



Diversity of and Implications from the Viral Genomes and Viral Proteins of Zika Virus

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Abstract

Zika virus (ZIKV) belongs to the Genus *Flavivirus* of Family *Flaviviridae* and is closely related to Dengue, West Nile, Japanese encephalitis and yellow fever viruses. Since 2007 ZIKV has caused a series of epidemics in Micronesia, the South Pacific, and, most recently, the Americas. Infection with ZIKV is likely linked to severe medical sequelae. Most recently, it has been observed that ZIKV infection is probably associated with microcephaly of neonates and this makes it urgent to investigate every aspect of the virus including its natural history, epidemiology, pathogenesis and interactions with hosts. In the present study, we analyzed the nucleotide acid (NA) and amino acid (Aa) sequences of the strains of Zika viruses responsible for the seven different epidemics. Sequence alignments and phylogenetic analysis revealed that the ZIKV can be divided into African and Asian types. Interestingly, we found that the Malaysia strain isolated in 1966 appears more divergent from other Asian strains and less divergent from the African type than other Asian strains. To understand why the recent ZIKV outbreak correlates with microcephaly in neonates, we analyzed the diversity of amino acid sequences between the French Polynesia (FP) strain and the Brazil strain. We found that they are more closely related to each other than to other strains being studied. This is consistent with the hypothesis of Brazil ZIKV being evolved from FP-type ancestors. Notably, there were 11 amino acid residues in the Brazil 2016 strain that were different from the consensus sequence. Further analysis of sequence divergence in individual proteins showed that the biggest difference between the FP strain and Brazil strain lies within the NS1 protein, which is related to neurovirulence as in the Dengue virus. Therefore, NS1 might be an important target for further investigation.

Keywords

Zika virus (ZIKV); Outbreak; Genomic diversity; Alignment; *Flaviviridae*

Background

The Zika virus (ZIKV), together with the West Nile virus, Yellow fever virus, Japanese encephalitis virus, Dengue fever virus, and other classified and unclassified viruses, forms the genus *Flavivirus* within

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the family *Flaviviridae*. The family *Flaviviridae* consists of many other viruses as summarized in a recent review [1]. This family of viruses has an enveloped icosahedral capsid containing a single strand, positive sense RNA genome (about 11,000 nucleotides) [2]. Therefore, the infected viral RNA can be directly translated to a large polyprotein precursor, which is co- and post-translationally processed by viral and cellular proteases into structural and non-structural proteins. The three structural proteins are critical for the formation of the envelope and capsid, and the seven non-structural (NS) proteins play important roles in virus replication. The three structural proteins are the enveloped, E; membrane precursor, PrM; and capsid, C. The seven non-structural (NS) proteins include NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5. Both the structural and non-structural proteins are needed to determine the pathogenicity of ZIKV [3].

ZIKV is a re-emerging *Flavivirus* transmitted both via the mosquito *Aedes aegypti* and sexually [4,5]. The ZIKV was first isolated in 1947 from a monkey of the Zika forest [6], which represents the African strain, and was later isolated in Southern Asia [7]. Therefore, two types of ZIKV are considered to be the ancestors of the contemporary virus [8]. It was not considered as a severe medical concern until recent outbreaks of ZIKV disease in the Pacific Islands and in region of the Americas [9,10]. However, epidemiological data and clinical findings on laboratory-confirmed Zika virus disease remain limited. Here we report our recent genomic analysis of the Zika viruses and discuss their potential biological implications.

Results and Discussion

ZIKV has caused several epidemics at different scales since it was first isolated. The geographic locations of viral isolation and epidemics include: South Africa [6], Malaysia [7], Cambodia [11], Yap island [12], French Polynesia [13] and Brazil [14]. Strains of the virus were isolated from each of the epidemics. RNA viruses keep varying to avoid host immunity, leading to generation of new strains. We wondered whether the Zika virus outbreaks are related to the continued mutations of viruses. To examine this possibility, we chose seven strains of ZIKV from different geographic isolations: an African strain (MR477) isolated in 1947 [6], an Asian strain [11], an Malaysian strain [7], a Yap island strain [12,15], a more recent African strain [16], an FP strain [17,18], and a Brazilian strain [19]. The complete nucleotide (Nt) and Aa sequences of all these strains are available in the PubMed database, their database accession numbers are shown in the figures. First, as shown in Figure 1S, we performed a multiple sequence alignment of the whole Aa sequence of the precursor of ZIKV protein that contains 3,421 amino acids in total. Conserved amino acid residues are shown in red and other colors (gray or blue) indicate where sequences differ from the consensus sequence. The mutations in the Brazil strain are shown in gray and highlighted in yellow. The consensus sequence, shown in the end of each block, was derived using the BoxShade program based on the majority rule (>=50% agreement). Overall, the seven sequences were highly conserved.

