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Chikungunya Encephalitis: an Inconsistently Reported Headache and Cause of Death in Patients with Pre-Existing Conditions

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Abstract
Chikungunya virus (CHIKV) is an alphavirus of the family Togaviridae with outbreaks occurring across Africa, Asia, parts of Europe, and South and Central America. There are three main lineages of CHIKV, including the West African lineage, the East Central South African (ECSA) lineage, and the Asian lineage. While CHIKV infection usually results in a self-limited febrile illness, there have been reports of concerning neurological manifestations, including encephalitis. Herein we discuss findings of over 700 cases of CHIKV encephalitis and risk factors for death. Additionally, we examined the genotypes of CHIKV associated with encephalitis and found that both the Asian and ECSA lineages were responsible for encephalitis but not the West African lineage. Protein analysis of consensus sequences of CHIKV strains associated with encephalitis identified mutations in the nsP1, nsP2, and nsP3 proteins. Reports and manuscripts of CHIKV encephalitis were inconsistent in reporting viral, demographic, and clinical features which complicated the delineation of risk factors associated with the disease and viral evolution. As climate change contributes to the range expansion of natural vectors, it is important for researchers and clinicians to consistently report patient and viral data to facilitate research and countermeasures for the ecology and epidemiology of CHIKV due to the lack of a targeted treatment or vaccine.

Keywords Chikungunya · Encephalitis · CHIKV · Meningoencephalitis · Death · Pre-existing conditions

Introduction

Chikungunya virus (CHIKV) is an arbovirus that is an alphavirus of the family Togaviridae [1, 2]. CHIKV outbreaks have occurred in the Americas, Africa, Asia, and Europe [3]. CHIKV was first isolated in 1952, but there are earlier suspected outbreaks in Africa and Asia [4]. CHIKV is vectored by the mosquitoes *Aedes aegypti* and *Aedes albopictus*, which are invasive to the western hemisphere [5•, 6, 7]. Climate change and urbanization are creating a favorable environment for the range expansion of *Aedes* mosquitos. In addition to mosquitoes, there is evidence CHIKV can be spread via vertical transmission from mother to child [6, 8].

There are three predominant lineages of CHIKV including West African, East Central South African (ECSA), and Asian [9, 10]. The Indian Ocean lineage (IOL) is a subtype descendant from the ECSA lineage [9]. The first major IOL sub lineage was identified during the 2005–2006 La Reunion outbreak, which was also associated with neurological complications [11]. The role of these viral lineages in the pathogenesis of CHIKV is unclear but the ECSA lineage and the IOL sub lineage has been associated with severe neurological disease as well as heart disease [4, 11]. Symptoms of CHIKV include a biphasic fever, muscle and joint pain, headache, a maculopapular rash, polyarthralgia, conjunctivitis, and fatigue, among other varying symptoms [1, 2, 12, 13]. In about 60% of patients, there are long lasting arthritis symptoms that can last for years [1, 12]. In recent years, CHIKV has been associated with neurological complications, including encephalitis [14, 15].

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CHIKV encephalitis is of interest due to the broad range of symptoms associated with it and the potential for long-term neurological sequelae. Broadly, encephalitis is "inflammation of the brain parenchyma" [16]. This inflammation typically results in neurological debilitation and encephalopathy [16]. Clinically, encephalopathy is an altered mental state that can occur regardless of brain inflammation status but is sometimes used reciprocally with encephalitis [16]. While encephalitis can occur for numerous reasons, approximately 20–50% of cases are most likely viral [17•, 18, 19]. Viruses typically responsible for encephalitis are herpes simplex virus (HSV), varicella-zoster virus (VZV), arboviruses, and enteroviruses [17•]. Viral encephalitis has been reported for alphaviruses with long-term neurological sequelae including cognitive deficits, confusion, mood changes, and memory decline [20, 21]. One study following patients with the alphaviruses Madariaga virus (MADV), Venezuelan equine encephalitis (VEEV), and Una virus (UNAV) found that long-term sequelae could include depression, insomnia, and dizziness [14, 20, 21].

While CHIKV encephalitis has been reported in the literature, it is unknown how common CHIKV encephalitis is, nor do we know who is most at risk. Thus, we searched peer-reviewed literature and case reports to determine if there were associations between CHIKV lineage, geographic location, or patient characteristics with a diagnosis of encephalitis and outcome of death.

Methods

Search Strategy

The literature and case reports used were sourced from Pubmed, Embase, LILACS, and Google Scholar. Additionally, other sources were pulled from the references of articles sourced from the aforementioned databases. The search terms utilized were "CHIKV + encephalitis," "CHIKV + brain," and "case report of CHIKV encephalitis brain."

Case Definition

Cases were included based on a clinical diagnosis of a CHIKV infection and a diagnosis of encephalitis. The clinical diagnosis was defined as having a laboratory confirmation either by ELISA for IgM or IgG or RT-PCR of serum or CSF. All forms of encephalitis were included, but it was noted which type of encephalitis the patient had. These included encephalopathy, encephalitis, meningoencephalitis, acute disseminated encephalomyelitis (ADEM), Bickerstaff's brainstem encephalitis, encephalomyelitis, acute necrotizing encephalopathy (ANE), and NMDAR antibody encephalitis. Case reports in which patients were pregnant or had altered immune systems such as an autoimmune condition (HIV, lupus), cancer, or malaria were excluded from the statistical analysis.

Statistical Analysis

Patient data was either reported in the literature as a case study or as part of a cohort study. The analysis for case studies and cohort studies was performed separately to avoid unintentional repeating of cases. For the case study analysis, logistic regression was performed comparing risk of certain outcomes based on sex, death, and age as a categorical variable. The age categories were 0–1, 1–10, 11–20, 21–30, 31–40, 41–50, 51–60, and 60 + . Patients younger than 1 and older than 60 have been shown to have a greater risk for CHIKV complications [22]. Statistical analyses were run on MedCalc version 17.9.7–64-bit. Odds ratios were calculated for each of the dependent variables with 95% confidence intervals. The threshold for significant for odds ratios was set at $p < 0.05$. ANOVA with Tukey–Kramer post-hoc test was performed when deemed appropriate.

Phylogenetic Analysis

Phylogenetic trees were developed using data from the NCBI virus software corresponding to year and location in which an encephalitis case was reported [23]. These data were selected from *homo sapiens* samples at least 10,000 base pairs long. The sequences were aligned using the multiple sequence comparison by log-expectation (MUSCLE) software via the molecular evolutionary genetics analysis (MEGA-X) software [24]. MEGA-X was used to create the phylogenetic trees using the bootstrap method with 100 repeats [24].

