Interactions between antenatal sulfadoxine-pyrimethamine, drug-resistant *Plasmodium falciparum* parasites and delivery outcomes in Malawi

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Summary: In Malawian pregnant women with placental malaria, the presence of parasites with the SP-resistance allele *dhps* A581G was associated with lower birthweights, but antenatal SP receipt did not exacerbate the adverse consequences of malaria in pregnancy.

ABSTRACT

Background. Sulfadoxine-pyrimethamine (SP) is used as intermittent preventive therapy in pregnancy (IPTp) for malaria in sub-Saharan Africa. The resistance marker *dhps* A581G has been associated with reduced IPTp-SP efficacy and enhanced morbidity in SP-recipients.

Methods. We measured SP-resistance allele frequencies in Malawian women participating in a trial (www.isrctn.com/ISRCTN69800930) comparing IPTp with SP against intermittent screening by rapid diagnostic tests (ISTp). We genotyped PCR-detected parasites using deep sequencing of SP-resistance alleles.

Results. Among 125 placental infections, A581G-bearing parasites were associated with reduced birthweight (mean difference[MD]:252g, 95% CI:46,457, p=0.017). Relative to ISTp, IPTp-SP was associated with higher birthweights in women with wildtype parasites (MD:116g, 95% CI:-40,272; p=0.142) and lower birthweights in women with A581G-bearing parasites (MD:192g, 95% CI:-264,648; p=0.385) ($p_{interaction}$=0.033). Similar associations were noted on gestational age ($p_{interaction}$=0.075). Amongst only IPTp-SP recipients, relative to women who last received SP >4 weeks before delivery, recent SP receipt was associated with lower birthweight in women with wildtype parasites (MD:118g, 95% CI:-376,139; p=0.361) and higher birthweight in women with A581G-bearing parasites (MD:783g, 95% CI:-20,1586; p=0.054) ($p_{interaction}$=0.005).

Conclusions. The effectiveness on birthweight of IPTp-SP is compromised by A581G-bearing parasites, but there was no evidence that the adverse effects of these parasites are exacerbated by antenatal SP.

Keywords: malaria, malaria in pregnancy, placental malaria, drug resistance, prevention

Background

Nearly all malaria-endemic African countries provide intermittent preventive therapy to pregnant women (IPTp) with monthly sulfadoxine-pyrimethamine (SP) beginning in the second trimester. Antenatal receipt of SP reduces the risk of anemia, antenatal infections, placental infections, and low birth weight (LBW),[1], and its benefit on LBW is only partially attenuated [2] by widespread *Plasmodium falciparum* resistance to SP across Africa.[3, 4] Resistance to SP is conferred by mutations in the parasite genes dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*).[5] The rapid spread across Africa of *dhfr* mutations at codons 51, 59, and 108 rendered SP unsuitable for malaria case management.[6] In contrast, *dhps* mutations at codons 437, 540, and 581 have been slower to spread across African settings. In East Africa, where the A437G and K540E mutations are largely fixed, SP as IPTp has remained beneficial to protect from LBW.[2, 4]

In recent years, the *dhps* A581G allele has been more commonly reported in East Africa, where it has been directly associated in pregnant women with increased placental parasite densities,[7] reduced birthweight,[8] and, in one study in northern Tanzania, with enhanced morbidity in women receiving SP.[9] In this observational study, the receipt of SP within 4 weeks of delivery among women with placental malaria was associated with a higher density of placental parasites and a higher fraction of parasites bearing the A581G allele. Also, ecological studies[4, 10] of IPTp-SP use and LBW in areas of varying prevalence of A581G alleles suggest that high prevalences of these resistant parasites may undermine IPTp-SP efficacy as a strategy to improve birthweight.[4] Overall, however, associations between A581G-bearing parasites and SP efficacy have been inconsistent, and there exists an ongoing need to characterize better the effects of A581G alleles on delivery outcomes and how these effects modify SP's impact on birthweight.

In this study, we investigated interactions between antenatal SP receipt, SP-resistant placental parasites, and delivery outcomes. We used parasites collected in a trial of pregnant Malawian women randomized to either standard IPTp-SP or to intermittent screening during pregnancy (ISTp) with a rapid diagnostic test (RDT) followed by treatment of RDT-positive infections with dihydroartemisinin-piperaquine (DP) (www.isrctn.com/ISRCTN69800930).[11] All three study sites had measurable frequencies of the *dhps* A581G allele in women at antenatal presentation (1-3.4%). Owing to the presence of this allele and a large population of SP-unexposed women, our study was uniquely able to analyze modifications of the effect of antenatal SP on delivery outcomes by resistant parasites. We hypothesized that parasites harboring the *dhps* A581G mutation in placental infections would be associated with higher parasite densities and worse birth outcomes and would also modify the effect of SP on birthweight.

