Whole-Genome Sequencing to Evaluate the Resistance Landscape Following Antimalarial Treatment Failure With Fosmidomycin-Clindamycin

Ann M. Guggisberg,¹ Sesh A. Sundararaman,^{3,4} Miguel Lanasa,^{5,6} Cinta Moraleda,^{5,6} Raquel González,^{5,6} Alfredo Mayor,^{5,6} Pau Cisteró,⁶ David Hutchinson,⁷ Peter G. Kremsner,^{8,9} Beatrice H. Hahn,^{3,4} Quique Bassat,⁵ and Audrey R. Odom^{1,2}

¹Department of Pediatrics, and ²Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri; ³Department of Medicine, and ⁴Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia; ⁵Centro de Investigação em Saúde de Manhiça, Mozambique; ⁶Barcelona Institute for Global Health, Barcelona Center for International Health Research, Hospital Clínic-Universitat de Barcelona, Spain; ⁷Jomaa Pharma, Hamburg, and ⁸Institut für Tropenmedizin, University of Tübingen, Germany; and ⁹Centre de Recherches Médicales de Lambaréné, Gabon

Novel antimalarial therapies are needed in the face of emerging resistance to artemisinin combination therapies. A previous study found a high cure rate in Mozambican children with uncomplicated *Plasmodium falciparum* malaria 7 days after combination treatment with fosmidomycin-clindamycin. However, 28-day cure rates were low (45.9%), owing to parasite recrudescence. We sought to identify any genetic changes underlying parasite recrudescence. To this end, we used a selective whole-genome amplification method to amplify parasite genomes from blood spot DNA samples. Parasite genomes from pretreatment and postrecrudescence samples were subjected to whole-genome sequencing to identify nucleotide variants. Our data did not support the existence of a genetic change responsible for recrudescence following fosmidomycin-clindamycin treatment. Additionally, we found that previously described resistance alleles for these drugs do not represent biomarkers of recrudescence. Future studies should continue to optimize fosmidomycin combinations for use as antimalarial therapies.

Keywords. Malaria; fosmidomycin; recrudescence; *Plasmodium*; clindamycin; whole genome amplification.

Malaria remains a serious global health concern, with approximately 214 million cases of and 483 000 deaths due to malaria in 2015 [1]. A majority of severe malaria cases occur in pregnant women and children <5 years of age and result from infection with the parasite *Plasmodium falciparum* [1]. Artemisinin combination therapies (ACTs) represent the current first-line treatment in areas of endemicity. Historically, the development of drug resistance has hindered malaria control. As delayed parasite clearance has emerged for ACTs in Southeast Asia and has continued to spread [1, 2], new antimalarials are urgently needed.

One such potential therapeutic is the phosphonic acid antibiotic fosmidomycin (FSM). FSM is a well-characterized inhibitor of the first committed step of isoprenoid precursor synthesis via the methylerythritol phosphate (MEP) pathway [3–5]. Because humans synthesize isoprenoids via an enzymatically distinct metabolic route (the mevalonate pathway), FSM is a highly specific inhibitor of malaria parasite growth [3, 5] and is well tolerated in humans [6, 7].

FSM was originally developed as an antibacterial [8] and is currently under evaluation as a partner agent for combination therapy against uncomplicated *P. falciparum* malaria (clinical trials registration NCT02198807). Several studies have paired FSM with the antibiotic clindamycin (CLN), a protein translation inhibitor (clinical trials registration NCT02198807, NCT01361269, NCT01002183, NCT00214643, and NCT00217451) [6, 9, 10]. One such phase 2 clinical trial, performed in Mozambique in 2010, evaluated the efficacy of a FSM-CLN combination against uncomplicated malaria in children aged 6–35 months, the youngest cohort tested to date [11, 12]. A high parasite cure rate was observed at day 7 after treatment with FSM-CLN (94.6%). Unfortunately, the cure was not durable, and the posttreatment polymerase chain reaction (PCR)-corrected day 28 cure rate was 45.9% (17 of 39), due to recrudescence infections. Parasite recrudescence is common in clinical studies of FSM efficacy, with an overall day 28 cure rate of approximately 70% in adults [12].

