Original Article

Molecular Characterization of Full-length Genome of Japanese Encephalitis Virus Genotype V Isolated from Tibet, China*

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Abstract

Objective To determine the molecular characterization of full-length genome of Japanese encephalitis virus (JEV) genotype V.

Methods The full-length nucleotide sequences of JEV strains isolated from different locations and sources were used in sequence and phylogenetic analysis.

Results The full-length genome of genotypes V JEV, XZ0934, and Muar strain were composed of 10 983 and 10 988 nucleotides respectively and shared a lower level of identity with JEV genotypes I-IV, ranging from 78.4% (G I, KV1899) to 79.7% (G III, JaGAr01), for the nucleotide sequences, and from 90.0% (G I, KV1899) to 91.8% (G III, JaGAr01) for the amino acid sequences. The open reading frame (ORF) of JEV genotype V spanned nucleotides 96 to 10 397 and encoded 3 433 amino acids. Interestingly, a comparison with JEV genotype I-IV revealed that 3 nucleotides (encoded with a serine residue) were inserted in the NS4A gene of JEV genotype V, and the insertion of nucleotides was also found in downstream of the ORF stop codon in 3'-untranslated region. Moreover, numerous amino acid mutations were observed in 3 functional domains of the E gene of JEV genotype V.

Conclusion The molecular characterization of JEV genotype V is significantly different from that of the known genotypes I-IV. The mutations located in the coding region and the non-coding region may be molecular markers of JEV genotype V and warrant further studies to determine their effects on biology and immunogenicity of genotype V strains.

Key words: Japanese encephalitis virus; Genotype V; Molecular characterization

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INTRODUCTION

Japanese encephalitis (JE) caused by mosquito-borne JE virus (JEV), one of the most significant viral encephalitides worldwide[1], is mainly prevalent in East Asia, South Asia, Southeast Asia, and is even found in the south Pacific region, including Australia[2]. It is estimated that about 3 billion people live in JE endemic areas[2-3].

JEV has a zoonotic transmission cycle between mosquitoes (especially the genus *Culex*) and

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vertebrate hosts, such as bats, water birds, and pigs[1,4]. Human beings who contact JEV when bitten by infected mosquitoes may develop viral encephalitis or die. About 35 000-50 000 JE cases are reported each year, of which 10 000-15 000 are fatal. Approximately 50% of persons who survive the infection experience severe neurological and mental sequelae[1,3-5].

JEV is a member of genus *Flavivirus*, family *Flaviviridae*. Its genome consists of a single-stranded, positive-sense RNA of approximately 11 kb in length. The genome capped at its 5'-end, is not polyadenylated at the 3'-end, and has a single open reading frame (ORF) that encodes a polyprotein. The ORF is flanked by the 5'- and 3'-untranslated region (UTR). The viral structural proteins encoded by the first one-third (from the 5'-end) of ORF, comprise the capsid (C), membrane (M) formed by proteolytic cleavage of its precursor protein PrM, and envelope (E) proteins. The remaining 3'-region encodes 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5)[1,6]. JEV strains are divided into 5 genotypes (I, II, III, IV, and V), based on the nucleotide (nt) sequences of the E gene, and each genotype has a distinct geographical distribution[7]. The sequences of full-length genomes of JEV genotypes I-IV have been reported[7-10].

The Muar strain isolated from the brain tissues of patients with viral encephalitis in Malaya in 1952, is regarded as the only example of JEV genotype V[7,11-13], and has not been detected since then. In 2009, the second strain of JEV genotype V (XZ0934) was isolated in China, indicating that JEV genotype V is re-emerging worldwide after a 57-year hiatus[14] and shares a lower identity than that of the known JEV strains. Thus, it is crucial to study the relationship between JEV genotype V and genotypes I-IV at the molecular level. In the present study, the characterization of nucleotide (nt) and predicted amino acid (aa) sequences of full-length genome of JEV genotype V XZ0934 strain was used to show the differences between JEV genotype V and sequenced JEV genotypes I-IV strains.

