Parasitology Research (2022) 121:167–175
https://doi.org/10.1007/s00436-021-07399-y

GENETICS, EVOLUTION, AND PHYLOGENY - ORIGINAL PAPER

Plasmodium falciparum msp1 and *msp2* genetic diversity in parasites isolated from symptomatic and asymptomatic malaria subjects in the South of Benin

Hamirath Odée Lagnika[1,2] · Azizath Moussiliou[2] · Romuald Agonhossou[1,2] · Pierre Sovegnon[1,2] · Oswald Yédjinnavênan Djihinto[1,2] · Adandé Assogba Medjigbodo[1,2] · Laurette Djossou[1,2] · Linda Eva Amoah[3] · Aurore Ogouyemi-Hounto[4] · Luc Salako Djogbenou[1,2]

Received: 13 July 2021 / Accepted: 29 November 2021 / Published online: 6 January 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract
Symptomatic and asymptomatic malaria patients are considered as the reservoirs of human *Plasmodium*. In the present study, we have evaluated the *Plasmodium falciparum* merozoite surface protein-1 (*Pfmsp1*) and protein-2 (*Pfmsp2)* genetic diversity among the symptomatic and asymptomatic malaria infection from health facilities in Cotonou, Benin Republic. A cross-sectional study recruited 158 individuals, including 77 from the asymptomatic and 81 from the symptomatic groups. The parasites were genotyped using Nested Polymerase Chain Reaction. Samples identified as *Plasmodium falciparum* were genotyped for their genetic diversity. No significant difference was observed in the overall multiplicity of infection (MOI) between the asymptomatic and symptomatic groups. In the symptomatic group, the overall frequency of K1, MAD20, and RO33 allelic family was more predominant (98.5%) followed by 3D7 (87.3%) and FC27 (83.1%). However, in asymptomatic group, the K1 alleles were the most prevalent (100%) followed by FC27 (89.9%), 3D7 (76.8%), MAD20 (60.5%), and RO33 (35.5%). The frequency of multiple allelic types (K1+MAD20+RO33) at the *Pfmsp1* loci in the symptomatic infections was significantly higher when compared to that of the asymptomatic ones (97% vs. 34%, $p<0.05$), whereas no difference was observed in the frequency of multiple allelic types (3D7 and FC27) at the *Pfmsp2* loci between the two groups. The high presence of *msp1* multiple infections in the symptomatic group compared to asymptomatic ones suggests an association between the genetic diversity and the onset of malaria symptoms. These data can provide valuable information in the development of a vaccine that could reduce the symptomatic disease.

Keywords Malaria · Asymptomatic · Symptomatic · Genetic diversity · South of Benin

Section Editor: Kevin S.W. Tan

✉ Luc Salako Djogbenou
ldjogbenou22002@yahoo.fr

[1] Tropical Infectious Diseases Research Centre, University of Abomey-Calavi, 01BP 526, Cotonou, Benin

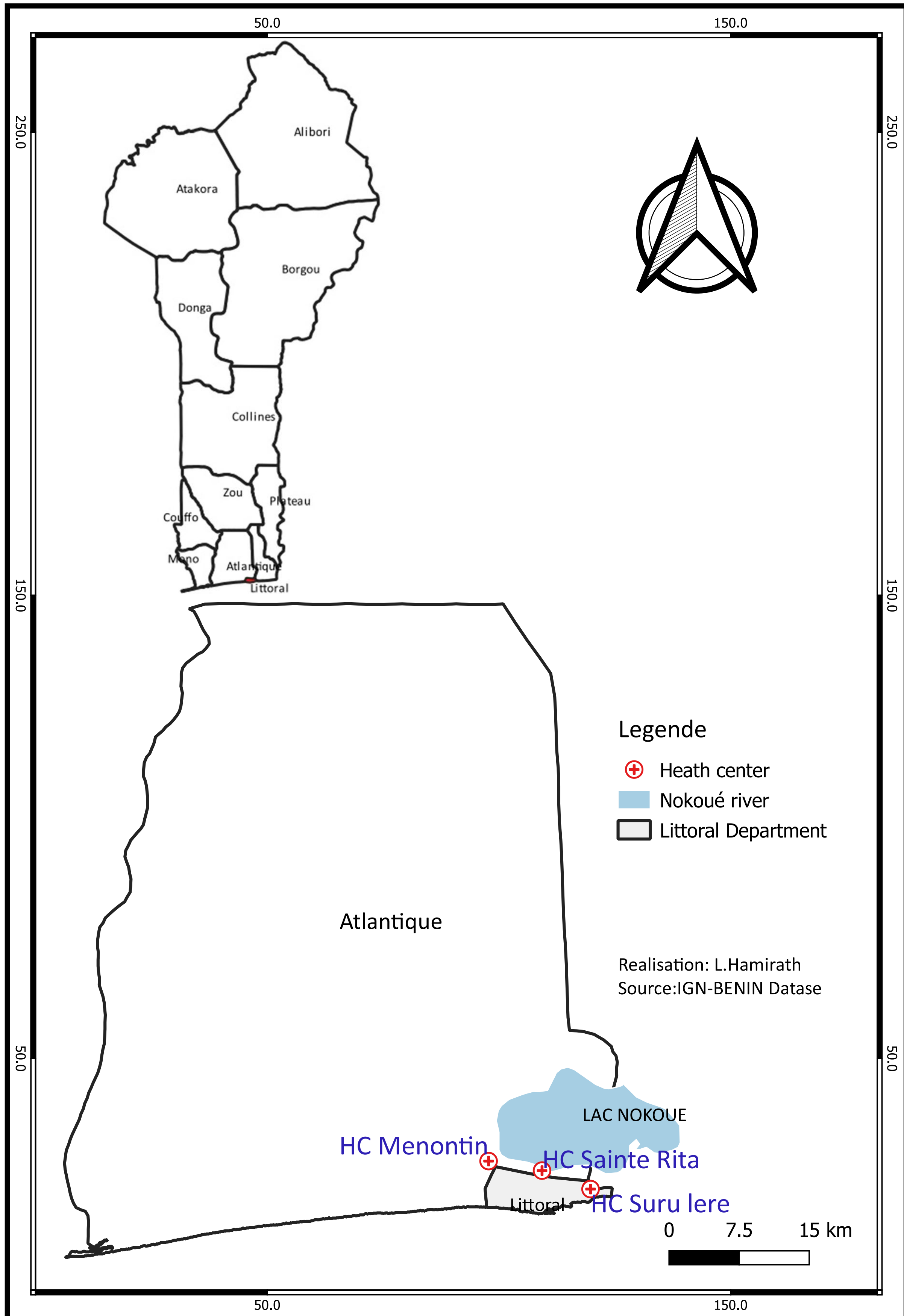
[2] Laboratory of Infectious Vector-Borne Diseases, Regional Institute of Public Health/University of Abomey-Calavi, BP 384, Ouidah, Benin

