

Disruption of human *astn2* function by ZIKV *ns4b* gene as a molecular basis for Zika viral microcephaly

Bhaskar Ganguly^{*1}, *Enakshi Ganguly*²

¹Animal Biotechnology Center, Department of Veterinary Physiology and Biochemistry, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar – 263145. Uttarakhand, INDIA.

²Department of Community Medicine, MediCiti Institute of Medical Sciences, Medchal – 501401. Telangana, INDIA.

*Corresponding author, address for correspondence: C/o Late Sri Bisweswar Gangopadhyay, D-04, Alliance Kingston Estate, Rudrapur – 263153. Uttarakhand. INDIA. E-mail: vetbhaskar@gmail.com

ABSTRACT

The present Zika virus (ZIKV) pandemic is being associated with increased incidence of microcephaly in newborns. However, a molecular basis for such pathogenesis is distinctly lacking. Comparative nucleic acid sequence analysis showed similarity between regions of non-structural protein 4B (*ns4b*) gene of ZIKV and human astrotactin2 (*astn2*) gene. Based on these findings, a molecular target of Zika viral microcephaly is being proposed.

Keywords: Zika virus, microcephaly, astrotactin 2, ns4b, parsimony, pathogenesis

1. INTRODUCTION

Given the present Zika virus (ZIKV) pandemic, there is an accentuated interest in its pathobiology. ZIKV is a mosquito-borne Flavivirus, whose natural transmission cycle involves mainly vectors from the *Aedes* genus and monkeys. The ZIKV genome consists of a single-stranded positive sense RNA molecule, about 11 Mb in size, with two flanking non-coding regions (5' and 3' NCR) and a single long open reading frame encoding a polyprotein: 5'-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3', that is cleaved into capsid (C), precursor of

membrane (prM), envelope (E) and seven non-structural proteins (NS 1 through 5). Humans were considered occasional hosts of the virus and till 2007 only 14 human cases had been reported. The potential of ZIKV as an emerging human pathogen was first realized during the year 2007 epidemic at Yap Island in Micronesia involving 49 confirmed cases of morbidity and 73% seropositivity in the adult population.¹ Before April, 2015, no case had been reported in Brazil. However, between April and November, 2015, 18 of the 27 Brazilian states reported ZIKV. The most arresting of all the present concerns regarding the manifestation of ZIKV is its association with microcephaly in newborns. After ZIKV emerged in Brazil, a 20-fold annual increase of microcephaly cases was observed.² A relationship between ZIKV and microcephaly has been confirmed, and WHO has issued an epidemiological alert about the association of ZIKV infection with microcephaly,³ although a molecular basis for associating the condition with the pathogen is still lacking.

Pathogens and hosts have evolved in each other's presence and parsimonious analysis of the two by nucleic acid sequence comparison can uncover hitherto unknown interaction networks.⁴ Therefore, it was posited that a parsimonious association between ZIKV and humans may lead to a patho-molecular basis for microcephaly.

2. METHODS

The ZIKV genome sequence [RefSeq: NC_012532.1]⁵ was used to query the human non-redundant nucleotide sequence database using blastn⁶ for finding 'somewhat similar sequences' keeping all search parameters at their default value. Sequences in the human genome that showed alignment with ZIKV genome sequences were manually annotated using extensive literature search for finding a possible role in pathogenesis of microcephaly.

3. RESULTS AND DISCUSSION

BLAST search revealed sequence matches of different parts of ZIKV genome with several different targets in the human genome. One of these matches of functional significance in the etiopathogenesis of microcephaly was that of ZIKV *ns4b* gene with *astrotactin 2* (*astn2*) gene (Figure 1).

Homo sapiens astrotactin 2 (ASTN2), RefSeqGene on chromosome 9				
Sequence ID: reflNG_021409.1 Length: 996814 Number of Matches: 1				
Range 1: 10334 to 10368 GenBank Graphics ▼ Next Match ▲ Previous Match				
Score	Expect	Identities	Gaps	Strand
42.8 bits(46)	9.2	32/37(86%)	2/37(5%)	Plus/Minus
Query 7112	GCCACACAAGCTGGAGTGCTGTTTGGCATGGGCAAAG	7148		
Sbjct 10368	GCCACACAGGCTGGAGTGCTG--TGGTATGGGCACAG	10334		

Figure 1: blastn output showing local alignment of ZIKV *ns4b* (query) with human *astn2* (subject)

An expect value of 9.2 for the alignment appears high but is likely due to the large disparity in the size of the query and the alignment with the subject. When a reciprocal blastn search was performed using the *astn2* gene sequence to query the ZIKV non-redundant sequences, the same alignment was achieved with an expect value of 0.049 (data not shown). The non-structural protein 4B (NS4B) protein is small and poorly conserved among the Flaviviruses. NS4B contains multiple hydrophobic potential membrane spanning regions. NS4B may form membrane components of the viral replication complex and could be involved in membrane localization of NS3.⁷ ASTN2 is a protein that is expressed at high levels in migrating, cerebellar granule neurons during developmental stages when glial-guided migration is ongoing and mutations of *astn2* gene are associated with microcephaly in humans.⁸

