An open dataset of *Plasmodium falciparum* genome variation in 7,000 worldwide samples

MalariaGEN *Plasmodium falciparum* Community Project

Contributing partner studies

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The Community Project (https://www.malariagen.net/projects/p-falciparum-community-project) is coordinated by the MalariaGEN Resource Centre and is comprised of partner studies – independent studies undertaken in malaria endemic areas. Each partner study is unique with their own research objectives. They have agreed to contribute samples to the Community Project on the understanding that this will not interfere with their research objectives. Prior to submitting samples, all partner studies complete a Partner Study Information Form that captures information about their study, and confirms that all relevant ethical and regulatory requirements have been met and that all stakeholders have agreed to contribute samples and data to the Community Project. Each partner study is represented on the Community Project website with a brief description of the study, and details of the study contact person, key associates and their affiliations. Below is a summary of the information presented at the time of publication.

1001-PF-ML-DJIMDE

Developing the Community Project with partners in Mali

Abdoulaye Djimde and colleagues worked with the MalariaGEN team to collect clinical parasite samples from three sites in Mali: Bamako, the capital, and Kolle and Faladje, rural villages approximately 60km and 80km away. These samples helped form the cornerstone of MalariaGEN's initial efforts to characterise global *Plasmodium* genome variation, and contributed to the development of field and lab-based sample handling methods, sequencing approaches, and data analysis pipelines.

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1004-PF-BF-OUEDRAOGO

Developing the Community Project with partners in Burkina Faso

In Burkina Faso, Jean-Bosco Ouedraogo and colleagues worked with the MalariaGEN team to collect samples from three urban clinics in Bobo-Dioulasso – Colsama, Ouezzin-ville and Sakaby – each up to 8km from the laboratory at the Institut de Recherche en Sciences de la Santé. These samples helped form the cornerstone of MalariaGEN's initial efforts to characterise global *Plasmodium* genome variation, and contributed to the development of field and lab-based sample handling methods, sequencing approaches, and data analysis pipelines. Jean-Bosco and team are keen to investigate signatures of population structure and other unique features of genome variation between the three locations where samples were collected.

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1006-PF-GM-CONWAY

Genome-wide analysis of genetic variation in The Gambia

Alfred Amambua-Ngwa, David Conway, and colleagues surveyed clinical *P. falciparum* isolates from The Gambia to assess several measures of genetic variation including allele frequency spectra and signatures of balancing selection, across geographical regions and developmental stages. Using Illumina sequencing data from these parasites, the team published the first population-based study of signatures of balancing selection throughout a pathogen genome — a landmark in understanding pathogen polymorphism. They continue to study the functional and immunological targets identified in this study, and to extend the geographical range of their population genetic studies. This study is contributing to the Plasmodium Diversity Network Africa (PDNA), an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* parasites across Africa.

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1007-PF-TZ-DUFFY

Mother Offspring Malaria Study (MOMS) in Tanzania

Led by Patrick Duffy, the Mother Offspring Malaria Study (MOMS) aims to understand the relationship between parasite phenotype and clinical outcomes, and to identify parasite ligands and soluble mediators involved in malaria infections during early life. Longitudinal and cross-sectional cohorts were recruited at Muheza Designated District Hospital and Morogoro Regional Hospital. Pregnant women, children and infants were recruited, and children were followed at regular intervals to capture parasite samples and clinical phenotypes from birth up to the age of 5 years. MOMS clinical activities have been completed, but sample and data analyses continue. Samples sequenced through the MalariaGEN *P. falciparum* Community Project are being analysed to understand the local diversity and population structure of malaria parasites from Tanzanian mothers and children. Subsets of sequenced samples have also been assayed by RNA-seq and microarray platforms, and these data provide an additional dimension of information for ongoing expression studies in the Duffy group.

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1008-PF-SEA-RINGWALD

Containment of artemisinin tolerant malaria parasites in South-East Asia (ARCE)

ARCE is a WHO-lead strategy for the containment of artemisinin resistant parasites in Southeast Asia, aiming to ultimately contribute to the elimination of *falciparum* malaria from the area. Samples collected as part of ARCE-lead studies of artesunate efficacy at sites in Myanmar, Laos and Viet Nam were contributed to the MalariaGEN *P. falciparum* Community Project for whole genome sequencing and subsequent analysis of genetic variation, population structure and signatures of selection. The genetic data is also being used in a replication genome-wide association study to validate and extend the outcomes of the ARC3 GWAS, with the hope of identifying molecular markers of artemisinin resistance.

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1010-PF-TH-ANDERSON

Genetic variation underlying drug resistance at the Thai-Burmese border

In one of the first partnerships of the MalariaGEN *P. falciparum* Community Project, Timothy Anderson and colleagues used whole genome sequencing and genotyping to identify genetic variants that underpin drug resistance in *P. falciparum*. Working with Francois Nosten at his field site in Mae Sot on the Thai-Burmese border, clinical parasite samples were collected, cloned and lab-adapted to use for both genetic and phenotypic analyses, namely evaluating drug resistance *in vitro*. The central aim of the project was to combine these analyses to investigate genotype-phenotype association using high-resolution SNP data generated by Illumina sequencing, as well as traditional genotyping platforms.

