Virology xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Multiple retroviral infection by HTLV type 1, 2, 3 and simian foamy virus in a family of Pygmies from Cameroon

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ARTICLE INFO

Article history:
Received 21 July 2010
Returned to author for revision
12 August 2010
Accepted 15 October 2010
Available online xxxx

Keywords:
Retrovirus
HTLV-1
HTLV-2
HTLV-3
Simian foamy viruses
Family transmission
Retroviral emergence
Hunting
Pygmies
Cameroon

ABSTRACT

To better understand the origins and modes of transmission of HTLV-3 and to search for other retroviral infections (HTLV-1, HTLV-2, foamy viruses), we studied the family of a HTLV-3-infected individual (Pyl43), from Cameroon.

Thirty-five persons were included. All adult men were still actively hunting nonhuman primates (NHP). All women were also butchering and cutting-up animals. Five persons reported a bite by an NHP. While HTLV-3 infection was only found in Pyl43, HTLV-1 and HTLV-2 infections were found, respectively, in 5 and 9 persons with one being co-infected by both retroviruses. Phylogenetic analysis suggested intra-familial transmission of HTLV-1 subtypes B and D and HTLV-2. One man was infected by a chimpanzee foamy virus, acquired probably 45 years ago, through a bite. Acquisition of retroviral infections still occurs in central Africa involving to various extent not only intra-familial transmission for HTLV-1/HTLV-2 but also direct interspecies transmission from NHP for foamy virus and possibly for HTLV-1 and HTLV-3.

Introduction

Human T cell lymphotropic virus (HTLV) type 1 and HTLV-2, as well as their simian counterparts (STLV-1 and STLV-2), are deltaretroviruses that belong to the primate T cell lymphotropic viruses (PTLVs), which share some common epidemiological and biological features (Salemi et al., 2000; Slattery et al., 1999; Verdonck et al., 2007). In 2005, we and others discovered two slightly different strains of a new human HTLV type that was named HTLV-3 (HTLV-3_{Pyl43} and HTLV-3_{2026ND}) (Calattini et al., 2005; Wolfe et al., 2005). Moreover, recently, we published the discovery of a third HTLV-3 strain (HTLV-$3_{Lobak18}$) (Calattini et al., 2009), and a novel and highly divergent HTLV-3 was found and described by others in a primate hunter from Cameroon (Zheng et al., 2010). All HTLV-3 strains were closely related to STLV-3, a simian retrovirus discovered in 1994, and shown, since then, to be prevalent in several monkey species living in different areas of the African continent (Goubau et al., 1994; Liegeois et al., 2008; Meertens and Gessain, 2003; Meertens et al., 2002; Sintasath et al., 2009b; Takemura et al., 2002; Van Dooren et al., 2001). HTLV-3 strains were discovered in persons living in Cameroon (Central Africa), a country where a highly diverse set of primate retroviruses exists. Indeed, not only lentiviruses (as SIV and HIV) and deltaretroviruses (as STLV and HTLV) but also simian foamy viruses (SFV) have been reported during the last two decades in human and nonhuman primates (NHPs) living in this country (Calattini et al., 2009, 2006a, 2004; Corbet et al., 2000; Gessain et al., 1995; Mauclere et al., 1997; Meertens et al., 2001; Nerrienet et al., 2004; Santiago et al., 2002; Sintasath et al., 2009a; Switzer et al., 2008; Wolfe et al., 2004).

The presence of such retroviruses in humans is considered to be the result of cross-species viral transmissions from established NHPs enzootic viruses (SIV, STLV, SFV) to the human host. For SIV_{CPZ} and

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doi:10.1016/j.virol.2010.10.025

STLV-1/2, the transmission to the human host was followed respectively by dramatic epidemic or sustained endemic host-to-host spread in the human population (Keele et al., 2006; Peeters, 2004; Slattery et al., 1999; Verdonck et al., 2007; Wolfe et al., 2007). On the contrary, more recent data about SFV transmissions seem to indicate that single episodes of transmission can occur from NHP to human, but with no onward dissemination in the human population (Boneva et al., 2002; Calattini et al., 2007; Heneine et al., 2003; Jones-Engel et al., 2008; Linial, 2000; Switzer et al., 2004, 2008; Wolfe et al., 2004). However, such assumption is still based on very few studies. Lastly, in the case of HTLV-3 infection, no data are available regarding the possible dissemination of such virus within the human population after the initial episode of cross-species transmission.

To get new insights into the origin and modes of transmission of HTLV-3 and to search for other retroviral infections (HTLV-1, HTLV-2, SFV) and associated risk factors for viral cross-species and human to human transmission, we conducted a study within the family of one individual (Pyl43) infected by HTLV-3, living in the rain forest area of South Cameroon.

Results

Studied population

The current study was performed on 35 persons, all family related to Pyl43 (Fig. 1). The family history of this 60-year-old man can be summarized as follows: both his parents are dead; he currently has 2 living sisters (Pyl44, Pyl107), who have living children. He has been married 3 times: his first wife (PW01), with whom he had one boy (Pyl112), is dead; his second wife (PW02), with whom he had two girls (Pyl42 and Pyl37) and one boy (Pyl119), is still alive but lives currently with another man (Pyl124) and could not unfortunately be sampled. His third wife (Pyl45), with whom he had no child, is still alive. This last woman had, from a first marriage, two living children (one girl, Pyl113, and one boy, Pyl36). All the 35 persons included in this study (18 women, 17 men, mean age 39 years, range 11–65 years) belong to the Bakola Pygmies tribe, even if from different clans.

