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Comparison of the Amino Acid Sequence and Phylogenetic Analysis of the Peplomer, Integral Membrane and Nucleocapsid Proteins of Feline, Canine and Porcine Coronaviruses

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Abstract: **Complete nucleotide sequences were determined by cDNA cloning of peplomer (S), integral membrane (M) and nucleocapsid (N) genes of feline infectious peritonitis virus (FIPV) type I strain KU-2, UCD1 and Black, and feline enteric coronavirus (FECV) type II strain 79-1683. Only M and N genes were analyzed in strain KU-2 and strain 79-1683, which still had unknown nucleotide sequences. Deduced amino acid sequences of S, M and N proteins were compared in a total of 7 strains of coronaviruses, which included FIPV type II strain 79-1146, canine coronavirus (CCV) strain Insavc-1 and transmissible gastroenteritis virus of swine (TGEV) strain Purdue. Comparison of deduced amino acid sequences of M and N proteins revealed that both M and N proteins had an identity of at least 90% between FIPV type I and type II. The phylogenetic tree of the M and N protein-deduced amino acid sequences showed that FIPV type I and type II form a group with FECV type II, and that these viruses were evolutionarily distant from CCV and TGEV. On the other hand, when the S protein-deduced amino acid sequences was compared, identity of only about 45% was found between FIPV type I and type II. The phylogenetic tree of the S protein-deduced amino acid sequences indicated that three strains of FIPV type I form a group, and that it is a very long distance from the FIPV type II, FECV type II, CCV and TGEV groups.**

Key words: **Feline infectious peritonitis virus, Feline enteric coronavirus, Amino acid sequence**

Feline infectious peritonitis virus (FIPV), family *Coronaviridae*, genus *Coronavirus*, causes a chronic, progressive, immunologically mediated disease in domestic and exotic cats.

The family *Coronaviridae* is divided into 3 distinct antigenic groups on the basis of serologic tests (18). One group contains mouse hepatitis virus, neonatal calf diarrhea coronavirus, human coronavirus OC43, hemagglutinating encephalomyelitis virus of swine and rat coronavirus. The second group contains avian infectious bronchitis virus. The third group consists of human respiratory coronavirus 229E, transmissible gastroenteritis virus (TGEV) of swine, porcine respiratory coronavirus, canine coronavirus (CCV), FIPV and feline enteric coronavirus (FECV). By using monoclonal antibodies we have shown the existence of at least 2 serotypes of FIPV, and the antigenicity of type II strain of FIPV and FECV were closer to TGEV and CCV than to type I FIPV (7, 8). Both types I and II FIPV cause infectious peritonitis in cats, and the pathogenicity of type II FIPV is greater than that of type I FIPV (25). However, in the field, the prevalence of FIPV type I is high, and about 70% of feline cases of FIP are due to infection with type I (9).

FIPV is an enveloped RNA virus with a single-stranded positive-sense RNA genome and the virions consist of three main structural proteins, peplomer (S) glycoprotein, integral membrane (M) glycoprotein and nucleocapsid (N) protein. The virus genome is at least 20 kilobases

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Abbreviations: CCV, canine coronavirus; fcwf 4, *Felis catus* whole fetus cells; FECV, feline enteric coronavirus; FIPV, feline infectious peritonitis virus; kb, kilobases; kDa, kilodaltons; M protein, integral membrane glycoprotein; M gene, integral membrane gene; N protein, nucleocapsid protein; N gene, nucleocapsid gene; ORF, open reading frame; RT, reverse transcription; S protein, peplomer glycoprotein; S gene, peplomer gene; TGEV, transmissible gastroenteritis virus of swine.

(kb) long and the three major structural protein genes are located in the 3′ half of the genome (4). The complete nucleotide sequences of these genes have been established with FIPV type II strain 79-1146 (3, 5, 31) and several other serologically related coronaviruses, such as TGEV and CCV (10, 12–14, 26). With regard to FECV strain 79-1683, the sequences of the peplomer (S) gene and 3′ end 1-kb gene have been reported (32, 33). Wesseling et al (33) have determined, from the phylogenetic tree constructed on the basis of the S protein-deduced amino acid sequences, that 2 strains of CCV (strain Insavc-1 and strain K378) are evolutionarily more closely related to FIPV strain 79-1146 and FECV strain 79-1683 than TGEV. We have recently determined the nucleotide sequence of the S gene of strain KU-2 classified as type I, and compared it with that of FIPV type II strain 79-1146, CCV strain Insavc-1 and TGEV strain Purdue (17). Deduced amino acid sequences of S protein showed identities of 90.9% and 80.9% between FIPV type II and CCV and between FIPV type II and TGEV, respectively. However, deduced amino acid sequences of S protein showed identity of only 45.9% between FIPV type II and FIPV type I. These findings supported the results of our study on antigenic relationships among feline, porcine and canine coronaviruses with monoclonal antibodies (7, 8). However, it is not clear whether a similar pattern would be obtained by phylogenic analysis by comparison of integral membrane (M) and nucleocapsid (N) genes or whether other strains of FIPV type I would be differentiated from the groups in a way similar to that for KU-2.