MATERIALS AND METHODS

JEV Strains Used in this Study

JEV genotype V XZ0934 strain, isolated from *Culex tritaeniorhynchus* mosquitoes collected in China in 2009, exerts cytopathic effects (CPE) on C6/36 (*Aedes albopictus*) and BHK-21 (mammal) cell line. The accession number for the full-length genome sequence of XZ0934 in GenBank is JF915894[14]. JEV genotype V Muar strain was isolated from the brain tissues of a 19-year-old male patient in Malaya in 1952[12,15] and its complete genome was recently sequenced[15]. To better understand the difference between JEV genotype V and other JEV genotypes, the full-length nt sequences of JEV strains isolated from different locations and sources, were downloaded from GenBank (Table 1) and used in sequence analysis.

Multiple Alignment and Sequence Analysis

The nt and aa sequence alignments were generated using the CLUSTALX ver. 2.0.9 software[16-17], and analyzed using the GeneDoc and MegAlign in the Lasergene software package (DNASTAR).

Phylogenetic Analysis

Phylogenetic trees were constructed using the nt sequences of selected JEV strains (Table 1) according to the complete genome and E gene sequences. Neighbor-joining phylogenetic trees were constructed using the MEGA ver. 4.0.2 software[18-19]. The phylogenetic construction robustness was assessed by bootstrapping with 1 000 replicates.

RESULTS

Analyses of JEV Full-length Nucleotide and Deduced Amino Acid Sequences

The genome of JEV genotype V XZ0934 and Muar contained 10 983 nt and 10 988 nt respectively, with a single ORF of 10 302 nt (nt 96 to nt 10 397) putatively encoding a polyprotein of 3 433 aa. The ORF of XZ0934 was flanked by a 95-nt 5'-UTR and a 586-nt 3'-UTR while that of Muar was flanked by a 95-nt 5'-UTR and a 591-nt 3'-UTR. The genome of JEV genotype V was longer than that of JEV genotypes I-V. Most importantly, the ORF of JEV genotype V (10 302 nt) was 3 nt longer than that of JEV genotypes I-IV (10 299 nt), which encode 3 432 aa[7-10].

Difference in Sequences between JEV Genotype V (XZ0934) and Other JEV Genotypes

To detect the nt and aa disparities for each gene segment between genotype V and genotypes I-IV, four

Table 1. Parameters of JEV Strains Used in this Study

Genotype	Strain	Year	Location	Source	GenBank Accession No.
I	K94P05	1994	Korea	Mosquito	AF045551
I	KV1899	1999	Korea	Pig	AY316157
I	Ishikawa	1998	Japan	Mosquito	AB051292
I	JEV/sw/Mie/41/2002	2002	Japan	Pig	AB241119
I	JEV/sw/Mie/40/2004	2004	Japan	Pig	AB241118
I	SC04-17	2004	China	Mosquito	GU187972
I	HEN0701	2007	China	Pig	FJ495189
I	XJ69	2007	China	Mosquito	EU880214
I	XJP613	2007	China	Mosquito	EU693899
I	SH17M-07	2007	China	Mosquito	EU429297
I	JX61	2008	China	Pig	GU556217
II	FU	1995	Australia	Human	AF217620
III	Vellore P20778	1958	India	Human	AF080251
III	GP78	1978	India	Human	AF075723
III	14178	2001	India	Human	EF623987
III	04940-4	2002	India	Mosquito	EF623989
III	57434	2005	India	Human	EF623988
III	Nakayama	1935	Japan	Human	EF571853
III	JaGAr01	1959	Japan	Mosquito	AF069076
III	JaOH0566	1966	Japan	Human	AY508813
III	JaTAn1/75	1975	Japan	Pig	AB551990
III	ML17	1981	Japan	Human	AY508812
III	JaOArS982	1982	Japan	Mosquito	M18370
III	JEV-AT31	IU	Japan	IU	AB196923
III	JEV-rAT	IU	Japan	JEV-AT31 derivative	AB196925
III	JEV-at222	IU	Japan	Vaccine strain	AB196924
III	K87P39	1987	Korea	Mosquito	AY585242
III	CNU/LP2	1987	Korea	K87P39 derivative	AY585243
III	p3	1949	China	Human	U47032
III	Beijing-1	1949	China	Human	L48961
III	SA14	1954	China	Mosquito	U14163
III	SA14-14-2	1954	China	Vaccine strain	AF315119
III	SA14-12-1-7	1954	China	SA14 derivative	AF416457
III	SA (A)	1954	China	SA14-14-2 derivative	D90195
III	B58	1986	China	Bat	FJ185036
III	HW	1988	China	Pig	AY849939
III	WHe	1988	China	Pig	EF107523
III	GB30	1997	China	Bat	FJ185037
III	SH0601	2006	China	Pig	EF543861
III	Ling	1965	Taiwan	Human	L78128
III	RP-2ms	1985	Taiwan	Mosquito	AF014160
III	RP-9	1985	Taiwan	Mosquito	AF014161
III	CH2195LA	1994	Taiwan	CH2195 derivative	AF221499
III	CH2195SA	1994	Taiwan	CH2195 derivative	AF221500
III	YL	IU	Taiwan	Vaccine strain	AF486638
III	HVI	IU	Taiwan	Mosquito	AF098735
III	TL	IU	Taiwan	Mosquito	AF098737
IV	JKT6468	1981	Indonesia	Mosquito	AY184212
V	Muar	1952	Malaysia	Human	HM596272
V	XZ0934	2009	China	Mosquito	JF915894

