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Phylogenetic analyses of the JEV gene sequence

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ABSTRACT

The E gene was amplified by semi-nested PCR.

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MATERIALS TEXT

mosquito grinding supernatant,CSF sample that shown positive by detection of JEV by quantitative real-time reverse-transcription(q-RT)-PCR;Tianlong Nucleic Acid Automatic Extractor ,Tianlong Nucleic Acid Extraction Kit ,Ready-To-Go kit ,random primers (pdN6) ,GoTaq Green Master Mix Kit (Promega, Madison, WI, USA) ,The primers used for the first round of E gene PCR were JEV-E-1F (TTCATAGAAGGAGCCAGTGGA) and JEV-E-1R (TCGTTTAAACTCGCGACTGA) ,the primers used for the second round were JEV-E-1F and JEV-E-2R (TTTCCCGAAAAGTCCACATC).The primers used for the first round of C+PrM gene PCR were JE-C+PrM-1F (CGTTCTTCAAGTTTACAGCATTAGC) and JE-C+PrM-1R (CCYRTGTTYCTGCCAAGCATCCAMCC), and the primers used for the second round were JE-C+PrM-1Fand JE-C+PrM-2R (CGYTTGGAATGYCTRGTCCG).

Nucleic acid extraction

1 Use Tianlong nucleic acid extraction instrument (automatic nucleic acid extraction instrument, model: np968. C, manufacturer: Suzhou Tianlong Biotechnology Co., Ltd.), and supporting nucleic acid extraction kit: Tianlong nucleic acid extraction kit (magnetic bead method EX-RNA/DNAvirus), manufacturer: Suzhou Tianlong Biotechnology Co., Ltd., for nucleic acid extraction. Perform the following operations in a biosafety cabinet. Add 200 µl of patient cerebrospinal fluid and serum specimens and mosquito grinding supernatant to each well in columns 1 and 7 of the 96-well plate provided with the nucleic acid extraction kit

cDNA library preparation

2 33 µl of RNA extract was placed in a 65 ° C water bath for 10 min.

3 Ice bath for 2 min

4 32ul of RNA samples were pipetted into the first chain reaction tube in a Ready-To-Go kit kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and allowed to stand at room temperature for 1 min.

5 1 ul of random primer (pdN6) (TaKaRa, Japan) was added to every sample.

6 37 ° C water bath for 1 h.

The E gene amplification

The E gene sequence of JE virus was amplified by semi-nested PCR. The first round of

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7 amplification , using primers: JEV 3F: TTCATAGAAGGAGCCAGTGGA and JEV 3R: TCGTTTAAACTCGCGACTGA.he gene amplification system was 25 ul, including: 2 µl of cDNA template, 12.5 µl of GoTaq ® Green Master Mix-2 × (Promega, Madison, WI), 1 µl of upstream and downstream primers at 10 umol/L, and 8.5 µl of RNase Free Water. The reaction program was: 94 ° C, 8 min for 1 cycle; 94 ° C, 1 min, 55 ° C, 1 min, 72 ° C, 1 min for 35 cycles; 72 ° C, 10 min, keep in 4 ° C. The second round of amplification sequence was 300 bp, using the first round of PCR product as a template with the forward primer JEV 3F and the reverse primer JEV 2R: TTTCCCGAAAAGTCCACATC.

8 The second round of amplification sequence was 300 bp, using the first round of PCR product as a template with the forward primer JEV 3F and the reverse primer JEV 2R: TTTCCCGAAAAGTCCACATC,the amplification system and reaction program was same as the first round.

C+PrM gene amplification

9 The C+PrM gene sequence was also amplified by semi-nested PCR according to the same amplification system and procedure as E gene. The primers used for the first round of PCR were JE-C+PrM-1F (CGTTCTTCAAGTTTACAGCATTAGC) and JE-C+PrM-1R (CCYRTGTTYCTGCCAAGCATCCAMCC)

10 primers used for the second round were JE-C+PrM-1Fand JE-C+PrM-2R (CGYTTGGAATGYCTRGTCCG). The product of the first round of PCR was used as the template of the second round.The second round also use the same amplification system and procedure as E gene.

agarose gel electrophoresis

11 5 µL of the amplification product was detected by 1% agarose gel electrophoresis .

Nucleotide sequence determination

12 Nucleotide sequence determination was done by Sangon Biotech Co. Ltd. (Shanghai, China, Beijing Sequencing Department).

Phylogenetic analyses

13 The viral gene nucleotide sequences were spliced and corrected using SeqMan II software (DNA Star, Madison, WI, USA).

14 The JEV gene sequences used for phylogenetic analysis were downloaded from GenBank(Table S1) .

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15 The ClustalW multiple sequence alignment was performed using BioEdit (Version 7.0, Hall 1999).

16 Neighbor-joining phylogenetic trees were drawn using MEGA6.0 with 1000 bootstrap replicates.

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