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Fc Gamma Receptor IIA and Merozoite Surface Protein 1 Gene Variants in Relation to Malaria Status in the Buea Health District

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ABSTRACT

Background: Malaria remains a major public health burden in sub-Saharan Africa, with Cameroon experiencing high endemicity and transmission intensity. While Plasmodium falciparum is the predominant species responsible for severe disease, host and parasite genetic factors are increasingly recognized as important determinants of malaria susceptibility and clinical outcomes. This study investigated the association between P. falciparum Merozoite Surface Protein 1 (MSP1) and human Fc gamma receptor IIA (FCγRIIA) gene polymorphisms with malaria status in the Buea Health District, Cameroon.

Methods: A total of 273 participants were enrolled and assessed for demographic and clinical characteristics. Nested PCR targeting P. falciparum confirmed species-specific infections. Genotyping of MSP1 block 2 (K1, MAD20, RO33) was conducted via allele-specific PCR, while FCγRIIA polymorphisms were analyzed using restriction fragment length polymorphism (RFLP). Statistical analyses, including chi-square tests and logistic regression, were used to assess associations between genetic markers, parasitaemia, and malaria status.

Results: Of the samples screened, 95 (97%) were confirmed as P. falciparum infections. The K1 allele was the most prevalent MSP1 genotype, followed by MAD20 and RO33, with several mixed infections, reflecting high transmission intensity. No significant association was found between MSP1 genotypes and malaria status, though high parasitaemia was linked to increased odds of symptomatic malaria (OR = 4.03; 95% CI: 0.97–16.83; p = 0.054). FCγRIIA analysis revealed that individuals with homozygous or heterozygous genotypes had higher odds of symptomatic malaria compared to the wild type, although results were not statistically significant. Trends also indicated increasing FCγRIIA expression with age and parasitaemia, suggesting possible immune modulation.

Conclusion: While no statistically significant associations were identified, trends observed in FCγRIIA and MSP1 polymorphisms suggest potential roles in influencing malaria status. The high prevalence of mixed-genotype infections indicates considerable genetic diversity of P. falciparum in Buea. These findings underscore the need for larger, longitudinal studies to validate genetic markers of disease status and inform targeted malaria interventions in Cameroon.

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Introduction

Malaria remains a significant global health threat, caused by Plasmodium parasites and transmitted to humans via the bite of infected female Anopheles mosquitoes. According to the World Health Organization (WHO), an estimated 263 million malaria cases and 597,000 related deaths were recorded globally in 2023, with sub-Saharan Africa bearing a disproportionate burden, accounting for 95% of cases and 96% of deaths [1]. This overwhelming prevalence in Africa is driven by factors such as widespread poverty, inadequate healthcare access, insecticide resistance in mosquito vectors, favourable climatic conditions for mosquito breeding, and persistently high transmission rates [2].

Malaria endemicity across Cameroon ranges from hyperendemic to holoendemic, varying by region and season. Environmental changes driven by urbanization, deforestation, and human migration have significantly influenced malaria transmission

dynamics and parasite diversity. For example, infrastructural expansion in Buea has altered local ecosystems, potentially affecting mosquito behavior and distribution, which in turn influences malaria epidemiology [3,4].

Over the years, several global and national initiatives have been implemented to combat malaria. Internationally, the Roll Back Malaria (RBM) Partnership, the Global Fund, the U.S. President's Malaria Initiative (PMI), and the WHO Global Technical Strategy for Malaria (2016–2030) have collectively promoted malaria control through increased funding, research, and strategic policy implementation. These interventions have contributed to measurable reductions in malaria incidence and mortality in many countries [5].

At the national level, Cameroon's National Malaria Control Program (NMCP) leads efforts to reduce malaria through interventions such as the widespread distribution of insecticide-treated bed nets (ITNs), intermittent preventive treatment in pregnancy (IPTp), indoor residual spraying (IRS), and strengthening of community-based health services [6,7]. Despite these initiatives, the persistence of malaria underscores the need for innovative approaches, particularly given the complex transmission patterns and the growing resistance of parasites and vectors to current tools [8].

One promising frontier in malaria research is the study of host genetic factors that influence susceptibility and disease status. Understanding these genetic determinants can inform the development of more effective diagnostics, targeted treatments, and vaccines. Among the most studied genetic elements are single nucleotide polymorphisms (SNPs), which represent the most common type of human genetic variation. These polymorphisms can significantly impact how individuals respond to malaria infection, particularly through genes involved in immune regulation [9,10].

Two such genes of interest are Merozoite Surface Protein 1 (MSP1) and Fc gamma receptor IIA (FCγRIIA). MSP1 is expressed on the surface of the merozoite stage of *P. falciparum* and is crucial for the parasite's invasion of red blood cells. It is highly polymorphic, especially in block 2, which comprises the K1, MAD20, and RO33 allelic families [11]. The diversity of MSP1 alleles is associated with immune evasion, transmission intensity, and clinical outcomes of malaria. In the Mount Cameroon region, high levels of MSP1 polymorphism have been reported, with more than 60% of infections involving multiple parasite clones, suggesting high transmission intensity and genetic complexity [12].

