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Sequence Note

Genetic Diversity of HIV Type 1 in Likasi, Southeast of the Democratic Republic of Congo

KAYOKO KITA,[1] NICAISE NDEMBI,[1] MICHEL EKWALANGA,[2] EIJI IDO,[3] RIGOBERT KAZADI,[2] BLAISE BIKANDOU,[4] JUN TAKEHISA,[1] TAICHIRO TAKEMURA,[3] SEIJI KAGEYAMA,[1] JUNJI TANAKA,[5] HENRI JOSEPH PARRA,[4] MASANORI HAYAMI,[3] and HIROSHI ICHIMURA[1]

ABSTRACT

To investigate the prevalence of subtypes A and C, and the existence of recombinants of both subtypes in the southeast of the Democratic Republic of Congo (DRC), blood samples were collected from 27 HIV-infected individuals in Likasi, located in an area bordering close to Zambia, and analyzed phylogenetically. Out of the 24 strains with a positive PCR profile for *pol*-IN and *env*-C2V3, 15 (62.5%) had a discordant subtype or CRF designation: one subtype A/G (*pol/env*), four A/U (unclassified), three G/A, one G/CRF01, three H/A, one J/C, one CRF02 (G)/A, and one U/A. Nine (37.5%) strains had a concordant subtype or CRF designation: five subtype A, two C, one D, and one CRF02/G. The remaining three samples negative for PCR with *env*-C2V3 primers used in this study were further analyzed with *env*-gp41 primers and revealed the presence of two profiles: two J/J (*pol*-IN/*env*-gp41) and one C/G. These data highlight the presence of a high proportion (16/27, 59.3%) of recombinant strains and a low prevalence (4.1 and 7.4%) of subtype C based on *env*-C2V3 and *pol*-IN analyses, respectively, in Likasi. In addition, this is the first report that CRF02_AG exists in DRC, though the epidemiological significance of the existence of CRF02_AG in DRC remains unknown.

HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 (HIV-1) has been classified into three major phylogenetic groups, termed M (major), N (non-M, non-O), and O (outlier).[1] The vast majority of variants found worldwide and responsible for the AIDS pandemic belong to group M.[2,3] Phylogenetic analysis of group M has further led to its subclassification into nine pure subtypes, A–D, F–H, J, and K and subsubtypes A1, A2, F1, and F2.[2] Recently, it was realized that a significant fraction of HIV-1 isolates, 10–40% or more, exhibit a shift in subtypes when different regions of their genome are analyzed.[3] Currently, some of the mosaic HIV-1 genomes play a major role in the global AIDS epidemics and are designated as circulating recombinant forms (CRFs), CRF01-CRF16. Although subtypes A, C, and CRF02_AG are most prevalent in Africa, the distribution of CRF/subtype is very heterogeneous.[2–7] The proportion of CRF02_AG among subtype A strains based on *env* sequences decreases from west to central Africa, with an absence of CRF02_AG in the Democratic Republic of Congo (DRC).[2,8,9] The profile of HIV-1 endemic in DRC, such as high number of cocirculating HIV-1 subtypes, possible recombinant viruses, and unclassified strains, is consistent with that of an old and mature epidemic of HIV-1.[5]

The DRC is bordered on the southeast by Zambia. The majority (95%) of the HIV-1 strains circulating in Zambia are subtype C, although HIV-1 group M subtypes A, D, G, and J as well as group O have been identified.[2,3,10,11]

[1]Department of Viral Infection and International Health, Graduate School of Medical Science, Kanazawa University, Kanazawa 920-8640, Japan.
[2]Laboratoire de virologie, INRB, Kinshasa, Republique Democratique du Congo.
[3]Laboratory of Viral Pathogenesis, Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan.
[4]Cité Louis Pasteur-Laboratoire National de Santé Publique, Brazaville 120, Congo.
[5]Department of Laboratory Science, School of Health Sciences, Kanazawa University, Kanazawa 920-8640, Japan.

The purpose of this study was to investigate the prevalence of subtypes A and C, and the existence of the recombinants of both subtypes in Likasi, located in the southeast of the DRC, 200 km from Lubumbashi in an area bordering Zambia.

