17 to 68, more than 95% were Senegalese, and about 60% were women. The main route of HIV transmission was heterosexual contact, homosexual transmission was documented in 1.5% of the cases,

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and transmission via blood transfusion was also documented in 1.5% of the cases studied. The mean $CD4^+$ cell count was 234 cells/mm^3 and the majority (53%) of the patients were at stage B according to Centers for Disease Control (CDC) classification, 15% were asymptomatic or stage A, and 32% had developed AIDS and were at stage C.

From each individual a 10-ml whole-blood sample was collected in an EDTA tube. Peripheral mononuclear blood cells were separated from plasma by Ficoll gradient centrifugation. Plasma and cell pellets were stored at −20°C. DNA was extracted from the dry cell pellets with an IsoQuick isolation kit (Microprobe, Garden Cove, CA) or a DNA extraction kit from Qiagen (Courtabeauf, France). A nested polymerase chain reaction (PCR) technique was used to amplify the V3–V5 region of the envelope for a heteroduplex mobility assay (HMA) as previously described by Delwart *et al.*[8] The reference strains to form the heteroduplexes were those previously described.[8] Samples indeterminate by HMA were characterized by direct sequencing of the same region (V3–V5) as studied by HMA. For the *gag* gene, the p24 region was sequenced. Amplifications were done with previously described primers[9]: G00–G01 for the first round, and G60–G25 for the second round. The purified PCR products were directly sequenced. Cycle sequencing was performed by fluorescent dye terminator technology (dye terminator cycle sequencing with AmpliTaq DNA polymerase FS (Perkin-Elmer, Foster City, CA) according to the instructions of the manufacturer. Electrophoresis and data collection were done on an automated DNA sequencer (model 373A Stretch; Applied Biosystems, Foster City, CA). Nucleotide sequences were aligned with CLUSTAL W[10] with mi-

