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Sequence Note

Genetic and Phylogenetic Analysis of *env* Subtypes G and H in Central Africa

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PHYLOGENETIC ANALYSIS of HIV-1 strains has resulted in a classification in which different subtypes, or *clades,* are distinguished. Determining which variants are circulating in a given area at a given time is important when considering vaccination trials. To classify new strains phylogenetically, they are compared at the genetic level with a set of "reference" or "probe" strains that are representative for the respective subtypes. To date, there is evidence for at least six *env* subtypes.[1] Although HIV-1 variants of certain subtypes may be prevalent in certain geographic areas, it does not exclude circulation of less prevalent subtypes. Some variants have been reported as "uncertain" or "pending" with respect to their phylogenetic classification.[2] This may be due to a suboptimal sequence length or lack of related reference strains.[1]

Here we report on HIV-1 strains that may be considered as representative of two new *env* subtypes G and H. Four HIV-1 isolates have been studied: two from Gabon (LBV21-7 and VI525), one from Cameroon (CA13), and one from Zaire (VI557). LBV21-7, VI525, and VI557 were chosen for study on the basis of their phylogenetic classification in *gag* subtypes G (LBV21-7) and H (VI557, VI525).[1,3] This *gag* subtype classification replaced the classification earlier reported by Louwagie et al.[3] whereby sequences formerly identified as "*gag* F" and "*gag* G" are called "*gag* G" and "*gag* H" respectively[1]. CA13 was part of a study on characterization of Cameroonian subtypes.[4] The full-length HIV-1 *env* gene of VI525 and LBV21-7 was amplified from cultured peripheral blood mononuclear cells; a 900-bp part of the *env* gene encoding V3, V4, V5, and the beginning of gp41 was amplified for VI557 and CA13. Virus culture, polymerase chain reaction, cloning, and sequencing of the 900-bp fragment were performed as described previously.[5] The nucleotide sequence data were deposited in the EBML, GenBank, and DDBJ Nucleotide Sequence Databases under the following accession numbers: U09664–U09667.

The 4 newly obtained HIV *env* sequences were aligned with 21 known sequences of HIV-1 isolates belonging to different subtypes, and the HIV-1 related chimpaneze isolate $SIV_{cpz\text{-}gab}$, on the basis of primary structure. Starting from the alignment, a distance matrix corrected for multiple mutations per site, according to Jukes and Cantor, was constructed. Tree topologies were inferred by neighbor joining. Confidence values for individual branches of the resulting tree were determined by a bootstrap analysis in which 1000 bootstrap trees were generated from resampled data. Distance calculation, tree construction, and bootstrap analysis were realized with the software package TREECON.[6] A phylogenetic tree based on the common 900-bp *env* fragments was generated. LVB21-7 and VI525, on the one hand, and VI557 and CA13, on the other hand, clustered significantly in two separate subgroups different from those described before (Fig. 1). On the basis of their genetic distances from the other reported subtypes they were assigned as new subtypes. LBV21-7, formerly described as subtype G on the basis of *gag* sequences, is now classified as *env* subtype G together with VI525. Remarkably, VI525 was earlier classified as *gag* subtype H, indicating that this virus is an *env* subtype G–*gag* subtype H recombinant. VI557, formerly described as *gag* subtype H, is classified as *env* subtype H together with CA13.

The predicted amino acid sequence of the V3 loop region for these strains is presented in Fig. 2. The GPGQ tetrameric tip of the V3 loop in the "global" consensus was also present in LBV21-7 and VI557 of subtypes G and H, respectively. The GTGR motif in VI525 (G) and the GRGQ tip in CA13 are rather unusual. The replacement of proline by threonine or arginine in the respective tetrapeptides disturbs the β turn and may result in an altered epitope in the principal neutralizing domain. Both subtype G isolates have a deletion of one amino acid proximal to the last consensus glycosylation site, which is also ab-