Methods

Study cohort. We used specimens collected 2011-2014 from a randomized clinical trial in Malawi.[11] In this study, HIV-seronegative women presenting for antenatal care to three sites were randomized (1:1) to ISTp-DP or IPTp-SP and followed through delivery. Overall, the median number of scheduled ANC visits and SP doses was 4. As previously reported,[11] the prevalence of PCR-positive infections was similar at baseline between ISTp-DP (44.4%) and IPTp-SP (43.0%) recipients, but higher in maternal or placental specimensat delivery in ISTp-DP recipients (30.1% v. 22.7%). Outcome assessments were as previously described for maternal, newborn, and placental measurements and molecular parasite detection.[12] For the latter, parasite densities were measured by reference to a standard curve on each reaction plate and expressed as ng/µL of strain 3D7 genomic DNA.[13]

Genotyping procedures. We used a two-stage genotyping approach: we first genotyped pools of parasites to identify those with non-fixed resistance alleles, and then we genotyped individual parasitemias within those pools with heterogeneous resistance alleles. For the first stage of pooled analyses, we defined 18 parasite populations on the basis of study arm (two), study site (three), and timing of specimen (antenatal booking, maternal peripheral blood at delivery, and placental blood) (**Supplemental Table 1**). The two largest populations (207 parasitemias each) were each divided into two, and therefore we created 20 pooled templates, each consisting of between 19 and 120 parasitemias. For genotyping in the second stage, the same gDNA specimens were used as PCR templates, but in unpooled fashion.

We amplified *P. falciparum* genes *dhfr* and *dhps* using nested PCR assays on pooled or individual gDNA templates.[14] Amplicons were prepared as barcoded sequencing libraries using the NEBNext Fast DNA Fragmentation and Library Prep Set for Ion Torrent (New England Biolabs, Ipswich, MA, USA) and Ion Xpress Barcode Adaptors (Thermo Fisher Scientific, USA). Barcoded libraries were

mixed in equimolar amounts by gene target and sequenced on an Ion Torrent PGM platform using 318 chips.

Sequence analyses. All *dhfr* and *dhps* reads were analyzed in Galaxy (usegalaxy.org):[15-17] reads were first aligned to 3D7 reference sequences for either *dhfr* (XM_001351443) or *dhps* (Z30654) using Bowtie2, and then variants at each position were quantified using MPileup. For quality filtering of allele frequencies, we allowed reads of any quality to be mapped to reference sequences but analyzed within those reads only bases with quality scores >q33 for *dhfr* or >q29 for *dhps*.[18] This enforces stringent quality while limiting false-discovery, resulting in expected per-base error probabilities of less than $5x10^{-4}$ (for *dhfr*) and $1.3x10^{-3}$ (for *dhps*). At resistance loci, the mutant allele frequency was defined as the proportion of reads at that locus that harbored the nucleotide substitution encoding the amino acid substitution conferring resistance; we censored frequencies in pooled parasitemias < 1% to mitigate the risk of false-discovery.[18] Read processing was performed by personnel masked to study data.

Statistical analyses. We used Poisson regression using robust standard errors to compute Prevalence Ratios (PRs) on dichotomous outcomes and linear regression to compute mean differences on continuous outcomes and 95% confidence intervals (CIs) for each. Results are presented for crude models, for multivariable models, adjustment for treatment arm, gravidity, maternal underweight (body-mass index < 18.5 kg/m^2), maternal bednet use the night before enrollment and conditioned on resistance allele using interaction terms. We estimated the significance of regression interaction terms between treatment arm and resistance allele using the Wald test. We performed sensitivity analyses of models on gestational age and birthweight by re-computing models after re-setting an outlier value for each variable to the lowest value of the 99^{th} percentile of the remainder values. We used the Kruskal-Wallis test to compare parasite densities. A p-value of <0.05 was considered statistically significant. All analyses were conducted in Stata/SE (v14.2, Stata Corp).

Ethics. Written informed consent was obtained from all participants before enrollment. Ethical approval was obtained from the Malawian National Health Science Research Committee and the Liverpool School of Tropical Medicine; molecular testing of parasites was approved by the ethical review boards of the University of North Carolina and Duke University.