Non-Structural Protein Analysis

Three separate consensus sequences were created using the NCBI viruses align software and was found to cover only the regions for the non-structural proteins [23]. The accession numbers for each are shown in Table 1. The consensus sequences were broken up by lineage and location, Asian for the Caribbean and South and Central American sequences, Brazilian for all the Brazil sequences, and Indian for all the India sequences. This split was conducted based on the phylogenetic analysis, where the strains found in the Caribbean and South and Central America were suspected to be of the Asian lineage of CHIKV, and the distinction found between the Brazil strains and the India strains, despite both being suspected to be of the ECSA lineage. The consensus sequences were then translated into amino acid sequences using the Expasy translate tool [25]. The frame with the

Table 1 Specific accession numbers for each of the consensus nucleotide sequences generated. Accession numbers and consensus sequences were taken from NCBI viruses [23]

Asian	Brazilian	Indian
LC259088.1	KY704951.1	MK286899.1
MH359139.1	MT877206.1	MK286895.1
MW656171.1	MW260512.1	MK286897.1
KR264951.1	KY704954.1	MW042255.1
KY272961.1	MH000704.1	MW574902.1
KU365372.1	MH000700.1	KX619424.1
KU365374.1	KX228391.1	MH124570.1
KX262994.1	MK156056.1	KX619422.1
LN898095.1	MK244643.1	FJ000062.1
LN898094.1		GQ428212.1
		FJ000069.1

largest coverage was chosen as the amino acid sequence for each of the sequences, specifically F2 for Asian, F3 for Brazilian, and F1 for Indian. Using BLASTp, it was determined that these sequences covered the non-structural proteins of the CHIKV genome [26].

Previously established protein structures for non-structural protein 1 (nsP1), non-structural protein 2 (nsP2), and non-structural protein 3 (nsP3), were found on the Protein Data Bank (PDB) under the IDs 6Z0V, 6JIM, and 6W8Y respectively [27–30]. The structure of non-structural protein 4 (nsP4) has yet to be confirmed and made publicly available and was not included in this study. Using BLASTp, the differences between amino acid sequences of the consensus sequences and the established sequences were recorded, and using the PyMOL software's mutagenesis function the protein differences were generated [26, 31].

Results

CHIKV Encephalitis

The literature search found 769 of cases of CHIKV encephalitis (Table 2). There were 111 individual cases analyzed, as well as 650 of patients reported in cohort studies. Eight of the individual case studies were excluded either due to a diagnosis of cancer, an autoimmune disease, malaria, or pregnancy. The country with the greatest number of cases was India ($n = 370$) while Brazil had the second greatest number of cases ($n = 98$) (Fig. 1).

Patient Demographics

For the individual case studies, of the 111 total patients, there were 57 males and 39 females. Fifteen of the patients had no sex reported. The average reported age was 43.06 years (+/− 27.61 years) (Fig. 2). The average age of female patients was 31.26 years (+/− 27.56 years), and the average age of male patients was 47.63 years (+/− 26.70 years) (Fig. 2) (Table 3). When a logistic regression was performed with death as the dependent variable, the model was not found to be significant (Table 5). According to this model, patients in age groups younger than 1 and older than 60 had 1.26 times greater odds to not survive CHIKV encephalitis than people who were not in those age groups (Table 5).

For the 650 patients with CHIKV encephalitis reported in the cohort studies, sex and age were not reported sufficiently to make any meaningful statistical inferences. Seven of the cohort studies reported on neonates or infants (< 12 months old) [59, 61, 62, 73, 87, 92, 96] (Table 3).

Table 2 Total numbers of patients with CHIKV encephalitis based on country

Country	*N*
Brazil [10, 32–57]	98
Cambodia [58]	23
Colombia [59–61]	4
Curacao [62]	6
Dominican Republic [59, 63]	2
Ecuador [64]	3
El Salvador [59]	10
French Guiana [65]	4
French Polynesia [66, 67]	6
Guadeloupe and Martinique [68–71]	46
Honduras [72, 73]	21
India [74–106]	370
Italy [107]	1
La Reunion [11, 108–113]	133
Pakistan [14, 114–116]	16
Puerto Rico [60, 117–119]	20
Singapore [120]	1
Tanzania [121]	1
Thailand [122]	2
Tonga [123]	1
Venezuela [124]	1
TOTAL	769

Pre-existing Conditions

Ninety-nine case studies reported pre-existing conditions in 27.27% of patients. The most commonly reported condition was diabetes followed by hypertension and heart disease (Table 4) [35, 37, 39, 44, 48, 49, 65, 66, 106, 109, 110, 117]. When a logistic regression was performed with death as the dependent variable, the model was not found to be significant. However, patients with a pre-existing condition were

