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Polymorphisms in *Plasmodium falciparum* Kelch 13 and *P. vivax* Kelch 12 Genes in Parasites Collected from Three South Pacific Countries Prior to Extensive Exposure to Artemisinin Combination Therapies

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ABSTRACT The South Pacific countries Solomon Islands, Vanuatu, and Papua New Guinea (PNG) adopted artemisinin-based combination therapies (ACTs) in 2008. We examined Kelch 13 and Kelch 12 genes in parasites originating from these countries before or at ACT introduction. Four Kelch 13 and two Kelch 12 novel sequence polymorphisms, not associated with artemisinin resistance, were observed in parasites from Solomon Islands and Vanuatu. No polymorphisms were observed in PNG parasites. The findings provide useful baseline information.

KEYWORDS Kelch 12, Kelch 13, *P. falciparum*, artemisinin combination therapy, artemisinin resistance, sequence polymorphism

Artemisinin combination therapies (ACTs) are adopted by all countries where malaria is endemic as first-line treatment for uncomplicated falciparum malaria (1), following WHO treatment guidelines (2), and have become the cornerstone of national malaria control and elimination programs. However, parasites resistant to the artemisinin class of drugs have been reported in western Cambodia since 2008–2009 (3, 4) and in several Southeast Asian countries (5–8). This has caused treatment failures of some potent ACTs (9), posing a serious threat to case management and malaria control and elimination programs. It is critical to contain resistance within the foci and to conduct surveillance for any spread or emergence of artemisinin-resistant parasites outside the foci.

Surveillance of malarial drug resistance can be achieved by studying molecular markers of resistance. The *Plasmodium falciparum* Kelch 13 gene was established as a molecular marker for artemisinin resistance in 2014 (10). Twenty-two genetic mutations in Kelch 13 correlated with *in vivo* delayed parasite clearance after artemisinin treatment (10–15), and four mutations have been validated *in vitro* to confer ring-stage survival after drug exposure (10). The association of Kelch 13 with artemisinin resistance was supported by a large genome-wide association study (16) and confirmed by genetic modification studies (12). After the report of Kelch 13, three large-scale surveillance studies of Kelch 13 mutations were conducted. The first was conducted in 12 sub-Saharan countries by examining 1,212 samples collected between 2013 and 2014 (17). Seven nonsynonymous mutations, not associated with artemisinin resistance, were observed at frequencies of 1% to 3%, the most frequent mutation being A578S.

An examination of 14,037 samples collected from 163 sites in 59 countries in Asia, Africa, South America, and Oceania, mostly after 2012 (18), found >100 nonsynonymous sequence polymorphisms, of which 9 had a frequency of >1% and 72 were only

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TABLE 1 Summary of *P. falciparum* isolates and nonsynonymous polymorphisms in the Kelch13 observed

Country and area	Collection yr(s)	Sample source (ref.)	No. of isolates	Wild-type sequences (*n* [%])	NS-SNPs[a] (*n* [%])	Resistant mutants (*n*)	NS-SNP
PNG							
Madang	1980s	(55)	13	13 (100)	0 (0)	0	NA[b]
Bougainville	1998–1999	Community survey (56)	61	61 (100)	0 (0)	0	NA
Solomon Islands							
Guadalcanal	1987	Culture adapted (57)	6	6 (100)	0 (0)	0	NA
Malaita	2008	Therapeutic efficacy study	74	71 (96)	3 (4)	0	I465F[c], F673Y[c], M460K[c]
Vanuatu							
Tanna	2008	Community survey (58)	18	17 (94)	1 (6)	0	S477F, T677I[c]
Total			169	165 (97.6)	4 (2.4)	0	

[a]NS-SNPs, nonsynonymous sequence polymorphisms.
[b]NA, not applicable.
[c]Novel sequence polymorphisms.

observed once. Artemisinin-resistant mutations were only observed in Southeast Asia and China. The study included 43 samples each from Papua New Guinea (PNG) and Solomon Islands of Oceania and revealed zero and one (G592R) nonsynonymous mutation in PNG and Solomon Islands, respectively.

The third study examined Kelch 13 mutations in 581 isolates from 14 countries, mostly before the introduction of ACTs (19). The number and frequency of mutations observed was low in pre-ACT compared to post-ACT samples, although post-ACT samples came mainly from three Southeast Asian countries where artemisinin resistance has been reported. This sample set included 60, 41, and 49 samples from PNG, Solomon Islands, and Vanuatu, respectively, collected between 1995 and 2003. No nonsynonymous mutations were seen in PNG and Solomon Islands, whereas A481T and N531D mutations were seen in Vanuatu. Other studies have been conducted in Asian (20–31), African (32–49), and South American countries (50, 51). There are no other reports from Oceania countries apart from the two worldwide studies.

