The Dana Foundation's "Design a Brain Experiment" Competition

2017 Second Place (tied)

"Identifying Sequences in the Zika Virus that Cause Microcephaly"

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Lay Summary (provided by the Dana Foundation):

The Zika Virus (ZIKV), known since 1950, caused great alarm in 2015 following outbreaks in Brazil and other South American countries. ZIKV belongs to the same family as the Yellow Fever and West Nile virus. Mosquitoes are the natural reservoir or long-term host of the pathogen. Importantly, ZIKV can cause pathologies in fetuses, including microcephaly. Babies born with microcephaly have abnormally small brains, resulting from underdevelopment of the fetal brain during pregnancy or the halt of brain development after birth.

ZIKV is a positive, single-stranded, mRNA virus. It contains a central core that is made up of a capsid that carries the viral genomic RNA. The central core is surrounded by membrane and envelope proteins. ZIKV infection leads to the dysregulation of the neural progenitor cell cycle that ordinarily allows neural progenitor cells to differentiate into an array of neural cells. Of ZIKV's 417 strains, only a few cause microcephaly in infected fetuses; the SZ01 strain of the virus causes microcephaly in mice.

Scientists have not developed a vaccine but they have mapped the complete genome of several strains of the virus, enabling them to deduce the functions of its proteins as well as the causes of microcephaly. Of the 14 proteins that make up the ZIKV, 11 damage living cells and may help produce microcephaly. There are 10,272 nucleotide bases and 3,424 codons in the genetic sequence of ZIKV SZ01. Prior studies of ZIKV strain SZ01 in Ifnar knockout mice that lack interferon α/β and are therefore more susceptible to viral infection, had high viral loads in the brain, spinal cord, and testes compared to wild type mice. The infected mice also experienced complete paralysis of at least one hind limb.

Using this information, Karen designed an experiment using alanine scanning mutagenesis, an in vitro process that involves mutating different, individual codons in a genetic sequence on the ZIKV strain SZ01. Karen's experiment is designed to determine whether alanine scanning mutagenesis of randomly selected codons of cytopathic protein regions of the ZIKV strain SZ01 prevents microcephaly in infected mice pups. The data could show that mice pups infected with ZIKV SZ01 with a mutation of protease were born with the same brain size as mice pups who did not contract ZIKV. In this study, the causes of microcephaly in ZIKV could be uncovered, providing information on ways to prevent it, and revealing promising targets for creating therapeutic drugs that prevent microcephaly in ZIKV.

Karen notes that many ethical problems could arise from randomly mutating the ZIKV strain. There is a possibility that the pathogen may acquire stronger virulence when substituting one of its codons with alanine. In addition, by creating so many versions of the virus, there is a risk of the pathogen being used to harm others.

Purpose:

The Zika Virus is a major threat as a result of its rapid spread around the world and the potential danger it poses to one's health. Since its discovery in the 1950s, Zika Virus has been prevalent throughout Africa and Asia. In 2015, the Zika Virus began to raise even greater alarm following the outbreaks in Brazil and other South American countries (Haddow, et al., 2012). In adult humans, Zika infection has resulted in mild symptoms like fever, rashes, and muscle ache to more severe conditions such as Guillain-Barré Syndrome, a rare disorder in which the body's immune system damages the peripheral nervous system (Li, et al., 2016). In human fetuses, Zika Virus infection has led an array of pathologies including microcephaly, a birth defect in which babies are born with brains that are abnormally small. Microcephaly can occur as a result of the underdevelopment of the fetal brain during pregnancy or the halt of brain development after birth.

While it is not a relatively new disease, scientists have not yet found a vaccine for the Zika Virus nor found effective drugs to treat its symptoms. However, as of today, scientists have completed ample research and mapped the complete genome of several strains of the virus. Coding the virus' genetic material allow scientists to deduce the functions of its proteins as well as the causes of microcephaly. Out of the 14 proteins that make up the Zika Virus, 11 (C, E, M, NS1, NS2A, Protease, MS3, 2k, NS4B, NS5, and Polymerase) are cytopathic or damage living cells. Hence, they may play a large role in causing Zika Virus induced microcephaly.

Question:

Does alanine scanning mutagenesis of randomly selected codons of cytopathic protein regions of the Zika Virus (strain SZ01) prevent microcephaly in infected mice pups?

