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VIEWPOINTS

Differential virulence between Asian and African lineages of Zika virus

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Zika virus (ZIKV) is a small enveloped positive-sense single-stranded RNA virus belonging to the genus *Flavivirus* of the *Flaviviridae* family that has reemerged in recent years as a human pathogen with epidemic potential. To date, the ongoing epidemic in the Americas has led to tens of thousands of confirmed cases in Brazil alone (Pan American Health Organization Zika-Epidemiological Report, 2 March 2017). More concerning are the reports of severe neurological disorders such as Guillain-Barré syndrome and congenital Zika syndrome (microcephaly and other neurodevelopmental defects), which led the World Health Organization to declare the ZIKV epidemic as a Public Health Emergency of International Concern in 2016.

Phylogenetic analyses show that ZIKV can be classified into 2 main lineages: the African lineage and the Asian lineage, the latter of which is responsible for the recent epidemics. Because recent ZIKV infections were associated with the development of congenital and neurological disorders, a key question was raised as to whether Asian-lineage ZIKV strains were phenotypically different from the African lineage strains. It is well described that mutations acquired during *Flavivirus* evolution can alter their virulence and/or tropism [1]. ZIKV is primarily transmitted by the mosquito species *Aedes aegypti* and *Aedes albopictus*. Several studies show that vector transmission can differ between ZIKV strains, as the overall ZIKV infection prevalence and transmission rates of African strains may be higher in *A. aegypti* than Asian strains [2], suggesting that viral adaptation may have occurred—similar to a single mutation in the chikungunya virus envelope protein that affected vector specificity and epidemic potential.

During the recent outbreak, it became clear that ZIKV is sometimes able to cause a prolonged infection. Numerous studies showed that Asian-lineage strains isolated in South America replicate at low levels in tissues months after the initial infection. For example, viral genome can be found in stillborn babies who were infected early during gestation [3], weeks after initial infection in sperm, and in rhesus monkeys, ZIKV can be found in the cerebral spinal fluid (CSF) 42 days after infection, weeks after the virus was cleared from the blood [4]. However, to date, no data are available on the ability of African-lineage strains to cause prolonged infections, but early studies in 2016 suggested that an African ZIKV strain was highly pathogenic and led to cell death; when the first reports on a potential link between microcephaly and ZIKV emerged, laboratories started to study the effect of ZIKV on human neural precursor cells (hNPCs)/human neural stem cells (hNSCs) with MR766, the most available strain at the time (Fig 1 and Table 1). These first studies showed strong tropism and deleterious effects on NSC homeostasis and growth (e.g., [5]). However, some inconsistencies between this rather strong virulence and the long-term developmental effects associated with ZIKV infections led researchers to question the biological validity of this strain, which has been