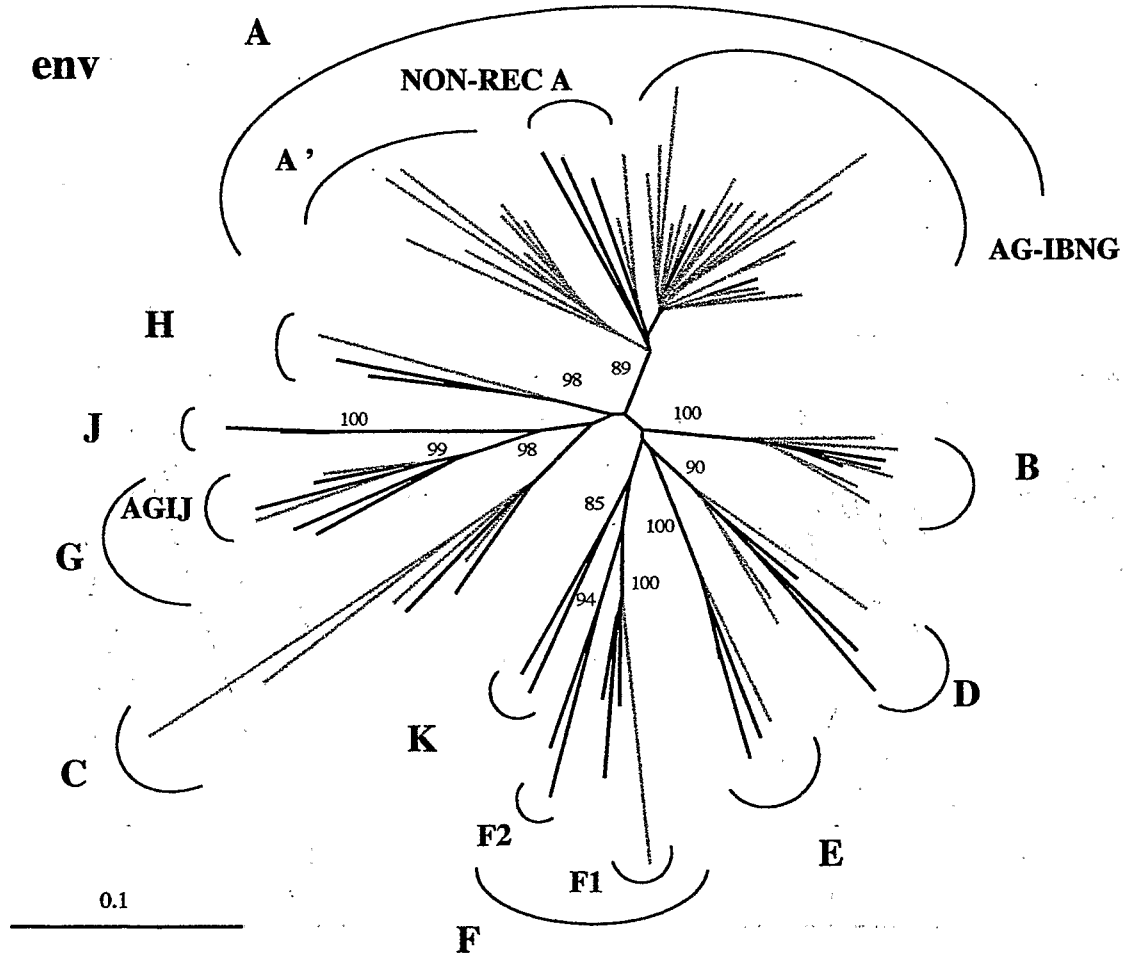

FIG. 1. Phylogenetic tree based on 486 unambiguously aligned nucleotides from the V3–V5 *env* region of the new HIV-1 isolates from Senegal and reference strains representing the different genetic subtypes: A-U455, A-SESO7253, A-92UG037, AG-IBNG, AG-DJ263, AG-DJ264, B-RF, B-JRFL, B-HBX2, C-ETH2220, C-92BR025, D-NDK, D-94UG114, D-ELI, E-90CR402, E-93TH253, E-CM240, F1-93BR020, F1-BE-VI850, F1-FI.FIN6393, F2-95CMMP255, F2-95CMMP257, G-HH8793, G-SE6165, G-92NG083, AGIJ-BFP90, AGIJ-95ML84, H-90CF056, H-BE.VI991, H-BE.VI997, J-SE91733, J-SE92809, K-96CM-MP535, K-97ZR-EQTB11. The analysis was performed as described, F1 and F2 correspond to subclades within subtype F,[13] and subtype K has been described.[14] The strains from Senegal are indicated in gray, the references in black.

TABLE 1. GENETIC SUBTYPES IN ENVELOPE REGION DETERMINED BY HMA AND/OR SEQUENCING, GENETIC SUBTYPES IDENTIFIED IN *gag* BASED ON PHYLOGENETIC TREE ANALYSIS OF p24 SEQUENCES AND GENBANK ACCESSION NUMBERS FOR THE VARIOUS *env* AND *gag* SEQUENCES[a]

