Changing antimalarial drug resistance patterns identified by surveillance at three sites in Uganda

Patrick Tumwebaze,[1a] Stephen Tukwasibwe,[1a] Aimee Taylor,[2] Melissa Conrad,[3] Emmanuel Ruhamyankaka,[1] Victor Asua,[1] Andrew Walakira,[1] Joaniter Nankabirwa,[1] Adoke Yeka,[4] Sarah G. Staedke,[5] Bryan Greenhouse,[3] Samuel L. Nsobya,[1] Moses R. Kamya,[1,6] Grant Dorsey,[3] and Philip J. Rosenthal[3]

[1]Infectious Diseases Research Collaboration, Kampala, Uganda; [2]Center for Communicable Disease Dynamics, Harvard T.H. Chan School of Public Health, Boston, MA, USA and the Broad Institute of MIT and Harvard, Cambridge, MA, USA; [3]Department of Medicine, University of California, San Francisco, California, USA; [4]Makerere University School of Public Health, College of Health Sciences, Kampala, Uganda; [5]London School of Hygiene and Tropical Medicine, London, UK; [6]Department of Medicine, Makerere University College of Health Sciences, Kampala, Uganda

[a]These authors contributed equally.

Running title: Antimalarial drug resistance in Uganda

Word count:

Abstract: 100

Text: 2,000

Keywords : Malaria; *Plasmodium falciparum*; drug resistance; artemisinin; chloroquine; artemether; lumefantrine; artesunate; amodiaquine; dihydroartemisinin; piperaquine; pfcrt; pfmdr1; K13

Footnote page

Potential conflicts of interest. All authors: No reported conflicts.

Financial support. This work was supported by grants from the National Institutes of Health (AI075045 and AI089674).

Corresponding author: Philip J. Rosenthal, Dept. of Medicine, University of California, San Francisco, CA 94143 USA; Phone: (415) 206-8845; FAX: (415) 648-8425; E-mail: philip.rosenthal@ucsf.edu

Abstract

We assessed *Plasmodium falciparum* drug resistance markers in parasites collected in 2012, 2013, and 2015 at 3 sites in Uganda. The prevalence and frequency of parasites with mutations in putative transporters previously associated with resistance to aminoquinolines, but increased sensitivity to lumefantrine (*pfcrt* 76T; *pfmdr1* 86Y and 1246Y), decreased markedly at all sites. Antifolate resistance mutations were common, with apparent emergence of mutations (*pfdhfr* 164L; *pfdhps* 581G) associated with high level resistance. K13 mutations linked to artemisinin resistance were uncommon and did not increase over time. Changing malaria treatment practices have been accompanied by profound changes in markers of resistance.

Drug resistance challenges the treatment and control of malaria. In Africa, use of artemisinin-based combination therapies (ACTs) has become standard to treat uncomplicated malaria, accompanied by changes in the selective pressure for resistance. In Uganda, the first line regimen to treat uncomplicated malaria changed from chloroquine to chloroquine + sulfadoxine/pyrimethamine in 2000 and artemether-lumefantrine (AL) in 2004, although implementation was slow. Elsewhere in Africa, artesunate-amodiaquine (AS/AQ) is first line to treat malaria in many countries and dihydroartemisinin-piperaquine (DHA/PQ) is under study for chemoprevention. SP remains the standard-of-care to prevent malaria in pregnant women.

Our understanding of antimalarial drug resistance is incomplete, but some *Plasmodium falciparum* genetic polymorphisms are clearly important. The 76T mutation in the putative transporter PfCRT is linked to decreased sensitivity to the aminoquinolines chloroquine and amodiaquine, and *pfcrt* mutations are selected in new infections that occur soon after treatment with aminoquinolines [1, 2]. Mutations in *pfmdr1*, which encodes another putative transporter, the p-glycoprotein homologue, are also associated with altered drug sensitivity. In Africa, the *pfmdr1* 86Y and 1246Y mutations are common, associated with decreased sensitivity to

aminoquinolines, and selected by prior treatment with AS/AQ and DHA/PQ [1, 3]. Interestingly, wild type sequences at these same alleles are associated with decreased sensitivity to lumefantrine and selected by recent treatment with AL, demonstrating opposite effects of the same polymorphisms on sensitivity to different drugs [1, 2].

Resistance to SP is well characterized, with 5 mutations in dihydrofolate reductase (51I, 59R, and 108N) and dihydropteroate synthetase (437G and 540E) now common in much of Africa and associated with an intermediate level of resistance [4]. Additional mutations, notably *pfdhfr* 164L and *pfdhps* 581G, lead to high level resistance. These mutations have been rare in African surveys, but recent studies have suggested emergence in some areas [5, 6].

Changing malaria treatment practices may lead to changes in drug sensitivity. In Malawi, the replacement of chloroquine with SP was followed by widespread *pfcrt* wild type parasites and excellent clinical efficacy of chloroquine [7]. In Uganda, increased prevalence of wild type *pfcrt* K76 and *pfmdr1* N86 and D1246 sequences was seen in Tororo from 2003-12 [2, 8], although analyses were complicated by numerous mixed genotypes. In ex vivo studies, increasing sensitivity to chloroquine and decreasing sensitivity to lumefantrine were documented [2]. Consistent with these changes, and in contrast to older studies that showed superiority of AL, in a recent 3-site trial, treatment with AS/AQ was followed by decreased recurrent malaria compared to AL [9]. Thus, parasites in Uganda are changing, and these changes appear to have clinical consequences. However, improved measures of parasite trends will be helpful, in particular utilizing randomly collected samples rather than those available from drug efficacy trials, and including analyses of frequency, which circumvent the complexities of polyclonal infections. We now report the prevalence and frequency of key polymorphisms utilizing samples collected by probability sampling.

