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GENOME SEQUENCES

Complete Genome Sequence of a Baboon Simian Foamy Virus Isolated from an Infected Human

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ABSTRACT We obtained the full-length genome of a simian foamy virus (SFV) from an infected human. This virus originated from a baboon (*Papio* species, strain SFVpxx_hu9406). The genome is 13,113 nucleotides long with the canonical SFV genome structure. Phylogenetically, SFVpxx_hu9406 clustered closely with SFVpan_V909/03F from a captive baboon and other Cercopithecidae SFVs.

Simian foamy viruses (SFVs) belong to the genus *Spumavirus* and family *Retroviridae* and are complex retroviruses that are ubiquitous in many nonhuman primates (NHPs), including apes, Old World monkeys (OWMs), New World monkeys (NWMs), and prosimians (1). SFV seroprevalence in primates is very high, exceeding 75% in some cases (1). A number of studies have described zoonotic transmission from primates to humans in various populations, such as persons working in zoos or primate research centers and persons exposed to primates in natural habitats, especially in many parts of Africa where hunting and butchering of primates is common (2–8). However, no cases of human-to-human transmission or disease have been reported to date (3, 5, 8, 9). As human populations expand and encroach upon NHP habitats, risks for SFV exposure and infection continue to increase. The monitoring of potential zoonotic infections will be facilitated by the development of sensitive molecular and serologic assays. The availability of full-length SFV sequences from a variety of primate species can benefit the development of improved diagnostic assays and the study of adaptive changes in SFVs following transmission to humans. We describe here the sequencing and characterization of the full-length SFV genome isolated from a human likely infected with a baboon (*Papio* species, strain SFVpxx_hu9406) variant. We also analyzed evolutionary relationships with other primate SFVs using nonsimian foamy viruses (FVs) as outgroups.

The SFVpxx_hu9406-infected NHP worker was identified in previous studies of primate research centers and zoo workers (10, 11). He was an animal care supervisor who had >30 years of exposure to NHPs, including baboons and chimpanzees. He reported a severe baboon bite prior to 1985 and first tested seropositive for SFV in 1988. The baboon species was not reported. We used the phenol-chloroform method to extract genomic DNA from infected *Mus dunni* fibroblast cells generated by coculture with the NHP worker's peripheral blood mononuclear cells. We PCR amplified seven overlapping subgenomic fragments (Table 1), which were then Sanger sequenced using an ABI 3100 instrument (Applied Biosystems, Foster City, CA) and assembled into the complete genome using Geneious v11.1.4. We used the gene annotation tools in Geneious to identify the group-specific antigen (*gag*), polymerase (*pol*), envelope (*env*), transcriptional activator (*tas*), and *bet* (between *tas* and *env*) coding regions of the SFVpxx_hu9406 genome. We determined the positions of the complete 5′ and 3′ long terminal repeats (LTRs) manually using the previously published complete SFVpan_V909/03F proviral genome (GenBank accession number MK241969) from a captive olive baboon as a reference (12). We used

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TABLE 1 Primers used for generation of overlapping subgenomic regions of the SFVpxx_hu9406 genome

Genomic region[a]	Primer	Amplification step	Sequence (5′ → 3′)
LTR	FVLTRF1	Primary	TAG IIA ATG AAG GAA CIC TII AIG AGT A
LTR	BABLR1	Primary	TAC ACC TCT TGG GAT AAG TGT AGT
LTR	FVLTRF2	Nested	AGT TAA TCC TTA GGI AGI ATT TGG T
LTR	BABLR2	Nested	AGC AAG GCT AAT ATA CAA TAT CTT TCA
LTR-*gag*	PBF1	Primary	CAC TAC TCG CTG CGT CGA GAG TGT
LTR-*gag*	SPUG1R	Primary	TTT GTC CTC TGG CAT TGA GGC CTA
LTR-*gag*	FVLGF1	Nested	TGT ICG AGA CTC TCC AGG ITT GGT AAG
LTR-*gag*	SPUG3R	Nested	TTC TTG ATC TAG ACG CTG ITG CA
gag-pol	SPUGF2b	Primary	CCT ATG TGG ATT GGA AGA AAT TCT GC
gag-pol	SPGR1	Primary	AAC CAW ACA AAT CCA GTC ATW CCR TC
Diagnostic integrase	DNHF1	Primary	GCC ACC CAA GGR AGT TAT GTG G
Diagnostic integrase	DNHR2	Primary	GCT GCM CCY TGR TCA GAG TG
Diagnostic integrase	DNHF3	Nested	CCT GGA TGC AGA GYT GGA TC
Diagnostic integrase	DNHR4	Nested	GAR GGA GCC TTW GTK GGR TA
pol-env	SPGF1	Primary	AAT TAC TAC AAG GAC AGT ATC CAA AAG GTT
pol-env	SFVenvR7	Primary	GII AGC TGC IGC AGG CCA AAC GTC
pol-env	SPGF1	Nested	AAT TAC TAC AAG GAC AGT ATC CAA AAG GTT
pol-env	BABenvR6	Nested	GGA TGT CTA GCC GAA GTA GCT GTG
env	SFVenvF3	Primary	CAT GAT ITI ICI ITI ATG GAA GGA ATG
env	SFVenvR8	Primary	GII GWI CCR AAT ATI CCI TGG GCA
env	SFVenvF3	Nested	CAT GAT ITI ICI ITI ATG GAA GGA ATG
env	SFVenvR7	Nested	GII AGC TGC IGC AGG CCA AAC GTC
ORFs	BABENVF7	Primary	TCG GCT AGA CCA YGA AGG AGA
ORFs	BABLR12N	Primary	GGA GCA CCG GCG TGA ATG AAC TGG

