

Genomic Surveillance of malaria reveals localized sporadic emergence of artemisinin partial resistance and wide-spread sulfadoxine-pyrimethamine resistance in Ghana

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Abstract

Plasmodium falciparum malaria accounts for a significant proportion of febrile illnesses in Ghana. Multiple first-line artemisinin combination therapies (ACTs) are available for treatment of uncomplicated malaria in Ghana. Sulfadoxine-pyrimethamine (SP) remains the backbone for prevention of malaria during pregnancy and seasonal malaria chemoprevention in young children. Although ACTs remain effective in West Africa, there are reports of partner drug resistance, and there is now stable transmission of artemisinin partial resistance in several countries across East Africa. Here, we utilize targeted amplicon sequencing data from nation-wide malaria molecular surveillance for 3,804 clinical isolates collected from 2019 to 2025 to characterize antimalarial drug-resistant mutations in *Pfkelch13* (K13), *Pfdhfr*, *Pfdhps*, *Pfmdr1* and *Pfcrt* across 32 health facilities in Ghana. Overall, the K13 P574L validated marker for artemisinin resistance (ART-R) and S522C previously linked to delayed parasite clearance *in vitro* was observed, albeit at very low frequency. Also, there was a high prevalence (54.4%) of AL associated resistance haplotype *Pfmdr1* N₈₆F₁₈₄D₁₂₄₆. In addition, we found high (78.7%) *pfdhfr* triple mutant haplotype allele (IRNI) across all regions, but low frequency of *pfdhfr/pfdhps* combined quintuple and sextuple haplotypes, suggesting some level of resistance to SP, but not high enough to undermine targeted SP interventions in Ghana. The chloroquine resistance associated allele (K76T) was also found at < 2% prevalence. Sporadic emergence of WHO validated K13 resistance markers highlights the need for continuous monitoring of *P. falciparum* populations for early detection of stable transmission of ART resistance in Ghana.

Introduction

Malaria remains a public health challenge globally with its impact most severe in disease-endemic areas like sub-Saharan Africa¹. Africa has borne the highest malaria burden with vulnerable population groups such as infants, young children and pregnant women being the most affected^{2,3}. In 2024, about 6.7 million malaria cases and a significant number of malaria associated deaths were reported in Ghana⁴. *Plasmodium falciparum* is the dominant and virulent species in sub-Saharan Africa, estimated to cause over 90% of malaria morbidity and mortality^{5,6}. A key challenge in malaria control and elimination is the parasite's inherent ability to rapidly develop biological mechanisms of antimalarial drugs resistance².

The hitherto wide spread *P. falciparum* chloroquine (CQ) resistance that resulted in significant treatment failures led to the adoption of artemisinin-based combination therapies (ACTs) as first-line treatment for uncomplicated malaria in the early 2000s^{7,8}. Ghana replaced CQ with multiple ACTs including artemether-lumefantrine (AL), artesunate -amodiaquine (AS-AQ) and dihydroartemisinin -piperaquine (DHA-PQ) as first-line treatment for uncomplicated malaria from 2004⁹. However, artemisinin resistance (ART-R) mediated by several mutations in the K13 propeller domain including the C580Y mutation have compromised ACTs in southeast Asia (SEA)¹⁰⁻¹². The high rates of treatment failure in SEA were due to concomitant circulation of multi-drug resistant genotypes¹³. More recently, several WHO validated K13 mutations occurring at variable frequencies have been reported in the east African countries of Tanzania¹⁴, Rwanda¹⁵, Uganda^{16,17} and Eritrea and Ethiopia in the horn of Africa^{18,19}. Thus far, there is no credible evidence of stable transmission of K13 mutants associated ART-R in West Africa. However, previous anecdotal reports show detection of isolates harbouring validated ART-R markers C580Y and Y493H at low frequencies in the central and northern regions

of Ghana respectively ²⁰ and several uncharacterized mutations in *K13* in Greater Accra ²¹. While artemisinin-based combination therapies (ACTs) remain effective in Ghana, there is fear that emergence of resistance to ACT partner drugs Lumefantrine, Amodiaquine and piperaquine would undermine the efficacy of ACTs. This is in lieu of the experience in SEA where the clinical impact of *K13* mutants such as C850Y were significantly exacerbated by ACT partner drug resistance, particularly so in West Africa where evolution of resistance to common ACT partner drugs is preceding the emergence of ART-R. Mutations in the legacy drug resistant genes, *P. falciparum* chloroquine resistance transporter (*Pfcr*t) and multidrug resistance gene 1 (*Pfmdr*1) have been linked to amodiaquine (AQ) resistance ^{22–24}. Recent treatment efficacy studies have reported a significant selection of wildtype *Pfmdr*1-N86 genotypes following treatment with AL ^{25,26}. Also, ex vivo drug efficacy studies in the Gambia showed parasite tolerance to AL in extant populations ²⁷.

Besides, in Ghana, Sulfadoxine-Pyrimethamine (SP) is used either as a prophylaxis to prevent malaria during pregnancy or combined with AQ for seasonal malaria chemoprevention (SMC) in the northern Savannah communities where malaria transmission is seasonal. Amino acid substitutions in the bifunctional *P. falciparum* dihydrofolate reductase protein (*Pfdhfr*) at codons 51, 59, 108 and dihydropteroate synthase protein (*Pfdhps*) at codons 437, 540, 581 and 613 have been associated with resistance to SP. However, increasing prevalence of mutations in *Pfdhfr* and *Pfdhps* genes and their association with reduced treatment efficacy have been observed in several malaria endemic countries including Ghana ²⁸. Thus, nationwide monitoring drug-resistant trends of *P. falciparum* is essential in detecting emergence of not only ART-R but also the spread of legacy SP and partner drug resistance markers to generate data that will support malaria control decision making and the shuffling of interventions in order to maximise outcomes.

This study assessed the nation-wide prevalence distribution of mutations in five (*Pfkelch13* (*K13*), *Pfmdr*1, *Pfdhfr*, *Pfdhps* and *Pfcr*t) *P. falciparum* known antimalarial drug resistant markers as part of a routine surveillance studies of anti-malarial drug resistance across 14 geopolitical regions of Ghana. By evaluating patterns of drug resistance in these markers, we report the emergence of a recently reported mutation in *K13* in Ghana linked to artemisinin resistance, increased prevalence of *mdr*1 mutations associated with reduced susceptibility to artemether-lumefantrine, while affirming the continued effectiveness of SP-IPTp. These findings highlight the need for continuous monitoring of antimalarial drug resistance providing evidence for decision making by policy makers including the National Malaria Elimination Program (NMEP), and early detection of emerging drug-resistant parasites to front-line ACTs in Ghana.

Results

Clinical and demographic characteristics

Of the samples collected from patients with confirmed *P. falciparum* infection across Ghana, a total of 3804 were successfully genotyped for five drug resistance genes by amplicon sequencing. The distribution of samples across all 14 regions; Bono, Bono East, Eastern, Ashanti, Central, Greater Accra, Northern, Upper East, Upper West, Volta, Western, Oti, Bono East, Savannah is shown in Fig. 1B. A strong seasonality is evident, with sharp peaks in sample collection during the peak malaria transmission season (rainy season, around May to November) and low numbers during the dry season (December to April) (Fig. 1C).

Complexity of Infection

Complexity of Infection (COI) across the surveillance period ranged from 1 to 13. There was pre-dominance of single clone *P falciparum* infections at a prevalence of 66.7% (2402/3804). However, over 30% of infections were polyclonal suggesting high malaria transmission across study regions (Fig. 2A). Overall, high COI (> 5) were prevalent in the Upper East, Upper West, Savannah and Western North region of Ghana (Fig. 2B), in tandem with the high parasite prevalence observed in these regions from other studies ^{29–32}.

