New PfATP6 mutations found in *Plasmodium falciparum*

isolates from Vietnam

Artemisinin and its derivatives have been used against malaria in Vietnam since 1991 (4). An increase in clinical artemisinin resistance would be disastrous for malaria treatment. All possible indicators of this potential resistance must be monitored. The sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase ortholog of *Plasmodium falciparum* (*PfATP6*) has been suggested to be the target of artemisinins (3). Consequently, the polymorphism of *PfATP6* is being monitored by several scientific research teams (2; 6; 7; 9; 10; 15). We report here the genotyping results of *PfATP6* from 98 *Plasmodium falciparum* (*P. falciparum*) field isolates collected in 2006-2007 in South Vietnam.

Parasite samples were taken from patients (28.82 ± 12.31 years old) with uncomplicated *P. falciparum* infections before drug treatment. They were collected in Binh Phuoc and Dak Nong provinces in South Vietnam. Patients did not follow a chemoprophylaxis before sampling. Diagnosis was carried out by microscopic examination and confirmed by real-time PCR as previously described (14). The whole *PfATP6* gene was sequenced once in both directions with 5 primer pairs (adapted from Jambou *et al.* (7)), and compared to the reference sequence of the 3D7 strain (PFA0310c in the genome annotation).

We found a total of 8 mutations (Table 1): 4 non-synonymous (I89T, N463S, N465S, N683K), 3 synonymous (N460N, I898I, C1031C) and 1 double deletion leading to the loss of two asparagines (Δ463-464). Five of these have not been described previously (N460N, N463S, Δ463-464, N465S, C1031C). All the mutations were detected on different isolates, except for the I898I which was found alone or associated with others. Like Mugittu *et al.* in Tanzania and...
Zhang et al. in China (10; 15), we did not find either the S769N mutation, or the A623E E431K double mutation, associated with reduced susceptibility to artemether (7). Previously, the N683K mutation was only found in Cambodia (2), suggesting that it may be specific to *P. falciparum* from South-East Asia. However, we did not detect this mutation in the South-East Asiatic strains W2, Dd2 (both from Indochina, Malaria Research and Reference Reagent Resource Center), IMT-A4 (Vietnam) and IMT-K2 (Cambodia; data not shown). Interestingly, the N460N, N463S, N465S and N683K mutations, and the Δ463-464 double deletion, are in a stretch of 9 asparagines located in the interspecies variable region of *PfATP6*, a domain specific for *Plasmodium* species (8). Consequently, these modifications could be adaptive changes that might alter susceptibility to artemisinins.

Cojean et al. found the S769N mutation in an isolate from Africa that was susceptible to dihydroartemisinin (1), while Noedl et al. did not find this mutation in Cambodian samples that were less susceptible to artesunate (11). Consequently, we speculated on whether the correlation between the S769N mutation and the increased artemether IC50, found in 6 isolates from French Guyana (7), should be regarded as a local case. Like others investigators, we did not detect any polymorphism in codon 263, described as the key amino acid for the interaction between *PfATP6* and artemisinins (13). Mutations observed in our sequences, and in those of previous studies (2; 6; 9) could be implicated indirectly in this interaction, in case of association with artemisinins susceptibility. Considering the development of artemisinin combined therapies, and the possible implication of *PfATP6* in artemisinin resistance, the molecular variability of this gene should be carefully monitored.

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REFERENCES


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TABLE 1. Diversity of PfATP6 in Plasmodium falciparum samples from Vietnam.  

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>Number of samples</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>T266C</td>
<td>I89T</td>
<td>13</td>
<td>Price 2004 (12)</td>
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<tr>
<td>T1380C</td>
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</tr>
<tr>
<td>A1388G</td>
<td>N463S</td>
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<tr>
<td>___</td>
<td>Δ463-464</td>
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<tr>
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<td>T2049A</td>
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<td>5</td>
<td>Dahlström 2008 (2)</td>
</tr>
<tr>
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<td>I898I</td>
<td>91</td>
<td>Ferreira 2007 (5)</td>
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<tr>
<td>C3093T</td>
<td>C1031C</td>
<td>3</td>
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*Total number of analyzed samples: 98