Results

Frequencies of dhfr *and* dhps *mutations in pooled parasite populations.* Median (Interquartile Range [IQR]) read depth at loci of interest was 1,251 (427, 2583) for *dhfr* and 2,397 (1351, 3781) for *dhps* (**Figure 1A, 1B**). Similar to parasites collected at antenatal booking,[11] across each study site, study arm, and specimen type, allele frequencies exceeded 92% for the *dhfr* substitutions N51I, C59R, and S108N, and 97% for the *dhps* substitutions A437G and K540E. (**Supplemental Tables 2 & 3**). We did not observe any pools harboring the *dhfr* I164L or the *dhps* I431V mutations.

The frequency of the *dhps* A581G mutation ranged between 0 and 23% between the 20 pools of parasites (**Figure 1C**) (antenatal booking: 0.6-6.5%, delivery: 0.1-2.5% in maternal peripheral parasites and 0-23.1% in placental parasites).

Frequencies of dhfr *and* dhps *mutations in individual placental parasitemias*. We genotyped all individual placental parasites across *dhfr* and *dhps* loci from Mpemba, the study site with the highest frequency of *dhps* A581G alleles in placental parasites. At this site, the frequency of A581G alleles was similar at enrollment between IPTp-SP (4.3%) and ISTp-DP (3.1%) recipients. In placental samples, 144 women had PCR-detectable parasites (IPTp-SP: 65, ISTp-DP: 79); after censoring samples with fewer than ten reads, we obtained allele frequencies for 121 and 125 parasitemias for loci in *dhfr* or *dhps*, respectively. Amongst women with placental infections, compared to those with genotype data, women missing genotype data did not differ significantly in randomization group or gravidity, but did have lower median placental parasite densities (0.14×10^{-3} ng/μL versus 0.02×10^{-3} ng/μL; p=0.0037 by Kruskal-Wallis).

In 125 placental infections, mean (SD) frequencies were high for the *dhfr* substitutions N51I (0.993 [0.05]), C59R (0.987 [0.09]), and S108N (0.999 [0.002]), and all less than 0.001 for

I164L. Similarly, in 121 placental infections, mean (SD) frequencies were high for the *dhps* substitutions A437G (0.998 [0.01]) and K540E (0.999 [0.011]) and less than 0.001 for I431V. In contrast, the overall mean (SD) frequency of the A581G substitution was 0.037 (0.15) (**Figure 1D**), and the prevalence of the A581G mutation was 14.4% (18/125) among placental infections. Among these 18 placental infections, the median (IQR) allele frequency was 0.20 (0.001, 0.464).

Associations of mutant alleles and clinical factors. Enrollment characteristics were similar in Mpemba between women who ultimately had placental infections with *dhps* A581G or wildtype parasites (**Table 1**). The prevalence of the A581G mutation in placental parasites was similar between women who had received IPTp-SP (9/58, 16%) and ISTp-DP (9/67, 13%) (PR=0.87; 95% CI 0.34-2.18, p=0.760). Amongst women infected with placental parasites bearing the A581G allele (n=18), there was no evidence of differences in median (IQR) allele frequencies between those who had received IPTp-SP (0.002 [0.001-0.273] or ISTp-DP: (0.311 [0.001-0.464]; p=0.354 by Kruskal-Wallis).

Effect of IPTp-SP vs. ISTp-DP by dhps*581 alleles*. Prior studies have suggested that A581G-bearing parasites modify the effect of antenatal SP to exacerbate placental morbidity.[9] We, therefore, tested among women with placental infection if the effects of the antenatal prevention strategy on delivery outcomes were modified by A581G-bearing placental parasites. Among these women in whom antenatal strategies failed to prevent placental malaria, compared to women allocated to ISTp-DP, receipt of IPTp-SP resulted in a non-significant 63g increase in birthweight (95% CI -87, 213; p=0.408) (**Table 2**). When conditioned on allele, IPTp-SP was associated with a 116g increase (95% CI -40, 272; p=0.142) in women with wildtype parasites and a 192g decrease (95% CI -648, 264; p=0.385) in women with A581G-bearing parasites (**Figure 2**) and this difference in effect was statistically significant ($p_{interaction}$=0.033). Furthermore, relative to ISTp-DP, IPTp-SP was associated with increased gestational age at delivery in those infected with wildtype

parasites (Mean Difference [MD]=0.64 weeks; 95% CI: 0.1, 1.2; p=0.032) but not among those infected with A581G-bearing parasites (MD=-0.44; 95% CI -2.7, 1.8; p=0.677) ($p_{interaction}$=0.075). We did not observe modification of the effect of antenatal SP receipt by allele on placental parasite density, placental inflammation, maternal hemoglobin concentration, or weight-for-age Z-score. (**Table 2**).

