

Short Communication

Natural simian foamy virus infection in wild-caught gorillas, mandrills and drills from Cameroon and Gabon

Sara Calattini,¹ Eric Nerrienet,² Philippe Mauclère,^{1,2}
Marie-Claude Georges-Courbot,³ Ali Saïb⁴ and Antoine Gessain¹

Correspondence
Antoine Gessain
agessain@pasteur.fr

¹Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes, Département EMMI, Institut Pasteur, Paris, France

²Centre Pasteur du Cameroon, Yaoundé, Cameroon

³Centre International de Recherches Médicales, Franceville, Gabon

⁴CNRS UPR 9051, Hôpital Saint Louis, Paris, France

A survey for the presence of simian foamy retroviruses (SFVs) was performed in 44 wild-caught apes and monkeys, including 27 gorillas, 11 mandrills and six drills, originating from south Cameroon or Gabon. Combined serological and/or nested-PCR assays indicated SFV infection among five *Gorilla gorilla gorilla*, seven *Mandrillus sphinx* and two *Mandrillus leucophaeus*. Sequences of a 425 bp fragment of the *integrase* gene were obtained for 11 animals. Phylogenetic studies indicated that strains from gorillas, mandrills and drills each formed a highly supported phylogenetic clade with, moreover, the existence of two different gorilla SFVs. This study demonstrates for the first time that these animals are naturally infected with specific SFVs. In the context of simian-to-human interspecies transmission, the results confirm that such viruses can also infect humans, as the SFVs identified in wild-caught animals were the same as those recently reported as infecting hunters living in the same geographical areas.

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The recognition that AIDS originated as a zoonosis heightened public health concerns associated with infection of humans by other simian retroviruses endemic in non-human primates (NHPs) (Switzer *et al.*, 2004). These retroviruses include several strains of simian immunodeficiency virus (SIV) (Hahn *et al.*, 2000; Peeters *et al.*, 2002), simian T-cell lymphotropic virus types 1, 2 and 3 (STLV-1/2/3) (Meertens & Gessain, 2003; Meertens *et al.*, 2001) and simian foamy retroviruses (SFVs or spumaretroviruses) (Bieniasz *et al.*, 1995; Broussard *et al.*, 1997; Hussain *et al.*, 2003; Switzer *et al.*, 2004; Verschoor *et al.*, 2003).

Recent papers have reported the presence of SFV infection in a small number of people occupationally exposed to NHPs (Brooks *et al.*, 2002; Heneine *et al.*, 1998; Sandstrom *et al.*, 2000; Schweizer *et al.*, 1997; Switzer *et al.*, 2004). Based on these studies, it seems that SFVs, which are highly endemic in captive NHPs (around 70%), are transmitted at a higher rate (approx. 2–5%) than are other primate retroviruses into people working in zoos and/or primate research centres. Wolfe *et al.* (2004) reported the first

evidence for the presence of naturally acquired infection with SFVs in three hunters from southern Cameroon. Based on a comparison of the viral sequences identified in the hunters with those known from different captive monkeys and apes, they concluded that the hunters had been infected by viruses originating from gorilla, mandrill and De Brazza's guenon, respectively.

As part of the ongoing studies on retroviruses (Nerrienet *et al.*, 2001, 2004) and herpesviruses (Lacoste *et al.*, 2000, 2001) in NHPs, we searched for SFVs in a series of plasma and/or DNA samples from wild-caught gorillas (*Gorilla gorilla gorilla*), mandrills (*Mandrillus sphinx*) and drills (*Mandrillus leucophaeus*) originating from Cameroon (36 animals) or Gabon (eight animals).

The animals from Cameroon were all wild-born, mostly in the southern rain-forest areas, and wild-caught, generally during hunting, after their mothers had been killed by hunters. Most of the drills and mandrills, after being brought to the zoos or sanctuaries, which housed only animals from the region, were kept in individual cages. Most of the gorillas were housed with animals of the same species only. This made the possibility of transmission of virus by contact with animals of another species during the period of captivity highly unlikely. Samples were taken from

The GenBank/EMBL/DDBJ accession numbers of the 11 novel SFV strains determined in this work are AY583775–AY583782 and AY603409–AY603411.

