Relationship Between Single Nucleotide Polymorphisms and Severe Dengue in a Brazilian Population

by

Dzigbordi Kamasa-Quashie

BS, Georgia State University, 2016

Submitted to the Graduate Faculty of the

Department of Infectious Diseases and Microbiology

Graduate School of Public Health partial fulfillment

of the requirements for the degree of

Master of Public Health

University of Pittsburgh

2020

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

This thesis was presented

by

Dzigbordi Kamasa-Quashie

It was defended on

April 16, 2020

and approved by

Thesis Advisor:
Jeremy Martinson, PhD
Assistant Professor
Infectious Diseases and Microbiology
Graduate School of Public Health
University of Pittsburgh

Committee Member:
Ernesto Marques Jr., PhD, MD
Associate Professor
Infectious Diseases and Microbiology
Graduate School of Public Health
University of Pittsburgh

Committee Member:
Joanne Russell, MPPM
Assistant Dean
Assistant Professor
Center for Global Health
Behavioral and Community Health Sciences
Graduate School of Public Health
University of Pittsburgh

Jeremy Martinson, PhD

Relationship Between Single Nucleotide Polymorphisms and Severe Dengue in a Brazilian Population

Dzigbordi Kamasa-Quashie, MPH

University of Pittsburgh, 2020

Abstract

Dengue virus has become one of the most important arboviral diseases of today. With nearly half of the global population at risk, this infectious disease carries great significance. The aim of this study was to determine the relationship between 18 single nucleotide polymorphisms (SNPs) and severe dengue in a population from Recife, Brazil. The SNPs of interest are as follows: TLR8 rs17256081, IFNG rs2069718, IFNG rs2069727, IRF1 rs2070729, OAS2 rs2072137, OAS2 rs2072138, OAS3 rs2240188, MX1 rs3737399, VEPH1 rs3911403, IRAK4 rs4251580, CLEC4C rs17199006, PLCE1 rs3740360, MRC1 rs606231248, MRC1 rs2296414, RNASEL rs486907, OASL rs3213545, MX1 rs7277299, and MICB rs3132468. A total of 450 DNA samples were pulled from two studies—a cohort study of dengue patients and a yellow fever vaccine cohort. Sample concentrations were tested using the Nanodrop 1000 Spectrometer. The concentrations of all samples were between 10-100 ng/μL, per the laboratory technician's request. Samples were transported to the University of Pittsburgh's Genomic Core Research Laboratory for genotyping using the iPlex MassARRAY system and results were analyzed using Microsoft Excel and R statistical software. Of the 18 SNPs, statistically significant results were observed for OAS2 rs2072137, OAS3 rs2240188, PLCE1 rs3740360, and MX1 rs7277299. For OAS2 rs2072137, the

CC genotype was shown to be significantly associated with severe dengue (OR=2.10, P=0.01). The CC genotype associated with OAS3 rs2240188 also appears to influence disease severity (OR=1.96, P=0.02). For PLCE1 rs3740360, calculations reveal a significant association between the AA genotype and severe dengue (OR=2.28, P=0.03). The last notable result was found in MX1 rs7277299 (OR=5.33, P=0.02) where the CC genotype was also significantly associated with severe disease. Though this is one of the largest dengue-related gene association studies, further research is necessary to validate the findings. The increasing burden of dengue disease signifies the public health importance of this research—to contribute to the advancement of dengue research, vaccine development, therapeutic strategies, and diagnostic tools.

Table of Contents

List of Tables

List of Figures

Acknowledgements

I would like to thank my committee members, Dr. Jeremy Martinson and Dr. Ernesto Marques Jr., for inviting me to participate in this study and for their guidance throughout the process. I would especially like to thank Dr. Martinson for providing ample space in his lab to complete the necessary tasks and assisting with lab work and data analysis. He truly played an integral role in the completion of this project. I would also like to thank my other committee member, Joanne Russell, for her unwavering guidance and support throughout my academic experience.

1.0 Introduction

The purpose of this project is to understand whether or not certain genetic polymorphisms increase an individual's risk of developing severe dengue. The single nucleotide polymorphisms (SNPs) in question were all selected from various studies that suggest an increased risk of severe dengue exists. The subset of DNA samples used in this project belong to a larger dengue study which was conducted at the Aggeu Magalhães Research Center by researchers in the Department of Virology in Recife, Brazil. Dengue cases are stratified into three categories—dengue fever, complicated dengue and dengue hemorrhagic fever. The controls are enrolled patients who were determined to be dengue free and dengue negative volunteers from a separate yellow fever vaccine cohort.

The concentration of each sample was tested prior to the genotyping process. Genotyping was performed on 450 samples by the University of Pittsburgh's Genomics Research Core Laboratory. Results were analyzed using Microsoft Excel and R statistical software. Two principal component analysis plots were created to visualize a relationship between SNPs and disease status. Hardy-Weinberg equilibrium (HWE) testing was performed to compare observed and expected allele and genotype frequencies, followed by significance testing using chi-squared calculations. Odds ratios were performed to uncover significant associations between genetic makeup and severe dengue. To my knowledge, this is the most comprehensive genetic analysis of these samples.

2.0 Background

2.1 Dengue

Flaviviruses are characterized by positive, single-stranded RNA genomes (2). Other commonly known viruses in this family include West Nile virus, Japanese encephalitis and Yellow Fever (2). These viruses are generally found in ticks and mosquitoes (2). Though the *Aedes aegypti* mosquito is the principal vector for dengue, research shows that *Aedes albopictus* is also capable of transmitting the virus, albeit less efficient (3). *A. aegypti* originated in Africa before it was spread throughout the world via trade and war (4). Its ability to adapt to urban environments, daytime feeding behavior, and preference for indoors contributes to its efficiency (4).

The cycle of viral transmission between human and mosquitos is contingent upon the frequency of human interaction, like most infectious diseases. Mosquitos contract the virus after feeding on an infected host. Viral replication occurs in the mosquito's midgut before spreading to secondary tissues (5). The extrinsic incubation period, the time between infection and transmission to a new host, lasts for roughly 8-12 days in adequate conditions—25-28 °C (5). Once infected, mosquitos harbor the virus for the remainder of their lifetime.

There are four phylogenetically distinct dengue serotypes—DENV-1, DENV-2, DENV-3, and DENV-4. Humans can be infected by all 4 serotypes during their lifetime. Infection with one of the four serotypes provides lifelong immunity against the infecting serotype and short-lived protection against the other three (8). However, a secondary infection with another serotype may result in more severe clinical presentations such as Dengue Hemorrhagic Fever or Dengue Shock Syndrome (6). This phenomenon is known as Antibody Dependent Enhancement (ADE). ADE

occurs when cross-reactive, non-neutralizing antibodies from a previous infection bind to the novel infecting serotype (8). This interaction boosts uptake of the virus by macrophages, resulting in the activation of the complement system and an augmented cytokine cascade (7). These immune responses result in hemorrhagic manifestations including plasma leakage and low platelet count (7).

2.2 Dengue Virus Susceptibility

The severity of dengue in humans is influenced by a range of factors, including the infecting serotype, genetic predisposition, pre-existing conditions, age, and nutritional status (9, 10). Figure X highlights environmental, viral, vector and human genetic determinants known to influence outcome.

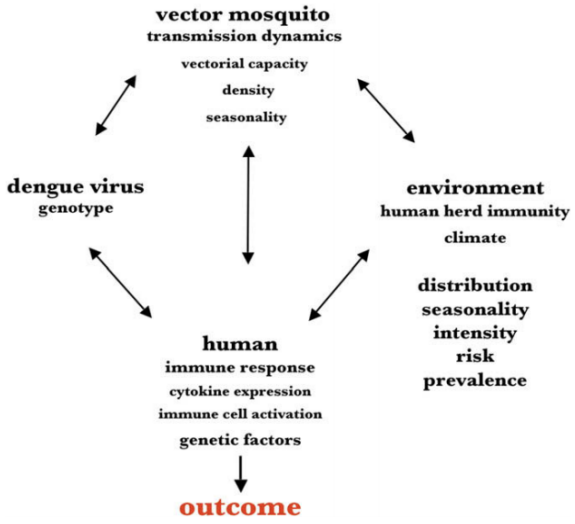

Figure 1. Risk Factors

Understanding human genetic susceptibility is key to understanding and predicting dengue pathogenesis. Moreover, this information can potentially aid in the development of anti-dengue

vaccines and therapies. Several human genetic polymorphisms, which will be discussed in later sections, have been associated with severe hemorrhagic manifestations.

The relationship between mosquitos and dengue virus is complex. The terms vector competence and vectorial capacity can be used to explain mosquito activity. Vector competence refers to the vector's ability to efficiently transmit a pathogen. Vectorial capacity refers to the potential number of bites on one host on a single day (11). Interactions between internal, external and viral factors such as the microbiota of the mosquito, regional climate, and viral genetics impact vector competence (12). The mosquito's vectorial capacity is ultimately influenced by vector density and the frequency of host interaction, in addition to feeding behavior and longevity, which are dependent upon its response to the virus (12).

Some dengue serotypes are suspected to be more virulent than others when comparing primary and secondary infections. Research from Fried et al. suggests that in the case of primary infections, DENV-1 and DENV-3 are more pathogenic serotypes (13). Furthermore, their research also suggests that DENV-2 and DENV-3 are twice as likely to result in DHF in secondary infections when compared to DENV-4 (13).

2.3 Clinical Classification

The evolving perception of dengue has shaped the clinical classification of the disease over the years. Initially, dengue was not considered to be a life-threatening illness. However, outbreaks of dengue hemorrhagic fever in Southeast Asian children in the late 1960s prompted the public to reconsider (14). Information obtained from these cases became the foundation of the World Health Organization's (WHO) guidelines for the clinical classification of dengue, published in 1975 and

updated in 1997 (14). Before 2009, dengue infection was classified into two categories: dengue fever and dengue hemorrhagic fever, as seen in figure 2 (14).