Difference in outcomes by dhps*581 alleles among SP recipients*. Among the 55 women with placental infections who received IPTp-SP, the mean (SD) birthweight among 9 women infected with parasites carrying the A581G mutation were significantly lower than those among 46 women infected with wildtype parasites: 2606gr (596) vs. 3018g (418), MD=412gr (84, 740), p=0.015 (**Table 2**). This reflected a difference in gestational age (MD=1.24 weeks, -0.04, 2.52, p=0.057) rather than z-score for weight-for-gestational age (MD=0.30, -0.30, 0.96, p=0.292) (**Table 2).**

Timing of SP and outcome by dhps*581 alleles.* We next tested if SP receipt within four weeks prior to delivery modified the association of the A581G allele with birth outcomes (**Table 3**). The median (IQR) number of days since the last dose of SP prior to delivery was similar between women infected with wildtype (n=49, 20 [11, 33] days) and A581G-bearing parasites (n=9, 23 [14, 30] days; p=0.940 by Kruskal-Wallis). Overall, the timing of SP receipt did not modify the effect of SP on mean (SD) birthweight (SP≥28 days: n=20, 2941g [610] vs <28 days: n=35, 2956g [379], MD=15g, -252, 282, p=0.911). However, this differed significantly by 581 allele: recent SP receipt <28 days before delivery was associated with a 783g increase in mean birthweight (95% CI -20, 1586, p=0.054) among the 9 women infected with A581G-bearing parasites and a 118gr decrease (-139, 375, p=0.361) among the 46 women infected with wildtype parasites ($p_{interaction}$=0.005) (**Table 3**, **Figure 2**). Similar effect modification by the timing of SP use and 581 allele was found for gestational age; recent SP intake was associated with longer duration of pregnancies (P=0.037) among women infected with parasites carrying the A581G mutation, but not among women infected

with wildtype parasites (p=0.590) ($p_{interaction}$=0.001). In addition, we did not observe associations between A581G-bearing parasites and significant increases in parasite density or intervillous inflammation with recent SP use (**Table 3**). Collectively, these findings suggest that recent SP use was not associated with increased morbidity among women carrying the A581G-bearing parasites at delivery.

Discussion

We investigated the impact of molecular SP resistance among *P falciparum* parasites on the effectiveness of IPTp-SP for antenatal malaria prevention in a large cohort of pregnant women in southern Malawi. Consistent with prior studies of antenatal malaria, parasites harboring the *dhps* A581G allele in placental infections were associated with reduced birth weight in IPTp-SP recipients relative to birthweights among IPTp-SP recipients infected with less resistant parasites; this observation was consistent with a similar association with a shorter gestational age. However, contrary to the previous trial in northern Tanzania suggesting SP use in the four weeks prior to delivery was associated with increased parasite densities and adverse pregnancy outcomes,[9] we found no such associations. Therefore, although failure of SP among women infected with parasites harboring the *dhps* A581G allele may manifest as placental infection with resistant parasites that are associated with worse birth outcomes, there was no evidence that recent SP use exacerbated malaria-associated morbidity.

Amongst recipients of SP with placental malaria, the presence of the *dhps* A581G allele was associated with a reduction in birthweight of 412g (**Table 2**). This association of the *dhps* A581G allele with reduced birthweight adds to the growing literature from antenatal studies,[8] delivery series,[7, 9] and ecological studies[4, 10] that parasites bearing the A581G allele partially undermine the improvements in birthweight following IPTp-SP. Notably, these associations were present despite the relatively low abundance of A581G alleles within placental infections: in 16 of the 18 women infected with A581G-bearing parasites, the A581G allele frequency within the placental infection was less than 50%, indicating minority variants within mixed populations (**Figure 1D**). Given the more frequent reporting of this mutation in the past decade,[3] these results further underscore the need to identify alternatives to IPTp-SP that can mitigate the deleterious effects of antenatal malaria.

