Genetic Predisposition Favors the Acquisition of Stable Artemisinin Resistance in Malaria Parasites

Dorothee Beez,1 Cecilia P. Sanchez,1 Wilfred D. Stein,2 and Michael Lanzer1*

Department of Infectious Diseases, Parasitology, Universitätsklinikum Heidelberg, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany,1 and Biological Chemistry, Life Sciences, Hebrew University, Jerusalem 91904, Israel2

Received 7 July 2010/Returned for modification 29 August 2010/Accepted 20 October 2010

The emergence of artemisinin-resistant Plasmodium falciparum malaria jeopardizes efforts to control this infectious disease. To identify factors contributing to reduced artemisinin susceptibility, we have employed a classical genetic approach by analyzing artemisinin responses in the F1 progeny of a genetic cross. Our data show that reduced artemisinin susceptibility is a multifactorial trait, with pfmdr1 and two additional loci (on chromosomes 12 and 13) contributing to it. We further show that the different artemisinin susceptibilities of the progeny strains affect their responses to selection with increasing concentrations of artemisinin. Stable, high-level in vitro artemisinin resistance rapidly arose in those parasites that were the least artemisinin susceptible among the F1 progeny, whereas progeny that were highly artemisinin susceptible did not acquire stable artemisinin resistance. These data suggest that genetic predisposition favors the acquisition of high-level artemisinin resistance. In vitro-induced artemisinin resistance did not result in cross-resistance to artesunate or artemether, suggesting that resistance to one derivative does not necessarily render the entire drug class ineffective.

Artemisinins are potent, rapidly acting antimalarial drugs that are widely used as first-line treatment in areas where malaria is endemic. To preserve their efficacy, the World Health Organization has recommended that artemisinins be deployed as combination therapies (artesinin combination therapies [ACTs]) together with partner drugs. Despite this precautionary measure, artemisinin resistance has emerged in western Cambodia and the bordering region with Thailand (1, 5, 6, 19, 20, 35), where the artemisinin derivative artesunate has been used unregulated as monotherapy since the mid-1970s. Fortunately, artemisinin resistance is still contained and does not seem to have spread (20), and although the artesunate-mefloquine combination used in Cambodia does not kill the human malaria parasite Plasmodium falciparum as quickly as it did in the past, it still remains effective in the treatment of malaria (5). Should resistance to artemisinins and ACTs heighten and should it spread, malaria-related infections and casualties are expected to rise beyond today’s 200 million clinical cases and 1 million deaths annually, since no other drug class that can match artemisinins’ efficacy and safety is licensed or in development.

The molecular basis of reduced artemisinin responsiveness is unclear. Artemisinins are endoperoxides that are thought to form free radicals that may kill malaria parasites by alkylating essential biomolecules (3). Alternatively, artemisinins may act by specifically inhibiting the P. falciparum endoplasmic Ca\(^{2+}\)-ATPase ATP6 (PfATP6) (8). A single point mutation in PfATP6 can abolish artemisinin sensitivity, as shown in a heterologous system, which has led to the model that artemisinin resistance is brought about by mutational changes in the artemisinin binding site of PfATP6 (32). However, in P. falciparum, polymorphisms within PfATP6 do not seem to correlate with altered artemisinin responsiveness, as suggested by molecular epidemiological studies (5, 14, 36), although one study found a single amino acid polymorphism, outside the putative artemisinin binding site, in field isolates with a reduced artemisinin responsiveness (16). Further, allelic exchange mutants expressing wild-type or mutant PfATP6 did not show clear differences in artemisinin responsiveness (33).

Equally unclear is the contribution of the parasite’s multidrug resistance transporter PIMDR1 to artemisinin resistance. PIMDR1 resides at the digestive vacuolar membrane of the parasite, where it transports solutes, including antimalarial drugs, into the digestive vacuole (25). Polymorphisms within and overexpression of PIMDR1 affect its transport properties and, in turn, drug responses (23, 26–28). Artemisinin interferes with PIMDR1 transport function (24), and some studies have correlated altered artemisinin responsiveness with mutational changes within pfmdr1 and an increased copy number of pfmdr1 (23, 27, 28), whereas other studies did not find evidence for such a correlation (5, 14, 16).

