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Molecular evolution of Japanese encephalitis virus isolates from swine in Oita, Japan during 1980–2009☆

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ABSTRACT

In order to identify the patterns of genetic change of Japanese encephalitis virus (JEV) strains circulating in Oita, the complete envelope (E) gene has been sequenced for 35 isolates from swine in a 30-year span. Based on nucleotide and deduced amino acid sequences, the genetic variation was examined, phylogeny was estimated and selection pressures were also analyzed. This study demonstrated that the major genotype (G) of JEV isolates had shifted from GIII to GI in the mid-1990s in Oita. The intensities of selection acting on the Oita GIII and GI strains were found to be almost same. It suggests that the intensity of selection might not be the reason for such a genotype shift observed in Oita. Pairwise comparisons revealed the high conservation of the E gene at the protein level. Compared with the Oita GIII strains, all the Oita GI strains shared four amino acid changes at E129 (T–M), E222 (A–S), E327 (S–T) and E366 (A–S). Among all 70 JEV isolates involved in this paper, the GI strains shared only one amino acid change at E222 (A–S) in comparison with the GIII strains. No strong evidence for positive selection was found, the JEV evolution has generally been subject to strong purifying selection, but one ongoing evolutionary pathway was found to be under relaxed purifying selection in Oita. This study is a localized example of JEV molecular evolution in nature.

1. Introduction

Japanese encephalitis virus (JEV) belongs to the genus *Flavivirus* within the family *Flaviviridae*. Many flaviviruses are significant human pathogens, including JEV, dengue viruses, West Nile virus, yellow fever virus, tick-borne encephalitis virus and Murray Valley encephalitis virus. Flaviviruses are small, enveloped viruses containing a single-stranded positive-sense RNA genome of approximately 11 kb. The 5′- and 3′-untranslated regions flank a single open reading frame, which encodes three structural proteins, capsid (C)–premembrane (prM)–envelope (E), and seven nonstructural (NS) proteins (NS1–NS2A–NS2B–NS3–NS4A–NS4B–NS5) (Sumiyoshi et al., 1987; Weaver and Barrett, 2004).

Japanese encephalitis, a mosquito-borne viral infection transmitted in Asia, is a serious disease in the region, accounting for more than 16,000 reported cases and 5000 deaths annually. Recently, transmission has intensified in certain countries and the disease has extended its geographical range to previously unaffected areas of Asia and to Australia (Tsai, 2000; Williams et al., 2000; Van Den Hurk et al., 2006). JEV infects a number of animal hosts. Because humans have transient and low-level viremias, they are “dead-end” hosts that do not normally transmit the virus (Weaver and Barrett, 2004; Solomon, 2006). In addition to its public health importance, JEV also causes encephalitis in horses and abortion in swine, which act as amplifying hosts (Chen et al., 1992).

Based on the nucleotide sequencing of C/prM and E genes, at least four genotypes of JEV have been identified (Chen et al., 1990, 1992; Williams et al., 2000). The E protein of flaviviruses is the major antigen for the viruses and is also believed to be associated with virus binding and entry to host cells. Thus, the majority of phylogenetic analyses of JEV have focused on the E protein (Nga et al., 2004; Nerome et al., 2007; Nitatpattana et al., 2008; Nabeshima et al., 2009). Despite the growing interest in the molecular epidemiology of JEV, there have been few informative attempts to detect selection pressures (Yang et al., 2000). Especially, few studies are restricted to a single host population with sufficient temporal sampling at a given geographic location.

In order to identify the patterns of genetic change of JEV strains circulating in Oita, the complete envelope gene has been sequenced for 35 isolates from swine sera over a 30-year period. This paper reports the results of the genetic variation examined, phylogeny estimated and selection pressures analyzed.

☆ *Note*: Nucleotide sequence data reported in this paper are available in the GenBank, EMBL and DDBJ databases under the accession numbers FJ943462–FJ943494, GU108334 and GU108335.

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Table 1
JEV isolates used for analysis in this study.

