Arch Virol (2003) 148: 783–791
DOI 10.1007/s00705-002-0954-8

Archives of Virology
Printed in Austria

Phylogenetic analysis of Vietnamese isolates of feline immunodeficiency virus: genetic diversity of subtype C*

Brief Report

K. Nakamura[1,5], **Y. Suzuki**[2], **K. Ikeo**[2], **Y. Ikeda**[1], **E. Sato**[1], **N. T. P. Nguyen**[3], **T. Gojobori**[2], **T. Mikami**[1,4], and **T. Miyazawa**[1,5,6]

[1]Department of Veterinary Microbiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[2]Center for Information Biology, National Institute of Genetics, Mishima, Shizuoka, Japan
[3]Department of Parasitology, Microbiology and Pathology, Faculty of Animal Science and Veterinary Medicine, College of Agriculture and Forestry, Ho Chi Minh City, Vietnam
[4]Laboratory of Veterinary Public Health, Department of Veterinary Medicine, College of Bioresource, Nihon University, Fujisawa, Kanagawa, Japan
[5]Research Center for Emerging Infectious Diseases, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan
[6]Host and Defence, PRESTO, Japan Science and Technology Corporation, Tokyo, Japan

Received August 19, 2002; accepted November 11, 2002
Published online March 13, 2003

Summary. Phylogenetic relationships of novel Vietnamese strains of feline immunodeficiency virus (FIV) were analysed. One Vietnamese strain was found to cluster with subtype D, which was previously known only in Japan, while the other seven strains were placed with members of subtype C. Calculation of the relative numbers of mutations resulting in amino acid and silent changes in FIV *env* subtypes suggested that subtype C isolates may be less structurally constrained (potentially more pathogenic) than subtype B.

*The nucleotide sequences reported here in this paper have been submitted to the DDBJ/EMBL/GenBank databases and assigned the accession numbers AB083502 to AB083509.

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Feline immunodeficiency virus (FIV) is a feline lentivirus associated with immunodeficiency and opportunistic infection in both domestic and wild members of the *Felidae* [1, 23]. FIV shares many biological, immunological and pathogenic properties with the human immunodeficiency viruses (HIVs) [2], such that, FIV is regarded as a useful animal model for the study of acquired immunodeficiency syndrome (AIDS) caused in humans by HIV. Efforts to develop vaccines against HIVs have been complicated by the extraordinary genomic diversity of the viral *env* gene. Nine variable regions (V1 to V9) have been identified in the HIV *env* gene [29] and at least nine phylogenetically distinct clades of HIV-1 isolates can be distinguished [17]. The development of vaccines which can induce a broadly cross-reactive immune response, capable of recognizing multiple HIV-1 subtypes, is clearly needed [6]. The *env* genes of FIV and HIV show many parallels. For example, the FIV *env* gene is also known to have nine variable regions (V1 to V9) [21], and FIV sequences have recently been separated into five phylogenetically distinct subtypes (A, B, C, D and E) based on the differences found in the V3 to V5 regions of the *env* gene [9, 22, 27]. This degree of similarity between HIV and FIV makes the FIV/cat system a potential animal model for use in field vaccine trials during development of 'cross-clade' vaccines against HIV.

An inactivated vaccine using subtypes A and D is commercially available at the moment. However, the development of FIV vaccine effective to all subtypes is still a major assignment. In order to develop effective vaccine strategies against FIV, it is important to know the diversities and geographical distributions of the FIV subtypes. The *env* sequences of FIV subtypes A and B are rather diverse and have a wide geographic distribution [1, 3, 7, 9, 19, 22, 24, 25, 27]. Subtype A is found in the U.S.A., Argentina, Nicaragua, Japan, Australia, U.K., Germany, Italy, Netherlands, France, Switzerland and South Africa, while subtype B has been reported in U.S.A., Canada, Argentina, Japan, Italy and Germany. Both subgroups A and B contain *env* sequences from a large number of FIV isolates, and recent genetic analyses have revealed that subtype B comprises a more diverse group of FIV *env* sequences than subtype A [1]. However, the extent of the sequence diversity and geographical distribution of *env* sequence subtypes C, D and E are still unclear, due to the limited number of FIV isolates which cluster into these phylogenetic groupings [1, 19, 22]. Subtype C was first defined when several divergent Canadian isolates were added to the FIV phylogeny in 1994 [27]. Subsequently, two more *env* sequences clustering in this part of the FIV tree were found in isolates from Germany [1] and Japan [19]. We recently isolated a further seven *env* sequence subtype C strains from animals located in Taiwan [8, 28]. Subtypes D and E are, at present, known only in Japan and Argentina, respectively [9, 19, 20, 22].

