International Scholars Journals

African Journal of Parasitology Research ISSN 2756-3391 Vol. 13 (8), pp. 001-009, August, 2025. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Polymorphism of the *Pfmdr1* and *Pfk13* genes of *Plasmodium falciparum* isolates in the cities of Abengourou, San Pedro, and Man

Gohoun WCZ[1,2]*, Kipre GR[1], Konate-Toure A[2], Gnagne AP[2], Bedia-Tanoh AV[2], Yavo W[2]

[1]Biology and Health Laboratory, Felix Houphouët Boigny University, Abidjan, Côte d'Ivoire.
[2]Malaria Research and Control Center (MRCC) of the National Institute of Public Health of Côte d'Ivoire (INSP), Abidjan, Côte d'Ivoire.

Abstract

Received 16 June, 2025; Revised 10 July, 2025; Accepted 17 July, 2025; Published 14 August, 2025

Despite efforts to control malaria, the disease remains a major public health problem, especially in Côte d'Ivoire, where it is the leading cause of morbidity. One of the factors limiting the efficacy of treatments is the emergence and spread of *Plasmodium falciparum* resistance to antimalarial drugs, including artemisinin-based combinations. This study aimed to determine the prevalence of resistance markers in the *Pfmdr1* and *Pfk13* genes in Côte d'Ivoire. Samples were collected from October 2021 to August 2022 from individuals who presented a thick drop positive for *Plasmodium falciparum* at Man, San-Pedro, and Abengourou. Plasmodial DNA was extracted using the ZYMO Quick DNA kit, followed by nested PCR amplification and Sanger sequencing. Of the 300 samples, successful sequencing rates were 91% (273/300) for *Pfmdr1* and 86.3% (259/300) for *Pfk13*. Analysis of sequencing data showed a high prevalence of the *Pfmdr1* 184F mutation (60.22%), while the 86Y mutation was infrequent (1.9%). For the *Pfk13* gene, no mutations associated with resistance to artemisinin derivatives as observed in Southeast Asia were detected. However, 14 mutations were identified (5.4%); of which 8 were synonymous mutations (3.1%) and 6 were non-synonymous mutations (2.3%). This study shows a decrease in the prevalence of mutant allele 86Y and a high prevalence of mutant allele 184F of the *Pfmdr1* gene. For the *Pfk13* gene, no known artemisinin resistance-conferring mutations were observed. Their high frequency could compromise the long-term effectiveness of currently recommended treatments. In terms of research, these results call for continued molecular surveillance to prevent the emergence of new resistance. From a policy perspective, this situation requires regular reassessment of treatment protocols.

Keywords: *Plasmodium falciparum*, Molecular monitoring, *Pfmdr1*, *Pfk13.*

INTRODUCTION

Malaria remains a major public health problem in the world, especially in sub-Saharan Africa. Progress in global malaria control has stagnated for some years, and business-as-usual approach will only further distance countries and their development partners from the goals they had set themselves (WHO, 2023). According to the WHO report, there were 263 million cases and 597,000 deaths in 2023. Nearly 76% of malaria deaths occurred in children under 5. Africa continues to pay the highest price. In 2023, it recorded 94% of the total number of malaria

Corresponding author. Email: gohouncarola@gmail.com

cases (246 million cases) and 95% of deaths (569,000 deaths). (WHO, 2024).

Because of the recurring phenomenon of resistance to antimalarial drugs for more than 20 years, most countries where *Plasmodium falciparum* malaria is endemic have gradually updated their treatment policies, switching from chloroquine, sulfadoxine-pyrimethamine and amodiaquine (which have had several therapeutic failures) leading to the currently recommended artemisinin derivative (ACT) combinations (WHO, 2015). The ACT has been adopted worldwide as a first-line treatment for uncomplicated *Plasmodium falciparum* malaria (WHO, 2022).

ACTs have been successful in reducing the global malaria burden; however, the emergence and spread of partial resistance to artemisinin threatens their effectiveness (WHO, 2022). Indeed, *Plasmodium falciparum* adapts more and continues to develop resistance, even against therapeutic combinations based on artemisinin derivatives. For more than a decade, studies have shown that *Plasmodium falciparum* can also develop forms of resistance, even to ACTs. Partial artemisinin resistance, characterized by the delayed elimination of the parasite from the bloodstream after artemisinin treatment, is prevalent in the Greater Mekong sub-region of Southeast Asia (WHO, 2018). However, WHO recently confirmed the presence of partial resistance to artemisinin in Rwanda (Uwimana et al., 2020), the United Republic of Tanzania, and Eritrea (WHO, 2024).