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1011-PF-KH-SU

Genome-wide scans of cultured adapted parasites in Cambodia

Xin-zhuan Su and colleagues provided samples from their field site in Pursat province, Cambodia at an early stage in the MalariaGEN *P. falciparum* Community Project. These samples were contributed to support the development of our sequencing and analysis pipelines, and to provide geographical representation of parasites from Cambodia for preliminary analysis of global population structure. For Su's analyses, the parasites were culture adapted and analysed for genome-wide scans for positive selection, recombination hot spots and resistance to antimalarial drugs.

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1012-PF-KH-WHITE

Developing the Community Project with partners in Cambodia

Several investigators contributed to the early stages of the MalariaGEN *P. falciparum* Community Project by providing samples that were not themselves the basis of a partner study, but were used in establishing our laboratory and analytical pipelines. For example, some samples were used to compare early Illumina sequencing outputs with capillary sequencing data, or for preliminary analysis of population structure.

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1013-PF-PEGB-BRANCH

Developing the Community Project with partners in Peru

Samples from Zungarococha, Peru were collected in 2005-06 as part of the Malaria Immunology and Genetics in the Amazon (MIGIA) project. Malaria caused by *Plasmodium falciparum* emerged near the Amazonian city of Iquitos in recent history in the 1990s. Since 2003, MIGIA has been following a cohort of approximately 2000 Peruvians longitudinally at least 6 times per year in active-household based visits, as well as seeing patients in clinics and hospitals at each febrile episode. A unique feature well-characterised in this study is the high proportion of asymptomatic infections given the low malaria transmission rate. Low transmission dynamics or host-parasite characteristics may explain the rapid development of immunity compared to observations in high transmission settings. This is a collaboration with Dr. Branch at New York University School of Medicine, Dr. Lastenia Ruiz at the Universidad Nacional de la Amazonia Peruana and Dr. Moises Sihuincha at the Peruvian Ministry of Health.

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1014-PF-SSA-SUTHERLAND

Analysis of *Plasmodium falciparum* samples from UK travellers returning from malaria endemic countries

In collaboration with Colin Sutherland and Tim Robinson, we sequenced samples obtained from travellers returning to the UK from malaria-endemic countries being treated for clinical malaria in the Hospital for Tropical Diseases, London. Malaria isolates were obtained from four patients, two of which had recently travelled to Ghana, one to Kenya and one to Mozambique. We have previously described parasite clearance dynamics in each of these patients while they were being treated (Beshir et al, 2010).

Analysis of the genome sequences obtained from these isolates resulted in the first manuscript published using data generated from the MalariaGEN *P. falciparum* Community Project: Drug-resistant genotypes and multi-clonality in *Plasmodium falciparum* analysed by direct genome sequencing from peripheral blood of malaria patients by Robinson T, Campino SG (co-first authors) et al.

Each patient was found to harbour multiple clone infections, and this was verified in each case using standard PCR genotyping of the original blood sample. Evidence was found for known and novel gene deletions and amplifications, and full–length sequence was analysed for eight known loci implicated in drug resistance. We were thus able to demonstrate that Illumina whole genome sequencing of peripheral blood *P. falciparum* taken directly from malaria patients provides high quality data useful for drug resistance studies, genomic structural analyses and population genetics, and also robustly represents clonal multiplicity.

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1015-PF-KE-NZILA

Genome-wide association study of in vitro drug resistance in Kenya

As a part of clinical studies being undertaken by the KEMRI-Wellcome Trust Research Programme, samples were collected in the Kilifi district of Kenya. Alexis Nzila and his colleagues adapted these field isolates to laboratory culture and tested their *in vitro* sensitivity to antimalarial drugs including lumefantrine, chloroquine, piperaquine and dihydroartemisinin (DHA). The phenotypic data are now being used in genome-wide association studies with parasite genotypes generated by Illumina sequencing to identify regions associated to decreased sensitivity to the antimalarial drugs.

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1016-PF-TH-NOSTEN

Developing the Community Project with partners in Thailand

Several investigators contributed to the early stages of the MalariaGEN *P. falciparum* Community Project by providing samples that were not themselves basis of a partner study, but were used in establishing our laboratory and analytical pipelines. For example, some samples were used to compare early Illumina sequencing outputs with capillary sequencing data, or for preliminary analysis of population structure.

