Genetic Analysis of VP2 Gene of Canine Parvovirus Isolates in Korea

Seok-Young JEOUNG1), So-Jeo AHN1) and Doo KIM1)*

1)School of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, Chunchon, Kangwon, 200–701, Republic of Korea

(Received 21 August 2007/Accepted 14 March 2008)

ABSTRACT. The purpose of this study was to genetically characterize CPV isolates from Korea. The VP2 gene of 31 isolates was characterized by DNA sequencing and their phylogeny. Among the 31 field CPV isolates, 28 isolates were classified as type 2a and other 3 isolates as type 2b. The isolates in 2a-I, II and III subclusters have unique mutations. The isolates in 2a-IV and V subclusters had similar amino acid sequences to type 2a isolates from other parts of the world. The isolates in type 2b had similar amino acid sequences to type 2b isolates from Asia, Italy, and U.S.A. The molecular analysis of VP2 gene of CPV provided the useful information for the identification of CPV types and the understanding of their genetic relationship.

KEY WORDS: canine parvovirus, Korea, phylogeny.

Canine parvovirus type 2 (CPV-2) was firstly recognized in the 1978, and since then it has been well established as an enteric pathogen of dogs throughout the world [1, 14]. CPV-2 was shown to be closely related, genetically and antigenically, to the feline panleukopenia virus (FPLV) and FPLV-like parvoviruses from wild carnivores [18, 19] from which it presumably originated by host species shift and subsequent rapid adaptation [22]. A few amino acid substitutions between CPV and FPLV determine the ability for each virus to replicate in dogs and cats and their cultured cells [16]. In the 1980s, two antigenic variants of CPV-2, distinguishable using monoclonal antibodies (MoAbs), emerged almost simultaneously and were termed CPV-2a and CPV-2b [10, 19]. Currently, CPV-2a is major field strain in India, Italy and Germany, while CPV-2b is common in USA, Taiwan, and Japan [2, 5, 7, 9, 15, 25].

Mutations affecting important residues of the capsid protein of CPV, such as residues 300 and 426, have been recognized recently, suggesting that CPV is still in the process of evolution [3, 13, 15, 17, 26]. Ikeda et al. reported that the CPV-2c(a) and CPV-2c(b)-type viruses emerged from CPV-2a- and CPV-2b-type viruses in domestic and leopard cats in Vietnam [13]. And a CPV-2b mutant, now named CPV type 2c (CPV-2c), with a change (Asp→Glu) occurring in a strategic residue for the antigenicity of CPV-2b has been detected in Vietnam [17], Italy [3], Spain [8], Germany [6], United Kingdom [6], and South America [22].

In Korea, CPV infections in dogs reported from the early 1980’s and loss of dogs from the CPV enteritis was enormous during the first few endemic years [11]. However, the antigenic and genetic relationships between the CPV isolates had not been reported. The purpose of this study was to genetically characterize CPV isolates from Korea and to compare them with reference CPV strains.

NOTE

Virology


Fecal specimens (n=31) were collected from diarrheic dogs with CPV enteritis which were diagnosed by polymerase chain reaction (PCR) from 2003 to 2006 in various areas of Korea (Table 1). CPVs were cultured with A-72 cells grown in Dulbecco’s minimal essential medium containing 5% fetal bovine serum [8]. DNA was extracted from the fecal specimens using QIAamp Mini DNA Purification System (QIAGEN Inc., Germany) according to manufacturer’s instructions. The CPV VP2 gene specific primer pairs used for sequencing except F53 (5’ GGT GCA CAA GTA AAA AG -3’) primer instead of F51 were as reported by Ikeda et al. [13]. F53 primer was designed in this study with the reference of CPV-b strain (GenBank accession no. M38245). The PCR amplification was performed as previously described with some modifications [12]. The sequences were directly determined from PCR products by the dideoxynucleotide chain termination method with the primer pair A [F53 and R1], primer pair B [F1 and R2], and primer pair C [F2 and R3] using Big dye terminator cycle sequencing kit (Applied Biosystems Inc., U.S.A.) according to manufacturer’s instructions. Alignment and phylogenetic tree of the nucleotide and deduced amino acid sequences were analyzed with Cluster V multiple sequence alignment algorithm of the software MegAlign (DNASTAR 5.0, Laser gene, U.S.A.). Phylogenetic tree was generated by Cluster V method with weighted residue weight table of DNASTAR software. In this study, the 31 Korean isolates and 32 reference strains were analyzed for comparison [Accession number of each strain; MEV-d (M24001), FPV-b CU4 (M38246), 56-00 (AY380577), 97-008 (ABi115504), Africa 2 (AJ007497), Africa 9 (A1007500), BR56–95 (DQ340431), BR183–85 (DQ340409), ChowChow (AJ002927), CPV-15 (M24003), CPV-31 (M24000), CPV-39 (M74849), CPV-133 (AY787927), CPV-339 (AY742933), CPV-407 (AY742949), CPV-431 (AY742951), CPV-435 (AY742953), CPV-632 (AF306445), CPV-699 (AF393506), CPV-b (M38245), CPV-d (M23255), CPV-N...
The amino acids that are identical to those in CPV-2 are indicated by dashes, while different ones are indicated by letters.

