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The new genotypic human calicivirus isolated in Seoul

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Abbreviations: FCV, feline calicivirus; HuCV, human calicivirus; LV, Lordsdale virus; MV, Manchester virus; NV, Norwalk virus; RDRP, RNA-dependent RNA polymerase; ORF, open reading frame; RHDV, rabbit hemorrhagic disease virus; SRSV, small round structured virus; SHV, Southampton virus; SMV, Snow mountain virus; SV, Sapporo virus

Abstract

A new type of human calicivirus (HuCV) showing the classic cup-shaped surface morphology was identified in the stool sample from a child with symptoms of acute gastroenteritis in Seoul, Korea (SK virus). Genomic RNA was extracted directly from the stool sample, and the nucleotide sequence of 3.2 kb of the 3' end of SK virus was determined from cDNA. This region spanned sequences from the RNA-dependent RNA polymerase (RDRP) region in the open reading frame 1 (ORF1) to the 3' poly A tail. The nonstructural and capsid protein coding sequences were fused in a single ORF as observed in Manchester type (Genogroup III). However, ORF2 of Manchester virus was missing in SK virus. In RDRP region, SK virus showed amino acid and nucleotide identities of 74-75% and 68-69% respectively, with those of Manchester virus, while showed 34-46% and 55-60% identities respectively with those of other human caliciviruses. However, capsid protein of SK virus showed a partial (29-46%) amino acid identity with those of other caliciviruses including Manchester type. The closest resemblance in amino acid (97-99%) and nucleotide sequence (85-86%) identities were found in RDRP region with Vanderbijlpark and Pretoria isolates recently found in South Africa. These results suggest that SK virus together with Vanderbijlpark and Pretoria isolates belong to a new type different from Manchester virus.

Keywords: SK virus, calicivirus, sequencing, RNA polymerase, capsid protein

Introduction

The family Caliciviridae is composed of a single-stranded, positive-sense RNA viruses whose nonenveloped, spherical virions are 35-40 nm in diameter and consist of a single major capsid protein (Schaffer, 1980). Members of this group include feline calicivirus (FCV), vesicular exanthema of swine virus (VESV), rabbit hemorrhagic disease virus (RHDV), and human cali-civirus (HuCV).

HuCVs cause sporadic cases and outbreaks of gastroenteritis (Madeley and Cosgrove, 1976). Typical HuCVs have the distinctive Star of David appearance and surface hollows (Appleton, 1987). Unlike other Caliciviridae, the Norwalk virus (NV) isolated from patients in an outbreak of gastroenteritis in Norwalk City (Adler and Zickl, 1969; Kapikian *et al*., 1972) did not show the characteristic Star of David appearance, and is called small round structured viruses (SRSVs) based on its unique morpholgy. NV has also been classified within the family Caliciviridae based on the genome data (Jiang *et al*., 1990; Matsui *et al*., 1991; Jiang *et al*., 1993). HuCVs have been classified into at least five distinct strains based upon their reactivity by immune electron microscopy or radio-immunoassay using acute and convalescent phase human sera (Cubitt *et al*., 1987); Norwalk, Hawaii, Snow Mountain, Tanton, and Sapporo type. Especially, Sapporo strains were antigenically distinct and its sequence in RNA-dependent RNA polymerase (RDRP) region was rather closer to animal caliciviruses than other known human caliciviruses (Matson *et al*., 1995). Recently, Sapporo type virus was isolated in Manchester city, UK and its genomic structure was thoroughly examined.

Human caliciviruses have a single-stranded positive sense RNA genome of about 7700 nucleotides (Meyers *et al*., 1991; Carter *et al*., 1997) that possesses two or three open reading frames (ORF). The ORF1 encodes a large polypeptide containing amino acid motifs found in the picornavirus 2C helicase, 3C protease and 3D RDRP regions. The ORF2 encodes a single viral capsid protein of about 58 kDa (Jiang *et al*., 1992a). Capsid protein sequence is fused to ORF1 in RHDV and Manchester virus. The function of ORF3 is unknown, although it is conserved in both human and animal caliciviruses where it encodes a small basic protein which may function as an RNA-binding protein (Neill *et al*., 1991).

Based on the sequence comparisons of the RDRP region, calicividridae was classified into three subgeno-

groups; I: (Norwalk related), II: (Snow Mountain or Hawaii virus related), and III: (Sapporo and Manchester viruses) (Lambden and Clark, 1995). The recent study showed that the genome organization of the genogroup III isolates was rather closely resembled that of RHDV. In this investigation, characterization of genome sequence of the calicivirus isolated from a patient with gastroenteritis in Seoul (SK virus) indicated a distinct genome structure from other known HuCVs.

