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Sequence Note

Genetic Variability of HIV Type 1 in Bénin

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In contrast to most sub-Saharan African countries, the Republic of Bénin has until recently been relatively spared from the AIDS epidemic. Heterosexual transmission is predominant and mostly the young and productive adult population is affected. Since the first case was reported in 1985, the number of cumulative AIDS patients tripled each year until 1989 and doubled from 1990 to 1992. Seroprevalence has remained below 1% in the general population.[1,2] A total of 856 cumulative AIDS cases was reported in Bénin (as of December 14, 1994), with a cumulative attack rate of 2.1 cases per 100,000 inhabitants.[3] Although these figures indicate a slow incidence rate, a rapid rise in HIV positivity rates is observed among tuberculosis (TB) and sexually transmitted disease (STD) patients and among prostitutes. HIV prevalence among prostitutes was 34.4% in 1993[1,2] but 53.3% in 1995.[4]

To our knowledge, this is the first study on HIV-1 genetic variability in Bénin. We examined the genetic variation of HIV-1 strains circulating among prostitutes in Cotonou, Bénin, where we previously documented a HIV-1 group O and group M dual infection.[5] Twenty-one HIV-1-infected prostitutes were enrolled in this study, and serum samples were taken between March 1993 and February 1994. These women were on average 30 years old; none were born in Bénin. Their country of origin was either Ghana (n = 14) or Togo (n = 7). They had on average 21 clients during the week before sampling. All declared to have used condoms at least during the year before sampling, with the exception of two, who used condoms only occasionally.

From each individual RNA was extracted from 100 μl of serum, followed by reverse transcription of the RNA and polymerase chain reaction (PCR)[6] for heteroduplex mobility assay (HMA)[7] purposes. The HMA resulted in HIV-1 subtype classification of 19 of 21 samples as subtypes A: 13 from Ghanaian and 6 from Togolese prostitutes. Two samples were classified as subtype G: 1 each from Ghanaian and Togolese prostitutes. For these samples, a 250-base pair (bp) fragment encoding the Env C2–V3 region was directly sequenced and analyzed. For four samples (BJ233, BJ251, BJ253, BJ366) no readable sequence information was obtained, and therefore these specimens were cloned. For sample BJ259, a 900-bp *env* fragment, encoding V3, V4, V5, and the start of gp41, was amplified, cloned, and sequenced, as described previously.[8] The newly determined HIV-1 *env* sequences were aligned with 19 previously known sequences of HIV-1 isolates representing HIV-1 group M subtypes A through I, and the sequence of the HIV-1-related chimpanzee isolate SIV*cpz-gab*, on the basis of primary structure. Distance calculation, tree construction, and bootstrap analysis were realized with the software package TREECON as previously described.[9] In the tree, shown in Fig. 1, 19 of 21 specimens of Bénin clustered with members of subtype A, but the clustering was not supported by 70% or more of the bootstrap tests. Two samples of Bénin clustered with subtype G strains, supported by 89.4% of the bootstrap trees. For Bénin specimens, belonging to subtype A, interhost distances at the nucleotide level were on average 17.1%, varying from 2.8% (between BJ241 and BJ281) to 33.4% (between BJ231 and BJ260). The interhost distance between the two subtype G Bénin samples, BJ43 and BJ259, was 21.9%.

The predicted amino acid sequence of the Env C2–V3 region for these strains is presented in Fig. 2. The tetrameric amino acid sequence observed at the apex of the V3 loop, the principal neutralizing domain, was GPGQ for both subtype A and G strains, except for subtype A strain BJ281, having GPGK. Octameric tips of the V3 loop–RIGPGQTF (n = 9), HIGPGQAF (n = 4), and RIGPGQAF (n = 3)—are frequently documented for subtype A.[10] In addition, octameric sequences RIGPGQSF, HIGPGQTF, and HIGPGKAF (subtype A), and HFGPGQAL and TFGPGQAF (subtype G), were documented once in this study.

This study on a limited number of samples reveals cocirculation of HIV-1 group M subtypes A and G, with subtype A

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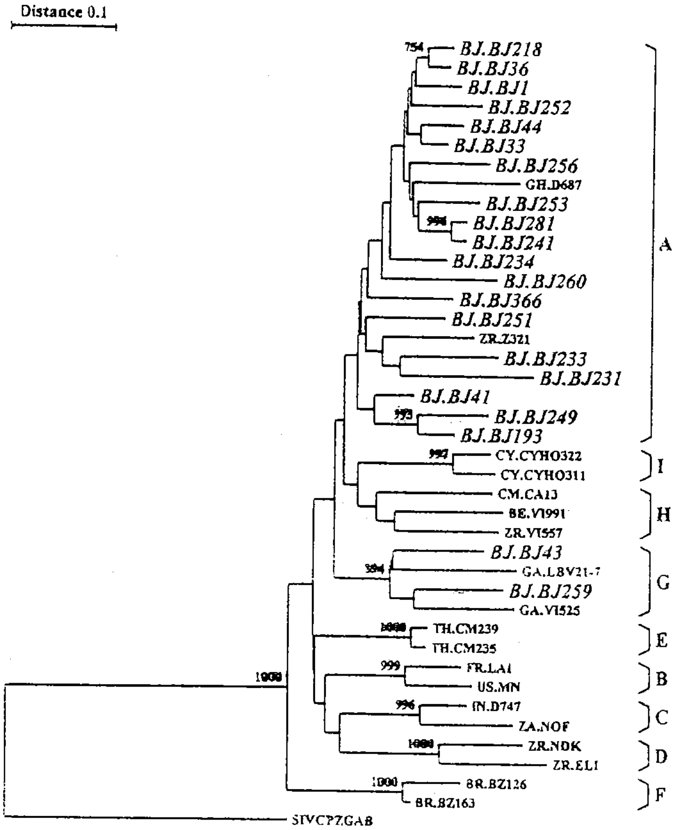