Several molecular markers of antimalarial resistance have been studied, in particular the *Pfmdr1* and *Pfk13* genes for CTA resistance. The *Pfmdr1* gene codes for a glycoprotein and is involved in resistance to several antimalarials such as amodiaquine, lumefantrine, mefloquine, etc. N86Y mutation points, Y184F, S1034C, N1042D, and D1246Y are involved in the resistance of the *Pfmdr1* gene to antimalarials. The *Pfk13* gene is involved in resistance to artemisinin derivatives. Several mutations are associated with partial resistance of *Plasmodium falciparum*, but to date, thirteen mutations are currently validated by the WHO as being related to artemisinin resistance. These are F446I, N458Y, C469Y M476I, Y493H, R539T, I543T, P553L, R561H, P574L, C580Y, R622I and A675V. Some of these changes have been observed recently in Africa, specifically in Rwanda, Eritrea, Ghana, Ethiopia, Uganda, Mali and Tanzania (Baina et al., 2024). In Côte d'Ivoire, none of these mutations has yet been observed (Konaté-Touré et al., 2024). This study aims to determine the prevalence of molecular markers of resistance in the *Pfmdr1* and *Pfk13* in Côte d'Ivoire.

MATERIAL AND METHOD

Site and period of study

This is a cross-sectional study that took place from October 2021 to August 2022. It was carried out in three localities of the Ivory Coast, namely Man, San-Pedro, and Abengourou.

Man is a city located in the west of the Ivory Coast, 658.8 km from Abidjan. The average temperature in the coldest month (August) is 24.8°C, the average temperature in the warmest month (March) is 28.5°C, and the average annual rainfall is 1500 mm. Sample collection took place at the Regional Hospital Centre of Man.

Located 368 km from Abidjan, the city of San-Pedro is in the southwest of the Ivory Coast. The rainfall in San-Pedro is much greater in June. Over the years, the average temperature in San-Pedro is 26.6°C and precipitation averages 902.2 mm. Sample collection took place at San-Pedro General Hospital.

Abengourou is located in eastern Côte d'Ivoire, 210 km from Abidjan. During the year, the temperature generally ranges from 20 to 35°C is rarely below 16°C or above 38°C, and rainfall averages 826.4 mm. Sample collection took place at the Abengourou Regional Hospital Centre.

These sites are being studied because of their high transmission rates throughout the year, but also because of their geographical, ecological, and epidemiological diversity. The molecular analyses were carried out at the Malaria Research and Control Center.

Study sites are shown in Figure 1.

Study population and inclusion criteria

The study population consisted of patients regardless of sex or age who came to the different health centers in Man, San Pedro, and Abengourou for suspicion of simple malaria. Patients with a temperature of 37°C or higher and *P. falciparum* mono-infestation with thick droplets and blood smear were included in this study.

Collection of samples

Two to three microlitres of venous blood were taken from an EDTA tube from each patient confirmed for malaria by microscopic examination. Three 25 µL blood spots were placed on Whatman 3 MM filter paper and then dried at room temperature, protected from dust and insects, for 24 to 48 hours. The samples were then transported to the National Institute of Public Health's Malaria Control and Research Centre for molecular analysis.

Extraction of plasmodial DNA and detection of polymorphisms of *Pfmdr1* and *Pfk13*

DNA was extracted using the ZYMO DNA® kit following the manufacturer's protocol and stored at -20°C.

The genes were amplified by nested PCR. The MDR1_PCR1_F/MDR1_PCR1_R and MDR1_PCR2_F/MDR1_PCR2_R primers for the Pfmdr1 gene and the K13_PCR_F/K13_PCR_R and K13_N1_F/K13_N1_R primers for the Pfk13 gene were used, respectively, for the first and second PCR (Table 1).

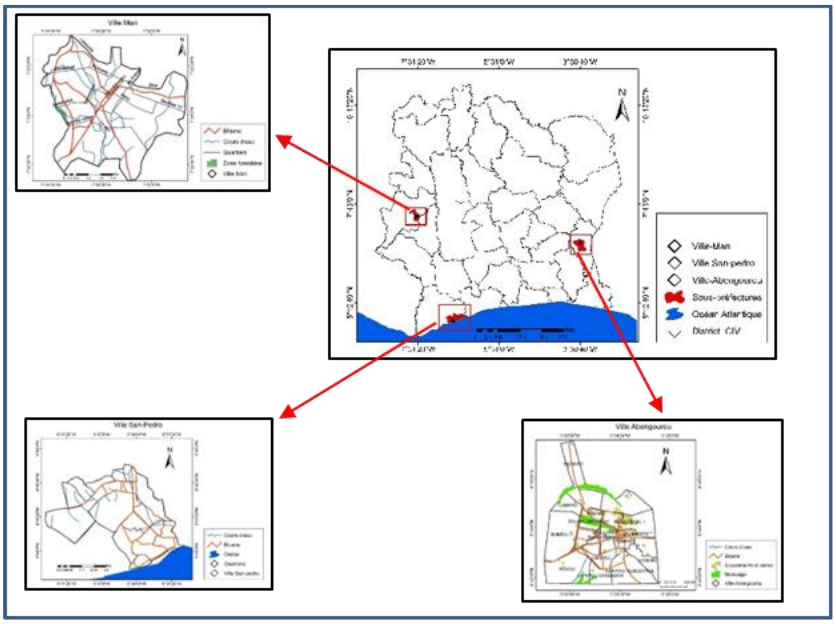

Figure 1: Map of Côte d'Ivoire with study site (source: CNTIG, 2020).