Background:

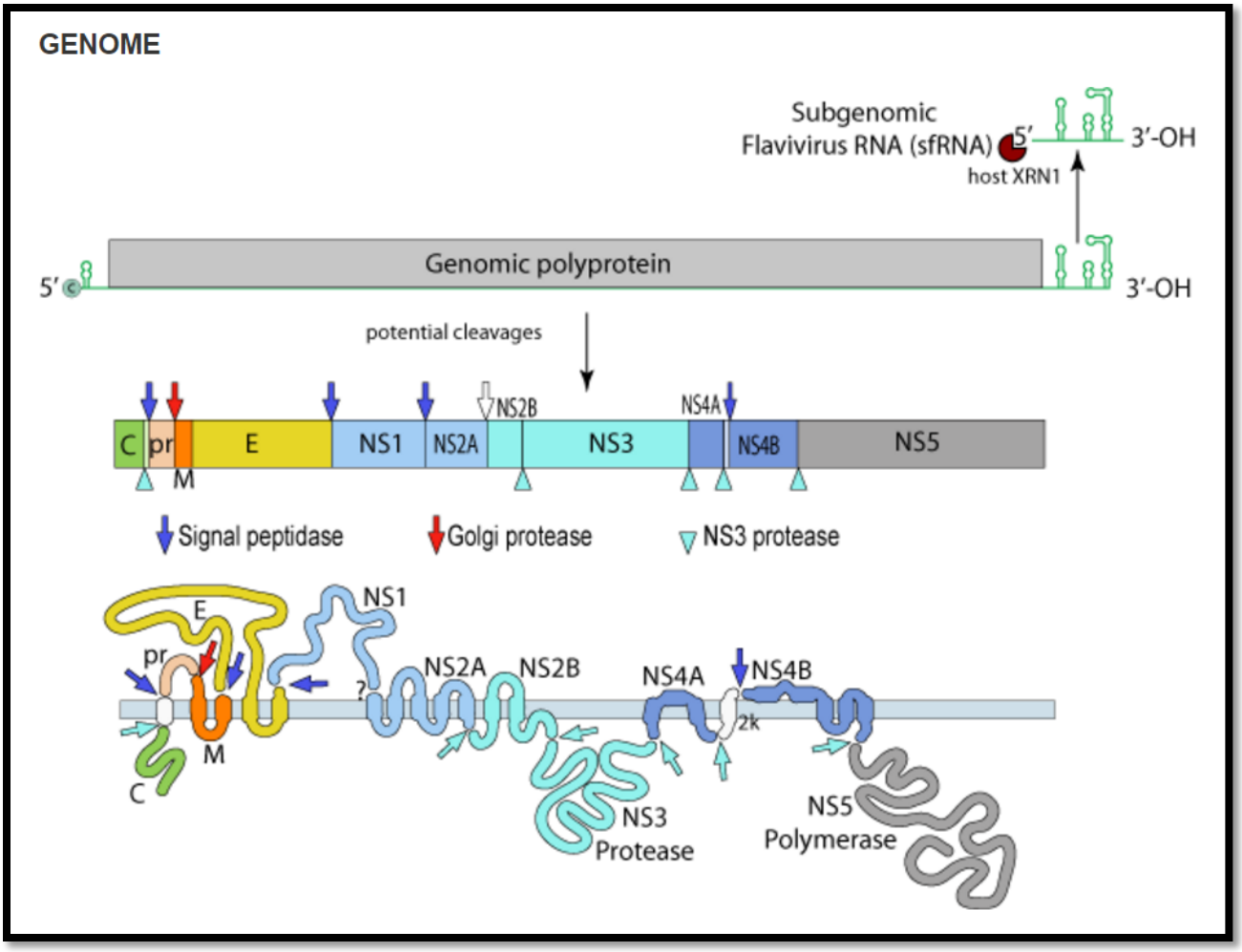
The Zika Virus (ZIKV) belongs to the Flaviviridae family along with the Yellow Fever virus and West Nile virus. It is characterized as positive, single-stranded, mRNA virus. The virion, otherwise known as the virus form when outside a host cell, is 40 nm long. It is composed by a central core that is made up of a capsid that carries the viral genomic RNA. The central core is

then surrounded by membrane and envelope proteins (Saiz, et al., 2016). Mosquitoes are the natural reservoir or long-term host of the pathogen. Neural stem cells, astrocytes, oligodendrocyte precursor cells, and microglia are more susceptible to ZIKV infection than neurons. Zika Virus infection additionally leads to the dysregulation of the neural progenitor cell cycle, an important process that allows neural progenitor cells to differentiate into an array of neural cells. (Retallack, et al., 2016). As of today, 417 strains of the Zika Virus have been discovered. However, only a few cause microcephaly in infected fetuses. The SZ01 strain of the virus causes microcephaly in mice (Li, et al., 2016).

