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Molecular characterizations of phosphoprotein of rabies virus circulating in Korea

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Abstract : Rabies is a major zoonotic disease that causes approximately 55,000 human deaths worldwide on an annual basis. The nucleocapsid protein and glycoprotein genes of the Korean rabies virus (RABV) have been subjected to molecular and phylogenetic analyses. Although the phosphoprotein (P) has several important functions in viral infection and pathogenicity, the genetic characterizations of the P of Korean RABV isolates have not yet been established. In the present study, we conducted genetic analyses of P genes of 24 RABV isolates circulating in the Republic of Korea (hereafter, Korea) from 2008 to 2011. This study revealed that the P genes of Korean RABVs are genetically similar to those of RABV strains of lyssavirus genotype I including V739 (dogs, Korea), NNV-RAB-H (humans, India), NeiMeng925 (raccoon dogs, China), and RU9.RD (raccoon dogs, Russia). Among Korean isolates, the RABV P genes showed low variability in the variable domains among Korean isolates; they had specific consensus sequences and amino acid substitutions capable of identifying geographic characteristics and retained specific sequences thought to be important for viral function. These results provide important genetic characteristics and epidemiological information pertaining to the P gene of the Korean RABV.

Keywords : molecular epidemiology, P gene, rabies virus

Introduction

Rabies is a fatal viral disease in animals and humans that each year causes approximately 55,000 human deaths worldwide [22]. Rabies can be transmitted by various host species such as bats, red foxes, skunks, raccoon dogs, dogs, wolves, and mongooses according to geographic region [11]. Prior to 1993, dogs were considered the main vector for transmission of the rabies virus (RABV) to humans or other animals in the Korea. Since 1993, the raccoon dog has been the main vector between domestic and wild animals.

The first case of rabies was reported in 1907. Since then, many rabies cases were reported in Korea up to 1945 [7]. The occurrence of rabies decreased dramatically to an average of 32 cases per year by 1984, with no case of rabies reported between 1985 and 1992 due to effective implementation of the RABV control program using live and inactivated vaccines [10]. However, since rabies was identified in a dog in Gyeonggi-do Province

in 1993, a few other rabies cases have been reported in Gyeonggi-do and Gangwon-do provinces each year [23]. Although a renewed national RABV control program that distributed bait vaccine was conducted in early 2000, rabies still occurs in some areas of Korea [23]. According to national data, no case was reported in Gyeonggi-do Province since 2007, whereas 18 rabies cases were reported in Gangwon-do Province (KAHIS program; Animal, Plant and Fisheries Quarantine and Inspection Agency, Korea).

The lyssavirus nucleocapsid protein (N) genes were divided into seven genotypes (GTs) by phylogenetic analysis: 1, rabies virus (RABV); 2. Lagos bat virus (LBV); 3. Mokola virus (MOKV); 4. Duvenhage virus (DUVV); 5. European bat lyssavirus (EBLV) group 1; 6. European bat lyssavirus (EBLV) group 2; and 7. Australian bat lyssavirus (ABLV) [1, 5].

The RABV contains approximately 12 kb of unsegmented negative-strand RNA for encoding the genes of the nucleocapsid protein (N), phosphoprotein

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(P), matrix protein (M), glycoprotein (G), and the polymerase (L) [11]. The N protein is involved in encapsidation of the genomic RNA and the formation of an active cytoplasmic ribonucleoprotein complex essential for viral replication [25]. The nucleotide (nt) sequence of the N gene has been used as a molecular marker to explain geographic characteristics of RABV on regional and global levels [19]. The G protein is critically important for attachment of the virus to the cell, pathogenicity, immunogenicity, and neurovirulence [11, 24]. The G gene has been used as another marker to study genetic diversity and antigenic typing because of having several major antigenic sites and relatedness with pathogenicity [24].

Along with the G protein, both the P and the M rabies proteins have been reported to be associated with the pathogenicity of RABV [12, 14]. The P protein forms a ribonucleoprotein (RNP) that wraps around the viral RNA along with the N and L proteins. It plays an important role in transcription and replication in conjunction with the L protein [11, 12, 14]. Moreover, the P protein counteracts the host's interferon responses in infected cells [2, 3].

