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Molecular characterization of full-length genome of Japanese encephalitis virus (KV1899) isolated from pigs in Korea

Dong Kun Yang[1,*], Byoung Han Kim[1], Chang Hee Kweon[1], Jun Hun Kwon[1], Seong In Lim[1], Hong Ryul Han[2]

[1]*National Veterinary Research and Quarantine Service, Ministry of Agriculture and Forestry, Anyang 430-824, Korea*
[2]*Department of Veterinary Internal Medicine, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea*

We have determined the complete nucleotide and deduced amino acid sequences of the Japanese encephalitis virus (JEV) strain KV1899, isolated from a fattening pig in Korea. In comparison with 22 fully sequenced JEV genomes currently available, we found that the 10,963-nucleotide RNA genome of KV1899 has a 13-nucelotide deletion in the 3' non-translated variable region and 53 unique nucleotide sequences including 3' non-translated region (NTR). Its single open reading frame has a total of 28 amino acid substitutions. Comparison of the KV1899 genomic sequence with those of the 21 fully sequenced JEV strains in published databases showed nucleotide homology ranging from 97.4% (Ishikawa strain) to 87.0% (CH2195 strain). Amino acid homology with KV1899 strain ranged from 96.4% (K94P05) to 91.0% (GP78). The KV1899 showed the highest nucleotide homology with Ishikawa strain and the highest amino acid homology with K94P05. We performed an extensive E gene based phylogenetic analysis on a selection of 41 JEV isolates available from the GenBank. Compared with Anyang strain, isolated from a pig in 1969, that is current live vaccine strain for swine in Korea, the homology of nucleotide sequence in envelope gene was only 87.1%. The prM gene of the isolate was closely related with those of Ishikawa and K94P05 strains, which were grouped into genotype I of JEV.

Key words: Japanese encephalitis virus, Complete genome sequence, Phylogenetic analysis

Introduction

Japanese encephalitis virus (JEV) is a member of the family *Flaviviridae*, genus *Flavivirus* containing a single open reading frame (ORF) encoding a polyprotein approximately 11 kb in length [3]. Its RNA is capped at the 5'-end and unpolyadenylated at the 3'-end. The polyprotein is co- or post-translationally processed into structural and non-structural proteins. It has three structural (C, M: a mature form of its precursor protein prM, E) and at least seven non-structural proteins, designated NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. Recently, based on analysis of highly variable nucleotide sequence in the prM, E and 3' NTR gene, several authors classified a number of JEV into 4 genotypes [1,4,19,27]. Since the JEV (Nakayama strain) was first isolated from human brain in 1935 Japan, a number of geographically diverse JEV strains have been isolated at different time from several sources and a lot of JEV isolates have been partially sequenced [33]. Previous phylogenetic analyses have mainly focused on partial sequences derived from either the prM or E gene. On the basis of prM gene, JEV strains can be subdivided into four genotypes [2,4,5]. Similar studies have been made with E gene [16,18,20]. Recently, Yun *et al*. compared the Korean strain K87P39 with 27 fully sequenced JEV genomes [33].

Several Korean isolates of JEV were isolated from circulating *Culex* mosquitoes in the 1980s and 1990s and their genomes were partially or completely sequenced [6,19,33]. In veterinary science, Anyang strain was obtained from piglets in 1969 [12], and partially sequenced over the structural region [22]. Nam *et al*. reported that the optimal pH of Korean strains was different compared with that of the Nakayama NIH in hemaglutination test [19]. Nakayama or Beijing-1 vaccine might not be effective in epidemic areas where antigenically different strain prevail [2,32]. Therefore, we need more detailed genetic information at the molecular level on the recent Korean isolates in pigs especially.

To investigate molecular characteristics of Korean JEV strain, KV1899 isolated from a fattening pig in 1999, we determined its complete nucleotide sequence. In addition, we have tried to characterize the KV1899 strain at the molecular level and to establish its relationships to the other fully sequenced JEV strains. So, we have discussed the genetic relationship of the KV1899 strain to the other 41 JEV strains isolated from different geographic regions world wide at different time periods.

*Corresponding author
Phone: 82-31-467-1794; Fax: 82-31-467-1797
E-mail: yangdk@nvrqs.go.kr

Materials and Methods

Preparation of virus

The JEV KV1899 strain was isolated from a fattening pig in Korean province of Gyeonggi in 1999. The virus was propagated in Vero cell and the infected culture fluid was frozen and thawed three times. After centrifugation, the supernatant was stored at −70°C until use.

