Short Communication

Identification and Characterization of CRF02_AG, CRF06_cpx, and CRF09_cpx Recombinant Subtypes in Mali, West Africa

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Abstract

Multiple HIV-1 subtypes and circulating recombinant forms (CRFs) are known to cocirculate in Africa. In West Africa, the high prevalence of CRF02_AG, and cocirculation of subtype A, CRF01_AE, CRF06_cpx, and other complex intersubtype recombinants has been well documented. Mali, situated in the heart of West Africa, is likely to be affected by the spread of recombinant subtypes. However, the dynamics of the spread of HIV-1 recombinant subtypes as well as nonrecombinant HIV-1 group M subtypes in this area have not been systematically assessed. Herein, we undertook genetic analyses on full-length *env* sequences derived from HIV-1-infected individuals living in the capital city of Mali, Bamako. Of 23 samples we examined, 16 were classified as CRF02_AG and three had a subsubtype A3. Among the remaining HIV-1 strains, CRF06_cpx and CRF09_cpx were each found in two patients. Comparison of phylogenies for six matched *pol* and full-length *env* sequences revealed that two strains had discordant subtype/CRF designations between the *pol* and *env* regions: one had A3^{pol}CRF02_AG^{env} and the other had CRF02_AG^{pol}A3^{env}. Taken together, our study demonstrated the high prevalence of CRF02_AG and complexity of circulating HIV-1 strains in Mali. It also provided evidence of ongoing virus evolution of CRF02_AG, as illustrated by the emergence of more complex CRF02_AG/A3 intersubtype recombinants in this area.

THE REMARKABLE VIRAL DIVERSITY OF HIV-1 has resulted in the classification of nine subtypes (A to D, F to H, J, and K), five subsubtypes (A1, A2, A3, F1, and F2), and a number of intersubtype recombinant strains within the major group M.^{1,2} Some recombinants have already attained considerable prevalence and geographic coverage worldwide and become important strains in the pandemic. Among the known 37 circulating recombinant forms (CRFs, CRF01 to CRF37), at least eight are believed to originate from West and Central Africa, making this region a hotspot for intersubtype recombination.³ In West and Central Africa, CRF02_AG is of great

importance in the pandemic, accounting for the majority of new HIV-1 infections in this region.^{1,4-9} The CRF02_AG, like pure subtypes, is also involved in recombination events. Indeed, the emergence of more complex intersubtype recombinants containing fragments of CRF02_AG, such as CRF09_cpx, CRF30_0206, or the recently described CRF36_cpx and CRF37_cpx, has been reported in certain West and Central African countries.¹⁰⁻¹³

Mali, a nation with an estimated population of 12.3 million,¹⁴ is one of a few sub-Saharan African countries with a low prevalence of the HIV/AIDS epidemic in the general

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population. The adult (aged 15–49 years) HIV prevalence was estimated to be at 1.7% (1.3–2.1%) in 2005.¹⁵ Despite the low rate of HIV/AIDS infection in Mali today, a wider spread of the epidemic is currently anticipated. Poverty, poor health conditions, and certain cultural practices are among a variety of factors that could possibly contribute to the spread of HIV infection in Mali. Migration of seasonal workers to and from neighboring countries such as Côte d'Ivoire and Burkina Faso with higher rates of HIV infection (7.1% and 2.0%, respectively) is another potentially significant contributor to the spread of HIV infection in this country.¹⁵

Mali is a landlocked country and shares its borders with Senegal and Mauritania on the west; Algeria on the northeast; Niger on the east; and Burkina Faso, Côte d'Ivoire, and Guinea on the south. High rates of HIV infection and high genetic diversity of HIV strains have been reported in some of the neighboring West and Central African countries.^{11,15–22} Given that Mali has important trade and travel links with such countries, it is likely that Mali is affected by the spread of recombinant subtypes that are prevalent in the West and Central African region. However, with the exception of the single survey conducted in 1995, covering commercial sex workers in Bamako, Mali,²³ the dynamics of the spread of HIV-1 recombinant subtypes as well as nonrecombinant HIV-1 group M subtypes in this area have not been systematically assessed.