The genetic characterization of RABV is considered important for developing diagnostic and preventive measures, including a more effective vaccine. Most recently, the genetic characterizations of the N and G proteins of Korean RABV were analyzed in comparison with those of other reported RABVs [24]. The N and G genes of 11 Korean RABV isolates collected from animals diagnosed with rabies between 2008 and 2009 were subjected to molecular and phylogenetic analyses [24].

Although the P protein has been shown to have several important functions in viral infection and pathogenicity, the genetic characterization of the P gene of Korean RABV isolates had not been analyzed. In the present study, we analyzed the epidemiological and genetic characteristics of the P gene of 24 RABV isolates circulating in Korea between 2008 and 2011.

Materials and Methods

Samples

Twenty-four isolates of RABV were obtained from brain samples collected from cattle, dogs, and raccoon dogs in Gangwon-do Province, Korea, from 2008 to 2011 (Table 1). These samples had previously been

Table 1. The P genes of Korean isolates detected and analyzed in this study

Isolate	Species of origin	Region	Year of isolation	Accession Number
KRVR0801	Raccoon dog	Sokcho	2008	JN786934
KRVC0802	Dog	Inje	2008	JN786930
KRVR0803	Raccoon dog	Sokcho	2008	JN786935
KRVR0804	Raccoon dog	Sokcho	2008	JN786936
KRVR0901	Raccoon dog	Goseong	2009	JN786937
KRVB0902	Cattle	Goseong	2009	JN786915
KRVB0903	Cattle	Hongcheon	2009	JN786916
KRVB0904	Cattle	Goseong	2009	JN786917
KRVB0905	Cattle	Inje	2009	JN786918
KRVR0906	Raccoon dog	Goseong	2009	JN786938
KRVB0907	Cattle	Inje	2009	JN786919
KRVB0908	Cattle	Goseong	2009	JN786920
KRVB0909	Cattle	Goseong	2009	JN786921
KRVB0910	Cattle	Goseong	2009	JN786922
KRVB1001	Cattle	Goseong	2010	JN786923
KRVB1002	Cattle	Goseong	2010	JN786924
KRVC1003	Dog	Goseong	2010	JN786931
KRVB1004	Cattle	Yangyang	2010	JN786925
KRVC1005	Dog	Goseong	2010	JN786932
KRVB1006	Cattle	Goseong	2010	JN786926
KRVC1007	Dog	Goseong	2010	JN786933
KRVB1008	Cattle	Goseong	2010	JN786927
KRVB1101	Cattle	Goseong	2011	JN786928
KRVB1102	Cattle	Inje	2011	JN786929

diagnosed as rabies-positive by the indirect fluorescence antibody test (FAT) using a monoclonal antibody against the N protein of RABV (Median Diagnostics, Korea).

RNA extraction and RT-PCR

Total RNA was extracted from the lysates of 24 samples using the Qiagen RNeasy Mini Kit (Qiagen, USA) according to the manufacturer's instructions. RT-PCRs were performed using a sequencing primer set for the P genes of 24 RABV isolates (Table 2). RT-PCR was conducted in a reaction mixture consisting of 10 μL of denatured RNA, 1 μL of each primer (50 pmol), 10 μL of 5× buffer (12.5 mM MgCl$_2$), 2 μL of dNTP mix, 2 μL of enzyme mix (reverse transcriptase and Taq polymerase), and 24 μL of distilled water (Qiagen, USA).

The cycling profile consisted of cDNA synthesis at 42°C for 30 min, followed by 35 cycles at 95°C for 45 sec, 50°C for 45 sec, and 72°C for 1 min, with a final extension at 72°C for 5 min. The PCR products were visualized using electrophoresis on 1.8% agarose gel containing ethidium bromide.

Cloning and DNA sequencing

RT-PCR products were purified using a QIAEX II Gel Extraction kit (Qiagen, USA) and were ligated with the pGEM-T easy vector (Promega, USA) according to the manufacturers' instructions. Plasmid DNA was isolated from amplified *Escherichia coli* (DH5α), and recombinant plasmids were identified by *EcoR*I enzyme digestion (Bioneer, Korea). DNA sequencing of the purified plasmids was carried out using an ABI 3730xl automated DNA sequencer (Applied Biosystems, USA).