Dengue fever patients presented with fever and at least two of the following symptoms: headache, pain behind the eye, myalgia, joint pain, rash, hemorrhagic manifestations and low white blood cell count (14). Additionally, serological or epidemiological (same location and timepoint as confirmed cases) confirmation was also required (14). Dengue hemorrhagic fever patients presented with all four of the following symptoms: fever, hemorrhagic manifestations, low platelet count, and plasma leakage. While the case definition of DHF may seem specific, many expressed difficulties diagnosing dengue in low-resource or primary care settings (14). Additional arguments include its exclusivity of patients experiencing more severe disease and its inapplicability to other regions with different epidemiological trends of dengue disease (14).

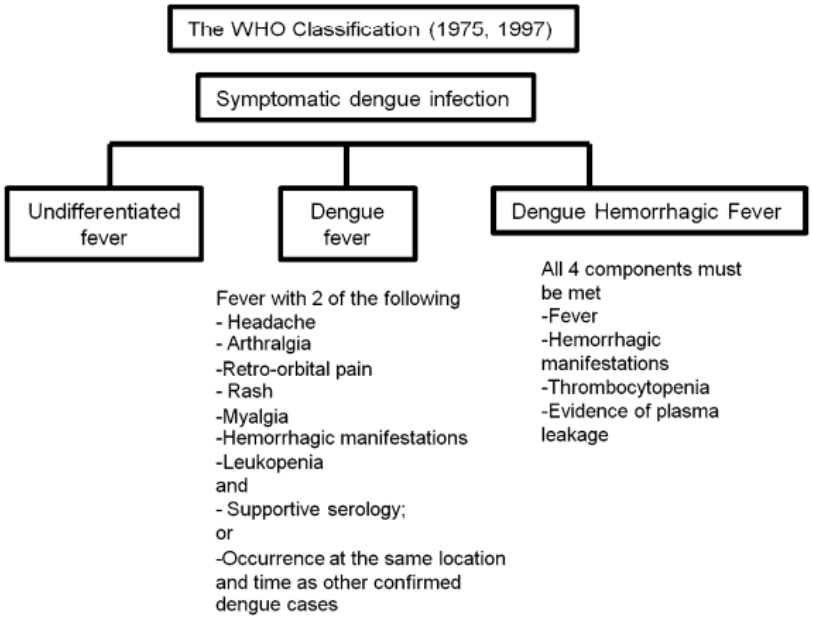

Figure 2. 1997 World Health Organization Classification of Dengue

In response to these concerns, WHO reclassified the categories of clinical dengue in 2009, as seen in Figure 3. Dengue is currently recognized as dengue, with or without warning signs, and severe dengue. Dengue is characterized by a fever with at least two of the following: vomiting, nausea, rash, myalgia, a positive tourniquet exam and any warning signs. Similar to the 1997 classification, serological and epidemiological evidence is required as well. Warning signs include abdominal pain and/or tenderness, persistent vomiting, hepatomegaly, mucosal bleeding, tiredness, and fluid accumulation. Patients exhibiting these signs should be closely monitored to prevent disease progression. Severe dengue is characterized by any of the following: severe plasma leaking, severe bleeding or severe organ impairment of the liver, heart or central nervous system (14). About 5% of all dengue fever cases will progress to the severe dengue stage (15). While the sensitivity of the current system is considered to be superior to the former, issues do exist (16). Determination of disease severity may differ by clinician because the current system fails to define the criteria for severe dengue (15). Moreover, presence of one of the three clinical components of severe dengue does not always indicate severe disease but could be the result of an unrelated diseases or conditions (14). Considering its over-inclusivity and use of nonspecific warning signs, it is clear that classification requires some modification in order to be effective worldwide.

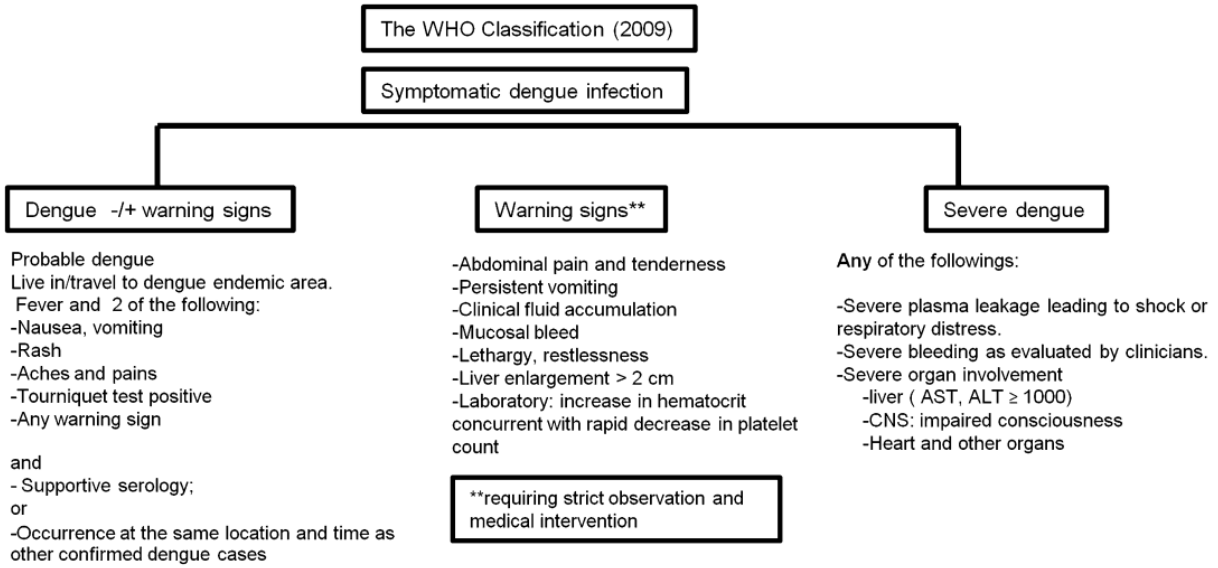

Figure 3. 2009 World Health Organization Classification of Dengue

2.4 Clinical Presentation

Clinical presentation of dengue ranges from non-severe to severe manifestations. Due to lack of a vaccine or a specific antiviral treatment, timely intervention and adequate understanding of the natural history of disease is key to recovery.

Dengue disease characterized by three distinct phases—febrile, critical, and recovery (Figure 4). Only patients with severe dengue will experience all three phases (17). Patients in the febrile stage develop a high-grade fever following incubation. This phase generally lasts for 2-7 days along with common acute febrile illness symptoms such as myalgia, headache, skin erythema, and facial flushing (18). The similarities between the symptoms of dengue and other acute febrile illnesses make it difficult to accurately diagnose infections in this stage. Since elevated viremia is a critical indicator of disease in this phase, serological testing should be performed if dengue is suspected.

Warning signs will surface in the late stages of the febrile phase. In the absence of medical intervention, the patient will progress to the critical phase. This phase generally lasts for 24-48 hours and begins at the time of defervescence (18). It is marked by an increase in hematocrit and capillary permeability, along with a decrease in platelet count (18). Most patients who experience this phase will recover however those with significant plasma leakage will develop severe dengue (15). During the recovery phase, patients begin to reabsorb extravascular fluids that were lost during the critical phase and overall health improves (15). Patients may experience fatigue and depression during the recovery phase (19).

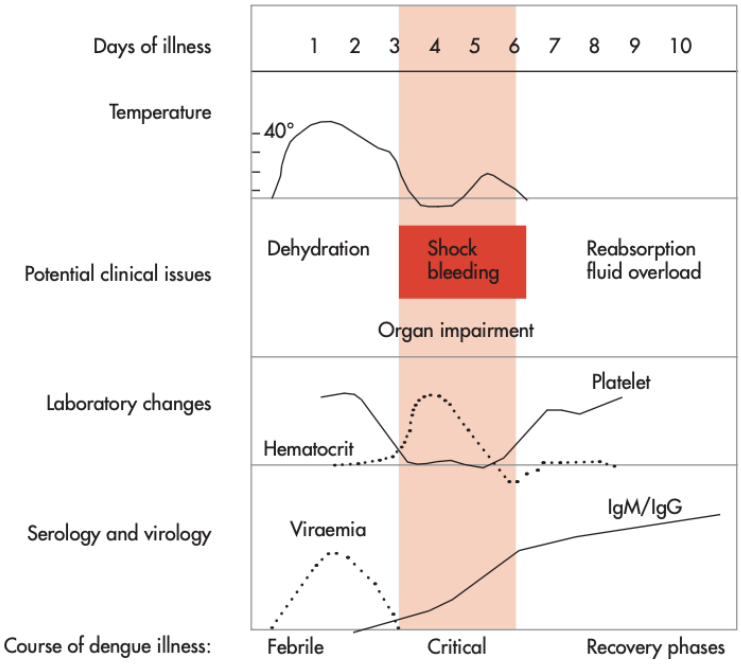

Figure 4. Course of Dengue Illness

2.5 Global Burden of Dengue

Researchers believe that dengue has existed for centuries. Despite the multiple dengue-like outbreaks in 1635, 1669, and 1780, a Chinese encyclopedia dated as early as A.D. 265 describes symptoms similar to dengue (4, 20). The Chinese referred to the disease as "water poison" and associated it with flying insects and water. However, the virus now known as DENV-1 was not isolated until 1943 by Ren Kimura and Susuma Hotta during an outbreak in Nagasaki, Japan. (21). A year later, Albert Sabin isolated DENV-2 (20). In 1956, William Hammon, the first chair of the Department of Infectious Diseases and Microbiology at the University of Pittsburgh Graduate School of Public Health isolated DENV-3 and DENV-4 (20). Before 1970, severe dengue epidemics were only recorded in 9 countries (5). Since then, the geographical distribution of dengue has expanded, and the virus is now present in every WHO region (5, 1). According to the WHO, it is now endemic in over 100 countries (5). While Asia carries the largest burden of disease, the South-East Asia, America and Western Pacific regions are also greatly affected (5).

Approximately half of the global population is at risk of contracting dengue. The WHO states that in the past two decades, the number of reported cases has increased exponentially (5). In 2013, researchers in a study by Bhatt et al predicted 390 million dengue infections occur every year and approximately 24% of these cases are symptomatic (6). Increases in global incidence and hyperendemicity in certain regions can be contributed a number of factors, including inefficient or lack of vector control, poor living conditions, international travel, and geographic expansion (7, 1).

