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 P. falciparum field isolates collected in 2006 to 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 drug treatment. They were monitored. The sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase is being monitored by several scientists. However, we did not see 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 five 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 eight mutations (Table 1): four nonsynonymous (I89T, N463S, N465S, and N683K), three synonymous (N460N, I898I, and C1031C), and one double deletion leading to the loss of two asparagines (Δ463–464). Five of these have not been described previously (N460N, N463S, Δ463–464, N465S, and C1031C). All of the mutations were detected on different isolates, except for I898I, which was found alone or associated with others. Like Mugittu et al. in Tanzania (10) and Zhang et al. in China (15), we did not find either the S769N mutation or the A623E E431K double deletion, 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 and 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 nine 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 50\% inhibitory concentration found in six isolates from French Guyana (7) should be regarded as a local case. Like other 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 the case of association with artemisinin 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 P. falciparum samples from Vietnam**

<table>
<thead>
<tr>
<th>Nucleotide mutation</th>
<th>Amino acid mutation</th>
<th>No. of samples</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T266C</td>
<td>I89T</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>T1380C</td>
<td>N460N</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>A1388G</td>
<td>N463S</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>A1395G</td>
<td>N465S</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>T2049A</td>
<td>N683K</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>T2064A</td>
<td>I898I</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>C3093T</td>
<td>C1031C</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

* The total number of samples analyzed was 98.


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