Prevalence of potential mediators of artemisinin resistance in African isolates of *Plasmodium falciparum*

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Abstract

The devastating public health impact of malaria has prompted the need for effective interventions. Malaria control gained traction after the introduction of artemisinin-based combination therapies (ACTs). However, the emergence of artemisinin (ART) resistance in South-East Asia and reports of delayed parasite sensitivity to ACTs in African parasites signal an imminent treatment failure. Monitoring the prevalence of mutations associated with artemisinin resistance in African populations is required to stop resistance in its tracks. *Plasmodium falciparum* Kelch-13 (*Pfk13*), *Pfcoronin* and *PfATPase6* gene mutations have been associated with ART resistance. In this review, we collated findings from published research articles to establish the prevalence of *Pfk13*, *Pfcoronin* and *PfATPase6* polymorphisms in Africa. We searched PubMed, Embase and Google Scholar for relevant articles reporting polymorphisms in these genes across Africa between 2014 to 2021. Seventy-two studies which passed the inclusion criteria reported 739 single nucleotide polymorphisms (331 unique variants) from 34,353 samples collected in 26 African countries. Four validated *Pfk13* resistant markers linked with delayed parasite clearance were identified in Africa: R561H in Rwanda and Tanzania, M476I in Tanzania, and F446I in Mali, and P553L in Angola. In Tanzania, three (L263E, E431K, and S769N) of the four mutations (L263E, E431K, A623E, and S769N) in *PfATPase6* gene previously associated with delayed parasite clearance in presence of artemisinin were reported. *Pfcoronin* polymorphisms were reported in Senegal, Gabon, Ghana, Kenya and Congo, with P76S being the most prevalent *Pfcoronin* mutation. Our findings demonstrate independent emergence and widespread distribution of *Pfk13*, *Pfcoronin* and *PfATPase6* mutations in Africa. Understanding the phenotypic consequences of these mutations can provide information on the efficacy status of artemisinin-based treatment of malaria in Africa.

Introduction

Malaria is a leading cause of mortality and morbidity especially among children under five years in Africa (WHO, 2020). Interventions such as insecticide-treated nets, space spraying, indoor residual spraying, larval control and antimalarial therapeutics have been adopted to reduce malaria burden across the continent (Bhatt et al., 2015). However, these strategies have been hampered by the emergence of resistant strains of both mosquitoes and parasites (Akinola et al., 2019; Baraka et al., 2018; Okell et al., 2008).

A major setback in malaria control is the emergence and spread of artemisinin-resistant strains of *Plasmodium falciparum* in South-East Asia (SEA) where decreased parasite susceptibility to artemisinin is associated with polymorphisms in the parasite Kelch-13 propeller gene (Amaratunga et al., 2012; Arieu et al., 2014; Dondorp et al., 2009; Noedl et al., 2008; Takala-Harrison et al., 2015). The Kelch-13 (*Pfk13*) gene mutations that have been associated with delayed artemisinin parasite clearance in SEA include C580Y, R561H, F446I, N458Y, I543T, M476I, R539T, Y493H, and P553L (Arieu et al., 2014). In addition, polymorphisms in *P. falciparum* coronin (*Pfcoronin*) gene have been linked artemisinin resistance (Sharma et al., 2020). The *Pfcoronin* protein belongs to the actin-binding protein family which has been associated with the motility of sporozoites (Olshina et al., 2015). *Pfcoronin* gene polymorphism (P76S)

has been identified in three different countries in Africa (Delandre et al., 2020; Velavan et al., 2019). This polymorphism has been suspected to have a correlation with delayed parasite clearance (Velavan et al., 2019).

Earlier reports also suspected the contribution of *PfATPase6* mutations to reduced sensitivity of African parasites (Li et al., 2016; Yobi et al., 2020). The dependency on artemisinin derivatives for falciparum malaria treatment has stressed the need for a synergistic effort to monitor the emergence and spread of artemisinin resistant strains of *P. falciparum* in Africa. Here, we dissected published research documents to determine the prevalence of *Pfk13*, *Pfcoronin* and *PfATPase6* mutations across Africa.

Methodology

This article followed the guidelines for systematic reviews and meta-analyses (Moher *et al.*, 2009). Two electronic biomedical databases (PubMed and Embase) were methodically explored for peer-reviewed articles published between 2014 and 2021 which had the relevant study population, study design and expected outcomes for this review. Google Scholar was also combed for relevant peer-reviewed articles. Both interventional and observational studies were retrieved and included in the review using the “MeSH” search terms “OR” and “AND”: “kelch13” OR “kelch-13” OR “*Pfk13*” OR “*Pfkelch13*” OR “*Pfkelch-13*” OR “*Plasmodium falciparum* drug resistance” OR “ATP6” OR “*Plasmodium falciparum* ATP6” OR “*Plasmodium falciparum* ATPase6” OR “*PfATP6*” “*PfATPase6*” OR “*Plasmodium falciparum* coronin” OR “*Pfcoronin*” OR “*Plasmodium falciparum* coronin” OR “molecular marker” OR “*Plasmodium falciparum*” “*P. falciparum*” OR “falciparum malaria” AND (“African” OR “Africa” OR with each name of the 54 countries in Africa). The citations of the individual search were saved and sent to the reference manager (EndNote version 9.0). The full text of retrieved citations was downloaded using EndNote. Articles with data from unknown countries and/or sampling sites as well as systematic reviews, conference presentations, letters or correspondence to editors and abstracts with insufficient information were removed.