Dengue is generally a non-fatal, self-limiting disease. According to the CDC, approximately 25% of those infected with the virus will actually develop an illness and about 5% of those cases will progress to severe dengue (3). The cost of treatment for these severe cases is often unaffordable for individuals or families in low-income countries. It is important to note that mild infections can

also negatively impact an individual's economic stability. Mild symptoms can limit an individual's ability to participate in daily activities, which then affects their ability to pay for necessary medications. A 2013 study estimated that the annual global burden of dengue in that year was 8.9 billion USD, though the true burden was most likely more expensive (23). Specifically, they estimated that the short-term costs per case ranged from 31-333 USD (23). This includes direct healthcare costs and indirect costs, money lost due to illness. Long-term costs for fatal cases ranged from 75,820-80,414 USD (23). As previously stated, it is difficult to estimate the true burden of disease due to the misdiagnosis and underreporting of dengue infections. Dengue treatment also places a strain on healthcare systems. Without adequate screening criteria for emergency settings in hyperendemic regions, overcrowding is likely to occur.

2.6 History of Dengue in Brazil

The first Brazilian dengue epidemic was reported in 1845, in Rio de Janeiro (3). Additional epidemics were reported from 1853-1851 and 1916-1923. The Pan American Health Organization's urban fever mosquito eradication program, which eliminated the presence of A. aegypti, was instrumental in the decline of dengue in Brazil until 1976 (3). DENV-1 and DENV-4 were the first serotypes found in Brazil, as they were discovered to be responsible for the 1981 outbreak (3). DENV-2 was later introduced in 1990 after an outbreak in Rio de Janeiro. DENV-3, the cause of the 2000 outbreak, was the last to appear in Brazil (3). In 2002, with 288,245 reported cases and 91 deaths, DENV-3 was responsible for one of the largest outbreaks in the country (3). From 1981 and 2006, there were 4,343,049 reported cases with 5,817 cases of severe dengue and 338 deaths (3). Figure 4 highlights the activity of all four serotypes in Brazil from 1845 to 2010. As of today, all

four serotypes are endemic in Brazil and continue to threaten the health and well-being of its citizens. In 2016, approximately 1.5 million of the total global dengue cases occurred in Brazil, a threefold increase from 2014 (3). Brazil's climate has sustained the mosquito population and continues to provide favorable conditions that allow replication of the species.

Table 1. Dengue Serotype Activity in Brazil from 1845-2010

Year (s)	Activity reported	Dengue serotype	Location	Reference
1845	1st dengue epidemic was reported	Unknown	Rio de Janeiro	[46]
1981	1st dengue epidemic in Brazil after *Ae. aegypti* reinfestation	DENV-1 DENV-4	Roraima	[46, 50]
1986-1987	Epidemic	DENV-1	Rio de Janeiro	[6, 48, 49, 51]
1990	First identification of DENV-2	DENV-2	Rio de Janeiro	[26, 48, 49, 54]
1990–2000	DENV spread intensified contributing to several outbreaks	DENV-1 DENV-2	Southeast and northeast region	[47, 55, 56]
2000	1st appearance of DENV-3 in Brazil	DENV-3	Rio de Janeiro	[48–51, 53]
2002	One of the largest dengue outbreaks in Brazil since the virus emergence	DENV-3	Rio de Janeiro	[48–51]
2000–2007	Brazil reported >60% of the cases registered in the world	DENV-1 DENV-2 DENV-3	All Brazilian states	[47, 54, 55]
2007-2008	Intense outbreak with high number of severe cases and fatalities	DENV-2	Rio de Janeiro	[6, 55]
2009	Large outbreak	DENV-2	Espírito Santo	[56]
2010	Several outbreaks	DENV-1 DENV-2 DENV-3 DENV-4	21 Brazilian states	[26]
2010	Reemergence of DENV-4	DENV-4	Roraima, Amazonas, Amapá, Pará, São Paulo, and Rio de Janeiro	[57, 58, 65, 68]

2.7 Management and Prevention

Efforts to reduce the global morbidity and mortality of dengue include but are not limited to the following: providing preventative resources and education to affected communities, improving vector control strategies, strengthening healthcare systems, training healthcare workers at all levels, and providing adequate treatment to severe cases (24). In 2012, the WHO implemented the Global Strategy for Dengue Prevention and Control. This eight-year plan was created to mitigate the effects

of dengue and reduce the overall global burden of disease (24). Specifically, this plan aimed to estimate the exact burden of dengue fever by 2015 (24). It also aimed to reduce global dengue morbidity and mortality by at least 25% and 50%, respectively, by 2020 (24). Unfortunately, global incidence rates of dengue remain high, despite the WHO's efforts. In 2019, the Region of the Americas recorded the highest number of reported cases to date (25). Barriers of change include urbanization, climate changes, and international travel and trade (26).

Other notable interventions include The PAHO Integrated Management Strategy for Dengue Prevention and the World Mosquito Program. PAHO aims to see a 30% reduction in the case fatality rate of dengue in the Region of the Americas by 2020. PAHO plans to achieve this through the following objectives: enhancing the detection and management of dengue, improving surveillance systems and genetically monitoring the virus (27). The World Mosquito Program has implemented a unique method to combat the spread of diseases via *A. aegypti* mosquitoes. Researchers have discovered that mosquitoes carrying *Wolbachia* are less likely to transmit viruses to humans (31). This program breeds *Wolbachia*-infected mosquitoes and releases them into communities which are highly impacted by mosquito-borne diseases (31). However, like many interventions, there are some limitations. Most notably, the *A. aegypti* is not the sole vector for all mosquito-borne diseases.

The current dengue vaccine, Dengvaxia, is licensed in 20 countries and is only administered to individuals between the ages of 9 to 45 (47). A major limitation of this vaccine stems from the fact that it can only be administered to dengue-seropositive individuals, those who have previously been exposed to the virus. Global controversy behind Dengvaxia arose in 2017 after Filipino children, who were suspected to have had a previous exposure to dengue, either experienced negative health outcomes or died after receiving the vaccine (46). Issues such as these signify the importance of continued vaccine research.

3.0 Methods

3.1 Research Question

Is there an association between the selected single nucleotide polymorphisms (SNPs) and host susceptibility to severe dengue in these samples?

3.2 Sample Selection

The DNA samples used in this project belong to a cohort of dengue patients in Recife, Brazil (34). This study was conducted at the Aggeu Magalhães Research Center by researchers in the Department of Virology (34). Patients were recruited from 2004-2006 at three different hospitals in Recife— the Hospital Esperança, Hospital Santa Joana and Instituto Materno Infantil. Each patient was admitted to one of the three hospitals with suspected dengue fever. Patients under the age of five were not eligible to participate (34). Blood samples were collected from all patients to perform necessary confirmatory laboratory testing. All positive cases were confirmed by testing to be caused by DENV-3. All suspected cases were not dengue positive. Patients found to be dengue-free were used as controls for this thesis project. Additionally, patients from a yellow fever vaccine cohort, which also took place in Recife, Brazil, were included in the control group as well. Eligible patients were required to be at least 10 years of age with no prior history of dengue infection (35). Serological testing was performed on all patients prior to immunization.

Dengue-positive patients were classified into three groups—classic dengue, complicated dengue and dengue hemorrhagic fever. Prior to the 2009 revision, researchers and physicians found that some cases did meet the all criteria for the WHO classifications of dengue fever, specifically for dengue hemorrhagic fever. In this case, the complicated dengue category was created for patients who presented with dengue fever symptoms, hemorrhagic manifestations and low platelet count, but did not meet the laboratory criteria required by the WHO (34).

Patients in the dengue cohort were also classified by infection type—primary or secondary. Primary cases lacked the presence of anti-dengue IgG antibodies following initial infection but were positive for anti-dengue IgM and IgG in convalescent serum samples (34). Secondary cases were characterized by the presence of anti-dengue IgG antibodies in acute serum samples and the absence of anti-dengue IgM antibodies (34). However, convalescent serum samples of secondary cases showed a presence of anti-dengue IgM antibodies.

3.3 Single Nucleotide Polymorphisms of Interest

As previously stated, human genetic variants can influence infectious disease susceptibility. Associations between genetic polymorphisms and susceptibility have been identified for infectious diseases such as hepatitis B and C, malaria, tuberculosis and HIV-1 (28). This study investigates the association between 18 single nucleotide polymorphisms (SNPs) and severe dengue. The polymorphisms in question, along with the corresponding gene, can be found in Table 1. The first 11 SNPs were identified in a study which predicted dengue fever severity using human genome data and machine learning (29). PLCE1 rs3740360 was shown to be significantly associated with Dengue Shock Syndrome in a Vietnamese pediatric study (30). MRC1 rs606231248, formerly rs34039386,

and MRC1 rs2296414 were found to be associated with severe dengue by former students Erin Cathcart and Hannah Polglase (39,40). OASL rs3212545 and RNASEL rs486907 have been shown to be associated with increased susceptibility to severe West Nile virus disease, a close relative of dengue virus (33). Due to this association, the aforementioned SNPs may be of interest in this analysis. MICB rs3132468 was found to be a risk factor for dengue shock syndrome in Thai children (32). Lastly, MX1 rs7277299 is associated with a gene that participated in the cellular antiviral response so variants of this gene may increase dengue susceptibility as well (45).

