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The Emergence and Spread of Antimalarial Drug Resistance based on Molecular Markers

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ABSTRACT

Malaria, which is caused by the *Plasmodium* parasite, causes an enormous disease burden in regions of Africa and Southeast Asia. Monotherapy drug treatment was used in the 20th century to treat malaria, but malaria quickly generated resistance to this treatment approach. Malaria mortality and morbidity has been significantly reduced since 2000 due to a different treatment, artemisinin-based combination therapy (ACT). But drug resistance to ACTs started to appear in 2008, causing scientists to employ various methods to prevent drug resistance from emerging and spreading. Analyzing parasite molecular markers associated with drug resistance play a major part in determining the best antimalarial drug or therapy for a region. Countries will change their national drug treatment policy based on drug efficacy studies and molecular marker frequency. Here, I analyze how seven molecular markers' frequencies have changed from 1995 to 2020 in six malaria endemic countries. The molecular marker frequencies are then compared to national treatment policy to see if frequencies reflect the policy change.

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Chapter 1: Introduction

Malaria is a dangerous infectious disease caused by *Plasmodium* parasites; during 2019, there were about 229 million cases of malaria resulting in 409,000 deaths.[1] It is a vector-borne disease that is transmitted to people through the bite of a female *Anopheles* mosquito. Malaria is characterized by acute febrile illness with the most common symptoms being vomiting and headaches. It can be categorized as either uncomplicated or severe malaria and if not treated, the disease can cause severe complications and lead to death. The most well-known *Plasmodium* species are *Plasmodium vivax* and *Plasmodium falciparum*. *P. falciparum* has a higher death burden; therefore, this paper will focus on *p. falciparum*.

An issue concerning malaria treatment is drug resistance. Drug resistance occurs when the pathogen evolves to surpass the drug molecular mechanism. In drug resistance, a drug that previously successfully treated a disease, now fails in a high percentage of patients. To track malaria drug resistance, therapeutic efficacy studies (TES) and molecular markers are utilized. TES give an overview of how effective a drug is in a certain population. Molecular markers are associated with antimalarial drugs and show the genomic composition of a parasite population. TES and molecular markers can be used to guide national treatment policy by creating a picture of what drugs could fail and succeed based off the parasite genotypes.

Drug resistance has played an important role in malaria treatment for most of malaria history. The first drug used to treat malaria was quinine, which is a compound that comes from cinchona bark. During World War II, chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) were created to combat malaria. After World War II, the World Health Organization (WHO) launched a global malaria eradication campaign which included the use of CQ, insecticide-

treated bed nets, and DDT, an insecticide. The WHO global eradication malaria campaign failed and resulted in increased prevalence of malaria and drug resistance to chloroquine and sulfadoxine-pyrimethamine.[2] Because of increasing treatment failure, a new treatment was developed to combat malaria, artemisinin-based combination therapies (ACTs). From 2000 to 2017, the incidence rate of malaria decreased by 36%, while the annual death rate decreased by 60%, a huge part of which can be attributed to the use of ACTs and vector control strategies.

1.1: Vector Control Strategies

The decrease in malaria can be attributed to two approaches, antimalaria drugs and vector control. Vector control refers to the methods used to control the intermediate organism carrying the pathogen in vector-borne diseases. In the case of malaria, the *Anopheles* mosquito is the vector for *Plasmodium falciparum*. The mosquito carries the *P. falciparum* parasite and transmits malaria between humans. Two vector control measures for malaria are insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS). Insecticide-treated bed nets are hung over the sleeping area since *Anopheles* tend to bite between dusk and dawn. The insecticide can repel, kill, or sterilize the mosquitos. In Africa, pyrethroid-insecticide treated mosquito nets reduced all-cause mortality by roughly 20% in children younger than 5 years.[3] Another study done in Africa found that ITNs were the most important intervention for the continent; an estimated 68% of the decline in *P. falciparum* prevalence from 2000 to 2015 could be attributed to ITNs.[4]

1.2: Artemisinin-based Combination Therapies

ACTs were introduced in the 2000s and is widely responsible for controlling malaria in Asia as it provides rapid clearance rates.[5,6] Dihydroartemisinin, artesunate, and artemether are drugs that are derived from artemisinin and belong in the artemisinin drug class. In ACTs, artemisinin and artemisinin derived drugs are used in combination with partner drugs from other drug classes. The purpose of combining two drugs for malaria treatment is that malaria parasites will have trouble evolving to resist the drug cocktail because there are two drug mechanisms to overcome. Scientists have done studies to determine what the most efficacious drug combinations are. The ACTs currently used globally are dihydroartemisinin-piperaquine (DHA-PPQ), artesunate-amodiaquine (ASAQ), artemether-lumefantrine (AL), and artesunate-mefloquine (ASMQ). From 2005 to the present, the WHO has recommended ACTs as the first line treatment for malaria globally.

Drug	**Abbreviation**	**Artemisinin derived or partner drug**
Chloroquine	CQ	
Sulfadoxine-pyrimethamine	SP	
Dihydroartemisinin	DHA	Artemisinin-derived
Artesunate	AS	Artemisinin-derived
Artemether	A	Artemisinin-derived
Mefloquine	MQ	Partner drug
Lumefantrine	L	Partner drug
Piperaquine	PPQ	Partner drug
ACTs	**Abbreviation**	
Artesunate-amodiaquine	ASAQ	
Artesunate-mefloquine	ASMQ	
Dihydroartemisinin-piperaquine	DHA-PPQ	
Artemether-lumefantrine	AL	

Table 1: Antimalarial Drug Treatment Abbreviations

1.3: Causes of Drug Resistance

The use of ACTs and insecticide-treated bed nets is a great approach to eliminating malaria but using ACTs and bed nets is not entirely foolproof. As seen in the past, with the use of the drugs as treatment and prevention, drug resistance can occur, which can result in a lack of control of malaria and emergence of new malaria genotypes.

Drug resistance by malaria, otherwise known as antimalarial drug resistance, can occur from a variety of factors. Antimalarial drug resistance is largely defined as drug resistance to ACTs. When counterfeit and substandard drugs are distributed and used to treat patients, it poses a threat to malaria control.[7] Since the drugs are not up to quality standards, the drug efficacy is lower, which leads to treatment failure. Treatment failure leads to a higher chance of the parasite evolving and gaining drug resistance since it has extended exposure to the drug environment.

Some scientists have concluded that low malaria transmission allows resistant parasite populations to emerge more easily because host immunity is lower and drug pressure is higher. However, this theory is not conclusive, as drug resistance has emerged in high transmission areas as well. Surveillance strategies may be adapted for low transmission areas to monitor the drug resistance genotype frequency.[8]

Monotherapy drug treatment can also result in quicker emergence of drug resistance in parasites. Using only a single drug can cause drug resistance to occur because the parasite must evolve to overcome the killing action of one molecule. For example, in Cambodia, expert opinion of individuals working in Southeast Asia at the time, (personal communication) suggest that the Chinese military used artemisinin as a prophylaxis in the 1980s. Unregulated antimalarial drugs in the form of artesunate or artemisinin monotherapy have been available in Cambodia since the 1970s.[9] Unregulated drugs lead to drug resistance because an unregulated

environment can mean that there are various amounts in doses, sales, and length of treatment that are recommended to patients. There are unknown risks to this approach as the treatment plans may not be based on clinical trial data and can result in selection pressure for drug resistance. To avoid unregulated monotherapy, public health professionals have changed the approach in which ACTs are distributed.

ACTs can be available as co-blistered packs or fixed dosed combinations. Co-blistered packs are where the two antimalarial drugs come together, but they are packaged separately. Fixed dose combinations have the ACTs in one tablet. When there are co-blistered packs, this allows for possible use as a monotherapy if only one of the drugs is taken from the blister pack. Although ACTs are the main cause of the decline in malaria morbidity and mortality in the past 20 years, to get closer to malaria elimination, the public health community must ensure that drug resistance does not emerge and spread.

1.4: Managing Drug Resistance

Currently, there are concerns that artemisinin resistance will spread across Africa, where there is the highest malaria incidence. Western Cambodia and the Thailand-Myanmar border are already regions of great concern due to the artemisinin resistance that is occurring there. To delay the spread of antimalarial resistance, public health officials have tested and deployed several strategies.

Currently, the majority of malaria drug treatments use ACTs, which poses a significant risk. If antimalarial drug resistance becomes widespread, there is no alternative group of drugs that can replace ACTs in the same way we were able to replace chloroquine and sulfadoxine

pyrimethamine.[9] A promising alternative are triple ACTs (TACTs), which were tested in a 3-year study in 2018.TACTs are a triple therapy that consists of an artemisinin derivative and two partner drugs. DHA-PPQ plus mefloquine and AL plus amodiaquine were found to have a 98% efficacy rate, be well tolerated, and proven as safe treatments.[10] Although there has not been significant wide-scale testing on TACTs, it is an avenue that may be used in the future if antimalarial drug resistance becomes a forefront problem.