Materials and Methods

Identification of virus and isolation of HuCV from stool specimens

The classic human enteric caliciviruses were identified by electron microscopy and RT-PCR in stool samples obtained from a 10 months old infant with an acute diarrhea. HuCV from 10 g stool sample was purified by the following procedure (Jiang *et al*., 1990). A 10% solution of stool samples in PBS was first washed by low speed centrifugation at 3,000 rpm for 15 min, and the washing step was repeated two times. The supernatant was recentrifuged at 15,000 rpm for 60 min in a SS34 rotor (Sorvall), the resultant supernatant was concentrated by pelleting at 40,000 rpm for 120 min in a RP70T rotor (Hitachi, SCP85H). The pellet was fractionated by cesium chloride gradient centrifugation according to Jiang *et al.* (1990) and each fraction was examined for HuCV by RT-PCR with primers p36/p51.

Electron microscopy of fecal specimens

A specimen obtained from a child with gastroenteritis was processed for negative contrast electron microscopy (Middleton *et al*., 1977). Negative-contrast stain EM was performed by mixing a small portion of crude stool with 1% ammonium acetate and then placing a drop of the suspension on an EM polyvinyl formal carbon-coated grid (Ted Pella Inc.). After a few seconds, excess fluid was removed with filter paper, and a drop of 2% phosphotungstic acid (pH 7.0) was added to the grid. Grids were placed under an ultraviolet light for 2 min and then observed in the EM (JEM-1200EX II).

Isolation of HuCV RNA

HuCVs RNA was isolated from fecal specimen or the samples fractioned by CsCl gradient centrifugation with TRIzol Reagent (Gibco BRL) according to the method of Levett *et al.* (1996). A 10% suspension of the stool specimen in PBS was washed by centrifugation at 3,000 rpm in a microcentrifuge for 30 min. The supernatant was recentrifuged for 15 min at 10,000 rpm and further washed by centrifugation at 10,000 rpm for additional 15 min. A 100 μl volume of the stool suspension was added to 1.0 ml of TRIzol reagent in a microcentrifuge tube. After incubation at room temperature for 5 min, the preparation was mixed by shaking with 0.2 ml of chloroform, and after centrifugation at 12,000 rpm for 10 min, the aqueous layer was removed and mixed with the same volume of isopropanol at room temperature for 10 min. The pellet obtained after centrifuging at 12,000 rpm for 10 min at 4°C was washed with 75% ethanol, dried, and resuspended in 20 μl of Rnase-free water.

Primers for RT-PCR and cDNA library.

The primers used for RT-PCR and cDNA library of HuCV genome are listed in Table 1. A primer for cDNA library was designed for HuCV cDNA synthesis using oligo d(T) sequence. A primer set p36/p51 which was used for detection of HuCV in the stool specimen was previously described by Ando *et al*. (1994). The other primer set, p6H/pM95 were referred from the nucleotide sequences of HuCV cDNAs obtained by RT-PCR and from cDNA library.

Construction of cDNA library

A single stranded cDNA for the construction of cDNA library was synthesized using the cDNA synthesis kit (Amersham Life Science) with the primer T25VN and HuCV RNA for template. After the synthesis of the second strand, the reaction mixture was extracted once with phenol-chloroform and once with chloroform followed by ethanol precipitation. The cDNA was ligated with the EcoRI adaptor, and unreacted adaptor was removed through spin column according to the procedure of cDNA rapid adaptor kit (Amersham Life Science).

To detect the sequences which contain HuCV cDNAs, cDNA library was constructed in pBluescript SK(+) with cohesive end ligation into the EcoRI site. White colonies from transformed *E. coli* TOP10' cells were picked, and minipreps of plasmid DNA were performed for each clone.

Table 1. Primers for RT-PCR and cDNA library

cDNA	Position of cDNA (Length)	Names(Polarity) and Sequences (Position) of primers	Methods
a	1-416(416)	p36(+); ATAAAAGTTGGCATGAACA p51(-); GTTGACACAATCTCTCATCATCATC	RT-PCR
b	263-1761(1499)	p6H(+); GCCATTCACTAGTGTTGTCAAC pM95(-);GCTCAATAGGCAGTGTTGACCAAC	RT-PCR
c	1665-3210(1546)	T_{25}VN(-);$(T)_{25}$(A/G/C)(A/G/C/T)	cDNA library

Clones containing insert were identified after electrophoresis of the plasmids DNA in an agarose gel, and plasmids with insert DNA were sequenced by the dideoxy-chain termination method (Sanger *et al.*, 1977) using the Sequenase version 2.0 (USB). Internal primers were synthesized to complete the sequencing of both strands of each clone.

