

Geographic Differences in Antimalarial Drug Efficacy in Uganda Are Explained by Differences in Endemicity and Not by Known Molecular Markers of Drug Resistance

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Background. Recent clinical trials from Uganda have shown that the risk of failure following antimalarial therapy varies geographically. We tested the hypothesis that geographic differences in the response to therapy could be explained by differences in the prevalence of known molecular markers of drug resistance.

Methods. Samples from 2084 patients treated with chloroquine (CQ) plus sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) plus SP were tested for the presence of known molecular markers of resistance. Differences in the risk of treatment failure across 6 sites were compared, and age and complexity of infection were controlled for.

Results. The prevalence of molecular markers of drug resistance was high at all of the sites: 61%–91% of patients were infected with parasites containing the *pfprt* Thr-76 mutation and *dhfr/dhps* quintuple mutation. The risk of treatment failure decreased with increasing transmission intensity for both CQ plus SP (73% to 19%) and AQ plus SP (38% to 2%). Restricting the analyses to patients infected with parasites containing all 6 mutations of interest did not affect these trends.

Conclusions. The risk of treatment failure was inversely proportional to transmission intensity and was not explained by differences in molecular markers of antimalarial drug resistance. Our findings strongly suggest that geographic differences in response to antimalarial therapy in Uganda are primarily mediated by acquired immunity associated with malaria transmission intensity, rather than by parasite factors.

Widespread resistance to chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) has forced many countries in Africa to reevaluate their antimalarial-treatment policies [1]. There is a growing consensus that anti-

malarial therapy in Africa should include combinations of drugs; however, it is not clear what combinations are best [2]. Programmatic decisions about antimalarial therapy are complex and involve many factors, including the efficacy and therapeutic life span of a regimen, as well as practical considerations, such as cost, availability, and safety [3].

Antimalarial-treatment policies in Africa are generally guided by the results of drug-efficacy trials. These trials are best done using standardized protocols to assess the response to therapy in patients representing the diversity of malaria risk within a country [4]. We recently conducted drug-efficacy trials at multiple sites in Uganda by comparing combinations of CQ plus SP and amodiaquine (AQ) plus SP and found considerable differences in the risk of treatment failure for both treatment arms across the study sites [5, 6]. Geographic

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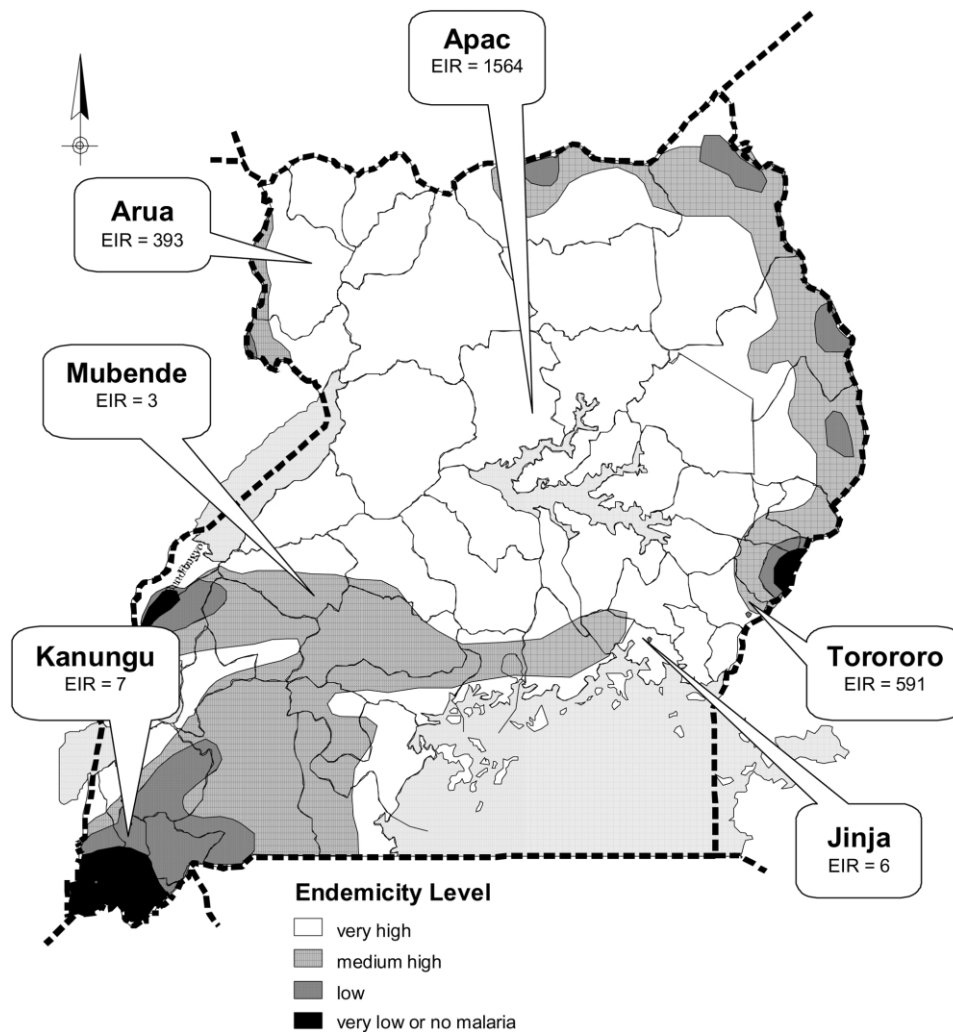