Appropriate treatment and early diagnosis would prevent transmission of malaria parasites and clinical symptoms from progressing. Appropriate treatment, without the use of monotherapies or substandard drugs, would reduce the likelihood of the parasites evolving drug resistance and decrease drug pressure. Many nations have placed rules to ban the sale of monotherapies or provide fixed dose combinations rather than co-blistered packs.[9] In some countries, the transmission of malaria can be primarily attributed to mobile populations. To combat the high percentage of malaria cases in mobile populations, proper access to detection and treatment resources must be given to these communities.[9]

The foundation for successful malaria control is a functioning primary health care system. A robust primary health care system should include knowledgeable, trained village malaria workers or community health workers since they are absolutely vital to delivering malaria-related interventions.[11] As different regions make malaria control plans, they will consider their capabilities for intervention depending on the epidemiology of malaria, health infrastructure, and political stability of the region. An analysis of four countries that all had successful reduction of malaria burden found that they had strong economic growth, political stability, and vertical implementation strategies in common.[12]

Setting goals and collaborating at all levels of government and communities is also part of the malaria strategy that can aid in malaria control. Malaria endemic areas have set malaria elimination goals on the national and regional level.[13] There are also major regional malaria elimination initiatives such as the African Leaders Malaria Alliance (ALMA) and Asia Pacific Leaders Malaria Alliance (APLMA) that have been established. Goal setting and collaboration are facets of public health communication since it shows the importance of malaria control to communities and keeps people active and engaged.

To track and compare the progress of drug efficacy and resistance, surveillance methods are used. Surveillance can be done through therapeutic efficacy studies and molecular monitoring. Therapeutic efficacy studies (TES) show how effective specific drug treatments are in a population. TES can either test the efficacy of one drug or compare a new drug to a currently used drug. In TES, the efficacy of drugs is assessed by the parasite clearance rate. The parasite clearance rate is the rate in which malaria parasite levels decline in the blood after drug treatment. A slow parasite clearance rate is a possible sign of parasite resistance. All modern ACT drug treatments are three days long. After the three days, there is typically a 28 or 42 day follow-up in which a blood sample is taken and the blood is analyzed for the presence of malaria parasites. There are three possible outcomes for these follow-ups. A patient can have an adequate clinical and parasitological response, a reinfection, or recrudescence. An adequate clinical and parasitological response is where the patient has been cured from malaria. Reinfection is where the patient has been bitten again by a different mosquito carrying malaria. Recrudescence occurs when the initial infection was not cleared, and the parasites were able to reproduce again. If a drug has a failure rate of 10%, according to the WHO's standards, the drug is no longer effective for that region and other drugs must be considered as the first line malaria treatment.

The information from TES helps policymakers and public health professionals make optimal decisions for their national malaria treatment programmes and regional guidelines. The purpose of TES is to give a good overview of the state of malaria and how the parasites respond to treatments during the period that the drugs were administered. Although TES can be useful, Ljojle et al. made a point that "therapeutic efficacy studies are not powered to test for the association between parasite genotypes and treatment outcome."[14] This is an important point because TES will show how a population responds to a drug, but it does not give enough insight into the genotype of the parasites that are circulating in the population.[14] Utilizing genotypes, public health professionals identify molecular markers associated with drug resistance to determine which ACT is best for a specific population.

1.5: Using Molecular Markers to Manage Drug Resistance

Using phenotype-genotype association studies, molecular markers can be associated with drug resistance. Once public health professionals see a high amount of treatment failure in response to a certain drug, scientists can use the parasite clinical isolates that were taken from patients' blood during TES and genotype them.

Scientists have identified many molecular markers associated with ACTs and their partner drugs. There are three main types of molecular markers: a mutation, a copy number variation (CNV) and a haplotype. A mutation is a mistake in gene transcription that causes a change in an amino acid in a protein, which creates a different allele. A copy number variation creates more than one copy of part of the genome, which results in a greater expression of the

gene because of the duplicate proteins created. A haplotype is a group of alleles that cannot be broken down by recombination and therefore are often inherited together.

There are dozens molecular markers that are associated with drug resistance, but we are going to focus on 6 molecular markers: *pfk13* C580Y, *pfmdr1* N86Y, *pfmdr1* Y184F, *pfmdr1* CNV, *pfcrt* K76T, and *plasmepsin 2* CNV.[15,16] To identify molecular markers, scientists used parasite isolates and survival assays to determine which genotypes have been selected by drugs. In this situation, the environment, which is the antimalarial drug treatment, exerts selection pressure on the parasite to evade the drug treatment. The selection pressure chooses alleles that give the parasites the best fitness. With malaria, the parasites will either choose the mutant or wild-type allele depending on the drug environment.

Pfkelch13 (pfk13) is a gene on chromosome 13, and mutations on this gene are associated with artemisinin resistance. It was identified when scientists analyzed parasite isolates from Cambodian patients collected in 2001-2012.[15] The molecular marker was prevalent in areas where artemisinin resistance has been established, supported by therapeutic efficacy data in Western Cambodia – Pailin, Battambang, and Pursat.[15] The predominant mutation is C580Y, in which a cysteine on the 580th amino acid changes into a tyrosine.

Pfmdr1 is a gene that has molecular markers that are CNV and mutations. The two major mutations are N86Y, which turns an asparagine into a tyrosine on the 86th amino acid, and Y184F, in which a tyrosine mutates into a phenylalanine on the 184th amino acid. Lumefantrine selects for the wild-type N86 allele and Y184 allele, while amodiaquine and chloroquine selects for the mutant 86Y allele.[17] *Pfcrt* is a gene that also has alleles associated with lumefantrine, chloroquine, and amodiaquine. The major mutation in the pfcrt gene is K76T, in which threonine mutates into a lysine on the 76th amino acid. Similar to pfmdr1 N86Y, lumefantrine selects for

the wild-type K76 allele, while chloroquine and amodiaquine select for the mutant 76T allele.[18,19]

The *plasmepsin 2 (pfpm2)* gene is associated with piperaquine. Due to increasing treatment failures with dihydroartemisinin-piperaquine in Cambodia, a molecular marker was needed so that large-scale surveillance programs could be launched in Cambodia and other countries in Southeast Asia to predict treatment failures.[20] The TRACII study was a multi-country randomized clinical trial conducted from 2015-2018 that was able to highlight the high rate of treatment failure of DHA-PPQ. Because of the molecular marker toolkit that was available by that time, they were able to show that the treatment failure was associated with *pfpm2* and *pfcrt* mutations and make recommendations on what drugs could replace DHA-PPQ.[21]

There have also been larger scale studies that map the prevalence of resistance across continents. Between 2011 and 2013, there was a study that was done across Southeast Asia in 10 different countries. The purpose of the study was to map the extent and severity of artemisinin resistance. They found that *pfk13* SNPs are predictive of slow parasite clearance and that artemisinin resistance is prevalent across Southeast Asia. Additionally, standard three day ACT drug treatments were starting to fail.[22]

Molecular markers are identified to save time, energy, and money to create a molecular toolkit. Molecular toolkits that contain many molecular markers have been created for public health programs to use to assess the best course of treatment in their regions. It can act as surveillance and inform if the drug resistance is contained. It also shows the nature of drug resistance and how it is spreading. Most importantly, it helps health departments decide the best drug choice to treat malaria patients by informing them which drugs the parasite may be resistant

to. This prevents continuous funds being poured into therapeutic efficacy programs and calling in patients to collect blood samples. The blood samples also require microscopic analysis to determine if there was parasite clearance. Instead, if specific SNP regions that are commonly associated with drug resistance of the parasite clinical isolates are analyzed, scientists can determine what drugs effectively against the parasite without testing them on the population.

There is no such thing as a resistance proof therapy. However, we can use genetics to combine drugs that generate opposite selection pressures on the same target.[8] Certain parasite genes are pleiotropic, meaning they have more than one phenotype. For example, as mentioned earlier, lumefantrine selects for K76, the wild-type allele, while amodiaquine selects for the the mutant 76T allele. Deploying AL and ASAQ would create opposite selection pressures for the 76th amino acid, delaying the emergence of resistance.

An alternative method to analyzing molecular markers is to rotate drug treatment options. In the past, countries have changed antimalarial drug treatments, but only after a high amount of treatment failure was shown. An example of this can be seen in Cambodia in 2008, where certain regions switched to DHA-PPQ because there was high treatment failure to ASMQ. Preplanning out the drug therapies to be switched instead of waiting until there is high drug failure to switch may be a more efficient strategy. Rotating drug therapies before there is high treatment failure could prevent the parasite from evolving drug resistance to multiple drugs since it would not have time to select for the specific drug resistant genotype.

