

An alternative dogma on reduced artemisinin susceptibility: A new shadow from east to west

Thirumalaisamy P. Velavan^{a,b,c,1}, David Nderu^{a,d}, Tsiri Agbenyega^{e,f,g}, Francine Ntoumi^{a,h}, and Peter G. Kremsner^{a,i}

In PNAS, Demas et al. (1) show, by long-term in vitro selection using culture-adapted *Plasmodium falciparum* isolates from Senegal, that the gene encoding the actin-binding protein *P. falciparum* coronin (*pfcoronin*) and its genetic variants (G50E, R100K, and E107V) can reduce the susceptibility of the parasite to the active metabolite of the fast-acting antimalarial drug artemisinin, dihydroartemisinin (DHA). Resistance to artemisinins is a global threat in malaria control and elimination efforts (2).

Artemisinin resistance, first reported in Southeast Asia and still extremely rare, was associated with the *P. falciparum* PfKelch13-propeller domain (*kelch13* mutations: Y493H, R539T, I543T, and C580Y) (3). PfCoronin, which is structurally similar to Kelch13, is believed to interact with F-actin via its N-terminal propeller domain and to mediate actin organization and motility in merozoites and sporozoites (4, 5). The worldwide map of the occurrence of *kelch13*, however, indicates absence of the Asian artemisinin-resistance alleles in Africa (6–8). So far, it is not clear whether the *pfcoronin* variants G50E, R100K, and E107V occur in natural *P. falciparum* populations—in particular, in clinical isolates from Africa.

We looked at a total of 353 *P. falciparum* patient isolates that were earlier characterized for the absence of *kelch13* gene mutations (7–10) from 4 African countries to verify whether these isolates carry the *pfcoronin* mutations G50E, R100K, and E107V, which were described by Demas et al. (1) to be associated with reduced susceptibility to DHA. A total of 297 samples

were successfully genotyped by direct Sanger sequencing. Details of the study groups from Gabon ($n = 102$), Congo ($n = 48$), Ghana ($n = 57$), and Kenya ($n = 90$) are described elsewhere (7–10). The *pfcoronin* mutations G50E, R100K, and E107V were not observed at all among the isolates. However, 14 distinct mutations, including several nonsynonymous substitutions, were identified in the *pfcoronin* exon-3 (Table 1). None of the isolates carried the Asian *kelch13* resistance alleles M476I, Y493H, R539T, I543T, and C580Y. The mutation P76S (DNA position C562T) was observed to be most frequent (>10%) among isolates from central and west Africa. There was no indication of artemisinin or artemisinin-based combination therapy resistance in these patients. The functional role of the observed *pfcoronin* P76S needs to be elucidated among central and west African *P. falciparum* isolates.

Much effort has been made in recent years to determine the genetic basis of artemisinin resistance, which still remains unclear to a large extent. There is an obvious difference in occurrence of *pfkelch13* and *pfcoronin* alleles between Asia and Africa, which may also cause differences in parasite clearance rates during treatment with artemisinin-containing antimalarial combinations. However, we should bear in mind that parasite clearance rate or failure of an artemisinin-containing antimalarial is also, and even most often, determined by the activity of the partner drug, such as lumefantrine, amodiaquine, piperaquine, and pyronaridine.

^aInstitut für Tropenmedizin, Universitätsklinikum Tübingen, 72074 Tübingen, Germany; ^bVietnamese-German Center for Medical Research (VG-CARE), Hanoi, Vietnam; ^cFaculty of Medicine, Duy Tan University, Da Nang, Vietnam; ^dSchool of Health Sciences, Kirinyaga University, 10300 Kerugoya, Kenya; ^eDepartment of Physiology, School of Medical Sciences, University of Science and Technology, 00233 Kumasi, Ghana; ^fDepartment of Child Health, Komfo Anokye Teaching Hospital, 00233 Kumasi, Ghana; ^gDepartment of Medicine, Komfo Anokye Teaching Hospital, 00233 Kumasi, Ghana; ^hFondation Congolaise pour la Recherche Médicale (FCRM), Brazzaville, Republic of Congo; and ⁱCentre de Recherches Médicales de Lambaréné, Lambaréné, Gabon

Author contributions: T.P.V. designed research; D.N. performed research; T.P.V., D.N., T.A., F.N., and P.G.K. contributed new reagents/analytic tools; T.P.V. and D.N. analyzed data; and T.P.V. and P.G.K. wrote the paper.

