



HAL
open science

Plasmodium falciparum population structure and genetic diversity of cell traversal protein for ookinetes and sporozoites (CelTOS) during malaria resurgences in Dielmo, Senegal

Amélé Nyedzie Wotodjo, Mary Aigbiremo Oboh, Cheikh Sokhna, Nafissatou Diagne, Fatoumata Diène-Sarr, Jean-François Trape, Souleymane Doucouré, Alfred Amambua-Ngwa, Umberto d'Alessandro

► To cite this version:

Amélé Nyedzie Wotodjo, Mary Aigbiremo Oboh, Cheikh Sokhna, Nafissatou Diagne, Fatoumata Diène-Sarr, et al.. Plasmodium falciparum population structure and genetic diversity of cell traversal protein for ookinetes and sporozoites (CelTOS) during malaria resurgences in Dielmo, Senegal. *Infection, Genetics and Evolution*, 2023, 116, pp.105535. 10.1016/j.meegid.2023.105535 . hal-04509995

HAL Id: hal-04509995

<https://hal.science/hal-04509995>

Submitted on 29 Mar 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



Plasmodium falciparum population structure and genetic diversity of cell traversal protein for ookinetes and sporozoites (CelTOS) during malaria resurgences in Dielmo, Senegal

Amélie Nyedzie Wotodjo^{a,b,*}, Mary Aigbiremo Oboh^{b,c,d}, Cheikh Sokhna^a, Nafissatou Diagne^a, Fatoumata Diène-Sarr^e, Jean-François Trape^f, Souleymane Doucouré^a, Alfred Amambua-Ngwa^b, Umberto D'Alessandro^b

^a VITROME, UMR 257 IRD, Campus UCAD-IRD, Dakar, Senegal

^b Medical Research Council Unit, The Gambia, at the London School of Hygiene and Tropical Medicine, Fajara, Gambia

^c Department of Biological Sciences, University of Medical Sciences, Ondo, Nigeria

^d Department of Biomedical Sciences, Rochester Institute of Technology, Rochester, NY, United States of America

^e Institut Pasteur de Dakar, 36 Avenue Pasteur, 220 Dakar, Senegal

^f UMR MIVEGEC, Laboratoire de Paludologie et Zoologie Médicale, IRD, Dakar, Senegal

ARTICLE INFO

Keywords:

PfCelTOS
Malaria transmission
Malaria resurgences
Population structure
Genetic diversity
Senegal

ABSTRACT

The ability to accurately measure the intensity of malaria transmission in areas with low transmission is extremely important to guide elimination efforts. *Plasmodium falciparum* Cell-traversal protein for ookinetes and sporozoites (*PfCelTOS*) is an important conserved sporozoite antigen reported as one of the promising malaria vaccine candidates, and could be used to estimate malaria transmission intensity. This study aimed at determining whether the diversity of *PfCelTOS* gene reflects the changes in malaria transmission that occurred between 2007 and 2014 in Dielmo, a Senegalese village, before and after the implementation of insecticide treated bed nets (ITNs). Of the 109 samples positive for *PfCelTOS* PCR, 96 (88%) were successfully sequenced and analysed for polymorphisms and population diversity. The number of segregating sites was higher during the pre-intervention period (13) and the malaria resurgences (11) than during the intervention period (5). Similarly, the number and diversity of haplotypes were higher during the pre-intervention period (16 and 0.914, respectively) and the malaria resurgences (6 and 0.821, respectively) than during the intervention period (4 and 0.758, respectively). Moreover, the average number of nucleotide differences was higher during the pre-intervention (3.792) and during malaria resurgences (3.467) than during the intervention period (2.189). The 3D7 KSSFNEP haplotype was only observed during the intervention period. Only two haplotypes were shared in both the pre-intervention and intervention periods while four haplotypes were shared between the pre-intervention and the malaria resurgences. The *Fst* values indicate moderate differentiation between pre-intervention and intervention periods (0.17433), and between intervention and malaria resurgences period (0.19198) as well as between pre-intervention and malaria resurgences periods (0.06607).

PfCelTOS genetic diversity reflected changes of malaria transmission, with higher polymorphisms recorded before the large-scale implementation of ITNs and during the malaria resurgences. *PfCelTOS* is also a candidate vaccine; mapping its diversity across multiple endemic environments will facilitate the design and optimisation of a broad and efficacious vaccine.

1. Introduction

In some countries, including Senegal (Trape et al., 2012; PNL, 2018), the burden of malaria has decreased significantly over the last 15

years (Noor et al., 2014), to the point that standard malaria indicators such as the prevalence of infection or the entomological inoculation rate cannot be reliably estimated (Tusting et al., 2014). Genomic tools can estimate transmission intensity by assessing the changes in parasite

* Corresponding author at: VITROME UMR 257 IRD, Campus UCAD-IRD, BP 1386 Dakar, Senegal.

E-mail address: amele-nyedzie.wotodjo@ird.fr (A.N. Wotodjo).

<https://doi.org/10.1016/j.meegid.2023.105535>

Received 13 April 2023; Received in revised form 20 November 2023; Accepted 25 November 2023

Available online 28 November 2023

1567-1348/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

population and the complexity of infections with molecular metrics (Tusting et al., 2014; Niang et al., 2016). Indeed, the intensity of malaria transmission reflects the level of *Plasmodium falciparum* genetic diversity and population structure, with low transmission resulting in lower genetic diversity (Anthony et al., 2005; Escalante et al., 2015; Nkhoma et al., 2013). As the Senegalese National Malaria Control Program (NMCP) aims to achieve malaria elimination by 2030, ability to measure accurately the intensity of transmission in areas where this is low is extremely important to guide elimination efforts. This would also allow measuring the effectiveness of malaria control interventions, for better planning and targeting. *Plasmodium* antigens are often used to monitor malaria parasites population structure. *P. falciparum* Cell-traversal protein for ookinetes and sporozoites (*PfCelTOS*) is an important conserved sporozoite antigen (Kariu et al., 2006) that could be used to estimate malaria transmission intensity (Doolan et al., 2003). *PfCelTOS* is a 25-KDa cell-traversal protein expressed both in the mosquito ookinete and sporozoite stages, playing a crucial role in the locomotory ability of both ookinetes and sporozoites in breaking through cellular barriers and establishing infection in the new host (Kariu et al., 2006). Because of its high immunogenicity (Doolan et al., 2003; Bergmann-Leitner et al., 2010), *PfCelTOS* is also a potential vaccine candidate. However, a major concern for its use as vaccine candidate is antigenic diversity, which has been reported in two studies so far (Oboh et al., 2022; Pirahmadi et al., 2018).

