In Vitro Sensitivities of Plasmodium falciparum Isolates from the China-Myanmar Border to Piperaquine and Association with Polymorphisms in Candidate Genes

Mingming Hao, a Dandan Jia, a Qing Li, b Yongshu He, b Lili Yuan, a Shuhui Xu, a Kexuan Chen, a Jia Wu, c Lijuan Shen, c Lin Sun, a Hongbin Zhao, a Zhaoqing Yang, a Liwang Cui* a

Department of Pathogen Biology and Immunology, a Department of Cell Biology and Genetics, a Department of Pathology, a and The First Affiliated Hospital, a Kunming Medical University, Kunming, Yunnan, China; Department of Entomology, Pennsylvania State University, University Park, Pennsylvania, USA

The recent reports of resistance in Plasmodium falciparum to artemisinin derivatives and their partner drugs demand intensive studies toward understanding the molecular mechanisms of resistance. In this study, we examined the in vitro susceptibility of 63 P. falciparum field isolates collected from the China-Myanmar border area to chloroquine (CQ) and piperaquine (PPQ). Parasite isolates remained highly resistant to CQ, with the geometric mean 50% inhibitory concentration (IC₅₀) of 252.7 nM and a range of 51.9 to 1,052.0 nM. In comparison, these parasites had a geometric mean IC₅₀ of 28.4 nM for PPQ, with a fairly wide range of 5.3 to 132.0 nM, suggesting that certain parasite isolates displayed relatively high levels of resistance to PPQ. Interestingly, within the 4 years of study, the parasites exhibited a continuous decline in susceptibilities to both CQ and PPQ, and there was a significant correlation between responses to CQ and PPQ (Pearson correlation coefficient = 0.79, P < 0.0001). Consistent with the CQ-resistant phenotype, all parasites carried the pfCRT K76T mutation, and most parasites had the CVIET type that is prevalent in Southeast Asia. In contrast, pfmdr1 mutations were relatively rare, and no gene amplification was detected. Only the pfmdr1 N1042D mutation was associated with resistance to CQ. For the pfmrp1 gene, four substitutions reached relatively high prevalence of >22%, and the I876V mutation was associated with reduced sensitivity to CQ. However, we could not establish a link between PPQ responses and the polymorphisms in the three genes associated with quinoline drug resistance.

To deal with multidrug-resistant Plasmodium falciparum and to delay antimalarial drug resistance development, artemisinin-based combination therapies (ACTs) have taken the main stage as the first line treatment for falciparum malaria (1, 2) and as a potential treatment for vivax malaria (3). Artemisinin derivatives, which have very short half-lives, efficiently kill the parasites and rapidly reduce the parasite biomass, whereas the partner drug, which has a longer duration of action, eliminates the residual parasites. Given the essential role of ACTs in the contemporary malaria control and elimination efforts, the development of resistance in malaria parasites to these drugs would be a disaster. Undoubtedly, the recent detection of artemisinin resistance in Cambodia and Thailand manifested as delayed parasite clearance is worrisome (4, 5). Moreover, there is evidence showing that P. falciparum is resistant to partner drugs such as mefloquine (MQ) and amodiaquine (6–8). Therefore, close surveillance of drug resistance and elucidation of the resistance mechanisms are urgently needed for resistance management with the goal of expanding the life span of current ACTs.

There are a number of commercially registered ACTs. Dihydroartemisinin plus piperaquine (DHA-PPQ), marketed as Artekin and Eurartesim, has been widely used in Southeast Asia. Clinical studies conducted in many malaria-endemic regions have shown that DHA-PPQ is safe and highly effective for uncomplicated P. falciparum malaria, with excellent cure rates (9–15). PPQ is a bisquinoline compound that was synthesized in the 1960s but only received wide applications for malaria treatment in China (16). In the late 1970s, in response to the increasing prevalence of chloroquine (CQ) resistance, PPQ was used to replace CQ as the first-line treatment of falciparum malaria in China. In addition, PPQ has also been extensively used in malaria prophylaxis in combination with the antifolate drug sulfadoxine. However, PPQ-resistant strains soon emerged in China (17–22), which together with the increasing popularity of artemisinins, led to the diminished use of PPQ in the late 1980s. Since 2005, DHA-PPQ combination has been officially used as the ACT for treating uncomplicated P. falciparum malaria in China.