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1017-PF-GH-AMENGA-ETEGO

Population genetics of natural populations in Northern Ghana

In collaboration with colleagues at the Navrongo Health Research Center, Lucas Amenga-Etego conducted his thesis research under the guidance of Dominic Kwiatkowski on genetic diversity in natural populations of *Plasmodium falciparum* sampled from seven clinical sites throughout the Kassena-Nankana Districts of the upper East region, Ghana. Based on ecological and epidemiological differences, the study delineates six sub-populations partitioned into three broad categories: 1) lowland savannah, comprising mainly the central district, mid-south villages and eastern cluster of villages; 2) rocky highlands, comprising Chiana and Kayoro; and, 3) forest, mainly the enclave of Naga. Whole genome sequencing and genotyping are being used to study the population genetics of parasites from the different micro-ecological zones of the study area, and to compare these to patterns observed across West Africa.

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1020-PF-VN-BONI

Measuring in vitro drug sensitivity in Vietnam

Samples were collected in the southern province of Binh Phuoc, Vietnam, as part of a set of clinical studies initiated by Professor Tran Tinh Hien and Christiane Dolecek in 2009 to measure in vivo and in vitro drug sensitivity in this region. In collaboration with Maciej Boni, Professor Hien's research team also worked towards characterizing signatures of sulfadoxine/pyrimethamine and chloroquine resistance, and *pfMDR* copy number in these samples using the Illumina sequence and genotype data. Where samples were found to contain *P. vivax* data, for example due to mixed infection, this data was contributed to the *P. vivax* Genome Variation project.

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1021-PF-PG-MUELLER

Building a national repository of malaria isolates in Papua New Guinea

Samples from Papua New Guinea were collected as part of ongoing malaria surveillance efforts to build a national repository of malaria isolates. Some of these samples were obtained as part of MalariaGEN Consortial Project 1. These samples helped form the cornerstone of MalariaGEN's initial efforts to characterise *Plasmodium* genome variation, and contributed to the development of field and lab-based sample handling methods, sequencing approaches, and data analysis pipelines. In a follow-up study, MalariaGEN is working with Dr. Alyssa Barry from the Walter and Eliza Hall Institute, Australia, to sequence *P. falciparum* isolates from two additional populations and to develop a PNG-specific assay for genotyping and population genetic studies of a nation-wide *P. falciparum* sample collection.

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1022-PF-MW-OCHOLLA

Genome variation and selection in clinical isolates from rural Malawi

As part of his PhD research Harold Ocholla worked with colleagues at the Liverpool School of Tropical Medicine and in Malawi to collect uncultured paediatric *P. falciparum* isolates from malaria patients in a region of high malaria transmission in the Chikwawa and Zomba districts of Malawi over multiple malaria seasons. The goal of his work is to map genome variation and genetic signatures of evolutionary selection in these parasite populations in space and time, to identify associations between genetic variants and important clinical malaria phenotypes.

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1023-PF-CO-ECHEVERRI-GARCIA

Comparative analysis of permeome genes and drug resistance in Colombia

Colombia is among the countries with the highest malaria burden outside of Africa, and one of the least successful in reducing case numbers, with approximately 100,000 cases per year. The emergence and evolution of antimalarial resistance started approximately 50 years ago, however this problem is poorly understood and no whole genome sequences are yet available for Colombian *P. falciparum* parasites. This project aims to use sequence data from *P. falciparum* parasites from the Colombian Pacific region, the predominant area for falciparum malaria in the country, to identify genomic regions with strong evidence for recent selection with a particular focus on variation in permeome drug transporters, potential candidates in the mechanism of action of multidrug resistance.

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1024-PF-UG-BOUSEMA

FightMal - Correlating protection from malaria with immune profile of infected individuals in Uganda

This study aims to correlate protection from malaria with the immune profile of infected individuals. Cross-sectional and longitudinal studies were conducted in all-age cohorts living in Apac, northern Uganda. This area is characterised by intense perennial malaria transmission with *P. falciparum* as major malaria species, and *P. malariae* and *P. ovale* as prevalent other species. Detailed immune-profiling is undertaken by protein microarray, and individuals protected and unprotected from clinical malaria episodes are compared. Site and cohort descriptions are given in http://www.ncbi.nlm.nih.gov/pubmed/23473542 and http://www.ncbi.nlm.nih.gov/pubmed/21540398

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1026-PF-GN-CONWAY

Effects of transmission intensity on population structure and signatures of selection in Guinea

This study was designed to identify malaria parasite genes under selection in the highly endemic forested area of southern Guinea, where very few studies on malaria have been conducted previously. These results were compared with a parasite population from the Greater Banjul Area where malaria transmission is highly seasonal and relatively low. The findings contributed to global research efforts to identify parasite genes involved in pathogenesis, susceptibility to immune responses, and therapeutic agents. This study also provided a training opportunity in population genomics and bioinformatics for Victor Mobegi as a PhD student. Some analyses of the data are given in the original publication: Mobegi, V.A. et al. (2014) Genome-wide analysis of selection on the malaria parasite *Plasmodium falciparum* in West African populations of differing infection endemicity. *Molecular Biology and Evolution*, 31:1490-1499.