Note. IU: information unavailable.

strains representing JEV genotypes I-IV were selected and compared with XZ0934 and Muar (Table 2). Three nt were inserted in the NS4A gene of JEV genotype V. Further analysis showed that the inserted nt were AGC for XZ0934 strain and AGT for Muar strain (Figure 1), implying that a serine (Ser) residue was inserted into the non-structural NS4A protein of JEV genotype V with the exception of the NS4A gene. The lengths of the remaining gene segments were identical between genotypes I-V (Table 2).

Table 2. Genome Sequences of JEV Strains*

Genotype	Strain	Length of Nucleotides (Amino Acids) in Genome Segment													
		Complete	5'UTR	C	PrM	M	E	NS1	NS2A	NS2B	NS3	NS4A	NS4B	NS5	3'UTR
I	Ishikawa	10 965	96	381	276	225	1500	1236	501	393	1857	867	345	2715	570
		(3 432)	(0)	(127)	(92)	(75)	(500)	(412)	(167)	(131)	(619)	(289)	(115)	(905)	(0)
II	FU	10 964	95	381	276	225	1500	1236	501	393	1857	867	345	2715	570
		(3 432)	(0)	(127)	(92)	(75)	(500)	(412)	(167)	(131)	(619)	(289)	(115)	(905)	(0)
III	SA14-14-2	10 976	95	381	276	225	1500	1236	501	393	1857	867	345	2715	582
		(3 432)	(0)	(127)	(92)	(75)	(500)	(412)	(167)	(131)	(619)	(289)	(115)	(905)	(0)
IV	JKT6468	10 978	95	381	276	225	1500	1236	501	393	1857	867	345	2715	584
		(3 432)	(0)	(127)	(92)	(75)	(500)	(412)	(167)	(131)	(619)	(289)	(115)	(905)	(0)
V	Muar	10 988	95	381	276	225	1500	1236	501	393	1857	870	345	2715	591
		(3 433)	(0)	(127)	(92)	(75)	(500)	(412)	(167)	(131)	(619)	(290)	(115)	(905)	(0)
V	XZ0934	10 983	95	381	276	225	1500	1236	501	393	1857	870	345	2715	586
		(3 433)	(0)	(127)	(92)	(75)	(500)	(412)	(167)	(131)	(619)	(290)	(115)	(905)	(0)

Note. *The nucleotide sequences of JEV strain were compared with those of JaOArS982 strain.