There are 10,272 nucleotide bases and 3,424 codons in the genetic sequence of ZIKV SZ01. The Zika Virus contains 14 proteins which are produced by an inducible promoter. All the structural proteins of the zika virus with an exception of Pr, a structural protein, and NS2B and MS4A, two nonstructural proteins were cytopathic or caused damage to living cells (Li, et al., 2016).

Mice are more susceptible to viral infection if they lack interferon α/β; they are identified as Ifnar1knockout mice. These organisms have commonly been used in past studies of the pathogenesis of Zika Virus. According to a paper published in Cell Host and Microbe, Ifnar1knockout mice were infected with ZIKV SZ01, a strain of the Zika Virus that originates from Samoa in French Polynesia. There were high viral loads present in the brain, spinal cord, and testes of the infected Ifnar1knockout mice in comparison to the wild type. Ifnar1knockout mice, when in contact with the virus, also experienced complete paralysis of at least one hind limb. (Lazear, et al., 2016).

Figure 1: The Protein Structure of the ZIKV SZ01 (SIB Swiss Institute of Bioinformatics) (Adapted from http://viralzone.expasy.org/all_by_species/6756.html)



In **Figure 1**, ZIKV is classified as a flavivirus that is made up of RNA genetic material. The virus contains one large polyprotein which is made up of 14 smaller, individual proteins: C, pr, E, M, NS1, NS2A, NSAB, Protease, NS3, NS4A, 2k, NS4B, NS5, and Polymerase.

Methodology:

In the experiment, alanine scanning mutagenesis, an in vitro process that involves mutating different, individual codons in a genetic sequence, will be conducted on the Zika Virus Strain SZ01 (ZIKV SZ01). One RNA codon will be randomly selected of each of the ZIKV SZ01 protein regions (C, E, M, NS1, NS2A, Protease, NS3, 2k, NS4B, NS5, and Polymerase). The RNA codon will be altered to GCU which codes for alanine, an α-amino acid. In total, 11 different mutations will be performed on the original ZIKV SZ01.

The mutated versions of the virus as well as the original strain will be studied in a model organism, the Ifnar1knockout mouse. A total of 60 female, pregnant Ifnar1knockout mice will be tested with one version of the Zika SZ01 virus. One variation of the virus will be tested on five mice. The virus will be injected subcutaneously or beneath the skin with a 2 ml solution with the modified ZIKV strain 10 days after conception. The experiment will also examine three pregnant Ifnar1knockout mice that will not be infected to any version of ZIKV SZ01.

Every 5 days after conception, the mouse fetus brains will be measured with an ultrasound scan. After birth, the mouse pup brains will continue to be measured with MRI scans every week.

Results:

The measurements of the mice fetuses after birth infected with genetic variations of ZIKV SZ01 could demonstrate the role of protease in causing microcephaly. The data could show that mice pups infected with ZIKV SZ01 with a mutation of protease were born with the same brain size (17mm) as mice pups who did not contract ZIKV. However, the pups infected with ZIKV with mutations of other proteins and the pups infected with the original virus were born with brains sized 9-10mm. They contracted microcephaly. Hence, the disablement of protease in ZIKV could have prevented the infected mice pups from developing microcephaly. (See Figure 2).

Figure 2: The Effect of Infection of Different Genetic Variations of ZIKV SZ01 and Microcephaly in Infected Fetus Mice Immediately After Birth

Type of Infection	Length of Infected Fetus Brain Along Longitudinal Fissure (mm)
Original ZIKV SZ01	10
ZIKV SZ01 with C mutation	10
ZIKV SZ01 with E mutation	10
ZIKV SZ01 with M mutation	10

ZIKV SZ01 with NS1 mutation	10
ZIKV SZ01 with NS2A mutation	10
ZIKV SZ01 with Protease mutation	17
ZIKV SZ01 with NS3 mutation	10
ZIKV SZ01 with 2k mutation	10
ZIKV SZ01 with NS4B mutation	10
ZIKV SZ01 with NS5 mutation	10
ZIKV SZ01 with Polymerase mutation	10
None	17

The MRIs of the pups infected with ZIKV SZ01 with Protease mutation along with non-infected pups may not demonstrate cortical thinning. Additionally, in the non-microcephalic pups, there may be no signs of cell death of neural progenitor cells. On the other hand, the brains scans of the microcephalic mice could show an accumulation of calcium salts in tissue between the cortical and subcortical white matter. There could additionally be hypoplasia of the cerebellum and the brainstem. In 80% of the microcephalic mice, the brain scans may show signs of ventriculomegaly, a brain condition in which lateral vesicles are dilated. As in most premature pups, there may also be delayed myelination, causing slower propagation of electrical impulses down an axonal tract. Moreover, there can be deformities of the cortical development occurring in the frontal lobes of the mouse brains as well as malformations of the corpus callosum (Li, et al., 2016).