Figure 1. Map of the study sites in Uganda. EIR, entomological inoculation rate

differences in drug efficacy might be explained by differences in parasite factors—such as the prevalence of drug-resistant parasites, multiplicity of parasite clones, and intrahost competition between malaria parasite strains [7]—or by differences in patient characteristics—in particular, levels of acquired antimalarial immunity. Host factors are an important determinant of the response to therapy [8], and age-related differences in drug efficacy among patients infected with resistant parasites demonstrate the specific importance of acquired immunity [9]. A better understanding of the factors responsible for geographic differences in drug efficacy will be helpful in guiding malaria control efforts in Africa.

To evaluate the importance of parasite factors in explaining differences in the risk of treatment failure across Uganda, we compared the prevalence of known molecular markers of CQ and SP resistance in pretreatment samples collected from patients with uncomplicated malaria who were enrolled in clinical

trials at 6 sites in Uganda with varying transmission intensities. We then explored the relationship between the risk of treatment failure, the level of transmission intensity, and the prevalence of molecular markers of drug resistance.

PATIENTS, MATERIALS, AND METHODS

Study sites and patients. Samples and clinical data for the present study were from antimalarial drug–efficacy studies conducted between December 2002 and May 2004 at 6 sites in Uganda. The study sites were selected to reflect the varying epidemiological profiles of malaria in Uganda (figure 1). Details of the clinical trials have been published elsewhere [5, 6]. Briefly, patients ≥ 6 months old who had uncomplicated falciparum malaria were randomized to receive CQ plus SP or AQ plus SP, according to weight-based guidelines for administration of fractions of tablets. Patients were followed for 28 days, and

Table 1. Study characteristics stratified by site.

Characteristic	Study site					
	Kanungu	Mubende	Jinja	Arua	Tororo	Apac
Relative transmission intensity ^a	Low	Low	Low	Medium	High	High
Patients with treatment outcomes, no.	357	351	333	351	334	358
Patients included in molecular analyses, ^b no. (%)	353 (99)	344 (98)	325 (98)	342 (97)	333 (100)	352 (98)
Patients treated with CQ + SP, no.	173	176	156	172	162	179
Risk of treatment failure with CQ + SP, ^c %	64	38	41	47	34	22
Patients treated with AQ + SP, no.	180	168	169	170	171	173
Risk of treatment failure with AQ + SP, ^c %	34	13	13	14	18	7
Age, median (range), years	1.92 (0.5–65)	1.75 (0.5–52)	3.83 (0.5–52)	1.42 (0.5–30)	1.25 (0.5–56)	1.92 (0.5–43)
Parasite density, geometric mean, parasites/ μ L	24,238	20,493	36,015	23,299	18,880	12,139
Complexity of infection, mean (SD)	2.64 (1.49)	3.01 (1.61)	2.62 (1.54)	2.12 (1.01)	4.46 (1.97)	4.16 (1.86)

NOTE. AQ, amodiaquine; CQ, chloroquine; SP, sulfadoxine-pyrimethamine.

^a Measured by entomological inoculation rate: low, 1–10; medium, 300–500; and high, >500 infective bites/person/year.

^b Includes patients with complete data for pretreatment complexity of infection and treatment outcomes, adjusted by genotyping results.

^c Estimated using survival analysis with censoring for new infections.

treatment outcomes were classified, according to World Health Organization guidelines, as either early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), or adequate clinical and parasitological response. Genotyping based on polymorphisms in the merozoite surface protein–2 gene (*mSP-2*) was performed on samples from all patients, to derive a conservative estimate of the complexity of infection (number of infecting strains) in pretreatment isolates and to distinguish recrudescence from new infections in those who developed recurrent parasitemia after day 4, as described elsewhere [10]. Treatment failure was defined as all ETFs and any LCF or LPF categorized as a recrudescence on the basis of genotyping results.