Blood samples (10 ml) were collected from 27 HIV-1 infected individuals in February and September 2001. Plasma and buffycoat were separated and stored at −80°C until use. The plasma samples were screened for HIV antibodies with a commercial particle agglutination (PA) test kit (Serodia-HIV, Fujirebio, Tokyo, Japan).

Genomic DNA was extracted from buffycoat of the seroreactive samples using a Qiagen DNA extraction kit (Qiagen, Hilden, Germany). A part of the *pol* gene coding integrase (IN) (corresponding to 4493–4780 nt in HIV-1_{HXB2}) and *env* gene covering C2V3 (corresponding to 6975–7520 nt in HIV-1_{HXB2}) was amplified with nested polymerase chain reaction (PCR) using the primers unipol 5 (5′-TGGGTACCAG-CACACAAAGGAATAGGAGGAAA-3′)/unipol 6 (5′-CCA-CAGCTGATCTCTGGCCTTCTCTGTAATAGACC-3′) and M5 (5′-CCAATTCCCATACATTATTGTGCCCCAGCTGG-3′)/M10 (5′-CCAATTGTCCCTCATATCTCCTCCTCCAGG-3′), respectively, in the first round, and unipol 1 (5′-AGTGGATT-CATAGAAAGCAGAAGT-3′)/unipol 2 (5′-CCCCTATTCC-TTCCCCTTCTTTTAAAA-3′), and M3 (5′-GTCAGCACAG-TACAATGACACATGG-3′)/M8 (5′-TCCTTGGATGGGAG-GGGCATACATTGC-3′), respectively, in the second round.[11] Nested PCR was performed with an AmpliTaq Gold PCR kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Amplification was done with one cycle at 95°C for 10 min, and 35 cycles at 95°C for 30 sec, 45°C for 30 sec (for the *pol* region) or 55°C for 30 sec (for the *env* region), and 72°C for 1 min, with a final extension of 72°C for 10 min. Samples that could not be amplified with the *env*-C2V3 primers were analyzed with *env*-gp41-specific primers.[12] PCR amplification was confirmed by visualization with ethidium bromide staining of the gel. The PCR products were cloned by using a TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA) and plasmid DNA for sequencing was prepared by a plasmid miniprep kit (Sigma, Hilden, Germany). DNA sequencing was carried out using dye-deoxy terminator chemistry on an ABI 310 automatic sequencer (Applied Biosystems, Foster City, CA). We sequenced at least 12 clones to obtain a consensus sequence. Sample DNA sequences were aligned with subtype reference sequences from the Los Alamos database by CLUSTAL W (version 1.81) with subsequent inspection and manual modification. The frequency of nucleotide substitution in each base of the sequences was estimated by the Kimura two-parameter method.[13] Phylogenetic trees were constructed by the neighbor-joining method, and its reliability was estimated by 1000 bootstrap replications.[14] All alignments were gap stripped for the generation of trees. The profile of the tree was visualized by Treeview version 1.6.5.

The phylogenetic trees based on *env*-C2V3 and *pol*-integrase sequences were constructed with representative HIV-1 strains of each subtype and CRF as a reference (Fig. 1). Out of the 27 samples from Likasi, 24 (88.9%) were found to be positive for HIV-1 PCR with *pol*-IN and *env*-C2V3 primers. The remaining 3 (11.1%) samples were negative for PCR with the *env*-C2V3 primers used in this study. The phylogenetic tree based on *env*-C2V3 sequences (Fig. 1A) showed that out of the 24 samples 13 were subtype A, three C, one D, two G, one CRF01_AE, and four U (unclassified). The outcome of the phylogenetic analysis of the *pol*-IN gene is shown in Figure 1B, and revealed that 10 were subtype A, three C, one D, four G, three H, three J, two CRF02_AG, and one U. Thus, 15 (62.5%) had a discordant subtype or CRF designation: one subtype A/G (*pol/env*), four A/U, three G/A, one G/CRF01, three H/A, one J/C, and CRF02/A, and one U/A (Tables 1 and 2). Nine (37.5%) strains had a concordant subtype or CRF designation: one subtype A, two C, one D, and one CRF02/G (Tables 1 and 2). In 14 of these 15 strains, subtype A was involved in recombination events, and among the strains with a discordant subtype or CRF designations A/U (*pol/env*) was by far the most common, followed by H/A and G/A recombination. Additional PCR analyses on three samples with a *pol/env* (+/−) profile were carried out with groups M, N, and O primers for *env*-gp41. Phylogenetic analysis based on *env*-gp41 sequences revealed the presence of two profiles: two J/J (*pol*-IN/*env*-gp41) and one C/G (Tables 1 and 2).