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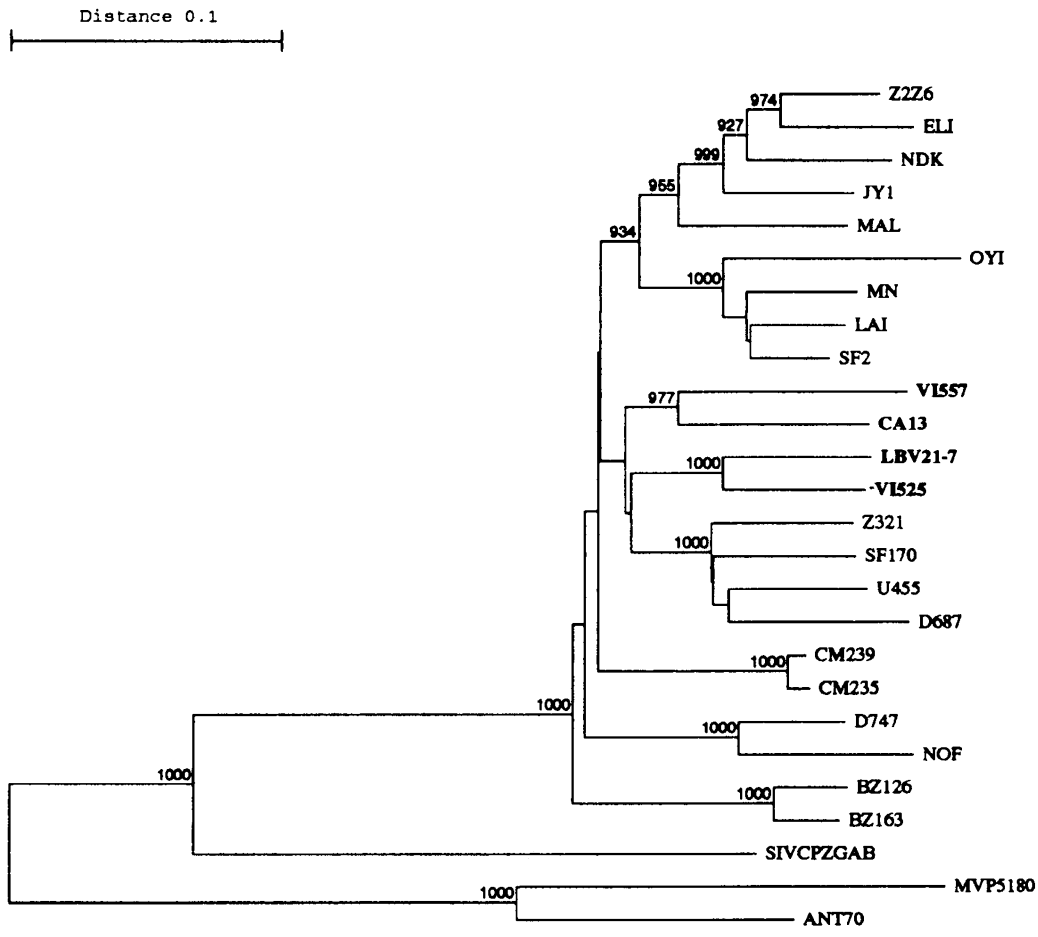

FIG. 1. Phylogenetic tree based on 872 unambiguously aligned positions of HIV-1 *env* gene sequences from 25 different subjects and the simian immunodeficiency virus $SIV_{cpz\text{-}gab}$. The sequences determined in this study are indicated in bold. The tree is unrooted. The distance between two sequences is obtained by summing the lengths of the connecting horizontal branches, using the scale at the top. The number of bootstrap trees out of 1000 replications supporting a particular phylogenetic group in more than 85% is placed alongside the node considered.

sent from the subtype A consensus. The subtype H isolates do not have the third consensus potential N-linked glycosylation site, which is also absent from the subtype A consensus.

To verify the presence of subtype G or H isolates in the Central African Republic, C2V3 sequence data of hereto unclassified strains from an earlier reported study by Murphy *et al.*[2] were aligned with the corresponding fragments of all isolates in this study and analyzed phylogenetically. Isolate U4056 was classified with high confidence in subtype H (data not shown).

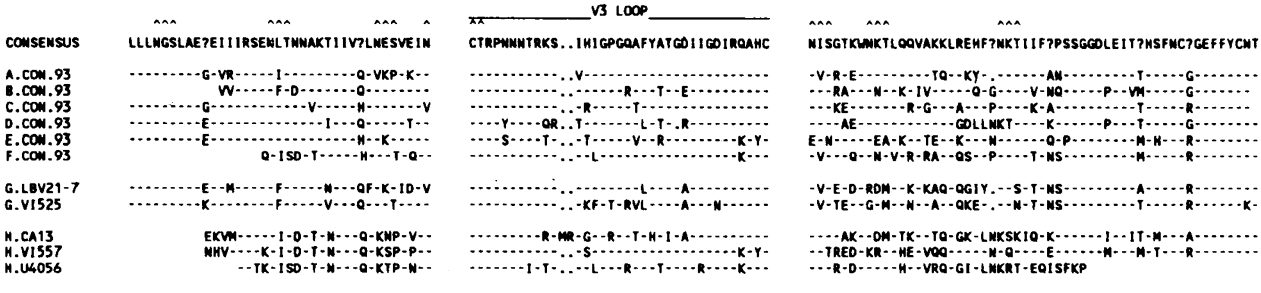

FIG. 2. Amino acid sequence alignment of *env* V3 regions of the "global" consensus and subtype consensus sequences A, B, C, D, E, and F,[1] as compared to the *env* subtype G and H sequences. Amino acid identity between sequences is represented by dashes; points are introduced to align the sequences. The positions of potential N-linked glycosylation sites are shown by carets.

Globally, eight HIV-1 *env* subtypes have now been documented, besides the HIV-1 outliers of clade O, that may eventually be representative for new subtypes.[7] When more sequences become available, additional subtypes may arise.

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