While most of the FIV *env* sequences included in phylogenetic analyses were isolated from cats in North America, Japan, Taiwan, Australia and several European countries, to date, there have been no reports of FIV isolation from animals in Southeast Asia. This presents the possibility that new FIV strains

remain to be identified from animals in this geographic area. Southeast Asian cats could harbour or could represent the progenitors of one or more novel subgroups. Recently, we carried out serological surveys for FIV in Vietnam, and revealed that 22% of domestic cats in southern Vietnam had FIV antibodies [16, 18]. In the study presented here, we analyse the genetic diversity of 8 FIV strains in Vietnamese cats, and show that these isolates considerably extend the diversity of previously described FIV subtypes C and D. We also discuss the mutational pattern of the V3-V5 domains of FIV *env* subtype C, and try to relate the degree of diversification observed between subgroups to the state of adaptation to the host.

FIV isolation from Vietnamese domestic cats was performed as described previously [14]. Briefly, peripheral blood mononuclear cells (PBMCs) from FIV-seropositive cats were purified by centrifugation over Ficoll-Paque (Amersham Pharmacia Biotech, Buckinghamshire, U.K.), and stimulated with 10 μg/ml of concanavalin-A (Amersham Pharmacia Biotech) for three days. The stimulated PBMCs were cocultured with MYA-1 cells (an interleukin-2-dependent feline T-lymphoblastoid cell line), which are highly sensitive to FIV [15]. Cell-free culture supernatants were tested for Mg^{2+}-dependent reverse transcriptase activity, and the cultures were examined for the expression of FIV antigens by indirect immunofluorescence assays, as described previously [14]. Using this methodology, we succeeded in isolating eight FIV strains which were designated VND1 to VND8.

The newly isolated FIV strains were inoculated onto MYA-1 cells and, 12 days after inoculation, high-molecular-weight DNA was extracted from infected cells using a QIAamp Blood Kit (QIAGEN, Hilden, Germany). The V3 to V5 region of the FIV *env* gene was amplified in each of the 8 novel Vietnamese strains using the polymerase chain reaction (PCR). The primer pair used in this PCR (HV3f and HV5r) has been described previously [22]. PCR-amplified fragments were then sequenced directly, using the dideoxynucleotide chain termination method and an automated DNA sequencer (ABI model 377A) with the same primers using a Big Dye Cycle Sequencing Kit (Applied Biosystems Inc. (ABI), CA, U.S.A.) according to the manufacture's instructions. To rule out the possibility of contamination of PCR products, three independent PCR amplifications were carried out for each of the DNA templates. Each PCR replicate yielded almost identical results. Unrooted phylogenetic trees were then constructed using the neighbor-joining method [26], with the number of nucleotide substitutions estimated using the two-parameter method [10]. The robustness of individual internal tree nodes was estimated using bootstrap resampling (1,000 bootstrap reiterations) [5]. With the exception of the Vietnamese sequences described in this report, all of the *env* nucleotide sequences included in our phylogenetic analyses were obtained from GenBank.

Phylogenetic analyses using the neighbour-joining approach revealed that all but one of the Vietnamese FIV strains formed a distinct subcluster placed within the previously described subgroup C clade (Fig. 1). The seven *env* sequence subtype C FIV isolates from Vietnam (VND2-7) were placed as sister taxa to the previously described isolates reported in Canadian cats [19, 27]. The Canadian and Vietnamese sequences formed a sister taxon to 7 Taiwanese isolates

[8, 28]. Bootstrap support of 98% was gained for the placement of Canadian, Taiwanese and Vietnamese *env* sequences into a monophyletic subtype C clade. However, the relationships between the groups of FIV subgroup C isolates from Taiwan, Vietnam and Canada differ in phylogenies generated from nucleotide, and amino acid datasets (data not shown). The *env* sequence of Vietnamese FIV strain VND1 clustered with the seven Japanese isolates comprising *env* subtype D, with this relationship supported by 83% of 1,000 bootstrap replicates [9, 19, 20]. Calculation of the degree of *env* sequence diversity present within FIV subtypes C and D (across the aligned V3 to V5 region) revealed pairwise divergences of 14% and 15% between the members of these two subtypes, respectively. These figures are as high as previously recorded within FIV *env* subtype A. These data indicate that, despite being known from a more restricted geographic range, *env* sequence subtypes C and D are both as diverse as subtype A.