Molecular characterization

P genes of the 24 Korean isolates were compared with those of the other RABV isolates (Table 3) submitted to NCBI using the DNAsis MAX DNA Basic module (MiraiBio, USA). Phylogenetic analyses were conducted using MEGA 4 [21]. Genetic distances were calculated using the Kimura 2-parameter correction at the nt level, and the phylogenetic tree was constructed using the neighbor-joining method with 1,000 bootstrap replicates. The nt and amino acid (aa) sequence similarities of P genes of the 24 Korean isolates were performed using the homology and distance matrices method of DNAMAN program (ver. 6.0, Lynnon BioSoft; Canada).

Results

The nts of 24 Korean RABV isolates were sequenced and all data were deposited in GenBank under accession numbers JN786915-JN786938. The P genes of all 24 isolates showed 894 nts encoding 297 aas, which is quite common for RABV isolates. The similarities of nt and aa sequences of the 24 Korean RABV P genes were found to be 98.9~100% and 98.6~100%, respectively, scoring high similarities regardless of the species of origin (Table 4).

The P gene open reading frame sequences of 24 Korean RABV isolates were compared with those of previously reported RABV isolates. Among the reported RABV isolates, the P genes of the Korean RABVs showed the highest nt and aa similarities (98.4~99.0% and 98.3~99.0%, respectively) with Korean dog RABV isolate V739 (Table 4). In addition, Korean RABVs showed high (> 95%) nt and aa similarities with NNV-RAB-H (humans, India), NeiMeng925 (raccoon dogs, China), and RU9.RD (raccoon dogs, Russia; Table 4).

Similar to previous reports [12], the phylogenetic tree of P gene nt sequences in this study showed two large genetic clusters (Chiroptera-related and Carnivora-related RABVs; Fig. 1). All 24 Korean RABVs were included in the Carnivora-related RABV cluster and were closely clustered with one another within the same lineage regardless of species origin. In addition, Korean isolates were most closely related with the V739 isolated from Korean dogs as well as being closely related to NeiMeng925 (raccoon dogs, China), RU9.RD (raccoon

Table 2. Oligonucleotide primers for amplifying the P genes of rabies viruses in this study

Primer name	Primer sequence (5'-3')	Region (nt)[*]	Size (bp)
RVPF1	GAGATCGCACATACGGAGAT	1321~1340	611
RVPR1	CTGGTCCCTGGAGGGAATCCT	1932~1908	
RVPF2	CAAATAGTCAGACAAATGA	1820~1838	833
RVPR2	AAGTTCCTCATGTTCTTCTTGC	2653~2631	

[*]The position of primers are based on the PV strain (GenBank accession no. M13215).

dogs, Russia), and NNV-RAB-H (humans, India). These findings are consistent with the similarities in nts. Finally, skunk (V211.SK and V216.SK) and raccoon dog (FL.RAC and NY.RAC) RABV isolates detected in the United States were included in the Chiroptera-related RABV cluster in this study, as previously reported [12] (Fig. 1).

Two conserved domains (CD1 and CD 2) and two variable domains (VD1 and VD2) have been identified based on aa sequence multiple alignments of RABV P gene (Fig. 2). Significant differences were not detected between the conserved and variable domains in the 24 Korean RABV P genes in this study (Fig. 2). However, specific consensus sequences of Korean RABVs lineage including V739, NeiMeng925, RU9.RD, and NNV-RAB-H in the phylogenetic tree were detected as KEPS (N/