FCγRIIA, by contrast, which exists in humans, is a low-affinity receptor for the Fc portion of immunoglobulin G (IgG). It plays a central role in antibody-mediated immune responses, including phagocytosis, cytokine production, and antibody-dependent cellular cytotoxicity. The H131R polymorphism in FCγRIIA alters the receptor's binding affinity to different IgG subclasses and has been linked to variations in immune response and malaria status [13,14].

While these genetic associations have been investigated in several malaria-endemic regions across Africa, South America, and Asia, there is a scarcity of data from Cameroon; particularly in the Buea Health District. Characterizing the local distribution and diversity of MSP1 and FCγRIIA polymorphisms could provide crucial insights into the molecular epidemiology of malaria in this region. Such knowledge can enhance our understanding of malaria pathogenesis and contribute to the design of more effective diagnostic tools, personalized treatment strategies, and vaccines tailored to local population genetics.

This study, therefore, aims to examine the genetic diversity of MSP1 and FCγRIIA SNPs and their association with malaria status among individuals in Buea. By identifying host and parasite genetic markers linked to clinical outcome of malaria, the research seeks to advance targeted malaria control efforts in Cameroon.

Materials and Methods

Study Area and Design

This study was a cross-sectional Hospital and community-based study that included 300 participants, The study was designed to investigate the genetic diversity of *Plasmodium falciparum* in relation to malaria status, with a focus on single nucleotide polymorphisms (SNPs) in the *MSP1* and *FCγRIIA* genes. The participants were recruited from the community or from Solidarity Health Foundation, Muea Health Center, and Buea Regional Hospital in the Buea Municipality of Cameroon.

Sick participants were those with parasitaemia; presence of malaria parasite in the blood, had fever; a body temperature > 37.5°C while semi-immune participants were those with parasitaemia and no fever; a body temperature ≤ 37.5°C.

A convenient sampling technique was used to recruit participants and explain the purpose of the questionnaires administered to them.

Blood samples were collected from participants who gave written consent directly or through their parents and analyzed for parasite identification and quantification, as well as for DNA extraction and genotyping.

Sample Size Calculation

The sample size for this cross-sectional study was Calculated using Cochran's formula, based on a malaria prevalence of 26.0% [15,16];

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where:
n = required sample size
Z = Z-score for 95% confidence level (1.96)
P = estimated prevalence (26.0% or 0.26)
d = margin of error (5% or 0.05)

$$n = \frac{(1.96)^2 \cdot 0.26 \cdot (1 - 0.26)}{(0.05)^2}$$

$$n = \frac{3.8416 \cdot 0.26 \cdot 0.74}{0.0025}$$

$$n = \frac{3.8416 \cdot 0.1924}{0.0025}$$

$$n = \frac{0.7395}{0.0025}$$

$$n = 295.8$$

The required sample size for this study, considering a prevalence of 26.0% and a 5% margin of error, is approximately 296 participants.

Data and Sample Collection

Malaria-suspected participants were educated on the purpose and procedures of the study, and only those who provided informed consent were included. Questionnaires were administered to collect socio-demographic information (such as age, sex, residence), clinical symptoms, past malaria history, and any history of antimalarial treatment. Participants were assisted in filling out the questionnaires through face-to-face interviews to ensure accurate data collection. A drop of blood by finger-pricking was used to prepare slides for malaria detection by microscopy. Two microscopists read each slide to confirm the diagnosis. Venous blood (3 ml) was collected from participants who were positive for malaria by microscopy.

Parasitaemia was classified as follows; low (1 – 1000 trophozoites/µl), moderate (1001 – 10000 trophozoites/µl), and high (> 10000 trophozoites/µl). The body temperature of the participants was also measured.

Identification of *Plasmodium Falciparum* by Nested PCR

Genomic DNA was extracted from Dot Blood Spot (DBS) prepared from the 3 ml venous blood collected from the participants using the Chelex method as described [17]. Detection of *Plasmodium falciparum* DNA was performed using nested polymerase chain reaction (nested PCR) targeting the 18S small subunit ribosomal RNA gene. The primary PCR utilized 100 ng genomic DNA, rPLU5 and rPLU6 primers, which amplify a 1,200 bp fragment of the Plasmodium genus. The reaction was carried out in a 10 µL volume containing 5 µL of One Taq Quick-Load 2X Master Mix, 0.25 µL of each primer (10 µM), 2 µL (100ng) of template DNA, and 2.5 µL of nuclease-free water. This was run for 30 cycles using the following parameters; pre-denaturation (94 °C for 3 min), denaturation (94 °C for 1 min), annealing (55 °C for 1 min), extension (68 °C for 1 min), final Extension (68 °C for 5 min).

