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Review Article

Zika virus: An emerging challenge for obstetrics and gynecology

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ABSTRACT

Microcephaly is a rare birth defect, however, the re-emerging mosquito and sexual transmitted *flavivirus*, Zika virus (ZIKV), had changed the situation and caused an urgent challenge for the obstetrics and gynecology. This review will brief summarize the epidemiology and virology of ZIKV. And compared the animal models that had developed for the ZIKV infections. These animal models will be benefit for the development of vaccines and anti-ZIKV drugs. Furthermore, the genes that are involved in the causation of microcephaly were also summarized. Finally, the Wnt signal is critical for the brain development as well as innate immune response. Based on previous literatures, we proposed that ZIKV-induced microcephaly might result from the influence of Wnt/β-catenin signaling pathway through the regulation of miRNA-34.

Introduction

Microcephaly is a rare birth defect in which the baby's cranium with a significantly decrease in occipito-frontal head circumference (OFC) of greater than two standard deviations (SD) below the normative mean, whereas those at least three SD below the mean may be described as “severe microcephaly” [1]. Microcephaly is divided into primary microcephaly (apparent congenitally) and secondary microcephaly (develops postnatally) and can be genetic or acquired (caused by environmental factors) [2]. Microcephaly can cause seizures, delay in development, and impair motor function and learning abilities. According the World Health Organization (WHO), the etiology of microcephaly is complex and occurs to be affected by several factors, including: (1) genetic abnormalities; (2) severe malnutrition during fetal life; (3) exposure to toxic chemicals such as arsenic, mercury, and radiation; (4) infection in the womb with the STORCH (syphilis, toxoplasmosis, other infections, rubella, cytomegalovirus (CMV) and herpes simplex) group [3]. The incidence of neonatal microcephaly, as reported in birth defect registers world-wide, differs from 1.3 to 150/100,000 live births, depending upon the population type and the range of SD used to define microcephaly [1]. Although the incidence of microcephaly in general was low, however, the incidence of microcephaly has unusually risen 20-fold in Brazil since 2015 (nearly 3000 cases of microcephaly have been reported) [4]. Epidemiological studies have linked these microcephaly cases to an old, *Aedes* mosquito-transmitted *flavivirus*, Zika virus (ZIKV). This re-emerging ZIKV epidemic may change the once rare microcephaly to be a new challenge issue of obstetrics and gynecology. In this brief review, we will describe the epidemiology and virology of ZIKV and propose the possible molecular mechanism that ZIKV might “hijack” Wnt signaling pathway on the neural stem cells and specifically impair fetal brain development and lead to microcephaly.

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The ZIKV pandemic

Before 21 century, only sporadic cases of ZIKV infection in humans were reported. However, in 2007, ZIKV emerged outside of Asia and Africa and caused the first large outbreak on Yap Island in the Federated States of Micronesia [5]. Unfortunately, a larger epidemic in French Polynesia during 2013–2014 was followed. This ZIKV outbreak in French Polynesia (FP), an overseas country of the French Republic, was estimated about 32,000 persons (11.5% of the population) with Zika-like symptoms [6,7]. Subsequently, ZIKV spread to New Caledonia [8], Japan [9], Norway [10], Easter Island [11], and continental France [12]. Recently, the ZIKV "fire" also burns to the Americas.

In 2015, there was a burst of ZIKV infection in the Americas. Brazil is the most ZIKV hit country, with estimates of 440,000–1,300,000 suspected cases of ZIKV have been reported [13,14]. Since then, ZIKV has rapidly spread far and wide across 50 other countries and territories in the Americas, including the Mexico and United States (World Map of Areas with Risk of Zika, according to the Centers for Disease Control and Prevention (CDC)). The current ZIKV epidemic provides evidence that ZIKV infection may be associated with severe neurological complications, such as Guillain–Barre syndrome (GBS) in adults in FP [15] and microcephaly in fetuses in Brazil [16]. Although, in the early 1970s, Bell et al. had showed that intracerebral infections of newborn and 5-week-old Swiss Webster mice with ZIKV can result in astrocyte hypertrophy and damage to hippocampal pyramidal neurons [17]. Furthermore, electron microscopy revealed that virions were present in both neurons and astroglial cells and morphogenesis in the endoplasmic reticulum (ER). These results have documented that ZIKV can infect and replicate in nervous system of mice, similar to other arboviruses [17].