[3] Immunology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana

[4] Programme Nationale de Lutte Contre Le Paludisme (PNLP), Cotonou, Benin

Introduction

Malaria is an infectious disease caused by one-celled parasites of the genus *Plasmodium.* Out of the five main species known to infect humans, *Plasmodium falciparum* is the most-deadly form (Josling and Llinás 2015). Malaria is one of the major vector-borne diseases affecting millions of people worldwide. Despite all the control efforts over several decades, malaria remains a paramount public health concern (Barat et al. 2004). In 2019, global malaria cases estimated by the WHO (2019) (World Health Organization) worldwide were 229 million and 409,000 deaths. National Malaria Control Programs (NMCPs) and international partners invested heavily in a series of malaria control interventions such as intermittent preventive treatment during pregnancy (IPTp), distribution of insecticide-treated bed nets (ITNs), use of


50.0
150.0
250.0
Alibori
Atakora
Borgou
Donga
Collines
Zou
Plateau
Couffo
Mono
Atlantique
Littoral
Legende
Heath center
Nokoué river
Littoral Department
Atlantique
Realisation: L.Hamirath
Source:IGN-BENIN Datase
LAC NOKOUE
HC Menontin
HC Sainte Rita
HC Suru lere
Littoral
0 7.5 15 km

◂**Fig. 1** Map of the samples collection

artemisinin-based combination therapy (ACT), indoor residual spraying (IRS), a test-and-treat policy, and the development of antimalarial vaccines (Gosling et al. 2010).

One of the difficulties in developing an effective universal vaccine against *Plasmodium falciparum* parasite is the extensive genetic diversity of vaccine targets allowing the parasites with mutated genes to escape from the host's immune response (Genton et al. 2002; Healer et al. 2004; Mahmoudi and Keshavarz 2018). Several candidate proteins such as Duffy-Binding Protein (DBP), Apical Membrane Antigen-1 (AMA-1), Thrombospondin-Related Anonymous Protein (TRAP), Circumsporozoite protein (CSP), Merozoite Surface Protein-1 (*msp1*), and Merozoite Surface Protein-2 (*msp2*) have been designed to induce immunity against malaria parasites (Richards and Beeson 2009). Several polymorphic genetic markers have widely been used to evaluate *Plasmodium falciparum*'s genetic diversity, especially *msp1* and *msp2* proteins (Kiwuwa et al. 2013; Yavo et al. 2016), which are two proteins challenging the human immune system (Patel et al. 2017). Additionally, these antigenic markers are used to investigate the level of malaria transmission intensities, to discriminate new from recrudescent infections in therapeutic efficacy monitoring studies and to determine the number of different strains of *Plasmodium falciparum* co-infecting the same host define as the multiplicity of infection (MOI) (Congpuong et al. 2014; Ferreira and Hartl 2007; Vafa et al. 2008).

In the ongoing fight against malaria, asymptomatic cases are often not included in clinical studies (Ramaswamy et al. 2020), thus representing a silent natural reservoir for malaria transmission. Consequently, asymptomatic malaria cases may be the precursors of symptomatic disease progression and in eventual spread to other human hosts (Bousema et al. 2014). It has been shown that asymptomatic malaria infections were four to five times more prevalent than clinically patent infections (Lindblade et al. 2013). Similarly, in Colombia, 37% of asymptomatic pregnant women were found to have parasitemia (Carmona-Fonseca et al. 2017). Therefore, asymptomatic plasmodial infections constitute a significant obstacle for malaria control and elimination efforts. As a result, asymptomatic infections need to be considered in endemic countries to achieve the malaria elimination goal in 2030.

Previous studies investigated the genetic diversity of *Plasmodium* by using *msp1* and *msp2* proteins from symptomatic cases (Beck et al. 2001; Somé et al. 2018), but our understanding of the genetic diversity of *Plasmodium* isolates from asymptomatic infections is limited. Studies comparing the genotype of asymptomatic infection to the symptomatic ones in different local endemic conditions will play a critical role in developing new generations of malaria control strategies. This could help to accelerate efforts to overcome control and move towards this disease elimination through combinations of interventions tailored to local contexts. This information will also enrich the data on parasite population diversity that is invaluable for designing and implementing an effective malaria vaccine.

In this study, we assessed and compared the genetic diversity of *Plasmodium falciparum* isolates among individuals with asymptomatic malaria infections and symptomatic patients attending health facilities in Cotonou (Republic of Benin) using two polymorphic markers: merozoite surface proteins markers *msp1* and *msp2*.