Our study design, which included an SP-unexposed population, allowed us to investigate if the A581G allele modified the effect of SP use on birthweight among women with placental infections. Compared with SP-unexposed women in the ISTp arm, the effect of IPTp-SP on mean birthweight differed significantly between women infected with wildtype parasites (a 116g increased relative to the ISTp arm) and those infected with A581G-bearing parasites (a 192 decrease) (p=0.033) (**Table 2**). Additionally, we observed similar interactions between the allele and SP receipt on gestational age at delivery (interaction p=0.075). Although this agrees with a prior reported study,[19] these results were largely driven by a single outlier birth of a 1,250g viable infant at 30 weeks gestation to a mother who received only a single dose of SP; in a sensitivity analysis of these interactions in which this participant's birthweight and gestational age were set to the lower limit of the 99th percentile of the overall distribution of each variable, these interactions remained but were not statistically significant (**Supplemental Table 4**). Taken together, these observations suggest that the lower birthweights observed in women infected with parasites harboring the A581G mutation in the SP recipients was driven by shorter gestation in these women rather than intrauterine growth retardation.

We did not observe the previously-reported phenomenon whereby the receipt of SP in the 4 weeks prior to delivery exacerbated the pathology of A581G-bearing parasites (**Table 3**).[9] On the contrary, we found that recent SP use was associated with higher mean birthweight and longer gestation among women infected with parasites harboring the *dhps* A581G allele at delivery, and these findings were maintained in sensitivity analyses (**Supplemental Table 5**). In addition, there was no evidence that recent SP use modified the effect of SP on parasite density, intervillous inflammation, malaria pigment, or maternal hemoglobin concentrations **(Table 3)** in women harboring A581G-bearing parasites, each of which could provide a mechanism for the previously observed association with reduced birthweight. Collectively therefore, there was no evidence that

SP use exacerbates the adverse effect of malaria infections in women infected with highly resistant parasites.

Several other observations were notable. Firstly, other than the Mpemba site, we did not observe consistent increases from enrollment to delivery in the frequency of SP-resistance alleles. This is surprising, given the presence at baseline of parasites bearing the A581G mutation, the large mean number of SP doses received per woman (3.3) in the IPTp-SP group, suboptimal efficacy of SP to clear parasites in Malawi,[2] the high prevalence of "breakthrough" parasites at delivery, andreported selection during pregnancy for drug resistance mutations in *dhfr* by SP[20, 21] and *pfmdr1* by mefloquine.[22] This lack of clear increase in A581G alleles may be the result of our ecological approach to genotyping, in which we compared pairings of allele frequencies between only 3 sites, or more mild selection on the A581G allele by sulfadoxine compared with that on *dhfr* alleles by pyrimethamine. Additionally, there was an absence of the *dhfr* I164L substitution, which confers a higher degree of pyrimethamine resistance and, for obscure reasons, has remained rare in African settings, as well as the lack of appearance of the *dhps* I431V substitution, which has been reported in West Africa in association with the A581G substitution,[23, 24] but is of uncertain clinical significance.

Why did pooled genotyping fail to detect any A581G alleles in the Mpemba placental specimens from ISTp-DP recipients? (**Figure 1C**) When tested individually, we identified A581G-bearing parasites in 9/66 of these placental specimens. To explore this, for each parasitemia, we computed the product of the molecular parasite density and the proportion of wildtype and mutant allele at codon 581; we then summed these absolute allele densities for each parasitemia within the ISTp group and computed an estimated proportion of alleles. Using this approach, we estimated that parasites bearing the A581G allele comprised 8.62% of the pooled population of placental parasites amongst IPTp recipients, but only 0.04% of

those parasites pooled from women who received ISTp. In prior applications,[18, 25] pooled amplification and sequencing has not been demonstrated to be sensitive to minority variants comprising below 1% of a mixed infection.

Our study has several limitations. Common *dhfr* and *dhps* mutant alleles are nearly fixed in Malawi, and we were able to analyze the effect of SP resistance only as a function of A581G allele frequency and only in one study site. However, the epidemiology of SP resistance markers in Malawi is similar to that in other settings in East and Southern Africa, enhancing the generalizability of our findings in this part of Africa. As noted above, some subgroup analyses were limited by the very few women infected with the A581G allele and with the specified co-incident outcomes. Therefore we analyzed a range of delivery outcomes to assess for consistency between effects. Finally, errors in Ion Torrent sequencing could have biased results, particularly if error rates are associated with template abundance; to mitigate this known risk, we only interrogated known resistance loci and analyzed only high-quality base calls.

Our results indicate that the effectiveness of IPTp with SP is compromised in women infected with A581G-bearing parasites. However, there was no evidence that SP use exacerbates the adverse effect of malaria infections in women infected with these resistant parasites. These findings, together with reports of the increased spread of this allele[3] and of its association in ecological studies with a loss of IPTp-SP efficacy to prevent LBW,[4] underscore the necessity to identify alternative strategies to IPTp-SP for the prevention of malaria-associated LBW in Africa.

