

across all the districts (Fst mean = 0.00238). Tajma's D analysis was done to look for signatures of selection and showed direction selection in both Muheza (-1.055) and Nachingwea (-0.676) while Muleba showed neutrality (Tjd= 0.151). More analysis is under way to map the diversity of the pfrh5 gene in all the districts. The preliminary results reveal low levels of genetic variation in the pfrh5 gene across the districts, these results correspond to the findings from previous studies conducted in 2020 in Nigeria. However, a broader investigation is required in some other parts of the country to support the potential of pursuing pfrh5 gene as a malaria vaccine antigen.

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POPULATION GENOMICS OF PLASMODIUM FALCIPARUM AND MALARIA CONTROL: IMPLICATIONS IN ABIDJAN (COTE D'IVOIRE)

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The onset of *Plasmodium falciparum* (*P. falciparum*) resistance to antimalarial drugs requires careful surveillance of African parasite population. Genomics tools are implemented to detect evolutionary changes that could impact on malaria control and elimination strategies. Here, we evaluate the genome-wide pattern of selection and sequence variation of *P. falciparum* populations in Abidjan, Côte d'Ivoire. The study was conducted in three localities of Abidjan from 2013 to 2014. We collected 70 blood samples following a written informed consent from patients above two years of age. After extracting *P. falciparum* and human DNA from isolates, we performed Whole Genome Sequencing and used population genomics approaches to investigate genetic diversity, complexity of infection and identify loci under positive directional selection. We observed an excess of rare variants in the population showing a clear mutation process in the isolates. Moderate Fst estimates (0.3) was detected for surfin, an immune invasion gene family. Seven iHS regions that had at least two SNPs with a score greater than 3.2 were identified. These regions code for genes that have been under strong directional selection. Two of these genes were the chloroquine resistance transporter (crt) on chromosome 7 and the dihydropteroate reductase (dhps) on chromosome 8. Our analyses showed a recent selective sweep in the erythrocyte membrane protein (Pfemp1). In conclusion, our analyses identified genes under selective drug pressure and balancing selection on protective immune-specific genes. These findings demonstrate the effectiveness of genomics analyses to follow malaria parasite evolution of parasite and adopt appropriate strategies to eliminate malaria in Côte d'Ivoire.

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ASSESSING TRANSMISSION DYNAMICS AND RELATEDNESS OF PLASMODIUM FALCIPARUM ON BIOKO ISLAND, EQUATORIAL GUINEA

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In 2019, the Bioko Island Malaria Elimination Project (BIMEP) conducted their annual malaria indicator survey on Bioko Island (BI), Equatorial Guinea, revealing 13.4% malaria prevalence by RDT. The challenge facing BIMEP, as BI approaches pre-elimination, is defining the sources that contribute to the persistence of malaria in the island. To this end, we are investigating transmission dynamics of *Plasmodium falciparum* (Pf), the predominant malaria species on BI, both within BI and between BI and continental Africa. Dried blood spot samples from participants with reported fever and a

positive RDT for Pf were selected for selective whole genome amplification and sequencing (n=74). Utilizing a variety of population genetics metrics and analyses, including nucleotide diversity (π), FST, admixture and identity-by-descent (IBD), the genetic diversity and population structure of the BI parasite population were compared to continental African countries. Initial results show BI parasites cluster with, and have similar ancestral background to, samples from its geographical neighbor Cameroon. This further supports previous epidemiological evidence of malaria importation to BI via human migration, and the observation of mixing between island and continental strains, despite a geographical barrier. Next, to determine whether Pf in BI forms a panmictic population, relatedness between BI samples was measured using IBD to characterize on-island transmission dynamics. Overall, BI samples appeared mostly unrelated (average IBD = 0.003). However, stratification by urban and rural communities revealed some differentiation (FST = 0.03) and higher IBD among rural infections (average IBD = 0.008) than among urban (average IBD = 0.002) consistent with a partially structured population. If progress continues, rural communities may be amenable to elimination interventions without the fear of constant reseeding from urban environments. Further investigation using epidemiological and genetic data will be utilized to provide greater detail of BI transmission among epidemiological subgroups within the context of malaria control management.