The safety and specificity of MEP pathway-targeting therapeutics such as FSM are highly desirable. However, the failure of FSM treatment to result in lasting cure has led to concern regarding the clinical utility of FSM or related antimalarials. We recently found that FSM resistance is readily achieved in culture owing to mutations in the metabolic regulator *PfHAD1* (PlasmoDB ID PF3D7_1033400) [13]. Additionally, CLN resistance, attributed to a mutation in the apicoplast 23S ribosomal RNA (rRNA) [14], has been reported in clinical isolates of *P. falciparum*.

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Correspondence: A. R. Odom, 660 S Euclid Blvd, Campus Box 8208, St. Louis, MO 63110 (odom_a@kids.wustl.edu).

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In this study, we address whether observed recrudescence is produced by selection for mutations in previously identified candidate genes or novel resistance loci. Using selective whole-genome amplification (SWGA) and sequencing, we characterized *P. falciparum* field isolates obtained from blood spot samples to evaluate the genetic landscape of drug resistance before and after FSM-CLN treatment. Specifically, we evaluated enrichment in genetic changes associated with decreased susceptibility to either FSM or CLN.

METHODS

Study Information

The study criteria have been previously described [11]. The study evaluated 37 children ages 6–35 months with uncomplicated malaria. Inclusion and exclusion criteria are outlined in the original study [11]. Patients were administered a 3-day, twice-daily course of oral FSM-CLN. Blood spots were collected at days 0, 7, 14, and 28 and, if applicable, upon recrudescence.

Clinical Trial Information

The original trial in Mozambique, sponsored by Jomaa Pharma, was conducted in 2010 according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use good clinical practice guidelines. The protocol was approved by the National Mozambican Ethics Review Committee and the Hospital Clinic of Barcelona Ethics Review Committee. The study is registered with ClinicalTrials.gov (NCT01464138).

Selective Whole Genome Amplification

Blood spotting and DNA isolation has been previously described [11]. SWGA of *P. falciparum* genomes was performed as described previously [15], including primer sets (Supplementary Table 2) and reaction conditions. Reactions contained 10 ng of template and 3.5 μ M of primer set 6A. Reactions were cleaned using Ampure beads (Beckmann Coulter) at a 1:1 DNA to bead ratio. Samples underwent a second round of SWGA, using primer set 8A and 15–30 ng of DNA.

PCR of SWGA Products

Selective amplification of *P. falciparum* genomes was verified by PCR, using *P. falciparum*- or human-specific primers (Supplementary Table 1). Human DNA and human-specific primers were kindly provided by Shiming Chen (Washington University). Reactions contained 2 μ M each primer, 25–50 ng of bead-cleaned SWGA product, and BIO-X-ACT Short PCR Mix (Bioline). Vendor-recommended cycling conditions were used, with modifications: annealing at 55°C and 60°C for *P. falciparum*- and human-specific primers, respectively, and extension at 68°C. Amplicons were visualized by agarose gel electrophoresis.

Whole-Genome Sequencing

Library preparation, Illumina sequencing, read alignments, and variant calling were performed by the Washington University

Genome Technology Access Center. Bead-cleaned SWGA product DNA (0.5–1.2 μ g) was sheared and subjected to end repair and adapter ligation. PCR-based libraries were sequenced on an Illumina HiSeq 2500 to generate 101-base pair paired end reads. Reads were aligned to the 3D7 reference (PlasmoDB v24) using Novoalign (V2.08.02) [16]. Duplicate reads were removed. Single-nucleotide polymorphisms (SNPs) were called using samtools (mpileup) [17], filtered (quality, ≥ 20 ; read depth, ≥ 5), and annotated using snpEff (3.3c, build 2013-06-28) [18].