Twenty-three patients were included in the present study. Blood specimens were randomly collected from HIV-1 seropositive, epidemiologically unlinked individuals who were seen at the University Hospital of Bamako in Mali between

2003 and 2005 (Table 1). All individuals provided written informed consent for the use of patient samples that was approved by the Institutional Review Boards at the University of Bamako, Mali, and the National Institute of Allergy and Infectious Diseases. HIV was reported to have been sexually acquired between 1998 and 2004. HIV-1 subtype assignments were determined based on the full-length *env* sequences. Additional analysis on phylogenies of the *pol* gene was carried out for six patients out of 23. For the remaining 17 patients, the *pol* gene sequencing was not done due to lack of biological specimens.

Peripheral blood was separated by centrifugation at 400×g for 5 min, and plasma was aliquoted and stored at –80°C. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples by Ficoll-Hypaque gradient density, pelleted, and stored at –80°C until use. Viral RNA was extracted from plasma samples using the QIAamp Viral RNA Mini kit (Qiagen, Inc., Valencia, CA). Due to limited plasma sample availability, proviral DNA derived from PBMCs instead of plasma RNA was used for amplification of the HIV-1 gene for the following 11 patients: MAL01, 04, 06, 07, 12, 13, 14, 019, 125, 144, and 154. Reverse transcription was performed with the Superscript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) as previously described with minor modifications²⁴ by using –9146 5'-CTGCCAATCAGGGAAGTAGCCTTGTGT-3' as the cDNA primer. DNA was extracted from PBMCs using the PureGene genomic DNA isolation kit (Gentra Systems, Minneapolis, MN). Nested polymerase chain reaction (PCR) amplification of a 3-kbp fragment, encompassing the entire

TABLE 1. PATIENT CHARACTERISTICS AND HIV-1 SUBTYPE INFORMATION^a

Patient ID	Gender	Profession	Year sampling	Probable year of infection	Probable location of HIV infection	Sample source	HIV-1 subtype	
							env	pol
MAL01	F	Housewife	2003	1998	Bamako, Mali	PBMC provirus	CRF02	
MAL04	F	Housewife	2003	1998	Bamako, Mali	PBMC provirus	CRF02	
MAL06	M	Driver	2003	1998	Bamako, Mali	PBMC provirus	A3	
MAL07	F	Housewife	2003	1998	Bamako, Mali	PBMC provirus	CRF02	
MAL12	F	Housewife	2003	1998	Bamako, Mali	PBMC provirus	CRF02	
MAL13	F	Trader	2003	1998	Bamako, Mali	PBMC provirus	CRF02	
MAL14	F	Trader	2003	1998	Côte d'Ivoire	PBMC provirus	CRF09	
MAL019	M	Trader	2003	1999	Bamako, Mali	PBMC provirus	CRF06	
MAL125	F	Artist	2003	1999	Bamako, Mali	PBMC provirus	CRF02	
MAL127	F	Housewife	2003	1999	Bamako, Mali	Plasma virus	CRF02	
MAL144	F	Housewife	2003	1999	Bamako, Mali	PBMC provirus	CRF02	
MAL152	M	Manager	2003	1999	Bamako, Mali	Plasma virus	CRF02	
MAL154	F	Housewife	2003	1999	Bamako, Mali	PBMC provirus	A3	
MAL155	M	Carpenter	2003	2000	n.a.	Plasma virus	CRF02	
MAL162	n.a.	n.a.	2003	n.a.	n.a.	Plasma virus	CRF02	
MAL164	n.a.	n.a.	2003	n.a.	n.a.	Plasma virus	CRF02	
MAL08	F	Housewife	2004	2004	Bamako, Mali	Plasma virus	CRF02	CRF02
MAL27	M	Military	2004	2004	Bamako, Mali	Plasma virus	CRF02	A3
MAL29	M	Carpenter	2004	2003	Bamako, Mali	Plasma virus	CRF09	CRF09
MAL30	F	Housewife	2004	2004	Bamako, Mali	Plasma virus	A3	CRF02
MAL40	F	Nurse	2004	2004	Bamako, Mali	Plasma virus	CRF02	CRF02
MAL41	F	Nurse	2004	2004	Bamako, Mali	Plasma virus	CRF02	CRF02
MAL56	F	Housewife	2005	n.a.	Bamako, Mali	Plasma virus	CRF06	n.a.

^aThe HIV-1 subtype/CRF designation of *env* and *pol* genes was determined based on the nearest reference strain found by the neighbor-joining tree method. n.a., not available.

