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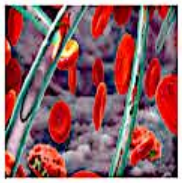


Genomic Epidemiology of Malaria
18–20 September 2024
Wellcome Genome Campus, UK and Virtual

Genomic Epidemiology of Malaria (GEM)–2024

MESA Correspondents Report

Written by Ambadiang Mae Marilene M., Emma Collins, Deborah Neumbe, Duru Vincent Chiagozie, Leen Vanheer, and Nkemngo Francis Nongley.

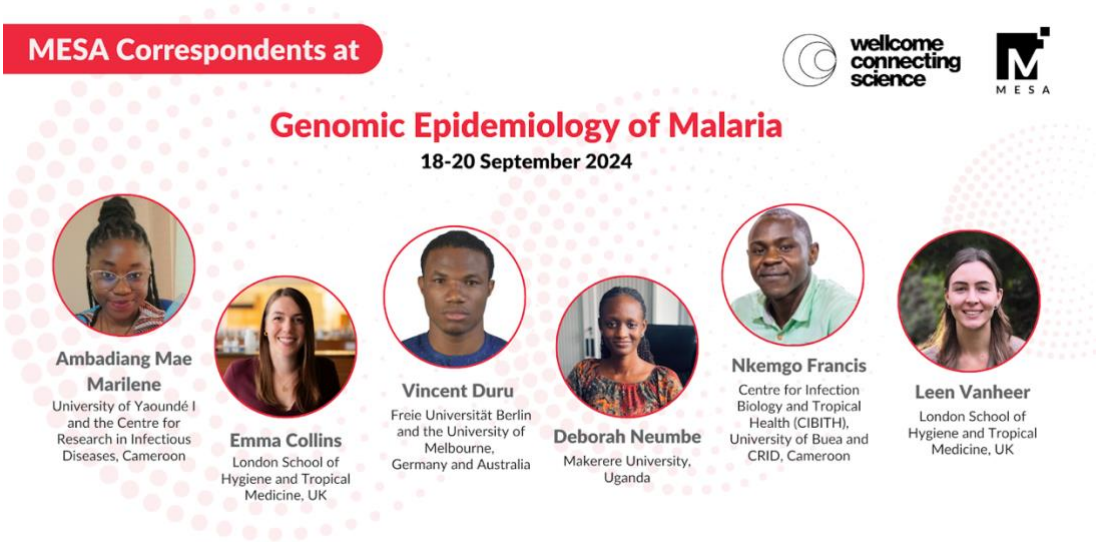
Senior editorial support has been facilitated by Andrew Balmer, Chiyun Lee, Jacob Tennessen, and Patricia Doumbe Belisse.

MESA Correspondents bring you cutting-edge coverage from the 9th Genomic Epidemiology of Malaria (GEM) Conference 2024

18 - 20 September 2024

London, United Kingdom and Virtual

MESA would like to thank Andrew Balmer (Wellcome Sanger Institute, United Kingdom), Chiyun Lee (Wellcome Sanger Institute, United Kingdom), Jacob Tennessen (Harvard T.H. Chan School of Public Health & Broad Institute, United States) and Patricia Doumbe Belisse (Liverpool School of Tropical Medicine, United Kingdom) for providing senior editorial support.



MESA - the malaria knowledge hub, would also like to acknowledge the MESA Correspondents Ambadiang Mae Marilene (University of Yaoundé I and the Centre for Research in Infectious Diseases, Cameroon), Emma Collins (London School of Hygiene and Tropical Medicine, United Kingdom), Deborah Neumbe (Makerere University, Uganda), Duru Vincent Chiagozie (Freie Universität Berlin, Germany and the University of Melbourne, Australia), Leen Vanheer (London School of Hygiene and Tropical Medicine, United Kingdom) and Nkemngo Francis Nongley (Centre for Infection Biology and Tropical Health, University of Buea and the Centre for Research in Infectious Diseases, Cameroon) for their coverage of the conference.

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Day 1: Wednesday, 18th September 2024

Welcome Remarks

The 9th Genomic Epidemiology of Malaria (GEM) conference took place at the Sanger Institute in Cambridge between September 18th – 20th, 2024. This was GEM's first hybrid conference, bringing together 570 participants from 63 countries, with 177 attending in person. The conference portal was highlighted for its collaborative features, such as the discussion forum, and attendees were encouraged to use the hashtag #GEM24 on social media. The conference began with remarks from members of the Scientific Programme Committee. **Daniel Neafsey** (Harvard T.H. Chan School of Public Health, United States) emphasized the travel bursary programme and its importance to make attendance as diverse as possible. **Mara Lawniczak** (Wellcome Sanger Institute, United Kingdom) delivered a heartfelt tribute to Professor Dominic Kwiatkowski† and emphasized his commitment to open data, mentorship and friendship, while warmly welcoming newcomers to the GEM community. **Sarah Auburn** (Menzies School of Health Research, Australia) introduced discussion prompts for the final day: What is your vision for the field of malaria genomic epidemiology in 2034? How can we improve translation of genetic data to inform treatment policy? How do we see the field moving forward as genomic data production becomes more decentralized? Finally, **Victoria Simpson** (Wellcome Sanger Institute, United Kingdom) outlined the various opportunities over the conference, including speed networking and a quiz.

