Plasmodium falciparum genetic diversity and antimalarial drug resistance prevalence across different transmission zones in Ghana

Cheikh Cambel Dieng[1], Maame-Esi Dawson-Amoah[2], Kareen Pestana[1], Lauren Gonzalez[1], Samuel Johnson[1], Linda Amoah[3], Yaw Afrane[2], Eugenia Lo[1]

[1]Biological Sciences, University of North Carolina at Charlotte, USA; [2]Medical Microbiology, School of Biomedical and Allied Health Sciences, University, Ghana; [3] Noguchi Memorial Institute For Medical Research, University, Ghana

Background

- *Plasmodium falciparum* is the most common human malaria parasite species in Sub-Saharan Africa, where the continent bears over 90% of the global *P. falciparum* burden [1].
- In the past decade, Ghana has made remarkable progress in malaria control. The percentage of outpatient attendance in public health facilities decreased sharply from 48% to 28% from 2008 to 2016.
- However, in some parts of the country, malaria still remains one of the leading cause of morbidity and mortality. For instance, previous studies showed a wide variation in parasite prevalence between the south (4%) and the north (51%) [2].
- Seasonal Malaria Chemoprevention (SMC) is one of the preventive measures aims to protect infants and young children in highly endemic areas with seasonal transmission.
- The SMC intervention has a potential to result in the emergence of antimalarial drug resistant parasites [3]. An accurate profile of genetic structure and diversity of the parasites is essential to infer where drug resistance genotypes originated and how fast they spread [4].

Objectives

- This study aims to genotype the parasite populations from different transmission zones using microsatellites and infer genetic diversity, population structure and underlying transmission patterns.
- Our goal is to generate baseline data that allows us to assess how current interventions influence the transmission dynamics and complexity of infections in the asymptomatic populations.

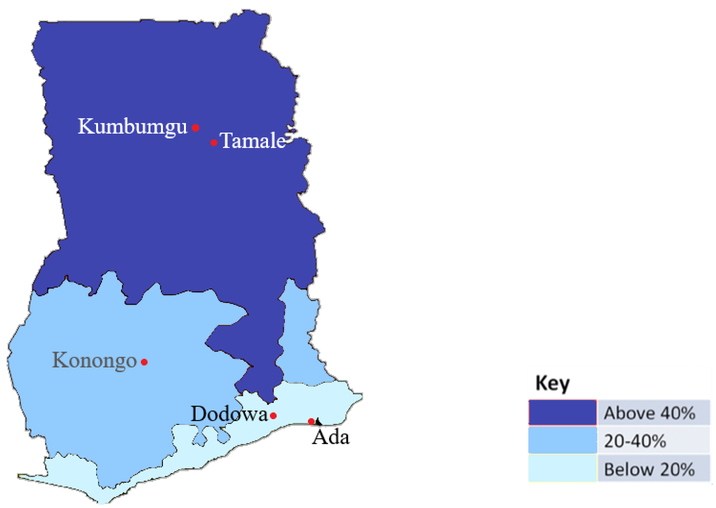


Figure 1. Malaria prevalence and study sites in Ghana

Methods

- Asymptomatic samples were collected from five sites representing the three distinct ecological zones including the northern savannah, the central forest, and the southern coastal regions.
- Fingerprick dried blood samples were collected from over 1,000 schoolchildren aged 3-12 during the rainy season (July-August) of 2017.
- Parasite DNA was screened by quantatitive real-time PCR based on 18S rRNA and *TARE*-2 gene regions [5].
- Six microsatellite loci with tri- or tetranucleotide repeats were typed for *P. falciparum* using the published oligonucleotide primers [6].
- Gene codons in *pfcrt* (K76T) and *pfmdr*1 (N86Y and Y184F) associate with amodiaquine (AQ) resistance; *pfdhfr* (N51I, C59R, S108N) and *pfdhps (*A437G and K540E) associate with sulfadoxine-pyrimethamine (SP) resistance were examined by sequencing.

