

Complete Genome Sequence of a Canine-Origin H3N2 Feline Influenza Virus Isolated from Domestic Cats in South Korea

Seong-Jun Park,^{a,b} Bo-Kyu Kang,^c Hye-Young Jeoung,^d Hyoung-Joon Moon,^c Minki Hong,^{a,e} Woonseong Na,^{a,e} Bong-Kyun Park,^b Haryoung Poo,^{a,e} Jeong-Ki Kim,^f Dong-Jun An,^d Dae-Sub Song^{a,e}

Viral Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, South Korea^a; Department of Veterinary Medicine Virology Laboratory, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul, South Korea^b; Research Unit, Green Cross Veterinary Products, Yongin, South Korea^c; Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, South Korea^d; University of Science and Technology, Daejeon, South Korea^e; Department of Pharmacy, College of Pharmacy, Korea University, Sejong, South Korea^f

A canine-origin Korean H3N2 feline influenza virus (FIV), A/feline/Korea/01/2010 (H3N2), was isolated in 2010 from a dead cat with severe respiratory disease. Here, we report the first complete genome sequence of this virus, containing 3' and 5' noncoding regions, which will help elucidate the molecular basis of the pathogenesis, transmission, and evolution of FIV.

Received 29 December 2012 Accepted 5 February 2013 Published 7 March 2013

Citation Park S-J, Kang B-K, Jeoung H-Y, Moon H-J, Hong M, Na W, Park B-K, Poo H, Kim J-K, An D-J, Song D-S. 2013. Complete genome sequence of a canine-origin H3N2 feline influenza virus isolated from domestic cats in South Korea. *Genome Announc.* 1(2):e00253-12. doi:10.1128/genomeA.00253-12.

Copyright © 2013 Park et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Dong-Jun An, andj67@korea.kr, or Dae-Sub Song, sds1@kribb.re.kr.

Influenza A virus (IAV), a member of the family *Orthomyxoviridae*, is an enveloped virus with eight segmented, negative-sense, single-stranded RNA genes. IAV is a clinically and economically important pathogen which infects many animals, including humans, pigs, horses, marine mammals, and poultry (1). Influenza had long been absent from the list of infectious diseases considered as possibilities in dogs and cats (2). However, this has been recently disproved by the isolation of IAVs in those animals (3–7). A canine-origin Korean H3N2 feline influenza virus (FIV) strain, designated A/feline/Korea/01/2010 (H3N2), was isolated at the beginning of 2010 from a lung specimen of a dead cat, which had suffered from a severe respiratory disease (6). To date, a complete genome sequence containing 3' and 5' noncoding regions (NCRs) of H3N2 FIV has not been reported despite multiple functions of NCRs in the replication of IAVs (8–11). Therefore, it is necessary to analyze the complete genome sequence containing both 3' and 5' NCRs of A/feline/Korea/01/2010 (H3N2) and elucidate its molecular characteristics.

Viral RNA was extracted from allantoic fluids of embryonated eggs infected with A/feline/Korea/01/2010 (H3N2) and circularized with T4 RNA ligase as described previously (12–15). To determine the complete genome sequence containing 3' and 5' NCRs, the PCR products produced by RNA ligation-mediated reverse transcriptase PCR (RT-PCR) were purified, cloned (16), and sequenced on an automated DNA sequencer (ABI System 3700; Applied Biosystems, Inc.) by utilizing universal primer sets (17) with slight modifications and newly designed segment-specific primers.

The complete genome of A/feline/Korea/01/2010 (H3N2) is 13,629 nucleotides (nt) long, a length identical to that of the avian-origin Korean H3N2 canine influenza virus, A/canine/Korea/01/2007 (H3N2) (13); segments 1 (Seg-1) through 8 (Seg-8) are 2,341, 2,341, 2,233, 1,765, 1,565, 1,467, 1,027, and 890 nt, respectively. They encode 12 viral proteins with amino acid lengths as follows: PB2, 759; PB1, 757; N40 (18), 718; PB1-F2, 90; PA, 716;

HA, 566; NP, 498; NA, 469; M1, 252; M2, 97; NS1, 230; and NS2 (nuclear export protein [NEP]), 121.

While the lengths of the 3' and 5' NCRs of the viral RNA of A/feline/Korea/01/2010 (H3N2) were variable (19 to 45 and 20 to 58 nt at the 3' and 5' NCRs, respectively) in the different genome segments, the terminal 12 and 13 nt of the 3' and 5' ends, respectively, were highly conserved (3'-UCGYUUUCGUCC- and -GGAACAAAGAUGA-5') among all eight genome segments, which is consistent with previous studies (13, 14, 19, 20). Surprisingly, 1 nt was changed between the start codon (UAC) and the conserved region (UCGYUUUCGUCC) in the 3' NCR of Seg-1 and also in Seg-6 of A/feline/Korea/01/2010 (H3N2) compared to A/canine/Korea/01/2007 (H3N2).

This is the first report of the complete genome sequence containing the 3' and 5' NCRs of H3N2 FIV. We hope that these data will provide important insights into the molecular basis of the pathogenesis, transmission, and evolution of FIV as well as other IAVs.

Nucleotide sequence accession numbers. The complete genome sequence of the A/feline/Korea/01/2010 (H3N2) strain described here has been deposited in GenBank under the accession numbers [KC422453](https://www.ncbi.nlm.nih.gov/nuccore/KC422453) to [KC422460](https://www.ncbi.nlm.nih.gov/nuccore/KC422460) for Seg-1 to Seg-8.