METHODS

Cross-sectional surveys and sample collection. Cross-sectional surveys were conducted in 200 randomly selected households each at 3 sites in 2012, 2013, and 2015 as previously described [10]**.** Samples were collected during the same period for each year of the study, January-February in Nagongera, Tororo District, in eastern Uganda near Kenya; March-April in Walukuba, Jinja District, in south-central Uganda on Lake Victoria; and May-June in Kihihi, Kanungu District, in southwestern Uganda. The sites varied greatly in malaria transmission intensity (annual entomological inoculation rates 3.8, 26.6, and 125.0 infectious bites per person year for Walukuba, Kihihi, and Nagongera, respectively). Households were randomly selected as described, and finger prick blood samples collected on filter paper from all children under 15 years of age and 1 randomly selected adult from each of 5 age categories; samples were collected regardless of whether symptoms were present [10].

Assessment of *P. falciparum* polymorphisms. Parasite DNA was extracted from dried blood spots from samples positive by microscopy, and sequences of alleles of interest in *pfcrt*, *pfmdr1*, *pfdhfr*, and *pfdhps* were determined using a ligase detection reaction-fluorescent microsphere assay, as previously described [11], with minor modifications, including nested PCR amplifications of templates, as described [12]. The K13 gene propeller domain was amplified and sequenced as previously described [13].

Frequency and linkage analyses. Parasite population frequencies were estimated using all 1466 samples and a previously described model that accounts for mixed infections [14]. Linkage disequilibrium was estimated for all samples, but results from alleles with mixed or missing genotyping outcomes were omitted from the analysis (see supplementary file for details).

RESULTS

Prevalence of resistance-mediating polymorphisms in putative transporters. A total of 1486 microscopy-positive samples were collected and analyzed. Sequences at all alleles of interest were classified as wild type (identical in sequence to the reference 3D7 strain), mutant, or mixed for the 1466 samples that yielded data for at least one polymorphism. Considering the key transporter polymorphisms *pfcrt* K76T, *pfmdr1* N86Y, and *pfmdr1* D1246Y, the prevalence of parasites with mutant sequences decreased steadily from 2012 to 2015 (Figure 1A). Results were similar at the 3 sites. For another polymorphic allele, *pfmdr1* Y184F, the prevalence of parasites with mutant alleles increased in Jinja, but was stable at other sites. The *pfmdr1* 1034C and 1042D mutations, generally seen only outside Africa, were identified rarely.

Prevalence of resistance-mediating polymorphisms in folate pathway enzymes. The prevalence of parasites with 5 mutations (*pfdhfr* 51I, 59R, 108N; *pfdhps* 437G, 540E) that were common in prior surveys remained high (Figure 1A). In addition, 2 mutations that have previously been rare in most surveys from Africa were seen, with *pfdhfr* 164L in Kanungu, and *pfdhps* 581G at all 3 sites in in 2015.

Frequency of resistance-mediating polymorphisms and haplotype analysis. Prevalence data did not take into account multiplicity of infection (MOI) and were complicated by many samples containing both wild type and mutant sequences. Therefore, we used a statistical model to estimate parasite population frequencies for the studied polymorphisms. Frequency results were broadly similar to those based on prevalence; point estimates for the mutant transporter alleles *pfcrt* 76T, *pfmdr1* 86Y, and *pfmdr1* 1246Y decreased markedly from 2012 to 2015 at all 3 sites (Figure 1B). Estimates of linkage disequilibrium suggested linkage between *pfmdr1* 86, 184, and 1246, and *pfcrt* 76; between *pfmdr1* 1246 and *pfcrt* 76; and between *pfdhps* 437 and 540 (Figure 2). Considering haplotypes, Y184F did not appear to impact trends,

with increases in both NYD and NFD haplotypes over time (Supplemental Figure 1); there was a marked increase in frequency of the *pfmdr1* N86/D1246 haplotype, suggesting that parasites with both wild type alleles have a selective advantage (Supplemental Figure 2); and the wild type *pfmdr1* N86/D1246 haplotype was associated with both *pfcrt* K76T alleles, while K76 was associated only with N86/D1246 (Supplemental Figure 3).

Prevalence of K13 polymorphisms. We randomly selected 20 samples from each site and year for analysis. Of the 153 sequences obtained, 4 samples had non-synonymous polymorphisms, with a total of 5 mutations, each identified once (V555A from Kihihi in 2012; M472V from Nagongera in 2013; A569S from Nagongera in 2015; K563E and A578S in a single sample from Walukuba in 2013). K13 mutations were seen in samples from all 3 sites and all 3 years of study. Mutations at 4 of the loci were reported previously in Africa, from Niger (M472I), Rwanda (V555A), Kenya and Cameroon (A569S), and 7 different countries, including Uganda (A578S), but to our knowledge K563E has not been reported (http://www.wwarn.org/molecular-surveyor-k13).

