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The F423Y Mutation in the pfmdr2 Gene and Mutations N51I, C59R, and S108N in the pfdhfr Gene Are Independently Associated with Pyrimethamine Resistance in Plasmodium falciparum Isolates

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Screening for in vitro susceptibility to pyrimethamine and sequencing of the pfmdr2 and pfdhfr genes were performed in 140 Plasmodium falciparum isolates. The risk of in vitro resistance to pyrimethamine was analyzed with a logistic regression model. The mutation F423Y in pfmdr2 (odds ratio [OR] = 2.12 [confidence interval [CI], 1.02 to 4.59]; \( P = 0.0489 \)) and the mutation N51I, C59R, or S108N in pfdhfr (OR = 42.34 [CI, 5.52 to 324.61]; \( P = 0.0003 \)) were independently associated with in vitro resistance to pyrimethamine.

Sulfadoxine and pyrimethamine (Pyr) (SP) were widely used as first-line drugs for the treatment of malaria until the spread of resistance in Plasmodium falciparum isolates all over the world. SP is still recommended in combination with artesunate or chloroquine as a second-line therapy for uncomplicated P. falciparum and P. vivax malaria (7, 17, 19). SP is also still widely used in Africa for the intermittent preventive treatment (IPT) of malaria in infants (6), children (3), and pregnant women (8). SP resistance has also been linked to SP resistance in Plasmodium vivax (1, 9).

The mean 50% inhibitory concentration (IC50) of Pyr for the 140 isolates was 3,093 nM (95% confidence interval [CI], 2,527 to 3,660; minimum and maximum, 9.43 and 19,654 nM). Of these isolates, 69 had a Pyr IC50 > 2,000 nM (mean Pyr IC50 = 5,687 nM; 95% CI, 4,941 to 6,432) and were considered resistant; 71 isolates had a Pyr IC50 < 2,000 nM (mean Pyr IC50 = 573.3 nM; 95% CI, 425.2 to 721.4) and were considered susceptible to Pyr. Regarding the pfdhfr gene polymorphism, 74% (104/140) of the isolates had a point mutation at codon 51 (N51I); 73% (102/140) of the isolates had a triple mutation in pfdhfr (N51I; C59R; and S108N). Of these isolates, 69% (97/140) of the isolates had a point mutation at codon 51 (N51I); 73% (102/140) had a mutation at codon 59 (C59R), and 79% (110/140) had a mutation at codon 108 (S108N). Further, 69% (97/140) of the isolates had a triple mutation in pfdhfr. All 140 isolates possessed only one copy of the pfmdr2 gene. The analysis of the sequence of pfmdr2 gene revealed three points of mutation: S208N, F423Y, and K924E. There was no significant association between the S208N or K924E mutation and in vitro resistance to Pyr (\( P = 1; \)
Fisher’s exact test). The difference between the median of the Pyr IC50 (2,598 nM) for isolates harboring the Y substitution at codon 423 (n = 59) and the median of the Pyr IC50 (890 nM) for wild-type isolates (n = 81) was statistically significant (P < 0.02; Mann-Whitney test).

There was no statistically significant correlation between the F423Y mutation in pfmdr2 and the mutation in pfdhfr, the mean Pyr IC50 for these isolates was 434.6 nM (95% CI, 0 to 1,014; minimum and maximum, 10 and 4,214 nM). A total of 13 isolates had the F423Y mutation in pfmdr2 and were wild type for pfdhfr; the mean Pyr IC50 for these isolates was 170.1 nM (95% CI, 0 to 459.3; minimum and maximum, 10 and 1,762 nM). A total of 43 isolates were wild type for pfmdr2 and mutant type for pfdhfr (with at least one of the mutations N51I, C59R, and S108N); the mean Pyr IC50 for these isolates was 3,229 nM (95% CI, 2,186 to 4,272; minimum and maximum, 9.43 and 13,294 nM). A total of 68 isolates were mutant type for both the pfmdr2 and pfdhfr genes; the mean Pyr IC50 for these isolates was 4,192 nM (95% CI, 3,362 to 5,022; minimum and maximum, 50 and 19,654 nM). There was no statistically significant association between the median of the Pyr IC50 (50 nM) for isolates with both pfmdr2 and the pfdhfr wild type and the median of the Pyr IC50 (50 nM) for isolates with the mutant type for pfmdr2 and the wild type for pfdhfr (P = 0.25; Mann-Whitney test). The difference between the median of the Pyr IC50 (1,993 nM) of pfmdr2 wild-type and pfdhfr mutant-type isolates and the median of the Pyr IC50 (3,255 nM) for isolates harboring the Y substitution at codon 423 and a pfdhfr mutation was statistically significant (P = 0.0438; Mann-Whitney test).

