Systematic Review

Influence of malaria transmission intensity and the 581G mutation on the efficacy of intermittent preventive treatment in pregnancy: systematic review and meta-analysis

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Abstract

OBJECTIVES To estimate where intermittent preventive treatment (IPTp) using sulphadoxine–pyrimethamine (SP) could be withdrawn as an intervention due to declining malaria transmission intensity, or due to increasing prevalence of the *Plasmodium falciparum* dihydropteroate synthetase resistance mutation at codon 581G.

METHODS We conducted a systematic review and meta-analysis of protection against the incidence of low birth weight (LBW) conferred by ≥2 doses of IPTp-SP. We matched these outcomes to a proxy measure of malaria incidence in women of the same studies, applied meta-regression models to these data and conducted sensitivity analysis of the 581G mutation.

RESULTS Variation in the protective effect of IPTp-SP against LBW could not be explained by malaria transmission intensity. Among primi- and secundigravidae, IPTp-SP protected against LBW where 581G was ≤10.1% [odds ratio (OR): 0.49; 95% confidence intervals (CI): 0.29, 0.81; P = <0.01] and 581G was >10.1% (OR = 0.73; 95% CI: 0.29, 1.81; P = 0.03). Random-effects models among multigravidae showed that IPTp-SP protects against LBW where 581G was ≤10.1% (OR = 0.56; 95% CI: 0.37, 0.86; P = 0.07), a finding of borderline statistical significance. No evidence of protection against LBW was observed where 581G was >10.1% (OR = 0.96; 95% CI: 0.70, 1.34; P = 0.47).

CONCLUSION There appears to be a prevalence of 581G above which IPTp-SP no longer protects against LBW. Pregnancy studies are urgently needed where 581G is >10.1% to define the specific prevalence threshold where new strategies should be deployed.

keywords malaria , pregnancy, sulphadoxine–pyrimethamine, drug resistance, transmission, sub-Saharan

Introduction

The World Health Organization (WHO) recommends the provision of intermittent preventive treatment (IPTp) using sulphadoxine–pyrimethamine (SP) at every scheduled antenatal care visit, from the second trimester until delivery, in areas of moderate (stable) to high transmission to protect pregnant women against the adverse consequences of malaria infection. Although sulphadoxine and pyrimethamine are synergistic and produce complimentary inhibition, several mutations are associated with decreased parasite sensitivity. Parasite resistance to SP is associated with mutation on the dihydrofolate reductase (*Pfdhfr*) and the dihydropteroate synthetase (*Pfdhps*) genes. Three *Pfdhfr* mutations, namely N51I, C59R and S108N, are commonly referred to as the triple mutation; *Pfdhps* mutations A437G and G540E are often described as the double mutation. Collectively, these constitute the quintuple mutations and compromise SP efficacy [1–3]. The effectiveness of IPTp-SP to prevent low birth weight (LBW) decreases with increasing population prevalence of *Pfdhps* K540E mutation – a proxy for the quintuple *Pfdhfr* and *Pfdhps* mutant – although some beneficial effect on birth

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weight remains evident in areas where *Pfdhps* K540E is even above 90% [4]. Importantly, however, IPTp-SP fails to inhibit parasite growth where the *Pfdhps* A581G mutation has emerged alongside the *Pfdhfr* and *Pfdhps* quintuple mutant to produce 'sextuple' mutant parasites [5]. Thus, the population prevalence of 581G has assumed a central role in IPTp-SP policy discussions [6].

With historic reductions in malaria transmission observed in some countries of sub-Saharan Africa over the past decade and increasing parasite resistance to SP during the same time period, two questions have come to the forefront related to IPTp-SP. Is there a level of malaria transmission intensity below which IPTp-SP no longer prevents LBW at the population level and can be withdrawn? Secondly, is there a prevalence threshold of 581G which renders falciparum parasites 'super resistant' to SP and above which IPTp-SP is no longer protective against LBW and can be withdrawn?

To answer these questions, we delineated three objectives for this study. The first objective was to conduct a systematic review and meta-analysis of IPTp-SP studies that have reported the protective effect against LBW by gravidae. The second objective was to use meta-regression analysis to determine whether there was a level of malaria transmission intensity below which IPTp-SP no longer conferred protection against LBW at the population level. The third objective was to use sensitivity analysis among these same IPTp-SP studies to determine whether 581G prevalence appeared to affect LBW outcomes.

These objectives could be achieved most directly with evidence from new, large, placebo-controlled randomised clinical trials conducted in endemic areas with narrowly different levels of low transmission or resistance. However, the use of placebo where IPTp-SP is policy, or the withholding of preventive treatment to create an unprotected comparison group, raises ethical concerns. Thus, analysis needs to be conducted using available data. This has its own set of challenges, particularly as it relates to our second objective. Maternal parasitaemia is highest between gestational weeks 9 and 16, and then tapers until term [7], but IPTp-SP studies almost exclusively measure parasitaemia only once, at or near delivery, producing a point estimate that has been influenced by the total amount of antimalarial drug administered during the antenatal period and by near-to-term therapy. Thus, antenatal exposure to malaria infection is often understated in IPTp-SP studies. A proxy measure for malaria transmission intensity is needed. This proxy should lend itself to stratification by gravidae because malaria infection in endemic areas is known to be more prevalent and intense in primi- and secundigravidae than among multigravidae [7]. The proxy we chose is the prevalence of malaria infection among children.