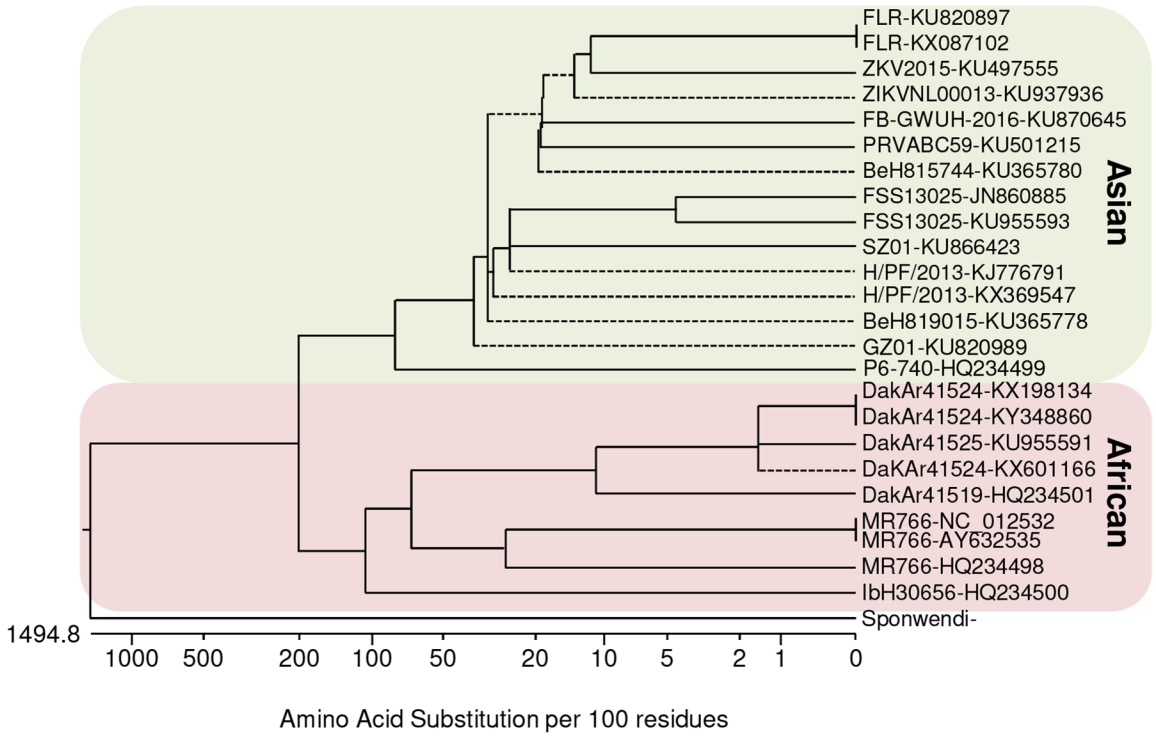

Fig 1. Phylogenetic analyses of ZIKV strains discussed.

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passaged many times in animals and in cells and acquired mutations and deletions at multiple positions during passaging (Table 1) [6–8]. The relevance of this strain was also questioned in a study that showed reduced toxicity and long-term persistence in human neural progenitors of an Asian-lineage ZIKV strain from Puerto Rico, which seemed more consistent with clinical manifestations [9]. Consequently, we compared the neurovirulence *ex vivo* of 2 low-passaged African- and Asian-lineage ZIKV strains [6]. The African ArB41644 strain was isolated in Central African Republic in 1989, whereas the Asian H/PF/2013 strain was isolated in 2013 during the French Polynesian epidemic (Table 1). We showed that the African ZIKV strain displayed a higher infectivity and replication rate in hNSCs than the Asian ZIKV strain. ArB41644 triggered more defects in proliferation and more apoptosis, which correlated with a stronger induction of the antiviral response [6]. In a subsequent study, we compared 2 Asian-lineage ZIKV strains (H/PF/2013 and ZIKVNL00013, isolated during the current epidemic in 2015) with 2 African-lineage ZIKV strains (MR766 and Uganda 927) in human neural progenitor cells and confirmed the phenotypic differences between ZIKV strains from the Asian and African lineages [10]. Both African strains infected more cells, replicated to higher titers, and induced early cell death more frequently than the Asian-lineage ZIKV strains [10]. The milder virulence of the Asian strains in these studies could be consistent with a persistent infection and effect of the Asian ZIKV lineage in developing neural cells. In addition, in dendritic cells, infection by African (MR766 and Dakar-1984) and Asian (PR-2015) strains led also to a difference in pathogenicity, as African strains triggered more cellular death [8]. Notably, this study also showed that despite differences in viral replication rates between the extensively passaged MR766 strain and the more recently isolated Dakar-1984 strain, the inductions of cell death were similar, suggesting that the ability to cause cell death seen in various cellular models with

Table 1. Passage history of ZIKV strains.

