Molecular characterization of the *L1* gene of papillomaviruses in epithelial lesions of cats and comparative analysis with corresponding gene sequences of human and feline papillomaviruses

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Objective—To characterize the *L1* gene of papillomaviruses detected in epithelial lesions of cats and to determine the relationship between those *L1* gene nucleotide sequences and known *L1* gene sequences of human and feline papillomaviruses.

Sample Population—10 tissue samples of epithelial lesions from 8 cats.

Procedures—DNA was extracted from tissue samples. Primers were designed to amplify the *L1* gene of papillomaviruses. Amplicons of DNA were sequenced; nucleotide sequences were compared with known *L1* gene nucleotide sequences of papillomaviruses and used for phylogenetic analysis.

Results—Tissue samples were obtained from lesions (diagnosed as dysplasia [n = 1], squamous cell carcinoma in situ [3], or squamous cell carcinoma [6]) of the skin (9) and oral mucosa [1]. Two amplicons had 99% homology with the *L1* gene nucleotide sequence of human papillomavirus type 38b subtype FA125. Another amplicon had 84% homology with the *L1* gene nucleotide sequence of human papillomavirus type 80 and was considered to be a new type of papillomavirus. Phylogenetic tree analysis revealed that these 3 papillomaviruses were grouped into 2 clades that were not similar to the clades of *Felis domesticus* papillomavirus type 1 or *F domesticus* papillomavirus type 2 (FdPV2). The remaining 7 amplicons had 98% to 100% homology with the *L1* gene nucleotide sequence of FdPV2. Phylogenetic tree analysis revealed that those 7 papillomaviruses were grouped into a single clade with FdPV2.

Conclusions and Clinical Relevance—Results support the likelihood of transmission of papillomaviruses between humans and cats. (*Am J Vet Res* 2010;71:1457–1461)

Papillomaviruses are a group of small, nonenveloped, double-stranded DNA viruses that are epitheliotropic. An approximately 8,000-nucleotide circular genome of these viruses encodes 7 to 9 open reading frames, depending on the genotype. Papillomaviruses express 3 regulatory proteins (*E1*, *E2*, and *E4*), 3 oncogenic proteins (*E5*, *E6*, and *E7*), and 2 viral capsid proteins (*L1* and *L2*). The *L1* protein is highly conserved, and papillomavirus classification is generally based on sequence analysis of the amino acids of this major capsid protein.[1]

These epitheliotropic viruses infect a wide range of birds and mammals, including humans,[2] and cause benign cutaneous and mucosal epithelial proliferations called papillomas (warts). Occasionally, some papillomaviruses can cause malignant epithelial lesions (carcinomas). Environmental factors, host genetic factors, or both may play a role in the progression of papillomavirus-associated malignancy.[3,4]

Typically, papillomaviruses are host and tissue specific, but there are some exceptions. Equine and feline sarcoids have been associated with bovine papillomavirus.[5,6] In 2 recent studies[7,8] in cats, HPV type 9

Abbreviations

CPV	Canine papillomavirus
FdPV1	*Felis domesticus* papillomavirus type 1
FdPV2	*Felis domesticus* papillomavirus type 2
FPV	Feline papillomavirus
HPV	Human papillomavirus
SCC	Squamous cell carcinoma

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DNA was extracted from a cutaneous papilloma, and the DNA from a papillomavirus present in an SCC of the oral cavity had 92% nucleotide sequence similarity with HPV type 76 DNA. However, those investigators analyzed approximately 25%[7] and 30%[8] of the *L1* gene; thus, the extent to which the HPVs could be genotyped was somewhat limited. Nonetheless, the host species fidelity of papillomaviruses appears not to be absolute, especially in humans and felids. The purpose of the study reported here was to characterize the *L1* gene of papillomaviruses detected in epithelial lesions of cats and to determine the relationship between those *L1* gene nucleotide sequences and known *L1* gene nucleotide sequences of human and feline papillomaviruses.

Materials and Methods

Samples and extraction of viral DNA—Ten tissue samples from 8 cats undergoing a biopsy procedure submitted for histopathologic examination to a pathology service[a] from 1999 through 2007 were used in this study. A commercially available kit[b] was used to extract viral DNA from these samples according to the protocol suggested by the manufacturer, and the DNA was stored at –20°C prior to analysis.

