Arch Virol (2013) 158:1765–1772
DOI 10.1007/s00705-013-1770-z

ORIGINAL ARTICLE

Complete genome sequence of a novel porcine parainfluenza virus 5 isolate in Korea

Yu Na Lee · Changhee Lee

Received: 10 April 2013 / Accepted: 8 May 2013 / Published online: 27 June 2013

Abstract A novel cytopathogenic paramyxovirus was isolated from a lung sample from a piglet, using continuous porcine alveolar macrophage cells. Morphologic and genetic studies indicated that this porcine virus (pPIV5) belongs to the species *Parainfluenza 5* in the family *Paramyxoviridae*. We attempted to determine the complete nucleotide sequence of the first Korean pPIV5 isolate, designated KNU-11. The full-length genome of KNU-11 was found to be 15,246 nucleotides in length and consist of seven nonoverlapping genes (3′-N-V/P-M-F-SH-HN-L-5′) predicted to encode eight proteins. The overall degree of nucleotide sequence identity was 98.7 % between KNU-11 and PIV5 (formerly simian virus 5, SV5), a prototype paramyxovirus, and the putative proteins had 74.4 to 99.2 % amino acid identity to those of PIV5. Phylogenetic analysis further demonstrated that the novel pPIV5 isolate is a member of the genus *Rubulavirus* of the subfamily *Paramyxovirinae*. The present study describes the identification and genomic characterization of a pPIV5 isolate in South Korea.

Introduction

Paramyxoviruses are important human and animal pathogens of the central nervous and respiratory systems. The family *Paramyxoviridae* contains enveloped, negative-sense, single-stranded RNA viruses and is divided into two subfamilies: *Paramyxovirinae* and *Pneumovirinae*. The subfamily *Paramyxovirinae* is further divided into seven genera, *Respirovirus*, *Rubulavirus*, *Avulavirus*, *Morbillivirus*, *Aquaparamyxovirus*, *Ferlavirus*, and *Henipavirus*, as well as a group of unclassified paramyxoviruses [17]. Many novel paramyxoviruses have emerged in humans and a wide range of animal species over the last few decades, and some animal pathogens have been shown to infect across species, leading to zoonotic outbreaks [3, 4, 24]. A number of porcine paramyxoviruses have also been reported in several countries. La Piedad Michoacan paramyxovirus (LPMV), which is the only well-studied neurotropic porcine rubulavirus, was first isolated in Mexico in the early 1980s [23]. Two novel parainfluenza virus 3 (PIV3) isolates were identified from the brains of pigs with interstitial pneumonia and encephalitis in the United States in 1981 and 1992 [7, 14]. A new paramyxovirus infectious for pigs, humans, and fruit bats was identified from stillborn piglets in Australia in 1997 [29]. In addition, parainfluenza virus type 5 (PIV5) was isolated from a case of concurrent infection with porcine reproductive and respiratory syndrome virus (PRRSV) in Germany in 1998, and subsequently named “SER” virus [9].

PIV5, a member of the genus *Rubulavirus*, previously known as simian virus 5 (SV5), was first identified in 1954 from primary monkey kidney cells. Since then, PIV5 has been isolated from different hosts, including humans, dogs, pigs, cats, and rodents [2, 13]. The full-length genome of PIV5 is 15,246 nucleotides long and composed of a 3′ leader region, seven genes (NP, V/P, M, F, SH, HN and L), and a 5′ trailer region. The PIV5 genome encodes eight proteins from seven genes because the V/P gene encodes two distinct structural proteins, V and P, as a consequence of a specific RNA editing mechanism, resulting in the addition of two G residues at the editing site [17, 37]. During the identification of porcine viral pathogens using

Y. N. Lee · C. Lee (✉)
Department of Microbiology, College of Natural Sciences, Kyungpook National University, Daegu 702-701, Republic of Korea
e-mail: changhee@knu.ac.kr

continuous porcine alveolar macrophage (PAM) cells, a novel isolate of PIV5 was isolated from the lung of a piglet with respiratory illness. Although PIV5 has been isolated from a stillborn piglet in Germany, its importance as a swine pathogen remains undetermined. As a first step toward understanding the significance of porcine PIV5 (pPIV5) for pig health, we aimed to initiate molecular characterization studies and perform a complete genomic sequence analysis of pPIV5 (strain KNU-11)

Materials and methods

Virus isolation

Lung tissues of piglets that were experiencing respiratory problems at the time of sampling were obtained from pig farms in Gyeongbuk province in 2011. The tissue samples were subsequently inoculated on PAM cells grown in RPM1 1640 medium supplemented with 10 % fetal bovine serum (FBS; Invitrogen) and 1 % antibiotic-antimycotic solution for virus isolation as described previously [34]. The inoculated cells were maintained at 37 °C under 5 % CO_2 and monitored daily for cytopathic effect (CPE). The culture supernatants were harvested when CPE appeared in 70 % of the cells and stored at −80 °C as the virus stock until use. The virus supernatants were purified through a 20 % sucrose cushion (wt/vol) prepared in TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA) by centrifugation at 40,000 rpm for 2 h at 4 °C in a P70AT rotor (model CP100WX; Hitachi), after which the purified sample was examined by transmission electron microscopy as described previously [19].

