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Genomic characterization of Zika virus isolated from Indonesia

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ABSTRACT

Zika virus (ZIKV) JMB-185 strain was isolated from a febrile patient in Jambi, Indonesia in 2014. To understand its genetic characteristics, we performed whole genome sequencing using the Ion Torrent PGM platform on the supernatant of the first passage. The phylogenetic analysis showed that the isolate was not closely related to the Brazilian ZIKV associated with microcephaly or isolates from the recent Singapore Zika outbreak. Molecular evolution analysis indicated that JMB-185 strain may have been circulating in the Southeast Asia region, including Indonesia since 2000. We observed high nucleotide sequence identity between Indonesia, Thailand, Singapore, and American strains although unique amino acid substitutions were also observed. This report provides information on the genomic characteristics of Indonesian ZIKV which may be used for further studies.

1. Introduction

The emergence of Zika virus (ZIKV, family *Flaviviridae*) has become one of the world's public health emergencies because of its association with Guillain-Barré syndrome and microcephaly. First discovered in 1947 in Uganda (Dick et al., 1952), the virus was relatively unknown for many decades. Isolated and sporadic cases of mild ZIKV-associated illness were reported in countries in Africa and Asia, such as in Uganda (Simpson, 1964), Nigeria (Fagbami, 1979), and Indonesia (Olson and Ksiazek, 1981). The ZIKV re-emerged in 2007 in Yap Island in the Federated States of Micronesia and now has a widespread distribution in Southeast Asia, Polynesia, and the Americas. ZIKV was known to be circulating in the Indonesian archipelago for decades (Olson and Ksiazek, 1981; Olson et al., 1983); however it was successfully isolated and characterized to be of the Asian lineage only recently (Perkasa et al., 2016).

Viral determinants of ZIKV infection are still unclear, including their contribution to infectivity, severity, and immunogenicity. Mutations in the flavivirus genome are known to influence virulence, replication efficiency, and antigen-antibody interaction with the host (Diamond, 2003; Holmes, 2009). Understanding the genome variants of ZIKV might be useful in deciphering its explosive spread as well as its potential link with microcephaly, leading to eventual preventive measures (Pybus et al., 2012). To further explore the genetic characteristics of ZIKV from Indonesia and its relationship with other isolates from around the world, especially those that were linked with congenital malformations, whole genome sequencing and analysis were performed on the JMB-185 Jambi isolate.

The ZIKV JMB-185 was isolated from a 27-year old man presenting to the hospital in Jambi, Sumatra in 31st Dec 2014, two days after illness onset with sudden high fever, headache, elbow and knee arthralgia, myalgia, and malaise. Some common clinical characteristics of ZIKV infection previously reported (Simpson, 1964), including maculopapular rash, conjunctivitis, and peripheral edema, were not seen. Hematological investigation revealed lymphocytopenia and monocytosis with a normal platelet count. All assays for dengue virus infection using Non-Structural Protein-1 (NS1) antigen detection, anti-dengue IgM and IgG ELISA and dengue real-time RT-PCR were negative. Chikungunya diagnosis using pan-alphavirus RT-PCR detection was also negative. The illness was self-limiting and the patient recovered 2 days after presentation without any complications (Perkasa et al., 2016).

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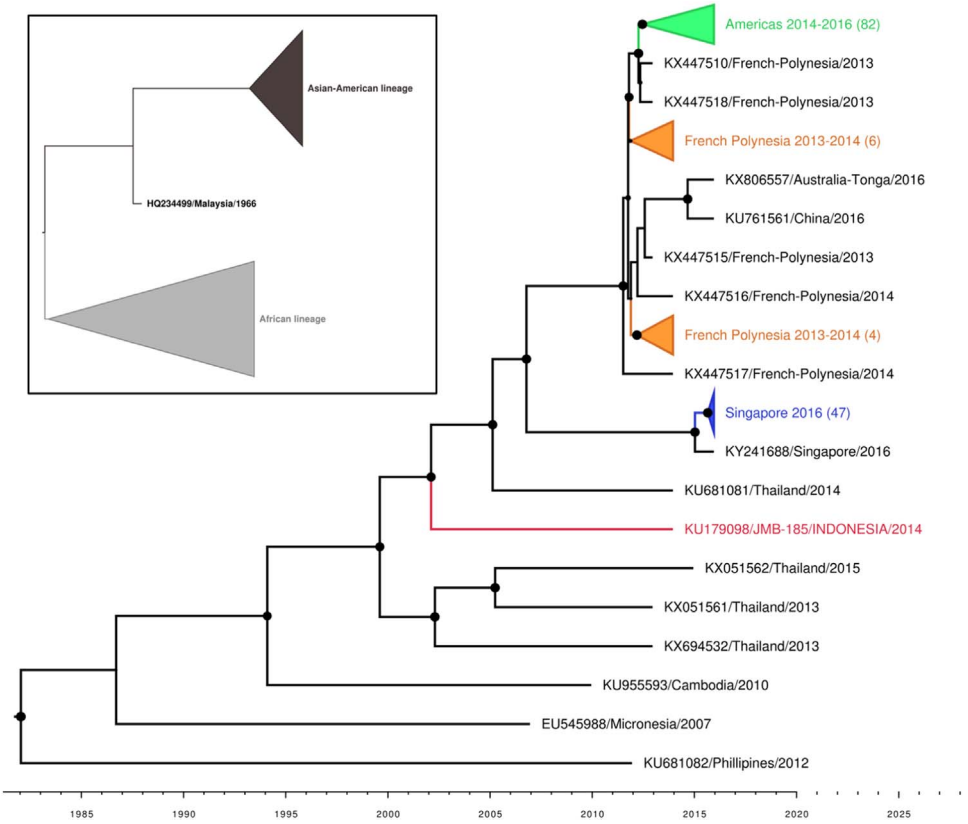