Information on molecular markers is continuously collected, tracked, and analyzed through molecular surveillance systems. However, molecular surveillance is not an easy system to maintain because there are several technical challenges in setting up a molecular surveillance system as it involved implementing genotyping in-country. There are molecular surveillance

projects such as SpotMalaria and GenRe-Mekong whose main objectives are to build in-country processing capacity for molecular surveillance.[6] A strong genetic surveillance system would extend the processing capacity in countries and deliver more timely products.[6] On a broader sense, understanding *P. falciparum's* genetics and keeping track of the changes in the genome could provide a map for monitoring molecular markers on a global level. The information can also be used for genetic modeling which would allow scientists to keep an eye on the progress towards malaria elimination.[23]

Antimalarial	Gene	Molecular Marker
Artemisinin derivatives	*pfk13*	C580Y
Piperaquine	*pfpm2*	CNV
Mefloquine	*pfmdr1*	CNV
Lumefantrine	*pfmdr1*	N86Y Y184F
	pfcrt	K76T
Chloroquine	*pfcrt*	K76T
	pfmdr1	N86Y
Amodiaquine	*pfcrt*	K76T
	pfmdr1	N86Y

Table 2: Molecular markers associated with antimalarial drugs

Molecular markers associated with antimalarial drug resistance. Underlined letter for molecular marker corresponds to the amino acid that is selected for under drug pressure. The letter before the number is the wild-type and the letter after is the mutant genotype.

1.6: National Malaria Treatment Programmes 1995-2020

On a national level, countries have implemented national malaria control programmes in which they will assess the malaria control strategy to decide what is working and what needs to change. Countries change their national malaria control programmes based on a variety of factors, including the amount of drug resistance and treatment failure there is to the current drug treatment. More specifically, the WHO recommends that if the first-line treatment from the national malaria treatment programme has 10% or more treatment failure, the treatment should be changed.[24] Looking at how drug policies have changed over time and comparing it to molecular marker prevalence can show how successful malaria control strategies are working. The six countries that I have analyzed are Rwanda, Senegal, Kenya, Vietnam, Cambodia, and Myanmar, which are all malaria endemic countries.

Starting in 2002, ACT was recommended nationally in Myanmar as the first-line treatment for malaria. In 2008, there were signs of artemisinin resistance, which prompted a change in the drug policy to recommend AL and DHA-PPQ instead of ASMQ.[25] In 2014, it was re-emphasized that AL, DHA-PPQ, and ASMQ be used as fixed-dosed combination ACTs for *P. falciparum* cases. Artemisinin monotherapy was also removed as a recommendation as second-line drug treatment. Today, AL, DHA-PPQ and ASMQ continue to be recommended as first-line treatments for malaria.[25]

Vietnam was one of the first countries that recommended artemisinin-based combination therapies as first line treatments on a national level.[26] Vietnam started their widespread antimalarial drug coverage in 1991 and implemented many treatment guidelines from 1991 to 2003. Uncomplicated malaria was treated with chloroquine and primaquine, but uncomplicated cases showing chloroquine resistance had other treatment guidelines, which included SP. From

2003 to 2007, the guidelines were changed to AS plus primaquine or DHA-PPQ. Since 2009, uncomplicated malaria has been treated with DHA-PPQ plus primaquine.[27] Vietnam is credited with carrying out a successful malaria program that included intense investment into malaria, a quick transition to artemisinin-based treatments, and a centralized system.[12,26] Due to Vietnam's malaria program, there was a 98.3% decrease in incidence from 1991 to 2014 and has since kept a low incidence rate.[27]

In 2000, Cambodia became the first country to switch to ASMQ. In 2007, artemisinin resistance was detected.[28] High failure rates with ASMQ were soon found, and in 2008, the national policy changed from co-blistered ASMQ to fixed dose DHA-PPQ in regions where ASMQ was failing. In 2009, DHA-PPQ was made the nationwide first-line treatment for malaria. The national treatment guidelines were updated again in 2014, substituting DHA-PPQ with ASMQ where DHA-PPQ had high treatment failures and switching from co-blistered to fixed dosed combination treatment methods.[29] Today, Cambodia utilizes ASMQ and the triple ACT, DHA-PPQ, and primaquine as the first line treatment for malaria.

Starting in 1999, Rwanda joined the East Africa network for Monitoring Antimalarial Treatment (EANMAT).[30] EANMAT found that there was a clinical failure of CQ and SP. Because of this discovery, the Rwandan Ministry of Health changed its national treatment policy from CQ to amodiaquine plus sulfadoxine pyrimethamine in 2001. In 2005, there was an ACT scale-up that aimed for universal drug coverage. In 2006, there was a change from amodiaquine plus sulfadoxine pyrimethamine to AL due to an increase in antimalarial drug resistance. As of 2018, Rwanda uses AL as the first line treatment for malaria.[31]

Chloroquine was used in Senegal until 2003 as the primary treatment and was replaced by amodiaquine plus sulfadoxine pyrimethamine. By 2006, Senegal initiated a nationwide

scaling up program of ACT. A reemphasis of AL and ASAQ as first line treatments were done in 2010.[32] From 2013 to 2018, AL and ASAQ were used as first-line treatments and DHA-PPQ was used as a second-line treatment for malaria.[33]

In Kenya, from 1998 to 2004, SP was used as the first line treatment for malaria. After 2004, the national policy was shifted to AL as the first line treatment. Even though there was distribution of AL, SP drug use continued and was at 11% in 2011 and 12% in 2016 of all antimalarial drug used according to a survey.[34] Since then, AL has continued to be the first line treatment in Kenya.[31] The most recent global WHO recommendation for treating uncomplicated *P. falciparum* malaria is for the use ACTs as the first-line treatment, of which include AL, ASAQ, ASMQ, DHA-PPQ, and AS+SP.[35] Figure 1 summarizes the changes in national antimalarial treatment policy from 1995 to 2020 for Myanmar, Vietnam, Cambodia, Senegal, Kenya and Rwanda.

Scientists have identified that artemisinin resistance is prevalent in Southeast Asia and has started spreading across Sub-Saharan Africa, but ACTs are currently still efficacious.[22] Understanding the frequency of molecular markers can help inform national control malaria programs, which then affects drug usage and controls drug resistance. Although the frequency of markers has been measured by country, a comparison and summary for countries is yet to be examined. Looking at data from Kenya, Rwanda, Senegal, Vietnam, Cambodia, and Myanmar, which are central to the malaria issue, can provide an overview of the current state of malaria. I sought to plot and fit a model for the frequency of *P. falciparum* genotypes in the six countries and correlate them to national antimalarial treatment policy changes from 1995 to 2020.