RT-PCR, DNA cloning and sequence analysis

To determine the full-length genomic sequence of the Korean pPIV5 isolate designated KNU-11, oligonucleotide primers were first selected based on published sequences of the prototype PIV5 (formerly SV5; GenBank accession no. NC_006430) to obtain RT-PCR fragments. Primers were then synthesized based on newly amplified KNU-11 sequences for RACE experiments and nucleotide sequencing (Table 1). Overlapping cDNA fragments spanning the entire viral genome were amplified by RT-PCR using gene-specific primer sets. Briefly, viral RNA was extracted from the purified virus stock using an RNeasy Mini Kit (QIAGEN) according to the manufacturer's instructions. Reverse transcription was performed by using 1 μg of viral RNA and specific reverse primers using a PrimeScript 1st strand cDNA Synthesis Kit (TaKaRa). PCR was carried out to amplify each cDNA fragment from the RT product using KOD Hot Start DNA polymerase (Novagen) according to the manufacturer's protocol. The individual cDNA amplicons were gel-purified, cloned into the pGEM-T Easy Vector (Promega), and sequenced in both directions using primers for T7 and SP6 promoters and KNU-11-specific primers.

The leader and trailer sequences of the viral genome were determined by rapid amplification of cDNA ends (RACE) as described previously, with some modification [20]. Briefly, virion RNA was reverse transcribed using 3′ leader and 5′ trailer RT primers. The resulting cDNA products were purified using a QIAquick PCR Purification Kit (QIAGEN), and 3′ and 5′ tailing reactions were conducted using terminal transferase (Roche) to add a poly(A) tail to each end of the purified cDNA products, followed by re-purification using a QIAquick PCR Purification Kit. A first round of PCR was performed using 10 μl of the poly(A)-tailed cDNA product with the adapter primer (AP-dT17) and leader R1 or trailer F1 primer. A second round of PCR was then conducted using 1 μl of a 1:50 dilution of the first reaction with the adapter primer and leader R2 or trailer F2 primer. The PCR products obtained from each reaction were gel-purified and cloned into pGEM-T Easy Vector (Promega), and two clones of each reaction were sequenced as described above. General DNA manipulation and cloning were performed according to standard procedures [36]. The complete genomic sequence of the KNU-11 virus was deposited in the GenBank database under accession number KC852177.

Multiple alignments and phylogenetic analysis

The phosphoprotein (P) gene sequences of 35 paramyxoviruses within the family *Paramyxoviridae* and the fusion (F) gene sequences of 12 PIV5 isolates were used independently in sequence alignments and phylogenetic analysis. The accession numbers of the viral sequences used were as follows: Atlantic salmon paramyxovirus (ASPV-Ro), EU646380; avian metapneumovirus (aMPV-15a), NC_007652; avian paramyxovirus 2, EU338414; avian paramyxovirus 6, NC_003043; Beilong virus (BeV), NC_007803; bPIV3-910N, D84095; bPIV3 strain Kanas/15,626/84, AF178654; bPIV3-SF, AF178655; bPIV3-Q5592, EU277658; bovine respiratory syncytial virus (bRSV), NC_001989; canine distemper virus (CDV), NC_001921; dolphin morbillivirus (DMV), NC_005283; Fer-de-Lance virus, NC_005084; Hendra virus (HeV), AF017149; human metapneumovirus, NC_004148; hPIV1 strain Washington/1964 (hPIV1-Wa), NC_003461; hPIV2, NC_003443; hPIV3, AB012132; hPIV3-GP2, NC_001796; hPIV3-JS, Z11575; human RSV (hRSV), NC_001781; J-virus, NC_007454; measles virus, NC_001498; Menangle virus, NC_007620; Mossman virus (MoV), NC_005339; mumps virus (MuV), NC_002200; Newcastle

