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Original Article

Zika Virus (ZIKV): a review of proposed mechanisms of transmission and associated congenital abnormalities

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Abstract: Zika virus (ZIKV) has been of major international public health concern following large outbreaks in the Americas occurring in 2015-2016. Most notably, ZIKV has been seen to pose dangers in pregnancy due to its association with congenital abnormalities such as microcephaly. Numerous experimental approaches have been taken to address how the virus can cross the placenta, alter normal fetal development, and disrupt specific cellular functions. Many areas concerning the mechanisms of transmission, especially from mother to fetus, are largely unknown but demand further research. Several promising new studies are presented that provide insight into possible mechanisms of transmission, different cell types affected, and immune responses towards the virus. By aiming to better understand the processes behind altered fetal neuronal development due to ZIKV infection, the hope is to find ways to increase protection of the fetus and prevent congenital abnormalities such as microcephaly. As ZIKV infection is spreading to increasingly more areas and bringing harmful outcomes and birth defects with it, it is imperative to identify the mechanisms of transmitting this infectious agent, consider different genetic backgrounds of hosts and strain types, and navigate methods to protect those affected from the detrimental effects of this newly emerging virus.

Keywords: Zika virus, congenital abnormalities, mechanisms of transmission, microcephaly, cellular function, genetic background

Introduction

Zika virus (ZIKV) is transmitted by a mosquito-borne infection of the flavivirus family and has gained worldwide attention following large outbreaks in Brazil during the 2015-16 epidemic. Since then, there has been international concern and investigation for comprehensive information regarding its pathogenesis, transmission, and most importantly, impact on pregnant women and neonates [13]. The size of these outbreaks and the resulting implications of severe neurological damage in the fetuses of infected pregnant women led to the World Health Organization to announce a Public Health Emergency of International Concern on February 1, 2016 [16].

The ZIKV was first discovered in 1947 during a research study on yellow fever in Uganda [24]. Following its discovery, reported infections have been observed in numerous countries in the African continent [11]. Additionally, reports of infections have been noted throughout Southeast Asia [24]. The first major outbreak that received significant attention occurred in 2007 on the Yap Island in the Federated States of Micronesia, where 73% of the island's inhabitants were infected with ZIKV; those infected were either asymptomatic or showed mostly mild and brief clinical presentations [16]. Following the 2007 Yap outbreak, subsequent notable cases included outbreaks in French Polynesia from October 2013 to April 2014 [16].

Another widespread outbreak in Brazil during late 2014, and the continuing spread of ZIKV recorded throughout South America, Central America, and the Caribbean led to ZIKV becoming of major international public health concern. Since December 2016, over 50 countries in the

Figure 1. Countries and Territories Reporting Active ZIKV Transmission. Shaded areas show countries and territories that have reported active ZIKV transmission. It does not mean that transmission is automatically appearing in the entire area or territory. Pregnant women are heavily advised to avoid traveling to these areas. [This figure was modified from CDC (https://www.cdc.gov/zika/geo/active-countries.html)].

Americas have reported active local transmission of ZIKV (https://www.cdc.gov/zika/geo/active-countries.html). **Figure 1** displays all countries and territories that have reported the active transmission of ZIKV.

The transmission of ZIKV infection is primarily through the bites of infected mosquitoes [16], though there have been other recorded but limited known methods of transmission via sex [12], blood transfusion [16], and perinatally [3]. The extent to which the latter three contribute to disseminating an outbreak is, however, still undetermined. The presentation of clinical symptoms for those affected with ZIKV infection typically occurs an average of 6 days after infection, with a range of asymptomatic to non-specific symptoms including fever, rash, and headaches. However, it must be noted that 80% of those infected with ZIKV do not display any clinical symptoms [24]. Thus, ZIKV presents with diagnostic and surveillance difficulties as it displays no characteristic clinical manifestations when compared with other arboviral diseases [24].