Table 3. Reference rabies virus isolates used in this study

Isolate	Species of origin	Country	Accession Number	Isolate	Species of origin	Country	Accession Number
Mokola	Unknown	Unknown	Y09762	ONT1.RFX	Arctic fox	Canada	AF369265
ABL	Human	Australia	AF418014	ONT5.RFX	Arctic fox	Canada	AF369269
ERA	Vaccine strain	Germany	EF206707	ARC5.RFX	Arctic fox	Canada	AF369270
PV	Unknown	Unknown	M13215	V280.FX	European fox	France	AF369278
NNV-RAB-H	Human	India	EF437215	V660.FX	Middle east fox	Israel	AF369280
08022MAR/ 2007	Bovine	Morocco	GU798462	V265.BFX	Bat-eared fox	Tanzania	AF369296
I15.DG	Dog	India	AF369309	V213.SK	Coyote	U.S.A.	AF369289
I19.DG	Dog	India	AF369310	FT2891.DG	Coyote	U.S.A.	AF369308
IR5.DG	Dog	Iran	AF369311	V211.SK	Skunk	U.S.A.	AF369287
V027.DG	Dog	Tunisia	AF369322	V216.SK	Skunk	U.S.A.	AF369291
V113.DG	Dog	Sri Lanka	AF369320	KY2877.SK	Skunk	U.S.A.	AF369292
V121.DG	Dog	Nepal	AF369317	V037.MG	Mongoose	Botswana	AF369300
V118.DG	Dog	Sri Lanka	AF369321	V264.MG	Mongoose	South Africa	AF369302
V461.DG	Dog	Nigeria	AF369326	3694.MYO	*Myotis* sp. (bat)	Canada	AF369349
V464.DG	Dog	Nigeria	AF369328	V089.LBB	*Myotis lucifugus* (bat)	Canada	AF369343
V739	Dog	Korea	DQ275562	88BC1319BB	*Eptesicus fuscus* (big brown bat)	Canada	AF369341
M29.DG	Dog	Mexico	AF369313	89SK1461BB	*Eptesicus fuscus* (big brown bat)	Canada	AF369340
DRV-Mexico	Dog	Mexico	HQ450386	93ON0058BB	*Eptesicus fuscus* (big brown bat)	Canada	AF369338
ZAMRAV5100	Dog	Zambia	AB285215	V230.BBB	*Eptesicus fuscus* (big brown bat)	U.S.A.	AF369342
08056MAR/ 2005	Dog	Morocco	GU798489	M4.VB	Vampire bat	Canada	AF369366
08116MAR/ 2006	Dog	Morocco	GU798414	P4.VB	Vampire bat	Canada	AF369364
09011Ceuta/ 1991	Dog	Spain	GU798429	V077.SHB	*Lasionycteris noctivagans* (silver-haired bat)	Canada	AF369346
NeiMeng925	Raccoon dog	China	FJ415313	SHBRV-18	*Lasionycteris noctivagans* (bat)	U.S.A.	AY705373
RU9.RD	Raccoon dog	Russia	AF369284	V103.HB	*Lasiurus cinereus* (hoary bat)	Canada	AF369347
FL.RAC	Raccoon	U.S.A.	AF369294	6832.RB	*Lasiurus borealis* (red bat)	Canada	AF369351
NY.RAC	Raccoon	U.S.A.	AF369293	V235.FTB	*Tadarida brasiliensis* (free-tailed bat)	U.S.A.	AF369359

S) LGGVTTA encoded by aas 61~72 within the VD1 (Fig. 2). In addition, a specific substitution between VDI and VD2 was found in aa 91 of Korean RABV P genes (Fig. 2). This aa was substituted by Val_{91} in only Korean RABVs and was not found in other isolates such as PV (Leu_{91}) and V739 (Ile_{91}) (Fig. 2).

Phosphoacceptors related to protein kinase C (PKC) or RABV protein kinase in the P protein of the CVS strain were reported as Ser_{63}, Ser_{64}, Ser_{162}, Ser_{210}, and Ser_{271} [6]. All but phosphoacceptor Ser_{63} were retained in all Korean RABVs. Instead, Ser_{63} was substituted with Pro_{63} in all Korean RABVs (Fig. 2). The four N-terminally truncated P proteins could be synthesized due to translational initiation at internal Met_{20}, Met_{53}, Met_{69}, and Met_{83} in the CVS strain [4, 14]. Of the four aforementioned methionine residues, Met_{69} was substituted by Val_{69} in all Korean RABVs (Fig. 2).

The binding site for the cellular protein, cytoplasmic dynein light chain (LC8), was recently detected in P protein residues 139~151 (N'-RSSEDKSTQTTGR-C') of the PV strain of RABV and is thought to be involved in viral RNP axoplasmic transport along the microtubule network with the D_{143} and Q_{147} residues being critical in this interaction [9, 17, 18]. Also, the consensus sequence (K/R)XTQT has been reported to be the common target-acceptor of LC8 and is a conserved LC8 binding motif [13]. This consensus sequence was located within the VD2 region and strongly conserved in all Korean RABVs examined in this study (Fig. 2). Finally, the lysine-rich motif (N'-FSKKYKF-C'), an important component of C-terminal N protein-binding, was also conserved in aas 209~215 in all Korean RABVs (Fig. 2).