Clinical examination of Pyl43 revealed no evident signs related to a particular ongoing illness, apart from regular body aches frequently found in most of the aged people in the area. Concerning hunting and butchering, field interviews indicated that all adult men and also some women have been and are still actively hunting. Furthermore, all women are still actively butchering and cutting-up animals. Such activities are constant features in Pygmies living in these areas, in contrast to the situation present in the different populations of Bantus leaving nearby, where only few men are still hunting actively. The most frequent game included rats, squirrels, porcupines, duikers, antelopes, and NHPs. Among the latter, monkeys as *Cercopithecus nictitans and Cercopithecus cephus* are by far the most frequent game but mandrills, baboons, as well as chimpanzees and gorillas are also hunted. During field works, we also asked specific questions regarding parenteral exposure to NHPs including bites, wounds, scratches, or

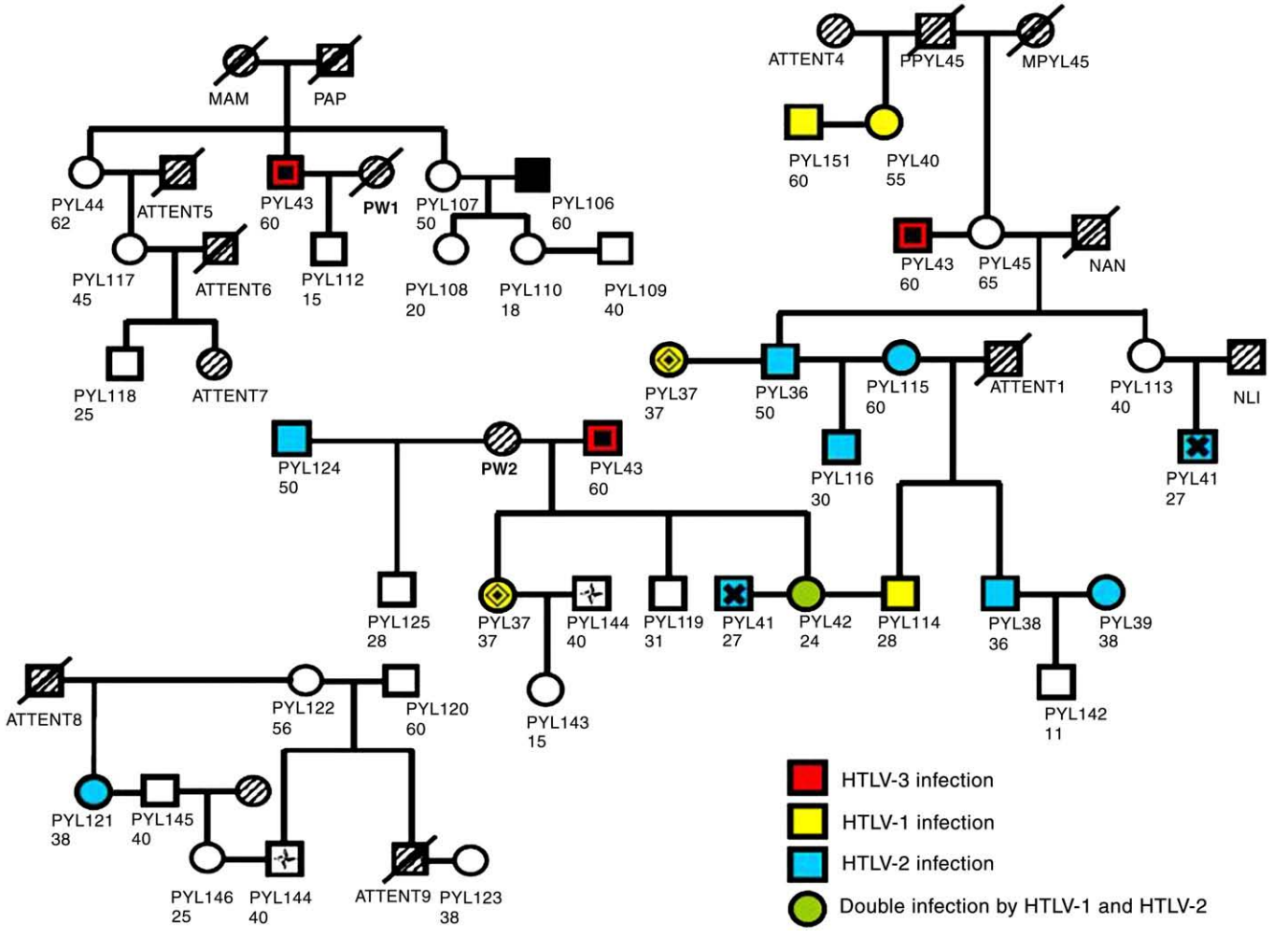

Fig. 1. Pedigree of the Pyl43 family. This was obtained through several field interviews performed from 2005 to 2008. Some individuals (Pyl43, Pyl37, Pyl144, and Pyl41) have been placed several times in this tree for a better comprehension.