A third candidate gene implicated in altered artemisinin responsiveness is an ubiquitin C-terminal hydrolase. Mutations in this enzyme are associated with artemisinin resistance in the mouse malaria parasite Plasmodium chabaudi (13). The significance of this finding for human malaria parasites is unclear (13, 14).

Several studies have attempted to generate artemisinin-resistant P. falciparum strains in vitro, however, with mixed results (2, 13, 15, 17). While strains with increased artemisinin half-maximal growth-inhibitory concentration (IC\(_{50}\)) values could initially be selected for, they were unstable. One study reported the selection of artelinic acid-resistant parasites over...
a period of 3 years (2). These parasites could subsequently be adapted to high artemisinin concentrations (2).

To explore the basis for altered artemisinin responsiveness, we have employed a classical genetic approach by investigating in vitro artemisinin responses in the F1 progeny of a genetic cross between \textit{P. falciparum} clones HB3 and Dd2 (30). Our data support the model that in vitro artemisinin responsiveness is genetically determined and that genetic predisposition favors the acquisition of stable, high-level artemisinin resistance when it is selected for.

\section*{MATERIALS AND METHODS}

\textbf{P. falciparum strains and cultures.} \textit{P. falciparum} was cultured as described previously (31) and synchronized using the sorbitol method (18). Cultures were maintained in type A-positive human erythrocytes suspended at a hematocrit of 5\% in RPMI 1640 supplemented with 2 mM l-glutamine, 25 mM HEPES, 20 \mu g/ml gentamicin, and 10\% human type A-positive serum. Cultures were incubated at 37\^\circ C under controlled atmospheric conditions of 5\% O\textsubscript{2}, 3\% CO\textsubscript{2}, and 92\% N\textsubscript{2}. The F1 progeny from the HB3 × Dd2 cross were obtained from MR4. The identities of all progeny were verified using eight polymorphic microsatellite markers (see the information in the supplemental material).

\textbf{pfmdr1 copy number.} The \textit{pfmdr1} copy number was determined by real-time PCR as described previously (24). The \textit{pfmdr1} copy numbers of the progeny from the HB3 × Dd2 cross have been described previously (21).

\textbf{In vitro artemisinin response assay.} Cell proliferation assays were performed in the presence of different drug concentrations as described previously (4, 29), with the following modifications. One hundred-microliter aliquots of a cell suspension containing highly synchronized rings (2 to 6 h postinvasion) at a parasitemia of 0.5\% and a hematocrit of 1.25\% were placed in the wells of 96-well microtiter plates, and the plates were incubated for 72 h in the presence of different drug concentrations (0, 3, 4, 10, and 20 nM and 0, 25, 46, and 100 nM, respectively). The parasite inoculum was 5 × 10\textsuperscript{12} infected erythrocytes (trophozoite stages) at the beginning of each selection step. In the case of GC06 and CH3-61, parasites were recovered after 8, 12, 18, and 26 days and 78, 94, and 122 days, respectively, following exposure to the next higher artemisinin concentration.

More information is available in the supplemental material.

\section*{RESULTS}

\textbf{Artemisinin responsiveness is genetically determined.} The in vitro artemisinin response, as defined by the IC\textsubscript{50} value, was determined for \textit{P. falciparum} clones HB3 (South America) and Dd2 (Southeast Asia) and 34 F1 progeny derived from a genetic cross between these two clones (30). Although the two parental clones have comparable artemisinin IC\textsubscript{50} values of 15.9 ± 0.9 nM (HB3) and 11.1 ± 0.5 nM (Dd2), their progenies' sensitivities covered a 9-fold range (3.2 ± 0.1 nM for GC06 to 28.8 ± 1.3 nM for CH3-61) (Fig. 1A and Table 1; see Table S1 in the supplemental material). The sensitivity to artemisinin was, in every case, unaffected by the presence of 0.89 \mu M verapamil (Fig. 1A; see also Table S1 in the supplemental material), a concentration sufficient to reverse chloroquine sensitivity when added to the culture medium.
In comparison, the Dd2 allele is, therefore, associated with altered artemisinin responsiveness (9, 10), Note that none of the parental clones or the progeny had been exposed to artemisinin prior to this study.