The reason why FIV *env* subtype C is prevalent in southern Vietnam is at present unknown. One possible explanation for the recent movement of FIV into Vietnam is the high immigration rate into southern Vietnam in the past few decades. It is possible that FIV might have been introduced into this geographic region by the movement of domesticated pets. FIV is transmitted mainly during fighting between male adult cats [30], and near-feral existence of animals in Vietnam may have promoted infection.

Recently, Nishimura et al., [20] reported the interspecies transmission of FIV from the domestic cat to the Tsushima cat (*Felis bengalensis euptilura*),

Fig. 1. Phylogenetic tree of 73 sequences of FIV from the V3-V5 region of FIV *env*. The tree was constructed from synonymous substitutions by the neighbor-joining algorithm (Saitou and Nei, 1987). Bootstrap values above 65 out of 100 are shown at the branch points. We examined eight FIV sequences (strains VND1 to VND8) determined in this study and 65 available sequences from GenBank. The GenBank accession numbers for the *env* sequences included in this study are AIC01 (AB010396), AIC02 (AB010397), Aomori-2 (D37817), CABCpady02C (U02392), CABCpbar01C (U02393), CABCpbar02C (U02394), CABCpbar03C (U02395), CABCpbar04C (U02396), CABCpbar07C (U02397), CABCpbar08C (U02398), CABCpbar09C (U02399), CA.Dixon (L00608), CA.PPR (M36968), DutchK1 (M73964), Dutch4 (X69498), Dutch6 (X69499), EngUK8 (X69496), FranceWo (L06312), Fukuoka (D37815), ItalyM3 (X69502), JN-BR1 (D67052), KUM01 (AB010398), KUM02 (AB010399), LP-3 (D84496), LP-9 (D84497), LP-20 (D84498), LP-24 (D84500), MC2 (D67062), MU-1 (AB016666), MU-2 (AB016667), MU-3 (AB016668), MY8 (D67063), OKA01 (AB010400), Petaluma (M25381), PTH-BM3 (AB010401), SAP01 (AB010402), SAP02 (AB010403), SAP03 (AB010404), ScotUK2 (X69494), Sendai-1 (D37813), Sendai-2 (D37814), Shizuoka (D37811), SwissZ1 (X57002), SwissZ2 (X57001), TAU01 (AB010405), TAU02 (AB010406), TI-1 (AB016025), TI-2 (AB016026), TI-3 (AB016027), TI-4 (AB016028), TM2 (M59418), TY1 (D67054), USCAhnky02A (U02400), USCAlemy01A (U02404), USCAlemy02A (U02405), USCAsam_01A (U02410), USCAtt_09A (U02413), USCAtt_10A (U02414), USCAzepy01A (U02417), USILbrny03B (U02418), USMAsboy03B (U02419), USMOglwd03B (U02420), USOKlgrl02B (U02421), USTXmtex03B (U02422), and WalesUK14 (X69497). Abbreviations: *AR*, Argentina; *CA*, Canada; *FR*, France; *IT*, Italy; *JP*, Japan; *NL*, Netherlands; *SW*, Switzerland; *TW*, Taiwan; *UK*, United Kindom; *USA*, United States of America; *VN*, Vietnam