Artemisinin partial resistance

Of the 3804 successfully sequenced samples, 98.3% (n = 3740) were successfully genotyped for the *PfK13* gene. Over the surveillance period, 30 isolates (0.8%) carried nonsynonymous mutations, all of which were homozygous. Of these isolates, 11 unique uncharacterized mutants within the PfK13 propeller and BTB/POZ domain were detected at low frequency, of which 7 (A557S, A676S, G665S, I590V, Q613H, V589I, V637I) had been reported previously in several Africa countries ^{33–37} while 4 mutations (C580S, E605D, G357V, T387A) were identified for the first time. Prevalence of uncharacterized mutations fluctuated minimally between 0.2 and 0.9% from 2019 to 2025, peaking at 0.9% in 2024, while the wildtype remained predominant throughout the study (Table 1). The pan-African mutant A578S, WHO validated but not associated with reduced artemisinin efficacy, was the most prevalent mutation, occurring in 0.32% (n = 12) of the isolates (Fig. 3B; Table 1) ^{38,39}. Also shown in Fig. 3B is the distribution of *PfK13* heterozygous calls (mixed genotypes with wildtype) detected across the study. Notably, P547L associated with ART resistance was observed in Kumbungu in the Northern region of Ghana in 2023 (Fig. 3A). Overall, three isolates had S522C substitution identified in the Upper west (Garu, 2019), Eastern region (Begoro, 2019), and Upper east (Wechiau, 2021), respectively (Fig. 3). The prevalence of all mutations from 2019 to 2025 is provided in Supplementary Table S1.

Table 1
Temporal trends in *Pfkelch13* among isolates from 2019 to 2025.

SNP	2019 n (%) [95%CI]	2020 n (%) [95%CI]	2021 n (%) [95%CI]	2022 n (%) [95%CI]	2023 n (%) [95%CI]	2024 n (%) [95%CI]	2025 n (%) [95%CI]	Total n (%) [95%CI]
P574L	0	0	0	0	1 (0.2) [0.0-0.6]	0	0	1 (0.0) [0.0-0.1]
S522C	2 (0.2) [0.0-0.4]	0	1 (0.7) [0.0-2.1]	0	0	0	0	3 (0.1) [0.0-0.2]
A578S	5 (0.5) [0.1–0.9]	0	0	3 (0.3) [0.0-0.6]	2 (0.4) [0.0-0.9]	2 (0.6) [0.0-1.4]	0	12 (0.3) [0.2–0.6]
Mixed	11 (1.0) [0.4–1.6]	1 (0.5) [0.0-1.4]	2 (1.4) [0.0-3.3]	8 (0.8) [0.2–1.3]	1 (0.2) [0.0-0.6]	2 (0.6) [0.0-1.4]	3 (0.7) [0.0-1.5]	28 (0.7) [0.5–1.1]
Wild-type	1049 (97.9) [97.1–98.8]	206 (99.0) [97.7-100.4]	139 (97.9) [95.5-100.3]	1029 (98.7) [98.0-99.4]	519 (98.9) [97.9–99.8]	324 (97.9) [96.3–99.4]	416 (99.0) [98.1–100.0]	3682 (98.4) [98.0-98.8]
Other*	4 (0.4) [0.0-0.7]	1 (0.5) [0.0-1.4]	0	3 (0.3) [0.0-0.6]	2 (0.4) [0.0-0.9]	3 (0.9) [0.0-1.9]	1 (0.2) [0.0-0.7]	14 (0.4) [0.2–0.6]
Total	1071	208	142	1043	525	331	420	3740
*Previously reported (A557S, A676S, G665S, I590V, Q613H, V589I, V637I) & unreported (C580S, E605D, G357V, T387A)								

Prevalence of multi-drug resistance

Four polymorphic sites of the *P. falciparum* multidrug resistance transporter 1 gene (*Pfmdr1*), codons 86, 184 and 1246 were analysed. The frequency of *Pfmdr1* wildtype allele N86 associated with lumefantrine resistance was observed at 98.5% (n = 3774). Prevalence of *Pfmdr1* 184F was 56.3% (2122/3771), with a year-on-year prevalence above 50% and increasing from 54.1% in 2019 to 60.8% in 2025 (p = 0.005, Cochran-Armitage trend test) (Supplementary Table S2). A total of 4 haplotypes of *Pfmdr1* were found as well as mixed types (Fig. 4A; Supplementary Table S4). *Pfmdr1* NFD, associated with reduced susceptibility to Artemether lumefantrine was identified in 55.2% (2071/3752) of infections while wildtype haplotype NYD was observed at a prevalence of 26.4% (990/3752). Distribution of haplotypes in successfully genotyped samples shows the *Pfmdr1* NFD haplotype increasing steadily from 2021 to 2025 (Fig. 4B; Table 2). The *pfmdr1* YYY haplotype associated with amodiaquine resistance was not identified in the current study.

Table 2
Prevalence of *Pfcrt* and *Pfmdr1* haplotypes detected in isolates from 2019 to 2025.

Mutation	2019	2020	2021	2022	2023	2024	2025	Total	p-value
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n/N (%)	
	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]	
<i>Pfcrt</i>									
CVMNK	1048 (97.4)	207 (99.0)	140 (98.6)	1024 (98.1)	495 (95.0)	324 (97.6)	412 (97.9)	3650 (97.5)	0.511
	[96.3– 98.2]	[96.6– 99.7]	[95.0– 99.6]	[97.1– 98.8]	[92.8– 96.6]	[95.3– 98.8]	[96.0– 98.9]	[96.9– 97.9]	
CVIET	8 (0.7)	1 (0.5)	0	9 (0.9)	12 (2.3)	8 (2.4)	5 (1.2)	43 (1.1)	0.016
	[0.4– 1.5]	[0.1– 2.7]		[0.5– 1.6]	[1.3– 4.0]	[1.2– 4.7]	[0.5– 2.7]	[0.9– 1.5]	
Mixed	20 (1.9)	1 (0.5)	2 (1.4)	11 (1.1)	14 (2.7)	0	4 (1.0)	52 (1.4)	
	[1.2– 2.9]	[0.1– 2.7]	[0.4– 5.0]	[0.6– 1.9]	[1.6– 4.5]		[0.4– 2.4]	[1.1– 1.8]	
<i>Pfmdr1</i>									
NFD	550 (51.2)	113 (53.3)	70 (49.6)	575 (55.1)	307 (58.3)	198 (59.5)	258 (61.3)	2071 (55.2)	< 0.001
	[48.2– 54.2]	[46.6– 59.9]	[41.5– 57.8]	[52.0– 58.1]	[54.0– 62.4]	[54.1– 64.6]	[56.5– 65.8]	[53.6– 56.8]	
NYD	268 (25.0)	60 (28.3)	39 (27.7)	287 (27.5)	138 (26.2)	98 (29.4)	100 (23.8)	990 (26.4)	0.689
	[22.5– 27.6]	[22.7– 34.7]	[20.9– 35.6]	[24.9– 30.3]	[22.6– 30.1]	[24.8– 34.5]	[19.9– 28.0]	[25.0– 27.8]	
YFD	17 (1.6)	0	0	4 (0.4)	1 (0.2)	0	0	22 (0.6)	< 0.001
	[1.0– 2.5]			[0.1– 1.0]	[0.0– 1.1]			[0.4– 0.9]	
NFY	1 (0.1)	0	0	0	0	0	0	1 (0.0)	0.198
	[0.0– 0.5]							[0.0– 0.2]	
NFD	550 (51.2)	113 (53.3)	70 (49.6)	575 (55.1)	307 (58.3)	198 (59.5)	258 (61.3)	2071 (55.2)	< 0.001
	[48.2– 54.2]	[46.6– 59.9]	[41.5– 57.8]	[52.0– 58.1]	[54.0– 62.4]	[54.1– 64.6]	[56.5– 65.8]	[53.6– 56.8]	
NYD	268 (25.0)	60 (28.3)	39 (27.7)	287 (27.5)	138 (26.2)	98 (29.4)	100 (23.8)	990 (26.4)	0.689

