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Sequence Note

Genetic and Phylogenetic Analysis of HIV Type 1 Strains from Southern Ghana

J.A.M. BRANDFUL,[1] W.K. AMPOFO,[1,2] W. JANSSENS,[3] Y. ADU-SARKODIE,[4] F. APEAGYEI,[5] F. ANYOMI,[6] S. AIDOO,[1] N. YAMAMOTO,[2] K. ISHIKAWA,[3,7] T. SATA,[7] and T. KURATA[8]

SINCE THE FIRST REPORTED AIDS cases in Ghana in 1986, the number of reported cumulative cases has gradually increased to 22,000 as of September 1997.[1] In the same period the proportion of AIDS due to a single infection with HIV-2 has decreased significantly, with HIV-1 being responsible for most of the current infections in Ghana.[2]

Two studies have been carried out since 1989 on a limited number of Ghanaian specimens to characterize the genetic subtype of HIV-1 strains, mainly from Akwatia[3,4] in the Eastern Region, which has a higher-than-average HIV prevalence level.[5] The present study focuses on subjects who were mainly from Kumasi and Accra, the two largest cities in southern Ghana. Twenty-six HIV-1-infected individuals sampled between January and May 1996: $n = 10$ from Accra (capital city of Ghana), $n = 13$ from Kumasi (272 km northwest of Accra) and its environs, and $n = 3$ from Dzodze (198 km east of Accra). The subjects had a mean age of 31 years. Eleven (42%) were male, including 2 babies born to HIV-1-seropositive mothers, and 15 (58%) were female. Informed consent was obtained from each individual (from mothers or guardians in the case of babies) and 10 ml of whole blood (<5 ml in the case of babies) was collected in EDTA tubes. This was then processed for plasma/serum and peripheral blood monocyte cells (PBMCs). All plasma samples were initially screened for HIV antibody status by particle agglutination (PA) test (Serodia HIV-1/2; Fujirebio, Tokyo, Japan). Reactive specimens were subsequently confirmed by synthetic peptide-based immunoassay (PEPTI-LAV 1–2) or Western blot (WB) analysis (New Lav Blot 1–2) (both kits from Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). The criterion for HIV antibody positivity by WB was the presence of at least two envelope and two core bands. Epidemiological, immunological, and clinical data on the subjects are shown in Table 1. The three-stage guideline for HIV/AIDS classification of adults, based on CD4 subset levels of T cells and clinical symptoms, from the Centers for Disease Control (CDC, Atlanta, GA; revised 1993),[6] was adopted in determining subject clinical status with respect to HIV/AIDS.

Genomic DNA was extracted from primary PBMCs of HIV-seropositive individuals. A 300-base pair (bp) fragment encoding the Env C2V3 region was amplified using a nested polymerase chain reaction (PCR), and direct sequencing was performed. The newly sequenced HIV-1 *env* variants were aligned with 18 previously documented HIV-1 strains, representing group M subtypes from A to I, and the sequence of $SIV_{cpz\text{-}gab}$, on the basis of primary structure. Distance calculation (Jukes and Cantor), tree construction (neighbor joining), and bootstrap analysis were realized with the software package TREECON.[7] In the tree, shown in Fig. 1, 24 Ghanaian subjects (92%) clustered with the HIV-1 subtype A representatives, but the clustering was not supported by 70% or more of the bootstrap tests. Analyzed separately, 21 of these 24 strains were supported by more than 70% of the bootstrap trees; 3 strains, NJS179, NJS187, and NJS245, were not. The latter may be representative of aberrant subtype A strains or intersubtype recombinants. Further analysis, including the sequencing of additional parts of the HIV-1 genome of these strains, is necessary to confirm this preliminary classification. Two Ghanaian samples clustered with members of subtype G, supported by 87.8% of the bootstrap trees. For Ghanaian specimens in this study belonging to subtype A, interhost distances at the nucleotide level were on average 14.4%, varying from 2.5% (between NJS66

[1]Virology Unit, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Ghana.
[2]Department of Microbiology, Tokyo Medical and Dental University, 115 Tokyo, Japan.
[3]Department of Microbiology, Institute of Tropical Medicine, 2000 Antwerp, Belgium.
[4]Department of Microbiology, Komfo Anokye Teaching Hospital, Kumasi, Ghana.
[5]37 Military Hospital, Accra, Ghana.
[6]St. Anthony's Catholic Hospital, Dzodze, Volta Region, Ghana.
[7]Laboratory of AIDS Pathology, National Institute of Infectious Diseases (NIID), 162 Tokyo, Japan.
[8]Department of Pathology, National Institute of Infectious Diseases (NIID), 162 Tokyo, Japan.

