Detection of novel ferret coronaviruses and evidence of recombination among ferret coronaviruses

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Abstract In an epidemiological study of ferret coronaviruses (FRCoVs), novel FRCoV strains (Saitama-1 and Aichi-1) were detected by reverse transcription-polymerase chain reaction (RT-PCR) and nucleotide sequence analysis of partial RNA-dependent RNA polymerase (RdRp) genes. Phylogenetic analysis indicated that these strains belonged to different clusters from other FRCoV strains. Next, the nucleotide sequence of the 3'-terminal region of Saitama-1 (8271 bases) strain was determined and compared with those of the other FRCoVs, indicating that the Saitama-1 strain differed from the previously reported MSU-1 and MSU-2 strains in the regions encoding spike (S) protein, nucleocapsid, and open reading frame 7b. Furthermore, the results of SimPlot analysis indicated that FRCoV (MSU-2 strain) emerged via a recombination event of S protein between the MSU-1 and Saitama-1 strains. This mechanism is similar to that responsible for the emergence of type II feline coronavirus. This information will be useful for understanding the pathogenesis of FRCoV in ferrets.

Keywords Ferret coronavirus (FRCoV) · Novel genotype · Recombination

Epizootic catarrhal enteritis (ECE) in ferrets (Mustela putorius furo) was first reported in the United States in the early 1990s as a new enteric disease [16]. A novel alpha-coronavirus, ferret coronavirus (FRCoV), was detected as the causative agent of ECE in 2000, and was designated as ferret enteric coronavirus (FRECV) [16, 17]. General clinical signs of ECE include vomiting, lethargy, anorexia, and foul-smelling, green mucous-laden diarrhea [17]. FRCoV was also reported as the causative agent of a feline infectious peritonitis (FIP)-like disease in 2006. This FRCoV was designated as ferret systemic coronavirus.
but not FRCoV-specific primers, even though the latter can detect FRCoV with more sensitivity than CoV-consensus primers [14].

The nucleotide sequences of partial RdRp genes were determined using a BigDye Terminator Ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. All sequences were deposited to the DNA Data Bank of Japan (DDBJ). A phylogenetic tree was constructed using the program MrBayes Ver. 3.2.2 [11] for MrModeltest analysis with a general time reversible (GTR) or WAG substitution matrices [15]. All trees were graphically represented using FigTree Ver. 1.4.2 [10]. The phylogenetic tree based on the partial RdRp genes showed that the FRCoV Saitama-1 and Aichi-1 strains belonged to a different cluster from other FRCoV strains (Fig. 1a).

In order to determine the 3′-terminal region of the Saitama-1 genome, further sequence analysis was performed. QIAGEN OneStep RT-PCR Kit (QIAGEN, Hilden, Germany) and TaKaRa RNA LA PCR Kit (AMV) Ver. 1.1 (TaKaRa, Shiga, Japan) were used to amplify each fragment of the Saitama-1 and Aichi-1 strains using the primer pairs listed in Supplementary Table S2. The 3′-terminal region, nt 6935-8271, of the Saitama-1 strain was amplified by 3′-RACE using TaKaRa RNA LA PCR Kit (AMV) Ver. 1.1 (TaKaRa) according to the manufacturer’s instructions. All sequences were deposited in the DDBJ. The nucleotide sequences from the S gene to poly A of the Saitama-1 strain (8271 bases) and N gene of Aichi-1 strain (1128 bases, 375 amino acids (a.a.)) were determined. The Saitama-1 strain had open reading frames (ORFs) encoding S (4308 bases, 1435 a.a.), ORF3 (744 bases, 247 a.a.), N (1128 bases, 375 a.a.), 3x-like (225 bases, 74 a.a.), membrane (M) (798 bases, 265 a.a.), envelope (E) (249 bases, 82 a.a.), and ORF7b (609 bases, 202 a.a.). The consensus transcription-regulating sequences (TRSs) of coronaviruses, 5′-CTAAAC-3′ [12], were observed upstream of each ORF, except ORF7b. Although we could not detect typical TRS of ORF7b, a possible TRS 5′-CTAAC-3′ was observed upstream of ORF7b.

The nucleotide sequence from the S gene to poly A was compared among FRECV MSU-2, FRSCV MSU-1, and Saitama-1 strain using SimPlot Ver. 3.5.1 [5]. SimPlot analysis showed that the Saitama-1 strain was different in some regions, including the N, 3x-like, and ORF7b genes, when compared with other strains (data not shown). Surprisingly, the 3′-terminal in two-thirds of S genes in the MSU-2 strain was similar to that in the Saitama-1 strain, but one-third of S genes in the MSU-2 strain was similar to that in the MSU-1 strain (Fig. 2a). Alignment of S proteins among Saitama-1, MSU-1, and MSU-2 strains also indicated that the MSU-2 strain was similar to the MSU-1 strain in the N-terminal one-third of S protein and to the Saitama-1 strain in the C-terminal two-thirds of the protein.
In addition, a low similarity in nucleotide sequences between the MSU-1 and MSU-2 strains and the Saitama-1 strain was observed in the N, 3x-like, and ORF7b genes, but the E and M genes were highly conserved among all strains (data not shown).

Phylogenetic analysis was performed based on the N-terminal one-third of the S protein (a.a. 1-438 of Saitama-1 S protein) (b), C-terminal two-thirds of S protein (a.a. 439-1435 of Saitama-1 S protein) (c), and N protein (d). Posterior probabilities are indicated above branches in all trees. Sequences reported in this study are shown in bold.

Therefore, we consider the Saitama-1 and Aichi-1 strains to comprise a novel FRCoV genotype. In addition, these results indicate that the MSU-2 strain emerged by recombination of the S protein between the MSU-1 and Saitama-1 strains. These recombination events often occurred among CoVs, resulting in the diversity of CoV genomes. Especially, the recombination of S protein caused the cross-species transmission or change of pathogenesis of SARS-CoV [4], porcine transmissible gastroenteritis virus (TGEV), canine coronavirus (CCoV) type II [1], and feline coronavirus (FCoV) [13]. Novel CoVs must emerge and drastically evolve by these recombination events. Further investigation will thus be required to determine the evolution of CoV including FRCoV.

In conclusion, novel FRCoV strains (Saitama-1 and Aichi-1) were detected in Japan. These FRCoVs appear to have emerged by recombination events among other FRCoVs. This information will be useful for understanding the pathogenesis of FRCoV in ferrets.
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