Much of the information needed to evaluate the degree to which ZIKV poses a global threat is poorly defined and remains largely unknown. This current review seeks to elaborate on the methods by which ZIKV is transmitted, and discuss the relationship between ZIKV and congenital abnormalities, particularly microcephaly. The aim is to provide scientists and clinicians an overview of current literature on the mechanisms involved, and to discuss the strengths and limitations of these studies. Several recently published studies will be presented that provide insight into mechanisms of transplacental and paraplacental means of transmission, the different cell types affected, and immune response towards the virus. Studies showing differential outcomes of ZIKV infection based on the genetic variability of host stem cells and the types of ZIKV strain used are also elaborated upon. The limitations of these studies and suggestions for future research are provided throughout. In a continuously more interconnected global society, it is imperative to recognize the methods of disseminating infectious agents, consider different genetic backgrounds of hosts and strain types in disease outcomes, and navigate methods to protect those infected from the detrimental effects of this newly emerging virus.

Fetal infection of ZIKV through intrauterine mechanisms

There are two primary strains of ZIKV that have been identified: (1) an African lineage of ZIKV and (2) an Asian lineage of ZIKV. The latter

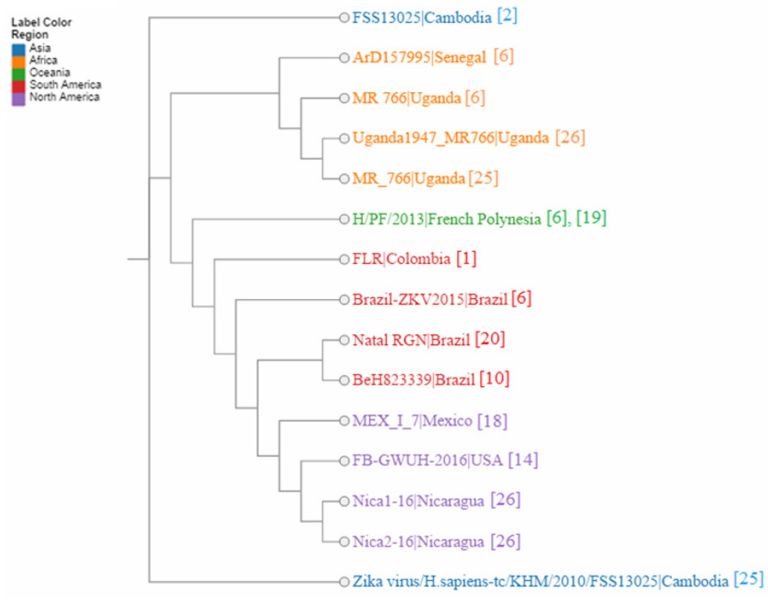

Figure 2. Phylogenetic tree comparing the genetic similarities of ZIKV strains. The strains are color coordinated by region. The reference of the paper in which they were mentioned follows the strain name. The African lineage is colored orange, and the rest are thought to be part of the Asian lineage, which shows increased genetic diversity. The Cambodian strain (FSS13025) is differentiated because each paper used a different genetic sequence referenced in GenBank. Although it is part of the Asian lineage, the Cambodian strain seems separated from the Asian lineage cluster, but that might be due to the database not having a complete genome of the strains. The strains from [20], [6] and [10] were from fetuses who displayed microcephaly, whereas the strain in Aagard was from a female who had been infected with the virus. [This is an original figure created by the authors of this paper].

Asian lineage of ZIKV is the one that has infected Brazil [2], which also share a common ancestry to the French Polynesian strain [10]; this can be seen in **Figure 2**. The Brazilian ZIKV strain "shares 97-100% of [its] genomic identity" with strain of ZIKV isolated in French Polynesia (Asian strain: H/PF/2013), but only 87-90% semblance to African strains (MR766 from Uganda and ArD157995 from Senegal); however, it should be noted that the Brazilian strain used in this comparison was based on amniotic fluid from one woman in Brazil [6]. This is relevant because Brazilian strains display genetic diversity interspersed with genomes isolated in other parts of the Americas [10]. Furthermore, the Asian lineage has been shown to cause brain developmental abnormalities. The African lineage is also able to infect human neural progenitor cells and may be more destructive to the early placenta due to its highly trophic behavior in the primitive trophoblast, and its increased ability to cause cell lysis [25]. Genetic differences are important to recognize because they affect the virus's transmission and symptoms. Accordingly, the strains that are mentioned throughout this paper were compared genetically using GenBank database and a phylogenetic tree was constructed with the Virus Pathogen Database and Analysis Resource (ViPR), displayed in **Figure 2**.