DISCUSSION

We surveyed the prevalence and calculated the frequency of key drug resistance polymorphisms in *P. falciparum* isolates collected at 3 sites in Uganda from 2012 to 2015. This study improved on prior evaluations by studying randomly collected isolates, rather than those collected in the context of a clinical trial, and by evaluating the frequency of polymorphisms, circumventing confounding by varied MOI and prevalence of mixed infections at different sites. We identified important changes over time. Notably, with increasing use of AL to treat malaria, the prevalence and frequency of mutant sequences at 3 key *pfcrt* and *pfmdr1* alleles decreased, consistent with decreasing sensitivity to lumefantrine, but increasing sensitivity to

aminoquinolines. In addition, mutations in *pfdhfr* and *pfdhps* that mediate high level antifolate resistance appear to be emerging.

Changes in the prevalence of parasites with drug resistance polymorphisms were not unexpected. In Malawi, discontinuation of chloroquine as the first-line antimalarial was accompanied by loss of the *pfcrt* 76T mutation and regaining of chloroquine antimalarial efficacy [7]. Multiple studies showed selection for mutant genotypes by amodiaquine-containing regimens and for wild type genotypes by AL [1]. In Uganda, wild type sequences at 3 key transporter alleles are increasingly common. Most parasites studied in Tororo were mutant at these 3 alleles through about 2010 [8], but wild type sequences have been increasingly prevalent since that time. The rate of change toward wild type transporter sequences was greater in a cohort treated with AL for every episode of malaria, compared to a cohort treated with DHA/PQ, documenting the contribution of selective pressure from AL to this process [12].

Mutations in *pfdhfr* and *pfdhps* have been common in Uganda for at least a decade. Use of SP to treat malaria, WHO-recommended SP for intermittent preventive therapy in pregnant women, antifolates to treat bacterial infections, and trimethoprim-sulfamethoxazole in HIV-infected individuals likely all offer continued selective pressure for antifolate resistance. It was thus of interest to see if additional mutations that have been seen primarily outside Africa [4] are emerging in Uganda. The *pfdhfr* 164L mutation was identified in an earlier survey in southwestern Uganda [5], and it was present in Kanungu, also in southwestern Uganda, over the course of our study. The *pfdhps* 581G mutation, which has been noted in Tanzania [6], was detected at all 3 study sites. These additional mutations will probably render SP useless for the treatment or control of malaria. Consideration of other regimens for the prevention of malaria, notably DHA/PQ, which recently showed outstanding efficacy in children and pregnant women [3, 15], is an urgent priority.

Frequency analyses clarified results by accounting for MOI and mixed infections, and were consistent with prevalence results. Linkage analyses demonstrated linkage between the

transporter polymorphisms *pfmdr1* 86, 184, 1246, and *pfcrt* 76. Haplotype analyses demonstrated an apparent selective advantage of parasites with the wild type alleles *pfmdr1* N86 and D1246, and that wild type *pfcrt* K76 was present almost exclusively with a background of *pfmdr1* N86/D1246. As *pfcrt* 76T is the main mediator of resistance to chloroquine, but decreased sensitivity to lumefantrine is linked to all 3 of these polymorphisms [1, 2], it seems likely that the evolution of transporter polymorphisms has been driven both by decreasing use of chloroquine and increasing use of AL over time.

Resistance to artemisinins, mediated principally by mutations in K13, is of great concern, but resistance does not appear to have yet spread to Africa. We identified a handful of K13 propeller domain mutations, but no evidence of geographic differences in prevalence or changes over time. K13 polymorphisms may be under selection from ACT use in Uganda, but they do not yet appear to be mediating artemisinin resistance.

Our study had some limitations. First, we studied only 3 sites over 4 years; important additional trends may be underway, but not evident over this short interval. Second, sample sizes were fairly small, especially for Jinja, where decreasing prevalence limited available samples over time. Third, we considered only a small number of well characterized resistance-mediating polymorphisms. Consideration of full sequences of genes of interest or of whole genomes might identify additional important trends in the evolution of drug resistance.

In summary, surveillance for *P. falciparum* drug resistance markers in Uganda has demonstrated marked changes in recent years, with a return to wild type transporter sequences that likely mediate decreased sensitivity to AL, the national regimen to treat malaria, emergence of mutations that mediate high level antifolate resistance, but no convincing evidence of artemisinin resistance. Continued surveillance for mediators of antimalarial drug resistance is warranted. Furthermore, as selective pressures of AS/AQ and DHA/PQ differ from those of AL [1-3, 9, 12], we suggest consideration of rotating treatment regimens to delay emergence of resistance.

Acknowledgments. We thank the study communities for their participation; the study team of Winnie Nuwagaba, David Ntege, Stephen Gama, Joseph Omara, Ronald Bampiga, Angella Bukirwa and Moses Were; and the staff of the Infectious Diseases Research Collaboration for administrative and technical support. This work was supported by grants from the National Institutes of Health (AI075045 and AI089674).

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Figure 1.