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Conflicts of Interest. All authors declare that they have no real or perceived potential conflicts of interest relevant to this work.

Previous Presentations. Preliminary results of the pooled genotyping data were presented by Dr. Taylor at the annual meeting of the American Society of Tropical Medicine and Hygiene in November 2016, in Atlanta, GA, USA.

Table 1. Maternal characteristics at antenatal enrollment among women in Mpemba with placental malaria by *dhps* codon 581 allele

	Placental parasites wild-type A581 (n=107)	**Placental parasites with A581G (n=18)**	**p-value[a]**
Allocated to IPTp-SP, % (n/N)	45.8 (49/107)	50 (9/18)	0.741
Paucigravidae, %, (n/N)	67.3 (72/107)	83 (15/18)	0.171
Maternal age, y, mean (SD)	21.9 (4.9)	20.9 (3.9)	0.413
Gestational age at enrollment, days, mean (SD)	146 (23)	147 (18)	0.872
Maternal weight, kg, mean (SD)	54.0 (6.0)	53.1 (5.0)	
Maternal height, cm, mean (SD)	153.7 (5.9)	153.4 (5.9)	0.890
Maternal BMI < 18.5 kg/m^2, % (n/N)	1.9 (2/107)	0	0.559
Used bednet last night, % (n/N)	4.7 (5/107)	6 (1/18)	0.871
P. falciparum infection at enrollment,[b] %, (n/N)	55.1 (59/107)	39 (7/19)	0.201

[a] Computed either with the chi-squared test or the student's t-test.

[b] Positive by either light microscopy or PCR..

Table 2. Comparisons of clinical and parasitological variables among women with PCR-positive placental malaria parasites in Mpemba study site with and without the *dhps* A581G mutation by study arm

	All women	**ISTp-DP**	**IPTp-SP**	**Mean difference (95% CI)**	**p-value[a]**	**Interaction term[b]**
Birthweight No., mean (SD), g						
All infected women	121, 2916 (415)	66, 2887 (364)	55, 2950 (471)	63 (-87, 213)	0.408	
dhps A581	103, 2953 (399)	57, 2901 (379)	46, 3018 (418)	116 (-40, 272)	0.142	
dhps A581G	18, 2702 (454)	9, 2797 (248)	9, 2606 (596)	-192 (-648, 264)	0.385	
Mean difference (95% CI)	-252 (-457, -46)	-104 (-365, 158)	-412 (-740, -84)			
p-value[a]	0.017	0.432	0.015			
						0.033

Parasite density,						
No., median (IQR), ng/uL x 10-3						
All infected women	125, 0.14 (0.03, 2.5)	67, 0.27 (0.04, 4.1)	58, 0.10 (0.03, 0.7)		0.110	
dhps A581	107, 0.13 (0.02, 2.5)	58, 0.26 (0.04, 4.1)	49, 0.09 (0.03, 0.6)		0.086	
dhps A581G	18, 0.26 (0.03, 3.0)	9, 0.27 (0.05, 0.99)	9, 0.11 (0.04, 3.0)		0.895	
p-value[a]	0.288	0.269	0.672			
						0.306
Intervillous inflammation leukocytes ≥ 5						
%, n/N						
All infected women	38 (47/124)	38 (25/66)	38 (22/58)		0.995	
dhps A581	40 (42/106)	40 (23/57)	39 (19/49)		0.869	
dhps A581G	28 (5/18)	22 (2/9)	33 (3/9)		0.599	
p-value[a]	0.338	0.297	0.757			
						0.769
Maternal hemoglobin at delivery						
No.,mean (SD), g/dL						
All infected women	125, 12.1 (1.7)	67, 11.9 (1.8)	58, 12.2 (1.6)	0.3 (-0.4, 0.9)	0.432	
dhps A581	107, 12.0 (1.6)	58, 11.9 (1.8)	49, 12.1 (1.4)	0.2 (-0.4, 0.8)	0.579	