A case study was conducted to observe the relationship between the ZIKV and microcephaly by studying the amniotic fluid of two pregnant women in Brazil who previously displayed ZIKV infection-like symptoms and whose fetuses were diagnosed with microcephaly [6]. Both women had ZIKV genome along with anti-ZIKV antibodies (IgM) in their amniotic fluid at 28 weeks of gestation, without any traces of dengue or chikungunya virus. The existence of the virus in the amniotic fluid before the child was born indicates that ZIKV vertically transmitted through an intrauterine, possibly transplacental, mechanism of infection [6]. Additional studies have reported incidents in which a fetus had microcephaly, there were dystrophic calcifications in cortex and subcortical white matter, as well as ZIKV and viral RNA in fetal brain tissue following the mother's infection of ZIKV [20].

Animal models are now being used to determine the mechanism of the causal relationship between ZIKV and congenital abnormalities, like microcephaly. Pregnant mice studies show that mice lacking type I interferon (IFN), a molecule which initiates an immune response, and are infected with ZIKV have increased fetal death due to the virus [19]. A transplacental transmission has been proposed in these studies as well [19]. Pregnant mice infected with ZIKV (Asian strain H/PF/2013, French Polynesia 2013) showed that the mouse placenta is susceptible to ZIKV, leading to damage of the pla-

cental barrier, resulting in fetal demise and intrauterine growth restriction (IUGR) from placental insufficiency [19]. Additionally, using immunofluorescent microscopy confirm ZIKV RNA in trophoblast cells of the mouse placenta, which may indicate possible features of human fetal infection of ZIKV [19].

However, use of a mouse model poses limitations when comparing to humans because of the "morphological differences between the human and mouse placenta", and the different immune responses between these two species [2]. In humans, during the end of the first trimester of fetal development, the hemochorial placenta is protected by syncytiotrophoblast cells, which arise from primary human trophoblasts (PHT). These PHTs have an innate immune system, proposed to protect them from ZIKV through type III interferon (IFN) IFNλ1 release. Additionally, IFNλ1 paracrine constitutive release of IFNλ1 is shown to protect non-trophoblast cells from ZIKV [2]. However, flow cytometry and immunofluorescence microscopy analysis reveal the infection and replication of ZIKV in JEG-3 and HTR8/SVneo human trophoblast cell lines, which corresponds to extravillous trophoblasts. As a result, it was proposed that ZIKV can infect the extravillous trophoblast cells before the placental barrier is fully developed [19]. Furthermore, the previously mentioned Bayer study supported these results and showed that PHTs were the only cells that were not susceptible to ZIKV infection testing two strains of ZIKV: the African strain (MR766), and the Asian strain (FSS13025, Cambodian origin) [2]. Conversely, this has been called into question by a new study, which will be explored further in the following paragraphs.

An experiment compared the human placental trophoblasts derived from "placental villi at term", representing cells at later periods of gestation, to trophoblasts from "embryonic stem cells", representing cells present at the beginning of conception [25]. This was done in order to compare the susceptibility of these two lines to ZIKV (strains: MR766 and FSS13025). The primitive trophoblast derived from embryonic stem cells was a better target for ZIKV because it had a low expression of genes that provided innate immunity, and the cells provided easy access to the virus [25]. These cells have the TAM family of tyrosine kinase receptors (TYRO3, AXL, and MERTK), which function to clear apoptotic cells [26]. From these data, it was concluded that the primitive trophoblast which surrounds the embryo in the beginning of the first trimester is particularly vulnerable to ZIKV infection [25]. One other observation is that ZIKV transmission to the fetus most likely does not involve syncytiotrophoblast infection, especially in the later stages of pregnancy, but may involve other trophoblast cells [2].

However, this does not explain the cases of fetal problems that arise when a mother is infected in her second or third trimester. One possibility is that ZIKV (FSS13025) has been shown to bind to Gas6, an AXL ligand that facilitates entry into the human umbilical vein endothelial cells (HUVEC) using AXL as a cofactor [23]. Other flaviviruses are unable to use this cofactor, which may be the reason that ZIKV has the ability to infect the fetus and cause congenital abnormalities such as microcephaly, while dengue and West Nile virus do not have these effects [23]. Although AXL was consistently expressed in HUVEC cells, another experiment showed that ZIKV (strains: MR766 and Nica 1-16 and Nica 2-16; the Nica strains were isolated in Nicaragua and are "closely related to the Brazilian 2015-2016 strains" (**Figure 2**)) infects primary human placenta cells, such as amniotic epithelial cells, endothelial cells, Hofbauer cells, cytotrophoblast etc. These data provide the evidence for transplacental and paraplacental routes of ZIKV infection of the fetus [26]. It was also suggested that TIM1 is the significant cofactor that allows ZIKV to be transported into the fetal compartment because of its ubiquitous expression [26].