FIG. 1. Phylogenetic classification of 23 Malian HIV-1 isolates based on the full-length *env* region. A representative sequence from each Malian patient (highlighted in red) and 116 reference subtypes and available CRFs (at least two representative sequences of subtypes, subsubtypes, and of CRF01 to CRF15, CRF18, CRF19 as well as a prototype sequence from CRF16, CRF17, CRF20–CRF25, CRF28–CRF33, and CRF35–CRF37) were initially used to construct the tree; some references have been omitted here for clarity. For a complete list of reference sequences used in the analysis, see Table 2. A phylogenetic tree was constructed by the neighbor-joining method with the HKY85 model of evolution. Bootstrap percentile values from 1000 replications are shown at nodes defining major grouping of sequences. Clustering of subtype/subsubtype sequences are delineated in the figure. Previously generated sequences from Mali, 95ML127 (CRF06), were included in the analysis. Recently classified CRF30_0206¹¹ and CRF32_06A1,³¹ both containing CRF06_cpx segments in their *env* region, were also included in the analysis and found within the CRF06_cpx radiation with a high bootstrap value of 100%.

env gene and 5'-portion of the *nef* gene, was performed with the Expand High Fidelity PCR System (Roche Applied Science, Indianapolis, IN) as previously described²⁵ using the following primer sets: +5954 (sense) 5'-GGCTTAGGCATC TCCTATGGCAGGAAGAA-3' and -9146 (antisense) 5'-CTGCCAATCAGGGAAGTAGCCTTGTGT-3' in a first round reaction; +6202 (sense) 5'-AGAAAGAGCAGAAGACAGTG GCAATGA-3' and -9068 (antisense) 5'-TAGCCCTTCCAGT CCCCCCTTTTCTTTTA-3' in a second round reaction. Each round of PCR consisted of 30 cycles, and amplifications were performed as follows: the initial denaturation at 94°C for 2 min; 10 cycles of amplification (94°C for 15 s, 63°C for

30 s, 68°C for 4 min); and an additional 20 cycles of amplification (94°C for 15 s, 63°C for 30 s, 68°C for 4 min + 5 s per cycle); and the final extension at 68°C for 7 min. The PCR products were gel-purified on 0.8% agarose gels using the S.N.A.P. UV-Free Gel Purification Kit (Invitrogen, Carlsbad, CA) and then cloned into pCR2.1-TOPO vector (TOPO TA Cloning it, Invitrogen, Carlsbad, CA) for sequence analysis of individual molecular clones. The DNA was sequenced with the ABI BigDye Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and analyzed with the ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). On average 8–10

molecular clones were generated, and one single clone for each patient has been sequenced and analyzed. Amplification and sequencing of the HIV-1 *pol* gene were performed with the TRUGENE HIV-1 Genotyping kit (Bayer). Sequence segments were assembled with the Sequencher (Gene Codes Corporation, Ann Arbor, MI).

The completed sequences were aligned with reference sequences of appropriate HIV-1 subtypes and circulating recombinant forms (CRFs) (obtained from the Los Alamos HIV Sequence Database, <http://www.hiv.lanl.gov>) by using the Se-Al (Sequence Alignment Editor, v2.0a11, Rambaut, A. Department of Zoology, University of Oxford, UK), taking into account the protein sequences. Phylogenetic relationships among different subtypes were estimated by use of the neighbor-joining method²⁶ with the PAUP* program (Swofford, DL. 2003. PAUP*. Phylogenetic analysis using parsimony and other methods. Version 4.0b10. Sinauer Associates, Sunderland, MA). Statistical support for various nodes in the neighbor-joining tree was obtained by 1000 replications of the bootstrap procedure.²⁷ Gaps were ignored for affected pairwise comparisons. The sequence data used in this study have been deposited in GenBank and are available under the accession numbers EU480455–EU480489.