Table 1 List of primers used in this study

Primer name	Nucleotide sequences	Purpose	Location (nt)
PIV5-NP-F	5′-ATGTCATCCGTGCTTAAAGC-3′	PCR	152-171
PIV5-NP-R	5′-CTAGATGTCAAGATCACCCA-3′	RT and PCR	1662-1681
KNU-11-3′ leader-R2	5′-CGGCTCATACCTGAACGAGC-3′	3′-RACE-PCR	503-522
KNU-11-3′ leader-R1	5′-GCATAGGCATTGATCTCTCC-3′	3′-RACE-PCR	524-543
KNU-11-3′ leader-F1	5′-GCAGAAGATCTACCTGACAC-3′	3′-RACE-PCR	551-570
KNU-11-3′ leader-F2	5′-CCATGCAACACCTCTCGTTG-3′	3′-RACE-PCR	557-577
KNU-11-3′ leader-RT	5′-GCAGTTCCCTCGACTTCGG-3′	3′-RACE-RT	600-618
KNU-11-NP/P junction-F	5′-TGCAGGCACCCATGATGATG-3′	PCR	1501-1520
KNU-11-NP/P junction-R	5′-GCATTGGTTAGTAGTCCTGT-3′	RT and PCR	1991-2010
PIV5-P-F	5′-ATGGATCCCACTGATCTGAG-3′	PCR	1850-1869
PIV5-P-R	5′-TCAAATTGCACTGCGGATGA-3′	RT and PCR	3007-3026
KNU-11-P/M junction-F	5′-GATTGAAGATCACACTAGAG-3′	PCR	2881-2990
KNU-11-P/M junction-R	5′-CTCCGACAATAGTATTCCCC-3′	RT and PCR	3341-3360
PIV5-M-F	5′-ATGCCATCCATCAGCATCCC-3′	PCR	3141-3160
PIV5-M-R	5′-TCATTCCAGCTCCGTCAGGT-3′	RT and PCR	4255-4274
KNU-11-M/F junction-F	5′-CTCTTATCGTGGAGACTACT-3′	PCR	4141-4160
KNU-11 M/F junction-R	5′-CAACAATGAATGCTGATGAG-3′	RT and PCR	4661-4680
PIV5-F-F	5′-ATGGGTACTATAATTCAATT-3′	PCR	4530-4549
KNU-11-F-801-F	5′-GCAGATGGTCATAAAAAT-3′	Sequencing	5330-5347
PIV5-F-R	5′-TTATTTATGATAAACAAAAT-3′	RT and PCR	6100-6119
KNU-11-F/SH junction-F	5′-TCTGTCTTGGATCGTTAGGT-3′	PCR	6001-6020
PIV5-SH-F	5′-ATGCTGCCTGATCCGGAAGA-3′	PCR	6303-6322
PIV5-SH-R	5′-TTAGGACAGCAAGTGTCTTA-3′	RT and PCR	6418-6437
KNU-11-SH/HN junction-R	5′-ATGATTTGCTTTCGGGTTAT-3′	RT and PCR	6701-6720
PIV5-HN-F	5′-ATGGTTGCAGAAGATGCCCC-3′	PCR	6584-6603
KNU-11-HN-801-F	5′-TGATACAATCGTGGAGCG-3′	Sequencing	7384-7401
PIV5-HN-R	5′-TTAGGATAGTGTCACCTGAC-3′	RT and PCR	8262-8281
KNU-11-HN/L junction-F	5′-AACTTGTTTTAGGGACACAG-3′	PCR	8161-8180
KNU-11-HN/L junction-R	5′-TCTTCATGTGCTATCTGATT-3′	RT and PCR	8561-8580
PIV5-L-part 1-1-F	5′-ATGGCTGGGTCTCGGGAGAT-3′	PCR	8414-8433
PIV5-L-part 1-1-R	5′-AGCACACATAGACTCGCG-3′	RT and PCR	9554-9571
PIV5-L-part 1-2-F	5′-CACCCAGGATGAATTAAG-3′	PCR	9409-9426
KNU-11-L-10159-F	5′-CCATGCTGGGAAGTTAAT-3′	Sequencing	10159-10176
KNU-11-L-10909-F	5′-TAGCAAGAGAATATTCTATCA-3′	Sequencing	10909-10929
PIV5-L-part 1-2-R	5′-GTTCACAGTAGCCCGATCCA-3′	RT and PCR	11941-11960
PIV5-L-part 2-F	5′-GCAATGACACTTGAAACATG-3′	PCR	11801-11820
KNU-11-L-12701-F	5′-GCTGTAGATATGACAGGT-3′	Sequencing	12701-12718
KNU-11-L-13447-F	5′-CAATTACTACCTGACCAG-3′	Sequencing	13347-13464
PIV5-L-part 2-R	5′-TTAGATTTCCTCGCCATCGA-3′	RT and PCR	15162-15181
KNU-11-5′ trailer-RT	5′-GGTTGATCCTCCCACCTTC-3′	5′-RACE-RT	14847-14865
KNU-11-5′ trailer-R2	5′-CCTGCTTCACGATCATCCG-3′	5′-RACE-PCR	14870-14888
KNU-11-5′ trailer-R1	5′-CCTGAATATGCCGAATTCC-3′	5′-RACE-PCR	14893-14911
KNU-11-5′ trailer-F1	5′-CCATCCTCAATTCTGATCG-3′	5′-RACE-PCR	14919-14937
KNU-11-5′ trailer-F2	5′-CCTGAGGCTTTCTCCAAATA-3′	5′-RACE-PCR	14941-14960

disease virus (NDV), NC_002617; Nipha virus (NiV), NC_002728; pestedes-petits-ruminants virus, NC_006383; porcine rubulavirus (LPMV), NC_009640; rinderpest virus (strain Kabete O), NC_006296; Sendai virus (SeV), NC_001552; simian PIV5 (SV5), NC_006430; Tioman virus (TioV), NC_004074; Tupia paramyxovirus (TPMV),

NC_002199; porcine PIV5-SER, AJ278916.1; PIV5-W3A, NC_006430; simian PIV5-WR, AB021962.1; PIV5-MEL, AJ749988.1; PIV5-LN, AJ749987.1; PIV5-MIL, AJ749989.1; PIV5-DEN, AJ749986.1; PIV5-T1, AB033629; PIV5-78524, AJ749990.1; PIV5-H221, AJ749991.1; PIV5-CPI+, AJ278916.1; PIV5-CPI-, AJ278916.1.

Multiple-sequencing alignments were conducted using ClustalX 1.83, and percent nucleotide sequence divergence was calculated using the same software application [38]. Phylogenetic trees were constructed from the aligned nucleotide sequences using the neighbor-joining method, after which they were subjected to bootstrap analysis with 1,000 replicates to determine percent reliability values at each internal node of the tree [35]. All tree figures were produced using the TreeView program [27].

Results and discussion

In the present study, a porcine viral pathogen was newly isolated from a lung sample from a suckling piglet, using continuous porcine alveolar macrophage (PAM) cells. This novel porcine isolate was remarkably cytopathogenic, showing distinct cell rounding and clumping evident in PAM cells within 12 h postinfection (Fig. 1A). An ultrastructural study of purified virus suspensions identified spherical to pleomorphic virions approximately 50-200 nm in diameter that were morphologically indistinguishable from paramyxoviruses (Fig. 1B). To confirm the presence of paramyxovirus-like pleomorphic virions in infected PAM cells, the viral genome was amplified by RT-PCR using parainfluenza virus NP-specific primers and then sequenced. The resulting sequences were subjected to sequence similarity searching using the Basic Local Alignment Search Tool (BLAST) of the NCBI nucleotide database. The data indicated that the amplified NP gene is almost identical to that of parainfluenza virus type 5 (PIV5), formerly known as simian virus 5 (SV5), demonstrating that the newly identified porcine paramyxovirus is a porcine isolate of PIV5.