Mutation analysis. Mutations of interest were the *pfprt* Thr-76 mutation, which has been associated with CQ resistance [11], and the *dhfr* Leu-164, Asn-108, Ile-51, Arg-59, *dhps* Gly-437, and Glu-540 mutations, which have been associated with SP resistance [12]. Mutations were identified using a nested polymerase chain reaction followed by restriction-enzyme digestion. Blood was collected on filter paper on the day of diagnosis, and parasite DNA was isolated using Chelex extraction [13]. Primers, amplification conditions, and restriction endonucleases for assays to detect mutations have been described elsewhere [14, 15]. Digestion products were visualized by gel electrophoresis, and results were classified as wild type (*wt*), pure mutant, or mixed (*wt* and mutant alleles present in the same sample) on the basis of the migration patterns of the fragments.

Statistical analyses. We compared patient characteristics at each site using the χ^2 test for categorical variables and 1-way analysis of variance for continuous variables. We compared the proportion of patients at each site with *wt*, mixed, or pure mutant infections for each molecular marker using the χ^2 test. On the basis of the assumption that the number of mutant strains in an individual patient follows a binomial distribution,

the probability of an individual parasite strain carrying mutations of interest at each site was estimated using maximum-likelihood estimation. We used the log-likelihood ratio test to compare the probability that an individual parasite strain carried mutations of interest at each study site. Associations between genotype and treatment failure were assessed for each treatment group separately using multivariate logistic regression, age and parasite density were controlled for, and the results were stratified by study site by use of an interaction term. Crude risks of treatment failure stratified by study site and treatment group were estimated using survival analysis, with censoring for new infections. To account for differences in age and complexity of infection across the study sites, estimates of the risks of treatment failure were adjusted for these variables using a Cox regression model. Data were entered and verified using Epi Info (version 6.04; Centers for Disease Control and Prevention) and SPSS (version 12.0; SPSS). Analyses were performed using STATA (version 8.0; Stata) and R statistical software (version 2.1.0; R Free Software Foundation).

RESULTS

Study sites and patient characteristics. A total of 2084 patients completed the clinical trials. Genotype-adjusted outcomes were available for 2049 patients (98%). Baseline patient characteristics differed between the 6 sites (table 1). At the 3 sites with the highest transmission intensities, >90% of patients were <5 years old, compared with 61%–80% at the 3 sites with the lowest transmission intensities ($P < .001$). Patients from the 2 sites with the highest transmission intensities also had lower parasite densities ($P < .001$) and higher complexity of infection ($P < .001$), compared with patients from the other sites. A total of 8407 (99%) of 8450 tests for the mutations of interest were

Table 2. Proportion of patients at each study site infected with parasites containing key *pfcr*, *dhfr*, and *dhps* mutations.

Mutation	Study site					
	Kanungu	Mubende	Jinja	Arua	Tororo	Apac
<i>pfcr</i> Thr-76						
Wild type	0 (0)	0 (0)	1 (1)	3 (4)	0 (0)	14 (4)
Mixed	5 (6)	3 (4)	1 (1)	8 (10)	1 (1)	94 (27)
Pure mutant	75 (94)	77 (96)	78 (98)	69 (86)	79 (99)	238 (69)
<i>dhfr</i> Leu-164						
Wild type	79 (99)	80 (100)	80 (100)	80 (100)	80 (100)	80 (100)
Mixed	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pure mutant	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>dhfr</i> Asn-108						
Wild type	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Mixed	1 (1)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Pure mutant	79 (99)	80 (100)	79 (99)	80 (100)	80 (100)	80 (100)
<i>dhfr</i> Ile-51						
Wild type	0 (0)	1 (1)	1 (1)	0 (0)	0 (0)	0 (0)
Mixed	5 (6)	3 (4)	2 (3)	0 (0)	5 (6)	0 (0)
Pure mutant	75 (94)	76 (95)	77 (96)	80 (100)	75 (94)	80 (100)
<i>dhfr</i> Arg-59						
Wild type	98 (28)	86 (25)	54 (17)	119 (36)	24 (7)	23 (7)
Mixed	41 (12)	90 (26)	55 (17)	48 (14)	122 (37)	140 (40)
Pure mutant	214 (61)	168 (49)	216 (66)	165 (50)	187 (56)	188 (54)
<i>dhps</i> Gly-437						
Wild type	52 (15)	73 (21)	20 (6)	21 (6)	5 (2)	16 (5)
Mixed	27 (8)	42 (12)	32 (10)	32 (9)	48 (14)	109 (31)
Pure mutant	274 (78)	225 (66)	272 (84)	287 (84)	280 (84)	227 (64)
<i>dhps</i> Glu-540						
Wild type	55 (16)	65 (19)	27 (8)	43 (13)	8 (2)	18 (5)
Mixed	32 (9)	71 (21)	47 (15)	45 (13)	86 (26)	130 (37)
Pure mutant	266 (75)	204 (60)	250 (77)	246 (74)	239 (72)	204 (58)
Six key <i>pfcr</i> and <i>dhfr/dhps</i> mutations ^a						
All mixed or mutant	219 (62)	209 (61)	252 (77)	193 (58)	303 (91)	297 (86)
All pure mutants	174 (49)	112 (33)	180 (55)	135 (40)	160 (48)	114 (33)

