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SHORT REPORT

First detection and complete genome analysis of the Lyon IARC polyomavirus in China from samples of diarrheic cats

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Abstract

Lyon IARC polyomavirus (LIPyV), a newly discovered polyomavirus (PyV), was first identified in 2017 in human skin samples in the USA. Later, it was detected in several other countries in samples of human and feline origin. Our aim was to find out if the virus occurs in China. To this end, 100 fecal samples were collected from cats with diarrhea in Guangxi Province during 2016 and 2018 and tested with polymerase chain reaction (PCR). Only 2 samples that originated from two related individuals were found to be positive. Based on the sequence identity of the 240-bp PCR products, the two positive samples supposedly contained identical viruses. Therefore, only one of them, which was designated as LIPyV-GXNN01, was selected for full genome amplification, cloning, sequencing and analysis. LIPyV-GXNN01, which comprises 5,263 nucleotides, has an early region that consists of small T antigen (ST-Ag) and large T antigen (LT-Ag) and a late region coding for the VP1, VP2, and VP3 structural proteins. Moreover, the LIPyV-GXNN01 strain structural proteins share 95.9–99.4%, 97.6–99.2%, and 97.1–99.2% nucleic acid identity with the VP1, VP2, and VP3of other LIPyV reference strains, respectively. A phylogenetic analysis revealed that GXNN01 clustered together with previously reported LIPyV strain. This present study is the first report of LIPyV in China.

Keywords Polyomaviruses (PyVs) · Genomic characterization · Cat

Introduction

Polyomaviruses (PyVs) are nonenveloped DNA viruses; a total of 86 PyV species have been classified [1]. PyVs are ubiquitous viruses that frequently infect cattle [2, 3], fish [4, 5], horses [6], pigs [7,8] and other vertebrates including humans [9, 10], and their effects on distinct hosts differ [11]. PyVs replicate in animal tonsils and adenoids [12] and may cause benign or malignant tumors in some animals [13].

Lyon IARC PyV (LIPyV) was first reported in 2017 in skin samples from humans. A genetic analysis indicated a 65% nucleotide identity with previously reported raccoon polyomavirus (RacPyV) [14]. In a study by Brostoff et al., LIPyV was found in 1/127 benign skin tumor samples from liver transplant recipients in Finland [14]. In another study by Gheit et al. 9 skin swabs (9/445; 2.0%) and 1 eyebrow hair sample (1/439; 0.2%) tested positive for LIPyV from 448 American skin cancer screening patients and 3/140 gargle samples from healthy French adults [15]. Recently, no evidence of LIPyV presence was found in 689 noncancerous human tonsil samples or 139 gargle samples from France [16]. The same result was reported in a serological survey of 156 people in the Netherlands [17]. LIPyV has also been found in the feces of diarrheic cats [18]; however, only two complete genomes of LIPyV strains have been sequenced to date. Furthermore, epizootiology and molecular data on LIPyV in China have yet to be reported. In this study, LIPyV

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was identified in fecal samples collected from cats with diarrhea from Guangxi Province, China between 2016 and 2018 by the Guangxi Center for Animal Disease Control and Prevention and further characterized by genomic similarity and phylogenetic analysis.

Stool supernatants were extracted according to the instructions of the DNA extraction kit. Polymerase chain reaction (PCR) amplification was performed by using the detection primers for LIPyV (F-R) (Table 1) (Fahsbender et al. 2019). The PCR products were purified from agarose gels and sequenced. A BLAST analysis of the sequences identified that 2% (2/100) of the samples contained a virus most closely related to LIPyV. The LIPyV-positive samples were also examined for feline panleukopenia virus (FPV) [19], feline bocavirus (FBoV) 1 to 3 [20], feline astrovirus (FeAstV) [21], feline kobuvirus (FeKoV) [22] and feline coronavirus (FCoV) [23] with the previously described PCR/RT-PCR assays. The LIPyV-positive samples were also evaluated according to specific primers (16S mam1/16S mam2) as previously described [24].

The sequences obtained from the two PCR-positive samples were identical. Moreover, the two cats were related, therefore it was assumed that most likely identical viruses were present in both cases, so only one was selected for analysis. Five pairs of PCR primers that cover the complete genome sequences of previously reported LIPyV strains were designed (Table 1). The target fragments were amplified by using 2 × VazymeLAmp Master Mix (Vazyme, China). DNA fragments of 1072, 1296, 1305, 1297 and 695 bp were produced. The PCR-amplified products were ligated into pMD18T vectors (Takara, Japan), and the positive plasmids were sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing. After obtaining the raw sequencing data, DNASTAR SeqMan was used to assemble the whole genomic sequences of LIPyV-GXNN01 (accession number: MW054655). A sequence analysis of the entire genome and the genomes of representative strains of mammalian PyVs in GenBank was performed (Table S1).