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1027-PF-KE-BULL

Genomics of severe malaria and low host immunity in Kenya

Plasmodium falciparum var genes are a large and diverse gene family that encode PfEMP1, an important set of antigenic virulence molecules that are inserted into the surface of parasite erythrocytes. Pete Bull and colleagues have a long-standing collaboration with the Sanger Malaria Programme using capillary sequence tag analysis to measure expression of *var* genes in clinical isolates from Kilifi, Kenya, and have identified genes that are associated with severe malaria and low levels of host immunity. Their current interests extend this work to whole transcriptome analysis, which will provide information on whole *var* genes that are associated with severe forms of malaria, as well as other variant antigens such as the RIFINS and STEVORS. Peter Bull and colleagues have kindly agreed to allow these samples to also be whole genome sequenced for analysis in the MalariaGEN *P. falciparum* Community Project.

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1031-PF-SEA-PLOWE

Artemisinin Resistance Confirmation, Characterization and Containment (ARC3)

ARC3, an international collaboration led by the World Health Organization, supported clinical trials of artesunate monotherapy in Cambodia, Thailand and Bangladesh. ARC3 aimed to confirm clinical resistance to artemisinin in South-East Asia, define *in vitro* drug resistance phenotypes, and identify molecular markers of resistance. One aspect of this work was developing methods to identify candidate markers of drug resistance using outputs from Illumina sequencing, laying the groundwork for large-scale studies to use whole genome sequencing and other high-throughput technologies to rapidly identify genetic loci associated with artemisinin resistance. This collaboration included investigating signatures of selection and genome-wide association studies of sensitive and resistant parasite isolates collected in the ARC3 trials, and resulted in improved handling and sequencing methods for field samples. Candidate markers discovered using this approach will be assessed for their ability to predict clinical drug resistance, and if validated, used to develop surveillance tools to guide containment efforts.

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1044-PF-KH-FAIRHURST

Genomics of parasite clearance and recrudescence rates in Cambodia

In field-based studies, Rick Fairhurst and colleagues investigated patient responses to artemisinin combination therapies (ACTs), in three Cambodian provinces, where artemisinin resistance is entrenched (Pursat), emerging (Preah Vihear), or uncommon (Ratanakiri). They provided samples from this study with to identify genetic markers of antimalarial drug resistance, use them in real time to define frontline treatments at the provincial level, and eliminate multidrug-resistant malaria in the Greater Mekong Subregion. All three sites provided *Plasmodium falciparum* samples, with Pursat additionally providing samples from patients presenting with *Plasmodium vivax*. In related laboratory-based studies, researchers aimed to elucidate the molecular mechanisms of *P. falciparum* artemisinin and partner-drug resistance, to develop point-of care diagnostics to identify drug-resistant parasites, and discover new compounds to treat drug-resistant malaria episodes. For *P. vivax*, they investigated whether red blood cell polymorphisms protected against *P. vivax* malaria, *P. vivax*-infected erythrocyte binding to monocytes, reticulocyte invasion, immune responses to candidate vaccine antigens, and efficacy of chloroquine against *P. vivax* malaria.

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1052-PF-TRAC-WHITE

Tracking Resistance to Artemisinin Collaboration (TRAC)

TRAC is investigating the scope and spread of parasite resistance to artemisinin-based therapies at sites across Asia and Africa. The first TRAC study has been completed. This multi-centre, open-label randomised trial studied the clearance rates of peripheral blood *P. falciparum* parasitaemias in patients with acute uncomplicated *falciparum* malaria treated with two different doses of artesunate. Findings were used to validate the recently discovered kelch13 marker of artemisinin resistance. Working with MalariaGEN, TRAC samples have been sequenced and analysed for features of population genetics and signatures of selection, and contributed to the genetic basis of a genome-wide associations study for genetic markers of artemisinin resistance. Where samples were found to contain *P. vivax* data, for example due to mixed infection, this data was contributed to the *P. vivax* Genome Variation project.

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1062-PF-PG-BARRY

Understanding malaria parasite populations and outbreaks in Papua New Guinea

Microsatellite analysis has previously demonstrated that *Plasmodium falciparum* populations on the north coast of PNG are organised into distinct subpopulations. If this pattern is observed throughout PNG, maps of population structure may guide malaria control programmes by identifying isolated populations and major routes of transmission. Moreover, the data will provide a framework upon which the origins of imported infections and outbreaks in non-endemic areas can be determined. In this first stage of a much larger study, we have two main objectives: (i) to investigate the population genomics of *P. falciparum* in PNG and (ii) to develop SNP markers for defining the population structure of *P. falciparum* in PNG on a fine scale. Where samples were found to contain *P. vivax* data, for example due to mixed infection, this data was contributed to the *P. vivax* Genome Variation project.