To identify determinants of altered artemisinin responsiveness, we conducted a QTL analysis by correlating the artemisinin IC\textsubscript{50} values with the established genetic maps of these strains (30). Three major QTLs were identified (with \( P < 0.01 \)), one each on chromosome 5, 12, and 13 (Fig. 1B). We did not detect linkage between artemisinin responsiveness and polymorphisms within \( pfjert \), the prime determinant of chloroquine resistance (10); orthologues of the \( P. chabaudi \) ubiquitin C-terminal hydrolase; or \( PfATP6 \). The last finding was not surprising, since neither of the parental strains contained polymorphisms in \( PfATP6 \) that are implicated in altered artemisinin susceptibility (16, 32).

The \( pfmdr1 \) genes of strains HB3 and Dd2 differ at three of the five polymorphic sites that are associated with altered drug responses (for HB3, F\textsuperscript{396}, F\textsuperscript{184}, S\textsuperscript{1034}, D\textsuperscript{1042}, and D\textsuperscript{1246}; for Dd2, \( \gamma^{\text{396}} \), \( \gamma^{\text{184}} \), S\textsuperscript{1034}, N\textsuperscript{1042}, and D\textsuperscript{1246} ) (9). Moreover, the \( pfmdr1 \) locus is amplified in Dd2, whereas in HB3 the \( pfmdr1 \) locus exists as a single copy (9, 24). From the sign of the regression coefficients in the QTL analyses, it was apparent that the presence of the HB3 \( pfmdr1 \) allele gave lower mean IC\textsubscript{50} values. Indeed, grouping the strains into those possessing \( pfmdr1 \) from HB3 and those possessing \( pfmdr1 \) from Dd2 gave mean IC\textsubscript{50} values of 7.2 \( \pm \) 0.7 nM and 12.4 \( \pm \) 1.4 nM, respectively (\( P = 0.001 \); Fig. 2A), so that the Dd2 allele is, therefore, associated with an increased (almost doubled) resistance to artemisinin. In comparison, the \( pfmdr1 \) copy number did not affect the artemisinin IC\textsubscript{50} values (\( P = 0.67 \)), not even if strains bearing the HB3 or the Dd2 \( pfmdr1 \) allele were considered separately (\( P = 0.067 \) and \( P = 0.84 \), respectively).

Loci from both parental strains contribute to artemisinin responsiveness. The locus on chromosome 12 detected in our QTL analysis centers around the C12M63 marker. It is 194 kb in size, and it contains 37 genes (see Fig. S1 in the supplemental material). The locus on chromosome 13 is 98 kb in size; contains 25 genes, excluding the subtelomeric variant gene families (see Fig. S2 in the supplemental material); and coincided with one part of a bifurcated peak that had previously been identified as being involved in altered quinine responsiveness in the same cross (9). Both the chromosome 12 and the chromosome 13 loci are characterized by negative regression coefficients in the QTL analysis, suggesting that inheritance of the contributing genes from HB3 resulted in increased resistance, whereas inheritance from Dd2 resulted in increased susceptibility (Fig. 2B and C). A multiple linear regression on all three loci suggested that the effect of the Dd2 \( PfMDR1 \) locus exerted its full doubling of the artemisinin IC\textsubscript{50} value only when it was combined with the C12M63 and the C13M73 loci from HB3 (\( P = 0.03 \); Fig. 2D).

Acquisition of stable artemisinin resistance. We next investigated whether the different artemisinin susceptibilities of the progeny strains might affect their responses to selection in artemisinin. To this end, we chose the two progeny with the most extreme artemisinin IC\textsubscript{50} values: strains GC06 and CH3-61 (Fig. 1A; Table 1). The artemisinin concentration in which the parasites were maintained was raised in steps from the strain’s IC\textsubscript{50} value and then to its IC\textsubscript{90} value and, after resistant strains had emerged, to final maintenance levels of 20 nM for GC06 and 100 nM for the initially more resistant CH3-61 strain. Clearly, both strains demonstrated a substantial (\( P < 0.001 \)) 2- to 4-fold increase in IC\textsubscript{50} values on selection in artemisinin (Fig. 3A and C; Table 1). No parasites were recovered.