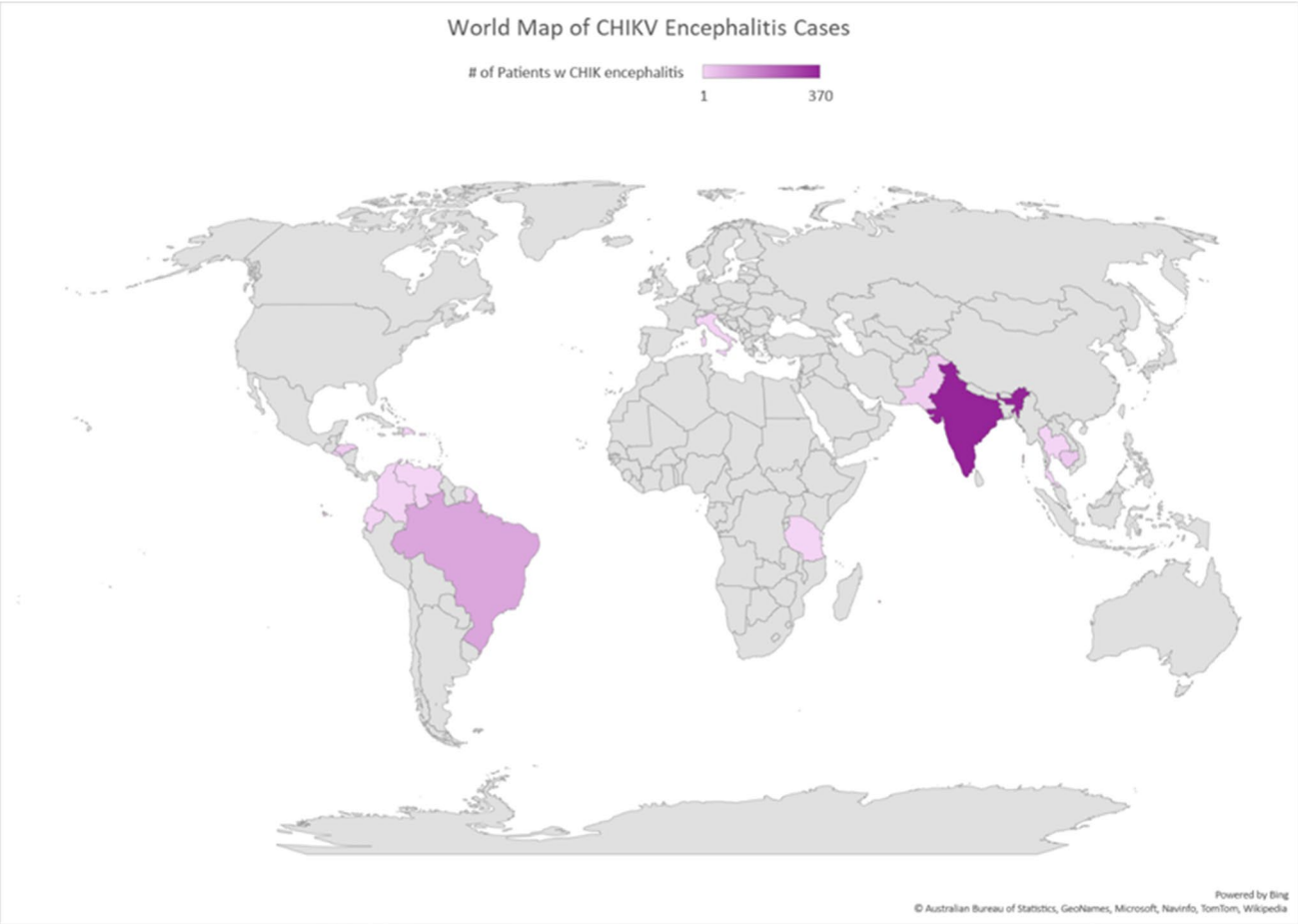

Fig. 1 World heatmap of total cases of CHIKV encephalitis

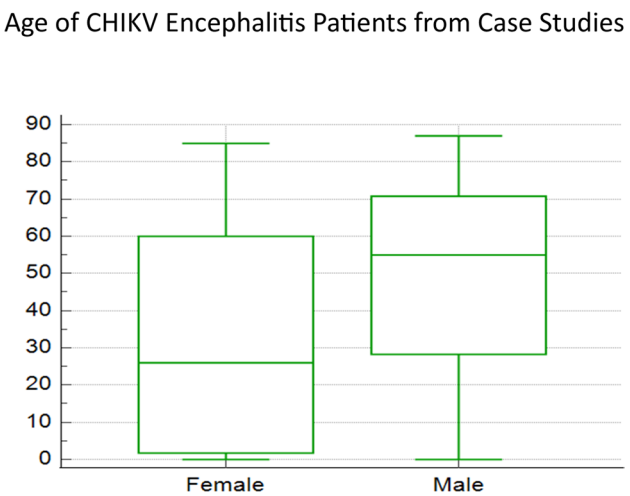

Fig. 2 Age distribution of patents with CHIKV encephalitis

10.73 times more likely to die as a result of CHIKV encephalitis than those without a pre-existing condition (Table 5). Case studies that reported arthralgia were 3.18 times more likely to result in death (Table 5). Of note, patients with a rash were no more likely to die from encephalitis than those without and patients with either a co-infection or change in mental status had a significant reduction in odds of dying from encephalitis (Table 5). Patients that suffered from seizures or tremors were 2.23 and 17.17 more times likely to die from encephalitis than those who did not have seizures or issues with movement, respectively (Table 5).

Pre-existing conditions were reported in the cohort studies. They included diabetes, sickle cell anemia, immunosuppression, pregnancy, hypertension, chronic heart failure, psoriasis, myasthenia gravis, and benign prostatic hyperplasia [32, 69, 71]. Pre-existing conditions were reported generally for the whole cohort, and no cohort patients were excluded due to pre-existing conditions. The nature of data reporting in the cohort studies was incomplete, and it was impossible to deduce whether patients with CHIKV encephalitis had a pre-existing condition or not. Thus, no meaningful statistical analysis could be performed on the data.

Table 3 Age and sex of patients with CHIKV encephalitis in the cohort studies as the average age reported above did not include this data. *M*, male; *F*, female; *NR*, not reported

Study	Age	Sex	Citation
de Lima et al. 2020	Median age of 63 years	43.6% F	[44]
de Lima et al. 2020	Median age of 28 years	68.7% F	[45]
Maria et al. 2018	Neonates	11 M, 2 F	[92]
Horwood et al. 2017	Median age of 7 years	16:1 (M:F)	[58]
Gérardin et al. 2016	Median age of 63.9 years	48.5% F	[11]
Gérardin et al. 2014	Median age of 1.6 months	48.5% F	[113]
Rueda-Lopes et al. 2021	All patients older than 65 years	NR	[55]
Mesquita et al. 2021	Median age of 77 years	41.82% M	[51]
Alves-Leon et al. 2021	NR	1:1 (M:F)	[32]
Gohel et al. 2019	Mean age of 54.2 years	1.5:1 (M:F)	[80]
Arocho et al. 2019	Median age of 3 months	52.4% F	[118]
Alam et al. 2018	Mean of age 45 years	NR	[114]
Maheshwari et al. 2018	0–16 years	NR	[89]
Koeltz et al. 2018	Mean age of 62 years	NR	[67]
dos Passos Cunha et al. 2017	44.9% 15–30 years	61.7% F	[10]
Samra et al. 2017	0–12 months	NR	[73]
Singh and Jain 2017	6–10 years	NR	[102]
Tami et al. 2016	Mean age of 59 years	NR	[124]
Lopez et al. 2016	Range of 0 to 97	NR	[119]
Torres et al. 2016	Neonates	NR	[59]
Van Enter et al. 2015	Neonates	NR	[62]
Villamil-Gomez et al. 2015	Neonates	NR	[61]
Thiery et al. 2015	Median age of 63 years	NR	[69]
Kumar et al. 2019	Neonates	NR	[87]
Sachdev et al. 2018	Neonates	NR	[96]
Ferreira et al. 2020	Median age of 48 years	53% F	[47]
Asha et al. 2008	50% older than 60	77% F	[76]
Dorleans et al. 2018	Mean age of 41 years	0.9:1 M:F	[71]
Silva de Lima et al. 2020	Mean age of 47.6 years	NA	[125]

Encephalitis Diagnoses and MRI Manifestations

Table 4 Pre-existing conditions reported in the case studies. Some patients had multiple pre-existing conditions

Pre-existing condition	Percentage ($n=27$)
Unspecified type of diabetes	37.04
Type 2 diabetes	7.41
Hypertension	37.04
Anxiety disorder	3.70
Dyslipidemia	3.70
Smoker	3.70
Alcohol drinker/alcoholic	14.81
Renal problems	14.81
Stroke	3.70
Heart disease	22.22
COPD	7.41
Pancreatitis	3.70
Uveitis	3.70

Table 5 Clinical features of patients in case studies

Dependent variable: death Overall model fit: $p=0.4678$		
Variable	**OR**	**95% CI**
Age	1.2614	
Sex	1.13×10^{8}	
Fever	4.36×10^{8}	
Arthralgia	3.1876	0.11 to 87.63
Rash	1.0581	0.05 to 21.43
Tremor	17.1751	0.52 to 564.51
Seizure	2.2382	0.07 to 62.98
Change in mental status	0.5154	
Co-infection	1.1588×10^{-9}	
Pre-existing condition	10.7338	0.62 to 182.9

There was a wide variety of neurological diagnoses reported by the authors. The most common diagnosis was encephalitis ($n=69$), while the least common diagnoses were NMDAR

Table 6 Diagnoses and demographics reported by authors in individual case studies

Encephalitis diagnosis	*N*	Age	M/F	Deaths
Encephalitis	69	43.4 (+/−27.5)	45/24	10
Meningoencephalitis	10	58.1 (+/−22.8)	4/10	1
Acute disseminated encephalomyelitis	5	21.3 (+/−11.8)	2/4	0
Perinatal encephalitis	3	0	0/3	0
Acute necrotizing encephalopathy	2	27.5 (+/−20.5)	1/1	0
Bickerstaff's encephalitis	2	70 (+/−5.7)	1/1	1
NMDAR encephalitis	1	5	1/0	0
Acute meningoencephalic syndrome	1	74	1/0	0
Brainstem encephalitis	1	30	1/0	0

Table 7 Logistic regression of comparing patients with a diagnosis of encephalitis to those with another type of encephalitis diagnosis

Dependent variable: encephalitis diagnosis Overall model fit: $p=0.7776$		
Variable	OR	95% CI
Age	1.0191	0.34 to 3.05
Sex	1.7129	0.59 to 4.95
Tremor		
Seizure		
Change in mental status		
Death	1.4290	1.42 to 7.66

encephalitis ($n=1$), acute meningoencephalic syndrome ($n=1$), and brainstem encephalitis ($n=1$) (Table 6). The diagnosis with the most reported deaths was encephalitis ($n=10$), while there was 1 meningoencephalitis and 1 Bickerstaff's encephalitis death (Table 6).