Table 1: Priming sequences for the amplification of *Pfmdr1* and *PfK13* genes.

Primers	Nucleotide sequences	Genes
MDR1_PCR1_F	5'-AGAGAAAAAAGATGGTAACCTCAG- 3'	
MDR1_PCR1_R	5'-ACC-ACA-AAC-ATA-AAT-TAA-CGG- 3'	
MDR1_PCR2_F	5'-TTTGTATGTGCTGTATTATCAGG- 3'	*Pfmdr-1*
MDR1_PCR2_R	5'-GTA-ATA-CAT-AAA-GTC-AAA-CGT-GC- 3'	
K13_PCR_F	5'- CGGAGTGACCAAATCTGGGA - 3'	
K13_PCR_R	5'- GGGAATCTGGTGGTAACAGC - 3'	
K13_N1_F	5'- GCCAAGCTGCCATTCATTTG - 3'	*PfK13*
K13_N1_R	5'- GCCTTGTTGAAAGAAGCAGA - 3'	

The first PCR was performed in a volume of 25 µL. For each amplification, the mixture contained 12.5 µL of the 2X Master Mix with Standard Buffer (NEW ENGLAND BioLabs Inc.) enzyme mix containing dNTP, 10X buffer, MgCl2, and buffer, as well as two commonly used tracking dyes for DNA gels; 0.5 µL of each gene-specific primer at 10 µM each, and 6.5 µL of DNase/RNase-free water. Five microlitres (5 µL) of *P. falciparum* DNA extract were added to the reaction mixture. The mixture was placed in a SimpliAmp TM 96-well plate thermocycler (Thermo Fischer Scientific, Waltham, MA, United States) according to the following amplification program: initial denaturation at 95°C for 15 min, followed by 30 cycles of denaturation at 92°C for 30s, hybridization at 58°C for 2 min and extension at 72°C for 2 min. Finally, a terminal extension at 72°C for 10 min.The second PCR was performed in a volume of 50 µL. The mixture for each amplification contained 25 µL of 2X Master Mix with Standard Buffer (NEW ENGLAND BioLabs Inc.) enzyme

Table 2: Socio-demographic and microscopic characteristics of patients.

	Man n (%)	San-Pedro n (%)	Abengourou n (%)	Total n (%)	p-value
Sex					
Male	51 (17)	50 (16,67)	35 (11,67)	136 (45,33)	
Female	49 (16,33)	50 (16,67)	65 (21,67)	164 (54,67)	0,036
Age group					
<5	56 (18,67)	54 (18,00)	46 (15,33)	156 (52)	
[5-14]	32 (10,67)	23 (7,67)	15 (5)	70 (23,33)	$2.e^{-16}$
≥15	12 (4)	23 (7,67)	39 (13)	74 (24,67)	
Parasite density					
Mean	45035,6	16044,06	11488,78	24189,48± 26653,11	

mix containing dNTP, 10X buffer, MgCl2, and buffer, as well as two commonly used tracking dyes for DNA gels; 1 µL of each gene-specific primer at 10 µM each, and 18 µL of DNase/RNase-free water. Five microliters (5 µL) of *P. falciparum* DNA extract were added to the reaction mixture. The mixture was introduced into the same SimpliAmp TM 96-well plate thermocycler (Thermo Fischer Scientific, Waltham, MA, United States) according to the following amplification program: initial denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 92°C for 30s, hybridization at 60°C for 1 min and extension at 72°C for 1 min. Finally, a terminal extension at 72°C for 10 min. The amplification products were visualized by electrophoresis on a 1.5% agarose gel. Subsequently, all samples that were successfully amplified were sent for sequencing.

Sequencing and sequence analysis

The successful PCR amplification products were sequenced by the Sanger method by BGI Tech®. The sequences were analyzed by alignment with BioEdit TM software. Identification involved the detection of codons of interest 86, 184 of the *Pfmdr1* gene. For the *Pfk13* gene, the analysis also looked at all codons involved in resistance to artemisinin derivatives identified in South-East Asia, as well as other mutations using the reference strain.