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1083-PF-GH-CONWAY

Alternative molecular mechanisms for erythrocyte invasion by *P. falciparum* in Ghana

The intensity of malaria transmission varies considerably among sites in Ghana due to differences in average temperatures, rainfall patterns, and urbanization. This study has selected two locations that have among the highest transmission rates in Ghana: Kintampo in central Ghana and Navrongo in northern Ghana. It will examine the relationship between ligand gene expression in parasite isolates and anti-ligand antibody titers in the infected hosts, and expects that immune pressure will select for parasites that most effectively evade antibody recognition. Genomes of parasite isolates with contrasting phenotypes, in the context of varying antibody titres and gene expression profiles, will be sequenced to explore if polymorphisms in or flanking the ligand genes, or elsewhere in the genome, are associated with parasite resistance to anti-EBA or Rh ligand antibodies.

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1093-PF-CM-APINJOH

Population genetics of *P. falciparum* parasites in South-Western Cameroon

Tobias Apinjoh is using whole genome sequencing to investigate the genetic diversity present in natural populations of *P. falciparum* parasites collected in different micro-ecological zones along the slope of Mount Cameroon in the South-Western region. This study examines the genetic structure of these parasite populations including looking for signatures of natural selection. This study is contributing to the Plasmodium Diversity Network Africa (PDNA), an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* parasites across Africa.

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1094-PF-GH-AMENGA-ETEGO

Population genetics of *P. falciparum* parasites in Northern Ghana

In collaboration with colleagues at the Navrongo Health Research Center, Lucas Amenga-Etego is investigating the genetic diversity and population structure of *P. falciparum* parasites collected in the Kassena-Nankana districts of Northern Ghana. *P. falciparum* isolates were sampled from individuals with a confirmed *falciparum* malarial fever. This study is contributing to the Plasmodium Diversity Network Africa (PDNA), an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* parasites across Africa.

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1095-PF-TZ-ISHENGOMA

Genome variation and its affect on ACT treatment outcome in Tanzania

This study aims to assess the diversity of *Plasmodium falciparum* in regions of varying endemicity in Tanzania, and to investigate parasite genetic factors which might affect antimalarial treatment outcome among patients treated with ACTs. As part of the Plasmodium Diversity Network Africa (PDNA), an additional aim of this work is to build and strengthen the capacity of African investigators to conduct clinical trials and genomic studies in their local contexts. Over 400 samples were collected at three sites in Muheza and Muleba (hypoendemic), and Nachingwea (hyper-endemic/holoendemic) districts. The findings will provide baseline data and built the capacity for tracking and detection of emergence of artemisinin resistance in Tanzania. The PDNA is an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* parasites across Africa.

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1096-PF-GH-GHANSAH

Population genetics of *P. falciparum* parasites in Southern Ghana

Anita Ghansah is investigating the population diversity and genetic structure of *P. falciparum* parasites collected from Cape-Coast in the coastal savanna zone of Ghana. The overall objective is to build capacity in the use of *P. falciparum* genomic data to describe the extent of genetic diversity of the parasite, discovery of new genes/SNPs that may have biological importance and to develop novel genetic analysis tools for studying well-defined phenotypes such as drug resistance, vaccine efficacy. This study is contributing to the Plasmodium Diversity Network Africa (PDNA), an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* parasites across Africa.

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1097-PF-ML-MAIGA

Detection of artemisinin-resistant *Plasmodium falciparum* parasites in Southern Mali

Abdoulaye Djimdé at the Malaria Research and Training Centre at the University of Bamako and Oumou Maïga-Ascofaré are investigating the genetic signature of drug pressure on *P. falciparum* parasites collected in a seasonal transmission areas in Southern Mali. Samples collected before and after the introduction of artemisinin-based combination therapy (ACT) are compared for signatures of a recent positive selection. This study is contributing to the Plasmodium Diversity Network Africa (PDNA), an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* parasites across Africa.

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1098-PF-ET-GOLASSA

The prevalence of asymptomatic carriage; emergence of parasite mutations conferring anti-malaria drug resistance; and G6PD deficiency in the human population, as possible impediments to malaria elimination in Ethiopia

Since 2004, Ethiopia has adopted a species-specific treatment policy for malaria: artemether-lumefantrine (AL) for the treatment of uncomplicated *P. falciparum* malaria and chloroquine (CQ) for *P. vivax* infections. *P. falciparum* and *P. vivax* are co-endemic in Ethiopia. Periodic assessment of mutant and susceptible genotypes would help towards a better understanding of the effects of the current regimens. In areas where *P. vivax* is endemic and primaquine is required for the radical cure, individual's G6PD status must be known before the recommendation of this drug. Indeed, G6PD-deficient individuals are at risk of haemolysis when exposed to primaquine and tafenoquine drugs. Apparently no measures are currently in place to ensure safe delivery of this drug within the context of G6PD deficiency risk in the country. Given the incomplete removal of CQ, co-transmission of *P. falciparum* and *P. vivax* in the country and use of primaquine for the radical cure of *P. vivax*, Lemu Golassa and colleagues are interested to explore the frequencies of *P. falciparum* clinical isolates carrying mutant and susceptible genotypes in Pfcrt and Pfmdr1 genes and to determine the prevalence of G6PD deficiency among endemic people. This study is contributing to the Plasmodium Diversity Network Africa (PDNA), an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* parasites across Africa.