Methods

Study sites

The samples used in this study were collected between April and July 2016 from the Mènontin and Sainte-Rita health facilities, located in Littoral province in Southern Benin (Fig. 1). Both health facilities are located around the Nokoué river, which constitutes suitable habitat for malaria-transmitting mosquitoes. Southern Benin is characterized by a sub-equatorial climate and a perennial malaria transmission with two peaks corresponding to the rainy seasons (April to July and mid-September to November) (Plan stratégique de lutte contre le paludisme au Bénin, 2010).

Study population and blood samples collection

Dried blood spots were prepared by dropping 50 μL of blood onto strips of Whatman® 3 mm paper from individuals willing to participate in the study and aged ≥ 2 years old. The blood samples were obtained from symptomatic subjects presenting suggestive symptoms of malaria (temporal temperature ≥ to 37.5 °C or history of fever 72 h preceding admission to the health facility, positive thick smear regardless of parasite density) and from asymptomatic residents (temporal temperature < 37.5 °C, absence of fever in the previous 2 weeks and at least 1 week after enrolment and positive thick smear regardless of parasite density). *Plasmodium falciparum* species identification. Only positive samples regardless of parasitemia from both asymptomatic and symptomatic malaria patients were included in this study. Samples from a total of 158 participants were collected and all of them were positive by microscopy.

Table 1: Primer sequences for *Plasmodium* species identification and *msp1* and *msp2*

Primer		Sequence (5′–3′)
Nested 1	rPLU6	TTA AAA TTG TTG CAG TTA AAA CG
	rPLU5	CCT GTT GTT GCC TTA AAC TTC
Nested 2	rFAL1	TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT
	rFAL2	ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC
Outer	M1-OF	CTA GAA GCT TTA GAA GAT GCA GTA TTG
	M1-OR	CTT AAA TAG TAT TCT AAT TCA AGT GGA TCA
K1	M1-KF	AAA TGA AGA AGA AAT TAC TAC AAA AGG TGC
	M1-KR	GCT TGC ATC AGC TGG AGG GCT TGC ACC AGA
MAD 20	M1-MF	AAA TGA AGG AAC AAG TGG AAC AGC TGT TAC
	M1-MR	ATC TGA AGG ATT TGT ACG TCT TGA ATT ACC
RO33	M1-RF	TAA AGG ATG GAG CAA ATA CTC AAG TTG TTG
	M1-RR	CAA GTA ATT TTG AAC TCA TGT TTT AAATCAGCGTA
Msp2		
Outer	M2-OF	ATG AAG GTA ATT AAA ACA TTG TCT ATT ATA
	M2-OR	CTT TGT TAC CAT CGG TAC ATT CTT
3D7/FC27	S1fw	GCT TAT AAT ATG AGT ATA AGG AGA A
FC27	M5rev	GCA TTG CCA GAA CTT GAA
3D7	N5rev	CTG AAG AGG TAC TGG TAG A

DNA extraction and *Plasmodium falciparum* identification after malaria diagnostic tests

Genomic DNA was extracted from 2 spots on Whatman paper dried blood spots using chelex-100® (Bio-Rad Laboratories, CA, USA) as previously described (Wooden et al. 1993). The DNA extracts were stored at −20 °C until used for the amplification reactions.

Plasmodium falciparum species were detected using the method described by Snounou et al. (1993). Briefly, *Plasmodium falciparum* species were determined by a nested PCR in a final volume of 20 µL. The first round of amplification was made up of genus-specific primers mix of rPlu 5 (0.5 µL) and rPlu 6 (0.5 µL), with 4.75 µL of sterile water, 4 µL of 5× PCR buffer, 1 µL of 5 mM dNTP, 4 µL of 25 mM $MgCl_2$, 0.25 µL of Go Taq DNA polymerase (BioLabs®Inc.), and 5 µL of genomic DNA. The second round of amplification was done using 3 µL of the first PCR products with 0.5 µL of *Plasmodium falciparum* specific (rFAL1 and rFAL2) primers (10 µM), and 6.75 µL of sterile water. Samples confirmed to be *Plasmodium falciparum* positive were then selected for other molecular characterization. The primary and secondary primers sequences are listed in Table 1.

Allelic genotyping of the *Plasmodium falciparum msp1* and *msp2* genes

A protocol previously described by Barry et al. (2013) was used to amplify the *msp1* and *msp2* genes of *Plasmodium falciparum* using specific primers summarized in Table 1. The polymorphic allelic families of the *msp1* (K1, MAD20, and RO33) and *msp2* (FC27 and 3D7) genes were amplified with a nested PCR amplification. Briefly, the primary amplification used 0.25 µL of each of primers M1-OF/M1-OR for *Pfmsp1* and M2-OF/M2-OR for *Pfmsp2* in the presence of 3.55 µL of water, 5 µL of buffer 5×, 4 µL of 25 mM $MgCl_2$, 1 µL of 5 mM dNTPs, 0.25 µL of Taq DNA Polymerase (BioLabs®Inc.), and 5 µL of DNA extract.

For the secondary PCR reaction, the primer pairs specific to each *Pfmsp1* and *Pfmsp2* allelic family were used in the presence of the same reaction constituents as the first PCR round but with different reaction volumes. The primer pairs used were K1/K2 for the K1 family, MAD20-1/ MAD20-2 for the MAD20 family, and RO33-1/RO33-2 for the RO33 family of *Pfmsp1*. For the allelic families of the *Pfmsp2* gene, the primer pairs FC27-1/FC27-2 and 3D7-1/3D7-2 were used for the FC27 and 3D7 families respectively. The primary and secondary primers set used are listed in Table 1. The cycling conditions for both amplification reactions were an initial denaturation at 94°C for 5 min followed by 35 cycles of 94 °C for 30 s, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. The amplification products from each secondary PCR were assessed using ethidium bromide staining 2% agarose gel followed by visualization under UV.

