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Therapeutic efficacy of generic artemether–lumefantrine in the treatment of uncomplicated malaria in Ghana: assessing anti-malarial efficacy amidst pharmacogenetic variations

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Abstract

Background Despite efforts made to reduce morbidity and mortality associated with malaria, especially in sub-Saharan Africa, malaria continues to be a public health concern that requires innovative efforts to reach the WHO-set zero malaria agenda. Among the innovations is the use of artemisinin-based combination therapy (ACT) that is effective against *Plasmodium falciparum*. Generic artemether–lumefantrine (AL) is used to treat uncomplicated malaria after appropriate diagnosis. AL is metabolized by the cytochrome P450 family of enzymes, such as CYP2B6, CYP3A4 and CYP3A5, which can be under pharmacogenetic influence. Pharmacogenetics affecting AL metabolism, significantly influence the overall anti-malarial activity leading to variable therapeutic efficacy. This study focused on generic AL drugs used in malarial treatment as prescribed at health facilities and evaluated pharmacogenomic influences on their efficacy.

Methods Patients who have been diagnosed with malaria and confirmed through RDT and microscopy were recruited in this study. Blood samples were taken on days 1, 2, 3 and 7 for parasite count and blood levels of lumefantrine, artemisinin, desbutyl-lumefantrine (DBL), and dihydroartemisinin (DHA), the active metabolites of lumefantrine and artemether, respectively, were analysed using established methods. Pharmacogene variation analysis was undertaken using iPLEX microarray and PCR–RFLP.

Results A total of 52 patients completed the study. Median parasite density from day 1 to 7 ranged from 0–2666/μL of blood, with days 3 and 7 recording 0 parasite density. Highest median plasma concentration for lumefantrine and desbutyl lumefantrine, which are the long-acting components of artemisinin-based combinations, was 4123.75 ng/mL and 35.87 ng/mL, respectively. Day 7 plasma lumefantrine concentration across all generic ACT brands was ≥ 200 ng/mL which potentially accounted for the parasitaemia profile observed. Monomorphism was observed for CYP3A4 variants, while there were observed variations in CYP2B6 and CYP3A5 alleles. Among the CYP3A5 genotypes, significant differences in genotypes and plasma concentration for DBL were seen on day 3 between 1/*1 versus *1/*6 ($p=0.002$), *1/*3 versus *1/*6 ($p=0.006$) and *1/*7 versus *1/*6 ($p=0.008$). Day 7 plasma DBL concentrations showed a significant difference between *1/*6 and *1/*3 ($p=0.026$) expressors.

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Conclusions The study findings show that *CYP2B6* and *CYP3A5* pharmacogenetic variations may lead to higher plasma exposure of AL metabolites.

Keywords Pharmacogenomics, Pharmacokinetics, Generic anti-malarials, Artemether–lumefantrine, *CYP2B6*, *CYP3A5*

Background

Despite the efforts and significant investment in malaria eradication in sub-Saharan Africa (SSA), the disease remains a major public health challenge [1, 2]. Africa, in general, carries a more significant proportion of the global malaria burden accounting for 95% of malaria cases and 96% of malaria-related mortality [3]. Recent publications and the reports on malaria of the World Health Organization (WHO) have documented a decline in malaria morbidity and mortality [4–7], although it continues to remain a public health challenge.

Malaria is endemic and perennial in Ghana, with a pronounced seasonal variation. Malaria is highly prevalent during the rainy season, providing the perfect environment for the female *Anopheles* mosquito. Ghana is one of the eleven countries that accounted for 70% of the global malaria cases and 71% of estimated deaths in 2017 [4]. Across Ghana, the incidence per 1000 people decreased by 7.8% each year from 2011–2018. However, there was a decline in incidence/1000 people to 2.6% from 2018–2020 [8].

Accurate diagnosis and timely treatment with effective anti-malarial medication are major tools in malaria control. Over the years, several anti-malarial medications have been rolled out and changed over time due to the emergence of *Plasmodium falciparum* resistance and undesirable side effects. Anti-malarial medications from quinine to chloroquine, mefloquine, sulfadoxine–pyrimethamine (SP) and currently artemisinin-based combination therapy (ACT) have been prescribed for malaria treatment over decades. Ghana implemented its ACT policy in 2004 with the rollout of artesunate–amodiaquine as the first-line drug to replace chloroquine which was no longer effective due to extensive *P. falciparum* resistance across the country and Africa [9]. In 2009, there was a change in the anti-malarial drug policy of ACT to include artemether–lumefantrine and dihydroartemisinin–piperaquine as alternative first-line treatment medications due to challenges with tolerability to artesunate–amodiaquine by a significant section of the Ghanaian population [10].

Some gains have been made since the roll out of the anti-malarial drug policy of ACT. However, the continued success of the ACT policy and its subsequent implementation will largely depend on the availability, quality and cost of ACT drugs and the ability of health professionals to adhere to treatment guidelines [11–13]. The Affordable

Medicine Facility for Malaria Initiative (AMFm) assisted in expanding access to ACT and promoting the appropriate use of antimalarial medication [14, 15]. From the AMFm's initiatives, which was largely seen as success, Coartem[®] (Novartis Pharma AG, Basel Switzerland) became an innovator product with significant anti-malarial activity used for malaria treatment [16]. However, the high cost and availability of Coartem[®] has called for alternative, equally efficacious but affordable, anti-malarial medications that can hopefully elicit enough anti-plasmodial activity. The Food and Drug Administration of Ghana has approved several generic artemether–lumefantrine (AL) anti-malarial medications for use in Ghana, including Lumether (20 mg/120 mg), Luzatil (20 mg/120 mg), Artetab (80 mg/480 mg), Lumetrust (80 mg/480 mg) and Shal'Artem (20 mg/120 mg). These generic drugs are prescribed at health facilities and pharmacies for malaria treatment after rapid detection tests and or microscopy. AL is prescribed in individuals of weight ≥ 35 kg or ≥ 12 years of age as an 8–12 hourly, 3-day dosage regimen of 80 mg/480 mg artemether/lumefantrine. The number of AL tablets are adjusted to the required 80 mg/480 mg for ALs of 20 mg/120 mg (4 tablets) and ALs of 40 mg/240 mg (2 tablets) depending on the brand.

Artemether has a fast absorption rate with a rapid plasma clearance ($T_{1/2}=2-3$ h) with its major metabolite dihydroartemisinin (DHA) following a similar pattern. Lumefantrine is slowly absorbed followed by a slow plasma clearance rate ($T_{1/2}=\text{upto } 10$ days). Intake of fatty food increases bioavailability of AL by approximately 2–tenfold [17, 18]. AL is metabolized by the CYP450 enzymes such as *CYP2B6*, *CYP3A4/5*, *CYP2A6* and UGTs to active components to exert their therapeutic effects. AL metabolism leads to active metabolites of desbutyl-lumefantrine (DBL) and dihydroartemisinin (DHA) which provides its therapeutic anti-malarial activity [19]. Most of the presently available evidence point to pharmacogenetic variations in these drug metabolizing enzymes, especially cytochrome P450, and have been shown to influence drug disposition and efficacy [20]. The importance of pharmacogenetics in AL is clearly seen in circumstances of resistance, shrinking narrow therapeutic window and efficacy [21]. Generic AL is critical in the management and control of malaria in low- and middle-income countries (LMICs) such as Ghana as they are affordable and readily available. However, substandard

generic drugs expose parasites to sub-therapeutic drug pressure, which enables *P. falciparum* resistance selection and treatment failures and threatens patients' safety [22, 23].

