A single mutation in the prM protein of Zika virus contributes to fetal microcephaly

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Zika virus (ZIKV) has evolved into a global health threat because of its unexpected causal link to microcephaly. Phylogenetic analysis reveals that contemporary epidemic strains have accumulated multiple substitutions from their Asian ancestor. Here we show that a single serine-to-asparagine substitution [Ser139→Asn139 (S139N)] in the viral polyprotein substantially increased ZIKV infectivity in both human and mouse neural progenitor cells (NPCs) and led to more severe microcephaly in the mouse fetus, as well as higher mortality rates in neonatal mice. Evolutionary analysis indicates that the S139N substitution arose before the 2013 outbreak in French Polynesia and has been stably maintained during subsequent spread to the Americas. This functional adaption makes ZIKV more virulent to human NPCs, thus contributing to the increased incidence of microcephaly in recent ZIKV epidemics.

Fig. 1. Neurovirulence phenotypes of the contemporary ZIKV strains and their ancestral Asian strain. (A) Neurovirulence tests of different ZIKV strains in neonatal mice. P1 BALB/c mice were intracerebrally injected with 10 plaque-forming units (PFU) of virus, and mortality was observed for 26 days. CAM/2010: n = 24; VEN/2016: n = 21; SAM/2016: n = 22; MTQ/2015: n = 23 (n, number of mice). Log-rank test was performed for statistical analysis. ****P < 0.0001. (B to D) Littermate embryonic brains were injected with CAM/2010, VEN/2016, or culture media containing 2% fetal bovine serum (mock) at E13.5 and inspected at E16.5 or E18.5. (B) Images of brains (E18.5). Red and yellow bars represent brain width and cerebral cortex length, respectively. (C) Nissl staining of E18.5 brains. CP, cortical plate; SP, subplate; IZ, intermediate zone; VZ, ventricle zone; SVZ, subventricle zone. (D) Images of E16.5 cortices stained with phosphorylated histone H3 (P-H3, red). Scale bars: 5 mm (B), 100 μm (C), 40 μm (D).

Fig. 2. Phylogenetic and molecular clock analysis of ZIKV strains of the Asian lineage. (A) Root-to-tip analysis using TempEst v1.5. The input was a maximum likelihood tree estimated using RAxML (Randomized Accelerated Maximum Likelihood) with 1000 bootstrap replicates (fig. S6). R², coefficient of determination. (B) Bayesian phylogenetic tree estimated using BEAST v1.8.4. The positions of CAM/2010, VEN/2016, SAM/2016, and MTQ/2015 are indicated with black (CAM/2010) and red (VEN/2016, SAM/2016, and MTQ/2015) arrows. Conserved amino acid changes were inferred using the CAM/2010 strain as the parental strain. The green bars indicate the 95% highest probability density intervals of the age of the lineage. The details of the tree are shown in fig. S7. V, Val; A, Ala; N, Asn; S, Ser; M, Met; L, Leu; P, Pro; T, Thr.
Guillain-Barré syndrome (24, 25). Our findings offer an explanation for the unexpected causal link of ZIKV to microcephaly and will help to clarify how ZIKV evolved from an innocuous mosquito-borne virus into a congenital pathogen with global impact.

Structural modeling based on dengue virus, a closely related flavivirus member, indicates that residue 139, referred to as residue 17 of prM protein, is fully exposed on the surface of prM-E heterodimers or immature particles (fig. S14). The prM protein of flavivirus is required for viral maturation, egress, and secretion, and the pr domain is thought to prevent premature fusion within the infected cells (26). Flavivirus-infected cells contain a mixture of immature, partially mature, and mature particles (27). A recent study showed that the first 40 amino acids of the pr domain are involved in the interactions within trimeric spikes in the immature virus particle and affect the dynamics of conformational changes (28). The S139N substitution might have some effects on the transition of ZIKV from the immature to the mature virion, and the heterogeneity in maturity of progeny virions might thus affect viral fitness as well as neurovirulence. Our results also show that the ancestral Asian strain CAM/2010 can result in a mild microcephaly phenotype in mouse fetus (Fig. 1), and the N139S reverse mutant virus of contemporary ZIKV strain retains some neuroviruclence to neonatal mice (Fig. 3B). Thus, further work will be required to identify additional viral genetic determinants.
and host factors that might affect ZIKV pathogenesis. In addition, enhancement of vector infectivity by specific amino acid substitutions has been reported in ZIKV and other mosquito-borne viruses (29, 30), but the potential relationship between epidemic potential and disease severity is still under investigation.

REFERENCES AND NOTES

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S14

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Mutation for microcephaly
Zika virus infections in humans have been known since 1947. Microcephaly and neuropathologies associated with Zika have only been reported recently, most prevalently in the Americas. Yuan et al. investigated recent stable mutations in the virus genome and engineered them into a low-virulence ancestral strain (see the Perspective by Screaton and Mongkolsumapaya). A single amino acid substitution (serine to asparagine, S139N) in the viral precursor membrane protein exacerbated symptoms in pregnant mice. The reverse mutation (N139S) was less virulent. The S139N mutation arose in 2013 in French Polynesia before the virus jumped to Brazil in 2015. In vitro, this amino acid change made the virus more infectious for mouse and human neural progenitor cells and promoted apoptosis. The terrible sequelae of infection during pregnancy could thus be the result of a simple viral mutation.

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