Reverse transcription polymerase chain reaction (RT-PCR) and sequencing of the JEV KV1899 genomic RNA

Viral genomic RNA was extracted from 300 μl of infected culture fluid by using RNA isolation reagent (Ultraspec II, Houston, USA) according to the manufacturers instruction. The precipitated RNA was dissolved in DEPC-treated water and stored at −70°C until use. The extracted RNA was denaturated at 95°C for 5 min. The denaturated RNA was incubated at 50°C for 50 min to obtain the first strand cDNA synthesis using reverse transcriptase reaction with primer. Oligonucletide primers (Table 1) were selected on the basis of the submitted sequence for the K94P05 strain [19]. PCR amplification was carried out in 30 cycles using denaturation at 95°C for 45 sec, annealing at 50°C for 45 sec and extension at 72°C for 60 sec using a thermal cycler (Whatman T gradient, Geottingen, Germany). The final extension step was done at 72°C for 5 min. The PCR products were detected by electrophoresing 15 μl in 1.5% agarose gels (GibcoBRL, New York, USA) containing 0.1 μg/ml of ethidium bromide and TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 7.5). The gels containing nucleic acid bands with expected size were excised and the DNAs were eluted using Gene purification system (Qiagen, Miami, USA). The DNAs were then ligated directly into TA cloning vector system (Promega, Madison, USA) and used to transform competent *Escherichia coli* strain, DH5α, following the manufacturer's instruction. The recombinant colonies were screened by blue-white color reaction on X-gal containing plates and the DNA inserts were confirmed by *Eco* RI digestion. After extracting plasmid from recombinant colony, sequencing reactions were performed on the plasmid DNAs as recommended in the ABI PRISM Big Dye Terminator Cycle Sequencing reaction kit (Perkin-Elmer Applied Bio system Inc., New Jersey, USA). The products were analyzed using an automated Applied Biosystems 377 DNA sequencer according to the manufacturers recommendations. Both directions of the DNA were sequenced to verify the sequences.

Table 1. Oligonucleotide primers for PCR amplification

Primer name	Oligonucleotide sequence (5-3)	Orientation
JEN(1-20)*	TGT GTC AAC TTC TTG GCT TA	Sense
JEN(520-539)	GCT TGC AAT GTC CGT GTT GT	Antisense
JEM(429-448)	ATC ATG TGG CTC GCA AGC TT	Sense
JEM(1,029-1,048)	TCC TTC TAG CAC CAA GTA CA	Antisense
JEE(960-979)	GTC GCT CCG GCT TAC AGT TT	Sense
JEE(2,482-2,501)	GAT GTC AAT GGC ACA GCC GT	Antisense
JENS(2,478-2,498)	GAC ACT GGA TGT GCC ATT GAC	Sense
JENS(3,512-3,533)	AGC ATC AAC CTG TGA TCT GAC G	Antisense
JENS(3,481-3,500)	TCA GAC CTG TTA GGC ATG AT	Sense
JENS(4,421-4,440)	CTA GCC TCC GGC TGC TTC CT	Antisense
JENS(4,381-4,400)	GGG CTG CCG ATA TCA GCT GG	Sense
JENS(5,351-5,370)	TTC CCT GAT GCT CCC TCT GC	Antisense
JENS(5,313-5,322)	TTG AGA GGA CTC CCA GTA CG	Sense
JENS(6,270-6,289)	CTG TCA GTG TAC TGA ATG CC	Antisense
JENS(6,171-6,190)	GGA GAG TAC CGT TCT AGA GG	Sense
JENS(7,041-7,060)	ACT GTG CTC CCC CCA TAC AG	Antisense
JENS(6,981-7,000)	CCG GAT TGC CAA GCA TGG CA	Sense
JENS(7,981-8,000)	CGC CCC ACC TTT CGT GTA CC	Antisense
JENS(7,921-7,940)	GCG GGC GCG GAA GCT GGA AC	Sense
JENS(8,931-8,950)	CAG GCC GTG CTC CAT TGA TT	Antisense
JENS(8,891-8,910)	CAA CAG CAA CGC GTC TCT CG	Sense
JENS(9,901-9,920)	TCC TGG GGA GAT GCG CGC CC	Antisense
JENS(9,861-9,880)	CTA CTC GTC CCG TGC AGA GG	Sense
JENS(10,944-10,963)	AGA TCC TGT GTT CTT CCT CA	Antisense

*Numbers in parenthesis indicate the nucleotide sequence of K94P05 strain.