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other injuries. Only five individuals (one woman—Pyl42, and 4 men) have been bitten during former hunting activities by NHPs including one by a gorilla (Pyl36), one by a mandrill (Pyl42), two by small monkeys (Pyl118 and Pyl41), and the last one by a chimpanzee (Pyl106).

HTLV serology, PCR, and sequence analyses

All 35 plasma samples were directly tested with the two confirmatory assays: the WB and the INNO-LIA, without any prior initial screening. The plasma of Pyl43 was collected at three different times (2004, 2005, 2007), and it exhibited in both tests the same HTLV indeterminate pattern, as originally found in 2004 (Calattini et al., 2005). Furthermore, presence of HTLV-3 provirus was confirmed by specific PCR analysis in his PBBC DNA (Table 1). No other person of this family was infected by HTLV-3, as determined by serological and molecular methods. Indeed, despite the fact that 15 persons had an indeterminate WB (including one HGIP (HTLV-1 Gag indeterminate pattern) pattern (Mauclere et al., 1997)) and/or INNO-LIA profile, none was PCR-positive with the primer pairs specific for HTLV-3.

An unexpected result was the finding of several HTLV-1 and HTLV-2 infections in members of this family. Indeed, HTLV-1 infection was found in 5 individuals, while HTLV-2 was present in 9 persons. Among them, one person was doubly infected by HTLV-1 and HTLV-2 (pyl42) (Table 1 and Fig. 1). As seen in Table 1, such infections were demonstrated by both specific serological assays (WB, LIA, and IFA titration of antibodies) and specific molecular means (PCR and sequences analyses). Sequences analysis of the PCR-positive samples showed that all the 9 HTLV-2 strains obtained from the 9 HTLV-2-infected persons belonged to the HTLV-2 subtype B and were nearly identical to each other in the 625 bp LTR fragment analyzed (only Pyl36 and Pyl115 have one base of difference compared to the other HTLV-2 sequences) (Fig. 2). In contrast, among the five HTLV-1-positive samples, we could identify two different strains: three people had an HTLV-1 subtype B virus, while the two others were infected by a typical subtype D strain (Fig. 3).

By analyzing more in detail the family pedigree, we can clearly identify nine different couples, infected by one or both of these retroviruses. Indeed, concerning HTLV-2 alone, we could identify 2 couples in which both husband and wife were infected (Pyl38/Pyl39 and Pyl36/Pyl115), as well as two mother–child HTLV-2-positive couples (Pyl115/Pyl38 and Pyl115/Pyl116). Concerning HTLV-1 alone, only one positive couple could be identified (Pyl40/Pyl151). Interestingly, they were the two only persons infected by HTLV-1 subtype D, and their strains were identical. The last 4 couples included two mixed cases; the first consisted of a husband infected by HTLV-2 and his second wife infected by HTLV-1 (Pyl36 and Pyl37), and the second one is about a mother infected by HTLV-2 and her child with HTLV-1 infection (Pyl115/Pyl114). The two last couples consisted of a wife doubly infected by HTLV-1 and 2 (Pyl42) and her two successive husbands, one being infected by HTLV-1 (Pyl114), the other by HTLV-2 (Pyl41).

All of the 35 individuals were also screened for foamy virus infection. The WB serology was found clearly positive (gag doublet

Table 1
HTLV and SFV serological and molecular results on the 35 individuals included in this study. Columns *IFHTLVMT2* and *IFHTLVC19* report the HTLV-1 or HTLV-2 antibody titers of the two immunofluorescence assays, respectively. HTLV WB indicates the Western blot 2.4 assay. INNO-LIA indicates the line immunoassay. The different HTLV PCRs are indicated with the name of the primers used for the amplification, as previously described (Calattini et al., 2006a; Calattini et al., 2005; Meertens et al., 2001; Switzer et al., 1995; Wolfe et al., 2005). B and D indicate subtypes B and D, respectively. FV: foamy virus; Int: *integrase*; LTR : long terminal repeat; Ind: indeterminate; N.D.: not done. The ages of the individuals are estimations in 2006/2007.