In this study, complete nucleotide sequences of the S, M and N genes of FIPV type I strain UCD1 and strain Black, and of the M and N genes of FIPV type I strain KU-2 and FECV strain 79-1683 were determined, in order to clarify the phylogenetic relationships among these viruses. Genetic relationships among feline coronaviruses, FIPV type I, FIPV type II and FECV type II, were also estimated from the phylogenetic tree of S, M and N proteins.

Materials and Methods

Viruses and their genomic RNA. FIPV strains KU-2, Black and UCD1, and FECV strain 79-1683 were used for cDNA cloning and sequencing. FIPV strain KU-2 was isolated by Hohdatsu et al (8). FIPV strain UCD1 was supplied by Dr. Niels C. Pedersen of the University of California, Davis (19, 21). FIPV strain Black was supplied by Dr. Janet K. Yamamoto of the University of Florida (1, 22). FECV strain 79-1683 was supplied by Dr. Alison J. McKeirnan of Washington State University, Pullman (16, 24). Genomic RNA was extracted from virus-infected *Felis catus* whole fetus cells (fcwf 4) by the method used by Motokawa et al (17).

DNA primers. DNA primers were custom-synthesized by Bex Corp. (Tokyo) by using di-amidite chemistry and an automatic DNA synthesizer. Table 1 shows the nucleotide sequences of the primers used in cloning. Recognition sites of restriction enzymes for cloning were included in the primers. The binding locations of primers are shown in Fig. 1. A minus-sense primer

Table 1. Nucleotide sequences of DNA primers used for cloning

Primer	Nucleotide sequence	Region amplified [d]	Sense	
ISPr-8F [a]	5′-CGCTGGATCCAATGGTAAGTTACTAAACT-3′	region S1	+	(position 44)[e]
ISPr-9R [a]	5′-GGGGAATTCTAGCCTAGTAATAGCTGTGCC-3′		−	(position 1537)
BUSPr-12F [b]	5′-GGGGGATCCTACACCAATTATACAGATGTAATG-3′	region S2	+	(position 1524)
BUSPr-13R [b]	5′-GGGGAATTCGGCTTGCACTGCAACATG-3′		−	(position 2674)
ISPr-10F [a]	5′-GGGGGATCCACAGGTAACATATCGATACC-3′	region S3	+	(position 2663)
ISPr-11R [a]	5′-GGGGGAATTCAAGGCATTAGCAAG-3′		−	(position 3310)
BUSPr-14F [b]	5′-GGGGGATCCGTGCAGGCTAGGCTTAATTATG-3′	region S4	+	(position 3268)
ISPr-2 [a]	5′-GGGGAATTCAGAGGTAAATAATACTTTAAGTG-3′		−	
IM5′F [a]	5′-CCCAAGCTTGAATTCTTGGTTTGAACTAAAC-3′	region MN2	+	
IIMPr-1 [c]	5′-TGGGGATCCGATTTTACGTAGTAAGCCCA-3′		−	
IMPr-3F [a]	5′-GGGGAATTCAATTAAAGGCAACTACTGCCA-3′	region MN3	+	
IN3′R [a]	5′-GGGGGATCCGCATGGAGGAAAACGAGCAT-3′		−	
IMPr-3F [a]	5′-CCCAAGCTTGAATTCTTGGTTTGAACTAAAC-3′	region MN4	+	
BEP(dT)$_{21}$	5′-CTGTGAATTCTGCAGGATCCTTTTTTTTTTTTTTTTTTTT-3′			−

[a] Primer prepared with reference to nucleotide sequence of FIPV strain KU-2. [b] Primer prepared with reference to nucleotide sequences of FIPV strains Black and UCD1. [c] Primer prepared with reference to nucleotide sequences of FIPV strain 79-1146. [d] Region amplified by RT-PCR. [e] Binding location in FIPV strain KU-2 S gene (Accession No. D32044).

was used for reverse transcription (RT) for synthesis of cDNA from genomic RNA.

cDNA cloning. The M and N genes of FIPV strain KU-2 were divided into the three regions (region MN1, MN2 and MN4) shown in Fig. 1. Region MN1 was sequenced by using the previously reported cDNA clone pFPSI-1 (17). Genomic template RNA was amplified by RT-polymerase chain reaction (PCR). The PCR products were cloned into pUC18, then were subcloned into M13mp18/19 for determination of the nucleotide sequence.