The rapid emergence of resistance to PPQ after deployment in China and ex vivo drug assays using isolates from Southern China suggest the presence of cross-resistance between PPQ and CQ (18–22). Similarly, significant correlation of response between PPQ and CQ was also observed in ex vivo studies using parasite isolates from Cameroon (23) and Papua New Guinea (24), albeit PPQ was equally active against CQ-sensitive and -resistant parasites. Studies using laboratory CQ-sensitive and -resistant lines further support the existence of cross-resistance between CQ and PPQ (25, 26). In contrast, recent ex vivo and in vitro drug assays using parasites of diverse origins (but mostly from Africa) did not notice significant correlation of responses to CQ and PPQ (27–30) but detected significant positive correlations in some studies between the responses to PPQ and other antimalarials such as DHA.
pyronaridine, amodiaquine, or doxycycline. Investigations of the PPQ resistance mechanism mostly using the candidate gene approach also produced conflicting results. Using laboratory parasite lines genetically modified at the \textit{pfcr} and \textit{pfmdr1} loci, Muangnoicharoen et al. found that resistance to PPQ was linked to mutations in \textit{pfcr} that are commonly associated with CQ resistance (26). However, two subsequent studies did not establish the association between PPQ \textit{in vitro} sensitivities and polymorphisms in \textit{pfcr}, \textit{pfmdr1}, \textit{pfnirp}, and \textit{pfhsd} genes (27, 29). In order to elucidate the genetic basis of PPQ resistance, Eastman et al. used an \textit{in vitro} scheme to select PPQ-resistant lines from Dd2 parasite and identified deamplification of a region in chromosome 5 encompassing \textit{pfmdr1} and amplification of an adjacent region associated with the selection, suggesting a potential link between \textit{pfmdr1} copy number and PPQ sensitivity (31). To corroborate this genetic study, Veiga et al. determined the \textit{in vitro} response to PPQ in parasite isolates from western Thailand, where \textit{pfmdr1} copy number has been increased due to MQ selection and found that \textit{pfmdr1} amplification was significantly associated with enhanced sensitivity to PPQ (32). It is worth noting that most of these \textit{in vitro} drug studies used parasite isolates that were not exposed or rarely exposed to PPQ. Thus, it might be difficult to link the known genes for quinoline resistance to PPQ resistance unless they share a similar resistance mechanism. In the present study, we sought to measure the \textit{in vitro} susceptibilities to PPQ of parasite isolates from the China-Myanmar border where PPQ has been used extensively in the past. We sought to determine their relationship with the polymorphisms in genes that are known to be associated with resistance to quinoline drugs.

\textbf{MATERIALS AND METHODS}

\textbf{Parasite collection and \textit{in vitro} adaptation.} \textit{P. falciparum} clinical isolates were collected from 2007 to 2010 from malaria patients with acute, uncomplicated \textit{P. falciparum} infections at two malaria clinics located in Laiza township near the Myanmar-China border. Provision of informed consent from the participants was approved by the Institutional Review Board of Kunming Medical University. After collection, blood cell pellets were mixed with glycerol and cryopreserved in liquid nitrogen. In the laboratory, they have been adapted to continuous culture according to a previously described procedure (33). Briefly, frozen parasite stocks were thawed, washed twice with RPMI 1640 medium at 37°C, and mixed with fresh type O human red blood cells (RBCs) suspended at a 5% hematocrit in a complete medium containing HEPES (5.94 g/liter), hyoxanthine (50 mg/liter), Albumax II (5 g/liter), RPMI 1640 (10.4 g/liter), gentamicin (5 mg/liter), NaHCO\textsubscript{3} (2.1 g/liter), and 9% AB serum. In order to select monoclonal infections, parasites were genotyped at three polymorphic genes—merozoite surface protein 1 (\textit{mssp1}), \textit{mssp2}, and glutamate-rich protein (\textit{glurp})—as previously described (33, 34). On average, parasites were continuously cultured for 3 to 4 weeks before the drug sensitivities were measured.