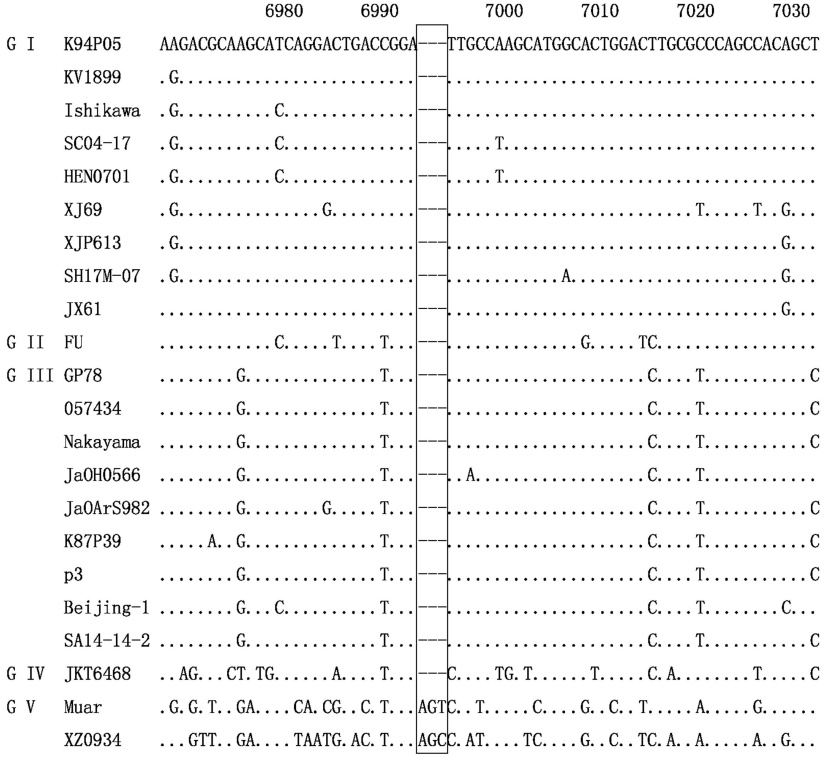

Figure 1. Insertion site of XZ0934 and Muar strains in NS4A gene. Dots indicate conserved nucleotide sequences, dashes indicate gaps, box indicates the nucleotide insertion site of XZ0934 and Muar in NS4A gene.

The lengths of 5'-UTR of genotype V and genotypes II-IV were identical at 95 nt (Table 2). However, JEV genotype V Muar strain had the longest 3'-UTR at 591 nt (Table 2), followed by XZ0934 at 586 nt. Furthermore, the insertion of nt was detected from the downstream of ORF stop codon in 3'-UTR of JEV genotype V (Figure 2). Ten and 14 nt were inserted in XZ0934 and Muar strain, respectively.

The wide distribution of JE is associated with the differences in vectors and host preferences. It was reported that the 20 aa proximal to the carboxyl terminus of capsid (C) protein is a variable region of JEV, in which the aa sequence divergence has been found in JEV with different genotypes[7]. This variable region may be associated with the differences in vector and host availabilities, thereby influencing the spread of different JEV genotypes. The aa sequence of genotype V strains XZ0934 and Muar these two viruses was divergent from that of JEV genotypes I-IV (Figure 3). Moreover, the JEV genotype V strains had specific signalase/protease cleavage motifs: Val-Ser-Ala. However, as far as this region was concerned, the aa sequence of the XZ0934 and Muar strains was different. In all, 5 aa differences were found with a divergence of 25% (Figure 3). These divergences may lead to worldwide re-emergence of JEV genotype V.