A recent study in Tanzania has explored the activity of generic drugs used in treating uncomplicated malaria in comparison to the innovator drug Coartem® [24]. Therapeutic efficacy of drugs, including artemisinin-based combinations, depends on specified plasma concentrations of active drugs or metabolites [25, 26]. Plasma concentration of drugs and metabolites depends on several pharmacokinetic considerations, including variations on genetic profiles of individuals. There is very little data on plasma metabolite profiles in most medications administered among the Ghanaian population. Bearing in mind the significant role of genetics on plasma concentration of reports on malaria and the economics of malaria management in the country *vis-a-vis* sub-optimal therapeutic outcomes in some generic anti-malarials, our study focused on potential pharmacogenetic variations of response to generic drugs used in anti-malarial treatment as prescribed at health facilities and by evaluating the pharmacogenomic influences on their efficacy.

Methods

Study design, site and participants

This was prospective open-label pharmacogenomic-pharmacokinetic study conducted to compare the metabolite profiles and treatment outcomes in patients being treated with generic artemether–lumefantrine medications for the treatment of uncomplicated *P. falciparum* malaria. Patients were selected after diagnosis for uncomplicated

malaria (defined as “a patient who presents with symptoms of malaria and a positive parasitological test (RDT and or microscopy), but with no clinical features of severe malaria”) [27], age 15 years and above and for females not pregnant. Patients were excluded if they had severe malaria [28], other significant health conditions, were taking medications that could potentially interact with the AL (e.g. rifampin, carbamazepine, phenytoin, St. John’s work) or had initiated treatment of malaria with either herbal or other ACTs prior to the recruitment. Of the 96 patients who presented with symptoms of malaria at the healthcare facilities, 75 met the eligibility criteria, provided both written and verbal consent, and were subsequently enrolled in the study. However, only 52 patients successfully completed the study (Fig. 1).

Sample size

It was estimated that the power of study posteriori based on the size of our sample applying the methods suggested by Ogungbenro and Aarons [29]. Based on the above the 52 subjects that completed the study provided adequate AL parent drug and metabolite data to demonstrate differential drug plasma levels.

Malaria diagnosis and AL treatment

Participants were recruited from the Cape Coast Teaching Hospital, Ewim Polyclinic and Moree Health Post, all within the Cape Coast Metropolis, Central Region, Ghana. Verbal and written informed consent were obtained from participants after the study had been explained to them in both English and local languages (Fante and Twi). Patients were initially diagnosed with

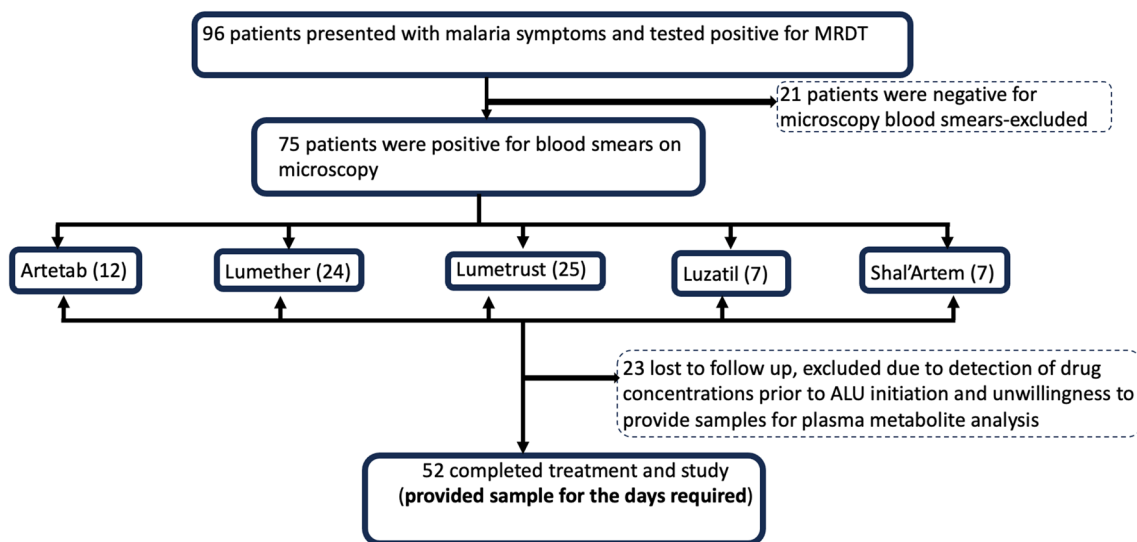


Fig. 1 Study recruitment, follow-up, and sampling procedure

malaria RDT at the Outpatient Department (OPD) of the recruiting facilities, confirmed with microscopy by a microscopist and placed on any of the available brands of generic artemisinin-based combinations for three days. Follow up calls were made to participants for them to take their medications on time. AL 80 mg/480 mg was taken after meals 8–12 hourly for a 3-day regimen. Thin and thick-smear blood films were stained with Giemsa stain and analysed under a microscope for the presence of parasites. Blood samples were taken on days 1, 2, 3 and 7 for parasitaemia and blood metabolite analysis. Samples were taken not more than 2-h post dosing due to the short half life of artemether. Plasma samples were stored in -80°C until ready for analysis.

Haematological analysis

Five millilitres of venous blood was taken into EDTA tubes for full blood count (FBC) analysis on a Sysmex-XN-350-15024 haematological analyzer.

DNA extraction and genotyping

DNA was extracted from whole blood using the EZNA DNA extraction kit (Omega Bio-tek, Inc. Norcross, USA) according to the manufacturer's instructions. The quality of DNA was established on a 1% agarose gel and nanodrop. Genotyping for *CYP2B6**6, *CYP2B6**18, *CYP3A4**2, *CYP3A4**17, *CYP3A4**22, *CYP3A5**2, *CYP3A5**3, *CYP3A5**6 and *CYP3A5**7 was undertaken using Iplex GOLD SNP genotyping protocol on the Agena MassARRAY® system (Agena Bioscience™, San Diego, CA, USA).

Analysis of plasma concentrations of artemether, dihydroartemisinin (DHA), lumefantrine and desbutyl-lumefantrine (DBL)

Sample preparation

For the extraction of lumefantrine and desbutyl lumefantrine from plasma, a volume of 400 μL of acetonitrile containing lumefantrine-d9 at 15 ng/mL was added to 100 μL of plasma. The sample was vortex-mixed for 30 s and centrifuged at $15,000\times g$ for 5 min at room temperature. A volume of 200 μL of the supernatant was transferred into a 96-well plate for analysis. For the extraction of artemether and its metabolite from plasma, a volume of 100 μL of plasma was added to a polypropylene microcentrifuge tube, together with 100 μL of 20 mM ammonium bicarbonate containing artemether-d3 and dihydroartemisinin-d3 at 100 ng/mL. Following the addition of 700 μL of ethyl acetate, the sample was vortex-mixed for 1 min and centrifuged at $16,000\times g$ for 5 min at 4°C . A volume of 650 μL of the top organic layer was transferred into a glass tube and dried under a gentle stream of nitrogen at 30°C . The sample was reconstituted with 150 μL

of methanol: 10 mM ammonium acetate (65:35; v:v) containing 0.1% acetic acid and vortex-mixed for 30 s prior to transfer into a 96-well plate for analysis.