\textbf{In vitro drug assay.} Routine culture of the parasites was maintained in type O RBCs in RPMI 1640 medium supplemented with 9% AB human serum under an atmosphere of 90% N\textsubscript{2}, 5% O\textsubscript{2}, and 5% CO\textsubscript{2}. CO was purchased from Sigma (St. Louis, MO), while CQ and PPQ were obtained from Chongqing Kangle Pharmaceutical Co., Ltd. The stock solution of CQ (3.0 mM) was prepared in distilled water and PPQ (0.3 mM) in methanol. Serial dilutions were made in a complete medium to obtain final concentrations of 0, 1, 4, 16, 64, 256, 1,024, and 4,096 nM for CQ and 0, 1, 3, 9, 27, 81, 243, and 729 nM for PPQ. For drug assays, synchronized late ring- or early trophozoite-stage parasite suspension was dispensed into the test wells of a 96-well microtiter plate to obtain a 5% hematocrit and 0.3% parasitemia. The microtiter plates were incubated at 37°C for 72 h. Measurement of parasite growth was performed using the SYBR green I-based fluorescence assay (33, 35). For each drug concentration and each parasite isolate, the assay included three technical replications and three biological replications. For consistency, the laboratory clone 3D7 was included throughout the study as an internal control.

\textbf{Analysis of genetic polymorphisms in candidate genes.} Polymorphisms of the candidate genes were determined using a PCR and sequencing method. parasite genomic DNA was isolated from cultured parasites using a QiAmp DNA minikit (Qiagen). Two \textit{pfmdr1} fragments were amplified by PCR to include the codons 86, 184, 1034, 1042, and 1246 (36). Similarly, the polymorphisms in codons 72 to 76 and codon 220 of the \textit{pfcr} gene were determined as described previously (37). The \textit{pfnp1} gene was amplified using primers and conditions described previously (38). Amplified DNA was purified using a High-Pure PCR Cleanup Microkit (Roche) and sequenced using BigDye Terminator v3.1. Sequences were aligned with the CLUSTAL X 2.0.12 with manual editing. The copy number of \textit{pfmdr1} was estimated as described previously (33).

\textbf{Statistical analysis.} The geometric mean of the half-maximal inhibitory concentration (IC\textsubscript{50}, standard error, and the 95% confidence interval (CI) were determined using GraphPad Prism 5.0 for Windows. Pearson correlation coefficients were calculated using SPSS 11.0 for Windows. The difference between the field samples and the laboratory clone 3D7 was compared using the One-Sample t-test. The differences in drug responses among the years were compared using analysis of variance (ANOVA). Differences in drug responses between different alleles were compared using the Student t-test.

\textbf{RESULTS}

\textbf{In vitro sensitivity of parasite isolates to CQ and PPQ.} We cultured adapted 22, 9, 22, and 10 parasite isolates with monoclonal \textit{P. falciparum} infection collected in 2007, 2008, 2009, and 2010, respectively, and assayed their \textit{in vitro} susceptibilities to CQ and PPQ using a SYBR green I method. Overall, parasites collected from the China-Myanmar border area showed high levels of CQ resistance. The mean IC\textsubscript{50} for CQ was 252.7 nM, ranging from 51.9 to 1,052.0 nM, with an ~20-fold difference between the most and least susceptible isolates (Table 1). Based on the criteria described earlier, CQ susceptibility was categorized into three levels, i.e., sensitive (IC\textsubscript{50} < 25 nM), moderately resistant (25 nM ≤ IC\textsubscript{50} < 100 nM), and highly resistant (IC\textsubscript{50} ≥ 100 nM) (39). Of the 63 samples assayed, 5 and 58 parasite isolates were moderately and highly resistant to CQ. The parasite isolates had a mean IC\textsubscript{50} of 28.4 nM for PPQ with a wide range of 5.3 to 132.0 nM (Table 1). There was an ~25-fold difference in PPQ IC\textsubscript{50} between the most and least susceptible isolates. Compared to the mean values of the laboratory clone 3D7, the mean IC\textsubscript{50} of all parasite isolates to both CQ and PPQ were significantly higher (P < 0.05, one-sample t test) (Table 1). Specifically, all field parasite isolates had higher IC\textsubscript{50}s to CQ than 3D7, whereas 62 of 63 parasite isolates had >20% higher IC\textsubscript{50}s to PPQ than 3D7. At present, no clinical data are available for the determination of the cutoff values of PPQ resistance. If we used a 3-fold decrease in susceptibility to PPQ versus 3D7 as the arbitrary resistance cutoff, 52 of 63 (83%) parasites would be considered PPQ resistant.