Table 2. Japanese encephalitis virus strains used in this study

Nation	Gene type	Strain	Isolated year	Source	GenBank No
Australia	II	**Fu***	1995	Human serum	AF217620
China	III	**SA14**	1954	Mosquito	U14163
China	III	**SA14-14-2**	1954	SA-14 derivative	AF315119
China	III	**Beijing-1**	1949	Human brain	L48916
China	III	P3	1949	Mosquito	U47032
China	III	SA14-2-8	1954	SA-14 derivative	U02367
India	III	**GP78**	1978	Human brain	AF075723
India	III	**P20778**	1958	Human brain	AF080251
Indonesia	IV	JKT7003	1981	Mosquito	U70408
Indonesia	II	JKT5441	1981	Mosquito	U70406
Indonesia	II	JKT6468	1968	Mosquito	U70407
Indonesia	II	JKT1749	1979	Mosquito	U70405
Indonesia	IV	JKT9092	1981	Mosquito	U70409
Japan	III	**JaOArs982**	1982	Mosquito	M18370
Japan	I	**Ishikawa**	1998	Mosquito	AB051292
Japan	III	**JaGAr01**	1959	Mosquito	AF069076
Japan	III	Kamiyama	1966	Human brain	S49265
Japan	III	Nakayama	1395	Human brain	U70413
Japan	III	JaNAr516	1999	NA**	AB028270
Japan	III	Oita100	1999	NA	AB028269
Japan	III	JaOH0566	1997	NA	AY029207
Korea	I	**K94P05**	1994	Mosquito	AF045651
Korea	I	**KV1899**	1999	Pig	AY316157
Korea	III	K87P39	1987	Mosquito	U34927
Korea	III	K82P01	1982	Mosquito	U34926
Korea	III	Anyang	1969	Pig	Unpublished
Korea	I	K91P55	1991	Mosquito	U34928
Malaysia	II	WTP7022	1970	Mosquito	U70421
Taiwan	III	**T1P1**	1997	Mosquito	AF254453
Taiwan	III	**CH2195LA**	1994	NA	AF221499
Taiwan	III	**CH1392**	1990	Mosquito	AF254452
Taiwan	III	**RP-2ms**	1985	Mosquito	AF014160
Taiwan	III	**RP-9**	1985	Mosquito	AF014161
Taiwan	III	**Ling**	1972	Mosquito	U70396
Taiwan	III	**YL**	NA	NA	AF486638
Taiwan	III	**TC**	NA	Mosquito	AF098736
Taiwan	III	**TL**	NA	Mosquito	AF098737
Taiwan	III	**HV1**	NA	Mosquito	AF098735
Taiwan	III	T263	1996	NA	U44972
Thailand	I	ThCMAr4492	1992	Mosquito	D45360
Thailand	I	ThCMAr6793	1963	Mosquito	D45363

*fully sequenced JEV strains are indicated in bold type.
**NA: Not available.

Multiple alignments and phylogenetic analysis

The JEV strains used in multiple alignments and phylogenetic analysis are shown in Table 2. This list includes the 22 JEV strains for which complete sequences are presently available from the NCBI nucleotide sequence databases. In the case of the viral E gene, our analysis was performed with a total of 41 strains that are available in the GenBank.

Multiple sequence alignments and sequence similarity calculations between aligned nucleotide and amino acid