Statistical analyses

The data was entered with the Epi-Info software and analyzed with R version 4.3.2. The different parameters were compared using the chi square test, and all differences were considered significant at $p < 0.05$. The analysis of *Pfmdr1* and *Pfk13* gene polymorphism was performed by identifying wild-type and mutant alleles present at specific loci.

Ethical considerations

The National Committee has approved this study for Ethics in Life and Health Sciences (CNESVS) under ref. 255/MSHPCMU/CNESVS-km. All participants gave free and informed consent before inclusion in the study.

RESULTS

Socio-demographic and microscopic patient data

A total of 300 *Plasmodium falciparum* positive samples were analyzed in this study. The patients' ages ranged from 2 months to 70 years, and the mean age was 11.32 years with a standard deviation of 15.15 years. Parasite densities ranged from 250 to 200,000 trophozoites/µL of blood, with mean densities of 45035.6, 16044.06, and 11488.78/µL of blood, respectively, in Man, San-Pedro, and Abengourou (Table 2).

Polymorphisms of *Pfmdr1*

Of the 273 amplified samples, 269 were successfully sequenced. The mutant allele Y184F was predominant, while the N86Y mutant allele was infrequent. Five mutant alleles 86Y (1.9%) and 162 mutant alleles 184F (60.22%) were observed in this study (Figure 2). A significant difference was observed between the prevalence of the 184F mutant allele and the prevalence of the Y184 sensitive allele ($p= 0.022$).

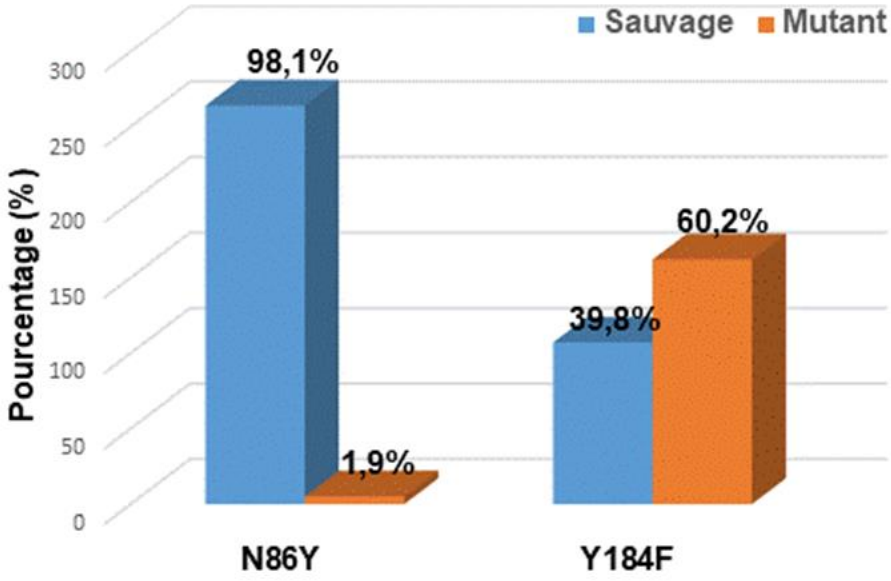

Figure 2: Prevalence of the N86Y and Y184F alleles of the Pfmdr1 gene.

Table 3: Synonymous and non-synonymous mutations of *Pfk13.*

Type of mutation	Codon	Reference nucleotide	AA	Mutant nucleotide	AA	Frequency (%)
	441	cca	P	ccc	P	1 (0,4)
	478	acc	T	acg	T	1 (0,4)
	595	ggt	G	ggc	G	1 (0,4)
	469	tgc	C	tgt	C	1 (0,4)
Synonymous	666	gta	V	gtc	V	1 (0,4)
	667	cca	P	ccc	P	1 (0,4)
	687	gga	G	ggt	G	1 (0,4)
	690	ggc	G	ggt	G	1 (0,4)
Non synonymous	522	agt	S	tgt	C	1 (0,4)
	532	tgt	C	tct	S	1 (0,4)
	578	gct	A	tct	S	2 (0,8)
	602	gaa	E	gat	D	1 (0,4)
	621	gct	A	tct	S	1 (0,4)

AA: amino acid AA:

Polymorphisms of *Pfk13*

Of the 259 samples that were successfully amplified, the entire K13 gene domain was successfully sequenced for 254 samples with a mono-infestation of *P. falciparum*. No mutations associated with artemisinin resistance, as seen in Southeast Asia, were observed. On the whole fragment of the *Pfk13* gene ranging from codon 432 to 705, 14 mutations were found in 13 isolates, including 5 non-synonymous mutations and 8 synonymous mutations.

Specific mutations were observed at the codons listed in Table 3.