NOM	Sex	Age	IF HTLV MT2	IF HTLV C19	HTLV WB	INNO-LIA WB	TAX SW	POL SC	TAX SC	PTLV-3 PCR	HTLV-1 PCR	HTLV-2 PCR	FV WB	FV PCR INT/LTR
Pyl36	M	50	1/160	1/640	HTLV-2	HTLV-2	+	+	+	−	−	+ B	+	−
Pyl37	F	37	1/160	1/160	HTLV-1	HTLV-1	+	+	+	−	+ B	−	−	N.D.
Pyl38	M	36	1/640	1/1280	HTLV-2	HTLV-2	+	+	+	−	−	+ B	+	−
Pyl39	F	38	1/160	1/160	HTLV-2	HTLV-2	+	+	+	−	−	+ B	Indet.	−
Pyl40	F	55	1/160	1/80	HTLV	HTLV	+	+	+	−	+ D	−	−	N.D.
Pyl41	M	23	1/80	1/320	HTLV-2	HTLV-2	+	+	+	−	−	+ B	−	N.D.
Pyl42	F	24	1/640	1/80	HTLV-1	HTLV-1	+	+	+	−	+ B	+ B	−	N.D.
Pyl43	M	60	1/320	1/20	Ind	Ind	−	+	−	+	−	−	−	N.D.
Pyl44	F	62	Neg	Neg	Ind	Ind	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl45	F	65	Neg	Neg	Ind	Ind	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl106	M	60	Neg	Neg	Neg	Neg	−	−	−	−	N.D.	N.D.	+	+
Pyl107	F	50	1/80	1/320	Ind	Ind	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl108	F	20	1/40	1/80	Ind	Neg	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl109	M	40	Neg	Neg	Ind	Neg	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl110	F	18	Neg	Neg	Neg	Neg	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl112	M	15	Neg	Neg	Ind	HTLV-2 faint	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl113	F	40	Neg	Neg	Ind	Ind	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl114	M	28	1/1280	1/320	HTLV-1	HTLV	+	+	+	−	+ B	−	−	N.D.
Pyl115	F	60	1/320	1/1280	HTLV-2	HTLV-2	+	+	+	−	−	+ B	−	N.D.
Pyl116	M	30	1/320	1/1280	HTLV-2	HTLV-2	+	−	+	−	−	+ B	−	N.D.
Pyl117	F	45	1/80	Neg	Ind	Neg	−	−	−	−	N.D.	N.D.	+	−
Pyl118	M	25	Neg	Neg	Neg	HTLV-1 faint	−	−	−	−	N.D.	N.D.	+	−
Pyl119	M	31	1/320	1/80	Ind	Neg	−	−	−	−	N.D.	N.D.	Indet	−
Pyl120	M	60	Neg	1/80	Ind	HTLV-1 faint	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl121	F	38	1/320	1/2560	HTLV-2	HTLV-2	+	+	+	−	−	+ B	−	N.D.
Pyl122	F	56	Neg	Neg	Neg	Neg	−	−	−	−	N.D.	N.D.	Indet	−
Pyl123	F	38	Neg	Neg	Ind	Neg	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl124	M	50	1/80	1/320	HTLV-2	HTLV-2	+	+	−	−	−	+ B	−	N.D.
Pyl125	M	28	1/320	1/320	Ind	Ind	−	−	−	−	N.D.	N.D.	Indet	−
Pyl142	M	11	Neg	1/40	HGIP	Neg	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl143	F	15	Neg	Neg	Neg	Neg	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl144	M	40	Neg	1/80	HTLV	HTLV-2	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl145	M	40	Neg	Neg	Ind	Neg	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl146	F	25	Neg	1/640	Ind	Neg	−	−	−	−	N.D.	N.D.	−	N.D.
Pyl151	M	60	1/80	1/80	HTLV-1	HTLV-1	+	−	−	−	+ D	−	−	N.D.

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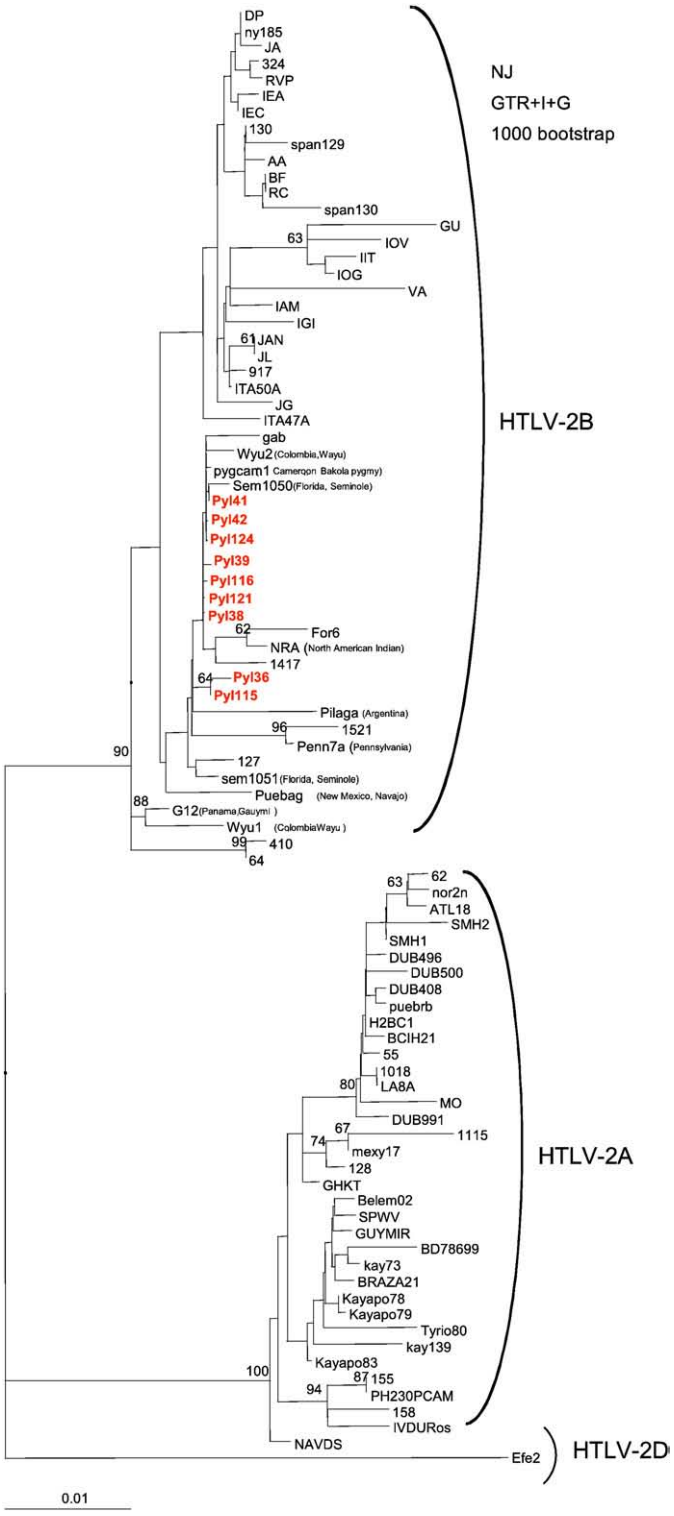

