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Leveraging Biotechnology to Investigate *Monocyte Chemoattractant Protein-1* and *Merozoite Surface Protein 2* Gene Polymorphisms in Relation to Malaria Status in an Endemic Setting in the Buea Health District

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ABSTRACT

Malaria remains a life-threatening mosquito-borne disease and a major global health concern, particularly in endemic regions such as sub-Saharan Africa. In these settings, individuals often harbor multiple genetically distinct strains of *Plasmodium falciparum*, a phenomenon known as multiplicity of infection (MOI). Genetic polymorphisms in both the parasite and host are believed to modulate malaria Status. In the parasite, polymorphic alleles of the Merozoite surface protein 2 (*MSP2*) gene, particularly the FC27 and 3D7 allelic families, have been associated with disease Status across varying transmission settings. Similarly, in the human host, a single nucleotide polymorphism in the *Monocyte Chemoattractant Protein 1 (MCP-1)* gene at position -2518 (A>G) has been implicated in susceptibility and inflammatory responses during Plasmodium infections, though existing evidence remains inconclusive.

This study investigated the association between *MCP-1* gene polymorphisms and malaria Status in individuals from Buea Municipality, a malaria-endemic region in southwestern Cameroon. A cross-sectional study design was employed, enrolling 350 individuals, of whom 273 consented to participate (56% female, 44% male; age range: 1–90 years). Participants were classified into symptomatic (46%) and asymptomatic (54%) groups based on clinical and laboratory criteria. Malaria infection was confirmed by RDT (31.5%), microscopy (42.5%), and PCR (34.8%), with PCR-positive cases comprising 61% symptomatic and 39% asymptomatic individuals.

Analysis of *MCP-1* polymorphisms revealed that the wild-type AA genotype was more frequent in symptomatic individuals (84.4%), whereas the AG and GG genotypes were more prevalent among asymptomatic participants. G allele frequency was higher in the asymptomatic group (17.4%) than in symptomatic individuals (9.4%). Genotype distributions were in Hardy-Weinberg equilibrium for both groups, and no significant association was found between *MCP-1* genotypes ($p = 0.21$) or alleles ($p = 0.15$) and malaria Status. Notably, MSP2 genotyping was not completed, highlighting challenges in assessing parasite polymorphisms in field settings.

These findings suggest a possible modulatory role of *MCP-1* polymorphisms in influencing malaria outcomes, potentially through altered monocyte recruitment and inflammatory responses. However, further studies incorporating both host and parasite genetic markers are needed to clarify these associations in malaria-endemic populations.

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Introduction

Malaria is a life-threatening infectious disease caused by protozoan parasites of the genus *Plasmodium*, transmitted to humans through the bites of infected female *Anopheles* mosquitoes. The disease remains a pressing public health concern, particularly in tropical and subtropical regions. Africa accounts for about 94% of these cases and deaths, reflecting the disproportionate vulnerability of sub-Saharan Africa (SSA) to malaria transmission and mortality [1]. Cameroon is among the countries where malaria is endemic,

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Malaria parasites exhibit a complex life cycle involving both vertebrate (human) and invertebrate (*Anopheles*) hosts, alternating between sexual and asexual reproductive phases [3]. This biological complexity underlies the parasite's ability to evade immune responses and poses major challenges to drug and vaccine development [4]. Five *Plasmodium* species infect humans; *P. falciparum, P. vivax, P. ovale, P. malariae*, and *P. Knowlesi*; with P. falciparum being the most virulent and predominant species in SSA [5]. While P. vivax is globally more widespread, the majority of severe cases and malaria-related deaths are attributable to *P. Falciparum* [6].

Infection outcomes vary widely, ranging from asymptomatic to life-threatening complications such as cerebral malaria. Severe malaria often presents with coma, seizures, respiratory distress, or metabolic acidosis and disproportionately affects young children, pregnant women, immunocompromised individuals, and those lacking prior exposure [7]. Interestingly, adults over 20 years are increasingly represented among severe malaria cases, with some reports indicating a higher prevalence in females. Specific *P. falciparum* alleles have been linked to symptoms such as delirium and diarrhea, suggesting a possible genotype-phenotype correlation [8].

One hallmark of malaria in endemic settings is the high multiplicity of infection (MOI); the presence of multiple genetically distinct *Plasmodium* clones within a single host. MOI reflects local transmission intensity and can result from bites by mosquitoes carrying multiple parasite clones or from successive bites by different infected mosquitoes [9]. The coexistence of diverse parasite clones may influence clinical Status, immune evasion, and drug resistance dynamics. MOI and the proportion of polyclonal infections are widely used in malaria genomic epidemiology to assess transmission patterns and inform control strategies [10,11]. High MOI is often associated with increased polymorphism and genetic diversity, which in turn are driven by immune selection pressures and recombination [12]

Genotyping of *P. falciparum* is commonly performed using polymorphic markers such as *merozoite surface protein 1 (MSP1*), *merozoite surface protein 2 (MSP2)*, and *glutamate-rich protein (glurp). Msp2*, in particular, encodes an immunodominant surface antigen with high allelic diversity, helping the parasite escape host immune responses. The two major allelic families of msp2, FC27 and 3D7, vary widely in prevalence and have been implicated in immune evasion and disease Status [13]. Co-occurrence of FC27 and 3D7 allele pairs, especially of similar molecular weights, has been suggested to reflect possible epistatic interactions or immune selection. Infections with multiple *msp2* clones or specific alleles have been associated with severe disease phenotypes in several endemic regions [14].