Session 1 – Spatiotemporal genomic epidemiology of parasites and vectors

Anastasia Hernandez-Koutoucheva (Wellcome Sanger Institute, United Kingdom) presented the Malaria Genome Vector Observatory (VObs), which is an innovative resource that integrates genetic variation and builds on the Ag1000G project. VObs aims to add genetic variation data from the Anopheles species, particularly the *Anopheles arabiensis* and *Anopheles funestus* subgroups, while also strengthening bioinformatics and data analysis capacity for vector genomics in Africa. She emphasized the importance of timely data access and quick turnaround to influence public health decisions, to maintain a ready-to-use resource for immediate public health applications. At present, there are over 25,000 genomes from 30 countries, derived from 67 partner studies, each addressing different primary questions. In addition, the last few years have seen a substantial increase in the number of genomes that have been added to the resource. Hernandez-Koutoucheva highlighted partnerships and studies that have contributed data to and utilized data from the Observatory, some of which have been able to identify new resistance mechanisms through genome-wide selection scans, providing key insights into population biology and resistance mechanisms as part of this collaborative effort. Additionally, analytical software and data analysis training courses are being provided to enhance knowledge sharing, training, and community engagement. Lastly, Hernandez-Koutoucheva shares their vision to accelerate the generation, utilization, and sharing of genomic insights to detect and characterize emerging evolutionary threats to malaria vector control in Africa by integrating analysis-ready data for both existing and new *Anopheles* taxa, maintaining and expanding analytical tools, sustaining data analysis and bioinformatics training initiatives.

Alyssa Barry (Deakin University, Australia) focused on using parasite population genetic data to monitor transmission dynamics and draw insights from two decades of malaria surveillance on the island and mainland of Papua New Guinea (PNG). PNG is hyperendemic, with variable malaria transmission throughout the country. All five human *Plasmodium* species circulate with *P. falciparum* and *P. vivax* being the most dominant. Two cross-sectional and cohort studies were conducted in East Sepik and Madang Province over two decades, both before and after the implementation of control measures. All

surveys tracked parasite prevalence, which decreased following the introduction of long-lasting insecticide nets (LLINs) in both locations. However, there was a rebound in prevalence in 2016 due to expanding residual lineages including increasing prevalence of K13 C580Y mutants, which later got established in the populations after 2019. The results of her studies also showed a subtle change in the population diversity, with an increase in the proportion of high identical by descent (IBD) pairs over time. Exploring the various factors that could contribute to the rebound, Barry and her team explored the role of expansion of residual strains, parasite adaptation (e.g., drug resistance), migration, and the effects of combinations of these factors. For this, they developed tools for monitoring population genetics indicators of malaria transmission dynamics, such as the use of 10 new microsatellites, VARcodes, Single Nucleotide Polymorphism (SNP) barcodes, and Whole Genome Sequencing (WGS).

Alfred Amambua Ngwa (MRC Unit The Gambia at London School of Hygiene and Tropical Medicine, The Gambia) focused his talk on malaria in West Africa. He highlighted that the far western region of Africa, near The Gambia and Senegal has relatively low malaria prevalence compared to most of West Africa, which includes Nigeria which has one of the largest *Plasmodium* parasite populations. To implement genomic surveillance of malaria in West Africa their project had three main aims: building infrastructure, expanding capacity and working with national malaria control programmes (NMCPs). Their project was launched in 2018 with the second phase commencing in 2022. Surveillance hubs were established in The Gambia and Ghana, processing samples from other West African sites. The scale-up between phases led to an increase from 1500 to over 12,000 samples processed, with over 10,000 from the Gambia and nearly 8,000 from Ghana. The project provided in-house amplicon sequencing training building local data processing capacity, and collaborated with MalariaGEN to share genomic data. Key findings included persistent chloroquine resistance across The Gambia, signified by a high prevalence of *Pfcrt mutation*, and the presence of the K67T *Pfcrt* mutation across Ghana. Data indicated mini-epidemics of resistant infections. Additional drug resistance markers were identified, including *dhfr* mutations (codons 51, 59, 108) and the emergence of K540E, 581 and 613 mutations in *Pfdhps*. Ngwa also emphasized the importance of vector surveillance, highlighting ongoing work on insecticide resistance marker discovery. Next steps include expanding genomic surveillance capacity, establishing regional sequencing hubs, and integrating genomic data into NMCP practices.

Sarah Volkman (Harvard T.H. Chan School of Public Health, United States) presented the strategic use of molecular data for malaria surveillance and decision-making in Senegal. Despite Senegal's progress in reducing malaria cases, challenges remain. The country's National Malaria Control Program (NMCP) focuses on threat mapping, transmission dynamics, and improving the impact of interventions. Volkman's group uses pathogen genomics to support public health decisions, focusing on threat detection, transmission surveillance, and decision-making models. They examine the impact of changing antimalarial drug use, particularly Artemisinin Combination Therapies (ACTs) and artesunate monotherapy, on resistance patterns. Seasonal malaria chemoprevention (SMC) has expanded geographically, with some regions transitioning to multidrug administration (MDA). Volkman's team has tracked resistance markers, noting a selection sweep for *Pfcrt* K76T after SMC was introduced in 2014, but no markers for artemisinin resistance were detected. They also studied natural isolates, identifying a highly fit parasite with double mutations (S436A and A437G in *Pfdhps)* but no triple mutations in *Pfdhfr*. This parasite demonstrated increased resistance to sulfadoxine-pyrimethamine (SP), contributing to a competitive fitness advantage. Transmission surveillance revealed related parasites across Senegal, with distinct patterns of parasite relatedness. However, Senegal faces "data strikes", whereby only 25% of data required for incidence estimation is available. A regression model was created to translate complex metrics from the genetic data into incidence. The model showed that genetic metrics generally correlated with reported incidence, except in particular settings

where incidence fell below 10%. To support decision-making, the team developed dashboards to visualize genomic surveillance data, integrating epidemiological, entomological, and genetic information for NMCP operational needs.