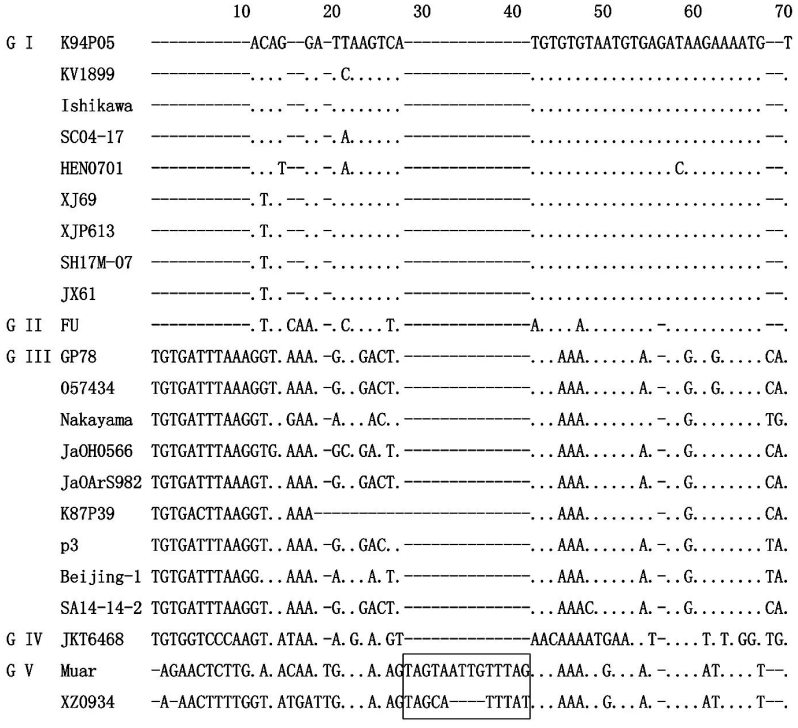

Figure 2. Nucleotide sequence alignment of variable regions in 3'UTR of JEV strains. Dots indicate conserved nucleotide sequences, dashes indicate gaps, box indicates the nucleotide insertion site of XZ0934 and Muar in 3'UTR.

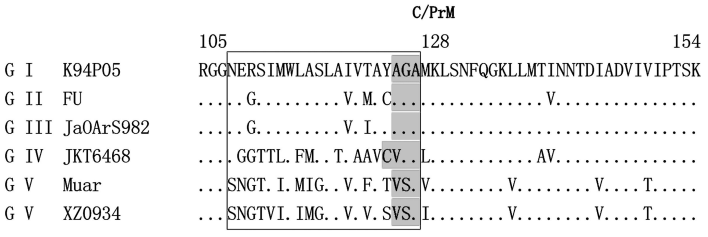

Figure 3. Variable regions in capsid (C) protein of JEV strains. The box indicates variable regions of 20 amino acids proximal to carboxy terminus of JEV, dots indicate conserved residues, shaded regions indicate predicted signalase/protease cleavage motifs.

Compared with the aa sequences of 48 selected JEV genotype I-IV strains (Table 1), the XZ0934 and Muar strains showed 117 unique aa differences (Table 3), representing a 3.4% difference between the aa sequences. These differences in aa dispersed throughout the protein segments, with 36 in the structural proteins and 81 in the non-structural proteins. The structural C protein contained the highest percentage of these aa differences (5.5%), followed by the PrM protein (5.4%). Twenty-one unique differences were found in the E protein sequence, constituting a 4.2% aa divergence. Regarding the non-structural proteins, the NS2A protein showed the highest percentage of aa difference (7.8%), whereas the NS4B protein had the lowest divergence (1.7%). The percentage divergence for the remaining non-structural proteins was 2.1%-3.8%.

Sequence Identity Analysis

In comparison with the complete genome sequences of genotype V XZ0934 and Muar strains and 48 fully sequenced genotype I-IV strains (Table 1), the nt sequence identities ranged from 78.4% (G I, KV1899) to 79.7% (G III, JaGAr01), and the aa sequence identities ranged from 90.0% (G I, KV1899) to 91.8% (G III, JaGAr01). Overall, the nt sequence divergence ranged from 20.3% to 21.6%, and the aa sequence divergence ranged from 8.2% to 10.0%.

The identity between XZ0934 and Muar strains of JEV genotype V was higher than that between XZ0934 and other strains of JEV genotype V. The identity of complete genome sequences was 90.6% in the nt and 98.3% in the aa (Table 4). When each gene segment was compared, the aa sequence was conserved between XZ0934 and Muar although the nt sequences in their non-structural genes NS2A, NS2B, NS4A, NS4B were divergent, demonstrating that the two strains of JEV genotype V are similar despite the span of their isolated time was 57 years (1952-2009).

The 5'-UTR nt sequence identities ranged from 91.7% to 96.9% between XZ0934 and other strains (Table 4). However, the other gene segments shared lower identities. Thus, for the structural genes (C, PrM, M, E), the nt sequence homologies ranged from 71.7% (PrM, JKT6468) to 84.0% (M, Ishikawa), and the aa sequence homologies ranged from 72.4% (C, JKT6468) to 94.7% (M, FU). For the non-structural genes (NS1-NS5), the nt sequence homologies ranged from 72.5% (NS2A, Ishikawa) to 81.4% (NS4B, JKT6468), the aa sequence homologies ranged from 80.2% (NS2A, Ishikawa) to 98.3% (NS4B, JKT6468), the 3'-UTR nt sequence identities ranged from 81.6% to 82.9% (Table 4), revealing a low similarity between JEV genotype V and genotypes I-IV.