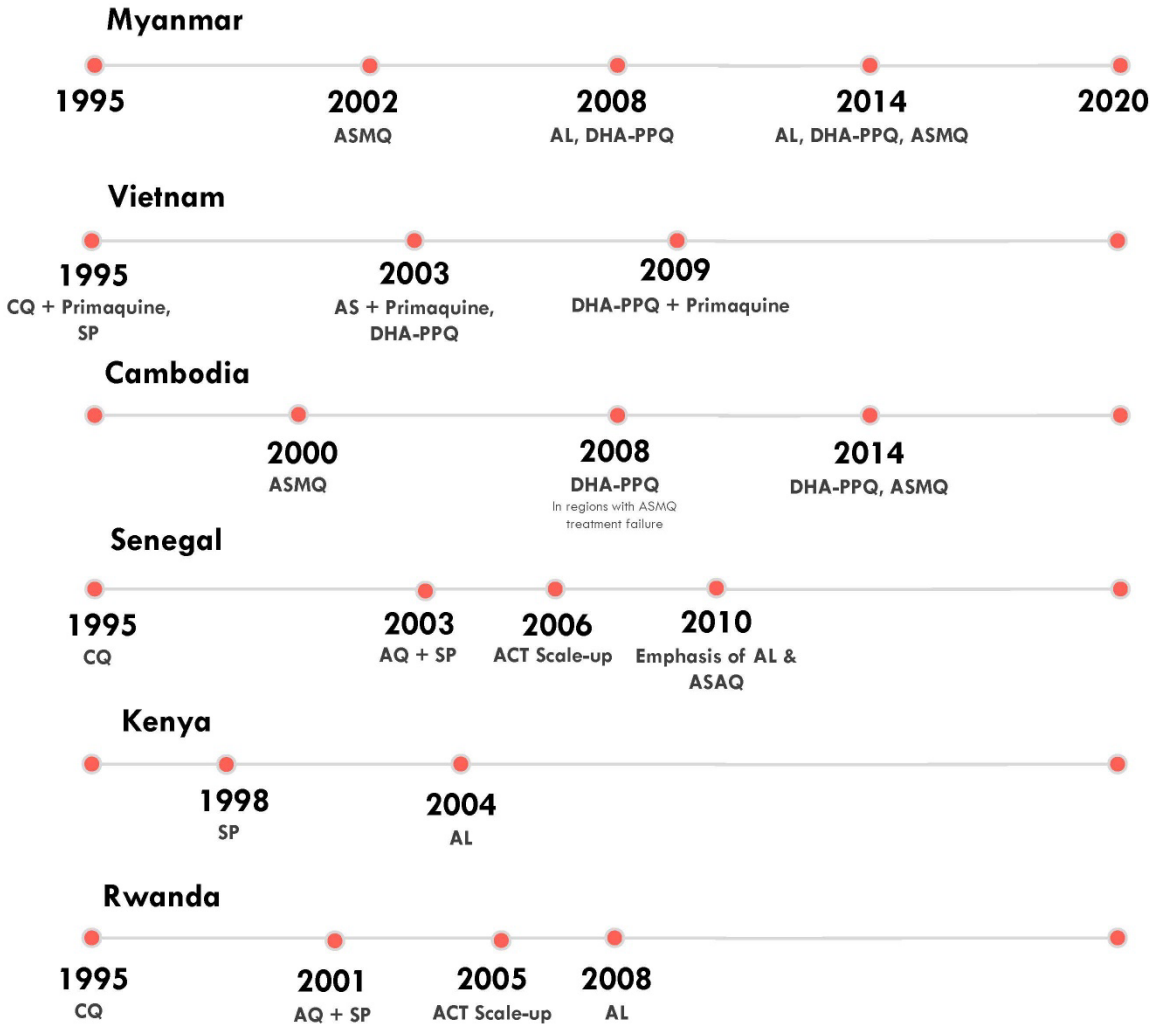

Figure 1: Summary of national malaria treatment policy changes in Myanmar, Vietnam, Cambodia, Senegal, Kenya, and Rwanda from 1995 to 2020.

Chapter 2: Genotype Level Results

The R-squared value is expected to be small for all linear regression models and that is normal because these samples were not collected systematically. Rwanda did not have enough data on the molecular markers studied to create a plot and fit a linear regression model.

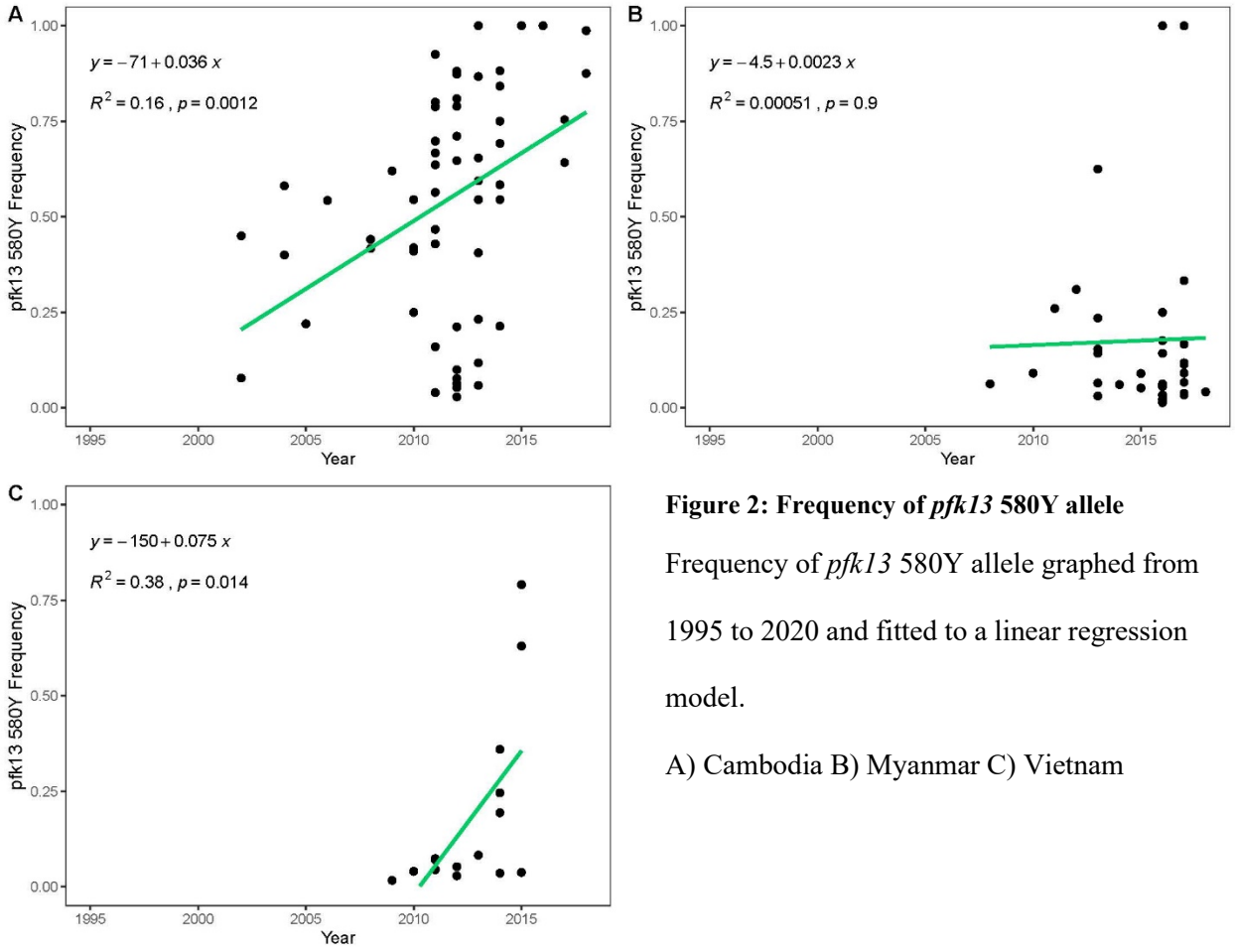

Figure 2: Frequency of *pfk13* 580Y allele

Frequency of *pfk13* 580Y allele graphed from 1995 to 2020 and fitted to a linear regression model.

A) Cambodia B) Myanmar C) Vietnam

2.1: *pfk13* 580Y Results

From 2000 to 2015, according to the line of best fit from linear regression, there was an increase in *pfk13* 580Y frequency in Cambodia (Figure 2A). After 2005, there was a sharp increase in *pfk13* 580Y frequency in Vietnam (Figure 2B). The relationship between the year and

pfk13 580Y frequency is significant in Cambodia (p = 0.0012) and Vietnam (p = 0.014). *pfk13* 580Y frequency points for Myanmar do not have relationship with the year (Figure 2B). The points are scattered and have a slope closer to 0 in comparison to the other two *pfk13* graphs. There is no significance between the year and frequency for Myanmar.

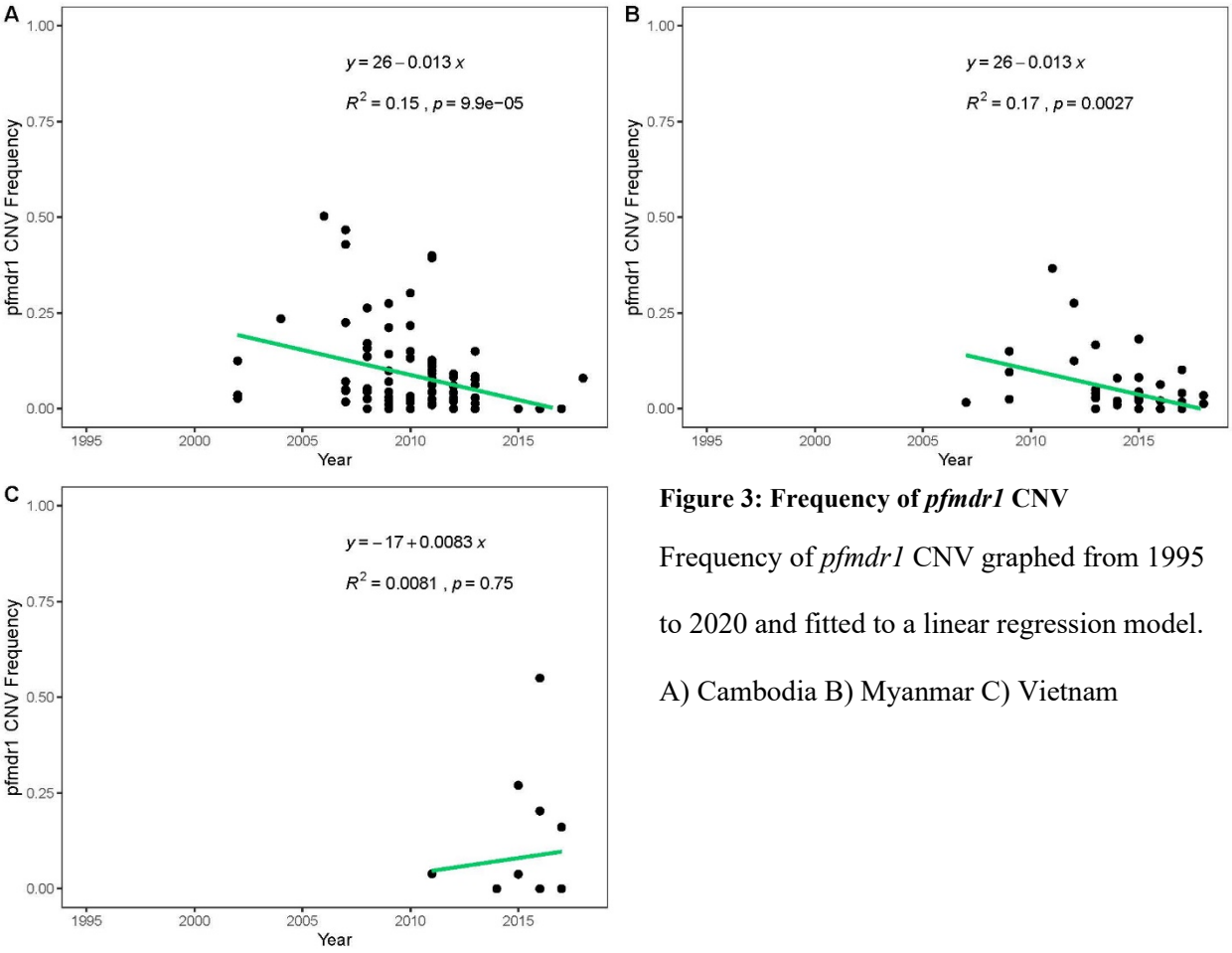

Figure 3: Frequency of *pfmdr1* CNV

Frequency of *pfmdr1* CNV graphed from 1995 to 2020 and fitted to a linear regression model. A) Cambodia B) Myanmar C) Vietnam