Results

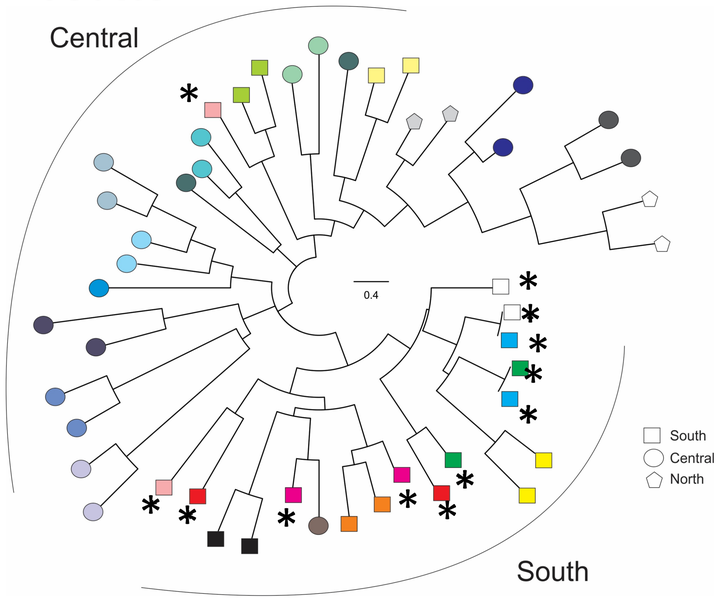


Figure 2. Within-host diversity by microsatellites

- Only samples of a single or bi-clonal infection were included. The majority of samples in the north contain more than two clones and thus were not included in the analyses.
- Six samples from the south (marked in asterisk) have clones that are genetically different from each other. The rest of the samples mostly from the central region have clones that are genetically similar.
- Ongoing work use *pfmsp1* to further infer multiplicity and diversity of polyclonal samples.

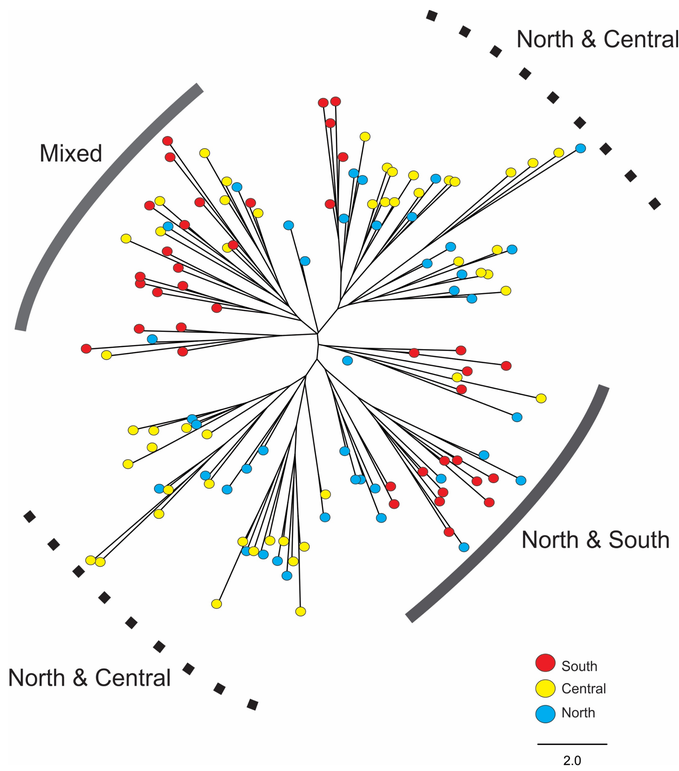


Figure 3. Genetic relatedness of *P. falciparum* samples from different transmission zones.

- Each individual contain either a single or multiple parasite strains.
- Phylogeny was constructed based on the presence or absence of an allele and does not reflect the genetic identity of individual clones.
- Parasites in the north and central are more closely-related compared to those in the south.