Session 2 – Drug, diagnostics and insecticide resistance

Abebe Fola (Brown University, United States) talked about the implications of using large-scale genomic surveillance in understanding drug and diagnostic resistance in malaria parasites, particularly *Plasmodium falciparum* for malaria control and elimination in Africa. This approach, as highlighted by Fola, helps identify genetic mutations linked to resistance, such as those in genes like *pfcrt*, *pfmdr1*, and *kelch13*, allowing researchers to monitor antimalarial efficacy and guide new treatment strategies. This study was conducted across several countries including Ethiopia, Tanzania, Rwanda, the DRC, and Zambia. He highlighted that despite the significant progress made in the elimination of malaria, increasing drug and diagnostic resistance remains a major challenge. With special emphasis on the geographic spread of the K13 622I mutation and its co-occurrence with *hrp2/3* deletions, and the independent emergence of the K13 C580Y mutation in Ethiopia revealed by genomic surveillance of malaria parasites revealed other rare emerging mutations, including P441L, P574L and A675V. Additionally, 8 other non-synonymous mutations were identified, further illustrating the complexity of resistance patterns.

Martin Donnelly (Liverpool School of Tropical Medicine, United Kingdom) presented research investigating the impacts of malaria control on insecticide resistance evolution. Although observational studies have suggested that the introduction of new insecticides, such as Clothianidin, led to emergence of resistance, his goal was to improve understanding and quantify this impact. A five-country trial was conducted, testing over 50,000 children for malaria, along with cluster-specific levels of insecticide resistance, with the aim of correlating malaria prevalence with bioassay mortality rates. No correlation was found between malaria prevalence and insecticide resistance. To explore this further, he integrated genomic screening for insecticide resistance into vector control trials. The CRTs-LLINEUP trial was a two-arm cluster-randomized trial, comparing piperonyl butoxide long-lasting insecticidal nets (PBO-LLINs) with non-PBO-LLINs. The primary outcome measured was malaria prevalence, while the secondary outcome was mosquito population size. After 25 months, malaria prevalence was 20% lower in PBO communities compared to non-PBO communities, although the effect was diminishing over time. The trial also impacted mosquito populations, with 73% fewer mosquitoes in PBO communities. The effect on insecticide resistance markers were mixed, with some markers increasing in frequency while others decreased.

Monica Mbabazi (Infectious Disease Research Collaboration, Uganda) presented research on the genetic diversity and resistance profiles of *P. falciparum* in Southwestern Uganda, a region with lower malaria transmission. The study aimed to understand the factors sustaining malaria in low transmission settings to inform effective control strategies, incorporating both epidemiological data, like travel history, and parasite genomics. Focusing on Muko and Maziba (low transmission) and Kamwezi (moderate transmission), they sequenced samples from confirmed malaria cases in 2023 using highly multiplexed amplicon sequencing. Analytical tools were used to assess the complexity of infection (COI) and identity by descent (IBD), with a transmission network analysis linking genomic and epidemiological data. Results indicated that malaria cases in low transmission sites were typically older and more likely to originate in male individuals, often reporting recent travel, suggesting imported cases as a major factor. Interestingly, higher COI was observed in low transmission areas, highlighting the impact of imported malaria. Three *Kelch13* mutations (A675V, P441L, and C469F) associated with artemisinin resistance were identified in all three sites, while the highest prevalence of R561H and P441L was found in Kamwezi. Mbazazi highlighted that the P441L

mutation was associated with the largest cluster in Kamwezi. In conclusion, malaria control interventions at these sites may require different approaches

Ethan Booth (Mahidol Oxford Tropical Medicine Research Unit, Thailand) shared insights from his team's work as part of the Genetic Reconnaissance of Malaria in the Greater Mekong Subregion (GenRe-Mekong Project), showcasing how policy changes in antimalarial drug use led to a drastic change in the spread of drug-resistance parasite population in the region. They observed that with a switch in frontline therapy away from dihydroartemisinin-piperaquine (DHA-PPQ) since 2020, there has been a rapid reduction in *P. falciparum* incidence in the region, alongside a decline of the KEL1/PLA1 lineage, which was previously dominant in the eastern GMS. Additionally, Booth reported a significant reduction in the frequency of plasmepsin2/3 amplifications, associated with piperaquine resistance, between 2017 and 2022. However, he noted that artemisinin resistance levels remained high in the subregion.

This report is brought to you by the MESA Correspondents Ambadiang Mae Marilene M., Emma Collins, Deborah Neumbe, Duru Vincent Chiagozie, Leen Vanheer, and Nkemngo Francis Nongley. Senior editorial support has been facilitated by Andrew Balmer, Chiyun Lee, Jacob Tennessen and Patricia Doumbe Belisse.

Day 2: Thursday, 19th September 2024

Session 3 – Genomic studies of species interactions

Megan Michel (Max Planck, Germany) presented an exploration of the history of human malaria using ancient DNA. Michel's research aimed to use ancient DNA to understand how these parasites spread and evolved. Archives of infectious diseases consist of textual sources, osteological data, or ancient DNA from human bones and teeth. Ancient DNA data for malaria has so far been limited, as ancient DNA is often damaged and highly fragmented, and pathogen DNA typically represents a very small percentage of the total DNA extracted from bone or teeth samples. However, methodological advancements, such as hybridization capture, have increased the percentage of ancient microbial DNA in samples, making it possible to reconstruct genome-wide data from pathogens preserved in ancient human skeletal remains. Michel highlighted that they identified 38 ancient individuals from around the world with evidence of malaria infection. They used hybridization capture to enrich *Plasmodium* DNA, which was successfully sequenced from 36 individuals. These ancient parasite genomes can shed light on disease histories. For example, an ancient *Plasmodium vivax* genome from Peru clusters with modern Latin American *P. vivax* populations in a PCA, as well as with Ancient European strains. Taken together, her results suggest that vivax was likely spread by European colonizers to the Americas. In contrast, for *P. falciparum*, ancient European strains are quite different from clusters of ancient American strains, suggesting that *P. falciparum* may have been transmitted during the trans-Atlantic slave trade. Michel concluded by explaining two more examples, using ancient parasite DNA from a cemetery in Belgium and an ancient individual from the Himalayas, to draw insights into how human mobility and trade in ancient history facilitated interactions between human and parasite populations.