TABLE 1. EPIDEMIOLOGICAL, IMMUNOLOGICAL, AND CLINICAL DATA ON SUBJECTS IN SEQUENCE STUDY

Sample (NJS)	*Place*	*Age/sex*	*HIV-1/2 serology*	*CD4 (cells/μl)*	*Clinical status*	*Associated infection*	*Travel history*	*HIV-1 env C2V3 subtype*
66	K'si	45/M	1	nd	Sympt	HBV, TB	—	A
69	Accra	—/M	Dual	739	A1	—	—	A
72	K'si	32/F	1	126	A3	—	—	A
74	K'si	26/F	Dual	319	C2	TB	—	A
75	K'si	26/M	1	102	C3	HBV, HTLV-1	—	A
147	K'si	30/F	Dual	245	C2	TP	—	A
156	Accra	28/M	Dual	122	Sympt	—	—	A
173	Dz	43/F	Dual	452	B2	—	Togo	G
174	Dz	34/F	Dual	225	C2	—	Togo	G
178	K'si	29/F	1	<50	B3	HBV, HCV	B. Faso C. d'Ivoire	A
179	K'si	40/F	1	193	C3	—	—	A
180	Accra	1/M	1	674	A1	—	—	A
182	K'si	36/M	1	86	A3	HBV, TB	C d'Ivoire	A
183	K'si	36/F	1	118	C3	HBV, TP, TB	Yes	A
187	Accra	<1 M	1	nd	na	—	—	A
190	K'si	24/F		<50	A3	—	—	A
199	Accra	—/M	1	400	A3	HBV	Liberia	A
202	Accra	—/M	1	717	A1	—	Liberia	A
203	Accra	39/M	Dual	367	A2	—	Liberia	A
204	Accra	—/—	1	465	A2	—	—	A
222	Accra	42/F	1	nd	na	HCV	—	A
232	Dz	25/F	Dual	724	A1	HTLV-I	—	A
245	Accra	33/F	1	nd	Sympt	—	—	A
247	K'si	40/M	Dual	<50	C3	—	—	A
255	K'si	32/F	1	215	A2	TP	—	A
VYA	Accra	36/F	1	465	A2	—	—	A

Abbreviations: K'si, Kumasi; Dz, Dzodze; na, not available; nd, not done; HBV, hepatitis B virus; HCV, hepatitic C virus; HTLV-I, human T-lymphotropic virus type I; TP, *Treponema pallidum*; TB, tuberculosis; B. Faso, Burkina Faso; C. d'Ivoire, Côte d'Ivoire.

and NJS204) to 20.4% (between NJS203 and NJS204). The interhost distance between the two subtype G Ghanaian samples, NJS173 and NJS174, was 24.5%.

The predicted amino acid sequence of the Env C2V3 region for these strains is presented in Fig. 2. Twenty-three subtype A and both subtype G strains possessed GPGQ tetrameric amino acid sequences at the apex of the V3 loop, except for subtype A strain NJS178, which had GPGR. Octameric tips of the V3 loop—RIGPGQTF ($n = 14$), HIGPGQTF ($n = 4$), and RIGPGQAF ($n = 2$)—are frequently documented for subtype A. In addition, octameric sequences GIGPGQTF ($n = 1$), NIGPGRAF ($n = 1$), HIGPGQAL ($n = 1$), and GIGPGQAF ($n = 1$) (subtype A), and TIGPGQAF ($n = 2$) and HIGPGQAF ($n = 1$) (subtype G) were documented in this study (Fig. 2). NIGPGAF, HIGPGQAL, and GIGPGQAF; and TIGPGQAF and HIGPGQAF were previously not reported for, respectively, subtype A and G.[8]

This study adds to the previous reports[3,4] on HIV-1 subtype distribution in Ghana. Akwatia exposed cocirculation of mainly subtype A variants, but also subtype G and D variants; Accra and Kumasi revealed subtype A; in Dzodze two female individuals that previously traveled to Togo were infected by subtype G variants. Subtype A is prevalent in West Africa. A high subtype G prevalence was reported for northern regions of Nigeria[9] (Maiduguri, 35%; Kano, 25%) and Mali[10] (Bamako, 15%). In addition, subtype G was demonstrated to cocirculate with subtype A in Benin,[11] Ghana,[3,4] Côte d'Ivoire,[12] and Senegal,[13,14] suggesting a similar course of the epidemic in these West African countries.