Table 2. Selected SNPs and Corresponding Genes

SNP	Gene	SNP	Gene
rs17256081	Toll-like receptor 8 (TLR8)	**rs4251580**	Interleukin-1 receptor-associated kinase 4 (IRAK4)
rs2069718	Interferon gamma (IFNG)	**rs17199006**	C-Type Lectin Domain Family 4 Member C (CLEC4C)
rs2069727	Interferon gamma (IFNG)	**rs3740360**	Phospholipase C Epsilon 1 (PLCE1)
rs2070729	Interferon regulatory factor 1 (IRF1)	**rs606231248**	Mannose Receptor C-Type 1 (MRC1)
rs2072137	Oligoadenylate synthase 2 (OAS2)	**rs2296414**	Mannose Receptor C-Type 1 (MRC1)
rs2072138	Oligoadenylate synthase 2 (OAS2)	**rs486907**	Ribonuclease L (RNASEL)
rs2240188	Oligoadenylate synthase 3 (OAS3)	**rs3213545**	Oligoadenylate Synthetase Like (OASL)
rs3737399	MX dynamin like GTPase 1 (MX1)	**rs7277299**	MX dynamin like GTPase 1 (MX1)
rs3911403	Ventricular zone expressed ph domain containing 1 (VEPH1)	**rs3132468**	MHC class I polypeptide-related sequence B (MICB)

3.4 Genotyping

Due to the large sample size, samples were sent to the University of Pittsburgh's Genomics Research Core Laboratory for SNP genotyping. Prior to plating and transport, the concentrations of all samples were tested using a Nanodrop 1000 Spectrometer. Concentrations ranged from 10-100 ng/μL, per the laboratory technician's request. Samples with concentrations over 100 μL were diluted within acceptable range using Tris-EDTA buffer. Samples were then plated on 96 well plates and stored at 4 degrees Centigrade until transport. Samples were then genotyped at the Genomics Research Core by polymerase chain reaction, single-base primer extension, and mass spectrometry using the iPlex MassARRAY system (Agena Bioscience).

3.5 Data Analysis

The demographic data of the total sample population was analyzed using Microsoft Excel. Samples were sorted by age, sex and dengue disease status — dengue fever (DF), complicated dengue (CD), and dengue hemorrhagic fever (DHF). Principal component analysis (PCA) was performed on these samples, using R statistical software, to group samples into clusters by SNPs and disease status (severe vs. mild disease). PCA is an unbiased approach that was used to cluster samples based on their overall similarities in genotype at each of the 18 SNPs for which we have data. If these SNPs collectively impact dengue disease outcome, then samples with the same disease classification should cluster together in the PCA. HWE and chi-square testing was performed using Microsoft Excel to check the reliability of the genotypes obtained. Using genotypic data, odds ratios (ORs) were performed for all SNPs to determine the odds of developing severe disease. Odds ratios

were calculated using Microsoft Excel. P-value and 95% confidence interval calculations were provided for each OR as well.

4.0 Results

4.1 Demographics of Total Population

Demographic data was analyzed using Microsoft Excel. Results can be found in Table 2. Of the 450 samples, 225 were confirmed dengue cases. Females represented 58.4% of the population and males represented 41.6%. When combining both sexes, patients within the 30-39 age group had the highest percentage of disease, followed by the 0-19 age group. Overall, younger populations were more affected by dengue in this sample.

Table 3. Distribution of Age and Sex in Total Population

	Age								
Sex	**0-19**	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**70-79**	**TOTAL**	**%**
Female	28	29	34	23	13	4	1	132	58.7%
Male	26	12	23	20	9	2	1	93	41.3%
TOTAL	54	41	57	43	22	6	2	225	
%	24.0%	18.2%	25.3%	19.1%	9.8%	2.7%	0.9%	100.0%	

Overall, there were 132 confirmed dengue cases in the female population, with the highest number of cases being seen in the complicated dengue (CD) group. The lowest number of cases were seen in the dengue hemorrhagic fever (DHF) group, which is expected due to the rarity of the condition. This group represented 47.73% of the total female population. Results can be seen in Table 3. Again, younger age groups were more affected in this population as well. Table 4 displays

the distribution of dengue cases by age group in the male population. The older populations for both sexes are not well represented in this sample. Similar to the female population, the complicated dengue group carries the largest burden of disease among males.

Table 4. Distribution of Disease by Age in Female Population

	Diagnosis			
Age	**DF**	**CD**	**DHF**	**Total**
0-19	16	12	0	28
20-29	11	7	11	29
30-39	8	23	3	34
40-49	5	13	5	23
50-59	7	6	0	13
60-69	1	2	1	4
70-79	0	0	1	1
Total	48	63	21	132
%	**33.36%**	**47.73%**	**15.91%**	**100.00%**

Table 5. Distribution of Disease by Age in Male Population

	Diagnosis			
Age	**DF**	**CD**	**DHF**	**Total**
0-19	13	10	3	26
20-29	5	7	0	12
30-39	1	20	2	23
40-49	7	12	1	20
50-59	4	5	0	9
60-69	1	0	1	2
70-79	0	1	0	1
Total	31	55	7	93
%	**33.33%**	**59.14%**	**7.53%**	**100.00%**

Infection type data was provided for 189 of the 225 dengue cases. Results can be found in Figure 5. The highest number of primary and secondary infections were observed in the CD group,

51 and 55 respectively. Interestingly, there were more primary infections in the DHF group and more secondary infections in the dengue fever group. In total, there were 94 primary infections and 95 secondary infections.

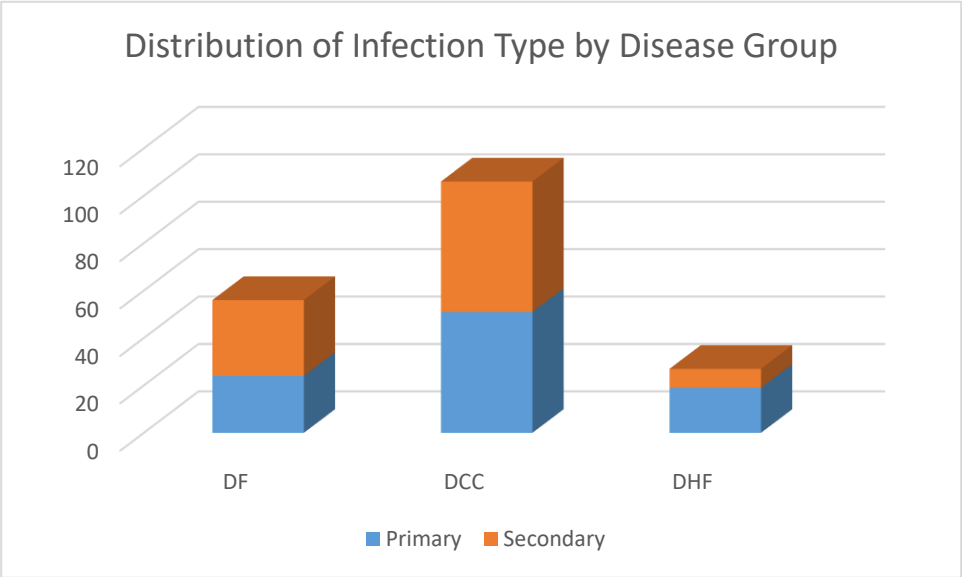

Figure 5. Distribution of Primary and Secondary Infections

4.2 Principal Component Analysis

Principal component analysis was used to group samples into two clusters by disease status, mild vs. severe dengue. However, the results were not very informative. Theoretically, if there were differences in in the distribution of genotypes for each disease group, two distinct clusters would be present in the plot. All 18 SNPs were used to create Figure 6. Figure 7 is based on the 11 SNPs that were predicted to be associated with severe dengue using human genome data and machine learning by Davi et al. Possible explanations for the lack of clusters will be discussed in later sections.