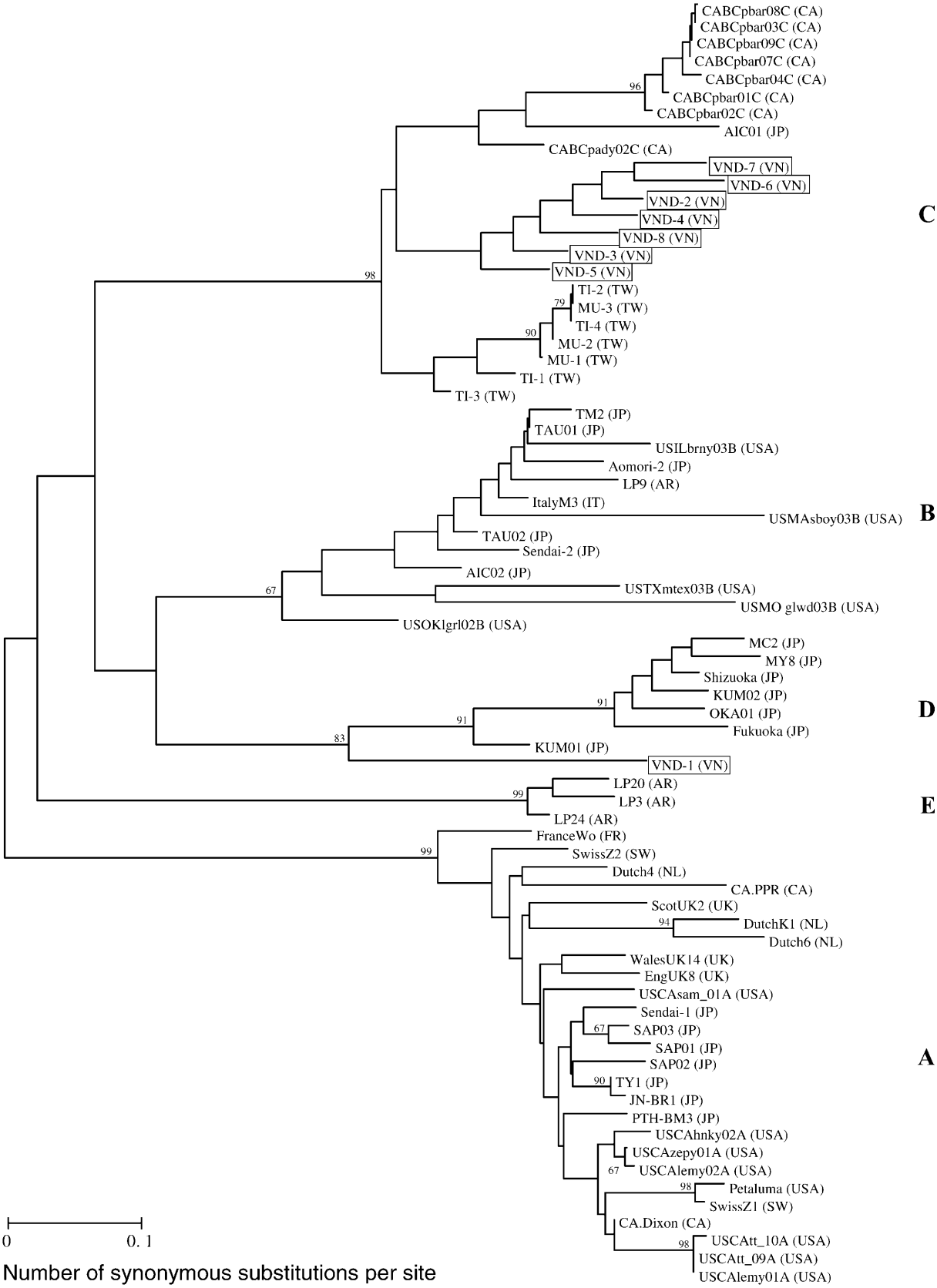
CABCpbar08C (CA)
CABCpbar03C (CA)
CABCpbar09C (CA)
CABCpbar07C (CA)
CABCpbar04C (CA)
CABCpbar01C (CA)
CABCpbar02C (CA)
AIC01 (JP)
CABCpady02C (CA)
VND-7 (VN)
VND-6 (VN)
VND-2 (VN)
VND-4 (VN)
VND-8 (VN)
VND-3 (VN)
VND-5 (VN)
TI-2 (TW)
MU-3 (TW)
TI-4 (TW)
MU-2 (TW)
MU-1 (TW)
TI-1 (TW)
TI-3 (TW)
TM2 (JP)
TAU01 (JP)
USILbrny03B (USA)
Aomori-2 (JP)
LP9 (AR)
ItalyM3 (IT)
USMAsboy03B (USA)
TAU02 (JP)
Sendai-2 (JP)
AIC02 (JP)
USTXmtex03B (USA)
USMO glwd03B (USA)
USOKlgrl02B (USA)
MC2 (JP)
MY8 (JP)
Shizuoka (JP)
KUM02 (JP)
OKA01 (JP)
Fukuoka (JP)
KUM01 (JP)
VND-1 (VN)
LP20 (AR)
LP3 (AR)
LP24 (AR)
FranceWo (FR)
SwissZ2 (SW)
Dutch4 (NL)
CA.PPR (CA)
ScotUK2 (UK)
DutchK1 (NL)
Dutch6 (NL)
WalesUK14 (UK)
EngUK8 (UK)
USCAsam_01A (USA)
Sendai-1 (JP)
SAP03 (JP)
SAP01 (JP)
SAP02 (JP)
TY1 (JP)
JN-BR1 (JP)
PTH-BM3 (JP)
USCAhnky02A (USA)
USCAzepy01A (USA)
USCAlemy02A (USA)
Petaluma (USA)
SwissZ1 (SW)
CA.Dixon (CA)
USCAtt_10A (USA)
USCAtt_09A (USA)
USCAlemy01A (USA)
C
B
D
E
A
0
0. 1
Number of synonymous substitutions per site

