Abstract

The emergence and spread of artemisinin-resistant *Plasmodium falciparum* is of huge concern for the global effort toward malaria control and elimination. Artemisinin resistance, defined as a delayed time to parasite clearance following administration of artemisinin, is associated with mutations in the *Pfkelch13* gene of resistant parasites. To date, as many as 60 non-synonymous mutations have been identified in this gene, but whether these mutations have been selected by artemisinin usage or merely reflect natural polymorphism independent of selection is currently unknown. To clarify this, we sequenced the *Pfkelch13*-propeller domain in 581 isolates collected before (420) and after (161) the implementation of artemisinin combination therapies (ACTs), from various endemic regions worldwide. Non-synonymous mutations were observed in 1% of parasites isolated prior to the introduction of ACTs. Frequencies of mutant isolates, nucleotide diversity and haplotype diversity were significantly higher in the parasites isolated from populations exposed to artemisinin compared to those from populations that had not been exposed to the drug. In the artemisinin-exposed population, a significant excess of dN compared to dS was observed, suggesting the presence of positive selection. In contrast, pairwise comparison of dN and dS and the McDonald and Kreitman test indicates that purifying selection acts on the*Pfkelch13*-propeller domain in populations not exposed to ACTs. These population-genetic analyses reveal a low baseline of *Pfkelch13* polymorphism, probably due to purifying selection in the absence of artemisinin selection. In contrast, various *Pfkelch13* mutations have been selected under artemisinin pressure.