In Dielmo, Central Senegal, malaria endemicity has significantly changed over the last 20 years, from holoendemic to hypoendemic, thanks to the scale up of insecticide treated bed nets (ITNs) in 2008, with mass distribution campaigns in 2011, 2014, 2016, and the use of artemisinin-based combination treatment (ACTs) for the management of malaria cases since 2006. In this area, malaria is currently in the pre-elimination phase with current malaria prevalence <1% and no local malaria cases observed between 2018 and 2021. Nevertheless, over the last 10 years, in 2010/2011 and 2013/2014, two malaria resurgences occurred (Trape et al., 2011; Wotodjo et al., 2015). The main objective of this study is to determine whether the genetic diversity, haplotype frequency and patterns of selection of *PfCelTOS* gene can adequately reflect the changes in malaria transmission that occurred in Dielmo between 2007 and 2014.

2. Methodology

2.1. Ethics approval and consent to participate

Written informed consent was obtained from all participants. The study was approved by the Ministry of Health of Senegal, the assembled village population, and the National Ethics Committee of Senegal.

2.2. Study area and sample collection

The study was carried out in Dielmo, a village in Central Senegal, where malaria surveillance of the whole population started in June 1990 (Trape et al., 1994). The village is at about 280 km south-east of Dakar, the capital city, on the marshy bank of the Nema, a small permanent stream where anopheline breeding sites can be found throughout the year. Malaria transmission was intense and perennial in the 1990s and early 2000s but decreased and became seasonal after the implementation of ITN universal coverage, in July 2008. From being holoendemic in 1990, Dielmo has been hypoendemic since 2010 (Trape et al., 2014). Nevertheless, two malaria resurgences occurred in 2010/2011 and 2013/2014 (Trape et al., 2011; Wotodjo et al., 2015). Since 2006, clinical malaria is treated with artesunate-amodiaquine (ASAQ). Malaria surveillance in this area includes quarterly cross-sectional surveys of all villagers, daily visits of all residents and systematic recording of body temperature three times a week, with blood test in case of fever or history of fever (Trape et al., 2014). During the surveys, blood samples were collected by fingerpick for thick blood film, malaria rapid

diagnostic test (RDT), and later molecular analysis. The samples used in this study are capillary blood collected during quarterly cross-sectional surveys to assess malaria prevalence in asymptomatic carriers.

The study period was divided in pre-intervention (2007), intervention (2008–2009, ITNs implementation) and malaria resurgences in 2010/2011 and 2013/2014.

2.3. DNA extraction

Genomic DNA was extracted from blood samples using Qiagen kit for Blood and Body Fluid Spin Protocol, following manufacturer recommendations, and eluted in a 200 µl final volume and stored at –20 °C until use.

2.4. qPCR for *Plasmodium falciparum* diagnostic, *PfCelTOS* amplification and sequencing

Molecular diagnostic with real time polymerase chain reaction (RT-PCR) targeting the var. gene multi-copy acidic terminal sequence (var-ATS) was performed (Hofmann et al., 2015) with the extracted DNA using a volume of 5 µl of TaqMan Universal PCR Master mix (Life Technologies LTD, UK), 0.5 µl of nuclease free water, 0.8 µl of the forward and reverse primer each, 0.4 µl of the probe and 2.5 µl of DNA for a total reaction volume of 10 µl.

P. falciparum positive DNA was used for *PfCelTOS* gene amplification using newly designed primers (*Pf CelTOS* forward 5'- TCAGAGGAAA-CAACGGACACA –3' and *Pf CelTOS* reverse 5'- TCACTTTCTGAGACGTCTGCTG –3') synthesised by IDT DNA (Corralville IA USA). The newly designed primers cover a 475 base pair fragment of *PfCelTOS* gene all within the coding region. *PfCelTOS* PCR amplification reaction was performed in a total reaction volume of 25 µl containing: 5 µl of 10XThermopol Reaction Buffer (New England BioLabs), 10 µl of nuclease free water, 2 µl of dNTPs, 5 µl of gDNA, 1.25 µl of each forward and reverse primer (which equates to 0.5 mM concentration of each primer) and 0.5 µl of Taq polymerase (New England BioLabs). The PCR cycling conditions were as follow: one step at 95 °C for 2 min; 35 cycles at 95 °C for 1 min, 58 °C for 2 min and 68 °C for 1min30s and extension at 68 °C for 5 min.

All PCR products were resolved on 2% agarose gel stained with safe green (biosharp, life sciences) and the products were visualized under ultraviolet trans-illumination (Biorad GelDoc™ XR + System with Image Lab). Only amplified samples were carried forward for subsequent purification and sequencing. The amplified PCR products were purified using the ExoSAP-IT™ PCR Product Cleanup Reagent enzyme (Thermo Scientific, MA, USA) prior to DNA sequencing as per manufacturer's instruction. Purified *PfCelTOS* PCR products were sequenced from both directions with *PfCelTOS* forward and reverse primers following Sanger dideoxy chain termination sequencing PCR and analysis on ABI SeqStudio automatic sequencer (Applied Biosystems).