The genome of LIPyV-GXNN01 is circular and 5263 bp in length, and it contains open reading frames (ORFs) for all the major PyV proteins (Fig. S1). An analysis of the complete nucleotide sequence showed that the LIPyV genome shares the features of other known PyVs, with an early region that consists of ST-Ag and LT-Ag and a late region coding for the VP1, VP2, and VP3 structural proteins with lengths of 1307, 743 and 515 aa, respectively, (Table S2). The G + C content of LIPyV-GXNN01 was 39.6%, and the VP1, VP2 and VP3 sequences of LIPyV-GXNN01 exhibited a 95.9–99.4%, 97.6–99.2%, and 97.1–99.2% identity, respectively, with their counterparts in other LIPyVs reported previously (Table 2). However, with the corresponding sequences of other PyVs, such as those from chimpanzees or raccoons, they shared less than a 65% identity.

In addition, compared with the reference LIPyV sequences identified in the USA, several amino acid substitutions (T41A in VP2; S334N in VP1 Y435F, I436F, T679I, and K692R in LT-Ag; and A126S in ST-Ag) were observed in the genomic sequences of LIPyV-GXNN01.A

Table 1 List of primers

Primer name	Primer sequence (5′–3′)	Size (bp)	Annealing temperature (°C)
LIPyV F	CAWGCTGTRTITAGTAATA	240	48
LIPyV R	RWTTATTMACHCCITTAC		
LIPyV1F	ACTAACAAATGCAAGGTAAAGGCGGGAGAT	1072	61
LIPyV1073R	AACTAAAGGTAAAAGCCAGTCAGGACAGGT		
LIPyV1038F	CAGCAAACCTGTCCTGACTGGCTTTTACCT	1296	64
LIPyV2334R	ATGTTCCTCACCCACACTATCACCCACATC		
LIPyV2292F	GCCTGTTGTGGGTGATGTGGGTGATAGTGT	1305	64
LIPyV3597R	CCTTTGAAGCCTCATGAAGCCCATAGGAAA		
LIPyV3544F	TATATACTTGGCATTTTCATAATTTTTCCT	1297	55
LIPyV4841R	TTACTGCTACTATTGCTTTGTTACTTGGT		
LIPyV4568F	CCATAGTGCTTGCGGTACCACCAAGCTCTA	695	64
LIPyV5263R	ATGGATGCTGTACTGACTACGCCTGAGAGA		

Table 2 Genetic sequence similarity among different polyomavirus hosts (%)

Virus-encoded protein		Type of virus						
		LyonIARCPyV(human)	LI PyV (cat)	Chimpanzee PyVBob(ape)	Raccoon PyVRac17(raccoon)	Chaere-phonPyV1 (bat)	Crow PyV (bird)	Murine PyV(mouse)
Structural protein	VP1	99.4	95.9	54.3	64.4	56.5	58.5	58.6
	VP2	99.2	97.6	50.8	59.0	50.4	38.3	34.3
	VP3	99.2	97.1	47.9	/	47.1	41.2	34.2
Nonstructural protein	LT	99.6	92.5	55.8	33.1	52.9	45.5	33.8
	ST	99.8	97.2	54.2	36.4	53.5	42.4	30.6
	MT	/	/	/	/	/	/	/

noncoding control region (NCCR, nucleotide positions1–401) that shared the characteristics of the ori regions of most mammalian PyVs was found. This region contains four LT-Ag-binding sites [15], specifically, one GAGGC and three reverse complement GCCTC motifs. To visually illustrate the evolutionary relations among the LIPyVs and other PyVs, after estimation with the Model Finder program, a maximum likelihood (ML) phylogenetic tree was created with gamma distributed with invariant sites' (G + I) substitution and Kimura 2-parameter models, respectively, with 1000 bootstrap replicates that used MEGA7 [25] (Fig. 1).The results based on full-length genomes demonstrated that LIPyV-GXNN01 formed a single clade with LIPyV reference strains LI polyomavirus and Lyon-IARC polyomavirus. Lyon-IARC polyomavirus strains clustered significantly with different mammalian PyVs including human polyomaviruses, bat polyomaviruses, and primate polyomaviruses.

Here, we present the first evidence for the occurrence and possible circulation of LIPyV among cats in China. The proportion of the positive samples (2%) is comparable to that reported in other countries, including the USA (0.2%), France (2.14%) and Finland (0.79%). Although the presence of other viral agents (FPV, FeBoV 1 and 3, FeAstV, FeKoV, and FeCoV) was excluded in these two samples, the eventual etiological role of the detected LIPyV in feline diarrhea has yet to be clarified. In previous investigations, LIPyV was identified during a metagenomics analysis of feline diarrheic samples in which FPV, FBoV 3 and numerous bacteria were also detected [18]. The effect of coin fection with other enteric viruses on pathogenesis is still unclear. Despite the apparent distribution of LIPyV in skin biopsies, the debate regarding the possibility of the environmental contamination of human skin biopsy samples remains. To answer this question, LIPyV isolations necessary; however, no study has reported the successful isolation of the virus. We failed to use F81 cells for isolation.