When the IC\textsubscript{50} data were stratified by years, a trend of increase in mean IC\textsubscript{50}s to both CQ and PPQ was observed from 2007 to 2010 (Fig. 1 and Table 1). For CQ, parasite collected in 2007 had significantly lower IC\textsubscript{50}s than those collected in 2009 and 2010, respectively (P < 0.001, ANOVA). Likewise, there were significant differences in response to PPQ between 2007 and 2009 (P < 0.05), between 2007 and 2010 (P < 0.001), between 2008 and 2010 (P < 0.05), and between 2009 and 2010 (P < 0.05).
of the IC50s to CQ and PPQ by Pearson correlation analysis (Fig. 1C). A significant correlation was detected between the responses to CQ and PPQ ($r$ = 0.79, $P$ = 0.0001) (Fig. 1C).

Polymorphisms in candidate target genes. For pfcrt, the key determinant of CQ resistance K76T was universally present in all isolates. Further, the A220S mutation was found in 62 of 63 parasite isolates. Only one parasite isolate contained the SVMNT genotype, which is the most prevalent CQ-resistant genotype in South America, whereas the rest of the isolates had the CVIET genotype, which is the predominant CQ resistant genotype found in Southeast Asia. We genotyped pfmdr1 by PCR and sequencing, converging on the widely cognitive amino acid substitutions at codons 86 (N/Y), 184 (Y/F), 1034 (S/C), 1042 (N/D), and 1246 (D/Y). About 68% of all of these clinic isolates contained the wild-type haplotype. The N86Y mutation was very rare and found only in one isolate (1.6%). The S1034C and D1246Y mutations were not detected in our samples. The Y184F and N1042D mutations occurred at frequencies of 25.4 and 6.3%, respectively. Similar to our previous study, all parasite isolates had one copy of pfmdr1 (data not shown). Sequencing the coding region of pfmrp1 from 63 parasite samples identified mutations at residues 191, 325, 437, 785, 876, 1007, and 1390. The H191Y, S437A and I876V substitutions were relatively abundant, occurring in 65.1, 61.9, and 60.3% of parasite isolates, respectively. In comparison, the T1007M, N325S, H785N, and F1390I substitutions were present in 22.2, 9.5, 9.5, and 6.3% of the parasite isolates, respectively.

Correlation between polymorphisms and in vitro drug responses. Since the pfcrt K76T and A220S mutations were fixed or near fixation and pfmdr1 amplification was not detected, we only analyzed the potential associations between point mutations in pfmdr1 and pfmrp1 and drug responses. We only found significant association of the pfmdr1 N1042D mutation and the pfmrp1 I876V mutation with reduced sensitivity to CQ (Fig. 2 and 3). In comparison, none of the substitutions in pfmdr1 and

### Table 1

<table>
<thead>
<tr>
<th>Year (no. of samples)</th>
<th>Chloroquine concn (nM)</th>
<th>Piperaquine concn (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>95% CI</td>
</tr>
<tr>
<td>3D7</td>
<td>18.6 ± 1.0</td>
<td>16.6–20.9</td>
</tr>
<tr>
<td>2007 (22)</td>
<td>167.9 ± 35.9</td>
<td>123.9–227.5</td>
</tr>
<tr>
<td>2008 (9)</td>
<td>253.2 ± 11.8</td>
<td>227.2–282.0</td>
</tr>
<tr>
<td>2009 (22)</td>
<td>315.8 ± 47.6</td>
<td>255.0–391.2</td>
</tr>
<tr>
<td>2010 (10)</td>
<td>379.3 ± 48.1</td>
<td>291.2–494.0</td>
</tr>
<tr>
<td>Total (63)</td>
<td>252.7 ± 23.9*</td>
<td>217.1–294.1</td>
</tr>
</tbody>
</table>

*The IC50 of 3D7 were included here for reference, and an asterisk (*) indicates significant differences between the mean of the total parasite samples and mean of 3D7 ($P < 0.05$, Student’s t-test).

![Graph of in vitro responses of parasites isolates collected from 2007 to 2010 to CQ (A) and PPQ (B).](http://example.com/graph.png)

**FIG 1** Dot plots of in vitro responses of parasites isolates collected from 2007 to 2010 to CQ (A) and PPQ (B). Lines indicate the geometric means; the error bars denote one standard error. The asterisks indicate significant differences in drug sensitivity between the years ($P < 0.05$, ANOVA). (C) Pearson correlation analysis between the IC50 of CQ and PPQ.
pfmrp1 were associated with altered responses of the parasites to PPQ.