In a logistic regression comparing type of encephalitis diagnosis, there were no significant differences between age ($p=0.97$), sex ($p=0.32$), and death as an outcome ($p=0.68$). None of the variables contributed significantly to the type of encephalitis diagnosed, but, in this model, patients with encephalitis were 1.7129 times more likely to be men than those with a different encephalitis subtype (Table 7). Patients with encephalitis were also 1.4290 times more likely to die (Table 7).

Location

Since the majority of individual case studies were reported in Brazil and India, an analysis was performed to compare the two locations. There were 22 cases from Brazil and 50 from India included in this analysis (Table 8). The average age of Brazilian cases was 41.3 (+/−32.3), while the average age of Indians was 35.6 (+/−27.9) (Table 8). Brazilian patients were equally male and female (21/22), while there were 29 males and 21 female Indian patients (Table 8). Age ($p=0.28$) nor sex ($p=0.62$) were significantly different

Table 8 Patient demographics from India and Brazil

Variable	Brazil	India
Age	41.3 (+/−32.3)	35.6 (+/−27.9)
Males/females	21/1	29/21
Co-infections	3	3
Pre-existing condition	5	9
Deaths	2	6
Total cases	22	50

Table 9 Logistic regression of case reports from Brazil and India ($n=55$) using location as the dependent variable

Dependent variable: country Overall model fit: $p=0.4233$		
Variable	OR	95% CI
Age	0.5048	0.14 to 1.73
Sex	1.3763	0.38 to 4.94
Fever	1.55×10^{9}	
Arthralgia	1.2	0.16 to 8.79
Rash	0.9065	0.22 to 3.67
Tremor	3.19×10^{8}	
Seizure	2.4731	0.59 to 10.23
Change in mental status	0.9101	
Coinfection	0.2566	
Pre-existing condition	1.4×10^{-9}	
Death	7.44×10^{-10}	

between the two countries (Table 9). Patients from India had 50% decrease in odds of being in an age group of younger than 1 and older than 65 (Table 9). Patients from India also had much greater odds of dying of CHIKV encephalitis and 1.38 times higher odds of being male than patients in Brazil (Table 9). A multiple logistic regression was performed with country of origin as the dependent variable. Here, patients from Brazil were 2.47 times more likely to experience seizures and 3.19×10^{8} more likely to have tremors than patients from India (Table 9).

There were three co-infections in each country. The Brazilian co-infections included dengue virus (DENV) and zika virus (ZIKV), while the co-infections in India included Japanese encephalitis virus (JEV), DENV, herpes simplex virus (HSV), cytomegalovirus (CMV), and rubella [35, 37, 88]. Though not statistically significant, Indian patients were 87% less likely to have a co-infection than Brazilian patients (Table 9).

There were two deaths reported in the Brazilian case reports, while there were six deaths reported in the Indian case reports (Table 8). The outcome of death was not significantly different between the two countries ($p=0.70$) (Table 9). Five of the 22 Brazilian patients had a pre-existing condition (Table 8). They were type 2 diabetes and unspecified diabetes, arterial hypertension and general hypertension, an anxiety disorder, and uveitis [35, 37, 39, 44, 48, 49]. Nine of the 50 Indian patients had a pre-existing condition (Table 8). The reported conditions were hypertension, diabetes, ischemic heart disease, stroke, and renal artery stenosis [106]. Though not statistically significant, Indian patients were 30% less likely to have a pre-existing condition than Brazilian patients ($p=0.67$) (Table 9).

When logistic regression was performed with death as the dependent variable, having a pre-existing condition was a significant predictor of the outcome of death ($p=0.029$), with patients with a pre-existing condition being 8.80 times more likely to not survive CHIKV encephalitis (Table 10). Patients in the most at-risk age groups (younger than 1 and older than 60) were 2.44 times more likely to die than those between the ages of 1 and 60. Patients with a co-infection and who were male were less likely to die than those without a co-infection and female (Table 10). There was no difference between countries for survival outcome (Table 10). While the country of origin was not a significant predictor of the outcome of death, patients in India were 1.45 (0.21 to 9.96) times more likely to die from CHIKV encephalitis than patients from Brazil (Table 9).

Analysis of Strains

Logistic regression performed using CHIKV lineage for the dependent variable and overall, the model fit was significant ($p=0.041$). Due to their co-circulation in relation to the case reports in India and Brazil, ECSA and Asian lineages were combined as a single group, and the IOL sub-type was analyzed as a separate group. The West African lineage was not associated with any reports of encephalitis and thus was not included in the analysis. Here, having a pre-existing condition was significant ($p=0.038$) with patients having 79% decrease in odds of being infected with ECSA/Asian lineages (Table 11). Patients less than 1 and older than 60 had a 40% decrease in odds of being infected with the ECSA and/or Asian strains (Table 11). Patients who died had 1.15 times greater odds of being infected with ECSA or Asian strains of CHIKV than with the IOL sub-type. Patients with a coinfection were also more likely to be infected with ECSA or Asian strains than the IOL subtype (Table 11). Sex was not a significantly contributing factor for CHIKV lineage (Table 11).

A model comparing lineage and using death as the dependent variable was significant ($p=0.035$) (Table 12). Having a pre-existing condition was a significant predictor of death ($p=0.014$) as these patients had 10.55 greater odds of dying than those without a pre-existing condition (Table 12). In this model, patients younger than 1 and older

Table 11 Logistic regression of individual case reports ($n=69$) using CHIKV lineage as the dependent variable

Dependent variable: lineage Overall model fit: $p=0.0414$			
Variable	OR	95% CI	p value
Age	0.6141	0.2 to 1.84	NS
Sex	1.0260	0.31 to 3.32	NS
Coinfection	1.42×10^{9}	NA	NS
Pre-existing condition	0.2068	0.04 to 0.91	0.0381
Death	1.1548	0.24 to 5.36	NS

Table 10 Logistic regression of case reports from Brazil and India ($n=55$) using death as the dependent variable

Dependent variable: death Overall model fit: $p=0.1757$			
Variable	OR	95% CI	p value
Age	2.44	0.37 to 15.96	0.3505
Sex	0.39	0.05 to 2.88	0.3591
Coinfection	3.80×10^{-9}		0.9982
Pre-existing condition	8.80	1.25 to 61.71	0.0287
Country	1.00	0.14 to 7.04	0.9960

Table 12 Logistic regression of individual case reports ($n=69$) using death as the dependent variable

Dependent variable: death Overall model fit: $p=0.0354$			
Variable	OR	95% CI	p value
Age	1.9012	0.42 to 8.58	NS
Sex	0.2143	0.03 to 1.18	NS
Coinfection	3.0214×10^{-9}	NA	NS
Pre-existing condition	10.5536	1.59 to 69.72	0.0144
Strain	1.2163	0.23 to 6.19	NS

than 60 had 1.90 times greater odds of dying, and patients infected with the ECSA or Asian strains had 1.21 greater odds of dying than those infected with the IOL lineage. Male patients had a 79% decrease in odds of dying than females when infected with the ECSA or Asian strain but were more likely to die when infected with the IOL subtype. Patients with a co-infection were also less likely to die from any lineage of CHIKV (Table 12).