Equipment

Analysis for artemether, DHA, lumefantrine and DBL was undertaken at the Division of Clinical Pharmacology, University of Stellenbosch, Cape Town, South Africa. Liquid chromatography–mass spectrometry (LC–MS/MS) analysis was conducted on a SHIMADZU 8040 triple quadrupole-mass spectrometer (SHIMADZU, Kyoto, Japan) connected to a SHIMADZU Prominence LC system. The system consisted of a LC-20ADXR solvent delivery system, Nexera XR SIL-20AXR autosampler and CTO-20A column oven. The analytes were chromatographically resolved on an Agilent Poroshell 120 EC-C18 (3.0×100 mm, $2.7\ \mu\text{m}$) column. Data acquisition and processing was performed using LabSolutions Version 5.109 software (Shimadzu Corporation, Kyoto, Japan).

Analysis

Artemether and dihydroartemisinin were quantified as described by Wiesner et al. [30], with modifications. Lumefantrine and desbutyl lumefantrine were quantified as described by Govender et al. [31], with modifications. The liquid chromatography-tandem mass spectrometry (LC–MS/MS) methods were validated according to Food and Drug Administration (FDA) [32] and European Medical Agency (EMA) [33] guidelines prior to sample analysis.

Statistical analysis

Descriptive statistics of participants including generic brands, parasitemia, medians and means full blood count parameters was performed. Continuous variables were expressed as mean \pm standard deviation or median (inter-quartile range), with categorical variables being expressed as absolute values and/or frequencies. Kruskal Wallis or Dunn's test was used to test for significance among various groups. Linkage disequilibrium, haplotype genotype and allele frequencies were calculated using a web based tool LDlink [34] and Shesis Plus [35]. A p value of $p < 0.05$ was considered statistically significant. Statistical analysis was performed using STATA v18 ((StataCorp, College Station, Texas, USA) and Graphpad v9 (Prisma, San Diego, California) statistical software packages.

Results

Clinico-demographic characteristics

Table 1 shows the clinicodemographic data for the study participants. There were more females (61.54%) than males (38.46%). Mean age was 34.83 ± 18.32 years

Table 1 General clinicodemographic of patients on ACT

Total number of participants, N = 52	
Gender	
Male	20 (38.46)
Female	32 (61.54)
Mean age in years ± SD (range)	34.83 ± 18.32 (15–78)
Median BMI in kg/m ² (IQR)	22.10 (19.36–26.85)
Mean Hb in g/dL ± SD (range)	11.37 ± 1.94 (7.1–15.8)
Mean HCT (%) ± SD (range)	36.43 ± 8.06 (9.8–55.90)
Mean PLT (10 ³ /μL) ± SD (range)	166.67 ± 88.20 (35–369)
Mean WBC (10 ⁹ /L) ± SD (range)	4.60 ± 2.76 (0.86–16.61)
ACT BRANDS n (%)	
Artetab	8 (15.38)
Lumether	15 (28.85)
Lumetrust	22 (42.31)
Luzatil	4 (7.69)
Shal'Artem	3 (5.77)
Parasite density (parasite/μL of whole blood), median, IQR	
Day 1	2666.67 (397.62–10,687.57)
Day 2	1529.89 (36.86–4804.24)
Day 3	0
Day 7	0

for both males and females. Lumetrust (42.31%) and Lumether (28.85%) were the most administered generic artemisinin-based combinations brands to participants. Full blood count (FBC) analysis showed a mean value for Hb (11.37 ± 1.94 g/dL), HCT (36.43 ± 8.06), PLT (166.67 ± 88.20) × 10³/μL and WBC (4.60 ± 2.76) × 10⁹/L. On day 1 of recruitment, the median parasite density for participants was 2666.67/μL of blood, while on day 2, the parasite count was 1529.89/μL; by day3, parasite density was 0.

Figure 2 shows the parasite density in relation to the various artemisinin-based combinations that participants took, and it shows that despite the differences in observed parasite density, the trend of 0 parasite density on day 3 and 7 was seen for all treatments.

Plasma artemether–lumefantrine concentration distribution among the generic brands

Plasma concentrations of artemether, dihydroartemisinin, lumefantrine and desbutyl lumefantrine influences parasitaemia response and treatment outcome in malaria. Parasite resistance can set in at significantly low levels, while higher plasma concentrations may also likely be associated with adverse drug events. This study successfully gathered comprehensive drug and metabolite plasma concentrations from all 52 samples. However, it is essential to note that for three of these

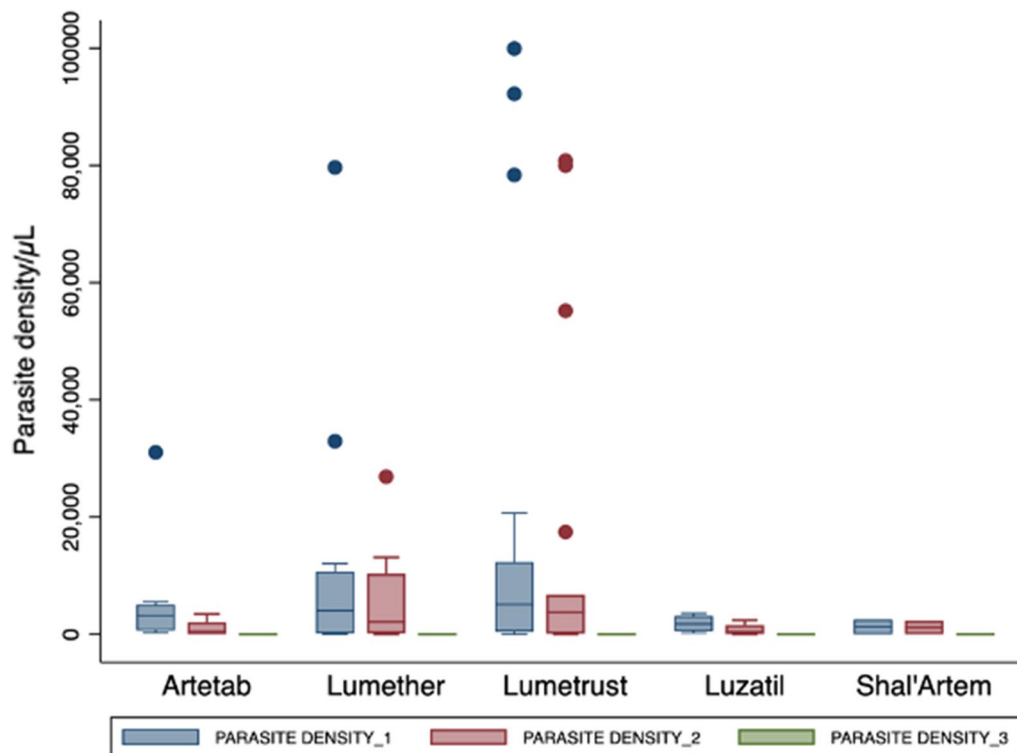


Fig. 2 Parasite density per generic ACT administered. Day 1 to 3 parasite density as quantified by microscopy per administered ACT

patients, the drug concentrations of some metabolites were below the detectable limit. High plasma concentrations of lumefantrine and desbutyl lumefantrine were observed across all brands of generic AL. However, the high plasma concentration for lumefantrine and desbutyl lumefantrine were observed in participants who were dosed with Artetab, 4123.75 (3056.34–4943.79) ng/mL and 35.87 (15.37–71.15) ng/mL, respectively. The day 7 plasma lumefantrine concentration for all brands of generic AL were above 200 ng/mL, which could account for the parasitaemia levels observed (Table 2).