Table 1. Synonymous and nonsynonymous mutation rates between FIV envelope subtypes[a]

FIV *env* subtype	n	dN	dS	dN/dS
A	26	0.058	0.175	0.331
B	13	0.044	0.225	0.194
C	23	0.063	0.221	0.286
D	8	0.037	0.221	0.166
E	3	0.035	0.081	0.430

[a]Mutation rates were calculated as the proportion of nonsynonymous (dN) or synonymous (dS) substitutions per potential synonymous or nonsynonymous site, respectively

on Tsushima Island off of the Japanese mainland. Phylogenetic analyses revealed the V3-V5 sequences from all FIV isolates harboured by Tsushima cat belonged to FIV *env* subtype D. The Tsushima cat sequences [20] are included in a cluster which previously comprised only isolates from Japanese domestic cats [20]. In our study, the one subtype D Vietnamese sequence revealed by our screening process (VND1) is positioned at the base of the clade of Japanese isolates in phylogenetic analyses (Fig. 1). This suggests that FIV strain VND1 is not a recent introduction into Vietnam from Japan.

To look at the mutational patterns underpinning diversification of the FIV *env* subgroups (including our new Vietnamese isolates), the numbers of synonymous (dS) and non-synonymous (dN) substitutions per synonymous or non-synonymous site, respectively, were estimated [13] (Table 1). Analyses of dN/dS ratios can provide information as to the selective forces affecting genetic evolution [11, 12, 13]. For example, Sodora et al. [27] compared the ratio of amino acid and silent changes calculated between pairs of FIV (V3 to V5) *env* gene sequences to the ratios observed across the equivalent *env* gene region of HIV-1. They used these calculations to try and predict the relative pathogenicities for FIV subtypes A and B. The *env* genes of pairs of FIV subtype B viruses were found to display a significantly higher degree of synonymous (silent) changes than subtype A, and a correspondingly low dN/dS ratio, suggesting purifying selection and a more advanced state of host-pathogen adaptation (*i.e.* reduced pathogenicity). Sodora and coworkers [27] note that the low dN/dS ratio of FIV subtype B is comparable to that of the relatively non-pathogenic simian immunodeficiency virus of African green monkeys, while FIV subtype A shows a higher dN/dS ratio, more reminiscent of that previously recorded in the more highly pathogenic HIV-2. The apparently ‘saturated’ state (reaching of an ‘upper limit’ of mutation at synonymous sites, while maintaining viral integrity) of subtype B also suggest it to be relatively more ancient than subtype A. While other studies [1, 24] do not unequivocally support the findings of Sodora et al. [27], that subtype B shows low dN/dS ratio, the suggestion of structural constraint and host adaptation of FIV subtype B (possible to avoid host immune surveillance) is recurrent [1].

The addition of 7 new FIV isolates to subtype C allowed re-analyses of the dN/dS ratios in this subgroup. Pairwise dN/dS ratios were calculated for the 23 subtype C FIV sequences included in phylogenetic analyses, and this revealed that subtype C has a lower proportion of silent changes than subtype B, and a dN/dS ratio which is significantly higher than that of subtype B ($P < 0.01$). In contrast, subtype C shows a lower dN/dS ratio than FIV subtype A although this difference is not statistically significant. If generation time and mutation rate are constant across FIV subtypes B and C, this would suggest that members of the subtype C clade are less host adapted than subtype B isolates. This, in turn, could suggest that subtype C viruses are more pathogenic than subtype B, but possibly slightly less pathogenic than members of subtype A. Intriguingly, a subtype C FIV strain was reported to induce accelerated immunodeficiency in cats during *in vivo* passage of acute phase viruses [4], which may support the hypothesis. However, to understand the different degrees of the pathogenicity caused by the five FIV subtypes, both molecular epidemiological surveys and experimental infections with viruses clustering into the five different subtypes are needed.