Phylogenetic analysis of 23 full-length *env* sequences revealed that all 23 clustered within the HIV-1 group M clade (Fig. 1). The majority of group M sequences (21 of 23) fell within the subtype A clade. Sixteen of the 21 subtype A sequences were further classified as CRF02_AG, three as subsubtype A3, and two sequences clustered with recently described CRF09_cpx (Fig. 1 and Tables 1 and 2). The remaining two sequences clustered together within the CRF06_cpx radiation with a bootstrap value of 100%. An additional pruning analysis, which consists of removing respective reference sequences from an alignment and rerunning the phylogenetic analysis, revealed that our CRF02_AG, subsubtype A3, CRF09_cpx, and CRF06_cpx sequences all maintained their distinct clustering patterns even when their respective reference sequences were absent, further confirming the stability of the tree topology and subtype assignments (data not shown). In addition, the CRF02_AG, CRF09_cpx, and subsubtype A3 sequences clustered separately and distantly from the crown groups of A1 viruses. Similarly, CRF06_cpx sequences clustered among themselves being outside the crown group of the pure subtype G reference sequences. Taken together, these data imply that CRF02_AG, CRF09_cpx, subsubtype A3, and CRF06_cpx have been in circulation for some time.

To confirm the clade assignments and the recombinant structure of newly generated Mali sequences, bootscanning analyses were performed with the use of SimPlot software.²⁸ All Mali CRF sequences: CRF02 (16 sequences), CRF06 (two sequences), and CRF09 (two sequences) were subjected to bootscanning analysis by being plotted against references of nonrecombinant subtypes and prototype CRFs. Bootscanning plots of two representative sequences for CRF02 (MAL08 and 27), CRF06 (MAL019 and 56), and CRF09 (MAL14 and 29) are shown in Fig. 2. CRF02 sequences found in Mali (represented by MAL08 and 27 in Fig. 2) showed a similar breakpoint profile of the CRF02 prototype: IbNG, having a mosaic genome structure, involving subtypes A and G. CRF06 sequences derived from

the Mali patients (MAL019 and 56) had multiple breakpoints, involving segments from subtypes G and J, which is in line with the mosaic pattern previously identified for the CRF06 prototype: BFP90. CRF09 sequences found in Mali patients (MAL14 and 29) had a complex genome structure, involving short stretch of segments derived from subtypes A and G with multiple breakpoints. The bootscanning analysis involving prototypic CRF sequences confirmed the similarity between the CRF09 sequences from Mali and the prototype CRF09: 96GH2911.

Next, we examined the *pol* and *env* gene fragments of the Mali specimens for concordance of subtypes, which supports the clade assignment. We observed that four of the six specimens studied (MAL08, MAL29, MAL40, and MAL41) had concordant subtypes in the *pol* and *env* (Fig. 3 and Table 3). On the other hand, the sequence of MAL30 was a mosaic of CRF02_AG and subsubtype A3. The analyses indicated that the sequence from subject MAL30 was grouped with high bootstrap values to CRF02_AG reference sequences in the *pol* gene, and the *env* portion of the genome clustered with high bootstrap values with subsubtype A3 reference sequences (Fig. 3). Similarly, the viral sequence from MAL27 was composed of a mosaic of A3 in the *pol* and CRF02_AG in the *env* regions (Fig. 3). Since amplification of the *pol* and *env* gene fragments was performed separately, it is not possible to determine whether these A3/CRF02_AG viruses truly represent intersubtype recombinants or dual infection in the individuals who carried discordant subtypes. Further analysis involving the full-length HIV-1 genome sequencing is warranted for the precise intersubtype assignments. In addition, no known major drug resistance-associated mutations were detected in these Mali isolates.

The current study revealed a broad HIV-1 genetic diversity in Mali, identifying genetic materials of CRF02_AG, CRF06_cpx, CRF09_cpx, and subsubtype A3. We also report the presence of the recently described CRF09_cpx sequences in Mali for the first time. The CRF09_cpx, an intersubtype recombinant composed of CRF02_AG, Z321 and some uncharacterized fragments, was originally identified in the Senegal cohort in 2004.²⁹ While initially thought to have limited spread, subsequent reports of the presence of CRF09_cpx in Côte d'Ivoire, Cameroon, and Nigeria,^{16,17,19} coupled with our present report of presence of CRF09_cpx in Mali, indicate that the CRF09_cpx may be more broadly distributed and at higher prevalence in West and Central Africa. Furthermore, we have identified two intersubtype recombinants involving CRF02_AG and subsubtype A3 in the *pol* and *env* segments. The subsubtype A3 sequences, which we describe in this study for the first time in Mali, were reported earlier to be circulating in other parts of West and Central Africa, where they had also recombined with CRF02_AG.²¹ The exact prevalence of the A3/CRF02_AG intersubtype recombinants is currently unclear. However, given that the A3-containing recombinants were possibly introduced to the population as early as 1989,²² A3/CRF02_AG could be more broadly distributed over West and Central African countries.