The nested PCR used 1 µL of the primary PCR product as the template and *P. falciparum*-specific primers rFAL1 and rFAL2 to amplify a 205 bp fragment. The reaction mix was similar to the primary PCR, with annealing temperature adjusted to 61 °C for *P. falciparum* specificity. All reactions were performed and loaded into a thermocycler under standard nested PCR conditions. Negative and positive controls were included in each batch to ensure reliability. The reaction was then run on Ethidium bromide agarose gel electrophoresis for visualization using a UV lamp. Table 1 below summarizes the primer sequences and expected band sizes for the genus and species-specific amplifications.

Table 1: Primer Sequences and Expected Band Sizes for *Plasmodium Falciparum*

Target	Primer	Sequence (5'–3')	Expected Product Size (bp)
Plasmodium genus	rPLU6 (sense)	TTAAAATTGTTGCAGTTAAAACG	1200
	rPLU5 (antisense)	CCTGTTGTTGCCTTAAACTTC	
P. *falciparum* species	rFAL1 (sense)	TTAAACTGGTTTGGGAAAACCAAATATATT	205
	rFAL2 (antisense)	ACACAATGAACTCAATCATGACTACCCGTC	

Genotyping of FCγRIIA Gene by PCR-RFLP

Genotyping of the Fc gamma receptor IIA (FCγRIIA) gene was performed using a modified Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique to detect the H131R single nucleotide polymorphism (SNP) associated with malaria status [18].

PCR amplification was done using the primer pair shown in Table 2 under the same parameters as shown in section 2.4 above.

Following successful amplification, the resulting PCR products were subjected to restriction digestion using the BstUI restriction enzyme, which recognizes the polymorphic site within the amplified region of the FCγRIIA gene. The digestion reaction was carried out in a total volume of 8.45 µL containing 4.5 µL of the PCR amplicon, 0.75 µL of the appropriate restriction enzyme buffer, 2.0 µL of nuclease-free water, and 1.2 µL of BstUI enzyme. The mixture was incubated at 60 °C for 15 minutes and then resolved on a 2% agarose gel stained with ethidium bromide and visualized under UV light.

Genotypes were determined based on the following banding patterns: individuals homozygous for the H allele showed a single undigested band of 337 bp; those homozygous for the R allele showed a digested product of 316 bp; and heterozygous individuals (HR) displayed two bands at 337 bp and 316 bp. Positive and negative controls were included in all reactions to validate the reliability of the PCR and digestion processes. Table 2 shows the sequence of the pair of primer used to amplify the *FCγRIIA* gene.

Table 2: Primer Sequences and Expected Band Sizes for FCγRIIA (H131R)

Target Gene	Primer	Sequence (5'–3')	Expected Product Size (bp)
FCγRIIA (H131R)	Forward Primer	5′-GGA AAA TCCCAGAAATTCTC-3′	337 bp, 316bp,
	Reverse Primer	5′-CAATTTTGCTGCTATGGGC-3′	

Genotyping of MSP1 Gene by Nested PCR

Genotype determination of the *Plasmodium falciparum* MSP1 gene was carried out using a two-stage nested PCR protocol targeting the polymorphic block 2 region, following the WHO-recommended genotyping assay [19]. This method enables identification of the K1, MAD20, and RO33 allelic families using strain-specific primers. The primary PCR (pPCR) reaction was set up in a total volume of 10 µL, comprising 2.5 µL of nuclease-free water, 5 µL of OneTaq Master Mix (containing buffer, dNTPs, and Taq polymerase), 0.25 µL each of forward and reverse outer primers (10 µM), and 2 µL (100ng) of extracted template DNA.

The PCR tubes were sealed and subjected to thermal cycling under the following conditions: an initial denaturation at 94 °C for 3 minutes, followed by 30 cycles of 94 °C for 30 seconds, 58 °C for 2 mins, and 68 °C for 60 seconds, with a final extension at 68 °C for 5 minutes.

For the second-stage nested PCR (nPCR), separate reactions were prepared for each allelic family using 2 µL of the pPCR product as template. Each 10 µL nested reaction consisted of 2.5 µL nuclease-free water, 5 µL OneTaq Master Mix, 0.25 µL each of the family-specific forward and reverse primers (10 µM), and 2 µL of the corresponding primary amplicon. The nested PCR was performed under similar cycling conditions.

The primers used are shown on Table 3. PCR products were resolved on a 2% agarose gel stained with ethidium bromide and visualized under UV transillumination.

Band sizes were compared against a 100 bp DNA ladder to assign genotypes: approximately 180 bp for K1, 220 bp for MAD20, and 160 bp for RO33. Positive and negative controls were included in every PCR run to ensure the specificity, reproducibility, and reliability of the results.