Methods

Systematic review

A systematic review of the literature on malaria in pregnancy was completed in August 2014. The protocol can be found in the International Prospective Register of Systematic Reviews (PROSPERO; CRD42014007618). In brief, we searched PubMed, MEDLINE, EMBASE, the WHO International Clinical Trials Registry and reference lists to identify pregnancy studies that reported: (i) the proportion of pregnant women who were exposed to ≥2 doses of IPTp-SP compared to placebo, or to no doses of IPTp-SP, and (ii) the incidence of LBW by treatment group. We imported records into EndNote X7 software (Thomson Reuters), removed duplicates and screened each record against pre-determined eligibility criteria. Discrepancies in eligibility assessment were settled by a third-party expert. We reviewed full-text articles against the same criteria. Studies were excluded if they were conducted: (i) before 1990, (ii) outside sub-Saharan Africa, (iii) without reporting the effect of IPTp-SP on LBW, (iv) with selective enrolment of pregnant women from high-risk groups such as those with HIV or (v) utilised active antimalarial drug comparators against IPTp-SP. Figure 1 illustrates our selection process, the number of studies excluded and the reasons for their exclusion in accordance with guidelines from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Table S1 is the PRISMA checklist. We extracted data without blinding to author or publication, but we did so in duplicate and independently, and then applied methods of Grading of Evidence, Assessment, Development and Evaluation (GRADE) to appraise data quality. RevMan 5.2 software was used to determine the risk of bias among individual pregnancy studies and across studies (Oxford, UK).

Meta-analysis

We conducted a standard meta-analysis employing Stata/IC version 13 software (Stata Corporation) using the results of our systematic review without regard for malaria transmission intensity on LBW outcomes. We pooled the incidence of LBW by gravidae and by exposure to ≥2 doses of IPTp-SP *vs.* placebo or no doses, and assessed our results with the Q statistic.

Estimating malaria transmission intensity

We employed two methods to estimate the malaria transmission intensity that was likely to have been present in each IPTp-SP study site. Our first approach was to use

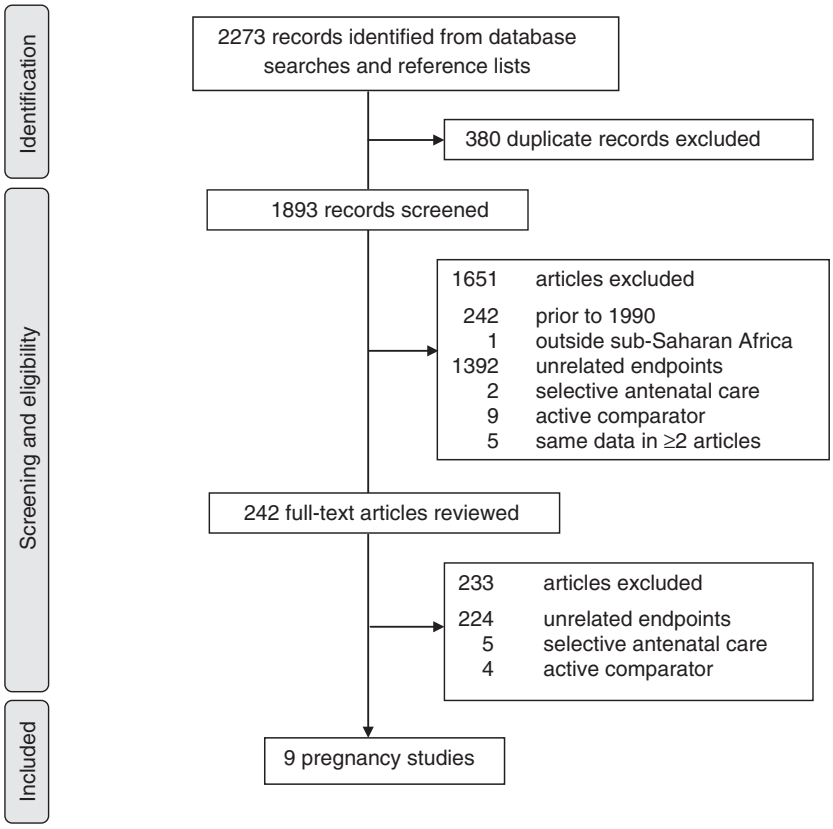

Figure 1 PRISMA flowchart that illustrates the systematic review of IPTp-SP studies.

the 2007 prevalence estimates of *Plasmodium falciparum* infection among children 2–10 years of age (2007 $Pf\text{PR}_{2-10}$) as calculated by the Malaria Atlas Project (MAP) for the same locations where IPTp-SP studies had been conducted. However, because all our IPTp-SP studies had been conducted prior to 2007, with the exception of one multi-year trial that ended in 2007, we sought prevalence estimates that better aligned with the years of the pregnancy studies. For this, we obtained national cross-sectional survey data sets from MAP of *P. falciparum* prevalence for each of the 12 countries where the IPTp-SP studies had been conducted. We then developed four matching rules shown in Table 1 to identify point estimates from the cross-sectional data that were most likely to reflect the malaria transmission intensity to which pregnant women were exposed.

