REPLY TO VELAVAN ET AL.:

Polymorphisms of *pfcoronin* in natural populations: Implications for functional significance

Aabha I. Sharma[a], Allison R. Demas[a], Daniel L. Hartl[a,b], and Dyann F. Wirth[a,c,1]

Velavan et al. (1) describe work that they have carried out based on our recent PNAS publication, "Mutations in *Plasmodium falciparum* actin-binding protein coronin confer reduced artemisinin susceptibility" (2). Among 297 patient samples from 4 countries in Africa, they found 12 polymorphic amino acid sites in *Pf*Coronin, 7 observed only among 48 parasites from the Congo (1). These findings are consistent with results reported in the Pf3k database (https://www.malariagen.net/projects/p-falciparum-communityproject), which we referenced in our original publication. The amino acid replacements of *Pf*Coronin that we found to be associated with artemisinin resistance after long-term in vitro selection (G50E, R100K, and E107V) have not yet been found in samples from Africa or elsewhere (2). These findings are reminiscent of those for *Pf*Kelch13, in which the M476I mutation discovered in the laboratory by in vitro selection was not initially found among artemisinin-resistant clinical isolates (3).

Widespread amino acid polymorphisms at other sites in *Pf*Coronin have been reported in Africa and elsewhere (https://www.malariagen.net/projects/p-falciparum-communityproject), including sites in the WD40 domain (S183G, T251A, and A263S, among others) as well as in the non-WD40 domain (V424I, E450K, A457V, and E519K, among others). The mutations mentioned by Velavan et al. (1) in their Letter include those previously observed; some mutations reported from the Congolese samples may be unique, but they are supported by a small number of reads and would require further validation.

It is reassuring that the polymorphisms reported by Velavan et al. (1) do not confer clinical resistance to artemisinin combination therapy. However, the standard measure for reduced artemisinin susceptibility is either to conduct controlled clinical trials with artemisinin treatment alone and monitor in vivo parasite clearance (4–7) or to perform ring-stage survival assay in vitro and measure parasite survival (3, 8). Given that the findings reported by Velavan et al. (1) are with artemisinin combination therapy, it may be premature to dismiss the potential that *Pf*Coronin polymorphisms contribute to artemisinin resistance.

It is also worth noting that there are important differences in the allele-frequency spectrum of polymorphisms in different functional domains of *Pf*Coronin. In particular, the allele-frequency spectrum of polymorphisms in the non-WD40 domain tends to be much more evenly distributed across populations than those in the WD40 domain (https://www.malariagen.net/projects/p-falciparum-communityproject). The 3-dimensional β-propeller structure of the WD40 domain and its biological role in coordinating multiprotein complex assemblies suggest that the unusual allele-frequency spectrum of the WD40 polymorphisms may be functionally significant.

1 T. P. Velavan, D. Nderu, T. Agbenyega, F. Ntoumi, P. G. Kremsner, An alternative dogma on reduced artemisinin susceptibility: A new shadow from east to west. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 12611–12612 (2019).

2 A. R. Demas *et al.*, Mutations in *Plasmodium falciparum* actin-binding protein coronin confer reduced artemisinin susceptibility. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 12799–12804 (2018).

3 F. Ariey *et al.*, A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* **505**, 50–55 (2014).

4 H. Noedl *et al.*, Artemisinin resistance in Cambodia: A clinical trial designed to address an emerging problem in Southeast Asia. *Clin. Infect. Dis.* **51**, e82–e89 (2010).

5 H. Noedl *et al.*; Artemisinin Resistance in Cambodia 1 (ARC1) Study Consortium, Evidence of artemisinin-resistant malaria in western Cambodia. *N. Engl. J. Med.* **359**, 2619–2620 (2008).

[a]Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA 02115; [b]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138; and [c]Infectious Disease and Microbiome Program, Broad Institute, Cambridge, MA 02142

Author contributions: A.I.S. and A.R.D. performed research; and A.I.S., D.L.H., and D.F.W. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence may be addressed. Email: dfwirth@hsph.harvard.edu.

Published online June 25, 2019.

6 P. Starzengruber *et al.*, Current status of artemisinin-resistant falciparum malaria in South Asia: A randomized controlled artesunate monotherapy trial in Bangladesh. *PLoS One* **7**, e52236 (2012).
7 E. A. Ashley *et al.*; Tracking Resistance to Artemisinin Collaboration (TRAC), Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* **371**, 411–423 (2014).
8 B. Witkowski *et al.*, Novel phenotypic assays for the detection of artemisinin-resistant *Plasmodium falciparum* malaria in Cambodia: In-vitro and ex-vivo drug-response studies. *Lancet Infect. Dis.* **13**, 1043–1049 (2013).