Mutation	2019	2020	2021	2022	2023	2024	2025	Total	p-value
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n/N (%)	
	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]	
	[22.5–27.6]	[22.7–34.7]	[20.9–35.6]	[24.9–30.3]	[22.6–30.1]	[24.8–34.5]	[19.9–28.0]	[25.0–27.8]	
Mixed	238 (22.2)	39 (18.4)	32 (22.7)	178 (17.0)	81 (15.4)	37 (11.1)	63 (15.0)	668 (17.8)	
	[19.8–24.7]	[13.8–24.2]	[16.6–30.3]	[14.9–19.5]	[12.5–18.7]	[8.2–14.9]	[11.9–18.7]	[16.6–19.1]	

Prevalence of legacy Chloroquine resistance

The *P. falciparum* chloroquine resistance transport (*Pfcr*t) gene was successfully sequenced in 98.4% (3745/3804) of samples. Overall, the chloroquine resistance associated allele, 76T was observed at a low prevalence of 1.15% (43/3745) (Supplementary Table S2). This indicates the expansion of parasite isolate carrying the sensitive CVMNK haplotype at a high prevalence year-on-year from 2019 (97.4%) to 2025 (97.9%) across Ghana (Fig. 5C; Table 2). Thus, confirming the consistently observed low frequency of the chloroquine resistant haplotype CVIET (< 2.0%), with no significant variation during the surveillance period (Fig. 5A; Fig. 5C). Interestingly, the persistence of chloroquine resistant haplotypes was not spatially homogeneous, with Greater Accra (0.35%), Northern (0.13%), Central (0.13%) and Upper West (0.13%) regions having the highest frequencies with a total frequency of 0.75% (28/3745) (Fig. 5B). Overall, the carrier (heterozygous) frequency of 76T was much higher (> 1.0%) across Ghana from 2019 to 2025.

Prevalence of Sulfadoxine -Pyrimethamine resistance alleles and haplotypes

Overall prevalence of the *Pfdhfr* triple mutant haplotype IRNI associated with SP resistance was high 78.7% (2941/3738). Also, the prevalence of double mutant NRNI was 6.6% (246/3738) (Fig. 6A). Other haplotypes included a double and single mutants ICNI and NCNI were found at low frequencies of 1.4% (54/3738) and 0.2% (7/3738), respectively.

The frequency of *Pfdhps* mutant alleles including 437G, 540E, 581G and 613S were observed to be 91.2% (3437/3768), 0.7% (25/3769), 1.4% (54/3760) and 9.7% (364/3758) respectively (Supplementary Table S3). There were 11 distinct haplotypes of *Pfdhps* identified. The frequency of the most observed haplotypes SGKAA and AGKAA were 36.9% (1380/3740) and 25.6% (956/3740) respectively (Fig. 6). Prevalence of the double mutant haplotype SGEAA, which includes the high capacity 540E mutation was over all low at 0.6% (24/3740) but, was predominant in Greater Accra and Upper East at 25.0% (6/24) and 16.7% (4/24) respectively. While SGEAA was not detected in 2020 and 2021, prevalence reached a peak of 1.1% in 2022 before declining to 0.3 by 2024, although these variations were not statistically significant ($p = 0.448$) (Fig. 6D; Table 3). Also, triple AGKGS and double SGKAS mutant haplotypes carrying high-capacity variants at codons 581G, 613S were observed at 1.4% and 0.16% respectively. Under 1.0% of infections were observed to carry

the completely sensitive haplotype, SAKAA. Other uncharacterized haplotype variants YAKAS, FAKAS involving codon 613S were detected across multiple regions. FAKAS was exclusive to northern Ghana (i.e Savannah, Upper West, Bono, Bono East, Upper East, and also Oti) while YAKAS showed broader distribution (i.e Central, Upper West, Oti, and Western regions), albeit at a prevalence of 0.4%.

Table 3
Prevalence of *Pfdhps* and *Pfdhfr* haplotypes detected in isolates from 2019 to 2025.

Mutation	2019 n (%) [95%CI]	2020 n (%) [95%CI]	2021 n (%) [95%CI]	2022 n (%) [95%CI]	2023 n (%) [95%CI]	2024 n (%) [95%CI]	2025 n (%) [95%CI]	Total n/N (%) [95%CI]	p-value
<i>Pfdhfr</i>									
IRNI	765 (71.5) [68.7–74.1]	164 (78.1) [72.0–83.2]	108 (76.1) [68.4–82.3]	838 (80.4) [77.9–82.7]	442 (84.2) [80.8–87.1]	274 (83.3) [78.9–86.9]	350 (83.3) [79.5–86.6]	2941 (78.7) [77.3–80.0]	< 0.001
NRNI	82 (7.7) [6.2–9.4]	16 (7.6) [4.7–12.0]	7 (4.9) [2.4–9.8]	66 (6.3) [5.0–8.0]	27 (5.1) [3.6–7.4]	21 (6.4) [4.2–9.6]	27 (6.4) [4.5–9.2]	246 (6.6) [5.8–7.4]	0.127
ICNI	22 (2.1) [1.4–3.1]	4 (1.9) [0.7–4.8]	3 (2.1) [0.7–6.0]	12 (1.2) [0.7–2.0]	1 (0.2) [0.0–1.1]	6 (1.8) [0.8–3.9]	6 (1.4) [0.7–3.1]	54 (1.4) [1.1–1.9]	0.074
NCSI	42 (3.9) [2.9–5.3]	6 (2.9) [1.3–6.1]	2 (1.4) [0.4–5.0]	30 (2.9) [2.0–4.1]	14 (2.7) [1.6–4.4]	4 (1.2) [0.5–3.1]	6 (1.4) [0.7–3.1]	104 (2.8) [2.3–3.4]	0.002
NCNI	3 (0.3) [0.1–0.8]	0	0	1 (0.1) [0.0–0.5]	1 (0.2) [0.0–1.1]	2 (0.6) [0.2–2.2]	0	7 (0.2) [0.1–0.4]	0.781
Mixed	156 (14.6) [12.6–16.8]	20 (9.5) [6.2–14.3]	22 (15.5) [10.5–22.3]	95 (9.1) [7.5–11.0]	40 (7.6) [5.6–10.2]	22 (6.7) [4.5–9.9]	31 (7.4) [5.2–10.3]	386 (10.3) [9.4–11.3]	
<i>Pfdhps</i>									
AGKAS	61 (5.7) [4.5–7.2]	10 (4.8) [2.6–8.6]	8 (5.7) [2.9–10.8]	84 (8.0) [6.5–9.8]	35 (6.7) [4.8–9.1]	34 (10.3) [7.5–14.1]	41 (9.8) [7.3–13.1]	273 (7.3) [6.5–8.2]	< 0.001
AGKAA	298 (27.8) [25.2–30.5]	70 (33.5) [27.4–40.1]	43 (30.5) [23.5–38.5]	274 (26.2) [23.6–29.0]	106 (20.2) [17.0–23.8]	75 (22.7) [18.5–27.5]	90 (21.6) [17.9–25.8]	956 (25.6) [24.2–27.0]	< 0.001