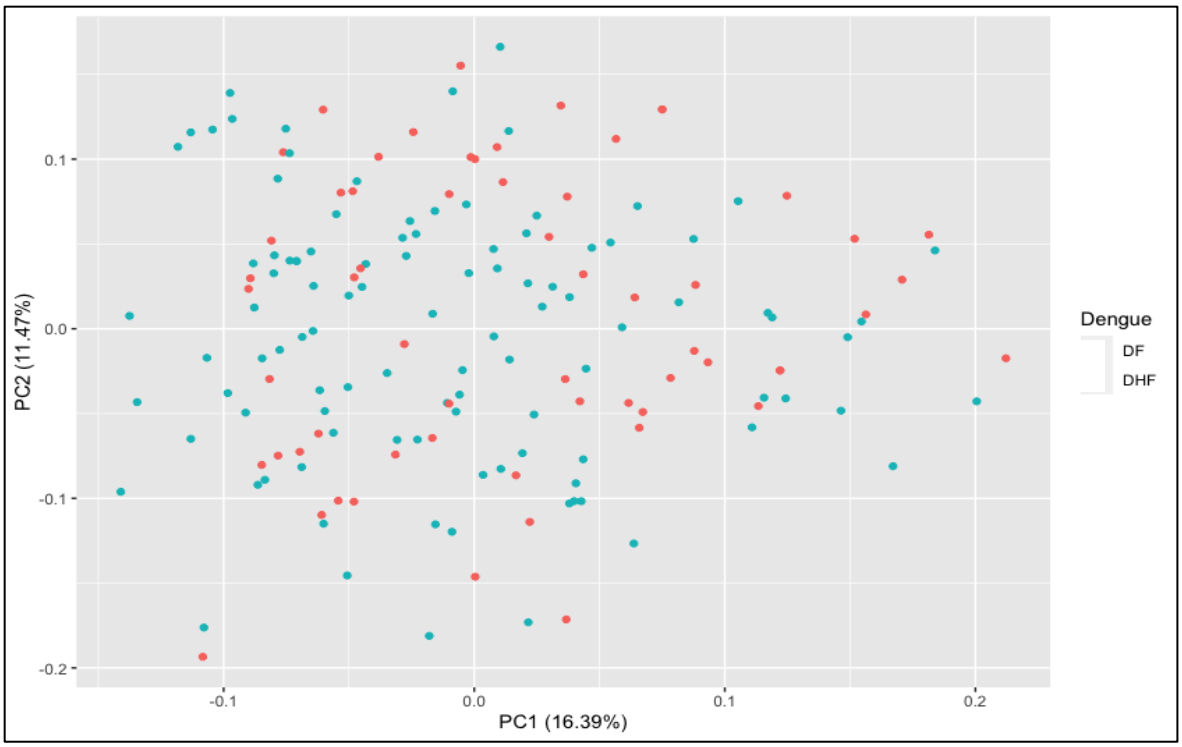

Figure 6. Principal Component Analysis of 18 SNPS

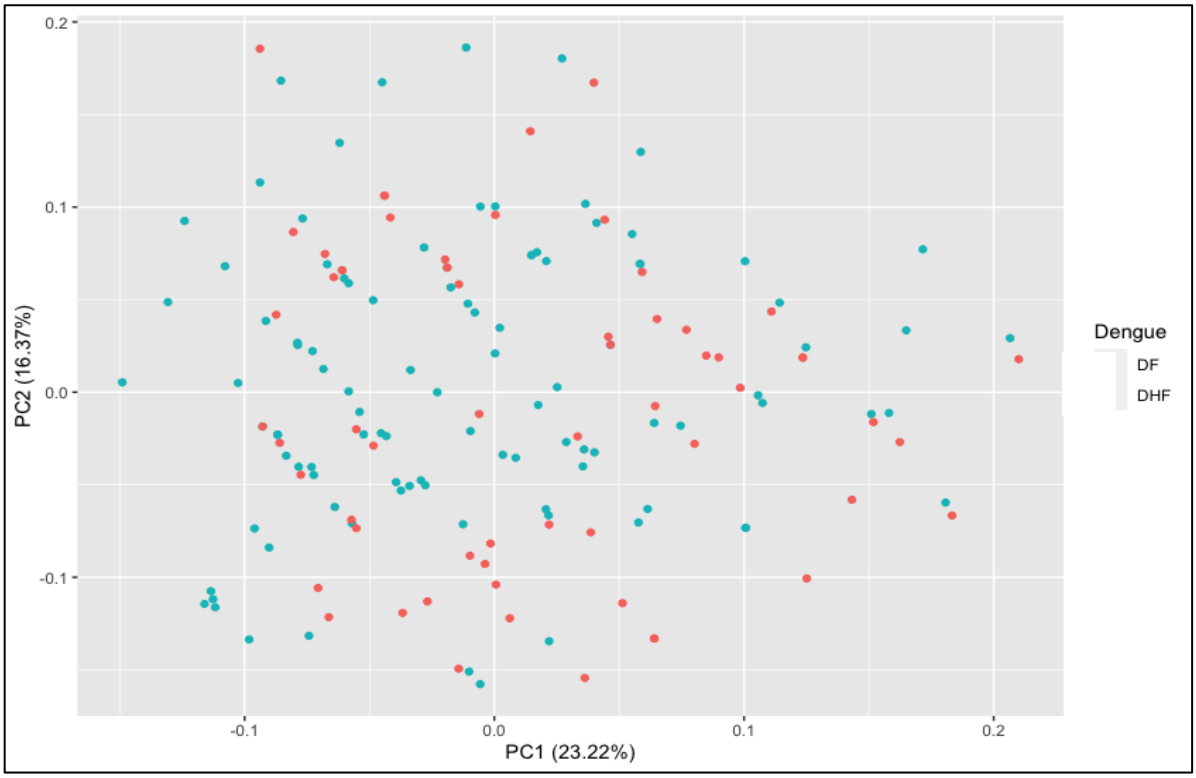

Figure 7. Principal Component Analysis of 11 Predicted SNPs

4.3 Hardy Weinberg Equilibrium

HWE testing was performed to compare the observed and expected allele frequencies. HWE is based upon the following principle: genotype frequencies among a population will remain constant in the absence of disrupting factors. HWE was calculated for the whole sample, cases, controls and by disease status. To evaluate the significance of the differences between the observed and expected genotype frequencies, chi-squared testing was performed. All observed genotypes were in HWE across all disease categories, cases and controls except for the following: rs606231248, rs3737399, rs17256081, rs2070729, rs3911403, and rs486907. Possible reasons for these discrepancies will be highlighted in the discussion section.

4.4 Genotype and Allele Frequencies

This section includes data for each SNP on the observed genotypes and alleles among this Brazilian population. Genotype and allele frequency data per SNP can be found in the appendix section in Table 1. The CD group was the most represented disease group and the DHF group was the least represented for all SNPs.

4.4.1 MRC1 rs2296414

The frequency of the CC genotype for the entire sample, including non-dengue cases, was 71.91%. The frequencies of the CT and TT genotypes were 25.91% and 2.18%, respectively. In terms of alleles, the T allele was more common, with a frequency of 84.87% in the total sample.

4.4.2 CLEC4C rs17199006

The frequencies of AA, GA and GG in the whole sample were 74.01%, 24.34%, and 1.64%, respectively. The frequency of the A allele was 86.18% and the frequency of the T allele was 13.82%.

4.4.3 TLR8 rs17256081

Results for this SNP show that the TT was the most frequent genotype in the entire population, with a frequency of 51.56%. The frequency of CC and CT was 27.60% and 20.83%, respectively. The T allele was the more represented allele among this population.

4.4.4 IFNG rs2069718

The most frequent genotype among the entire sample was AG, with a proportion of 54.04%. The frequencies of AA and GG in the total sample were 17.42% and 28.53%, respectively. The G allele was more common among this population.

4.4.5 IFNG rs2069727

The frequencies of the CC, CT and TT genotypes in the entire population were 14.43%, 48.17%, and 37.41%, respectively. The distribution of the T allele was higher than the C allele.

4.4.6 IRF1 rs2070729

The frequencies of the AA, CA and CC genotypes in the entire population were 23.02%, 54.42%, and 22.56%, respectively. The C and A alleles were evenly distributed across all samples.

4.4.7 OAS2 rs2072137

The frequencies of the CC, TC, and TT genotypes in the entire population were 15.65%, 43.28%, 41.08%, respectively. The frequency of the T allele was 62.71% and the frequency of the C allele was 37.29%.

4.4.8 OAS2 rs2240188

The distribution of the CC and CT genotypes among this sample were very similar. The frequency of the CC genotype was 44.03% and the frequency of the CT genotype was 44.50%. The TT genotype is least represented in this sample, with a frequency of 11.48%. This finding explains the high percentage of C alleles in the population.

4.4.9 MICB rs3132468

The distribution of the T allele is significantly higher than that of the C allele in this population. The CC genotype was present in a small percentage of the confirmed and non-dengue cases. The frequency of the TC and TT genotypes was 31.67% and 64.52%.

4.4.10 OASL rs3213545

Similar to the last, the distribution of the G allele is significantly higher than that of the A allele in this population. The AA genotype was present in a small percentage of the confirmed and non-dengue cases. The frequency of the GA and GG genotypes was 38.15% and 56.64%.

4.4.11 MX1 rs3737399

The distribution of the CC genotype among the entire population was significantly higher than that of the CT and TT genotypes. The frequency of the CC genotype was 77.12%. The CT genotype was not detected in the CD or DHF groups and the frequency of the genotype in the entire population was 1.03%. The frequency of the C allele was 77.63%.

4.4.12 PLCE1 rs3740360

The CC genotype was not detected in this sample. The distribution of the AA genotype, 81.19%, was significantly higher than that of the CA genotype, 18.81%. As expected, the frequency of the A allele was also higher than the C allele.

4.4.13 VEPH1 rs3911403

The frequencies of the AA, TA and TT genotypes were 3.03%, 23.48%, and 73.48%. The frequency of the T allele, 85.23%, was significantly higher than the A allele, 14.77%.

4.4.14 IRAK4 rs4251580

The there is a greater presence of the C allele in this sample. The frequency of the CC genotype was 76.26%, followed by 22.35% for the CT genotype. The lowest frequency was observed for the TT genotype at 1.40%. As expected, the frequency of the C allele, 87.43%, was significantly higher than the T allele, 12.57%.

4.4.15 RNASEL rs486907

The highest proportion of samples was seen in the CC genotype, followed by CT. The frequencies were 50.63% and 39.35%, respectively. The frequency of TT was 10.02%.

4.4.16 MRC1 rs606231248

The frequencies of the AA, AG and GG genotypes were 8.02%, 26.07%%, and 65.91%. The frequency of the G allele, 78.95%, was significantly higher than the A allele, 21.05%.

4.4.17 MX1 rs7277299

The proportion of the A allele is very low in this population. The AA genotype was not present at all and the CA genotype was only present in 26 samples. The frequency of the CC genotype was 93.55% and the frequency of the C allele was 96.77%.

4.4.18 OAS2 rs2072138

The genotyping results for this SNP revealed the presence of three alleles, A, C and G. The highest percentage of samples were seen in the CC group at 45.89%, followed by the GC group at 39.27%. The frequencies of the AA, GG and CA genotypes were 6.85%, 6.39% and 1.60%. The C allele had the highest frequency among the population with 71.03%, followed by the G allele (24.45%) and the A allele (4.52%).

4.5 Odds Ratios

Odds ratios were calculated for each of the 18 SNPs in order to determine if certain genotypes were associated with severe dengue. Additionally, these calculations will uncover which alleles act in dominant and recessive manners. Severe dengue (CD+DHF) and dengue fever (DF) groups were used for all odds ratios. Insignificant odds ratio results can be found in the appendix section in Table 2.

There were 4 significant results from this analysis (Table 22). The first was found for rs2072137. The CC genotype was shown to be significantly associated with severe dengue (OR=2.10, P=0.01). The next significant result (OR=1.96, P=0.02) was found for rs2240188, where the CC genotype also appears to influence disease severity. The odds ratios for rs3740360 reveal a significant association between the A allele and severe dengue (OR=2.28, P=0.03). The last notable result was found in rs7277299 (OR=5.33, P=0.02) where the CC genotype was also significantly associated with severe disease.

Table 6. Significant Odds Ratios

rs2072137		**Severe Dengue (CD+DHF)**	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
	TT+TC	116	60	1.55	0.75 – 3.20	0.2401
	CC	20	16			
	TT	67	24	2.10	1.17 – 3.79	**0.0133**
	TC+CC	69	52			
rs2240188		**Severe Dengue (CD+DHF)**	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
	CC+CT	123	64	1.67	0.75 – 3.71	0.2123
	TT	15	13			
	CC	69	26	1.96	1.10 – 3.50	**0.0224**
	CT+TT	69	51			
rs3740360		**Severe Dengue (CD+DHF)**	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
	AA+CA	129.5	74.5	1.74	0.03 – 88.52	0.7828
	CC	0.5	0.5			
	AA	112	55	2.28	1.10 – 4.72	**0.0272**
	CA+CC	17	19			
rs7277299		**Severe Dengue (CD+DHF)**	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
	CC+CA	135.5	74.5	1.82	0.04 – 92.61	0.7655
	AA	0.5	0.5			
	CC	132	66	5.33	1.37 – 20.77	**0.0158**
	CA+AA	3	8			

5.0 Discussion

5.1 Demographics

Results show that more women were infected with dengue virus than men in this population. While this is a small sample size, the results correlate with Cordeiro's findings on dengue distribution across sexes (34) and that of other Latin American countries (34, 37). The male to female ratio in Brazil from 2001-2010 was 0.75 to 0.82 (37). Further analysis of health data records in Brazil show a higher percentage of confirmed dengue cases in women, suggesting that they may seek care more often than men (34). Reasoning behind these differences in Brazil should be studied further.