Acknowledgements

We thank Professors K. Doi, K. Ono and H. Nakayama (The University of Tokyo, Tokyo, Japan) for supporting our investigations. We also thank Dr. M. Hattori (Kyoto University, Kyoto, Japan) for providing human IL-2-producing Ltk$^-$IL-2.23 cells. This work was supported by a Grant-in-Aid for International Scientific Research (Field Research) (grant no. 09041150) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by Host and Defence, PRESTO, Japan Science and Technology Cooperation. The authors are grateful to Dr. J. Martin (Imperial College, London, U.K.) for discussion and help in preparing this manuscript. K. Nakamura is supported by Research Fellowships from the Japanese Society for the Promotion of Science for Young Scientists.

References

1. Bachmann MH, Mathiason-Dubard C, Learn GH, Rodrigo AG, Sodora DL, Mazzetti P, Hoover EA, Mullins JI (1997) Genetic diversity of feline immunodeficiency virus: dual infection, recombination, and distinct evolutionary rates among envelope sequence clades. J Virol 71: 4241–4253
2. Bendinelli M, Pistello M, Lombardi S, Poli A, Garzelli C, Matteucci D, Ceccherini-Nelli L, Malvaldi G, Tozzini F (1995) Feline immunodeficiency virus: an interesting model for AIDS studies and an important cat pathogen. Clin Microbiol Rev 8: 87–112
3. Carpenter MA, Brown EW, MacDonald DW, O'Brien SJ (1998) Phylogeographic patterns of feline immunodeficiency virus genetic diversity in the domestic cats. Virology 251: 234–243
4. Diehl LJ, Mathiason-Dubard CK, O'Neil LL, Obert LA, Hoover EA (1995) Induction of accelerated feline immunodeficiency virus disease by acute-phase virus passage. J Virol 69: 6149–6157
5. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791
6. Gotch F (1998) Cross-clade T cell recognition of HIV-1. Curr Opin Immunol 10: 388–392

