WITHDRAWN: Complete Genome Sequence of Unusual Strain of Zika Virus From West Africa

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Research Article

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EDITORIAL NOTE:

The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be

directed to the corresponding author.

Abstract

In 2018, a Zika infection was detected in patients in the Republic of Guinea. We determined the complete genome sequence of the Faranah/18 strain of Zika virus (ZIKV) isolated using C6/36 cells. The genome sequences are significantly different from those of other African ZIKVs. This isolate has 13 aa substitutions in nonstructural polypeptides, an additional glycosylation site on protein E, and unusual localization of MSI1 binding sites in the 3'-UTR of the African lineage. Additionally, Faranah/18 formed a separate lineage into African genotypes with divergence times near 94 and 646-861 years from Senegal and Uganda ZIKVs, respectively.

Full Text

Zika virus (ZIKV) is an arbovirus that belongs to the genus *Flaviviruses*, the *Flaviviridae* family [1]. Zika virus was first detected in 1947 in a sentinel rhesus monkey in the Zika forest in Uganda, and the virus was isolated from *Aedes africanus* approximately one year later. The ZIKV genome is a positive-sense single-stranded RNA approximately 11 kb in length. The viral genome has a typical flavivirus structure and is flanked by highly structured untranslated regions (UTRs). Currently, ZIKV has spread widely and is actively circulating in human populations in practically all countries with a tropical climate. The total number of countries with Zika virus transmission reached 70 in 2017 [2, 3]. Recently, it was shown that African ZIKV strains display higher transmissibility in mosquitoes and higher lethality for mouse models than their Asian counterparts [4].

The aim of this study was to sequence the whole genome of a novel isolate of ZIKV from Guinea with subsequent annotation of the ZIKV genome as a putative new African lineage with possible epidemic potential.

The C6/36, HEK-293 and Vero E6 cells were grown in DMEM/F12 (1:1) medium supplemented with 5% foetal bovine serum (Gibco BRL, USA) and 40 µg/ml gentamicin and incubated at 28°C or 37°C. A series of successive passages of primary isolate 15555 of ZIKV (GenBank ID: MN025403) were carried out on C6/36 cells. Viral infectivity was determined using 96-well plates in triplicate in C6/36, HEK-293 and Vero E6 cells as previously described [5]. RNA extraction, PCR amplification, and sequencing using an ABI 3130xl genetic analyser were performed as previously described [6]. The nucleotide sequence derived from isolated ZIKV, called Faranah/18, was assembled and aligned with other sequences of ZIKV using MEGA 10 [7]. An evolution analysis was performed using the BEAST package [8]. Bayesian Markov chain Monte Carlo (MCMC) was used to estimate the time to the most recent common ancestor (tMRCA) for the Zika viruses [9]. The MCMC was run for 100 million iterations, and convergence was evaluated using the TRACER program. A maximum clade credibility tree was built using the Tree Annotator program [10]. The secondary structure of the 3'-UTR of ZIKV genomic RNA was predicted using the MFOLD 3.4 server [11].

The adapted toC6/36 cells Faranah/18 ZIKV was easily cultivated on C6/36, HEK-293, and Vero E6 cells with CPE. The viral infectious titre was $3x10^6$ $TCID_{50}$/ml in HEK-293 cells and $1x10^6$ $TCID_{50}$/ml in Vero E6

and C6/36 cells. The complete genome sequence was determined after the 3rd passage in C6/36 cells (OL414716).

The identity of Faranah/18 ZIKV was closest to that of Senegal ZIKV (MF510857) from *Aedes taylori* (97% with 193 nucleotide substitutions and 99% with 13 aa substitutions). Amino acid substitutions were located in the nonstructural polypeptides NS1, NS2A, NS2B, NS3, NS4B, and NS5 (Table S1), with most substitutions clustered in NS3 and NS5. The detected substitutions were not changes that happened during the virus adaptation to cells from the primary human 15555 isolate of ZIKV. Additionally, African ZIKV isolates from Uganda and Nigeria have short deletions located at 153-156 aa or 155-160 aa positions for protein E, and the potential glycosylation site may be eliminated by this deletion (Fig. S1). However, the potential glycosylation site for Faranah/18 is available and similar to that of modern isolates of American and Asian genotypes of ZIKV associated with the last pandemic of ZIKV infection. Therefore, it is important to note that the N-glycosylation site may be associated with flavivirus virulence and neuroinvasiveness [12, 13].