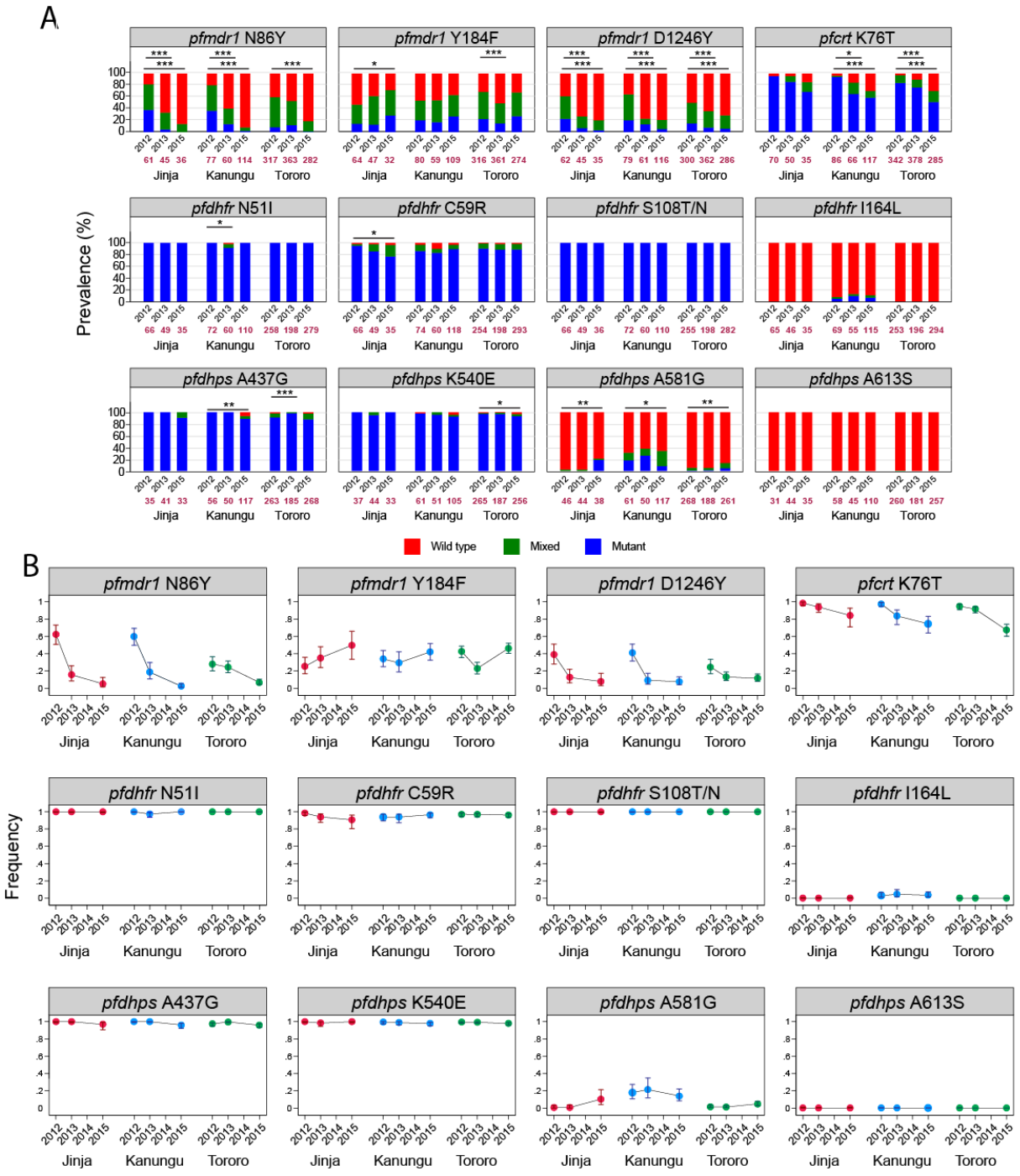

Figure 1. A. Prevalence of wild type, mixed, or mutant sequences at alleles of interest at the indicated sites and years. The numbers under the dates represent the number of samples generating results. Asterisks represent p-values based on comparisons with pure wild type infections for *pfmdr1* and *pfcrt* and pure mutant infections for *pfdhfr* and *pfdhps* using the Fisher's exact test (***<0.001, **<0.01,* <0.05). **B. Frequency of mutant sequences.** Error bars represent 95% credible intervals.

Figure 2.