NOTE. Data are no. (%) of patients. All samples were assumed to have the *pfcr* Thr-76 mutation, except those from Apac.

^a *pfcr* Thr-76, *dhfr* Asn-108, Ile-51, Arg-59, *dhps* Gly-437, and Glu-540 were considered. All samples were assumed to have the *dhfr* Asn-108 and Ile-51 mutations.

successfully performed; they characterized genotypes for 7 key mutations.

Prevalence of the *pfcr* Thr-76 mutation. Previous work has suggested that the *pfcr* Thr-76 mutation is very common in Uganda [16]. Therefore, we initially tested samples from 80 randomly selected patients from each site (40 patients/treatment arm), and tested all samples from patients from Apac only, where a lower than expected prevalence of pure mutants was found in the initial 80 samples. At all of the sites, >95% of samples tested were mixed or pure mutants for *pfcr* Thr-76 (table 2). However, the prevalence of mixed genotypes was significantly higher in samples from Apac (27%) than in those from the other sites (range, 1%–10%) ($P < .001$).

Prevalence of *dhfr* mutations. Previous work has also sug-

gested that, in Uganda, the *dhfr* Asn-108 and Ile-51 mutations are very common and the *dhfr* Leu-164 mutation is very rare [17]. Therefore, we tested samples from 80 randomly selected patients from each site (40 patients/per treatment arm) for each of these mutations. A total of 478 of 480 samples tested were found to be pure mutants for *dhfr* Asn-108; the remaining 2 samples had mixed genotypes. At least 94% of samples were found to be pure mutants for *dhfr* Ile-51, and at least 99% were found to be mixed or pure mutants. None of the 480 samples was found to be a pure mutant for *dhfr* Leu-164, and only 1 sample had a mixed genotype (table 2).

We tested all samples for the presence of the *dhfr* Arg-59 mutation. The proportion of samples with at least 1 mutant strain (mixed or pure mutant) was highest at the 2 sites with