DISCUSSION

The worldwide adoption of ACTs is instrumental in the global reduction of malaria incidence in recent years, which has motivated many nations where malaria is endemic to consider malaria elimination. Ideally, selection of a particular ACT in a given region where malaria is endemic should be based on sensitivity of the parasites to both drugs. However, resistance to the partner drugs of ACTs currently in clinical use has already been reported, and it is one of the factors that might increase ACT treatment failure rates (40). For example, MQ has been used extensively in Thailand and resistance to this drug is evident (6), but artesunate-MQ has been the major ACT for this country. This could be one of the reasons responsible for the decline of ATS-MQ efficacy in treatment of falciparum malaria in the Cambodia-Thailand border area (41). Similarly, despite the fact that PPQ has been heavily used in China in the past as a monotherapy and PPQ resistance has emerged (16,17), DHA-PPQ is the ACT for falciparum malaria in China and at the China-Myanmar border area. Although the use of ACT could reverse resistance to the partner drug as reported in Thailand (42), selection of an ACT with a failing partner drug is undoubtedly a risk that should be avoided. Furthermore, due to potential cross-resistance between drugs, selection of an ACT should be based on solid clinical efficacy studies. Although DHA-PPQ have shown excellent efficacy for uncomplicated falciparum malaria in most clinical trials, results from the one conducted in Papua New Guinea was not promising (43). Therefore, understanding the genetic basis of resistance to ACTs and continued monitoring of parasite’s responses to both artemisinin derivatives and the partner drugs at the sites of extensive deployment are necessary.

The China-Myanmar border area has a long history of use of artesiminins and PPQ either as monotherapies or ACT. Although there is no report of reduced efficacy of this ACT, increased resistance to both components of this ACT has been documented in the past (17–22,44). Recently, in vitro assays of culture-adapted parasite isolates from this region did not detect significantly elevated resistance to DHA, ATS, MQ, and lumefantrine, although a 12-fold range in in vitro susceptibility to ATS was noted (45). Currently, the mechanisms of resistance to artemisinins and PPQ are not understood. It is suspected that cross-resistance might occur between PPQ and other quinoline drugs because of structural similarities of these drugs and possibly shared modes of ac-

![FIG 2](image1) Association of SNPs in pfmdr1 with responses to CQ (A) and PPQ (B). *, Significant difference \((P < 0.05)\) in sensitivity between the two alleles.

![FIG 3](image2) Association of SNPs in pfmrp1 with responses to CQ (A) and PPQ (B). *, Significant difference \((P < 0.05)\) in sensitivity between the two alleles.
In most clinical trials in Burkina Faso showed that, unlike amodiaquine, prior use of DHA–PPQ did not select for parasites with pfcrt and pfmdr1 polymorphisms associated resistance to CQ and amodiaquine (49). We also analyzed single nucleotide polymorphisms (SNPs) in the pfmrp1 gene, which were associated with altered sensitivity to quinoline drugs (50). Genetic analysis of PfMRP1 indicates that it may contribute to parasite responses to multiple antimalarial drugs, including CQ and PPQ. Clinical trials with ACTs detected a specific selection of the I876V mutation in pfmrp1 in the recurrent infections (51), while treatment with pyrimethamine and sulfadoxine selected the R1466K mutation in pfmrp1 (38), supporting the proposed role of pfmrp1 in responses to multiple drugs. In the parasite populations under study, pfmrp1 I876V, with 60.3% prevalence, was associated with increased CQ resistance. Thus, this globally prevalent mutation could have been selected by extensive use of CQ in the past. However, consistent with previous findings (27), we did not detect an association of pfmrp1 SNPs with altered sensitivities to PPQ.

To elucidate the molecular mechanism of PPQ resistance, Eastman et al. generated PPQ-resistant parasite lines with a 100-fold increase from Dd2 parasite after continuous exposure of the parasites to PPQ selection (31). Microarray analysis showed de-amplification of the pfmdr1 allele and amplification of an adjacent region on chromosome 5. Analysis of parasite samples from the Thai-Myanmar border area confirmed the role of pfmdr1 amplification with enhanced sensitivity to PPQ (32). pfmdr1 amplification is normally associated with resistance to amino-alcoholic drugs such as MQ (6). In our study area, MQ has never been used before and consistent with this, pfmdr1 amplification was not detected. Therefore, the wide range of PPQ responses in parasites with the background of CQ-resistant pfcr haplotype and mostly wild-type pfmdr1 suggests that PPQ resistance might be controlled by other unknown factors other than pfcr and pfmdr1. It is also highly possible that the association in pfmdr1 copy number and PPQ sensitivity observed from in vitro selection and field isolates from Thai-Myanmar border may depend on the genetic background of the parasites.