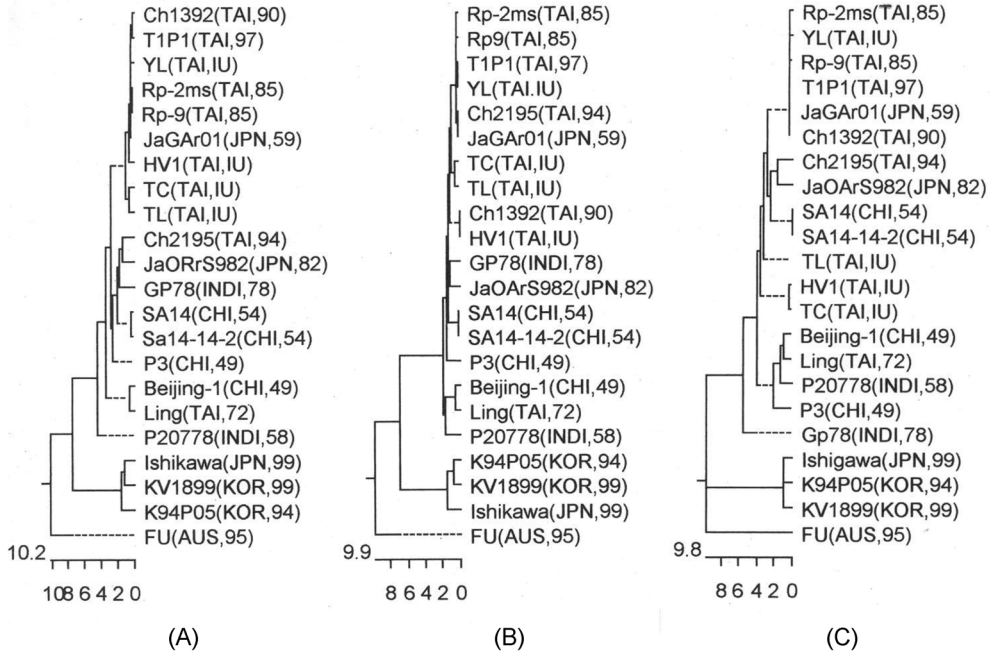

Fig. 1. Phylogenetic trees based on the full-length genome (A), the E gene (B), and prM (C) of all 22 available JEV strains. The multiple sequence alignments were obtained by CLUSTAL method and trees were constructed by the neighbor-joining method. Trees were drawn with DNASTAR software. Taiwan (TAI), Japan (JPN), India (INDI), Thailand (THA), China (CHI), Korea (KOR), Indonesia (INDO), Malaysia (MAL) and Australia (AUS).

sequences were performed using computer software program (DNASTAR Inc., Madison, WI, USA). Phylogenetic trees were reconstructed on aligned nucleotide sequences by using the Clustal X method.

Results

Full-length nucleotide and deduced amino acid sequence analysis

We determined the complete nucleotide sequence of KV1899 isolated from a pig to investigate molecular characteristics. The complete sequence of KV1899 determined in this study was deposited in GenBank (accession number AY316157). The RNA genome of KV1899 was 10,963-nucleotide in length and consisted of a 95-nucleotide 5' NTR followed by a 10,299-nucleotide single ORF and terminated by a 569-nucleotide 3' NTR. The length of the deduced amino acid sequence of KV1899 was 3,433 residues.

We compared the whole KV1899 genomic sequence with sequences of all 21 JEV strains available in GenBank (Table 2) to characterize the molecular structure of KV1899 genome and to determine how it is related to other fully sequenced JEV strains. Several candidate strains isolated from the same country in the same year showed very high levels of nucleotide homology. So, a representative sequence was selected for analysis in the manner previously described [31]. Comparison of the KV1899 genomic sequence with 21 fully sequenced JEV strains in published databases showed nucleotide homology ranging from 97.4% (Ishikawa strain) to 87.0% (CH2195 strain). Amino acid homology with KV1899 strain ranged from 96.4% (K94P05) to 91.0% (GP78). KV1899 showed the highest nucleotide homology with Ishikawa strain and highest amino acid homology with K94P05 (Fig. 1).

No unique nucleotide change was found in the 5' NTR of the isolate. Compared with the other 21 JEV strains, a total of 28 unique amino acid differences were found in full genome of the KV1899 strain, but there was no unique change in E gene (Table 3). However, the 13 base-pair deletion immediately downstream of the ORF stop codon was found in the Ishikawa, K94P05 as well as KV1899 strains. This deletion was considered a potential molecular fingerprint [17]. The 11 base pair deletion in 3' NTR was found in FU strain, but not found in any other JEV sequences.