2.5. Sequences analysis

BioEdit Sequence Alignment Editor v.7.2.5 (Hall, 1999) was used for the visualization of DNA sequence chromatograms and for alignments. The nucleotide sequences were aligned against the corresponding 3D7 reference strain sequence (Pf 3D7_1216600) from the GenBank (<https://www.ncbi.nlm.nih.gov>) by using ClustalW multiple alignments followed by single nucleotide polymorphisms (SNP) identification with BioEdit. All identified SNPs were carefully checked by revisualization of DNA sequence chromatograms. The nucleotide sequences were translated into amino acid sequences and the frequencies of different amino acid haplotypes circulating during the three study periods were determined.

The haplotypes were classified based on DNA and amino acid sequences of the studied samples in comparison with the 3D7 reference strain. Sequences generated will be submitted to NCBI database.

2.6. Population genetic analysis

DNA sequences of *PfCelTOS* gene in FASTA format were grouped according to the three study periods (period before and during ITNs implementation and malaria resurgences) and analysed using DnaSP v6.12.03 (Librado and Rozas, 2009). The amino acid sequence haplotype frequencies were determined for the study periods. Genetic test approaches were performed to evaluate the SNPs/segregating sites, the number of haplotypes, the haplotype (Nei, 1987) (H_d = the probability that two randomly sampled alleles are different) and nucleotide (as estimated by Π and Θ_w) diversities and the minimum number of recombination parameters (R_m). The average number of pairwise nucleotide difference per period was estimated by π (Tajima, 1989), and the Θ_w value was based on the number of segregating sites in a population (Watterson, 1975). Neutrality tests such as Tajima's D (TD), Fu & Li's F^* and D^* statistics were performed for the 3 periods to determine the mode of evolutionary selection acting on the gene. The TD test provides the normalized difference between Θ_w and π , where a negative value generally indicates abundance of low frequency polymorphism showing directional selection or population size expansion and a positive value indicates balancing selection or a reduction in the population size. Analyses were performed on a sliding window of 100 in 25 nucleotides to assess diversity of *PfCelTOS* gene segments.

In addition, to demonstrate the genealogical relatedness of the different haplotypes during the 3 periods, a network based on the method described by Templeton, Crandall, and Sing (TCS) (Templeton et al., 1992) was constructed in the PopArt program (Leigh and Bryant, 2015).

To determine the genetic differentiation and the gene flow among the parasites population during the three periods, the Wright fixation index (F_{st}) which evaluates the proportion of average diversity in relation to differences within populations and the haplotype statistic index (H_{st}) were estimated. Statistical significance was evaluated by non-parametric permutation-base test with 1000 iterations of samples. F_{st} values are interpreted as low genetic differentiation or high gene flow for 0 to <0.05, moderate differentiation or gene flow for 0.05 to <0.25, and high differentiation or low gene flow if ≥ 0.25 (Balloux and Lugon-Moulin, 2002). Finally, linkage disequilibrium (LD) test was carried out to ascertain if neighbouring SNPs present at different codons are associated in a parasite isolate. The r^2 value of the SNPs was plotted against the positions of the different SNPs.

3. Results

3.1. Evaluation of genetic diversity, haplotype frequency and patterns of selection of the *P. falciparum CelTOS* gene

Of the 109 samples positive for *PfCelTOS* PCR, 96 (88%) were successfully sequenced. Among the 96 sequences with uniform length of 370 bp, 36 were from the pre-intervention period, 20 from the intervention period, and 40 from the malaria resurgences period (Table 1). Among them, 46% (44/96) were from males and 54% (52/96) from females. Their age varied between 1.4 and 64.5 years old, with a mean of 17.9 years.

For the pre-intervention period, there were 13 segregating sites with

Table 1
Characteristics of individuals by period.

	2007	2008–2009	2010–2011/2013–2014
Number	36	20	40
Age (years)			
Mean	16.59	15.24	20.38
Range	1.4–64.4	7.8–20	4.8–64.5
Sex			
Male	12	12	20
Female	24	8	20

16 haplotypes while for the intervention period only 5 segregating sites with 4 haplotypes were observed. For the period of malaria resurgences, there were 11 segregating sites and 6 haplotypes. Haplotype diversity was higher (0.914) during the pre-intervention than during intervention (0.758) and malaria resurgences (0.821) period. A similar trend was observed for the nucleotide diversity values, which was higher during the pre-intervention ($\pi = 0.01025$, $\Theta_w = 0.01043$) than during the intervention ($\pi = 0.00592$, $\Theta_w = 0.00381$) and the malaria resurgences ($\pi = 0.00937$, $\Theta_w = 0.00699$) (Table 2). Likewise, the average number of nucleotide difference was higher during the pre-intervention (3.792) and during malaria resurgences (3.467) than during the intervention period (2.189). There was the same minimum number of putative recombination events in the pre-intervention and malaria resurgences periods ($R_m = 2$) while no recombination event was observed during the intervention period ($R_m = 0$).

LD analysis which helps to determine pair-wise SNPs association was evaluated. The r^2 value of each pairwise SNP association was computed separately. High LD was observed between SNPs located around the 0–50 base pair; interestingly, additional higher number association was noticed in SNPs pair around the 50–75 base pair (Fig. 1).

Quality-trimmed nucleotide sequences were translated into amino acid (AA) and the sequences were aligned with the protein reference strain to obtain the haplotype. Mutations at AA positions 100, 104, 116, 117, 119, 123 and 140 against a reference KSSFNEP haplotype were used to characterise population haplotypes. The most common haplotype during the 3 study periods was KSSVKEP (Table 3). The number of different haplotypes was higher (13) during the pre-intervention period than during the intervention (4) and malaria resurgences period (5). The 3D7 KSSFNEP haplotype was only observed during the intervention period, with 40% prevalence. Only two haplotypes (KSSVKEP and RSSVKEP) were shared by both the pre-intervention and intervention periods; while four haplotypes (NLSFKEP, KSSVKEP, NSSFKEP and KSPVKEP) were shared between the pre-intervention and the malaria resurgences period (Table 3).