Correlation between CYP2B6 and CYP3A5 genotypes with plasma concentrations of artemether, DHA, lumefantrine and desbutyl lumefantrine metabolites

Table 3 provides a detailed analyses of the *CYP2B6* and *CYP3A5* genotypes, highlighting the variations in plasma DHA concentrations across different genotypes. On day 3, a significant difference in plasma DHA concentrations was noted among the various *CYP2B6**6 genotypes ($p=0.0031^*$). Additionally, on day 7, there was a notable difference in plasma DBL concentrations ($p=0.0019^*$) between *CYP3A5**6 carriers. Desbutyl lumefantrine is the potent metabolite of lumefantrine with significant anti-malarial activity. The median day 3 plasma lumefantrine and desbutyl lumefantrine concentrations were 2979.25 (1869.30–4123.75) ng/mL and concentration of desbutyl lumefantrine 27.79 (14.28–44.76) ng/mL, respectively while that of day 7 was 578.18 (367.62–1057.01) ng/mL and 24.24 (14.60–39.32) ng/mL, respectively.

Correlation between CYP2B6 and CYP3A5 expressor status with plasma concentrations of artemether, DHA, lumefantrine and desbutyl lumefantrine metabolites

The study observed considerable inter-individual variability in the plasma concentrations of artemether, dihydroartemisinin, lumefantrine and desbutyl lumefantrine although most variations were not statistically significant. This lack of significance could be attributed to the sample sizes used in the analyses. Median plasma concentrations of artemether and its metabolite, dihydroartemisinin, ranged from 8.98–69.24 ng/mL and 16.30–90.90 ng/mL, respectively, while median plasma concentrations of lumefantrine and desbutyl lumefantrine ranged from 2664.17–3029.94 ng/mL and 17.23–47.45 ng/mL respectively for day 3 and 469.43–689.49 ng/mL and 14.23–35.43 ng/mL, respectively, on day 7. There were 4 samples where the metabolite concentration for LUM and DBL for day 7 were below the lower limit of quantification. The analysis combined non-expressor *CYP2B6**6/*6 and *18/*18 due to their numbers and functional effects. Figure 3 illustrates plasma artemether and DHA concentrations in relation to *CYP2B6* and *CYP3A5* expressors. There were significant differences in plasma artemether drug concentrations between *CYP2B6**1/*6 and *1/*1 carriers ($p=0.039$) with another significant difference observed between plasma DHA metabolite concentrations between *CYP2B6**1/*6 versus *CYP2B6**1/*1 ($p=0.000$) and combined *CYP2B6**6/*6 + *18/*18 versus *CYP2B6**1/*1 ($p=0.012$).

Plasma lumefantrine and DBL in relation to *CYP2B6* is shown in Fig. 4. No significant difference was observed between normal expressor (*1/*1) and reduced expressors. However, it was observed that the median plasma concentration for normal expressor *1/*1 was relatively

Table 2 Generic brands of ACT parent drug and metabolite concentrations

ACT and metabolites (ng/mL)	Artetab Median (IQR)	Lumether	Lumetrust	Luzatil	Shal/Artem	P value
Artemether	23.90 (17.70–30.95)	22.37 (9.89–49.40)	12.20 (6.09–17.10)	8.75 (N/A)	16.03 (N/A)	0.4832
Dihydro artemisinin (DHA) (2-h postdose)	35.87 (15.37–71.15)	15.84 (9.62–32.31)	32.98 (12.02–64.94)	N/A	31.47 (N/A)	0.4161
Lumefantrine concentration—day 3	4123.75 (3056.34–4943.79) ^{a,b,c}	3024.97 (2118.00–3926.77)	2538.58 (1727.43–3742.91)	1940.07 (N/A)	3235.86 (500.00–5971.7)	0.0202*
Desbutyl lumefantrine—day 3	44.10 (24.76–77.42) ^{d,e}	24.07 (14.19–40.28)	23.58 (14.11–43.28) ^f	19.44 (14.28–24.6)	27.79 (14.28–54.90)	0.0664
Lumefantrine concentration—day 7	678.83 (456.44–1171.07)	583.0 (454.01–1585.06)	550.77 (351.14–1151.70)	692.03 (N/A)	606.16 (250.00–962.33)	0.3323
Desbutyl lumefantrine (DBL)—day 7	39.32 (24.29–60.03) ^{g,h}	32.64 (14.60–51.82)	17.05 (11.30–24.24)	39.4 (N/A)	36.37 (34.00–38.73)	0.0715

N/A-some values were below lowest limit of quantification (LLOQ), Statistically significant comparisons among the generic drugs—^{a,b,c}comparison between Artetab, Lumether, lumetrust, Shal/Artem; ^{d,e}comparison between Artetab, Lumether, Lumetrust; ^fLumether, Shal/Artem; ^{g,h}comparison between Artetab, Lumetrust

Table 3 Plasma artemether–lumefantrine and metabolite concentrations stratified by genotypes

Genotype	n (%)	Median (IQR)	ART (ng/mL)	p-value	DHA (ng/mL)	p-value	LUM-DAY 3 (ng/mL)	p-value	DBL-DAY 3 (ng/mL)	p-value	LUM-DAY 7 (ng/mL)	p-value	DBL-DAY 7 (ng/mL)	p-value
CYP2B6 rs2839499 (*18)														
T/T	47	48.50 (14.46–99.14)	29.68 (11.45–52.80)	0.1627	3004.72 (1823.08–4502.25)	0.7937	26.28 (14.28–44.43)	0.3699	573.36 (381.45–1151.70)	0.2887	22.90 (14.60–39.32)	0.7231	22.90 (14.60–39.32)	0.9587
C/T	5	8.98 (6.03–71.61)	51.08 (4.75–97.40)		2029.04 (1929.00–2573.97)		41.8 (24.60–55.75)		613.00 (339.52–693.03)		29.09 (16.04–36.58)		29.09 (16.04–36.58)	
CYP2B6 rs3745274 (*6)														
T/T	15	40.47 (9.89–111.40)	29.43 (16.30–54.20)	0.8509	2989.45 (1823.08–4893.64)	0.0031*	17.64 (15.70–61.48)	0.9610	469.34 (353.78–689.49)	0.7244	14.23 (11.30–42.00)	0.3315	14.23 (11.30–42.00)	0.5937
G/T	23	32.86 (13.68–86.07)	13.32 (8.12–32.30)		3029.94 (1903.77–3926.77)		26.77 (14.28–47.12)		693.45 (500.00–1262.36)		23.57 (18.28–33.75)		23.57 (18.28–33.75)	
G/G	14	69.24 (10.71–113.95)	90.90 (44.35–111.33)		2488.28 (1929.00–4335.37)		34.20 (24.60–41.80)		613.00 (495.72–918.00)		33.43 (16.54–39.40)		33.43 (16.54–39.40)	
CYP3A5 rs776746 (*3)														
T/T	38	42.39 (12.28–102.79)	29.68 (9.67–66.57)	0.7335	2926.18 (1869.30–4123.74)	0.8102	27.79 (14.28–50.14)	0.5594	583.00 (353.78–1151.70)	0.3506	24.29 (15.19–39.40)	0.7214	24.29 (15.19–39.40)	0.1776
T/C	14	55.77 (9.21–92.80)	29.26 (16.30–34.37)		3648.10 (1970.54–4238.41)		22.59 (13.04–33.14)		559.91 (381.45–918.00)		15.46 (11.97–25.46)		15.46 (11.97–25.46)	
CYP3A5 rs41303343 (*7)														
–/–	37	37.37 (9.89–111.40)	16.30 (5.90–49.68)	0.8287	3020.00 (1869.30–4547.00)	0.6569	29.48 (14.28–50.14)	0.2991	583.00 (342.81–1220.00)	0.5114	24.69 (13.93–40.66)	0.7613	24.69 (13.93–40.66)	0.5094
–/T	15	59.17 (16.52–93.91)	30.25 (8.36–44.35)		2664.17 (1940.07–3056.34)		24.68 (16.91–32.33)		573.36 (550.39–692.03)		18.99 (16.54–33.42)		18.99 (16.54–33.42)	
CYP3A5 rs10264272 (*6)														
C/C	39	48.50 (10.9–99.14)	19.75 (5.89–49.68)	0.6084	2989.45 (1903.88–3926.77)	0.9800	17.23 (14.28–36.79)	0.8284	573.36 (454.01–1220.00)	0.0019*	19.44 (13.27–33.75)	0.9353	19.44 (13.27–33.75)	0.3424
C/T	13	28.34 (10.24–80.96)	18.78 (8.08–49.83)		2795.56 (1592.93–4893.64)		47.45 (31.60–77.42)		689.49 (342.81–962.33)		35.43 (17.57–39.32)		35.43 (17.57–39.32)	