For nucleotide sequences analysis of the E protein gene, the complete nucleotide sequence of the E protein gene of the isolate was aligned and compared with temporally and geographically diverse JEV strains. The nucleotide differences were scattered throughout the length of the gene and there was no particular region of hyper-variability. The nucleotide sequences of the KV1899 isolate revealed 81.1 to 98.5% nucleotide identity (average divergence 11.0%) with other published JE virus strains. This result indicated that the isolate KV1899 is similar to K94P05, a strain isolated from a

Table 3. Amino acid substitutions in the JEV KV1899 strain relative to available full-length JEV genomes

Protein	Amino acid Position	Amino acid Substitution*	Protein	Amino acid position	Amino acid substitution
C	32	Arg-Lys	NS4	2,163	Gly-Ser
	111	Ser-Thr		2,192	Gly-Arg
prM/M	236	Glu-Lys		2,205	Gly-Glu
	243	Lys-Arg		2,343	Thr-Pro
NS1	987	Ala-Thr		2,469	Thr-Ser
	992	Leu-Val		2,473	Glu-Lys
	1,006	Leu-Arg		2,786	Glu-Lys
	1,012	Gly-Glu	NS5	2,820	Glu-Gly
	1,109	Ser-Asn		3,068	Thr-Pro
NS2	1,311	Leu-Phe		3,109	Lys-Gln
NS3	1,547	Glu-Gln		3,111	Val-Phe
	1,629	Ala-Thr		3,153	Leu-Phe
	1,945	Glu-Lys		3,158	Glu-Lys
	1,946	Gly-Glu		3,159	Ala-Val

*Amino acid substitutions were based on the consensus sequence of the 22 full-length genomes.

mosquito pool in Korea in 1994 (98.5% nucleotide identity). Because Anyang strain is the current vaccine strain for swine, we attempted to compare this strain with the new isolate. The homology of the envelope gene nucleotide sequence between Anyang and KV1899 strain was only 87.1%.

The E protein of the isolate contained 12 highly conserved cysteine residues that form six disulfide bridges [21], and the RGD (Arg-Gly-Asp) motif at position E387-389 was also conserved in the KV1899 as well as other strains [15,24]. The strain, KV1899 showed 95.3-99.5% amino acid identity with other published strains. Thus, it again indicated that the KV1899 was most distantly related to JKT7003 strain (95.3% identity), whereas it was also very closely related to ThCM6793 strain (99.5% identity).

For further genotype determination, 240 nucleotide sequences in the prM gene were analyzed from the isolate. The isolate was found to possess similar nucleotide sequence with the reference Ishikawa and ThCMAr4492 strain. In order to find out any similarity among new isolate and JE virus genotypes as reported by Chen *et al.* [4] homology comparison was carried out between their sequences. The results revealed that this new isolate possessed the highest nucleotide sequence homology with other strains of the genotype I with 98.0 to 98.7%, and amino acid sequence homology between 98.7 to 100% (Table 4). The homology to genotype II and III strains was similar: genotype II, 84.5-86.2% in the nucleotide and 91.2-93.7% in the amino acid; genotype III, 82.5-86.2% in the nucleotide and 91.2-96.2% in amino acid. In contrast, homology to genotype IV strains appeared relatively lower, 74.5-76.2% in the nucleotide and 82.5-87.5% in amino acid, respectively.

A region of 100 nucleotides immediately downstream of the open reading frame stop codon of KV1899 showed high sequence variability when compared with other 21 JEV isolates (Fig. 2). Deletions in the 3' NTR make KV1899 strain getting shorter sequence in genomes between KV1899 and other JEV strain. KV1899, FU, Ishikawa and K94P05 strains showed similar form just downstream of the stop codon in 3' NTR.

Phylogenetic analyses

To better understand the genetic relationships and evolution of JEV strains, we performed a phylogenetic analysis of the 22 fully sequenced JEV strains, including the KV1899 strain. Construction of a phylogenetic tree revealed that there were two distinct phylogenetic groups of JEV with nucleotide divergence ranging from 12.7 to 14.2% (Fig. 1). One major cluster included the Korean K94P05, Australian FU, Japanese Ishikawa and KV1899 strain. The other major group consisted of 17 other closely related JEV strains and branches into several minor subgroups. Interestingly, the Korean KV1899 isolate was distantly related to Japanese representative immunotype JaGAr01 isolate.