On the host side, chemokines such as monocyte chemoattractant protein-1 (MCP-1); also known as CCL2; play a pivotal role in immune regulation by recruiting monocytes, memory T cells, and basophils to infection sites. MCP-1 levels rise in response to inflammation, and recent studies have explored its involvement in malaria pathogenesis. Elevated MCP-1 levels have been linked to severe malaria and increased mortality in both *P. falciparum* and *P. vivax* infections [15]. However, findings remain inconsistent, with some studies reporting no association between MCP-1 polymorphisms and clinical outcomes. These discrepancies may stem from population heterogeneity, genetic background, and study design differences.

Given the extensive genetic variability in both the *P. falciparum* parasite and the human immune response, it is crucial to examine how host-parasite genetic interactions contribute to malaria pathogenesis. In malaria-endemic regions such as the Buea municipality in Cameroon, where clinical presentations vary significantly, understanding the association between *MSP2* allelic diversity, *MCP-1* polymorphisms, and disease Status can inform targeted interventions and improve patient outcomes. This study seeks to explore these associations, contributing to a better understanding of the molecular and immunological factors underlying malaria Status in an endemic setting.

Materials and Method

Study Site and Design

This hospital-based cross-sectional study was conducted between November 2024 and May 2025. This study was conducted in Buea, located in the Fako Division of the South West Region of Cameroon. As of 2023, Fako Division has an estimated population of approximately 800,000 residents (Ngum et al., 2023). Situated at the base of Mount Fako, Buea experiences a humid climate, with cooler temperatures in the higher altitude neighborhoods and warmer conditions in the lower areas (Helders and Stefan, 2013). Similar to much of Cameroon, Buea has two main seasons: the rainy season, which lasts from March to October, and the dry season, occurring from November to February. Samples for this research were obtained from several health facilities, including Buea Regional Hospital, Solidarity Hospital and Muea Health Centre.

Study participants were between the age of 1 month and 90 years. Participants who gave their consent, malaria positive from microscopy with/without symptoms were included in the study while those who did not give their consent, had other illnesses as per their medical records or had severe anaemia were excluded from the study.

Samples were subjected to DNA extraction, Polymerase Chain Reaction (PCR), restriction enzyme digestion, and gel electrophoresis.

Sample Size Calculation

The Sample size for this study was calculated using the formula for sample calculation described by Cochrans

$$n=\frac{Z^2\cdot p\cdot(1-p)}{e^2}$$

Where,
n= Minimum sample size
z= confidence level
p= Prevalence of malaria in Cameroon (Ngum *et al*., 2023).
e= error rate
P=30%
At 95% confidence level, Z=1.96
e=0.05
Therefore, n= $[(1.96)^2\times0.30(1-0.30)]/ 0.05^2 = 322.7$.

This implies that the minimum sample size required to obtain statistically significant results is 323 participants.

Sample Collection

Venous blood (2 ml) was collected from participants who were admitted into the study for Dot Blood Spot preparation and DNA extraction for Polymerase Chain Reaction and Restriction Enzyme Digestion.

Fisher-brand filter paper (Whatmann No. 3) was cut into triangle shape, wrapped with aluminium foil and autoclaved, after which they were placed on a clean disinfected work surface spread with table napkin, and the edge of the filter paper was labelled according to the number code on the collected blood sample. About 150 µl of whole blood was spotted on the labelled filter paper and allowed to air dry for completely. The DBS paper was stored inside sterile air-tied bags alongside desiccants at room temperature until further use.

DNA Extraction

Genomic DNA was extracted from the DBS using the chelex 100™ (BIORAD) method. Chelex-100 resin captures positively charged ions, freeing DNA to bind elsewhere. Upon heating to 99°C, Chelex released DNA into solution. Vortexing facilitated DNA release, and centrifugation separated the DNA-containing supernatant, which was then transferred to a sterile tube for storage (Simon *et al*., 2020).

Prior to extraction, the equipment (puncher and forcep) were sterilized by dipping in 2% detergent and rinsed with distilled water. In a sterile eppendorf tubes, three 6mm DBS discs were punched using sterile punchers, picked using the forceps, and placed inside the labelled tubes. The puncher and forcep were sterilized each time it was used to punch a small piece of the DBS, and 1ml of 0.5% Tween 20 in 1XPBS was added to the tubes containing DBS and vortexed to mix well. The tubes were incubated at 4°C overnight.