* Significant

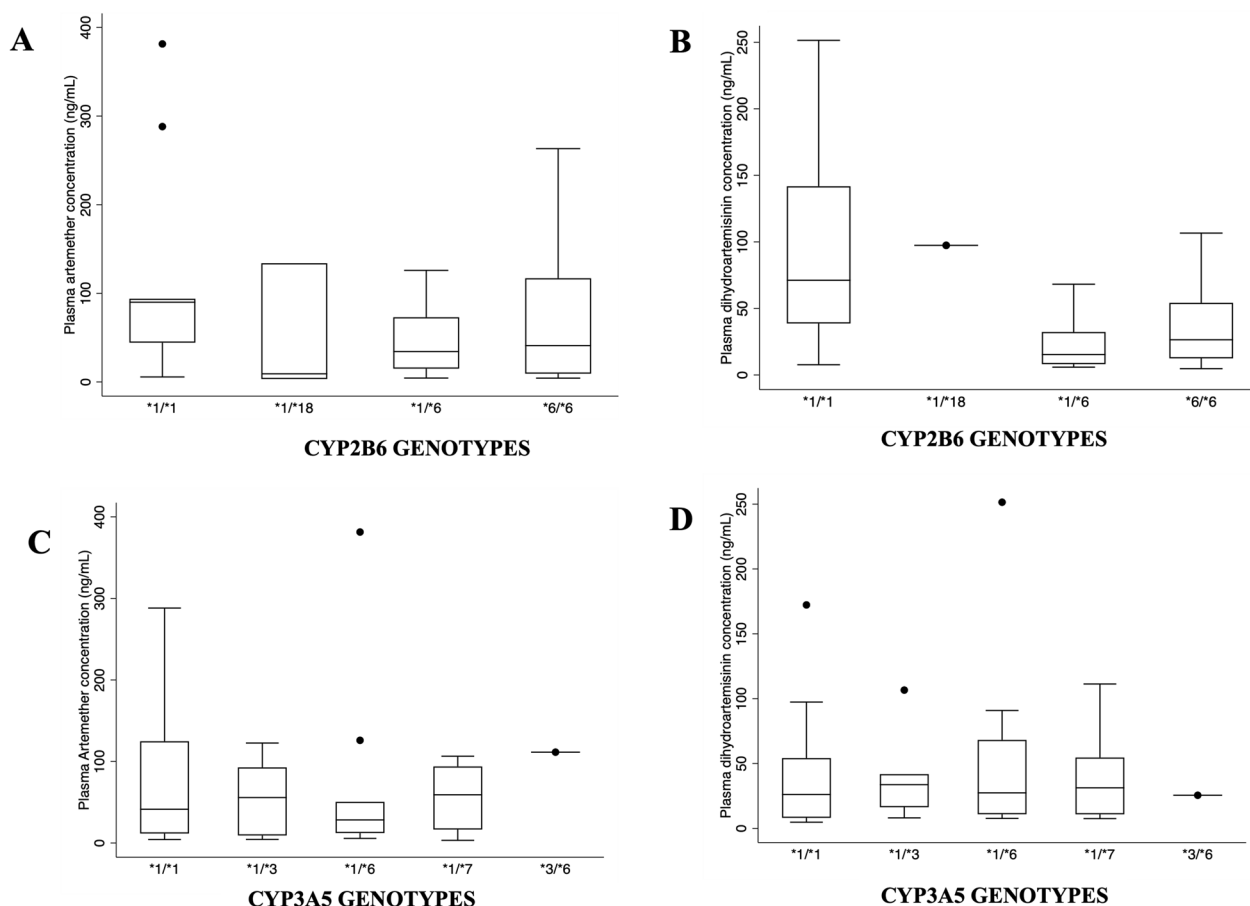


Fig. 3 Plasma drug concentration of artemether and dihydroartemisinin and CYP2B6 and CYP3A5 expressors. **A** Observed significance between *1/*6 vs *1/*1 ($p=0.039$). **B** observed significance between *1/*6 vs *1/*1 ($p=0.000$), *6/*6 + *18/*18 vs *1/*1 ($p=0.012$). **C** There were no observed significant differences. **D** There were no observed significant differences

higher for day 7 plasma lumefantrine and desbutyl lumefantrine in comparison to reduced expressors.

Figure 5 shows the plasma lumefantrine and DBL for CYP3A5 expressor status. There were significant differences in plasma DBL concentrations on day 3 between *1/*1 versus *1/*6 ($p=0.002$), *1/*3 versus *1/*6 ($p=0.006$) and *1/*7 versus *1/*6 ($p=0.008$). There was an observed significance on day 7 desbutyl plasma concentrations among *1/*6 and *1/*3 ($p=0.026$) expressors.

The linkage disequilibrium (LD) observed among CYP3A4 and CYP3A5 variants were from low to high (Fig. 6). CYP3A4*22 and CYP3A5*6 were in high LD while all the CYP3A5 variant alleles (*3, *6,*7) occur in high LD which is similar to observations in a study undertaken in pregnant women in Tanzania [36].

Discussion

The therapeutic efficacy of AL is largely dependent on the systemic bioavailability of active metabolites [25, 26]. Clinical dosing guidelines for AL are based on body

weights with a fixed artemether to lumefantrine ratio of 1:6. However, plasma drug and metabolite concentrations and their effect on parasitaemia have not been thoroughly studied in most exposed populations [37]. In Ghana, there is no data on the plasma drug and metabolite concentration for patients who are prescribed AL and how it influences parasitaemia. This study is the first to evaluate cytochrome P450 variation on plasma drug and metabolite concentration of generic AL and its effects on parasitaemia in Ghanaians. This study, therefore, report the first data on the effects of pharmacogenetic variations on plasma concentrations of artemether and lumefantrine in patients who presented with uncomplicated malaria at health facilities. The most notable finding is that although pharmacogenetic variations influence plasma concentrations of AL and its metabolites, parasitaemia significantly reduces with strict adherence to AL.