Table 1. Percentage nucleotide identities for a 425 bp fragment of the *integrase* gene between the three novel *Gorilla* foamy virus strains and previously published SFV sequences from *Gorilla*

Strain	<i>G. gorilla gorilla</i>		
	GorGabColSFV	GorGabOmoSFV	GgoCam7SFV
GorGabColSFV	–	100·0	96·2
GorGabOmoSFV	100·0	–	96·2
GgoCam7SFV	96·2	96·2	–
CAM1083	96·2	96·2	98·6
SFVggo	95·5	95·5	97·9
Ggobabs	96·2	96·2	100·0

Table 2. Percentage nucleotide identities for a 425 bp fragment of the *integrase* gene between the eight novel *Mandrillus* foamy virus strains and previously published SFV sequences from *Mandrillus*

Strain	<i>M. sphinx</i>						<i>M. leucophaeus</i>	
	Mnd13SFV	Mnd14SFV	Mnd18SFV	Mnd15SFV	Mnd2eSFV	Mnd301SFV	Mnd203SFV	Mnd205SFV
Mnd13SFV	–	100·0	100·0	99·3	97·1	96·9	82·1	89·6
Mnd14SFV	100·0	–	100·0	99·3	97·1	96·9	82·1	89·6
Mnd18SFV	100·0	100·0	–	99·3	97·1	96·9	82·1	89·6
Mnd15SFV	99·3	99·3	99·3	–	96·4	96·2	81·6	89·2
Mnd2eSFV	97·1	97·1	97·1	96·4	–	98·3	83·0	90·1
Mnd301SFV	96·9	96·9	96·9	96·2	98·3	–	83·3	90·6
Mnd203SFV	82·1	82·1	82·1	81·6	83·0	83·3	–	82·8
Mnd205SFV	89·6	89·6	89·6	89·2	90·1	90·6	82·8	–
MspSFVmnd	97·9	97·9	97·9	97·1	96·9	97·1	83·0	90·3
Msp5440	96·9	96·9	96·9	96·2	98·3	99·0	83·0	90·6
MleSFVdrl	88·2	88·2	88·2	87·7	88·4	88·9	82·8	93·9
CAM1465	97·4	97·4	97·4	96·7	98·3	98·6	83·0	90·6

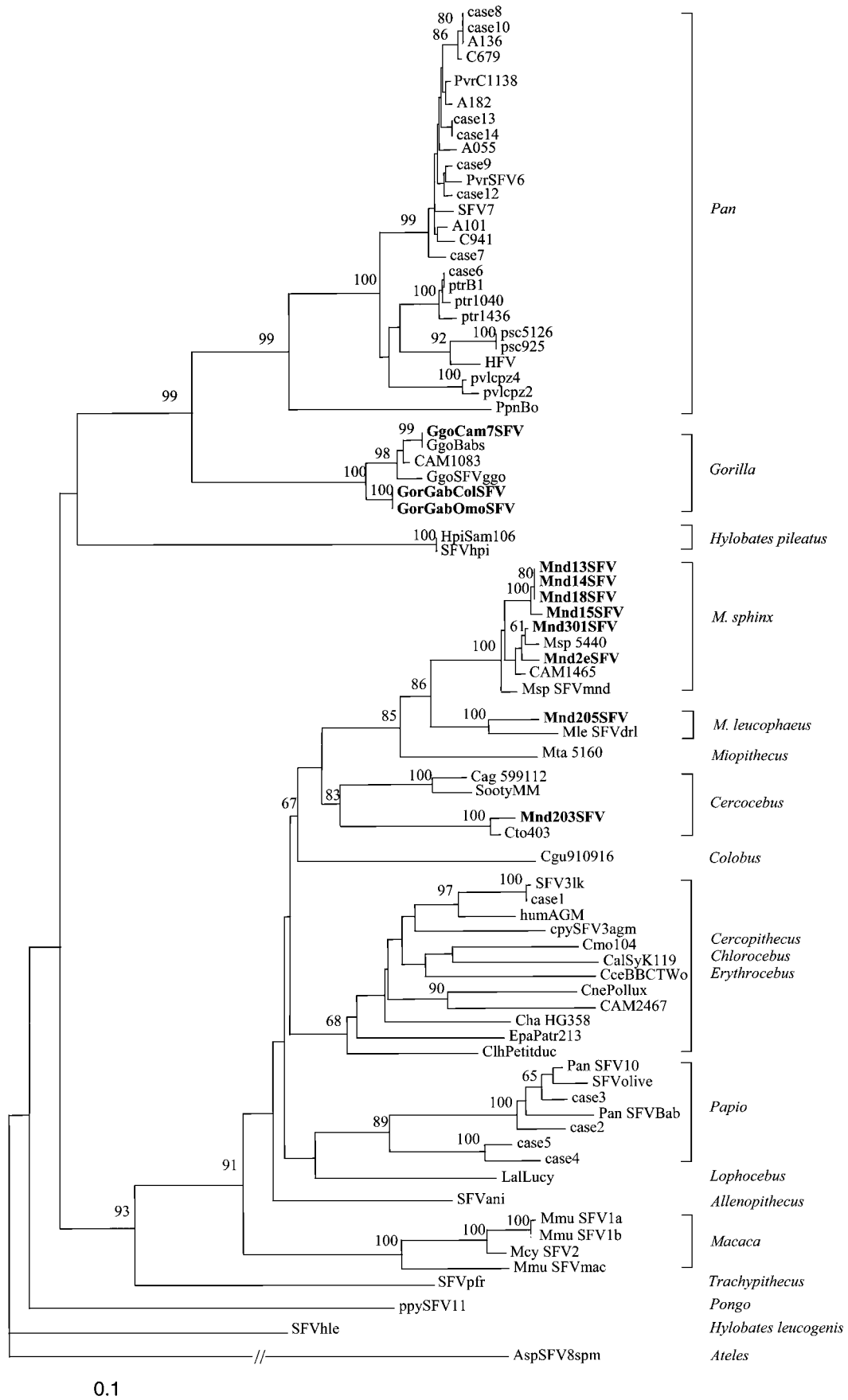
the animals several weeks to several years after their arrival in the sanctuaries/zoos (Nerrienet *et al.*, 2001, 2004). In Gabon, most of the animals originated from a semi-free-ranging colony of mandrills, living in rain-forest enclosures, which was established in 1983 at the Centre International de Recherches Médicales de Franceville (Georges-Courbot *et al.*, 1996).