Before applying these matching rules, we extracted geospatial coordinates for each IPTp-SP study and cross-sectional survey using the GEOnet Names Server [8] and then used ArcGIS 10 software (Environmental Systems Research Institute) to calculate straight-line distances between the locations of IPTp-SP studies and cross-sectional surveys. This allowed us to restrict matching of IPTp-SP studies to survey data that had been collected within <100 miles of each other as described in rule 1, a distance used in previous malaria modelling of pregnancy and paediatric data [9]. Similarly, we obtained elevation estimates from the Consortium for Spatial Information [10] for locations of the IPTp-SP studies and survey data. We used these estimates as part of an elevation criterion in rule 3 that took into account the fact that malaria infection declines sharply at altitudes ≥1200 m compared to lower elevations [11]. We also included a temporal criterion in the matching rules, stipulating that survey data needed to have been collected within ±2 years of the IPTp-SP studies to be paired. All four matching rules required that cross-sectional surveys and pregnancy studies had to have been conducted in areas from the same malaria transmission category as designated by MAP: high (>40% *P. falciparum* prevalence among 2–10 year olds), intermediate (5–40%) and low (<5%). This accounted for the possibility that IPTp-SP studies and cross-sectional surveys may have been conducted in proximate locations that had very different intensities of malaria transmission. To reflect the gravidae-specific nature of

Table 1 Rules used to match low birth weight outcomes in IPTp-SP studies with point estimates from prevalence surveys of *Plasmodium falciparum* infection among children

Rules	Criteria
1	Prevalence estimates will be paired to IPTp-SP studies if they were both conducted: In an area with the same risk of malaria infection (high, intermediate or low), AND Within 2 years (+ or −) of each other, AND <100 miles of each other
2	Prevalence estimates will be paired to IPTp-SP studies if they were both conducted: In an area with the same risk of malaria infection (high, intermediate or low), AND Within two years (+ or −) of the pregnancy study, AND In the same country
3	Prevalence estimates will be paired to IPTp-SP studies if they were both conducted: In an area with the same risk of malaria infection (high, intermediate or low), AND At the same elevation, either ≤1200 m OR >1201 m, AND In the same subregion of Africa (East/Southern OR West/Central)
4	If no prevalence estimates can be paired, then IPTp-SP studies will be matched to the 2007 *Pf*PR$_{2-10}$ estimate

parasitaemia among pregnant women in endemic areas when pairing prevalence estimates of malaria in children, we made some assumptions based on reports from Sierra Leone [12] and Kenya [13] that suggest the prevalence of parasitaemia among children is comparable to maternal parasitaemia as presented in Table 2.

We then applied our matching rules sequentially. If survey data could not be matched to an IPTp-SP study using rule 1, then rule 2 was applied, and so on until we identified all point prevalence estimates from survey data that could be considered the best possible matches for each IPTp-SP study as illustrated in Figure 2. Using random-effects models, we pooled survey data that were paired with IPTp-SP studies under rules 1, 2 or 3. If survey data could not be matched under these rules, we imposed the 2007 *Pf*PR$_{2-10}$ estimate for the same location where the IPTp-SP study had been conducted.

Table 2 Age structure of paediatric data matched to gravidity

Paediatric description	Age structure	Gravidity
Infancy	Birth to <1	Primigravidae
Childhood	1–4	Segundigravidae
School-aged	5–15	Multigravidae

Meta-regression analysis

With data from IPTp-SP studies paired to malaria prevalence estimates, whether using 2007 *Pf*PR$_{2-10}$ or our pooled estimates, we applied standard meta-regression models [14, 15] to LBW outcomes reported in the IPTp-SP studies. This allowed us to correct for the variances of treatment effect within pregnancy studies, and the residual heterogeneity across pregnancy studies, and to estimate the effect of malaria transmission intensity on the protection conferred by IPTp-SP against LBW.

Potential effect modification of *Pfdhps* 581G on outcomes

Because the *Pfdhps* 581G mutation may have acted as an important effect modifier, we used the geographical database of molecular biomarkers as described elsewhere [16] to obtain point prevalence estimates of the 581G mutation associated with 'super resistance' to SP among parasites from the same locations where IPTp-SP studies had been conducted. We included mutation prevalence estimates if they had been collected from <100 miles of the

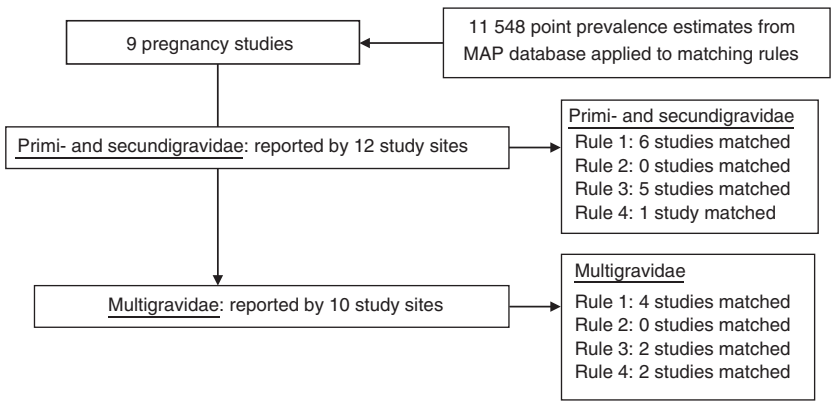

Figure 2 Flow diagram that illustrates the pairing of pregnancy studies to paediatric malaria prevalence estimates.

Table 3 Studies that reported by gravidae the incidence of low birth weight following exposure to two or more doses of IPTp-SP *vs.* placebo or no doses in order of malaria prevalence

