Novel Point Mutations in the Dihydrofolate Reductase Gene of *Plasmodium vivax*: Evidence for Sequential Selection by Drug Pressure

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Abstract

Mutations in the dihydrofolate reductase (*dhfr*) genes of *Plasmodium falciparum* and *P. vivax* are associated with resistance to the antifolate antimalarial drugs. *P. vivax dhfr* sequences were obtained from 55 *P. vivax* isolates (isolates Belem and Sal 1, which are established lines originating from Latin America, and isolates from patient samples from Thailand [n = 44], India [n = 5], Iran [n = 2], and Madagascar [n = 2]) by direct sequencing of both strands of the purified PCR product and were compared to the *P. vivax dhfr* sequence from a *P. vivax* parasite isolated in Pakistan (isolate ARI/Pakistan), considered to represent the wild-type sequence. In total, 144 *P. vivax dhfr* mutations were found at only 12 positions, of which 4 have not been described previously. An F→L mutation at residue 57 had been observed previously, but a novel codon (TTA) resulted in a mutation in seven of the nine mutated variant sequences. A new mutation at residue 117 resulted in S→T (S→N has been described previously). These two variants are the same as those observed in the *P. falciparum dhfr* gene at residue 108, where they are associated with different levels of antifolate resistance. Two novel mutations, I→L at residue 13 and T→M at residue 61, appear to be unique to *P. vivax*. The clinical, epidemiological, and sequence data suggest a sequential pathway for the acquisition of the *P. vivax dhfr* mutations. Mutations at residues 117 and 58 arise first when drug pressure is applied. Highly mutated genes carry the S→T rather than the S→N mutation at residue 117. Mutations at residues 57 and 61 then occur, followed by a fifth mutation at residue 13.

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