Phylogenetics

Sequence data for the phylogenetic trees was taken from NCBI viruses, and the trees were developed using the MEGA-X software [23, 24]. Bootstrap values were evaluated by the MEGA-X software with 100 repeats. Based upon these bootstrap values, there is strong evidence with a bootstrap value of 100 that separates the sequences found in Central and South America (with the exception of Brazil), from the sequences found in Asia, Europe, and Brazil (Fig. 3). The bootstrap of 100 supports that the strains from Brazil are genetically distinct from the rest of the strains in Asia and Europe. The specific breakdown of individual strains is more variable, with a large range of bootstrap values from 16 to 100. Based on this data and data from past studies, it is likely that the sequences in Asia, Europe, and Brazil are from the ECSA strain, while the remainder are of the Asian lineage [38, 67, 126–137].

Further phylogenetic analysis was performed with the addition of more sequences from NCBI viruses that do not correspond to CHIKV encephalitis cases (where the overlapping sequences are highlighted) (Fig. 4) [23]. This was done to add additional information to the tree to further break down the potential strain lineages. Again, there is strong evidence to separate the sequences found in South and Central America (excluding Brazil) with a bootstrap value of 100, while the sequences found in Brazil are distinct from those found in Asia and Europe, also with a bootstrap value of 100. The data show that the Brazil sequences are more closely related to the sequences from Africa found in the late 1970s and early 1980s. With the additional data, it is further supported that the sequences found in Asia, Europe, and Brazil are of the ECSA lineage, and the sequences from South and Central America, excluding Brazil, are of the Asian lineage.

Analysis of Non-Structural Proteins

In order to explore the impact of viral genotype on viral protein structure, CHIKV sequences were divided into three categories from which consensus sequences were created: Asian (from the suspected Asian strain sequences), Brazilian (including all the sequences from Brazil), and Indian (including all the sequences from India) (Table 1) [23]. After the consensus sequences were acquired and translated into amino acids using the Expasy translate tool, BLASTp analysis revealed that the sequences only covered the non-structural proteins of the CHIKV genome; thus, no other proteins were included in this analysis [25, 26]. Using BLASTp, 17 amino acid differences between the consensus sequences and recognized sequences for nsP1, nsP2, and nsP3 were found (Table 13) [27–30].

On nsP1, there was a total of nine mutations, five of which changed the class of the side chain (Table 13). Using PyMOL software and the published protein structure of nsP1 from PDB ID 6Z0V, each mutation was visualized (Figs. 5 and 6) [27, 28]. nsP1 had the greatest number of mutations of the non-structural proteins, with the Asian consensus sequence having the most mutations and Brazilian having the fewest (Figs. 5B, C, and 6B, C). All Brazilian mutations were also found in the Asian and Indian consensus sequences. The nsP2 protein had six amino acid substitutions, three of which altered the class of the side chain (Table 13). The consensus sequence with the most mutations from the previously established nsP2 protein with the PDB ID 6JIM was the Asian consensus, with a single overlapping mutation from the Brazilian sequence and none from the Indian sequence (Fig. 7) [27, 29]. The nsP3 protein had two amino acid substitutions, one of which altered the side chain class (Table 13). Both mutations were at the same location but consisted of different amino acid changes from the original sequence from PDB ID 6W8Y (Fig. 8) [27, 30]. As with nsP2, the Indian consensus sequence did not have any amino acid differences from the previously established sequence.

Discussion

Chikungunya is primarily vectored by *Aedes* mosquitoes which have an expanding global range [9, 138, 139]. As climate change further contributes to the proliferation of these mosquitoes, additional disease risk is confronted by immunologically naive populations across the world, similarly to how West Nile virus spread throughout the USA [139–142]. Since there is no known treatment or vaccine for CHIKV, it is important for clinical information about CHIKV to be recognized by physicians across the world as disease risk increases [15, 143]. The data show that patients with a pre-existing condition are most likely to die from CHIKV encephalitis. This is concerning as pre-existing conditions are more common in Europe, Russia, and especially the USA where *Aedes* mosquitos are expected to increase their range by over 30% by 2050 [144, 145]. The data also showed that persons over 60 were more likely to die from CHIKV encephalitis than younger individuals. This is also of concern since in the USA alone, the number of individuals over the age of 65 will increase by 50% by the year 2050 putting 90 million people in this at risk age group with