Phylogenetic analysis showed that the ZIKV most closely related to Faranah/18 was isolated in Senegal in 2018 (Fig. 1). The estimated divergence time for the basal node is 861 years for the African genotype, 109 years for the Asian genotype and 5.5-21.7 years for the American genotype.

The length of the 3’-UTR of Faranah/18 is 428 nucleotides, and the secondary structure of the 3’-UTR is conservative for ZIKV (Fig. 2). Only 3 nucleotide substitutions were detected in the SL-2 structure of the 3’-UTR. The Faranah/18 ZIKV has five canonical MSI1 (Musashi binding element 1) sites. The MSI1 (UAG) site at position 10637 typical for the African genotype is absent in the 3’-UTR of Faranah/18. Another UAG site at position 10774 associated with the Asian lineage was detected in the 3’-UTR of Faranah/18. It was previously shown that the MSI1 site mediates the maintenance and self-renewal of stem cells and acts as a translational regulator that has been associated with the level of ZIKV replication and foetal neuropathogenesis during this viral infection [14–16]. A change in the location (10637 to 10774) of the MSI1 sites for Faranah/18 ZIKV may indicate an increase in its neurotropism. Additionally, 3 nucleotide substitutions were detected in the SL-2 structure of the 3’-UTR that is responsible for binding to NS2A, as well as one amino acid substitution in NS2A. These substitutions may change the local conformation of NS2A, which is critical for the interaction of the NS2A protein with the SL-2 structure and the effectiveness of viral replication.

Thus, the complete genome sequence for the novel isolate Faranah/18 ZIKV was sequenced and analysed. Phylogenetic analysis showed that Faranah/18 significantly differed from other African ZIKVs and formed a separate lineage into African genotypes. The isolate has numerous amino acid substitutions into nonstructural polypeptides, nontypical for African isolate glycosylation sites of protein E and unusual MSI1 binding sites of the 3’-UTR of the viral genome. This low-passage ZIKV strain presenting circulating viral genetic diversity for African lineage and may be associated with future outbreaks of human ZIKV infections.

Declarations

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Compliance with ethical standards I have read and abided by the statement of ethical standards for manuscripts submitted to Archives of Virology.

Conflict of interest All authors declare they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