Table 3: Primer Sequences for MSP1 Genotyping

Allelic Family	Primer Type	Sequence (5'–3')	Expected Product Size (bp)
MSP1 – General	N1 Forward (pPCR)	GCAGTATTGACAGGTTATGG	
MSP1 – General	N2 Reverse (pPCR)	GATTGAAAGGTATTTGAC	
K1	Forward (nPCR)	ROX/AATGAAGAAGAAATTACTACAAAAGGTGC	140–500 bp (common: 170–300 bp)
K1	Reverse (nPCR)	gtgtcttGCTTGCATCAGCTGGAGGGCTTGCACCAG	
MAD20	Forward (nPCR)	6FAM/AAATGAAGGAACAAGTGGAACAGCTGTTAC	100–390 bp (common: 150–300 bp)
MAD20	Reverse (nPCR)	gtgtcttATCTGAAGGATTTGTACGTCTTGAATTACC	
RO33	Forward (nPCR)	HEX/TAAAGGATGGAGCAAATACTCAAGTTGTTG	~160–240 bp (often single conserved band around 160–200 bp)
RO33	Reverse (nPCR)	gtgtcttCAAGTAATTTTGAACTCTATGTTTTAAATC	

pPCR = primary PCR, nPCR = nested PCR

Data Management and Analysis

After initial data entry and cleaning in Excel, the dataset was imported into SPSS version 20.0 (Statistical Package for the Social Sciences) for statistical analysis. Descriptive statistics such as frequencies, percentages, means, and standard deviations were used to summarize demographic and clinical outcome of malaria of the study population.

For inferential analysis, Chi-square tests were conducted to examine associations between categorical variables, and clinical outcome of malaria (sick vs. semi-immune). Independent sample t-tests were employed to compare means of continuous variables like age and parasitaemia between clinical groups and Logistic regression analysis was also performed to assess the predictive power of diagnostic methods and genetic markers in relation to malaria status. Statistical significance was set at a p-value less than 0.05.

Ethical Considerations

An ethical Clearance was obtained from the Institutional Review Board of the Faculty of Health Sciences, University of Buea; reference number: 2024/2575-08/UB/SG/IRB/FHS of 30 October 2024. After explaining the purpose of the study, voluntary written consent was obtained before enrolling into the study.

Results

Sociodemographic Features of the Study Population

Table 4 shows the socio-demographic characteristics of the study population. Out of the 273 participants admitted into the study, majority were recruited from the General Hospital (n = 115), females were the majority (52.0%) and 125 participants were classified as symptomatic, that is, they have parasitaemia (malaria parasite in the blood) and fever; body temperature above 37.5°C.

Table 4: Sociodemographic Characteristics of the Study Participants

Characteristic	Number (n)	Percentage (%)
Gender Distribution (n = 273)		
Female	142	52.0
Male	131	48.0
Clinical outcome of malaria (n = 273)		
Sick (parasitaemia + fever)	125	45.8
Semi-immune (parasitaemia + no fever)	148	54.2

***Plasmodium Falciparum* Nested PCR**

Out of the 98 samples that were positive for the Plasmodiun genus using primary PCR (results not shown), nested PCR analysis, which targets a 205 bp fragment specific to *Plasmodium falciparum*, confirmed 95 positive cases (Figure 1). This implies that 97% of Plasmodium infection were of the falciparum species. The presence of distinct bands at this size indicated the presence of *P. falciparum* DNA in the samples. PCR remains the most specific and sensitive method used in this study, capable of detecting low-level parasitemia and differentiating among *Plasmodium* species. Negative controls for the nested PCR were samples that were negative for the primary PCR.

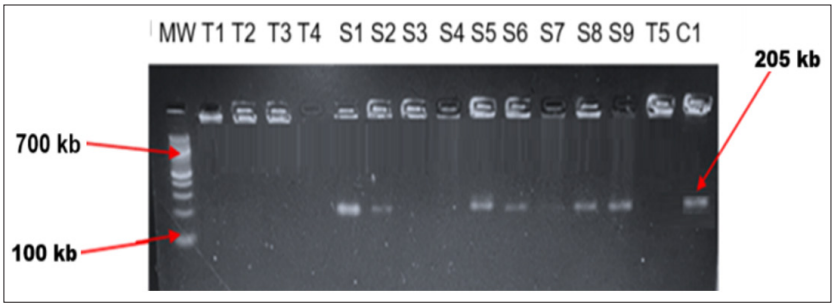

Figure 1: Representative Agarose Gel Electrophoregram Showing *P. Falciparum*
MW = molecular weight marker, T1-T4, and T5 = negative controls. S1 – S9 = test samples, C1 = Positive Control.

Genotyping of FCyRIIa

The distribution of FCγRIIA gene polymorphisms was analyzed across age groups, and parasitaemia among sick and semi-immune participants.