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PLASMODIUM FALCIPARUM POPULATION STRUCTURE IN SOUTHWESTERN AFRICA, USING WHOLE GENOME SEQUENCE DATA: INITIAL GENOME-WIDE SEQUENCE DATA FROM ANGOLA

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Malaria continues to be the principal cause of morbidity and mortality in Angola, primarily due to *Plasmodium falciparum* (Pf) infection. Angola ranked 9th worldwide in number of malaria deaths in 2021 but the distribution of disease is remarkably heterogeneous, with provinces varying between <1% and >50% malaria prevalence. Despite malaria's heavy toll on public health in Angola, Pf genetic diversity and demography in the country remain largely unexplored. Here we aimed to characterize malaria infections in six provinces in Angola, two each in regions where malaria transmission is hyperendemic (Cabinda, Uíge), mesoendemic stable (Luanda, Cuanza Sul) and seasonal with low prevalence (Cunene, Namibe). We hypothesize that (1) multiplicity of infection is positively correlated with transmission intensity and (2) that Pf transmission among provinces conforms to a model of isolation by distance. Finally, (3) Pf genetic diversity in Angola will be contrasted with that found in neighboring countries. To address these questions, parasite DNA was isolated from 150 dried blood spots collected in 2022, and subjected to selective whole genome amplification, and sequencing in an Illumina NovaSeq 6000 platform. The sequencing data was mapped against the *P. falciparum* reference genome, single nucleotide polymorphisms (SNPs) were identified according to best practices, and joint SNPs calling was done together with WGS data from several hundred publicly available Pf samples from East, West and Central Africa, as well as Brazil and French Guiana. A Principal Component Analysis (PCA) done on the SNP calls revealed that Pf samples from Angola cluster with others from Central Africa. Admixture analyses are still ongoing, to determine the extent to which the ancestry of the Angolan Pf population differs from those of neighboring countries. In addition, average multiplicity of infection and overall nucleotide diversity will be estimated for each province, and

population differentiation between provinces will be estimated with Wright's fixation index (FST). To test isolation by distance, FST will be compared with geographic distance using a Mantel test.

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SEQUENCE POLYMORPHISMS IN THE PFS47 6-CYSTEINE PROTEIN IN PLASMODIUM FALCIPARUM ISOLATES FROM ANGOLA, 2019

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The survival and transmission of the *Plasmodium falciparum* parasite is dependent on the gene encoding the 6-cysteine protein Pfs47 (PF3D7_1346800) that plays a crucial role in the parasite avoiding the mosquito vector's immune system. Allelic variants of the Pfs47 sequence constitute haplotypes that vary globally and are adapted to evade the immune system of specific *Anopheles* mosquitoes found in each region. We utilized a targeted amplicon deep sequencing (TADS) protocol to evaluate the highly polymorphic Pfs47 gene for its ability to determine the geographic origin of *P. falciparum*. A total of 56 samples from three provinces in Angola (11 from Benguela, 14 from Lunda Sul, and 31 from Zaire) were individually sequenced and evaluated for single nucleotide polymorphisms (SNPs) in Pfs47. From these, 52 samples (11 from Benguela, 14 from Lunda Sul, and 27 from Zaire) had sufficient alignments to Pfs47 (PF3D7_1346800) and were evaluated further. There was a total of 12 haplotypes observed in Angola made up of 11 unique amino acid changes. Two haplotypes were exclusively found in Zaire, two in Lunda Sul, and one in Benguela. Major SNPs, those with a weighted allele frequency of 25% or more, were P194H, N272I, E188D, E27D, and P369H. Apart from E27D (Domain 1) and P369H (Domain 3), the other three polymorphisms occur in Domain 2 of Pfs47. Interestingly, E27D is most prevalent in Lunda Sul, E188D is most prevalent in Zaire, and N272I is more prevalent in Zaire and Benguela than in Lunda Sul. Finally, we utilized 535 publicly available consensus sequences for Pfs47 (99 from Asia, 59 from Americas, and 377 from Africa) and compared them with our Angola samples. Through hierarchical clustering, the representative haplotype for Angola is most similar to haplotypes found in Western African countries, as expected. This evaluation strengthened the observation that E27D and N272I are distinctly African SNPs. Although Pfs47 haplotypes can discern the geographic origin of *P. falciparum* among Africa, Asia, and the Americas, more power is needed to evaluate whether Pfs47 polymorphisms can discern parasite origin among countries or provinces/regions.

