# POLYMORPHISMS IN PLASMODIUM FALCIPARUM PfAMA1ANDPfRH5 GENES IN ISOLATES FROM SELECTED CENTRES IN LAGOS, NIGERIA.

by

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### DECLARATION

We hereby declare that this thesis titled "Polymorphisms in *Plasmodium falciparumPfAMA1* and *PfRH5* Genes in Isolates from Selected Centres in Lagos, Nigeria" submitted to the School of Postgraduate Studies, University of Lagos, Nigeria for the award of Doctor of Philosophy in Biochemistry is a record of original research work carried out by **AJIBAYE**, Olusolain Biochemistry, Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria.

The work has not been submitted previously in whole or in part to qualify for any academic award.

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This is to certify that the Thesis:

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Submitted to theSchool of Postgraduate Studies, University of Lagos

For the award of the degree of

# DOCTOR OF PHILOSOPHY (Ph.D.),

is a record of original research carried out

by

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In the Department of Biochemistry

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### DEDICATION

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# TABLE OF CONTENTS

Page
TITLE PAGEi
DECLARATIONii
CERTIFICATIONiii
ACKNOWLEDGEMENTSiv
DEDICATIONvi
TABLEOF CONTENTSvii
LIST OF FIGURESxiv
LIST OF TABLESxvi
LISTOF PLATESxviii
LIST OF EQUATIONSxix
LIST OF APPENDICESxx
ABSTRACTxxi
CHAPTER ONE
1.0 INTRODUCTION
1.1 BACKGROUND OF THE STUDY1
1.2 STATEMENT OF THE PROBLEM
1.3 AIM OF THE STUDY7

1.4 SPECIFIC OBJECTIVES OF THE STUDY
1.5 SIGNIFICANCE OF THE STUDY
1.6 DEFINITION OF TERMS9
1.7 LIST OF ABBREVIATIONS11
CHAPTER TWO
2.0 LITERATURE REVIEW
2.1 Malaria: Transmission and Burden12
2.1.1 Malaria In Children and Pregnancy13
2.2 Parasite Life Cycle, Biology and Genomics16
2.2.1 Parasite Life Cycle16
2.2.2 Parasite Biology16
2.2.2.1. The Hepatic Stage
2.2.2.2 Erythrocytic Stage
2.2.2.3 The Sexual Stage
2.2.3. <i>Plasmodium falciparum</i> Parasite Genomics21
2.2.3.1 Homology, OrthologyandParalogy23
2.3 Malaria Diagnosis25
2.3.1 Clinical/Presumptive Diagnosis and Treatment25
2.3.2 The Quantitative Buffy Coat (QBC)

2.3.3 Antigen Detection Tests	26
2.3.4 Serological Tests	28
2.3.5 Molecular Diagnostic Methods	28
2.3.6 Polymerase Chain reaction (PCR) Technique	29
2.3.7 Loop-Mediated Isothermal Amplification (LAMP) technique	
2.3.8 Microarrays	
2.3.9 Flow Cytometry (FCM) Assay	30
2.4 Anti-Malarial Drugs	32
2.4.1 Quinine	32
2.4.2 Chloroquine (CQ)	
2.4.3 Amodiaquine	35
2.4.4 Mefloquine	
2.4.5 Atovaquone	
2.4.6 Primaquine	
2.4.7 Halofantrine	
2.4.8 Antifolate drugs	
2.4.8.1 Sulphadoxine Pyrimethamine (SP)	40
2.4.8.2. Proguanil	41
2.4.9Antibiotics	42

2.4.9.1 Tetracycline	42
2.4.9.2 Clindamycin (7-Chlorolincomycin)	43
2.4.9.3 Rifampicin	44
2.4.9.4 Artemisinin Compounds	
2.5 OVERVIEW OF ANTIMALARIAL RESISTANCE	48
2.6 Immunologic Responses in Malaria	50
2.6.1. T-cell andHumoral Responses in Malaria	52
2.6.1 HOST-PARASITE RELATIONSHIP	
2.6.2. <i>Plasmodium falciparum</i> Apical Membrane Antigen 1 (PfAMA1)	62
2.6.3 <i>Plasmodium falciparum</i> Reticulocyte binding Protein Homolog 5 (PfRH5)	63
2.6.4 Merozoite Surface Protein 2 (MSP2)	63
2.7.0 Multiplicity of Infection	66
2.8.0 Malaria Pathogenesis	66
2.9.0. Malaria Vaccine-types and Status of Vaccines/Current Issues	67
2.9.1 Major Vaccine Types	68
2.10.0 Plasmodium falciparum Parasite Antigenic Variation And Genetic Divers	ity68
2.11.0 Parasite Genetic Structure	70