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1100-PF-CI-YAVO

Drug resistance and *Plasmodium falciparum* diversity in forest zone of Côte d'Ivoire

William Yavo is investigating *P. falciparum* parasite population structures according to epidemiological facies and their impact on artemisinin combination therapy (ACT) treatment failure. Samples of the parasites were collected in the urban and suburban areas of Abidjan (economic capital of Côte d'Ivoire), the forest and coastal zone of San Pedro as well as from Abengourou (transition forest zone in the East of the country), during clinical trials comparing Artesunate-Amodiaquine vs Artemether-Lumefantrine according to WHO protocol. This study is contributing to the Plasmodium Diversity Network Africa (PDNA), an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* parasites across Africa.

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1101-PF-CD-ONYAMBOKO

Efficacy of three ACTs in treating *falciparum* malaria in the Democratic Republic of Congo

This clinical study aims to assess the efficacy of amodiaquine-artesunate for the treatment of uncomplicated *P. falciparum* malaria in children in Kinshasa, DRC, five years after its introduction as a first line treatment. It also compares this therapy with the efficacies of dihydroartemisinin-piperaquine and artemether-lumefantrine. Parasite DNA from samples collected from infected patients will be genotyped to perform and association studies with observed clinical phenotypes.

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1102-PF-MG-RANDRIANARIVELOJOSIA

Genotyping *P. falciparum* and *P. vivax* in Madagascar

The island of Madagascar is geographically situated in the south western region of the Indian Ocean and amongst malaria-endemic countries, its situation is unique: historically, human migration has occurred from both Africa and Asia; Duffy negative people can be susceptible to *P. vivax*; there is an absence of *pfcrt* mutant *P. falciparum* despite the official use of chloroquine to treat malaria for six decades (1945 - 2005). This study is mainly investigating *Plasmodium* samples collected directly from patients with uncomplicated malaria, as well as tracking malaria parasites and genetic markers of drug resistance in these parasites. This long-term study is contributing to the Plasmodium Diversity Network Africa (PDNA), an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* across Africa.

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1103-PF-PDN-GMSN-NGWA

Population genetics of cross-border *P. falciparum* parasites in West Africa

In collaboration with colleagues at the MRC Unit The Gambia, Alfred Amambua Ngwa is investigating the population structure and signatures of selection from immunity and drugs in isolated and cross-border *P. falciparum* populations. He uses whole genome sequencing and targeted genotyping to analyse such populations across geopolitical borders and islands in West Africa. This study is contributing to the Plasmodium Diversity Network Africa (PDNA), an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* parasites across Africa.

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1107-PF-KEN-KAMAU

Population genetics of *P. falciparum* parasites in Kenya

Together with colleagues at the United States Army Medical Research Directorate in Kenya, Edwin Kamau is investigating population structure and signals of selection from anti-malarial drug resistance of *P. falciparum* in Kenya. The aim is to analyse the parasite's genetic diversity to inform malaria control policy in sub-Saharan Africa. Specific focus will be on finding molecular markers of antmalarial drug resistance, measuring frequencies or novel markers of resistance to artemisinin, comparing heterozygosity by conventional MoI and FWs metric and monitor the emergence and spread of artemisinin resistance in sub-Saharan Africa. This study is contributing to the Plasmodium Diversity Network Africa (PDNA), an African-led network investigating the genetic diversity and drug resistance of *Plasmodium* parasites across Africa.

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1125-PF-TH-NOSTEN

Investigating artemisinin resistance emergence on Thai-Burmese border

P. falciparum resistance to artemisinin derivatives emerged on the Thai Myanmar border between 2000 and 2010. The Shoklo Malaria Research Unit (SMRU) has collected phenotypic data on more than 3,000 patients with uncomplicated hyperparasitaemia and stored packed red blood cells for over 600 at the time of admission. This constitutes a unique collection of samples available for genomic analysis to determine changes in the parasite population structures and to identify potential molecular markers associated with resistance to artesunate.

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1127-PF-ML-SOULEYMANE

Genetic analysis of *P. falciparum* before and after artemether-lumefantrine treatment in Mali

Although artemisinin combination therapy (ACT) resistance has not yet been found in Mali, previous studies have shown that cases of recurring *P. falciparum* parasites within a month of infection after treatment with artemether-lumefantrine seem to be increasing. The Molecular Epidemiology and Drug Resistance Unit, at the Malaria Research and Training Center, University of Science, Techniques and Technologies of Bamako, Mali is conducting this study to characterise the phenotype and genotype of these recurrent parasites, with parasites being collected before and after artemether-lumefantrine treatment and stored for ex vivo drug efficacy studies and subsequent genotyping by next generation sequencing.