Table 4. The similarities (%) of nucleotide (nt) and amino acid (aa) sequences

Isolate	P genes of 24 Korean RABVs[*]		Isolate	P genes of 24 Korean RABVs[*]	
	nt similarities	aa similarities		nt similarities	aa similarities
Mokola	55.6~55.9	45.6~46.3	ONT1.RFX	90.4~90.9	92.2~92.9
ABL	69.0~69.4	72.9~73.9	ONT5.RFX	89.8~90.3	92.9~93.6
ERA	85.2~85.9	88.8~89.8	ARC5.RFX	89.5~90.0	92.9~93.6
PV	84.9~85.6	88.2~89.2	V280.FX	86.1~86.6	90.5~91.6
NNV-RAB-H	96.5~97.0	96.3~97.3	V660.FX	86.1~86.6	88.9~89.9
08022MAR/2007	86.9~87.4	91.2~91.9	V265.BFX	84.5~85.0	89.5~90.5
I15.DG	83.0~83.4	87.5~88.5	V213.SK	85.5~85.9	88.9~89.5
I19.DG	91.7~92.3	93.9~94.6	FT2891.DG	85.0~85.6	89.2~89.8
IR5.DG	86.6~87.1	90.2~91.2	V211.SK	78.9~79.3	84.1~84.8
V027.DG	86.3~86.9	91.9~92.9	V216.SK	78.9~79.3	84.1~84.8
V113.DG	83.4~84.0	89.5~90.2	KY2877.SK	84.7~85.3	89.8~90.8
V121.DG	91.5~91.9	94.2~94.9	V037.MG	83.8~84.3	87.1~88.1
V118.DG	83.3~83.8	89.2~89.8	V264.MG	83.3~83.8	85.5~86.5
V461.DG	83.3~83.9	88.9~89.5	3694.MYO	82.4~83.0	86.8~87.5
V464.DG	83.3~83.9	88.9~89.5	V089.LBB	81.9~82.5	86.1~87.1
V739	98.4~99.0	98.3~99.0	88BC1319BB	79.4~79.9	83.1~83.7
M29.DG	86.7~87.1	89.5~90.2	89SK1461BB	80.6~81.3	86.4~87.1
DRV-Mexico	86.8~87.2	90.8~91.5	93ON0058BB	80.6~81.1	85.8~86.4
ZAMRAV5100	85.5~86.0	90.2~91.2	V230.BBB	79.4~79.9	83.8~84.5
08056MAR/2005	86.2~86.8	90.5~91.5	M4.VB	78.6~79.5	82.8~83.4
08116MAR/2006	86.3~86.9	90.8~91.9	P4.VB	78.1~78.7	82.8~83.4
09011Ceuta/1991	86.9~87.4	90.8~91.9	V077.SHB	80.2~80.6	83.7~84.7
NeiMeng925	95.5~95.9	95.9~97.0	SHBRV-18	79.7~80.0	84.1~85.1
RU9.RD	96.5~97.0	96.3~97.3	V103.HB	78.5~78.9	82.4~83.4
FL.RAC	78.5~78.9	84.4~85.4	6832.RB	79.5~79.9	83.1~84.1
NY.RAC	78.1~78.5	83.1~84.1	V235.FTB	78.6~79.2	83.8~84.5