7. Greene WK, Meers J, Chadwick B, Carnegie PR, Robinson WF (1993) Nucleotide sequences of Australian isolates of the feline immunodeficiency virus: comparison with other feline lentiviruses. Arch Virol 132: 369–379
8. Inada G, Miyazawa T, Inoshima Y, Kohmoto M, Ikeda Y, Liu C-H, Lin JA, Kuo T-F, Mikami T (1997) Phylogenetic analysis of feline immunodeficiency virus isolated from cats in Taiwan. Arch Virol 142: 1459–1467
9. Kakinuma S, Motokawa K, Hohdatu T, Yamamoto JK, Koyama H, Hashimoto H (1995) Nucleotide sequence of feline immunodeficiency virus: classification of Japanese isolates into two subtypes which are distinct from non-Japanese subtypes. J Virol 69: 3639–3646
10. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111–120
11. Li W-H (1993) Unbiased estimation of the rates of synonymous and nonsynonymous substitution. J Mol Evol 36: 96–99
12. Li W-H, Tanimura M, Sharp PM (1988) Rates and dates of divergence between AIDS virus nucleotide sequence. Mol Biol Evol 5: 313–330
13. Li W-H, Wu C-I, Luo C-C (1985) A new method for estimating synonymous and nonsynonymous rates of nucleotide substitution considering the relative likelihood of nucleotide and codon changes. Mol Biol Evol 2: 150–174
14. Miyazawa T, Furuya T, Itagaki S, Tohya Y, Nakano K, Takahashi E, Mikami T (1989) Preliminary comparisons of the biological properties of two strains of feline immunodeficiency virus (FIV) isolated in Japan with FIV Petaluma strain isolated in the United States. Arch Virol 108: 59–68
15. Miyazawa T, Furuya T, Itagaki S, Tohya Y, Takahashi E, Mikami T (1989) Establishment of a feline T-lymphoblastoid cell line highly sensitive for replication of feline immunodeficiency virus. Arch Virol 108: 131–135
16. Miyazawa T, Ikeda Y, Maeda K, Horimoto T, Tohya Y, Mochizuki M, Vu D, Vu GD, Cu DX, Ono K, Takahashi E, Mikami T (1998) Seroepidemiological survey of feline retrovirus infections in domestic and leopard cats in northern Vietnam in 1997. J Vet Med Sci 60: 1273–1275
17. Moore JP, Parren PWHI, Burton DR (2001) Genetic subtypes, humoral immunity, and human immunodeficiency virus type 1 vaccine development. J Virol 75: 5721–5729
18. Nakamura K, Miyazawa T, Ikeda Y, Sato E, Nishimura Y, Nguyen NTP, Takahashi E, Mochizuki M, Mikami T (2000) Contrastive prevalence of feline retrovirus infections between northern and southern Vietnam. J Vet Med Sci 62: 921–923
19. Nishimura Y, Goto Y, Pang H, Endo Y, Mizuno T, Momoi Y, Watari T, Tsujimoto H, Hasegawa A (1998) Genetic heterogeneity of *env* gene of feline immunodeficiency virus obtained from multiple districts in Japan. Virus Res 57: 101–112
20. Nishimura Y, Goto Y, Yoneda K, Endo Y, Mizuno T, Hamachi M, Maruyama H, Kinoshita H, Koga S, Komori M, Fushuku S, Ushinohama K, Akuzawa M, Watari T, Hasegawa A, Tsujimoto H (1999) Interspecies transmission of feline immunodeficiency virus from the domestic cat to the Tsushima cat (*Felis bengalensis euptilura*) in the wild. J Virol 73: 7916–7921
21. Pancino G, Fossati I, Chappey C, Castelot S, Hurtrel B, Moraillon A, Klatzmann D, Sonigo P (1993) Structure and variations of feline immunodeficiency virus envelope glycoproteins. Virology 192: 659–662
22. Pecoraro MR, Tomonaga K, Miyazawa T, Kawaguchi Y, Sugita S, Tohya Y, Kai C, Etcheverrigaray ME, Mikami T (1996) Genetic diversity of Argentine isolates of feline immunodeficiency virus. J Gen Virol 77: 2031–2035

23. Pedersen NC, Ho EW, Brown ML, Yamamoto JK (1987) Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. Science 235: 790–793
24. Pistello M, Cammarota G, Nicoletti E, Matteucci D, Curcio M, Del Mauro D, Bendinelli M (1997) Analysis of the genetic diversity and phylogenetic relationship of Italian isolates of feline immunodeficiency virus indicates a high prevalence and heterogeneity of subtype B. J Gen Virol 78: 2247–2257
25. Rigby MA, Holmes EC, Pistello M, Mackay A, Leigh Brown AJ, Neil JC (1993) Evolution of structural proteins of feline immunodeficiency virus: molecular epidemiology and evidence of selection for change. J Gen Virol 74: 425–436
26. Saitou N, Nei M (1987) The neighbor-joining methods: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425
27. Sodora DL, Shpaer EG, Kitchell BE, Dow SW, Hoover EA, Mullins JI (1994) Identification of three feline immunodeficiency virus (FIV) *env* gene subtypes and comparison of the FIV and human immunodeficiency virus type 1 evolutionary patterns. J Virol 68: 2230–2238
28. Uema M, Ikeda Y, Miyazawa T, Lin JA, Chen M-C, Kuo T-F, Kai C, Mikami T, Takahashi E (1999) Feline immunodeficiency virus subtype C is prevalent in northern part of Taiwan. J Vet Med Sci 61: 197–199
29. Willey RL, Rutledge RA, Dias S, Folks T, Theodore T, Buckler CE, Martin MA (1986) Identification of conserved and divergent domains within the envelope gene of the acquired immunodeficiency syndrome retrovirus. Proc Natl Acad Sci USA 83: 5038–5042
30. Yamamoto JK, Hansen H, Ho EW, Morishita TY, Okuda T, Sawa TR, Nakamura RM, Pedersen NC (1989) Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission. J Am Vet Med Assoc 194: 213–220

Author's address: Dr. T. Miyazawa, Research Center for Emerging Infectious Diseases, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan; e-mail: takavet@mc.kcom.ne.jp