The S genes of FIPV strains Black and UCD1 were divided into four regions (region S1 through S4), and the M and N genes of FIPV strains Black and UCD1 and FECV strain 79-1683 were divided into two regions (region MN2 and MN3), as shown in Fig. 1. The RT-PCR products were directly cloned into M13mp18/19 for determination of the nucleotide sequence.

DNA sequencing and analysis. The single-stranded DNA was sequenced by means of dideoxynucleotide chain termination using the Dye Primer cycle sequencing kit (Applied Biosystems Inc., Foster City, Calif., U.S.A.). The sequence was resolved with an automated DNA sequencer (Applied Biosystems model 373S). At least eight clones for one type of cDNA were sequenced to avoid artifact mutations due to misreading by reverse transcriptase and *Taq* polymerase for PCR.

The sequences determined were then analyzed with the GENETYX computer program (Software Development Co., Ltd., Tokyo). Homology including the deleted sequence was calculated (30). Multiple sequence alignment and evolutionary distances between amino acid sequences were estimated with the PAM250 matrix (2), which compares amino acid changes according to empirically determined probabilities of change. The phylogenetic tree was prepared by the UPGMA method (29).

Results

Nucleotide Sequence of 3' End 4.5 Kilobases of FIPV Type I Strain KU-2

Two new cDNA clones, pFPMI (region MN2) and pFPNI (region MN4), of FIPV strain KU-2 were obtained in the present experiment (Fig. 1). The distance between the 3' end of the S gene and the poly A tail of FIPV type I strain KU-2 was 4,496 bases. The M gene, of 789 bases, coded 263 amino acids, and it was estimated to express proteins of 29.9 kilodaltons (kDa) (Fig. 2). The N gene, of 1,131 bases, coded 377 amino acids, and it was estimated to express proteins of 42.5 kDa (Fig. 3).

S, M and N Genes of FIPV Strains Black and UCD1, and FECV Strain 79-1683

The M genes of FIPV strain Black and FECV strain 79-1683 were 789 bases, which is the same as that of strain KU-2. The M gene of strain UCD1 was 786 bases, which is less than that of the above viruses by 3 bases. The M gene of FIPV strain Black and FECV strain 79-1683 coded 263 amino acids and the M gene of FIPV strain UCD1 coded 262 amino acids. They were estimated to express proteins of about 29.9 kDa (Fig. 2).

The N genes of FIPV strain Black and strain UCD1 were 1,131 bases, which is the same as that of strain KU-2. The N gene of FECV strain 79-1683 was 1,128 bases, which is less by 3 bases than that of these viruses. FIPV strain Black and strain UCD1 N genes coded 377 amino acids, and FECV strain 79-1683 N gene coded 376 amino acids, and they were estimated to express proteins of 42.3, 42.7 and 42.4 kDa, respectively (Fig. 3).

FIPV strain Black S gene was 4,386 bases, and strain UCD1 S gene was 4,371 bases. They were less than the