Fig. 2. Phylogenetic tree built on a fragment of 625 bp of the *LTR* of HTLV-2.The tree was generated with the Neighbor-joining method and performed in the PAUP program using the 9 new sequences generated in this study (shown in red), as well as 80 other HTLV-2 sequences available in GenBank. The tree was rooted using an HTLV-2 subtype D sequence (Efe). Numbers at each node indicate the percentage of bootstrap samples (1000 replicates); only values greater than 60% are shown. The branch lengths are drawn to scale, with the bar indicating 0.01 nucleotide replacement per site. In brackets, we reported the geographical origin of some of the source of the HTLV-2. The GenBank accession numbers of the LTR sequences generated in this study are GQ330488 through GQ330496. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

reactivity for p70 and p74) in 5 samples (Pyl36, Pyl38, Pyl106, Pyl117, Pyl118), and indeterminate, in 4 samples (with only a single band of the same expected size). Then, DNAs extracted from PBBC of these 9 individuals were tested by two PCR, specific for fragments of the *integrase* gene and of the LTR region. Only one sample (Pyl106) was found to be PCR-positive in both assays. Sequence analysis of the *integrase* gene fragment (GenBank accession number GQ337063) indicated that this 60-year-old man (the brother in law of Pyl_{43}) was infected by a chimpanzee foamy virus (of the *Pan troglodytes troglodytes* subspecies) exhibiting between 94% to 96% of nucleotide similarity with other *P. troglodytes troglodytes* strains. Interestingly, during field interview, this man reported parenteral contact with a chimpanzee, around 45 years ago when he was bitten on a finger while hunting with his father.

Discussion

The search for understanding the initial mechanisms and factors at the origin of retroviral emergence in humans remains a major challenge. A way to try to reach such goal is to study high-risk populations, as human groups living in central African regions in close contact with NHPs, known to be potentially infected by several retroviruses and being considered as their natural reservoir. Several points should be, however, raised concerning this search for retroviral family transmission in such an environment. First, large genealogic trees with several generations of individuals are very difficult to obtain in Pygmy population, due to their very high mortality both in children and in adults, even as compared to the Bantus population leaving nearby (life expectancy estimated in 1980 to be around 25 years for Pygmies versus 55 in the Bantus, A.F. personal data). Secondly, such a genealogic tree reflects only the current family status and not the lifetime exposures of each person, especially regarding their past sexual contacts (number of lifetime partners, frequency of sexual intercourse), which can be at the origin of the acquisition/transmission of such retroviruses. Furthermore, in such small remote groups, several intra-familial contacts may be present, as it is reflected by the complexity of the connections between some of the individuals of this family (Fig. 1). Cultural practices (as for example levirate marriages: a type of marriage in which the widow is obligated to marry her deceased husband's brother) have also to be considered. Lastly, contacts with body fluids of NHPs are frequently reported in such a population, which still remains today, a group of active hunters–gatherers.

The initial goal of this study was to obtain new insights into the acquisition and transmission of HTLV-3, as nothing is currently known on such a topic. In our study, we did not find any other case of HTLV-3 infection in the Pyl43 family. However, only few of these 34 individuals were closely related to Pyl43 and thus potentially at risk, through him, for such a virus infection. Indeed, as very close relatives, we could test only his third and current wife and 4 of his children. Other potentially infected persons as his mother and his first wife were dead. Furthermore, his second potentially informative wife refused any blood sampling. How HTLV-3 infection was acquired, by this old hunter, remains then an unanswered question. In a recent study, we did not find any other HTLV-3 infection in the wife of the third person ($HTLV\text{-}3_{Lobak18}$) known to be infected by such a virus (Calattini et al., 2009). Furthermore, no data were available on the family of the other HTLV-3-infected persons including $HTLV\text{-}3_{2026ND}$ (Switzer et al., 2006; Wolfe et al., 2005) and Cam2013AB (Zheng et al., 2010). An HTLV-3-infected person may acquire the virus either from a STLV-3-infected NHP, probably through contacts with infected blood, or from another HTLV-3-infected human, through blood transfusion and/or sexual contact and/or breast-feeding. Pyl43 had hunted extensively as the large majority of Bakola Pygmy men. He frequently butchered wild game (including several NHPs) but did not recall any severe event that may have led to a wound or a bite involving blood,