DISCUSSION

Molecular markers like Pfmdr1 and Pfk13 are crucial for monitoring drug resistance in malaria control to identify mutations and guide treatment policies for malaria. The ACTs have been adopted as a first-line treatment in Côte d'Ivoire since 2007, and to date, resistance has not yet been confirmed. This study aims to evaluate the prevalence of N86Y and Y184F mutant alleles of the *Pfmdr1* gene and the *Pfk13* gene.

The *Pfmdr1* gene contributes to a multigenic resistance profile through mutations in different codons. Analysis of mutations in the N86Y, Y184F, and D1246Y codons were associated with 4-aminoquinoline resistance and clinical failure to amodiaquine treatment (Venkatesan et al., 2014). In our study, we studied the simple nucleotide polymorphisms N86Y and Y184F.

The cessation of chloroquine use and the adoption of ACTs as first-line treatment led to an increase in the prevalence of wild-type K76 mutations of the *Pfcrt* and N86 and D1246 genes in Africa (Asua et al., 2021; Wamae et al., 2019). The results of this study are consistent with these observations. Our results are also similar to those observed by Konaté-Touré et al. (2024), who found a prevalence of 86Y and 184F mutations of 5.9% and 64.2%, respectively.

The low prevalence of 86Y mutant alleles observed in this study may simply reflect the relatively common use of AL. Indeed, some studies have shown that lumefantrine positively selects the mutant N86 allele in contrast to amodiaquine, which positively selects the mutant 86Y allele (Sondo et al., 2016). For example, in Uganda, AL is the treatment of national choice, and its widespread use has led to both an increase in the prevalence and frequency of wild *Pfmdr1* N86 alleles and a parallel increase in mutant *Pfmdr1* 184F alleles. This was accompanied by a decrease in sensitivity to lumefantrine and an increase in sensitivity to aminoquinolines over time (Tumwebaze et al., 2015). In addition, the infrequent use of AS-AQ as a first-line alternative treatment for simple malaria in Uganda may also partly explain this decline in prevalence of mutant alleles (Achol et al., 2019).

The prevalence of the mutant allele *Pfmdr1* 184F was high in our study. This trend was observed in a study conducted in Abidjan, which revealed a frequency of 57% of this mutant allele (Trebissou et al., 2014). Several authors have shown that the high frequency of *Pfmdr1* 184F could indicate increased resistance to piperaquine (PPQ), especially when associated with the wild-type allele *Pfmdr1* N86. Previous studies have established significant resistance to PPQ in NFD parasites carrying the haplotype IET in Southeast Asia (Veiga et al., 2016). In addition, reports indicated that the *Pfmdr1* 184F mutation decreased sensitivity to lumefantrine (Leski et al., 2022). The widespread use of artemether-lumefantrine (AL) therapy has also led to the selection of strains containing *Pfmdr1* Y184F. According to studies, the *Pfmdr1* 184F mutation does not directly cause phenotypic resistance, but it modifies the kinetics of drug transport, resulting in a decrease in sensitivity. When combined with the *Pfmdr1* N86Y and *Pfcrt* mutations, the *Pfmdr1* 184F mutation contributes to resistance. Avci et al. found in their work that although this mutation is the most widespread, its presence alone does not directly affect resistance to antimalarial drugs, which could pose a risk of relapse (Avcı et al., 2024). The role of the Pfmdr1 184F mutation in drug resistance remains unclear. Other studies suggest that the use of CQ would have favoured the selection of various haplotypes of *Pfmdr1* at different locations, based on the predominant alleles of *Pfcrt* and other genes present in these populations. An analysis of the genetic diversity around the *Pfmdr1* gene could thus provide evidence that selective scanning has promoted the spread of these alleles, as shown for *Pfdhfr* and *Pfdhps* alleles that are associated with resistance to the sulfadoxine/pyrimethamine (SP) combination (Humphreys et al., 2007).

The study of a molecular marker such as the *Pfk13* gene is crucial for monitoring drug resistance in malaria control programs. This allows mutations to be detected and appropriate treatment policies to be adapted (Ikegbunam et al., 2021). The resistance mechanisms associated with KELCH are not clearly understood. In Côte d'Ivoire, artemisinin-based therapies have been introduced as first-line treatment for malaria since 2007 (Konate et al., 2018), and to date, resistance has not yet been confirmed. First-line antimalarial drugs administered in Côte d'Ivoire include artemether lumefantrine (AL), artesunate amodiaquine (ASAQ). Others are commonly used dihydroartemisinin-piperaquine (DHA-PPQ) and artesunate-pyronaridine (ASPY) (). In this study, the *Pfk13* gene sequence data of codons 432 to 705 were studied. No known mutations in the *Pfk13* gene associated with reduced susceptibility of parasites to ART were identified. However, it is necessary to report all SNPs observed in this gene because these new mutations detected could signal the emergence of drug resistance, especially when the molecular markers of resistance in Africa have not yet been revealed (Matrevi et al., 2022).