Zika virus virology

ZIKV is an arbovirus of the Flaviviridae family, which includes dengue virus (DENV), West Nile virus (WNV), yellow fever virus, and tick-borne encephalitis virus (TBEV) [18], and is transmitted to humans by *Aedes* mosquitoes [19]. It was first isolated in Uganda in 1947 from a Rhesus monkey (strain MR-766) [20] and later the first human-infected case was detected in Nigeria in 1954 [21]. Before the 2007 outbreak in Yap, ZIKV has only been found to commonly circulate in tropical regions of Africa and Asia [22]. During outbreaks, humans serve as primary hosts for ZIKV [23], and both urban and sylvatic viral transmission have been demonstrated [24,25]. Otherwise, epizootics of ZIKV in monkeys had also been documented [26], but it is unclear whether primates are the only reservoir in the transmission of ZIKV to humans. Intriguingly, an unexpected finding is that ZIKV can be transmitted by sexual activity had been documented in 2011 [27]. And this may imply that both mosquito bites and human sexual activity could play a role in the recent ZIKV outbreak.

Like other member of the Flaviviridae family, ZIKV is constituted by a single positive sense RNA genome (+ssRNA), which is initially translated into a single polyprotein. This polyprotein is then cleaved post-translationally into individual proteins via host and viral proteases [28]. Three structural proteins named capsid (C), membrane precursor (PrM), and envelope (E) form capsids and seven nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) are involved in both viral transcription and replication and also modulate the host antiviral response [29,30]. Among nonstructural proteins, the glycosylated NS1 exists in two major forms, one is a membrane-bound dimer (mNS1) involved in the viral replication, and the other is a secreted hexamer (sNS1) that functions in pathogenesis and immune evasion via interacting with immune components [31–34]. Furthermore, it can also serve as a biomarker for early detection of flavivirus infection [35]. The other six NS proteins, NS2A to NS5, are thought to be involved in the formation of a replication complex on the cytoplasmic side of the ER membrane [36]. Two major lineages of ZIKV had been revealed by phylogenetic analyses (genomic sequencing): one includes the African lineage come from the original 1947 Ugandan strain (MR-766) [37] and the Asian lineage which are confirmed to be responsible for current outbreak [38].

The common clinical manifestations of ZIKV infection resembles that of DENV and chikungunya virus (CHIKV) may include fever, headache, arthralgia, myalgia, and maculopapular rash [39]. For these complex of symptoms that also hampers clinical diagnosis. Although 80% of human ZIKV infections are asymptomatic or may not get sick at all [5]. However, cases of neurological manifestations and the GBS were reported in FP during the 2013–14 ZIKV outbreak [12]. GBS is a rare autoimmune disorder in which the body's immune system attacks the peripheral nerves [40]. Often, the first symptoms of this disorder are weakness and tingling sensations in legs [41]. The exact cause of GBS is unknown and a new paradox was added that why this neurological disorder can be caused by ZIKV infection. However, it is learned that GBS is frequently preceded by an infectious illness such as acute respiratory or gastrointestinal infection [42]. There were 42 cases of GBS occurring during ZIKV outbreak in FP was recorded [43]. The observation of GBS in ZIKV cases implied an increase in the potential clinical severity of the disease [44]. More bizzare is that the Brazil Health Ministry reported a dramatically increased number of neonatal microcephaly cases (approximately 20 times higher than previous years) in the northeast region of Brazil and concerned this unusual epidemic linked with ZIKV [16]. And after Brazil raised the alarm, health officials in FP also identified more than a dozen babies born with neural defects [4]. Based on these epidemic observations, an emerging issue to clarify is that: Does the ZIKV is the only pathogen that lead to neurological manifestations, especially the microcephaly?

Zika virus caused microcephaly in animals and human

As aforementioned, the unusual increased microcephaly cases in the "hot zone" of ZIKV in Brazil, which may imply that ZIKV is the pathogenic factor. Moreover, causality relationship between the ZIKV epidemic and microcephaly had been experimentally confirmed and mentioned in a clinical case.