[a] LTR, long terminal repeat; *gag*, group-specific antigen; *pol*, polymerase; *env*, envelope; ORFs, open reading frames between *env* and 3′ LTR (including the transcriptional activator [*tas*] and *bet* [between *tas* and *env*]).

MAFFT v7.017 within Geneious to align *gag-pol-env* concatemers from representative SFVs with complete genomes from four apes, two OWMs, four NWMs, one prosimian, and one FV each from equine, bovine, and feline hosts. We determined phylogenetic relationships using Bayesian inference (BEAST v1.8.4).

The SFVpxx_hu9406 genome is 13,113 nucleotides (nt) long with a GC content of 33.3%. The complete proviral genome comprises all expected structural, enzymatic, and auxiliary gene-coding regions flanked by the long terminal repeats (Fig. 1A). The gene lengths are 1,899 nt for g*ag*, 3,468 nt for *pol*, 2,973 nt for *env*, 602 nt for *tas*, and 1,269 nt for *bet*. Interestingly, the *tas* gene was 303 nt shorter than that of SFVpan_909/03F due to a large deletion that removed the *bet* intron that is within the *tas* coding sequence such that the two *bet* exons are now directly linked in the same frame. FVs with this deletion have been termed Δ*tas* genomes, are believed to replicate poorly since they do not produce Tas, and are involved in SFV persistence (13, 14). SFVpxx_hu9406 persistently infected the *M. dunni* cell line to constitutively express virus without cytopathic effect. The *bet* sequence also has a single adenine insert that causes a frameshift mutation toward the end of the protein, which truncates it by 71 amino acids. Comparison of SFVpan_hu9406 gene sequences to those from other OWM SFVs showed that SFVpxx_hu9406 was distinct but shared the highest nucleotide identity with SFVpan_V909/03F (*gag*, 93.4%; *pol*, 91.2%; *env*, 78.5%; *tas*, 62.6%; *bet*, 89.1%; and the whole genome, 81.1%).

Phylogenetic trees generated using Bayesian inference of the *gag-pol-env* concatemer showed that FV sequences from a broad range of genetically diverse NHPs and nonsimians formed monophyletic lineages and distinct clusters (Fig. 1B). SFVpxx_hu9406 clustered closely with SFVpan_V909/03F and in a clade with other OWMs with strong posterior probability (>1) support.