Pfk13 single-nucleotide polymorphisms (SNPs) that are related to delayed parasite clearance after treatment with ACTs are now widespread in Southeast Asia (WHO, 2022). Until recently, they had not been a problem on the African continent (Bwire et al., 2020). Validated SNPs used as molecular markers to predict clinical treatment outcomes and track partially artemisinin-resistant malaria

parasite strains include F446I, N458Y, C469Y, M476I, Y493H, R539T, I543T, P553L, R561H, P574L, C580Y, R622I, and A675V (WHO 2018). Recent studies have demonstrated the emergence of novo and clonal expansion of the *Pfk13* R561H mutation in some East African countries, notably Rwanda and Tanzania (Bwire et al., 2020; Uwimana et al., 2020).

Thirteen SNPs were detected at low frequency in this study, of which eight were synonymous mutations and five were non-synonymous mutations. The A578S mutation, the most common Pfk13 gene in Africa, was observed in two isolates. This mutation is particularly prevalent in Uganda, Kenya, and the Republic of the Congo (Ménard et al., 2016), as well as on the Ivory Coast (Konaté-Touré et al., 2024). However, it is important to note that A578S is not associated with partial resistance to either clinical or in vitro artemisinin (Ménard et al., 2016). The S522C mutation observed in this study has already been described in several African countries, specifically in Gabon, the Democratic Republic of the Congo, the Central African Republic, Kenya, and Togo (Ménard et al., 2016) and in Côte d'Ivoire (Konaté-Touré et al., 2024). Some studies in Africa have revealed mutations in *P. falciparum* parasites even before the introduction of ACTs in malaria treatments, such as E602D in Côte d'Ivoire (Djaman et al., 2017) and Y588C, E556K (Leang et al., 2015). E602D was also observed. The observed A621S and C532S mutations are not involved in decreased sensitivity to artemisinin derivatives.

The high prevalence of the mutant allele of the Pfmdr1 gene observed in Côte d'Ivoire suggests continuous drug selection pressure. This could gradually reduce the effectiveness of currently recommended ACTs by selecting resistant strains and promoting treatment failure. As for the Pfk13 gene, the non-synonymous mutations observed could be precursors of emerging resistance to artemisinin derivatives in Africa.

Although this study provides valuable data on the prevalence of mutations associated with antimalarial resistance, it has certain limitations related to geographical scope, the absence of clinical data, and the lack of functional analyses.

CONCLUSION

More than a decade after the introduction of ACTs in Côte d'Ivoire, the present study showed a significant decrease in the prevalence of mutant allele 86Y and a high prevalence of mutant allele 184F in isolates of *P. falciparum* for the *Pfmdr1* gene. For the *Pfk13* gene, no known mutations that confer resistance to artemisinin derivatives were observed. These findings underscore the need for ongoing molecular surveillance to inform malaria treatment policies in Côte d'Ivoire and prevent the emergence of new resistance.

Functional studies of these non-synonymous mutations are necessary because they could represent emerging local forms of resistance. These results highlight the importance of maintaining molecular surveillance in order to guide malaria treatment policies in Côte d'Ivoire and prevent the emergence of new resistance.

ACKNOWLEDGEMENT

We would like to thank all the participants in the study and the directors of the Man, San-Pedro and Abengourou health centres. We would also like to thank all the staff of the Malaria Research and Control Center (MRCC) of the National Institute of Public Health of Côte d'Ivoire (INSP) and the various health centres and University of Félix Houphouët Boigny, Abidjan (Côte d'Ivoire).

REFERENCE

Achol E, Ochaya S, Malinga GM, Edema H, Echodu R (2019). High prevalence of Pfmdr-1 N86 and D1246 genotypes detected among febrile malaria outpatients attending Lira Regional Referral Hospital, Northern Uganda. BMC Res. Notes 12: 235.

Asua V, Conrad MD, Aydemir O, Duvalsaint M, Legac J, Duarte E, Tumwebaze P, Chin DM, Cooper RA, Yeka A, Kamya MR, Dorsey G, Nsobya SL, Bailey J, Rosenthal PJ (2021). Changing prevalence of potential mediators of aminoquinoline, antifolate, and artemisinin resistance across Uganda. J. Infect. Dis. 223: 985–994.

Avcı KD, Karakuş M, Kart Yaşar K (2024). Molecular survey of pfmdr-1, pfcrt, and pfk13 gene mutations among patients returning from Plasmodium falciparum endemic areas to Turkey. Malar. J. 23: 286.

