RESEARCH NOTE

# Predicted protein interactions of IFITMs which inhibit Zika virus infection [version 1; referees: 1 approved]

Madhavi K. Ganapathiraju

Department of Biomedical Informatics, University of Pittsburgh, Pittsburgh, PA, 15213, USA

## Abstract

After the first reported case of Zika virus in Brazil, in 2015, a significant increase in the reported cases of microcephaly was observed. Microcephaly is a neurological condition in which the infant's head is significantly smaller with complications in brain development. Recently, two small membrane-associated interferon-inducible transmembrane proteins (IFITM1 and IFITM3) have been shown to repress members of the flaviviridae family which includes the Zika virus. However, the exact mechanisms leading to the inhibition of the virus are yet unknown. Here, we assembled an interactome of IFITM1 and IFITM3 with known protein-protein interactions (PPIs) collected from publicly available databases and novel PPIs predicted using High-confidence Protein-Protein Interaction Prediction (HiPPIP) model. We analyzed the functional and pathway associations of the interacting proteins, and found that there are several immunity pathways (interferon signaling, cd28 signaling in T-helper cells crosstalk between dendritic cells and natural killer cells), neuronal pathways (axonal guidance signaling, neural tube closure and actin cytoskeleton signaling) and developmental pathways that are associated with these interactors. These results could help direct future research in elucidating the mechanisms underlying the viral immunity to Zika virus and other flaviviruses.

This article is included in the Zika & Arbovirus Outbreaks channel.

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**Referee Status:** ☑

| | Invited Referees |
|---|---|
| | **1** |
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1   **Judith Klein-Seetharaman**, University of Warwick UK

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**Competing interests:** No competing interests were disclosed.

## Introduction

The Zika virus (ZIKV) is a flavivirus that was initially isolated from rhesus monkeys in 1947 and was first reported in humans in 1952[1]. Until recently, reports of this virus had been limited to Africa and Asia[2] but currently there is an ongoing, wide-spread Zika epidemic[3]. The virus has rapidly spread across the Americas and has been declared a 'global emergency' by the World Health Organization[4]. It is mostly transmitted by mosquitoes and clinical manifestations include rash, mild fever, arthralgia, conjunctivitis, myalgia, and headaches. In addition, it has been reported recently that the virus can be transmitted sexually, with the risk of infection persisting for several months after initial contact[5]. Earlier, the symptoms of ZIKV had been reported to be mild[1], but, the virus has been recently linked to two more serious afflictions: Guillen-Barré syndrome[6,7] and microcephaly[8–12], both of which are serious neurological conditions. Microcephaly results in reduced head circumference measurement in infants, exhibiting complications in brain development. Of particular concern is the attribution of microcephaly to infection with ZIKV occurring between the first two trimesters of pregnancy[11,12]. Evidence linking ZIKV to microcephaly includes detection of ZIKV RNA in tissue such as the placenta and amniotic fluid of pregnant women with ZIKV, as well as in the brains of stillborn infants with microcephaly[13]. In a study with human induced pluripotency stem cells, the mechanism of ZIKV related cell death has been elucidated. This study demonstrated that ZIKV infects human embryonic cortical neural progenitor cells (hNPCs), ultimately leading to attenuated population growth mediated by virally induced caspase-3-mediated apoptosis and cell-cycle dysregulation[14]. Mice studies showed that ZIKV infection can lead to nerve degeneration, softening of the brain and porencephaly[15].

Very recently, two small membrane-associated interferon-inducible transmembrane proteins (IFITMs) IFITM1 and IFITM3 were discovered to have a protective role against the Zika virus infection by inhibiting replication of the virus and preventing cell death induced by Zika virus[5]. IFITMs were shown to have an inhibitory role against other flaviviruses also, such as West Nile and dengue virus. Type 1 interferon (IFN) signaling inhibits Zika virus pathogenesis. Prior to induction of IFN-stimulated genes, IFITMs may provide initial defense against the infection[5]. However, since the exact mechanism of IFITM1 and IFITM3 mediated restriction are yet unknown, computational methods could accelerate research by presenting testable hypotheses.