A study was conducted to examine the effects of ZIKV infection in neurospheres and brain organoids that generated from human iPSC-derived neural stem cells. This *in vitro* model demonstrates that ZIKV directly infects the brain cells and impairs their viability and grows in human neurospheres and brain organoids. These results implied that ZIKV interferes neurogenesis during human brain development [45]. To further confirm the observations of ZIKV infection in an *in vitro* brain organoids model, the establishment of animal models is necessary and critical. And these animal models should also be benefit for the development of the anti-ZIKV drugs and vaccines. At present, two kind of murine models of ZIKV infection have been established and published, including adult models [46,47] and pregnancy models [48–50], are summarized in Table 1. Moreover, several non-human primate models to study ZIKV are also under way [51]. These studies provide solid evidences that ZIKV can replicate in the embryonic brain and cause severe fetal abnormalities, which is consistent with the reported Zika-related microcephaly cases during the 2015–2016 American outbreak [39].

To test whether ZIKV can infect the embryonic mouse brain, $ZIKV^{SZ01}$ (Asian ZIKV strain) were directly injected into one side of

Table 1
The murine models used in clarify linkage between ZIKV and microcephaly.

ZIKV Strain used	Strain of mice	Inoculation routes	Virus titers	Infected cells (or tissue) identified	Reference
Adult model					
1. Asian-lineage ZIKV (FSS13025)	3-week-old, 5-week-old, or 11-week-old A129 mice 3-week-old AG129	Intraperitoneal (IP) route Intradermal (ID) route	1.0×10^4 PFU/mouse 1.0×10^5 PFU/mouse	Heart, lung, liver, kidney, and muscle Spleen, brain, and testes	[47]
2. Asian-lineage ZIKV (H/PF/2013) African-lineage ZIKV (MR-766, Uganda)	4- to 6-week-old *Ifnar1*$^{-/-}$ mice *Irf3*$^{-/-}$ *Irf5*$^{-/-}$ *Irf7*$^{-/-}$ TKO mice (C57BL/6 background)	Subcutaneous (footpad) route Intravenous (IV) route	1.0×10^2 FFU/mouse 1.0×10^3 FFU/mouse 1.0×10^4 FFU/mouse	Serum, spleen, liver, and kidney Brain, spinal cord, and testes	[46]
Pregnant model					
1. Asian-lineage ZIKV (SZ01/2016/China)	E13.5 ICR mice	Directly injected into one side of the cerebroventricular space/LV	6.5×10^2 PFU/mouse	Fetal brain Neural progenitor cells (NPCs)	[49]
2. Asian-lineage ZIKV (H/PF/2013)	E6.5~7.5 *Ifnar1*$^{+/-}$ mice (C57BL/6 background)	Subcutaneous (footpad) route	1.0×10^3 FFU/mouse	Serum, spleen, fetal brain, and placenta Trophoblast cells (including glycogen trophoblasts and spongiotrophoblasts)	[50]
3. Asian-lineage ZIKV (Brazilian ZIKV strain)	Day 10–13 of gestation SJL mice	Intravenous (IV) route	2.0×10^2 PFU/mouse 8.0×10^9 PFU/mouse 2.0×10^{11} PFU/mouse	Brain Cortical progenitor cells	[48]

the cerebroventricular space/lateral ventricle (LV) of embryonic day 13.5 (E13.5) ICR mouse brains and inspected 3–5 days later [49]. Real-time PCR and immunohistochemistry verified that the brains were readily infected and the number of ZIKV RNA copies increased in 300 times was detected 3 days after infection. Besides, the sizes of ZIKV infected embryonic brains are smaller as compared to those of their mock infected control. Similar to the *in vitro* human brain organoids model, this *in vivo* model also found that ZIKV leads to cell-cycle arrest, apoptosis, and inhibition of neural progenitor cell (NPC) differentiation in the infected embryonic brain. These results may provide pathological evidences that ZIKV infection will cause cortical thinning and microcephaly [49].

During the period of 2013–2015 Brazil outbreak, ZKIV has been linked with microcephaly and other severe neurological diseases, such as GBS [52,53]. Besides the suspected clinical evidences, direct experimental data confirming that the ZKIV^{BR} (Brazilian ZIKV strain which was isolated from a baby born with microcephaly in Paraiba, a state in the Northeast Region of Brazil) is the only pathogen that causes microcephaly and other neurological syndromes was also reported recently [48]. To understand the relationship between ZIKV^{BR} infection and birth defects, a pregnant mouse model (10–13 days of gestation) in which SJL (a immunocompetent mice with high susceptibility to several different viral infections [54]) and C57BL/6 (control) mice (6–8 weeks of age) were infected with ZIKV^{BR} via an intravenous route [48]. These results clearly demonstrated that ZIKV^{BR} can cross the placenta and infect fetuses and display intrauterine growth restriction (IUGR), which caused congenital malformation (including microcephaly) in the pregnant SJL mice. Additionally, ZIKV^{BR} selectively infects cortical progenitor cells of fetal brain, inducing cell death through apoptosis and autophagy as well as impairing neurodevelopment [48].