DNA amplification—For sequence analysis and virus classification, primers were designed to amplify the *L1* gene of papillomaviruses (**Appendix**). Each PCR mixture contained 1 µL each of a forward and reverse primer (concentration, 50µM), 8 µL of nuclease-free water, 12.5 µL of a *Taq* premix,[c] and 2.5 µL of DNA template. The PCR reactions were conducted in an automated thermocycler[d] with the following temperature cycle conditions: 5 cycles of 1 minute at 95°C, 1.5 minutes at 50°C, and 2 minutes at 72°C and then followed by 35 cycles of 1 minute at 95°C, 1 minute at 55°C, and 2 minutes at 72°C. The PCR products were analyzed by electrophoresis in a 1.4% agarose gel containing ethidium bromide.

Nucleotide sequence alignment and phylogenetic tree analysis—For sequencing of the PCR products, single-stranded DNA was enzymatically digested with a kit[e] according to the manufacturer's instructions or appropriately sized bands were excised from agarose gels and the DNA purified by use of a gel extraction kit.[b] Digested and purified samples were sequenced at a molecular biology facility[f] by use of a sequencing reaction kit[g] and a capillary electrophoresis DNA analyzer.[h] Nucleotide sequence results were compared with *L1* gene nucleotide sequences reported in GenBank by use of a bioinformatic search tool.[i] Alignment of gene nucleotide sequences and construction of a phylogenetic tree were performed by use of a commercially available software program.[j] A gene nucleotide sequence of an amplicon that had < 90% homology with known *L1* gene nucleotide sequences was considered a new papillomavirus. Any new papillomaviruses identified in the study were to be submitted for inclusion in the GenBank database.

Results

Of the 10 tissue samples, 9 (designated as samples 18, 37, 38, 52, 61, 132, 134, 138, and 162) were collected from the skin and 1 (designated as sample 160) was collected from a lesion of the oral mucosa overlying the mandible. In the 9 skin samples, 5 (samples 18, 61, 132, 134, and 138), 3 (samples 37, 38, and 52), and 1 (sample 162) of the lesions were diagnosed as SCC, SCC in situ (ie, tumor did not penetrate the epidermal basement membrane), and dysplasia, respectively. In the tissue sample obtained from the mandible, the lesion was diagnosed as an oral SCC. Amplicons of the *L1* gene were successfully made from all 10 tissue samples. Nucleotide sequences of amplicons were compared with known sequences of papillomaviruses.

Sequence alignment of the *L1* gene of 7 amplicons (samples 18, 37, 38, 52, 61, 132, and 134 derived from 3 SCCs in situ and 4 SCCs) had 98% to 100% nucleotide sequence homology with the *L1* gene nucleotide sequence of the reported FdPV2 gene nucleotide sequence (GenBank accession No. EU796884); however, 27 nucleotides from the 5′ end of the amplicon were not amplified in 3 (samples 38, 52, and 61) of these 7 amplicons. Phylogenetic tree analysis based on nucleotide sequence alignment (ie, nucleotides 6,205 through 7,692) of the *L1* gene of these 7 amplicons and the *L1* gene of FPV genomes (FdPV1 [GenBank accession No. AF480454], FdPV2 [GenBank accession No. EU796884], *Panthera leo persica* papillomavirus type 1 [GenBank accession No. AY904724], *Lynx rufus* papillomavirus type 1 [GenBank accession No. AY904722], *Puma concolor* papillomavirus type 1 [GenBank accession No. AY904723], and *Uncia uncia* papillomavirus type 1 [GenBank accession No. DQ180494]) revealed that these papillomaviruses were grouped into a single clade with the reported FdPV2 *L1* gene nucleotide sequence (GenBank accession No. EU796884; **Figure 1**).

Sequence alignment of the nucleotide and deduced amino acid sequences of the amplicons derived from 2 samples, which were an oral SCC (sample 160) and a cutaneous dysplasia (sample 162), had 99% nucleotide sequence homology (ie, nucleotides 5,260 to 7,174)