In summary, we examined the in vitro susceptibilities of P. falciparum isolates to CQ and PPQ. Interestingly, we found that parasites from the China-Myanmar border area were highly resis-

### TABLE 2 Comparison of in vitro IC_{50}s to PPQ of P. falciparum isolates from different areas where this organism is endemic

<table>
<thead>
<tr>
<th>Parasite origin (no. of strains)</th>
<th>Assay</th>
<th>Type</th>
<th>Method</th>
<th>3D7 IC_{50} (nM)</th>
<th>Parasite isolates (nM)</th>
<th>Fold difference</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cameroon (103)</td>
<td>Ex vivo</td>
<td>[3H] isotope</td>
<td>6.3–14.5(^a)</td>
<td>38.9</td>
<td>7.8–78.3</td>
<td>~10</td>
<td>23</td>
</tr>
<tr>
<td>Lab strains (8)</td>
<td>In vitro</td>
<td>[3H] isotope</td>
<td>3.4</td>
<td>3.9–15.8</td>
<td>~4</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Lab strains (10)</td>
<td>In vitro</td>
<td>[3H] isotope</td>
<td>3.4</td>
<td>3.9–15.8</td>
<td>~4</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Papua New Guinea (57)</td>
<td>Ex vivo</td>
<td>Chlorimetric LDH</td>
<td>11.7</td>
<td>10–13(^a)</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uganda (199)</td>
<td>Ex vivo</td>
<td>Hrp2 ELISA</td>
<td>27</td>
<td>32</td>
<td>~3</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Kenya (115)</td>
<td>In vitro</td>
<td>[3H] isotope</td>
<td>58</td>
<td>57.8</td>
<td>~3</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Multiple (22)</td>
<td>In vitro</td>
<td>[3H] isotope</td>
<td>58</td>
<td>57.8</td>
<td>~3</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Thailand-Myanmar (46)</td>
<td>In vitro</td>
<td>Hrp2 ELISA</td>
<td>22.5</td>
<td>39.4</td>
<td>~8</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>China-Myanmar (63)</td>
<td>In vitro</td>
<td>SYBR green</td>
<td>4.5</td>
<td>28.4</td>
<td>~25</td>
<td>This study</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) That is, the fold difference between the most and least susceptible parasite strains or isolates.

\(^b\) 95% CI.

\(^c\) Range for four reference strains (D6, HB3, K1, and W2.).
tant to CQ and that the in vitro responses to CQ and PPQ were highly correlated. The sustained high-level CQ resistance in spite of the withdrawal of CQ from treating falciparum malaria could have resulted from continued CQ use for treating P. vivax infections in this region. We did not establish here a positive association between PPQ sensitivity with the pfcrt, pfdnrd1, or pfmrp1 genes. Nonetheless, the identification of parasites with significantly decreased sensitivity to PPQ from an area with a long history of PPQ use provides excellent opportunities for future dissection of the PPQ resistance mechanism. In addition, the trend of decreasing PPQ sensitivity by year is also worrisome, and future continuous monitoring in this region is warranted. Our study is limited by the rather small set of data from 9 to 22 parasite isolates per year, and our findings may not be representative of the trends elsewhere. Therefore, sample sizes need to be expanded in future studies. Furthermore, the results from both Asia and Africa suggest complex mediators of resistance to aminooquinolines, and future studies should focus on the elucidation of the resistance mechanism. In addition, since PPQ is used as a partner drug in ACTs, the evaluation of drug sensitivity to other ACT components will be useful for predicting the overall responses of parasites to the treatments. Comprehensive drug sensitivity profiling of archived parasite isolates to commonly used antimalarials is under way and will hopefully reveal a more complete picture of the drug sensitivities in P. falciparum populations at the China-Myanmar border.

ACKNOWLEDGMENTS

This study was supported by a National Institutes of Health (NIH) international grant (1R01AI075429) to Z.Y., the National Natural Science Foundation of China (grants 8116120421, 31260508, and U1202226), and grant U19AI089672 from the National Institute of Allergy and Infectious Diseases, NIH.

REFERENCES