# **CHAPTER THREE**

3.0 MATERIALS AND METHODS71
3.1.0 Materials71
3.1.1 Methods71
3.1.2 Description of Study Areas and Population71
3.2 Ethical Consideration74
3.3. Inclusion Criteria74
3.4. Exclusion Criteria74
3.5 Sample Size Determination75
3.6 Sampling75
3.6.1 Microscopy76
3.6.2 Anthropometric Measurements77
3.6.3 Immunological Assays77
3.6.4 Hematology
3.7Molecular Determination of Haplotypic Variations in AMA1and Pfrh578
3.7.1 Malaria Parasite DNA Extraction From Dried Blood Spots (DBS)78
3.7.2 Parasite DNA Yield and Quality Determination
3.7.3 Parasite GenotypingBy <i>Pfmsp2</i> 80
3.7.4 Agarose Gel Electrophoresis

3.7.5 Multiplicity of Infection (MOI) or Number of Genotypes Per Infection
3.7.6 Heterozygosity
3.7.7 Amplification of <i>Pfama1</i> Gene83
3.7.8 Amplification of <i>Pfrh5</i> Gene82
3.7.9.1 Gene Clean-Up
3.7.9.2 Nucleotide Sequencing and Sequence Analyses
3.8 Bioinformatics
3.9 Statistical and Genetic Analyses85

# **CHAPTER FOUR**

RESULTS
4.1 Demographic Profile of Participants
4.2 Malaria Parasite Density Distribution
4.3 BMI Status of Participants
4.4 Relationship BetweenBMI Status and Malaria Parasitaemia92
4.5 Evaluation of Cytokine Response92
4.6 Evaluation of Haematological Parameters of Participant
4.7 DNA Yield and Purity By <i>Nanodro</i> p 1000 Spectrophotometer97
4.8 Parasite Clonal Distribution and Genetic Diversity101
4.8.1 Multiplicity of Infection101

4.8.2 At	mplification	of Apical	Membrane	Antigen-I	(AMA1)	and	Reticulocyte	Binding
Protein I	Homology 5	(RH5)			•••••			106
4.8.3 Ge	netic Differe	ntiation, Nu	cleotide and	Haplotypic	Diversity	v of P	fama1	112
4.8.4 Ge	netic Differe	ntiation, Nu	cleotide and	Haplotype	Diversity	of P	frh5	113
4.8.5 <i>P</i> . j	falciparum G	enetic Strue	cture, Evider	nce of Selec	ction and l	Reco	mbination	117
4.8.6 Eff	fect of Polym	orphisms of	n MOI and C	Cytokine Re	esponse			119

# **CHAPTER FIVE**

5.0 DISCUSSION	126
5.1CONCLUSION	134
5.2 SUMMARY OF FINDINGS	136
5.3 CONTRIBUTION TO KNOWLEDGE	138
5.4 RECOMMENDATIONS	139
REFERENCES	140
APPENDICES	176