2.2: *pfmdr1* CNV Results

There was a decrease in *pfmdr1* CNV frequency from 2000 to 2015 in Cambodia and Myanmar (Figure 3A-B). The decline in *pfmdr1* CNV frequency started after 2000 in Cambodia and after 2005 in Myanmar. The linear regression shows significance for the relationship

between year and frequency for both Cambodia (p = 0.000099) and Myanmar (p = 0.0027). The points on the graph for Vietnam are grouped towards 2010 to 2020, resulting in a short linear regression line. The *pfmdr1* CNV frequency data from Vietnam does not fit a linear regression model well. The linear regression model for Vietnam does not have significance (Figure 3C).

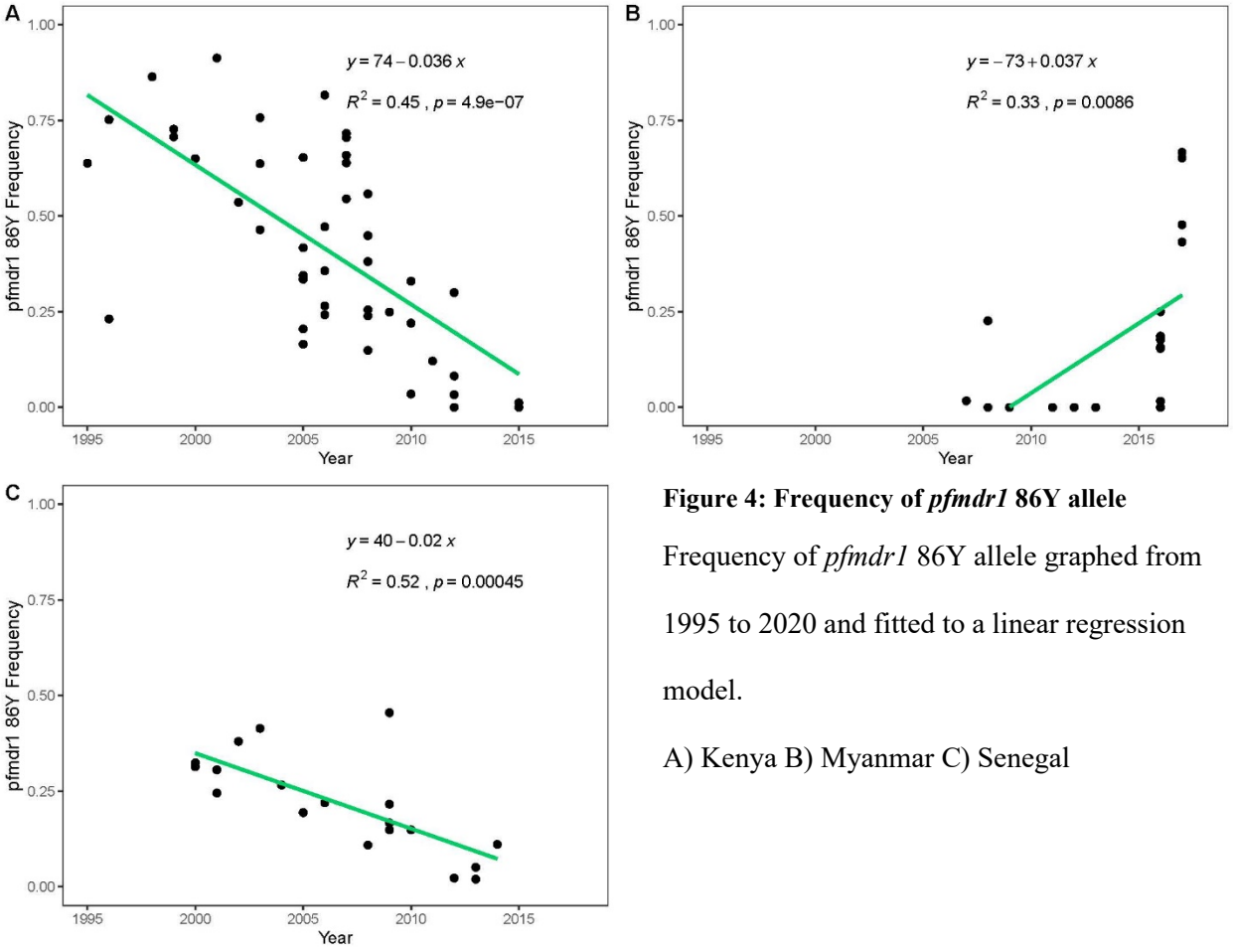

Figure 4: Frequency of *pfmdr1* 86Y allele

Frequency of *pfmdr1* 86Y allele graphed from 1995 to 2020 and fitted to a linear regression model.

A) Kenya B) Myanmar C) Senegal

2.3: *pfmdr1* 86Y Results

The *pfmdr1* 86Y graphs for Kenya and Senegal have negative trendlines (Figure 4A, 4C). The *pfmdr1* 86Y graph for Kenya contains a steeper slope and more points in comparison to Senegal's (Figure 4A, 4C). The linear regression model for Myanmar has a positive trend (Figure

4B). Myanmar did not see in increase in prevalence until after 2005. All three graphs displaying *pfmdr1* 86Y frequencies are significant ($p < 0.05$).