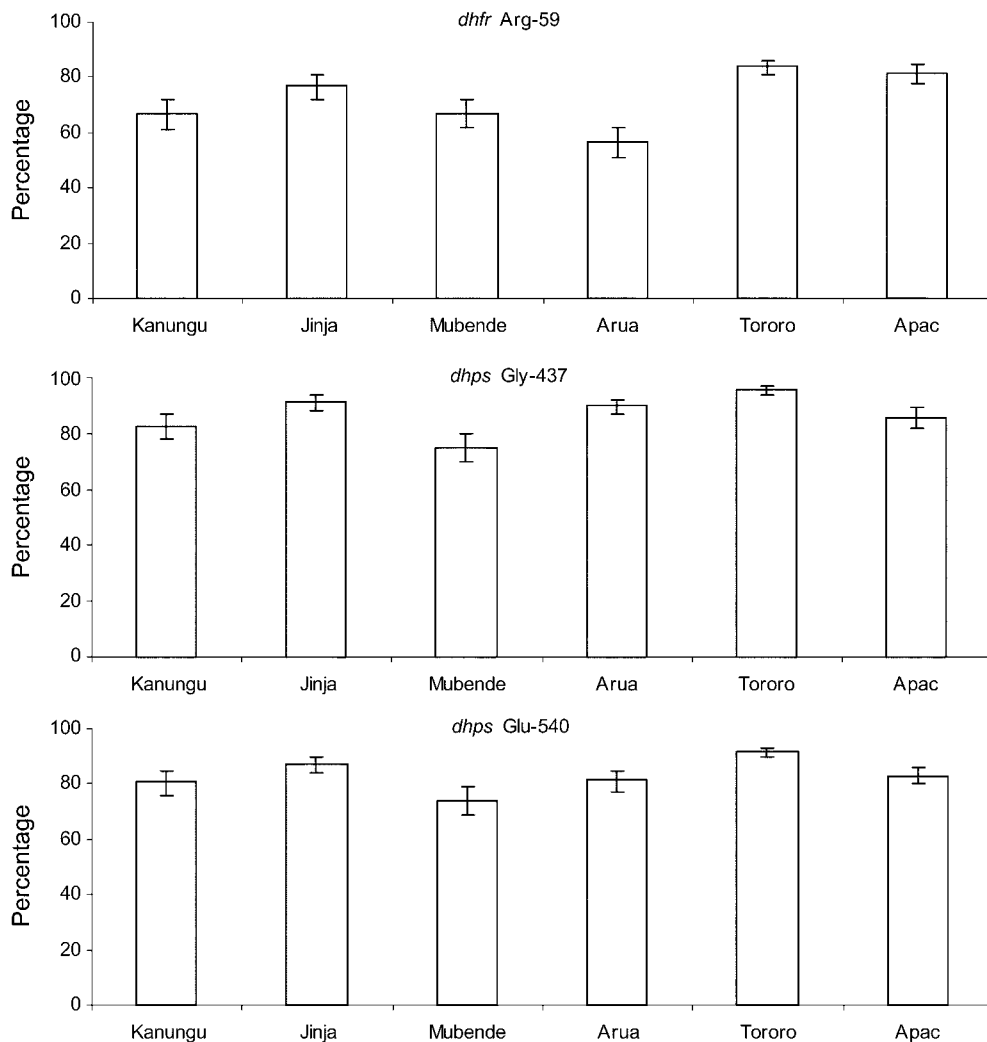


Figure 2. Estimated probability that an individual parasite strain carried selected *dhfr/dhps* mutations at each site

the highest transmission intensities (range, 93%–94%) (table 2). To account for differences in complexity of infection across the sites, the probability that an individual parasite strain had the *dhfr* Arg-59 mutation was determined, and this probability was higher at the 2 sites with the highest transmission intensities than at the other sites (range, 81%–84% vs. 57%–77%; $P < .001$) (figure 2).

Prevalence of *dhps* mutations. All samples were tested for the presence of both the *dhps* Gly-437 and the Glu-540 mutation. Tororo and Apac had the highest prevalences of infection with at least 1 mutant strain (98% and 95%, respectively) (table 2). The probabilities that an individual parasite strain had the *dhps* Gly-437 mutation (average, 86% [range, 75%–95%]) or the Glu-540 mutation (average, 83% [range, 74%–92%]) were high at all of the sites, and there was no obvious relationship between these probabilities and transmission intensity (figure 2 and table 2).

Combinations of mutations. When evaluating combina-

tions of mutations, we made the assumption that all samples contained the *dhfr* Asn-108 and the Ile-51 and *pfcr* Thr-76 mutations, except in Apac, where individual patient data for *pfcr* Thr-76 were used. The proportion of patients with infections with parasites containing all 6 mutations of interest ranged from 58% to 91% when mixed or pure mutant genotypes were considered and from 33% to 55% when only pure mutant genotypes were considered (table 2). There was no clear relationship between the prevalence of infections containing all 6 mutations of interest and transmission intensity.

Individual-level predictors of treatment failure. Decreasing age and increasing pretreatment parasite density were independent predictors of treatment failure for both treatment groups (table 3). At the 4 sites with the lowest transmission intensities, but not at the 2 sites with the highest transmission intensities, patients infected with parasites containing all 6 mutations of interest were significantly more likely than those without all 6 mutations of interest to experience treatment

Table 3. Predictors of treatment failure stratified by study drug and site.

Predictor	CQ + SP		AQ + SP	
	OR (95% CI)	P	OR (95% CI)	P
Age ^a	0.95 (0.92–0.98)	<.001	0.96 (0.92–1.00)	.08
Parasite density ^b	1.21 (1.07–1.38)	.003	1.25 (1.06–1.47)	.009
All 6 mutations of interest present ^c				
Kanungu	1.83 (0.98–3.43)	.06	1.65 (0.82–3.32)	.16
Mubende	5.80 (2.50–13.48)	<.001	2.73 (0.85–8.78)	.09
Jinja	4.82 (1.73–13.42)	.003	2.03 (0.56–7.36)	.28
Arua	2.32 (1.19–4.53)	.01	1.21 (0.43–3.42)	.72
Tororo	2.26 (0.27–18.91)	.45	2.42 (0.53–11.17)	.26
Apac	0.76 (0.28–2.07)	.59	1.46 (0.18–11.95)	.73

NOTE. AQ, amodiaquine; CI, confidence interval; CQ, chloroquine; OR, odds ratio; SP, sulfadoxine-pyrimethamine.