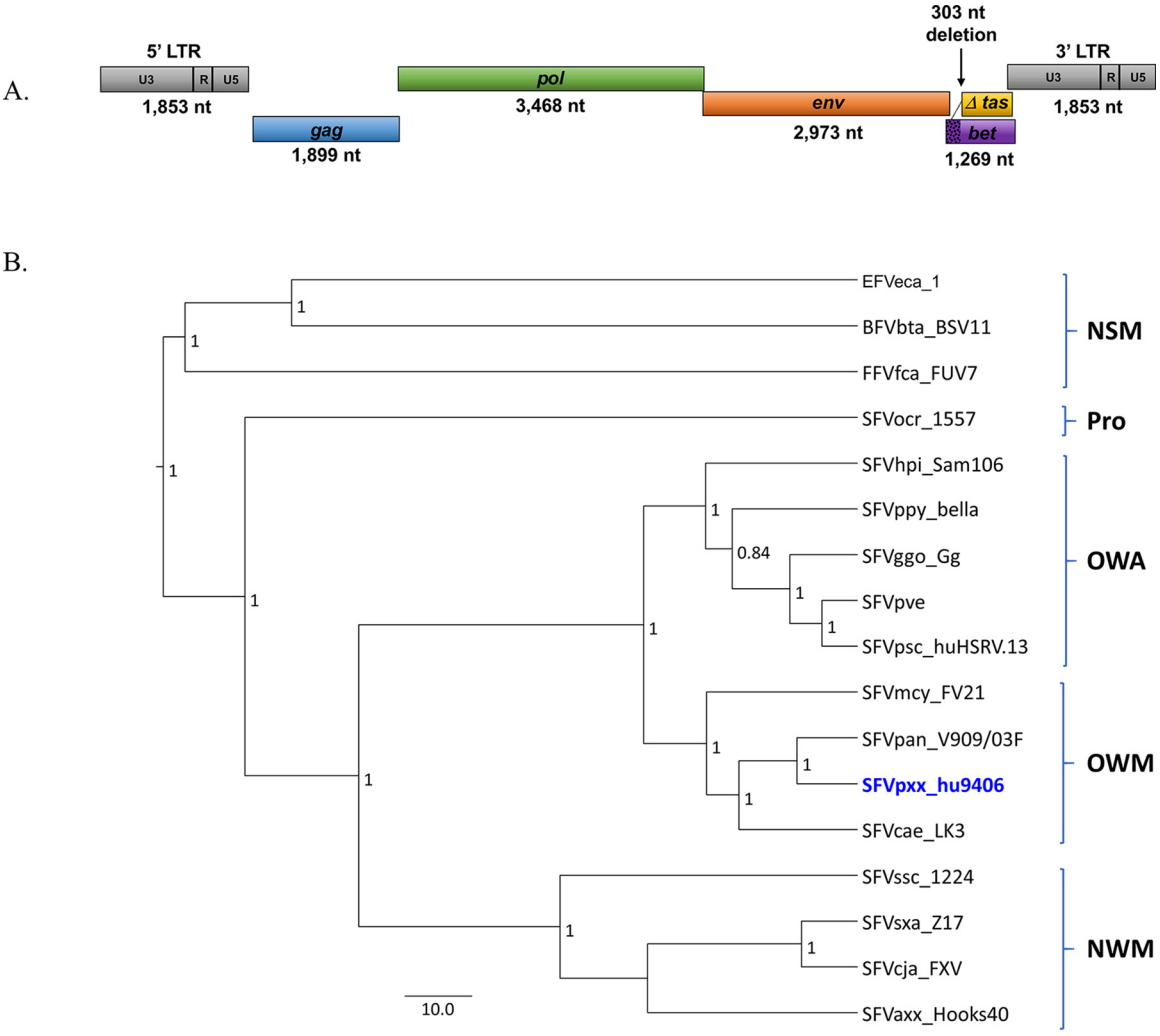

FIG 1 (A) Genomic structure of SFVpxx_hu9406. LTR, long terminal repeat; *gag*, group-specific antigen; *pol*, polymerase; *env*, envelope; Δ*tas*, defective transactivator gene due to 303-nt deletion; *bet*, between *env* and *tas* genes; U3, unique 3′ region of the LTR; R, repeat region of the LTR; U5, unique 5′ region of the LTR. The Bet protein is typically translated from a spliced RNA and residues from the 5′ part of *tas* indicated by the speckled region. However, the 303-nt deletion of the *bet* intron results in a contiguous *bet* coding region in SFVpxx_hu9406. The dotted line indicates that the *tas* reading frame shared with *bet* (purple speckled region) would require RNA slicing to the 2nd *tas* exon in another reading frame. (B) Evolutionary relationships of foamy viruses (FVs) from various mammals inferred by BEAST analysis of the *gag-pol-env* concatemer (~7.0 kb). Posterior probabilities are provided on the branch to the right of the node. Branch lengths are proportional to median divergence times in years estimated from the postburn in trees, with the scale at the bottom indicating 10 million years. Old World apes (OWA): SFVpve, *Pan troglodytes verus* (chimpanzee), GenBank accession number U04327; SFVpsc_huHSRV.13, *Pan troglodytes schweinfurthii* (chimpanzee), Y07725; SFVppy_bella, *Pongo pygmaeus* (orangutan), AJ544579; SFVggo_Gg, *Gorilla* (gorilla), NC_039029; SFVhpi_SAM106, *Hylobates pileatus* (pileated gibbon), MF621235. Old World monkeys (OWM): SFVpxx_hu9406 (human infected with baboon SFV, MF472626) ("xx" in the SFV name indicates that the simian species is unknown); SFVpan_V909/03F, *Papio anubis* (olive baboon), MK241969; SFVcae_LK3, *Cercopithecus aethiops* (African green monkey), M74895; SFVmcy_FV21, *Macaca cyclopsis* (macaque), X54482. New World monkeys (NWM): SFVcja_FXV, *Callithrix jacchus* (common marmoset), GU356395; SFVsxa_Z17, *Sapajus xanthosternos* (capuchin), KP143760; SFVaxx_Hooks40, *Ateles* species (spider monkey), EU010385; SFVssc_1224, *Saimiri sciureus* (squirrel monkey), GU356394. Prosimian (Pro): SFVocr_1557, *Otolemur crassicaudatus* (brown greater galago), KM233624. Nonsimian mammals (NSM): EFVeca_1, *Equus caballus* (equine), AF201902; BFVbta_BSV11, *Bos taurus* (bovine), U94514; FFVfca_FUV7, *Felis catus* (feline), Y08851.

Data availability. The SFVpxx_hu9406 sequence is available in GenBank under the accession number MF472626.

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We declare no conflicts of interest.

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V. Shanmugam performed the PCR testing and sequencing. A. Shankar and W. M Switzer conducted the sequence and phylogenetic analyses and wrote the manuscript.

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