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## Spread and Distribution of Drug Resistance and Compensatory Mutations in *Plasmodium falciparum*

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# Abstract

In 2010 there were an estimated 216 million cases of malaria worldwide. In Honduras there were ~9000 cases of which 88% were due to *Plasmodium vivax* mono-infection. Chloroquine (CQ) resistant *Plasmodium falciparum* have spread throughout the world curtailing its use. The only exception appears to be north of Panama where CQ reportedly remains efficacious and the drug of choice for treating both *P. falciparum* and *P. vivax*. Resistance to antimalarials is associated with specific genetic polymorphisms and recently a putative H<sup>+</sup> pump (*pfvp2*) has been suggested to be linked to CQ resistant *P. falciparum*. The aim of this thesis was to identify resistance associated genetic polymorphisms in *P. falciparum* and *P. vivax* from Honduras and to describe the worldwide distribution of *pfvp2* polymorphisms and their correlation to CQ resistance.

Resistance associated genetic polymorphisms in *P. falciparum* and *P. vivax* multidrug resistance gene (*pfmdr1* and *pvmdr1*), dihydrofolate reductase (*pfdhfr* and *pvdhfr*), *P. falciparum* chloroquine resistance transporter (*pfcr1*), dihydropteroate synthase (*pfdhps*) and V-type H<sup>+</sup> pyrophosphatase (*pfvp2*) were identified in field samples using PCR based methods. From Honduras, 37 *P. falciparum* and 64 *P. vivax* samples, collected from symptomatic patients were used. In addition, 50 samples from each of Colombia, Liberia, Guinea-Bissau, Tanzania, Iran, Thailand and Vanuatu were used. The samples represented a time period from 1978 to 2009 and areas with different prevalence of CQ resistant *P. falciparum*.

In samples from Honduras no genetic polymorphisms associated with CQ or sulphadoxine-pyrimethamine (SP) resistance were found in *P. falciparum*. In *P. vivax*, the CQ resistance associated *pvmdr1* 976F allele was found in 7/37 samples and the SP resistance associated *pvdhfr* 57L+58R alleles were found in 2/57 samples. When analysing the worldwide collection of samples, the *pfvp2* 405V, 582K and 711P haplotype was associated with the for CQ resistance essential allele, *pfcr1* 76T (P=0.007). Samples with *pfvp2* 405I and/or 582R and/or 711S were significantly more common in Liberia in 1978-1980 (P=0.01), all African countries (P=0.004) and all African countries + Honduras (P=0.01) compared to the rest of the world.

Our results suggest that *P. falciparum* and *P. vivax* in Honduras are sensitive to CQ and SP. However, small numbers of *P. vivax* had genetic polymorphisms suggesting a degree of tolerance to CQ and SP. The association between *pfcr1* 76T and the *pfvp2* 405V, 582K and 711P haplotype suggest that this haplotype is associated with CQ resistance. This is in line with previous research that has described increased expression of *pfvp2* during CQ exposure. The higher frequency of *pfvp2* 405I and/or 582R and/or 711S in CQ sensitive settings in Africa and Honduras suggests a larger variation in the *pfvp2* genome prior to the spread of CQ resistance further supporting the association between *pfvp2* and CQ resistance.