The next morning the tubes containing the DBS were spanned for 15s at 4000 rpm to remove precipitation from the inner lead. The Tween 20 was removed, and 1ml of 1XPBS was added, vortexed for few 5s and incubated at 4oC for 30min to wash out the Tween 20. The 1mL PBS was then removed leaving the DBS in the tube 5% chelex resin was pre-heated with nuclease-free water at 95°C and 150ul of the hot 5% chelex resin solution was added to the tubes containing the PBS. The tubes were then incubated in the heat block for 15 minutes at 99°C and the samples were vortexed at 5minutes interval during the 15minutes heating and then centrifuge at a speed of 4000rpm for 5 minutes. The supernatant containing the DNA was removed carefully without disturbing the chelex beats (pellet) and transferred to freshly labelled tubes and stored at -20°C until further use. The DNA concentration was measured to be 50 ng/µl.

Identification of *Plasmodium Falciparum* by Nested PCR

Plasmodium falciparum speciation was performed by amplification of the 18s ribosomal RNA gene using genius specific primers (primary PCR (pPCR)) and species-specific primers (secondary PCR (sPCR)). The primer sequences used for gene amplification are shown in Table 1.

Table 1: Primer Sequence for the Nested PCR Amplification of the 18sRNA of *Plasmodium falciparum*

Organism	Primer	Primer Sequence	Expected Band Size
Plasmodium	Forward rPLU6	5’ TTAAAATTGTTGCAGTTAAAACG 3’	1,200bp
	Reverse rPLU5	5’CCTGTTGTTGCCTTAAACTTC 3’	
Plasmodium falciparum	Forward rFAL1	5’TTAAACTGGTTTGGGAAAACCAAATATATT 3’	205bp
	Reversed rFAL 2	5’ACACAATGAACTCAATCATGACTACCCGTC 3’	

The pPCR was carried out in a total volume of 10 µL consisting of 5 µL 2x PHire Green Hot Start II PCR master mix (New England BioLabs), 2.5 µL of Nuclease free water, 0.25 µL each of forward and reverse primers and 2 µL of the genomic DNA extracted. The PCR reaction was carried out using a thermo-cycler (Eppendorf Mastercycler) and conditions were as indicated (Table 2).

The sPCR was also carried out in a total volume of 10 µL consisting of 5 µL 2x PHire Green Hot Start II PCR master mix (New England BioLabs), 2.5 µL Nuclease free water, 0.5 µL each of the forward and reverse primers and a 1.5 µL of the primary amplicons. The PCR reaction was carried out using a thermo-cycler (Eppendorf master cycler) and conditions were the same as that of the outer PCR except for the annealing temperature (Table 2).

Table 2: Thermo Cycling Conditions for the Nested PCR Amplification of the 18sRNA of *Plasmodium falciparum*

PCR Conditions	Initial Denaturation	Denaturation	Annealing	Extension	Number of Cycles	Final Extension
pPCR	94°C for 3minutes	94°C for 30seconds	55°C for 1minute	72°C for 1minute	35	72°C for 5 minutes
sPCR	94°C for 3minutes	94°C for 30seconds	61°C for 1minute	72°C for 1minute	35	72°C for 5 minutes

Identification of *MCP-1 Polymorphism*

The *MCP-1* gene polymorphisms were detected by Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) as earlier described by (Sirisabhabhorn *et al*., 2021) with slide modifications.

The lyophilized *MCP-1* primer pair (Table 3) gotten from *Inqaba biotec* were re-suspended according to the manufacturer instructions as follows: The tubes were briefly centrifuged before opening to prevent the loss of the pellet. 722.6 µL and 542.77 µL of sterile deionized water were added to 100 µM stock solution of the *MCP-1* forward and reverse primers, respectively. The pH of the deionize water was maintained at 7.0 to prevent oligo depurination and subsequent loss of activity. Aliquots were prepared from the stock solution and stored together with the stored solution at -20°C for future use.

MCP-1 Gene Amplification by Polymerase Chain Reaction
The PCR conditions (total volume of 15µL) were set as follows: 2.75 µl of gDNA, 3.75µl of nuclease water, 7.5µl of 2x HPire Green Hot Start II PCR master mix and 0.5 µl each of the forward and reverse primers. The primers used are presented in table 1. Gene amplification, denaturation, annealing and extension steps (30 cycles) were set as 94°C for 3 min, 94°C for 30s, 64°C for 1 min, 72°C for 1 min and final extension at 72°C for 5 min. The amplified gene product was separated by a 2% agarose gel electrophoresis.