				Accession number	
Name	*Env-HMA*	*Env-SEQ*	*Gag-SEQ*	*Gag*	*Env*
96SE-1018	A	-	AG-IBNG	AJ274542	-
96SE-1030	A	AG-IBNG	-	-	AJ272642
96SE-1047	A	-	AG-IBNG	AJ274547	-
96SE-1103	A	-	AG-IBNG	AJ274553	-
96SE-1121	A	-	AG-IBNG	AJ274559	-
96SE-1124	A	-	AG-IBNG	AJ274560	-
96SE-1142	A	-	AG-IBNG	AJ274563	-
96SE-1146	A	AG-IBNG	AG-IBNG	AJ274565	AJ272649
96SE-1149	A	-	AG-IBNG	AJ274566	-
97SE-1031	*A*	-	*G*	*AJ274544*	-
97SE-1041	A	A	A	AJ274545	AJ272643
97SE-1043	A	-	AG-IBNG	AJ274546	-
97SE-1055	A	-	A	AJ274548	-
97SE-1090	A	-	AG-IBNG	AJ274552	-
97SE-1107	A	A'	-	-	AJ272646
97SE-1113	A	-	AG-IBNG	AJ274555	-
97SE-1117	A	-	AG-IBNG	AJ274556	-
97SE-1127	A	A'	-	-	AJ272648
97SE-1145	A	-	A'	AJ274564	-
97SE-1148	A	AG-IBNG	-	-	AJ272650
97SE-1161	A	-	AG-IBNG	AJ274567	-
97SE-1171	A	-	AG-IBNG	AJ274568	-
97SE-1181	*A*	*A*	*H*	*AJ274570*	*AJ272651*
97SE-1183	A	AG-IBNG	AG-IBNG	AJ274571	AJ272652
97SE-1187	A	AG-IBNG	-	-	AJ272654
97SE-1197	A	-	AG-IBNG	AJ274573	-
97SE-1210	A	-	AG-IBNG	AJ274576	-
97SE-1216	A	A'	A'	AJ274577	AJ272656
97SE-1217	A	-	AG-IBNG	AJ274578	-
97SE-1219	A	-	AG-IBNG	AJ274579	-
97SE-1228	A	-	AG-IBNG	AJ274581	-
97SE-1254	A	-	A	AJ274583	-
97SE-13FANN	A	AG-IBNG	-	-	AJ272664
97SE-14FANN	*A*	-	*C*	*AJ274586*	-
97SE-25FANN	*A*	-	*C*	*AJ274590*	-
97SE-4HALD	*A*	-	*G*	*AJ274596*	-
97SE-6HPD	A	-	AG-IBNG	AJ274600	-
97SE-7HPD	A	-	AG-IBNG	AJ274615	-
97SE-Ko1051	A	-	AG-IBNG	AJ274614	-
98SE-106HPD	A	-	AG-IBNG	AJ274549	-
98SE-111HPD	*A*	-	*G*	*AJ274558*	-
98SE-120HPD	A	-	AG-IBNG	AJ274575	-
98SE-1238	A	AG-IBNG	-	-	AJ272660
98SE-1268	A	AG-IBNG	-	-	AJ272662
98SE-27HALD	A	-	AG-IBNG	AJ274591	-
98SE-46HALD	A	-	A'	AJ274595	-
98SE-61HPD	A	-	A	AJ274598	-
98SE-65HALD	A	-	AG-IBNG	AJ274616	-
98SE-72HPD	A	-	A	AJ274601	-
98SE-74HALD	A	-	AG-IBNG	AJ274602	-
98SE-79HALD	A	-	AG-IBNG	AJ274603	-
98SE-83HPD	A	-	AG-IBNG	AJ274605	-
98SE-87HALD	A	-	AG-IBNG	AJ274607	-
98SE-Ko1212	A	-	A'	AJ274613	-
99SE-123HPD	A	-	A'	AJ274582	-
99SE-125HPD	A	-	A'	AJ274584	-
99SE-128HPD	A	-	AG-IBNG	AJ274585	-

(*continued*)

TABLE 1. GENETIC SUBTYPES IN ENVELOPE REGION DETERMINED BY HMA AND/OR SEQUENCING, GENETIC SUBTYPES IDENTIFIED IN *gag* BASED ON PHYLOGENETIC TREE ANALYSIS OF p24 SEQUENCES AND GENBANK ACCESSION NUMBERS FOR THE VARIOUS *env* AND *gag* SEQUENCES[a] (*continued*)