Lineage	Strain name	GenBank	Country of origin	Year	Reference	Passage histories
African	MR766	NC_012532; HQ234498.1; AY632535	Uganda	1947	[8]	Unknown + 1x Vero
					[11]	Not indicated
					[12]	Not indicated
					[13]	Not indicated
					[10]	Not indicated
					[14]	Not indicated
					[15]	Not indicated
					[16]	Not indicated
					[17]	Not indicated
					[18]	146x SM, 1x C6/36, 1x Vero
					[19]	146x SM, 3x Vero
					[20]	Not indicated
					[21]	Not indicated
	Uganda 976		Uganda	1962	[10]	6x Vero
	P6-740	HQ234499.1	Malaysia	1966	[8]	Unknown + 1x Vero
					[18]	6x SM, 1x BHK, 1x C6/36, 3x Vero
	IbH30656	HQ234500	Nigeria	1968	[16]	Not indicated
	Dakar41519	HQ234501	Senegal	1984	[14]	Not indicated
					[18]	1x AP61, 1x C6/36, 3x Vero
	Dakar 41671		Senegal	1984	[14]	Not indicated
	Dakar 41677		Senegal	1984	[14]	Not indicated
	DakAr41524	KX198134; KY348860; KX601166	Senegal	1984	[8]	Unknown + 1x Vero
	DakAr41525	KU955591	Senegal	1985	[22]	5x Vero, 1x C6/36, 2x Vero
					[2]	1x AP61, 1x C6/36
	ArB41644		Central African Republic	1989	[10]	5x Vero
					[6]	5x Vero
	HD78788	KF383039	Senegal	1991	[13]	Not indicated
	MP1751		Uganda	1962	[23]	3x SM,4x unknown methods, 1x Vero
Asian	FSS13025	KU955593; JN860885.1	Cambodia	2010	[15]	Not indicated
					[22]	1x AP-1, 1x C6/36, 5x Vero
					[2]	1x Vero, 1x C6/36
					[17]	Not indicated
					[18]	5x Vero
					[21]	Not indicated
	H/PF/2013	KX369547; KJ776791	French Polynesia	2013	[12]	Not indicated
					[10]	4x Vero
					[14]	Not indicated
					[24]	6x C6/36
					[6]	5x Vero
					[20]	Limited passages in Vero
	BeH815744	KU365780	Brazil	2015	[24]	3x C6/36
	BeH819015	KU365778.1	Brazil	2015	[20]	Not indicated
	FB-GWUH-2016	KU870645	Guatemala	2015	[12]	Not indicated
	FLR	KU820897; KX087102	Colombia	2015	[16]	Not indicated
	Mex1-7		Mexico	2015	[22]	3x Vero
					[2]	4x Vero
	PRVABC59	KU501215; KU501215.1	Puerto Rico	2015	[8]	Unknown + 1x Vero

(*Continued*)

Table 1. (*Continued*)

Lineage	Strain name	GenBank	Country of origin	Year	Reference	Passage histories
					[23]	4x Vero
					[15]	Not indicated
					[16]	Not indicated
					[18]	3x Vero
					[19]	
	ZIKV^{BR}		Brazil	2015	[11]	Not indicated
	ZKV2015	KU497555	Brazil	2015	[4]	Not indicated
	ZIKVNL00013	KU937936	Surinam	2016	[10]	4x Vero
	GZ01	KU820898	Venezuela	2016	[15]	Not indicated
	SZ01	KU866423	Samoa	2016	[16]	Not indicated

Abbreviations: BHK, baby hamster kidney, SM suckling mice

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MR766 could still in certain circumstances be representative for African ZIKV lineage strains [8].

Since infection with ZIKV strains from the Asian lineage is associated with microcephaly in humans, it is likely that ZIKV crosses the placental barrier early in gestation, when the brain is starting to develop. Some studies showed that cells of the placenta are susceptible to ZIKV infection, in line with the congenital infections and neurological symptoms associated. Because infections early in gestation seem to be more linked to microcephaly [25], it was hypothesized that the placenta vulnerability could differ depending on the gestational stage. While the placental syncytiotrophoblast, which is found in mature placenta, appears mostly resistant to ZIKV, a study that compared an African strain (MR766) to an Asian strain (FSS13025, Cambodia) showed that embryonic stem cell (ESC)-derived trophoblasts, which recapitulate primitive placenta cells during implantation, are highly susceptible to ZIKV infection [17]. Interestingly, infection with the African strain again resulted in higher viral replication and cell lysis. These data might indicate that infection with African-lineage ZIKV strains—if they enter the fetus—could result in an early termination of pregnancy, while infection with an Asian-lineage ZIKV strain would be less destructive and more chronic, thereby allowing the pregnancy to continue, leading to the development of congenital malformations [17].