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1131-PF-BJ-BERTIN

Identification of virulence factors in cerebral malaria in Benin

Gwladys Bertin is interested to facilitate the identification of variants associated to cerebral malaria in the proteomic analysis. She is correlating proteomic data from cerebral malaria samples with whole genome sequencing of these samples. The whole genome sequencing will allow adding new sequences and to facilitate the identification of variants associated to cerebral malaria in the proteomic analysis. The aim of this study is to correlate proteomic data from cerebral malaria samples with whole genome sequencing of these samples. The data from the whole genome sequencing will be used for analysis of proteomic and RNA-sequencing. The whole genome sequencing will allow adding new sequences and to facilitate the identification of variants associated to cerebral malaria in the proteomic analysis.

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1134-PF-ML-CONWAY

Population Genetics of *P. falciparum* in West Africa

This project has involved studying the population genomics of malaria parasites in Mali, to compare the signatures of selection in the parasite genome with patterns seen elsewhere in West Africa. The work was supported by an ERC Advanced Grant on 'Parasite population genomics and functional studies towards development of a blood stage malaria vaccine', and some analyses of the data are reported already: Duffy, C.W. et al. (2018) Multi-population genomic analysis of malaria parasites indicates local selection and differentiation at the *gdv1* locus regulating sexual development. *Scientific Reports*, 8:15763. doi: 10.1038/s41598-018-34078-3.

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1135-PF-SN-CONWAY

Parasite adaption in Senegal at molecular, functional and population level

This project has involved studying the population genomics of malaria parasites in an area of low infection endemicity in Senegal, to compare the signatures of selection in the parasite genome with patterns seen elsewhere in West Africa. The work was supported by an ERC Advanced Grant on 'Parasite population genomics and functional studies towards development of a blood stage malaria vaccine', and some analyses of the data are reported already: Duffy, C.W. et al. (2018) Multi-population genomic analysis of malaria parasites indicates local selection and differentiation at the *gdv1* locus regulating sexual development. *Scientific Reports*, 8:15763. doi: 10.1038/s41598-018-34078-3.

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1136-PF-GM-NGWA

Plasmodium falciparum anti-malarial drug resistance in the Gambia: Identification of potential genetic markers by retrospective whole genome approaches

In The Gambia, malaria transmission has substantially declined in the last 5-10 years and access to treatment is good. The relatively high drug pressure, reduced transmission and consequent waning of population immunity would increase opportunities for parasite inbreeding, possibly favoring the establishment of ART/ACT resistant parasite genotypes. Therefore, this setting offers a unique opportunity to understand the evolution of the natural variation of *Plasmodium falciparum* populations and the potential for the emergence of ART resistance. For this reason, we propose to characterize the *P. falciparum* genomic and phenotypic variations that occurred after the large-scale implementation of ACTs to identify novel genetic mechanisms of antimalarial drug resistance.

We will analyse patterns of genome-wide temporal SNP and microsatellite diversity to identify evolving genomic loci that have been involved in recent adaptations. To our knowledge, this will be the first attempt of retrospective genome scanning to identify genome-wide signatures of directional selection in a natural *P. falciparum* population following ACT implementation. The project proposes to analyse 540 *P. falciparum* specimens from archival and recent sampling.

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1137-PF-GM-DALESSANDRO

Malaria transmission dynamics in The Gambia: Defining the spatial and temporal spread of malaria at micro-level (village)

The overall objective of this study is to understand the determinants of malaria heterogeneity and the spatial and temporal spread of malaria infections. Alfred Amambua Ngwa and Umberto d'Alessandro will analyse *P. falciparum* specimens from 3 consecutive years in the country to describe infection, complexity and identify parasite genotypes adapting to current interventions and environmental changes in the population. The aim is to integrate high-throughput genomic technologies into detailed field epidemiology and entomology studies to increase our understanding of what drives the heterogeneities in host-parasite-vector interactions and ultimately transmission, providing the basis for the rational application of interventions and the development of evidence-based plans for elimination.

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1138-PF-CD-FANELLO

Parenteral artesunate compared to quinine as a cause of late post-treatment anaemia in African children with hyperparasitaemic *P. falciparum* malaria (DHART)

Caterina Fanello and colleagues investigated the incidence of late onset anaemia in children with uncomplicated hyperparasitemic *P. falciparum* malaria treated with intravenous (IV) artesunate or IV quinine. Two blood samples were taken from patients in the Democratic Republic of Congo. The first to examine the parasite DNA to establish information on the frequency of the major alleles associated to drug resistance in the area and the other to analyse the human DNA for the presence of Sickle Cell Disease, thalassemia, G6PD deficiency and other hemoglobinopathies or enzyme deficiencies that might have affected the primary outcome of the clinical trial.