RT-PCR

RT-PCR was performed as described previously (Jiang *et al.*, 1992b). 5 μl of purified viral RNA was reverse transcribed for 1 h at 42°C with 10 U of avian myeloblastosis virus reverse transcriptase in 25 μl of PCR buffer containing 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 3.3 mM each dATP, dCTP, dGTP, and dTTP, 1.0 M primer, and 40 U of RNasin. PCR was performed by adding 70 μl of PCR buffer containing the second primer and 5 U of Ex. Taq DNA polymerase (Takara Biomedicals) to the RT reaction mixture. A negative control in which distilled water was substituted for cDNA was included in all sets of PCRs. DNA was amplified for 35 cycles consisted of denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 1 min/500 bp at 72°C. The first denaturation time was increased to 3 min, and the final extension time was increased to 3-5 min. The PCR products were analyzed by agarose gel electrophoresis and visualized by staining with ethidium bromide and UV illumination. PCR products were separated on 1.0% SeaKem agarose (FMC Bio Products, MD), and a portion of the gel that corresponded to the position of expected PCR products was cut out. cDNAs was purified by binding to silica ('MERmaid' Bio101) following extraction from agarose gels. Extracted DNA

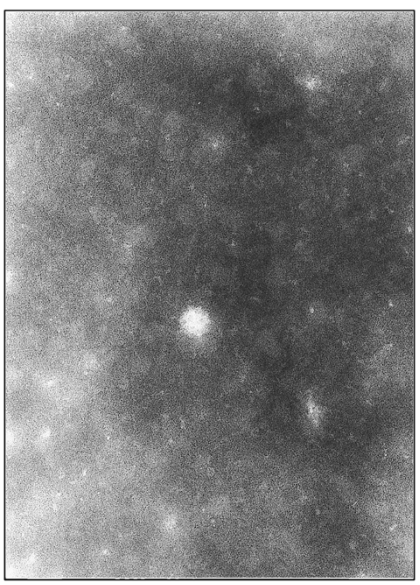

Figure 1. Electron microscopic picture of SK virus. Virus was visualized by staining with 2% phosphotungstic acid (pH 7.0). Scale bar is 50 nm.