Mutation	2019 n (%) [95%CI]	2020 n (%) [95%CI]	2021 n (%) [95%CI]	2022 n (%) [95%CI]	2023 n (%) [95%CI]	2024 n (%) [95%CI]	2025 n (%) [95%CI]	Total n/N (%) [95%CI]	p-value
<i>Pfdhfr</i>									
SGKAA	354 (33.0) [30.2– 35.9]	61 (29.2) [23.4– 35.7]	41 (29.1) [22.2– 37.0]	392 (37.5) [34.6– 40.5]	240 (45.7) [41.5– 50.0]	134 (40.6) [35.4– 46.0]	158 (37.9) [33.4– 42.6]	1380 (36.9) [35.4– 38.5]	< 0.001
AAKAA	37 (3.4) [2.5– 4.7]	4 (1.9) [0.7– 4.8]	5 (3.5) [1.5– 8.0]	33 (3.2) [2.3– 4.4]	18 (3.4) [2.2– 5.4]	12 (3.6) [2.1– 6.2]	12 (2.9) [1.7– 5.0]	121 (3.2) [2.7– 3.9]	0.907
AGKGS	11 (1.0) [0.6– 1.8]	0	0	6 (0.6) [0.3– 1.2]	18 (3.4) [2.2– 5.4]	7 (2.1) [1.0– 4.3]	11 (2.6) [1.5– 4.7]	53 (1.4) [1.1– 1.8]	< 0.001
SGEAA	5 (0.5) [0.2– 1.1]	0	0	12 (1.1) [0.7– 2.0]	3 (0.6) [0.2– 1.7]	1 (0.3) [0.1– 1.7]	3 (0.7) [0.2– 2.1]	24 (0.6) [0.4– 1.0]	0.448
FAKAS	1 (0.1) [0.0– 0.5]	0	2 (1.4) [0.4– 5.0]	2 (0.2) [0.1– 0.7]	2 (0.4) [0.1– 1.4]	1 (0.3) [0.1– 1.7]	0	8 (0.2) [0.1– 0.4]	0.747
SAKAA	10 (0.9) [0.5– 1.7]	1 (0.5) [0.1– 2.7]	0	10 (1.0) [0.5– 1.8]	3 (0.6) [0.2– 1.7]	4 (1.2) [0.5– 3.1]	5 (1.2) [0.5– 2.8]	33 (0.9) [0.6– 1.2]	0.619
SGKGS	0	0	0	0	0	1 (0.3) [0.1– 1.7]	0	1 (0.0) [0.0– 0.2]	0.250
SGKAS	0	0	0	3 (0.3) [0.1– 0.8]	2 (0.4) [0.1– 1.4]	0	1 (0.2) [0.0– 1.3]	6 (0.2) [0.1– 0.3]	0.154
YAKAS	2 (0.2) [0.1– 0.7]	4 (1.9) [0.7– 4.8]	0	2 (0.2) [0.1– 0.7]	0	0	0	8 (0.2) [0.1– 0.4]	0.055
AAKAS	1 (0.1)	0	0	0	0	0	0	1 (0.0)	0.198

Mutation	2019	2020	2021	2022	2023	2024	2025	Total	p-value
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n/N (%)	
	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]	[95%CI]	
<i>Pfdhfr</i>									
	[0.0-0.5]							[0.0-0.2]	
Mixed	293 (27.3)	59 (28.2)	42 (29.8)	227 (21.7)	98 (18.7)	61 (18.5)	96 (23.0)	876 (23.4)	
	[24.7–30.0]	[22.6–34.7]	[22.9–37.8]	[19.3–24.3]	[15.6–22.2]	[14.7–23.0]	[19.2–27.3]	[22.1–24.8]	

A total of 7 *Pfdhfr* and *Pfdhps* combined haplotypes were observed (Supplementary

Table S5). Overall, the most prevalent combined haplotypes were the quadruple mutants, IRNI-SGKAA detected at 56.3%, (1112/1975) and the quintuple mutant, IRNI-AGKAA at 39.2%, (774/1975). The sextuple mutant haplotype, IRNI-AGKGS and quintuple mutant, IRNI-SGEAA were found at lower prevalences of 2.5% (50/1975) and 1.1%, (21/1975) respectively. These long haplotypes involving codons 540E, 581G and 613S have been characterized to confer high level resistance to SP. The combined frequency of IRNI and all 437G containing haplotype was identified at high frequencies across all regions with overall prevalence of 96.1%.

Discussion

In West Africa, stable transmission of artemisinin partial resistance (APR) genotypes have not been detected and characterised. This is within the context of sporadic reports of emergence of K13 variants (i.e candidate or WHO validated) in various settings within these landscapes^{40,41}. This is against the backdrop of expanding APR genotypes in East Africa reported in Rwanda⁴², Uganda¹⁰ and Tanzania^{43,44}, the horn of African among other settings^{17,45}. Therefore, malaria genomic surveillance to track known validated markers of resistance to front-line antimalarial drugs, including sulfadoxine-pyrimethamine that remains the mainstay for targeted interventions such as seasonal malaria chemoprevention (SMC) and intermittent preventive treatment of malaria during pregnancy (IPTp) is crucial for generating data to support malaria control programme decisions. We conducted genomic surveillance of malaria (GSM) across fourteen regions of Ghana from 2019 to 2025, sampling *P. falciparum* clinical infections to monitor single nucleotide polymorphisms in five well characterized drug resistance genes. Since the early 2000s when Ghana replaced chloroquine with ACTs, the Ghana malaria elimination programme adopted a multiple first-line approach to the treatment of uncomplicated malaria with current first-line ACTs being artesunate-amodiaquine (AS-AQ), artemether-Lumefantrine (AL) and dihydroartemisinin-piperaquine (DHAP).

By and large, after seven years of surveillance, we report the absence of stable transmission of WHO validated K13 markers of APR in Ghana. However, sporadic emergence of a candidate resistance K13 mutation P574L was observed in homozygous infection in Kumbungu in the northern region of Ghana, albeit in isolate in 2019. However, continuous monitoring did not detect this variant in these communities or other settings in the

country in subsequent years. Thus, suggesting that the P574L mutation may not confer a strong fitness advantage in the presence of artemisinin derivatives used in ACTs in Ghana. More recently, Tandoh et al. reported P574L in the forest ecological zone of Ghana at a low frequency, representing the first report of this variant in clinical isolates from that zone ⁴⁶. This further confirms the sporadic nature of P574L emergence across Ghana and highlights the importance of continued molecular surveillance to track its distribution. Indeed, the lack of strong selection for the P574L variant in Ghana is consistent with its observed low prevalence (< 3.0%) in the Central-East African countries of Equatorial Guinea and Rwanda ^{47,48}. We also found the K13 S522C mutation, which associated with delayed parasite clearance, but at low frequency. This candidate variant has been previously reported in studies conducted in Kenya⁴⁹ and Mali ⁵⁰, but also at low frequency, 2 samples in each study. Interestingly, in Ghana, unlike the P574L variant, the S522C variant was identified in two geopolitical regions (ie Northern & Eastern) located over 690km apart. We note that this mutation has been reported across sub-Saharan Africa ^{17,39,51,52}. Therefore, the mutation though not currently a validate APR marker, is pan-African and when selected would be a major threat to effective malaria treatments across Africa, particularly in context of increasing reports of ACT partner drug resistance ^{17,53,54}.