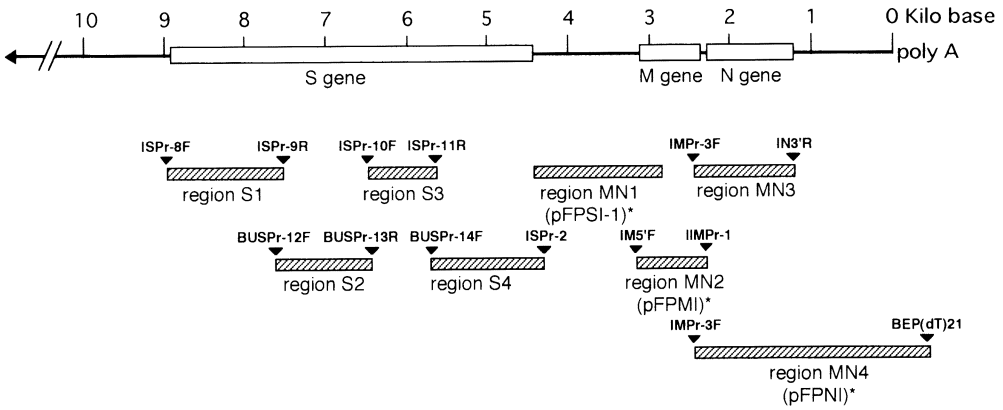

Fig. 1. Map of the binding location of cloning regions and DNA primers. Hatched boxes show the regions of RT-PCR and cDNA cloning. Arrowheads indicate the binding location of DNA primers which are shown in Table 1. *In FIPV strain KU-2, cDNA clones pFPMI and pFPNI were obtained by recombination of regions MN1 and MN4 with pUC18.

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KU-2      MKYVVFILACILACAFGE--RYCAMQDAVSTSCVNKTDNSCQTCFERGDLIWHLANWNFSWSVILIVFITVLQYGRPQFSWLVYGIKMLIMWLLWPIVLA 98
Black     ....LL.....I..V...--.....K.DS.NT.I.G.NS............................................................. 98
UCD1      ...LL..F...I..VY..--.....-SDSG.Q..YG.NST............................................................ 97
FIPV II   ...ILL.....I..VY..--.....-QDSGLQ.I.G.NSR............................................................ 97
FECV II   ..-ILL....AV..VY..QI.....-QETGL..R.G.ASD.ES..NG...............I..................F.................. 98
CCV       ..KIL.L...AI..VY..--.....-TES....R.S.AGN.AS...T...................I..............F.C................ 97
TGEV      .-KILL....VI...C..--.....KSDTDL..R.S.ASD.ES..NG...............I..................FA.............V... 97

KU-2      LTIFNAYSEYQVSRYVMFGFSVAGAVVTFALWMMYFVRSIQLYRRTKSWWSFNPETNAILCVNAVGRSYVLPLDGTPTGVTLTLLSGNLYAEGFKMAGGL 198
Black     .....................I..........................................L................................... 198
UCD1      ..........................I.....................................L..R................................ 197
FIPV II   .......................................V........................L................................... 197
FECV II   ..........E.....................................................L................................... 198
CCV       .......L..R..............T...I..I.......................S.....S.L........E.V.............C.....I...M 197
TGEV      .....................I...I...V..I.......................K.....S.L........E.V...................I...M 197

KU-2      TIEHLPKYVMIATPSRTIVYTLVGKQLKATTATGWAYYVKSKAGDYSTEARTDNLSEHEKLLHMV                                   263
Black     .................................................................                                   263
UCD1      ....................................................P............                                   262
FIPV II   .................................................................                                   262
FECV II   ...........R..N..................................................                                   263
CCV       N.DN......V.L.V..........K...SS..................D...............                                   262
TGEV      N.DN......V.L............K...SS...........................Q......                                   262
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Fig. 2. Deduced amino acid sequence alignment of M protein of FIPV type I strains KU-2, Black and UCD1 FIPV type II strain 79-1146, FECV type II strain 79-1683, CCV strain Insavc-1 and TGEV strain Purdue. ...: indicates the same amino acid as that in KU-2. -: indicates a gap.

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KU-2      MATQGQRVNWGDEPSKRRDRSNSRGRKNNNIPLSFFNPTTLEQGAKFWYVCPRDFVPKGIGNKDQQIGYWNRQARFRIVKGQRKELPERWFFYFLGTGPH 100
Black     ............................G......Y..I..S..S...NI.......................V.Y...R..............S..... 100
UCD1      ..................G.....V....D....YY..I.QP..S...N..........................Y........................ 100
FIPV II   ..................G..........D.....Y..I.....S...NL....L..................I.Y..........A............. 100
FECV II   ..................G..........D....Y...I..D..S...NL.........................Y.......V................ 100
CCV       ..S.....S....ST...G..........D........I.....S...DL.......................T.Y.M...R..N...K....Y...... 100
TGEV      ..N.....S....ST.T.G...................I..Q..S...NL............R..........T.Y.M...............Y...... 100