The large polyprotein precursor must be cleaved to generate active functional proteins. The distribution of mutations varies in 10 proteins. As summarized in Figure 1, PrM protein presents the highest number of the mutations (7.2%), and NS2b has the smallest number of mutations (1.5%). Interestingly, the region spanning

NS2b, NS3, and NS4a seems to be more conserved than the other areas. The functional consequence of these mutations awaits further investigation.

We then performed phylogenetic analysis for both the Aa and Nt sequences (Figures 2 and 3) [20-23]. The neighbor-joining (NJ) trees inferred from Aa and Nt sequences yielded a consistent topology.

Brazil 2016 and FP 2014 were most closely related, forming a clade in the trees with strong bootstrapping support. Both strains are most closely related to an Asian strain, the Cambodia strain, and the Yap island strain. The African strains (1947 and 2014) appear to be highly homologous. However, Malaysia 1966, a previously identified Asian strain, appears to be divergent from the other Asian strains. Notably,

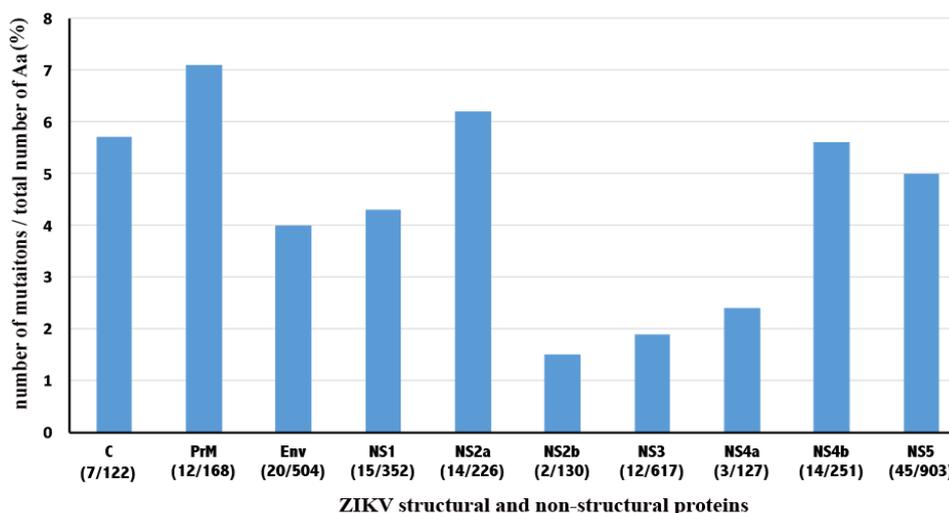


Figure 1: Summary of the mutations of ZIKV genes: There are a total of 144 mutations in the ZIKV proteins, most of which are substitutions except one deletion in the NS5 protein of the African 2014 strain. The percentages were shown by dividing the mutation number to the total number of amino acid residues of each protein. The numbers were shown under the name of each protein. Abbreviations: C (capsid), PrM (precursor of M), Env (envelope), NS (non-structure protein).