				Accession number	
Name	*Env-HMA*	*Env-SEQ*	*Gag-SEQ*	*Gag*	*Env*
99SE-42FANN	A	-	AG-IBNG	AJ274593	-
99SE-84HALD	A	-	AG-IBNG	AJ274606	-
99SE-90HALD	A	-	AG-IBNG	AJ274608	-
99SE-92HALD	A	-	A'	AJ274609	-
99SE-95HALD	A	-	AG-IBNG	AJ274610	-
98SE-MP1211	A	AG-IBNG	AG-IBNG	AJ251056	AJ251056
98SE-MP1213	A	AG-IBNG	AG-IBNG	AJ251057	AJ251057
96SE-1109	B	-	B	AJ274554	-
97SE-1140	B	-	B	AJ274562	-
97SE-16FANN	B	-	B	AJ274587	-
97SE-17HALD	B	B	-	-	AJ272666
97SE-45HPD	B	B	-	-	AJ272672
98SE-1224	B	B	B	AJ274580	AJ272657
98SE-21HALD	B	-	B	AJ274588	-
98SE-22HALD	B	-	B	AJ274589	-
98SE-47HPD	B	B	-	-	AJ272673
98SE-59HPD	B	-	B	AJ274597	-
98SE-95HPD	B	-	B	AJ274611	-
98SE-Ko2010	B	B	-	-	AJ272681
96SE-1083	C	-	C	AJ274551	-
97SE-1084	C	C	-	-	AJ272645
97SE-1119	C	C	C	AJ274557	AJ272647
97SE-1176	*C*	-	*AG-IBNG*	*AJ274569*	-
97SE-1189	C	-	C	AJ274572	-
97SE-31FANN	*C*	-	*AG-IBNG*	*AJ274592*	-
96SE-1029	D	-	D	AJ274543	-
96SE-1116	D	-	D	AJ274541	-
97SE-1207	D	-	D	AJ274574	-
97SE-9HPD	D	D	D	AJ274612	AJ272678
98SE-112HPD	D	-	D	AJ274561	-
98SE-63HPD	G	-	G	AJ274599	-
97SE-1020	IND	A'	-	-	AJ272641
97SE-1078	*IND*	*AGIJ-BFP90*	*AGIJ-BFP90*	*AJ274550*	*AJ272644*
97SE-1186	IND	C	-	-	AJ272653
97SE-1212	IND	H	-	-	AJ272655
97SE-1234	IND	E	-	-	AJ272658
97SE-1235	IND	A'	-	-	AJ272659
97SE-1FANN	IND	AG-IBNG	-	-	AJ272668
97SE-36HPD	IND	D	-	-	AJ272671
97SE-7FANN	IND	AG-IBNG	AG-IBNG	AJ274604	AJ272676
97SE-8FANN	IND	F1	-	-	AJ272677
98SE-1267	IND	A'	-	-	AJ272661
98SE-12HALD	IND	C	-	-	AJ272663
98SE-14HALD	IND	AG-IBNG	-	-	AJ272665
98SE-18HALD	IND	AG-IBNG	-	-	AJ272667
98SE-32HALD	IND	A'	-	-	AJ272669
98SE-33HALD	IND	AG-IBNG	-	-	AJ272670
98SE-40HPD	IND	AGIJ-BFP90	-	-	AJ272640
98SE-58HPD	IND	AG-IBNG	-	-	AJ272674
98SE-62HPD	IND	D	-	-	AJ272675
98SE-Ko1011	IND	AG-IBNG	-	-	AJ272679

Abbreviation: IND, Indeterminate.

[a]In italic are shown the samples with discordant subtype designations between *env* and *gag*. A dash (-) indicates sequences not done or accession numbers not applicable.

nor manual adjustments, bearing in mind the protein sequences. The newly determined HIV-1 *env* and *gag* sequences were aligned with known HIV-1 sequences representing the different genetic subtypes. Phylogenetic trees, created by the neighbor-joining method, and the reliability of the branching orders, determined by the bootstrap approach, were implemented by CLUSTAL W. Genetic distances were calculated by the Kimura two-parameter method.[11]

Overall, the genetic subtype distribution among the 343 samples characterized in the envelope by HMA and/or sequencing was as follows: 290 (84.6%) were subtype A, 22 (6.5%) subtype B, 16 (4.7%) subtype C, 8 (2.5%) subtype D, 1 (0.03%) subtype E, 1 (0.03%) subtype F, 4 (1.2%) subtype G, and 1 (0.03%) subtype H. Of the 343 samples, 323 (94.2%) were characterized by HMA and 20 (5.8%) were indeterminate by this technique. Sequence and phylogenetic tree analysis of the HMA-indeterminate samples identified them as follows: 11 subtype A, 2 subtype C, 2 subtype D, 1 subtype E, 1 subtype F (F1), 2 subtype G but forming a separate cluster with the complex AGIJ-BFP90 and 95ML84 strains,[12] and 1 subtype H. No significant changes in subtype distribution were observed during the 3 years of follow-up (data not shown).

In addition, 23 samples identified by HMA were also sequenced in the envelope region, in order to verify the subtype obtained by HMA. All subtype identifications between HMA and sequencing were concordant. The phylogenetic tree analysis of the 43 envelope sequences (HMA indeterminates and HMA confirmations) is shown in Fig. 1.[13,14] From the phylo-