[*]The similarities (%) of nt and aa sequences of 24 Korean RABV P genes were found to be 98.9~100% and 98.3~100%, respectively.
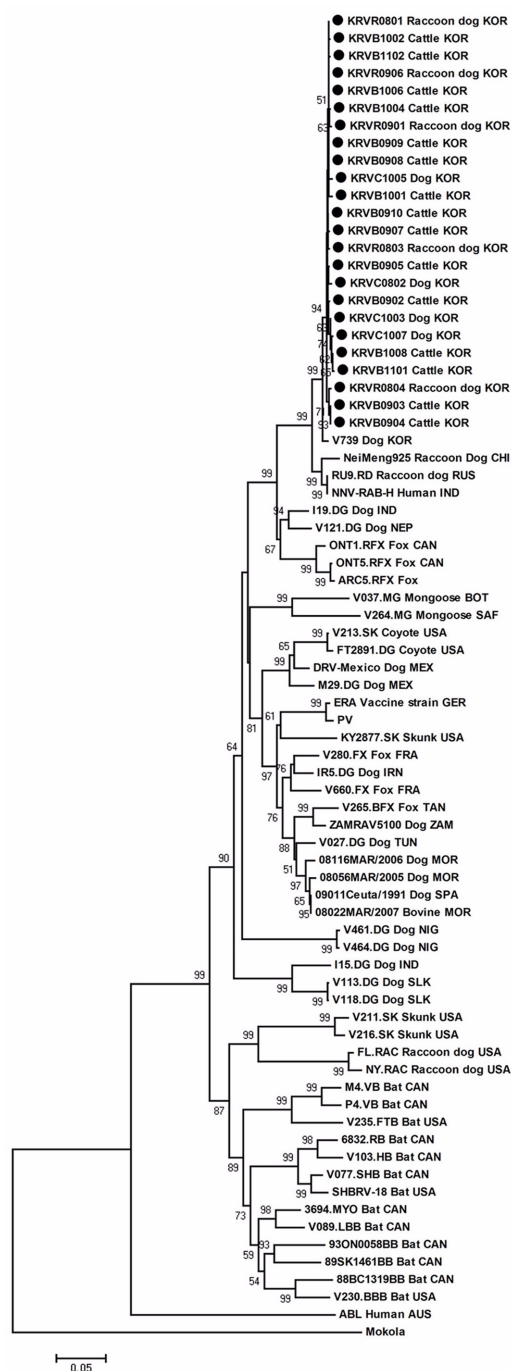
Fig. 1. Phylogenetic analysis based on the ORF nucleotide sequences of P gene of 24 Korean rabies virus (RABV) isolates with other RABV isolates submitted to NCBI GenBank. Mokola virus used as an outgroup. Genetic distances were calculated using the Kimura-2 parameter model at the nucleotide level and the phylogenetic tree was constructed using the neighbor-joining method with 1,000 bootstrap replicates using MEGA 4. Bootstrap values above 50 are shown. The abbreviations of countries are as follows: AUS, Australia; BOT, Botswana; CAN, Canada; CHI, China; FRA, France; GER, Germany; IND, India; IRN, Iran; ISR, Israel; KOR, Korea; MEX, Mexico; MOR, Morocco; NEP, Nepal; NIG, Nigeria; RUS, Russia; SAF, South Africa; SLK, Sri Lanka; SPA, Spain; TAN, Tanzania; TUN, Tunisia; USA, United States; ZAM, Zambia.
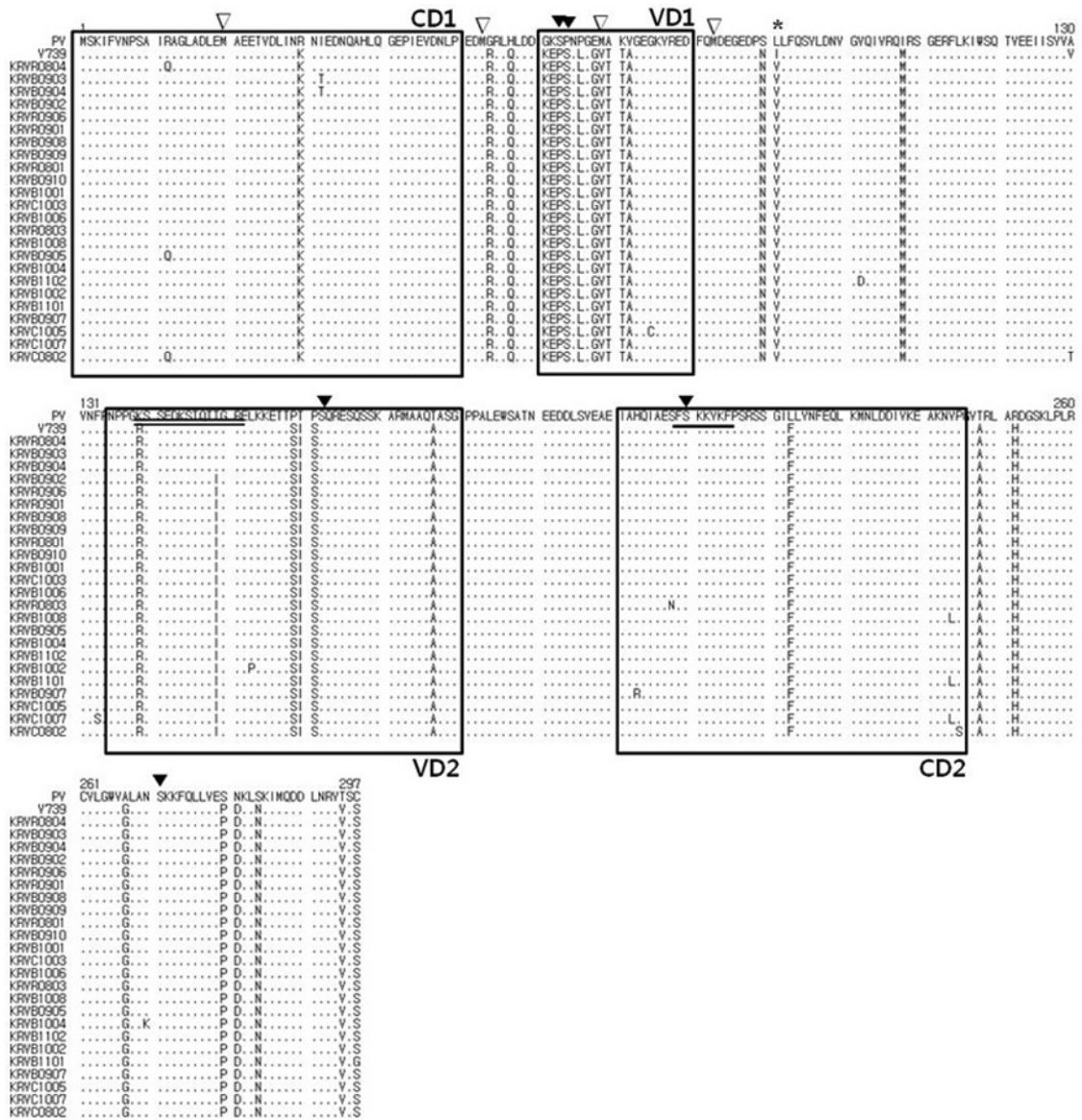
Fig. 2. Comparison of P protein amino acid sequences of 24 Korean RABVs with those of PV strain and V739 (Korean dog) isolate. Only different amino acids from the PV sequence are indicated and dots indicate amino acids that are in agreement with the sequence of PV strain. Boxes with CD1 and CD2 indicate conserved domains, while boxes with VD1 and VD2 delimitate variable domains. Black triangles indicate the positions of serine residues identified as phosphoacceptors in the P protein of the CVS strain. White triangles indicate the positions of the four methionines used for internal translation initiation in the CVS strain. Asterisk indicates specific amino acid substitutions of P genes of only 24 Korean RABVs found in amino acid 91 (valine). The lysine-rich motif (FSKKYKF) and LC8 binding motif (RSSEDKSTQTTGR) were shown as continuous and double-continuous underlining, respectively.