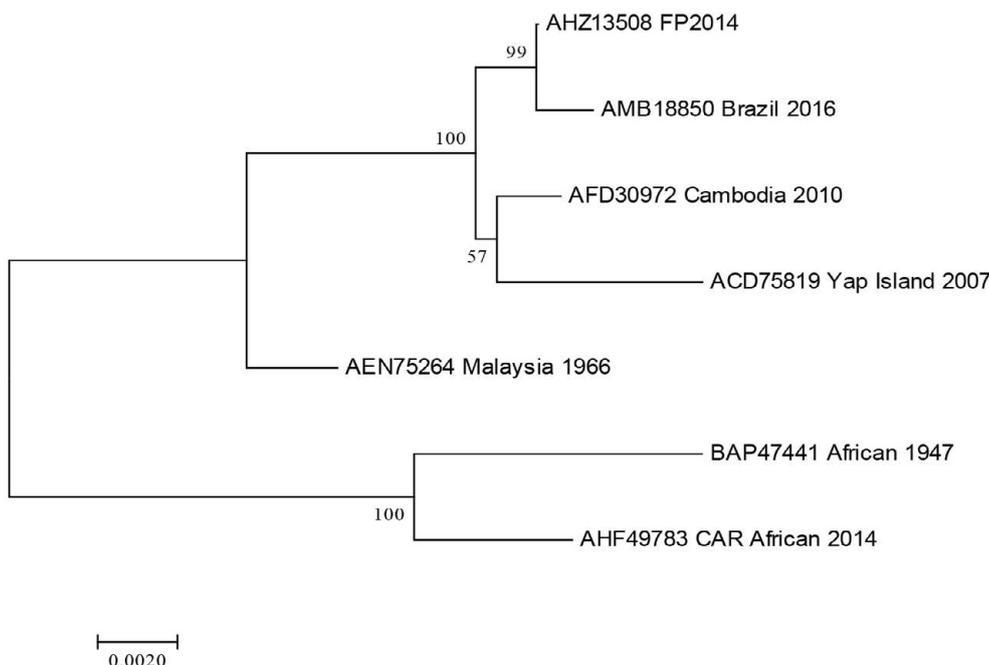
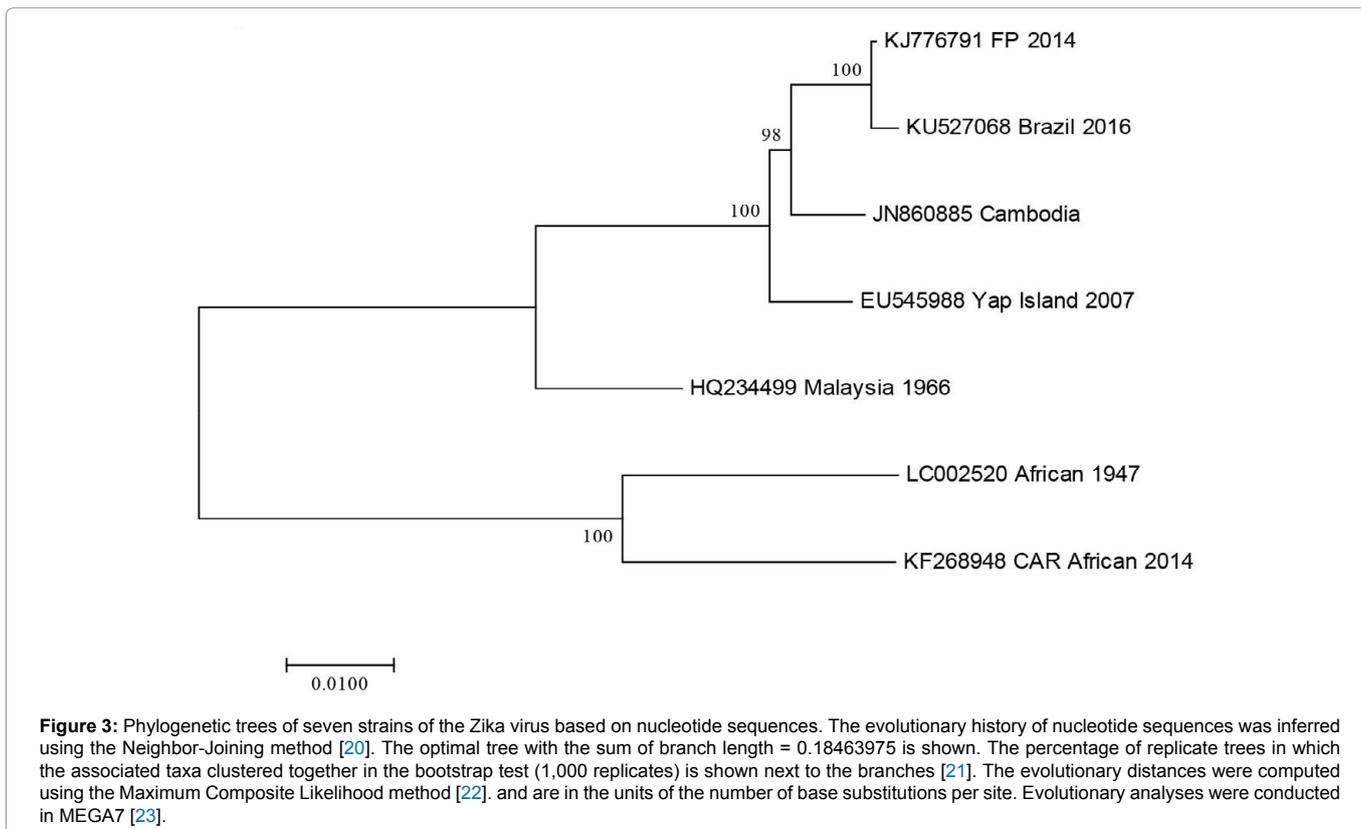