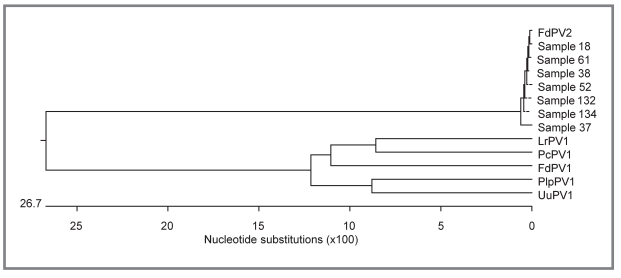

Figure 1—Results of phylogenetic tree analysis that was based on sequence alignment (ie, nucleotides 6,205 through 7,692) of *L1* gene nucleotide sequences of 7 amplicons derived from papillomaviruses in 3 SCCs in situ and 4 SCCs in skin samples (samples 18, 37, 38, 52, 61, 132, and 134 [obtained from a group of 8 cats]) with the *L1* gene nucleotide sequences of FPV genomes (FdPV1 [GenBank accession No. AF480454], FdPV2 [GenBank accession No. EU796884], *Panthera leo persica* papillomavirus type 1 [GenBank accession No. AY904724], *Lynx rufus* papillomavirus type 1 [GenBank accession No. AY904722], *Puma concolor* papillomavirus type 1 [GenBank accession No. AY904723], and *Uncia uncia* papillomavirus type 1 [GenBank accession No. DQ180494]). These 7 papillomaviruses were grouped into 1 clade with the reported *L1* gene nucleotide sequence of FdPV2. LrPV1 = *Lynx rufus* papillomavirus type 1. PcPV1 = *Puma concolor* papillomavirus type 1. PlpPV1 = *Panthera leo persica* papillomavirus type 1. UuPV1 = *Uncia uncia* papillomavirus type 1.

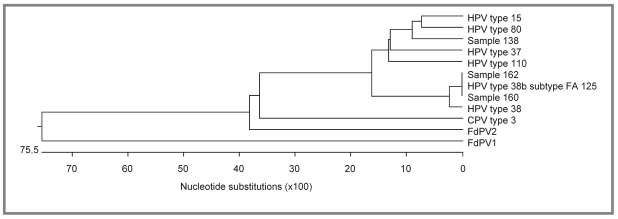

Figure 2—Results of phylogenetic tree analysis that was based on sequence alignment (ie, nucleotides 5,260 through 7,109) of a 1,850-bp sequence of the *L1* gene amplicon derived from papillomaviruses in a cutaneous dysplastic lesion, cutaneous SCC, and SCC of the oral mucosa in feline samples (samples 138, 160, and 162 [each obtained from a different cat]) with *L1* gene reference sequences (HPV type 38b subtype FA125 [GenBank accession No. DQ090005], type 38 [GenBank accession No. U31787.1], type 110 [GenBank accession No. EU410348.1], type 15 [GenBank accession No. X74468.1], type 37 [GenBank accession No. U31786.1], and type 80 [GenBank accession No. Y15176] and FdPV1 [GenBank accession No. AF480454], FdPV2 [GenBank accession No. EU796884], and CPV type 3 [GenBank accession No. DQ295066]). Gene nucleotide sequences of these papillomaviruses were grouped into 2 clades that were not similar to the clades of FdPV1, FdPV2, or CPV.

with the *L1* gene nucleotide sequence of HPV type 38b subtype FA125. Alignment of the *L1* gene nucleotide sequence derived from sample 138 (the remaining skin-associated SCC) had 84% nucleotide sequence homology (ie, nucleotides 5,260 through 7,105) with the *L1* gene nucleotide sequence of HPV type 80, and the nucleotide sequence was submitted for inclusion in the GenBank database (accession No. GQ916646) as a novel papillomavirus. Phylogenetic tree analysis based on the alignment of 1,850-bp sequences of these 3 amplicons and the most closely aligned *L1* gene nucleotide sequences (HPV type 38b subtype FA125 [GenBank accession No. DQ090005], type 38 [GenBank accession No. U31787.1], type 110 [GenBank accession No. EU410348.1], type 15 [GenBank accession No. X74468.1], type 37 [GenBank accession No. U31786.1], and type 80 [GenBank accession No. Y15176] and FdPV1 [GenBank accession No. AF480454], FdPV2 [GenBank accession No. EU796884], and CPV type 3 [GenBank accession No. DQ295066]) of papillomaviruses reported in GenBank revealed that sequences of these 3 papillomaviruses were grouped into 2 clades that were not similar to the clades of FdPV1, FdPV2, or CPV (**Figure 2**).