The 1995 study conducted in Mali reported a predominance of subtype A and subtype G virus with a few strains belonging to subtypes C and D.²³ The subsequent sequence analysis revealed that some of the subtype G sequences from the 1995 study clustered with CRF06_cpx. In the

TABLE 2. CLASSIFICATION BY SUBTYPE

<i>Subtype</i>	<i>Reference strain</i>	<i>Accession no.</i>	<i>Country code</i>	<i>Country</i>
A1	Q23	AF004885	KE	Kenya
A1	SE7253	AF069670	SE	Sweden
A1	92UG037	U51190	UG	Uganda
A1	98UG57136	AF484509	UG	Uganda
A2	97CDKTB48	AF286238	CD	Congo
A2	94CY017	AF286237	CY	Cyprus
A3	DDJ360	AY521630	SE	Sweden
A3	DDI579	AY521629	SE	Sweden
A3/02	DDJ362	AY521632	SE	Sweden
A3/02	DDJ364	AY521633	SE	Sweden
B	HXB2	K03455	FR	France
B	671_00T36	AY423387	NL	Netherlands
B	BK132	AY173951	TH	Thailand
B	1058_11	AY331295	US	USA
C	BR025-d	U52953	BR	Brazil
C	ETH2220	U46016	ET	Ethiopia
C	95IN21068	AF067155	IN	India
C	SK164B1	AY772699	ZA	South Africa
D	ELI	K03454	CD	Congo
D	4412HAL	AY371157	CM	Cameroon
D	A280	AY253311	TZ	Tanzania
D	94UG114	U88824	UG	Uganda
F1	VI850	AF077336	BE	Belgium
F1	93BR020_1	AF005494	BR	Brazil
F1	FIN9363	AF075703	FI	Finland
F1	MP411	AJ249238	FR	France
F2	0016BBY	AY371158	CM	Cameroon
F2	MP255	AJ249236	CM	Cameroon
F2	MP257	AJ249237	CM	Cameroon
F2	CM53657	AF377956	CM	Cameroon
G	DRCBL	AF084936	BE	Belgium
G	HH8793	AF061641	KE	Kenya
G	92NG083	U88826	NG	Nigeria
G	SE6165	AF061642	SE	Sweden
H	VI991	AF190127	BE	Belgium
H	VI997	AF190128	BE	Belgium
H	90_056	AF005496	CF	Central African Republic
J	SE7887	AF082394	SE	Sweden
J	SE7022	AF082395	SE	Sweden
K	EQTBI1C	AJ249235	CD	Congo
K	MP535	AJ249239	CM	Cameroon
01_AE	CM240	U54771	TH	Thailand
01_AE	90CF11697	AF197340	CF	Central African Republic
01_AE	90CF402	U51188	CF	Central African Republic
01_AE	90CF4071	AF197341	CF	Central African Republic
02_AG	IBNG	L39106	NG	Nigeria
02_AG	DJ263	AF063223	FR (DJ)	Djibouti
02_AG	DJ264	AF063224	FR (DJ)	Djibouti
02_AG	SE7812	AF107770	SE	Sweden
03_AB	KAL153_2	AF193276	RU	Russian Federation
03_AB	98BY10443	AF414006	BY	Belarus
03_AB	RU98001	AF193277	RU	Russian Federation
04_cpx	CY032	AF049337	CY	Cyprus
04_cpx	97PVCH	AF119820	GR	Greece
04_cpx	97PVMY	AF119819	GR	Greece
05_DF	VI1310	AF193253	BE	Belgium
05_DF	VI961	AF076998	BE	Belgium
05_DF	X492	AY227107	ES	Spain
06_cpx	BFP90	AF064699	AU	Australia
06_cpx	95ML84	AJ245481	ML	Mali
06_cpx	95ML127	AJ288982	ML	Mali
06_cpx	97SE1078	AJ288981	SN	Senegal

(Continued)

TABLE 2. (CONTINUED)