dhps A581G	18, 12.5 (2.3)	9, 12.2 (2.2)	9, 12.8 (2.6)	0.6 (-1.8, 3.0)	0.610
Mean difference (95% CI)	0.5 (-0.4, 1.4)	0.3 (-1.0, 1.6)	0.7 (-0.4, 1.9)		
p-value[a]	0.237	0.643	0.219		
					0.538
Gestational age at delivery					
No.,mean (SD), weeks					
All infected women	122, 37.6 (1.6)	66, 37.3 (1.5)	56, 37.8 (1.8)	0.46 (-0.1, 1.0)	0.123
dhps A581	104, 37.7 (1.5)	57, 37.4 (1.5)	47, 38.0 (1.4)	0.64 (0.1, 1.2)	0.032
dhps A581G	18, 37 (2.2)	9, 37.2 (1.0)	9, 36.8 (3.0)	-0.44 (-2.7, 1.8)	0.677
Mean difference (95% CI)	-0.67 (-1.5, 0.2)	-0.16 (-1.2, 0.9)	-1.24 (-2.5, 0.04)		
p-value[a]	0.106	0.756	0.057		
					0.075
Weight-for-age Z-score					
No., mean (SD)					
All infected women	121, 0.26 (0.87)	66, 0.23 (0.88)	55, 0.30 (0.86)	0.07 (-0.24, 0.39)	0.650
dhps A581	103, 0.31 (0.90)	57, 0.28 (0.93)	46, 0.36 (0.87)	0.08 (-0.28, 0.43)	0.674
dhps A581G	18, -0.04 (0.66)	9, -0.10 (0.45)	9, 0.02 (0.85)	0.12 (-0.56, 0.80)	0.706
Mean difference (95% CI)	-0.35 (-0.79, 0.08)	-0.23 (-0.45, 0.25)	-0.30 (-0.96, 0.30)		

p-value[a]	0.113	0.232	0.292	
				0.427

CI: confidence interval; SD: standard deviation; g: grams; dL: deciliter; IQR: interquartile range; ISTp-DP: Intermittent screening during pregnancy and treatment with dihydroartemisinin-piperaquine; IPTp-SP: Intermittent preventive therapy during pregnancy with sulfadoxine-pyrimethamine.

[a] Computed by linear regression or the Kruskall-Wallis test (for continuous variables) or the chi-squared test (for categorical variables).

[b] Computed by the Wald test.

Table 3. Effect of *dhps* A581G mutation on the impact of recent SP on birth outcomes among women receiving IPTp-SP

	All women	SP ≥ 28 days before delivery	SP < 28 days before delivery	Mean difference (95% CI)	p-value[a]	Interaction term[b]
Birthweight						
No., mean (SD), g						
Overall	55, 2950 (471)	20, 2941 (610)	35, 2956 (379)	15 (-252, 282)	0.911	
dhps A581	46, 3018 (418)	17, 3092 (463)	29, 2974 (390)	-118 (-375, 139)	0.361	
dhps A581G	9, 2606 (596)	3, 2083 (722)	6, 2867 (339)	783 (-20, 1586)	0.054	
Mean difference (95% CI)	-412 (-740, -84)	-1009 (-1665, -353)	-107 (-457, 242)			
p-value[a]	0.015	0.005	0.536			
						0.005
Parasite density						
No., median (IQR), ng/µL x 10^{-3}						
Overall	58, 0.10 (0.03, 0.70)	22, 0.09 (0.03, 3.4)	36, 0.10 (0.03, 0.53)		0.737	
dhps A581	49, 0.09 (0.03, 0.60)	19, 0.11 (0.02, 3.4)	30, 0.08 (0.03, 0.31)		0.594	
dhps A581G	9, 0.11 (0.04, 3.0)	3, 0.04 (0.03, 125)	6, 1.10 (0.04, 3.0)		0.796	
p-value[a]	0.269	0.811	0.188			
						0.615

Intervillous inflammation leukocytes ≥ 5						
%, n/N						
Overall	38 (22/58)	36 (8/22)	39 (14/36)		0.847	
dhps A581	39 (19/49)	42 (8/19)	37 (11/30)		0.703	
dhps A581G	33 (3/9)	0 (0/3)	50 (3/6)		0.134	
p-value[a]	0.757	0.159	0.541			
						0.811
Maternal hemoglobin at delivery						
No.,mean (SD), g/dL						
Overall	58, 12.2 (1.6)	22, 12.1 (1.4)	36, 12.8 (2.6)	0.35 (-0.53, 1.2)	0.427	
dhps A581	49, 12.1 (1.4)	19, 12.0 (1.1)	30, 12.1 (1.5)	0.08 (-0.73, 0.89)	0.836	
dhps A581G	9, 12.8 (2.6)	3, 11.6 (0.9)	6, 13.4 (3.1)	1.75 (-2.7, 6.2)	0.379	
Mean difference (95% CI)	0.72 (-0.44, 1.9)	-0.39 (-1.8, 0.99)	1.3 (-0.40, 2.9)			
p-value[a]	0.219	0.560	0.130			
						0.273
Gestational age at delivery						
No., mean (SD), weeks						
Overall	56, 37.8 (1.8)	21, 37.6 (2.3)	35, 38.0 (1.4)	0.40 (-0.60, 1.4)	0.426	