Table 3: Primers and Analysis Condition of _MCP-1_ Gene and Amino Acid/Nucleotide Position with Mutation Under Investigation

Mutation		NCBI Number	Primer	Sequence (5'-3')	Geno Type	Description	Fragment bp
-2518	A>G	Rs1024611	MCP 1_F	CTTTCCCTTGTGTGTCCCC	AA	Homozygous wild type	940
			MCP 1_R	TTACTCCTTTTCTCCCCAACC	AG GG	Heterozygous mutant Homozygous mutant	940, 650, 290 650, 290

Identification of _MCP-1_ SNPs and Genotypes by Restriction Fragment Length Polymorphism
The genetic polymorphism of *MCP-1* results from a nucleotide change from adenine to guanine at nucleotide position -2518. The reaction mixture (15µL) consisted of 9 µL of PCR amplicon, 1.5µL of 10X NEBuffer (rCutSmart$_{TM}$), 4.1 µL of nuclease free water and 0.4 µL of the restriction enzyme PvuII-HF (*Proteus vulgaris* II High Fidelity). The mixture incubated at 37°C for 2 hours and the RFLP products were analyzed by electrophoresis on a 3% agarose gel (fig4b). PvuII a type II restriction endonuclease commonly used in molecular biology for DNA digestion with optimal activity at 37°C in the appropriate buffer recognizes the DNA sequence 5'-CAGCTG-3', cleaving it between the G and C bases (CAGˆCTG, producing blunt ended DNA fragments (Horton *et al*., 1998; Nasri and Thomas, 1987). Its high-fedility (HF) forms offer reduced star activity with improved performance in a single buffer system.

PCR Amplification of Merozoite Surface Protein 2 Gene
This was done using oligonucleotide primers to specifically amplify block of the *MSP-2* gene (Smythe *et al*., 1990). The gene was amplified by nested PCR with the conserved or family-specific primers pairs being amplified separately, as previously described (Foley *et al*., 1992), with slide modifications. The oligonucleotide primers used for the primary and nested PCR are shown in Table 4 below. The primary PCR was carried out in a total volume of 10 µL consisting of 5 µL OneTaq 2X MM W/V (#M0489S Master Mix, New England BioLabs), 2.5 µL of Nuclease free water, 0.25 µL each of forward and reverse primers and 2 µL of the genomic DNA extracted. The PCR reaction was carried out using a thermo-cycler (Eppendorf Mastercycler) and conditions were as indicated (Table 6). The master mix is formulated for quick loading onto gels thereby aiding in the visualization of the amplicons on agarose gel electrophoresis. OneTaq 2X MM master mix is suitable for routine PCR from various templates such as pure DNA, bacterial colonies and cDNA products.

Table 4: Primer Sequence for the PCR Amplification and Nested PCR Amplification of _MSP2_ Allelic Family Genes (FC27 and 3D7)

Primer	Primer Sequence	Expected Band Size
MSP2: S2-fwd	GAAGGTAATTAAAACATTGTC	
MSP2: S3-rev	GAGGGATGTTGCTGCTCCACAG	
MSP2: Stail rev	gtgtcttGCTTATAATATGAGTATAAGGAGAA	
MSP2: M5 (FC27) fwd MSP2: N5 (3D7) fwd	6FAM/GCATTGCCAGAACTTGAA HEX/CTGAAGAGGTACTGGTAGA	approx. 370 bp product approx. 260 bp product

The secondary PCR was also carried out in a total volume of 10uL consisting of 5 µL 2 OneTaq 2X MM W/V #M0489S Master Mix (New England BioLabs), 2.5 µL Nuclease free water, 0.5 µL each of the forward and reverse primers and a 1.5 µL of the primary amplicons. The sPCR reaction was carried out using a thermo-cycler (Eppendorf master cycler) and conditions were the same as that of the pPCR except for the annealing as shown (Table 5). The nested PCR amplicons for the FC27 and 3D7 alellic families of the *MSP2* gene where then electrophoresed on a 2% agarose gel and visualized using ultraviolet transillumination after staining with ethidium bromide

Table 5: ***MSP2*** **pPCR and sPCR Cycling Conditions**

PCR Conditions	Initial Denaturation	Denaturation	Annealing	Extension	Number of Cycles	Final Extension
Outer PCR	94ºC 3minutes	94ºC 30seconds	58ºC 2minutes	68ºC 1minute	25	68ºC 5 minutes
Inner PCR	94ºC 3minutes	94ºC 30seconds	58ºC 1minute	68ºC 1minute	30	68ºC 5 minutes

Ethical Consideration

Ethical approval to conduct this study was obtained from the Faculty of Health Sciences Institutional Review Board (Ref: 2024/2575/-08/UB/SG/IRB/FHS of 30 October 2024) at the University of Buea. After explaining the objective of the study, blood samples were collected from participants that met the inclusion criteria.

Data Analysis

Chi-square test was used to compare genotype and allele frequencies between symptomatic and asymptomatic groups, and the distribution of alleles across parasitaemia levels. The Hardy-Weinberg Equilibrium (HWE) was used to determine a possible deviation of allele distribution in the study population (https://dr.petrek.edu>documents>HWE.xls). Logistic regression analysis was used to assess the relationship between *MCP-1* polymorphisms and malaria status. Fisher's exact test was used to compare genotype distribution with parasitaemia levels. Statistical significance was set at 5%.

Results

Socio-Demographic Characteristics of Study Population

Consent to participate in the study was requested from 350 participants, and 273 participants agreed to take part in the study. In the consented group, 56% (142/273) were females and 44% (130/273) were males, with ages ranged from 0 to >40 years. 46% (125/273) of the participants recorded an axial temperature greater than or equal to 37.5℃, and 54% (148/273) recorded an axial temperature less than 37.5℃, making a total of 125 symptomatic and 148 asymptomatic participants respectively.