**TABLE 1.** In vitro responsiveness to different antimalarial drugs of GC06 and CH3-61 pre- and postselection in 20 and 100 nM artemisinin, respectively.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Antimalarial drug</th>
<th>IC\textsubscript{50} (nM)</th>
<th>Preselection</th>
<th>Postselection</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC06</td>
<td>ART</td>
<td>3.1 ( \pm ) 0.1 (9)</td>
<td>12.5 ( \pm ) 1.6 (5)</td>
<td>( &lt; 0.001 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATM</td>
<td>2.4 ( \pm ) 0.3 (7)</td>
<td>2.1 ( \pm ) 0.5 (7)</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATS</td>
<td>1.5 ( \pm ) 0.5 (6)</td>
<td>1.4 ( \pm ) 0.3 (6)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AQ</td>
<td>12.7 ( \pm ) 0.9 (5)</td>
<td>11.8 ( \pm ) 1.4 (5)</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LUM</td>
<td>10.3 ( \pm ) 1.1 (6)</td>
<td>9.3 ( \pm ) 1.0 (6)</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CQ</td>
<td>135.4 ( \pm ) 7.0 (7)</td>
<td>137.6 ( \pm ) 9.3 (7)</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>CH3-61</td>
<td>ART</td>
<td>28.8 ( \pm ) 1.3 (9)</td>
<td>58.3 ( \pm ) 4.5 (5)</td>
<td>( &lt; 0.001 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DHA</td>
<td>3.0 ( \pm ) 0.5 (9)</td>
<td>5.8 ( \pm ) 0.7 (6)</td>
<td>( &lt; 0.03 )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATM</td>
<td>7.0 ( \pm ) 1.2 (7)</td>
<td>7.8 ( \pm ) 0.4 (7)</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATS</td>
<td>3.2 ( \pm ) 0.4 (6)</td>
<td>4.5 ( \pm ) 0.4 (6)</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AQ</td>
<td>10.3 ( \pm ) 2.6 (5)</td>
<td>8.1 ( \pm ) 0.8 (5)</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LUM</td>
<td>48.1 ( \pm ) 3.2 (6)</td>
<td>42.6 ( \pm ) 4.6 (6)</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CQ</td>
<td>19.9 ( \pm ) 1.9 (7)</td>
<td>23.7 ( \pm ) 2.4 (7)</td>
<td>0.25</td>
<td></td>
</tr>
</tbody>
</table>

\( a \) The means \( \pm \) standard errors of the means of the indicated number (in parentheses) of independent determinations are shown. Cells were maintained under the conditions described in Materials and Methods. Cells were transferred from the preselection to the postselection step until the IC\textsubscript{90} values of the parasites had been increased by at least one part of a bifurcated peak (Fig. 2B and C).

\( \text{IC}_{50} \) values for GC06 and 100 nM for the initially more resistant CH3-61 strain. Clearly, both strains demonstrated a substantial (\( P < 0.001 \)) 2- to 4-fold increase in IC\textsubscript{50} values on selection in artemisinin (Fig. 3A and C; Table 1). No parasites were recovered.

\( ** \) all comparisons; \( * \) \( P < 0.03 \) compared to the data in the first column for all comparisons.
When these resistant strains, henceforth termed GC06<sup>ART</sup> and CH3-61<sup>ART</sup>, respectively, were then maintained in the absence of drug, the artemisinin IC<sub>50</sub> value of GC06<sup>ART</sup> rapidly (by 22 days) reverted to the preselection value (Fig. 3B). In contrast, CH3-61<sup>ART</sup> maintained its enhanced artemisinin resistance undiminished even after 97 days of continuous in vitro culture in the absence of artemisinin (Fig. 3D). Freezing and thawing did not affect the artemisinin IC<sub>50</sub> value of CH3-61<sup>ART</sup>. These data suggest that the CH3-61 strain, which had inherited the pfmdr1 locus from Dd2 and both the C12M63 and the C13M73 loci from HB3, is genetically predisposed to acquiring genetically stable, high-level artemisinin resistance.