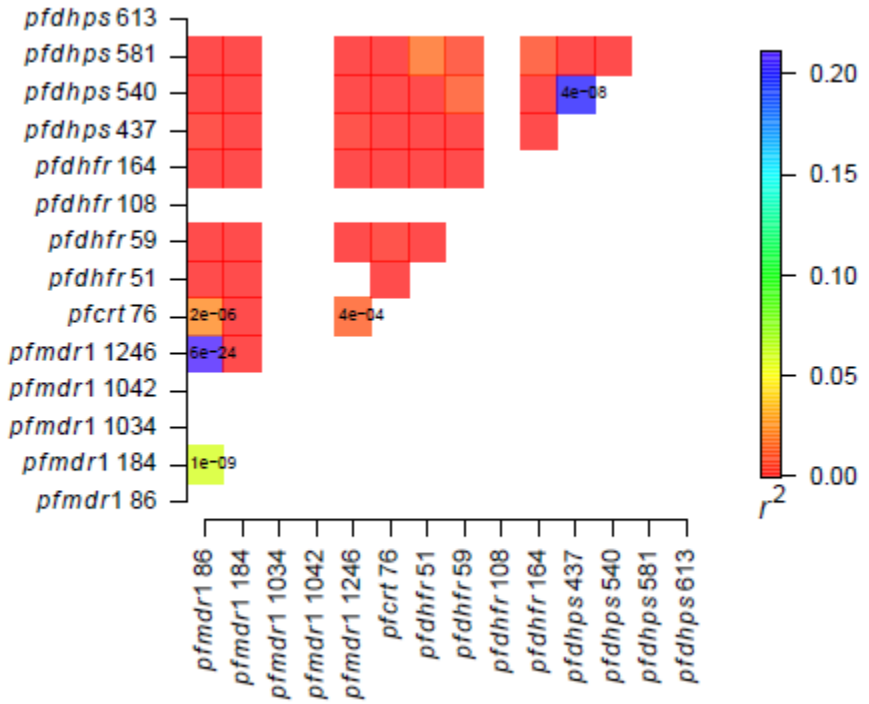

Figure 2. Plot of linkage disequilibrium for pairwise comparisons of all studied *pfcrt, pfmdr1, pfdhfr, and pfdhps* polymorphisms. Linkage disequilibrium, r^2, captures the non-random association of alleles within one gene or in different genes, with values ranging from 0 (no correlation) to 1 (perfect correlation). Colors indicate r^2 values (see supplementary file for details), calculable for polymorphic markers only; white denotes non-polymorphic markers. P-values of statistically significant r^2 values are shown. These were calculated using Fisher's exact test, with a Bonferroni correction for 45 tests over 10 polymorphic markers, placing significance at 0.001.

Supplement to Tumwebaze, et al.: Changing antimalarial drug resistance patterns identified by surveillance at three sites in Uganda

SUPPLEMENTAL METHODS

Frequency analyses

Frequencies were estimated using a previously published model [1] applied to data from different sites and years separately. We used a Dirichlet prior with concentration parameter equal to 0.1 over frequencies and a truncated geometric prior over each multiplicity of infection (MOI), $\rho(MOI) = \frac{\lambda\,(1-\lambda)^{MOI}}{\sum_{MOI=MOI_{min}}^{MOI_{max}} \lambda\,(1-\lambda)^{MOI}}$, where $MOI_{min} = 1$, or 2 if the sample was discernibly multiclonal, $MOI_{max} = 20$, and λ was based on experimentally derived MOI estimates by genotyping the *msp2* locus via nested PCR and capillary electrophoresis [2]. To account for ambiguity in MOI estimates greater than or equal to 5, the frequency results were generated by combining a posteriori samples from two separate runs of the model, one with λ equal to the reciprocal of the sample mean MOI per site and year, and one with λ equal to the reciprocal of the mean MOI over samples with MOI less than or equal to 5 per site and year.

Linkage analyses

Linkage disequilibrium, r^2 [3], was estimated for all studied *pfcrt, pfmdr1, pfdhfr, and pfdhps* polymorphisms, using data from all 1466 samples yielding results, but discarding counts with one or more mixed or missing genotyping outcomes from within-calculation quotients. P-values were calculated using Fisher's exact test. For 45 tests over 10 polymorphic markers, a Bonferroni correction on uncorrected significance at 0.05 placed significance at 0.001.

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Supplemental Table 1. Prevalence of wild type, mixed, or mutant sequences at transporter alleles of interest at the indicated sites and years. N = number of samples.

Locus	Site	Year	N	Wild type	Mixed	Mutant
***Pfmdr1* N86Y**	Jinja	2012	61	11 (18.0%)	27 (44.3%)	23 (37.7%)
	Jinja	2013	45	30 (66.7%)	13 (28.9%)	2 (4.4%)
	Jinja	2015	36	31 (86.1%)	5 (13.9%)	0
	Kanungu	2012	77	15 (19.5%)	34 (44.2%)	28 (36.4%)
	Kanungu	2013	60	36 (60.0%)	16 (26.7%)	8 (13.3%)
	Kanungu	2015	114	105 (92.1%)	7 (6.1%)	2 (1.8%)
	Tororo	2012	317	128 (40.4%)	163 (51.4%)	26 (8.2%)
	Tororo	2013	363	170 (46.8%)	151 (41.6%)	42 (11.6%)
	Tororo	2015	282	229 (81.2%)	51 (18.1%)	2 (0.7%)
***Pfmdr1* Y184F**	Jinja	2012	64	34 (53.1%)	21 (32.8%)	9 (14.1%)
	Jinja	2013	47	18 (38.3%)	23 (48.9%)	6 (12.8%)
	Jinja	2015	32	9 (28.1%)	14 (43.8%)	9 (28.1%)
	Kanungu	2012	80	37 (46.3%)	27 (33.8%)	16 (20.0%)
	Kanungu	2013	59	27 (45.8%)	22 (37.3%)	10 (17.0%)
	Kanungu	2015	109	40 (36.7%)	40 (36.7%)	29 (26.6%)
	Tororo	2012	316	99 (31.3%)	147 (46.5%)	70 (22.2%)
	Tororo	2013	361	184 (51.0%)	124 (34.4%)	53 (14.7%)
	Tororo	2015	274	88 (32.1%)	112 (40.9%)	74 (27.0%)
***Pfmdr1* S1034C**	Jinja	2012	72	72 (100%)	0	0
	Jinja	2013	47	47 (100%)	0	0
	Jinja	2015	15	15 (100%)	0	0
	Kanungu	2012	81	81 (100%)	0	0
	Kanungu	2013	67	67 (100%)	0	0
	Kanungu	2015	59	59 (100%)	0	0
	Tororo	2012	174	174 (100%)	0	0
	Tororo	2013	374	374 (100%)	0	0
	Tororo	2015	158	158 (100%)	0	0
***Pfmdr1* N1042D**	Jinja	2012	72	72 (100%)	0	0
	Jinja	2013	47	46 (97.9%)	1 (2.1%)	0
	Jinja	2015	15	15 (100%)	0	0
	Kanungu	2012	81	81 (100%)	0	0
	Kanungu	2013	55	54 (98.2%)	1 (1.8%)	0
	Kanungu	2015	65	65 (100%)	0	0
	Tororo	2012	167	167 (100%)	0	0
	Tororo	2013	363	361 (99.5%)	2 (0.6%)	0
	Tororo	2015	159	159 (100%)	0	0
***Pfmdr1* D1246Y**	Jinja	2012	62	24 (38.7%)	24 (38.7%)	14 (22.6%)
	Jinja	2013	45	33 (73.3%)	9 (20.0%)	3 (6.7%)
	Jinja	2015	35	28 (80.0%)	6 (17.1%)	1 (2.9%)
	Kanungu	2012	79	28 (35.4%)	35 (44.3%)	16 (20.3%)
	Kanungu	2013	61	47 (77.1%)	6 (9.8%)	8 (13.1%)
	Kanungu	2015	116	92 (79.3%)	18 (15.5%)	6 (5.2%)
	Tororo	2012	300	149 (49.7%)	107 (35.7%)	44 (14.7%)
	Tororo	2013	362	233 (64.4%)	103 (28.5%)	26 (7.2%)
	Tororo	2015	286	205 (71.7%)	65 (22.7%)	16 (5.6%)
***Pfcrt* K76T**	Jinja	2012	70	3 (4.3%)	0	67 (95.7%)
	Jinja	2013	50	2 (4.0%)	5 (10.0%)	43 (86.0%)
	Jinja	2015	35	5 (14.3%)	6 (17.1%)	24 (68.6%)