The evaluation of genealogy relatedness and similarity within and between the *PfCelTOS* population during the three periods by TCS further confirmed the number of haplotypes for each period, i.e., 16 for pre-intervention period, 4 for intervention period and 6 for malaria resurgences (Table 2). The haplotype network revealed a total of 6 shared haplotypes (Fig. 2). Hap 13, the most represented, is found throughout the study period while Hap 1, Hap 3, Hap 9 and Hap 11 were found in both the pre-intervention and malaria resurgences periods (Fig. 2). Only two haplotypes (Hap 13 and Hap 14) are found in both the pre-intervention and intervention periods.

3.2. *PfCelTOS* selective signatures, genetic differentiation, and gene flow across the three periods

To determine if the pattern of diversity of *PfCelTOS* from each period deviated from neutral expectations, the TD test was performed. A low negative TD value (−0.05638) was noted during the pre-intervention period while the TD values during the intervention (1.69373) and malaria upsurges (1.03326) periods were highly positive (Table 2). Nevertheless, none of the values reached statistical significance ($p > 0.10$). Similar results were obtained by the Fu & Li's (D & F) tests whose values did not reach statistical significance except for that during the malaria resurgences period with the D test ($P < 0.05$) (Table 2).

Genetic differentiation indices were significantly different between the three periods ($\text{Chi}^2 = 112.585$; $p < 0.001$; $df = 36$). H_{st} values for *PfCelTOS* show significant differences between the three study periods. This is confirmed by the F_{st} values that show moderate differentiation between pre-intervention and intervention periods (0.17433), and between intervention and malaria resurgence periods (0.19198); there was also moderate differentiation between pre-intervention and malaria resurgences periods (0.06607) (Table 4).

Table 2
Genetic diversity and pattern of selection of *Plasmodium falciparum* Celts by study period in Dielmo and in two states of Nigeria.

Genetic metric	Dielmo (Senegal)			Nigeria (Oboh et al., 2022)	
	2007	2008–2009	2010–2011/2013–2014	Cross River State	Edo State
Number of isolates	36	20	40	25	25
SNPs/Segregating sites	13	5	11	11	34
Number of Haplotypes	16	4	6	10	4
Haplotype diversity	0.914	0.758	0.821	0.89	0.757
Nucleotide diversity					
π	0.01025	0.00592	0.00937	0.01033	0.03993
Θ_w	0.01043	0.00381	0.00699	0.0093	0.02505
Average number of Nucleotide difference	3.792	2.189	3.467	3.823	14.773
Minimum number of recombination events (Rm)	2	0	2	2	0
Test of Neutrality					
Tajima's D	-0.05638 ^b	1.69373 ^b	1.03326 ^b	0.37982	2.24344
Tajima's D(Syn):	0.74772 ^b	n.a.	0.96335 ^b	-	-
Tajima's D(NonSyn):	1.19656 ^b	1.69373 ^b	0.82398 ^b	-	-
Tajima's D (NonSyn/Syn) ratio	1.60027	n.a.	0.85533	-	-
Fu and Li's D	-0.01245 ^b	1.18636 ^b	1.44072 ^a	0.65893	1.72336
Fu and Li's F	-0.03147 ^b	1.53213 ^b	1.54031 ^a	0.67066	2.21302

* = $P < 0.05$; a = $0.10 > P > 0.05$ and $b = p > 0.10$; not significant. All analyses were carried out with a sliding window of 100 in a 25 nucleotides stepwise length. The Singleton variable sites are 2 at positions 295 and 345.

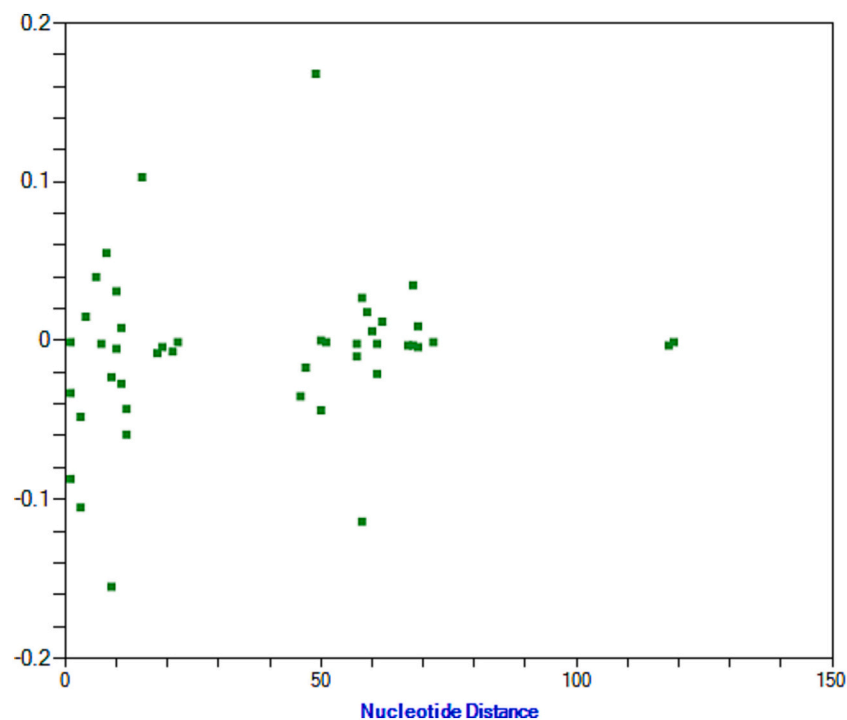


Fig. 1. Linkage disequilibrium (LD) between the SNPs of *Plasmodium falciparum* Celts gene from malaria parasite isolates between 2007 and 2014 in Dielmo.