Furthermore, a new study looked at prolonged exposure of ZIKV (ZIKV FLR, isolated in Colombia in 2015) on primary human trophoblast cells (PHT), and it reports that prolonged exposure facilitates low-levels of viral infection in the trophoblast cells, especially in comparison to dengue (DENV) [1]. Interestingly, the virus does not cause cell death, and so the cells could be a reservoir for the virus, leading to later abnormalities [1]. Moreover, another theory denotes that women previously infected with dengue may have antibodies which cross-react with ZIKV, allowing it to enter the protective syncytiotrophoblast layer of the mature placenta [25]. These recent studies question the conclusion reached by the aforementioned

Bayer study which stated PHT cells cannot be infected by introducing new variables such as length of exposure and previous exposures to different flavivirus.

Some limitations noticed in the aforementioned studies include the specific focus on identifying a certain receptor or cell type affected during ZIKV infection and how it could lead to microcephaly, as well as the use of different ZIKV strains. Focusing only on the AXL or TIM1 cofactor as mentioned above brings up a question of whether too narrow of a focus could overlook more broad physiological connections, especially when considering that cell types change through fetal development, and thus the receptors expressed also change. For example, AXL receptors are primarily found in human umbilical vein endothelial cells, and this limited scope could thwart research on ZIKV transmission through other receptors, such as TIM1, which are found more dispersed through different cells. Additionally, the debate of the vulnerability of PHT needs to be taken into account when discussing the length of exposure that pregnant women normally experience.

Mechanisms of ZIKV-induced microcephaly

Now that CDC researchers have reviewed the extensive evidence and concluded that ZIKV infection is a cause of microcephaly, the task remains to better understand the mechanisms behind the transmission of ZIKV [22]. A study conducted at the University of Texas Medical Branch shows that different human genetic backgrounds could contribute to cell strain-dependent neuronal deficits following ZIKV infection [18]. The unique feature of this study involves establishing an *in vitro* system using primary human fetal brain-derived neural stem cells (NSCs) from three different male donors, K048, G010, and K054 [18]. While the study focuses on characterizing the effects of ZIKV infection, the varied origin of their donors and the experimental findings bring up questions of whether ZIKV affects NSCs from different human origins in the same way. In the aforesaid study, a cell strain-dependence was found when it came to the ZIKV impairment of neural stem cell differentiation. "Specifically, ZIKV infection significantly reduced neuronal differentiation of K054 and K048 strains but not G010" [18]. An interesting point made by the researchers is the correlation between cell-strain-dependent neuronal impairment and ZIKV-induced alteration of global gene expression pattern [18]. It was noted that K054 and K048 have similarities in transcriptome and neuronal phenotype that are distinct from G010 [18]. The genes that are responsible for these differences remain to be identified. Both K054 and K048 shared similar innate immune response patterns that include interferons, complements, and cytokines [18]. These results raise questions of how different genetic backgrounds could contribute to the cell-strain-dependent neuronal deficits after ZIKV infection occurs [18]. The increased number of ZIKV-correlated microcephaly cases in Brazil and the surrounding geographical region raises issues of whether genes play a role in the extent to which ZIKV can cause damage to the fetus.

Another study from the University of California San Diego (UCSD) used a different model to look at ZIKV infection and located genes that are crucial for neuronal development. This group used human embryonic stem cell-derived cerebral organoids to represent first-trimester fetal brain development [8]. The 3D organoid models represented regions of the "developing neocortex, ganglionic eminence, and retinal tissue as evidenced by immunohistochemistry and transcriptomic analyses" [8]. To infect the organoids, the MR766 prototype strain of ZIKV was used to better understand the transcriptomic response during early stage neural development [8]. The infected organoids showed a significant decrease in overall size and neuroepithelium [8]. One limitation to consider is use of only the African MR766 strain of ZIKV for the infection. One of the highest number of reported cases of microcephaly occurred in Brazil, where the predominant virus strain is from the Asian lineage of ZIKV [2]. By not using the Asian strain however the ability of the study to recognize how different strains might cause variable outcomes might cuase some trepidation. A more systematic approach would have been to conduct testing on both strains, or at least on the strain more prominent in Brazil, which displayed greater incidences of microcephaly.