P. falciparum reads are available through the National Center for Biotechnology Information BioProject database (PRJNA315887) and Sequence Read Archive (SRP072442).

For some analyses (comparison of SNPs in resistance genes and gene ontology [GO] analysis), only sample pairs with both exomes showing sufficient coverage ($\geq 60\%$ covered at $\geq 5X$, as indicated in Supplementary Table 3) were used. Given a per-base error rate of 0.1%–0.5% for Illumina sequencing [19, 20], 5X coverage equates to $>99.9\%$ accuracy. Studies have indicated reasonable sensitivity and accuracy for our variant caller at this cutoff [21].

SNPhylo Plot Generation

Multisample variant call formats were converted to hapmap format. SNPhylo [22] was run with the default settings, with a linkage disequilibrium cutoff of 0.8.

Multiplicity of Infection (MOI) Determination

MOI was determined using the World Health Organization (WHO)-recommended PCR-based genotyping procedures at the *MSP1* (PF3D7_0930300), *MSP2* (PF3D7_0206800), and *GLURP* (PF3D7_1035300) loci [23].

Sequencing of the Apicoplast 23S rRNA Locus

The apicoplast 23S rRNA (PlasmoDB ID PCF10_API0010: rRNA) locus was amplified by PCR. Reactions contained 2 μ M of each primer 23S_1 and 23S_6 (Supplementary Table 1), 4–5 μ L of blood spot DNA, and CloneAmp HiFi PCR Premix (Clontech). Vendor-recommended cycling conditions were used (55°C primer annealing).

Amplicons were Sanger sequenced using primers 23S_1–23S_6 (Supplementary Table 1).

GO Analysis

GO term enrichment of genes containing SNPs unique to post-treatment samples was determined using the PlasmoDB Gene Ontology Enrichment tool [24] (Bonferroni-corrected $P < .01$).

RESULTS

SWGA of Blood Spot DNA Generates Parasite Templates for Whole-Genome Sequencing

We evaluated blood spot DNA (mixed human and *P. falciparum*) from 12 patient samples with microscopic recrudescence infection (12 pretreatment and posttreatment pairs, for a total of 24 samples). Pilot unmodified, low-input library preparation

methods resulted in <5% reads mapping to the *P. falciparum* genome. For this reason, we used SWGA to amplify *P. falciparum* DNA. This method uses the processive Φ 29 DNA polymerase and genome-specific primers to selectively amplify a target genome from a mixed sample [25–27] and has been recently used to characterize chimpanzee *Plasmodium* genomes [15].

Enrichment of the *P. falciparum* genome was verified by PCR (Figure 1). We amplified *P. falciparum* genomes from a broad range of parasite densities (18–315 064 parasites/ μ L whole blood). Amplified samples were used to prepare libraries for Illumina sequencing.

The samples displayed varying degrees of sequencing success (Supplementary Table 3). An average ($\pm$ standard error of the mean [SEM]) of $55.1\% \pm 2.9\%$ of reads mapped to the *P. falciparum* genome, resulting in $48.1\% \pm 3.5\%$ of the genome covered at $\geq 5X$. This is comparable to findings of previous studies that used SWGA to sequence microbial genomes [27, 28]. The genome coverage we obtained was consistent with very low proportions (<0.01%) of parasite DNA in the blood spot samples [15]. While more reads will increase genome coverage, including typically low-coverage intragenic regions, coverage is likely to be limited by incomplete genome representation in the sample, as very low proportions of parasite DNA are likely to result in incomplete or inefficient SWGA of some genome regions. Given the low coverage of AT-rich intragenic regions, our analyses focused on protein-coding regions of the *P. falciparum* genome. We observed an average ($\pm$ SEM) of $69.0\% \pm 4.4\%$ of the exome covered $\geq 5X$, with as much as 90% of the exome covered $\geq 5X$ (Supplementary Table 3).