4. Discussion

New preventive tools such as an efficacious malaria vaccine are needed to progress towards elimination and eventually eradication of malaria. The currently available malaria vaccines, RTS,S / AS01, recently recommended for use by WHO, is based on the *P. falciparum* circumsporozoite protein, and provides modest protective efficacy (Castelli and Tomasoni, 2022), although when used in combination with seasonal malaria chemoprevention can significantly decrease the incidence of clinical malaria (Chandramohan et al., 2021). A major concern for the development of a malaria vaccine is the high and diverse variations of the target antigens that could reduce its efficacy by selecting “resistant” parasites (Genton et al., 2002). Therefore, understanding antigenic diversity and the natural dynamics of any vaccine candidate antigen against interventions and changes in malaria epidemiology is

important for vaccine development (Escalante et al., 2002; Volkman et al., 2012). Moreover, monitoring accurately changes in transmission intensity is important to guide elimination efforts. *PfCelTOS* is both a candidate malaria vaccine and a marker to measure transmission intensity (Kariu et al., 2006; Doolan et al., 2003). Understanding its genetic diversity is thus important as it could inform the use of this marker for vaccine development and surveillance. Nevertheless, there is a paucity of information on *PfCelTOS* genetic diversity. To our knowledge, only two studies evaluated *PfCelTOS* genetic diversity from global isolates and in two Nigerian sites (Oboh et al., 2022; Pirahmadi et al., 2018). The diversity of *PfCelTOS* from Dielmo reflected changes in malaria prevalence, with significant high genetic diversity in the pre-implementation, declining to moderate to low diversity during intensive malaria intervention implementation period and then recovered following malaria resurgences. The number of segregating sites was

Table 3
Distribution of different haplotypes in *P. falciparum* isolates according to the different periods of intervention in Dielmo, Senegal.

Amino acid haplotypes	2007 N (%)	2008–2009 N (%)	2010–2011/2013–2014 N (%)
KSSFNEP	–	8 (40.0)	–
NLSFKEP	8 (22.2)	–	4 (10.0)
KSSVKEP	8 (22.2)	4 (20.0)	16 (40.0)
NSSFKEP	4 (11.1)	–	4 (10.0)
KHSVKEP	4 (11.1)	–	–
RSSVKEP	3 (8.3)	4 (20.0)	–
KISVKEP	2 (5.6)	–	–
KSSVKET	1 (2.8)	–	–
RSSVNAP	1 (2.8)	–	–
KSSFKEP	1 (2.8)	–	–
KSPVKEP	1 (2.8)	–	12 (30.0)
NLSFKQP	1 (2.8)	–	–
KISVNEP	1 (2.8)	–	–
KSSVNEP	1 (2.8)	–	–
NSSFNEP	–	4 (20.0)	–
RFSVNEP	–	–	4 (10.0)

The consensus nucleotides of isolates were translated into amino-acid sequence and compared with the sequence of the reference strain protein. Mutations were located at amino acid positions 100, 104, 116, 117, 119, 123, 140. The reference amino acids at those positions is KSSFNEP. “–” shows haplotype is absent.

substantial in the pre-intervention (13) and the malaria resurgences (11) periods, although they were lower than those previously observed in Nigeria (34 in Edo), Senegal (26), Gambia (25), Mali (20) and Uganda

(14) (Pirahmadi et al., 2018; Oboh et al., 2022). During the intervention period, the number of segregating sites was low (5), of similar magnitude than the one reported from Iran (9), a low endemic area (Pirahmadi et al., 2018). Similarly, haplotype diversity was higher in the pre-intervention (0.914) and malaria resurgences periods (0.821), and comparable to those reported from Senegal (0.940), Gambia (0.897), Mali (0.857) and Nigeria (Edo = 0.890), while during the intervention period, haplotype diversity was markedly lower (0.758) and comparable to that of Cross-River State (0.757), Nigeria, which is known to be meso-endemic (Oboh et al., 2022). Other parameters such as nucleotide diversity show a similar pattern, i.e., higher transmission in the pre-intervention and malaria resurgences periods than in the intervention period. This is to be expected as high transmission results in an increased parasite recombination and ultimately increased diversity (Mohd Abd

Table 4
Genetic differentiation of *PfCelTOS* gene by study period (n = 96).

Periods	2007	2008–2009	2010–2011/2013–2014
Hst values			
2007	–	0.05653***	0.03119**
2008–2009	–	–	0.08719***
Fst values			
2007	–	0.17433	0.06607
2008–2009	–	–	0.19198