Discussion

In the P gene, intragenotypic nt and aa similarities of 73.5% and 79.3%, respectively, were indicated while intergenotypic similarities between RABV and other virus GTs ranged from 65.9% and 69.7% (for ABLVs) down to 56% and 45.5% (for MOKVs) [14]. Among previously reported RABV isolates, the P genes of the

Korean RABVs showed the highest nt and aa similarities (95.5~99.0% and 95.9~99.0%, respectively) with V739 (dogs, Korea), NNV-RAB-H (humans, India), NeiMeng 925 (raccoon dogs, China), and RU9.RD (raccoon dogs, Russia) of GT I of lyssavirus.

In the phylogenetic tree, we found all 24 Korean RABV isolates to be categorized in the Carnivora-related RABV cluster. All 24 were closely clustered together within the same lineage regardless of species origin. The Korean RABV isolates of this study were most closely related to the V739 isolated from Korean dogs, as well as being close to NeiMeng925 (raccoon dogs, China), RU9.RD (raccoon dogs, Russia), and NNV-RAB-H (humans, India).

Based on the nt sequences of the N and G genes, the Korean RABV isolates were confirmed as GT I of lyssavirus, were clustered into four distinct subgroups (Gangwon I, II, III, and Gyeonggi) with high similarities. The isolates were most closely related to the NeiMeng1025B (raccoon dogs, Eastern China), and 857r (raccoon dogs, Russia) [24].