The in vitro resistance to Pyr (Pyr IC50 > 2,000 nM) was analyzed using a logistic regression model (Table 1). The mutation F423Y in pfmdr2 (OR = 2.12 [95% CI, 1.02 to 4.59]; P = 0.0489) and the mutation N51I, C59R, or S108N in pfdhfr (OR = 42.34 [95% CI, 5.52 to 324.61]; P = 0.0003) (Hosmer-Lemeshow goodness-of-fit test; P = 0.77) were independently associated with in vitro resistance to Pyr.

MDR2 is associated with heavy metal ion efflux and resistance to cadmium (14). MDR2 may be involved in the transport of organic anions, such as folate or pABA, as has been suggested for

![FIG 1] Pyrimethamine IC50s (in nanomoles) of the 140 Plasmodium falciparum isolates according to their genotypes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. (%) of Pyr-susceptible isolates</th>
<th>No. (%) of Pyr-resistant isolates</th>
<th>Crude OR (95% CI)</th>
<th>P value</th>
<th>Adjusted OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pfmdr2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F423</td>
<td>37 (26.4)</td>
<td>22 (15.7)</td>
<td>1 (reference)</td>
<td></td>
<td>1 (reference)</td>
<td>0.0489</td>
</tr>
<tr>
<td>Y423</td>
<td>34 (24.3)</td>
<td>47 (33.6)</td>
<td>2.31 (1.11–4.91)</td>
<td>0.017</td>
<td>2.12 (1.02–4.59)</td>
<td>0.0489</td>
</tr>
<tr>
<td>pfdhfr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>28 (20)</td>
<td>1 (0.7)</td>
<td>1 (reference)</td>
<td>&lt;0.0001</td>
<td>1 (reference)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Mutant type</td>
<td>43 (30.7)</td>
<td>68 (48.6)</td>
<td>43.36 (6.67–1,819.1)</td>
<td></td>
<td>43.36 (5.52–324.61)</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

*OR, odds ratio; CI, 95% confidence interval.
*Fisher’s exact test.
*Multivariate logistic regression model.
MRP1 (4); it may be also implicated in the transport of sulfadoxine, as suggested by Martinelli et al. (10). Interestingly, in the latter study, an association between the in vivo resistance of Plasmodium chabaudi to sulfadoxine and a mutation in the mdr2 gene has been shown. The same investigation performed with Plasmodium falciparum would be really interesting, but no laboratory is able to shown. The same investigation performed with particular and also to evaluate the potential association between would be necessary to confirm these data with field isolates in of interest from this point of view. But further investigations for the IPT of malaria in Africa. The results of the present study are underlying mechanisms to be elucidated, because SP is still used tance to SP are probably multigenic; it is important for all of these AA modification concerns a cytoplasmic loop of the MDR2 protein and does not represent a significant alteration in terms of size and charge characteristics of the side chain, as phenylalanine and tyrosine are both hydrophobic. But this tyrosine could be a phosphorylation site, and it is known that protein structure and activity can be modulated by phosphorylation of tyrosine residues. The mechanisms of P. falciparum resistance to SP are probably multigenic; it is important for all of these underlying mechanisms to be elucidated, because SP is still used for the IPT of malaria in Africa. The results of the present study are of interest from this point of view. But further investigations would be necessary to confirm these data with field isolates in particular and also to evaluate the potential association between this pfmdr2 mutation and the resistance of P. falciparum to sulfadoxine.

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REFERENCES