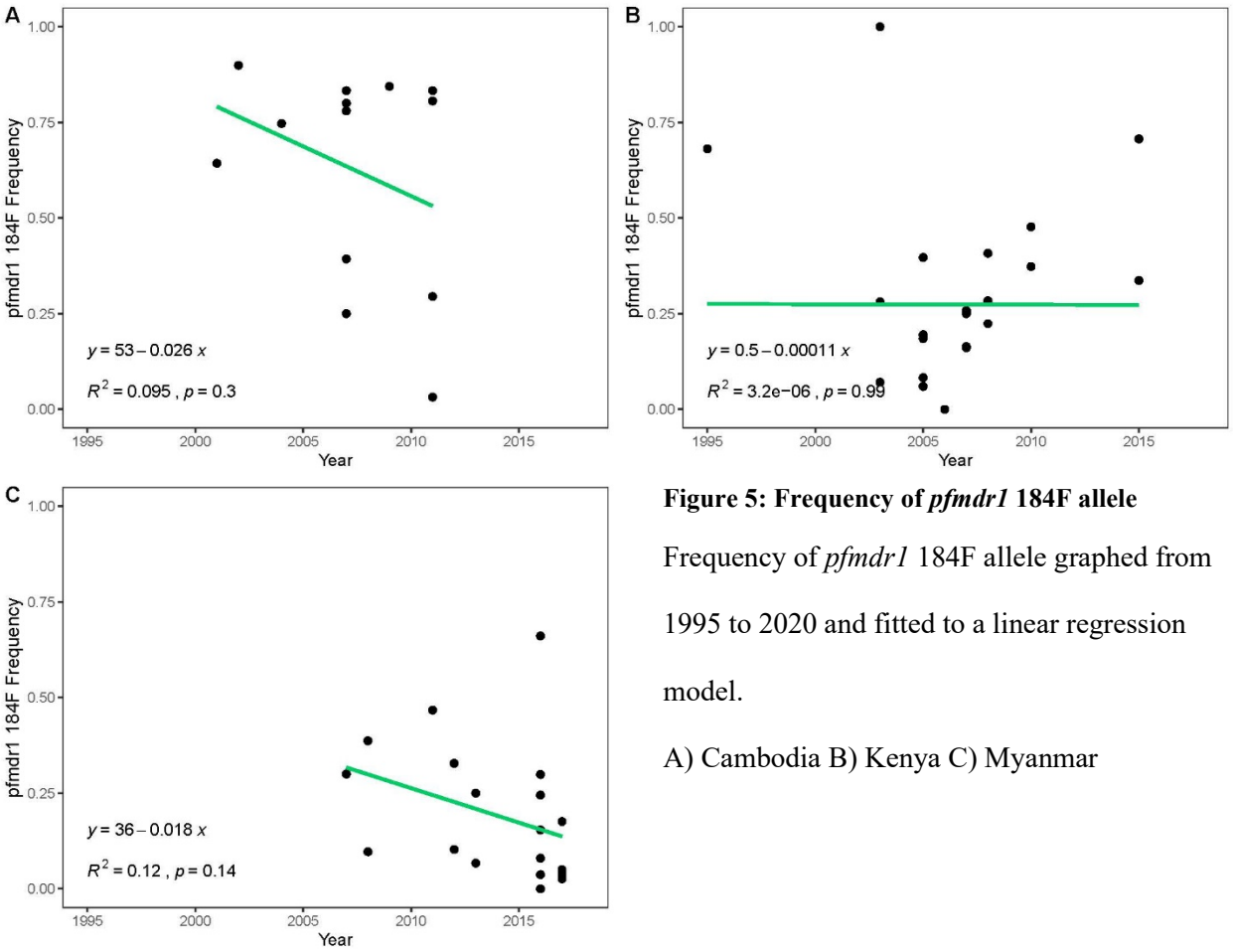

Figure 5: Frequency of *pfmdr1* 184F allele

Frequency of *pfmdr1* 184F allele graphed from 1995 to 2020 and fitted to a linear regression model.

A) Cambodia B) Kenya C) Myanmar

2.4: *pfmdr1* 184F Results

There was not much change in frequency in Kenya, as shown by the linear regression line of best fit (Figure 5B). The *pfmdr1* 184F data from Cambodia and Myanmar shows a slight decrease in frequency (Figure 5A, 5C). None of the *pfmdr1* 184F graphs contain significance (Figure 5).

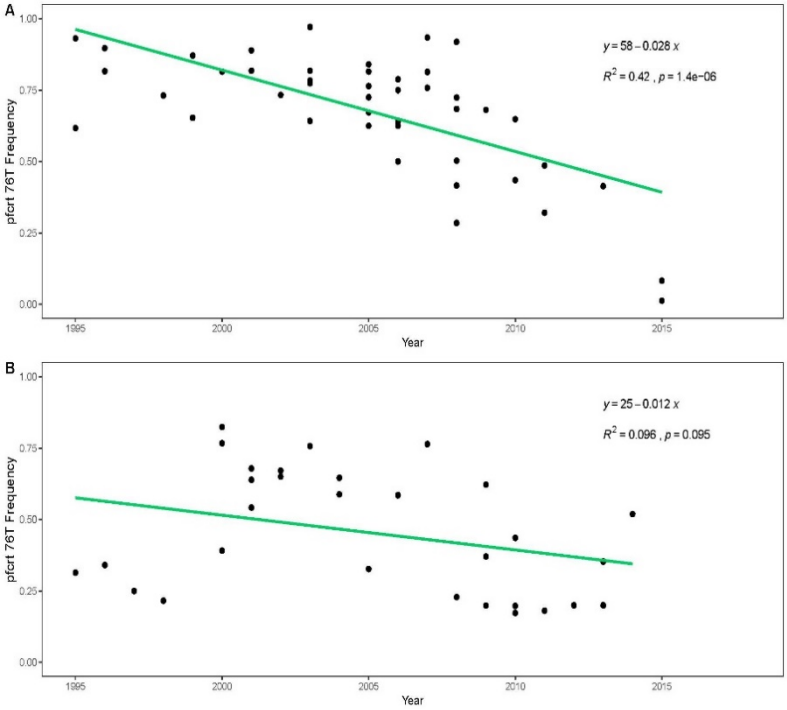

Figure 6: Frequency of *pfcrt* 76T allele

Frequency of *pfcrt* 76T allele graphed from 1995 to 2020 and fitted to a linear regression model.

A) Kenya B) Senegal

2.5: *pfpm2* CNV & *pfcrt* 76T Results

Only the *pfcrt* 76T frequency plot for data from Kenya is significant (p = 0.0000014). The *pfcrt* 76T frequency graphs have a decrease in molecular marker frequency from 1995 to 2015 in both countries (Figure 6). *Pfpm*2 CNV frequency in Vietnam had a significant large increase after 2010 (Figure 7). The linear regression model for *pfpm2* CNV has the steepest slope out of all the genotype graphs (Figure 7).

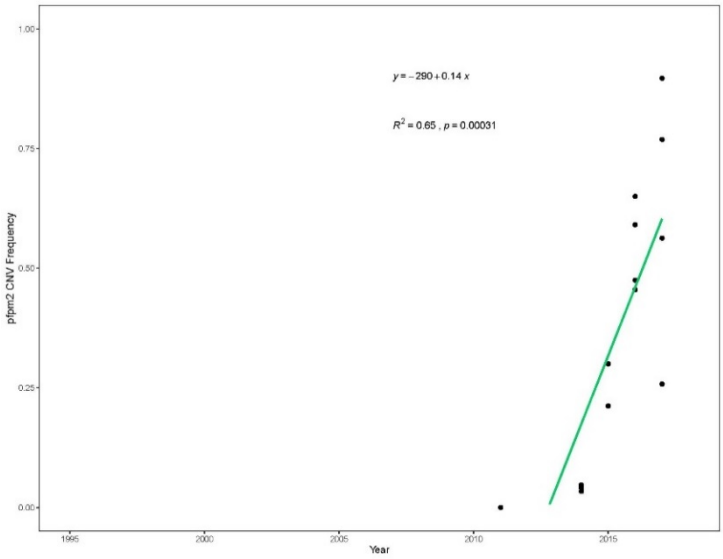

Figure 7: Frequency of *pfpm2* CNV

Frequency of *pfpm2* CNV in Vietnam graphed from 1995 to 2020 and fitted to a linear regression model.

2.6: Results by Country

In Cambodia, there was an increase in *pfk13* 580Y frequency and a decrease in *pfmdr1* CNV frequency, both starting in 2002 (Figure 2, Figure 3). In Kenya, there were decreases in *pfmdr1* 86Y and *pfcrt* 76T frequency, both beginning in 1995 (Figure 4, Figure 6). In Myanmar, there was an increase in *pfmdr1* 86Y frequency, and decrease in *pfmdr1* CNV frequency, which started in 2007 (Figure 2, Figure 4). In Senegal, there was a decrease in *pfmdr1* 86Y frequency beginning in 2000 (Figure 4). In Vietnam, there was an increase in *pfk13* 580Y and *pfpm2* CNV, which began in 2009 and 2011, respectively (Figure 2, Figure 7).

		Cambodia	Kenya	Myanmar	Senegal	Vietnam
Artemisinin	*pfk13* 580Y	Increase*		No correlation		Increase*
Mefloquine	*pfmdr1* CNV	Decrease*		Decrease*		Increase
Amodiaquine	*pfmdr1* 86Y		Decrease*	Increase*	Decrease*	
Piperaquine	*pfpm2* CNV					Increase*
Chloroquine, Amodiaquine	*pfcrt* 76T		Decrease*		Decrease	

Table 3: Relationship between molecular marker and year

A summary of the direction of the linear regression coefficients referring the relationship between molecular marker and year from 1995 to 2020. The drug that is associated with the molecular marker is in the leftmost column. An asterisk denotes that the data is significant.

Chapter 3: Discussion

Based off the available public data, scatterplots fitted with linear regressions for molecular marker frequencies were able to be made for six malaria endemic countries in Southeast Asia and Africa. The linear regression trends correlated well when compared to national treatment policy from 1995-2020. Due to the lack of data available for all molecular markers examined, there were not any plots created on molecular markers in Rwanda.

According to their national treatment policy, Cambodia has recommended either DHA-PPQ or ASMQ as their first-line treatment based on the amount of treatment failure in the region. An increase in *pfk13* 580Y is supported by the national treatment policy and is expected. This is an expected result because artemisinin-based drugs, whether it be dihydroartemisinin or artesunate, have been used in Cambodia since 2000; therefore, an increase in the associated molecular marker is not surprising. A decrease in mefloquine-associated *pfmdr1* CNV is plausible, since the scatterplot points decrease after 2008, which is when the first-line treatment switched from ASMQ to DHA-PPQ in some areas.[29]

During 2004, in Kenya, AL was recommended as the first line treatment. Lumefantrine selects for the wild-type for *pfmdr1* N86 allele and the wild-type *pfcrt* K76 allele (Table 2). The linear regression trends for Kenya show a decrease in the mutant alleles, *pfmdr1* 86Y and *pfcrt* 76T. The trends match up with the policy since AL puts selection pressure on the wild-type, therefore decreasing the frequency of the mutant allele. In Myanmar, according to the national treatment policy, in 2008, there was a shift to AL and DHA-PPQ from ASMQ. Therefore, it would be expected that there could be a decrease in *pfmdr1* CNV and *pfmdr1* 86Y because of the decrease in mefloquine use and increase in lumefantrine use in the years after. My data shows

that there was a significant decrease in *pfmdr1* CNV, but an increase in *pfmdr1* 86Y, which does not match the expected results based off national treatment policy. On the other hand, while the increase in *pfmdr1* 86Y is significant, there are not many data points before 2016. Thus, the data may not be representative of the molecular marker frequency during those years.