Amplification of *P. falciparum* 18sRNA

The results of the amplification of the 18s ribosomal RNA gene of malaria parasite using genus and specific primers for *P. falciparum* are shown in Figure 1 below. The presence of a band at 205 bp on the gel indicates a positive identification of *Plasmodium* by PCR. Conversely, the absence of a band at this position suggests the absence of *P. falciparum* DNA, and the sample is therefore classified as negative. This result is the same as reported in Dinga *et al*., 2025 [16]. Reproduced with written permission.

Prevalence and Distribution and of MCP1 Polymorphisms

Out of the 95 P. falciparum positive samples confirmed by nested PCR amplification of the 18s RNA of the parasite, 55 (57.9%) were found to be positive for the MCP-1 gene, as evident by the presence of a 940 bp band on agarose gel electrophoresis. This included 9 (64.3%) asymptomatic heterozygous individuals and 5 (35.7%) symptomatic heterozygous individuals. The homozygous individuals consist of 17 (41.5%) asymptomatic participants and 24 (58.5%) symptomatic participants. Polymorphisms were ascertained by the presence of either 2 bands at 650 bp and 290 bp (homozygous mutant) or 3 bands at 940 bp, 650 bp and 290 bp (heterozygous mutant). A single band at 940 bp indicated no mutation, hence the presence of the wild type (Figure 2).

MCP-1 mutations result in an A-to-G transition at amino acid position -2518. This result in three distinct allelic forms: homozygous wild type (AA), heterozygous mutant (AG) and homozygous mutant (GG). With respect to participant's age, the wild type is predominant in all age groups (Table 6).

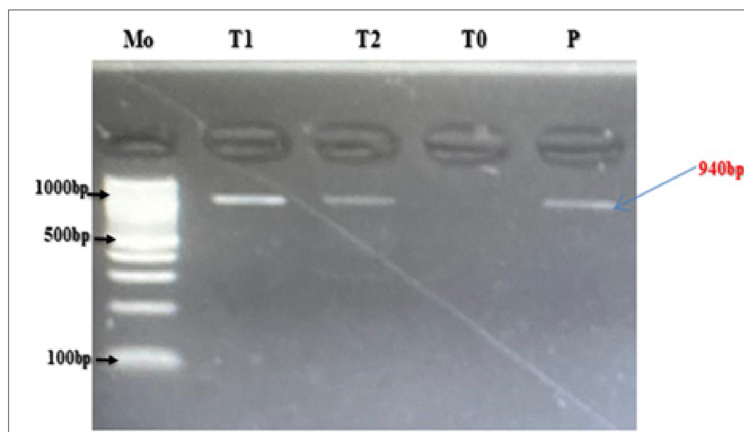

Figure 1: Agarose gel electrophoregram of MCP1 Gene amplicons

Mo = Molecular weight marker. T1 and T2 = MCP1 amplicons. T0 = Negative control; P = Positive control

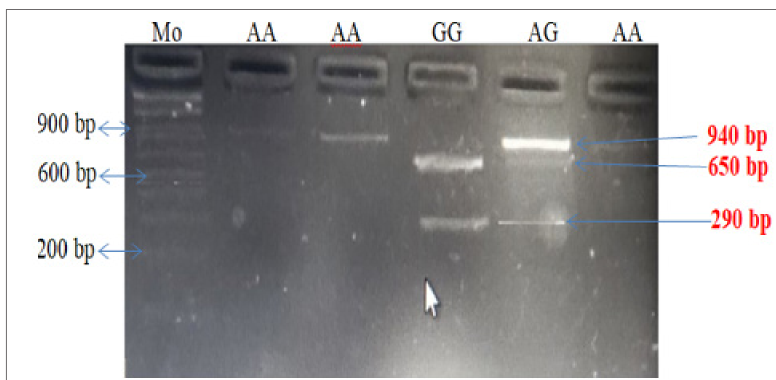

Figure 2: Agarose gel electrophoregram of MCP1 amplicons Digested with PvuII restriction enzyme

Mo = Molecular weight marker, AA(940 bp), AG (940 bp. 650 bp and 290 bp) and GG(650 bp and 290 bp)

Chi-Square goodness-of-fit test for Hardy-Weiberg Equilibrium (HWE) shows that the two study groups (symptomatic and asymptomatic groups) are in HWE, with statistical values (p = 0.73) and (p = 0.82) respectively.