Apart from aforementioned studies, two mouse models, one with genetic knockout approach and another with antibody mediated “functional knock-down”, were also developed to study in utero ZIKV transmission and infection [50]. In the gene knockout approach, pregnant females *IFNαβR*$^{-/-}$ mice that lacking type I interferon (IFNs) signaling were mated to wild-type (WT) males (C57BL/6) to generate *IFNαβR*$^{+/-}$ (heterozygous) pups which display similar immune status to human fetuses, whereas in a complementary approach using pregnant WT females treated with an anti-ifnar-blocking antibody (MAR1-5A3) to functional knock-down of IFN-α receptor 1 by intraperitoneal injection. Using these mice as an animal model for ZIKV infection, pregnant dams were inoculated with ZIKV (H/PF/2013), which was isolated from a clinical patient in FP, on E6.5 or E7.5 via a subcutaneous (footpad) route. These results showed that ZIKV can replicate in placental trophoblasts and induce cellular apoptosis, which is involved in disruption of the placental barrier, as well as subsequent trans-placental infection of the developing fetus. Thus, most fetuses suffered in utero demise, while surviving fetuses exhibited significant IUGR and high levels of viral RNA in fetal brains [50].

Similarly, there are two recent reports using IFN-related transgenic mice models to study the association between ZIKV infection and neurological disorders [46,47]. To characterize ZIKV in immunocompetent mice, Lazrar et al. subcutaneously injected with 10^3 or 10^4 FFU of ZIKV strain H/PF/2013 into 4- to 6-week-old WT C57BL/6 mice and transgenic mice lacking key components of innate antiviral immunity. *Ifnar1*$^{-/-}$ mice which lacking the interferon receptor cannot respond to IFN-α/β and *Irf3*$^{-/-}$ *Irf5*$^{-/-}$ *Irf7*$^{-/-}$ triple knockout (TKO) mice which produce almost no IFN-α/β were highly susceptible to ZIKV infection and presented with neurological disease [46]. In contrast, single *Irf3*$^{-/-}$, *Irf5*$^{-/-}$, and *Mavs*$^{-/-}$ knockout mice exhibited no signs of illness when infected with ZIKV. Noteworthy, *Ifnar1*$^{-/-}$ mice sustained high viral loads in the brain and spinal cord and developed highest levels of ZIKV infection in the testes, which is relevant to sexual transmission of ZIKV. These results provide solid evidence implying that ZIKV infection can affect neurodevelopment in human fetuses and can be contracted either by mosquito biting or through sexual transmission [46]. Additional, immunocompromised mice A129 (lacking the type I IFN-receptor) and AG129 (lacking both type I and type II IFN-receptors) have also observed similar results, when challenged with a low-passage Cambodian isolate (FSS13025), an Asian ZIKV strain [47]. These animal models, as summarized in Table 1, will be valuable for the study of ZIKV pathogenesis as well as assessing the efficacy of therapeutic drugs and vaccines.

Besides the animal studies implied the association between ZIKV infection and microcephaly, a direct human case of ZIKV in Brazil was recorded in 2016 [39]. In this case, an expectant mom presented a febrile illness with rash was probably infected with ZIKV at the end of the first trimester of pregnancy and the fetus exhibited microcephaly and severe brain injury. This report provides the evidence that fetal brain abnormalities related to ZIKV infection with vertical transmission [39]. Ultrasonographic examination that was performed at 32 weeks of gestation showed small head circumference (microcephaly) and numerous calcifications in the brain and placenta. A fetal autopsy was conducted 3 days after the end of the pregnancy which showed several brain

abnormalities including micrencephaly (an abnormally small brain), internal hydrocephalus, almost complete agyria, spotty calcifications in the cortex and subcortical white matter, cortical displacement, and focal inflammation. In addition, reverse transcriptase-polymerase chain reaction (RT-PCR) assays showed high levels of ZIKV in the fetal brain tissue. Furthermore, the complete genome of this ZIKV was recovered from the fetal brain, named ZIKV strain Bahia, Brazil (KU527068) is consistent with the strain circulating in Brazil comes from the Asian lineage [39]. This study also showed that placental calcification and low placental–fetal weight ratio of 0.136 (<3rd percentile), consistent with previous studies that ZIKV could damage the placenta [48,50]. More interesting, there were no virus and no pathological effects in other fetal organs, suggests a specific neurotropism of the ZIKV [39]. This open a question that the neurotropism of the virus is restricted through the entry pathways (e.g., with a neuronal specific receptor) or the specific replication strategy of the virus in the brain that may be interfered with the production of ZIKV in other tissues.