<i>Subtype</i>	<i>Reference strain</i>	<i>Accession no.</i>	<i>Country code</i>	<i>Country</i>
07_BC	CN54	AX149771	CN	China
07_BC	97CN001	AF286226	CN	China
07_BC	98CN009	AF286230	CN	China
07_BC	CNGL179	AF503396	CN	China
08_BC	GX_6F	AY008715	CN	China
08_BC	98CN006	AF286229	CN	China
09_cpx	96GH2911	AY093605	GH	Ghana
09_cpx	96SN1795	AY093603	SN	Senegal
09_cpx	99DE4057	AY093607	US	USA
09_cpx	00IC_10092	AJ866553	CI	Côte d'Ivoire
10_CD	BF061 (TZBF061)	AF289548	TZ	Tanzania
10_CD	BF071	AF289549	TZ	Tanzania
10_CD	BF110	AF289550	TZ	Tanzania
11_cpx	GR17	AF179368	GR	Greece
11_cpx	0186ND	AY371149	CM	Cameroon
11_cpx	1816	AF492624	CM	Cameroon
11_cpx	MP1298	AJ291719	FR	France
12_BF	ARMA159	AF385936	AR	Argentina
12_BF	X1241	AY536238	ES	Spain
12_BF	URTR17	AY037272	UY	Uruguay
13_cpx	1849 (96CM_1849)	AF460972	CM	Cameroon
13_cpx	3226MN	AY371154	CM	Cameroon
13_cpx	4164	AF460974	CM	Cameroon
14_BG	X397	AF423756	ES	Spain
14_BG	X475	AF423758	ES	Spain
14_BG	X605	AF450096	ES	Spain
15_01B	MU2079	AF516184	TH	Thailand
15_01B	OUR1331	AF529572	TH	Thailand
15_01B	R2399	AF530576	TH	Thailand
16_A2D	97KR004	AF286239	KR	Korea
18_cpx	CM53379	AF377959	CM	Cameroon
18_cpx	CU76	AY586540	CU	Cuba
19_cpx	CU38	AY588970	CU	Cuba
19_cpx	CU7	AY894994	CU	Cuba
O	ANT70	L20587	BE	Belgium
CRF17_BF	ARMA038	AY037281	AR	Argentina
CRF20_BG	CB228	AY900577 (gag-pol)	CU	Cuba
CRF21_A2D	99KE_KER2003	AF457051 (KER2003)	KE	Kenya
CRF22_01A1	CM53122	AY037284S2	CM	Cameroon
CRF23_BG	CB118	AY900571	CU	Cuba
CRF24_BG	CB378	AY900574	CU	Cuba
CRF25_cpx	02CM_1918LE	AY371169 (1918LE)	CM	Cameroon
CRF26_AU	Pending			
CRF27	Pending			
CRF28_BF	BREPM12609	DQ085873	BR	Brazil
CRF29_BF	BREPM16704	DQ085876	BR	Brazil
CRF30_0206	00NE36	AJ508597 (NE36)	NE	Niger
CRF31_BC	04BR142	AY727527	BR	Brazil
CRF32_06A1	EE0369	AY535660	EE	Estonia
CRF33_01B	05MYKL007	DQ366659	MY	Malaysia
CRF34_01B	Pending		TH	Thailand
CRF35_AD	AF026	EF158040	AF	Afghanistan
CRF36_cpx	00CMNYU830	EF087994	CM	Cameroon
CRF37_cpx	00CMNYU926	EF116594	CM	Cameroon

present study, we have shown that all isolates do indeed cluster with subtypes A and G in the *pol* and *env* regions. However, two significant differences emerged. First, non-recombinant subtype A, or “pure” subtype A, viruses were no longer found, and all the subtype A viruses were pri-

marily CRF02_AG. Similarly, all the subtype G viruses were intersubtype recombinant: CRF06_cpx. Second, in addition to the aforementioned CRF02_AG, we found other subtype A sequences, including subsubtype A3 and CRF09_cpx. These findings indicate a shift in the HIV-1 virus population

FIG. 2. Bootscanning analyses of 2.6-kb *env* regions of HIV-1 sequences of CRF02 (MAL08 and 27), CRF06 (MAL019 and 56), and CRF09 (MAL14 and 29). Two sets of bootscanning were performed: The first set of bootscanning plots depicts relationship of the Mali sequences to the representative strains of HIV-1 nonrecombinant subtypes (A: 94SE_Q23, G: DRCBL, and J: 93SE_7887). The second set of bootscanning plots delineates relationship of the Mali sequences to the prototype strain of CRF02 (IbNG), CRF06 (BFP90), and CRF09 (96GH2911). For the bootscanning plots, the SimPlot software performed bootscanning on neighbor-joining trees by using SEQBOOT (100 replicates), DNADIST (with the Kimura's two-parameter method and a transition/transversion ratio of 2.0), and CONSENSE from the PHYLIP package,³² for a 200 bp window moving along the alignment with increments of 20 bp.