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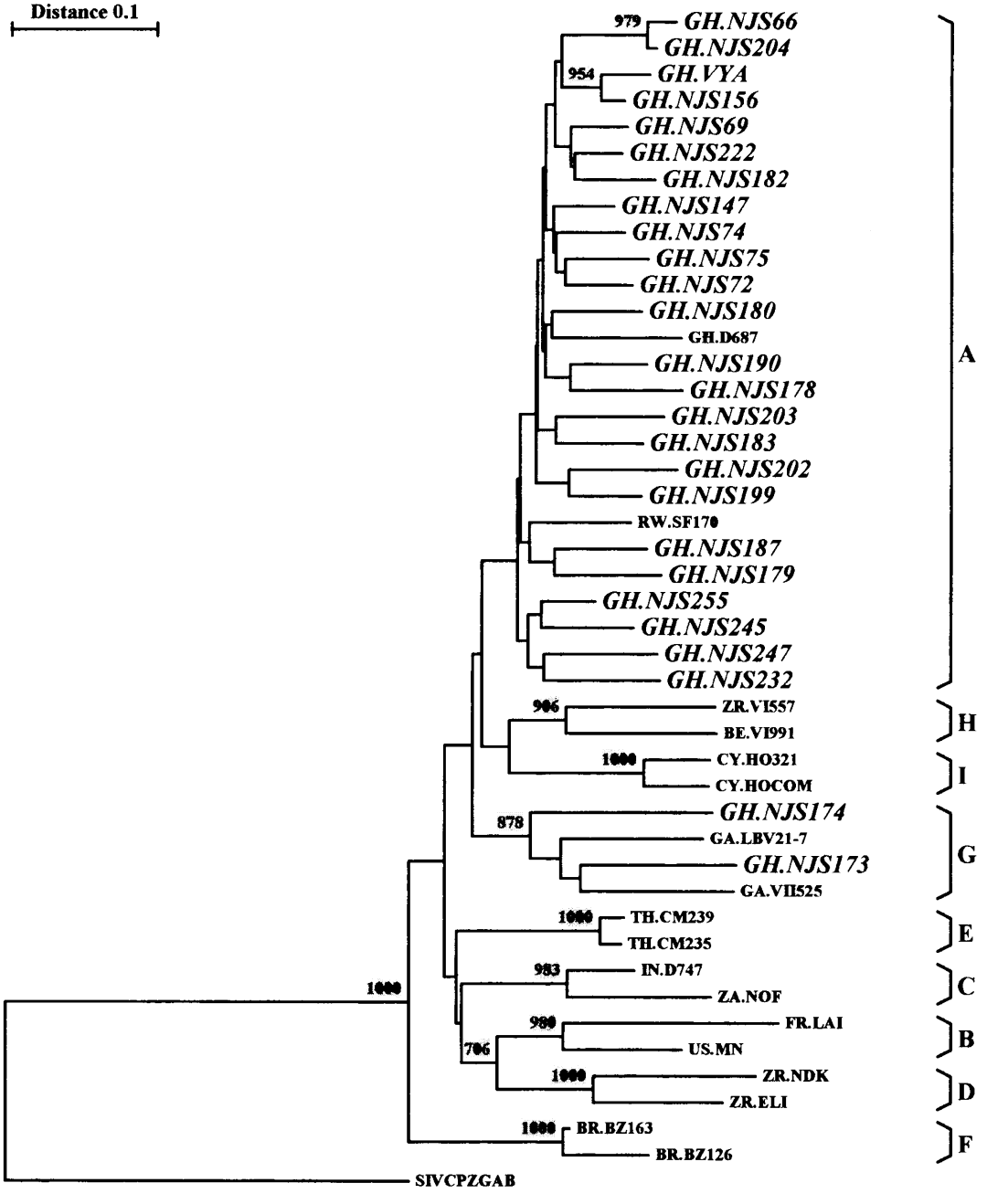