To assess whether the induced artemisinin resistance phenotypes displayed by GC06<sup>ART</sup> and CH3-61<sup>ART</sup> were associated with mutational changes in pfmdr1, pfcr, or pfatp6, we cloned and sequenced these genes and compared the sequences obtained with those of the parental strains GC06 and CH3-61. No mutational changes were found in these genes (data not shown). In addition, we determined the pfmdr1 copy number. Again, we found no changes (Fig. 4).

GC06<sup>ART</sup> and CH3-61<sup>ART</sup> did not display altered susceptibilities to the artemisinin derivates artemether and artesunate, suggesting different mechanisms of resistance.

FIG. 3. Selection of artemisinin-resistant <i>P. falciparum</i> parasites. Artemisinin IC<sub>50</sub> values of parasites recovered at the artemisinin concentrations indicated are shown. (A) GC06; (C) CH3-61. Values are means ± standard errors of the means of at least five independent determinations. *, <i>P</i> < 0.05 compared to unselected controls; **, <i>P</i> < 0.01 compared to unselected controls; ***, <i>P</i> < 0.001 compared to unselected controls. GC06 (B) and CH3-61 (D) selected in 20 and 100 nM artemisinin, respectively, were transferred to artemisinin-free medium, and their in vitro artemisinin IC<sub>50</sub> values were determined at the time points indicated after removal of the drug pressure. d, day.

nM and 100 nM for GC06<sup>ART</sup> and CH3-61<sup>ART</sup>, respectively. Thus, in vitro-induced artemisinin resistance did not extend to artemether and artesunate, suggesting different mechanisms of resistance.

DISCUSSION

Our data suggest that altered artemisinin responsiveness is a multigenetic trait, with loci on chromosomes 5, 12, and 13 (pfmdr1, C12M63, and C13M73, respectively) contributing to it. This finding corroborates the hypothesis that altered artemisinin susceptibility is genetically determined. The contributing genes on chromosomes 12 and 13 have not yet been identified. Both the C12M63 and the C13M73 loci are large and contain more than two dozen genes. The locus on chromosome 5 centers on pfmdr1. pfmdr1 is a known player in drug resistance in <i>P. falciparum</i>. Polymorphisms within pfmdr1 and overexpression of pfmdr1 can affect responses to a wide range of structurally and functionally diverse antimalarial drugs, including chloroquine, quinine, halofantrine, mefloquine, and lumefantrine (7, 23, 27, 28). Whether polymorphisms, copy number, or both also affect the response to artemisinin has been controversially discussed (5, 14, 16, 23, 27, 28). Our own data suggest that polymorphisms within pfmdr1, but not copy number, contributed to decreased susceptibility in the HB3 × Dd2 genetic cross. The result may differ if parasites with different genetic backgrounds, including different pfmdr1 polymorphisms, were to be investigated (2, 27).

How polymorphisms within PIMDR1 affect artemisinin responsiveness is unclear. In vivo transport studies using live parasites have shown that PIMDR1 transports solutes, including fluorochromes and antimalarial drugs, into the digestive vacuole (24, 25), where some of the drugs, including chloroquine, quinine, and, possibly, artemisinin, are thought to interfere with endogenous heme detoxification pathways (3, 11, 25). The described polymorphisms can drastically change the transport properties and the substrate specificity of PIMDR1, as has been shown by functionally expressing different pfmdr1 variants in <i>Xenopus laevis</i> oocytes (26). Extrapolating these data to artemisinin may suggest a model in which polymorphisms within PIMDR1 affect handling of this drug by PIMDR1, which in turn would reduce the susceptibility to artemisinin. In sup-
port of PIMDR1 interacting with artemisinin, it was found that artemisinin can block PIMDR1 transport activity in live parasites (24).

Given that none of the parental clones or the progeny had been exposed to artemisinin prior to this study, the differences in artemisinin susceptibility observed were not drug induced. Rather, they were the result of meiotic recombination and gene assortment and segregation processes. Such meiotic events are known to contribute to genetic diversity in natural P. falciparum populations (22) and may explain the wide range of in vitro artemisinin IC50 values currently observed in P. falciparum field isolates (16).