*** P < 0.001.
** P < 0.01.

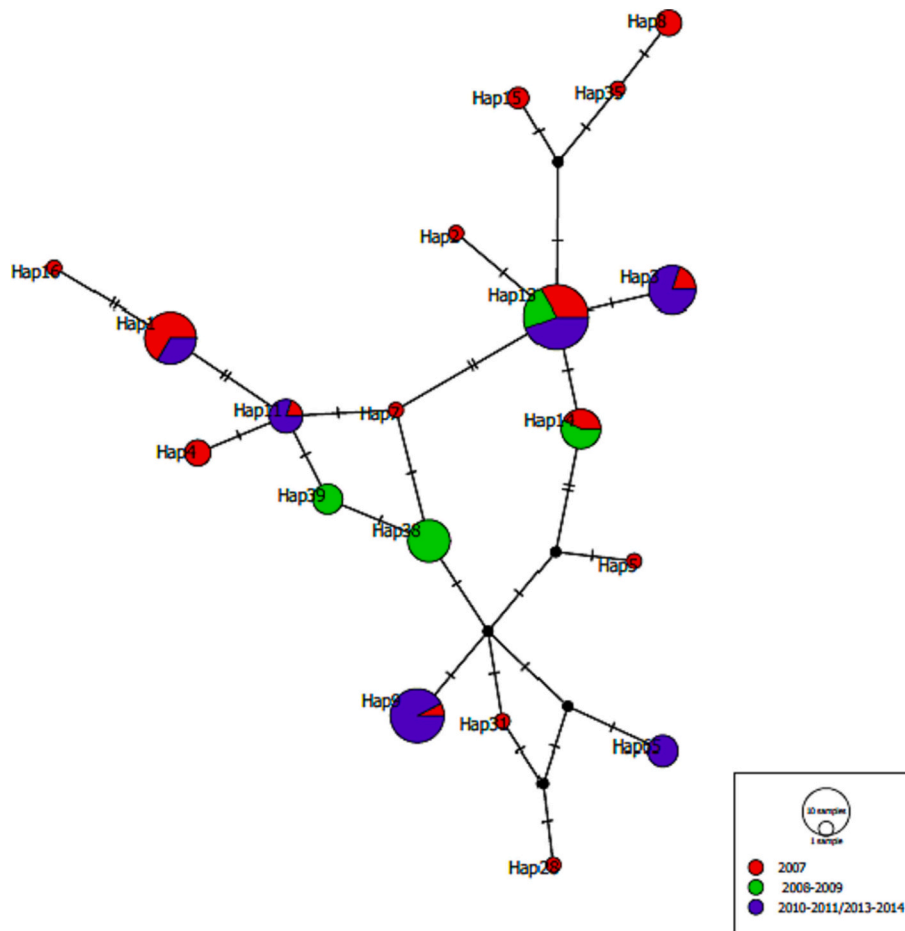


Fig. 2. Templeton, Crandall, and Sing (TCS) network providing a summary of the diversity of *PfCelTOS* haplotypes obtained from 96 DNA sequences in Dielmo during 3 periods.

Circles represent each *PfCelTOS* haplotype and are scaled according to the haplotypes' frequency. Each haplotype is denoted as “Hap”. Haplotypes from pre-intervention (2007), intervention (2008–2009) and malaria resurgences (2010–2011/2013–2014) periods are colour-coded red, green and blue, respectively.

Razak et al., 2016), while low transmission is associated with lower parasite genetic diversity (Anthony et al., 2005; Escalante et al., 2015; Nkhoma et al., 2013). Similarly, nucleotide polymorphisms are also correlated with transmission intensity (Escalante et al., 2001; Garg et al., 2007). Thus, genetics parameters such as number of segregating sites and haplotypes, haplotype diversity, average pairwise nucleotide diversity and average number of pairwise nucleotide seem to be associated with transmission intensity in Dielmo as they decreased following mass distribution of ITNs, which coincided with a significantly lower number of clinical cases (Wotodjo et al., 2017). With reduced opportunities for parasite outcrossing in the vector, the predicted *PfCelTOS* recombination events were zero during massive ITN interventions against the vector.

The observation of significant LD between the mutations at different codons is an evolutionary challenge for malaria elimination especially in the areas that are in the pre-elimination phase in Senegal. Hence, it is important that continuous monitoring of parasite diversity be carried out in the study area and other malaria endemic regions where pre-elimination is the next malaria milestone.

Most of the amino-acid substitutions, 13 out of 16, were detected during the pre-intervention period, reflecting the higher genetic diversity and in line with the nucleotide diversity value. Only one haplotype (KSSVKEP) was present across the three study periods while 2 haplotypes were shared between the pre-intervention and intervention period, and 4 between the pre-intervention and malaria resurgences period, reflecting the difference in transmission intensity between the intervention and the other 2 periods. This reduction in haplotype diversity translated into high differentiation between the temporal populations as indicated by moderate *F_{st}* values. Interventions and reduction of population size can therefore result into significant drift in a population. The *F_{st}* value was higher (0.255599) between Edo State and Cross river isolates (Oboh et al., 2022) than that observed in Dielmo, regardless the period. However, this value from Nigeria was lower than that reported between Iran and Senegal (0.31628), Gambia (0.39380), Mali (0.31819) and French Guiana (0.80723) isolates but higher than that from Ugandan isolates (0.18168) (Pirahmadi et al., 2018) which was explained by the geographical heterogeneity.

PfCelTOS is antigenic and interacts with immune responses, which can exert a selective pressure on allelic forms of the antigen. This was evident with Tajima's *D* measurements, which were low at the time of higher malaria prevalence prior to the implementation of ITNs. The low values are explained by the abundance of low frequency SNPs, an indication that balancing selection from immunity was affecting *PfCelTOS* diversity. However, more positive *TD* values were obtained following population collapse, and this did not return to low values with the malaria resurgences due to the demographic event from the bottleneck imposed by interventions (Tetteh et al., 2009; Weedall and Conway, 2010). The census sizes for the various study periods were small and may not accurately reflect the allele frequency spectrums interrogated. As *PfCelTOS* is involved in parasite-host interactions, and is a vaccine target, additional population genetic studies across different malaria endemicities will be helpful.

In conclusion, *PfCelTOS* genetic diversity seems to vary with intensity of transmission, with higher polymorphisms recorded before the large-scale implementation of ITNs and during the malaria resurgences. This suggests *PfCelTOS* antigen is a good and reproducible malaria transmission marker. Data from other areas in Senegal and other African countries are needed to confirm the potential use of *PfCelTOS* as a marker of transmission intensity. Moreover, given *PfCelTOS* is a candidate vaccine, it will be important to generate data on its antigenic diversity from different endemic regions.

Authors' contributions

UDA and AAN conceptualized this work. ANW and MAO performed the laboratory work and analysed the results. ANW wrote the first draft of the manuscript. MAO, UDA, SD and AAN carried out critical review of

the manuscript. CS, FDS, ND, and JFT supervised the data collection. All authors read and approved the final manuscript.