KU-2      ADAKFKDKIDGVFWVAKDGAMNKPTSLGTRGTNNESEPLRFDGKIPPQFQLEVNRSRNNSRGSQSRSGSRNRSQSRGR-QQSNNQNTNVEDTIVAVLQK 199
Black     ................R........T..........K..K.........................P..V..S......-..................S. 199
UCD1      ................R........T..........K...L...........................V.....F...-.......ND........... 199
FIPV II   ................R........T..........K...............................V.........-HH.....N..........E. 199
FECV II   ......A.........R...................K..K............................V.........-.................... 199
CCV       ......Q.L...V...RGDS.T...T..........KA.K..V.V.SE.H....QL.D....R.....Q.........QLSN.KKDD...QAVL.A.K. 200
TGEV      ........L...V............T..S..A....KA.K....V.GE......Q..D....R.....R.........Q.FN.KKDDS..QAVL.A.K. 200

KU-2      LGV-TDK--QRSRSKSRDRSDSKSRDTTPKNANKHTWKKTAGKGDVTNFFGARSASANFGDSDLVANGNAAKCYPQIAECVPSVSSVLFGSQWSAEEAGD 296
Black     ...-...--.......GE.NQ..P.........................Y....S............................A..I............. 296
UCD1      ...-...--.S......E....QL..............R..........Y..........................L....................... 296
FIPV II   ...-...--......P.E.....P.......................T.Y....S...............................II............ 296
FECV II   ...-...--.......GE..Q............................Y....S...............................I............. 296
CCV       ...D.E.-Q.......KE..S..T.......E......R........K.Y....S..............G..H...L.........I....H.T.K.D.. 299
TGEV      ...D.E.QQ.......KE..N..T.......E....S.R........R.Y....S......T......SS..H...L.........I....Y.TSK.D.. 300

KU-2      QVKVTLTHTYYLPKGDAKTSQFLEQIDAYKRPSQVAKEQRKPKPRSKSADKKPEE-LSVTLVEAYTDVFDDTQVEMIDEVTN                   377
Black     ..............D...............Q..E...D..QR.S...........-..........................                   377
UCD1      ..............D..................E...D..SR.S...........-..........................                   377
FIPV II   ..............D..................E...D..QRRS...........-..........................                   377
FECV II   ........N.....D..................E...D..QR.S...........-.....-....................                   376
CCV       .IE..F..K.H...D.P..G...Q..N..A...E......QR.A.....VERVEQ.VVPDA.T.N..........I......                   381
TGEV      .IE..F..K.H...D.P..G...Q..N..A...E.......R.S......ERSEQDVVPDA.I.N..........I......                   382
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Fig. 3. Deduced amino acid sequence alignment of N protein of FIPV type I strains KU-2, Black and UCD1, FIPV type II strain 79-1146, FECV type II strain 79-1683, CCV strain Insavc-1 and TGEV strain Purdue. ...: indicates the same amino acid as that in KU-2. -: indicates a gap.

4,392 bases of strain KU-2 by 6 and 21 bases, respectively. FIPV strain Black S gene coded 1,462 amino acids, and strain UCD1 S gene coded 1,457 amino acids, and these two were estimated to express proteins of about 164 kDa (Fig. 4). There were 37 *N*-glycosylation sites in strain Black as well as in strain UCD1. The number of the sites was smaller by 4 sites than the 41 sites in strain KU-2.

Homology Analysis

Deduced amino acid sequences of M protein showed identity of at least 82% among FIPV type I strains KU-2, Black and UCD1, FIPV type II strain 79-1146, FECV strain 79-1683, CCV strain Insavc-1 and TGEV strain Purdue (Table 2). Furthermore, among FIPV strains including type II, high homology was found to have been maintained, and the amino acid sequences showed identity of at least 92%. The M gene had sequences specific for individual viruses in the N-terminal 50 amino