Positive results were confirmed by the presence of clear DNA bands of expected size (337 bp, 316bp,) under UV illumination, indicating successful digestion. Negative samples showed no visible bands as illustrated in (Figure 2). Figure 2 is a representative picture of the gel showing RFLP analysis of FCYRIIa polymorphisms.

Stratification by age showed an increasing trend in mean FCγRIIA expression with advancing age, with the highest mean observed in participants aged 41 years and above. However, these differences were not statistically significant ($p > 0.20$), indicating limited impact of age on FCγRIIA polymorphism distribution in the studied population (Table 5).

Regarding parasitaemia, there was a gradual increase in mean FCγRIIA levels from low to high parasite densities. While this increase approached significance in the high parasitaemia group ($p = 0.05$), it did not meet the conventional threshold, suggesting a possible association that requires further investigation (Table 5).

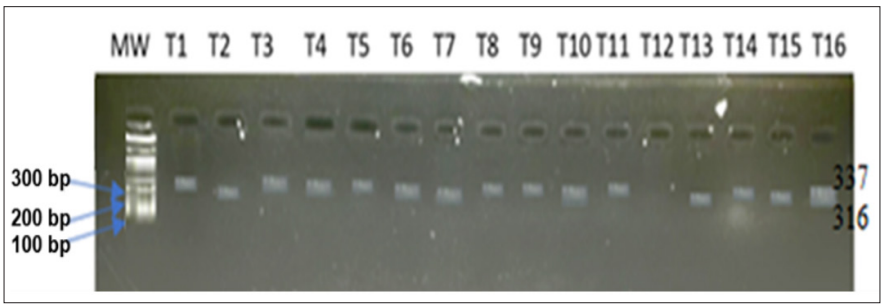

Figure 2: Representative Gel Showing Agarose Gel Electrophoregram of RFLP of FCYRIIa. MW = molecular weight (100 – 1000 bp). Lanes T1, T3, T4, T6, T10, T16 = Heterozygous HR (316 bp and 337 bp). Lanes T2 T7, T13, T15 = homozygous RR (316 bp). Lanes T5, T8, T9, T11, T14 = wild type HH (337 bp). Lane T12 = negative sample

Table 5: Distribution of Clinical Outcomes of Malaria by Age Group, and Parasitaemia with Corresponding Mean Values and *p*-values

Variable	*Category*	*Sick (n, %)*	*Semi-immune (n, %)*	*Mean ± SD*	*p-value*
Age Group	≤25	18 (50.0%)	22 (44.0%)	20.2 ± 3.60	0.705
	26–40	13 (36.1%)	17 (34.0%)	32.8 ± 4.10	0.530
	≥41	5 (13.9%)	11 (22.0%)	50.3 ± 4.20	0.220
Parasitaemia	Low	6 (16.7%)	4 (8.0%)	1.3 ± 0.40	0.400
	Moderate	8 (22.2%)	6 (12.0%)	2.1 ± 0.05	0.780
	High	22 (61.1%)	40 (80.0%)	2.8 ± 0.30	0.055

FCyRIIa Genetic Variation vs. Malaria Status

Regarding age distribution, participants aged 26–40 years had a slightly higher proportion of symptomatic malaria, followed by those aged ≤25 years, but the difference across age groups was not statistically significant (p = 0.757). This indicates that age group in this study population was not a strong determinant of symptomatic malaria.

Gender-wise, more males than females presented with symptomatic malaria; however, the difference was not statistically significant (p = 0.912). This suggests no meaningful gender-based disparity in clinical outcome of malaria among participants (Table 6).

Lastly, the relationship between parasitaemia levels and disease status showed that individuals with high parasitaemia were mostly sick and semi-immune participants. Equally, the chi-square test did not reveal a statistically significant difference between parasitaemia and malaria infection outcome status (p = 0.436) (Table 6).

Table 6: Combined Table of Malaria Infection Outcome and Genetic Variables vs. Malaria Status (n=95 for Age, Gender, Parasitaemia; n=86 for Genotyping)

Variable	*Sick (n)*	*Semi-immune (n)*	*p-value*
FCγRIIA Genotype			0.938
Homozygous	18 (42.9%)	22 (57.9%)	
Heterozygous	10 (23.8%)	12 (31.6%)	
Wild Type	8 (19.0%)	4 (10.5%)	
FCγRIIA Allele			0.765
RR	7 (21.9%)	11 (34.4%)	
HR	9 (28.1%)	13 (40.6%)	
HH	16 (50.0%)	8 (25.0%)	
Age Group			0.757
≤25	32 (47.8%)	22 (32.8%)	
26–40	21 (31.3%)	29 (43.3%)	
≥41	14 (20.9%)	16 (23.9%)	
Gender			0.912
Female	45 (47.4%)	38 (40.0%)	
Male	50 (52.6%)	57 (60.0%)	
Parasitaemia			0.436
Low	6 (6.3%)	6 (6.3%)	
Moderate	18 (18.9%)	22 (23.2%)	
High	71 (74.7%)	67 (70.5%)	