Lucas Amenga-Etego (West African Centre for Cell Biology of Infectious Pathogens, Ghana) presented his work on malaria drug resistance in Ghana. The study outlined how Ghana's malaria burden is decreasing, although it remains a public health issue, and prevalence is heterogeneous across the country. Additionally, although the dominant parasite is *Plasmodium falciparum*, *P. malariae* and *P. ovale* are also circulating. He is working with the National Malaria Elimination Programme (NMEP) to implement genomic surveillance of malaria to support evidence-based decision making. His team have collected over 7,000 positive malaria dried blood spots and sequenced around 1,000 of them. They identified that CQ resistance (CVIET) is under 2% and mainly found in the south. Investigating the *Pfmdr1* mutations showed that the sensitive haplotype was prevalent, although there are some resistant haplotypes. Similarly, an examination of *dhfr* and *dhps* markers found few highly resistant parasites. There were seven *dhps* haplotypes, with the dominant haplotype being AGKAA (A437G). He revealed that they had not discovered Kelch13 mutations among their samples, although they identified low-frequency uncharacterised variants (Q613H, V637I, T387A). He also investigated the role of treatment with herbal compounds in the selective pressure for drug resistance. They identified 20 compounds that had been used to treat malaria. IC50 analysis identified six products that were effective, and testing against various 3D7 life stages indicated efficacy, particularly against trophozoites. Finally, whole genome sequence data showed that asymptomatic infections had a lower mean multiplicity of infection (fws) compared to symptom cases.

Paolo Bareng (Burnet Institute, Australia) presented on defining immune escape polymorphisms in *P. vivax*, using insights from the analysis of allelic turnover of 16 antigens in a longitudinal cohort of Papua New Guinean children. Bareng's work aimed to use population genetics to guide malaria vaccine design by analyzing the genetic diversity

of *Pv* antigen vaccine candidates and identifying immune escape polymorphisms in these candidates. A multiplexed long-read amplicon sequencing assay was performed to amplify and sequence the target genes in 603 isolates obtained from a longitudinal cohort study in Papua New Guinea. The findings identified several polymorphisms in the target genes that were strongly associated with symptomatic malaria. For Ama-1, a blood-stage antigen, these polymorphisms were found within domains under strong balancing selection pressure, close to essential binding sites for erythrocyte invasion, further supporting their potential as targets of immune selection. Additionally, classifying these immune escape polymorphisms could help predict specific serotypes. When compared to a global database of *P. vivax* gene sequences, the study revealed a low prevalence of Sal-1 variants used in current vaccine formulations, alongside the widespread presence of non-vaccine strains. This highlights the potential challenges for vaccine efficacy across diverse populations.

Gavin Band (Oxford University, United Kingdom) shared his work on the geographical analysis of *P. falciparum* sickle haemoglobin association gene (*Pfsa*) at local and continental scales. Their study investigated the *Pfsa* mutations found in three regions (*Pfsa1* and *Pfsa2* in two regions of chromosome 2 and *Pfsa3* on chromosome 11) of the *P. falciparum* genome, which are speculated to overcome the protective advantage of sickle cell carriers (HbS). Band observed that 45 out of 49 patients with severe *P. falciparum* infections and Hbs genotypes had parasites carrying the *Pfsa* alleles, implying that these parasites are able to overcome the protective effects of sickle haemoglobin (Hbs). Furthermore, their work also showed that the *Pfsa* + parasites are able to infect people who do not have the sickle allele. Geospatial analysis and spatial simulations were carried out using data gathered from both Hbs and parasite information collected from global populations. The correlation between the *Pfsa* allele and sickle haemoglobin (HbAS or SS) was positive in Mali, Gambia, Senegal, Ghana, and Tanzania while in the DRC, there was a negative correlation. The *Pfsa2* allele was absent in West Africa but present in East Africa with a positive correlation, while the *Pfsa4* allele, not present in East Africa, had a positive relationship in West Africa. To achieve the same intermediate frequencies of the *Pfsa* alleles as observed in field data, the simulation required a fitness cost to be added to the mutation. This indicates that the *Pfsa* allele confers a selective advantage to the parasite by overcoming Hbs protection, however a potential fitness cost is preventing it from becoming fixed in the population.

Antoine Claessens (University of Montpellier, France) presented the var gene expression switching rate in chronic *Plasmodium falciparum* infections. His study explores how *Plasmodium falciparum* evades the immune system to maintain chronic asymptomatic infections. Regular blood samples from the same host were collected in the Gambia to characterize host-pathogen interaction at a population level, the individual level, and the molecular level. Patterns of var gene expression were investigated in 26 *P. falciparum*-infected individuals during the wet season and in 16 chronically infected individuals with 6 monthly blood samples. Var gene transcription was analyzed using RNA sequencing and amplicon sequencing of the DBLalpha domain of var genes. Alongside this, parasites were cultured in vitro, and PacBio long-read sequencing was used to retrieve the full-length var genes. His findings highlight that the entropy index, which measures the diversity of var gene expression within an isolate, was significantly lower in chronic infections in the dry season, compared to more recent infections in the wet season. Findings from the longitudinal data showed different var gene transcription patterns from month to month, indicating a high turnover rate. Surprisingly, some var genes were found to express at least twice during a chronic infection, indicating recurrent var genes.