In the current study we found that a high proportion (16/27; 59.3%) of HIV-1 strains in Likasi were intersubtype recombinants. This is higher than that reported in other regions of the country (29–44%).[5,8,15] In Lubumbashi, a city on the southeast border of the DRC with Zambia, subtype C was reported to be predominant (51.9%), followed by subtype A (22.1%).[15] Our data highlight a high prevalence of subtype A (37.0% and 52.5%) and low prevalence of subtype C (7.4% and 12.5%) in Likasi based on *env*-C2V3 and *pol*-IN analyses, respectively. The persisting civil war and population displacement from the east on the border with Ruanda and Burundi to the southern area close to Zambia could be linked to the change of HIV-1 distribution in the southeast of the DRC.[11]

Two HIV-1 strains from Likasi (00CD009 and 01CD208) significantly clustered with CRF02_AG reference strains (with 97.4% bootstrap value) (Fig. 1B). This is the first report of CRF02_AG in the DRC, suggesting that CRF02_AG is spreading into Central Africa. CRF02_AG and subtype A represent 70–80% of circulating HIV-1 strains in West and West-Central Africa.[2] However, the epidemiological significance of CRF02_AG in the DRC is yet to be investigated. The high proportion of unclassified strains (16.6%) and intersubtypic recombinants (52.3%) among HIV-1 strains circulating in the DRC indicates an old and mature epidemic of HIV-1 in the DRC.

SEQUENCE DATA

The nucleotide sequences in this study were submitted to GenBank and are available under the following accession numbers: *pol*-IN (288 bp), AY661750-AY661776; *env*-C2V3 (approximately 550 bp), AY675589–AY675612; and *env*-gp41 (405 bp), AY673112–AY673114.

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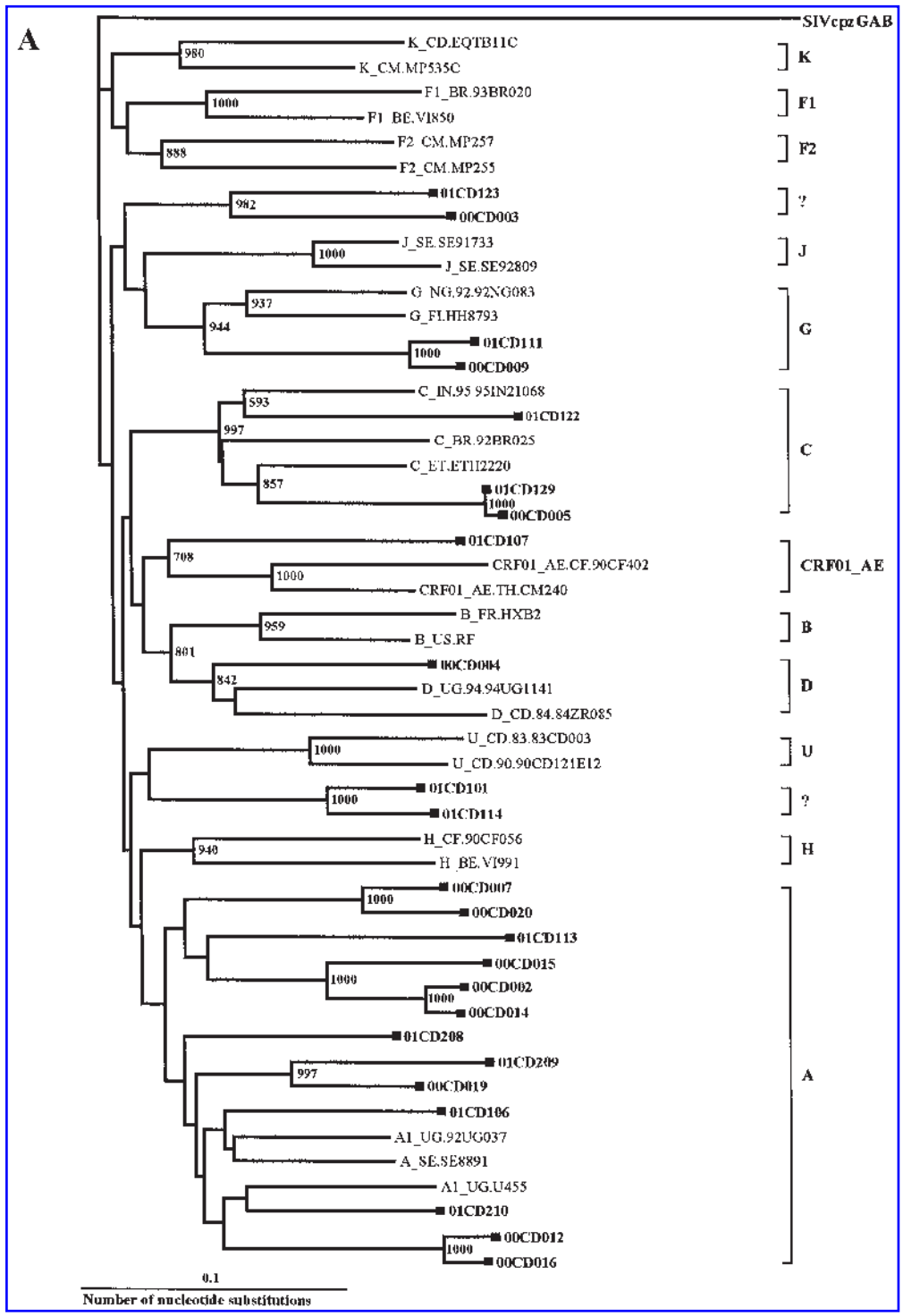