We observed an average ($\pm$ SEM) of $35\,451 \pm 1220$ total genome SNPs in our sequenced samples, consistent with recent studies of African field isolates [29, 30]. Of these, an average ($\pm$ SEM) of $15\,862 \pm 534$ were exome SNPs. We found an

average ($\pm$ SEM) of $10\,755 \pm 349$ nonsynonymous SNPs in our samples with sufficient exome coverage ($\geq 60\%$ covered at $\geq 5X$).

SNP Profiling Confirms Recrudescence Infections

Maximum-likelihood phylogenetic tree construction from our exome SNP profiles from the 12 pretreatment and posttreatment sample pairs demonstrated clustering by patient of origin (Figure 2). These data support the conclusion that all infections analyzed indeed reflected failure to completely clear the original infection (recrudescence), as opposed to novel, independent infections. Pretreatment and posttreatment samples shared a majority (average [$\pm$ SEM], $66.0\% \pm 3.4\%$) of nonsynonymous SNPs, while independent (between-patient) pretreatment infections were less related and shared only $24.6\% \pm 0.4\%$ of nonsynonymous SNPs (strains with $\geq 60\%$ of the exome at $\geq 5X$; $n = 8$). These data confirm the genotyping findings reported in the original study [11], which established recrudescence through WHO-recommended PCR-based genotyping at 3 *P. falciparum* loci: *MSP1*, *MSP2*, and *GLURP* [23].

MOI Before and After Treatment

In high-transmission areas, patients may be simultaneously infected with multiple parasite strains. This genetic variation provides a potential reservoir for the development of resistance to antimalarials, both within a given patient and in the larger *P. falciparum* population. The MOI has been shown to decrease after recrudescence following chloroquine treatment [31], and, unsurprisingly, an increased MOI is correlated with treatment failure [32]. However, during any individual infection, selective pressures during infection (such as immune evasion) and drug

Figure 1. Selective whole-genome amplification (SWGA) causes enrichment of *Plasmodium falciparum* DNA from mixed samples that can be detected by polymerase chain reaction. Shown is an agarose gel of SWGA amplicons obtained using *P. falciparum*-specific (top) and human-specific (bottom) primers. Samples are numbered by infection and were obtained before or after treatment. Markers indicate DNA size in base pairs.

Figure 2. Samples cluster by patient, indicating recrudescence infections. SNPhylo [22] was used to construct a maximum likelihood phylogenetic tree of single-nucleotide polymorphism (SNP) profiles from initial (before treatment) and recrudescence (after treatment) infections. Bootstrap values were 100 for all pretreatment and posttreatment branch points. Samples are numbered by infection. Scale bar represents units of substitution.

treatment are expected to decrease genetic diversity. We therefore assessed MOI before and after recrudescence.

All infections were polyclonal, with a mean pretreatment MOI ($\pm$ SEM) of 3.9 ± 0.3 . This is slightly higher than MOIs reported in other pediatric studies in Mozambique [33, 34]. MOIs decreased modestly in two thirds of the samples after treatment and recrudescence, with a mean posttreatment MOI ($\pm$ SEM) of 3.2 ± 0.2 ($P = .0065$, by the paired t test). This reduction was not a result of lower parasitemia densities in recrudescence infections, as the average posttreatment parasite density was approximately equal to the average ($\pm$ SEM) pretreatment parasite density ($76\,225 \pm 33\,944$ vs $70\,977 \pm 20\,498$ parasites/ μ L). Additionally, MOI and parasite density were not correlated (Pearson $r = -0.041$; $P = .849$).

Of note, loss-of-function mutations in the FSM-resistance gene *PfHAD1* have been infrequently reported in the genomes of field *P. falciparum* isolates (PlasmoDB; accessed April 2016), suggesting that such alleles are rare [24]. Therefore, if resistance emerges during the course of infection in a given patient, recrudescence due to outgrowth of a rare resistant clone should result in a dramatic decrease in MOI. This was not observed in our population, suggesting that FSM resistance alone is unlikely to account for recrudescence of patients treated with FSM-CLN.