Accordingly, this study is the first report of LIPyV in China and analyzes the presence of LIPyV, which provides a basis for research on PyV etiology and epidemiology. The threat of this virus to human health remains unknown. To investigate the pathogenicity of LIPyV, further studies including viral isolation are necessary.

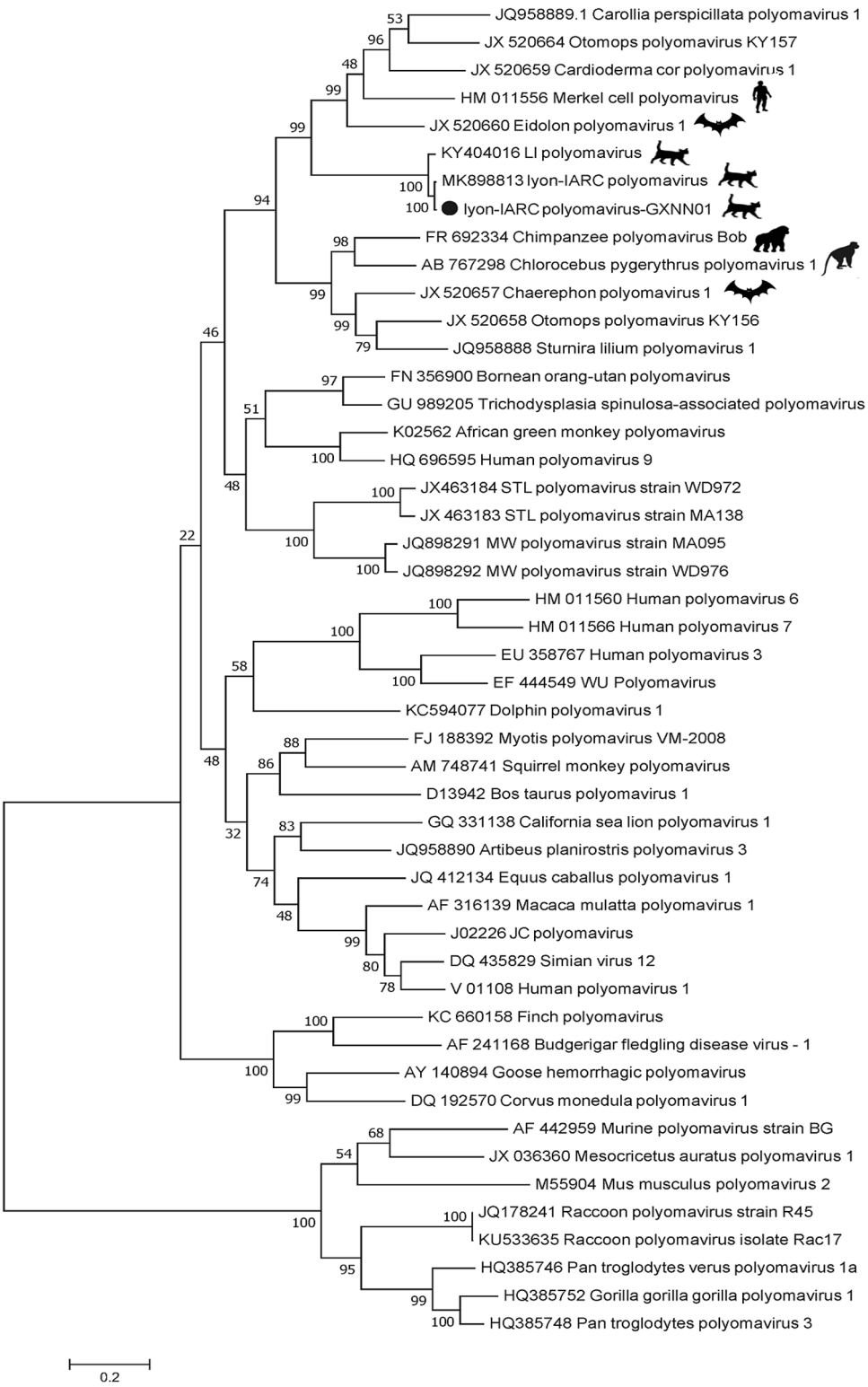

Fig. 1 Phylogenetic analyses of PyVs based on the full-length genomes using the maximum likelihood algorithm and 1000 bootstrap replications in a heuristic search with MEGA 7. Black circles represent sequenced strains from this study. Animal graphical indicate the virus host

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11262-021-01840-1.

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Declarations

Conflict of interest The authors declare that they have no competing interests.

Research involving human and animal rights All clinical samples used in this study were collected with consent from veterinarians and farm owners. All experiments were carried out in strict accordance with the regulations in the Guide for the Care and Use of Laboratory Animals of Guangxi University and the Guangxi Center for Animal Disease Control and Prevention, China.

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