To better understand the molecular characteristics of the porcine PIV5 (pPIV5), designated KNU-11, we sought to conduct full-length genome sequence analysis. To accomplish this, RT-PCR cDNA amplicons covering the entire RNA genome were cloned and sequenced in both directions. In addition, RACE experiments were performed to determine termini of the KNU-11 genome, and the KNU-11 genome contained the same 3′ and 5′ end nucleotides as those found in PIV5. The results revealed that the complete genomic sequence of KNU-11 was 15,246 nucleotides (nt) long and consisted of a 55-nt 3′ leader, a 14,701-nt protein-coding region (96.4 % coding capacity), and a 31-nt 5′ trailer. The viral genome length was consistent with the

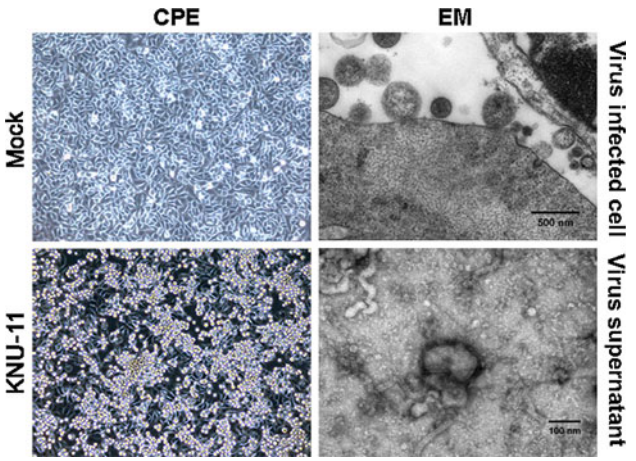

Fig. 1 Identification of porcine paramyxovirus. A. CPE formation due to porcine paramyxovirus infection. PAM-KNU cells were inoculated with porcine paramyxovirus, and virus-specific CPE was photographed at 24 hpi using an inverted microscope at a magnification of 100×. B. Ultrastructure of porcine paramyxovirus. Purified virions (upper panel; 100,000×) and an ultrasection of virions budding from a cultured cell (lower panel; 30,000×) were negatively stained with 2 % phosphotungstic acid and viewed under a transmission electron microscope

"rule of six" as described for other members of the family *Paramyxoviridae*, with a hexamer phase pattern of 2-1-6-1-2-1-6, which was the same as that of PIV5 [16]. This pattern is thought to be involved in nucleocapsid organization, in which each N monomer interacts with six nucleotides of the viral genome. The genome of KNU-11 contains seven non-overlapping genes (3′-N-V/P-M-F-SH-HN-L-5′) that can potentially encode eight proteins. The virus genome has conserved sequences for gene starts (GS) and gene ends (GE) at the beginning and end of each gene and intergenic regions that vary greatly from 1 to 22 nt in length between the gene boundaries. Full-length sequence analysis showed that the genome of KNU-11 shares 98.7 % homology with the prototype PIV5 strain at the nucleotide level.

The 3′ leader and 5′ trailer regions of KNU-11 were found to have 90.9 % and 93.5 % identity (5 and 2 nucleotide differences), respectively, to the prototype strain,. Comparison of the deduced amino acid sequences revealed that the predicted gene products, the N, V/P(V), V/P(P), M, F, SH, HN, and L proteins, of KNU-11 exhibited 99.2 %, 98 %, 97.8 %, 98.4 %, 98.3 %, 74.4 %, 96.8 %, and 99 % amino acid sequence identity, respectively, to those of the prototype PIV5 (Table 2). In addition, the P, M, F, and HN proteins of KNU-11 were shown to have 98.5 %, 100 %, 99.5%, and 99.1 % amino acid sequence identity, respectively, to the previously identified porcine isolate of PIV5, SER virus, (Table 2).

The nucleoprotein (N) gene in KNU-11 was 1,732 nt long and encoded a protein of 510 amino acids (aa) with a

Table 2 Nucleotide and amino acid sequence identity between proteins from the Korean pPIV5 isolate (KNU-11) and other viruses belonging to the genus *Rubulavirus*

Virus	Pairwise % nucleotide (nt) and amino acid (aa) sequence identity															
	NP		P		V		M		F		SH		HN		L	
	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa
PIV5	99.3	99.2	98.8	98.0	98.7	97.8	98.8	98.4	98.6	98.3	84.4	74.4	98.3	96.8	99.2	99.0
SER	-[a]	-[a]	99.4	98.5	-[a]	-[a]	99.7	100	99.7	99.5	-[a]	-[a]	99.5	99.1	-[a]	-[a]
LPMV	59.3	53.6	51.5	32.1	48.1	32.6	53.9	36.8	54.7	45.5	-[b]	-[b]	53.6	41.7	60.2	54.8
MuV	58.7	52.9	52.8	36.9	52.0	40.8	55.1	41.2	54	42.6	40.2	20.6	56.5	43.9	61.7	58.3
hPIV2	60.1	56.1	56.6	41.4	56.4	43.6	58.7	48.8	56.4	45.7	-[b]	-[b]	55.4	45.4	63.1	61.7
MENV	57.5	47.0	53.0	33.0	50.1	33.1	52.8	38.3	52.3	35.8	-[b]	-[b]	49.0	21.1	58.2	49.7
TioV	58.0	49.4	51.2	33.1	48.7	32.9	54.3	37.0	55.0	37.4	-[b]	-[b]	48.8	20.7	58.5	49.8

[a] The data are not presented, since complete sequence information on only the P, M, F, and HN genes of SER is currently available (accession nos. AJ278914-16 and AJ749981)

[b] The data are not available, since these viruses (LPMV, hPIV2, MENV, and TioV) do not encode the SH protein

predicted molecular mass of 56.5 kDa and an isoelectric point (pI) of 5.0. As a component of a viral ribonucleoprotein (RNP) complex, the paramyxovirus N proteins contain a highly conserved stretch in the central domain, F-X4-Y-X3-Ø-S-Ø-A-M (where X is any residue and Ø is an aromatic amino acid), which is involved in N-N self-assembly and the N-RNA interaction process [17, 25]. The KNU-11 N protein was also found to possess this motif as ^{323}FAAANYPLLYSYAM^{336}.