Since the original identification of JEV in 1935, a lot of JEV strains have been reported from different regions at different times. To find out the genetic relationships of KV1899 strain, we performed phylogenetic analyses of individual virus genes from the 41 sequenced JEV strains that represented a 50 year time span. These analyses showed that the phylogenetic tree based on the E gene corresponded well with the tree based on the full-length genome, with a minor difference (Fig. 1). In the E gene-based phylogenetic tree, KV1899 strain was still closely related to that of K94P05 strain. As shown in Fig. 3, the E gene based phylogenetic tree constructed with 41 JEV isolates consisted

Table 4. Homology comparison of nucleotide and amino acid sequences of prM, E and full length gene of KV1899 with reference strains

Strain	Nucleotide			Amino acid		
	prM	E	Full-length	prM	E	Full-length
Beijing-1	85.4	87.1	87.7	95.0	98.5	91.6
CH2195	82.5	87.4	87.0	91.2	98.7	91.3
CH1392	86.2	88.0	87.9	95.0	98.7	92.9
GP78	85.8	87.7	87.5	95.0	98.4	91.0
HV1	86.6	88.0	87.7	95.0	98.7	91.4
JaGAr01	86.2	88.0	88.0	95.0	98.7	92.9
JaOArS982	83.4	87.7	87.7	92.5	97.2	91.9
K94P05	98.7	98.5	96.4	98.7	99.2	96.4
Ishikawa	97.5	97.0	97.4	100	97.3	93.6
FU	84.5	88.6	89.0	93.7	98.4	92.5
Ling	85.4	87.3	87.6	93.7	97.7	92.4
P20778	86.2	86.7	87.6	95.0	98.0	92.1
P3	84.1	87.0	87.9	93.7	97.3	91.5
RP-2ms	85.8	87.4	87.7	93.7	98.4	91.5
RP-9	85.8	87.9	87.8	93.7	98.7	91.7
SA-14	86.2	87.2	88.0	95.0	97.2	91.8
SA-14-14	86.2	87.1	87.6	95.0	97.0	91.1
T1P1	86.2	87.8	87.9	95.0	98.7	92.0
TC	87.0	88.0	87.5	96.2	98.0	91.1
TL	86.6	88.1	87.5	96.2	98.5	91.2
YL	86.2	87.5	87.6	95.0	98.4	91.2
Anyang	85.0	87.1	NA*	92.5	98.0	NA
K91P55	NA	94.2	NA	NA	98.4	NA
WTP7022	85.8	90.0	NA	91.2	99.0	NA
JKT5441	85.8	89.4	NA	92.5	98.4	NA
JKT1749	86.2	88.5	NA	92.5	97.7	NA
JKT7003	76.2	81.1	NA	87.5	95.3	NA
JKT9092	74.5	85.5	NA	82.5	98.0	NA
ThCM4492	98.3	97.1	NA	100	98.7	NA
ThCM6793	98.3	97.1	NA	100	99.5	NA

NA: Not available.

of four distinct phylogenetic groups. The first major group, GIII, comprised a majority of the JEV strains and was divided into five clusters. The second group, GI, contained recent isolates from Japan, Korea and Thailand. The third group, GII, contained a single cluster of 4 strains (FU, WTP7022, JKT5441 and JKT1749 strain) and consistent with previously reports [17,31]. The last group, GIV, contained Indonesian isolates in 1981 as described previously [5].

A region of 100 nucleotides immediately down stream of the open reading frame stop codon of KV1899 showed high sequence variability as compared with 22 JEV strains (Fig. 2). Deletions in the 3' NTR made KV1899 strain getting shorter sequence in genomes between KV1899 and other JEV strains. KV1899, FU, Ishikawa and K94P05 strains showed a similar form just down stream of the stop codon in 3' NTR.

Discussion

Genomic hetrogenecity among various JE viruses isolated from geographical regions, chronological periods and several sources including human, mosquitoes and pig blood has been reported in previous studies [4,16,19,31]. We determined the full-length nucleotide and deduced amino acid sequences of new JEV isolate KV1899 from swine. In comparison with 22 available fully sequenced JEV strains from GenBank, we found that KV1899 strain has 53 unique nucleotide sequences including the deletion of thirteen nucleotides in 3' NTR region. These nucleotide deletions

