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Sequence Note

Identification of a New HIV-2 Subtype Based on Phylogenetic Analysis of Full-Length Genomic Sequence

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ABSTRACT

Human immunodeficiency virus type 2 (HIV-2) and simian immunodeficiency virus from sooty mangabey (SIV_{SM}) form one of the six primate lentivirus lineages. The close phylogenetic relationship and geographic coincidence indicate that HIV-2 originated from cross-species transmission of SIV_{SM} to humans. HIV-2 exhibits considerable genetic diversity, with subtypes A–F identified. Previously, we reported the partial *gag* and *env* sequences of an unusual HIV-2 isolate, Abt96. Abt96 was collected in Ivory Coast from an asymptomatic blood donor. Here we describe the near full-length genomic sequence of Abt96. The genome was assembled from overlapping PCR fragments amplified from viral RNA isolated from plasma. Phylogenetic analysis of sequences derived from segments of the Abt96 genome demonstrate that the Abt96 isolate branches independently of all other characterized HIV-2 isolates. On the basis of the phylogenetic data being presented, we propose that Abt96 is a new HIV-2 subtype and designate it subtype G.

SINCE THE DISCOVERY of human immunodeficiency virus type 2 (HIV-2) in West Africa,[1] phylogenetic analysis has shown that HIV-2 is related closely to simian immunodeficiency virus from sooty mangabey (SIV_{SM}); HIV-2 and SIV_{SM} form one of six primate lentivirus lineages.[2,3] The close phylogenetic relationship and geographic coincidence of HIV-2 and SIV_{SM} support the hypothesis that HIV-2 originated from cross-species transmission of SIV_{SM} from sooty mangabeys into humans.[3] By analogy to HIV-1 group M, HIV-2 isolates have been classified into genetic subtypes, designated A–F.[3–6] The majority of HIV-2 sequences in the database are subtypes A and B; currently the only full-length genomic sequences available for HIV-2 (13 isolates) are subtypes A or B.[6] Subtypes C, D, E, and F are each represented by a single isolate with sequences limited to small regions of the viral genome.[3–5]

The partial characterization of a unique HIV-2 isolate, Abt96, was described previously.[7] Abt96 was collected in Ivory Coast from an asymptomatic blood donor between 1992 and 1994. Phylogenetic analysis of sequences derived from segments of the Abt96 genome, *gag* p26, *env* gp105 V3, and *env* gp36 IDR (immunodominant region), demonstrated that the Abt96 isolate did not group closely with any other characterized HIV-2 isolate. To expand our understanding of the HIV-2 virus family and facilitate a more thorough analysis of Abt96, we sequenced the complete genome of the Abt96 isolate.

The full genomic sequence of Abt96 was generated by polymerase chain reaction (PCR) amplification of viral RNA, using methods previously described.[7] Briefly, total nucleic acids were extracted from plasma with a QIAamp blood kit (Qiagen, Chatsworth, CA). Overlapping fragments were generated by nested reverse transcription (RT)-PCR amplification. The sequences of primers used for PCR amplification were based on specific Abt96 sequence data and on the Los Alamos alignment of HIV-2/SIV sequences.[6] Primers used for amplification and sequencing are not shown but sequences are available on request. PCR fragments were purified with a QIAquick PCR purification kit or isolated by agarose gel electrophoresis and then purified with a QIAquick gel extraction kit (Qiagen). Generally, fragments were sequenced