Amodiaquine and lumefantrine are key partner drugs in ACTs in Ghana and across the West Africa region ⁵⁵⁻⁵⁷. Our data suggests a st increase in prevalence of the *Pfmdr1* NFD haplotype from 49.6% in 2021 to 61.3% in 2025. This haplotype has been associated with reduced lumefantrine susceptibility ^{58,59}. Decline in mutant allele 86Y over our surveillance period is consistent with findings from a continent-wide systemic review by Walker A et al⁶⁰ suggesting the selection of the *Pfmdr1* N86 over the 86Y allele in areas where AL is key first line treatment. In the absence of validated markers for lumefantrine resistance, it is important to continue to monitor such trends as possible early warning signs of poor response to lumefantrine. On the hand, the absence of *Pfmdr1* YYY (N86Y-Y184Y-D1246Y) triple mutant haplotype across Ghana and the near absence of parasite isolates that carry both the *Pfmdr1* YYY haplotype plus the *Pfcr1* 76T (ie CVIET haplotype) is in indicative of extant parasites to amodiaquine in Ghana. Over all these data suggest that amodiaquine remains effective as first-line treatment alternative and for targeted interventions such as seasonal malaria chemoprevention (SMC) in northern Ghana. We believe the rapid expansion of chloroquine sensitive infections in Ghana compared to other West African countries such as the Gambia ⁶¹, may be driven by the early adoption of multiple first-line treatment alternatives for uncomplicated malaria in the Ghana in 2005 ⁶².

Our study replicates the high levels of resistance to pyrimethamine mediated by the *Pfdhfr* triple mutant haplotype IRNI, reported across sub-Saharan Africa ⁶³⁻⁶⁵. For this bi-functional locus, the *Pfdhps* A437G mutation linked to sulfadoxine resistance was equally high(> 90.0%) resulting in high prevalence of the combined (dhfr/dhps) quadruple mutant haplotype linked to reduced response to SP. Overall, the prevalence of high capacity mutations such as 540E (0.7%), 581G (1.4%) and 613S (9.7%) were low, leading to the overall low prevalence of quintuple and sextuple mutation characterized to confer high SP resistance. Therefore, across Ghana, overall SP resistance remains below the WHO 50% threshold for discontinuing targeted interventions such as IPTp and SMC ⁶⁶. Taken together, our surveillance data suggest continuous nation-wide monitoring these markers, particularly the *Pfk13* uncharacterised mutants as early warning indicators of response of parasite populations to front-line treatments such as ACTs, including legacy resistance markers such as *Pfcr1* 76T and targeted interventions with SP to ensure rapid identification of poor treatment efficacy.

Conclusion

The current study observed the sporadic emergence of a WHO validated *Pfk13* resistance in northern Ghana and other uncharacterized nonsynonymous *Pfk13* mutations linked to delayed clearance of artemisinin derivatives observed in multiple regions. Overall, ACTs remain efficacious in Ghana. However, the high prevalence of the combined *Pfdhfr* and *Pfdhps* quadruple mutant haplotype across all regions associated with resistance to SP signals that SP treatments may not be optimal, and there is the need to monitor the prevalence of these markers in SP intervention groups to ascertain the impact of this wide-spread haplotype, including high capacity variants (i.e K540E, 581G, 613S) with a potential to impart high-level SP resistance.

Methods

Study Site and sampling

This was a longitudinal cross-sectional genomic surveillance study of *P. falciparum* drug resistance alleles and haplotypes from clinical sources. Thirty (32) health facilities across 14 administrative regions of Ghana were sampled from March 2019 to November 2025 (Fig. 1A). Patients of all ages reporting with fever (or history of fever within last 24hrs) were screened for malaria using OnSite® Malaria Pf/Pan Ag rapid diagnostic test kit (CTK Biotech, Inc. USA) according to manufacturer's instruction. Dried blood spots (DBS) were prepared for malaria positive cases using Whatman® (Grade 31ET Chr) cellulose chromatography papers.

DNA Extraction and Targeted Amplicon Sequencing

Genomic DNA was extracted using the QIAamp DNA Investigator kit (Qiagen, UK) and quantified using Quanti-iT™ PicoGreen™ dsDNA Assay kit following the manufacturer's instructions. Selective Whole Genome Amplification (sWGA) was performed to enrich for *P. falciparum* DNA⁶⁷. Protocols for Genotyping were as described in *P. falciparum* Amplicon Toolkit⁶⁸. The sWGA product was further amplified to generate amplicons targeting *K13*, *dhfr*, *dhps*, *mdr1*, and *crt* and species markers using Taq PCR Master Mix (Qiagen, UK). Barcoding of isolates were done using Illumina P5 and P7 flow cell oligo sequences. Pooled libraries were purified and size selected using AMPure beads and confirmed with the Agilent TapeStation 4200. Libraries were quantified using a set of six pre-diluted DNA standards using KAPA SYBER FAST qPCR Master Mix (Roche, SA). Pooled libraries was sequenced on an illumina MiSeq system using a MiSeq reagent kits v2 300 cycles.

Data processing and resistance mapping

Raw reads were demultiplexed, filtered for high quality reads and aligned to the *P. falciparum* 3D7 reference sequence to generate a Genetic Report Cards (GRC) (details may be reviewed in⁶⁹). For each sample genotyped, variants were called and further processed to determine known haplotypes associated with drug resistance. SNP and haplotype variants were analysed across space and time to reveal trends of emerging resistance across the country. Data analysis was done using R version 4.3.1. Mutations were further classified as mutant (resistant), wild-type (sensitive) and mixed types for infections containing genomes carrying both

mutant and wild-type alleles. Haplotypes linked to resistance against specific antimalarial drugs (ie artemisinin and its partner drugs in ACTs) were determined. Population structure and complexity of infection were determined using principal component analysis and McCOIL ⁷⁰ using genetic barcodes containing 101 SNP alleles respectively ⁷¹. The trend in prevalence of specific mutations over the study period was compared using the Cochran-Armitage trend test, with p-value < 0.05 considered statistically significant.

Declarations

Data availability

The raw sequence data generated for this study is available at the European Nucleotide Archive with project ID PRJEB2136 and PRJEB96789. The genotype datasets are open source and available in the MalariaGEN Community Project (www.malariaGEN.net).

Ethics statement

Ethical approval was obtained from the Ghana Health Service Ethics Review Committee (#GHS-ERC:007/08/24) and all methods were performed in accordance with relevant guidelines and regulations. Written informed consent was documented for all participants or their legal guardians prior to enrollment into the study.

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Author Contributions

L.N.A., G.A.A., A.N and C.M.M conceptualized and designed the study. Funding was acquired by L.N.A, A.N and G.A.A. Sample processing and sequencing were performed by C.M., M.S., R.B., E.D., F.M., E.M.A., J.M.N., K.B., D.Y.A., M.E.M., and C.M.K.K., respectively. Field coordination and sample curation by F.D., C.M., A.Y.K., and O.A. Data curation was performed by I.N.D. and C.M.M. Data analysis and interpretation were performed by I.N.D, C.M.M, E.K.A. and E.U. Project administration and Supervision were done by L.N.A, J.M.N, C.M.M., F.D., and D.S.Y.A. The initial manuscript was written by I.N.D, C.M.M, and L.N.A. All the authors read, reviewed and approved this manuscript.

Competing interests

The authors have declared that they have no competing interests.