Coartem® (Novartis Pharma AG, Basel Switzerland) is the pioneer innovator of artemether lumefantrine approved by the WHO in 2006, which has been a very

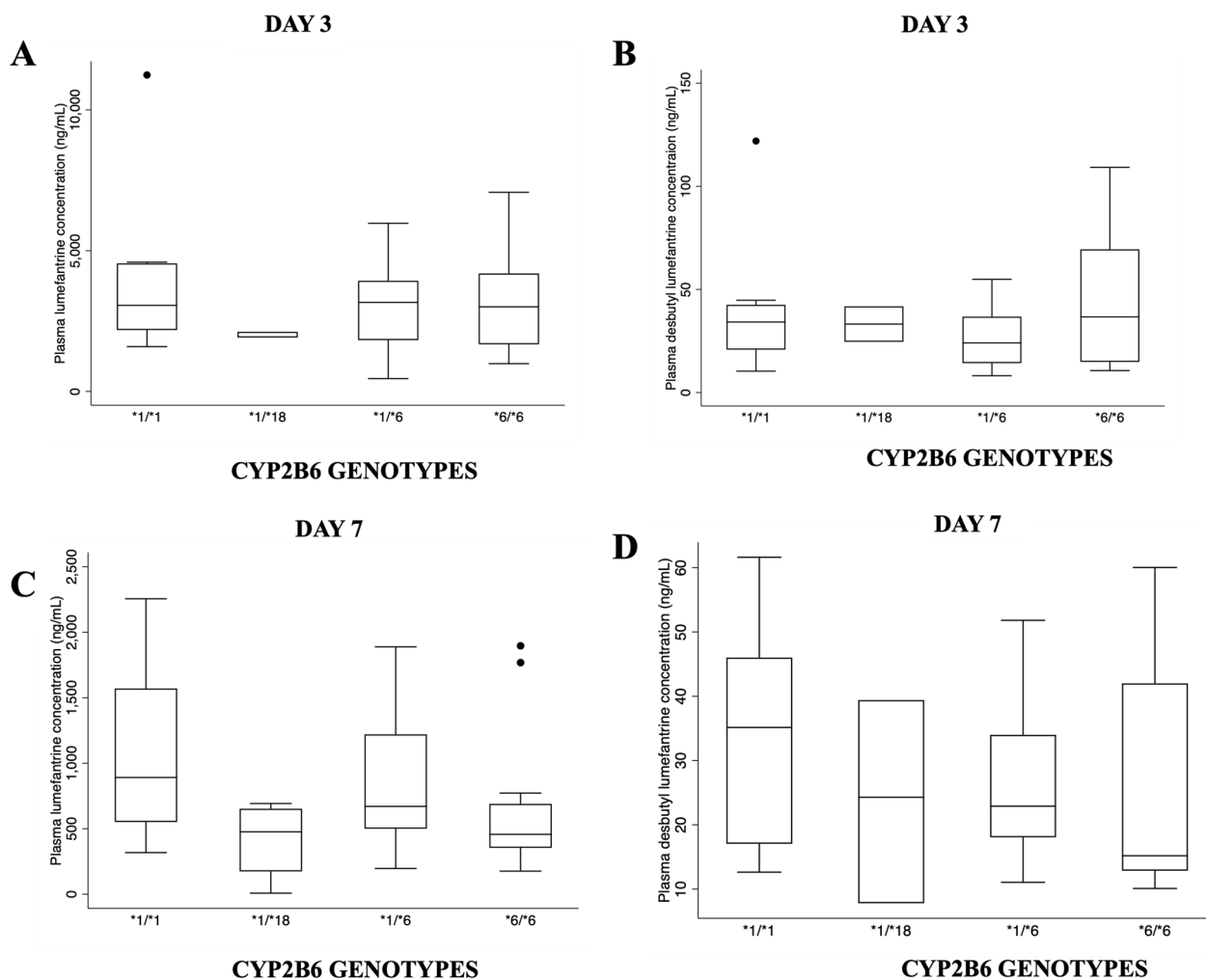


Fig. 4 Plasma drug concentration of lumefantrine and desbutyl lumefantrine and CYP2B6 expressors on day 3 and 7. **A** There were no observed significant differences. **B** There were no observed significant differences. **C** There were no observed significant differences. **D** There were no observed significant differences

effective anti-malarial drug [38]. However Coartem® is sold at an average price of US\$ 4.5–6, which poses financial barriers affecting its availability and affordability negatively [39]. Generic ALs have been prequalified to be used in the treatment of uncomplicated malaria in several African countries. In this current study, the pharmacogenetics of generic AL plasma drug and metabolite concentration and treatment outcome in uncomplicated malaria is investigated in patients who are on generic AL, which is mostly prescribed at health facilities in Ghana. Functional variants of cytochrome P450 enzymes which are responsible for AL metabolism, namely *CYP2B6*, *CYP3A4* and *CYP3A5*, were evaluated. Major findings from the study are that *CYP3A5* genotypes influences plasma AL metabolism and thus plasma concentrations whereas *CYP3A5* *3 and *6 is associated with elevated levels of AL and metabolites. While several studies

have explored effect of pharmacogenetics on AL [20, 36, 40] treatment in different populations, this study is unique in its evaluation of cytochrome P450 variation on plasma metabolite concentrations of generic AL and its effects on parasitaemia in Ghanaians. Understanding the role of pharmacogenetics in drug metabolism is crucial, especially in discerning how variations in key drug-metabolizing enzymes involved in AL metabolism might influence drug disposition and parasite clearance. This knowledge is vital for optimizing malaria treatment and ensuring effective patient care.

Artemether continues to be one of the most administered anti-malarial medications globally over the past 20 years, having been adopted by the majority of National Malaria Control Programmes in Africa [41]. Artemether metabolism is primarily mediated by *CYP2B6* [42, 43], with secondary contribution by *CYP3A4/5*. Our study