Genotyping of Known Resistance Loci Does Not Reveal a Change in the Resistance Landscape Between Pretreatment and Posttreatment Samples

To evaluate whether FSM-CLN recrudescence parasites have genetic changes associated with drug resistance, we investigated the genomes of paired pretreatment and posttreatment parasites for genetic markers of antimalarial resistance. We evaluated loci

previously implicated in resistance to FSM or CLN, as well as loci documented to contribute to resistance to other clinically available antimalarials.

FSM targets the isoprenoid synthesis enzyme 1-deoxy-D-xylulose 5-phosphate reductoisomerase (*PfDXR*, PF3D7_1467300). Following FSM-CLN treatment, recrudescence samples were not enriched in alternative *PfDXR* alleles (Figure 3 and [Supplementary Table 4](#)). One sample (114) possessed the Q361R *PfDXR* allele, previously reported in African isolates [24]. The homologous residue (R276) in FSM-susceptible *Escherichia coli* *DXR* (NCBI reference NP_414715.1) is identical to the mutated residue (361R). Therefore, the *DXR* Q361R variant is unlikely to confer FSM resistance.

We have recently identified a metabolic regulator, *PfHAD1*, whose loss results in FSM resistance in cultured *P. falciparum* [13]. No *PfHAD1* loss-of-function alleles were identified in our samples (Figure 3 and [Supplementary Table 4](#)), consistent with a known lack of variation in this gene in field samples [24]. The N70S allele seen in a number of strains has been previously reported as a common wild-type variant [24], which does not confer FSM resistance [13]. Two pretreatment samples (103 and 118) possessed additional polymorphisms in *PfHAD1*, S265L and E150K, respectively (Figure 3 and [Supplementary Table 4](#)). These alleles are not predicted to affect *PfHAD1* function (PolyPhen-2 scores, <0.005 [benign]) [35] and are not enriched in the recrudescence samples. Altogether, our data indicate that known markers of FSM resistance in *PfDXR* and *PfHAD1* do not emerge during the course of FSM treatment. Thus, known mechanisms of FSM resistance observed in the laboratory are unlikely to represent the underlying mechanism

Figure 3. Resistance landscape before and after recrudescence. Only infection pairs with sufficient exome coverage were analyzed ($\geq 60\%$ at $\geq 5\times$; $n = 8$). Shown are the percentages of pretreatment and posttreatment samples with indicated nonreference (3D7) alleles at resistance loci. Any codons not shown match the 3D7 reference in all samples.

behind FSM-CLN failure. Since recrudescence parasites were not culture adapted, we cannot distinguish whether these parasites were resistant or just failed to be cleared. Therefore, it is possible that resistance-causing mutations in *PfDXR*, *PfHAD1*, or other loci might occur if parasites were exposed to drugs for longer periods.

Resistance to the FSM partner agent, CLN, has also been reported in *P. falciparum* [14]. In this previous study, mutation (A1875C) in the 23S rRNA locus (PFC10_API0010) was associated with an approximately 100-fold increase in the clindamycin half-maximal inhibitory concentration for clinical isolates [14]. Insufficient coverage of the plastid genome was obtained from whole-genome sequencing; we therefore used targeted gene amplification and sequencing to interrogate for polymorphisms in PFC10_API0010. No samples possessed the A1875C variant. All sequenced samples matched the FSM- and CLN-susceptible 3D7 control strain (Supplementary Data 1). Notably, from our sequencing of laboratory 3D7 and HB3 strains, we identified variations not reported in the genome reference (T451A, A454T, and 401insG; Supplementary Data 1) [24]. These changes may have emerged over time during culturing of laboratory isolates.

Finally, we interrogated the prevalence of other drug resistance markers before and after FSM-CLN treatment (Figure 3 and Supplementary Table 4). Specifically, we evaluated variation in the locus encoding the multidrug transporter PfMDR1 (PF3D7_0523000), which modulates parasite susceptibility to hydrophobic antimalarials, such as mefloquine and halofantrine [36]. Strains possessing *PfMDR1* mutations are susceptible to FSM, and *PfMDR1* mutations are not predicted to influence FSM effectiveness [3, 9]. Three PfMDR1 variants (wild type, Y184F, and N86Y) were identified in our analyzed strains, but *PfMDR1* haplotype frequencies were not significantly different in pretreatment and posttreatment populations ($P > .5$).