In the same UCSD study, it was also observed that "the innate immune receptor Toll-like-Receptor 3 (TLR3) was upregulated after ZIKV infection of human organoids" [8]. Data analysis showed that TLR3 is most likely responsible for cell-type specific functions. It could activate

downstream anti-viral responses as well as play a role in the "dysregulation of signaling networks directing apoptosis and neurogenesis" [8]. Going off these findings, TLR3 receptor activation caused gene expression changes which, with the use of pathway analysis, helped pinpoint 41 genes related to neuronal development [8]. Some of the candidate genes included (NTN1, EPHA3, ADGRB3, EPHB2, SLITRK5, SYT11, and GRIK2) [8]. Many of these TLR3-activated genes are important in early brain cell fate decisions and may be responsible for the reduction of neural progenitor cell (NPC) population [8]. Focusing on these genes could help explain the mechanisms contributing to the disrupted neurogenesis seen in microcephaly [8].

A group in Germany conducted a similar study using human-derived organoids. Instead of infecting with the MR766 strain of ZIKV, they used a more recently isolated American strain from an infected fetal brain (FBGWUH-2016) and an Asian strain (H/PF/2013) of ZIKV [14]. This addressed the concerns from the previous paper of only using the African MR766 strain for the organoid infections. In fact, different outcomes were observed using these ZIKV strains. The infection pattern and cellular outcome showed differences from the results seen using the African MR766 strain of ZIKV [14]. Experimental findings showed that both the American and Asian ZIKV strains target and replicate in the "proliferating ventricular zone (VZ) apical progenitors" [14]. They also saw premature differentiation of neural progenitors, centrosome perturbation, and impaired neurogenesis [14]. Looking at these results showed that the structural changes observed with the organoid infection using the American and Asian strains more closely resemble the outcomes of microcephaly associated with ZIKV [14]. This helps highlight the importance of experimental design and how different strains, genetic makeup, and multiple compounding variables should be considered when conducting studies aimed to represent the population that is affected.

A study from Columbia University Medical Center focused on genes that once mutated, have been implicated in causing a particularly severe form of microcephaly. One such gene is NDE1 and the data from the study showed that "acute NDE1 knockdown arrested radial glia progenitors (RGPs) before mitotic entry", contributing to the severe form of microcephaly [9]. Further analysis of the genes NDE1 and NDEL1 showed their involvement in neocortical development [9]. This study helps to emphasize the significance of looking at genetics in order to reveal information behind the mechanisms needed in normal development that become impaired in microcephaly.

Teratogenic effects-microcephaly, other congenital malformations and their outcomes

The Brazil Ministry of Health declared a 20-fold rise in newborns with microcephaly in October 2015 [24]. Additionally, a case series study of congenital ZIKV syndrome completed in Brazil noted a 100-fold increase in newborns with microcephaly from November 2015 to February 2016 [13]. These noted instances, as well as the increasing circulation of ZIKV throughout the Central and South Americas, highlight the threat posed by ZIKV on newborns. Conspicuously, 80% of infected pregnant mothers with ZIKV do not display remarkable clinical symptoms, highlighting the difficulties in diagnosis and surveillance [13]. The great increase in rates of conditions including but not limited to microcephaly and brainstem dysfunction in fetuses and neonates show a correlation between the observed birth defects and ZIKV [4].