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1     GACAGTTACCAGGTAGAGGTGCTCAATGAATCCCTCAAGGGCGGTGTCGTGTATTGCCTGGACTACAGTAAGTGGGACTCCACTCAACAC
      D  S  Y  Q  V  E  V  L  N  E  S  L  K  G  G  V  V  Y  C  L  D  Y  S  K  W  D  S  T  Q  H
91    CCGGCCGTTACAGCTGCATCCTTGGCAATCCTTGAGCGGCTGTCTGAAGCGACACCAATAACAACATCAGCCGTTGAGCTGCTCTCATCA
      P  A  V  T  A  A  S  L  A  I  L  E  R  L  S  E  A  T  P  I  T  T  S  A  V  E  L  L  S  S
181   CCAGCACGTGGCCACTTAAATGACATAATCTTTGTCACAAAGTCTGGACTCCCTTCGGGCATGCCATTCACTAGTGTTGTCAACTCTCTT
      P  A  R  G  H  L  N  D  I  I  F  V  T  K  S  G  L  P  S  G  M  P  F  T  S  V  V  N  S  L
271   AACCATATGACATACTTTGCGGCTGCAGTGCTGAAGGCCTATGAACAACATGGGGCACCTTACACTGGTAATGTGTTCAGGTGGAAACT
      N  H  M  T  Y  F  A  A  A  V  L  K  A  Y  E  Q  H  G  A  P  Y  T  G  N  V  F  Q  V  E  T
361   GTTCACACATATGGTGATGATTGCATATACTCTTTGTGCCGGCAACCGCCTCCATCTTTGAAACTGTTCTGGCCAACCTCAGTGCCTTT
      V  H  T  Y  G  D  D  C  I  Y  S  L  C  P  A  T  A  S  I  F  E  T  V  L  A  N  L  S  A  F
451   GGTTTACGGCCCACCGCTGCCGACAAAACAGACAAAATAGCCCCTACCCACACACCAGTCTTTCTAAAACGAACTCTCACCTGCACACCG
      G  L  R  P  T  A  A  D  K  T  D  K  I  A  P  T  H  T  P  V  F  L  K  R  T  L  T  C  T  P
541   AGAGGTATTCGTGGGTTACTCGACATCACGTCAATCAGGCGGCAATTTTTCTGGATCAAGGCCAACCGCACCACAGACATCTCATCCCCG
      R  G  I  R  G  L  L  D  I  T  S  I  R  R  Q  F  F  W  I  K  A  N  R  T  T  D  I  S  S  P
631   CCCGCCTACGACAGGGAAGCCCGGAGTGTGCAGCTTGAAAATGCCCTGGCTTACGCTTCACAACACGGCCACGCAATCTTTGAGGAGATT
      P  A  Y  D  R  E  A  R  S  V  Q  L  E  N  A  L  A  Y  A  S  Q  H  G  H  A  I  F  E  E  I
721   GCCGAAATTGCCAAGAGGACGGCACAGTCAGAAGGGTTAGTGCTCACCAATGTGAATTATGACCAGGCTCTGGCCACCTACGAAGCGTGG
      A  E  I  A  K  R  T  A  Q  S  E  G  L  V  L  T  N  V  N  Y  D  Q  A  L  A  T  Y  E  A  W
811   TTTATAGGTGGTACAGGCACCGGCCAAGATAGCGCCAGTGAAGAGACTACCAAATTAGTGTTTGAAATGGAGGGCTTGGGCCAGCCACAG
      F  I  G  G  T  G  T  G  Q  D  S  A  S  E  E  T  T  K  L  V  F  E  M  E  G  L  G  Q  P  Q
901   TCCCAAAGGGACCAACAGGTTATGGAACAGGTTGTCACCCCCCAGGACACCATTGGACCAACGAGTGCACTTTTACTGCCCACTCAAGTT
      S  Q  R  D  Q  Q  V  M  E  Q  V  V  T  P  Q  D  T  I  G  P  T  S  A  L  L  L  P  T  Q  V
991   GAGACACCAAACGCTAGTGCTCAGCGTGTGGAACTTGCAATGGCCACAGGAGCAGTGACCAGCAATGTGCCCAACTGTATCCGGGAATGT
      E  T  P  N  A  S  A  Q  R  V  E  L  A  M  A  T  G  A  V  T  S  N  V  P  N  C  I  R  E  C
1081  TTGCTGCGGTCACCACAATACCATGGACCACCCGCAAGCAGCAAATACCTTTCTCGGTGCCATACACCTGGGCCCACGCATCAATCCC
      F  A  A  V  T  T  I  P  W  T  T  R  Q  A  A  N  T  F  L  G  A  I  H  L  G  P  R  I  N  P
1171  TACACTGCACACCTGAGCGCGATGTTTGCTGGTTGGGGGGGAGGCTTTCAGGTGCGGTCACAATCTCAGGTTCCGGGCTCTTTGCTGGT
      Y  T  A  H  L  S  A  M  F  A  G  W  G  G  G  F  Q  V  R  V  T  I  S  G  S  G  L  F  A  G
1261  CGAGCCATCACTGCCATCCTGCCACCAGGTGTCAACCCAGCTGCTGTTCAGAACCCGGGTGTCTTCCCCCATGCGTTTATTGCTCGCATC
      R  A  I  T  A  I  L  P  P  G  V  N  P  A  A  V  Q  N  P  G  V  F  P  H  A  F  I  D  A  R
1351  ACAACTGACCCCATACTGATCAACTTACCTGACATCCGGCCCATTGATTTTCACCGTGTAGATGGGGACGACGCGACAGCGTCTGTGGGG
      T  T  D  P  I  L  I  N  L  P  D  I  R  P  I  D  F  H  R  V  D  G  D  D  A  T  A  S  V  G
1441  TTATGGGTCGCGCAGCCCTTGATCAACCCGCTTCAAACTGGCTCCGTATCCACATGTTGGCTTAGTTTGAAACACGGCCTGGCCCTGAC
      L  W  V  A  Q  P  L  I  N  P  L  Q  T  G  S  V  S  T  C  W  L  S  F  E  T  R  P  G  P  D