While these *ex vivo* data suggested that African ZIKV lineage strains are more virulent, it was important to confirm the difference in (neuro)virulence of these 2 lineages *in vivo*. Interferon-α/β receptor (*Ifnar*)$^{-/-}$ mice injected subcutaneously with African ZIKV strains (MR766 and 3 strains from Senegal) or with the Asian-lineage H/PF/2013 strain all presented with weight loss and succumbed to the infection after 6 (for Senegal strains) to 10 (H/PF/2013) days. Interestingly, infection with the African-lineage strain MR766 resulted in 20% survival at 2 weeks [14]. Another study compared the symptoms induced by African (Uganda and Senegal) and Asian (Cambodia, Malaysia, and Puerto Rico) ZIKV strains in signal transducer and activator of transcription (*Stat2*)$^{-/-}$ and *Ifnar*$^{-/-}$ mice infected subcutaneously [18]. Importantly, infections with African strains resulted in more weight loss, mortality, and severe prolonged neurological symptoms compared to Asian strains. Moreover, induction of type 1 and 2 interferon (IFN) were higher following infection with African strains associated with enhanced levels of inflammatory cytokines such as interleukin 6 (IL6) or tumor necrosis factor (TNF). These observations were confirmed in another study showing that an African strain (MP1751) is pathogenic in A129 mice, contrary to an Asian strain (PRVABC59) that did not cause signs of illness [26]. These studies confirm the *ex vivo* results in neural and nonneural cells regarding

Table 2. Comparative analyses of ZIKV African and Asian lineages.

	Model	*Lineage*: Strain	Findings	Reference
Ex vivo				
	Endothelial cells	*African*: MR766, IbH30656; *Asian*: PRVABC59, FLR	Asian-lineage strains replicate faster and isolates induced significant cell death compared to African-lineage strains.	[16]
	Astrocytes	*African*: HD78788, ArB41644; *Asian*: H/PF/2013	African-lineage strains led to a higher infection rate and viral production infection. Asian-lineage strain led to the expression of these innate immune response genes early, while their induction by the African strain was delayed.	[6][13]
	Neuronal stem cells	*African*: DakAr41525, MR766, ArB41644, Uganda 976; *Asian*: H/FP/2013, Mex1-7, FSS13025, FB-GWUH-2016, ZIKVNL00013	African-lineage strains led to a higher infection rate and virus production as well as stronger cell death and antiviral response compared to Asian-lineage strains. Protein 53 (p53) plays an important role in apoptosis induced by Asian-lineage strains but not by African-lineage strains.	[22][12][6]. [21][10]
	Cerebral organoids	*African*: MR766; *Asian*: $ZIKV^{BR}$	Asian-lineage strain led to a higher viral production as well as stronger cell death compared to the African strain.	[11]
	Embryonic stem cell–derived trophoblast	*African*: MR766; *Asian*: FSS13025	African-lineage strain led to a higher viral production as well as stronger cell death compared to Asian-lineage strains.	[17]
	Monocyte-derived dendritic cells	*African*: DakAr41524, P6-740, MR766; *Asian*: PRVABC59	African-lineage strains led to a higher infection rate and viral production as well as stronger cell death and antiviral response compared to Asian-lineage strains.	[8]
	Neuronal cell lines (SK-N-SH, U87 MG)	*African*: *MR766*, *Uganda 976*; *Asian*: *H/FP/2013*, *ZIKVNL00013*	African-lineage strains led to a higher infection rate and virus production.	[10]
In Vivo				
	Mosquito: *Aedes aegypti*	*African*: DAKAR41525, MR766; *Asian*: MEX1–7, PRVABC59, GZ01, FSS13025, H/PF/2013, BeH815744	Infection prevalence and transmission rate of African-lineage strains is higher compared to Asian-lineage strains. Infection prevalence and transmission rate of Asian-lineage strains from the Americas are higher compared to Asian-lineage strains from Asia.	[15][24][2] [19]
	Mouse: *A129*	*African*: MR766, P6-740, DAKAR41519,41667,41671, MP1751; *Asian*: H/PF/2013, PRVABC59, FSS13025, BeH819015, PRVABC59	African lineage is pathogenic whereas Asian lineage does not cause sign of illness.	[23]
	Ifnar1$^{-/-}$ *Irf3*$^{-/-}$ *Irf5*$^{-/-}$ *Irf7*$^{-/-}$		African-lineage strains induced higher mortality and severe neurological symptoms in a short period as compared to Asian-lineage strains.	[14]
	Stat2$^{-/-}$ *Ifnar1*$^{-/-}$		Among Asian-lineage strains, the Cambodia strain presented more severe symptoms including front limb paralysis and lethality, followed by the Malaysia and Puerto Rico strains. The Brazilian strain is least severe. The Uganda strain showed orders of magnitude higher viral RNA level compared to all other strains including Senegal, especially in brain tissue.	[18]
	Ifngr1$^{-/-}$		Higher levels of interferon type I and type II and inflammatory cytokine induction by African-lineage strains as compared to the Asian-lineage strains.	[20]
	Nonhuman primate	*Asian*: ZKV2015, PRVABC59	No differences observed	[4]