In our earlier work, we developed a computational model called 'High-confidence Protein-Protein Interaction Prediction' (HiPPIP) model that identifies novel protein-protein interactions (PPIs) in the human interactome[16], motivated by the fact that PPIs prove to be valuable in understanding the function of a gene, and specifically in how it plays a role in causing or preventing disease. One example of the impact of these computational predictions is the PPI that we predicted between OASL and RIG-I[16], which was validated to be a true PPI through co-immunoprecipitation[16,17]. This led to the formulation of a hypothesis about its significance and led to the discovery of its functional relevance, namely that upon viral infection, OASL triggers the immune system by activating the RIG-I pathway, thus inhibiting virus replication[18]. functional studies initiated solely by this predicted PPI showed that human OASL binds to dsRNA to

enhance RIG-I signaling, and that boosting OASL can help inhibit viral infection[18]. In this work, we applied HiPPIP model to discover novel PPIs of IFITM1 and IFITM3, to potentially accelerate the discovery of the mechanism by which they inhibit ZIKV and other viral infections.

## Methods

PPIs were assembled by collecting known PPIs from the Human Protein Reference Database (HPRD)[19] and Biological General Repository for Interaction Datasets (BioGRID)[20], and by computing novel PPIs using the HiPPIP model that we developed[16]. Computationally discovered PPIs have been shown to be highly accurate by computational evaluations and experimental validations of a few PPIs[16]. Interactome figures were created using Cytoscape[21]. Pathways associated with proteins in the interactome were collected using Ingenuity Pathway Analysis® suite (www.ingenuity.com). Gene Ontology terms enriched among the interacting partners (including the candidate genes IFITM1 and IFITM3) were computed using the BiNGO plugin of Cytoscape[22].

## Results and discussion

We assembled the PPIs of IFITM1 and IFITM3 (Figure 1) by computing novel PPIs using HiPPIP model and collecting known PPIs from publicly available databases, Human Protein Reference Database (HPRD) and Biological General Repository for Interaction Dataset (BioGRID)[23,24]. We found that both proteins have known PPIs with proteins involved in immunity, and several novel (predicted) PPIs with proteins that seem to have relevant functions. DEAF1 is involved in neural tube closure, embryonic skeletal development and anatomic structure morphogenesis, and other functions. FNDC3B was found to be associated with heart rate, height and corneal structure through genome-wide association studies. It is a membrane protein, and, while its own functions are unknown, its interactors are involved in regulation of glial cell apoptotic process, regulation of ion transport (sodium, potassium, calcium) and several cardiac processes. SPTA1 is involved in neural functions of actin filament organization, neurite outgrowth and axon guidance. RASSF7 is localized to microtubule organizing center. While its function is unknown, it interacts with proteins that are involved in cell proliferation in brain, regulation of neuroblast proliferation, nervous system development, synaptic vesicle fusion to presynaptic membrane, and viral budding and assembly. TSSC4 interacts with both IFITM1 and IFITM3. TSSC4's functions are unknown but its
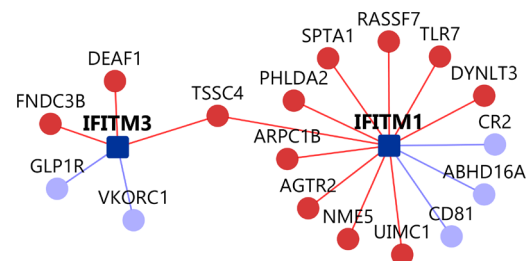


