Molecular Characterization of a Natural Mutant of Feline Calicivirus in China

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Short Report

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Abstract

During epidemiological surveillance of Feline calicivirus (FCV) isolates in Shanghai, China, a natural mutant of FCV, designated SH1909, was successfully isolated from a stray cat. The complete genome sequence of SH1909 was determined in this study. Sequence comparison and analysis showed that thirteen unique aa residues substitutions and single-aa insertion of N or Y were observed in SH1909, which indicated that SH1909 was a novel and natural mutant of FCV. Interestingly, phylogenetic analysis based on LC-VP1 showed SH1909 could be clustered into an independent evolutionary branch with some Chinese isolates and was more distantly related to vaccine strains, indicating its potential to escape from vaccine-elicited immunity. According to the predicted B-cell epitopes of LC-VP1, amino acid mutation sites and positive selective sites, peptide C (aa sites: 445–460) and, peptide D (aa sites: 425–440) located in the hypervariable regions of LC-VP1, may result in the decreased immunological protection. Moreover, amino acid sites 439 and 449 may be responsible for the potential immune escape of SH1909. This study provides an important insight into genetic variations of FCV and vaccine development.

Introduction

The *Calicivirus* family currently consists of eleven genera: *Bavovirus*, *Lagovirus*, *Minovirus*, *Nacovirus*, *Nebovirus*, *Norovirus*, *Recovirus*, *Salovirus*, *Sapovirus*, *Valovirus*, and *Vesivirus* ([https:// www.caliciviridae.com/index.html](https://www.caliciviridae.com/index.html)). Feline calicivirus (FCV) is a member of the genus *Vesivirus* in the family *Caliciviridae* (Green et al. 2000). Its genome is a single-stranded, positive-sense RNA molecule with a length of about 7.6 kb and a relative molecular mass of $(2.6\text{--}2.8)\times 10^6$, and contains three open reading frames (ORFs): ORF1 coding for non-structural proteins (NSPs), ORF2 coding for a capsid precursor that is posttranslationally processed to release the major capsid protein (VP1) and the leader of the capsid (LC) with 124 amino acids (aa) (Bhella and Goodfellow 2011; Glenn et al. 1999), and ORF3 coding for a minor capsid protein (VP2), respectively. The LC protein plays an important role in viral spread and can cause an activation of caspases and a cytopathic effect (CPE) in cell cultures (Abente et al. 2013). VP1 capsid protein has a functional role during the early replication of the calicivirus. The capsid precursor protein (LC-VP1) can further be divided into six regions, termed A to F. Regions B, D and F are more conserved, whereas regions A, C and E are variable. Region E contains two hypervariable regions that are separated by a conserved domain (Seal et al. 1993). The E region contains the major B cell epitopes and plays an important role in the formation of viral particle antigen structures. And conformational epitopes are probably more important than linear epitopes in viral neutralization (Radford et al. 2007; Sun et al. 2017). VP2 is essential for productive replication that results in the synthesis and maturation of infectious virions (Brunet et al. 2019; Sosnovtsev et al. 2005).

Since FCV was firstly isolated and identified in 1957, it has been distributed worldwide in all feline species, such as cats, tigers, lions, and cheetahs, with cats under one year old being the most susceptible (Kadoi et al. 1997; Radford et al. 1998). FCV infection is closely related to upper respiratory tract disease, acute mouth ulceration, and chronic stomatitis. Moreover, most infected or clinically recovered cats can persistently excrete the virus. During the widespread use of commercial vaccines, the cross-protection between the FCV wild strains and the live vaccine strain F9 has been gradually weakened (Smith et al. 2020), and immunization failure occurs frequently. Although existing vaccines can reduce clinical symptoms and virus excretion, they cannot prevent infection from occurring (Berger et al. 2015). Therefore, the persistent surveillance should be performed for the prevalence of FCV isolates and can provide an important insight into the development of novel vaccines. In recent years, there have been some reports of Chinese FCV strains, but few reports of FCV strains from the Chinese stray cats were reported (Guo et al. 2018; Sun et al. 2017). In view of the larger sphere of activity for stray cats, it is easier to spread viruses. So, the primary aim of this study was to isolate FCV strains from some stray cats in Shanghai, China and analyze their genomic and evolutionary characterization.