1531  TTTGATTTTTGTCTCCTCAAAGCCCCCGAGCAGGAAATGGACAATGGGATATCACCTGCCAACCTGTTGCCACGCGCCCTCGGGCGTTC
      F  D  F  C  L  L  K  G  P  R  A  G  N  G  Q  W  D  I  T  C  Q  P  V  A  T  R  P  R  A  F
1621  TCGGGGCAACGCCTCGGCGGTCGTGTTGTGGGGCTGGTTGTGGTAGCTGCGGCCGAGCAAGTCAACCACCACTTCGGCGCAAACTCCACC
      S  G  Q  R  L  G  G  R  V  V  G  L  V  V  V  A  A  A  E  Q  V  N  H  H  F  G  A  N  S  T
1711  ACACTTGGTTGGTCAACACTGCCTATTGAGCCAATTGCAGGGGCAGTTTCTTGGTACGATGACAACAATGAACATACCAAGATACGGGGC
      T  L  G  W  S  T  L  P  I  E  P  I  A  G  A  V  S  W  Y  D  D  N  N  E  H  T  K  I  R  G
1801  CTTTGAGTGCACAAGGAAAGGGCATCATCTTCCCAAACATTGTCAACCACTGGACAGATGTGTCATTGTCCGCCAAGACCTCGGGGCAG
      L  L  S  A  Q  G  K  G  I  I  F  P  N  I  V  N  H  W  T  D  V  S  L  S  A  K  T  S  G  Q
1891  ACAACTATCCCCATAGCTGCGGACAACCTCAACAACAGCCCTGGTGCAGCTGGTCCGGTAGTTATGTTTGAAACAATGGAGATGTCAAT
      T  T  I  P  I  A  A  D  N  L  N  N  S  P  G  A  A  G  P  V  V  M  F  E  N  N  G  D  V  N
1981  GAGTCCACGGCAAACCATGGCATACTAACCGCTGCGTCGCATGACTTCACATCGCTGTCCCAAACTTTCGATGCAGCCGGGTTGTGGGTT
      E  S  T  A  N  H  G  I  L  T  A  A  S  H  D  F  T  S  L  S  Q  T  F  D  A  A  G  L  W  V
2071  TGGATGCCATGGACCCGGAACAAACCTGACGGGCGTTCAAACACAAATGTCTACATTACTCCAACTTGGATTAATGGCAACCCTGCCCGT
      W  M  P  W  T  R  N  K  P  D  G  R  S  N  T  N  V  Y  I  T  P  T  W  I  N  G  N  P  A  R
2161  CCCATACATGAGAAATGTACCAACATGGTTGGGACTAATTTTCAATTCGGTGGCACTGGGACAAACAACATCATGTTATGGCAGGAGCAA
      P  I  H  E  K  C  T  N  M  V  G  T  N  F  Q  F  G  G  T  G  T  N  N  I  M  L  W  Q  E  Q
2251  CACTTCACATCTTTCCCAGGTGCTGCAGAGGTGTACTGTTCACAATTGGAGAGCACAGCAGAAATGTTCCAAAACAATGTGGTCAACATT
      H  F  T  S  F  P  G  A  A  E  V  Y  C  S  Q  L  E  S  T  A  E  M  F  Q  N  N  V  V  N  I
2341  CCAGCAAACCAAATGGCAGTCTTCAACGTAGAAACTGCAGGTAACACATTCCAGATTGGGATCTTGCCAAACGGCTATTCAGTCACCAAT
      P  A  N  Q  M  A  V  F  N  V  E  T  A  G  N  T  F  Q  I  G  I  L  P  N  G  Y  S  V  T  N
2431  GCAGCAATTGGCACACATCAACTCCTTGACTACGAGACTAGTTTTAGATTTGTTGGACTTTTCCCCCAAAGCACTTCACTTCAAGGGCCC
      A  A  I  G  T  H  Q  L  L  D  Y  E  T  S  F  R  F  V  G  L  F  P  Q  S  T  S  L  Q  G  P
2521  AATGGGAACGCTGGACGGGCCGTCAGGTTCTTAGAATAATGTCTTGGTTTACTGGAGCAGCCTTGGCCGCCGGCTCTGTCGTGGACATG
      N  G  N  A  G  R  A  V  R  F  L  E  *  M  S  W  F  T  G  A  A  L  A  A  G  S  V  V  D  M
2611  GCCGGCACAATATCTTCAATTGTTGCACAACACAGACAACTTGATTTGATGGCACAGGCAAACCCATACAACAGGATTGGGTAAACAGA
      A  G  T  I  S  S  I  V  A  Q  H  R  Q  L  D  L  M  A  Q  A  N  R  I  Q  Q  D  W  V  N  R
2701  CAAGAGGCACTCATCACTCGAGGACAGGACATCTCCCGCGACCTTGCTGTTAATGGAACGGCGCAACGCGTCGACTCACTGGTCAAAGCG
      Q  E  A  L  I  T  R  G  Q  D  I  S  R  D  L  A  V  N  G  T  A  Q  R  V  D  S  L  V  K  A
2791  GGGTTCACCCCTGTTGATGCTCGCAGATTGGCGGGCGGCACGGAGAGCGTGCAGTACGGCCTGCTGGACAGGCCTATACTGCAACGTTCG
      G  F  T  P  V  D  A  R  R  L  A  G  G  T  E  S  V  Q  Y  G  L  L  D  R  P  I  L  Q  R  S
2881  GTGCTGTCGGGCATCTCAGAGACTCGGCACTTGCAGTCTATGCAAGGTGCCTTGAGTGCTTTAAAAATGGATCCAGCTACGGAGCCCCT
      V  L  S  G  I  S  E  T  R  H  L  Q  S  M  Q  G  A  L  S  A  F  K  N  G  S  S  Y  G  A  P
2971  CCGCCCCAGCGGGCTTCGTTAACCCAAATTATCAGGCTTCACCACCAAAGCTAACACTTGGCCCCAGACCCCCGGTGACTAATGTATAAT
      P  P  Q  R  A  S  L  T  Q  I  I  R  L  H  H  Q  S  *                    154
3061  GATGATTAGGTTTTCCCAAGATAATCAAATTTCCACTCTCAGATATCTTTACTTTTCTTTTCTTTTCCCATGGTACCACACGCGTTCGGG