Sophie Berube and **Christine Markwalter** (University of Florida, United States) talked about investigating the effect of mosquito biting behaviour on infectious reservoirs of malaria. This study was done utilising two methodologies: a longitudinal cohort study along with a

transmission model. The longitudinal study was carried out in Kenya within 75 households across 5 villages. Indoor resting mosquito and blood spots of participants from households were collected. *Plasmodium falciparum* infection was detected by PCR along with human short tandem repeats (STR) to allow human to mosquito to human bite matching. Out of the 3000 female *Anopheles* mosquitoes collected, 662 mosquitoes' blood meals were matched to participants in the study. Among the matched bloodmeals about 22% were positive for *P. falciparum* sporozoites and about 17% of the matched blood meals were multi sourced. The data reveals that *P. falciparum* within mosquitoes had a higher multiplicity of infection (MOI) than the parasites in humans. It was also revealed that mosquito infection with *Pf* sporozoites increased the likelihood three-fold of biting infected hosts; the authors speculate that the sporozoite infection may be altering the mosquitoes' biting behaviour. This work was followed up with a transmission model which indicated that you require both heterogeneity in mosquito biting choice and heterogeneity in human attractiveness preference to reflect the same differences in MOI that were observed in the cohort longitudinal study with higher MOI in mosquitoes than humans.

Session 4 – Harmonizing data standards and analysis resources for public health

Aimee Taylor (Pasteur Institute, France) presented research on how reinfections can downwardly bias treatment efficacy estimates and interfere with drug resistance monitoring in malaria-endemic settings, as reinfection can be mistaken for recrudescence. She further explained that in order to classify a recurrent parasitic infection as a reinfection rather than recrudescence, genetic data must show that the parasite in the recurrent infection is distinct from the original strain. However, this distinction can be complicated by factors such as polyclonal infections, where genetically diverse strains coexist within the same host, and biological challenges like persistent gametocytes or multiple blood meals from different sources within households. Various molecular techniques and analytical methods are available to differentiate between recrudescence and reinfection. The case of *Plasmodium vivax* presents even greater complexity due to the potential for relapses caused by dormant liver hypnozoites. These relapses represent delayed activation of parasites from earlier infections, and the parasites may have complex relationships to each other, being clonal, related (siblings), or genetically distinct (strangers). Taylor then introduced the R package, Pv3Rs, developed in her lab, which aims to identify patterns in allelic data compatible with different *P. vivax* recurrent states, visualize data and outputs, and more accurately infer whether *P. vivax* recurrences are relapses, recrudescence, or reinfections using genetic data. It uses a Bayesian model to compute the probable cause of recurrences. She concluded by outlining plans for further enhancements, noting that the current version of Pv3Rs is still under development.

Session 5 – Transmission and genetic surveillance of malaria: new methods, analyses and resources

Bokretsion Gidey Brhane (Ethiopian Public Health Institute, Ethiopia) explained that Ethiopia aims to eliminate local malaria transmission by 2030, despite a recent resurgence in malaria cases due to multiple factors, including the emergence of partial resistance to artemisinin. Genomic surveillance is therefore essential to monitor the evolution of resistance markers. Dried blood spots were collected from febrile outpatients aged ≥1 year with microscopically confirmed *Plasmodium falciparum* malaria at 12 sentinel sites across 5 regions between 2019 and 2022. Molecular inversion probe sequencing targeted mutations associated with artemisinin and partner drug resistance, including the *k13*, *mdr1, crt, dhfr,* and *dhps* genes, along with genome-wide markers to assess the complexity of infection and parasite relatedness. The study revealed monogenetic infections and a regionally variable high

prevalence (15.7%, range 0-58.8%) of the WHO-validated *k13* R622I mutation. Importantly, the validated *k13* A675V mutation was detected for the first time in Ethiopia (4.5%), alongside several partner drug resistance markers, including mutations in *mdr1, dhps, dhfr,* and *crt*, which were nearly fixed across the country. This highlights the need for therapeutic efficacy trials to detect early failure of artemisinin-based combination therapies.

Shahiid Kiyaga (Makerere University, Uganda) presented on the genomic advances in understanding the genetic diversity and transmission intensity of *P. falciparum* in Uganda. They analysed 2,245 dried blood spot samples collected from symptomatic malaria cases between January and March 2023 across Uganda. Malaria prevalence varied widely, ranging from 6.0% to 57.4% depending on the location. Infected samples were subjected to multiplex amplicon sequencing to examine the haplotypes, estimate the complexity of infection (COI), and assess clonal relatedness. Data revealed a high level of genetic diversity within the parasite population in Uganda, with a mean COI of 3.88. Furthermore, the genomic metrics of parasite diversity at the health facility level were found to correlate with malaria prevalence, suggesting that genomic data could play a crucial role in improving the stratification of regions by transmission intensity.

William Hamilton (Wellcome Sanger Institute, United Kingdom) presented recent progress on using nanopore sequencing for *P. falciparum* surveillance, and discussed the significant 'implementation gap' in scaling this tool from research to clinical diagnostic applications. In his study, Hamilton showcased ongoing efforts to develop a *P. falciparum* nanopore assay that could be used in both clinical and public health settings. Following initial implementation in Ghana, the study has (i) expanded the amplicon panel to include additional drug resistance markers, polymorphic surface antigens, and *Plasmodium* species detection, (ii) designed a set of commercial plasmid inserts that serve as cost-effective positive controls, with both 'test' and 'control' single nucleotide polymorphisms (SNPs) engineered to assess assay performance, (iii) established robust systems for assay quality control, based on differences in amplicon coverage between positive and negative controls across a range of parasitaemia and sample types, and (iv) developed training resources for use in endemic settings, including Cameroon.

Edwin Sutanto (Exeins Health Initiative, Indonesia) highlighted the challenges that polyclonal infections pose to the study of *Plasmodium vivax* biology and epidemiology. Nearly one-third of the isolates in the MalariaGEN *Pv4* dataset are polyclonal infections, which are traditionally excluded from the analysis to avoid confounding molecular epidemiology results. Sutanto introduced DEploid, a software optimized for *Plasmodium vivax,* to deconvolve the genomes of over 300 polyclonal infections from Africa, the Asia-Pacific, and South America. Originally developed for *Plasmodium falciparum*, the software required modifications to address the unique challenges of *Plasmodium vivax*, such as low parasitemia and limited genomic read depth.