Discussion

Papillomaviruses have been associated with various diseases of felids, including cutaneous fibropapilloma (eg, sarcoid),[6] viral papillomas,[9] viral plaques,[10] invasive SCC, and bowenoid SCC in situ.[10,11] Domestic and nondomestic felids, like many species of mammals and birds, are susceptible to infection with 1 or more types of papillomavirus.[12,13]

Although papillomaviruses are generally considered to be species specific, the ability of bovine papillomavirus to cross the host-species barrier in horses[5] and domestic felids has been reported.[6] Investigators of previous studies[7,8] of papillomaviruses in cats have examined a portion of the *L1* gene. However, papillomavirus classification is usually based on full-length or nearly full-length *L1* gene nucleotide sequences. Papillomaviruses are classified into type, subtype, and variant on the basis of the extent to which their highly conserved L1 gene nucleotide sequences differ (ie, sequences differences of ≥ 10%, 2% to 10%, and ≤ 2%, respectively).[1]

In the study reported here, further nucleotide sequence analysis of the entire *L1* gene of the papillomaviruses derived from a cutaneous dysplastic lesion (sample 162) and an oral SCC (sample 160) in samples from cats revealed robust nucleotide sequence homology (ie, 99%) to the *L1* gene nucleotide sequence of the β-papillomavirus HPV type 38b subtype FA125. This β-papillomavirus has been associated with skin cancer in humans and is a member of a group of rare HPVs that are heterogeneous and contain subtypes.[14] The *L1* gene nucleotide sequence derived from another skin-associated SCC (sample 138) in the present study revealed 84% nucleotide sequence homology with the *L1* gene nucleotide sequence of HPV type 80. This papillomavirus sequence was submitted to GenBank as a new papillomavirus because the nucleotide sequence identity (homology) of this amplicon was < 90% across almost the entire known *L1* genome.

Phylogenetic tree analysis of the 3 aforementioned samples revealed that these sequences were grouped into a clade with HPV and were distinct from the FPV clade. The FPVs FdPV1[1] and FdPV2[15] phylogenetically belong to the λ-papillomavirus genus and a new, as yet unnamed genus, respectively. In contrast, HPVs 38 and 80 are phylogenetically distant from both FdPV1 and FdPV2 and are classified within the β-papillomavirus genus.

Among the papillomaviruses detected in cats, most of those that are closely related to HPV (eg, HPV type 9, 38, 76, and 80) belong to the cutaneous β-papillomavirus genus. The genetic organization of β-HPVs differs from that of other HPVs. In humans, the β-HPVs are highly prevalent in the general population and are associated with SCC development.[16] In combination with previous reports,[7,8] results of the present study have provided supportive evidence of papillomavirus transmission between humans and cats. Cats are often in close contact with humans; thus, the potential for interspecies transmission exists. The mechanism associated with the ability of these viruses to cross the host-species barrier is unknown, but a common viral receptor may exist on both human and feline epithelial cells.

Papillomavirus DNA has been amplified not only from samples of normal (nonlesioned) skin of humans,[17] but also from samples of normal (nonlesioned) skin of a cat.[18] Because both FPV and HPV can be present in apparently normal skin in humans and cats, it is difficult to determine in which species the virus originated. These findings suggest a potential use of cats in HPV research. Contamination with HPV was unlikely in the present study because all samples were collected and examined in a veterinary laboratory and because no human tissues were examined. Because the present study was restricted to the detection of a single nucleotide sequence of a gene and not an entire genome, detection of the *L1* gene nucleotide sequence provides indirect evidence for the presence of papillomavirus.

Seven samples were classified as FdPV2 because amplicons of their *L1* gene nucleotide sequence had

98% to 100% homology with the FdPV2 *L1* gene nucleotide sequence. Phylogenetic tree analysis of these samples grouped them into 1 clade with FdPV2, whereas other FPVs were grouped into a separate clade. The conserved FdPV2 *L1* gene nucleotide sequence has 51.1% nucleotide sequence homology with the *L1* gene nucleotide sequence of FdPV1 and has been suggested as a new genus of papillomavirus.[15]

Papillomaviruses that cause malignancy (ie, high-risk viruses) are different from those that cause benign warts (ie, low-risk viruses).[1] Specific types of α-HPV and β-HPV have been detected in cervical and cutaneous cancers, respectively, in humans. Similarly, analysis of the results of the study reported here suggested that FPVs that cause malignant lesions differ from those that cause benign lesions (eg, FdPV1). The FdPV2s detected in the present study were similar to those in previous studies[15,18,19] and were amplified from premalignant and malignant lesions. In addition, the detection of the entire FdPV2 *L1* gene in cats in the United States in the present study and on different continents[11,20] suggests that, in a geographic sense, FdPV2 is distributed widely.