					IPTp-SP (2 doses or more)				Placebo or no IPTp-SP			
References	Countries	Sites	Study years	Study type	No. LBW	No. weighed	LBW %	95% CI	No. LBW	No. weighed	LBW %	95% CI
Primi- and secundigravidae												
Gies [20]	Burkina Faso	Boromo	2004–06	1	104	812	12.8	10.5, 15.1	19	52	36.5	23.5, 49.6
Likwela [17]	DR Congo	Kisangani	2007	2	2	28	7.1	−2.4, 16.7	7	12	58.3**	30.4, 86.2
Likwela [17]	DR Congo	Mikalayi	2007	2	1	28	3.6	−3.3, 10.5	11	56	19.6**	9.2, 30.0
Likwela [17]	DR Congo	Rutshuru	2007	2	11	94	11.7	6.0, 20.0	5	15	33.3**	9.5, 57.2
Menendez [19]	Mozambique	Manhiça	2003–05	1	29	133	21.8	14.8, 28.8	25	121	20.7	13.5, 27.9
Ndyomugyenyi [18]*	Uganda	Kabale	2004–07	1	16	313*	5.1	2.7, 7.6	18	329	5.5	3.0, 7.9
Ramharter [21]	Gabon	Lambaréné	2006	2	4	49	8.2	0.5, 15.8	6	30	20.0	5.7, 34.3
Ramharter [21]	Gabon	Libreville	2006	2	14	168	8.3	4.2, 12.5	19	106	17.9	10.6, 25.2
Ndyomugyenyi [18]†	Uganda	Kabale	2004–07	1	22	287†	7.7	4.6, 10.7	16	277	5.8	3.0, 8.5
Sirima [23]*	Burkina Faso	Koupéla	2004	2	21	173*	12.1	7.3, 17.0	22	183	12.0	7.3, 16.7
Sirima [23]†	Burkina Faso	Koupéla	2004	2	6	46†	13.0	3.3, 22.8	23	186	12.4	7.6, 17.1
van Eijk [22]	Kenya	Kisumu	1999–2000	1	10	122	8.2	3.3, 13.1	78	513	15.2	12.1, 18.3
Subtotals					241	2253			249	1880		
Multigravidae												
Likwela [17]	DR Congo	Kisangani	2007	2	4	59	6.8	0.4, 13.2	9	37	24.3	10.5, 38.1
Likwela [17]	DR Congo	Mikalayi	2007	2	1	86	1.2	−1.1, 3.4	24	307	7.8	4.8, 10.8
Likwela [17]	DR Congo	Rutshuru	2007	2	28	397	7.1	4.5, 9.6	11	162	6.8	2.9, 10.7
Mbaye [24]	The Gambia	Farafenni	2003–04	1	51	931	5.5	4.0, 6.9	63	917	6.9	5.2, 8.5
Menendez [19]‡	Mozambique	Manhiça	2003–05	1	29	361‡	8.0	5.2, 10.8	34	375	9.1	6.2, 12.0
Ndyomugyenyi [18]§	Uganda	Kabale	2004–07	1	40	610§	6.6	4.6, 8.5	37	631	5.9	4.0, 7.7
Ndyomugyenyi [18]¶	Uganda	Kabale	2004–07	1	21	347¶	6.1	3.5, 8.6	27	325	8.3	5.3, 11.3
Rogerson [25]	Malawi	Blantyre	1997–99	2	30	291	10.3	6.8, 13.8	50	218	22.9	17.4, 28.5
Sirima [23]	Burkina Faso	Koupéla	2004	2	2	17	11.8	−3.6, 27.1	7	61	11.5	3.5, 19.5
van Eijk [22]	Kenya	Kisumu	1999–2000	1	2	36	5.6	−1.9, 13.0	23	232	9.9	6.1, 13.8
Sub-totals					208	3135			285	3265		

IPTp-SP, intermittent preventive treatment of malaria in pregnancy using sulphadoxine–pyrimethamine; LBW, low birth weight; CI, confidence interval; NA, not applicable; study type 1 = randomised trial and type 2 = observational study.

Additional notes: (i) Studies are presented in alphabetical order based on reference; (ii) Ndyomugyenyi *et al.* assigned pregnant women to one of three treatment groups in which they received either IPTp-SP, or an insecticide-treated bednet (only), or IPTp-SP plus an insecticide-treated bednet. Results used were stratified by gravidae for the first two groups only; (iii) Likwela *et al.* categorised as placebo recipients were given ≤1 dose of IPTp.

*Primigravidae.

†Secundigravidae.

‡1–3 pregnancies (likely to have contained some secundigravidae) and 4 or more pregnancies.

§2–4 pregnancies.

¶5 or more pregnancies.

**Women who received ≤ 1 dose of IPTp were classified in the placebo group

IPTp-SP studies and within ±2 years of when the IPTp-SP studies had been conducted. This radius was expanded to 100–250 miles if no data were available from <100 miles. If multiple point estimates were found, we pooled them using standard meta-analysis. We then conducted a sensitivity analysis of the 581G mutation on our results.

Results

Systematic review

Studies of IPTp-SP stratified by gravidae that met inclusion criteria are summarised in Table 3 [17–25]. Nine studies involving 10 279 pregnant women were included in our analysis. Seven studies reported LBW outcomes from 12 unique sites for primi- and secundigravidae who were exposed to ≥2 doses of IPTp-SP (N = 2314) *vs.* placebo or no doses (N = 1954). In addition, seven studies from 10 unique sites reported LBW outcomes among multigravidae who received ≥2 doses of IPTp-SP (N = 2941) compared to placebo or no doses (N = 3070).

Funnel-plot analysis in Figure S1 suggests that relatively small IPTp-SP studies failing to protect against LBW may have been under-represented in our sample. Our appraisal of evidence using GRADE methods is summarised in Table S2. Figure S2 illustrates the risk of bias within individual IPTp-SP studies, and Figure S3 shows the combined risk of bias across IPTp-SP studies. Five of the nine studies were randomised clinical trials, whereas four were observational studies. There is an unclear risk of selection bias and detection bias in one-half of the studies.