As seen in Figure 5, the number of primary and secondary infections in the complicated dengue group are similar. In terms of secondary infections, the data is consistent with the literature on dengue pathogenesis. Researchers have concluded that secondary infections increase severe dengue risk through ADE. However, it is interesting to see such a similar number of primary infections in this group. Moreover, there was a larger percentage of primary infections in the dengue hemorrhagic group. One explanation for these finding could be the pathogenesis of the infecting serotype. As previously stated, certain serotypes elicit a more severe reaction than others. The results in the dengue fever group are noteworthy as well. There was a larger percentage of secondary infections in this group, which contradicts the ADE phenomenon. While these findings are intriguing, it is important to note that infection type data was not provided for all 225 dengue cases.

5.2 Principal Component Analysis

The results of the PCA plot do not reveal any significant correlation between disease groups and SNPs. There was no clear difference in the distribution of genotypes across mild and severe disease groups. One explanation for this could include the restrictions of the PCA algorithm—it requires complete data. In order to create a plot, genotype data must be present for all samples. Any sample that did not produce genotype data for a particular SNP was excluded from the algorithm. Out of the 225 dengue cases, only 164 were included in the plot (60 dengue fever and 104 severe dengue). These exclusions may have affected the clustering of samples. It would be better to test the predictions made by Davi et al using a larger sample size with complete genotype data.

5.3 Hardy Weinberg Equilibrium

All observed genotypes were in HWE across all disease categories, cases and controls except for the following: MRC1 rs606231248, MX1 rs3737399, TLR8 rs17256081, IRF1 rs2070729, VEPH1 rs3911403, and RNASEL rs486907. Deviations from HWE could be due to the following: mutations, nonrandom mating, small sample size, gene flow or migration, and natural selection. Deviations can also be caused by genotyping errors. No genotyping technique is completely accurate and there may have been faults with the particular method used in this study. No significant results were observed for these 6 SNPs. Deviations from HWE may impact the ability to detect significant associations between the disease groups and SNPs.

5.4 Genotype/Allele Frequencies

Observed allele frequencies were compared to global frequencies from the National Library of Medicine's Reference SNP database. Comparison of the two revealed similarities in the observed frequencies in this project and those listed in the database. Among the aforementioned HWE deviations, IRF1 rs2070729 was the only SNP that did not follow allelic trends in the database. The library's records indicate that the frequency of the C allele should be higher than the A allele, however, that is not the case in this project. The frequencies of the C and A alleles were 49.77% and 50.23%, respectively, indicating an almost even distribution of both alleles. Several reasons could explain this difference—genotyping errors, genetic differences between populations, and small sample size. Information on the allele and genotype frequencies for all other SNPs can be found below.

5.5 Significant Odds Ratios

5.5.1 OAS2 rs2072137 and OAS3 rs2240188

The odds ratio calculations for OAS2 rs2072137 revealed a significant association between severe dengue and the TT genotype in this population (OR=2.10, p=0.0133). Calculations for OAS3 rs2240188 revealed a significant association between the CC genotype and severe dengue (OR=1.96, p=0.0224). The OAS gene family, located on chromosome 12, has been shown to encode for interferon-inducing proteins, which play a crucial role in the innate immune system's antiviral response via the OAS/RNase L pathway (41). Specifically, 2'-5' oligoadenylate production occurs

after the 2'-5' oligoadenylate synthetases recognize viral RNA (41). The 2'-5' oligoadenylates then bind to RNase L which cleaves viral and cellular RNA, resulting the inhibition of viral protein synthesis and replication (42). Given its antiviral activity, variations of the OAS gene could have significant effects on the body's ability to fight dengue infection.

5.5.2 PLCE1 rs3740360

A significant association was seen between the AA genotype and severe dengue (OR=2.28, p=0.0272) for PLCE1 rs3740360. Mutations in the PLCE1 gene, located on chromosome 10, are associated with nephrotic syndrome, a kidney disorder that causes hypoproteinemia and the presence of protein in urine (43). Severe symptoms lead to edema and a decrease in vascular oncotic pressure (43). Given that proteinuria and plasma leakage are also characteristics of severe dengue, it is possible that the physiological processes of severe dengue and nephrotic syndrome share similarities. Additionally, expression of the PLCE1 encodes a phospholipase enzyme that is responsible for the production of inositol 1,4,5-triphosphate and diacylglycerol, which regulate several cellular processes (44).

5.5.3 MX1 rs7277299

For MX1 rs7277299, a significant association was seen between the CC genotype and severe dengue (OR=5.33, p=0.0158). The MX1 gene, located on chromosome 4, inhibits viral replication by encoding guanosine triphosphate-metabolizing proteins, which are induced by type I and II interferons (45).

6.0 Public Health Significance

As climate change continues to alter the normalcy of our lives, it may also affect the spread of disease-carrying insects. Specifically, climate change may contribute to the proliferation of the mosquito population. As a result of this increase, mosquito-borne diseases may be spread to areas where they were previously eradicated or nonexistent. Climate change threatens the health and well-being of global populations and without a safe dengue vaccine, communities will lack complete protective immunity.

Vaccine development for dengue has been ongoing for at least 90 years (36). However, like other vaccines, it is challenging to create effective products without proper understanding of the virus and how it interacts with the human immune system. Genetic association studies are used to understand and predict how the human immune system will respond after vaccine exposure. They may also aid in the identification of vaccine targets. A better understanding of the genetic implications in dengue pathogenesis are necessary in order to develop a tetravalent vaccine that is safe for all populations, regardless of prior exposure to dengue.

Health clinics and emergency departments in dengue-endemic areas are burdened with a high number of patients daily. In many of these areas, it is difficult to diagnose dengue cases due to the presence of other acute febrile illnesses. However, those exhibiting dengue symptoms must be closely monitored to prevent progression to severe dengue, often causing unnecessary, long-term hospital admittances. This places a major strain on the staff and increases healthcare spending for the hospital and the patient. To mitigate these effects, genetic association studies can be used to develop triage tools that can accurately identify patients at an increased risk of developing severe dengue.

7.0 Conclusion

With over 50 percent of the global population at risk of infection, dengue carries significant global importance. Contributing factors to the spread of dengue include urbanization, inadequate vector control, climate change, and increased international travel. A large percentage of cases are asymptomatic, making it difficult to estimate the true burden of dengue worldwide. With the increasing global burden, it is more important than ever to develop an effective antiviral treatment and vaccine against dengue.

This study found a significant association between severe dengue and the following SNPs: OAS2 rs2072137, OAS3 rs2240188, PLEC1 rs3740360, and MX1 rs7277299. The OAS and MX1 genes have been shown to influence to the immune response against viral infections by restricting viral replication (41, 45). The PLCE1 gene is suspected to play a role in the clinical outcome of infection and possibly the integrity of the endothelial function (43). However, these findings should be evaluated using a larger sample size.

Several limitations exist in this study. Since all samples were collected in Recife, Brazil, this study not representative of the entire Brazilian population. Results cannot be generalized to the entire Brazilian or global population since the epidemiology of dengue varies by region and possibly by race/ethnicity. This population also lacked diversity in age group. As stated in the demographics section, the older population was not represented well. If this study were to be replicated, it should be done with a larger and more diverse population. Another limitation of this study is the small sample size, which reduces the statistical power and increases the margin of error, thus affecting the reliability of the study**.** This limitation may also lower the reproducibility of the results. A third

limitation is the lack of infection type data for all samples. There was not enough information on the samples to examine the relationship between infection type and disease status.

Researchers have been working to understand the nature of dengue for the past decade. The complexity of this virus poses a great barrier to the advancement of prevention measures, treatments, and vaccine development. As the epidemiology of dengue continues to change, further research is necessary in order to better understand the nature of the virus. There is still much to be discovered about dengue fever, but it is possible that these results can provide greater insight into dengue pathogenesis and susceptibility.

Appendix Supplemental Tables

Table 7. Genotype and Allele Distribution of SNPs

rs2296414					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
CC	51	80	16	150	297
CT	25	29	7	46	107
TT	0	4	2	3	9
No Data	3	5	3	26	37
Informative:	76	113	25	199	413
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
C	127	189	39	346	701
T	25	37	11	52	125
T frequency	16.45%	16.37%	22.00%	13.07%	15.13%
C frequency	83.55%	83.63%	78.00%	86.93%	84.87%
rs17199006					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
AA	50	58	18	99	225
GA	12	26	3	33	74
GG	0	1	0	4	5
No Data	17	33	7	89	146
Informative:	62	85	21	136	304
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
A	112	142	39	231	524
G	12	28	3	41	84
G frequency	9.68%	16.47%	7.14%	15.07%	13.82%
A frequency	90.32%	83.53%	92.86%	84.93%	86.18%
rs17256081					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
CC	19	21	8	58	106
CT	19	25	7	29	80
TT	34	59	9	96	198
No Data	7	13	4	42	66
Informative:	72	105	24	183	384
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
C	57	67	23	145	292
T	87	143	25	221	476
T frequency	60.42%	68.10%	52.08%	60.38%	61.98%
C frequency	39.58%	31.90%	47.92%	39.62%	38.02%