The authors declare no conflict of interest.

This open access article is distributed under [Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 \(CC BY-NC-ND\)](https://creativecommons.org/licenses/by-nc-nd/4.0/).

¹To whom correspondence may be addressed. Email: velavan@medizin.uni-tuebingen.de.

Published online June 25, 2019.

Table 1. pfcoronin mutations observed in 4 African countries

Amino acid change	SNP position	Gabon, n (%) (n = 102)	Ghana, n (%) (n = 57)	Kenya, n (%) (n = 90)	Congo, n (%) (n = 48)	Total, n (%) (N = 297)
I53I	C495T	0	1 (2)	0	0	1 (0.3)
V62M	G520A	1 (1)	1 (2)	0	0	2 (0.7)
K69K/I/R	A542A/T/G	0	3 (5)	0	11 (23)	14 (5)
P76S	C562T	11 (11)	9 (16)	4 (4)	8 (17)	32 (11)
N110N/Y/D	A664A/T/G	0	1 (2)	0	5 (10)	6 (2)
N112N/Y/D	A670A/T/G	0	0	0	1 (2)	1 (0.3)
K115K/stop/E	A679A/T/G	0	0	0	1 (2)	1 (0.3)
L121L/F/L	A699A/T/G	0	0	0	1 (2)	1 (0.3)
K127K/stop/E	A715A/T/G	0	0	0	1 (2)	1 (0.3)
K127K/I/R	A716A/T/G	0	0	0	5 (10)	5 (2)
V128V	A720A/T/G	0	0	0	1 (2)	1 (0.3)
N134N/Y/D	A736A/T/G	0	0	0	4 (8)	4 (1)
N137N/Y/D	A745A/T/G	0	0	0	10	10 (3)
N137N/I/S	A746A/T/G	0	0	0	2 (4)	2 (0.7)

SNP, single nucleotide polymorphism.

- 1 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).
- 2 WHO, *World Malaria Report 2018* (World Health Organization, Geneva, 2018).
- 3 F. Arley et al., A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* **505**, 50–55 (2014).
- 4 M. A. Olshina et al., *Plasmodium falciparum* coronin organizes arrays of parallel actin filaments potentially guiding directional motility in invasive malaria parasites. *Malar. J.* **14**, 280 (2015).
- 5 K. S. Bane et al., The actin filament-binding protein coronin regulates motility in *Plasmodium* sporozoites. *PLoS Pathog.* **12**, e1005710 (2016).
- 6 D. Ménard et al.; KARMA Consortium, A worldwide map of *Plasmodium falciparum* K13-propeller polymorphisms. *N. Engl. J. Med.* **374**, 2453–2464 (2016).
- 7 P. G. Kremsner et al., Intramuscular artesunate for severe malaria in African children: A multicenter randomized controlled trial. *PLoS Med.* **13**, e1001938 (2016).
- 8 C. N. Nguetse et al., Molecular markers of anti-malarial drug resistance in Central, West and East African children with severe malaria. *Malar. J.* **16**, 217 (2017).
- 9 F. Koukouikila-Koussounda et al., Molecular surveillance of *Plasmodium falciparum* drug resistance in the Republic of Congo: Four and nine years after the introduction of artemisinin-based combination therapy. *Malar. J.* **16**, 155 (2017).
- 10 D. Nderu et al., *Plasmodium falciparum* histidine-rich protein (PfHRP2 and 3) diversity in Western and Coastal Kenya. *Sci. Rep.* **9**, 1709 (2019).