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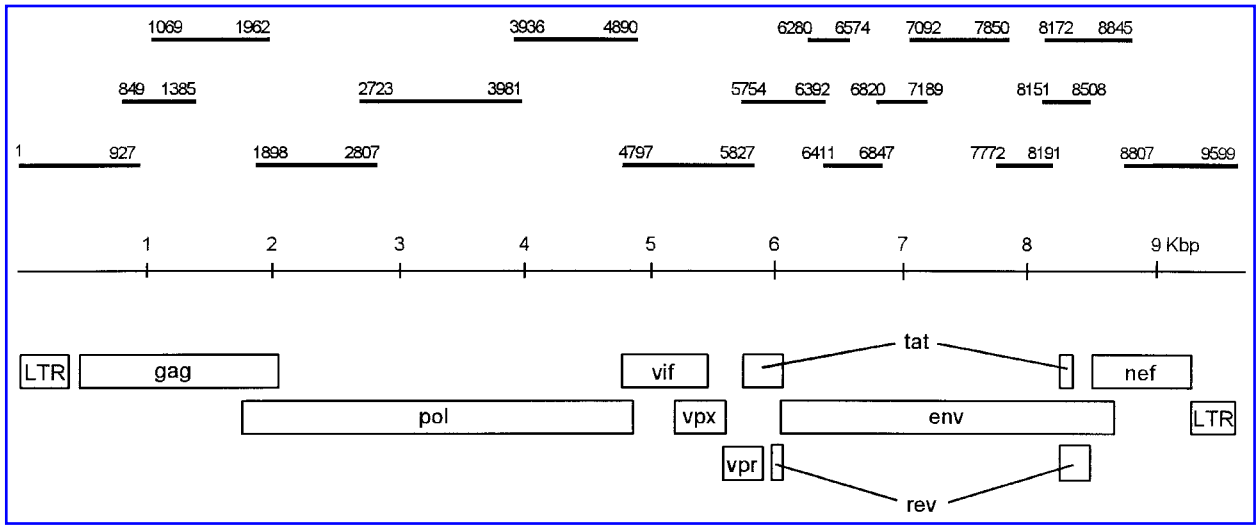


FIG. 1. Schematic diagram of the Abt96 genome. Overlapping PCR fragments used to generate full-length Abt96 sequence are shown as solid lines above the ruler, with the numbers indicating their nucleotide positions in the Abt96 full genome. The locations of structural genes and regulatory elements are shown below the ruler; the genome was mapped on the basis of open reading frames and by sequence homology to other HIV-2 isolates.

directly. However, when insufficient fragment was available or a heterogeneous fragment population resulted in ambiguous sequence data, fragments were cloned into the cloning vector PCR2.1 (InVitrogen, San Diego, CA) for sequencing. DNA sequencing was performed with Big-Dye terminator labeling kits on an automated DNA sequencer (ABI model 377; Perkin-Elmer, Norwalk, CT).

For phylogenetic analysis, regions of the Abt96 sequence were aligned with the corresponding sequences from other HIV-2 and SIV isolates, using GeneWorks release 2.4 (IntelliGenetics, Mountain View, CA), and manually edited. The nucleic acid alignments were evaluated with the PHYLIP software package (University of Washington, Seattle, WA). Evolutionary distances were estimated with Dnadist (Kimura two-parameter method) and phylogenetic relationships were determined with Neighbor (neighbor-joining method). The reproducibility