Analysis of E Gene-encoded Amino Acid Sequences

Overall, 21 unique aa changes found in the E gene of JEV genotype V were located at E58, E64, E66, E84, E96, E120, E122, E141, E149, E188, E196, E204, E219, E232, E238, E329, E340, E348, E365, E382, and E402 (Table 3). The envelope protein (E protein) encoded by the E gene plays an important role in the virulence of JEV. It has been shown that certain aa residues in the E protein are crucial for the neurovirulence of JEV[19-20]. The residues are important for the conserved neurovirulence between XZ0934, Muar and a virulent strain (Beijing-1) isolated from the brain tissues of a patient (Table 5), indicating that XZ0934 and Muar are members of the JEV strain family with a high pathogenicity.

Phylogenetic Analysis

To establish the phylogenetic relationship between JEV genotypes V and I-IV strains, a phylogenetic tree was constructed using the complete genome sequences of XZ0934, Muar and selected JEV genotypes I-IV strains (Figure 4). Murray valley encephalitis virus (MVEV) sequence was used as an out-group. Five distinct phylogenetic groups were identified based on the full-length genome sequences. The XZ0934 and Muar strains, members of JEV genotype V strains, formed a branch that was divergent from that of JEV genotype I-IV strains.

DISCUSSION

It is well known that JEV contains only a single ORF of 10 299 nt and encodes a polyprotein of 3 432 aa[7-10], suggesting that the number of different polyproteins encoded by JEV (genotypes I-IV) is limited. However, JEV genotype V has a single ORF of 10 302 nt, which putatively encodes a polyprotein of 3 433 aa. Three additional nt encoding a Ser residue in the NS4A gene of JEV genotype V may represent the main molecular marker of JEV genotype V. The NS4 gene shows multiple functions in the flavivirus life cycle. The central region of NS4A is a cofactor for serine protease, whereby it completes the folding of protease domain. Furthermore, NS4A participates in the recognition of RNA substrates by NS3 protease/helicase. NS4A and NS4B can also block type

Table 3. Amino Acid Substitutions in JEV XZ0934 and Muar Strains

Protein	Amino Acid Position	Amino Acid Substitution
C	25	Phe → Ser
	34	Val → Ile
	36	Ser → Asn
	67	Gly → Ser
	93	Asp → Asn
	108	Asn → Ser
	126	Gly → Ser
PrM	137	Leu → Val
	146	Ile → Val
	151	Val → Thr
	158	Glu → Thr
	192	Val → Ile
M	239	Leu → Met
	263	Phe → Leu
	264	Leu → Val
E	352 (E58)	Ser → Thr
	358 (E64)	Ser → Thr
	360 (E66)	Thr → Ala
	378 (E84)	Lys → Arg
	390 (E96)	Phe → Tyr
	414 (E120)	Ser → Val
	416 (E122)	Thr → Ser
	435 (E141)	Ile → Val
	443 (E149)	Ser → Ala
	482 (E188)	Leu → Met
	490 (E196)	Leu → Phe
	498 (E204)	Met → Leu
	513 (E219)	His → Asn
	526 (E232)	Ala → Asn
	532 (E238)	Leu → Ile
	623 (E329)	Ser → Thr
	634 (E340)	Val → Ser
	642 (E348)	Met → Leu
	659 (E365)	Ser → Thr
	676 (E382)	Tyr → Phe
	696 (E402)	Thr → Ser
NS1	885	Asn → Asp
	893	Ser → Pro
	896	Lys → Leu
	929	Val → Ile
	985	His → Asn
	986	Val → Met
	1066	Gly → Glu
	1068	Val → Thr
	1082	Thr → Ala
	1084	Asp → Gly
	1141	Arg → Lys
	1201	Ala → Val
NS2A	1221	Leu → Val
	1241	Leu → Ala
	1262	Leu → Met
	1268	Gln → Glu
	1269	Ile → Phe
	1271	Val → Ile
	1284	Ile → Val
	1319	Ile → Phe
	1327	Ser → Ala
	1350	Thr → Ser
	1358	Thr → Ala
	1359	Thr → Ile
	1360	Ile → Met
NS2B	1400	Glu → Asp
	1403	Ser → Ala
	1437	Met → Val
	1483	Leu → Phe
	1489	Val → Ile
NS3	1516	Pro → Val
	1517	Cys → Tyr
	1537	Thr → Val
	1596	Thr → Val
	1601	Val → Met
	1613	Ile → Val
	1636	Arg → Ser
	1806	Ala → Ser
	1949	Gly → Ser
	1988	Ser → Thr
	2 005	Met → Leu
	2 042	Leu → Ile
	2 087	Val → Ile
NS4A	2 129	Ile → Leu
	2 219	Ala → Met
	2 283	Ala → Glu
	2 285	Leu → Ile
	2 286	Lys → Arg
	2 291	Gly → Asn
	2 299	Thr → Pro
	2 301	Insert Ser
	2 303	Pro → Ser
	2 359	Val → Met
	2 392	Thr → Val
NS4B	2 456	Val → Ile
	2 521	Ala →Val
NS5	2 549	Arg → Lys
	2 557	Arg → Lys
	2 561	Ile → Val
	2 602	Ile → Val
	2 653	Ser → Thr
	2 696	Thr → Ala
	2 698	Asp → Glu
	2 725	Val → Thr
	2 737	Leu → Val
	2 812	Gln → Glu
	2 818	Phe → Tyr
	2 820	Thr → Ala
	2 905	Asn → Asp
	2 926	Lys → Arg
	2 942	Val → Met
	2 951	Thr → Ser
	3 042	Val → Ile
	3 078	Glu → Asp
	3 160	Ile → Val
	3 164	His → Asp
	3 174	Ile → Phe
	3 193	Ile → Val
	3 231	His → Gln
	3 409	Lys → Asn
	3 425	Val → Thr