ACTs, introduced in Mozambique in 2004 [37], may have selected for parasites able to withstand drug treatment, such as FSM-CLN. Alleles of *PfK13* (Kelch13, PF3D7_1343700) have been implicated in artemisinin resistance. We identified 2 *PfK13* variants in our population (K189T and A578S), neither of which has been implicated in laboratory artemisinin resistance. The K189T variant is common in African isolates [38, 39] and is not believed to cause increased clearance times following ACT. While the A578S variant has been associated with increased clearance times [40], this mutation was only observed in a small fraction of our strains. Additionally, strains possessing known resistance mutations in *PfK13* are FSM susceptible (R. L. Edwards et al, unpublished data) suggesting that selection for artemisinin resistance does not result in FSM resistance.

In our small study population, selection with FSM-CLN did not appear to alter the frequency of *PfMDR1* alleles or alleles of additional known genetic loci associated with antimalarial resistance, including *PfCRT* (chloroquine), *PfATP4* (multiple drug

Table 1. Gene Ontology (GO) Analysis of Genes Containing Single-Nucleotide Polymorphisms (SNPs) Unique to Recrudescence Samples

GO Term ID	Description	P Value
GO:0051704	Multi-organism process	1.5×10^{-4}
GO:0044403	Symbiosis, encompassing mutualism through parasitism	1×10^{-3}
GO:0044419	Interspecies interaction between organisms	1×10^{-3}
GO:0016337	Cell-cell adhesion	3.6×10^{-3}
GO:0009405	Pathogenesis	4.6×10^{-3}

classes), *PfDHFR* (antifolates), and *PfDHPS* (antifolates; Figure 3 and Supplementary Table 4) [41–45].

Our approach permitted an unbiased search for any novel nonsynonymous SNPs that are associated with recrudescence following FSM-CLN treatment. To better understand SNPs that were unique to or enriched in recrudescence samples, we also subtracted pretreatment nonsynonymous SNPs from post-treatment nonsynonymous SNPs. The 8 recrudescence strains analyzed had an average ($\pm$ SEM) of 3448 ± 604 unique nonsynonymous SNPs (approximately 33% of their total nonsynonymous SNPs).

Sixty-eight SNPs were shared in $\geq 50\%$ of the samples (Supplementary Table 5). However, no nonsynonymous SNPs were shared between all 8 recrudescence samples, demonstrating that, in this small population, a genetic marker of recrudescence was not present.

Selective pressures in vivo are likely to be distinct from those described in vitro. We hypothesized that certain biological processes may be enriched for genetic variation in our posttreatment samples. We therefore performed GO analysis on the genes with SNPs shared in $\geq 50\%$ of recrudescence samples (Supplementary Table 5) to understand the functions of genes containing SNPs enriched upon recrudescence. Our analysis revealed enrichment for immune evasion and parasitism-related functions (Table 1). This result has been observed in other studies of variation in *P. falciparum* populations [14, 30]. Because these genes are among the most variable in a population, they are likely to display changes in allele frequency following a population bottleneck, such as recrudescence. Notably, GO analysis did not reveal enrichment in pathways associated with drug resistance or with the mechanism of action of FSM (isoprenoid synthesis) or CLN (protein translation). While a novel genetic route to FSM or CLN resistance is possible, we see no evidence for enrichment of new SNPs or pathways in our unbiased genome analysis.