Statistical analysis

Plasmodium falciparum infections were classified as either monoclonal if it yielded a single band at either of the

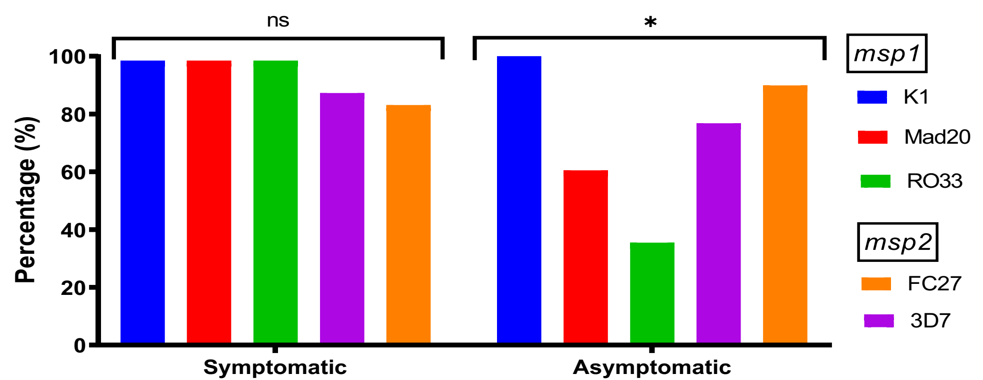


Fig. 2 Frequencies of *msp1* and *msp2* allelic families in *Plasmodium falciparum* isolates from symptomatic and asymptomatic malaria patients group. Frequencies were determined as the percentage of individuals within a given symptomatic and asymptomatic group among positive samples. Statistical differences were reckoned using Chi-squared exact test. ns and * indicate respectively *p*-value > 0.05 and *p*-value < 0.05

two *msp2* PCRs as well as a single band at either of the three *msp1* PCRs, otherwise the sample was classified as polyclonal, with more than one allele in at least one locus (Kiwuwa et al. 2013).

The multiplicity of infection (MOI) was calculated by dividing the total number of distinct *msp1* and *msp2* fragments by the number of samples positive for the same gene (Mohd Abd Razak et al. 2016). The mean MOI was calculated by dividing the total number of alleles detected in both *msp1* and *msp2* by the total number of positive samples for both markers. Chi-squared exact test was used to compare the distribution of the allelic frequencies of K1, MAD20, RO33, 3D7, and FC27 allele families between symptomatic and asymptomatic groups using the package "stats" in R Core Team, 2017 software version 3.4.4. The student's *t*-test was used to assess the statistical differences of MOI values between the two groups. Graphical representations were performed using GraphPad Prism 8.0.2 software (San Diego, California USA). All the analysis was set at a significance threshold of $p < 0.05$.

Table 2: Multiplicity of Infection (MOI) of *msp1, msp2*, families allelic, and overall MOI from symptomatic and asymptomatic malaria patients

Allelic family	MOI symptomatic	MOI asymptomatic	*P* value
K1	2.9	4.59	< 0.0001
MAD20	3.15	3.19	0.9
RO33	2.45	2.55	0.7
msp1	2.8	3.79	0.3
3D7	2.24	2.41	0.5
FC27	1.35	1.3	0.15
msp2	2	1.8	0.6
Overall	2.4	2.8	0.5

Results

Prevalence of *Plasmodium falciparum infection*

Samples from a total of 158 individuals were analyzed (81 asymptomatic and 77 symptomatic patients). All blood samples used were positive by *Plasmodium falciparum* infection using microscopy and confirmed by PCR 100% (158/158).

Allelic polymorphism of *msp1* and *msp2*

All three allelic types (K1, MAD20, and RO33) of *msp1* were detected in 89.3% (141/158) of the *Plasmodium falciparum* isolates, including 65 samples from the symptomatic group and 76 from the asymptomatic group. A total of 88.6% (140/158) *Plasmodium falciparum* isolates displayed a positive PCR outcome for *msp2*, including 71 samples from the symptomatic group and 69 from the asymptomatic group. In the symptomatic group, 98.5 % of *Plasmodium falciparum* isolates belonged to the K1, MAD20, and RO33 allelic family while 83.1% and 87.3% of the parasite isolates carried FC27 and 3D7 allelic types, respectively (Fig. 2). The distribution of all five allelic families was high and approximately the same across the symptomatic group. For samples collected from the asymptomatic group, 76 (100%), 46 (60.5%), and 27 (35.5%) isolates were found to have K1, MAD20, and RO33 allelic types respectively while 89.9% and 76.8% of the parasite isolates had FC27 and 3D7 allelic types respectively. The distribution of all five allelic families varies across the asymptomatic group. The predominant allelic family in both groups was K1, while the frequencies of MAD20 and RO33 were significantly higher in the symptomatic group ($p < 0.01.10^{-2}$) (Fig. 2).

The MOI of the five allelic families and the overall MOI of each gene are displayed in Table 2. The overall MOI of *msp1* and *msp2* in the symptomatic group were 2.8 (95% CI: 2.66–2.94) and 2 (95% CI: 1.9–2.1) respectively, whereas, in the asymptomatic group, the MOI of *msp1* and *msp2* were 3.79 (95% CI: 3.2–3.5) and 1.8 (95% CI: 1.71–1.9). There was no statistical difference in the MOI of *msp1* and