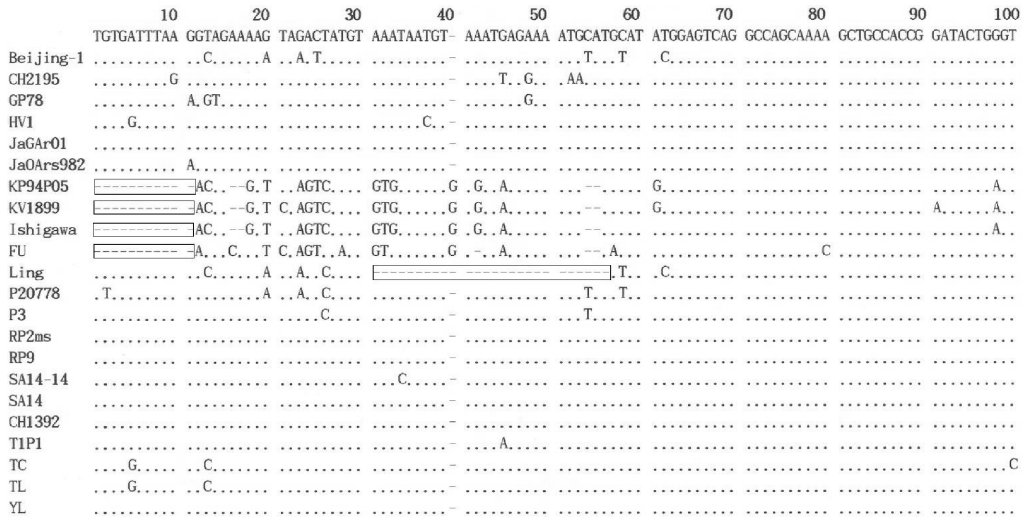

Fig. 2. Nucleotide sequence alignment of the variable region in the 3 non- translated region (NTR) of 22 available JEV strains including KV1899 sequenced in this study. The concensus sequence of 100 nucleotides immediately downstream of the ORF stop codon is shown on top, and only differences from that sequence are indicated for the other strains. Deletions are indicated by hyphens and boxes.

were previously reported in several JEV strains [19,26,31] and tick born encephalitis viruses [7]. These deletions have suggested that the variable region immediately down stream of the ORF stop codon be not only required for viral replication [33], but also does not exhibit RNA secondary structure [23]. Recently, this region in the 3' NTR shows high sequence variability and may play a role in the rate of viral RNA replication [19].

A number of available JEV strain have been partially sequenced for prM and E gene region, which are important for induction of protective immunity [3]. Using the prM and E gene, a lot of analyses have been reported about genetic variation among JEV strains [2,4,5,20,19,32].

Phylogenetic analyses of JEV have previously focused on highly variable sequence from 240 nucleotide of prM gene [2,4,5,8] and divided the JEV into four genotypes. Epidemic isolates were found to group together in genotype I and III, while endemic strains grouped together in cluster forming genotype II and IV. However, the genetic relationships based on the short sequence may need to consider the biological significance [20] and should be considered with caution [30].

The viral envelope (E) protein has been demonstrated to be a reliable phylogenetic marker. The E protein plays an important role in tissue tropism, cell fusion and infection, virus maturation, and protection [29]. Its corresponding protein established phylogenetic markers for JEV [1,16]. New JEV isolate-KV1899 genomic RNAs showed a nucleotide sequence similar to genotype I (Fig. 3). But Anyang strain [12,13], isolated in 1969, belonged to genotype III. Because Anyang strain has been the live vaccine strain for pig in Korea, we attempted to compare this strain with the KV1899 isolate. The envelope gene nucleotide sequence of Anyang strain was similar to JaOArS982 and Beijing-1 (98 and 99% homology). However, the homology of the envelope gene nucleotide sequence between Anyang strain and KV1899 strain was 87.1%. We could find that Korean isolate turned genotype III into genotype I in 1991 (Fig. 3). Although there exists a correlation between genotype and virus activity, this investigation suggests that the genotype pattern of virus may be a function of time and the immune status of host populations rather than genetic potential of the virus. In addition, the variable region in 3' NTR of JEV could be useful to assay the genetic relationship among various JEV isolates [19].