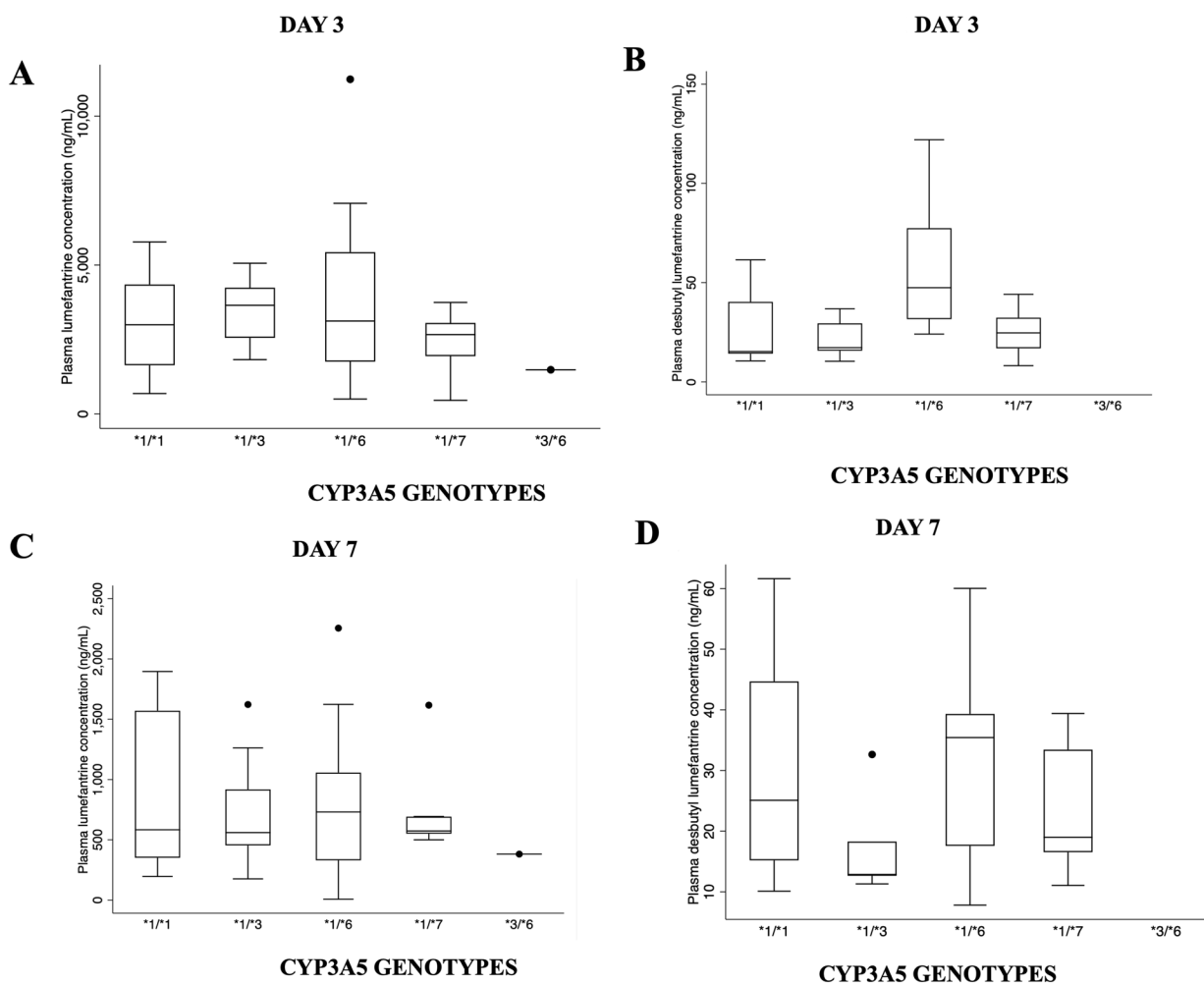


Fig. 5 Plasma drug concentration of lumefantrine and desbutyl lumefantrine and CYP3A5 expressors on day 3 and 7. **A** There were no observed significant differences. **B** Observed significance between *1/*6 vs *1/*1 ($p=0.002$), *1/*6 vs *1/*3 ($p=0.006$), *1/*7 vs *1/*6 ($p=0.008$). **C** There were no observed significant differences. **D** Observed significance between 1A/*6 vs 1A/*3 ($p=0.026$)

showed there was substantial interindividual variability among the participants. As part of the analysis and looking at the genotype frequencies obtained for *CYP2B6**6/*6 and *CYP2B6**18/*18, they were combined in our analysis since they both have similar functional effects. Day 3 and 7 plasma metabolite concentrations for variations in *CYP2B6* showed reduced artemether and DHA metabolite concentrations for the combined *CYP2B6* *6/*6+*CYP2B6* *18/*18 genotypes (Fig. 2). The effect on metabolic ratio is that these genotypes *6/*6+*18/*18 have higher metabolic ratios than *1/*1 carriers. The observation in our study is similar to a study which found a significant increase in the metabolic ratio of artemether-to-dihydroartemisinin of *CYP2B6**6/*6 volunteers over their *1/*1 counterparts [40]. There is less pharmacokinetic data on dihydroartemisinin (DHA), which is considered a very potent metabolite

of artemether, and in this study, we quantified plasma DHA and observed a median concentration range of 11.45–90.90 ng/mL. A previous study has shown declining artemether and DHA concentrations over time, with these compounds becoming undetectable at ≤ 18 h [44]. In contrast, another 12-h post-dose analysis study found the median concentration of DHA ranging from 54–158 ng/mL for patients with sensitive responses [37], which was important for recrudescence although it has a very short half-life.

Plasma lumefantrine concentration for *CYP3A5* *1/*1 was lower than other genotypes such as *1/*3, *1/*6, *1/*7 and *3/*6. There have been limited studies undertaken on *CYP3A4-CYP3A5* genotypes and their effects on AL, however, the role of *CYP3A4* and *CYP3A5* in artemether and DHA metabolism is highly elucidated [45, 46]. One of the determinants of plasma artemether

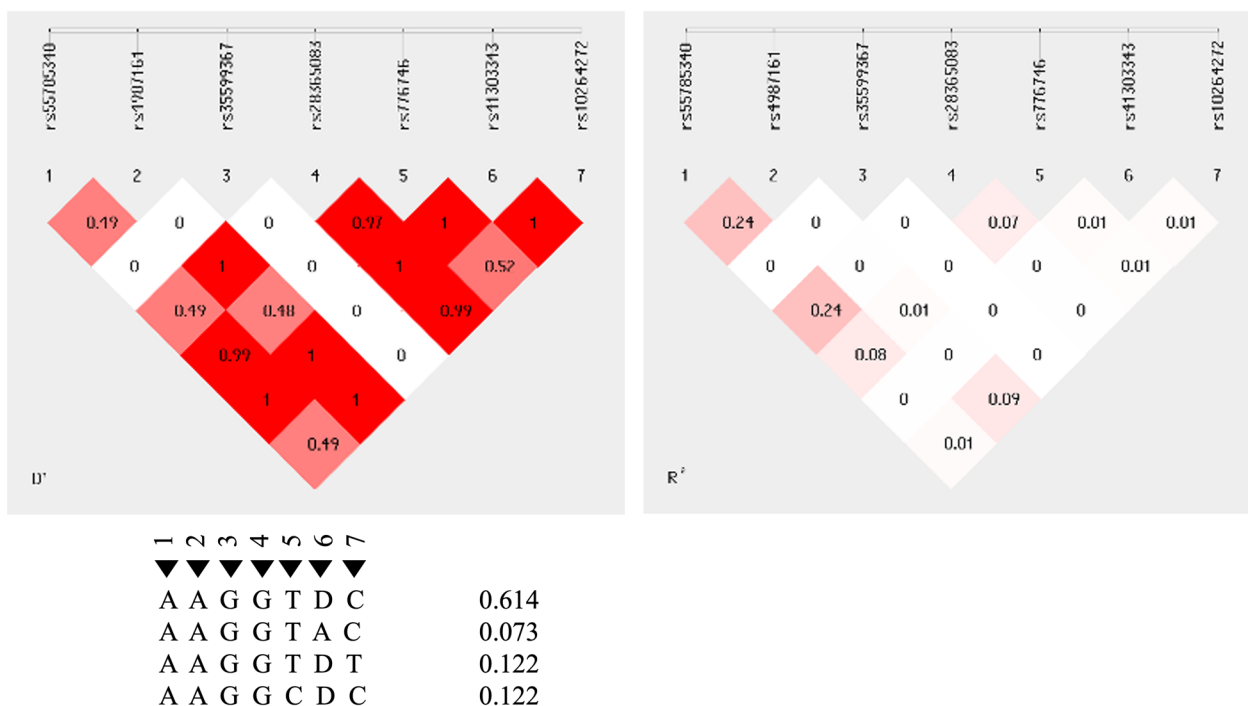


Fig. 6 Linkage disequilibrium (LD) plot of CYP3A4 (g.15713 T>C), CYP3A4 (T15615C), CYP3A4 (g.15389 C>T), CYP3A5 (C27289A), CYP3A5 (A6986G), CYP3A5 (g.14690 G>A), CYP3A5 (g.27131-27132insT) and observed D' and R' values. The pair-wise LD association between two SNPs and the corresponding D' and R' values. The colour gradient from red to white reveals higher to lower LD (D' 1–0; R' 1–0)

and LUM concentrations is the *CYP3A* pharmacogenetic status of an individual. *CYP3A4*1B* has been shown to be significantly associated with especially day 7 plasma LUM concentrations affecting treatment outcome [36]. All the *CYP3A4* genotypes studied, including *2, *17 and *22, were monomorphic and, therefore, excluded from the analysis. A previous study in Ghana also genotyped *CYP3A4*1B* and found monomorphism [47], which could mean variation in this enzyme in Ghanaian population could be limited. A future study would incorporate other *CYP3A4* variations for consideration.