Myo Naung (Burnet Institute, Australia) presented his research on predicting antigen serotypes. Naung emphasized that malaria eradication will likely require vaccines to achieve elimination, but designing effective vaccines has been challenging due to the diversity of antigens and the possibility of breakthrough infections. To address this, Naung developed a model that accounts for genetic diversity and other antigen properties, including epitope mapping, to predict immune escape. Using around 500 samples as inputs, the model hypothesized that Single Nucleotide Polymorphism (SNPs) with high turnover rates, often linked to asymptomatic infections, are associated with immune escape. The *Ama1* and *csp* genes were used as positive controls. The model identified several SNPs that may be markers of immune escape, most commonly found in merozoite genes. This

study emphasized the importance of evaluating the qualities highlighted by the model when selecting new vaccine candidates.

Olivo Miotto (Mahidol Oxford Tropical Medicine Research Unit, Thailand) reported the discovery of a rare *Plasmodium falciparum* cryptotype, named AF1, which circulates at low frequencies across Africa. Using data from 4,376 clonal African parasite genomes in the *Pf7* MalariGEN dataset, Miotto identified this cluster through principal component analysis, where it comprised about 1.2% of samples from 13 African countries. Further analysis revealed that AF1 parasites had highly correlated variants across seven chromosomes, indicating a common ancestry with high identity-by-descent (IBD) within the cluster. Miotto hypothesized that, since several of the genes containing these mutations are involved in red blood cell invasion and egress, AF1 parasites may be adapted to an evolutionary niche, potentially linked to host erythrocyte interactions.

Ashley Osborne (Menzies School of Health Research, Australia) presented an overview of Ethiopia's unique and structurally divergent *Plasmodium falciparum* populations using genomics. The study used whole genome sequencing (WGS) data from two trials evaluating treatment measures for *P. vivax* in Ethiopia, conducted in 2017 and 2021. A total of 116 high-quality WGS samples from 98 isolates were analyzed. Osborne's study revealed high IBD connectivity between parasites collected in 2017 and 2021, indicating a strong genetic link The study also showed a high prevalence of drug resistance biomarkers (*pfDHFR* and *pfDHPS*), which are associated with reduced susceptibility to sulfadoxine-pyrimethamine. The R622I Kelch13 variant was absent in the population. They finally found that the *P.falciparum* population in Ethiopia is genetically distinct from other sub-Saharan African populations.

Varanya Wasakul (Mahidol Oxford Tropical Medicine Research Unit, Thailand) presented her work exploring the use of pregnant women attending antenatal care (ANC) services as a sentinel population for malaria genomic surveillance in the Democratic Republic of Congo (DRC). Given the challenges and costs of setting up genomic surveillance in high-burden countries, especially among children, the study aimed to determine if pregnant women could serve as a more feasible sentinel group. Wasakul sampled over 4,000 pregnant women and 2,794 children in Kinshasa and used an amplicon sequencing platform to compare the *Plasmodium falciparum* genomes and drug-resistance markers. The findings revealed a malaria prevalence of 19% among pregnant women mostly asymptomatic and 49% among children (6-14 years) predominantly symptomatic. The study showed similar allele frequencies in both cohorts, with no significant differences in drug resistance mutations, including sulphadoxine-pyrimethamine-resistant haplotypes or kelch13 mutations. The complexity of infection was also comparable. The study concluded that integrating genomic surveillance into routine ANC activities is feasible, cost-effective, and beneficial for improving malaria surveillance and treatment for pregnant women in the DRC.

Annie Forster (University of Oxford, United Kingdom) focused her talk on *Plasmodium falciparum* sickle-associated loci across global parasite populations. She highlighted that malaria parasites have evolved to better infect individuals carrying the sickle cell allele (HbS). *Plasmodium falciparum* sickle associated (Pfsa) are regions in the malaria parasite genome found to be strongly associated with the human HbS polymorphism, that help the parasite overcome the protective effect of HbS. There are four Pfsa regions, however, this study specifically looked at Pfsa3 which was observed to have structural variants in its locus. Forster's study aimed to catalogue structural variants present and assess them in global populations, to determine whether HbS-associated alleles are linked to structural variants, and how these structures might affect gene expression. Forster's analysis revealed 11 different structural haplotype forms at this locus, including both gene deletions and duplications. The

presence of these structural variants varied across Africa and some were more common in certain populations. The duplications were found to be closely associated with Pfsa3+ alleles, suggesting that the structures, rather than nucleotide variation, may underlie the region's association with sickle cell disease.

Petra Korlevic (Wellcome Sanger Institute, United Kingdom) shared insights on population genomics in the African malaria vector *Anopheles funestus* over the past century. Samples were obtained from 10 partner institutions and the general population structure was examined. Principal component analysis (PCA) was conducted on 656 samples. Principal component 1 (PC1) showed an approximate geographical distribution from south to southeast, equatorial, and north. Several samples were found to be distributed approximately 4000 km around the equator. When 45 historic *Anopheles funestus* samples were sequenced and added to the current data set, they observed that the PCA was similar to that of the present-day counterparts implying there has been no major change in the past years. Furthermore, signals of selection for the resistance to dieldrin (GABA gene) were evaluated and a haplotype tree was constructed using current data showing a high frequency of the A296S mutation in samples from Ghana, Ashanti, and Nigeria while a few historic samples also had the same mutation. Finally, the A296S mutation commonly found in the *Anopheles gambiae* complex was also found in *Anopheles funestus*.

Luisa D. P. Rona (Federal University of Santa Catarina, Brazil) presented her research on the diversity of *Anopheles* species within the Brazilian Atlantic Forest. Her study was driven by the hypothesis that *Anopheles cruzii* may represent a species complex rather than a single species. To explore this, fixation index (Fst) analysis was conducted on various genes across the chromosomes to assess genetic differences among mosquito populations collected from distinct regions of the country, particularly those inhabiting environments at varying altitudes. She reported that the geographical distances between these populations did not account for the observed levels of genetic differentiation. Further analysis through phylogenetic tree construction revealed that *Anopheles cruzii* comprises five distinct subgroups, with the most significant genetic differentiation occurring on the X chromosome.