Table 6: Prevalence of *MCP-1* Genotypes and Gene Alleles in Symptomatic and Asymptomatic Groups

Category	Symptomatic, n (%)	Asymptomatic, n (%)	Total	% Total
Genotype Wild Type (AA)	27 (84.4)	16 (69.6)	43	78.2%
Heterozygous mutant (AG)	4 (12.5)	6 (26.1)	10	18.2%
Homozygous mutant (GG)	1 (3.1)	1 (4.3)	2	3.6%
Allele				
A	58 (90.6)	38 (82.6)	96	87.3%
G	6 (9.4)	8 (17.4)	14	12.7%

Malaria Symptomatic individuals (participants whose axial body temperature were greater than or equal to 37.5°C) recorded the highest distribution of the *MCP-1* wild type (AA), with asymptomatic individuals (participants whose axial body temperature was strictly less than 37.5°C) recording the highest distribution of the heterozygous mutant (AA). However, the wild type predominates in both symptomatic (84.4%) and asymptomatic (69.6%) groups, and an equal distribution of the homozygous mutant (GG) in both symptomatic and asymptomatic malaria cases (Table 7). There was a significant difference (p = 0.03) in the distribution of genotypes between symptomatic and asymptomatic groups. Similarly, the distribution of allele A predominates in both groups (90.6% in the case of symptomatic and 82.6% in the case of asymptomatic). The distribution of allele G was the least in both groups, with the G allele more frequent in the asymptomatic group. Despite the trend towards association, it is not statistically significant (p = 0.08).

Table 7: Association of *MCP-1* Genotypes and Gene Alleles with Demographic Data

Category	AA (n, ¬%)	AG (n,%¬)	GG (n, %)	Total (n)	p-value
Parasitaemia					0.07*
mean	3200±4500	5000±800	1000±1500		
Low	10 (50)	8 (40)	2 (10)	20	
Moderate	8 (61.5)	4 (30.8)	2(7.7)	14	0.21
High	25 (80.6)	1 (3.2)	5 (16.1)	31	
Gender					
Male	25 (75.8)	6 (18.2)	2 (6)	33	
Female	18 (81.8)	7 (18.2)	1 (4.5)	22	0.69
Age (years)					
0–25	15 (75%)	4 (20%)	1 (5%)	20	
26–40	20 (83.3%)	3 (12.5%)	1 (4.2%)	24	0.12
>40	8 (72.7%)	2 (18.2%)	1 (9.1%)	11	

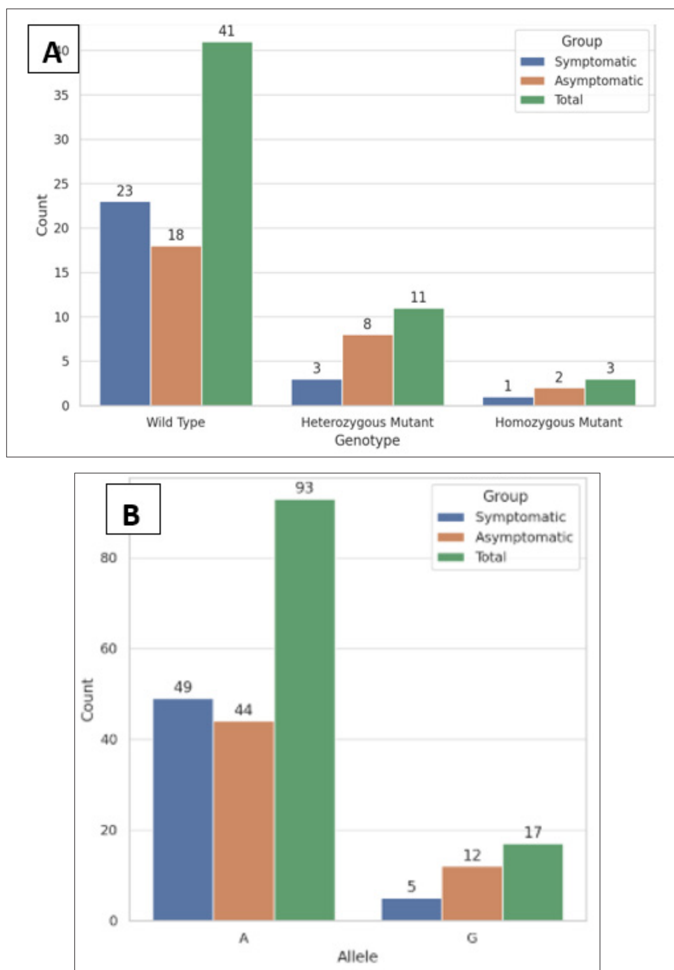

Figure 3: Distribution of *MCP-1* genotypes and alleles in the study population. A: Wild Type is most common among symptomatic individuals (85.2%, $p = 0.03$), while Heterozygous mutants are more prevalent in the asymptomatic group (28.6%). Suggests a potential protective role for the heterozygous mutation. B: G allele is more frequent in asymptomatic individuals (21.4%) compared to symptomatic (9.3%), indicating a possible protective trend, though not statistically significant ($p = 0.08$)

Association of *MCP-1* Gene Polymorphisms and Symptomatic Status in *P. falciparum* Infection

There was a significant difference ($p = 0.04$) in parasitaemia levels across genotypes, with wild type carriers having higher parasitaemia (Figure 3). However, parasitaemia levels in the study groups (symptomatic vs asymptomatic) showed no significant association with *MCP-1* genotypes ($p = 0.21$), and *MCP-1* gene alleles ($p = 0.15$) (Figure 4). Similarly, after adjusting for age and gender, logistic regression analysis showed a lower odd of symptoms with the heterozygous mutant ($p = 0.02$) (Figure 5).