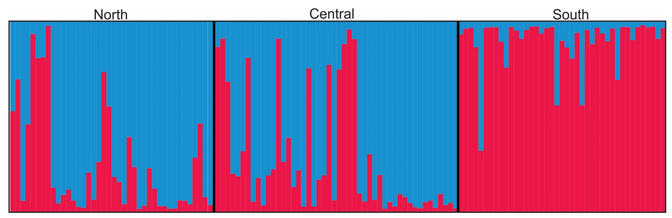
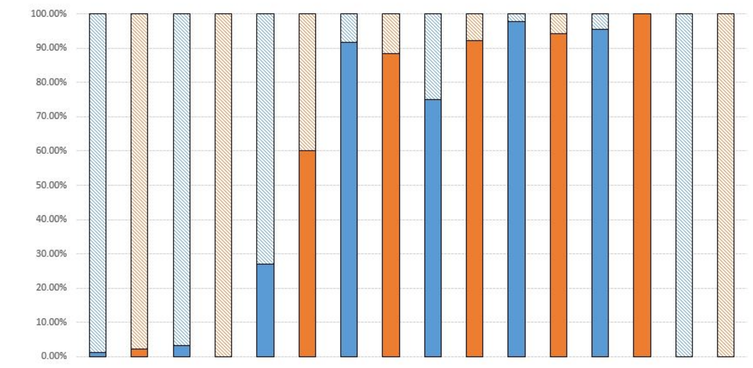


Figure 4. Bayesian barplot showing genotyping structure among *P. falciparum* samples.

- Two most probable genetic clusters (pink and blue) were identified based on Bayesian inference.
- Parasites in the north and central populations shared predominantly the blue cluster, whereas parasites in the south had the pink cluster.



Codon Position	K76T	N86Y	Y184F	N51I	C59R	S108N	A437G	K540E
Gene	*Pfcrt*	*Pfmdr1*		*Pfdhr*			*Pfdhps*	
Resistance to...	*Chloroquine*	*Amodiaquine*		*Pyrimethamine*			*Sulfadoxine*	
North	1.30%	3.15%	27.05%	91.70%	74.95%	97.85%	95.45%	0.00%
Central	2.20%	0.00%	60.00%	88.50%	92.30%	94.20%	100.00%	0.00%

Figure 5. Resistance marker prevalence in Ghana.

- Less than 5% of the samples harbor the mutant genotype at *pfcrt* codon 76 and *pfmdr*1 codon 86.
- Over 90% of the samples carried a triple mutant *pfdhfr* genotype (108N, 51I, and 59R). A high prevalence of *pfdhfr* 108N mutation suggested strong resistance to SP.
- Nearly 90% of the samples showed *pfdhps* mutation at codon 437 but not 540.

Summary

- A higher rate of polyclonality was observed in the north compared to the south and central regions.
- Parasites in the northern and central populations are genetically closely related, possibly due to frequent gene flow.
- Prevalence of the resistant gene codons were also similar between the north and central populations.
- A large percentage of samples observed with *pfdhfr* and *pfdhps* mutations, suggestive of increased resistance could be due to selective pressure by the SMC implemented since 2015.
- By contrast, very few samples had *pfcrt* mutation associated with CQ resistance.

References

1. Snow RW, Omumbo JA. Malaria. In: Jamison DT, Feachem RG, Makgoba MW, et al., editors. Disease and Mortality in Sub-Saharan Africa. 2nd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2006. Chapter 14.
2. Ghana. (n.d.). Retrieved from https://www.unicef.org/ghana/media_12060.html
3. Maigi H, Lasry E, Diarra M, Sagara I, Bamadio A, Traore A, et al. (2016) Seasonal Malaria Chemoprevention with Sulphadoxine-Pyrimethamine and Amodiaquine selects *Pfdhfr-dhps* quintuple mutant genotype in Mali. *PLoS ONE* 11:e0162718.
4. Lo, E.Y.Y., Hemming-Schroeder E., Yewhalaw D., Ngyuyen J., Kebede E., Zemene E., Getachew S., Tushune K., Zhong D., Zhou G., Petros B., Yan G. (2017) Transmission dynamics of co-endemic *Plasmodium vivax* and *P. falciparum* in Ethiopia and prevalence of antimalarial resistant genotypes. *PLoS Neglected Tropical Diseases* 11, e5806.
5. Hofmann N, Mwingira F, Shekalaghe S, Robinson LJ, Mueller I, Felger I. (2015) Ultra-Sensitive Detection of *Plasmodium falciparum* by amplification of multi-copy subtelomeric targets. *PLoS Med* 12:e1001788.
6. Anderson TJ, Su XZ, Bockarie M, Lagog M, Day KP. (1999) Twelve microsatellite markers for characterization of Plasmodium falciparum from finger-prick blood samples. *Parasitology* 119:113-25.

Acknowledgements

We thank the team at the University of Ghana for their assistance in sample collection and processing. This work is supported by the UNC Charlotte Faculty Research Grant (Lo).