One potential caveat that may arise from the experiment is that the mutations may not result in differences in the expression of ZIKV SZ01. Regardless of the alanine scanning mutagenesis, the different versions of the Zika Virus could unanimously result in microcephaly in infected mice. A solution would be to randomly select other codons in the viral sequence and conduct the same procedure.

Conclusion:

Zika Virus and microcephaly is a serious health concern that affects the lives of people all over the world. This experiment is imperative in potentially eradicating ZIKV induced microcephaly. Many ethical problems could arise from randomly mutating the Zika Virus strain. There is a possibility that the pathogen may acquire stronger virulence when substituting one of its codons with alanine. In addition, by creating so many versions of the virus, there is a risk of the pathogen being used to harm others.

As of October 2013, alarmingly, 73 countries and territories reported mosquito-borne ZIKV transmission since 2007 (J & F, 2016). With virus spreading quickly throughout the world, the percentage of children born with microcephaly increases. Microcephaly obstructs a child's cognitive abilities to learn or perform basic life functions. Children with microcephaly experience learning difficulties and often are unable to perform basic functions like movement, balance, and feeding. With such severe symptoms, babies infected with ZIKV induced microcephaly must undergo rigorous examination with frequent doctor check-ups. The costs of those medical appointments pose yet another problem, costing families thousands of dollars to ensure the health of their child. However, ZIKV induced microcephaly most dramatically affects familial bonds. It is devastating to raise a child who has been stripped of their opportunities as a result of a disease.

In this study, the causes of microcephaly in the Zika Virus could be uncovered, providing information on ways to prevent it. Therefore, these findings are a very promising target for creating therapeutic drugs that prevent microcephaly in ZIKV positive human subjects.

Bibliography:

1. Haddad, R., Rabe, A., Laqueur, G., Spatz, M., & Valsamis, M. (1969). Intellectual deficit associated with transplacentally induced microcephaly in the rat. Science , 163 (3862), 88-90.
2. Haddow, A. D., Schuh, A. J., Yasuda, C. Y., Kasper, M. R., Heang, V., Huy, R., et al. (2012, February). Genetic Characterization of Zika Virus Strains: Geographic Expansion of the Asian Lineage. PLoS; Neglected Tropical Diseases , 2.
3. Hughes, B. W., Addanki, K. C., Sriskanda, A. N., McLean, E., & Bagasra, O. (2016). Infectivity of Immature Neurons to Zika Virus: A Link to Congenital Zika Syndrome. EBioMedicine , 10, 65-70.
4. J, W., & F, L. (2016). Zika Virus Infection and Microcephaly: Evidence for a Causal Link. The International Journal of Environmental Research and Public Health , 13 (10), 13.
5. Lazear, H. M., Govero, J., Smith, A. M., Platt, D. J., Fernandez, E., Miner, J. J., et al. (2016). A Mouse Model of Zika Virus Pathogenesis. Cell Host & Microbe , 19, 720-730.
6. Li, C., Ye, Q., Hong, Y., Liu, X., Zhang, N., Shi, L., et al. (2016). Zika Virus Disrupts Neural Progenitor Development and Leads to Microcephaly in Mice. Cell Stem Cell , 19 (1), 120-126.
7. Li, G., Poulsen, M., Fenyvuesvolgyi, C., Yashiroda, Y., Yoshida, M., Simard, J. M., et al. (2016, December 1). Characterization of cytopathic factors through genome-wide analysis of the Zika viral proteins in fission yeast. Proceedings of the National Academy of Sciences of the United States of America .
8. Saiz, J.-C., Vazquez-Calvov, A., Blazquez, A. B., Merino-Ramos, T., Escribano-Romero, E., & Martin-Acebes, M. A. (2016, April 19). ZIka Virus: the Latest Newcomer. Frontiers in Microbiology , 496.
9. SIB Swiss Institute of Bioinformatics. (n.d.). Zika Virus (Strain Mr 766). Retrieved from ExpASy: Bioinformatics Resource Portal.
10. Zika virus cell tropism in the developing human brain and inhibition by azithromycin.2016Proceedings of the National Academy of Sciences of the United States of America 1135014408-14413