Table 4. Identities between XZ0934 and Other JEV G I-G V Strains

Genotype	Strain	Identity Comparison (%) of Nucleotides (Amino Acids) in Genome Segment													
		Complete	5'UTR	C	PrM	M	E	NS1	NS2A	NS2B	NS3	NS4A	NS4B	NS5	3'UTR
I	Ishikawa	79.1	93.8	79.5	78.3	84.0	77.0	79.9	72.5	79.1	79.5	75.4	81.2	80.2	81.6
		(90.7)	-	(77.2)	(87.0)	(93.3)	(89.4)	(91.0)	(80.2)	(91.6)	(94.5)	(89.3)	(96.5)	(92.3)	-
II	FU	79.0	96.9	81.4	74.3	81.8	77.5	80.1	74.1	79.1	79.0	74.5	80.9	79.9	82.7
		(91.2)	-	(78.7)	(84.8)	(94.7)	(90.6)	(91.0)	(82.0)	(93.9)	(94.7)	(89.7)	(98.3)	(92.3)	-
III	SA14(14(2	79.3	94.8	81.6	76.8	83.1	77.7	79.5	73.5	80.4	79.5	74.9	81.2	80.5	82.9
		(90.7)	-	(79.5)	(87.0)	(93.3)	(89.6)	(90.8)	(80.8)	(91.6)	(94.2)	(90.7)	(98.3)	(91.5)	-
IV	JKT6468	78.9	91.7	78.2	71.7	80.0	77.5	80.2	73.5	79.6	79.8	74.6	81.4	80.1	82.4
		(90.6)	-	(72.4)	(81.5)	(85.3)	(90.6)	(91.5)	(82.0)	(93.9)	(94.5)	(88.3)	(98.3)	(92.4)	-
V	Muar	90.6	99.0	92.1	92.4	96.0	90.2	90.4	88.6	89.6	90.6	88.0	89.9	90.4	95.0
		(98.3)	-	(92.1)	(98.9)	(100.0)	(98.8)	(98.8)	(98.8)	(98.5)	(98.9)	(96.6)	(100.0)	(98.2)	-

Table 5. Key Amino Acid Residues in E Protein of JEV

Strain	Amino Acid Residues Relative to Neurovirulence							
	E107	E138	E176	E177	E264	E279	E315	E439
SA14-14-2	Phe	Lys	Val	Ala	His	Met	Val	Arg
Beijing-1	Leu	Glu	Ile	Thr	Gln	Lys	Ala	Lys
Muar	Leu	Glu	Ile	Thr	Gln	Lys	Ala	Lys
XZ0934	Leu	Glu	Ile	Thr	Gln	Lys	Ala	Lys

I interferon[1]. No specific impact of a serine residue inserted in the NS4A protein of JEV genotype V was found on the virus life cycle and pathogenicity, which warrants further study.