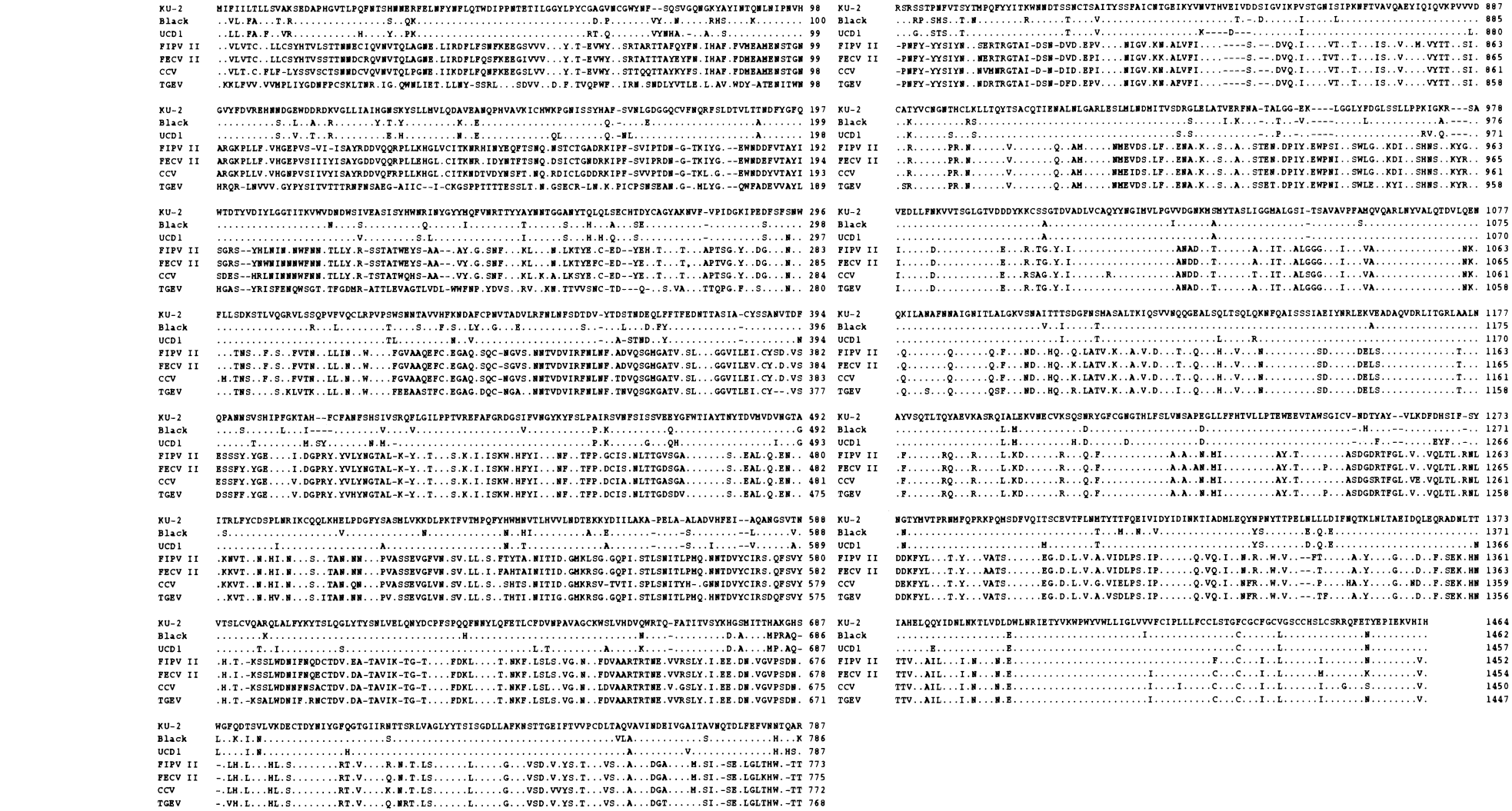

Fig. 4. Deduced amino acid sequence alignment of S protein of FIPV type I strains KU-2, Black and UCD1, FIPV type II strain 79-1146, FECV type II strain 79-1683, CCV strain Insavc-1 and TGEV strain Purdue. ...: indicates the same amino acid as that in KU-2. -: indicates a gap.

Table 2. Homology (%)* of deduced amino acid sequence of M and N proteins

Strain	FIPV type I			FIPV type II [a]	FECV 79-1683	CCV [b]	TGEV [c]
	KU-2	Black	UCD1				
KU-2	100	94.3	92.4	93.2	88.7	82.5	82.5
Black	90.2	100	93.9	96.2	89.8	82.9	84.8
UCD1	91.5	91.0	100	95.4	89.4	82.4	82.5
FIPV type II	91.0	92.3	93.1	100	92.1	83.6	84.4
FECV	92.3	93.9	93.4	93.9	100	84.9	86.7
CCV	74.0	74.8	75.3	76.4	77.2	100	88.3
TGEV	75.2	75.1	76.5	76.2	77.3	89.5	100

M protein amino acid sequence homology is shown at the upper right from the 100% diagonal, and N protein amino acid sequence homology at the lower left from the 100% diagonal. *Percentage of identical amino acid residues in amino acid sequences including deleted sequences. [a] FIPV strain 79-1146 (Accession No. X56496). [b] CCV strain Insavc-1 (Accession No. D13096). [c] TGEV strain Purdue (Accession No. M21627, M14878).