Genes that correlates microcephaly

Before the 2015 outbreak of ZIKV in Brazil which is indeed causing the severe epidemic of microcephaly, this abnormal brain development is an unusual event in obstetrics and gynecology [15]. However, there are many genetic studies that identified the genes that may associated with microcephaly and are summarized in Table 2. Thus, it is interesting to learn whether the ZIKV infection have any effect on the expression of these microcephaly associated genes. In the "sequence everything" approach of next generation sequencing (NGS) era, it is easy and straightforward to search for genes that were altered upon ZIKV infection [55–57]. Global gene expression analysis of infected brain organoids might provide insights into the link between ZIKV infection and microcephaly and further to figure out the potential underlying mechanisms and management of ZIKV-related pathological effects during brain development [58,59]. Li et al. (2016) carried out global transcriptome analyses determined by RNA-seq and identified several differentially expressed genes at 3 days in ZIKV-infected ICR mouse [49]. Gene ontology (GO) analyses showed that genes associated with microcephaly, including ASPM, CASC5, MCPH1, RBBP8, STIL, and TBR2 (all are listed in Table 2) were significantly downregulated. In addition, genes involved in organ development as well as participated in cell proliferation, differentiation, and migration were also significantly downregulated. In contrast, GO analysis revealed enrichment of upregulated genes related to immune response and apoptosis pathways. Among these upregulated genes, several genes involved in production of cytokines and the response to cytokines were notable. Thus, it is suggested that cytokines may play a significant role in the pathogenesis of ZIKV infection [49].

Zika virus regulates the Wnt signal during brain development-a hypothesis

The Wnt/β-catenin signaling pathway has been shown to be involved in regulation of development, cellular growth, cell–cell interactions, tumorigenesis, and stem cell biology [60–64]. Wnt signaling is initiated through the binding of soluble Wnt ligands to cell surface receptors of the Frizzled (Fz) family and co-receptor of the low-density lipoprotein receptor-related protein (LRP) family. This receptor-ligand complex recruits the scaffold protein, Dishevelled (DVL), and in turn recruits the Axin-glycogen synthase kinase 3β (GSK3β) complex, resulting in the phosphorylation of LRP by GSK3β [65–67]. This signalosome-mediated recruitment of more Axin from the β-catenin destruction complex to the plasma membrane [68,69]. The "β-catenin destruction complex" is a multi-domain scaffold phospho-protein complex, that includes Axin, adenomatous polyposis coli (APC), glycogen synthase kinase 3β (GSK3β), Casein Kinase 1 (CK1), protein phosphatase 2A (PP2A), and E3-ubiquitin ligase [70]. Recruitment of Axin lead to disassembly of the destruction complex resulting in the repression of GSK3β activity. Inhibition of GSK3β-mediated phosphorylation leads to stabilization and accumulation of cytosolic β-catenin [71]. The rise of cytoplasmic levels of β-catenin then translocates to the nucleus where interacting with the TCF/LEF transcription factors and thereby switch on Wnt-target gene transcription. In the absence of Wnt, GSK3β constitutively phosphorylates β-catenin, marking it for ubiquitination and subsequent proteasome-mediated degradation [72,73].

Previous studies have explored the role of Wnt signaling in the regulation of cerebral cortical size [74,75]. To examine whether Wnt signal could regulate mammalian brain development, a genetic approach is employed to generate transgenic mice that overexpress a stabilized form of β-catenin in neuronal progenitor cells [74]. β-catenin can act as an important component of adherens junctions [76], also serve as a transcriptional co-activator of Wnt target gene expression [77], and is highly expressed in mammalian neural progenitors [74]. These studies showed that over activating β-catenin influences cell fate decisions during brain development and leads the neural precursors to re-enter the cell cycle after mitosis. Thus, overexpression of β-catenin results in enlarged brain size which has a greatly increased cerebral cortical surface area without increased cortical thickness [74]. In contract, inactivation of Wnt signals leads to specific developmental brain defects, including reduction of hippocampal development [62] or absence of midbrain and cerebellum [60]. Based on these observations, we hypothesized that ZIKV-induced microcephaly might result from the influence of Wnt signaling pathway.