The differences in 3'-UTR length of JEV genotypes I-IV ranged from 570 nt to 584 nt. In addition, an 11-nt deletion in 3'-UTR was found in JEV genotypes I and II, as previously described[9]. The 3'-UTR of JEV genotype V was longer than that of other JEV genotypes with inserted nt in downstream of the ORF stop codon. It has recently reported that the variable region in 3'-UTR plays a role in viral RNA replication and regulation of transcription and translation initiation of the virus[21-23]. Therefore, further study is needed to determine whether the inserted nt affects the replication of JEV genotype V.

It has been shown that the divergence of the 20 aa proximal to carboxyl terminus of C protein in Muar strain is the molecular determinant that explains why JEV genotype V has not spread[7]. The newly isolated JEV genotype V XZ0934 strain differs from the Muar strain. The 5 aa differences were found for XZ0934 with a divergence of 25%. These divergences may be the molecular basis for the re-emergence of JEV genotype V worldwide after 57 years.

In the present study, the molecular characterization of JEV genotype V strains is described, the insertion mutations were identified in the ORF and 3'-UTR of JEV genotype V but not in JEV genotypes I-IV. These divergences may be a molecular marker of JEV genotype V. Furthermore, 117 unique aa differences were identified in JEV genotype V strains, representing a 3.4% difference in the aa sequences, which is greater than that detected JEV genotypes I-IV[7-10]. Moreover, the aa changes have spread to the 3 known domains of E protein. Their effect on viral biology of unique aa differences in JEV genotype V, particularly in E protein need to be further studied. The effect of existing attenuated (SA14-14-2) and inactivated (Nakayama or Beijing-1) vaccines against JEV genotype V strains should be investigated without delay.

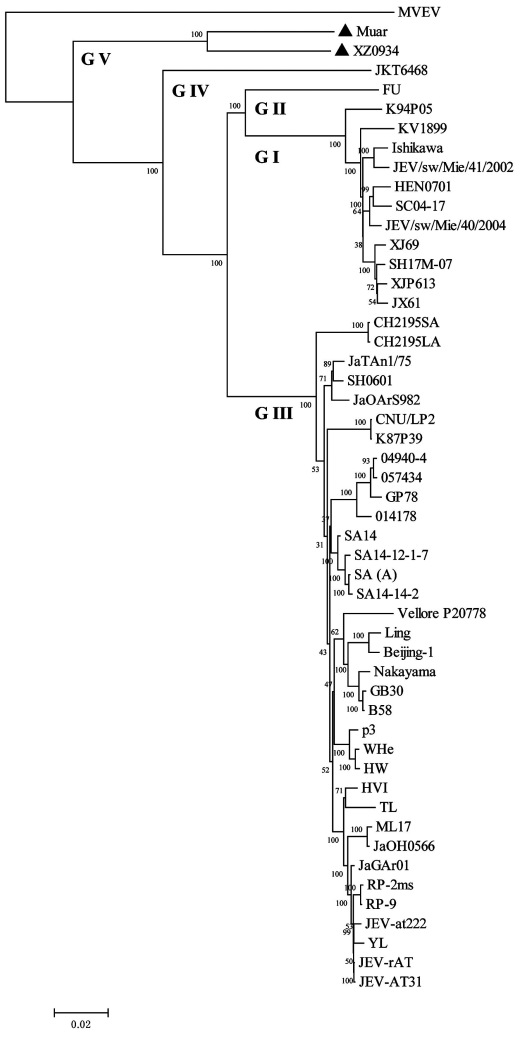

Figure 4. Phylogenetic tree based on complete genome of JEV. Phylogenetic analysis was performed with the neighbor-joining method using MEGA version 4.0.2 software package (www.megasoftware.net). The tree was rooted using Murray valley encephalitis virus (MVEV) strain MVE-1-51 as an out-group. Bootstrap probabilities of each node were calculated using 1 000 replicates. Scale bars indicate the number of nucleotide substitutions per site.

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