Authors' information

1 VITROME, UMR 257 IRD, Campus UCAD-IRD, Dakar, Senegal. 2 Medical Research Council Unit, The Gambia, at the London School of Hygiene and Tropical Medicine, Fajara, the Gambia. 3 Department of Biological Sciences, University of Medical Sciences, Ondo, Nigeria. 4 Department of Biomedical Sciences, Rochester Institute of Technology, Rochester, NY, United States of America. 5 Institut Pasteur de Dakar, 36 Avenue Pasteur, 220 Dakar, Senegal. 6 UMR MIVEGEC, Laboratoire de Paludologie et zoologie médicale, IRD, Dakar, Senegal.

Ethics approval and consent to participate

The study was approved by the Ministry of Health of Senegal, the assembled village population and the National Ethics Committee of Senegal.

Consent for publication

All authors read and approved the final manuscript and agree to its submission.

Funding

Institut de Recherche pour le Développement and the Pasteur Institute of Dakar funded the data collection. Amélé Nyedzie Wotodjo received research development fellowship AREF-318-WOTO-F-C0872 of Africa Research Excellence Fund (AREF).

Data statement

Sequences generated will be submitted to NCBI database.

CRediT authorship contribution statement

Amélé Nyedzie Wotodjo: Data curation, Formal analysis, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. **Mary Aigbiremo Oboh:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Cheikh Sokhna:** Funding acquisition, Project administration, Resources, Supervision. **Nafissatou Diagne:** Data curation, Resources. **Fatoumata Diène-Sarr:** Data curation, Resources. **Jean-François Trape:** Data curation, Resources, Supervision, Funding acquisition. **Souleymane Doucouré:** Data curation, Supervision, Writing – review & editing. **Alfred Amambua-Ngwá:** Conceptualization, Supervision, Writing – review & editing. **Umberto D'Alessandro:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The author(s) declare that they have no competing interests.

Data availability

Sequences generated will be submitted to NCBI database.

Acknowledgements

We are grateful to Dielmo residents for their participation in the project. We thank all the staff of IRD and Pasteur Institute of Dakar for their help during data collection. Amélé Nyedzie Wotodjo thanks Africa Research Excellence Fund (AREF) for their research development fellowship AREF-318-WOTO-F-C0872 she received.