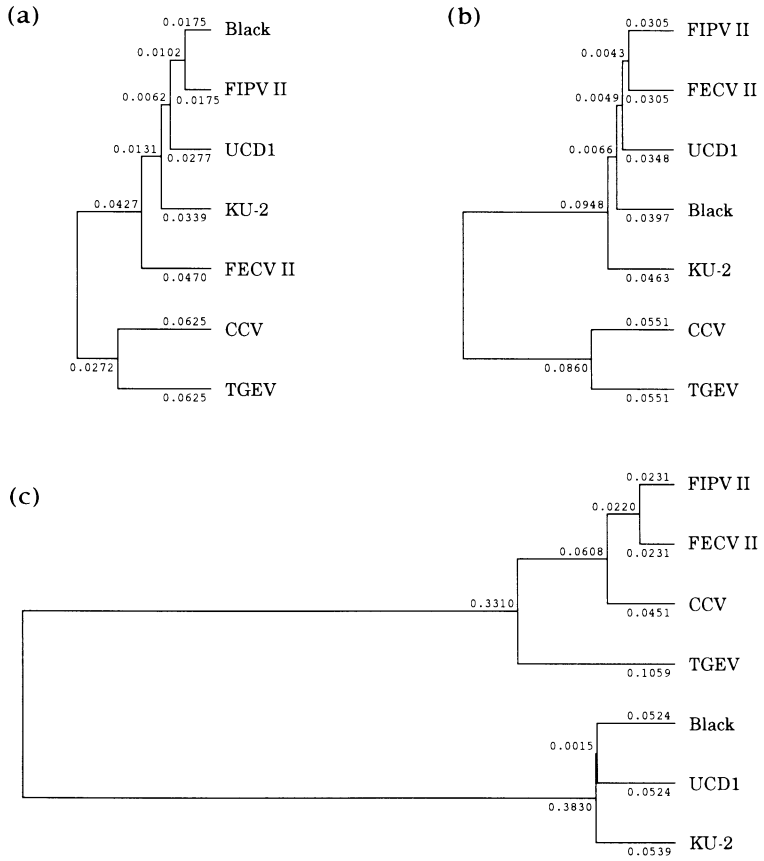

Fig. 5. Phylogenetic tree based on amino acid sequence of FIPV type I strains KU-2, Black and UCD1, FIPV type II strain 79-1146, FECV type II strain 79-1683, CCV strain Insavc-1 and TGEV strain Purdue. Evolutionary distances between amino acid sequences were estimated with the PAM250 matrix. The phylogenetic trees were prepared by the UPGMA method. (a) Phylogenetic tree of M gene. (b) Phylogenetic tree of N gene. (c) Phylogenetic tree of S gene. Numbers in the figure indicate evolutionary distance.

acids, and the sequence of residues 23–45 showed particularly low homology (Fig. 2). The phylogenetic tree prepared from the deduced amino acid sequence of M protein showed the shortest evolutionary distance among FIPV strains (Fig. 5a).

The deduced amino acid sequence of N protein showed identity ranging from 90.2 to 93.9% among three strains of FIPV type I, FIPV type II and FECV strain 79-1683 (Table 2). Deduced amino acid sequences of CCV and TGEV N proteins showed identity of about 75% with those of the feline coronaviruses. The phylogenetic tree prepared from the deduced amino acid sequence of N protein showed that individual feline coronaviruses have approximately equivalent evolu-

Table 3. Homology (%)* of deduced amino acid sequence of S protein

Strain	FIPV type I			FIPV type II [a]	FECV 79-1683 [b]	CCV [c]	TGEV [d]
	KU-2	Black	UCD1				
KU-2	100	89.6	89.2	46.0	46.2	45.7	45.2
Black		100	89.6	45.4	45.4	45.1	44.0
UCD1			100	46.4	46.4	45.7	45.3
FIPV type II				100	95.4	90.9	80.9
FECV					100	91.6	81.2
CCV						100	79.3
TGEV							100

*Percentage of identical amino acid residues in amino acid sequences including deleted sequences. [a] FIPV strain 79-1146 (Accession No. D00150). [b] FECV strain 79-1683 (Accession No. Q25539). [c] CCV strain Insavc-1 (Accession No. D13096). [d] TGEV strain Purdue (Accession No. M21950).

tionary distances, that FECV strain 79-1683 is not different from FIPV and that the feline coronavirus group is distinctly different from CCV and TGEV (Fig. 5b).