Baina MT, Djontu JC, Mbama Ntabi JD, Mfoutou Mapanguy CC, Lissom A, Vouvoungui CJ, Boumpoutou RK, Mouanga AM, Nguimbi E, Ntoumi F (2024). Polymorphisms in the Pfcrt, Pfmdr1, and Pfk13 genes of Plasmodium falciparum isolates from southern Brazzaville, Republic of Congo. Sci. Rep. 14: 27988.

Bwire GM, Ngasala B, Mikomangwa WP, Kilonzi M, Kamuhabwa AAR (2020). Detection of mutations associated with artemisinin resistance at k13-propeller gene and a near complete return of chloroquine susceptible falciparum malaria in Southeast of Tanzania. Sci. Rep. 10: 3500.

Djaman JA, Dagnogo O, Ako AB, Roman J, Ngane VF, Basco LK, Tahar R (2017). Molecular Epidemiology of Malaria in Cameroon and Côte d'Ivoire. XXXI. Kelch 13 Propeller Sequences in Plasmodium falciparum Isolates before and after Implementation of Artemisinin-Based Combination Therapy. Am. J. Trop. Med. Hyg. 97: 222–224.

Humphreys G, Merinopoulos I, Ahmed J, Whitty C, Mutabingwa T, Sutherland C, Hallett R (2007). Amodiaquine and Artemether-Lumefantrine Select Distinct Alleles of the Plasmodium falciparum mdr1 Gene in Tanzanian Children Treated for Uncomplicated Malaria. Antimicrob. Agents Chemother. 51: 991–997.

Ikegbunam M, Ojo JA, Kokou K, Morikwe U, Nworu C, Uba C, Esimone C, Velavan TP, Ojurongbe O (2021). Absence of Plasmodium falciparum artemisinin resistance gene mutations eleven years after the adoption of artemisinin-based combination therapy in Nigeria. Malar. J. 20: 434.

Konaté-Touré A, Gnagne AP, Bedia-Tanoh AV, Menan EIH, Yavo W (2024). Increase of Plasmodium falciparum parasites carrying lumefantrine-tolerance molecular markers and lack of South East Asian pfk13 artemisinin-resistance mutations in samples collected from 2013 to 2016 in Côte d'Ivoire. J. Parasit. Dis. 48: 59–66.

Leang R, Taylor WRJ, Bouth DM, Song L, Tarning J, Char MC, Kim S, Witkowski B, Duru V, Domergue A, Khim N, Ringwald P, Menard D (2015). Evidence of Plasmodium falciparum Malaria Multidrug Resistance to Artemisinin and Piperaquine in Western Cambodia: Dihydroartemisinin-Piperaquine Open-Label Multicenter Clinical Assessment. Antimicrob. Agents Chemother. 59: 4719–4726.

Leski TA, Taitt CR, Colston SM, Bangura U, Holtz A, Yasuda CY, Reynolds ND, Lahai J, Lamin JM, Baio V, Ansumana R, Stenger DA, Vora GJ (2022). Prevalence of malaria resistance-associated mutations in Plasmodium falciparum circulating in 2017–2018, Bo, Sierra Leone. Front. Microbiol. 13.

Matrevi SA, Tandoh KZ, Bruku S, Opoku-Agyeman P, Adams T, Ennuson NA, Asare B, Hagan OCK, Abuaku B, Koram KA, Fox A, Quashie NB, Letizia AG, Duah-Quashie NO (2022). Novel pfk13 polymorphisms in Plasmodium falciparum population in Ghana. Sci. Rep. 12: 7797.

Ménard D, Khim N, Beghain J, Adegnika AA, Shafiul-Alam M, Amodu O, Rahim-Awab G, Barnadas C, Berry A, Boum Y, Bustos MD, Cao J, Chen J-H, Collet L, Cui L, Thakur G-D, Dieye A, Djallé D, Dorkenoo MA, Eboumbou-Moukoko CE, Espino F-E-CJ, Fandeur T, Ferreira-da-Cruz M-F, Fola AA, Fuehrer H-P, Hassan AM, Herrera S, Hongvanthong B, Houzé S, Ibrahim ML, Jahirul-Karim M, Jiang L, Kano S, Ali-Khan W, Khanthavong M, Kremsner PG, Lacerda M, Leang R, Leelawong M, Li M, Lin K, Mazarati J-B, Ménard S, Morlais I, Muhindo-Mavoko H, Musset L, Na-Bangchang K, Nambozi M, Niaré K, Noedl H, Ouédraogo J-B, Pillai DR, Pradines B, Quang-Phuc B, Ramharter M, Randrianarivelojosia M, Sattabongkot J, Sheikh-Omar A, Silué KD, Sirima SB, Sutherland C, Syafruddin D, Tahar R, Tang L-H, Touré OA, Tshibangu-wa-Tshibangu P, Vigan-Womas I, Warsame M, Wini L, Zakeri S, Kim S, Eam R, Berne L, Khean C, Chy S, Ken M, Loch K, Canier L, Duru V, Legrand E, Barale J-C, Stokes B, Straimer J, Witkowski B, Fidock DA, Rogier C, Ringwald P, Ariey F, Mercereau-Puijalon O (2016). A Worldwide Map of Plasmodium falciparum K13-Propeller Polymorphisms. N. Engl. J. Med. 374: 2453–2464.