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DRUG RESISTANCE PROFILE OF PLASMODIUM FALCIPARUM IN THE COMMUNITIES OF CONDORCANQUI, AMAZONAS, PERU

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Malaria is a serious health problem in the native communities of Condorcanqui in the Amazonas region of Peru, reporting a 2.5-fold increase in the number of cases since 2019. Resistance to antimalarial drugs hampers malaria control and elimination; suspected resistance to artemisinin, chloroquine, sulfadoxine, pyrimethamine, and mefloquine in

Plasmodium falciparum can be explored by analyzing polymorphisms in the Pfk13, Pfcrt, Pf dhps, Pf dhfr, and Pfmdr1 genes, respectively. In this study, *P. falciparum* positive cases, collected during 2019 to 2022, from native communities of Condorcanqui were evaluated. Genomic DNA was isolated from fifty-one blood samples on filter paper, collected from 2019 to 2022 and species confirmation was performed by real-time PCR. Polymorphisms of Pfk13, Pfcrt, Pf dhps, Pf dhfr and Pfmdr1 genes were analyzed by nested PCR followed by Sanger sequencing. Electropherograms were analyzed in Geneious Prime and then compared to the 3D7 reference sequence obtained from the NCBI database. All samples had the same genotype, carrying mutant alleles for Pfcrt (C72S and K76T), Pf dhfr (A16V, C50R, N51I and S108N/T), Pf dhps (A437G, K540E, A581G) and Pfmdr1 (Y86N and Y184F). However, no mutations were found in the Pfk13 propeller domain. These results are consistent with a recent clonal expansion, due to a *P. falciparum* outbreak reported in the area in 2019. Continued surveillance of polymorphisms associated with antimalarial resistance is recommended to guide the formulation of rational drug policies and the mitigation of risk of *P. falciparum* artemisinin resistance.

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MOLECULAR EPIDEMIOLOGY OF NON-FALCIPARUM PLASMODIUM INFESTATIONS IN DIFFERENT AREAS OF THE IVORY COAST

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Malaria is a major public health problem, particularly in the tropical regions of America, Africa and Asia. *Plasmodium falciparum* is not only the most widespread but also the most deadly species. The share of *Plasmodium* infestations caused by the other species (*P. ovale* and *P. malariae*) is clearly underestimated. General objective was to determine the molecular epidemiology of plasmodial infestations due to *P. malariae* and *P. ovale* in Côte d'Ivoire. This is a cross-sectional study which took place from February to March 2021 at the Centre de Recherche et de Lutte contre le Paludisme (CRLP) of the Institut National de Santé Publique (INSP). The collection of samples took place from May 2015 to April in different malaria epidemiological facies in Côte d'Ivoire. Analysis of the collected samples was performed. In each patient, we collected blood by venipuncture at the elbow on EDTA tubes. These samples were used to make confetti on Wathman paper for the molecular diagnosis of malaria. Molecular diagnosis as well as differential diagnosis of plasmodial species using the nested PCR technique. A total of 360 samples were tested with a success rate of 72.5% (261 out of 360). The sex ratio was 0.84. The overall plasmodic index was 72.5%. The specific index was 77.4%; 1.5% and 0% for *P. falciparum*, *P. malariae* and *P. ovale* in mono-infestation, respectively. There was also 15% *P. falciparum* and *P. malariae* co-infestation, 3.4% *P. falciparum* and *P. ovale* co-infestation and 2.3% *P. falciparum*, *P. malariae* and *P. ovale* triple-infestation. After exclusion of *P. falciparum* monoinfestation cases, 59 samples were finally retained to evaluate the molecular epidemiology of non-falciparum *Plasmodium* infestations. Typing of *P. ovale* subspecies showed a clear predominance of *P. ovale curtisi* (81.2% of cases). *P. falciparum* remains the most prevalent malaria species in Côte d'Ivoire, but *P. malariae* and *P. ovale* are endemic at a low rate. The elimination of malaria requires a better understanding of the specific epidemiological characteristics of *P. malariae* and *P. ovale* with a particular emphasis on the identification of asymptomatic carriers.