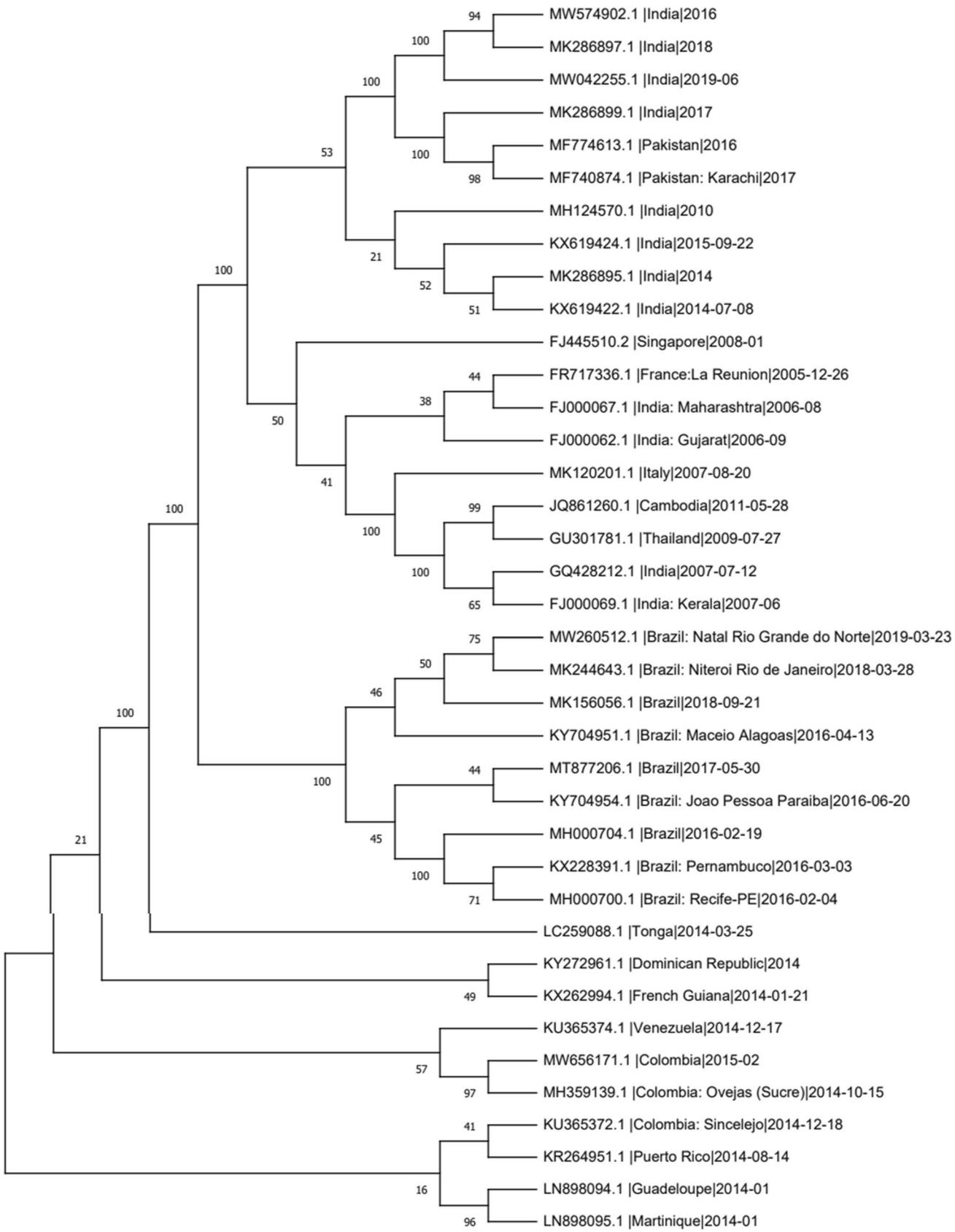

Fig. 3 Phylogenetic tree of locations and years in which a case of CHIKV encephalitis was reported. Data was gathered from NCBI viruses, and the tree was generated using the MEGA-X software [23, 24]

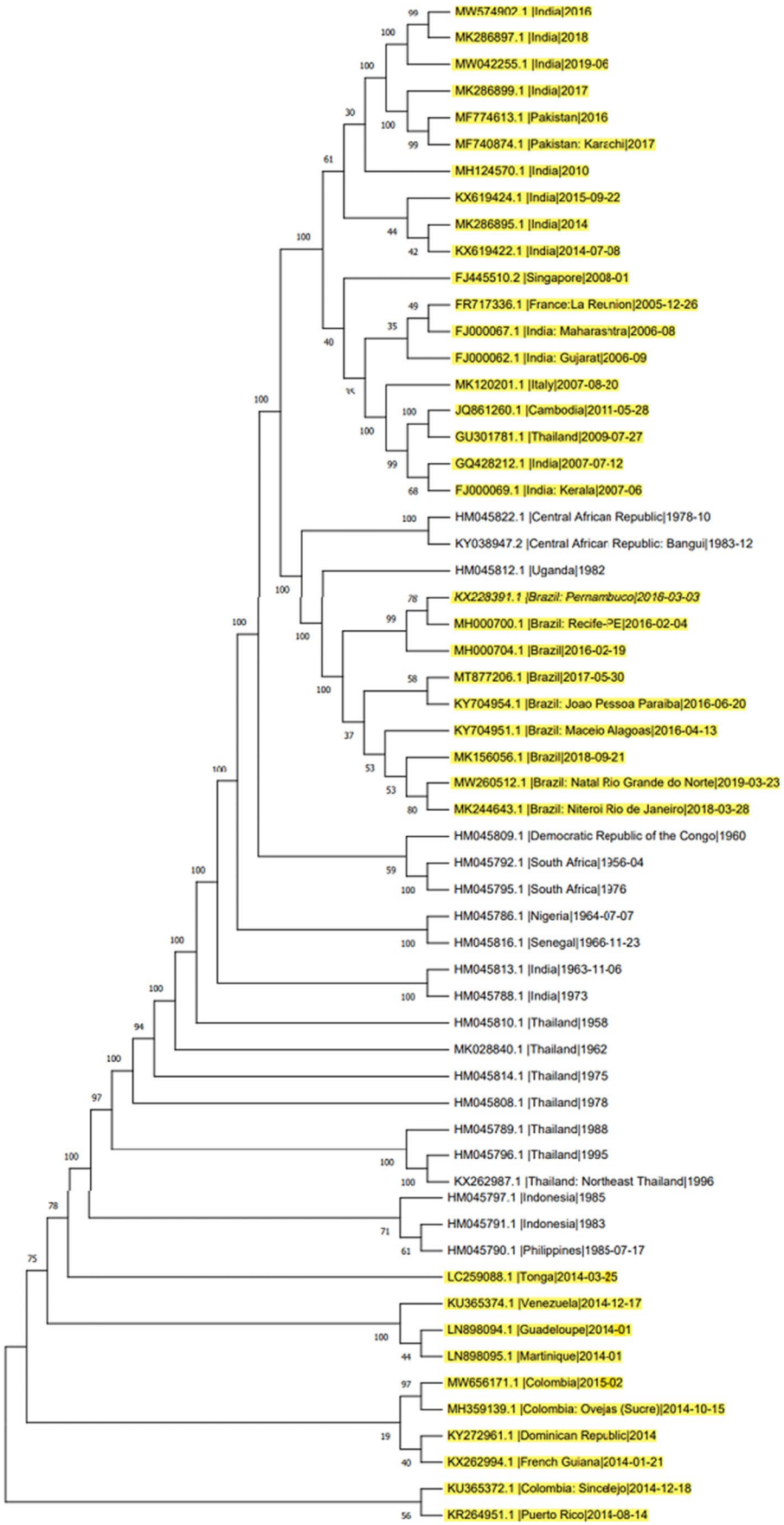

Fig. 4 Phylogenetic tree with additional outbreak data from other outbreaks up to 2005. The overlapping data is highlighted. These data were [23, 24] taken from NCBI viruses, and the tree was generated using the MEGA-X software

Table 13 Amino acid substitutions between consensus sequences and previously archived amino acid sequences of non-structural proteins from The Protein Data Bank (PDB) [27–30]. Mutations in bold indicate a change in class of side chain

6Z0V, nsP1		6JIM, nsP2		6W8Y, nsP3	
Sequence	Mutation	Sequence	Mutation	Sequence	Mutation
Asian	**P34S**	Asian, Brazilian	N54S	Asian	S77T
Asian	**A121E**	Asian	**P16L**	Brazilian	**S77L**
Asian, Brazilian, Indian	L172V	Asian	T218S		
Asian, Brazilian, Indian	**E234K**	Asian	**Q273L**		
Asian	**K253M**	Asian	**K338M**		
Asian, Brazilian, Indian	M383L	Asian	**Y374H**		
Asian, Brazilian, Indian	I384L				
Indian	T128K				
Indian	**T376M**				