^a Per 1-year increase in age.

^b Per 1 natural log increase in pretreatment parasite density.

^c Mixed or mutant genotypes for all 6 mutations of interest (*pfprt* Thr-76, *dhfr* Asn-108, Ile-51, Arg-59, *dhps* Gly-437, and Glu-540) vs. any other combination of genotypes.

failure with CQ plus SP (table 3). Patients infected with parasites containing all 6 mutations of interest were more likely to experience treatment failure with AQ plus SP, but these associations failed to achieve statistical significance at any of the 6 sites (table 3). It should be noted that the precision of the measure of association between parasite genotypes and treatment failure was limited because of the high prevalence of

patients infected with parasites containing all 6 mutations of interest at the 2 sites with the highest transmission intensities.

Comparisons of the risk of treatment failure across the study sites. The unadjusted risk of treatment failure was highest at the site with the lowest transmission intensity (Kanungu) and lowest at the site with the highest transmission intensity (Apac) in both treatment arms (table 1). We were interested in com-

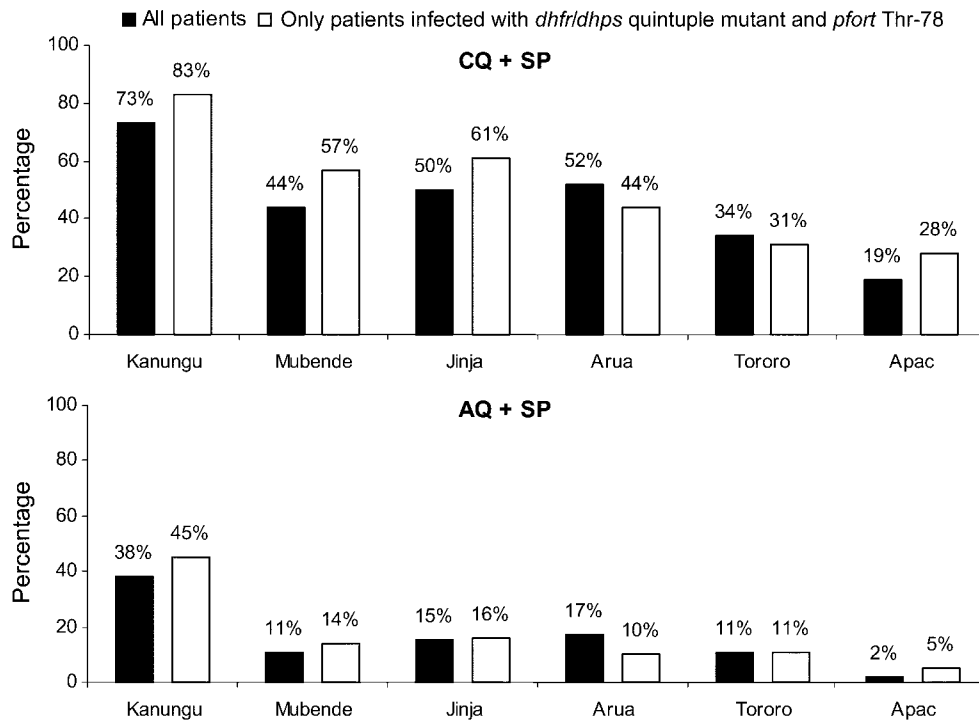


Figure 3. Risk of treatment failure at each site, with age and complexity of infection for all patients and for patients infected with the *dhfr/dhps* quintuple pure mutant (pure mutant for *dhfr* Asn-108, Ile-51, and Arg-59 and *dhps* Gly-437 and Glu-540) and the *pfprt* Thr-76 pure mutant controlled for. Results are stratified by treatment group. AQ, amodiaquine; CQ, chloroquine; SP, sulfadoxine-pyrimethamine.

paring the risk of treatment failure across the 6 study sites, with age and complexity of infection controlled for, because we felt that these were potential confounding factors. We did not control for differences in parasite density, because this may be an intermediate step in the causal pathway between site-specific factors related to the acquisition of immunity and treatment failure. The predicted risks of treatment failure for patients of median age (2 years old) and complexity of infection (3 strains) are presented in figure 3. When patients treated with CQ plus SP were considered, the risk of treatment failure was highest in Kanungu (73%), a site with a low transmission intensity and unstable malaria. The risk of treatment failure generally decreased with increasing transmission intensity; it decreased to 19% at Apac, which was the site with the highest transmission intensity. When our analysis was restricted to only those patients infected with parasites carrying all 6 mutations of interest, a similar trend was seen; the risk of treatment failure decreased from 83% to 28% between the site with the lowest and the site with the highest transmission intensity. Therefore, differences in the efficacy of CQ plus SP across the study sites were not explained by differences in the prevalence of molecular markers of drug resistance.

