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Dengue Virus Type 1 Strain Isolated in Indonesia Shows a Close Phylogenetic Relation with the Strains That Caused the Autochthonous Dengue Outbreak in Japan in 2014

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Dengue virus belongs to genus Flavivirus of family Flaviviridae and has been classified into 4 serotypes (DENV-1 to DENV-4) (1). Dengue infections currently occur in 128 countries, and 3.97 billion people are at risk of this arboviral infection (2). Although many advances have been made in reducing the burden of dengue infection, epidemics still occurred worldwide in the last decade (3–6). For example, an autochthonous dengue outbreak occurred in Japan in 2014 for the first time in 69 years (7,8).

Southeast Asia is one of the regions most affected by dengue (5). Among the countries in Southeast Asia, the incidences of dengue fever (DF) and dengue hemorrhagic fever (DHF) in Indonesia have been the highest since 2004 (9). Various studies showed dengue cases exported from Indonesia to China, Japan, and Australia (10–12). Therefore, epidemiological studies need to be conducted in Indonesia to clarify the evolution and migration of dengue virus. As part of dengue virus research in Indonesia, we conducted surveillance in Bogor, West Java. This city is located approximately 55 km south of the Indonesian capital Jakarta (13). Bogor has been identified as one of the cities in the West Java Province with large numbers of DHF cases (14).

In the present study, blood samples were collected between January and October 2014 from patients with suspected DF or DHF who visited the Insani clinic (Bogor, Indonesia). Written informed consent was obtained from the patients or their parents. Ethics approval was provided by the Ethics Committees of Airlangga University (Approval number: 24-934/UN3.14/PPd/2013) and Kobe University Graduate School of Medicine (Approval number: 784). Virus isolation and sequencing analyses of the envelope (E) protein-coding region were performed as described in the previous study (15).

Thirty-seven serum samples were subjected to virus isolation, and 5 strains of DENV-1 and 1 strain of DENV-3 were successfully isolated. The clinical information available for the DENV-1-infected patients is shown in Table 1. The five DENV-1 Bogor strains showed identical nucleotide sequences in the E region. Of these, 2 strains were randomly selected for a nearly full-genome analysis (16) and again showed identical nucleotide sequences. Thus, D1/BO15/14 (GenBank accession number: KT831765) was selected as a representative strain and subjected to a phylogenetic analysis, as described previously (17). Briefly, a preliminary neighbor-joining tree was constructed using all DENV-1 sequences deposited in GenBank (as of June 12, 2015; data not shown). Most strains were removed to preferentially show Indonesian strains as well as those closely related to the Bogor strain. The purpose of this preliminary tree was to select the best data-set for presenting simple phylogeny. Then, phylogenetic trees were reconstructed by the Bayesian Markov Chain Monte Carlo (MCMC) method to estimate the time point of divergence of the most recent common ancestor (MRCA).

An MCMC analysis using the E coding sequence grouped DENV-1 isolated in Bogor into Genotype I, clustered with viruses from Bali Island in Indonesia (in 2010/2011), Singapore (in 2013/2014), Malaysia (in 2013), China (in 2013/2014), and the major strain that caused the Japanese dengue outbreak in 2014 (LC011945; Fig. 1A). In the present study, LC011945 strain isolated from a patient who visited Yoyogi Park, Tokyo, served as a major epidemic strain because most of the strains of the Japanese autochthonous outbreak of 2014 showed identical E coding sequences (except for LC011949 isolated from a patient in Shizuoka) (7). Nucleotide and amino acid similarities in the E coding sequence between the isolates from Bogor and Japan (LC011945) were 99.6% (1479/1485) and 100%, respectively. The Singapore or Malaysian strains showed higher nucleotide similarities than the Bogor strain did (more than 99.6%). These results implied that the Japanese outbreak strain was imported from one of
Table 1. Clinical information of patients from whom DENV-1 was isolated

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>D1/BO23/14</th>
<th>D1/BO33/14</th>
<th>D1/BO13/14</th>
<th>D1/BO15/14</th>
<th>D1/BO4/14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>W</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Age (yr)</td>
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<td>30</td>
<td>35</td>
<td>37</td>
<td>32</td>
</tr>
<tr>
<td>Days after onset</td>
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<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Clinical diagnosis</td>
<td>DF</td>
<td>DF</td>
<td>DF</td>
<td>DF</td>
<td>DF</td>
</tr>
</tbody>
</table>

M, man; W, woman; DF, dengue fever.

1): Clinical information was provided by the clinic.

these Southeast Asian countries, particularly from Singapore or Malaysia.

An MCMC analysis using nearly a full genome is depicted in Fig. 1B. The Bogor strain showed the closest phylogenetic relation to the Japanese outbreak strain in 2014 among the 266 sequences covering nearly the full genome of DENV-1 (10,674 bp) deposited in GenBank (as of June 12, 2015; data not shown). Nucleotide and amino acid similarities between D1/BO15/14 and Japanese outbreak strains (LC011945, LC011948, and LC016760) were 99.5% (10620 or 10622/10674) and 99.8% (3384 or 3385/3392), respectively. Amino acid substitutions were distributed in non-structural proteins: H256Y in NS1, M168I in NS2A, P439S in NS3 (except for LC01670), M14L in 2k peptide, and I114V, V135I, T265A, and E824K in NS5. This result indicated that the Bogor strain was slightly different from the Japanese outbreak strain although the E region was identical at the amino acid level.

The MRCA of the Bogor and Japanese isolates of 2014 (except for LC011949) was found to be around 2010, as revealed by in both MCMC trees (Fig. 1). Among DENV-1 genotype I isolated in 2010, Indonesian strains showed the highest nucleotide similarities to the Japanese isolate of 2014. Although the strains showed the highest nucleotide similarity to the Japanese strain, those isolated in other Southeast Asian countries (Singapore, Malaysia, Laos, and Cambodia) in 2010 were included in the analysis (Fig. 1A). This result suggested that the origin of these strains is linked to Indonesia in 2010. In that year, Indonesia experienced the highest epidemic peak of DHF in the last 45 years, and DENV-1 genotype I was identified as the circulating and predominant strain in some cities in Indonesia in 2010 and subsequent years (15,17–19). Taken together, we can hypothesize that the ancestor of the Japanese out-
break strain was located in Indonesia around 2010 and may have spread to neighboring countries including Singapore and Malaysia because of the major epidemic of dengue in Indonesia. Then, a descendant of that strain entered Japan and caused the autochthonous outbreak in 2014.

In conclusion, we herein reported the nearly full genome sequence of DENV-1, which is phylogenetically close to the Japanese outbreak strain of 2014. This finding indicates that the Southeast Asian region was the source of the dengue autochthonous outbreak in Japan in 2014. We consider the results of the present study to be useful for retrospective analyses of dengue outbreaks in Japan.

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Conflict of interest None to declare.

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