FIG. 1. Phylogenetic trees based on a part of the *env*-C2V3 gene (approximately 550 bp) (**A**) and *pol*-IN gene (288 bp) (**B**) of 24 HIV-1 strains from the southeastern Democratic Republic of Congo with reference sequences of representative subtypes/CRF. The bootstrap value at each node represents the number among 1000 bootstrap replicates that supports the branching order. Bootstrap values of 70% or higher are shown. Brackets on the right represent the major group M subtypes. Newly derived sequences (shown in bold) are marked with a filled square (■).

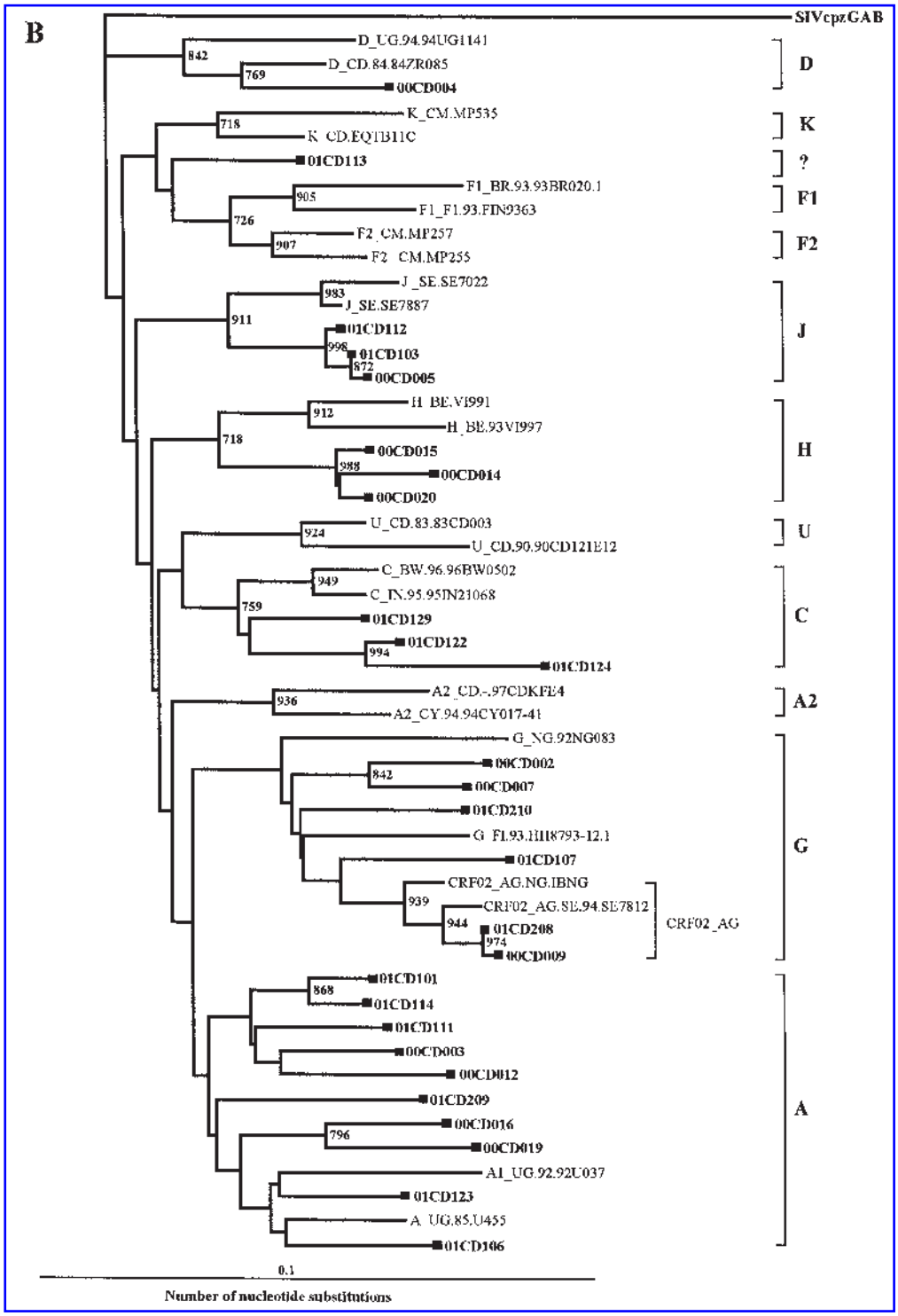

FIG. 1. *Continued*

TABLE 1. SUBTYPES IN *pol*-INTEGRASE AND *env*-(C2V3/ OR -gp41) FOR 27 HIV-1 STRAINS

ID number DRC	pol-*IN (288 bp)*	env-*C2V3 (550 bp)*	env-*gp41 (405 bp)*
00CD002	Subtype G	Subtype A	
00CD003	A	U	
00CD004	D	D	
00CD005	J	C	
00CD007	G	A	
00CD009	CRF02_AG (G)	G	
00CD012	A	A	
00CD014	H	A	
00CD015	H	A	