Based on the above finding, it first appears that both ZIKV NS4B and host ASTN2, being trans-membrane proteins, may co-localize and interact with each other in such a manner as to annul the physiological role of ASTN2 but a closer analysis of the alignment reveals that the part of

astn2 gene exhibiting sequence identity with ZIKV *ns4b* is located within a 123 kB intron (intron 1) coding region rather than forming a part of the exon coding region (Figure 2). Thus, it can be predicted that ZIKV *ns4b* alters *astn2* function through pre-translational interactions to cause microcephaly. The exceptional ability of ZIKV as a Flavivirus to exist within the host cell nucleus strengthens this model of pathogenesis.⁹ However, it is very difficult to speculate on the nature of the exact pre-translational interaction between the two genes that results in disruption of *astn2* function.

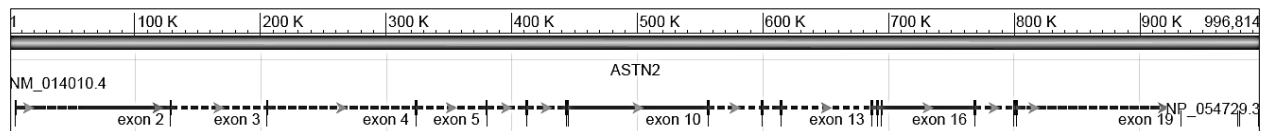


Figure 2: The gene structure of human *astn2*; the gene comprises of 22 exons. ZIKV *ns4b* is likely to interact with the first intron, about 123 kB in size, close to the first exon at about 10·3 K on the scale shown.

The 123 kB intron commonly features in many proteins of the epidermal growth factor receptor (EGFR) family,¹⁰ and the alignment site within the intron is unusual in having a high GC content ($22/37 = 59\%$); higher GC content is typically associated with shorter introns.¹¹ The presence of long non-coding, anti-sense RNA gene, PAPPAS1, which is differentially expressed in placenta and brain, juxtaposed with *astn2* on human chromosome number 9,¹² may also bear exciting implications in the molecular pathogenesis of ZIKV microcephaly. Biological studies abounding the determination of the copy numbers of functional *astn2* mRNA and ASTN2 protein in developing fetuses that are at risk of developing microcephaly due to maternal ZIKV exposure would be required to confirm this model of pathogenesis. If the proposed model of pathogenesis for zika viral microcephaly involving *astn2* and ZIKV *ns4b* is confirmed, it would allow development of new medical interventions for complementation of *astn2* function towards successful prevention of ZIKV microcephaly.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Faye O, Freire CCM, Iamarino A, et al. Molecular Evolution of Zika Virus during Its Emergence in the 20th Century. *PLoS Negl Trop Dis* 2014; **8(1)**: e2636.
2. Ventura CV, Maia M, Bravo-Filho V, Góis AL, Belfort Jr R. Zika virus in Brazil and macular atrophy in a child with microcephaly. *Lancet* 2016; **387**: 228.
3. WHO. Epidemiological alert: neurological syndrome, congenital malformations, and Zika virus infection. Implications for public health in the Americas. http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=32405&lang=en (accessed Feb 05, 2016).
4. Patro R, Kingsford C. Predicting protein interactions via parsimonious network history inference. *Bioinformatics* 2013; **29**: i237–46.
5. Kuno G, Chang GJ. Full-length sequencing and genomic characterization of Bagaza, Kedougou and Zika viruses. *Arch Virol* 2007; **152(4)**: 687-696.
6. States DJ, Gish W, Altschul SF. Improved sensitivity of nucleic acid database searches using application-specific scoring matrices. *Methods* 1991; **3(1)**: 66–70.
7. Chambers TJ, Hahn CS, Galler R, Rice CM. Flavivirus genome organization, expression, and replication. *Ann Rev Microbiol* 1990; **44(1)**: 649–88.

8. Zheng C, Ross E, Alder J, et al. Mechanisms of Neuronal Migration. In: Galaburda AM, Christen Y (Eds.) Normal and Abnormal Development of the Cortex 1997; pp 57-65. Springer Berlin Heidelberg. doi:10.1007/9783642608612_3.
9. Buckley A, Gould EA. Detection of virus-specific antigen in the nuclei or nucleoli of cells infected with Zika or Langat virus. *J Gen Virol* 1988; **69**(8): 1913–20.
10. Fenstermaker RA, Ciesielski MJ. EGFR Intron Recombination in Human Gliomas: Inappropriate Diversion of V(D)J Recombination? *Current Genomics* 2007; **8**(3): 163–70.
11. Amit M, Donyo M, Hollander D, et al. Differential GC Content between Exons and Introns Establishes Distinct Strategies of Splice-Site Recognition. *Cell Reports* 2012; **1**: 543–56.
12. García J, Castrillo JL Identification of two novel human genes, DIPLA1 and DIPAS, expressed in placenta tissue. *Gene* 2005; **344**: 241–50.