Fig. 1. MCC (Maximum Clade Credibility) phylogenetic tree of whole open reading frame (ORF) of ZIKV JMB-185 (red label) and other ZIKV Asian-American strains. For clarity, the tree only showed the Asia-America lineage (black clade, inset), truncated at HQ234499 Malaysian 1966 strain from the overall tree. Highly similar strains were condensed with numbers in brackets denoting number of strains in the condensed clades. Strains associated with microcephaly resided with other American strains in the green condensed Americas 2014–2016 clade. Strains associated with recent Zika outbreak in Singapore depicted as blue condensed clade. The condensed French Polynesia clades were labeled orange. Visible black circles at each node represented posterior support about or greater than 0.85 for the particular node.

Table 1
Amino acid (AA) substitutions observed in JMB-185, Indonesian Zika virus isolate.

No.	AA Substitution	Gene (AA position)	Remarks
1.	A131T	prM (8)	Small non-polar to polar AA
2.	P220L	M (4)	Rigid to flexible AA
3.	I1102V	NS1 (311)	Similar non-polar AA
4.	F1326L	NS2A (183)	Non-polar to non-polar
5.	R1353K	NS2A (210)	Similar positive charge AA
6.	R1368K	NS2A (225)	Similar positive charge AA
7.	I1411T	NS2B (42)	Non-polar to polar
8.	V2261A	2 K (18)	Similar non-polar AA
9.	T2294V	NS4B (28)	Similar hydrophobic AA

2. Results

2.1. ZIKV sequence and evolutionary analysis

The near-complete genome of JMB-185 (10,668 nt), GenBank Accession No. KU179098, has been successfully sequenced. The phylogenetic tree generated using the Bayesian MCMC method employing complete ORF of ZIKV showed the clear distinction of Asian and African lineages, with a most common recent ancestor (TMRCA) between these two clades that existed since year 1895 (95% HPD values ranging from year 1799 to 1951) (inset in Fig. 1). The overall evolutionary rates were estimated as 9.95×10^{-4} nucleotide substitutions per site per year (95% HPD: $4.37–15.28 \times 10^{-4}$ subs/nt/yr). This Jambi isolate was classified as an Asian lineage that was closely related to all isolates from Thailand (2013, 2014 and 2015) and shared a common ancestor around year 2000 (95% HPD: 1998–2005) (Fig. 1). Previous reports (Wang et al., 2016) observed that all human strains identified in recent years appeared to be more closely related to French Polynesia (2013) strain than Micronesia (2007) strain. The same observation was confirmed for the Jambi isolate which was also more related to the French Polynesia strains. In regards to recent Singapore ZIKV outbreak (Singapore Zika Study Group, 2017), we observed that the Jambi isolate was not directly related to those Singaporean strains although they shared the same monophyletic clade.

2.2. Amino acid (AA) comparison and 5′ and 3′ untranslated (UTR) region analyses

Analysis of the Jambi isolate revealed nine unique AA changes within the viral polyprotein that were not observed in other published genomes in our dataset from the Asian-American lineage, including genomes from ZIKV that caused microcephaly (Calvet et al., 2016; Mlakar et al., 2016) (Table 1 and Fig. 2). The majority of the unique variations (3 out of 9) were located in the NS2A, a protein without any clear function or known enzymatic motifs. No unique mutations were observed in E glycoprotein, a protein known for humoral immune responses against flaviviruses.

In addition to the AA analysis, we performed alignment of 5′ and 3′ UTR of the genomes, the most conserved regions. We did not observe any differences in the 5′UTR region of the Jambi isolate compared with other known 5′UTR regions of published sequences. We observed one nucleotide difference in the 3′UTR region of the Jambi isolate (data not shown).