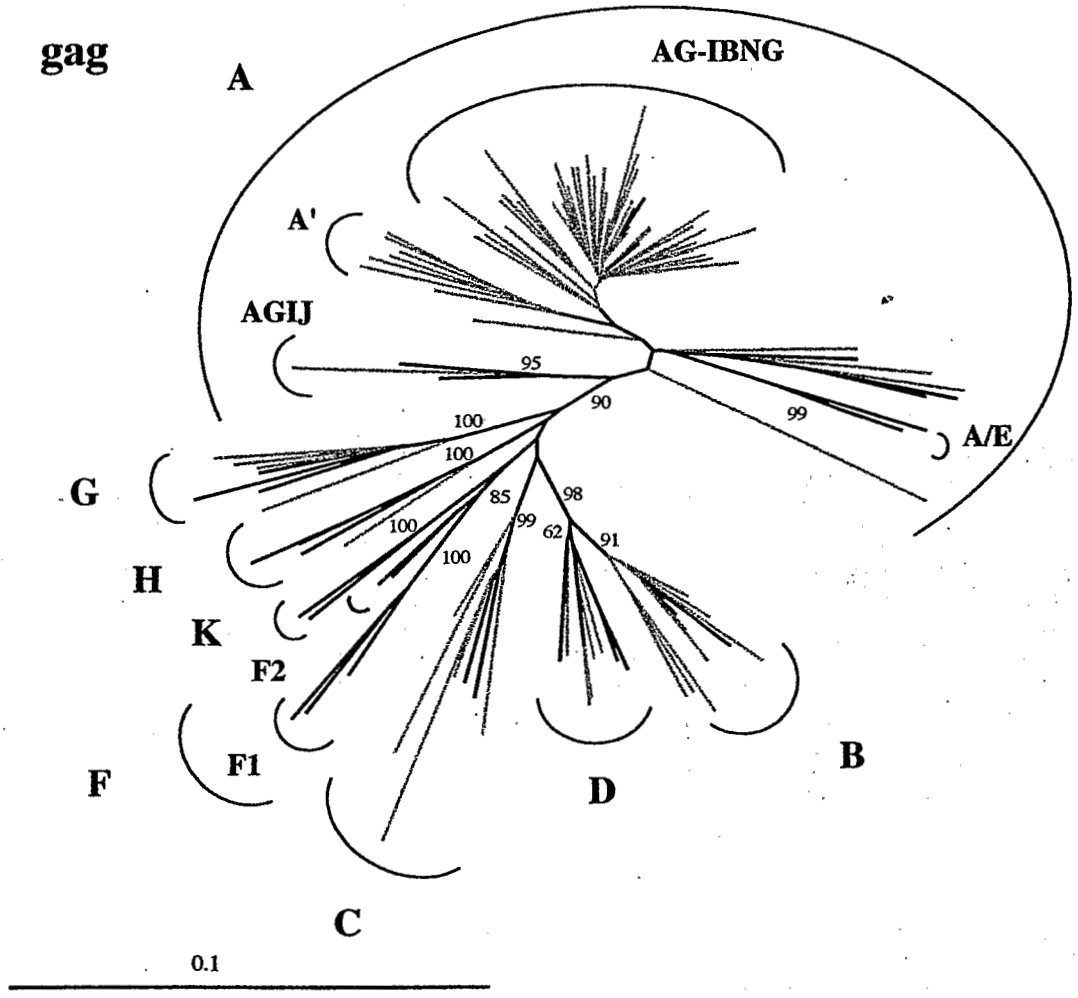

FIG. 2. Phylogenetic tree based on 600 unambiguously aligned nucleotides from the p24 *gag* region of the new HIV-1 isolates and reference strains representing the different genetic subtypes: A-U455, A-SESO7253, A-92UG037, AG-IBNG, AG-DJ263, AG-DJ264, B-RF, B-JRFL, B-HBX2, C-ETH2220, C-92BR025, D-NDK, D-94UG114, D-ELI, E-90CR402, E-93TH253, E-CM240, F1-93BR020, F1-BE-VI850, F1-FI.FIN6393, F2-95CMMP255, F2-95CMMP257, G-HH8793, G-SE6165, G-92NG083, AGIJ-BFP90, AGIJ-95ML84, H-90CF056, H-BE.VI991, H-BE.VI997, J-SE91733, J-SE92809, K-96CM-MP535, K-97ZR-EQTB11. The analysis was performed as described, F1 and F2 correspond to subclades within subtype F,[13] and subtype K has been described.[14] The strains from Senegal are indicated in gray, the references in black.

genetic tree it is evident that within subtype A, subclusters can be identified: 17 strains cluster with the previously described prototype strain of the circulating recombinant form AG-IBNG, a complex A/G recombinant HIV-1 virus[15]; 2 strains cluster with the nonrecombinant prototype subtype A strains; and 8 strains form a separate single cluster within subtype A, preliminarily called A′, and in this cluster only Senegalese sequences are present. Table 1 shows the results of the sequence analysis of the HMA-indeterminate samples, as well as the concordance between HMA and sequence data in the envelope region.

