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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.

Keywords

Zika virus; Genome; Amino; acid substitution; Indonesia

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

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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).

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).

3. Discussion

Recently, ZIKV was isolated from a patient with dengue-like illness in Jambi, Sumatra (Perkasa et al., 2016). Our study is the first to report the complete genome of an autochtonous ZIKV isolate in Indonesia which currently is the only isolate from Indonesia. In this study, we obtained a near-complete genome sequence of the Indonesian isolate (JMB-185) and analyzed its genetic variability among the whole genome reference strains selected from GenBank. Phylogenetic analysis of ZIKV complete ORF sequences classified the isolates into two distinct lineages, the Asian-American and African. The Indonesian isolate was grouped into Asian lineage together with other isolates from Southeast Asia including Singapore, the Pacific, China, and the Americas. The phylogenetic inference analysis employing Bayesian MCMC and molecular clock showed similar estimated overall evolutionary rate with other flaviviruses, as previously reported (Twiddy et al., 2003) and in concordance with the ZIKV in the Americas (Faria et al., 2016). The Indonesian isolate was found to share a common ancestry and TMRCA with ZIKV isolated from a Canadian traveler returning from Thailand in 2013 (Fonseca et al., 2014) and febrile Thailand patients in 2014 (Buathong et al., 2015). This result confirmed and complemented our previous finding of phylogenetic inference targeting only partial NS5 gene (Perkasa et al., 2016).

The estimated TMRCA for the Jambi isolate together with Thailand strains was ca. 2000, suggesting that it has an older lineage than the isolate from French Polynesia in 2013 (Baronti et al., 2014) and the recent Brazilian ZIKV strains (Faria et al., 2017, 2016). The TMRCA result indicates that the ZIKV Jambi strain may have been circulating in the Southeast Asia region, including Indonesia, for the last decade. Additionally, we included the recent Singaporean ZIKV outbreak strains in our analysis to explore the relationship between those strains and the Indonesian isolate. Although the Indonesian isolate share common ancestry with the Singaporean strains, the isolate has much older lineage and hence they are not directly related.

One of the interesting findings is that although there were nine AA variations unique to the Jambi isolate, none of these changes were located in the E or NS5 protein regions. Instead,

the majority of the unique variations were located in the NS2A. Although this nonstructural protein is not known to function as part of the virus production, NS2 protein is essential in the final step of viral replication cycle in vitro and in vivo (Bollati et al., 2010; Khromykh and Westaway, 1997; Murray et al., 2008). The majority of the nine AA variations involved similar physico-chemical properties, with the exception of A131T (prM region) and P220L (M region). The contribution of these substitutions to the viral virulence and human pathogenesis warrants further experimental studies. Although the rate of mutation in arboviruses is low compared to those directly infecting vertebrate hosts (Jenkins et al., 2002), more Indonesian isolates need to be analyzed to confirm whether these variations would be seen consistently.

In addition to polyprotein analysis, we also aligned the 5′ and 3′ genome untranslated regions important for flavivirus replication and protein translation (Brinton and Basu, 2015). No nucleotide variation was observed in the 5′ region, whereas in the 3′UTR region, we observed one nucleotide difference in the Jambi isolate. This is likely to be insignificant for the structure of the 3′UTR region, as the structure prediction we performed did not show any changes (results not shown).

The complete genome described in this study highlights the circulation of a unique strain of ZIKV in the region, although the potential importance of the AA changes remains unknown. This information could provide some insight into the evolution of ZIKV, virulence determination, and be valuable for future vaccine and drug development.

4. Methods

4.1. Zika virus isolation

ZIKV was isolated from the serum of a febrile patient during a dengue outbreak in Jambi in 2014, as previously described (Perkasa et al., 2016). ZIKV was propagated in Vero CCL-81 cell line and RNA extraction was performed from 0.22 μm filtered low passage culture supernatant. 140 μl of supernatant was extracted using QIAamp Viral RNA mini kit (Qiagen), according to manufacturer's instructions.

4.2. ZIKV whole genome sequencing

ZIKV RNA was treated first with DNAse I (Thermo Scientific) followed by cDNA library construction using the Ovation RNA-Seq System V2 (NuGEN) and the Ion Xpress Plus™ gDNA and Amplicon Library Preparation kit (Thermo Scientific). The sheared and purified cDNA library was analyzed using Bioanalyzer (Agilent Technologies). The cDNA template (100 pM) was then prepared and enriched using Ion PGM Hi-Q OT2 kit (Thermo Scientific). To reach a targeted depth coverage of sequence, the enriched ion spheres were then sequenced using the Ion Torrent Personal Genome Machine Sequencer and the Ion PGM Hi-Q Sequencing kit, with a 316 Chip (Thermo Scientific). SMALT (http://www.sanger.ac.uk/science/tools/smalt-0) was used to map the PGM reads to ZIKV genome reference (acc. no. NC_012532). All mapped reads were then filtered using PRINSEQ (http://prinseq.sourceforge.net) for only high-quality reads (mean Q > 30) with length range of 100 – 250 bp, and subjected to 5′/3′ dereplication. The filtered reads were assembled using

SPAdes (http://bioinf.spbau.ru/spades) in *metagenome mode*, and the resulting contig was used as an untrusted contig for the next consecutive SPAdes assembling in *single cell and careful mode*, which employed mismatch correction using BWA internally.