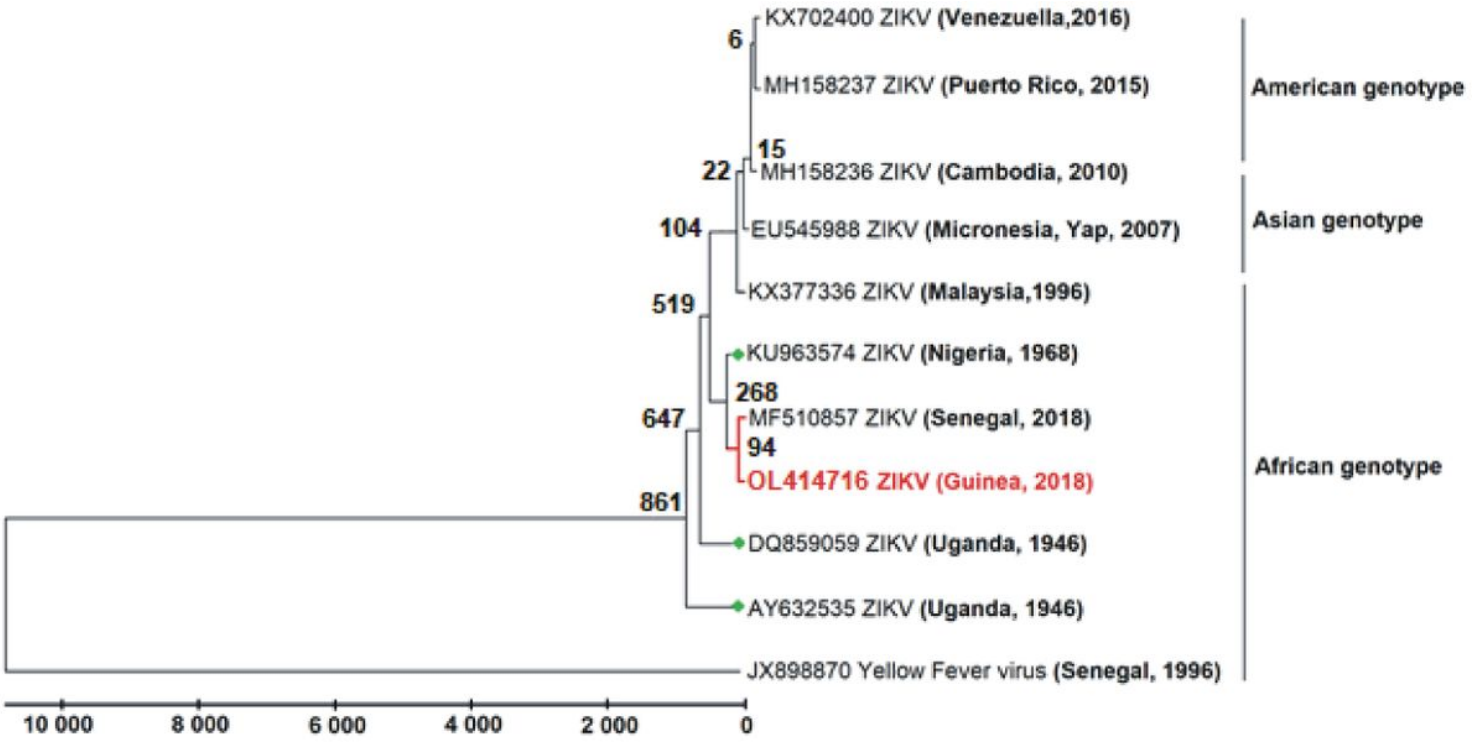

Figure 1

Maximum clade credibility tree of ZIKV. The node labels indicating the time (years) of divergence from ancestors (tMRCA, with the 95% HPD) and the time scale (years) are presented below. The Faranah/18 strain of ZIKV is highlighted in red. The strains that had deletions in the glycosylation site of protein E are highlighted by green rhombi.

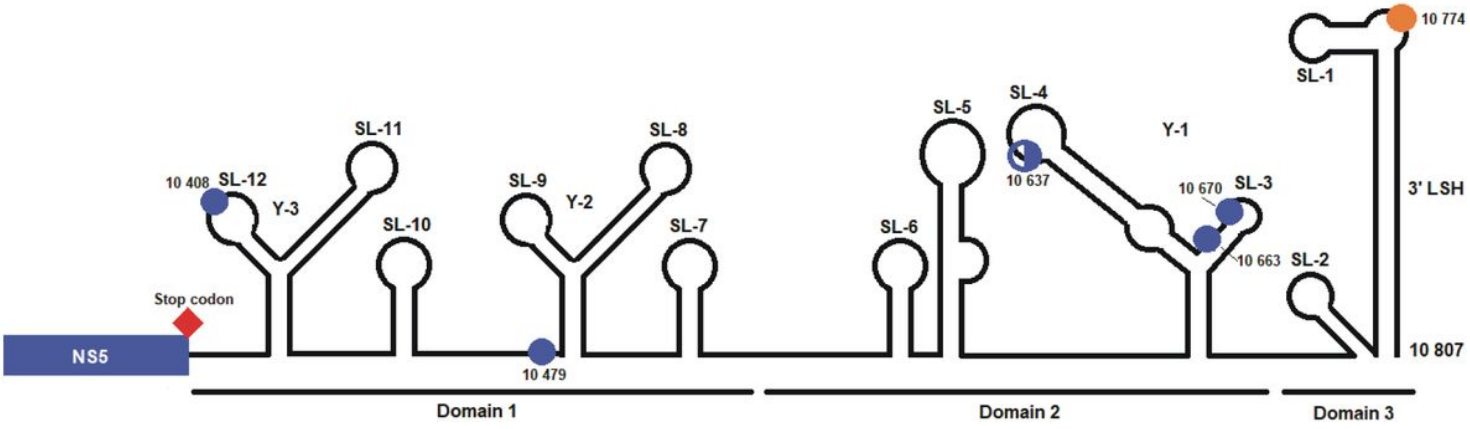

Figure 2

Location of MSI1 binding sites in a secondary structure of the 3’ UTR Faranah/18 strain of ZIKV. MSI1 sites shared with both Asian and African lineages are blue circle; the site specific to the Asian lineage is orange circle.

Supplementary Files

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