The *pfmdr1* 86Y frequency for Senegal decreased starting in 2000. This trend does not match the national treatment policy, as AQ + SP was recommended in 2003 and ASAQ was recommended in 2010. Both drug treatments contain amodiaquine, which is associated with *pfmdr1* 86Y (Table 2). A possible explanation is that since there was a scale-up of ACT in 2006 and an emphasis of AL in 2010, lumefantrine selected for the *pfmdr1* N86 allele, thus affecting the *pfmdr1* 86Y frequency. However, since those policy changes were not in place until well after 2000, it does not explain the decrease in frequency from 2000 to 2006. Vietnam was one of the first countries to start using ACTs; therefore it is expected that there would be an increase in *pfk13* 580Y frequency, which is what my figure shows. In addition, the increase in *pfpm2* CNV is expected since Vietnam has been using DHA-PPQ since 2003, and piperaquine is associated with *pfpm2* CNV.

Statistical analysis was unable to be performed between national treatment policy and molecular marker frequency because a proper experimental study cannot be done. Looking at the relationship between treatment policy changes and frequency would require multiple countries with the exact same conditions. While comparisons can be made between countries, there are many confounding factors such as political stability, health infrastructure and the epidemiology of malaria in the region.

The limitations of this study were that there was a lack of data in certain years especially before 2005. Only linear and logistic regression models were tested on the data, there are other

models that could be fitted to the data and may fit better. In the future, a deeper analysis based on regions within countries could be done because geographical features could be barriers for transmission across areas of the country, resulting in variations in molecular marker frequencies.

The molecular markers are a good reflection of antimalarial resistance and drug pressures. The national antimalarial drug treatment policy changes generally matched the changes in molecular marker frequency. Molecular markers are an important part of the malaria control toolkit. With knowledge of molecular markers, health professionals can determine the important genetic components of the malaria parasites circulating the population and decide the best course of action for antimalarial drug treatment. Prevention of emergence and spread of malaria drug resistance is crucial to eliminating malaria.

Chapter 4: Methods

When looking for data, I was looking for a database with *P. falciparum* molecular marker frequency data that was stratified by country from 1995 to 2020. The WorldWide Antimalarial Resistance Network (WWARN) collaborative platform was used to access to two databases: the ACT Partner Drug Molecular Surveyor and the Artemisinin Molecular Surveyor. These two databases were used to access genotype frequency data for *P. falciparum*.

4.1: ACT Partner Drug Molecular Surveyor

The ACT Partner Drug Molecular Surveyor provides resistance marker data for ACT partner drug treatments.[36] The database was filtered to include all the drugs and the markers *pfcrt* 76T, *pfcrt* K76, *pfcrt* 76K/T, *pfmdr1* 86Y, *pfmdr1* N86, *pfmdr1* 86N/Y, *pfmdr1* 184F, *pfmdr1* Y184, *pfmdr1* 184Y/F, *pfmdr1* copy number >1, and *pfpm* copy number >1. The database was also filtered to include only study sites in Kenya, Senegal, Rwanda, Vietnam, Cambodia, and Myanmar. Additionally, only studies from 1995 to 2020 with a minimum sample size of 20 were included. Table 4 outlines the columns that were removed because the information was unnecessary for the objective of this paper. The ACT Partner Drugs that were used in these studies were either amodiaquine, lumefantrine, piperaquine, or mefloquine. Most of the drug treatments were administered following a 3-day course following WHO guidelines.

Category	Removed from dataset
study ID	X
site number	X
country	

site	
longitude	X
latitude	X
study start date	
study end date	
marker type	
tested	
present	
mixed present	X
author	
publication year	
publication URL	
title	X
notes	X
PubMed Id	
drug	
marker	
group	
percentage	
included or excluded	X

Table 4: ACT Partner Drug Molecular Surveyor Data Categories

4.2: Artemisinin Molecular Surveyor

The Artemisinin Molecular Surveyor provides resistance marker data for artemisinin-derived drug treatments.[37] The database was filtered to include data that was associated with slow clearance and study sites in Kenya, Senegal, Rwanda, Vietnam, Cambodia, and Myanmar. Additionally, only studies from 1995 to 2020 with a minimum sample size of 20 were included. It provides the data stratified by continent, val, year, tested, previous, longitude, title, PubMed

ID, SID, mutation, site, estLoc, present, latitude, and authors. Table 5 outlines the columns that were removed because the information was unnecessary for the objective of this paper.

Category	Removed from dataset
continent	
val	X
year	
tested	
previous	
longitude	X
title	
PubMed ID	X
SID	X
mutation	
site	
estLoc	X
present	
latitude	X
authors	

Table 5: Artemisinin Molecular Surveyor Categories

4.3: Fitting Data to Linear Regression

The data was separated by country and genotype using R software.[38] From there, the data was analyzed and datasets that had less than 10 points were not used. The remaining datasets were graphed on scatterplots and fitted to the linear regression model. The packages reshape2, ggplot2, ggpubr and patchwork were used to create plot and fit the data to a linear regression model in R.[39,40,41] The study start date was the independent variable and the percentage was the dependent variable for all the models. Percentage was converted to frequency in the models by

dividing percentage by 100 for all the models. The following is a line of R code used to create one of the graphs; the same base code was used for all the datasets:

```
p76t.k = ggplot(pfcrt_K76T.AQ.K, aes(x=study.start, y = real.percentage/100)) +
geom_point(size = 2, color = "black") +xlab('Year')+ylab('pfcrt 76T Frequency')+theme_bw() +
my_theme +geom_smooth(method= "lm", se = FALSE , color = "springgreen3")+ ylim(0,1)+
xlim(1995, 2018)+ stat_cor(label.x = 2013, label.y = .8, aes(label = paste(..rr.label.., ..p.label..,
sep = "~`,`~")))+ stat_regline_equation(label.x = 2013, label.y = .9)
```

References

1. Malaria. Published 2020. https://www.who.int/news-room/fact-sheets/detail/malaria#:~:text=Disease burden,411 000 deaths in 2018.
2. Payne D. Spread of chloroquine resistance in Plasmodium falciparum. *Parasitol Today*. 1987;3(8):241-246. doi:https://doi.org/10.1016/0169-4758(87)90147-5
3. Phillips-Howard PA, Nahlen BL, Kolczak MS, et al. Efficacy of permethrin-treated bed nets in the prevention of mortality in young children in an area of high perennial malaria transmission in western Kenya. *Am J Trop Med Hyg*. 2003;68(4 SUPPL.):23-29. doi:10.4269/ajtmh.2003.68.23
4. Bhatt S, Weiss DJ, Cameron E, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. *Nature*. 2015;526(7572):207-211. doi:10.1038/nature15535
5. Adjuik M, Babiker A, Garner P, Olliaro P, Taylor W, White N. Artesunate combinations for treatment of malaria: meta-analysis. *Lancet (London, England)*. 2004;363(9402):9-17. doi:10.1016/s0140-6736(03)15162-8
6. Noviyanti R, Miotto O, Barry A, et al. Implementing parasite genotyping into national surveillance frameworks: Feedback from control programmes and researchers in the Asia-Pacific region. *Malar J*. 2020;19(1):1-20. doi:10.1186/s12936-020-03330-5
7. Newton PN, Fernández FM, Plançon A, et al. A collaborative epidemiological investigation into the criminal fake artesunate trade in South East Asia. *PLoS Med*. 2008;5(2):0209-0219. doi:10.1371/journal.pmed.0050032
8. Blasco B, Leroy Di, Fidock DA. Antimalarial drug resistance: Linking Plasmodium falciparum parasite biology to the clinic. *Nat Med*. 2017;23(8):917-928.

doi:10.1038/nm.4381

9. Dondorp AM, Yeung S, White L, et al. Artemisinin resistance: Current status and scenarios for containment. *Nat Rev Microbiol*. 2010;8(4):272-280. doi:10.1038/nrmicro2331

10. van der Pluijm RW, Tripura R, Hoglund RM, et al. Triple artemisinin-based combination therapies versus artemisinin-based combination therapies for uncomplicated Plasmodium falciparum malaria: a multicentre, open-label, randomised clinical trial. *Lancet*. 2020;395(10233):1345-1360. doi:10.1016/S0140-6736(20)30552-3

11. Dondorp AM, Smithuis FM, Woodrow C, Seidlein L von. How to Contain Artemisinin- and Multidrug-Resistant Falciparum Malaria. *Trends Parasitol*. 2017;33(5):353-363. doi:10.1016/j.pt.2017.01.004