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the higher virulence of African strains and could suggest that different ZIKV strains may also display phenotypic differences in human subjects (Fig 1) (Table 2). Many of the findings from immunocompromised mice have been recapitulated in nonhuman primates, such as rhesus and cynomolgus macaques. The development of these different animal models led to new knowledge concerning the pathogenesis of ZIKV. However, these systems have limitations, in particular, studying viral pathogenesis using animals lacking a key component of antiviral immunity, which makes it still difficult to derive definitive conclusions regarding ZIKV virulence in humans. Therefore, many unanswered questions remain, in particular, regarding the mechanisms of host restriction, immune evasion, and inflammatory response as well as the long-term neurodevelopmental implications of congenital infection in humans.

However, one major challenge in interpreting and comparing the data from these studies is the lack of harmonization of the virus strains used. In this review alone, in which we focused on studies that compared multiple ZIKV strains, at least 12 African and 13 Asian strains were found (Fig 1, Tables 1 and 2). While most studies include the African lineage MR766 strain, this strain cannot be considered as a standard, as it has been extensively passaged and at least 3 MR766 strains exist with genetic differences, including a 4–6 codon deletion in the envelope (E) protein [7]. For other strains, multiple sequences exist, or in some cases, no sequence data are available (Table 1). Therefore, when comparing ZIKV lineages, the strains should be sequenced and experiments more standardized to allow proper comparison between studies. Ideally, more recent African ZIKV strains should become available (as the most recent isolate so far is from 1991) (Table 1).

In conclusion, the data reviewed here indicate that there are intrinsic differences in the pathogenicity/virulence of African- and Asian-lineage ZIKV strains. Whether these differences are responsible for differences in clinical presentations should be confirmed, but one can speculate that the phenotype of Asian ZIKV strains (lower infection rate, less virus production, and poor induction of early cell death) might contribute, at least in part, to the ability to cause persistent infections within the CNS of fetuses, while African-lineage ZIKV strains could result in more acute infection. However, even though *ex vivo* and *in vivo* data point towards a stronger virulence for African strains, it is still premature to conclude that if an African epidemic declares itself (due to an African ZIKV strain), neurological symptoms with similar or worse gravity than those observed in South America will be found. If confirmed, recent reports of African ZIKV strain infection and potential microcephaly in Guinea Bissau may be proof of what could be expected in this continent [27]. Determining the virulence factors for ZIKV using reverse genetic systems that have now been developed for both African and Asian strains will be crucial to identify the molecular and cellular mechanisms behind differences in ZIKV pathogenicity and also those of other emerging arboviruses.

Author Contributions

Conceptualization: Yannick Simonin, Sara Salinas.

Funding acquisition: Yannick Simonin, Philippe Van de Perre, Sara Salinas.

Writing – original draft: Yannick Simonin, Debby van Riel, Barry Rockx, Sara Salinas.

Writing – review & editing: Philippe Van de Perre.

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