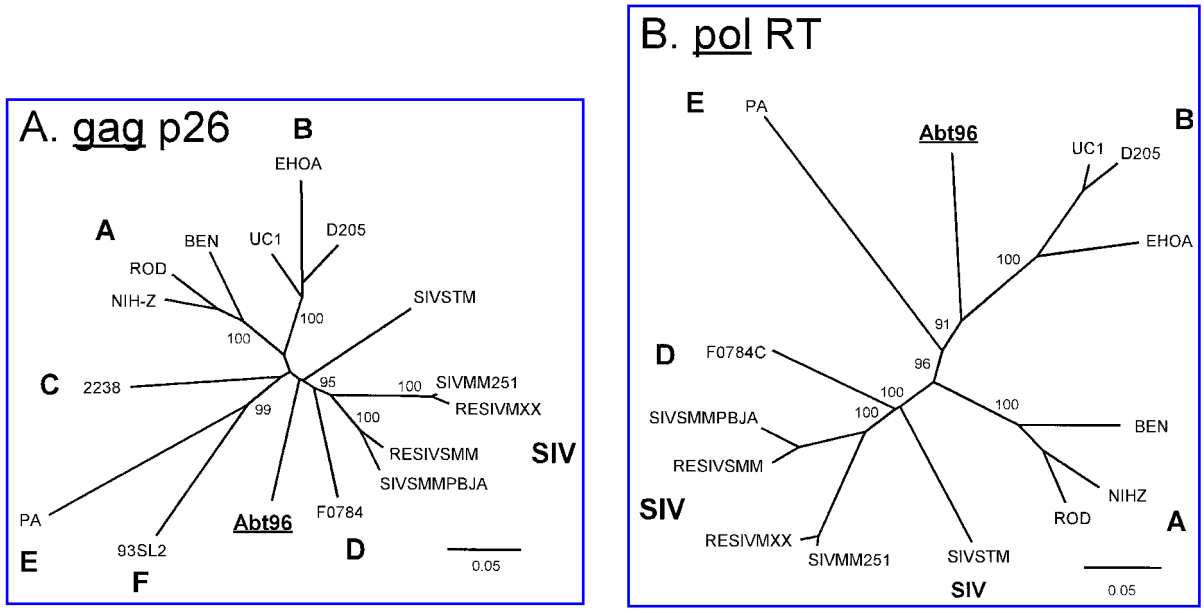
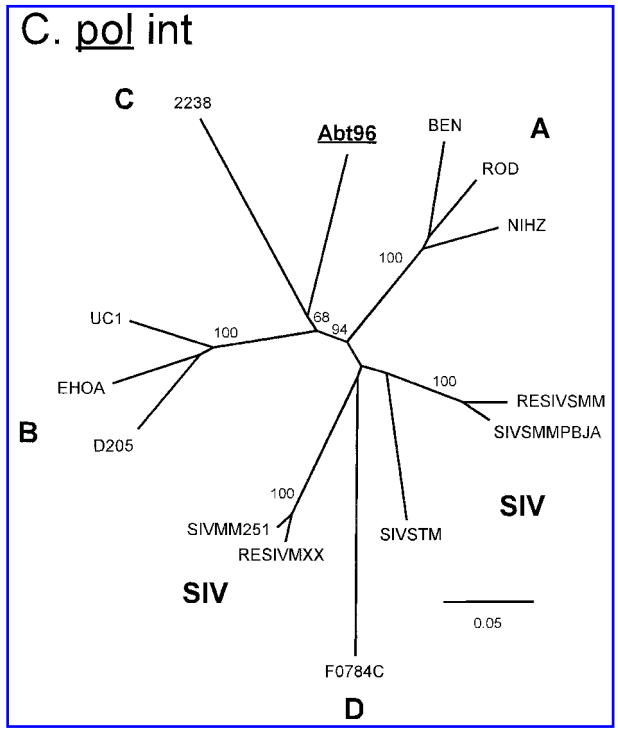
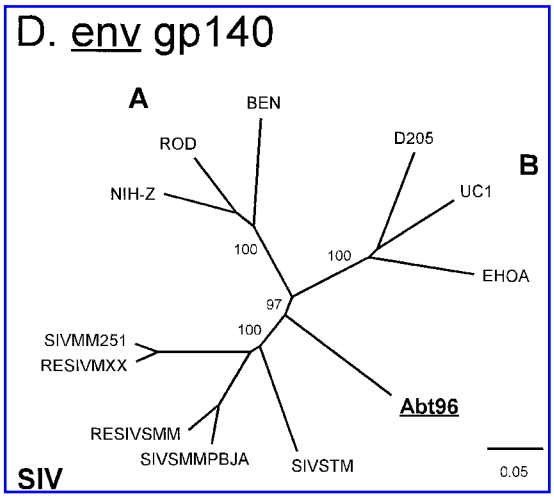


FIG. 2. Phylogenetic trees. Presented are the phylogenetic relationships between Abt96 and reference strains of HIV-2 and SIV. Reference sequences are designated by the isolate name with the subtype shown above the appropriate branch. The numbers at the nodes represent the percent reproducibility of the branch point. (**A**) *gag* p26, 780 bp; (**B**) *pol* reverse transcriptase, 995 bp; (**C**) *pol* integrase, 708 bp; (**D**) *env* gp140, 2600 bp; (**E**) *nef* LTR, 704–756 bp.





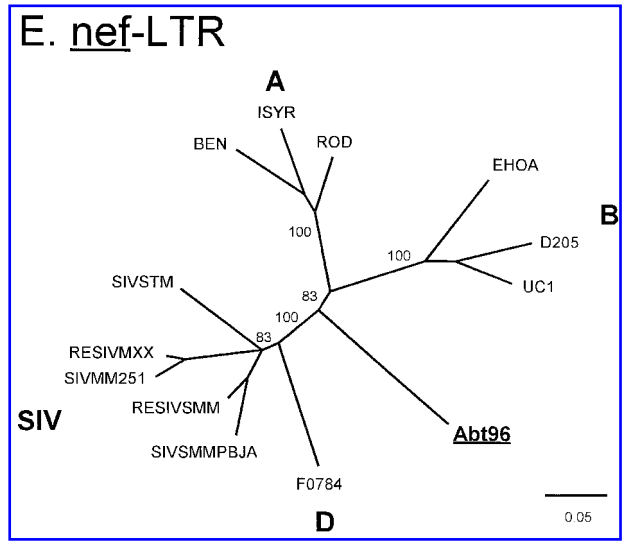


FIG. 2. *Continued*.

of the branching pattern was done with Seqboot (bootstrap, 100 replicates). Programs were run with default parameters.