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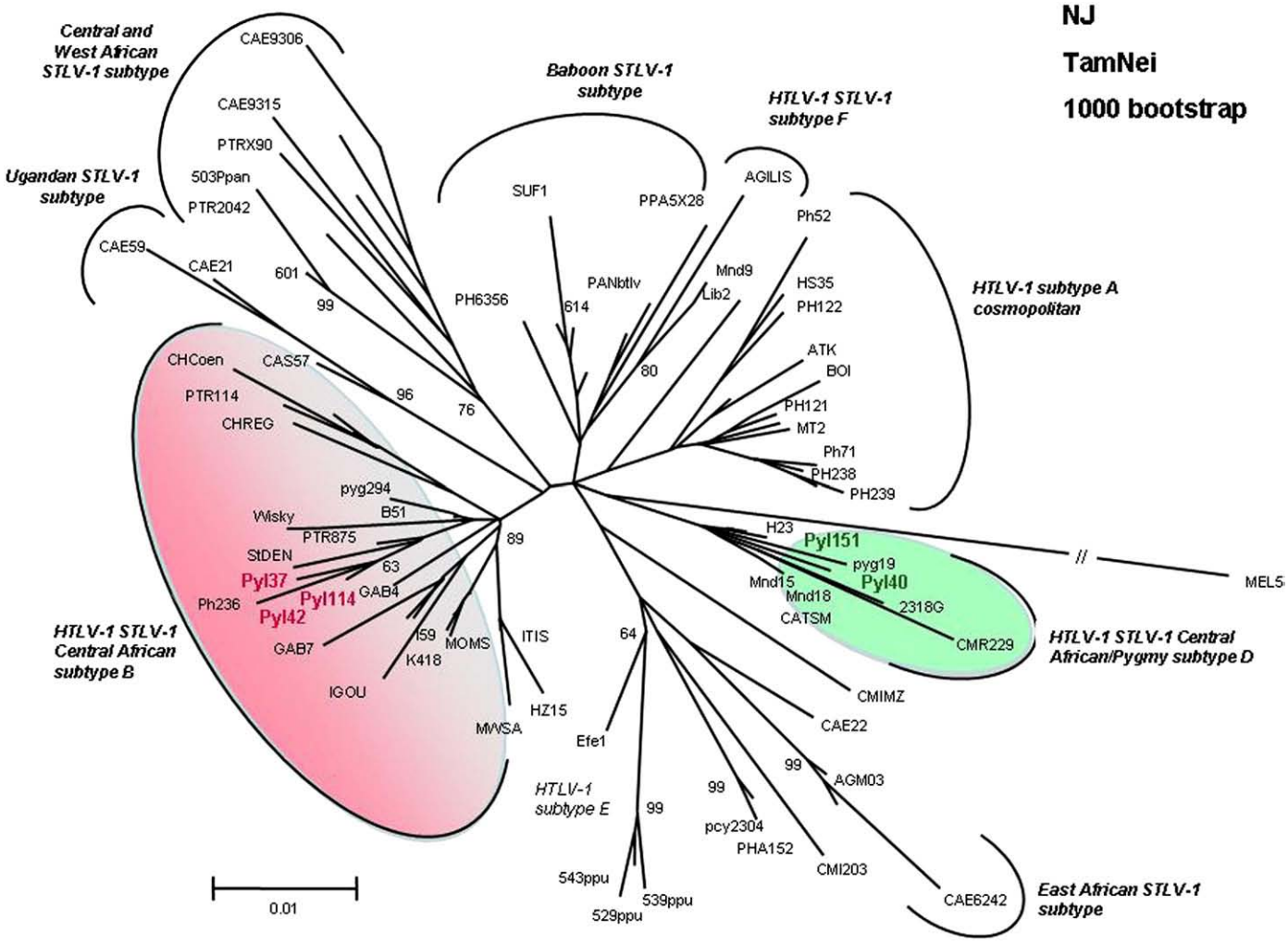

Fig. 3. Unrooted phylogenetic tree built on a fragment of 522 bp of the HTLV-1 *env* gene. The tree was generated with the Neighbor-joining method and performed in the PAUP program using the 5 new sequences generated in this study (shown in red), as well as 137 other *env* sequences available in GenBank. HTLV-1 subtypes B are shown in red, and subtypes D are in green. Numbers at each node indicate the percentage of bootstrap samples (1000 replicates); only values greater than 60% are shown. The branch lengths are drawn to scale, with the bar indicating 0.01 nucleotide replacement per site. The GenBank accession numbers of the *env* sequences generated in this study are GQ330497 through GQ330501. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

saliva, or other tissue fluids from a monkey. Furthermore, as the HTLV-3 strain infecting Pyl43 is nearly identical to other STLV-3 and HTLV-3, we cannot determine with confidence a simian or a human origin of this virus in this case.

We can nevertheless presume that the presence of HTLV-3 in this 60-year-old man can possibly be an isolated zoonotic infection that is not easily transmissible via sexual or any other contact within a family.