To our knowledge, this is the first study to detect the *L1* gene of HPV type 38b subtype FA125 in cats. On the basis of this finding, it appears that papillomaviruses can cross the host-species barrier. In addition, these results provide support for the possibility that other types of HPVs might be detected in cats and the likelihood that a broader association can be made between FdPV2 and malignant lesions of cats.

a. Department of Pathology, College of Veterinary Medicine, University of Tennessee, Knoxville, Tenn.
b. QIAquick kit, Qiagen, Valencia, Calif.
c. Ex *Taq* premix, TakaRa Bio Inc, Otsu, Shiga, Japan.
d. Eppendorf Mastercycler gradient, Perkin Elmer Inc, Norwalk, Calif.
e. ExoSAP-IT, USB, Cleveland, Ohio.
f. Molecular Biology Resource Facility, College of Arts and Sciences, University of Tennessee, Knoxville, Tenn.
g. ABI prism dye terminator cycle sequencing reaction kit, Perkin Elmer Inc, Foster City, Calif.
h. ABI 373 DNA, Perkin Elmer Inc, Foster City, Calif.
i. BLAST, National Center for Biotechnology Information, National Institutes of Health, Bethesda, Md. Available at: blast.ncbi.nlm.nih.gov/. Accessed Aug 12, 2009.
j. Lasergene, DNASTAR Inc, Madison, Wis.

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Appendix appears on the next page

Appendix

Design of the primers used in the PCR assays for the detection of the *L1* gene derived from papillomaviruses in a dysplastic lesion (sample 162 [skin lesion]) and each of 2 SCCs (samples 138 [skin lesion] and 160 [mucosal lesion]) in 3 tissue samples collected from 3 cats (1 sample/cat).

Primer	Oligonucleotide sequence	Amplimer size (nucleotides)	Nucleotide sequence location
Samples 160 and 162			
HPV1			
Forward	5′–AGGGTTTGGCAACATAAACAAC–3′	492	6,269–6,290*
Reverse	5′–AGCATCATATTCCTGAGCACCT–3′	492	6,761–6,740*
HPV2			
Forward	5′–AAGGTTCCTTTGACTGCTGAAG–3′	465	6,828–6,849*
Reverse	5′–GGTTGCTGGTGTTGACTTGTT–3′	465	7,293–7,273*
HPV4			
Forward	5′– GGGGACAGCCATTAGGAGTT–3′	369	5,989–6,008*
Reverse	5′–TCTGGATATTTGCAGGTTTCA–3′	369	6,358–6,338*
HPV7			
Forward	5′–TGTCAGATCACAGGATGGTCA–3′	345	5,816–5,836*
Reverse	5′–TTCTCCCAGACAAGGAGTGC–3′	345	6,161–6,142*
HPV8			
Forward	5′–TATCCCGAAAGCAGAGAACG–3′	392	5,507–5,526*
Reverse	5′–CTGGAAAGGTTACCCGGAAT–3′	392	5,898–5,879*
HPV10			
Forward	5′–GGTGCACAAATAGGGTCACA–3′	391	5,135–5,154*
Reverse	5′–CGTTCTCTGCTTTCGGGATA–3′	391	5,526–5,507*
Sample 138			
P1			
Forward	5′–CTTTCCCACAGTTAGTGGTTCTTT–3′	320	6,605–6,628†
Reverse	5′–CCTGAATTCATAGCATTAATTTGTGT–3′	320	6,925–6,900†
P6			
Forward	5′–AATAGDTTTGCWTTAGCAGAT–3′	470	5,946–5,966†
Reverse	5′–CATNGTTARRAAATCTGGATAVTT–3′	470	6,416–6,399†
P8			
Forward	5′–CAAARCACGGAYGADTAYRT–3′	310	5,778–5,797†
Reverse	5′–TCTCTAACTTATTTGAATAGTGGAT–3′	310	6,088–6,064†

*Nucleotide sequences are based on GenBank accession No. DQ090005 for the HPV type 38b genome.
†Nucleotide sequences are based on GenBank accession No. X74468.1 for the HPV type 15 genome.