Multiple attempts to amplify fragments greater than 1500 base pairs from plasma failed. This may have been due to low RNA titer, poor RNA condition, or the presence of inhibitory agents. Thus the complete genomic sequence was determined by PCR amplification and sequence analysis of 16 overlapping fragments ranging in length from 300 to 1300 base pairs (Fig. 1). Structural genes and regulatory elements were identified on the basis of homology with HIV-2 isolates ROD,[8] GH1,[9] and ST.[10] The final genomic sequence represents the predominant or consensus sequence of the virus population present in the Abt96 plasma sample. A BLAST search (BLASTN 2.0.10, August 26, 1999), using the complete Abt96 sequence, showed significant sequence homology only with sequences from HIV-2 isolates and SIVs from mangabey and macaque monkeys. The highest similarity scores are to HIV-2 isolates UC1 and BEN and to RESIVSMM, all of which were included in the phylogenetic analysis. The GenBank accession number for Abt96 is AF208027.

Phylogenetic analysis was performed on several regions of the genome, including *gag* p26, *pol* reverse transcriptase, *pol* integrase, *env* gp140, and *nef* long terminal repeat (LTR). Evaluation of genomic regions of Abt96 generally was restricted to partial genes in order to include subtypes C, D, E, and F. On the basis of phylogenetic analysis of a partial region of *gag* p26

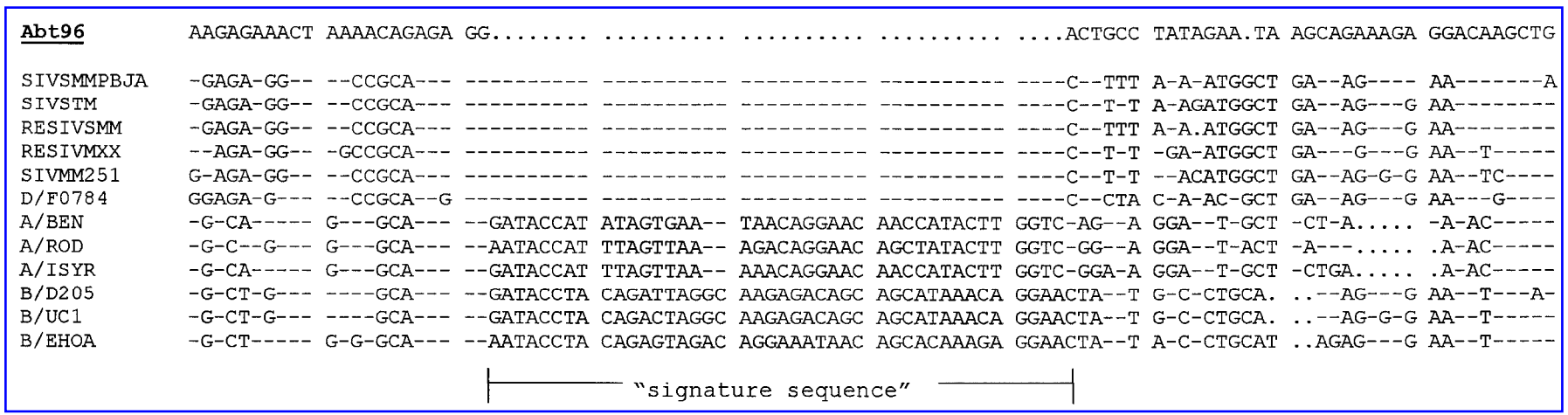


FIG. 3. Nucleic acid alignment of the *nef* LTR region. The *nef* LTR region of Abt96 is compared with reference HIV-2 and SIV sequences. Dashes indicate sequence identity with Abt96 and dots indicate gaps introduced for optimal alignment. The first base in the alignment corresponds to base 9290 in reference sequence HIV-2_{ROD} and to base 9238 in Abt96.