A Kelch 13 homolog in *Plasmodium vivax*, Kelch 12, was identified, and a V552I polymorphism was observed in 0.7% of isolates from Cambodia (52). Three nonsynonomous polymorphisms were identified in Cambodian isolates in a separate study, and none were orthologs of artemisinin-resistant K13 mutations (53).

In 2008, Solomon Islands, Vanuatu, and PNG adopted ACT as the national policy for treatment of *P. falciparum* and *P. vivax* malaria. Although limited information on Kelch 13 is available for limited sites in these countries, no information is available on Kelch 12. To fill this gap, we examined Kelch 13 and Kelch 12 in parasite samples originating from three South Pacific countries before or at the time of ACT introduction.

A total of 169 *P. falciparum* and 59 *P. vivax* isolates were examined retrospectively. These were collected through community surveys, therapeutic efficacy studies, and laboratory-adapted parasite lines. Tables 1 and 2 summarize the sources, number, and collection year of *P. falciparum* and *P. vivax* samples, respectively.

The storage and use of samples collected from community surveys in Solomon Islands and Vanuatu were approved by the Australian Defense Human Ethics Committee (ADHREC 505-07). The use of samples collected from Bougainville, PNG, for this study was approved by ADHREC (835-16) and the Government of Papua New Guinea Medical Research Advisory Committee (16.40). The use of culture-adapted parasitic isolates was approved by the Australian Defense Joint Health Command Low Risk Ethics Panel (LREP15-014).

The Kelch 13 gene was amplified and sequenced (codons 435 to 680) using a published protocol to determine genetic polymorphisms (10). All 74 *P. falciparum*

TABLE 2 Summary of *P. vivax* isolates and nonsynonymous sequence polymorphisms (NS-SNP) in Kelch12 observed

Country and area	Collection yr	Sample source (ref.)	No. of isolates	Wild-type sequences (*n* [%])	NS-SNPs[a] (*n* [%])	Resistant mutants (*n*)	NS-SNP
Solomon Islands							
Malaita	2008	Therapeutic efficacy study	26	24 (92)	2 (8)	0	V652L
Vanuatu							
Tanna	2008	Community survey (58)	11	11 (100)	0 (0)	0	NA
Epi Island	2011	Therapeutic efficacy study	22	21 (95)	1 (5)	0	I537V
Total			59	56 (95)	3 (5)	0	

[a]NS-SNP, nonsynonymous sequence polymorphisms.

isolates from PNG were of wild-type Kelch 13 sequences (Table 1). The lack of nonsynonymous polymorphisms in PNG samples reinforces earlier observations that *P. falciparum* parasites in PNG appeared to have few sequence polymorphisms in K13 genes before the introduction of ACTs (19).

Among 74 *P. falciparum* isolates from Malaita Province, Solomon Islands, three nonsynonymous polymorphisms (I465F, F673Y, and M460K) were observed, each identified only once from a single isolate. One of the 18 *P. falciparum* isolates from Tanna Island, Vanuatu, carried two nonsynonymous polymorphisms (S477F and T677I). Interestingly, all five nonsynonymous polymorphisms were observed in parasite samples collected in 2008, and all except S477F appeared to be novel. They are not known to be associated with artemisinin resistance.

Sequencing of the Kelch 12 gene was performed for 59 *P. vivax* samples using published procedures (52). Of the 26 samples collected from Solomon Islands, 2 exhibited nonsynonymous polymorphism V652L (Table 2). Of the 33 samples from Vanuatu, collected in 2012, 4 years after introduction of ACT, 1 carried nonsynonymous polymorphism I537V, and it is not clear whether this polymorphism may have resulted from ACT selective pressure. Both polymorphisms have not been reported elsewhere. Unfortunately, due to a lack of samples, we could not examine Kelch 12 from PNG parasites. The limited polymorphisms observed in this Pacific sample set agree with findings in Southeast Asia (52, 54).

In summary, we observed few genetic polymorphisms in Kelch 13 and Kelch 12 in parasite samples collected from three South Pacific countries mostly before or at the time of ACT introduction. The findings provide baseline information on both Kelch 13 and Kelch 12 in parasite populations before extensive exposure to ACT and will serve as a reference for future molecular surveys of ACT resistance in these countries.

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K.G., K.A., and C.P. processed and analyzed samples and performed sequence analyses. N.W. and Q.C. conceived the study. Q.C. prepared the manuscript. We all reviewed and approved the manuscript.

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