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Figures

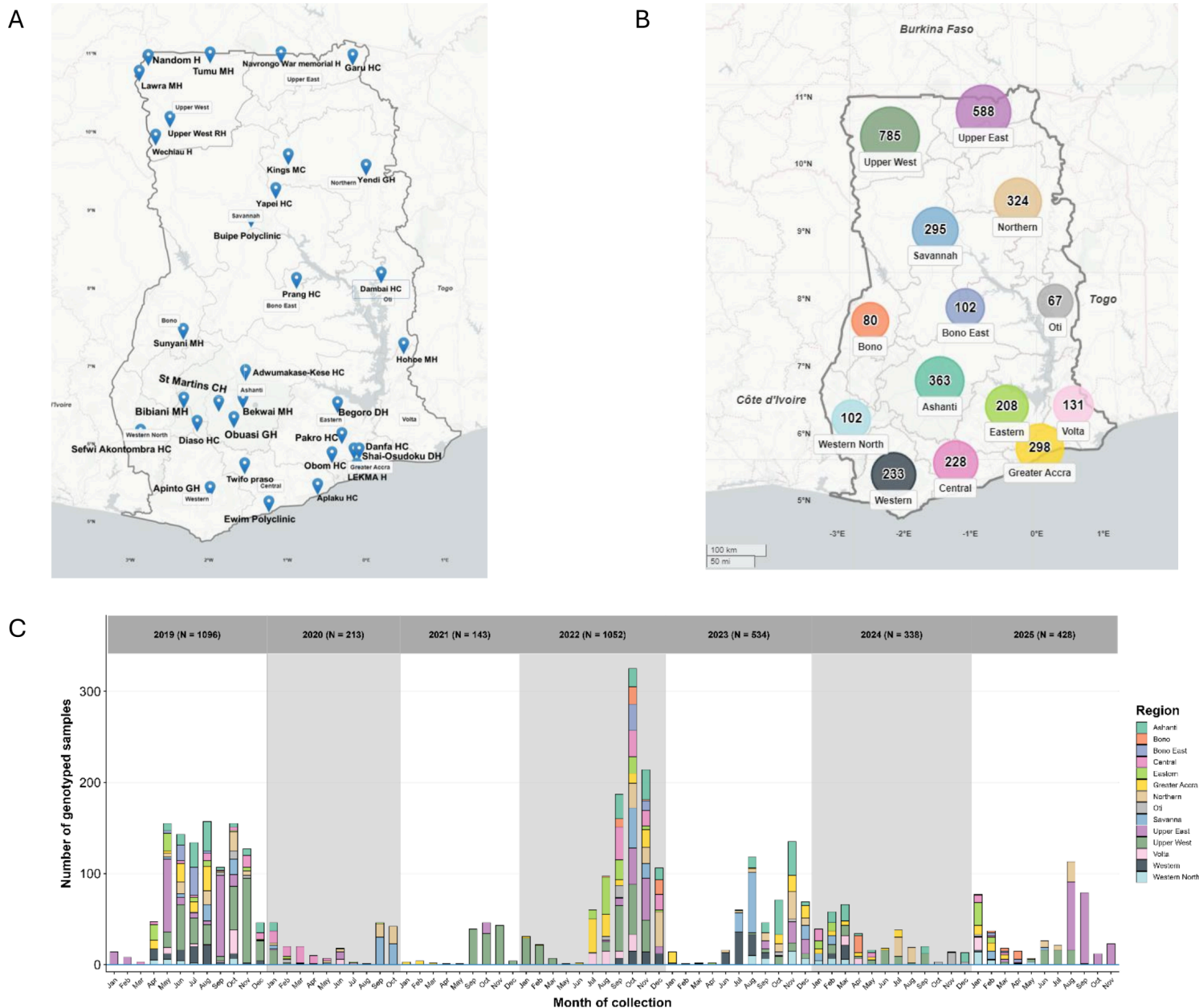


Figure 1

Sample collection sites and distribution of successfully genotyped samples. A) Location of all 32 sample collection sites across 14 regions of Ghana. **B)** Spatial distribution of genotyped *P. falciparum* isolates across Ghana from 2019 to 2025 ($n = 3804$). **C)** Temporal distribution of genotyped samples by month and region of collection. Colors represent geographic regions as indicated in the legend. Alternating white and light gray background bands separate years.

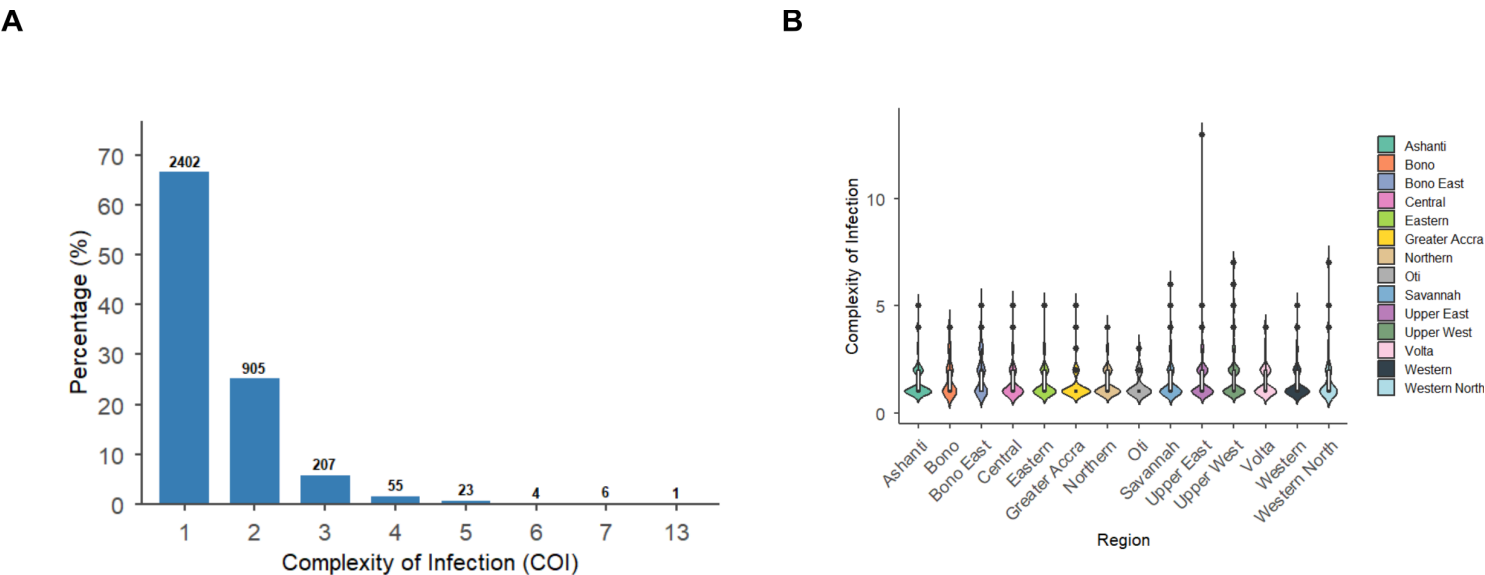


Figure 2

Population structure and complexity of infection of *P. falciparum* in Ghana

A) Overall *P. falciparum* complexity of infection. **B)** Regional complexity of infection.