	Kanungu	2012	86	3 (3.5%)	2 (2.3%)	81 (94.2%)
	Kanungu	2013	66	10 (15.2%)	13 (19.7%)	43 (65.2%)
	Kanungu	2015	117	35 (29.9%)	13 (11.1%)	69 (59.0%)
	Tororo	2012	342	9 (2.6%)	45 (13.2%)	288 (84.2%)
	Tororo	2013	378	39 (10.3%)	51 (13.5%)	288 (76.2%)
	Tororo	2015	285	85 (29.8%)	54 (19.0%)	146 (51.2%)

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Supplemental Table 2. Prevalence of wild type, mixed, or mutant sequences at antifolate alleles of interest at the indicated sites and years. N = number of samples.

Locus	Site	Year	N	Wild type	Mixed	Mutant
***Pfdhfr* N51I**	Jinja	2012	66	0	0	66 (100%)
	Jinja	2013	49	0	0	49 (100%)
	Jinja	2015	35	0	0	35 (100%)
	Kanungu	2012	72	0	0	72 (100%)
	Kanungu	2013	60	1 (1.7%)	4 (6.7%)	55 (91.7%)
	Kanungu	2015	110	0	0	110 (100%)
	Tororo	2012	258	0	0	258 (100%)
	Tororo	2013	198	0	0	198 (100%)
	Tororo	2015	279	0	0	279 (100%)
***Pfdhfr* C59R**	Jinja	2012	66	1 (1.5%)	2 (3.0%)	63 (95.5%)
	Jinja	2013	49	1 (2.0%)	6 (12.2%)	42 (85.7%)
	Jinja	2015	35	1 (2.9%)	7 (20.0%)	27 (77.1%)
	Kanungu	2012	74	2 (2.7%)	8 (10.8%)	64 (86.5%)
	Kanungu	2013	60	6 (10.0%)	4 (6.7%)	50 (83.3%)
	Kanungu	2015	118	3 (2.5%)	9 (7.6%)	106 (89.8%)
	Tororo	2012	254	2 (0.8%)	22 (8.7%)	230 (90.6%)
	Tororo	2013	198	3 (1.5%)	19 (9.6%)	176 (88.9%)
	Tororo	2015	293	3 (1.0%)	29 (9.9%)	261 (89.1%)
***Pfdhfr* S108T/N**	Jinja	2012	66	0	0	66 (100%)
	Jinja	2013	49	0	0	49 (100%)
	Jinja	2015	36	0	0	36 (100%)
	Kanungu	2012	72	0	0	72 (100%)
	Kanungu	2013	60	0	0	60 (100%)
	Kanungu	2015	110	0	0	110 (100%)
	Tororo	2012	255	0	0	255 (100%)
	Tororo	2013	198	0	0	198 (100%)
	Tororo	2015	282	0	0	282(100%)
***Pfdhfr* I164L**	Jinja	2012	65	65 (100%)	0	0
	Jinja	2013	46	46 (100%)	0	0
	Jinja	2015	35	35 (100%)	0	0
	Kanungu	2012	69	64 (92.8%)	2 (2.9%)	3 (4.4%)
	Kanungu	2013	55	48 (87.3%)	2 (3.6%)	5 (9.1%)
	Kanungu	2015	115	103 (89.6%)	5 (4.4%)	7 (6.1%)
	Tororo	2012	253	253 (100%)	0	0
	Tororo	2013	196	196 (100%)	0	0
	Tororo	2015	294	294 (100%)	0	0
***Pfdhps* A437G**	Jinja	2012	35	0	0	35 (100%)
	Jinja	2013	41	0	0	41 (100%)
	Jinja	2015	33	0	3 (9.1%)	30 (90.9%)
	Kanungu	2012	56	0	0	56 (100%)
	Kanungu	2013	50	0	0	50 (100%)
	Kanungu	2015	117	7 (6.0%)	6 (5.1%)	104 (88.9%)
	Tororo	2012	263	4 (1.5%)	18 (6.8%)	241 (91.6%)
	Tororo	2013	185	0	2 (1.1%)	183 (98.9%)
	Tororo	2015	268	5 (1.9%)	26 (9.7%)	237 (88.4%)
***Pfdhps* K540E**	Jinja	2012	37	0	0	37 (100%)
	Jinja	2013	44	0	2 (4.6%)	42 (95.5%)
	Jinja	2015	33	0	0	33 (100%)