At all of the sites, the risk of treatment failure was significantly lower for patients treated with AQ plus SP than for patients treated with CQ plus SP ($P < .001$). However, trends for differences in treatment efficacy across the sites were similar for both treatment arms. For AQ plus SP, the risk of treatment failure decreased from 38% to 2% as transmission intensity increased when all patients were considered and from 45% to 5% when only those patients infected with parasites carrying all 6 mutations of interest were considered (figure 3).

DISCUSSION

In the present study, we sought to explain the differences in drug efficacy across 6 sites in Uganda. We found that known molecular markers of CQ and SP resistance were common throughout the country. The *pfprt* Thr-76, *dhfr* Ile-51, and *dhfr* Asn-108 mutations were nearly ubiquitous, so our analysis concentrated on the 3 mutations that most clearly predicted treatment outcomes in prior studies [12, 15, 17]. The *dhfr* Arg-59 mutation was common at all sites and most prevalent at high-transmission sites. The latter observation is consistent with that of a previous study that demonstrated a higher frequency of the *dhfr* Arg-59 mutation at sites with high levels of transmission of malaria [18]. The *dhps* Gly-437 and Glu-540 mutations were also common, and differences in prevalence were small and not clearly associated with transmission intensity. At the level of the individual patient, the presence of resistance-conferring mutations was generally associated with a higher risk of treatment failure. However, levels of mutations did not explain differences in the risk of treatment failure at the popu-

lation level. Specifically, across 6 sites in Uganda, differences in the risk of treatment failure were not related to the prevalence of molecular markers of resistance. Thus, geographic differences in treatment efficacy appeared to be mediated by factors independent of parasite mutations known to predict drug resistance. Rather, we found a trend toward decreasing risk of treatment failure with increasing transmission intensity for both treatment arms, suggesting that acquired immunity plays a critical role in the clearance of mutant parasite strains.

The spread of resistance is dictated, in part, by patterns of antimalarial drug use [19]. When CQ resistance was considered, the higher proportion of mixed infections for *pfprt* Thr-76 at Apac may have been due to low access to antimalarial medications at this site, which is partially isolated from the rest of the country by conflict and geography. Indeed, both the prevalence of the *pfprt* Thr-76 mutation and CQ usage were lowest at Apac in an earlier comparison of the same sites [18]. Despite these differences, it is clear that parasites containing the *pfprt* Thr-76 mutation are now virtually ubiquitous in the studied regions of Uganda. When resistance to SP was considered in a prior study from southeastern Africa, the geographic spread of resistant alleles, rather than the spontaneous generation of new mutations, was the most common source of antifolate resistance in the regions studied [20]. Our results indicate that 2 of these alleles, the *dhfr* Asn-108 plus Ile-51 double mutant and the Asn-108 plus Ile-51 plus Arg-59 triple mutant, have almost completely replaced the *wt dhfr* allele in the studied regions of Uganda. At all sites, the *dhps* Gly-437 and Glu-540 mutations were more common than the *dhfr* Arg-59 mutation, suggesting that the *dhps* double mutant allele has spread through Uganda more quickly than has the *dhfr* triple mutant allele. This is consistent with the pattern of spread documented elsewhere in southeastern Africa [20].

The risk of treatment failure was lowest at sites with the highest transmission intensity, yet we found a high prevalence of resistant genotypes at all sites, with a higher prevalence of the *dhfr* Arg-59 mutation at the 2 sites with the highest transmission intensities. Restricting our analysis to patients with all 6 mutations of interest did not change these trends; the risk of treatment failure remained lowest at the high-transmission sites for both treatment arms. Thus, differences in outcomes were associated with differences in transmission intensity rather than with differences in parasite populations. In a previous study that explored the relationships between malaria transmission intensity, drug use, and response to monotherapies at these same sites, it was observed that the risk of failure of SP treatment was highest at the sites with the highest transmission intensities, whereas that for CQ was highest at the extremes of the transmission-intensity spectrum [21]. However, these studies had relatively few study participants. In vivo response to antimalarial therapy is a complex phenomenon that is depen-