TABLE 1. AVERAGE INTRA- AND INTERSUBTYPE GENETIC DISTANCES FOR THE gag^{p26} REGION[a]

Subtype	*A*	*B*	*C*	*D*	*E*	*F*	*SIV*	***Abt96***
A	0.10							
B	0.19	0.10						
C	0.21	0.21	NA					
D	0.20	0.20	0.22	NA				
E	0.32	0.30	0.30	0.28	NA			
F	0.26	0.27	0.24	0.24	0.27	NA		
SIV	0.21	0.22	0.21	0.16	0.29	0.24	0.13	
Abt96	**0.20**	**0.21**	**0.21**	**0.18**	**0.29**	**0.24**	**0.18**	**NA**

[a]Distances were generated by Dnadist for the sequences used to construct the gag^{p26} phylogenetic tree (Fig. 2A). NA, Not applicable.

(780 bp), which is the only region where sequences from all HIV-2 subtypes A–F are available, Abt96 forms a branch independent of the known HIV-2 subtypes (Fig. 2A). The average genetic distances from the *gag* p26 phylogenetic analysis are shown in Table 1. For subtypes with more than one isolate available (A, B, and SIV) the average intrasubtype distance is 0.10–0.13. In contrast, the intersubtype distances range from 0.16 to 0.32. The genetic distances between Abt96 and the other isolates range from 0.18 to 0.24. Thus, the average genetic distance between Abt96 and other HIV-2 isolates is significantly greater than intrasubtype distances and Abt96 is roughly equidistant to each subtype and SIV.

Partial gene sequences are available in *pol* reverse transcriptase for subtypes A, B, D, E, and SIV. As shown in Fig. 2B, in the phylogenetic analysis of the sequences for *pol* reverse transcriptase (995 bp) Abt96 continues to branch independently of the other known isolates. Partial gene sequences are available in *pol* integrase for subtypes A, B, C, D, and SIV. As shown in the analysis of *pol* integrase (708 bp) (Fig. 2C), Abt96 is associated with subtype C isolate 2238 by a bootstrap value of 68%. However, the genetic distance between the two isolates, 0.22, is comparable to the distances between Abt96 and subtype A, B, and SIV isolates, (0.24, 0.21, and 0.23, respectively).

The phylogenetic tree of full-length *env* gp140 contains only Abt96, subtypes A and B, and SIV; consistent with analysis of other genomic regions, the Abt96 isolate forms a branch independent of subtypes A and B, and SIV (Fig. 2D). In the *env* IDR tree reported previously,[7] Abt96 branched separately from subtypes A, B, C, D, and SIV; inclusion of subtype F isolate 93SL2,[5] identified since publication of the Abt96 IDR sequence, does not change the shape of the tree with both 93SL2 and Abt96 continuing to branch independently (data not shown).

Although the average intra- and intersubtype distances are not shown for the phylogenetic trees in Fig. 2B–E, the results are similar to those obtained for *gag* p26 (Table 1). For all regions analyzed Abt96 is roughly equidistant to each subtype and SIV, the genetic distance between Abt96 and the other isolates is in the range of intersubtype distances, and the intersubtype distances are significantly greater than the intrasubtype distances.

A characteristic feature of HIV-2 is a 40- to 44-bp "signature sequence" located in the LTR, which previously distinguished HIV-2 (human) isolates from SIV_{SM}/SIV_{MAC} (simian) isolates.[4] An alignment of this region of the LTR shows that this signature sequence is absent in Abt96 (Fig. 3). Until now the only other HIV-2 isolate that lacked this signature sequence was the subtype D isolate F0784,[4] which is closely related to SIV isolates. Unlike F0784, Abt96 does not cluster with SIV (Fig. 2A–E), making the absence of this sequence notable.

Phylogenetic analysis indicates that Abt96 forms a separate branch within the HIV-2/SIV cluster for all regions of the genome analyzed. On the basis of these data we propose that Abt96 is a new HIV-2 subtype and designate it subtype G. Discovery of variants such as Abt96 emphasize the divergent nature of HIV-2 and may lead to a better understanding of lentivirus evolution and its cross-species transmission into humans.

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