### LIST OF FIGURES

# pages

Figure 1: Geographical Distribution and Economic Importance of Malaria14
Figure 2: <i>Plasmodium falciparum</i> Parasite Life Cycle (CDC 2012)17
Figure 3: The chemical structure of artemisinin47
Figure 4: Regulation of Adaptive Immunity to Blood-stage Malaria by Cytokines54
Figure 5: Schematic representation of Erythrocyte invasion by Plasmodium falciparum60
Figure 6: Model for the sequence of Interactions duringerythrocyte invasion by
P.falciparum61
Figure 7: Schematic structure and full-length alignment sequence of MSP265
Figure 8:Map of Lagos State73
Figure 9:Distribution of msp2 Alleles in isolates from the sites studied104
Figure 10: Mean number of genotypes inmerozoite surface protein -2 (MSP2)108
Figure 11: Distribution of msp-2 families during the cross-sectional sample collection at the
three sites109
Figure 12: Molecular Phylogenetic Tree of AMA1 Haplotypes121
Figure13: Molecular Phylogenetic Tree of RH5 Haplotypes122
Figure 14: Linkage disequilibrium (LD) plot showing non-random association between
nucleotide variants at different polymorphic sites on AMA1 Domain I123

### LIST OF TABLES

### pages

Table 1:Geographical Prevalence of Plasmodium Parasites    15
Table 2: Merozoite Surface Protein-2 (MSP2) PCR Primer Sequences
Table 3: Demographic Profile of Study Population
Table. 4: Malaria Parasite Density of Participants
Table 5: BMI Status of Infected Participants from the Three Sites
Table 6: BMI Distribution among Participants90
Table 7: Classification of Participants <20 years
Table 8: Relationship between BMI Status and Malaria Parasitaemia
Table 9: Cytokine Response of Test Participants from Study Sites
Table 10: Levels of Pro-inflammatory Cytokines of Participants
Table 11: Effect of Malaria Parasite Density on Cytokine Response of Participants
Table 12: Haematological Parameters of Participants
Table 13: Correlation of IL-12, IL-1 $\beta$ and TNF- $\alpha$ with Platelet, MCH, MCHC, WBC
&Granulocyte
99

 Table 15: Distribution of Merozoite Surface Protein (msp)-2 in Isolates from 195

Table 14: Multiple Regression Analysis on MCH, GRA & Platelet with IL-12.....100

Nigerians Presenting with <i>P. falciparum</i> malaria105
Table 16: Relationship of MOI with Age, TNF, IL-1B and IL-12107
Table 17: Measures of DNA sequence polymorphisms at Domain I of AMA1
and RH5 among Nigerian <i>P.falciparum</i> isolates114
Table 18: Measures of DNA sequence polymorphisms at Domain I of
AMA-I among Nigerian <i>P. falciparum</i> populations115
Table 19: Measures of DNA Sequence Polymorphisms at The HAPBs 36718,
36727 and 36728 of Rh5116
Table 20 Inter-Population Genetic Differentiation of P. falciparum
Table 21: Relationship between pro-inflammatory cytokines and Polymorphisms in P.
<i>falciparum</i> antigenic proteins120

### LIST OF PLATES

Plate	Pages
Plates 1: Agarose gel electrophoregram of MS	SP2/ FC27 family resolved on 1.2% gel102
Plates 2: Agarose gel electrophoregram of MS	SP2/ 3D7 family resolved on 1.2% gel103
Plates 3: Agarose gel electrophoregram of AM	AA1 resolved on 1.2% Agarosegel109
Plates 3: Agarose gel electrophoregram of RH	I5 resolved on 1.2% Agarose gel111

# LIST OF EQUATIONS

### pages

Equation 1:	Sample size Formula	75
Equation 2	Parasite density Calculation	76
Equation 3	Formula for Anthropometric status	77
Equation 4	Formula for Multiplicity of Infection (M	OI)82
Equation 5	Formula for expected heterozygosity (	( <i>He</i> ) 8

### LIST OF APPENDICES

#### Pages

Appendix 1: Approval of the Nigerian Institute of Medical Research, Institutional ......176 Ethics Review Board.

Appendix 3: Plasmodium falciparum Apical Membrane Antigen-I (PfAMA1) .....179

Amino Acid Sequences from the study sites.

Appendix 4: Plasmodium falciparum Reticulocyte-binding protein homologue-5 ......188

(PfRH5) Nucleotide sequences (Haplotypes) from the study sites.

Appendix 5: Published Data on Pro-inflammatory Cytokine Response and Genetic ......189

Diversity in MerozoiteSurface Protein 2 of *Plasmodium falciparum*Isolates from Nigeria.