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1141-PF-GM-CLAESSENS

Genomic characterization of *P. falciparum* from asymptomatic infections in The Gambia

The dry season in The Gambia presents a bottleneck for the total parasite population as there is little or no malaria transmission. Some individuals maintain a malaria infection through this period, usually at very low densities, without having any symptoms, and are the source from which the transmission restarts the following season. In this study, Antoine Claessens and his colleagues from the MRC Unit The Gambia at the London School of Hygiene and Tropical Medicine aim to test the hypothesis that some *P. falciparum* "strains" are more likely to survive the ~8 month long dry season. More specifically, the frequency of alleles conferring dry season growth advantage will be high in June but lower towards the end of the transmission season in December. In practice, fingerpick and veinous blood samples are collected every three months from ~1000 participants in a village in the eastern part of The Gambia. All blood samples are filtered through a cellulose column to remove white blood cells. *Plasmodium falciparum* status is determined by nested-PCR or qPCR. All *P. falciparum* positive samples are shipped to the Wellcome Sanger Institute for whole genome sequencing, after a DNA amplification step. This work will provide important information for the possible elimination of malaria in The Gambia, as the last remaining parasites are likely to show a similar phenotype of infection to parasites in the dry season (low parasitaemia and asymptomatic, i.e. "the last parasite standing is the strongest" hypothesis).

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1145-PF-PE-GAMBOA

Genotype-phenotype study of erythrocyte invasion in Peruvian *P. falciparum* isolates

Dionicia Gamboa, Joseph Vinetz and colleagues are analysing Peruvian *P. falciparum* host-parasite interactions during invasion of the parasite into the human red blood cell. Samples for this study were collected as part of a study of erythrocyte invasion phenotypes by the Universidad Peruana Cayetano Heredia, New York Blood Center and the University of California San Diego. The team will use whole genome sequencing on these phenotyped strains as part of broader genotype-phenotype studies of invasion both locally and globally. Samples from Peru that were collected as part of a study of erythrocyte invasion phenotypes by Universidad Peruana Cayetano Heredia, New York Blood Center and University of California San Diego. *Plasmodium falciparum* parasites were collected and tested for invasion phenotypes, as described in PMID 23118907. Strains were later retested for invasion and prepared for genome sequencing at the Sanger Institute. This project is led by Dr. Dionicia Gamboa and Dr. Joe Vinetz, with sequencing carried out in collaboration with Dr. Julian Rayner. Lead investigators are interested in analysing host-parasite interactions during invasion, as well as broader studies in genomic variation within Peru as part of the Peruvian/Brazilian Center of Excellence in Malaria Research (Amazonia ICEMR). Anyone interested in analysing these samples should contact Drs. Vinetz and Gamboa.

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1146-PF-MULTI-PRICE

Characterisation of drug resistance in Indonesian *P. falciparum* populations

Ric Price and Rintis Noviyanti are the principal investigators in a genome-wide study aiming to characterise the molecular profile of drug resistance-conferring variants in Indonesian *P. falciparum* populations. The study entails genome-wide scans to identify novel resistance variants as well as characterising known variants in *P. falciparum* field isolates with *ex vivo*-determined drug sensitivity profiles for a range of antimalarial drugs. Samples are contributed by consenting patients attending local health centres and hospitals in Indonesia. The study is conducted alongside a genome-wide scan to identify and characterise drug-resistance conferring variants in *P. vivax* field isolates sourced from co-endemic sites in Indonesia and from Thailand. These studies are coordinated by Sarah Auburn and Jutta Marfurt.

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1147-PF-MR-CONWAY

Population genetics of *P. falciparum* parasites in Mauritania

This project is led by LSHTM and the Institut National de Recherches en Santé Publique (INRSP), Mauritania, and supported by an MRC Project Grant entitled 'Malaria parasite population structure and adaptation on the edge of endemic distribution in Africa'. Some of the analyses of data are reported already: Duffy, C.W. et al. (2017) Population genetic structure and adaptation of malaria parasites on the edge of endemic distribution. *Molecular Ecology*, 26:2880-2894. doi: 10.1111/mec.14066. This indicates that discrete foci of infection on the edge of the Sahara are genetically highly connected to the wider parasite population in Africa, and local elimination would be difficult to achieve without very substantial reduction in malaria throughout the region.

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1151-PF-GH-AMENGA-ETEGO

Testing the effectiveness of selective whole genome amplification on samples collected in Northern Ghana

For this study, Lucas Amenga-Etego and colleagues collected dried blood spot (DBS) and venous blood samples from patients in the Navrongo Health and Demographic surveillance area in the Upper East Region of Northern Ghana. In collaboration with the Malaria Programme at the Wellcome Sanger Institute, these samples were used to compare the effectiveness of whole genome sequencing of *P. falciparum* from DBS, using selective whole genome amplification, as opposed to other well-established methods using leucodepleted venous blood samples. The study showed that this technique overcomes a major limiting factor in *P. falciparum* genome sequencing from field samples, and paves the way for large-scale epidemiological applications. The collected samples will also be used to track drug resistance genes in the area.

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