Meta-analysis

Random-effects models produced statistically strong evidence among pregnancy studies; among primi- and

Table 4 Pooled prevalence estimates and Malaria Atlas Project (MAP) estimates at study sites by primi- and secundigravidae and multigravidae

References	Countries	Sites	Elevation	Matching rule used	Pooled prevalence (%)	2007 *Pf*PR$_{2–10}$ (%)
Primi- and secundigravidae						
Likwela [17]	DR Congo	Rutshuru	1212	4	29.6*	29.6
Ndyomugyenyi [18]†	Uganda	Kabale	2118	4	29.6*	25.1
Ndyomugyenyi [18]‡	Uganda	Kabale	2118	4	29.6*	25.1
Likwela [17]	DR Congo	Kisangani	401	1	30.4	40.2
Menendez [19]	Mozambique	Manhiça	21	1	31.2	48.1
Gies [20]	Burkina Faso	Boromo	258	1	44.2	57.4
Likwela [17]	DR Congo	Mikalayi	618	3	55.8	38.5
Ramharter [21]	Gabon	Libreville	13	3	55.8	37.1
Ramharter [21]	Gabon	Lambaréné	39	3	55.8	42.8
van Eijk [22]	Kenya	Kisumu	1166	1	57.5	20.5
Sirima [23]†	Burkina Faso	Koupéla	290	1	59.1	66.4
Sirima [23]‡	Burkina Faso	Koupéla	290	1	59.1	66.4
Multigravidae						
Ndyomugyenyi [18]§	Uganda	Kabale	2118	3	6.7	25.1
Ndyomugyenyi [18]¶	Uganda	Kabale	2118	3	6.7	25.1
Mbaye [24]	The Gambia	Farafenni	25	1	8.3	23.9
Rogerson [25]	Malawi	Blantyre	989	3	11.3	24.9
Menendez [19]‖	Mozambique	Manhiça	21	3	11.3	48.1
van Eijk [22]	Kenya	Kisumu	1166	1	25.0	20.5
Likwela [17]	DR Congo	Rutshuru	1212	4	29.6*	29.6
Sirima [23]	Burkina Faso	Koupéla	290	1	32.9	66.4
Likwela [17]	DR Congo	Kisangani	401	3	34.0	40.2
Likwela [17]	DR Congo	Mikalayi	618	1	39.3	38.5

Studies are presented in ascending order of malaria transmission intensity.
*Under rules 1–3, no matches were made and, therefore, the 2007 *Pf*PR$_{2–10}$ estimates from MAP are used.
†Primigravidae.
‡Secundigravidae.
§2–4 pregnancies.
¶5 or more pregnancies.
‖1–3 pregnancies (likely contained some secundigravidae) and 4 or more pregnancies.

secundigravidae, ≥2 doses of IPTp-SP *vs.* placebo or no IPTp-SP reduced the odds of delivering a LBW newborn by one-half [odds ratio (OR) = 0.54; 95% CI: 0.35, 0.84; I^2 = 69.0%; P < 0.00]. The reduction was 30% among multigravidae (OR = 0.70; 95% CI: 0.51, 0.95; I^2 = 48.9%; P = 0.04). To explore whether heterogeneity could be reduced, we stratified LBW outcomes as reported in observational studies *vs.* RCTs. We found less heterogeneity among the observational studies (I^2 = 34.7%; P = 0.190) than among RCTs (I^2 = 76.5%; P < 0.000) for primi- and secundigravidae. In contrast, there was less heterogeneity in RCTs among studies in multigravidae (I^2 = 38.8%; P = 0.133) compared to observational studies (I^2 = 63.7%; P = 0.064).

Estimating malaria transmission intensity

We used 12 national cross-sectional data sets from MAP containing 11 548 surveys to estimate the malaria transmission intensity at each IPTp-SP study site. Table 4 shows the matching rules that we used and our pooled prevalence estimates of malaria parasitaemia. Among primi- and secundigravidae, the pooled prevalence ranged between 25.1% and 59.1%, whereas MAP estimates were between 20.5% and 66.4%. Among multigravidae, pooled prevalence estimates ranged from 6.7% to 39.3%, in contrast to MAP estimates which were between 20.5% and 66.4%.

Meta-regression analysis

To explore the potential effect of malaria transmission intensity on the association between IPTp-SP and LBW, we produced four separate meta-regression models. In Model 1, we used our pooled prevalence estimates among primi- and secundigravidae for the measure of malaria transmission intensity. In our second model, also for primi- and secundigravidae, we applied MAP estimates for malaria transmission intensity. In Models 3 and 4 for multigravidae, we used our pooled prevalence estimates and MAP estimates, respectively. None of the models suggested that variation in the ORs of LBW could be explained by malaria transmission intensity: Model 1 (P = 0.83), Model 2 (P = 0.78), Model 3 (P = 0.30) and Model 4 (P = 0.93). Sensitivity testing among matching rules did not produce any statistically significant difference.

Effect modification of *Pfdhps* 581G

Using a geographical database of molecular biomarkers as described elsewhere [16], we obtained prevalence estimates of *Pfdhps* 581G from 44 locations that had been measured within ±2 years of the IPTp-SP studies. There were 20 locations within <100 miles of the pregnancy study sites and 24 locations within 100–250 miles as shown in Table 5 [26–40].