Predictors of Malaria Infection Outcome in Relation to FCγRIIA Genotype

Binary logistic regression was conducted to examine the association between demographic characteristics (age group), parasitological factor (parasitaemia), and FCγRIIA genotypes with malaria status among participants. Although none of the predictors reached statistical significance (p > 0.05), several variables showed notable trends. Participants with high parasitaemia had more than three times the odds of developing symptomatic malaria compared to those with low parasitaemia (OR = 3.35, 95% CI: 0.82 – 13.66, p = 0.093), suggesting a potential link between parasite burden and clinical outcome of malaria (Figure 3). Similarly, individuals with homozygous (OR = 2.22, 95% CI: 0.68 – 7.31, p = 0.188) and heterozygous (OR = 2.40, 95% CI: 0.64 – 9.01, p = 0.194) FCγRIIA genotypes had higher odds of being symptomatic compared to those with the wild-type genotype, though the wide confidence intervals indicate considerable uncertainty. Participants aged 26 – 40 and ≥41 years also had increased odds of symptomatic malaria compared to those aged ≤25 years, but these associations were not statistically significant (Figure 3). Overall, while the model did not identify statistically significant predictors, the observed trends suggest potential roles for parasitaemia and FCγRIIA polymorphisms in influencing malaria status, warranting further investigation in larger sample sizes

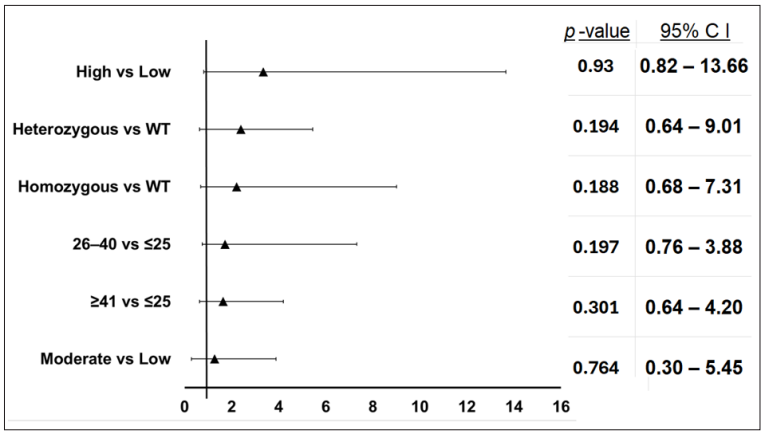

Figure 3: Binary Logistic Regression to identify predictors of clinical outcome of malaria. The following category of variables was used; FCyRIIa genetic variation (Homozygous vs WT and Heterozygous vs WT), age group (26–40 vs ≤25 and ≥41 vs ≤25), and parasitaemia (Moderate vs Low and High vs Low) as predictors of the clinical outcome of malaria

Distribution of MSP1 Genotyped Samples by Study Variables

Age-wise, the ≤25 age group accounted for the highest number of infections. Although a trend of increasing symptomatic cases with age was observed, particularly in the ≥41 group, the association between age and clinical outcome of malaria was not statistically significant (Table 7).

For parasitaemia, most symptomatic individuals had high parasite loads, with a mean density score of 2.9 ± 0.31. Although this was higher than in other categories, the association was only marginally significant (p = 0.052), indicating a potential link between parasitaemia and malaria symptoms (Table 7). Below is a picture showing the band sizes of MSP1 genotypes. Figure 4 below is a picture of the gel showing MSP1 Block 2 Alleles.

Table 7: Distribution of MSP1 Genotyped Samples by Study Variables (n= 86)

Variable	*Category*	*Sick (n, %)*	*Semi-immune* (n, %)	*Mean ± SD*	*p-value*
Age Group	≤25	18 (50.0%)	22 (44.0%)	20.0 ± 3.54	0.710
	26–40	13 (36.1%)	17 (34.0%)	33.0 ± 4.24	0.532
	≥41	5 (13.9%)	11 (22.0%)	50.0 ± 4.24	0.217
Parasitaemia	Low	6 (16.7%)	4 (8.0%)	1.2 ± 0.42	0.398
	Moderate	8 (22.2%)	6 (12.0%)	2.0 ± 0.00	0.776
	High	22 (61.1%)	40 (80.0%)	2.9 ± 0.31	0.052