Appendix 6: Equipment and Reagents......190

#### ABSTRACT

Plasmodium falciparummalaria remains a disease of global andnational health importance due to spread of multi-drug resistance strains of the parasite and insecticide resistance in the mosquito vector resulting in high morbidity and mortality rates. Erythrocyte invasion by P. falciparum is a complex process, of which P. falciparum apical membrane antigen I (PfAMA1) and reticulocyte binding protein homolog 5 (PfRH5) are key proteins, hence, targeted for malaria vaccine development. Immune responses against merozoite attachment and invasion may be associated with genetic variations observed in these vaccine candidate antigens. This study assessed polymorphisms in AMA1 and RH5 among P. falciparum isolates from Nigeria. One thousand eight hundred and eighty-three (1,883) febrile subjects were examined, out of which 384 (20.39%) were microscopically positive for P. falciparum mono-infection. Three hundred of these volunteers were included from three health facilities in Lagos state: Ijede General Hospital, Ikorodu (IJE), Ajeromi General Hospital, Ajegunle (AJE)and St. Kizito Mission Hospital, Lekki, (100 from each site) and Eighty age-matched apparently healthy controls from the same communities were included in the study. Giemsastained thick and thin blood films of participants were used for P. falciparum identification and quantitation. Blood collected on Whatmann 3.0 filter paper was used for DNA extraction and assessed for DNA quality and yield. Molecular genotyping was carried out using the block 3 of Merozoite Surface Protein-2 to determine parasite genetic diversity and Multiplicity of Infection (MOI). One hundred and ninety-five samples which were PCR positive for msp2 gene were used for PCR amplification and sequencing of pfama1 and Pfrh5 genes to determine the genetic structure and polymorphisms on AMA1andRH5. Serum levels of IL-12, TNF- $\alpha$  and IL-1 $\beta$  were determined by Enzyme Linked Immunosorbent Assay (ELISA). Sequence alignment was done usingBioedit and Clustal W in MEGA softwareswhile sequences were analysed using DnaSP. Results revealed that malaria prevalence among the febrile patients was 20.39 %, highest in Ijede and lowest in Lekki. Levels of the pro-inflammatory cytokines studied were higher (p < 0.05) in the infected participants than the controls. Eighteen different alleles were observed for MSP-2 loci, FC27 family being more prevalent. Mean MOI was  $1.54 \pm 0.2$ . Heterozygosity (H<sub>E</sub>) values ranged from 0.77 - 0.87 and highest for IJE (0.87). Cytokine response was significantly associated with MOI (P < 0.05) but not parasite density. Genetic sequence analysis revealed 93 different haplotypes (H) for AMA1 Domain I. Forty-eight of these haplotypes are new with 34 segregating sites, Haplotype diversity of  $0.992 \pm 0.004$ , mean nucleotide diversity of 0.02468 $\pm$  0.00076 and Total number of mutations being 52. Tajima's D anddN-dS (non-synonymous minus synonymous mutations) were positive showing positive selection on AMA1. Decline in Linkage Disequilibrium was observed showing high recombination events taking place, however, the High Activity Binding Peptide (HABP) sequences of rh5 gene revealed three haplotypes of RH5 with negative Tajima's D anddN-dS values showing no selection on RH5. A Single Nucleotide Polymorphism (SNP) (G A), on nucleotide position 608 was observed on the RH5 sequences based on 3D7 reference sequence. Phylogenetic analyses of the AMA1 and RH5 sequences from this study showed clustering and evidence of evolutionary relationship with 3D7, Guinean AMA1, PAS-2 and FCB-2 RH5 strains using P. reichenowi as the out-group at 1000 bootstraps replications. The study indicates that the flow of any resistance alleles among *P. falciparum* isolates in Lagos may be relatively high, with negative impacts on control measures owing to low fstvalues. New AMA1 haplotypes were found suggesting that Nigerian P. falciparum should be given consideration in the design and development of control strategies. The study shows that globally efficacious RH5-based vaccines may be potentially applicable in Nigeria, inspite of the complex genetic diversity of *P. falciparum* isolates from Nigeria.

Key words: Merozoite, polymorphisms, synonymous mutations, haplotypes, heterozygosity.