The highest prevalence estimates of 581G were calculated using data from four biomarker studies – Karema [32], Alker [30], Lynch [31] and Taylor [29] – that could be paired with two pregnancy studies – Likwela *et al.* [17] and Ndyomugyenyi [18]. Specifically, these four biomarker studies had 581G prevalence data from six locations that were related to the Likwela [17] pregnancy study (Rutshuru). We combined these point estimates using random-effects models to produce a pooled estimate of 52.4% [95% confidence intervals (CI): 47.5–57.4%). We were able to relate five biomarker studies that reported measurements of the 581G mutation to the Ndyomugyenyi [18] pregnancy study. We pooled these using random-effects models to generate an estimate of 52.6% (95% CI: 47.6–57.5%). The biomarker study by Karema [32] that reported the population prevalence of 581G to be 61.1% also observed *dhfr*-164L at a frequency of 11.4%. Similarly, population prevalence estimates of 581G detected by Lynch [31] were 45.8% (Rukungiri) and 45.0% (Kabale), whereas lower frequencies, 4.2% and 13.7%, were identified for 164L in the same locations, respectively. The *dhfr*-164L mutation is also associated with the failure of SP to clear malaria parasites and is more commonly found in South American and South-East Asia. Twelve biomarker studies reported the 581G mutation that could be related spatially and temporally to the remaining pregnancy studies. These prevalence estimates ranged between 0.0% and 10.1% (95% CI: 3.5, 16.8). Overall, 581G prevalence points presented a binomial structure – frequencies of 581G ≤10.1% and those >10.1% – which we used to stratify IPTp-SP studies.

Random-effects models shown in Figure 3 indicate that ≥2 doses of IPTp-SP reduced the odds of LBW among primi- and secundigravidae (OR = 0.49; 95% CI: 0.29, 0.81; I^2 = 67.7%; P < 0.00) and multigravidae (OR = 0.56; 95% CI: 0.37, 0.86; I^2 = 48.7%; P < 0.03) in areas where the prevalence of 581G was ≤10.1%. Where the prevalence of 581G was >10.1%, the protective effect of IPTp-SP persisted among primi- and secundigravidae, although at lower levels, as illustrated in Figure 4. Among multigravidae, random-effects models showed that IPTp-SP confers protection against LBW in areas where the prevalence of 581G was ≤10.1% (OR = 0.56; 95% CI: 0.37, 0.86; P = 0.07), a finding of borderline statistical significance. In contrast, there was

Table 5 Prevalence of the 581G resistance mutation at or near the IPTp-SP study sites

Pregnancy studies			*Pf*dhps codon 581G surveys								
References	Countries	Sites	Distance from IPTp-SP study (m)	No. sites	No. tested	No. positive	Prevalence (%)	95% CI	Heterogeneity (I^2), %	Prevalence across sites (%)	References
Gies [20]	Burkina Faso	Boromo	100–250	3	250	26	1.7	0.2 to 3.2	93.8	0.0–37.8	Mockenhaupt, Thera, Ibrahim [26–28]
Likwela [17]	DR Congo	Kisangani	100–250	1	18	1	5.6	−5.0 to 16.1	NA	NA	Taylor [29]
Likwela [17]	DR Congo	Mikalayi	100–250	0	0	0	0.0	NA	NA	NA	Taylor [29]
Likwela [17]	DR Congo	Rutshuru	<100	6	747	367	52.4	47.5 to 57.4	79.6	28.6–61.1	Taylor, Alker, Lynch, Karema [29–32]
Mbaye [24]	The Gambia	Farafenni	<100	1	27	0	0.0*	NA	NA	0.0	Noranate [33]
Menendez [19]	Mozambique	Manhiça	100–250	4	279	0	0.0*	NA	NA	0.0	Raman [34]
Ndyomugyenyi [18]	Uganda	Kabale	<100	5	740	365	52.6	47.6 to 57.5	83.3	30.2–61.1	Taylor, Alker, Lynch, Karema [>29–32]
Ramharter [21]	Gabon	Lambaréné	100–250	2	118	0	0.0	NA	NA	0.0	Kun, Aubouy [35, 36]
Ramharter [21]	Gabon	Libreville	100–250	2	236	4	2.0†	0.06 to 3.9†	NA	0.0–2.0	Alker, Aubouy [30, 36]
Rogerson [25]	Malawi	Blantyre	<100	3	318	3	3.4†	−0.4 to 7.1†	100	0.0–3.4	Alker, Bell [37, 38]
Sirima [23]	Burkina Faso	Koupéla	<100	1	79	8	10.1†	3.5 to 16.8†	NA	10.1	Ibrahim [28]
van Eijk [22]	Kenya	Kisumu	<100	7	1005	4	2.7†	0.1 to 5.4†	100	0.0–2.7	Omar, Zhong, Iriemenam [39, 40, 51]

NA, not applicable.
*No mutations were detected and, therefore, the prevalence shown is the point estimate of the one study that did yield positive samples.
†Only one of the studies detected mutations; models exclude studies in which there are no positive tests and, therefore, the prevalence shown is a point estimate of the one study that did yield positive samples.