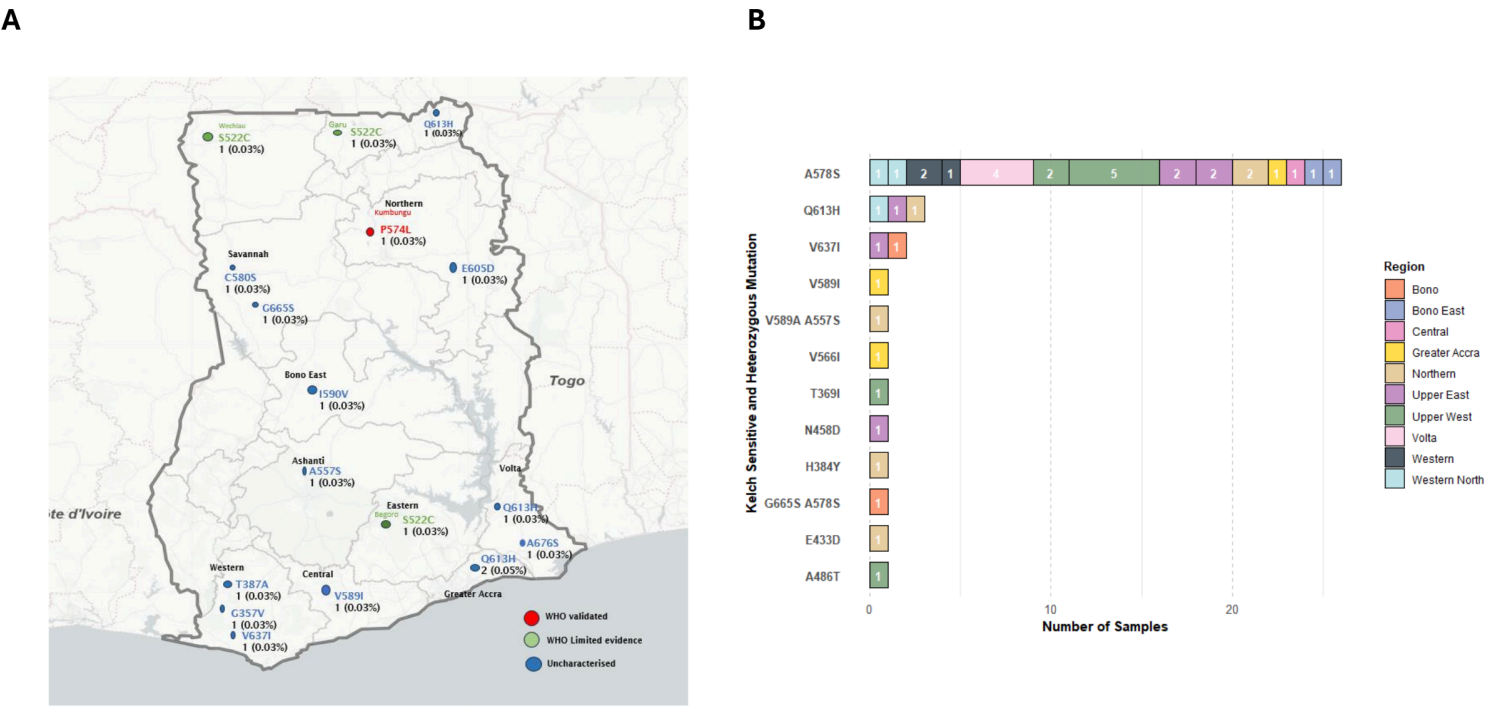
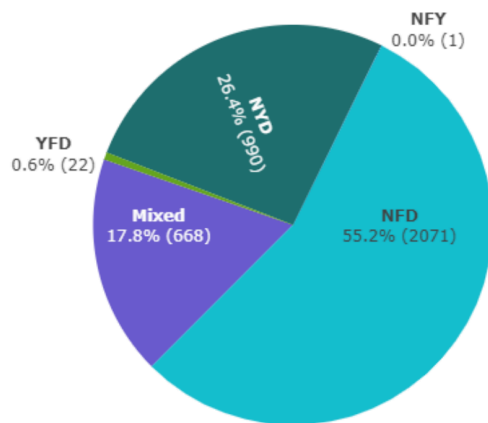


Figure 3

***Pfk13* mutations observed across regions in Ghana. A)** Distribution of homozygous mutations for categories (WHO-validated mutations denoted by red, WHO-limited evidence denoted by light green and uncharacterized mutations denoted by blue) identified across 14 regions in Ghana. **B)** Distribution of heterozygous mutations and the WHO-sensitive A578S mutation across regions in Ghana

A



B

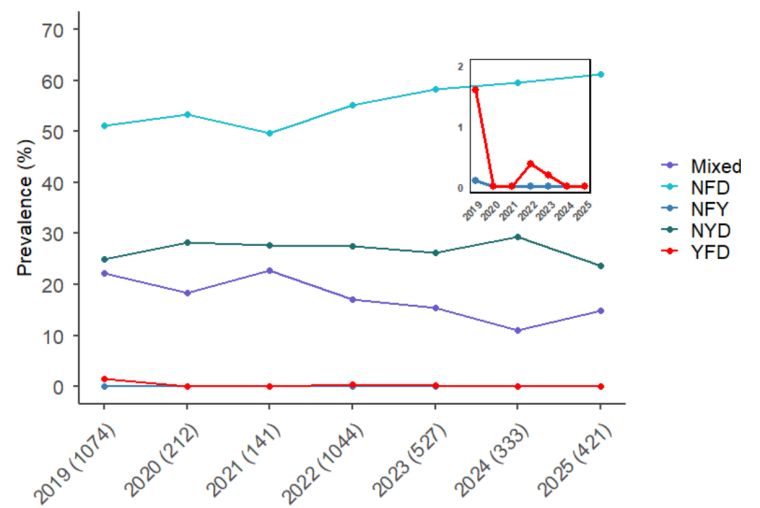
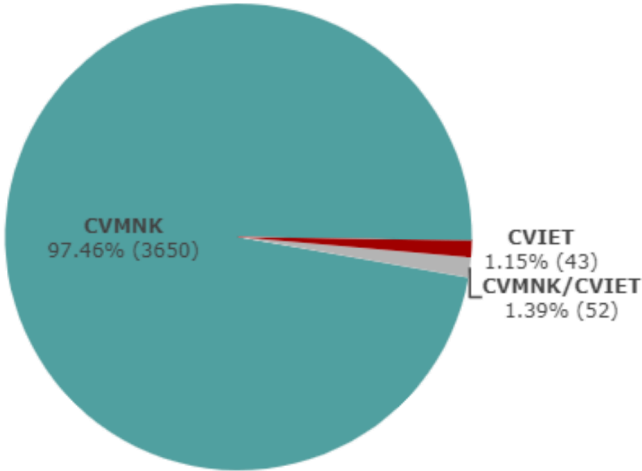


Figure 4

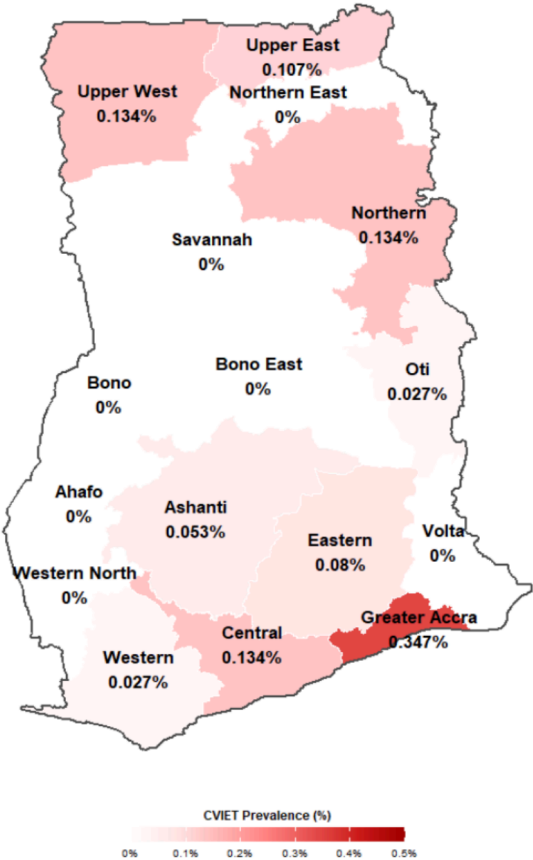
Distribution of *Pfmdr1* haplotypes from 2019 to 2025. A) Overall prevalence of *Pfmdr1* haplotypes.