Sondo P, Derra K, Nakanabo SD, Tarnagda Z, Kazienga A, Zampa O, Valéa I, Sorgho H, Owusu-Dabo E, Ouédraogo J-B, Guiguemdé TR, Tinto H (2016). Artesunate-Amodiaquine and Artemether-Lumefantrine Therapies and Selection of Pfcrt and Pfmdr1 Alleles in Nanoro, Burkina Faso. PLOS ONE 11: e0151565.

Trebissou JND, Yavo W, Tano KD, N'Cho M, Yapi HF, Djaman AJ (2014). In vitro susceptibility of Plasmodium falciparum to lumefantrine and analysis of polymophysims in Pfmdr-1 gene isolates from Abidjan (Côte d'Ivoire). Int. J. Pharm. Sci. and Res. 5: 1732–1738.

Tumwebaze P, Conrad MD, Walakira A, LeClair N, Byaruhanga O, Nakazibwe C, Kozak B, Bloome J, Okiring J, Kakuru A, Bigira V, Kapisi J, Legac J, Gut J, Cooper RA, Kamya MR, Havlir DV, Dorsey G, Greenhouse B, Nsobya SL, Rosenthal PJ (2015). Impact of antimalarial treatment and chemoprevention on the drug sensitivity of malaria parasites isolated from ugandan children. Antimicrob. Agents Chemother. 59: 3018–3030.

Uwimana A, Legrand E, Stokes BH, Mangala Ndikumana JL, Warsame M, Umusila N, Ngamije D, Munyaneza T, Mazarati J-B, Munguti K, Campagne P, Criscuolo A, Ariey F, Murindahabi M, Ringwald P, Fidock DA, Mbituyumuremyi A, Ménard D (2020). Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda. Nature Medicine 26: 1602–1608.

Veiga MI, Dhingra SK, Henrich PP, Straimer J, Gnädig N, Uhlemann A-C, Martin RE, Lehane AM, Fidock DA (2016). Globally prevalent PfMDR1 mutations modulate Plasmodium falciparum susceptibility to artemisinin-based combination therapies. Nat. Commun. 7: 11553.

Venkatesan M, Gadalla NB, Stepniewska K, Dahal P, Nsanzabana C, Moriera C, Pric, RN, Mårtensson A, Rosenthal PJ, Dorsey G, Sutherland CJ, Guérin P, Davis TME, Ménard D, Adam I, Ademowo G, Arze C, Baliraine FN, Berens-Riha N, Björkman A, Borrmann S, Checchi F, Desai M, Dhorda M, Djimdé AA, El-Sayed BB, Eshetu T, Eyase F, Falade C, Faucher J-F, Fröberg G, Grivoyannis A, Hamour S, Houzé S, Johnson J, Kamugisha E, Kariuki S, Kiechel J-R, Kironde F, Kofoed P-E, LeBras J, Malmberg M, Mwai

L, Ngasala B, Nosten F, Nsobya SL, Nzila A, Oguike M, Otienoburu SD, Ogutu B, Ouédraogo J-B, Piola P, Rombo L, Schramm B, Somé AF, Thwing J, Ursing J, Wong RPM, Zeynudin A, Zongo I, Plowe CV, Sibley CH (2014). Polymorphisms in Plasmodium falciparum Chloroquine Resistance Transporter and Multidrug Resistance 1 Genes: Parasite Risk Factors that Affect Treatment Outcomes for P. falciparum Malaria after Artemether-Lumefantrine and Artesunate-Amodiaquine. Am. J. Trop. Med. Hyg. 91: 833–843.

Wamae K, Okanda D, Ndwiga L, Osoti V, Kimenyi KM, Abdi AI, Bejon P, Sutherland C, Ochola-Oyier LI (2019). No Evidence of Plasmodium falciparum *k13* Artemisinin Resistance-Conferring Mutations over a 24-Year Analysis in Coastal Kenya but a Near Complete Reversion to Chloroquine-Sensitive Parasites. Antimicrob. Agents Chemother. 63: e01067-19.

WHO, 2015. Guidelines for the treatment of malaria, 3rd ed. ed. World Health Organization, Geneva.

WHO, 2018. Paludisme : résistance partielle à l'artémisinine.

WHO, 2022. World malaria report 2022.

WHO, 2023. World malaria report 2023.

WHO, 2024. World malaria report 2024.