	Kanungu	2012	61	1 (1.6%)	0	60 (98.4%)
	Kanungu	2013	51	0	2 (3.9%)	49 (96.1%)
	Kanungu	2015	105	4 (3.9%)	3 (2.9%)	98 (93.3%)
	Tororo	2012	265	2 (0.8%)	4 (1.5%)	259 (97.7%)
	Tororo	2013	187	1 (0.5%)	4 (2.1%)	182 (97.3%)
	Tororo	2015	256	7 (2.7%)	9 (3.5%)	240 (93.8%)
***Pfdhps* A581G**	Jinja	2012	46	45 (97.8%)	1 (2.2%)	0
	Jinja	2013	44	43 (97.7%)	1 (2.3%)	0
	Jinja	2015	38	30 (79.0%)	1 (2.6%)	7 (18.4%)
	Kanungu	2012	61	42 (68.9%)	8 (13.1%)	11 (18.0%)
	Kanungu	2013	50	31 (62.0%)	6 (12.0%)	13 (26.0%)
	Kanungu	2015	117	77 (65.8%)	31 (26.5%)	9 (7.7%)
	Tororo	2012	268	254 (94.8%)	13 (4.9%)	1 (0.4%)
	Tororo	2013	188	179 (95.2%)	7 (3.7%)	2 (1.1%)
	Tororo	2015	261	226 (86.6%)	24 (9.2%)	11 (4.2%)
***Pfdhps* A613S**	Jinja	2012	31	31 (100%)	0	0
	Jinja	2013	44	44 (100%)	0	0
	Jinja	2015	35	35 (100%)	0	0
	Kanungu	2012	58	58 (100%)	0	0
	Kanungu	2013	45	45 (100%)	0	0
	Kanungu	2015	110	110 (100%)	0	0
	Tororo	2012	260	259 (99.6%)	1 (0.4%)	0
	Tororo	2013	181	181 (100%)	0	0