3151  TGGATAATTGTGGTTAAGCGACCACAGCCATGATCCTCTGGAAAAGGGAAAAAAG
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Figure 2. Partial nucleotide and amino acid sequences of SK virus. The nucleotide and its deduced amino acid sequences of the 3' end with 3.2 kb was determined. Conserved sequences, GLPSG and YGDD, of RDRP and GSGVFAGR of capsid protein are underlined. Amino acid sequences of capsid protein and ORF2 start with new numbering and the first amino acid of capsid protein was denoted with a bold character. Termination codons of each ORF1 and ORF2 are marked with *.

fragments were ligated with pMOSBlue T-vector, and transformed to *E. coli* competent cells according to manufacturer's protocol (Amersham Life Science).

Nucleotide sequence accession numbers and analysis

Sequence data from this study have been deposited to EMBL/GenBank data libraries. The accession numbers for the various viruses are M86379 (FCV), M87611 (NV/8FiiA/68/US), L07418 (Southampton/91/UK), L23828 (KY-89/Japan), L23831 (SMA/76/US), L23830 (Oth-25/89/Japan), U22498 (MX/89/Mexico), U73124 (Parkville), X86559 (Plymouth/92/UK), X86557 (Lordsdale virus), U50825 (Pretoria/MK17/94/SA), U43287 (Vanderbijlpark/313616/SA), and X86560 (Manchester virus/93/UK). Nucleotide sequences and amino acid sequences were analyzed by computer program DNA-SISTM and PROSISTM (Hitachi-Amersham Ltd).