Recently, Solomon *et al.* [25] suggested that JEV originated from its ancestral virus in the Indonesia-Malaysia region and evolved there into the different genotypes which then spread across Asia. They postulated that tropical southest Asia, like Africa, might be an important region for emerging pathogens. Several JEVs belonged to genotype I and III have been isolated from mosquitoes and swine in Korea [12,19,22,33]. In regard to the evolution of JEV, genotype I has been reported in recent Korean isolates including KV1899 strain [18,19]. The reasons for emergence of genotype I in Korea are uncertain, but may include changes of in agricultural practice, animal husbandry and distribution of migrating birds such as heron and egret. Therefore, genotype II strain will be found in Korea according to ecological changes.

Phylogenetic analyses were performed on fully sequenced JEV genome to find out genetic relationships and origin [19,25,28,31,33]. Phylogenetic analyses of the full-length KV1899 genome and 21 fully sequenced JEVs isolated from several countries revealed the highest nucleotide

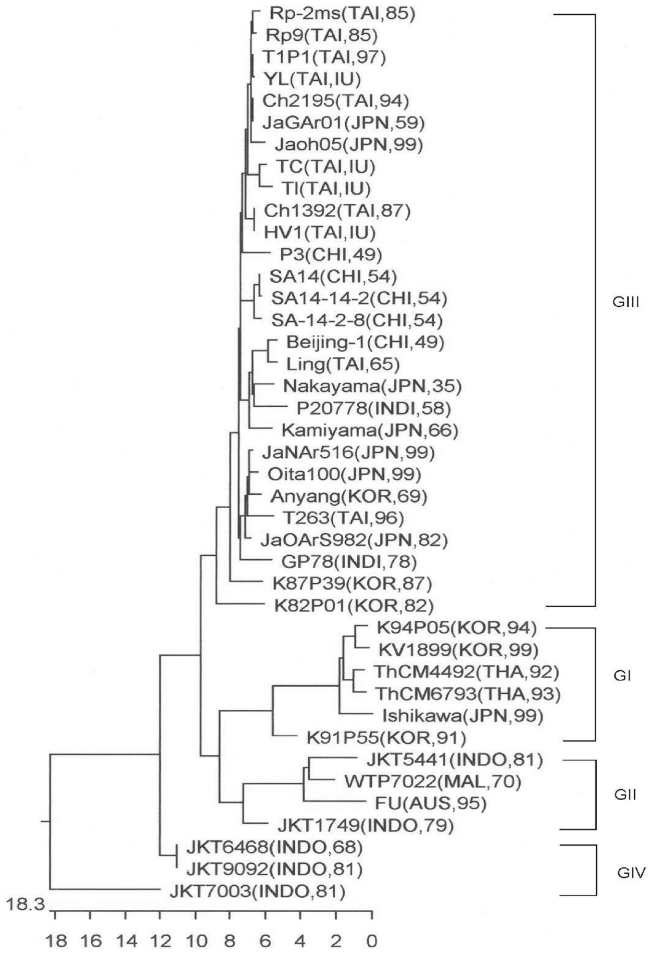

Fig. 3. Phylogenetic tree constructed with the E genes of 41 JEV strains isolated from different geographical regions worldwide at different time periods. Genotypes are given on the right of tree. Taiwan (TAI), Japan (JPN), India (INDI), Thailand (THA), China (CHI), Korea (KOR), Indonesia (INDO), Malaysia (MAL) and Australia (AUS).

homology with Ishikawa strain and the highest amino acid homology with K94P05 strain. In addition, our analyses of the full-length, E and prM gene revealed two clusters. The one cluster consists of recent four isolates (K94P05, Ishikawa, KV1899 and FU strain). Geographical correlations were found within these strains. The other comprises 18 strains with minor branches.

On the basis of nucleotide and amino acid sequence homology and phylogenetic data, KV1899 was genetically similar to Ishikawa and K94P05 strains compared with other JEV isolates, including Anyang strain, the current live vaccine strain used for livestock in Korea. The previous result from the plaque reduction neutralization test (PRNT) demonstrated that some sera from Taiwanese vaccine, after three dose of intramuscular vaccination, were unable to efficiently neutralize certain local JEV isolate [11,14]. We suggest that current live vaccine for swine need to investigate the protection against JEV challenge of recent isolate.

Characterization of the KV1899 strain at molecular level would be an important step towards identifying those properties of the virus, which may aid in the design and construction of recombinant JEV vaccine that are based on the E, NS1 protein. Future studies may be aimed to investigate the efficacy of current JEV vaccine against the KV1899 strain.

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