The KNU-11 V/P gene was 1,304 nt long, encoding both V and P proteins due to a specific RNA editing mechanism that is a common feature in paramyxoviruses. The first open reading frame (ORF) of 669 nt becomes the V mRNA, being a primary transcript of the genomic RNA, whereas the second ORF generated by insertion of two non-templated G residues at the editing site synthesizes the P mRNA [37]. To confirm the editing site in the KNU-11 virus, the P mRNA was amplified by RT-PCR from virus-infected cells and sequenced. We found that the P gene in KNU-11 has two G insertions at an mRNA editing site, 5′-^{545}AAGAGGGG^{552}-3′ (mRNA sense), which are identical to those in other PIV5 strains. As a result, the insertion of G residues during mRNA synthesis can shift the translational reading frame and thus potentially generate a P protein of 393 aa in length with a predicted size of 56.5 kDa and a pI of 5.0. The V protein of KNU-11 was composed of 223 aa and had a calculated size of 23.9 kDa with a pI of 7.55. The V protein in paramyxoviruses is known as a multifunctional protein that inhibits the host antiviral response by suppressing interferon (IFN) production and IFN signaling pathways, controls virus replication and encapsidation, and regulates RNA synthesis [1, 28, 31, 33, 40]. The C-terminal V unique (Vu) domain clustered in three regions is highly conserved in all members of the subfamily *Paramyxovirinae* and is characterized by a zinc-finger-like motif containing 15 aa residues involved in zinc binding [6]. Their conservation among paramyxoviruses indicates their importance for the structure and function of the V protein. In KNU-11, the C-terminus of the V protein had the well-conserved Vu domain at aa positions 171-221, including all seven cysteine residues and the motifs ^{171}H-R-R-E^{174} and ^{189}W-C-N-P^{192}. The presence of these domains implies a function for the V protein of KNU-11 similar to those of V proteins of other paramyxoviruses.

The matrix (M) gene is 1,370 nt in length, including a single ORF of 1,134 nt. The encoded protein is 378 aa long with a predicted molecular mass of 42.1 kDa and a pI of 9.46. The parainfluenza virus M protein is the most abundant and conserved virion structural protein and lines the inner surface of the virus envelope [15]. The M protein interacts with the cytoplasmic tails of membrane-associated proteins and the nucleocapsids and plays a pivotal role in virion assembly and release [17]. The levels of amino acid sequence identity to members of the genus *Rubulavirus* ranged from 38.3 % to 100 %.

The F gene of the KNU-11 strain was 1,718 nt in length with a single ORF of 1,656 nt beginning at position 28, capable of encoding a 551-aa protein. Although the length of the KNU-11 F gene was identical to that of a prototype strain of PIV5, it included a longer ORF and a shorter 5′ UTR (75 nt) than those of the prototype PIV5. The uncleaved F_0 protein of KNU-11 had a predicted molecular weight of 56.8 kDa and an estimated pI of 8.21. This was due to a natural mutation at position 1,589 that replaces the stop codon of the F gene with a triplet coding for serine and extends the ORF into the extragenic region. Thus, the KNU-11 F protein was found to be longer than that of PIV5 by 22 aa residues in the cytoplasmic tail domain, resulting in its molecular weight being higher than that of PIV5, as reported previously for SER virus [2, 39]. Like the F protein of other

paramyxoviruses, the F proteins of KNU-11 was predicted to be a type I membrane protein composed of an extracellular domain, a transmembrane (TM) region near the carboxyl terminus, and a 42-aa cytoplasmic tail. The F protein of paramyxoviruses mediates fusion of viral and cellular membranes for virus entry. Fusion activation is dependent on the intracellular cleavage of the F_0 protein into disulfide-like subunits (F_2-s-s-F_1) by the furin protease [26]. The consensus motif P-X-K/R-R is known to be the cleavage domain recognized by furin, which is conserved in the majority of the members of the subfamily *Paramyxovirinae* [12]. In the KNU-11 virus, the F cleavage motif was identified as RRRRR at aa positions 98 to 102, and cleavage appears to occur between residues R (102) and F (103), generating a cleaved F1 protein of approximately 46.5 kDa in size. A 20-aa hydrophobic fusion peptide is then located immediately following the predicted F cleavage site, which is highly conserved in all paramyxovirus F proteins [11]. In addition, the six conserved potential N-linked glycosylation sites (N65, N73, N352, N427, N431, N457) were identified in the F protein of KNU-11.

PIV5 contains an SH protein gene between the F and HN genes that is not present in all paramyxoviruses [17]. The SH protein of PIV5 is a type II membrane protein of 44 aa residues composed of a predicted 5-aa C-terminal ectodomain, a 23-aa TM domain, and an N-terminal 16-aa cytoplasmic region [10]. The genome of KNU-11 was also found to encode the SH gene, which is 292 nt in length and contains a single ORF 135 nt long, identical to that of PIV5. However, the SH protein shared the lowest similarity (74.4 %) with that of the prototype strain due to the high level of nucleotide sequence divergence. The most interesting nucleotide differences were observed in the predicted start and stop codons of the KNU-11 SH gene when compared to the ATG and TAA triplets of the PIV5 SH gene (Fig. 2). These were identified as ACG and CAA at the respective triplets, indicating that disruption of the open reading frame would likely lead to a lack of SH gene expression by KNU-11.