4.3. ZIKV sequence and evolutionary analysis

The near-complete genome sequence of Jambi ZIKV isolate was assembled and aligned with all available ZIKV sequences retrieved from the GenBank database as of June 2017, including strains associated with microcephaly and recent cases in America and Singapore (Faria et al., 2017; Singapore Zika Study Group, 2017). Sequence alignment was performed using USEARCH, which allows multiple alignment from sequences with varying length (Edgar, 2010). The resulting alignment was trimmed to generate a dataset of ~10.2 kb of complete open reading frame (ORF) of ZIKV. After removal of sequences covering less than 8 kb of ORF and other non-isolate sequences, the alignment was refined using MUSCLE to obtain a dataset of 458 ZIKF near-complete ORF sequences (Edgar, 2004). A phylogenetic tree was inferred based on the selection of a statistical model for likelihood calculation using jModelTest version 2.1.4. ZIKV ORF sequences was analyzed for their evolutionary rate and the time to most recent common ancestors (TMRCA) using the Bayesian Markov chain Monte Carlo (MCMC) method as implemented in BEAST version 1.8.4 (Drummond et al., 2012). Data were prepared using BEAUti graphical interface and the tip of each isolate was calibrated using the year of isolation as the calibration point. Runs were performed using GTR model with four gamma parameters and invariant sites (GTR + Γ4 + I) as inferred by jModelTest and relaxed uncorrelated lognormal molecular clock using the initial estimated evolutionary rate of 1×10^{-3} substitutions per site per year, as previously described. Tree prior was set as coalescent Bayesian skygrid which allowed for flexible demographic assumption. Two hundred million chains were run and sampled for every 1000^{th} iteration, with 10% burn-in employed. The convergence of parameters was analyzed using Tracer (version 1.6). A maximum clade credibility (MCC) tree was created using TreeAnnotator (version 1.8.4) and visualized in FigTree (version 1.4.3). The TMRCA in each node was estimated as median year with 95% Highest Posterior Density (HPD). Amino acid comparison analysis was performed using ORF dataset consisted of strains from Asian-American lineage.

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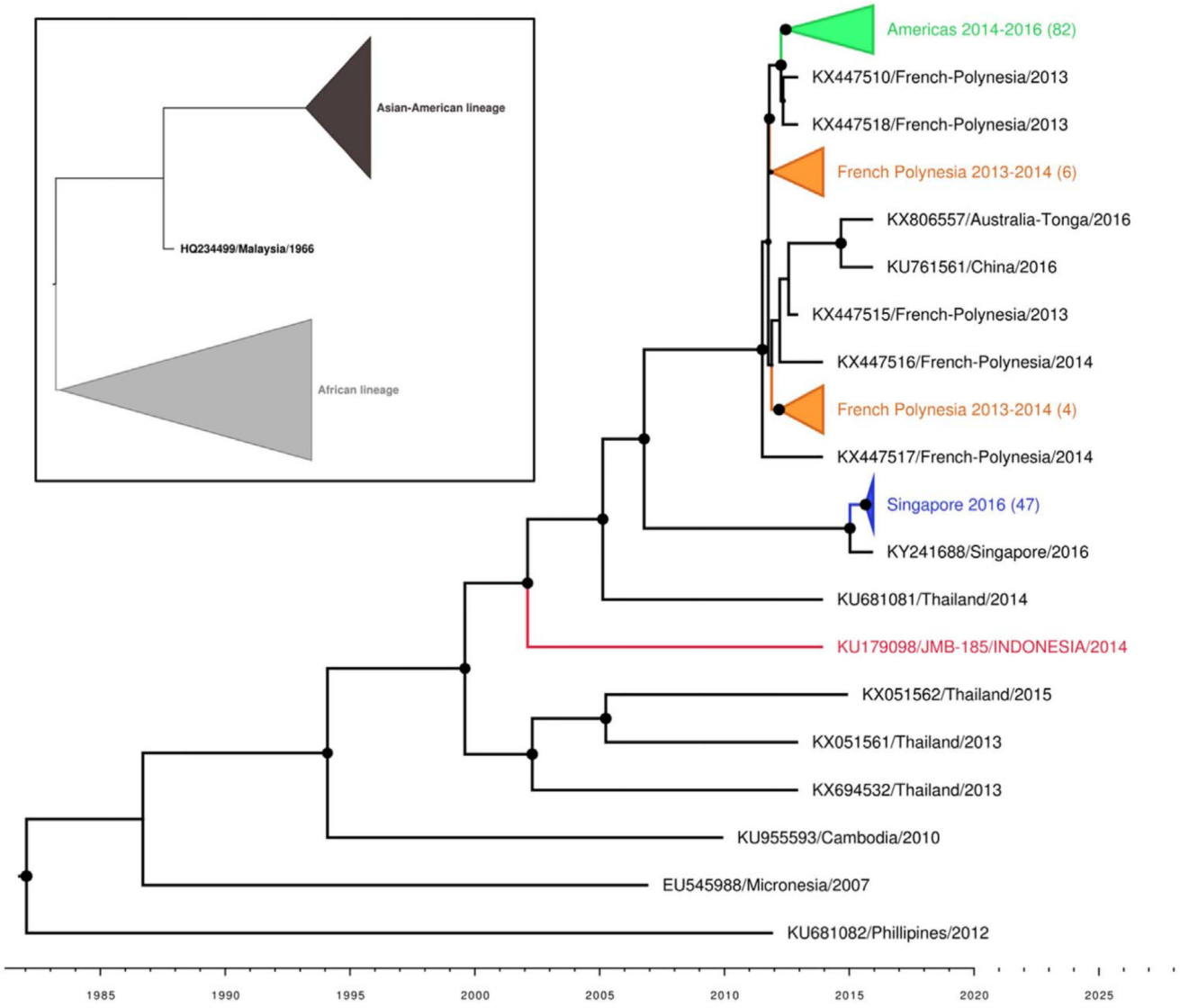