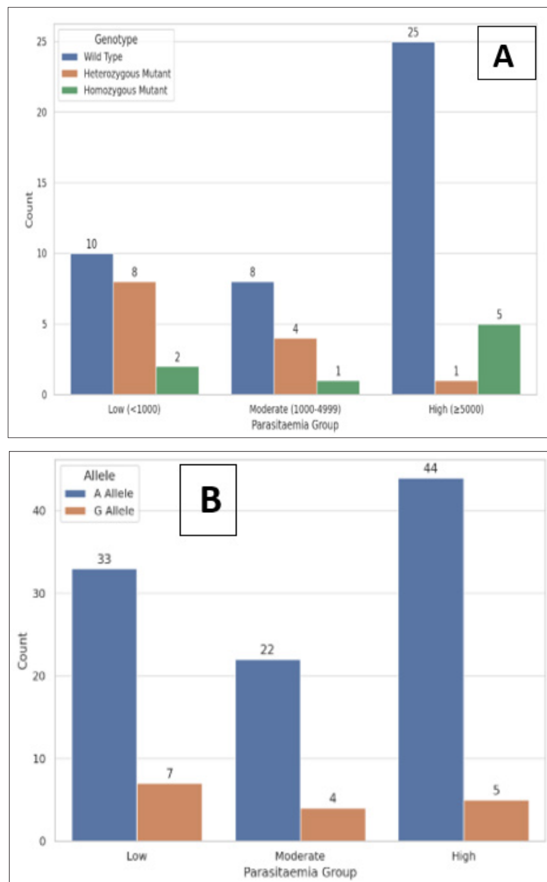

Figure 4: *MCP-1* genotype and allele distribution across parasitaemia levels. A: Wild Type (AA) increases with parasitaemia. Heterozygous genotype (AG) is more common in low/moderate parasitaemia, suggesting a potential role in reducing parasite burden, although the association is not significant ($p = 0.21$). B: Similarly, A allele dominates across all parasitaemia levels, but the G allele is relatively more present in low/moderate parasitaemia, hinting at a potential protective effect. There was, however, no significant association ($p = 0.15$)

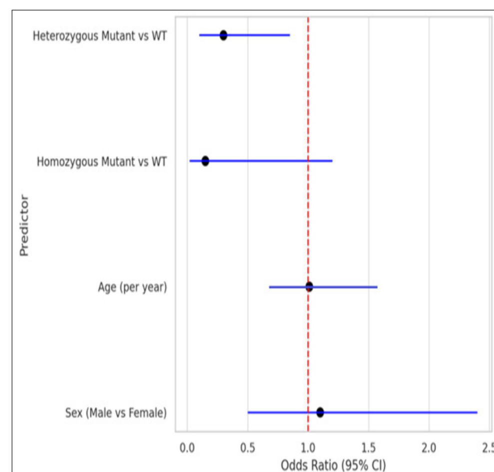

Figure 5: Logistic regression analysis of *MCP-1* polymorphism vs symptomatic malaria. The logistic regression curve (after adjusting for confounders like age and gender) shows that heterozygous individuals are significantly less likely to be symptomatic (OR = 0.30, $p = 0.02$), supporting a protective association. Homozygous mutation, however, also trends protective (OR = 0.15), but not statistically significant ($p = 0.07$)

Prevalence and Association of MSP2 Allelic Gene Families and Status of Malaria

In this study, efforts to genotype the *Plasmodium falciparum* merozoite surface protein 2 (*MSP2*) gene, specifically targeting block 3 and its allelic families (3D7 and FC27), consistently resulted in smears with no distinct bands on agarose gel electrophoresis. This occurred despite extensive optimization attempts including optimization of primer volumes, use of different DNA templates that worked well with other primers, gradient PCR for annealing temperature adjustment, and adjustment of agarose gel electrophoresis migration time and voltage.

Discussion

Monocyte chemoattractant protein-1 (MCP-1), also known as CCL2, plays a pivotal role in the immune system by recruiting monocytes, macrophages, and other immune cells to sites of inflammation; a process vital for effective immune responses against infections, including malaria [15]. The *MCP-1* gene is a key component of the host's immunological signaling network, and variations within it may influence individual susceptibility or resistance to malaria. However, studies investigating associations between *MCP-1* polymorphisms and malaria status have produced inconsistent or inconclusive results across different populations.

In this study, we explored the relationship between the *MCP-1* -2518A>G polymorphism and malaria status in individuals from Buea Municipality, Cameroon. A higher frequency of the wild-type AA genotype was observed among symptomatic individuals (84.4%) compared to asymptomatic cases (69.6%). Conversely, the heterozygous AG and homozygous GG genotypes were more frequent in the asymptomatic group. This distribution suggests a potential protective influence of the G allele against symptomatic malaria, possibly by modulating the host's inflammatory response. However, the observed difference in allele frequency (G allele: 17.4% in asymptomatic vs. 9.4% in symptomatic individuals) did not reach statistical significance ($p = 0.07$), which tempers the strength of this conclusion.