Isolate	Year of isolation	Location	Source	GenBank accession no.
FU	1995	Australia	Human	AF217620
TS00	2000	Australia	Swine	EF434785
Beijing-1	1949	China	Human	L48961
SA14	1954	China	Mosquito	U14163
YN79-Bao83	1979	China	Mosquito	DQ404128
YNDL04-1	2004	China	Mosquito	DQ404137
SH04-10	2004	China	Mosquito	DQ404107
SH05-24	2005	China	Mosquito	DQ404108
GP78	1978	India	Human	AF075723
JKT1724	1979	Indonesia	Mosquito	U70404
JKT5441	1981	Indonesia	Mosquito	U70406
JKT6468	1981	Indonesia	Mosquito	AY184212
JKT9092	1981	Indonesia	Mosquito	U70409
Nakayama	1935	Japan	Human	S75726
JaGAr01	1959	Japan	Mosquito	AF069076
JaOH0566	1966	Japan	Human	AY029207
JaOArS982	1982	Japan	Mosquito	M18370
Sw/Hiroshima/25/2002	2002	Japan	Swine	AB231465
Sw/Mie/40/2004	2004	Japan	Swine	AB231463
K87P39	1987	Korea	Mosquito	AY585242
K94P05	1994	Korea	Mosquito	AF045551
WTP-70-22	1970	Malaysia	Mosquito	U70421
B2524	1985	Nepal	Human	U70392
PhAn1242	1984	Philippines	Swine	U70417
H49778	1987	Sri Lanka	Human	U70395
CH2195	1994	Taiwan	Mosquito	AF030550
Chiang Mai	1964	Thailand	Human	U70393
KPP034-35CT	1982	Thailand	Mosquito	U03693
B2239	1984	Thailand	Swine	U70391
ThCMAr4492	1992	Thailand	Mosquito	D45362
ThCMAr6793	1993	Thailand	Mosquito	D45363
JE-CP-67	2004	Thailand	Swine	DQ087972
JE-KK-580	2005	Thailand	Swine	DQ238600
VN206	1986	Vietnam	Human	AY376460
VN22	2002	Vietnam	Swine	AY376465
80P136	1980	Oita, Japan	Swine	This paper; FJ943462
80P205	1980	Oita, Japan	Swine	This paper; FJ943463
81P241	1981	Oita, Japan	Swine	This paper; FJ943464
81P244	1981	Oita, Japan	Swine	This paper; FJ943465
89P49	1989	Oita, Japan	Swine	This paper; FJ943466
89P131	1989	Oita, Japan	Swine	This paper; FJ943467
89P141	1989	Oita, Japan	Swine	This paper; FJ943468
89P149	1989	Oita, Japan	Swine	This paper; FJ943469
89P160	1989	Oita, Japan	Swine	This paper; FJ943470
95P99	1995	Oita, Japan	Swine	This paper; FJ943471
97P82	1997	Oita, Japan	Swine	This paper; FJ943472
99P103	1999	Oita, Japan	Swine	This paper; FJ943473
99P104	1999	Oita, Japan	Swine	This paper; FJ943474
03P113	2003	Oita, Japan	Swine	This paper; FJ943475
03P120	2003	Oita, Japan	Swine	This paper; FJ943476
03P126	2003	Oita, Japan	Swine	This paper; FJ943477
03P145	2003	Oita, Japan	Swine	This paper; FJ943478
03P189	2003	Oita, Japan	Swine	This paper; FJ943479
05P75	2005	Oita, Japan	Swine	This paper; FJ943480
06P152	2006	Oita, Japan	Swine	This paper; FJ943481
06P169	2006	Oita, Japan	Swine	This paper; FJ943482
06P183	2006	Oita, Japan	Swine	This paper; FJ943483
06P212	2006	Oita, Japan	Swine	This paper; FJ943484
07P83	2007	Oita, Japan	Swine	This paper; FJ943485
07P90	2007	Oita, Japan	Swine	This paper; FJ943486
07P127	2007	Oita, Japan	Swine	This paper; FJ943487
08P37	2008	Oita, Japan	Swine	This paper; FJ943488
08P38	2008	Oita, Japan	Swine	This paper; FJ943489
08P42	2008	Oita, Japan	Swine	This paper; FJ943490
08P48	2008	Oita, Japan	Swine	This paper; FJ943491
08P49	2008	Oita, Japan	Swine	This paper; FJ943492
08P54	2008	Oita, Japan	Swine	This paper; FJ943493
08P62	2008	Oita, Japan	Swine	This paper; FJ943494
09P123	2009	Oita, Japan	Swine	This paper; GU108334
09P141	2009	Oita, Japan	Swine	This paper; GU108335