The HN gene was 1,876 nt in length with a single ORF beginning at position 68, coding for a 565-amino-acid protein. The HN protein of KNU-11 had a predicted molecular weight of 62.3 kDa and an estimated pI of 7.79. KNU-11 shared 98.3 % and 96.8 homology with a prototype PIV5 strain at the nucleotide and amino acid level, respectively. As a type II membrane glycoprotein, the major TM region of the KNU-11 HN protein was expected to extend from amino acid residues 17 to 37 of the protein. Predicted N-glycosylation sites were conserved in the HN protein as found in other members of the subfamily *Paramyxovirinae*. In KNU-11, potential N-linked glycan sites observed at all predicted sites (N110, N139, N267, N497 and N504) were the same as those in the prototype PIV5. Furthermore, the KNU-11 virus contained the conserved NRKSCS neuraminidase active site motif that has been identified in all analyzed members of the genera *Respirovirus* and *Rubulavirus* [18, 32].

The large polymerase (L) gene in KNU-11 is 6,810 nt long with a major 6,768-nt ORF encoding a 2,256-aa protein with a molecular mass of 255.9 kDa and a pI of 6.24. Since the L proteins of parainfluenza viruses are one of the major RNA polymerase components, they are involved in nucleotide polymerization, mRNA capping and methylation, and viral mRNA polyadenylation [17]. The L proteins of paramyxoviruses are divided into six highly conserved domains (domains I to VI) that appear to be independently responsible for each of its multiple functions [30]. Pairwise sequence alignment of the L protein of KNU-11 with those of other paramyxoviruses revealed the presence of the six domains in KNU-11 (data not shown). In addition, the highly conserved GDNQ motif, the active site for nucleotide polymerization [22, 32], was also present in domain III of the KNU-11 L protein at positions 772 to 775.

To establish genetic relationships, phylogenetic analysis was performed using the nucleotide sequences of the full-length genome, NP, P, or M protein of pPIV5 KNU-11 and other representative members of five genera of the family *Paramyxoviridae*. Our data demonstrated that all of the phylogenetic trees were similar and that KNU-11 is closely clustered phylogenetically in the genus *Rubulavirus* within the subfamily *Paramyxovirinae*. The result of a

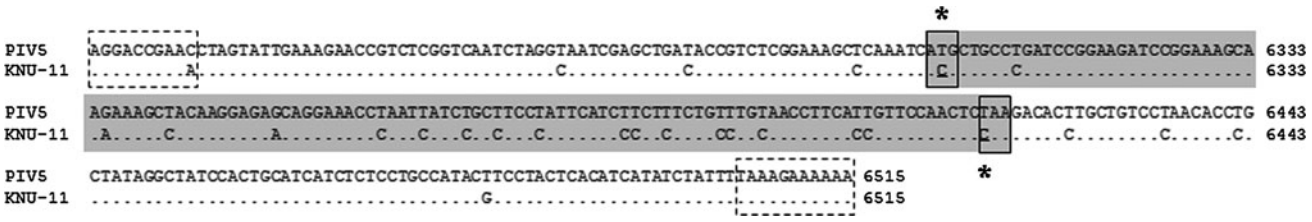

Fig. 2 Nucleotide sequence alignment of the SH genes of PIV5 and KNU-11. The numbers indicate the nucleotide position of the PIV5 genome. The conserved gene start and stop transcriptional regulatory sequences for the SH gene are indicated in dotted-line boxes, and the ORF of the SH gene is shaded. Translational start and stop codons of the SH genes from both viruses are shown in solid boxes, and mutations in the start and stop triplets of KNU-11 are indicated in underlined boldface type with an asterisk