These findings are biologically plausible considering *MCP-1*'s immunological function [15]. MCP-1-mediated recruitment of monocytes can enhance parasite clearance but may also contribute to inflammation-induced tissue damage, especially in severe malaria [17]. Thus, individuals with the G allele may experience attenuated monocyte recruitment, reduce inflammation and potentially leading to asymptomatic or milder infections. Despite this, our Hardy-Weinberg Equilibrium (HWE) analysis revealed no significant deviation in genotype distributions for either group, suggesting no recent selection pressure on this gene variant within the studied population; similar to findings from other African cohorts (Abramovs, Brass, & Tassabehji, 2020) [10,18,19].

Other studies have similarly reported a lack of significant association between *MCP-1* polymorphisms and malaria outcomes. For instance, Deckhum et al. (2006) found no meaningful relationship between *MCP-1* genotypes and the status of malaria in Thai populations [20]. The consistency of such results across divergent populations supports the hypothesis that while *MCP-1* protein expression may correlate with malaria status, genetic variants within *MCP-1* may not independently determine disease outcome [21]. Notably, Driss et al., 2011 emphasized the distinction between gene polymorphism and protein expression, suggesting that elevated MCP-1 protein levels in severe malaria reflect inflammatory responses rather than being directly driven by gene variants [22].

The broader context of malaria as a multifactorial disease further complicates the interpretation of single-gene effects. Malaria status results from an interplay of host genetic background, parasite genotype, immune response, co-infections, environmental conditions, and treatment accessibility [18,19]. Host polymorphisms in genes such as TNF, CR1, and G6PD have shown more robust associations with malaria status, possibly because their biological effects are more direct or better understood in the context of red blood cell physiology or inflammation [22].

Interestingly, *MCP-1* polymorphisms have demonstrated strong disease associations in non-malarial contexts. For example, in tuberculosis, the -2518 GG genotype was linked to increased susceptibility and higher MCP-1 expression [23]. These disease-specific effects underscore the importance of context, both immunological and genetic, when interpreting polymorphism-disease relationships.

Although our data showed no statistically significant association between MCP-1 polymorphisms and malaria status, the trend toward a higher G allele frequency in asymptomatic individuals aligns with the hypothesis of a potential immunomodulatory role. Larger sample sizes and more granular disease classifications (e.g., mild vs. severe vs. cerebral malaria) may be necessary to clarify subtle genetic effects. Furthermore, studies incorporating additional host genetic markers and gene–gene interactions (epistasis) may uncover more nuanced determinants of malaria susceptibility [24].

Technical challenges in the genotyping of the MSP2 gene highlight another important limitation of this study. Despite repeated attempts using nested PCR, we were unable to reliably amplify and resolve MSP2 allelic variants, likely due to the gene's highly polymorphic nature and the limitations of standard agarose gel electrophoresis. As previously documented (Felger et al., 1999; Falk et al., 2006), MSP2 is characterized by variable tandem repeats and complex secondary structures that can hinder accurate amplification [25]. Smearing and overlapping bands in the gel electrophoresis suggest issues related to primer specificity or the resolution limits of the gel, rather than DNA degradation or contamination, as multiple optimization steps (gradient PCR, voltage adjustments, template dilution) yielded consistent patterns.

This methodological constraint emphasizes the need for more robust genotyping platforms, such as fluorescently labeled nested PCR followed by capillary electrophoresis or next-generation sequencing, which can overcome the resolution and specificity limitations encountered in this study [26]. The inability to obtain reliable MSP2 genotyping data limited our capacity to assess multiplicity of infection (MOI) and parasite diversity; factors that are increasingly recognized as important contributors to malaria clinical outcomes.

The absence of comparative data from Central African countries like Cameroon further limits regional context for our findings. Most genetic association studies have been concentrated in West or East Africa. Our study therefore contributes valuable region-specific data, offering a starting point for more comprehensive investigations of host-pathogen genetic interactions in Central Africa. The genetic diversity of P. falciparum strains across Africa may also influence host–parasite dynamics, with certain alleles potentially exerting stronger effects in specific contexts [10].

Discrepancies among MCP-1 studies may stem from a range of factors including population heterogeneity, small sample sizes, variations in phenotype classification, and unmeasured confounders. Genetic associations can be masked or exaggerated by age, sex, co-infections, nutritional status, or host immunity. Moreover, MCP-1's role may be more prominent when analyzed in combination with other chemokine or cytokine gene variants (e.g., IL10, TGFβ1, ICAM1), rather than as an isolated determinant.

In summary, this study found no statistically significant association between the MCP-1 -2518A>G polymorphism and malaria symptom status, despite trends suggesting a potential protective role of the G allele. These findings are consistent with similar studies in other populations and reinforce the view that *MCP-1* gene variation alone is unlikely to serve as a strong genetic marker for malaria status. However, the potential role of *MCP-1* in modulating inflammation remains biologically relevant, and future studies with larger sample sizes, improved genotyping platforms, and integrative multi-gene approaches are necessary to fully elucidate its contribution to malaria pathogenesis.

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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