Lumefantrine is the long acting component of AL and is metabolized to a more active component, desbutyl lumefantrine, which *CYP3A4/5* principally metabolizes with an extensive anti-malarial activity [17]. Lumefantrine is absorbed and cleared more slowly with a half-life of 3–4 days and its concentration accumulates with successive doses, therefore preventing recrudescence [18]. Evaluation of the data showed monomorphism in the three *CYP3A4* and *CYP3A5*2* variants, so the effect of *CYP3A5* *3, *6 and *7 variants on the plasma metabolite levels of lumefantrine from dosed generic AL were rather analysed. However, with the knowledge that *CYP2B6* is capable of metabolizing 25%–30% of currently known clinical drug substrates of *CYP3A4/5* [48, 49], analysis of the effects of *CYP2B6* variation on lumefantrine

metabolism as part of this study was undertaken. There were observed variations in *CYP2B6* genotype effects on lumefantrine and desbutyl lumefantrine concentrations (Fig. 3). The median plasma lumefantrine concentration on day 3 was 2773.74 (1401, 3811.20) ng/mL and there was significance in day 3 median plasma desbutyl lumefantrine concentration between *1/*1 carriers and *1/*6 carriers.

The influence of *CYP3A5* haplotypes on lumefantrine plasma concentrations is shown in Fig. 4. There were variable effects on the lumefantrine plasma metabolite levels where there were significant differences between *1/*1 and *1/*6 carriers. *CYP3A5* *1/*1 carriers had reduced plasma lumefantrine and DBL concentrations on day 3 compared to other variations. Similar patterns of reduced lumefantrine and DBL concentrations were observed on day 7. A recent study in Tanzania reported that *CYP3A5* *1/*1 genotypes are significantly associated with low plasma lumefantrine concentrations [50] while another study also reported defective alleles, such as *CYP3A5*3* variant, are associated with high plasma lumefantrine concentrations [51]. A previous study reported that *CYP3A5* *1/*1 genotype had a significantly higher risk of having plasma lumefantrine concentration of <600 ng/mL [36] and it is observed in this study that *1/*1 carriers had a median plasma lumefantrine concentration

of < 600 ng/mL (Fig. 5) on day 7 which may be associated with risk of recurrent parasitaemia.

Several studies have previously reported that day 7 plasma lumefantrine concentrations significantly influence recrudescence. A systematic review using individual patient data concluded that day 7 plasma concentrations of ≥ 200 ng/mL is associated with greater cure rates [52] while other studies reported that day 7 lumefantrine concentrations < 600 ng/mL is associated with treatment failure [36]. This study observed that the median day 7 plasma LUM concentrations for all generic lumefantrine medications prescribed to our patients was 578.17 (367.62–1057.01) ng/mL. Irrespective of the medication that was prescribed, there were observed significant decline in parasite density from day 2 through day 7 (Fig. 2). This implies that these generic artemisinin-based combinations prescribed could have enough anti-malarial activity to clear the malaria parasite when there is adherence to the prescribed dosage regimen.

Desbutyl-lumefantrine, the metabolite of lumefantrine, has shown greater anti-malarial potency and synergy with lumefantrine and artemisinin. A study showed DBL has effective activity against field isolates and laboratory strains of *P. falciparum* at a concentration of 15.5 ng/mL (0.6–58.20) [19]. In this study, the median plasma DBL concentration observed ranged from 17.23–47.45 ng/mL on day 3 and 14.23–35.43 ng/mL on day 7 (Table 3). From these observations, there should be a certain plasma lumefantrine concentration to reach sufficient DBL concentrations to influence treatment outcomes.

Results therefore show that pharmacogenetic variations in *CYP2B6* and *CYP3A5* influence plasma disposition of artemether and lumefantrine and likely affect malaria treatment outcome if medication is not adhered to. Knowing the significant influence of *CYP3A4* and *CYP3A5* on AL metabolism, the interaction between these two *CYP3A* genotypes may largely determine eventual plasma exposure and parasite clearance *vis-a-vis* treatment outcome. Given that *CYP3A4* and *CYP3A5* are found within the same gene block, and there could be several major haplotype combinations in the *CYP3A4-CYP3A5* block [53], observed effects in terms of plasma concentrations that might be attributed to *CYP3A4* allele might actually be due to the influence of a *CYP3A5* allele in LD. There was linkage disequilibrium (LD) among *CYP3A4* and *CYP3A5* alleles, and following some of the monomorphisms observed in *CYP3A4* in this study, it can be hypothesized that the association between *CYP3A4* and *CYP3A5* play a critical role in plasma AL concentration.

This study faced limitations, particularly in terms of sample size, which, while adequate for assessing

anti-malaria drug efficacy, could have been larger for more comprehensive results. Additionally, different brands of ACT were used and there was an uneven distribution of patients across different generic AL brands due to the study's reliance on health facilities for recruitment, leaving the researchers with no control over the specific ACT brands dispensed. Despite these challenges, the study successfully underscores the significance of pharmacogenetic variations in influencing plasma AL concentrations demonstrating the effectiveness of the generic AL brands used in this study when the dosage regimen is properly followed.

Conclusion

The study findings show that variations in *CYP2B6* and *CYP3A5* enzymes may lead to higher lumefantrine exposure across selected generic artemether–lumefantrine brands dispensed. It is, therefore, important to investigate the impact of pharmacogenetic variations, including *CYP3A* haplotypes, on artemether–lumefantrine in a larger cohort in addition to other parameters such as pregnancy, concomitant drug use and comorbidities/infections in the Ghanaian population.

Abbreviations

ACT	Artemether–lumefantrine combination therapy
RDT	Rapid detection test
AL	Artemether–lumefantrine
DHA	Dihydroartemisinin
DBL	Desbutyl-lumefantrine
AMFm	Malaria Initiative
LMIC	Low- and middle-income countries
OPD	Outpatient Department
LC–MS/MS	Liquid chromatography–tandem mass spectrometry
FDA	Food and Drug Administration
EMA	European Medical Agency
LLOQ	Lower limit of quantification

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Author contributions

NET—Conceptualization, supervision, laboratory and statistical analysis, funding, project administration writing original draft, review and editing; TK—laboratory analysis, supervision, review and editing; RPB—supervision, statistical analysis, review and editing; CD—laboratory analysis; method validation, data collection, review; ME—Supervision; review and editing GBK—Supervision, review and editing.

Author information

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Availability of data and materials

Raw data from this study is available upon reasonable request from the corresponding author.

Declarations**Ethics approval and consent to participate**

This study obtained ethical approval from the Cape Coast Teaching Hospital ethical review Board (CCTHERC/EC/2020/2020/109). This study was conducted in accordance with the Helsinki declaration and all ethical protocols followed to ensure confidentiality.

Consent for publication

Written or verbal informed consent was obtained from each participant or legal guardian.

Competing interests

The authors declare that there are no competing interests.

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