Table 7 Continued

rs2069718					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
AA	12	22	3	32	69
AG	38	55	16	105	214
GG	25	29	5	54	113
No Data	4	12	4	34	54
Informative:	75	106	24	191	396
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
A	62	99	22	169	352
G	88	113	26	213	440
G frequency	58.67%	53.30%	54.17%	55.76%	55.56%
A frequency	41.33%	46.70%	45.83%	44.24%	44.44%
rs2069727					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
CC	13	14	4	28	59
CT	39	51	15	92	197
TT	24	46	6	77	153
No Data	3	7	3	28	41
Informative:	76	111	25	197	409
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
C	65	79	23	148	315
T	87	143	27	246	503
T frequency	57.24%	64.41%	54.00%	62.44%	61.49%
C frequency	42.76%	35.59%	46.00%	37.56%	38.51%
rs2070729					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
AA	20	20	7	52	99
CA	37	70	13	114	234
CC	19	24	6	48	97
No Data	3	4	2	11	20
Informative:	76	114	26	214	430
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
A	77	110	27	218	432
C	75	118	25	210	428
C frequency	49.34%	51.75%	48.08%	49.07%	49.77%
A frequency	50.66%	48.25%	51.92%	50.93%	50.23%

Table 7 Continued

rs2072137					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
CC	16	17	3	28	64
TC	36	39	10	92	177
TT	24	55	12	77	168
No Data	3	7	3	28	41
Informative:	76	111	25	197	409
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
C	68	73	16	148	305
T	84	149	34	246	513
T frequency	55.26%	67.12%	68.00%	62.44%	62.71%
C frequency	44.74%	32.88%	32.00%	37.56%	37.29%
rs2240188					
	DF	**CD**	**DHF**	**ND**	**TOTAL**
CC	26	55	14	93	188
CT	38	43	11	98	190
TT	13	14	1	21	49
No Data	2	6	2	13	23
Informative:	77	112	26	212	427
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
C	90	153	39	284	566
T	64	71	13	140	288
T frequency	41.56%	31.70%	25.00%	33.02%	33.72%
C frequency	58.44%	68.30%	75.00%	66.98%	66.28%
rs3132468					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
CC	3	4	1	8	16
TC	30	39	8	56	133
TT	43	70	17	141	271
No Data	3	5	2	20	30
Informative:	76	113	26	205	420
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
C	36	47	10	72	165
T	116	179	42	338	675
T frequency	76.32%	79.20%	80.77%	82.44%	80.36%
C frequency	23.68%	20.80%	19.23%	17.56%	19.64%

Table 7 Continued

rs3213545					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
AA	3	6	1	12	22
GA	27	39	14	81	161
GG	46	68	10	115	239
No Data	3	5	3	17	28
Informative:	76	113	25	208	422
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
A	33	51	16	105	205
G	119	175	34	311	639
G frequency	78.29%	77.43%	68.00%	74.76%	75.71%
A frequency	21.71%	22.57%	32.00%	25.24%	24.29%
rs3737399					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
CC	59	82	18	141	300
CT	1	0	0	3	4
TT	15	26	5	39	85
No Data	4	10	5	42	61
Informative:	75	108	23	183	389
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
C	119	164	36	285	604
T	31	52	10	81	174
T frequency	20.67%	24.07%	21.74%	22.13%	22.37%
C frequency	79.33%	75.93%	78.26%	77.87%	77.63%
rs3740360					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
AA	55	92	20	148	315
CA	19	13	4	37	73
CC	0	0	0	0	0
No Data	5	13	4	40	62
Informative:	74	105	24	185	388
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
A	129	197	44	333	703
C	19	13	4	37	73
C frequency	12.84%	6.19%	8.33%	10.00%	9.41%
A frequency	87.16%	93.81%	91.67%	90.00%	90.59%

Table 7 Continued

rs3911403					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
AA	1	5	0	6	12
TA	19	18	4	52	93
TT	55	84	20	132	291
No Data	4	11	4	35	54
Informative:	75	107	24	190	396
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
A	21	28	4	64	117
T	129	186	44	316	675
T frequency	86.00%	86.92%	91.67%	83.16%	85.23%
A frequency	14.00%	13.08%	8.33%	16.84%	14.77%
rs4251580					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
CC	51	84	17	121	273
CT	20	15	6	39	80
TT	0	2	0	3	5
No Data	8	17	5	62	92
Informative:	71	101	23	163	358
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
C	122	183	40	281	626
T	20	19	6	45	90
T frequency	14.08%	9.41%	13.04%	13.80%	12.57%
C frequency	85.92%	90.59%	86.96%	86.20%	87.43%
rs486907					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
CC	33	48	10	111	202
CT	34	51	12	60	157
TT	7	10	2	21	40
No Data	5	9	4	33	51
Informative:	74	109	24	192	399
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
C	100	147	32	282	561
T	48	71	16	102	237
T frequency	32.43%	32.57%	33.33%	26.56%	29.70%
C frequency	67.57%	67.43%	66.67%	73.44%	70.30%

Table 7 Continued

rs606231248					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
AA	5	12	2	13	32
AG	16	28	6	54	104
GG	51	66	16	130	263
No Data	7	12	4	28	51
Informative:	72	106	24	197	399
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
A	26	52	10	80	168
G	118	160	38	314	630
G frequency	81.94%	75.47%	79.17%	79.70%	78.95%
A frequency	18.06%	24.53%	20.83%	20.30%	21.05%
rs7277299					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
AA	0	0	0	0	0
CA	8	2	1	15	26
CC	66	108	24	179	377
No Data	5	8	3	31	47
Informative:	74	110	25	194	403
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
A	8	2	1	15	26
C	140	218	49	373	780
C frequency	94.59%	99.09%	98.00%	96.13%	96.77%
A frequency	5.41%	0.91%	2.00%	3.87%	3.23%
rs2072138					
Genotypes	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
AA	3	5	2	20	30
CC	32	54	13	102	201
GG	6	10	0	12	28
CA	0	3	1	3	7
GC	38	43	11	80	172
No Data	0	3	1	8	12
Informative:	41	69	15	134	438
Alleles	**DF**	**CD**	**DHF**	**ND**	**TOTAL**
A	3	8	3	23	37
C	102	154	38	287	581
G	44	53	11	92	200
A frequency	2.01%	3.72%	5.77%	5.72%	4.52%
C frequency	68.46%	71.63%	73.08%	71.39%	71.03%
G frequency	29.53%	24.65%	21.15%	22.89%	24.45%

Table 8. Insignificant Odds Ratios

		Severe Dengue (CD+DHF)	Dengue Fever (DF)	Odds Ratio	95% CI	P-Value
rs17199006	**GG+GA**	30	12	1.64	0.77 - 3.51	0.1986
	AA	76	50			
	GG	1.5	0.5	1.78	0.07 - 44.30	0.7260
	GA+AA	105.5	62.5			
		Severe Dengue (CD+DHF)	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
rs17256081	**CC+CT**	61	38	0.80	0.45 – 1.43	0.4556
	TT	68	34			
	CC	29	13	1.18	0.57 – 2.46	0.6548
	CT+TT	100	53			
		Severe Dengue (CD+DHF)	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
rs2069718	**AA+AG**	96	50	1.41	0.76 – 2.62	0.2751
	GG	34	25			
	AA	25	12	1.25	0.59 – 2.66	0.5629
	AG+GG	105	63			
		Severe Dengue (CD+DHF)	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
rs2069727	**TT+CT**	118	63	1.35	0.62 – 2.94	0.2751
	CC	18	13			
	TT	52	24	1.34	0.74 – 2.43	0.3331
	CT+CC	84	52			
		Severe Dengue (CD+DHF)	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
rs2070729	**AA+CA**	110	57	1.22	0.63 – 2.36	0.5498
	CC	30	19			
	AA	27	20	0.69	0.36 – 1.34	0.2787
	CA+CC	109	56			

Table 8 Continued

		Severe Dengue (CD+DHF)	Dengue Fever (DF)	Odds Ratio	95% CI	P-Value
rs2296414	**CC+CT**	132.5	76.5	0.13	0.01 – 2.40	0.1717
	TT	6.5	0.5			
	CC	96	51	1.12	0.61 – 2.04	0.7104
	CT+TT	42	25			
		Severe Dengue (CD+DHF)	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
rs3132468	**CC+CT**	52	33	0.78	0.44 – 1.38	0.3892
	TT	87	43			
	CC	5	3	0.91	0.21 – 3.91	0.8968
	CT+TT	134	73			
		Severe Dengue (CD+DHF)	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
rs3213545	**AA+AG**	60	30	1.18	0.67 – 2.09	0.5702
	GG	78	46			
	AA	7	3	1.30	0.33 – 5.18	0.7097
	GA+GG	131	73			
		Severe Dengue (CD+DHF)	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
rs3737399	**CC+CT**	100	60	0.81	0.40 – 1.62	0.5439
	TT	31	15			
	CC	100	59	0.87	0.44 – 1.73	0.7014
	CT+TT	31	16			
		Severe Dengue (CD+DHF)	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
rs3911403	**AA+A**	27	20	0.71	0.37 – 1.39	0.3201
	TT	104	55			
	AA	5	1	2.94	0.34 – 25.62	0.3297
	TA+TT	126	74			

Table 8 Continued

		Severe Dengue (CD+DHF)	Dengue Fever (DF)	Odds Ratio	95% CI	P-Value
rs4251580	**CC+CT**	122.5	71.5	0.34	0.02 – 7.24	0.4913
	TT	2.5	0.5			
	CC	101	51	1.72	0.87 – 3.42	0.1212
	CT+TT	23	20			
		Severe Dengue (CD+DHF)	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
rs486907	**CC+CT**	121	67	1.05	0.40 – 2.80	0.9169
	TT	12	7			
	CC	58	33	0.96	0.54 – 1.70	0.8911
	CT+TT	75	41			
		Severe Dengue (CD+DHF)	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
rs606231248	**AA+AG**	48	21	1.42	0.76 – 2.64	0.2666
	GG	82	51			
	AA	14	5	1.62	0.33 – 4.69	0.3761
	GA+GG	116	67			
		Severe Dengue (CD+DHF)	**Dengue Fever (DF)**	**Odds Ratio**	**95% CI**	**P-Value**
rs2072138	**AA+CA**	11	3	2.13	0.58 – 7.86	0.2666
	CC+GG+GC	131	76			
	GG+GC	64	44	0.65	0.38 – 1.14	0.1308
	AA+CA+CC	78	35			
	CC+CA+GC	125	70	0.95	0.40 – 2.23	0.8981
	AA+GG	17	9			

Bibliography

1. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

2. Viral Hemorrhagic Fevers (VHFs). (2013, June 18). Retrieved March 31, 2020, from https://www.cdc.gov/vhf/virus-families/flaviviridae.html

3. Fares, R. C., Souza, K. P., Añez, G., & Rios, M. (2015). Epidemiological scenario of dengue in Brazil. *BioMed research international*, *2015*.