dent not only on interactions between the drug and the parasite but also on host-parasite and host-drug interactions. However, *in vivo* treatment failure reflects the response to therapy in a milieu of factors and is probably the most useful parameter in the later stages of the evolution of resistance. We have demonstrated a striking relationship between transmission intensity and treatment failure and, on the other hand, combination therapy. Our studies were probably performed late in the evolution of drug resistance; the mutations were approaching a saturation point at most of the sites. Relationships between response to therapy, transmission intensity, and molecular markers of drug resistance may differ at an earlier time in the evolution of drug resistance. Nevertheless, these data confirm that malaria transmission intensity is a critical indirect determinant of response to antimalarial therapy. High transmission intensity leads to more-frequent exposure to *Plasmodium falciparum* and faster acquisition of antimalarial immunity [22, 23]. Our results suggest that differences in the rate of acquisition of immunity, rather than differences in parasite populations, best explain the lower risk of treatment failure at sites with higher transmission intensities. The relative importance of immunity in determining the response to therapy has been demonstrated in other studies that showed that age, a surrogate marker of acquired immunity, is associated with the ability to clear parasites with resistant genotypes [9, 24].

The inverse relationship between transmission intensity and the risk of treatment failure could also be explained by unknown markers of drug resistance that are more prevalent at low-transmission sites. A recent study from Afghanistan speculated that similarly high levels of resistance to both AQ and CQ were associated with a specific *pfcr* genotype that was present in all isolates [25]. Polymorphisms in *pfmdr-1* have been associated with CQ treatment outcomes in some studies [26]. We cannot rule out the possibility that a high prevalence of certain alleles that were not characterized in the present study might explain the higher risk of failure at Kanungu in both treatment arms, as well as the high risk of failure following treatment with AQ at a nearby site in Rwanda [27, 28]. It is unlikely that there is an unidentified marker of SP resistance that is more prevalent at low-transmission sites, since the 5 *dhfr* and *dhps* mutations we measured correlate best with clinical outcomes in Africa following SP therapy [12, 15, 17, 29–31] and other mutations in these genes have only rarely been identified in Africa [32].

The risk of failure in patients treated with CQ plus SP was unacceptably high at all sites [5, 6], which is consistent with previous findings [33]. In contrast, AQ plus SP had a relatively low risk of treatment failure at 5 of the 6 sites. Using data from the present study, we can examine the efficacy of AQ plus SP when the prevalence of genetic determinants of resistance to SP is very high. At 4 of the sites that also included an AQ plus

artesunate (AS) treatment arm, for patients infected with parasites carrying all 6 mutations of interest, the predicted risk of treatment failure with AQ plus SP was 5%–16% (figure 3), compared with 5%–11% for patients treated with AQ plus AS. A recent study concluded that the useful therapeutic life span of AQ plus SP would be very short if the combination were introduced after the prevalence of the *dhfr* triple mutant allele (Asn-108 plus Ile-51 plus Arg-59) was >5% [34], but our data show that AQ plus SP is effective in Ugandan populations even when all infections have the *dhfr* triple mutant allele and the *dhps* double mutant allele (Gly-437 plus Glu-540). These results suggest that the useful therapeutic life span of AQ plus SP may be longer than predicted in areas where malaria is highly endemic and immunity is high.

Despite evidence for continued good efficacy, several important factors may limit the promise of AQ plus SP as a treatment for malaria in Africa. First, the *dhfr* Leu-164 mutation, which is associated with high levels of resistance to antifolates in both South America and Asia, was rare in samples from the present study and others in Africa [35], but, if it were to spread, it is likely that the incidence of treatment failure with AQ plus SP would increase. Second, AQ resistance in some parts of Africa is an important concern. In coastal Tanzania, where AQ resistance is particularly prevalent, AQ plus SP was less effective than 2 artemisinin-based combinations [36]. Third, decreasing transmission intensity through control measures may lead to slower acquisition of antimalarial immunity and reduced efficacy of regimens, such as AQ plus SP, that rely on host responses for optimal efficacy.

In summary, molecular markers of resistance were very common at all 6 study sites in Uganda. Geographic differences in the response to therapy were best explained by differences in acquired immunity rather than by identifiable differences in drug-resistance markers. Perhaps most importantly, at high-transmission sites, where acquired immunity is presumably the highest, AQ plus SP was efficacious even in patients infected with parasites that had all mutations known to predict SP resistance in Africa. Because of resistance to multiple drugs and the limited supply and high cost of highly effective artemisinin-based combinations, many countries now face the possibility of a substantial increase in malaria-related morbidity and mortality. In this situation, it is prudent to continue to monitor the efficacy of AQ plus SP, which, despite its limitations, is likely the only available low-cost combination therapy that remains efficacious for the treatment of uncomplicated malaria in most of Africa.

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