DISCUSSION

FSM is an antimalarial with a novel, parasite-specific mechanism of action; a well-characterized target; and exceptional clinical safety. Despite this promise, FSM combination treatment of uncomplicated *P. falciparum* infection in children was unsuccessful due

to unacceptably high rates of parasite recrudescence [11]. Parasite FSM resistance arises readily in culture and has been attributed to loss-of-function mutations in *PfHAD1* [13]. These in vitro studies suggested that selection for FSM resistance alleles during clinical infection and/or FSM treatment may represent a mechanism to explain clinical failures following FSM treatment.

To address this concern, we surveyed the genetic diversity in Mozambican pediatric *P. falciparum* infections before and after treatment failure with FSM-CLN. Overall, our data indicate that drug resistance does not account for treatment failures following FSM-CLN therapy. Our results confirm that treatment and recrudescence represent a population bottleneck, as the MOI is decreased in recrudescence samples. Since resistance alleles are thought to represent only a minuscule proportion of the pre-treatment population, the modest decrease in MOI that we observed is inconsistent with the selection of resistant strains from the population.

Importantly, we did not find evidence of SNPs enriched in parasites following FSM-CLN treatment. Specifically, we did not identify enrichment for alleles already experimentally implicated in FSM or CLN resistance. Our use of whole-genome sequencing permitted an unbiased screen for additional SNPs that may contribute to resistance, regardless of whether these alleles are directly responsible for or otherwise associated with a recrudescence phenotype. While we were limited by the retrospective nature of our study and our inability to phenotype culture-adapted recrudescence parasites, both FSM and CLN have single, well-characterized targets and known SNPs underlying resistance [13, 14]. We therefore conclude that neither FSM nor CLN resistance was responsible for the clinical failure of FSM-CLN.

Our study was designed to evaluate the hypothesis that a simple coding mutation may underlie recrudescence in FSM-CLN-treated parasites. The results of our study cannot exclude other potential routes to resistance, such as noncoding mutations resulting in regulatory variation or nongenetic changes in gene expression or homeostatic responses. Further studies may address whether and how these mechanisms contribute to resistance to FSM, CLN, and other antimalarials.

Our study highlights an important caution in applying the results of forward genetic screening in cultured parasites to clinical populations. As resistance alleles are identified in vitro, it is important to recognize that selective pressures during natural human infection (immune pressure and metabolic requirements) are likely to be distinct. Our data indicate that mutation in *PfHAD1* is not readily achieved in clinical populations. Perhaps mutation of *PfHAD1* comes at a fitness cost in *P. falciparum*, similar to what has been found for other resistance loci, such as *PfCRT* and *PfATP4* [46, 47]. However, *PfHAD1* mutation appears to reduce fitness during human infection and not during culture, as loss of *PfHAD1* is easily achieved in laboratory selections.

Finally, our study illustrates the usefulness of SWGA for the analysis of *P. falciparum* genomes from blood spot samples. This method has applications for future field studies, as blood spots are easier to acquire than whole blood. Further optimization will facilitate the extraction of more data from these types of samples. Furthermore, our data provide additional validation of PCR-based strategies to determine MOI and recrudescence. We found that the high rate of recrudescence following FSM-CLN treatment in children was not overestimated owing to use of the standard 3-locus PCR genotyping protocol [11]. Eventual use of whole-genome sequencing for genotyping field populations will provide more information regarding variation within a geographic region and within patients.

This work supports the current hypothesis that the disappointing clinical efficacy of FSM combinations is likely due to challenges of partner drug selection and formulation [12]. The short serum half-life of both FSM and CLN (1–3 hours) limits parasite exposure and presumably reduces the selective pressure for resistance [8, 48]. However, this limited serum exposure almost certainly contributes to decreased clinical efficacy. Currently, FSM is being evaluated in combination with the bisquinolone piperaquine in a phase 2 clinical trial in Gabon (clinical trials registration NCT02198807). Piperaquine has a notably long half-life (>20 days) [49], which holds promise to limit recrudescence when paired with FSM. Our findings support the continued development of antimalarials targeting PfDXR and the MEP pathway, as well as the development of alternative FSM combinations.

Supplementary Data

Supplementary materials are available at <http://jid.oxfordjournals.org>. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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