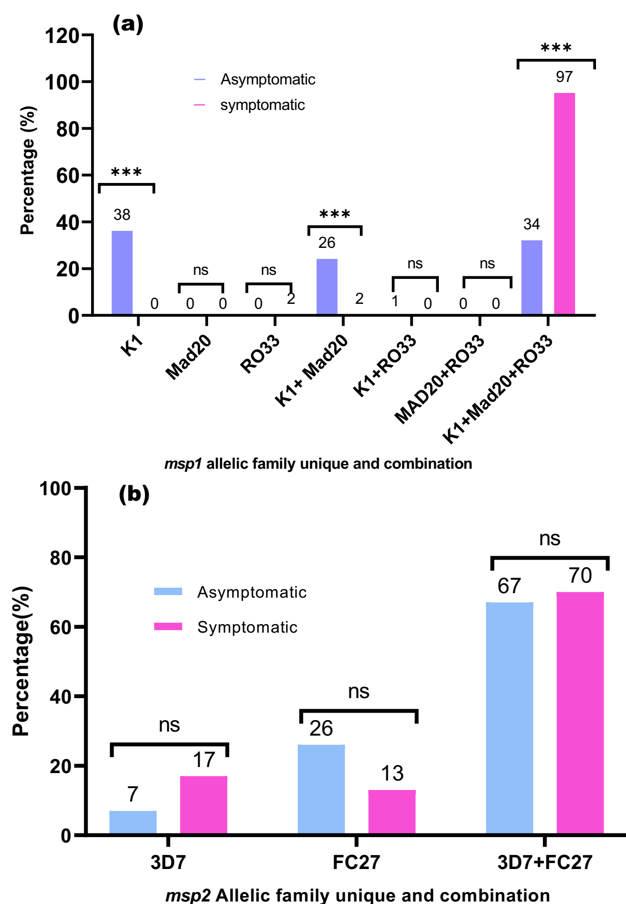


Fig. 3 Combinations of *msp1* and *msp2* allelic families in symptomatic and asymptomatic malaria patients. Alleles of the *msp1* (**a**) *and msp2* (**b**) genes were classified by the presence of a fragment after respectively the *msp1* and *msp2* allele's specific PCR amplifications. The number above each bar indicates the exact frequency. Statistical differences were determined using Chi-squared exact test

msp2 between the two groups ($p > 0.05$). The allelic family K1 displayed a significant difference of MOI between the asymptomatic and symptomatic groups (4.59 vs. 2.9) ($p<0.01.10^{-2}$) (Table 2).

For individual infections among the symptomatic group, 2% carried only RO33 type while 98% of individuals were found to have multiple allelic types (i.e., K1/MAD20, K1/RO33, MAD20/RO33, and K1/MAD20/RO33). In contrast, 38.2% of single-clone infections were recorded in the asymptomatic group with K1 type, and 61.8% were multiple allelic types (i.e., K1/MAD20, K1/RO33, and MAD20/RO33, and K1/MAD20/RO33) (Fig. 3a). The symptomatic group displayed a significantly higher frequency of multiclonal infections related to the *msp1* gene (mostly with K1 family alleles) compare to that observed in the asymptomatic group ($p < 0.05$). Concerning *msp2* gene, 13% of the parasite isolates had only FC27 and 17% had only 3D7 allelic type among the symptomatic group. The remaining 70% had the two allelic types (i.e., FC27/3D7) (Fig. 3b). In asymptomatic individuals, 26% had only FC27 allelic type, 7% were found to have only 3D7 type, and the remaining 67% had multiple allelic types (i.e., FC27/3D7). No statistically significant difference was observed when comparing 3D7, FC27 allelic family, and multiclonal (i.e., FC27/3D7) infections ($p > 0.05$) of two groups.

Discussion

Determining *Plasmodium falciparum* genetic diversity from field samples is crucial for understanding the population structure of *Plasmodium falciparum* genotypes. This information is a crucial component in adjusting malaria control strategies against the target parasites species in a specific endemic area. Since the parasite reservoir consists of symptomatic and asymptomatic individuals, we investigated the polymorphism of *Plasmodium falciparum* subpopulations between two categories of malaria infection (symptomatic and asymptomatic cases). Surface proteins 1 and 2 of merozoites were used as the marker because of their well-known genetic diversity, and also, they provide information on MOI and *Plasmodium falciparum* alleles frequency, which are most often correlated with the level of transmission in the study area (Ayanful-Torgby et al. 2016; Daubersies et al. 1996). Comparing the genetic diversity of *Plasmodium falciparum* isolates among individuals with asymptomatic malaria infections and symptomatic patients can provide clues to understand protection against clinical (symptomatic) malaria and more insights into parasite virulence mechanisms.

Here, we observed that the allelic families of both *msp1* (K1, MAD20, and RO33) and *msp2* (3D7 and FC27) were present in the symptomatic group at a relatively similar frequency (frequency >80%) whereas, in the asymptomatic group, the K1 *msp1* allelic family was the most prevalent, while a similar prevalence was observed among both allelic families of *msp2*. The MAD20 and RO33 *msp1* allelic families were significantly higher in the symptomatic group than the asymptomatic group. Furthermore, we observed that 98.5% of the symptomatic patient samples carried all the three *msp1* allelic families (K1, MAD20, and RO33) instead of 34% of the samples in the asymptomatic group that displayed the same allelic families. This suggests that the infections in our study area with the parasites belonging to the MAD20 and RO33 *msp1* allelic families would more likely result in the exhibition of malaria symptoms. However, studies on the association between allelic families of *msp1* or *msp2* and malaria clinical status, extended to all disease categories, displayed conflicting reports. Specifically, for the RO33 allelic family, some authors demonstrated that RO33 alone or in co-infection was found to be

strongly associated with fever and anemia (Anong et al. 2010; Sondo et al. 2019; Ofosu-Okyere et al. 2001; Robert et al. 1996; Ghanchi et al. 2015). Conversely, another study reported no association between RO33 allelic family and malaria clinical status, including symptomatic/asymptomatic (al-Yaman et al. 1997). These inconsistent results may be attributed to several factors like complex interactions of the host, parasite, and environment (Miller et al. 2002; Kiwuwa et al. 2013).