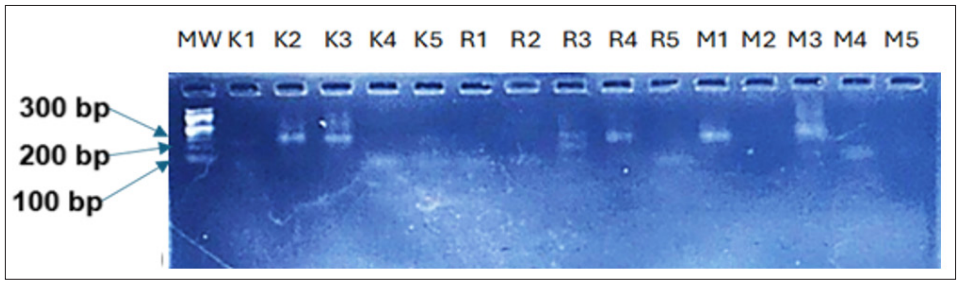

Figure 4: Agarose Gel Electropherogram Showing MSP1 Block 2 Alleles in Malaria Sick and Semi-Immune Participants in the Buea Health District. MW; Molecular Weight Marker, K1-K5: K1 Alleles, R1-R5: RO33 Alleles, M1-M5: MAD20 Alleles

Distribution of the MSP1 Genotype in the Study Population

Of the 86 participants that were successfully genotyped for MSP1, sick participants (**n = 50; 58.1%**) were more common than semi-immune participants (**n = 36; 41.9%**), suggesting that a significant portion of participants experienced active malaria infection.

Statistical comparison using Pearson's chi-square test revealed no significant differences in genotype distribution in the study site. Specifically, the K1 genotype showed a χ^2 value of 0.68 (p = 0.71), and MAD20 had a χ^2 value of 1.25 (p = 0.54). Due to small sample sizes, chi-square tests were not performed for RO33 and mixed genotypes. These results indicate that while some variation in genotype frequencies exists by location and clinical outcome of malaria, these differences are not statistically significant. This suggests a relatively homogeneous distribution of MSP1 allelic families within the Buea Health District, regardless of site of collection or disease phenotype.

The K1 genotype was the most prevalent (n = 29), followed by **MAD20 (n = 23)** and **RO33 (n = 19). Mixed infections** such as **K1 + MAD20** and **K1 + RO33** were also documented, indicating polyclonal infections and supporting the evidence of high transmission intensity. The relatively even spread of genotypes underscores the parasite's genetic variability in Buea, which has implications for vaccine development and malaria control strategies.

Predictors of the Clinical Outcome of Malaria in Relation to MSP1 Genotype, Age Group and Parasitaemia

logistic regression was performed to identify whether demographic characteristics (age group), parasitaemia, and MSP1 genotypes predicted malaria status among genotyped individuals (n = 86). Although no predictor reached statistical significance, several trends emerged. Participants with high parasitaemia had over four times the odds of being symptomatic compared to those with low parasitaemia (OR = 4.03, 95% CI: 0.97–16.83, p = 0.054), suggesting a strong but borderline significant association. Age also appeared to influence the clinical outcome of malaria; those aged ≥41 years had higher odds of symptomatic malaria compared to those ≤25 years (OR = 2.40, 95% CI: 0.71–8.16, p = 0.158), though not statistically significant. Regarding genotypes, individuals with MAD20 or RO33 alleles did not show significantly different odds of being symptomatic compared to those with the K1 genotype

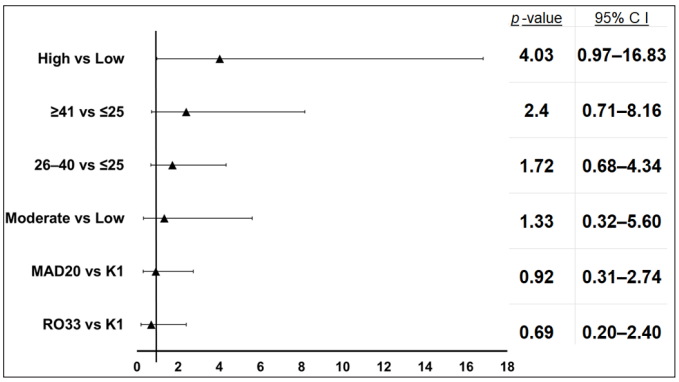

Figure 5: Predictors of Clinical Outcome of Malaria in Terms of MSP1 Genotype (MAD20 vs K1 and RO33 vs K1), Age Group (26–40 vs ≤25 and ≥41 vs ≤25), and parasitaemia (Moderate vs Low and High vs Low)

Discussion

This study investigated the genetic diversity of *P. falciparum* Merozoite Surface Protein 1 (MSP1) and host Fc gamma receptor IIA (FCγRIIA) polymorphisms and their association with malaria status in the Buea Health District, Cameroon. The findings provide important insights into the molecular epidemiology of malaria in this region, with implications for disease surveillance, diagnosis, and vaccine development.