An unexpected finding of this study was to discover several HTLV-1 and HTLV-2 cases of infection within this family. Despite being one of the largest endemic areas for such retroviruses, only very few data are known on risk factors for acquisition and modes of transmission for HTLV-1/2 in central Africa and in such a natural setting (Gessain et al., 1995; Jeannel et al., 1995; Tuppin et al., 1996). However, based on what is known in other high endemic areas (Roucoux et al., 2005; Sullivan et al., 1993; Verdonck et al., 2007; Vitek et al., 1995), these viruses may be acquired through sexual contacts (mainly but not exclusively from men to women), by prolonged breast-feeding, and by transmission of infected blood cells (transfusion and intravenous drug use). Based on several field interviews, we found in this family no evidence for blood transfusion or intravenous drug use. In contrast, analysis of the genealogic data indicated a likely transmission of HTLV-2 by breast-feeding from Pyl115 to two of her children, Pyl116 and Pyl38, the latter probably then transmitted (by sexual contact) the virus to his wife Pyl39. The origin of HTLV-2 infection in Pyl115 remains, however, less clear. She could have been infected either from her husband Pyl36 (currently infected by an identical HTLV-2 strain) or by sexual contact with another man before, or even by breast-feeding from her mother nearly 60 years ago. Furthermore, it is important to note that no STLV-2, closely related to the viruses present in humans, has ever been found. Thus, the HTLV-2 present in this family has very probably been acquired from HTLV-2-infected humans and not from monkeys.

Regarding HTLV-1, Pyl151 has probably transmitted the infection to his wife (Pyl40). This is strengthened by the fact that they are both infected by an identical strain of the rarely found HTLV-1 subtype D, which has never been so far described in a possible sexual transmission process. Furthermore, the two sisters, Pyl42 and Pyl37, who are both infected by the same HTLV-1 subtype B strain, have probably been infected through breast-feeding by their mother (currently dead and not tested) and Pyl42 has possibly transmitted his HTLV-1 to her husband Pyl114, as such transmission from woman to man is not as rare as initially thought (Roucoux et al., 2005).

The HTLV-1 strains present in this family are closely related to STLV-1 found in chimpanzees (subtype B) or mandrills' subtype D genotype, whose presence is here demonstrated for the first time in

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Bakola Pygmies. It is thus not impossible that the viruses present in humans may have been acquired (in some cases) from monkeys directly through contact with infected body fluids.

It is worth noting that to our knowledge, we report here the first case of a co-infection with HTLV-1 and -2 in a naturally infected person. Indeed, only few cases have been previously reported in US intravenous drug users.

Studies of the HTLV-1/2 proviral load in such couples would have been of interest, but unfortunately sufficient DNA (extracted from peripheral blood buffy-coat and not PBMCs) was not available from most of them.

Concerning infection by foamy virus, it is very likely that Pyl106 has been infected, nearly 45 years ago, through a severe bite by a chimpanzee while hunting. The concordance of the past history of this hunter with the virologic findings (strong SFV seropositivity and presence of a *P. troglodytes troglodytes* strain in his peripheral blood cells) strongly strengthens such a hypothesis. Interestingly, two other hunters (Pyl36 and Pyl118), bitten, respectively, by a gorilla and a small monkey during past hunting activities, have been found SFV-seropositive but PCR-negative for both primers set. Such a situation has already been recently noticed and may be related to low (pro)viral load in the blood samples or less likely to either presence of divergent viral strain or of non-specific sero-reactivity of unknown origin. Clearance of such infection is a less likely possibility (Calattini et al., 2007; Wolfe et al., 2004). Concerning the possible transmission of such zoonotic-acquired SFV in humans, our work did not demonstrate the presence of SFV in the spouse and 2 children of Pyl106 despite a probable very long-term chronic infection. Such data strengthen the suggestion that SFV transmission among humans does not occur easily by sexual contact, saliva exposure, or casual contact (Boneva et al., 2002, 2007; Calattini et al., 2007; Heneine et al., 2003; Jones-Engel et al., 2008; Linial, 2000; Switzer et al., 2004; Wolfe et al., 2004).

We demonstrated here the presence of infection by four different retroviruses in members of a family living in the rain forest area of South Cameroun, an area of high diversity for nonhuman primates and associated retroviruses. Furthermore, our data indicate that episodes of acquisition of retroviral infections still occur in central Africa mostly because of intra-familial transmission for HTLV-1 and HTLV-2, and also because of direct interspecies transmission from NHPs for foamy virus. However, several fundamental questions remain regarding the natural history of HTLVs in endemic areas for NHPs and associated STLVs: what are the specific risk factors and necessary contacts between retrovirally infected NHPs and humans for such viral interspecies transmission? What is the relative contribution of inter-species and inter-human transmissions to the overall distribution of HTLVs in Central African populations? Does the current increase of NHPs hunting and demand for bush meat also increase the emergence of STLVs in humans? Large ongoing field studies including epidemiological, anthropological, and virological approaches are ongoing to gain new insights into the fascinating natural history of retroviral emergence and associated risk factors in the human population.