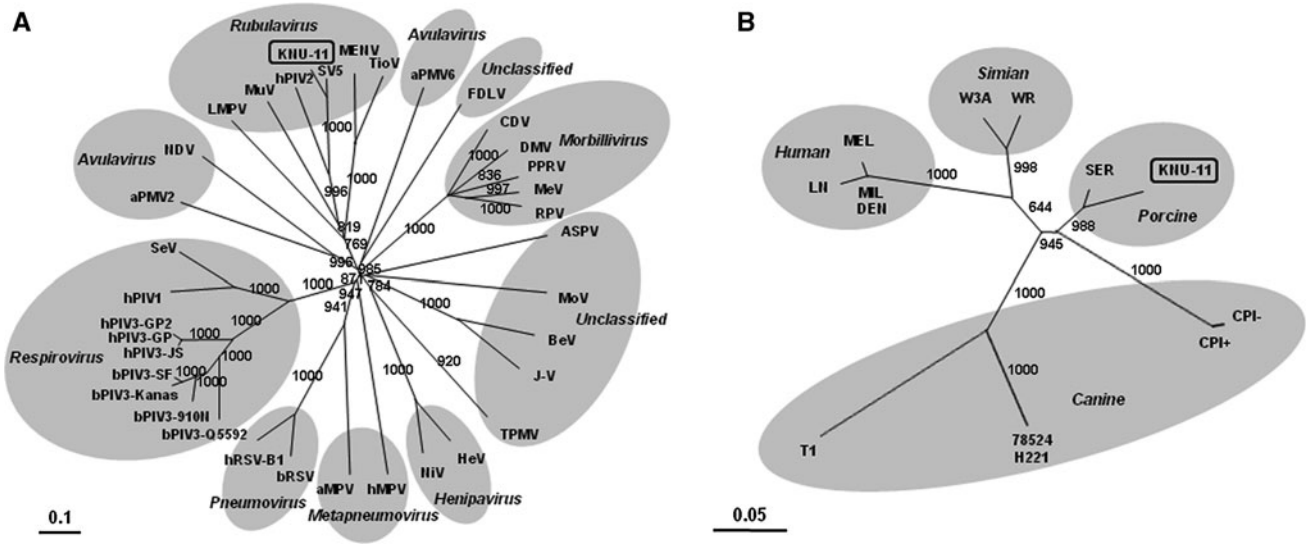

Fig. 3 Phylogenetic analysis using nucleotide sequences of the phosphoprotein (P) genes of 36 viruses belonging to the family *Paramyxoviridae* (**A**) and the fusion (F) gene sequences of 13 PIV5 isolates (**B**). Multiple sequence alignments were performed using the ClustalX program, and phylogenetic trees were constructed from the aligned nucleotide sequences using the neighbor-joining method. The numbers at each branch represent bootstrap values higher than 500 of 1000 replicates. The scale bars represent 0.1 inferred substitutions per site

phylogenetic study based only on the P proteins is shown in Fig. 3A. Phylogenetic analysis was further extended to the nucleotide sequences of F proteins from 13 other published PIV5 isolates (Fig. 3B). The F-gene-based phylogenetic tree revealed that the newly emerging PIV5 isolate is closely related to SER virus.

In the present study, the genome of the first Korean pPIV5 isolate, KNU-11, was fully sequenced in order to investigate its molecular characteristics. The entire length of the KNU-11 genome was determined to be identical to that of the prototype PIV5 genome. Nucleotide sequence comparison demonstrated that KNU-11 shared 84.4 to 99.3 % identity with PIV5 at the genome level. A novel finding of our genomic study was that unique nucleotide mutations are naturally present in both the start and stop codons of the KNU-11 SH gene, resulting in the potential absence of SH protein expression. This observation is further evidence that the SH protein is dispensable for paramyxovirus replication, as described previously [5, 8, 21]. Although the emergence of pPIV5 was first described in the late 1990s in Germany [9], its epidemiologic significance and other cases of pPIV5 have not been reported to date. Furthermore, despite being able to identify the presence of pPIV5 in the Korean pig industry, we did not elucidate the origin and prevalence of pPIV5, or its importance as a swine pathogen, in this study. Therefore, it is important that this novel virus be studied further to understand its prevalence in domestic pig populations as well as its association with porcine diseases, and accordingly, these issues are currently under investigation.

Acknowledgments This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2013R1A2A2A01004355) and Technology Development Program for Bio-industry, Ministry for Agriculture, Food and Rural Affairs, Republic of Korea (311007-05-1-HD120).

References

1. Baron MD, Barrett T (2000) Rinderpest viruses lacking the C and V proteins show specific defects in growth and transcription of viral RNAs. J Virol 74:2603–2611
2. Chatziandreou N, Stock N, Young D, Andrejeva J, Hagmaier K, McGeoch DJ, Randall RE (2004) Relationships and host range of human, canine, simian and porcine isolates of simian virus 5 (parainfluenza virus 5). J Gen Virol 85:3007–3016
3. Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, Ksiazek TG, Rollin PE, Zaki SR, Shieh W, Goldsmith CS, Gubler DJ, Roehrig JT, Eaton B, Gould AR, Olson J, Field H, Daniels P, Ling AE, Peters CJ, Anderson LJ, Mahy BW (2000) Nipah virus: a recently emergent deadly paramyxovirus. Science 288:1432–1435
4. Field HE, Breed AC, Shield J, Hedlefs RM, Pittard K, Pott B, Summers PM (2007) Epidemiological perspectives on Hendra virus infection in horses and flying foxes. Aust Vet J 85:268–270
5. Fuentes S, Tran KC, Luthra P, Teng MN, He B (2007) Function of the respiratory syncytial virus small hydrophobic protein. J Virol 81:8361–8366
6. Fukuhara N, Huang C, Kiyotani K, Yoshida T, Sakaguchi T (2002) Mutational analysis of the Sendai virus V protein: importance of the conserved residues for Zn binding, virus pathogenesis, and efficient RNA editing. Virology 299:172–181
7. Goyal SM, Drolet R, McPherson S, Khan MA (1986) Parainfluenza virus type 3 in pig. Vet Rec 119:363
8. He B, Leser GP, Paterson RG, Lamb RA (1998) The paramyxovirus SV5 small hydrophobic (SH) protein is not essential for virus growth in tissue culture cells. Virology 250:30–40

