ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2009, p. 4570–4571 Vol. 53, No. 10
0066-4804/09/$08.00+0 doi:10.1128/AAC.00684-09

New Pf*ATP6* 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* (Pf*ATP6*) has been suggested to be the target of artemisinins (3). Consequently, the polymorphism of Pf*ATP6* is being monitored by several scientific research teams (2, 6, 7, 9, 10, 15) We report here the genotyping results of Pf*ATP6* 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 chemoprophylaxis before sampling. Diagnosis was carried out by microscopic examination and confirmed by real-time PCR as previously described (14). The whole Pf*ATP6* 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 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 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 Pf*ATP6*, a domain specific for *Plasmodium* species (8). Consequently, these modifications could be adaptive changes that might alter susceptibility to artemisinins.

TABLE 1. Diversity of Pf*ATP6* in *P. falciparum* samples from Vietnam

Nucleotide mutation	Amino acid mutation	No. of samples[a]	Reference
T266C	I89T	13	12
T1380C	N460N	1	
A1388G	N463S	3	
	Δ463–464	1	
A1395G	N465S	2	
T2049A	N683K	5	2
T2694A	I898I	91	5
C3093T	C1031C	3	

[a] The total number of samples analyzed was 98.

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 Pf*ATP6* 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 Pf*ATP6* in artemisinin resistance, the molecular variability of this gene should be carefully monitored.

We thank Yannick Le Priol for discussions; Nicolas Benoit, Julien Cren, and Marc Desbordes for technical help; and the Military Institute of Hygiene and Epidemiology of Hanoi, the Military Center of Analysis and Research of Hanoi, and the Military Center of Preventive Medicine of Ho Chi Minh for helpful collection of samples.

This study was supported by the French Ministry of Foreign Affairs and by the French and Vietnamese Military Health Services.

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▽ Published ahead of print on 17 August 2009.