Lastly, regarding HTLV-3, studies on large populations, especially among different ethnic groups living in Cameroon, Central African Republic, and Gabon are currently underway in order to identify additional HTLV-3 strains. Based on the fact that the three known HTLV-3 strains were discovered in three geographically distant areas and from three different ethnic groups, and the high prevalence and wide diversity of STLV-3 in African monkeys (Calattini et al., 2009, 2005; Goubau et al., 1994; Liegeois et al., 2008; Meertens and Gessain, 2003; Meertens et al., 2002; Sintasath et al., 2009b; Takemura et al., 2002; Van Dooren et al., 2001; Wolfe et al., 2005), it is tempting to speculate that such viruses might be widespread in several areas of the African continent.

Materials and methods

The Pyl_{43} family

Pyl_{43} is a 60-year-old Bakola Pygmy living currently with his family in a remote settlement near Campo in the Ocean Division of South Cameroon. An active search for all his family members was performed, between 2004 and 2008, through different field missions. Bakola Pygmies are an ethnic group of 4000/5000 individuals living mainly in the Ocean Division of South Cameroon (Abega and Bigombe, 2006; Koppert et al., 1996). This group, traditionally hunters–gatherers, is spread throughout their rainforest territories for hunting. They now practice rudimentary agriculture but also exchange game for staple food and manufactured goods with neighboring Bantu farmers. This survey was approved by national (Ministry of Public Health and the Ethic committee of Cameroon) and local authorities (villages/settlements chiefs) with information to each participant. Informed consent was obtained from adults or parents of minors. Furthermore, a simple general medical examination was performed on site to comply with the participants' needs whenever necessary. The examined participants were treated for common simple infectious diseases including malaria, simple bacterial infections, and intestinal parasitosis, according to local procedures on site, or sent to local medical facilities. For each participant, a brief epidemiological questionnaire was filled and a 5/10 ml blood sample was taken on EDTA.

HTLV serology

All samples were directly tested by two confirmatory assays: a Western blot (HTLV Blot 2.4; MP Diagnostics/Biomedicals/Singapore) and a line immunoassay (INNO-LIA HTLV Confirmation/Immunogenetics/Belgium (Varma et al., 1995; Zrein et al., 1998)). Serial twofold dilutions of the plasma samples were performed for HTLV antibody titration with an immunofluorescence assay (IFA).

HTLV PCR, sequencing, and phylogenetic analyses

High molecular weight DNA was extracted from peripheral blood buffy-coat (PBBC) and was first subjected to PCR using human B-globin-specific primers, to ensure that DNA was amplifiable.

The DNA was then subjected to three nested PCRs and one single PCR with degenerate primers that are highly conserved in all PTLVs (Calattini et al., 2005; Wolfe et al., 2005). Samples showing at least one positive PCR result after this screening were then subjected to 3 different nested PCR specific for HTLV-1 (Meertens et al., 2001), HTLV-2 (Switzer et al., 1995) or HTLV-3 (Calattini et al., 2006a). PCR products were purified using the Qiaquick gel extraction kit (Qiagen), cloned into the pCR2.1 vector (TA cloning, Invitrogen), and sequenced using the BigDye terminator 3.1cycle kit and an ABI 3100 automated sequencer. Pylogenetic analyses were performed as previously described (Calattini et al., 2006a).

Foamy virus serology and PCR

A serological assay was performed, as previously described (Calattini et al., 2006c), on plasma samples in order to investigate the presence of specific SFV antibodies. High molecular weight DNA, from PBBC, was tested by 2 different nested PCR using generic primers able to amplify a fragment of 425 bp of the *integrase* gene and a fragment of 109 bp of the LTR region, as described (Calattini et al., 2006c). The PCR products were purified, cloned, sequenced, and analyzed as described (Calattini et al., 2006b).

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Competing interests

There are no competing interests reported to this work.

Authors' contribution

S.C. carried out part of the serological and all of the molecular and phylogeny work for HTLV-1/2/3; E.B. carried out the field work for epidemiologic data collection with A.F. and A.G., he also performed the serological and molecular work on SFV. S.B. carried out most of the WB for HTLVs. S.A.C. and R.M. participated in the serological and molecular tests and analyses of the data. P.T. conceived and drew the family pedigree. A.G. participated to the field missions, wrote the article, and supervised all the steps of the whole work.

All authors read and approved the final manuscript.

Acknowledgments

This work was supported by the Institut Pasteur and by grants from the Programme interdisciplinaire CNRS Maladies infectieuses émergentes and from National Institute of Health (AI072495-01) to R. M. and fellowships from la Ligue Contre le Cancer to S.C., from le Ministère de la Recherche to S.A.C., and from the Association Virus Cancer Prevention and the Service de la Coopération Française au Cameroon to E.B. R.M. is supported by INSERM.

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Please cite this article as: Calattini, S., et al., Multiple retroviral infection by HTLV type 1, 2, 3 and simian foamy virus in a family of Pygmies from Cameroon, Virology (2010), doi:10.1016/j.virol.2010.10.025

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Please cite this article as: Calattini, S., et al., Multiple retroviral infection by HTLV type 1, 2, 3 and simian foamy virus in a family of Pygmies from Cameroon, Virology (2010), doi:10.1016/j.virol.2010.10.025