Consistent with national and regional trends, *P. falciparum* was confirmed as the dominant species, accounting for 97% of malaria-positive cases. This reinforces prior reports indicating that *P. falciparum* remains the primary cause of malaria in Cameroon, responsible for the majority of severe cases and deaths [4]. The high specificity and sensitivity of nested PCR allowed for the accurate identification of infections, including those with low parasitaemia, thus affirming PCR's value in research and reference diagnostics, particularly in areas with overlapping febrile illnesses.

The genotyping of FCγRIIA revealed notable trends despite the absence of statistically significant associations. An increasing trend in FCγRIIA expression with age was observed, though not significant, suggesting age-related modulation of immune responses to malaria. This aligns with earlier studies indicating that repeated exposure to malaria in endemic settings contributes to the development of partial immunity with age [20]. Interestingly, higher mean FCγRIIA levels in individuals with high parasitaemia approached statistical significance (p = 0.05), hinting at a potential functional role of this polymorphism in modulating susceptibility or response to heavy parasite burden. Although these findings require confirmation in larger cohorts, they suggest that FCγRIIA genotypes may influence immune response intensity during malaria infection [21].

When examined as predictors of symptomatic malaria, both homozygous and heterozygous FCγRIIA genotypes were associated with higher odds of malaria symptoms compared to the wild-type genotype. While these associations were not statistically significant (p > 0.05), the observed odds ratios (ORs above 2) point to a potentially meaningful relationship between FCγRIIA variation and disease status. This aligns with earlier research linking FCγRIIA polymorphisms with altered IgG subclass binding affinity and differential clearance of parasitized red blood cells [20,22]. Thus, even in the absence of statistical significance, these trends warrant further exploration, particularly given the high endemicity of malaria in Buea and the possibility of population-specific genetic influences.

The MSP1 genotyping results revealed a high prevalence of the K1 allelic family, followed by MAD20 and RO33, with mixed infections also detected. The predominance of K1 is consistent with findings from other regions in Cameroon and suggests that this genotype remains widely distributed in the Mount Cameroon area [23]. Mixed-genotype infections further underscore the intense malaria transmission in this area and the complexity of parasite populations, a situation likely sustained by favorable climatic conditions, population mobility, and vector resistance to control measures [24].

Notably, no significant differences were found in MSP1 genotype distribution with respect to age, clinical outcome of malaria, or site of recruitment. The lack of significant variation across sites suggests a relatively homogeneous parasite population in Buea, possibly due to gene flow facilitated by human movement and shared vector habitats [25]. However, logistic regression revealed that individuals with high parasitaemia had over four times the odds of symptomatic malaria (OR = 4.03, p = 0.054), a borderline significant finding that supports the intuitive link between parasite density and clinical outcome of malaria.

These findings echo prior work indicating that high parasitaemia is a strong predictor of clinical outcome of malaria [26]. They also reinforce the hypothesis that parasite genotype diversity may influence disease status, albeit indirectly and in conjunction with host factors such as immune genetics. The absence of strong associations between individual MSP1 genotypes and disease status suggests that while allelic variation exists, no single allele confers significant clinical risk in this study population. However, the presence of polyclonal infections complicates this picture, as competition among parasite strains could modulate virulence and immune evasion strategies [27].

The logistic regression models used to explore predictors of symptomatic malaria further emphasize the multifactorial

nature of disease manifestation. Though no variables achieved conventional statistical significance, the combined trends from FCγRIIA and MSP1 analyses suggest a complex interplay between host immunity, parasite genetics, and environmental exposure. For example, older participants appeared to have slightly higher odds of symptomatic malaria, contradicting the expectation that increased age confers greater immunity. This may reflect other confounding factors such as occupational exposure, co-morbidities, or variability in prior immunity [28].

Importantly, the study's limitations must be acknowledged. First, the relatively small sample size may have reduced the statistical power to detect significant associations, especially for sub-group analyses. Second, the cross-sectional nature of the study limits causal inference and may not capture temporal variations in genotype distribution or immune responses. Third, while genotyping MSP1 and FCγRIIA provides valuable molecular insight, malaria status is likely influenced by a broader array of genetic, environmental, and socio-behavioral factors not assessed here.

Despite these limitations, the study provides novel and context-specific data on malaria host-parasite genetics in the Buea Health District. These findings support the growing recognition that both host immune genetics and parasite diversity play key roles in malaria pathogenesis. The identification of non-significant but consistent trends highlights the need for larger, longitudinal studies to more robustly delineate genetic risk profiles and inform locally adapted malaria control strategies.

In conclusion, this study contributes to the understanding of malaria epidemiology in Cameroon by profiling key genetic polymorphisms in **P. falciparum** and human immune genes. While definitive associations with disease status were not established, the findings suggest potential roles for FCγRIIA and MSP1 diversity in modulating the clinical outcome of malaria. Future research involving larger cohorts and broader genetic panels is needed to validate these observations and support the integration of molecular tools into malaria control and vaccine design programs in endemic regions like Buea.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The research data are available upon request from the corresponding author.

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