Results and Discussion

Electron microscopic examination

The HuCV was identified with the characteristic mor-

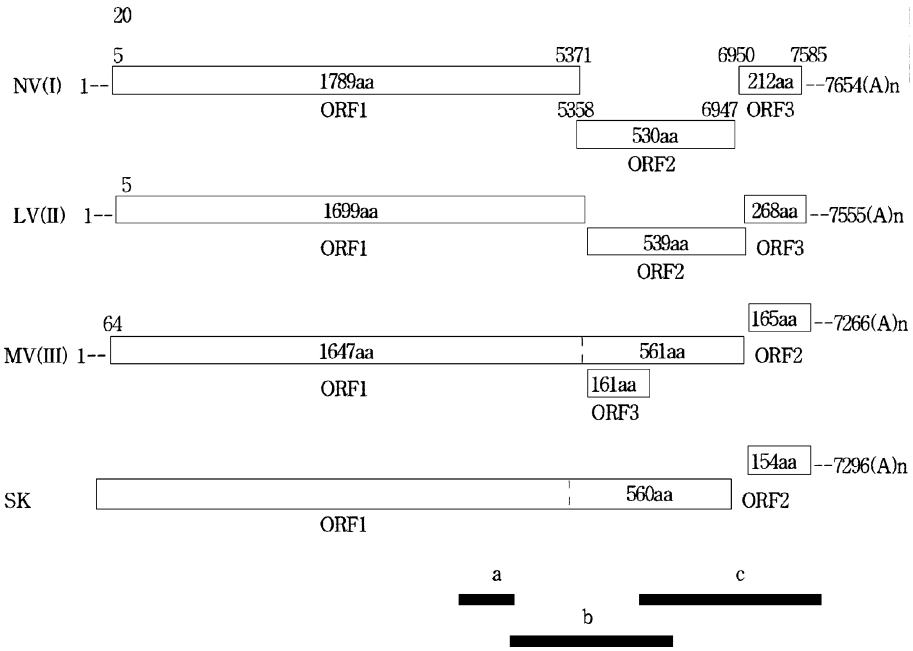

Figure 3. Comparison of the genomic organization of SK virus with HuCVs. Genomic organization of genogroup I (Norwalk virus, NV), II (Lordsdale virus, LV), III (Manchester virus, MV) (EMBL/GenBank data libraries) and SK virus was predicted from the analysis of nucleotide sequence. The bold lines at the bottom of figure indicate the locations of the 3' partial cDNAs of SK virus obtained from RT-PCR and cDNA libraries (a, b, and c denotes cDNA obtaind by each primers of Table 1). All cDNAs were obtained from the stool specimen of a single child with gastroenteritis, and cloned into T-vector and pBlueScript KS.

Table 2. Comparison of percentages of nucleotides and amino acids identity of the RNA-dependent RNA polymerase region of SK virus to other calicivirus

	FCV	RHDV	NV	SHV	SMA	MX	OTH	Sa/82	MV	Vanderbijl	Pretoria	SK
FCV		54	53	55	55	56	57	59	58	58	57	57
RHDV	40		52	54	54	55	56	58	59	57	58	60
NV (I)	37	35		77	62	61	62	55	52	55	54	55
SHV (I)	36	36	93		65	66	67	56	52	55	54	55
SMA (II)	35	35	57	60		78	79	55	56	55	55	55
MX (II)	37	34	57	61	88		96	55	56	53	53	55
OTH (II)	37	34	58	62	90	96		55	56	53	54	56
Sa/82 (III)	47	38	34	36	36	36	37		89	70	68	68
Manchester (III)	48	38	35	36	36	36	37	99		69	69	69
Vanderbijl	46	41	34	35	36	36	36	74	73		95	85
Pretoria/SA	46	42	34	36	35	36	36	75	74	97		86
SK	46	42	34	36	35	36	36	75	74	97	99	

The numbers in the upper triangle show the percent identity of the aligned nucleotide sequences, and the number in the lower triangle show the percent identity of the aligned amino acids. Sequences of viruses were obtained EMBL/GenBank Data Libraries. Roman number in paranthesis indicates the type of genogroup.

phology by electron microscopic examination of stool that was positive for HuCV by RT-PCR. SK (Seoul, Korea) virus showed the typical HuCV appearance with a diameter of around 35 nm (Figure 1).

Cloning and partial genome organization of SK virus

Nucleotide sequence of 3.2 kb fragment of the 3' end from three overlapping cDNA clones which were obtained by RT-PCR and cDNA library of RNA were determined (Figure 2). Sequence analysis showed that cDNA of the clones spanned from the RNA polymerase region in ORF1 to the 3' poly A tail. Figure 3 shows the genomic organization of SK virus compared with other types of HuCVs. The nonstructural and capsid protein coding sequence were fused in a single ORF as observed in Manchester type or RHDV. The ORF2 was located at the 3' end of genome. The AUG initiation codon of ORF2 is overlapped by one base by the termination codon of ORF1. The size of ORF2 (462 nucleotides, 154 amino acids) is larger than that of RHDV (117 amino acids), but smaller than those of the most HuCVs (165-268 amino acids). The ORF3 shown in Manchester virus was not found in SK virus.

Sequence analysis of SK virus in RDRP region and capsid region (Table 2) compares the nucleotide and predicted amino acid identity of the RDRP region of SK virus and other caliciviruses. SK virus showed amino acid and nucleotide identities of 74-75% and 68-69% respectively with those of genogroup III viruses, while showing 34-36% and 55-56% identities respectively with those of other human caliciviruses. It should be noted that the SK virus displays higher percentage of sequence similarity with animal viruses (FCV, RHDV) than genogroup I (NV) and genogroup II (SMA, MX, OTH), which is a typical feature of genogroup III. Vanderbijlpark and Pretoria isolates recently found in South Africa but ungrouped due to insufficient data which comprise only about the short region of RDRP showed the highest identities (97% and 99% in amino acid sequences, 85% and 86% in nucleotide sequences) with SK virus.