In studies exploring *Plasmodium falciparum* genetic diversity among or between malaria clinical status, MOI is used to measure malaria transmission level (Tusting et al. 2014; Arnot 1998; Chen et al. 2018; Koepfli et al. 2011). Consequently, the highest value of MOI recorded in some areas means that the transmission is particularly at the highest level (Abdel-Wahab et al. 2002; Paul et al. 1998). However, no significant difference was observed between the overall MOI of the asymptomatic and symptomatic groups, considering all allelic families of both *msp1* and *msp2* locus, suggesting no association between the different genotypes of the clinical malaria status in the study area. On the contrary, high MOI values were linked to the asymptomatic category compared to symptomatic in Ghana (Agyeman-Budu et al. 2013). However, some authors did not find any association between malaria asymptomatic/symptomatic status and MOI in other African countries such as Madagascar (Durand et al. 2008) and Gambia (Conway et al. 1991). Consequently, the discrepancy between the MOI level and the clinical malaria status might be due to distinct factors not yet well understood. Our results show that when considering only the K1 allelic family, the MOI reported in the asymptomatic group was higher than that of the symptomatic group. Furthermore, by comparing monoclonal infections of *msp1* locus between the asymptomatic and symptomatic groups, we found 38% of the asymptomatic individuals had only K1 variant. Taken together, these results suggest that in our study area, infections from parasites belonging to only the K1 allelic family are likely to be asymptomatic and not associated with the signs and symptoms of clinical malaria.

Our study did not show any significant difference in the prevalence of infections containing parasites belonging to both *msp2* allelic families (3D7-FC27 co-infection) among the asymptomatic and symptomatic patients. This observation here supports results from a previous study by A-Elbasit et al. (2007) and suggests that the occurrence of both *msp2* alleles (3D7 and FC27) in infection does not influence the occurrence of malaria symptoms.

This study had some limitations including the fact that allelic frequencies may have been underestimated due to the limited ability of the genotyping assay to work at low parasite densities, which would be more prevalent in the asymptomatic group and may have biased our results. This limitation was expected in previous studies (Contamin et al. 1995; Färnert et al. 2001). Earland et al. (2019) have shown that minor alleles are undetectable in low-density asymptomatic infections using genotype assay. In addition, capillary electrophoresis would assess allelic diversity more accurately than the agarose gel electrophoresis used in the current study which had intrinsically limited by the use of two genetic markers. Also, it would be better to use Next-Generation Sequencing (NGS) technologies which provide much more accurate data on genetic diversity in *Plasmodium* populations and have an increased sensitivity and specificity in detecting minority clones within multiclonal infections (Juliano et al. 2010; Lin et al. 2015).

Regardless of the above limitations, this study showed that the two *msp1* allelic families MAD20 and RO33 were more prevalent in symptomatic individuals and may be associated with the clinical signs. Although these results have to be taken with caution, they highlight the need to further explore this subject by implementing longitudinal studies in different geographical contexts with the standardized collection and genotyping methods.

Conclusion

The present study's findings provide an overview of the genetic variability of *Plasmodium falciparum* in symptomatic and asymptomatic infections. In the asymptomatic group, the predominant genotype was K1, while MAD20 and RO33 were most common in the symptomatic patient group. However, infections with the K1-MAD20-RO33 complex were higher in symptomatic than asymptomatic individuals. In addition, infections with the 3D7-FC27 complex were similarly distributed in the symptomatic and asymptomatic groups. The MOI was similar between the asymptomatic and symptomatic groups. Our findings provide an overview of the genetic variability of *Plasmodium falciparum* in symptomatic and asymptomatic infections.

Acknowledgements The authors thank all malaria patients who participated in the study. Also, we would like to thank the team of Tropical Infectious Diseases Research Centre and Laboratory of Infectious Vector-Borne Diseases, University of Abomey-Calavi, for participating in the field and laboratory work.

Author contribution Hamirath Odée Lagnika, Azizath Moussiliou, and Luc Salako Djogbenou conceived and designed the study. Hamirath Odée Lagnika, Azizath Moussiliou, and Laurette Djossou performed the experiments. Hamirath Odée Lagnika analyzed the data. Hamirath Odée Lagnika, Romuald Agonhossou, Pierre Sovegnon, Oswald Yédjinnavênan Djihinto, Adandé Assogba Medjigbodo, Aurore Ogouyemi-Hounto, Linda Eva Amoah, and Luc Salako Djogbenou performed drafting and substantial revision of the manuscript. All authors read and approved the final version of the manuscript.

Funding This work was supported by Wellcome Trust (intermediate fellowship in public health and tropical medicine n° 109917/Z/15/Z) awarded to Luc Salako DJOGBENOU.

Data availability All data generated or analyzed during this study are included in the article.

Declarations

Competing interests The authors declare no competing interests.

References

Abdel-Wahab A, Abdel-Muhsin A-MA, Ali E et al (2002) Dynamics of gametocytes among Plasmodium falciparum clones in natural infections in an area of highly seasonal transmission. J Infect Dis 185:1838–1842. https://doi.org/10.1086/340638

A-Elbasit IE, Elghazali G, A-Elgadir TME et al (2007) Allelic polymorphism of MSP2 gene in severe P. falciparum malaria in an area of low and seasonal transmission. Parasitol Res 102:29–34. https://doi.org/10.1007/s00436-007-0716-3

Agyeman-Budu A, Brown C, Adjei G et al (2013) Trends in multiplicity of Plasmodium falciparum infections among asymptomatic residents in the middle belt of Ghana. Malar J 12:22. https://doi.org/10.1186/1475-2875-12-22

Al-Yaman F, Genton B, Taraika J et al (1997) Cellular immunity to merozoite surface protein 2 (FC27 and 3D7) in Papua New Guinean children. Temporal variation and relation to clinical and parasitological status. Parasite Immunol 19:207–214. https://doi.org/10.1046/j.1365-3024.1997.d01-198.x