Interestingly, progeny that combined inheritable traits from the Latin American strain HB3 and the Southeast Asian strain Dd2 were among the strains that displayed high artemisinin IC50 values. Moreover, these progeny were genetically predisposed to rapidly acquiring even higher levels of stable artemisinin resistance under selective pressure. An example is the CH3-61 strain that had inherited the pfmdr1 locus from Dd2 and both the C12M63 and the C13M73 loci from HB3. CH3-61 displayed the highest artemisinin IC50 value among the progeny, and it rapidly acquired even higher levels of resistance when it was selected in artemisinin. In comparison, the GC06 strain that contained the pfmdr1 locus from HB3 and both the C12M63 and the C13M73 loci from Dd2 displayed the lowest artemisinin IC50 value among the progeny. Unlike CH3-61, GC06 did not develop stable artemisinin resistance. On the basis of these data, it is tempting to conclude that mating of parasites from geographic regions that have experienced different antimalarial drug histories poses a major threat to artemisinin’s efficacy since this can lead to parasites whose genetic backgrounds favor the acquisition of high-level artemisinin resistance.

The molecular basis of the high artemisinin resistance acquired by CH3-61 is presently unknown. Mutations in pfmdr1, pfcrt, and pfatp6 as well as changes in pfmdr1 copy number could be excluded as primary causes. Sequencing these genes from the progenitor clone CH3-61 and from CH3-61ART found no evidence for genetic changes (data not shown). The pfmdr1 copy number was determined by real-time PCR, and again, no changes were found (Fig. 4). The temporary increase in artemisinin IC50 values seen in the GC06 strain could have been due merely to a metabolic adaptation or a quiescence mechanism while the strain was under artemisinin selection (34).

Some of the inheritable traits that affected artemisinin susceptibility are shared with other antimalarial drugs, as demonstrated for polymorphisms within PIMDR1 that also contribute to increased resistance to artemisinin partner drugs, including quinine, amodiaquine, and mefloquine (25). Similarly, the C13M73 locus identified on chromosome 13 is shared with the C12M63 and the C13M73 loci from HB3. CH3-61 strain that had inherited the pfmdr1 locus from Dd2 and both the C12M63 and the C13M73 loci from HB3. CH3-61 displayed the highest artemisinin IC50 value among the progeny, and it rapidly acquired even higher levels of resistance when it was selected in artemisinin. In comparison, the GC06 strain that contained the pfmdr1 locus from HB3 and both the C12M63 and the C13M73 loci from Dd2 displayed the lowest artemisinin IC50 value among the progeny. Unlike CH3-61, GC06 did not develop stable artemisinin resistance. On the basis of these data, it is tempting to conclude that mating of parasites from geographic regions that have experienced different antimalarial drug histories poses a major threat to artemisinin’s efficacy since this can lead to parasites whose genetic backgrounds favor the acquisition of high-level artemisinin resistance.

The molecular basis of the high artemisinin resistance acquired by CH3-61 is presently unknown. Mutations in pfmdr1, pfcrt, and pfatp6 as well as changes in pfmdr1 copy number could be excluded as primary causes. Sequencing these genes from the progenitor clone CH3-61 and from CH3-61ART found no evidence for genetic changes (data not shown). The pfmdr1 copy number was determined by real-time PCR, and again, no changes were found (Fig. 4). The temporary increase in artemisinin IC50 values seen in the GC06 strain could have been due merely to a metabolic adaptation or a quiescence mechanism while the strain was under artemisinin selection (34).

Some of the inheritable traits that affected artemisinin susceptibility are shared with other antimalarial drugs, as demonstrated for polymorphisms within PIMDR1 that also contribute to increased resistance to artemisinin partner drugs, including quinine, amodiaquine, and mefloquine (25). Similarly, the C13M73 locus identified on chromosome 13 is shared with the low-level quinine resistance (9). This raises worries about the spread of artemisinin resistance to one artemisinin derivative does not necessarily render the entire drug class ineffective. Our study now provides the opportunity to identify the genetic mutations that bring about artemisinin resistance, so that we can prepare in advance to battle the almost certain spread of resistance to this lifesaving drug.

ACKNOWLEDGMENTS

This work was supported by the SFB 544 Control of Tropical Infectious Diseases. M.L. is a member of the CellNetwork Cluster and the EU-funded Network of Excellence EviMalaR.

REFERENCES