The first goal of this study was to detect and characterize SFVs in the wild-born, wild-caught animals. To date, the majority of data on SFVs present in NHPs has concerned captive animals (Bieniasz *et al.*, 1995; Blewett *et al.*, 2000; Broussard *et al.*, 1997; Hussain *et al.*, 2003), in which

interspecies transmission of viruses may occur (Voevodin *et al.*, 1996). The second goal was to compare these novel NHP viruses with those recently found in the three hunters referred to above, who live in the same geographical area (lowland forest sites of southern Cameroon), in order to obtain new insights into the possibility of simian-to-human transmission of such retroviruses in a natural setting.

Specific serological and nested PCR analyses were performed as described previously (Hussain *et al.*, 2003; McClure *et al.*, 1994; Schweizer & Neumann-Haefelin, 1995). Briefly, all plasma/sera were tested by a Western blot assay using human foamy virus (HFV)-infected U373-MG

Fig. 1. Phylogenetic tree for all available *integrase* gene 425 bp fragments (GenBank accession nos AY278774–AY278792, AY442339, X83290–X83298, X54482, X58484, M54978, AF049077–AF049086, AF516484–AF516487, AY195673–AY195683, AY195685–AY195698) including the 11 novel SFV strains (bold) (GenBank accession nos AY583775–AY583782 and AY603409–AY603411). The phylogeny was derived by the neighbour-joining method applied to pairwise sequence distances calculated using the Kimura two-parameter method (transition/transversion ratio set to 2) and with a bootstrap value of 1000. Horizontal branch lengths are drawn to scale, with the bar indicating 0·1 nucleotide replacements per site. Numbers at each node indicate the percentage of bootstrap samples in which the cluster to the right is supported. Only values greater than 60% are shown. The tree was rooted using the New World spider monkey (AspSFV8spm) sequence.



cells (Tobaly-Tapiero *et al.*, 2000). The criterion for Western blot seropositivity was clear reactivity to both p70 and p74 ape proteins (Gag doublet). PCR was performed on high-molecular-mass DNA extracted from either buffy-coat or peripheral blood mononuclear cells. Two SFV proviral genomic regions were studied using generic, nested primers. The first consisted of a 425 bp fragment of the *integrase* gene, the second of a 109 bp fragment of the LTR region (McClure *et al.*, 1994; Schweizer & Neumann-Haefelin, 1995). The PCR sensitivity of our nested assay ranged from one to ten copies detected in 500 ng of cellular DNA.