References

- Anthony, T.G., Conway, D.J., Cox-Singh, J., Matusop, A., Ratnam, S., Shamsul, S., Singh, B., 2005. Fragmented population structure of *Plasmodium falciparum* in a region of declining endemicity. *J. Infect. Dis.* 191, 1558–1564.
- Balloux, F., Lugon-Moulin, N., 2002. The estimation of population differentiation with microsatellite markers. *Mol. Ecol.* 11, 155–165.
- Bergmann-Leitner, E.S., Mease, R.M., De La Vega, P., Savranskaya, T., Polhemus, M., Ockenhouse, C., Angov, E., 2010. Immunization with pre-erythrocytic antigen CelTOS from *Plasmodium falciparum* elicits cross-species protection against heterologous challenge with *Plasmodium berghei*. *PLoS One* 5, e12294.
- Castelli, F., Tomasoni, L.R., 2022. New insights on malaria. *New Microbiol.* 45, 83–98.
- Chandramohan, D., Zongo, I., Sagara, I., Cairns, M., Yerbanga, R.S., Diarra, M., Nikiema, F., Tapily, A., Sompongou, F., Issiaka, D., et al., 2021. Seasonal malaria vaccination with or without seasonal malaria chemoprevention. *N. Engl. J. Med.* 385, 1005–1017.
- Doolan, D.L., Southwood, S., Freilich, D.A., Sidney, J., Graber, N.L., Shatley, L., Bebris, L., Florens, L., Dobano, C., Witney, A.A., et al., 2003. Identification of *Plasmodium falciparum* antigens by antigenic analysis of genomic and proteomic data. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9952–9957.
- Escalante, A.A., Grebert, H.M., Chaiyaroj, S.C., Magris, M., Biswas, S., Nahlen, B.L., Lal, A.A., 2001. Polymorphism in the gene encoding the apical membrane antigen-1 (AMA-1) of *Plasmodium falciparum*. X. Asembo Bay cohort project. *Mol. Biochem. Parasitol.* 113, 279–287.
- Escalante, A.A., Grebert, H.M., Isea, R., Goldman, I.F., Basco, L., Magris, M., Biswas, S., Kariuki, S., Lal, A.A., 2002. A study of genetic diversity in the gene encoding the circumsporozoite protein (CSP) of *Plasmodium falciparum* from different transmission areas—XVI. Asembo Bay Cohort Project. *Mol. Biochem. Parasitol.* 125, 83–90.
- Escalante, A.A., Ferreira, M.U., Vinez, J.M., Volkman, S.K., Cui, L., Gamboa, D., Krogstad, D.J., Barry, A.E., Carlton, J.M., van Eijk, A.M., et al., 2015. Malaria molecular epidemiology: lessons from the international centers of excellence for malaria research network. *Am. J. Trop. Med. Hyg.* 93, 79–86.
- Garg, S., Alam, M.T., Das, M.K., Dev, V., Kumar, A., Dash, A.P., Sharma, Y.D., 2007. Sequence diversity and natural selection at domain I of the apical membrane antigen 1 among Indian *Plasmodium falciparum* populations. *Malar. J.* 6, 154.
- Genton, B., Betuela, I., Felger, I., Al-Yaman, F., Anders, R.F., Saul, A., Rare, L., Baisor, M., Lorry, K., Brown, G.V., 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.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41, 95–98.
- Hofmann, N., Mwingira, F., Shekalaghe, S., Robinson, L.J., Mueller, I., Felger, I., 2015. Ultra-sensitive detection of *Plasmodium falciparum* by amplification of multi-copy subtelomeric targets. *PLoS Med.* 12, e1001788.
- Kariu, T., Ishino, T., Yano, K., Chinzei, Y., Yuda, M., 2006. CelTOS, a novel malarial protein that mediates transmission to mosquito and vertebrate hosts. *Mol. Microbiol.* 59, 1369–1379.
- Leigh, J.W., Bryant, D., 2015. Popart: full-feature software for haplotype network construction. *Methods Ecol. Evol.* 6, 1110–1116.
- Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451–1452.
- Mohd Abd Razak, M.R., Sastu, U.R., Norahmad, N.A., Abdul-Karim, A., Muhammad, A., Muniandy, P.K., Jelip, J., Rundi, C., Imwong, M., Mudin, R.N., Abdullah, N.R., 2016. Genetic diversity of *Plasmodium falciparum* populations in malaria declining areas of Sabah, East Malaysia. *PLoS One* 11, e0152415.
- Nei, M., 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Niang, M., Loucoubar, C., Sow, A., Diagne, M.M., Faye, O., Faye, O., Diallo, M., Toure-Balde, A., Sall, A.A., 2016. Genetic diversity of *Plasmodium falciparum* isolates from concurrent malaria and arbovirus co-infections in Kedougou, southeastern Senegal. *Malar. J.* 15, 155.
- Nkhoma, S.C., Nair, S., Al-Saai, S., Ashley, E., McGready, R., Phyto, A.P., Nosten, F., Anderson, T.J., 2013. Population genetic correlates of declining transmission in a human pathogen. *Mol. Ecol.* 22, 273–285.
- Noor, A.M., Kinyoki, D.K., Munda, C.W., Kabaria, C.W., Mutua, J.W., Alegana, V.A., Fall, I.S., Snow, R.W., 2014. The changing risk of *Plasmodium falciparum* malaria infection in Africa: 2000–10: a spatial and temporal analysis of transmission intensity. *Lancet* 383, 1739–1747.
- Oboh, M.A., Isaac, C., Schroeter, M.N., Morenikeji, O.B., Amambua-Ngwa, A., Meremikwu, M.M., Thomas, B.N., 2022. Population genetic analysis of *Plasmodium falciparum* cell-traversal protein for ookinetes and sporozoite among malaria patients from Southern Nigeria. *Infect. Genet. Evol.* 105, 105369.
- Pirahmadi, S., Zakeri, S., Mehrizi, A.A., Djadid, N.D., 2018. Analysis of genetic diversity and population structure of gene encoding cell-traversal protein for ookinetes and sporozoites (CelTOS) vaccine candidate antigen in global *Plasmodium falciparum* populations. *Infect. Genet. Evol.* 59, 113–125.
- PNLP, 2018. Bulletin Epidemiologique Annuel 2017 du Paludisme au Senegal. In: Programme National de Lutte contre le Paludisme.
- Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123, 585–595.
- Templeton, A.R., Crandall, K.A., Sing, C.F., 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132, 619–633.
- Tetteh, K.K., Stewart, L.B., Ochola, L.I., Amambua-Ngwa, A., Thomas, A.W., Marsh, K., Weedall, G.D., Conway, D.J., 2009. Prospective identification of malaria parasite genes under balancing selection. *PLoS One* 4, e5568.
- Trape, J.F., Rogier, C., Konate, L., Diagne, N., Bouganali, H., Canque, B., Legros, F., Badji, A., Ndiaye, G., Ndiaye, P., et al., 1994. The Dielmo project: a longitudinal study of natural malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. *Am. J. Trop. Med. Hyg.* 51, 123–137.
- Trape, J.F., Tall, A., Diagne, N., Ndiath, O., Ly, A.B., Faye, J., Dieye-Ba, F., Roucher, C., Bouganali, C., Badiane, A., et al., 2011. Malaria morbidity and pyrethroid resistance after the introduction of insecticide-treated bednets and artemisinin-based combination therapies: a longitudinal study. *Lancet Infect. Dis.* 11, 925–932.
- Trape, J.F., Sauvage, C., Ndiaye, O., Douillot, L., Marra, A., Diallo, A., Cisse, B., Greenwood, B., Milligan, P., Sokhna, C., Molez, J.F., 2012. New malaria-control policies and child mortality in Senegal: reaching millennium development goal 4. *J. Infect. Dis.* 205, 672–679.
- Trape, J.F., Tall, A., Sokhna, C., Ly, A.B., Diagne, N., Ndiath, O., Mazonot, C., Richard, V., Badiane, A., Dieye-Ba, F., et al., 2014. The rise and fall of malaria in a west African rural community, Dielmo, Senegal, from 1990 to 2012: a 22 year longitudinal study. *Lancet Infect. Dis.* 14, 476–488.
- Tusting, L.S., Bousema, T., Smith, D.L., Drakeley, C., 2014. Measuring changes in *Plasmodium falciparum* transmission: precision, accuracy and costs of metrics. *Adv. Parasitol.* 84, 151–208.
- Volkman, S.K., Ndiaye, D., Diakite, M., Koita, O.A., Nwakanma, D., Daniels, R.F., Park, D. J., Neafsey, D.E., Muskavitch, M.A., Krogstad, D.J., et al., 2012. Application of genomics to field investigations of malaria by the international centers of excellence for malaria research. *Acta Trop.* 121, 324–332.
- Watterson, G.A., 1975. On the number of segregating sites in genetical models without recombination. *Theor. Popul. Biol.* 7, 256–276.
- Weedall, G.D., Conway, D.J., 2010. Detecting signatures of balancing selection to identify targets of anti-parasite immunity. *Trends Parasitol.* 26, 363–369.
- Wotodjo, A.N., Diagne, N., Gaudart, J., Richard, V., Raoult, D., Sokhna, C., 2015. Malaria risk factors in Dielmo, a Senegalese malaria-endemic village, between October and November of 2013: a case-control study. *Am. J. Trop. Med. Hyg.* 92, 565–568.
- Wotodjo, A.N., Doucoure, S., Gaudart, J., Diagne, N., Diene Sarr, F., Faye, N., Tall, A., Raoult, D., Sokhna, C., 2017. Malaria in Dielmo, a Senegal village: is its elimination possible after seven years of implementation of long-lasting insecticide-treated nets? *PLoS One* 12, e0179528.