Several studies have found that microRNAs (miRNAs) might regulate components of the Wnt signaling pathways to affect various cellular processes during embryo development [78]. MiRNAs are small (20- to 23-nucleotide [nt]) and noncoding RNAs that play significant roles in regulating a broad of cellular processes through the posttranscriptional modulation of gene expression. More interestingly, there are reports indicating that viral infection induces significant changes in the expression levels of cellular miRNAs [79–83]. These observations may imply that the manipulation Wnt signaling pathways by the virus or the host response to viral infection through miRNAs. Recently, a microRNA screen approach was designed to understand the relationship between flavivirus infection and Wnt signaling pathway [84]. These results show that miR-34 family have the ability to repress Wnt signaling and exhibit antiviral effects in transfected cells infected with flaviviruses, including DENV, WNV, and ZIKV (strain PRVABC059). Further studies demonstrate that miR-34a repression the Wnt signaling, which inhibits the phosphorylation of GSK3β, results in a direct interaction between GSK3β and TANK-binding protein 1 (TBK1) that promotes interferon regulatory factor 3 (IRF3) phosphorylation/homodimerization and subsequently allows its translocation into the nucleus to activate the interferon responsive genes as well as to promote antiviral responses [84]. Consistent with previous established concepts that the cross talk between the Wnt pathway and the interferon-based innate immune pathway (the type I IFN signaling pathway) during viral infection [85–89]. Using locked nucleic acid (LNA)-anti-miR to knock-down endogenous miR-34a a significant increase in viral replication was observed, suggesting that endogenous miR-34a might function to suppress flaviviral replication through its ability to dampen Wnt signaling, allowing GSK3β-TBK1-mediated activation of IRF3 in response to pathogen-associated molecular pattern (PAMP) detection. However, the endogenous levels of miR-34a was unaffected by DENV

Table 2
The genes that are correlated to microcephaly.

Gene	Protein	Function	Chromosome location	References
MCPH1 (*BRIT1*)	Microcephalin	DNA damage repair; chromosome condensation; Transcriptional regulation of DNA damage genes	8p23	[92–94]
WDR62 (*MCPH2*)	WDR62	Play a role in cerebral cortical development; For centriole duplication	19q13.12	[95,96]
CDK5RAP2 (*MCPH3, Cep215*)	CDK5RAP2	Regulating microtubule dynamics; Centrosome maturation and cohesion	9q33.2	[97–99]
KNL1 (*CASC5, MCPH4*)	KNL1 (CASC5)	Essential for spindle-assembly checkpoint signaling; For correct chromosome alignment; involved in centriol duplication	15q15.1	[100–102]
ASPM (*ASP, MCPH5*)	ASPM	Orientation and organization of mitotic spindles During embryonic neurogenesis	1q31.3	[103–105]
CENPJ (*CPAP, MCPH6*)	CENPJ	Centriole biogenesis and length control; Microtubule dynamics	13q12.12	[106–108]
STIL (*SIL, MCPH7*)	STIL	Spindle organization; Hh/Shh signaling; Cell cycle progression	1p33	[109–111]
CEP152 (*MCPH9*)	CEP152	Necessary for centrosome duplication	15q21.1	[112]
CENPE (*MCPH13*)	CENP-E	For the maintenance of chromosomal stability; For the movement of chromosomes toward the metaphase	4q24-q25	[113]
SASS6 (*SAS6, MCPH14*)	SASS6	For centrosome biogenesis and duplication	1p21.2	[114,115]
MFSD2A (*NLS1, MCPH15*)	NLS1	For blood–brain barrier (BBB) formation and function	1p34.2	[116,117]
ANKLE2 (*MCPH16*)	ANKLE2	Mitotic nuclear envelope reassembly; Facilitating nuclear envelope assembly	12q24.33	[118,119]
CEP63 (*SCKL6*)	CEP63	For normal spindle assembly; plays a key role in mother-centriole-Dependent centriole duplication and in DNA damage response	3q22.2	[120,121]
DYRK1A (*DYRK*)	DYRK1A	Regulating cell proliferation; involved in brain development	21q22.13	[122,123]
EOMES (*TBR2*)	eomesodermin (TBR2)	Required for trophoblast development and mesoderm formation; Key regulator of neurogenesis in the SVZ	3p24.1	[124–126]
KIF11 (*EG5*)	KIF11	Establishing a bipolar spindle during mitosis; Chromosome positioning; centrosome separation	10q23.33	[127,128]
RBBP8 (*CTIP*)	RBBP8	Bind directly to retinoblastoma protein, regulates cell proliferation; DNA damage signaling and repair	18q11.2	[129–131]
WDFY3 (*ALFY*)	WDFY3	For selective autophagy (aggrephagy); Formation and degradation of cytoplasmic ubiquitin-positive inclusions	4q21.23	[132,133]