00CD016	A	A	
00CD019	A	A	
00CD020	H	A	
01CD101	A	A	
01CD103	J	ND[a]	J
01CD106	A	A	
01CD107	G	CRF01_AE (E)	
01CD111	A	G	
01CD112	J	ND	J
01CD113	U	A	
01CD114	A	A	
01CD122	C	C	
01CD123	A	U	
01CD124	C	ND	G
01CD129	C	C	
01CD208	CRF02_AG (G)	A	
01CD209	A	A	
01CD210	G	A	

[a]ND, not detected.

TABLE 2. SUBTYPES IN *pol*-INTEGRASE AND *env*-(C2V3) AND/OR -(gp41) GENE FOR 27 HIV-1 STRAINS

Genotypes	pol-*IN*	env-*C2V3*	n	*Total (%)*
Concordant	A	A	5	
	C	C	2	
	D	D	1	11 (40.7)
	J	J[a]	2	
	CRF02 (G)	G	1	
Discordant	A	U	4	
	A	G	1	
	C	G[a]	1	
	G	A	3	
	G	CRF01 (E)	1	
	H	A	3	16 (59.3)
	J	C	1	
	CRF02 (G)	A	1	
	U	A	1	

[a]*env*-gp41 (approximately 405 bp).

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Address reprint requests to:
Hiroshi Ichimura
Department of Viral Infection and International Health
Graduate School of Medical Science
Kanazawa University
13-1 Takara-machi
Kanazawa 920-8640, Japan

E-mail: ichimura@med.kanazawa-u.ac.jp