Reports on small pools of cases show fetuses and newborns exposed to ZIKV in utero presenting with congenital and postnatal defects, including decreased brain size, cerebral lesions, craniofacial disproportion, improper development of the cortex, intracranial calcifications, and neurologic dysfunction [27]. Although the initial thought was that infection of pregnant women with ZIKV was associated with congenital microcephaly in newborns, there have been many cases of infants in Brazil with evidence of infection by ZIKV without microcephaly at birth, suggesting that microcephaly is not a necessary feature of congenital Zika syndrome. The newborns described presented instead with other neurological malformations, including decreased brain volume, dilated lateral ventricles, malformations of the cortex, and calcification [27]. In many infants, postnatal microcephaly developed due to a decline in cephalic growth over the months after birth. The postnatal microcephaly was also associated with neurologic dysfunctions

such as epilepsy, increased and uncontrollable muscle tension, weakness of one side of the body, inability to swallow, and persistence of primitive reflexes. Though it is difficult to assess cognitive deficits in infants, it was found that those without microcephaly showed greater social interaction, measured through social smiling and ability to hold eye contact [27].

Additionally, there was a greater proportion of microcephaly in fetuses whose mothers showed symptoms of infection and had ZIKV present in amniotic fluid, when compared to newborns with mothers who were asymptomatic and displayed no ZIKV in amniotic fluid. There seems to be a correlation between the transmission of the virus through the placenta and maternal symptoms; these effects could likely be due to higher concentrations of the virus in the maternal blood [4]. A study suggested that mothers infected with ZIKV in the third trimester of pregnancy showed infection of the placenta, with ZIKV RNA generally absent in the fetus [4]. However, it seems that pregnant women who showed symptoms of ZIKV infections such as rashes earlier in pregnancy were more likely to have fetal congenital anomalies. There are a few theories postulating the reasoning behind this. It could be attributed to the fact that a significant amount of development occurs in the first trimester of pregnancy, at which point, the placenta has not matured completely, allowing viruses to pass to the embryo or the early fetus [21]. As mentioned earlier, primitive trophoblast showed less immunity to ZIKV compared to mature placental cells [25]. Another plausible explanation could be that viral infection occurring during organogenesis of the brain in the first trimester could interfere with neuronal growth and migration, causing "severe microcephaly and neuronal cell migration disorders" [4]. On the other hand, infections later in gestation can cause brainstem dysfunction including sucking deficiency and problems with breathing, without apparent damage to the brain or impaired intellectual functioning [4]. This theory is supported by another study in which human iPS-derived NSCs were exposed to ZIKV and grown *in vitro* mimicking the development of the brain in the first trimester [15]. Compared to the controls, ZIKV-infected NSCs displayed abnormalities and cell detachment; ZIKV was bound to membranes, observed in the mitochondria and vesicles, and caused apoptosis of cells. The growth area of ZIKV-exposed organoids was reduced by 40% compared to the controls, supporting that ZIKV infection in the first trimester may be related to pronounced hindrance in brain development [15].

A study using CT scans and MRI of 13 newborns born at full term with microcephaly suggests that there is damage of the brain parenchyma and abnormal migration of neural cells, leading to intracranial hypotension causing microcephaly and a lemon shape of the skull in the anterior portion [7]. Evidence of calcification in the regions between the cortex and the ventricles suggests that the parenchymal destruction is due to vasculopathy. These scans also showed lissencephaly, the absence of developed folds, in the cerebral cortex, likely due to infection in the first trimester [7]. Autopsies on patients with congenital ZIKV infection who died after birth also showed pulmonary hypoplasia, intra-alveolar hemorrhage and stiffness of joints, in addition to the external craniofacial abnormalities and malformations in the CNS mentioned above. It was also found that ZIKV was present only in the brain tissues [17].

Each of these studies has noted limitations in their findings due to small sample sizes. Larger studies with a higher number of cases must be performed in order to reach more definitive conclusions. Looking holistically at these studies, there is a great lack of cohesive research thus far, and the issues and mechanisms associated with ZIKV infection are still considered novel in the scientific community. It is difficult to characterize set symptoms of congenital ZIKV infection, especially because so few infections are symptomatic. The absence and lack of characteristic symptoms also undermines the argument that women infected earlier in pregnancy tend to have children with more severe congenital defects because many of them may have been infected multiple times throughout pregnancy without showing symptoms. Nonetheless, the importance of neuroimaging of infants exposed to ZIKV prenatally is emphasized, due to the current lack of evidence [27]. Those who are or may become pregnant are advised to avoid traveling to the countries with known ZIKV transmission shown in **Figure 1**, and to practice protected intercourse with partners who have recently traveled to these areas (https://www.cdc.gov/zika/geo/active-countries.html).