FIG. 1. Phylogenetic tree based on 264 unambiguously aligned positions of 44 HIV-1 sequences and the sequence of SIVcpz-gab. Tree topologies were inferred by neighbor joining, using the software program TREECON.[7] Sequences determined in this study are represented in boldface–italic. The root of the tree is placed so as to equalize its distance to the outgroup sequence SIVcpz-gab and its average to the HIV-1 sequences. The distance between two sequences is obtained by summing up the lengths of the connecting horizontal branches, using the scale provided (*top*). The number of bootstrap trees out of 1000 replications supporting a particular phylogenetic group in more than 50% of the replicates is placed alongside the node considered. Countries from which the strains are collected are indicated by code and precede the strain names: BE, Belgium; GH, Ghana; ZR, Zaire (now Democratic Republic of Congo); CM, Cameroon; GA, Gabon; TH, Thailand; FR, France; IN, India; ZA, South Africa; BR, Brazil; CY, Cyprus; US, United States. The nucleotide sequence data were deposited in EMBL, GenBank, and DDBJ nucleotide sequence databases under accession numbers AJ225655–AJ225659 and AJ225661–AJ225681.

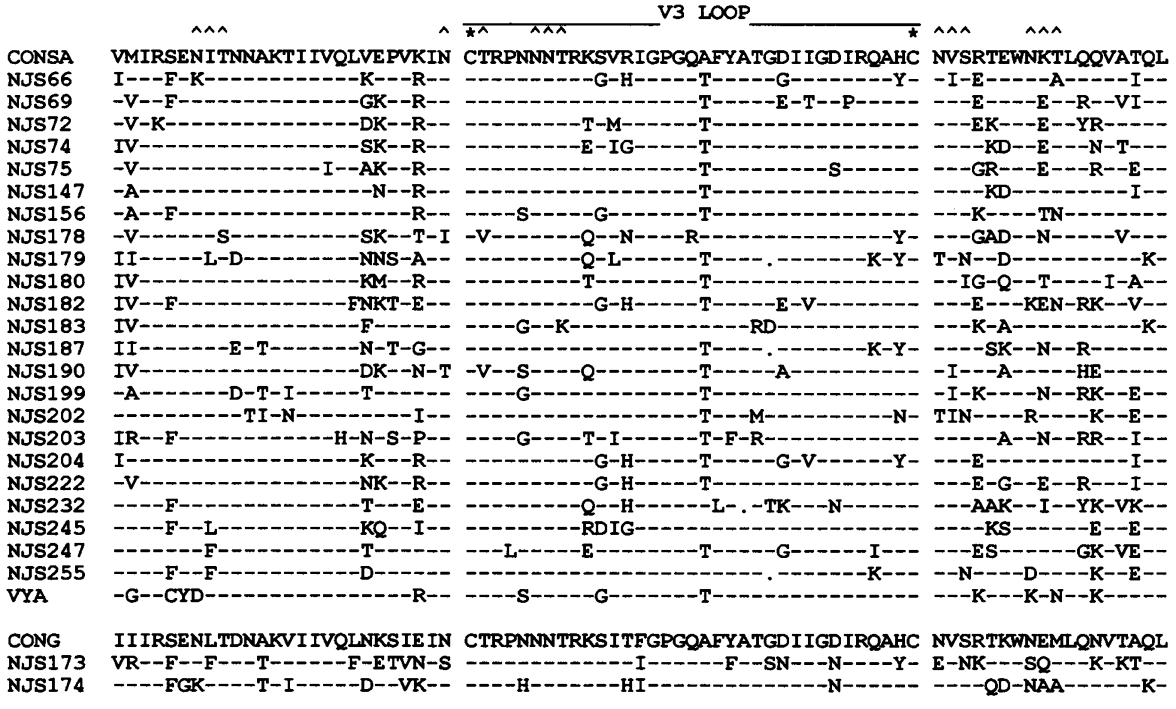

FIG. 2. Amino acid sequence alignment of Env C2V3 regions of the Ghanaian strains as compared with subtypes A and G consensus sequences. Amino acid identity is represented by dashes; points are introduced for alignment of the sequences. Carets show positions of potential N-linked glycosylation sites in the consensus sequence. Asterisks delimit the 35 amino acid residues (cysteine to cysteine) in the V3 loop of the *env* gene under comparison.

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Address reprint requests to:
James Brandful
Noguchi Memorial Institute for Medical Research (NMIMR)
University of Ghana
Legon, Ghana
E-mail: noguchi@ncs.com.gh

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