dhps A581	47, 38.0 (1.4)	18, 38.2 (1.5)	29, 37.9 (1.4)	0.24 (-1.1, 0.64)	0.590	
dhps A581G	9, 36.8 (3.0)	3, 34.0 (3.5)	6, 38.2 (1.6)	4.2 (0.33, 8.0)	0.037	
Mean difference (95% CI)	-1.2 (-2.5, 0.04)	-4.2 (-6.6, -1.8)	0.24 (-1.1, 1.5)			
p-value[a]	0.057	0.002	0.714			
						0.001
Weight-for-age Z-score						
No., mean (SD)						
Overall	55, 0.30 (0.9)	20, 0.31 (0.9)	35, 0.30 (0.8)	-0.01 (-0.50, 0.48)	0.967	
dhps A581	46, 0.36 (0.9)	17, 0.47 (0.8)	29, 0.29 (0.9)	-0.18 (-0.72, 0.35)	0.495	
dhps A581G	9, 0.02 (0.8)	3, -0.62 (1.2)	6, 0.34 (0.4)	0.96 (-0.28, 2.2)	0.109	
Mean difference (95% CI)	-0.33 (-0.96, 0.30)	-1.1 (-2.3, 0.07)	0.05 (-0.71, 0.82)			
p-value[a]	0.292	0.064	0.886			
						0.254

CI: confidence interval; SD: standard deviation; g: grams; dL: deciliter; IQR: interquartile range; SP: sulfadoxine-pyrimethamine.

[a] Computed by linear regression or the Kruskall-Wallis test (for continuous variables) or the chi-squared test (for categorical variables).

[b] Computed by the Wald test.

Figure 1. Frequencies of *P. falciparum dhps* A581G alleles in pooled (A) and individual placental (B) parasite templates.

A. Frequencies of *dhps* A581G allele in pooled parasitemias, by study site, study arm, and specimen type. * The sequencing of pooled maternal peripheral parasites from Chikwawa IPTp-SP recipients failed to yield analyzable reads.

B. Frequency distribution within individual placental parasitemias of *dhps* A581G alleles in participants receiving ISTp-DP or IPTp-SP in the Mpemba site

Figure 2. Comparisons of continuous delivery outcomes among women with PCR-positive placental malaria parasites with and without the *dhps* A581G mutation by study arm and, among SP recipients, by timing of most recent SP dose

White boxes: *dhps* A581; Dark gray boxes: *dhps* A581G. Thick line within each plot indicates median, dotted lines are quartiles. Overlying dots are individual values. Vertical dotted line on each sub-graph indicates overall population mean.

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Figure 1

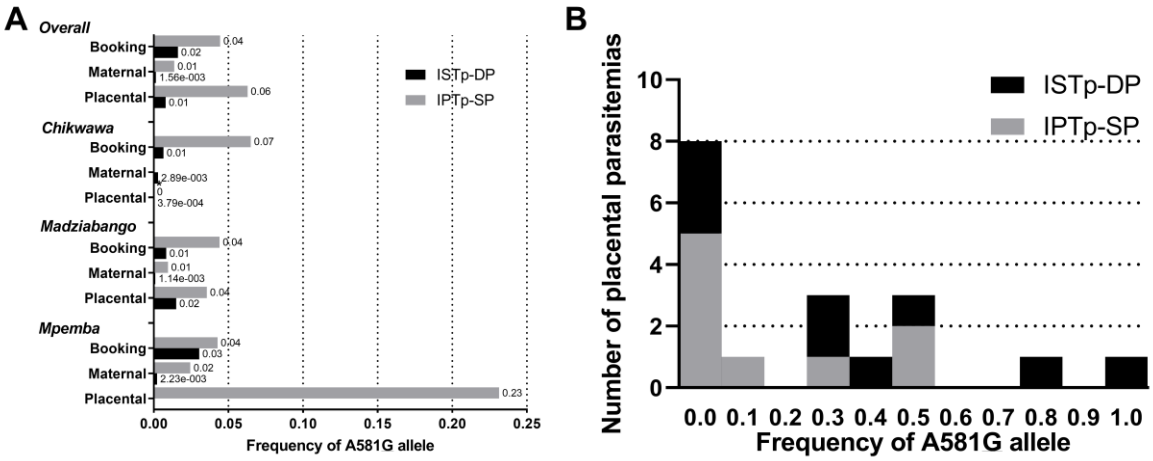

Figure 2