Figure 2: Phylogenetic tree of seven strains of the Zika virus based on amino acid sequences: The evolutionary history of amino acid sequences was inferred using the Neighbor- Joining method [20]. The optimal tree with the sum of branch length = 0.04561554 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches [21]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method [22], and are in the units of the number of amino acid substitutions per site. Evolutionary analyses were conducted in MEGA7 [23].



there were 11 amino acid residues in the Brazil 2016 strain that were different from the consensus sequence, as shaded in yellow color in Figure 1S. The possibility that some of these mutations are related to the pathogenicity of the ZIKV is intriguing.

Finally, we examined the types of amino acid mutations. The mutations are diverse, most of which are substitutions from Isoleucine to Valine, and Valine to Alanine. The change from Lysine to Arginine is also observed. Most mutations are not continuously distributed in the genome, except that six amino acid mutations were observed in the capsid gene of the Yap island strain, from kksqgf to EEIRRI. All the mutations are substitutions except one deletion mutation in the NS5 protein of African strain 2014.

It would be highly interesting to know whether the observed mutations affect the biological functions of the proteins. The cleavage of the polyprotein precursor is a sophisticated process and is completed collaboratively by cellular proteases of the PACE (Paired basic Amino acid Cleaving Enzyme)-type or other Golgi-localized proteases and the viral serine protease embedded in the N-terminal domain of non-structural protein 3 (NS3Pro), which requires NS2b for its activity [1]. A distinct feature of genus *Flavivirus* from other genera of *Flaviviridae* is that the 5'-end of the (+) ssRNA genome of genus *Flavivirus* is decorated with an RNA cap structure (N7meGpppA2'Ome-RNA). The 5' end capping of the viral RNA is as important as that for eukaryotic mRNAs, not only to initiate the process of translation but also to protect the viral RNA from degradation by endogenous RNA exonucleases. The protein translation happens immediately after the uncoating of viral particle in the cytoplasm. The (+) ssRNA genome is used as a template not only for gene expression but also for viral genome

replication. Both viral RNA replication and gene translation occur in the cytoplasm. For RNA replication, viral non- structural (NS) proteins and cellular proteins interact to form a replication compartment (RC).

During the period of viral RNA replication in the cytoplasm, the RC consists of morphologically distinct, membrane-bound compartments that also differ with respect to both function and NS proteins composition [24]. The NS3 and NS5 proteins are central to the viral RC, as together, they harbor most, if not all, of the catalytic activities required to both cap and replicate the viral RNA. Following replication, the protected genomic RNA is packaged by the C protein to form a capsid in a host-derived lipid bilayer in which the E protein is embedded and later integrated into viral envelope. The mature particles subsequently exit from the host cell by exocytosis.

Recent events in Brazil have earned the Zika virus the world's attention. It has become another member of Genus *Flavivirus* to move to the center of virological research. There is an urgent need to solve this problem, but time is needed to achieve a better understanding of its pathogenesis, prevention, and treatment. The genomic analyses presented here have identified mutations that may have driven the conversion of Zika virus from an infectious agent of little concern to a virulent pathogen, and it is our hope that these results will help guide the research community to a full understanding of ZIKV.

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