B) Temporal trends in *Pfmdr1* haplotype prevalence in Ghana. An inset plot at the top right shows low-prevalence haplotypes (magnifying the 0–2% prevalence range). The total number of genotyped samples per year is shown in parentheses (n = sample size per year). Missing haplotypes were excluded. Mixed haplotypes represent samples with heterozygous or mixed codon patterns at key *Pfmdr1* codons (86, 184, 1034, 1042, and 1246) (detailed in Supplementary Table S4).

A



B



C

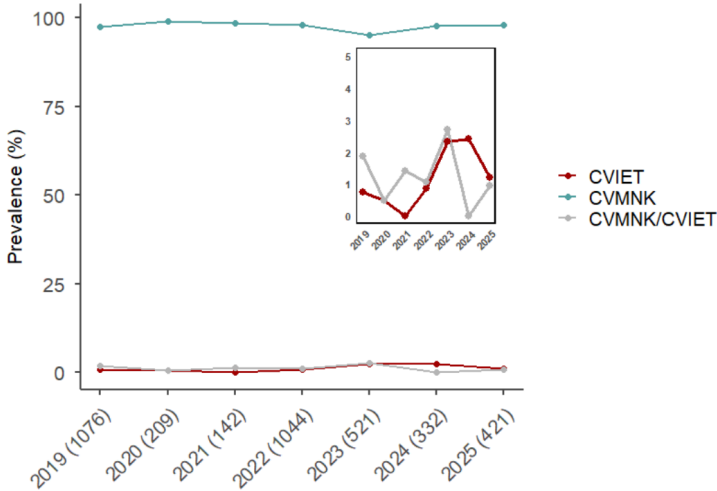


Figure 5

Prevalence of haplotypes in *PfCRT* from 2019 to 2025. **A)** Overall prevalence of *PfCRT* haplotypes. **B)** Density plot showing geographic distribution of *PfCRT* CVIET haplotype across Ghana. Colors represent the transition from wild-type CVMNK (white) to mutant CVIET (deep red), with increasing red intensity indicating higher CVIET prevalence. **C)** Temporal trends in *PfCRT* haplotype prevalence. The total number of genotyped samples per year is shown in parentheses (n = sample size per year). Haplotypes with missing genotype calls were excluded from the analysis.

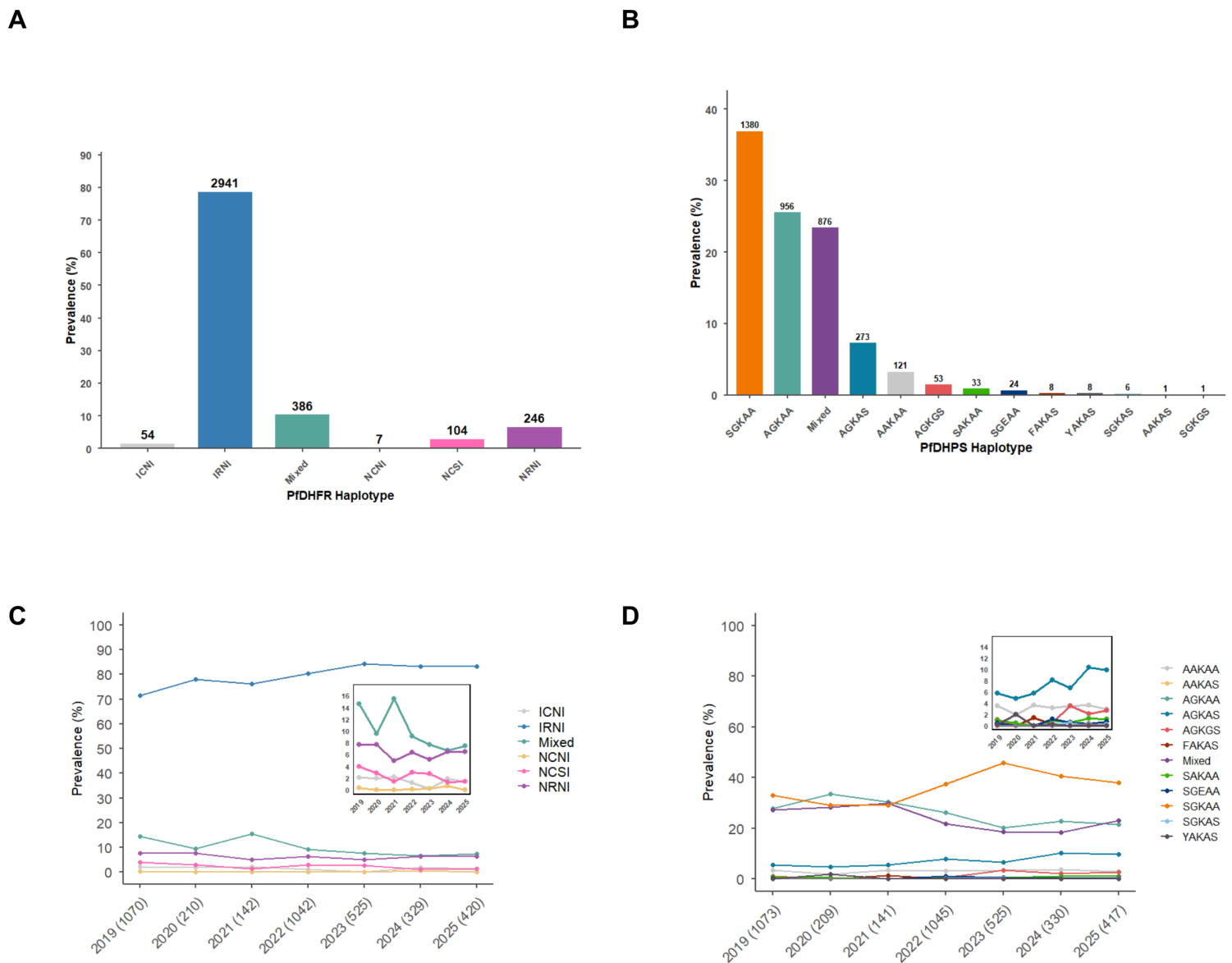


Figure 6

Trends in *Pfdhfr* and *Pfdhps* haplotype prevalence from 2019 to 2025. **A)** Overall prevalence of *Pfdhfr* haplotypes. **B)** Overall prevalence of *Pfdhps* haplotypes. **C)** Temporal trends in *Pfdhfr* haplotype prevalence. **D)** Temporal trends in *Pfdhps* haplotype prevalence. The total number of genotyped samples per year is shown in parentheses (n = sample size per year). Missing haplotypes were excluded. Mixed haplotypes represent samples with heterozygous or mixed codon patterns at key *Pfdhfr* codons (51, 59 and 108) and *Pfdhps* codons (436, 437, 540, 581 and 613) (detailed in Supplementary Table S4).

Supplementary Files

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- [Supplementarymaterials20260603.docx](#)