FIG. 3. Subregion tree analysis of the *pol* and full-length *env* sequences. Four representative patterns are shown. Each gene segment was subjected to separate phylogenetic analyses based on the neighbor-joining method to identify the subtype or circulating recombinant form (CRF) origin of the segment. The stability of the nodes was assessed by the use of bootstrap resampling of 1000 replications. Bootstraps values above 70% are shown. The clusters for subtype/subtypes and CRF01_AE, CRF02_AG, CRF06_cpx, and CRF09_cpx are outlined with a vertical bar on the right of each tree. The genotype assignments of each Mali strain in the respective phylogenetic analysis are highlighted in red in each tree. All the HIV-1 reference sequences were retrieved from the HIV sequence database, Los Alamos National Library. For a complete list of reference sequences used in the analysis, see Table 3.

TABLE 3. *POL* AND *ENV* TREES

Pol tree					env tree				
Subtype	Sequence name	Accession no.	Country code	Country	Subtype	Sequence name	Accession no.	Country code	Country
A1	Q23	AF004885	KE	Kenya	A1	Q23	AF004885	KE	Kenya
A1	SE7253	AF069670	SE	Sweden	A1	SE7253	AF069670	SE	Sweden
A1	U455	M62320	UG	Uganda	A1	98UG57136	AF484509	UG	Uganda
A1	92UG037	U51190	UG	Uganda	A1	92UG037	U51190	UG	Uganda
A2	97CDKTB48	AF286238	CD	Congo	A2	97CDKTB48	AF286238	CD	Congo
A2	94CY017	AF286237	CY	Cyprus	A2	94CY017	AF286237	CY	Cyprus
A3	DDJ360	AY521630	SE	Sweden	A3	DDJ360	AY521630	SE	Sweden
A3	DD1579	AY521629	SE	Sweden	A3	DD1579	AY521629	SE	Sweden
A3/02	DDJ362	AY521632	SE	Sweden	A3/02	DDJ362	AY521632	SE	Sweden
A3/02	DDJ364	AY521633	SE	Sweden	A3/02	DDJ364	AY521633	SE	Sweden
B	HXB2	K03455	FR	France	B	HXB2	K03455	FR	France
B	RF	M17451	US	USA	B	671_00T36	AY423387	NL	Netherlands
B	JFRL	U63632	Us	USA	B	BK132	AY173951	TH	Thailand
B	WEAU160	U21135	US	USA	B	1058_11	AY331295	US	USA
C	BR025-d	U52953	BR	Brazil	C	BR025-d	U52953	BR	Brazil
C	ETH2220	U46016	ET	Ethiopia	C	ETH2220	U46016	ET	Ethiopia
C	96BW0502	AF110967	BW	Botswana	C	95IN21068	AF067155	IN	India
C	95IN21068	AF067155	IN	India					
D	ELI	K03454	CD	Congo	D	ELI	K03454	CD	Congo
D	NDK	M27323	CD	Congo	D	4412HAL	AY371157	CM	Cameroon
D	84ZR085	U88822	CD	Congo	D	A280	AY253311	TZ	Tanzania
D	94UG114	U88824	UG	Uganda	D	94UG114	U88824	UG	Uganda
F1	VI850	AF077336	BE	Belgium	F1	VI850	AF077336	BE	Belgium
F1	93BR020_1	AF005494	BR	Brazil	F1	93BR020_1	AF005494	BR	Brazil
F1	FIN9363	AF075703	FI	Finland	F1	FIN9363	AF075703	FI	Finland
F1	MP411	AJ249238	FR	France	F1	MP411	AJ249238	FR	France
F2	MP255	AJ249236	CM	Cameroon	F2	MP255	AJ249236	CM	Cameroon
F2	MP257	AJ249237	CM	Cameroon	F2	MP257	AJ249237	CM	Cameroon
G	DRCBL	AF084936	BE	Belgium	G	DRCBL	AF084936	BE	Belgium
G	HH8793	AF061641	KE	Kenya	G	HH8793	AF061641	KE	Kenya
G	92NG083	U88826	NG	Nigeria	G	92NG083	U88826	NG	Nigeria
G	SE6165	AF061642	SE	Sweden	G	SE6165	AF061642	SE	Sweden
H	VI991	AF190127	BE	Belgium	H	VI991	AF190127	BE	Belgium
H	VI997	AF190128	BE	Belgium	H	VI997	AF190128	BE	Belgium
H	90_056	AF005496	CF	Central African Republic	H	90_056	AF005496	CF	Central African Republic
J	SE7887	AF082394	SE	Sweden	J	SE7887	AF082394	SE	Sweden
J	SE7022	AF082395	SE	Sweden	J	SE7022	AF082395	SE	Sweden
K	EQTb11C	AJ249235	CD	Congo	K	EQTb11C	AJ249235	CD	Congo
K	MP535	AJ249239	CM	Cameroon	K	MP535	AJ249239	CM	Cameroon
01_AE	90CF11697	AF197340	CF	Central African Republic	01_AE	90CF11697	AF197340	CF	Central African Republic
01_AE	90CF402	U51188	CF	Central African Republic	01_AE	90CF402	U51188	CF	Central African Republic
01_AE	90CF4071	AF197341	CF	Central African Republic	01_AE	90CF4071	AF197341	CF	Central African Republic
01_AE	CM240	U54771	TH	Thailand	01_AE	CM240	U54771	TH	Thailand
02_AG	MP807	AJ286133	CM	Cameroon	02_AG	DJ263	AF063223	FR (DJ)	Djibouti
02_AG	DJ264	AF063224	FR (DJ)	Djibouti	02_AG	DJ264	AF063224	FR (DJ)	Djibouti
02_AG	IBNG	L39106	NG	Nigeria	02_AG	IBNG	L39106	NG	Nigeria
02_AG	SE7812	AF107770	SE	Sweden	02_AG	SE7812	AF107770	SE	Sweden
06_cpx	BFP90	AF064699	AU	Australia	06_cpx	BFP90	AF064699	AU	Australia
06_cpx	95ML127	AJ288982	ML	Mali	06_cpx	95ML127	AJ288982	ML	Mali
06_cpx	95ML84	AJ245481	ML	Mali	06_cpx	95ML84	AJ245481	ML	Mali
06_cpx	97SE1078	AJ288981	SN	Senegal	06_cpx	97SE1078	AJ288981	SN	Senegal
09_cpx	96SN1795	AY093603	SN	Senegal	09_cpx	96SN1795	AY093603	SN	Senegal