Table 3. Comparison of percentages of amino acid identity of the capsid proteins of SK virus and the other caliciviruses

	NV	LV	FCV	RHDV	MV	Parkville	Plymouth	SV
NV								
LV	47							
FCV	23	29						
RHDV	24	33	28					
MV	24	32	29	26				
Parkville	23	32	27	24	78			
Plymouth	24	32	29	26	99	78		
SHV	24	32	29	26	99	78	98	
SK	31	32	29	32	44	46	44	

The numbers represent the percent identity of the aligned amino acids of capsid region

Table 3 shows an overall sequence identity of the capsid protein of SK virus and those of animal and human calicivirus. The capsid protein of SK virus (560 amino acids) was longer than SRSVs (530-548 amino acids) but shorter than Manchester type (561 amino acids) (Numata *et al.*, 1997). Amino acid identities between SK virus and other caliciviruses range 29-46%. Although the sequence identities between SK and Manchester strains (genogroup III) are higher than other caliciviruses, the identities are only 44-46%, which is quite low to be placed in the same genogroup. Figure 4 shows a comparison of direct amino acid between the capsid proteins of SK virus and genogroup III. N-terminus region (Region 1, 54-59%) and C-terminus region (Region 3, 50-52%) are more conserved than the central region (Region 2, 15-18%). There is a conserved motif of GSGVFAGR in

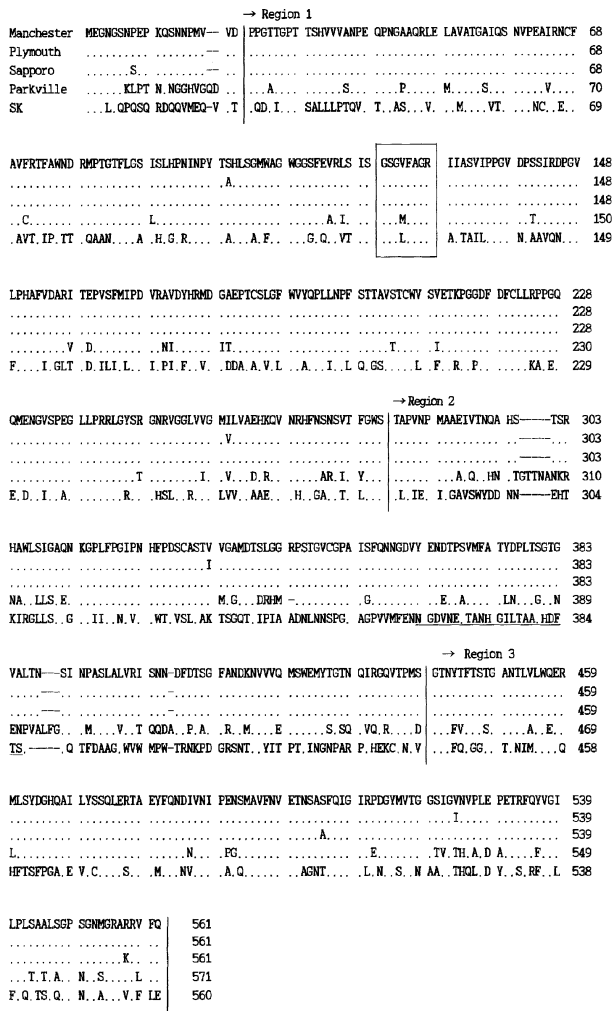

Figure 4. Alignment of the amino acid sequences of capsid regions of SK virus and other strains included in HuCV genogroup III. Identical amino acid residues shown as dots and dashes are used to adjust alignment. The open box indicates conserved regions among HuCV genogroup III and animal caliciviruses. The underlined region indicates an additional amino acid insertion site in the variable region.

N-terminus region. However, V in this region was changed to L and M, in SK and Parkville strain. As in Manchester virus, capsid protein of SK virus was longer by 30 amino acids than that of other SRSVs, and these additional amino acids are present in central variable region.

Compared to RDRP region which showed a relatively high amino acid identity (74%) to Manchester virus, deduced amino acid sequence of capsid region of SK virus showed only 44% identity to that of Manchester virus. In addition, ORF3 appeared in Manchester virus was not found in SK virus. In order to place virus strains into the same genogroup, amino acid sequence identity of >82% in the RNA polymerase region, and of >65% in capsid region is generally required (Lew *et al*., 1994; Estes *et al*., 1997). Vanderbijlpark and Pretoria isolates recently found in South Africa was classified as Manchester-like type in EMBL/Genbank data library. Present investigation revealed that Vanderbijlpark and Pretoria isolates showed very high identities (>97% in amino acids and >85% in nucleotides) with SK virus. These may suggest that SK virus together with Vanderbijlpark and Pretoria isolates could be a new type different from Manchester type.

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