**Figure 1. Protein-protein interactions (PPIs) of IFITM1 and IFITM3: Known PPIs were assembled from HPRD and BioGRID databases and novel PPIs were predicted using HiPPIP model.** Novel interactors of IFITM1 and IFITM3 are shown as red colored nodes while previously known interactors are shown as light blue colored nodes.

own interactions suggest that it may be involved in viral penetration into host nucleus, protein import into nucleus and immune response signaling, among other processes. TLR7 is involved in several functions and pathways related to innate immunity. ARPC1B is part of actin related protein 2/3 complex; its interactions suggest that it may be involved in neuronal development such as axono-gensis and development, neuron differentiation, nervous system development, and immune related terms such as innate immune

response, regulation of immune response, etc. These functional annotations are sourced from Schizo-Pi[16,25]; for example, see: http://severus.dbmi.pitt.edu/schizo-pi/index.php/gene/view/10522.

Pathways associated with IFITM interactome computed with Ingenuity Pathway Analysis Suite® are given in Table 1. Gene Ontology biological process terms associated with the interactome, compiled with BiNGO[22] are shown in Figure 2 and Table 2.

**Table 1. Pathways associated with IFITMs and their interactor.** Pathway associations were computed with Ingenuity Pathway Analysis Suite ®. Novel interactors are shown in bold.