9. Heinen E, Herbst W, Schmeer N (1998) Isolation of a cytopathogenic virus from a case of porcine reproductive and respiratory syndrome (PRRS) and its characterization as parainfluenza virus 2. Arch Virol 143:2233–2239
10. Hiebert SW, Richardson CD, Lamb RA (1988) Cell surface expression and orientation in membranes of the 44-amino-acid SH protein of simian virus 5. J Virol 62:2347–2357
11. Horvath CM, Lamb RA (1992) Studies on the fusion peptide of a paramyxovirus fusion glycoprotein: roles of conserved residues in cell fusion. J Virol 66:2443–2455
12. Hosaka M, Nagahama M, Kim WS, Watanabe T, Hatsuzawa K, Ikemizu J, Murakami K, Nakayama K (1991) Arg-X-Lys/Arg-Arg motif as a signal for precursor cleavage catalyzed by furin within the constitutive secretory pathway. J Biol Chem 266:12127–12130
13. Hsiung GD (1972) Parainfluenza-5 virus. Infection of man and animal. Prog Med Virol 14:241–274
14. Janke BH, Paul PS, Landgraf JG, Halbur PG, Huinker CD (2001) Paramyxovirus infection in pigs with interstitial pneumonia and encephalitis in the united states. J Vet Diagn Invest 13:428–433
15. Karron RA, Collins PL (2007) Parainfluenza viruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE (eds) Fields Virology, 5th edn. Lippincott Williams & Wilkins, Philadelphia, pp 1497–1526
16. Kolakofsky D, Pelet T, Garcin D, Hausmann S, Curran J, Roux L (1998) Paramyxovirus RNA synthesis and the requirement for hexamer genome length: the rule of six revisited. J Virol 72:891–899
17. Lamb RA, Parks GD (2007) Paramyxoviridae: the viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE (eds) Fields Virology, 5th edn. Lippincott Williams & Wilkins, Philadelphia, pp 1449–1496
18. Langedijk JP, Daus FJ, van Oirschot JT (1997) Sequence and structure alignment of Paramyxoviridae attachment proteins and discovery of enzymatic activity for a morbillivirus hemagglutinin. J Virol 71:6155–6167
19. Lee CH, Yoo DW (2006) The small envelope protein of porcine reproductive and respiratory syndrome virus possesses ion channel protein-like properties. Virology 355:30–43
20. Li JG, Wang SY, Huang YM, Wang CY (2008) Full-length cDNA cloning and biological function analysis of a novel gene FAMLF related to familial acute myelogenous leukemia. Zhonghua Yi Xue Za Zhi 88:2667–2671
21. Li Z, Xu J, Patel J, Fuentes S, Lin Y, Anderson D, Sakamoto K, Wang LF, He B (2011) Function of the small hydrophobic protein of J paramyxovirus. J Virol 85:32–42
22. Malur AG, Gupta NK, De Bishnu P, Banerjee AK (2002) Analysis of the mutations in the active site of the RNA-dependent RNA polymerase of human parainfluenza virus type 3 (HPIV3). Gene Expr 10:93–100
23. Moreno-Lopez J, Correa GP, Martinez A, Ericsson A (1986) Characterization of a paramyxovirus isolated from the brain of a piglet in Mexico. Arch Virol 91:221–231
24. Murray K, Selleck P, Hooper P, Hyatt A, Gould A, Gleeson L, Westbury H, Hiley L, Selvey L, Rodwell B (1995) A morbillivirus that caused fatal disease in horses and humans. Science 268:94–97
25. Myers TM, Pieters A, Moyer SA (1997) A highly conserved region of the Sendai virus nucleocapsid protein contributes to the NP-NP binding domain. Virology 229:322–335
26. Ortmann D, Ohuchi M, Angliker H, Shaw E, Garten W, Klenk HD (1994) Proteolytic cleavae of wild type and mutants of the F protein of human parainfluenza virus type 3 by two subtilisin-like endoproteases, furin and Kex2. J Virol 68:2772–2776
27. Page RD (1996) Treeview: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358
28. Parisien JP, Lau JF, Horvath CM (2002) STAT2 acts as a host range determinant for species-specific paramyxovirus interferon antagonism and simian virus 5 replication. J Virol 76:6435–6441
29. Philbey AW, Kirland PD, Ross AD, Davis RJ, Gleeson AB, Love RJ, Daniels PW, Gould AR, Hyatt AD (1998) An apparently new virus (family Paramyxoviridae) infectious for pigs, humans, and fruit bats. Emerg Infect Dis 4:269–271
30. Poch O, Blumberg BM, Bougueleret L, Tordo N (1990) Sequence comparison of five polymerases (L proteins) of unsegmented negative-strand RNA viruses: theoretical assignment of functional domains. J Gen Virol 71:1153–1162
31. Poole E, He B, Lamb RA, Randall RE, Goodbourn S (2002) The V proteins of simian virus 5 and other paramyxoviruses inhibits induction of interferon-beta. Virology 303:33–46
32. Qiao D, Janke BH, Elankumaran S (2009) Molecular characterization of glycoprotein genes and phylogenetic analysis of two swine paramyxoviruses isolated from United States. Virus Genes 39:53–65
33. Randall RE, Bermingham A (1996) NP:P and NP:V interactions of the paramyxovirus simian virus 5 examined using a novel protein:protein capture assay. Virology 224:121–129
34. Sagong M, Park CK, Kim SH, Lee KK, Lee OS, du Lee S, Cha SY, Lee C (2012) Human telomerase reverse transcriptase-immortalized porcine monomyeloid cell lines for the production of porcine reproductive and respiratory syndrome virus. J Virol Methods 179:26–32
35. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
36. Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor
37. Thomas SM, Lamb RA, Paterson RG (1988) Two mRNAs that differ by two nontemplated nucleotides encode the amino coterminal proteins P and V of the paramyxovirus SV5. Cell 54:891–902
38. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality tools. Nucleic Acids Res 25:4876–4882
39. Tong S, Li M, Vincent A, Compans RW, Fritsch E, Beier R, Klenk C, Ohuchi M, Klenk HD (2002) Regulation of fusion activity by the cytoplasmic domain of a paramyxovirus F protein. Virology 301:322–333
40. Wansley EK, Parks GD (2002) Naturally occurring substitutions in the P/V gene convert the noncytopathic paramyxovirus simian virus 5 into a virus that induces alpha/beta interferon synthesis and cell death. J Virol 76:10109–10121