ACKNOWLEDGMENTS

This work was supported by a National Agenda Project grant from the Korea Research Council of Fundamental Science & Technology and the KRIBB Initiative program (KGM3121221). This study was supported by a grant from the Korea Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (grant no. A103001).

REFERENCES

1. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. 1992. Evolution and ecology of influenza A viruses. *Microbiol. Rev.* 56:152–179.
2. Beeler E. 2009. Influenza in dogs and cats. *Vet. Clin. North Am. Small Anim. Pract.* 39:251–264.
3. Crawford PC, Dubovi EJ, Castleman WL, Stephenson I, Gibbs EP,

- Chen L, Smith C, Hill RC, Ferro P, Pompey J, Bright RA, Medina MJ, Johnson CM, Olsen CW, Cox NJ, Klimov AI, Katz JM, Donis RO. 2005. Transmission of equine influenza virus to dogs. *Science* 310:482–485.
4. Desvaux S, Marx N, Ong S, Gaidet N, Hunt M, Manuguerra JC, Sorn S, Peiris M, Van der Werf S, Reynes JM. 2009. Highly pathogenic avian influenza virus (H5N1) outbreak in captive wild birds and cats, Cambodia. *Emerg. Infect. Dis.* 15:475–478.
 5. Song D, Kang B, Lee C, Jung K, Ha G, Kang D, Park S, Park B, Oh J. 2008. Transmission of avian influenza virus (H3N2) to dogs. *Emerg. Infect. Dis.* 14:741–746.
 6. Song DS, An DJ, Moon HJ, Yeom MJ, Jeong HY, Jeong WS, Park SJ, Kim HK, Han SY, Oh JS, Park BK, Kim JK, Poo H, Webster RG, Jung K, Kang BK. 2011. Interspecies transmission of the canine influenza H3N2 virus to domestic cats in South Korea, 2010. *J. Gen. Virol.* 92:2350–2355.
 7. Songserm T, Amonsin A, Jam-on R, Sae-Heng N, Pariyothorn N, Payungporn S, Theamboonlers A, Chutinimitkul S, Thanawongnuwech R, Poovorawan Y. 2006. Fatal avian influenza A H5N1 in a dog. *Emerg. Infect. Dis.* 12:1744–1747.
 8. Hagen M, Chung TD, Butcher JA, Krystal M. 1994. Recombinant influenza virus polymerase: requirement of both 5' and 3' viral ends for endonuclease activity. *J. Virol.* 68:1509–1515.
 9. Lee MT, Klumpp K, Digard P, Tiley L. 2003. Activation of influenza virus RNA polymerase by the 5' and 3' terminal duplex of genomic RNA. *Nucleic Acids Res.* 31:1624–1632.
 10. Tiley LS, Hagen M, Matthews JT, Krystal M. 1994. Sequence-specific binding of the influenza virus RNA polymerase to sequences located at the 5' ends of the viral RNAs. *J. Virol.* 68:5108–5116.
 11. Zheng H, Palese P, García-Sastre A. 1996. Nonconserved nucleotides at the 3' and 5' ends of an influenza A virus RNA play an important role in viral RNA replication. *Virology* 217:242–251.
 12. de Wit E, Bestebroer TM, Spronken MI, Rimmelzwaan GF, Osterhaus AD, Fouchier RA. 2007. Rapid sequencing of the non-coding regions of influenza A virus. *J. Virol. Methods* 139:85–89.
 13. Park SJ, Moon HJ, Kang BK, Hong M, Na W, Kim JK, Poo H, Park BK, Song DS. 2012. Complete genome sequence of an avian-origin H3N2 canine influenza virus isolated from dogs in South Korea. *J. Virol.* 86:9548–9549.
 14. Park SJ, Park BK, Song DS, Poo H. 2012. Complete genome sequence of a mammalian species-infectious and -pathogenic H6N5 avian influenza virus without evidence of adaptation. *J. Virol.* 86:12459–12460.
 15. Szymkowiak C, Kwan WS, Su Q, Toner TJ, Shaw AR, Youil R. 2003. Rapid method for the characterization of 3' and 5' UTRs of influenza viruses. *J. Virol. Methods* 107:15–20.
 16. Park SJ, Song DS, Ha GW, Park BK. 2007. Cloning and further sequence analysis of the spike gene of attenuated porcine epidemic diarrhea virus DR13. *Virus Genes* 35:55–64.
 17. Obenauer JC, Denson J, Mehta PK, Su X, Mukatira S, Finkelstein DB, Xu X, Wang J, Ma J, Fan Y, Rakestraw KM, Webster RG, Hoffmann E, Krauss S, Zheng J, Zhang Z, Naeve CW. 2006. Large-scale sequence analysis of avian influenza isolates. *Science* 311:1576–1580.
 18. Wise HM, Foeglein A, Sun J, Dalton RM, Patel S, Howard W, Anderson EC, Barclay WS, Digard P. 2009. A complicated message: identification of a novel PB1-related protein translated from influenza A virus segment 2 mRNA. *J. Virol.* 83:8021–8031.
 19. Desselberger U, Racaniello VR, Zazra JJ, Palese P. 1980. The 3' and 5'-terminal sequences of influenza A, B and C virus RNA segments are highly conserved and show partial inverted complementarity. *Gene* 8:315–328.
 20. Robertson JS. 1979. 5' and 3' terminal nucleotide sequences of the RNA genome segments of influenza virus. *Nucleic Acids Res.* 6:3745–3757.