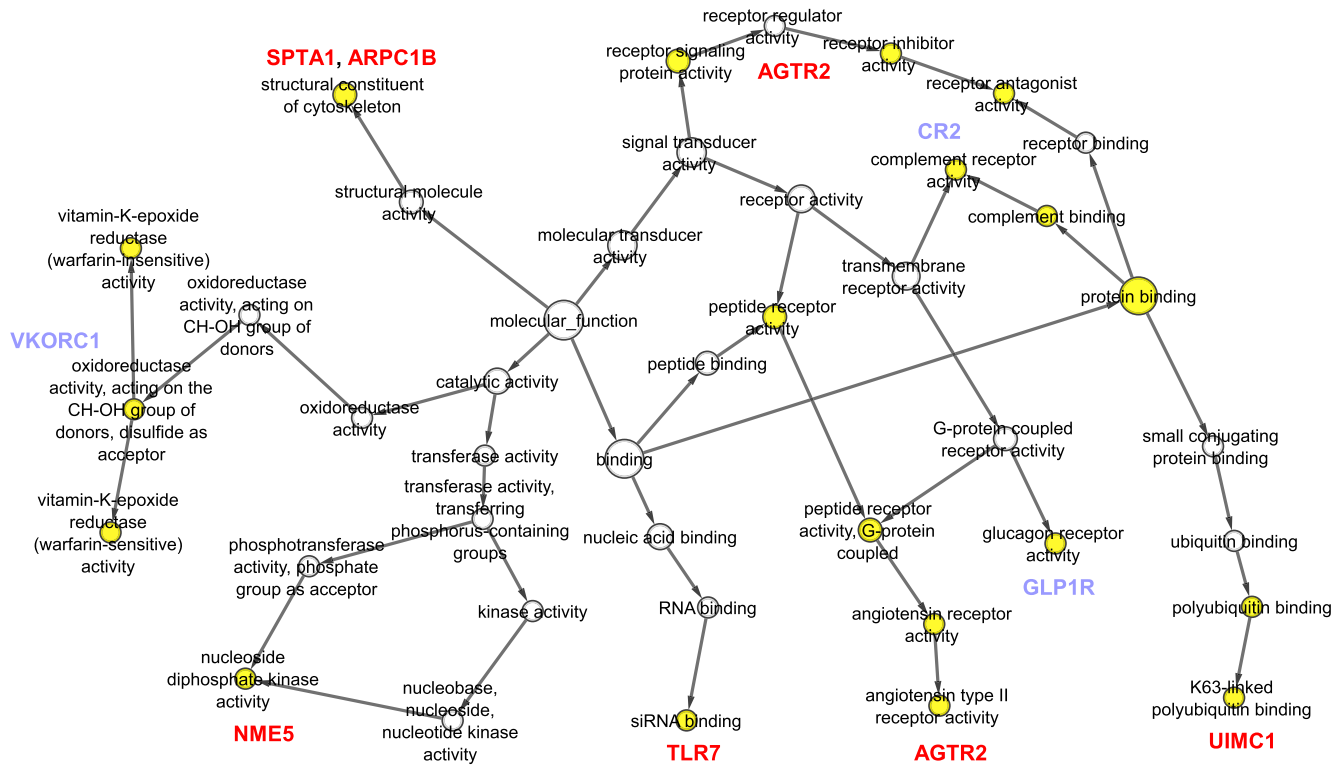| Gene | Associated pathways |
|---|---|
| **AGTR2** | Gαi Signaling<br>Renin-Angiotensin Signaling |
| **ARPC1B** | Axonal Guidance Signaling<br>Signaling by Rho Family GTPases<br>Actin Cytoskeleton Signaling<br>Integrin Signaling<br>Clathrin-mediated Endocytosis Signaling<br>Ephrin Receptor Signaling<br>RhoGDI Signaling<br>Cdc42 Signaling<br>Epithelial Adherens Junction Signaling<br>RhoA Signaling<br>CD28 Signaling in T Helper Cells<br>fMLP Signaling in Neutrophils<br>Rac Signaling<br>Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes<br>Regulation of Actin-based Motility by Rho<br>Remodeling of Epithelial Adherens Junctions<br>Actin Nucleation by ARP-WASP Complex |
| CD81,CR2 | PI3K Signaling in B Lymphocytes |
| CR2 | IL-8 Signaling<br>NF-κB Activation by Viruses<br>Complement System |
| GLP1R | Gαs Signaling<br>GPCR-Mediated Integration of Enteroendocrine Signaling Exemplified by an L Cell |
| GLP1R<br>**AGTR2** | G-Protein Coupled Receptor Signaling<br>cAMP-mediated signaling |
| IFITM3, IFITM1 | Interferon Signaling |
| **NME5** | Salvage Pathways of Pyrimidine Ribonucleotides<br>Pyrimidine Ribonucleotides *De Novo* Biosynthesis<br>Pyrimidine Ribonucleotides Interconversion<br>Pyrimidine Deoxyribonucleotides *De Novo* Biosynthesis I |
| **SPTA1** | Sertoli Cell-Sertoli Cell Junction Signaling |
| TLR7 | Role of Macrophages<br>Fibroblasts and Endothelial Cells in Rheumatoid Arthritis<br>Colorectal Cancer Metastasis Signaling<br>Systemic Lupus Erythematosus Signaling<br>NF-κB Signaling<br>Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses<br>phagosome formation<br>Communication between Innate and Adaptive Immune Cells<br>Crosstalk between Dendritic Cells and Natural Killer Cells<br>Altered T Cell and B Cell Signaling in Rheumatoid Arthritis<br>TREM1 Signaling<br>Toll-like Receptor Signaling |
| **UIMC1** | Role of BRCA1 in DNA Damage Response |

**Figure 2. Gene Ontology terms enriched in the interactome of IFTIM1 and IFTIM3.** Yellow color signifies statistically significant enrichments. Novel interactors that are associated with the GO terms are shown in red and known interactors in blue. See Table 1 for a complete list of terms associated with the genes.