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Day 3: Friday, 20th September 2024

Session 6 – Vector and parasites: neglected and emerging species

Nguyen Thanh Thuy Nhien (Oxford University Clinical Research Unit, Vietnam) presented her research on genomic surveillance of malaria in Vietnam. Cases of malaria in Vietnam have been decreasing in recent years, and the aim is to eliminate malaria by 2030. As cases decline, surveillance is focusing on hotspots of transmission, with Nhien's team advocating a high throughput genotyping approach. Their surveillance efforts have screened 30-40% of annual malaria cases (>1000 samples), and have conducted multiple analyses to examine how these samples have changed over time. Findings showed that the prevalence of *pvDHPS* and *pvMDR1* varied over time, and that *pvMDR1* amplification has not yet been observed outside the target loci of the protocol. Additionally, the proportions of different malaria species have changed over time, with a decrease in *Plasmodium vivax* and increase in *Plasmodium malariae,* particularly in Khanh Hoa province. Khanh Hoa is currently a hot spot of malaria cases, indicated by clonal expansion of parasites, particularly in *P.vivax*. Nhien then highlighted ongoing challenges to elimination, such as implementation of drug resistance monitoring, integration of surveillance data with the National Malaria Control Program (NMCP), and addressed technical and operational limitations in relation to resources, infrastructure, costs and sustainability.

Dionicia Gamboa (Universidad Peruana Cayetano Heredia, Peru) talked about the molecular surveillance of *P. vivax* in Peru, and the last milestones for malaria elimination in hard-to-reach communities. *P. vivax* is the predominant species circulating in the area (over 82% prevalence), mostly affecting children and adults with co-morbidities in Loreto, a peri-urban city. A three-year cohort study using active case detection demonstrated micro-heterogeneity of malaria within and between communities. The work suggested this could be due to the high human mobility in the region, which is associated with economic activities. A high proportion of submicroscopic and asymptomatic infection cases were also observed, with two-three asymptomatic submicroscopic cases for every *P. vivax* case (~67% of total cases). High proportions of outdoor feeding among *An. darlingi* mosquitoes were also noted, which was associated with professions involving work outdoors. Gamboa also emphasised how the use of tools such as Loop-mediated Isothermal Amplification will be important in generating adequate molecular surveillance data to inform policy in Peru, and in the wider global context to aid malaria control. She then highlighted the Malaria zero plan & National malaria elimination plan (2022-2030) that incorporates molecular testing for asymptomatic individuals and seroepidemiology to detect malaria re-introduction.

Jacob Tennessen (Harvard T.H. Chan School of Public Health, United States) presented research on *Anopheles darlingi: the* primary endemic malaria vector in South America. Following extensive studies on the main African malaria vectors *Anopheles gambiae* and *Anopheles funestus,* this study was established to investigate if key characteristics observed in these species, such as the complex taxa, high genetic diversity and distinct evolutionary histories are also observed in *An. darlingi.* Tennessen's team sequenced over 1000 *An. darlingi* specimens from 15 sites across six countries. No evidence of sympatric cryptic taxa was observed. Principal Component Analysis (PCA) and ancestral groupings observed could mostly be explained by geography, indicating low gene flow between populations. Notably samples from Sifontes, Venezuela and Guyana separated from each other by only 200 KM appear in two distinct genetic clusters. Samples had high genetic diversity, with more than 2% heterozygosity for most samples. Long distance linkage disequilibrium was observed, likely maintained by inversions, which also correlated with peaks in heterozygosity. One significant finding was a signal of low heterozygosity at a P450 gene locus, associated with metabolic

insecticide resistance. This is notable, because target site insecticide resistance polymorphisms have not been detected so far in *An. darlingi*.

Zach Popkin-Hall (University Of North Carolina, Chapel Hill, United States) presented on population genomics of *Plasmodium malariae* from four African countries. *P. malariae* has a lower prevalence compared to *P. falciparum* but may become more prevalent as *P. falciparum* declines. 77 *P. malariae* isolates from Cameroon, Democratic Republic of the Congo, Nigeria and Tanzania were collected between 2015 and 2021, and subjected to whole genome sequencing. In addition, *P. falciparum* isolates from the same areas were also selected from the Pf7 release. Complexity of infection (COI) in *P. malariae* was found to be much lower than in the *P. falciparum* samples. In addition, Popkin-Hall found that the nucleotide diversity was lower in *P. malariae* isolates compared to *P. falciparum*, and that the linkage disequilibrium (LD) was higher in *P. malariae* with larger LD blocks. An analysis of demographic history showed that the *P. malariae* population is in recovery from a bottleneck. Genome-wide selection scans determined six putative drug resistance markers showed evidence of selective sweeps.

Hoseah Akala (Kenya Medical Research Institute/Walter Reed Army Institute of Research - Africa, Kenya) shared insights on the burden and drug resistance profiles of *P. ovale*, which present challenges for malaria elimination. Their study established the burden of non-falciparum infections in symptomatic and asymptomatic infections in Kenya, and further determined the frequency of *P. ovale* DHFR/DHPS mutations in these samples, alongside those from other regions of Africa. Findings showed that individuals tested with malaria in hospital settings were more likely to have mixed species infections than those in households. Analysis on 518 *P. ovale curtis*i and *P. ovale wallikeri* samples collected from the French National Malaria Reference Centre were combined with field samples from several countries, including Gabon, Benin and Kenya. These samples showed the presence of two mutations: Ala15Ser-Ser58Arg in *P. ovale curtis*i and Phe57Leu-Ser58Arg mutations in *P. ovale wallikeri*. These mutations were most prevalent in Central and East Africa, and were fixed among Kenyan isolates. Whole-genome sequencing and microsatellite analyses revealed reduced genetic diversity around the mutant *P. ovale curtis*i and *P. ovale wallikeri* DHFR genes. The mutant DHFR proteins showed structural changes at the pyrimethamine binding site *in-silico*, confirmed by a 4-fold increase in pyrimethamine half-maximal inhibitory concentration for Phe57Leu-Ser58Arg and 50-fold increase for Ala15Ser-Ser58Arg, compared with the wild-type counterparts.