The deduced amino acid sequence of S protein showed identity ranging from 79.3 to 95.4% among FIPV type II, FECV strain 79-1683, CCV and TGEV (Table 3). However, FIPV type I showed identity of only 45% with these viruses in the S protein amino acid sequence of any of the strains KU-2, Black and UCD1. The homology among strains of FIPV type I showed 89.2% identity between strains KU-2 and UCD1, and 89.6% identity between KU-2 and Black and between UCD1 and Black. The phylogenetic tree prepared from the deduced amino acid sequence of S protein showed that three strains of FIPV type I were a very long evolutionary distance from FIPV type II, FECV strain 79-1683, CCV and TGEV groups (Fig. 5c).

Discussion

FIPV forms one antigenic cluster with FECV, CCV and TGEV, and the antigenic structures of these viruses are serologically closely related to each other (6, 11, 20, 28). Some studies have shown that S protein amino acid sequences of FIPV type II, FECV strain 79-1683, CCV and TGEV have identity of about 75% or more (10, 33). However, we have recently reported that the S protein amino acid sequence of FIPV type I strain KU-2 has only about 45% identity with FIPV type II, CCV and TGEV (17). In the present study, S proteins of FIPV type I strain UCD1 and strain Black showed results similar to those of strain KU-2. The phylogenetic tree prepared from the deduced amino acid sequence of S protein showed that three strains of FIPV type I were a very long evolutionary distance from FIPV type II, FECV strain 79-1683, CCV and TGEV groups. However, the phylogenetic tree newly prepared from the M and N proteins showed a pattern which differed from that of the S protein; the phylogenetic tree of the M and N proteins suggested that feline coronaviruses, i.e., FIPV type I, FIPV type II and FECV strain 79-1683, constitute a single group.

FIPV type I and type II can be said to belong to the same group of viruses in that both induce FIP. In addition, the length of the open reading frame (ORF) 6b of FIPV type I was the same as that of FIPV type II ORF 6b (data not shown), and there was not as much deletion as in FECV strain 79-1683 (32). However, the homology of the deduced amino acid sequence of FIPV type I and type II S proteins was very low, and it was impossible to regard the two virus types as members of the same group from the aspect of nucleotide sequence. S protein plays an important role in neutralization and adsorption to cellular receptors, suggesting that the function of type I is very different from that of type II. The results of our analysis of neutralizing epitopes using monoclonal antibodies support this suggestion (7). On the other hand, FECV type II strain 79-1683 causes only mild enteritis, and the homology of its S, M and N genes is the highest with FIPV type II. The homology of the S protein amino acid sequence between FIPV type II and FECV strain 79-1683 was higher than that among three strains of FIPV type I. That is, on the basis of pathogenicity, FIPV causing FIP is divided into type I and type II, and another virus, FECV, that does not cause FIP is thought to exist. If pathogenicity is neglected, and nucleotide sequence is considered as the basis, FIPV type II and FECV type II will be included in the same group of viruses. One study has shown that a type I virus strain is present in FECV as well as in FIPV (23). However, since it is impossible to culture FECV type I *in* vitro, its study has been delayed. The viruses including this type should be analyzed in the future.

The homology of the S protein amino acid sequence among FIPV type I strains was determined. There was 89.2% identity between strains KU-2 and UCD1, and 89.6% identity between KU-2 and Black and between UCD1 and Black. However, it has been reported that in

strains FIPV type II S protein amino acid sequences showed identity of 99.6% between strain 79-1146 and strain DF2 (27). The homology of the S gene amino acid sequence among FIPV type II strains was almost completely conserved, as compared to that of the S gene amino acid sequence among FIPV type I strains. Does FIPV type II that is currently prevalent throughout the world have such homology? If so, FIPV type II may be a virus with very high conservation of the nucleotide sequence of genomic RNA or it may be a virus that has recently appeared and become prevalent. It has to be shown in the future to what degree FIPV type II strains are different from each other. In the field, FIPV type I is significantly more prevalent than FIPV type II (9). FIPV type I may be the prototype of FIPV. Some reports have shown that CCV infects cats (15). FIPV type II was considered to be a new FIPV type, which may have arisen from recombinants between FIPV type I and CCVs.

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