**Table 2. Gene Ontology Biological Process terms associated with interactors.** Novel interactors are shown in bold.

| Interactor | Gene Ontology Terms |
| --- | --- |
| **AGTR2** | Angiotensin receptor activity<br>Angiotensin type ii receptor activity<br>Receptor antagonist activity<br>Receptor inhibitor activity<br>Glucagon receptor activity<br>Peptide receptor activity, G-protein coupled<br>Peptide receptor activity<br>Receptor signaling protein activity |
| **ARPC1B** | Structural constituent of cytoskeleton |
| CR2 | Complement receptor activity<br>Complement binding |
| GLP1R | Glucagon receptor activity<br>Peptide receptor activity, g-protein coupled<br>Peptide receptor activity |
| **NME5** | Nucleoside diphosphate kinase activity |
| **SPTA1** | Structural constituent of cytoskeleton |
| **TLR7** | Sirna binding |
| **UIMC1** | K63-linked polyubiquitin binding<br>Polyubiquitin binding |
| VKORC1 | Oxidoreductase activity, acting on the CH-OH group of donors, disulfide as acceptor<br>Vitamin-K-epoxide reductase (warfarin-sensitive) activity<br>Vitamin-K-epoxide reductase (warfarin-insensitive) activity |

There is only one study that presents altered gene expression under ZIKV infection available in Gene Expression Omnibus[14]. The study with eight samples (four infected and four control samples) showed that the infection of human neural progenitor cells (hNPCs) with the virus caused increased cell death and cell-cycle dysregulation[14]. We examined whether any of the interacting genes were differentially expressed in that study and found five genes that were differentially expressed with a small fold-change but with significant p-value ($< 0.005$) (Table 2): CD81, NME5, and RASSF7 were found to be under-expressed and FNDC3B and UIMC1 were found to be over-expressed (Table 3).

## Other resources

See http://severus.dbmi.pitt.edu/schizo-pi for annotations of individual proteins that are compiled from various databases. Also see the following link to our LENS webserver, where we present annotations of all the genes in the IFITM1-IFITM3 interactome and also annotations of proteins that further interact with interactors (i.e. 2nd level connectors of IFITMs). Under each tab, 'candidate genes' refers to IFITMs and their interactors shown in Figure 1 of the paper, while entire interactome includes all of their interactors. Note that the database behind LENS does not include novel protein-protein interactions; therefore, they are not shown as edges in the network diagram. The sources of the pathways and disease associations shown on this website are given in 25,26.

http://severus.dbmi.pitt.edu/LENS/index.php/results/view/57649c2516f9a/admin_57649c251737d

## Data availability

All pertaining data are provided in the manuscript.

**Table 3. Interacting genes that are differentially-expressed under Zika virus infection, along with fold-change and significant p-values.**

| Interactor | Log2 Fold Change | p-value |
|---|---|---|
| FNDC3B | 0.92 | 0.00005 |
| NME5 | -1.55 | 0.00005 |
| RASSF7 | -0.46 | 0.00215 |
| UIMC1 | 0.73 | 0.00005 |
| CD81 | -0.28 | 0.00325 |

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# Open Peer Review

## Current Referee Status: ☑

☑ **Judith Klein-Seetharaman**
Division of Metabolic and Vascular Health, University of Warwick, Warwick, UK

The title, abstract and article overall are well written and clear. The design, methods and analysis are mostly well described, although some detail could be added. In particular, given that the IFITM interactome contains a large number of previously unknown PPI's, it would be useful to give a little more detail on the methodology on how these predictions were obtained, rather than just stating that HiPPIP was used. In particular, it would be useful to understand what cut-off was used and a brief mentioning of the prediction methodology in general. An estimate of false positive and false negative errors for the prediction in Figure 1 would be particularly helpful.

In the analysis, the legend for Figure 2 needs expansion. It is not clear what the edges signify and in particular what is the meaning of directionality in the arrows.

I object to the wording used on page 2 "which was validated to be a true PPI", as any PPI evidence is debatable. I would reword to "which was experimentally validated".

I also object to the wording used on page 2 "Computationally discovered PPIs have been shown to be highly accurate". Such a blanket statement is clearly not true, as there are many predictions out there that are highly inaccurate. A more specific example needs to be provided here.

A minor suggestion is to replace "HiPPIP model" with "the HiPPIP model".

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

*Competing Interests:* No competing interests were disclosed.