Similar to the N and G genes, P gene sequence analyses revealed that all of the Korean isolates were classified into GT I of the lyssavirus. However, unlike the N and G genes, the P gene was not separated into distinct subgroups because the 24 isolates were collected only from Gangwon-do Province. In the phylogenetic tree, P genes of Korean RABV isolates were most closely related with NeiMeng925 (raccoon dogs, China) and RU9.RD (raccoon dogs, Russia). These findings are consistent with the results found for the N and G genes. Overall, the Korean RABV isolates show genetic closeness with RABV strains from Northeastern Asia (China and Russia) and are pathogenic in several hosts including dogs, raccoon dogs, and cattle.

Previous reports have suggested that two conserved domains (CD1 and CD2) and two variable domains (VD1 and VD2) have been identified based on aa sequence multiple alignments of the RABV P gene [14, 15]. The variable domains located on the surface structure of the P protein are believed to be involved in host/viral interactions and adaptation to the host environment, and are expected to be useful in elucidating the adaptive evolution of the rabies virus [12, 14, 16]. However, the VD and CD of the P genes among the 24 Korean RABV isolates examined in the current study were found to be highly conserved. Prior to the current findings, a commonly held belief was that variants of Korean RABV

in the variable domains occurred at low frequencies. We found that the Korean RABV maintained a conserved sequence of the P gene during transmission.

In addition, specific consensus sequences of Korean RABVs lineage including V739, NeiMeng925, RU9.RD, and NNV-RAB-H in the phylogenetic tree were detected as KEPS(N/S)LGGVTTA encoded by aas 61~72 within VD1. Moreover, a specific substitution was found in aa 91 of Korean RABV P genes between VD1 and VD2. These findings suggest that the specific consensus sequences and aa substitution using P genes are helpful in identifying epidemiological characteristics like geographic origins of Korean RABVs lineage or Korean isolates.

Of the five serine phosphoacceptors identified for the CVS strain, Ser_{210} and Ser_{271} within the PKC phosphoacceptor were conserved in all lyssavirus GTs (1~7) [12, 14]. The phosphoacceptors of Ser_{64}, Ser_{162}, Ser_{210}, and Ser_{271} were retained in all Korean RABVs, while Ser_{63} was substituted with Pro_{63} in Korean RABVs. Variation in P phosphorylation patterns, especially within the N-terminal region, is predicted and might form the basis of subtle differences in viral-host interactions [14]. Further analyses of phosphorylation patterns would likely offer explanations for the function of potential variations.

Four N-terminally truncated P proteins could be synthesized due to four methionine residues located in-frame of single P protein sequences in the CVS strain. In particular, approximately one-half of the RABVs examined, along with the GT 4 lyssaviruses, retained Met_{69} [4, 14]. In addition, the Met_{20} is retained in all lyssavirus GTs [14]. In this study, among the four methionine residues, Met_{69} is substituted with Val_{69}, and Met_{20} is retained in all Korean RABVs. Further studies are needed to identify the biological characteristics and potential functions of truncated P proteins synthesized by methionine residues.

The binding motif for cytoplasmic dynein light chain (LC8) involving viral RNP axoplasmic transport along the microtubule network was recently found in P proteins of the PV strain [8, 17, 18]. The consensus sequence (K/R)XTQT of the P gene was thought to be a conserved motif and the common binding site of LC8 [13]. Although consensus sequence was located within the VD2 region, it is still strongly conserved in all Korean RABVs examined in the current study. In addition, the lysine-rich motif (N'-FSKKYKF-C')

identified as an important C-terminal component for N protein binding [9], was also conserved in aas 209~215 in all Korean RABVs. These findings suggest that the functional binding sites of the P gene are conserved in Korean RABV. This conservation of GTs is clearly functionally important for Korean RABV to transport viral RNP with LC8 and to bind the N protein, which are essential for RABV pathogenicity and replication.

In conclusion, this study revealed that the P genes of Korean RABVs are genetically close to those of RABV strains of the lyssavirus GT I from Northeastern Asia, including V739 (dogs, Korea), NNV-RAB-H (humans, India), NeiMeng925 (raccoon dogs, China), and RU9.RD (raccoon dogs, Russia). The P genes of Korean RABVs showed low variability in the variable domains; they had specific consensus sequence and aa substitution capable of identifying geographic characteristics and retained specific sequences thought to be important for viral function in the P genes of RABV. This study will be helpful in understanding the genetic characteristics and viral functions of Korean RABV on the basis of the P gene.

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