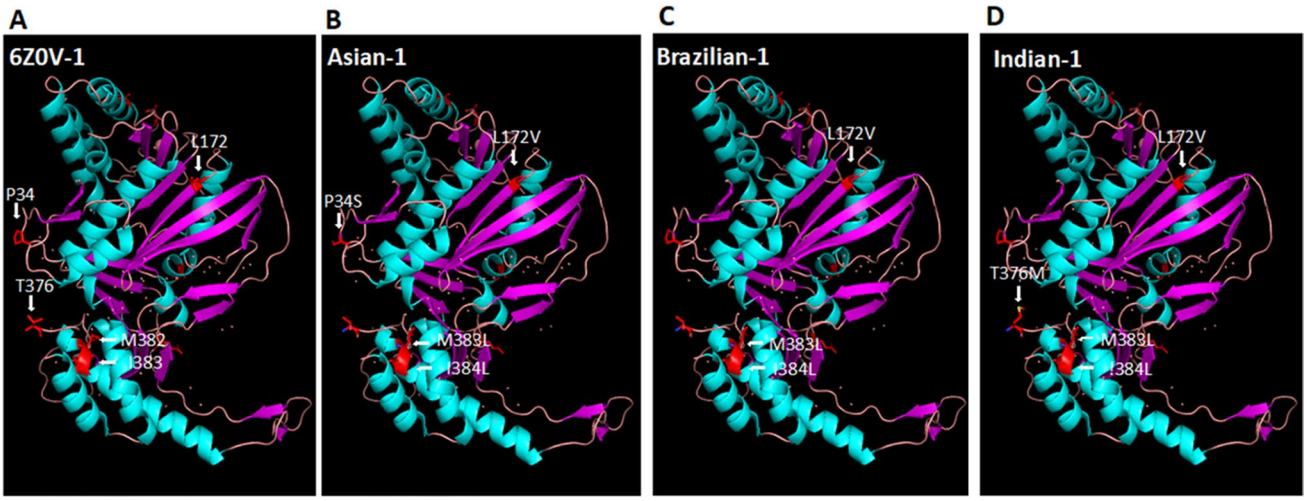

Fig. 5 PyMOL renditions of CHIKV nsP1, first half. **A** The base nsP1 from PDB ID 6Z0V with labeled amino acids of interest [28]. **B** nsP1 with the Asian amino acid sequence. Mutations from the base are labeled. **C** nsP1 with the Brazilian amnio acid sequence. **D** nsP1 with the Indian amino acid sequence

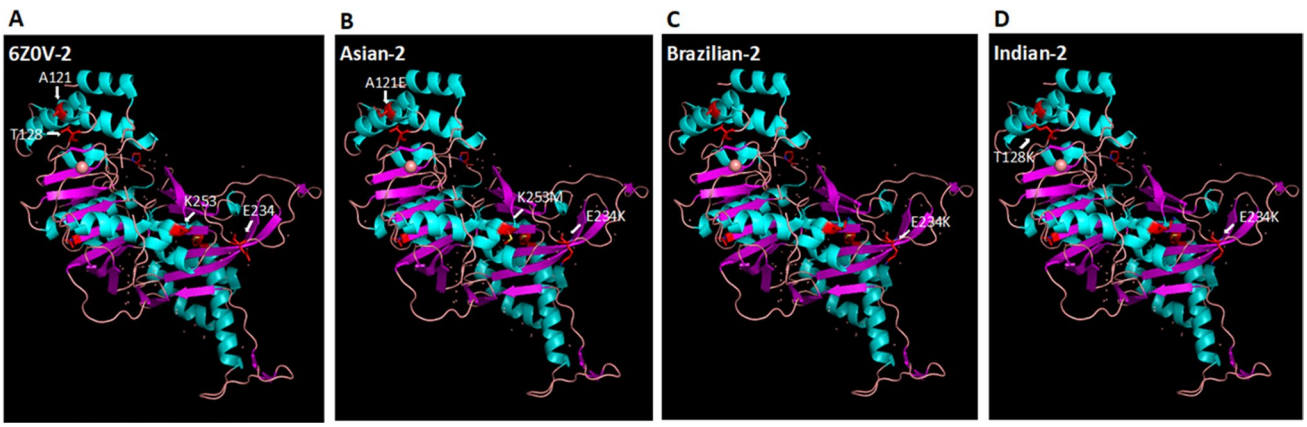

Fig. 6 PyMOL renditions of CHIKV nsP1, second half. **A** The base nsP1 from PDB ID 6Z0V with labeled amino acids of interest [28]. **B** nsP1 with the Asian amino acid sequence. Mutations from the base are labeled. **C** nsP1 with the Brazilian amnio acid sequence. **D** nsP1 with the Indian amino acid sequence

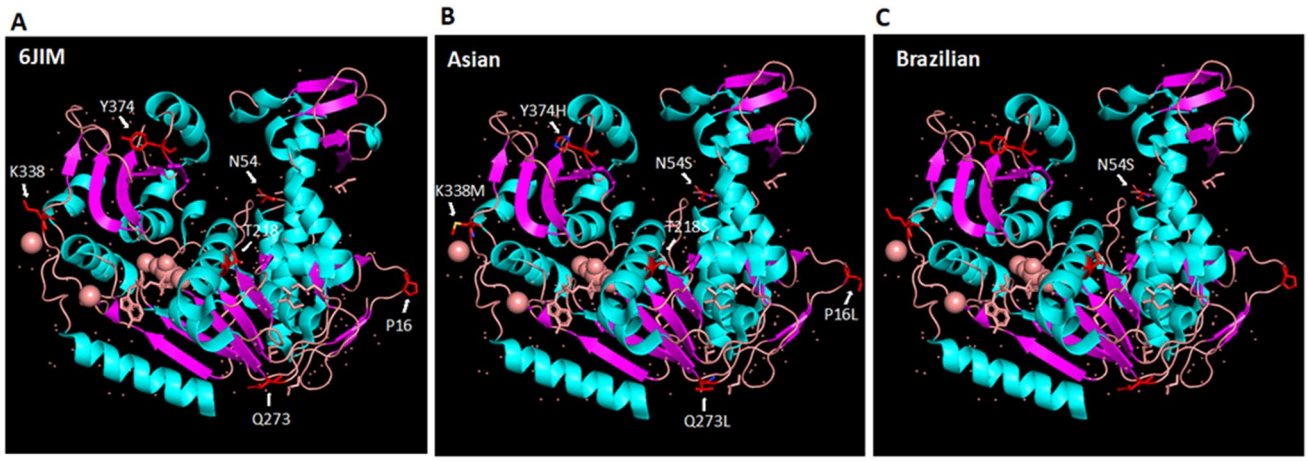

Fig. 7 PyMOL renditions of CHIKV nsP2. **A** The base nsP2 from PDB ID 6JIM with labeled amino acids of interest [29]. **B** nsP2 with the Asian amino acid sequence. Mutations from the base are labeled. **C** nsP2 with the Brazilian amnio acid sequence

Fig. 8 PyMOL renditions of CHIKV nsP3. **A** The base nsP3 from PDB ID 6W8Y with labeled amino acids of interest [30]. **B** nsP3 with the Asian amino acid sequence. Mutations from the base are labeled. **C**. nsP3 with the Brazilian amnio acid sequence

Europe, Russia, and Asia also having increased number of persons over 65 [146•, 147].