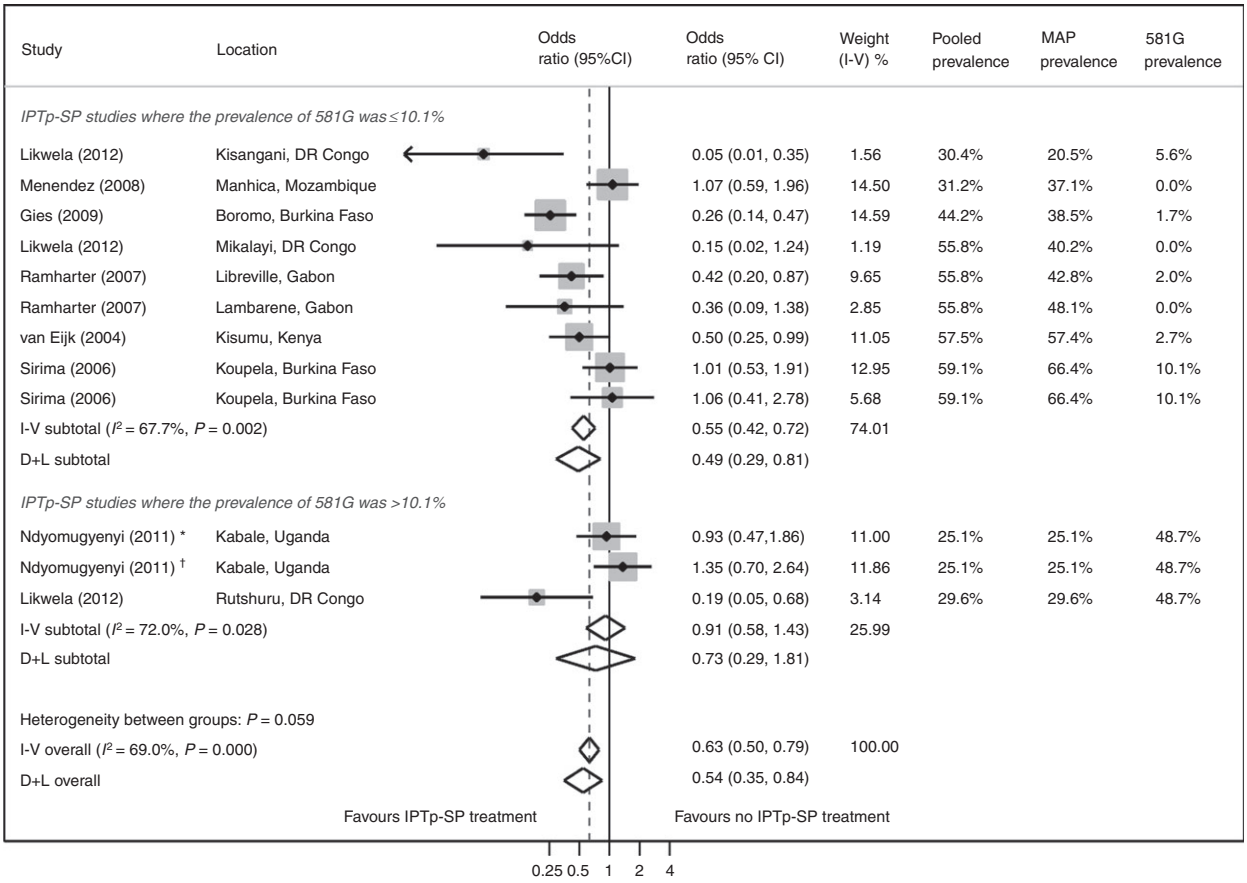

Figure 3 Odds ratio of low birth weight delivery among primi- and secundigravidae following two or more doses of IPTp-SP *vs.* placebo or no IPTp-SP stratified by estimates of the 581G resistance mutation among malaria parasites at study sites.

no evidence from fixed-effects or random-effects models that multigravidae were protected against LBW at sites where the prevalence of 581G was >10.1% (OR = 0.96; 95% CI: 0.70, 1.34; I^2 = 72.0%; P < 0.47). The interaction between malaria transmission intensity and the prevalence of 581G was borderline significant: P = 0.06 among primi- and secundigravidae, and P = 0.04 among multigravidae. Thus, meta-regression of malaria transmission intensity, stratified by the prevalence of 581G, was only able to explain some of the variation in protective effect of IPTp-SP against LBW.

Discussion

To our knowledge, this is the first use of age-stratified rates of paediatric parasitaemia to model the gravidity-specific relationship of malaria in pregnancy in endemic settings, and our results conform to established epidemiological patterns among pregnant women. In addition, IPTp-SP studies identified through our systematic review were protective against LBW to levels that are comparable to other studies. We do not consider the age of the data to be a limitation because we paired LBW outcomes to parasite prevalence estimates from the same time periods.

Lacking prevalence estimates for the 581G mutation between 10.1% and 52.4%, we can only conclude that the prevalence threshold of this mutation is >10.1% above which IPTp-SP no longer protects against the incidence of LBW. It is tempting to state the cut-off should be >52.4%, but the threshold may be much lower, especially considering the alarming results from Tanzania where placental infection was significantly higher in 84% of women (n = 104) given any dose of IPTp-SP compared to 16% of women (n = 104) who received none (P = 0.03) [41, 42]. The prevalence of the 581G mutation in this study area was 55% (95% CI: 44.7, 65.2; N = 87) [43].