Combined serological and/or PCR findings indicated SFV infection in 14 animals, including five out of 27 gorillas, seven out of 11 mandrills and two out of six drills.

For the 42 DNA samples available, there was perfect concordance between the results of the two nested-PCR analyses, with 11 positive samples. For the 20 animals for which plasma/sera and DNA samples were available, there was perfect concordance between the serological and the PCR results except for one sample originating from a gorilla from Cameroon, which exhibited a faint seropositivity but a negative PCR result with both primer sets.

Sequence fragments of 425 bp of the *integrase* gene were obtained for three gorillas, including one from Cameroon and two from Gabon (the only three of the five SFV-positive gorillas for which DNA was available) (Table 1), six mandrills (one from Cameroon and five from Gabon) and two drills from Cameroon (Table 2).

Genetic comparison of the gorilla foamy virus sequences indicated that of the three novel strains, the two sequences originating from the two Gabonese gorillas (GorGabColSFV and GorGabOmoSFV) were identical to each other, but were different (96.2% nt identity) from that of the novel strain (GgoCam7SFV) originating from the gorilla from Cameroon. The latter was either identical, or very closely related, to the three other previously known gorilla virus sequences, including sequence CAM1083 from one of the hunters from Cameroon (98.6% nt identity) (Table 1).

Genetic comparison of the mandrill sequences indicated that the six novel strains were related to each other and to the three other virus sequences known from mandrills, including sequence CAM1465 from one of the Cameroon hunters (96.2–100% nt identity) (Table 2).

Of the two novel drill sequences, one (Mnd205SFV) was closely related to the only previously known drill sequence (MleSFVdrl; 93.9% nt identity). The second sequence (Mnd203SFV) was, surprisingly, related to that of a strain originating from a red-capped mangabey (*Cercocebus torquatus*) (98.1% nt identity). However, *C. torquatus* are highly endemic in southern Cameroon and cross-species transmission cannot be excluded (Table 3).

Phylogenetic analyses, using both neighbour-joining (Fig. 1) and maximum-likelihood methods (not shown),

were performed on all the available SFV *integrase* sequences, including the 36 from Wolfe *et al.* (2004) together with our 11 novel SFV strains. Very similar trees were obtained by both methods. Analysis of these trees strongly reinforced the known presence of species-specific virus lineages, suggesting long-standing co-existence and co-evolution between the different SFVs and their NHP hosts (Switzer *et al.*, 2004). This contrasts with the situation observed for other NHP retroviruses such as STLV-1 (Meertens *et al.*, 2001; Nerrienet *et al.*, 2004; Slattery *et al.*, 1999), in which several episodes of interspecies transmission have occurred *in vivo* between numerous African NHPs, especially among chimpanzees (Leendertz *et al.*, 2004), leading to the present-day geographical distribution of STLV-1 biodiversity.

Regarding the novel sequences, analyses of the tree clearly indicated that the strains from gorillas, mandrills and drills (with the exception of Mnd203SFV) each formed a highly supported phylogenetic clade (bootstrap values of 100%) (Fig. 1). Furthermore, among the gorilla strains, there were two clearly different, highly supported phylogenetic clades (bootstrap values of 100 and 98%). The first comprised the two identical viruses from Gabonese gorillas; the second comprised two SFVs from Cameroon (our novel gorilla strain and the hunter CAM1083 strain) and two other strains from captive gorillas of unknown origin. Such data suggest the existence of two different SFVs in gorillas living in the wild, with approximately 4% nt diversity in this *integrase* gene fragment.

In conclusion, this study demonstrates for the first time that gorillas, mandrills and drills are naturally infected in the wild with specific and different foamy viruses. However, only non-invasive studies, on faeces and urine, or studies done on wild animals sampled as bush meat, will permit new insights into the real prevalence and geographical and subspecies distribution of such NHP foamy viruses in the wild. Similar studies have been recently performed with success for SIV and STLV-1 infection in feral chimpanzees and in a wide diversity of apes and monkeys in Cameroon (Courgnaud *et al.*, 2004; Santiago *et al.*, 2002). Finally, in the context of simian-to-human interspecies transmission, our data are complementary to those recently published by Wolfe *et al.* (2004), as we identified, in wild-caught animals, the same foamy virus strains as were recently reported to infect hunters living in the same geographical areas and to be in frequent contact with blood or body fluids of such animals.

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