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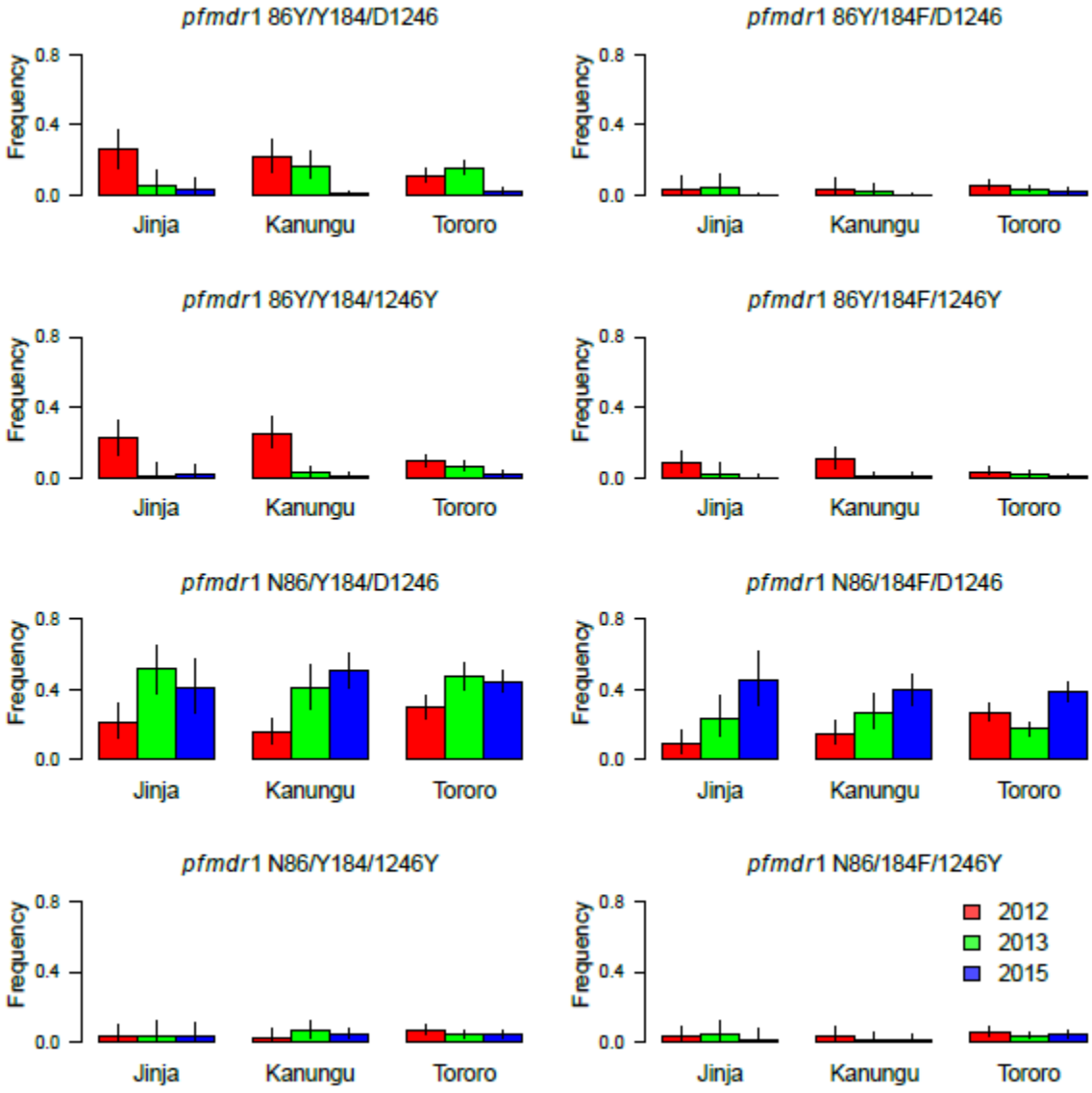
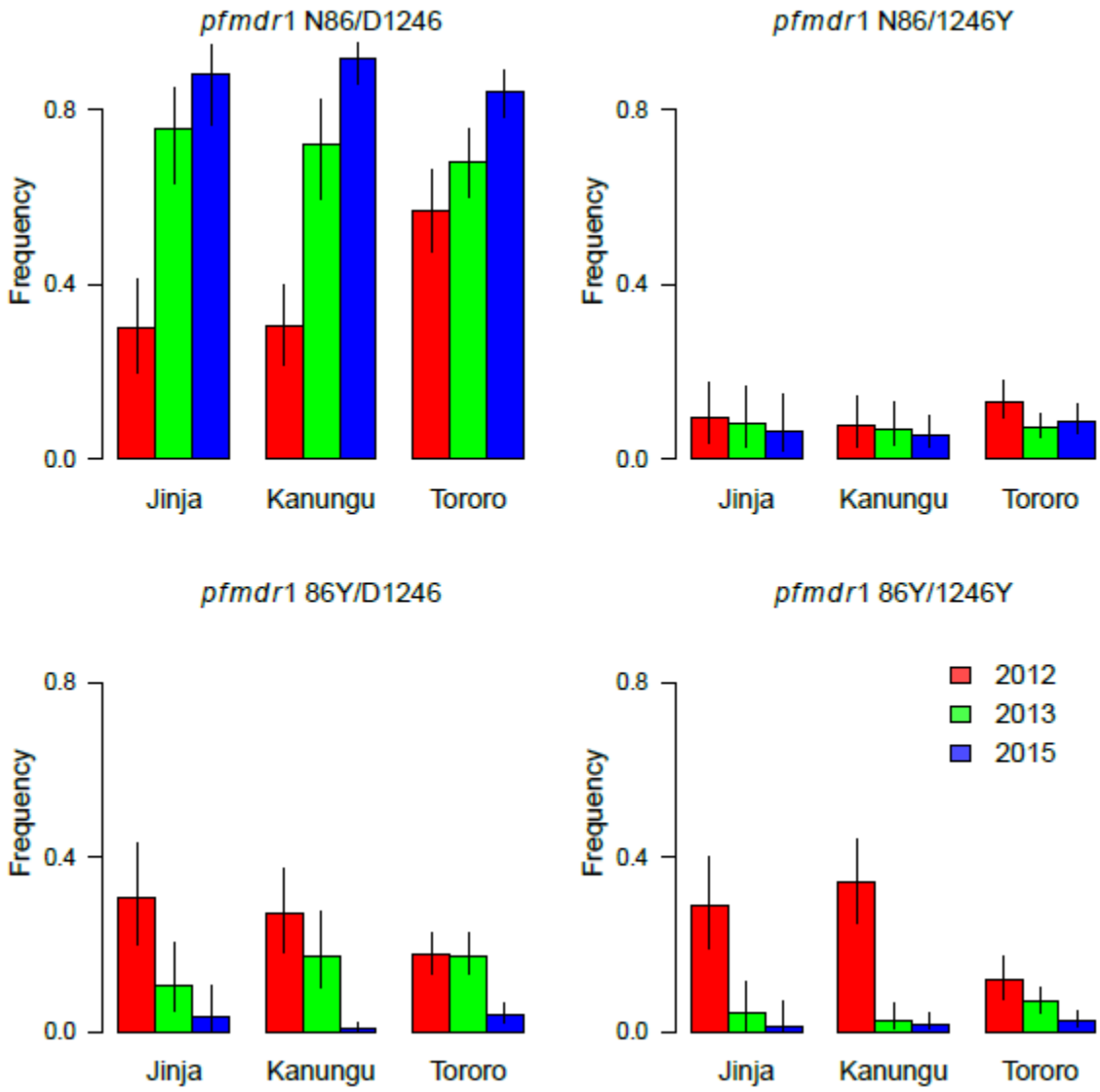
Supplemental Figure 1. Frequency point estimates of *pfmdr1* N86Y/Y184F/D1246Y haplotypes. Error bars represent 95% credible intervals.



Supplemental Figure 2. Frequency point estimates of *pfmdr1* N86Y/D1246Y haplotypes. Error bars represent 95% credible intervals.



Supplemental Figure 3. Frequency point estimates of *pfmdr1* N86Y/D1246Y haplotypes with *pfcrt* K76T. Error bars represent 95% credible interval.