(Continued)

TABLE 3. (CONTINUED)

Pol tree					env tree				
Subtype	Sequence name	Accession no.	Country code	Country	Subtype	Sequence name	Accession no.	Country code	Country
09_cpx	95SN7808	AY093604	SN	Senegal	09_cpx	95SN7808	AY093604	SN	Senegal
09_cpx	96GH2911	AY093605	GH	Ghana	09_cpx	96GH2911	AY093605	GH	Ghana
09_cpx	99DE4057	AY093607	US	USA	09_cpx	99DE4057	AY093607	US	USA
09_cpx	00IC_10092	AJ866553	CI	Côte d'Ivoire	09_cpx	00IC_10092	AJ866553	CI	Côte d'Ivoire

circulating in Mali and demonstrated the recombination-prone nature of HIV-1 in areas such as Mali where CRFs have a high prevalence.

Although the rate of HIV-1 infections in Mali is, at present, fairly low in the general population, compared to other West and Central African countries, a high HIV prevalence in bridge populations, such as truck drivers, street vendors, or commercial sex workers (3.9%, 4.6%, and 31.9%, respectively), has been reported in urban areas of Mali.³⁰ This may foreshadow a wider epidemic and potential HIV diversification in Mali. In the present study, our cohort was restricted to HIV-infected individuals living in the capital city of Mali, Bamako. Extensive surveys covering all regions of the country with a sufficient number of samples will enhance our understanding of viral diversity, which is critical to the development of relevant diagnostic testing, treatment strategies, and vaccine candidates.

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Disclosure Statement

No competing financial interests exist.

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