4. Kyle, J. L., & Harris, E. (2008). Global spread and persistence of dengue. *Annu. Rev. Microbiol.*, *62*, 71-92.

5. Dengue and severe dengue. (2020, March 2). Retrieved March 31, 2020, from https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue

6. Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., Moyes, C. L., ... & Myers, M. F. (2013). The global distribution and burden of dengue. *Nature*, *496*(7446), 504-507.

7. Wilder-Smith, A., & Gubler, D. J. (2008). Geographic expansion of dengue: the impact of international travel. *Medical Clinics of North America*, *92*(6), 1377-1390.

8. Guzman, M. G., Gubler, D. J., Izquierdo, A., Martinez, E., & Halstead, S. B. (2016). Dengue infection. *Nature reviews Disease primers*, *2*(1), 1-25.

9. Vaughn, D. W., Green, S., Kalayanarooj, S., Innis, B. L., Nimmannitya, S., Suntayakorn, S., ... & Nisalak, A. (2000). Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *The Journal of infectious diseases*, *181*(1), 2-9.

10. Whitehorn, J., & Simmons, C. P. (2011). The pathogenesis of dengue. *Vaccine*, *29*(42), 7221-7228.

11. Anderson, J. R., & Rico-Hesse, R. (2006). Aedes aegypti vectorial capacity is determined by the infecting genotype of dengue virus. *The American journal of tropical medicine and hygiene*, *75*(5), 886-892.

12. Kramer, L. D., & Ciota, A. T. (2015). Dissecting vectorial capacity for mosquito-borne viruses. *Current opinion in virology*, *15*, 112-118.

13. Fried, J. R., Gibbons, R. V., Kalayanarooj, S., Thomas, S. J., Srikiatkhachorn, A., Yoon, I. K., ... & Cummings, D. A. (2010). Serotype-specific differences in the risk of dengue hemorrhagic

fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. *PLoS neglected tropical diseases*, *4*(3).

14. Srikiatkhachorn, A., Rothman, A. L., Gibbons, R. V., Sittisombut, N., Malasit, P., Ennis, F. A., ... & Kalayanarooj, S. (2011). Dengue—how best to classify it. *Clinical Infectious Diseases*, *53*(6), 563-567.

15. Dengue - Clinical Presentation. (2019, May 3). Retrieved March 31, 2020, from https://www.cdc.gov/dengue/healthcare-providers/clinical-presentation.html

16. Hadinegoro, S. R. S. (2012). The revised WHO dengue case classification: does the system need to be modified?. *Paediatrics and international child health*, *32*(sup1), 33-38.

17. Kularatne, S. A. (2015). Dengue fever. *Bmj*, *351*, h4661.

18. World Health Organization, Special Programme for Research, Training in Tropical Diseases, World Health Organization. Department of Control of Neglected Tropical Diseases, World Health Organization. Epidemic, & Pandemic Alert. (2009). *Dengue: guidelines for diagnosis, treatment, prevention and control*. World Health Organization.

19. Whitehorn, James; Farrar, Jeremy; (2010) Dengue. British medical bulletin, 95 (1). pp. 161-173.

20. Gubler, D. J. (1998). Dengue and dengue hemorrhagic fever. *Clinical microbiology reviews*, *11*(3), 480-496.

21. Dengue Viruses. (n.d.). Retrieved March 31, 2020, from https://www.nature.com/scitable/topicpage/dengue-viruses-22400925/

22. Dengue Symptoms and Treatment. (2019, September 26). Retrieved March 31, 2020, from https://www.cdc.gov/dengue/symptoms/index.html

23. Shepard, D. S., Undurraga, E. A., Halasa, Y. A., & Stanaway, J. D. (2016). The global economic burden of dengue: a systematic analysis. *The Lancet infectious diseases*, *16*(8), 935-941.

24. World Health Organization. (2012). Global strategy for dengue prevention and control 2012-2020.

25. Dengue, PAHO. Epidemiological Update. 7 February 2020.

26. Moloo, A. (2019, November 21). WHO Region of the Americas records highest number of dengue cases in history; cases spike in other regions. Retrieved March 31, 2020, from https://www.who.int/news-room/detail/21-11-2019-who-region-of-the-americas-records-highest-number-of-dengue-cases-in-history-cases-spike-in-other-regions

27. San, J. M., & Brathwaite-Dick, O. (2007). Integrated strategy for dengue prevention and control in the Region of the Americas. *Revista Panamericana de Salud Pública*, *21*(1), 55-63.

28. Chapman, S. J., & Hill, A. V. (2012). Human genetic susceptibility to infectious disease. *Nature Reviews Genetics*, *13*(3), 175-188.

29. Davi, C., Pastor, A., Oliveira, T., de Lima Neto, F. B., Braga-Neto, U., Bigham, A. W., ... & Acioli-Santos, B. (2019). Severe dengue prognosis using human genome data and machine learning. *IEEE Transactions on Biomedical Engineering*, *66*(10), 2861-2868.

30. Khor, C. C., Chau, T. N. B., Pang, J., Davila, S., Long, H. T., Ong, R. T., ... & Gan, T. T. (2011). Genome-wide association study identifies susceptibility loci for dengue shock syndrome at MICB and PLCE1. *Nature genetics*, *43*(11), 1139.

31. World Mosquito Program: How it works. (n.d.). Retrieved April 2, 2020, from https://www.worldmosquitoprogram.org/en/work/wolbachia-method/how-it-works

32. Dang, T. N., Naka, I., Sa-Ngasang, A., Anantapreecha, S., Chanama, S., Wichukchinda, N., ... & Ohashi, J. (2014). A replication study confirms the association of GWAS-identified SNPs at MICB and PLCE1 in Thai patients with dengue shock syndrome. *BMC medical genetics*, *15*(1), 58.

33. Yakub, I., Lillibridge, K. M., Moran, A., Gonzalez, O. Y., Belmont, J., Gibbs, R. A., & Tweardy, D. J. (2005). Single nucleotide polymorphisms in genes for 2′-5′-oligoadenylate synthetase and RNase L in patients hospitalized with West Nile virus infection. *The Journal of infectious diseases*, *192*(10), 1741-1748.

34. Cordeiro, M. T., Silva, A. M., Brito, C. A., Nascimento, E. J., Magalhães, M. C. F., Guimarães, G. F., ... & Marques Jr, E. T. (2007). Characterization of a dengue patient cohort in Recife, Brazil. *The American journal of tropical medicine and hygiene*, *77*(6), 1128-1134.

35. de Melo, A. B., Maria da Paz, C., Magalhães, M. C. F., Gil, L. H. V. G., de Carvalho, E. M. F., Braga-Neto, U. M., ... & Cordeiro, M. T. (2011). Description of a prospective 17DD yellow fever vaccine cohort in Recife, Brazil. *The American journal of tropical medicine and hygiene*, *85*(4), 739-747.

36. Thisyakorn, U., & Thisyakorn, C. (2014). Latest developments and future directions in dengue vaccines. *Therapeutic advances in vaccines*, *2*(1), 3-9.

37. Teixeira, M. G., Siqueira, J. B., Germano Jr, L. C., Bricks, L., & Joint, G. (2013). Epidemiological trends of dengue disease in Brazil (2000–2010): a systematic literature search and analysis. *PLoS neglected tropical diseases*, *7*(12).

38. Coffey, L. L., Mertens, E., Brehin, A. C., Fernandez-Garcia, M. D., Amara, A., Després, P., & Sakuntabhai, A. (2009). Human genetic determinants of dengue virus susceptibility. *Microbes and infection*, *11*(2), 143-156.

39. Polglase, H. (2015). Validation of the relationship between MRCI polymorphisms with dengue disease in a Brazilian population. Master Thesis, University of Pittsburgh. (Unpublished)

40. Cathcart, E. (2013). Association of single nucleotide polymorphisms of the MRC1 gene and dengue fever in a Brazilian population. Master Essay, University of Pittsburgh.

41. Kristiansen, H., Gad, H. H., Eskildsen-Larsen, S., Despres, P., & Hartmann, R. (2011). The oligoadenylate synthetase family: an ancient protein family with multiple antiviral activities. *Journal of Interferon & Cytokine Research*, *31*(1), 41-47.

42. Thamizhmani, R., & Vijayachari, P. (2014). Association of dengue virus infection susceptibility with polymorphisms of 2'-5'-oligoadenylate synthetase genes: a case–control study. *Brazilian Journal of Infectious Diseases*, *18*(5), 548-550.

43. Whitehorn, J., Chau, T. N. B., Nguyet, N. M., Kien, D. T. H., Quyen, N. T. H., Trung, D. T., ... & Hibberd, M. L. (2013). Genetic variants of MICB and PLCE1 and associations with non-severe dengue. *PloS one*, *8*(3).

44. PLCE1 phospholipase C epsilon 1 [Homo sapiens (human)] - Gene - NCBI. (n.d.). Retrieved April 29, 2020, from https://www.ncbi.nlm.nih.gov/gene/51196

45. MX1 MX dynamin like GTPase 1 [Homo sapiens (human)] - Gene - NCBI. (n.d.). Retrieved April 29, 2020, from https://www.ncbi.nlm.nih.gov/gene/4599

46. Lo, C. (2019, December 16). Dengvaxia in the Philippines: timeline of the dengue dilemma. Retrieved May 6, 2020, from https://www.pharmaceutical-technology.com/features/dangvaxia-philippines/

47. Questions and Answers on Dengue Vaccines. (2018, April 24). Retrieved May 6, 2020, from https://www.who.int/immunization/research/development/dengue_q_and_a/en/