Abbreviations. MCPH: Autosomal recessive primary microcephaly; MCPH1: Microcephalin; BRIT1: BRCA1 C terminus-repeat inhibitor of human telomerase expression 1; WDR62: WD repeat domain 62; CDK5RAP2: CDK5 regulatory subunit associated protein 2; KNL1: kinetochore scaffold 1; CASC5: Cancer susceptibility candidate 5; ASPM: abnormal spindle microtubule assembly; CENPJ: Centromere protein J; CPAP: Centrosomal P4.1-associated protein; STIL: SCL/TAL1 interrupting locus; CEP152: Centrosomal protein 152 (kDa); CENPE: centromere protein E; SASS6: SAS-6 centriolar assembly protein; MFSD2A: major facilitator superfamily domain containing 2A; NLS1: Sodium-dependent LPC symporter 1; ANKLE2: ankyrin repeat and LEM domain containing 2; WDFY3: WD repeat and FYVE domain containing 3; ALFY: Autophagy-Linked FYVE Protein; CEP63: centrosomal protein 63; SCKL6: Seckel syndrome-6; DYRK1A: dual specificity tyrosine phosphorylation regulated kinase 1A; KIF11: Kinesin family member 11; RBBP8: Retinoblastoma-binding protein 8; CTIP: CtBP-interacting protein; EOMES: Eomesodermin; TBR2: T-box brain protein 2.

infection [84]. It is interesting to know whether the ZIKV infection can up-regulate the levels of miR-34a to down regulation Wnt signaling pathway. Based on these observations mentioned above, we hypothesized that ZIKV infection might contribute to microcephaly through (1) induction of changes in miRNA expression levels, like up-regulate the levels of miR-34a. (2) This changes might repression of Wnt/β-catenin signaling pathway. (3) Inactivation of Wnt signals would result in brain defects in the fetus during pregnancy. According this model, inactivation of Wnt signals would up-regulation of IFN-related genes (e.g. type I IFNs and interferon-stimulated genes (ISGs)) will promote antiviral activity for women in pregnancy, but will cause brain developmental defect, such as the microcephaly, in infants.

However, other mechanisms may also exist to explain the causal relationship between the ZIKV infection and microcephaly. For example, computational prediction of miRNAs (genome-wide) binding sites in the prototypic ZIKV (MR-766) whole genome had discovered six miRNAs, miR-627–5, miR-4646, miR-1304, miR-6771, miR-4528, and miR-3198 that share mutual homology with the microcephaly related genetic sequences MCPH1, 2, 3, 4, 5, 6, 7, and 9 (see Table 2), respectively. These findings also suggest that ZIKV may modulate host miRNAs that are involved in regulating MCPH genes during fetal brain development, resulting in microcephaly [90]. Noteworthy, miR-1304, which has homology to six different MCPH genes (MCPH 3, 4, 8, 9, 10, and 12), might be associated with gene disorders, neurological diseases, and nervous system development had been documented [91].

Conclusion

The re-emerging mosquito and sexual transmitted ZIKV is an urgent challenge for the obstetrics and gynecology. ZIKV and the so-called STORCH factors will be the main reasons that compromise brain development in utero and cause microcephaly. Thanks to the modern biotechnology, various animal models for the ZIKV infection have been established and will facilitate the development of vaccine and anti-ZIKV drugs. We proposed that ZIKV-induced microcephaly might result from the influence of Wnt signaling pathway through the regulation of miRNA and this may be verified through experimental test and also provided a route for the antiviral research. A critical issue to be answered will be: If a woman once has contracted with ZIKV but recovered, are future pregnancies safe?

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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