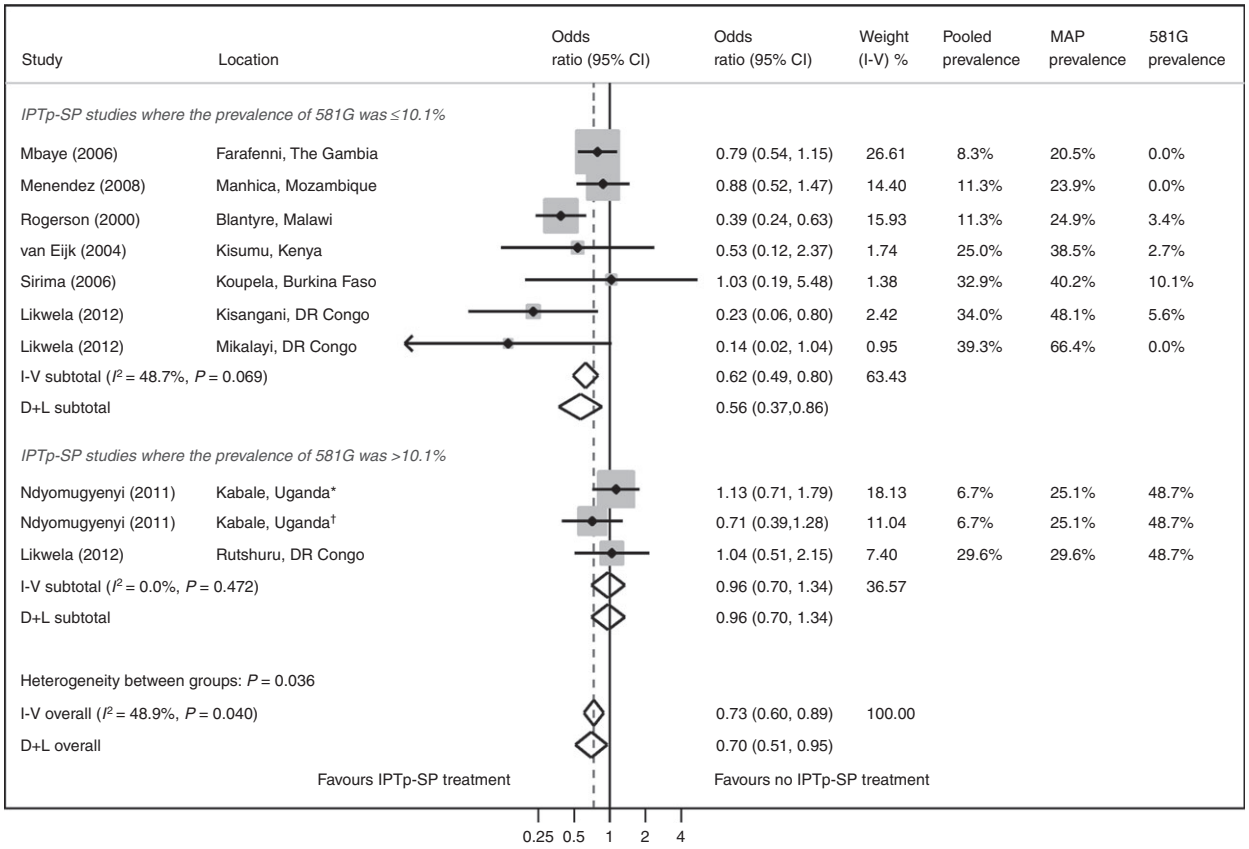

Figure 4 Odds ratio of low birth weight delivery among multigravidae following two or more doses of IPTp-SP *vs.* placebo or no IPTp-SP stratified by estimates of the 581G resistance mutation among malaria parasites at study sites.

Our results should be interpreted with caution. We were limited by the relative paucity of data that relate the efficacy of IPTp-SP to the intensity of malaria exposure during pregnancy that has been stratified by gravidae. Moreover, there may have been selection bias in six of the nine studies we identified because of the absence of random sequence generation or allocation concealment. In addition, there was considerable heterogeneity among pregnancy studies. We are, however, hesitant to draw any conclusions from stratifying studies by design, and doing so did not consistently reduce heterogeneity.

We were unable to detect a malaria transmission threshold below which IPTp-SP is no longer protective against LBW, a possible consequence of there being too few data points. However, an unpublished analysis of Multiple Indicator Cluster Surveys also found that IPTp-SP continued to protect against LBW until very low levels of transmission and, similar to our analysis, could not define a cut-off point of transmission below which IPTp-SP is no longer protective against LBW (Eisele TP, personal communication).

Our inability to detect a transmission threshold may be due to other factors. We do not know the prevalence of placental infection in very low-transmission settings and it is entirely possible that declines in peripheral parasitaemia are not reflected in equivalent reductions of placental infection. If that is the case, then IPTp-SP may provide important and continued protection against placental carriage and the incidence of LBW in areas of very low endemicity. Another possible explanation is that the causal pathway to LBW is multifactorial and that IPTp-SP offers some protection against other causes of LBW. SP has been used as chemoprophylaxis against *Toxoplasma gondii* infection and *Pneumocystis carinii* pneumonia [44]. Sulphadoxine is related to sulphamethoxazole, the partner compound used with trimethoprim to form co-trimoxazole which is commonly prescribed for urinary tract infections [45]. Sulphonamide has also been used to treat *Gardnerella vaginalis* [46], a bacterium found in women

with bacterial vaginosis which can double the odds of having a LBW baby (OR = 2.0; 95% CI: 1.3, 2.9) compared to pregnant women without bacterial vaginosis [47]. A meta-analysis reported the prevalence of bacterial vaginosis to be 50.8% (95% CI: 43.3, 58.4) among pregnant women attending antenatal care in East and Southern Africa and 37.6% (95% CI: 18.0, 57.2) in West and Central Africa [48].

Additional insight could be gained from prospective case–control studies that measure the incidence of LBW among women who had received ≥2 doses of IPTp-SP *vs.* women who had not to have received IPTp-SP during their pregnancies. Such studies, however, can readily be confounded.

Although the 581G mutation is not widely found throughout sub-Saharan Africa, the codon has been detected in 10 countries [49] and selection can be alarmingly rapid. A study in Kenya reported the prevalence of 581G to be 85.1% (95% CI: 80.0%, 89.4%) where no parasites had expressed the mutation 3 years prior [50]. Before declaring a specific level of malaria transmission or 581G prevalence at which IPTp-SP no longer provides a cost-effective benefit, placebo-control trials may be needed in a range of low-transmission settings that have sufficient power for subanalysis by gravidae and include robust micro-biological testing that would enable exploration of potential protection conferred by IPTp-SP against infections apart from malaria. To our knowledge, there are no such studies underway.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. PRIMSA checklist.

Table S2. Risk of bias among individual pregnancy studies.

Figure S1. Funnel plot.

Figure S2. Risk of bias among individual pregnancy studies.

Figure S3. Risk of bias across pregnancy studies.

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