One of the largest limiting factors of the statistical analysis was a lack of consistency in available data from the clinical sources. Few reports included data such as vital statistics, complete blood count, metabolic panels, and CSF changes. Several reports did not even include major symptoms of CHIKV infection, such as fever or arthralgia, or even the number of male and female patients. Furthermore, for cohort data, most of the demographic and clinical information was generalized for the entire cohort, so it was impossible to evaluate these data for CHIKV encephalitis patients specifically. Unfortunately, due to these limitations, few insights or patterns could be evaluated thus perpetuating the knowledge gap on CHIKV pathogenesis.

Data from clinical reports are valuable for developing models and estimating risk, and it is suggested that the following data should be included with clinical manuscripts: age, sex, pre-existing conditions, temperature, pulse, blood pressure, complete blood count, complete metabolic panel, and a list of all physical symptoms (arthralgia, myalgia, malaise, etc.), and when appropriate, CSF composition and protein level, MRI and radiology reports, blood culture and any treatment approach [148, 149]. It would be helpful for authors to include data on any patient deaths as many reports were missing this information which complicates estimating risk and mortality. It would be helpful for cohort studies to break down specific patient information when available, especially since the vast majority of CHIKV encephalitis cases were found in these cohort studies. It is possible that there are additional patterns or risk factors that have yet to

be elucidated in predicting CHIKV encephalitis due to the lack of data. It is important to note that the phylogenetic data was taken from NCBI viruses corresponding to location and year in which CHIKV encephalitis cases were reported, and are not the exact clinical sequences [23, 38, 67, 126–137]. Full length clinical sequences were not reported in any cases.

The nsP1 protein is a capping protein that works with nsP2, which has multiple functions [150]. A total of 17 amino acid changes were discovered across non-structural proteins 1, 2, and 3. NsP1 had the highest total number of mutations, and the Asian consensus sequence had the most changes [27, 28]. The L172V, M383L, and I384L mutations all maintain hydrophobic side chains indicating low clinical significance [151, 152]. It is more likely that mutations that change the class of the amino acid could change the protein structure [153]. For instance, P34S occurs at a region with no secondary structure of nsP1, where the lack of a proline could change the twist of this region [151]. A121E changes to a polar negatively charged chain in an alpha helix of nsP1. While this may or may not change the alpha helix structure, it could change the stability of the protein by losing the hydrophobic nature of the alanine [151]. E234K changes the negatively charged side chain to a positive charge which could alter the interactions and stability of the molecule [151]. K253M becomes hydrophobic in an alpha helix and could force the protein to change in order to hide that hydrophobic molecule [151, 152]. While these changes are more likely to alter the protein in a clinically significant way, that is not guaranteed and is outside of the scope of this review [151, 153]. Furthermore, while the amino acid changes that maintain class are likely of low clinical significance, that is not always the case, as exemplified by the A226V mutation on the E1 protein of CHIKV [151, 154]. The P34S mutation of the nsP1 mutation has previously been described in an antiviral study; the A121E mutation was previously found in the Philippines; the L172V, E234K, M383L, and I384L mutations were found in Cameroon; and the T128K and T376M mutations were found in India [155–158]. To our knowledge, the K253M mutation is novel.

The nsP2 protein has a few roles, including a helicase, ATPase, and protease [29, 150, 159]. A total of six amino acid changes were found on the nsP2 protein based on the previously established PDB ID 6JIM [27, 29]. The Indian sequence did not have any amino acid substitutions while the Brazilian sequence only had N54S, and the Asian sequence had P16L, N54S, T218S, Q273L, K338M, and Y374H. The N54S and the T218S mutations maintain hydrophobic side chains and are likely of low clinical significance [151, 152]. The P16L mutation loses the proline to a hydrophobic leucine, which could make the protein twist to hide the hydrophobic molecule [151]. The Q273L and K338M mutations lose the uncharged polar glutamine to the hydrophobic leucine and the positively charged polar lysine to the hydrophobic methionine, which could have similar consequences [151]. Finally, the Y374H adds the positive charge of the histidine to the already polar threonine, which could alter how the protein folds [151, 152]. Any of these amino acid changes could have varying degrees of clinical significance. The P16L, T218S, Q273L, K338M, and Y374H mutations have been previously reported in India but not characterized [160, 161]. To our knowledge, the N54S mutation found in this analysis is novel.

The role of nsP3 in the viral genome is not well understood, but some publications have proposed that it may play a role in escaping the host immune system, a role in viral replication, and possible others [30, 162, 163]. The nsP3 protein had the least number of total amino acid differences, consisting of S77T (Asian) and S77L (Brazilian). As shown in the previously established 3D model of nsP3 from PDB ID 6W8Y, the 77th position is the end of an alpha-helix [27, 30]. The serine to threonine mutation of the Asian sequence maintains an uncharged polar sidechain while adding a methyl group, which may have a fairly low clinical significance [151, 152]. However, the serine to leucine mutation from the Brazilian sequence changes to a hydrophobic side chain, and given the location of the side chain, this could alter the protein [151, 152]. To our knowledge, the S77L and S77T mutations found in this analysis are novel.

While the mutations on these proteins and their potential clinical significance are interesting, they have not been linked to CHIKV encephalitis. The consensus sequences were created using genetic information corresponding to year and location in which a CHIKV encephalitis case was identified, as full sequence data was not available from the clinical studies [23].

There were several CHIKV coinfections reported in the clinical data, including DENV, ZIKV, JEV, HSV, CMV, HIV, malaria, and rubella [35, 37, 39, 44, 47, 63, 64, 66, 74, 75, 79, 88, 94]. When examining the connections between coinfection and death, it was shown that patients with a coinfection were less likely to die of CHIKV encephalitis. However, it is important to note that these tests were run with a limited number of data points, therefore lacking statistical power. A more in-depth analysis would be required to see if coinfections have any protective effect against CHIKV encephalitis.

Conclusion

CHIKV has been documented to cause encephalitis across all age ranges, from neonates to the elderly, and presents a risk for death in patients less than a year old and those older than 60. Individuals with pre-existing conditions, most commonly reported being diabetes, hypertension, and heart disease, were also at risk of death via CHIKV encephalitis. Based on phylogenetic analysis, both the Asian and ECSA

lineages of CHIKV cause encephalitis more frequently than the IOL subtype. Since these two lineages are responsible for recent and ongoing outbreaks, it is important for clinicians to be aware of the potential neurological complications of infection, or long-term sequelae of encephalitis [9, 20, 21, 164]. While several non-structural protein mutations were found in this analysis, their clinical significance is unknown. Additional research should be conducted to see if any of these mutations increases pathogenicity, as the previously established A226V mutation of the E1 protein has shown [165]. CHIKV is a neglected disease and as such is under-reported; thus, it is of vital importance for researchers and clinicians to include complete clinical and demographic data to facilitate downstream modeling and epidemiological research.

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Declarations

Conflict of Interest Authors declare no competing interests.

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