To estimate the proportion of recombinant HIV-1 viruses circulating in Senegal, a subset of 77 samples was also sequenced in the p24 region from the *gag* gene, and for 9 (11.6%) of these samples, the subtype distribution between *env* and *gag* was different. The following discordant profiles were seen: *gag* G/*env* A ($n = 3$), *gag* C/*env* A ($n = 2$), *gag* A/*env* C ($n = 2$), *gag* A/*env* G ($n = 1$), *gag* H/*env* A ($n = 1$). Table 1 shows the subtype designations between *env*, either done by HMA or sequencing, and the subtypes in *gag* after phylogenetic tree analysis, and the accession numbers for the *env* and *gag* sequences in GenBank. Figure 2 shows the phylogenetic tree analysis of the *gag* sequences. Like the envelope sequences, we can observe within subtype A a subcluster of viruses around the AG-IBNG strain, one around the nonrecombinant subtype A strains, and another cluster consisting only of Senegalese sequences, preliminarily called A′. One sample, 97SE-1216, was sequenced in *gag* and *env*, and clustered in both regions with the A′ group. The majority (42 of 54, 77.7%) of the *gag* subtype A sequences cluster with the AG-IBNG prototype virus, 7 sequences were in the A′ cluster, 3 clustered with the nonrecombinant A strains, 1 with the complex AGIJ-BFP90/95ML84 viruses, and 1 formed a separate, single branch.

As in the other West and West-Central African countries, subtype A is predominant[6,16]; but in the majority of these previous studies no distinction was made between nonrecombinant subtype A and AG-IBNG-like viruses. We and others have previously shown by full-length genome sequencing that viruses that cluster with the AG-IBNG prototype strain have a similar complex A/G mosaic genome.[15,17] Therefore we can conclude that AG-IBNG-like viruses are predominant in the AIDS epidemic in Senegal and it is important to consider the AG-IBNG-like viruses as a separate group of viruses. Studies of subtype distribution in Guinea-Bissau and Nigeria revealed that in these countries AG-IBNG-like viruses are also predominant among *env* or *gag* subtype A viruses.[18,19] It will also be important to better characterize the Senegalese subcluster, called A′, among the subtype A viruses, in further studies.

In contrast to many other African countries, a relatively high number of subtype B viruses circulates in Senegal, more than 6% of the samples tested. To verify any possible contamination with HMA plasmids, the majority of these samples have been sequenced either in *gag* or in *env*; phylogenetic tree analysis (Figs. 1 and 2) confirmed that none of these samples was closely related to any of the reference strains used in the HMA. Moreover, the genetic distances observed among these subtype B sequences are comparable to those observed among epidemiologically nonlinked individuals. For only a limited number of these 22 subtype B patients was information available to trace the origin of their HIV infection. For six of them a sexual encounter in Europe, or in Senegal with a European sex partner, was documented, and for eight an HIV-positive Senegalese sex partner initially infected in Europe or by a European contact was seen; among them two intrafamilial infections were found (a husband and his spouse and a couple with their daughter, 11 years old). For two subtype B patients no foreign contacts could be tracked and for six no information was available. These preliminary epidemiological data on the subtype B-infected patients from Senegal suggest an introduction of these viruses by travel to Europe or by Europeans in Senegal. Further follow-up is needed and the presence of HIV-1 subtype B should be further monitored, especially in high-risk populations around the tourist hotels in Senegal.

Despite the low and stable HIV prevalence in Senegal, less than 1% in the general population, all the different genetic subtypes are documented in Senegal. The HMA proved to be a useful method to allow genetic characterization of HIV-1 strains in a developing country. More than 94% of the samples were correctly identified, and only 5.8% were indeterminate. This method is relatively simple and requires less sophisticated technical facilities compared with sequencing but in the actual configuration of the HMA assay, discrimination between the nonrecombinant subtype A and the recombinant AG-IBNG viruses is not possible. The most common subtype in Senegal is the recombinant AG-IBNG form and therefore a modification of the HMA or the development of an other nonsophisticated technique is necessary to continue to monitor the subtype distribution in Senegal and other African countries with limited technical facilities.

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SEQUENCE DATA

The new sequences have been deposited in the GenBank Data Library under the accession numbers shown in Table 1.

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