Sunil Kumar Dogga (Wellcome Sanger Institute, United Kingdom) presented a new chapter in the Malaria Cell Atlas, uncovering insights into neglected malaria parasites, *Plasmodium ovale* and *Plasmodium malariae* using single-cell RNA-sequencing in Mali. While *P. ovale* and *P. vivax* are less common than *P. falciparum*, understanding their biology has been difficult due to the lack of a complete reference genome or transcriptomic studies. Dogga's team performed short- and long-read single cell RNA sequencing (scRNA seq) on blood material from Malian individuals infected with *P. ovale* and *P. malariae* during the 2021-2022 transmission seasons. They then investigated transcriptional, isoform, and genotypic diversity of these parasites, both within and between hosts. A single cell reference atlas of *P. ovale* and *P. malariae* was generated, allowing for the exploration of the species biology, such as the ordering of cells by their developmental time, alongside investigation of stage-specific gene expression patterns and gene expression clusters. Higher genetic diversity was observed among *P. malariae* parasites compared to *P. ovale*, suggesting lower levels of recombination in *P. ovale*. Pacbio long-read sequencing was then used to make new reference genomes for the samples, which led to a significant improvement compared to previous reference genomes. These insights are available on the Malaria cell atlas website: www.malariacellatlas.org.

Session 7 – What next? Priorities for genomic epidemiology of malaria – Panel discussion

The audience-based discussion, introduced by **Abdoulaye Djimdé** (University of Bamako, Mali), revolved around two key questions initially raised in the welcome remarks:

1. How can we improve the translation of genomic data to inform treatment policy?

2. How do we see the field moving forward as genomic data production becomes more decentralized?

The discussion centred on advancing Genomic Epidemiology of Malaria research, to achieve the malaria elimination goal of 2034. The discussion underscored the importance of whole genome sequencing (WGS), and there was a particular emphasis on the sustainability of funding. Furthermore, emphasis was placed on the importance of genetic data to be translated into treatment policy. An especially important factor is the need for resistance markers for lumefantrine and amodiaquine combination drugs, as artemether-lumefantrine is currently used in ~80% of ACT treatments across Africa. Participants also discussed contingency plans for when these partner drugs fail and highlighted the value of combining drug resistance markers with treatment failure data to provide a complete epidemiological picture.

Effective communication was also stressed as essential, particularly in translating complex genomic data in a way that is accessible to Ministries of Health, National Malaria Control Programmes, local leaders and the general public. While simplifying these complex messages can be tempting, it is crucial to retain the nuances of the information. In line with this, it was suggested that meetings such as GEM should extend invitations to wider stakeholders not just researchers, as this would be valuable to get varied points of view and inputs.

The discussion also addressed improvement in capacity and development in the field, including issues with sustainability of research, such as maintaining equipment and ensuring long-term data storage. Participants emphasised the need to ensure data is stored well, in a way that is both open-source and accessible, and to be accompanied by metadata for future analysis. Data sharing was highlighted as critical for the development of future vaccines and therapeutics, both of which are essential for malaria elimination. However, it was noted that while investment in training is increasing, the fact that much of it is conducted in English may be a barrier for many researchers.

Another topic was integrating WGS into routine clinical practice and pairing WGS data with phenotypic data to better understand how resistance markers translate into treatment failure. To minimize “opportunistic sequencing”, studies investigating the genomics of clinically relevant phenotypes must be carefully planned and integrated into clinical trials from the outset, ensuring they have sufficient statistical power to detect novel drug resistance markers.

The need for a streamlined data analysis platform was also discussed. This platform should enable sequencing to be performed in the field, with real-time sequence uploads, and run necessary analyses with just a few simple clicks to generate reports that are easy to interpret.

In summary, better molecular markers for partner drugs resistance monitoring are needed. While amplicon sequencing is adequate for surveillance of known genetic markers, WGS has the potential to contribute additional information which will be relevant in malaria eradication and therefore more whole genome data should be generated. There is a need for easy and

standardised ways to generate, analyse and report genomic data. Lastly, the translation from research findings to governmental level should be accelerated.

Closing remarks and prize presentation

Sarah Auburn (Menzies School of Health Research, Australia) introduced the various awards starting with the Dominic Kwiatkowski Genomic Epidemiology of Malaria 2024 Prize award. This £5,000 cash prize, established by Dominic's family and close friends, was awarded to two exceptional early- and mid-career researchers who demonstrated great potential to become future leaders in the field of genomic epidemiology. The prizes were presented by **Alfred Amambua Ngwa** (MRC Unit The Gambia at London School of Hygiene and Tropical Medicine, The Gambia), with the first prize going to **Moses Ikegbunam** (Nnamdi Azikiwe University, Nigeria) and the second to **Eniyou Cheryll Oriero** (London School of Hygiene & Tropical Medicine, United Kingdom). Poster prizes were awarded to **Seri Kitada** (Wellcome Sanger Institute, United Kingdom), **Kyong-shin R. F.** (University of North Carolina, Chapel Hill, United States & the National Biomedical Research Institute, Democratic Republic of the Congo) and **Nelly Tatchou-Nebangwa** (University of Buea, Cameroon & Centre for Research in Infectious Diseases, Cameroon). **Deus Ishengoma** (National Institute for Medical Research, Tanzania) concluded the conference by expressing his gratitude to everyone who participated in this year's GEM conference in-person and virtually. He extended his thanks to the presenters, organisers and the support team from the Welcome Connecting Science Institute for contributing to the success of the conference.

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