Materials And Methods

Sample description and DNA extraction

27 fecal samples were collected from the stray cats of Shanghai in September 2019. All samples were respectively homogenized in 1 mL of phosphate-buffered saline and centrifuged at 12,000 × g for 10 min. The clarified supernatant was filtered through 0.22 μm filters (Millipore). Viral RNA was then extracted from the clarified suspension using a TIANamp Virus DNA/RNA Kit (Tiangen, Beijing, China) and reverse transcribed into cDNA using the FastQuant RT Kit (with gDNase) (Tiangen) according to the manufacturer's instructions

Primer design and Polymerase Chain Reaction(PCR) amplification of FCV

Identification of viruses was performed by PCR using the highly specific primers (Table 1). The results showed three out of 27 samples were positive for FCV. And Crandell feline kidney (CRFK) cells were respectively inoculated with the positive supernatant at 37°C under 5% CO² in 25 cm² flasks. When more than 80% of cells show obvious cytopathic effect, cell suspension was collected and stored at -80°C until use. To amplify the complete genome of FCV, 7 pairs of specific primers (Table 1) were designed based on the conserved regions of the FCV genome sequences retrieved from NCBI. Reverse transcription PCR was performed in a 50 μL reaction mixture comprising 25 μL of Premix Taq (Takara Bio Inc., Shiga, Japan), 2 μL of each primer (10 μM), 2 μL of cDNA template, and 19 μL of ddH₂O. The reaction conditions were as follows: initial denaturation at 95°C for 5 min; 33 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 2 min; and a final elongation step of 72°C for 10min. In addition, the 3' end of the genome was determined by using the method of rapid amplification of cDNA ends (RACE). The PCR products were visualized through electrophoresis on a 1% agarose gel and then purified using the FastPure Gel DNA Extraction Mini Kit (Vazyme) per the manufacturer's instructions. The purified amplicons were cloned into the pMD19-T vector (Takara) for subsequent sequencing (TSINGKE).

Table 1 Primers used for identification and PCR amplification of the complete genome of FCV.

Results And Discussion

After three serial passages, a mutated strain of FCV, referred to as SH1909, was isolated from the fecal specimen of a stray cat. The full-length genome sequence of SH1909 consisted of 7770 nucleotides (nt) with a 5'-terminal untranslated region (UTR) of 19 nt and a 3'-terminal UTR of 123 nt. Three potential ORFs, including ORF1 (nt 20 to 5320), ORF2 (nt 5321 to 7342), and ORF3 (nt 7339 to 7646), were predicted within the genome of SH1909. The complete genome sequence of FCV strain SH1909 has been submitted to the GenBank database under accession number MW356260. Multiple sequence alignments were conducted between SH1909 and other referenced FCV isolates retrieved from NCBI database using Phylosuite v1.2.1. The results showed that NSPs of SH1909 shared 88.6%-95.8% and 88.0%-89.1% aa identities with those of other FCV field isolates from China and vaccine strains, respectively, and the highest aa identity (95.8%) with those of FB-NJ-13. As for the LC-VP1 capsid precursor of FCV, SH1909 shared 83.1%-89.2% and 82.2%-85.4% aa identities with the Chinese FCV isolates and vaccine strains, respectively, and the highest aa identity (89.2%) with HB-S4. The above results indicated that SH1909 has evolved significantly when compared with other FCV isolates and less similarity to the FCV vaccine strains than the field isolates.

Phylogenetic trees based on the complete aa sequences of LC-VP1. As shown in Fig. 1, all Chinese FCV isolates can be divided into different evolutionary branches based on the phylogenetic analysis of LC-VP1. However, some FCV isolates including SH1909 were clustered in a monophyletic clade, which was distantly related to the FCV vaccine strains, indicating the genotype diversity of FCV isolates and the potential of some Chinese endemic isolates including SH1909 to escape from immune response induced by vaccine.