Anong DN, Nkuo-Akenji T, Fru-Cho J et al (2010) Genetic diversity of Plasmodium falciparum in Bolifamba, on the slopes of Mount Cameroon: influence of MSP1 allelic variants on symptomatic malaria and anaemia. Ann Trop Med Parasitol 104:25–33. https://doi.org/10.1179/136485910X12607012373876

Arnot D (1998) Unstable malaria in Sudan: the influence of the dry season. Clone multiplicity of Plasmodium falciparum infections in individuals exposed to variable levels of disease transmission. Trans R Soc Trop Med Hyg 92:580–585. https://doi.org/10.1016/s0035-9203(98)90773-8

Ayanful-Torgby R, Oppong A, Abankwa J et al (2016) Plasmodium falciparum genotype and gametocyte prevalence in children with uncomplicated malaria in coastal Ghana. Malar J 15:592. https://doi.org/10.1186/s12936-016-1640-8

Barat LM, Palmer N, Basu S, et al (2004) Do malaria control interventions reach the poor? A view through the equity lens. American Society of Tropical Medicine and Hygiene

Barry AE, Schultz L, Senn N et al (2013) High levels of genetic diversity of Plasmodium falciparum populations in Papua New Guinea despite variable infection prevalence. Am J Trop Med Hyg 88:718–725. https://doi.org/10.4269/ajtmh.12-0056

Bousema T, Okell L, Felger I, Drakeley C (2014) Asymptomatic malaria infections: detectability, transmissibility and public health relevance. Nat Rev Microbiol 12:833–840. https://doi.org/10.1038/nrmicro3364

Carmona-Fonseca J, Agudelo OM, Arango EM (2017) Asymptomatic plasmodial infection in Colombian pregnant women. Acta Trop 172:97–101. https://doi.org/10.1016/j.actatropica.2017.04.030

Chen J-T, Li J, Zha G-C et al (2018) Genetic diversity and allele frequencies of Plasmodium falciparum msp1 and msp2 in parasite isolates from Bioko Island. Equatorial Guinea Malaria Journal 17:458. https://doi.org/10.1186/s12936-018-2611-z

Congpuong K, Sukaram R, Prompan Y, Dornae A (2014) Genetic diversity of the msp-1, msp-2, and glurp genes of Plasmodium falciparum isolates along the Thai-Myanmar borders. Asian Pac J Trop Biomed 4:598–602. https://doi.org/10.12980/APJTB.4.2014APJTB-2014-0156

Contamin H, Fandeur T, Bonnefoy S, et al (1995) PCR typing of field isolates of Plasmodium falciparum. J Clin Microbiol 33:944–951. https://doi.org/10.1128/jcm.33.4.944-951.1995

Conway DJ, Greenwood BM, McBride JS (1991) The epidemiology of multiple-clone Plasmodium falciparum infections in Gambian patients. Parasitology 103(Pt 1):1–6. https://doi.org/10.1017/s0031182000059217

Daubersies P, Sallenave-Sales S, Magne S et al (1996) Rapid turnover of Plasmodium falciparum populations in asymptomatic individuals living in a high transmission area. The American journal of tropical medicine and hygiene 54:18–26. https://doi.org/10.4269/ajtmh.1996.54.18

Durand R, Ariey F, Cojean S et al (2008) Analysis of circulating populations of Plasmodium falciparum in mild and severe malaria in two different epidemiological patterns in Madagascar. Tropical Med Int Health 13:1392–1399. https://doi.org/10.1111/j.1365-3156.2008.02156.x

Earland D, Buchwald AG, Sixpence A, et al (2019) Impact of multiplicity of plasmodium falciparum Infection on clinical disease in malawi. Am J Trop Med Hyg 101:412–415. https://doi.org/10.4269/ajtmh.19-0093

Färnert A, Arez AP, Babiker HA, et al (2001) Genotyping of Plasmodium falciparum infections by PCR: a comparative multicentre study. Trans R Soc Trop Med Hyg 95:225–232. https://doi.org/10.1016/s0035-9203(01)90175-0

Ferreira MU, Hartl DL (2007) Plasmodium falciparum: worldwide sequence diversity and evolution of the malaria vaccine candidate merozoite surface protein-2 (MSP-2). Exp Parasitol 115:32–40. https://doi.org/10.1016/j.exppara.2006.05.003

Genton B, Betuela I, Felger I et al (2002) A recombinant blood-stage malaria vaccine reduces Plasmodium falciparum density and exerts selective pressure on parasite populations in a phase 1-2b trial in Papua New Guinea. J Infect Dis 185:820–827. https://doi.org/10.1086/339342

Ghanchi NK, Hasan Z, Islam M, Beg MA (2015) MAD 20 alleles of merozoite surface protein-1 (msp-1) are associated with severe Plasmodium falciparum malaria in Pakistan. J Microbiol Immunol Infect 48:213–218. https://doi.org/10.1016/j.jmii.2014.01.004

Gosling RD, Cairns ME, Chico RM, Chandramohan D (2010) Intermittent preventive treatment against malaria: an update. Expert Rev Anti-Infect Ther 8:589–606. https://doi.org/10.1586/eri.10.36

Healer J, Murphy V, Hodder AN et al (2004) Allelic polymorphisms in apical membrane antigen-1 are responsible for evasion of antibody-mediated inhibition in Plasmodium falciparum. Mol Microbiol 52:159–168. https://doi.org/10.1111/j.1365-2958.2003.03974.x

Josling GA, Llinás M (2015) Sexual development in Plasmodium parasites: knowing when it's time to commit. Nat Rev Microbiol 13:573–587. https://doi.org/10.1038/nrmicro3519