Fig. 1.

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.

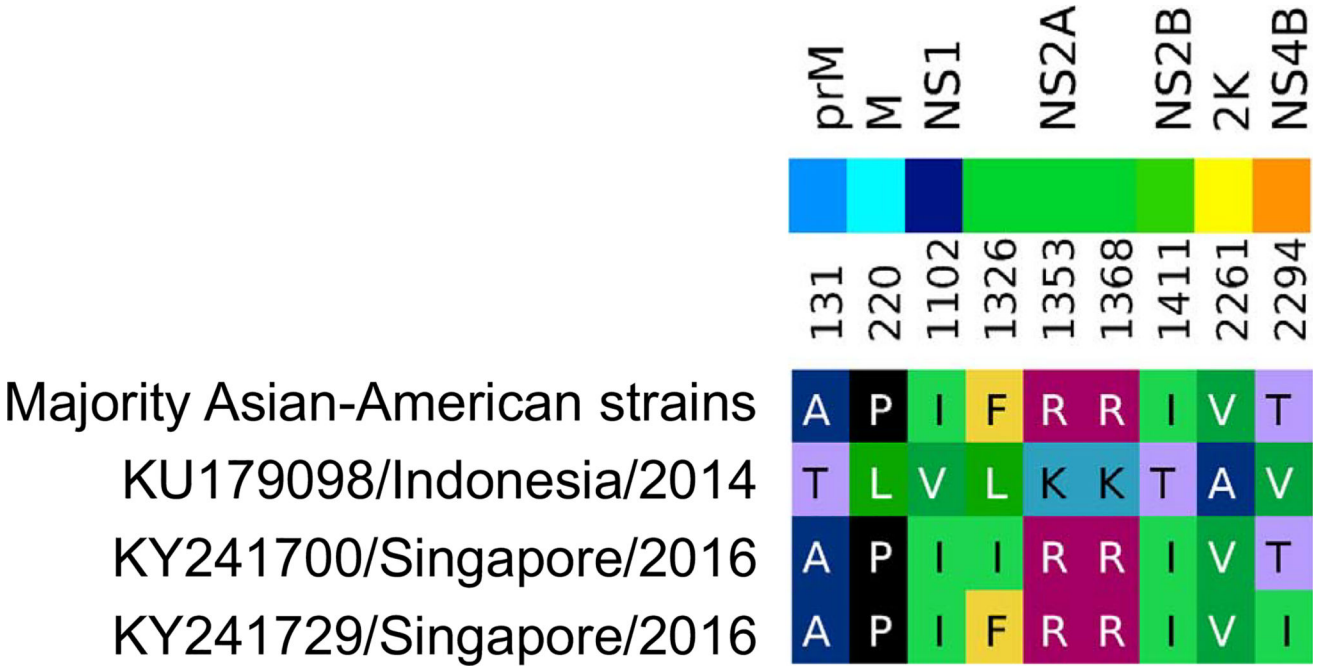

Fig. 2.

Comparative analysis of amino acid substitutions in the 9 positions unique for Indonesian isolate among Zika viruses. Dataset include strains from Asian-American lineage only. The first row represented almost all of the Asian-American strains, including the Brazilian strains that caused microcephaly are represented. The Indonesian isolate is represented in second row. The gene location and amino acid positions depicted above the sequences.

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