12. Barat LM. Four malaria success stories: How malaria burden was successfully reduced in Brazil, Eritrea, India, and Vietnam. *Am J Trop Med Hyg*. 2006;74(1):12-16. doi:10.4269/ajtmh.2006.74.12

13. Feachem RGA, Chen I, Akbari O, et al. Malaria eradication within a generation: ambitious, achievable, and necessary. *Lancet*. 2019;394(10203):1056-1112. doi:10.1016/S0140-6736(19)31139-0

14. Ljolje D, Dimbu PR, Kelley J, et al. Prevalence of molecular markers of artemisinin and lumefantrine resistance among patients with uncomplicated Plasmodium falciparum malaria in three provinces in Angola, 2015. *Malar J*. 2018;17(1):1-7. doi:10.1186/s12936-018-2233-5

15. Ariey F, Witkowski B, Amaratunga C, et al. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. *Nature*. 2014;505(7481):50-55. doi:10.1038/nature12876

16. Witkowski B, Duru V, Khim N, et al. A surrogate marker of piperaquine-resistant Plasmodium falciparum malaria: a phenotype–genotype association study. *Lancet Infect Dis*. 2017;17(2):174-183. doi:10.1016/S1473-3099(16)30415-7

17. Veiga MI, Dhingra SK, Henrich PP, et al. Globally prevalent PfMDR1 mutations modulate Plasmodium falciparum susceptibility to artemisinin-based combination therapies. *Nat Commun*. 2016;7(May). doi:10.1038/ncomms11553

18. Tinto H, Guekoun L, Zongo I, Guiguemdé RT, D'Alessandro U, Ouédraogo JB. Chloroquine-resistance molecular markers (Pfcrt T76 and Pfmdr-1 Y86) and amodiaquine resistance in Burkina Faso. *Trop Med Int Heal*. 2008;13(2):238-240. doi:10.1111/j.1365-3156.2007.01995.x

19. Warhurst DC. A Molecular Marker for Chloroquine-Resistant Falciparum Malaria. *N Engl J Med*. 2001;344(4):299-302. doi:10.1056/nejm200101253440411

20. Amato R, Lim P, Miotto O, et al. Genetic markers associated with dihydroartemisinin–piperaquine failure in Plasmodium falciparum malaria in Cambodia: a genotype–phenotype association study. *Lancet Infect Dis*. 2017;17(2):164-173. doi:10.1016/S1473-3099(16)30409-1

21. van der Pluijm RW, Imwong M, Chau NH, et al. Determinants of dihydroartemisinin-piperaquine treatment failure in Plasmodium falciparum malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. *Lancet Infect Dis*. 2019;19(9):952-961. doi:10.1016/S1473-3099(19)30391-3

22. Ashley EA, Dhorda M, Fairhurst RM, et al. Spread of Artemisinin Resistance in Plasmodium falciparum Malaria . *N Engl J Med*. 2014;371(5):411-423. doi:10.1056/nejmoa1314981

23. Daniels RF, Schaffner SF, Wenger EA, et al. Modeling malaria genomics reveals transmission decline and rebound in Senegal. *Proc Natl Acad Sci U S A*. 2015;112(22):7067-7072. doi:10.1073/pnas.1505691112

24. *No Title*.; 2015.

25. WHO. *External Evaluation of the National Malaria Control Programme Myanmar 2016*.; 2017. https://apps.who.int/iris/handle/10665/272395

26. Peak CM, Thuan PD, Britton A, et al. Measuring the association between artemisinin-based case management and malaria incidence in southern Vietnam, 1991-2010. *Am J Trop Med Hyg*. 2015;92(4):811-817. doi:10.4269/ajtmh.14-0461

27. Goldlust SM, Thuan PD, Duy Hoang Giang D, et al. The decline of malaria in Vietnam, 1991-2014. *bioRxiv*. Published online 2017. doi:10.1101/151456

28. Novotny J, Singh A, Dysoley L, Sovannaroth S, Rekol H. Evidence of successful malaria case management policy implementation in Cambodia: results from national ACTwatch outlet surveys. *Malar J*. 2016;15(1). doi:10.1186/s12936-016-1200-2

29. The National Institute of Malaria in Cambodia. National Treatment Guidelines for Malaria in the Kingdom of Cambodia. *Management*. 2014;(May):1-60.

30. Karema C, Wen S, Sidibe A, et al. History of malaria control in Rwanda: Implications for future elimination in Rwanda and other malaria-endemic countries. *Malar J*. 2020;19(1):1-12. doi:10.1186/s12936-020-03407-1

31. Country antimalarial drug policies: by region; WHO African region. Published 2018. https://www.who.int/malaria/am_drug_policies_by_region_afro/en/

32. Ndiaye M, Faye B, Tine R, et al. Assessment of the molecular marker of Plasmodium falciparum chloroquine resistance (Pfcrt) in Senegal after several years of chloroquine

withdrawal. *Am J Trop Med Hyg*. 2012;87(4):640-645. doi:10.4269/ajtmh.2012.11-0709

33. Sylla K, Abiola A, Tine RCK, et al. Monitoring the efficacy and safety of three artemisinin based-combinations therapies in Senegal: Results from two years surveillance. *BMC Infect Dis*. 2013;13(1). doi:10.1186/1471-2334-13-598

34. Hemming-Schroeder E, Umukoro E, Lo E, et al. Impacts of antimalarial drugs on plasmodium falciparum drug resistance markers, Western Kenya, 2003-2015. *Am J Trop Med Hyg*. 2018;98(3):692-699. doi:10.4269/ajtmh.17-0763

35. *Guidelines for the Treatment of Malaria, 3rd Edition*. World Health Organization; 2015.

36. ACT Partner Drug Molecular Surveyor. Worldwide Antimalarial Resistance Network. http://www.wwarn.org/molecular/surveyor/#1

37. Artemisinin Molecular Surveyor. Worldwide Antimalarial Resistance Network. http://www.wwarn.org/molecular/surveyor/k13/index.html?t=201608031200#0

38. R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

39. Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

40. Pedersen, Thomas Lin (2020). patchwork: The Composer of Plots. R package version 1.1.1. https://CRAN.R-project.org/package=patchwork.

41. Kassambara, Alboukadel (2020). ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.4.0. https://CRAN.R-project.org/package=ggpubr

ACADEMIC VITA

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EDUCATION

The Pennsylvania State University — **University Park, PA**
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RESEARCH EXPERIENCE

Boni Laboratory — **University Park, PA**
Undergraduate Research Assistant — *Mar 2020 – Present*
- Analyzed scientific literature relating to clinical trial data that report the efficacy of artemisinin-based combination therapies
- Utilized molecular marker prevalence to approximate the efficacy of artemisinin-based combination therapies for a range of plasmodium falciparum genotypes

Charles H. "Skip" Smith Laboratory — **University Park, PA**
Undergraduate Research Assistant — *Mar 2018 – Dec 2019*
- Worked with Dr. Yuchen Chen to examine in vivo cell conversion after the use of the ischemic stroke model on mice and refine existing methods to more effectively convert reactive astrocyte cells into neurons
- Photographed, analyzed, and quantified confocal images to determine the optimal conversion combination
- Evaluated efficiency of collagenase damage and subsequent neuron regeneration with various transcription factors
- Assisted with the regeneration of tau-based neuron loss and investigated the impact of tau in Alzheimer's Disease

LEADERSHIP EXPERIENCE

Schreyer Honors College Student Council — **University Park, PA**
Executive Board | Service Director — *Aug 2017 – Present*
- Represented the students in the Schreyer Honors College by acting as a liaison between students and the administration
- Communicated with the local community and organized a wide variety of philanthropic activities to create and provide donations such as blankets, bracelets, and toys for sick children, senior citizens, and war veterans
- Spearheaded the Giving Tuesday event and raised $5.60 k for financially struggling Schreyer peers
- Collaborated with the Executive Board to manage an organization of ~40 Schreyer students

Penn State Health Summer Treatment Program — **Harrisburg, PA**
Treatment Counselor — *Jun 2019 – Aug 2019*
- Implemented intensive behavioral modification treatment program to 10 children with ADHD, ODD, and CD
- Tracked daily behavior of children in order to design treatment modifications to meet the needs of individual children
- Planned and directed recreational games to assess children's sports skills and knowledge of game rules
- Provided weekly social skills training and conducted problem-solving discussions when necessary
- Communicated with parents to update on developments and make any necessary changes applicable to the home setting

Penn State Eco Rep Program — **University Park, PA**
Assistant Program Coordinator — *Nov 2018 – Present*
- Organized a six-week educational program aimed at educating first-year students to implement recycling norms in the halls
- Supervised a group of student leaders as they promoted environmental solutions to students, faculty, and the community
- Presented educational workshops inform fellow students and encourage sustainable behaviors in campus facilities
- Strategized with Resident Assistants (RAs) and residential life to inform freshmen on reducing waste at Penn State

HONORS & SKILLS

Honors: Dean's List, Schreyer Academic Excellence Scholarship, David S. Rocchino Family Foundation Scholarship
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