FIG. 1. Phylogenetic tree based on 270 unambiguously aligned positions of 40 HIV-1 sequences and the sequence of $SIV_{cpz-gab}$. Tree topologies were inferred by neighbor joining, using the software package TREECON.[9] The sequences determined in this study are indicated in boldface–italic. The root of the tree is placed so as to equalize its distance to the outgroup sequence $SIV_{cpz-gab}$ and its average to the HIV-1 sequences. The distance between two sequences is obtained by summing the lengths of the connecting horizontal branches, using the scale on top. The number of bootstrap trees out of 1000 replications supporting a particular phylogenetic group in more than 70% is placed alongside the node considered. Countries from which the strains are collected are indicated by a code and precede the strain names: BJ, Bénin; BE, Belgium; GH, Ghana; ZR, Zaire; 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 U61854 to U61874.

being predominant. These prostitutes having Ghanaian or Togolese nationality may have been infected before arrival in Bénin. On the basis of current knowledge of HIV-1 subtype prevalence in West African countries, subtype A is highly predominant. Although the available data on subtype prevalence in West Africa are still limited, this knowledge of subtypes based on sequence data is helpful for designing rapid subtyping strategies such as initial screening by V3 peptide serology,[11] followed by HMA.[7]

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                                                     ___________V3 LOOP___________
                    ^^^                   ^  *^      ^^^                      *  ^^^      ^^^
ALL_CONSENSUS    IIIRSENITDNAKTIIVQLNESVEIN CTRPNNNTRKSIRIGPGQAFYATGDIIGDIRQAHC NISRTKWN?TLQQVA?KLR.EHF

A_CONSENSUS_95   VMIRSENITNNAKTIIVQLVKPVKIN CTRPNNNTRKSVRIGPGQAFYATGDIIGDIRQAHC NVSRTEWNKTLQQVATQLR.KYF
BJ1              IT-----------------NQ----- -S--G-------------T---------------- -IX-----N------K---.---
BJ33             IV------I--------------R-- ----G-------------T---------------- --NG----N----------R---
BJ36             II---K------N------D---R-- ------------------T---------------- ---G--------E--K--K.---
BJ41             ----A--F-----------NES-P-- -----------I-----------.----------- ---E-K-IN---R--XZ--.RH-
BJ44             -V---K--------------T----- ----G-------------T---------------- ---Q-----------A---.---
BJ193            -I-----F-----------NES-T-D -I---------I-------------------L-Y- --T--N--N--KR-VAK--.QQ-
BJ218            I----K-----------------R-- ----S-------------T-----E---------- ---------T-----K--N.---
BJ231            -QV----F-D-T-N-----AEA-S-- -----------I------S---A-E---K----X- -INGER--N--H---EK--.EQ-
BJ233            -------L-----I--A---E--N-T -I--------GIH---------ADEV--N----Y- E-NAEK--E--Y---E---.QH-
BJ234            -V--------------A---T----D -I----------------T----.---------Y- ---GA---EA-----EK--.-E-
BJ241            IV-----------------AT--R-- ----G-----G-H--------T--E--------Y- --N------------I---.EH-
BJ249            -V--A--L-----------NEA-P-F ----S------IH---------I----------Y- T-N--S--N---R-VVK--.QQ-
BJ251            -------L----------F-E--Q-- ----------G-H---------------N--K-Y- DI---D---A-GK--E---.QH-
BJ252            -A---K---D----L----AA--G-- -----------I------T---------------- ---G----N--G--V----.EH-
BJ253            IV----D--S---N-----A---R-- ----G-------------T-----A---------- K-NG----N------A---.-EL
BJ256            IV----------------F-T--S-- ---------R--------T---------------- ---KG--DNN-R---VK--.E--
BJ260            IV------S-------I--KE--N-T -I--------G-H-----T-----G--------Y- ---KVK--N--KG--R--G.-H-
BJ281            IV------------------T--R-- ----G-----G-H----K---T--E---------- -------------------.-H-
BJ366            -K-----------------N-T-E-- ---------------------------------Y- ----AD------G--N--K.S--

G_CONSENSUS_95   IMIRSENFTDNAKVIIVQLNKSIEIN CTRPNNNTRKSITFGPGQAFYATGDIIGDIRQAHC NVSRTKWNQMLQNVKAQLRKIY
BJ43             -I-K---I-----I---------V-M ------------H------L----E--------Y- ----KD--D-----T-K-KNSL
BJ259            -----K------T-------ETV--- ----------------------TN----------- --NK---Y----K--T--KG--
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FIG. 2. Amino acid sequence alignment of Env C2V3 regions of the Bénin strains as compared to the "global" consensus, subtype A and G consensus sequences. Amino acid identity is represented by dashes; points are introduced to align the sequences. Presence of two different amino acids in a 50:50 ratio in a certain position is indicated by "X" or "Z" (BJ1: X = N and S; BJ41: X = E and D, Z = K and E; BJ231: X = H and Y).

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