Juliano JJ, Porter K, Mwapasa V et al (2010) Exposing malaria in-host diversity and estimating population diversity by capture-recapture using massively parallel pyrosequencing. PNAS 107:20138–20143. https://doi.org/10.1073/pnas.1007068107

Kiwuwa MS, Ribacke U, Moll K et al (2013) Genetic diversity of Plasmodium falciparum infections in mild and severe malaria of children from Kampala, Uganda. Parasitol Res 112:1691–1700. https://doi.org/10.1007/s00436-013-3325-3

Koepfli C, Ross A, Kiniboro B et al (2011) Multiplicity and diversity of Plasmodium vivax infections in a highly endemic region in

Papua New Guinea. PLoS Negl Trop Dis 5:e1424. https://doi.org/10.1371/journal.pntd.0001424

Kolakovich KA, Ssengoba A, Wojcik K et al (1996) Plasmodium vivax: favored gene frequencies of the merozoite surface protein-1 and the multiplicity of infection in a malaria endemic region. Exp Parasitol 83:11–19. https://doi.org/10.1006/expr.1996.0044

Lin JT, Hathaway NJ, Saunders DL et al (2015) Using amplicon deep sequencing to detect genetic signatures of Plasmodium vivax relapse. J Infect Dis 212:999–1008. https://doi.org/10.1093/infdis/jiv142

Lindblade KA, Steinhardt L, Samuels A et al (2013) The silent threat: asymptomatic parasitemia and malaria transmission. Expert Rev Anti-Infect Ther 11:623–639. https://doi.org/10.1586/eri.13.45

Mahmoudi S, Keshavarz H (2018) Malaria vaccine development: the need for novel approaches: a review article. Iran J Parasitol 13:1–10

Miller LH, Baruch DI, Marsh K, Doumbo OK (2002) The pathogenic basis of malaria. Nature 415:673–679. https://doi.org/10.1038/415673a

Mohd Abd Razak MR, Sastu UR, Norahmad NA et al (2016) Genetic diversity of Plasmodium falciparum populations in malaria declining areas of Sabah, East Malaysia. PLoS One 11:e0152415. https://doi.org/10.1371/journal.pone.0152415

Ofosu-Okyere A, Mackinnon MJ, Sowa MP et al (2001) Novel Plasmodium falciparum clones and rising clone multiplicities are associated with the increase in malaria morbidity in Ghanaian children during the transition into the high transmission season. Parasitology 123:113–123. https://doi.org/10.1017/s0031182001008162

Patel P, Bharti PK, Bansal D et al (2017) Genetic diversity and antibody responses against Plasmodium falciparum vaccine candidate genes from Chhattisgarh, Central India: implication for vaccine development. PLoS One 12:e0182674. https://doi.org/10.1371/journal.pone.0182674

Paul RE, Hackford I, Brockman A et al (1998) Transmission intensity and Plasmodium falciparum diversity on the northwestern border of Thailand. Am J Trop Med Hyg 58:195–203. https://doi.org/10.4269/ajtmh.1998.58.195

Ramaswamy A, Mahabala C, Basavaiah SH et al (2020) Asymptomatic malaria carriers and their characterization in hotpops of malaria at Mangalore. Tropical Parasitology 10:24. https://doi.org/10.4103/tp.TP_71_18

Richards JS, Beeson JG (2009) The future for blood-stage vaccines against malaria. Immunol Cell Biol 87:377–390. https://doi.org/10.1038/icb.2009.27

Robert F, Ntoumi F, Angel G et al (1996) Extensive genetic diversity of Plasmodium falciparum isolates collected from patients with severe malaria in Dakar, Senegal. Trans R Soc Trop Med Hyg 90:704–711. https://doi.org/10.1016/s0035-9203(96)90446-0

Snounou G, Viriyakosol S, Zhu XP et al (1993) High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol Biochem Parasitol 61:315–320. https://doi.org/10.1016/0166-6851(93)90077-b

Somé AF, Bazié T, Zongo I et al (2018) Plasmodium falciparum msp1 and msp2 genetic diversity and allele frequencies in parasites isolated from symptomatic malaria patients in Bobo-Dioulasso. Burkina Faso Parasites Vectors 11:323. https://doi.org/10.1186/s13071-018-2895-4

Sondo P, Derra K, Lefevre T et al (2019) Genetically diverse Plasmodium falciparum infections, within-host competition and symptomatic malaria in humans. Sci Rep 9:127. https://doi.org/10.1038/s41598-018-36493-y

Tusting LS, Bousema T, Smith DL, Drakeley C (2014) Measuring changes in Plasmodium falciparum transmission: precision, accuracy and costs of metrics. Adv Parasitol 84:151–208. https://doi.org/10.1016/B978-0-12-800099-1.00003-X

Vafa M, Troye-Blomberg M, Anchang J et al (2008) Multiplicity of Plasmodium falciparum infection in asymptomatic children in Senegal: relation to transmission, age and erythrocyte variants. Malar J 7:17. https://doi.org/10.1186/1475-2875-7-17

WHO technical consultation to review the role of drugs in malaria prevention for people living in endemic settings (2019). https://www.who.int/publications/m/item/who-technical-consultation-to-review-the-role-of-drugs-in-malaria-prevention-for-people-living-in-endemic-settings. Accessed 25 May 2021

Wooden J, Kyes S, Sibley CH (1993) PCR and strain identification in Plasmodium falciparum. Parasitol Today 9:303–305. https://doi.org/10.1016/0169-4758(93)90131-x

Yavo W, Konaté A, Mawili-Mboumba DP et al (2016) Genetic Polymorphism of msp1 and msp2 in Plasmodium falciparum Isolates from Côte d'Ivoire versus Gabon. J Parasitol Res 2016:3074803. https://doi.org/10.1155/2016/3074803

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.