Compared with other Chinese FCV isolates and vaccine strains, SH1909 possesses own unique aa mutation sites, involving seven aa mutations at sites 31 (E/D31K), 214 (K214R), 668 (C/H/N 668F), 751 (V/I751T), 1139 (T/S 1139N), 1416 (E1416D) and 1728 (Y1728F) in the NSPs and six aa residue substitutions at positions 10 (K10T), 55 (N/D55K), 402 (K402R), 405 (I/T405Y), 439 (D/N/S/T439K), 497(N/K/T/S/T/E/Q497R) in LC-VP1. It is worth mentioning that aa substitutions at residues 439 and 497, which located in the N-terminal half and C-terminal half parts of region E respectively, may affect the formation of the antigenic structure of the virus particles. In addition, SH1909, similar to CH-JL4 and TIG-1, possesses an insertion of an Asparagine at site 495 of LC-VP1, whereas the deletion of a Tyrosine locates at site 1034 of NSPs regions in CH-JL4 and TIG-1. Positive selection analysis of individual amino acid site. We found the amino acid residues at sites 449 and 493 were supported by more than one method, with P -values < 0.05 . In B cell epitopes predicted, four peptides, ranked top three in score, were selected as possible B cell epitopes for analysis (peptide A: sites 247–262, peptide B: sites 218–233, peptide C: sites 445–460, peptide D: sites 425–440). Four B cell epitopes were all displayed on the structural surface of VP1 protein (Fig. 2). According to the alignment of amino acid sequences, it showed that peptides A and B were located in the conserved N-terminal of VP1, whereas peptides C and D were displayed in its hypervariable C-terminal (Fig. 3). Specific amino acid mutation site 439 and the positive selection site 449 for SH1909 was included in the peptides D and C, respectively. These results indicated that amino acid mutations with high frequency in the predicted B cell epitopes (peptide C and D) may weaken the capacity of the cross-protection of vaccine. Moreover, the mutations at amino acid sites 439 and 449 may be closely related with SH1909 escaping from the immune response to FCV vaccine.

Conclusion

In conclusion, the complete genome sequence of a FCV strain SH1909 from a stray cat in China, was determined in this study. Phylogenetic analysis revealed that SH1909 was clustered into an independent evolutionary branch with some Chinese isolates and possessed the potential to escape from vaccine-elicited immunity. Sequence comparison and analysis showed that thirteen unique aa residues substitutions and single-aa insertion of N or Y were respectively observed in ORF1 and ORF2 of SH1909, which indicated that SH1909 was a novel and natural mutant of FCV. In addition, two (peptides C and D) out of four predicted B cell epitopes, located in the hypervariable regions of VP1, may result in the decreased immunological protection. Moreover, two specific mutation sites 439 and 449 may be closely related with SH1909 escaping

from the immune response to FCV vaccine. These results will provide an important insight into genetic variations involved in viral evolution and a reference for design of future FCV vaccines.

Declarations

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Data availability All data generated or analyzed during this study are included in this published article.

Ethics declarations No experimental work with animals or humans was done in this study.

Consent to Participate All authors participated voluntarily in the research.

Consent to publish All authors read and approved the manuscript.

Conflict of interests The authors declare no conflict of interest.

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Figures

Figure 1

Phylogenetic analysis of 60 FCVs based on LC-VP1 amino acid sequences using the neighbor-joining (NJ) method with the Jones-Taylor-Thornton (JTT) model with 1,000 bootstrap replicates. The reference sequences were retrieved from GenBank. SH1909 isolated in this study is labeled with a filled circle (●). The FCV field isolates from China were indicated by a filled diamond (◆), the FCV vaccine strains are indicated by a filled triangle (▲). GenBank accession numbers, names of viruses and nations were indicated in the branches. Scale bars indicate amino acid substitutions per site. Clade 1: FCV field strains and vaccine strains from different countries were assigned into a major branch of Clade 1. Clade 2: FCV field isolates only from China were grouped into a minor branch of Clade 2.

Figure 2

The 3D model of LC-VP1 of the FCV strain SH1909. The 218-233 peptides (blue), 247-262 peptides (red), 425-440 peptides (purple), 445-460 peptides (green). The blue sticks: positive sites of ALA449 and ASN493, SH1909 specific mutation site 439. Four possible B cell epitopes of LC-VP1 of the FCV strain SH1909 were predicted by using ABCpred. Peptide A: sites 247- 262 (red border), peptide B: sites 218-233 (blue border), peptide C: sites 445-460 (green border), peptide D: sites 425-440 (purple border)