a) Deduced amino acid sequences of the VP2 gene were obtained from the GenBank. FPV: FPV-b (accession number: M38246), CPV-2: CPV-b (M38245), original CPV-2a: CPV-15 (M24003), original CPV-2a: CPV-39 (M74849), CPV-2b: Taichung (AY869724), CPV-2c(a): LCPV-V139 (AB054222), CPV-2c(b): LCPV-V203 (AB054224), and CPV-2c: NHI-4-1 (AB120727).

b) Deduced amino acid sequences of Korean isolates were deposited in the GenBank. K001 (accession number: EU009200), K014 (EU009201), K015 (EU009202), K022 (EU009205), K026 (EU009204), K029 (EU009205), and K031 (EU009206).

(M19296), CPV-U6 (AY742935), LCPV-T1 (AB054214), LCPV-V139 (AB054222), LCPV-V203 (AB054224), NHI-4-1 (AB120727), T4 (U72695), Taichung (AY869724), Taiwan 9 (AB054213), V120 (AB054215), V203 (AB054224), and V209 (AB054219). The complete nucleotide sequences of VP2 gene of the typical 7 isolates from this study were submitted to the GenBank and were assigned accession numbers from EU009200 to EU009206. The full amino acid sequences of VP2 gene of 31 Korean isolates and 9 reference strains were determined and their
amino acid changes were summarized (Table 1). Alignment with deduced amino acid sequences exhibited 98.8 to 100% similarity among 31 Korean isolates. Korean isolates had deduced amino acid sequence similarities ranging from 98.3 to 100% with the reference strains of CPV. There were unique mutations of VP2 gene at amino acid Tyr324Ile, Asp413Asn, Ile418Thr, and Pro435Ser in Korean isolates. Korean isolates belong to CPV-2a were subclassified according to the following categories: (i) all the type 2a isolates had Ser297Ala substitution; (ii) subcluster 2a-I isolates (GenBank accession no. EU009200) had Asp413Asn, Ile418Thr and Thr440Ala substitution, and one isolate (K009) had Gln7His substitution; (iii) subcluster 2a-II isolates (EU009201) had Ile418Thr and Thr440Ala substitution; (iv) subcluster 2a-III isolates (except K020) (EU009202) had Ile418Thr, Pro435Ser, and Thr440Ala substitution, and K419 had a additional substitution of Ile463Val substitution; (v) subcluster 2a-IV isolates (K021) had a only Ser297Ala substitution, and the other three isolates (K026, K027, and K028) had Ile324Tyr substitution. Korean isolates belong to CPV-2b were subclassified according to the following categories: (i) subcluster 2b-
I isolates (EU009205) had the substitution at position Ser297Ala; (ii) subcluster 2b-II isolate (EU009206) had the substitution at position Gln7His and at position Ser297Ala. However, the substitutions of residue 300 and 426 were not observed in this study.

A phylogenetic tree was constructed from deduced amino acid sequences of 31 Korean isolates and 32 reference strains (Fig. 1). The 8 isolates in 2a-I subcluster, the 6 isolates in 2a-II subcluster, and the 6 isolates in 2a-III subcluster were presented as the different subclusters in the phylogenetic tree independently with other isolates. The 2 isolates in 2a-IV subcluster placed together with U.S.A. isolates [2]. All Korean type 2a and 2b isolates were located different branches in the phylogenetic tree with the prototype type 2a and 2b strains.

In conclusion, this study provided data on the molecular genetic aspect of CPV prevalent in Korea. Korean CPV isolates follow the same evolution as observed in other countries. However, there is an indication of the separate lineage. Since CPV continues to show an ongoing evolution, monitoring of further isolates to detect genetic and antigenic changes in CPV will be needed.

ACKNOWLEDGMENT. This study was supported by Institute of Veterinary Science, Kangwon National University, Korea.

REFERENCES