Conclusion

In this review, some of the most recent and promising findings that provide insight into the mechanisms involved in ZIKV transmission are examined and possible future study directions are elaborated upon. Only now are we beginning to uncover the complexities behind the pathology, transmission and neurological damage this virus is linked to cause. Nevertheless, the progress in research that has been made so far presents various observed limitations, and only through review and addressing such observations can more tailored experiments be conducted to lead the path forward. One thing known for certain is the need for further and more extensive studies on ZIKV transmission and its relationship to congenital abnormalities such as microcephaly.

One of the limitations encountered during the review of these recent publications is the use of different strains of ZIKV virus when it came to infecting various types of human cells. These strains vary in origin, genetic makeup, and prevalence to what regions of the world they are found in. This makes it difficult for us to understand the different effects of each strain on varied global populations (**Figure 2**). The strains of ZIKV found in Brazil share greater genomic identity with the Asian lineage than the African lineage of ZIKV [6]. Conducting experiments to study ZIKV correlated with cases of microcephaly found in Brazil, but using the African ZIKV strain that is not commonly found in that region, suggest limitations to the Dang, Tiwari study [8]. While the data found may offer interesting results, its applicability to actual health outcomes seen in Brazil remains to be addressed. The Gabriel paper used the more closely related Asian ZIKV strain to the one in Brazil in a similar study to the Dang, Tiwari paper, and found significant differences [14]. This brings the question of how various other experimental designs conducted on ZIKV using the extensively passaged and widely available African MR766 strain are limited when it comes to applicability of their results. To better understand the causes of microcephaly found in Brazil, where most cases occurred during the 2015-16 ZIKV outbreak [6], we argue that using the strain most representative in that region is of vital importance.

Several studies focused their experiments on specific time periods during pregnancy. Though these studies have provided interesting results, there is a clear need for research on larger groups of pregnant women with ZIKV infections during all trimesters of a pregnancy. Additionally, looking at the longevity of the ZIKV infection in pregnant women is important in determining the effects of the virus because the immunity of PHT cells against ZIKV seems to vary with time, leading to contradictory theories being proposed on its transmission. This might be difficult to do because most people (80%) do not display symptoms, the ones that do show symptoms are mild and nonspecific, and the extent of research done on pregnant women only distinguishes rashes in the first trimester as a potential marker for microcephaly and birth defects [16]. Absence of symptoms makes it difficult for researchers to track infections, which may be a factor in the dearth of subjects, and therefore poor understanding of the causation of neurological defects in neonates. Due to the inconsistencies in the presentation of symptoms among newborns with congenital ZIKV syndrome, there is a lack of well-defined parameters to classify this condition.

The spread of ZIKV, like any virus, is dependent on the overlying population's vulnerability, history of the virus, and local ecology. In order to ensure public health readiness in countries susceptible to ZIKV outbreaks, more extensive and larger-scale studies must be conducted on the potential for ZIKV transmission, especially in countries that are both more susceptible to outbreaks and more vulnerable to the detrimental effects of a public health crisis. Moreover, a recent modeling study examining the potential for ZIKV transmission in resource-limited countries in Africa and Asia-Pacific region highlighted the factor of warmer climates and large numbers of travelers facilitating means of virus propagation [5]. Researchers of the study noted that "because resource-limited countries in Africa and the Asia-Pacific region might have sub-optimum surveillance capacity to readily detect ZIKV infections, [their] validation efforts were retrospective and focused on the Americas" [5]. Their conclusions and the current lack of studies examining the effects of ZIKV outbreaks in economically and socially vulnerable areas reveal the pronounced need for studies focused not just on the Americas. As ZIKV infection is seen to spread to increasingly more areas and brings with it harmful links to birth defects, the consequences brought on to the health, economy, and soci-

ety of affected regions must also be considered and further assessed.

Evidently, more extensive and long-term studies need to be conducted on the ZIKV in detail to further reveal the complexities and transience of the virus and its capabilities. Many unanswered queries are apparent, hopefully to be answered with future research on ZIKV's impact on various areas of the world. By aiming to better understand the processes behind altered fetal neuronal development due to ZIKV infection, the hope is to find ways to increase protection of the fetus and prevent congenital abnormalities.

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None.

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