Inclusion criteria

The articles included in this review strictly reported *P. falciparum* artemisinin resistance markers, single nucleotide polymorphisms (SNPs) in African countries, polymorphisms in *Pfk13*-gene, *Pfcoronin* and *PfATPase6* confirmed through targeted or whole-genome sequencing. Articles from cross-sectional studies such as clinical or community surveys were included in addition to longitudinal studies of treatment efficacy. Specific studies reporting synonymous and nonsynonymous SNPs in *Pfk13*, *PfATPase6* and *Pfcoronin* were eligible for this meta-analysis.

Exclusion criteria

Articles reporting molecular markers other than *Pfk13*, *PfATPase6*, and *Pfcoronin* were excluded from this review. In addition, studies with no definite *Pfk13*, *PfATPase6*, and *Pfcoronin* SNPs reported either in the

main manuscript or supplementary files were excluded. Studies reporting *Pfk13*, *PfATPase6*, and *Pfcoronin* polymorphisms without sequencing techniques were not included.

Definitions

P. falciparum artemisinin resistance is defined as delayed, slow or decreased parasite clearance rate (WHO, 2019). This delayed parasite clearance results in partial resistance that affects ring-stage parasites (WHO, 2019). *P. falciparum* artemisinin resistance mutations are described polymorphisms in the major genes (*Pfk13*, *Pfcoronin*, and *PfATPase6*) associated with ACT resistance *in vitro*, *ex vivo* or *in vivo*. This review utilized the World Health Organisation (WHO) updated list of *P. falciparum* artemisinin resistance SNPs (WHO, 2019) classifying *Pfk13* mutations into validated and candidate SNPs. *Pfk13* validated SNPs are significantly associated with reduced drug susceptibility in laboratory assays and a slow parasite clearance rate in field studies. Validated *Pfk13* SNPs include C580Y, R561H, F446I, N458Y, I543T, M476I, R539T, P553L and Y493H (WHO, 2019). On the other hand, candidate SNPs are mutations strongly associated with slow parasite clearance in clinical trials but not confirmed *in vitro*. These include A675V, P441L, P574L, G449A, C469F, N537I, P527H, G538V, V1568G, F673I, and A481V (WHO, 2019).

Data extraction

The extracted data from each article captured first and last author affiliations, the year the studies were conducted (Figure 1), year of article publication (Figure 2), geographic location of the study area, duration of the study, age of the participants and the type of study design (that is, interventional vs observational). Data involving sampling strategies, molecular assays performed, clinical status of the study population, markers (synonymous and non-synonymous mutations) and publication affiliation were also reported (Figures 3 and 4).

Results

This review searched PubMed, Embase, and Google Scholar databases for all the relevant articles. Our search yielded a total of 421 articles of which 284 articles on *P. falciparum k13*, *Pfcoronin* and *PfATPase6* genes single nucleotide polymorphisms (SNPs) met inclusion conditions. Following an adjustment for duplication, redundant articles were discarded. Eleven articles with unobtainable full texts were removed. After prior screening and filtering, a total number of 72 articles (66 on *Pfk13*, two on *Pfcoronin* and six on *PfATPase 6*) from 34,353 samples collected in 26 African countries were included in this review (supplementary data sheet).

Structure of Kelch-13 protein

P. falciparum Kelch-13 encodes 726 amino acids. The polypeptide consists of a poorly conserved N-terminal region (*Apicomplexa*-specific; amino acid from 1 to 211) and three highly conserved regions (Ariey *et al.*, 2014). It has a coiled-coil-containing (CCC; amino acid from 212–341), broad-complex, tram track, and bric-a-brac (BTB; amino acid from 350–437) and a C-terminal kelch-repeat

propeller (KREP; amino acid from 443–726) which harbours virtually all *Pfk13* allelic variants associated with artemisinin resistance (Anderson *et al.*, 2017) (Figure 5). Kelch-13 gene is putatively associated with intraerythrocytic growth and proliferation of both *P. falciparum* asexual parasites (Bushell *et al.*, 2017; Zhang *et al.*, 2017).

Sample pre-processing and *Pfk13* genotyping

Majority of the studies collected blood samples for genotyping on filter paper (Abubakar *et al.*, 2020; Ahouidi *et al.*, 2021; Balikagala *et al.*, 2017; Conrad *et al.*, 2019; Djaman *et al.*, 2017) while others did not report the method used for collection (Uwimana *et al.*, 2020; Velavan *et al.*, 2019). *P. falciparum* polymerase chain reaction positive (PCR+) samples were 18,292 out of 32,406 total samples collected (Mayengue *et al.*, 2018; Nzoumbou-Boko *et al.*, 2020; Oboh *et al.*, 2018; Ocan *et al.*, 2016; Pacheco *et al.*, 2020; Tornyigah *et al.*, 2020; Uwimana *et al.*, 2020). Five studies did not report the number of PCR+ samples (Castaneda-Mogollon *et al.*, 2019; Chebore *et al.*, 2018; de Laurent *et al.*, 2018; Hemming-Schroeder *et al.*, 2018; Ikeda *et al.*, 2018). *Pfk13* gene was successfully genotyped in 15,861 (86.71%) samples. The variant calling algorithms and data analysis software used include Mega software, Jalview, phylo, DnaSp, Genescan, Genome Assembly Program, PROVEAN and R software (Gaye *et al.*, 2020; Guerra *et al.*, 2017; Gupta *et al.*, 2020; Hussien *et al.*, 2020)

Polymorphisms in *Pfk13* gene

Three hundred and thirty-one (331) non-synonymous mutations were reported, of which 291 (87.92%) occurred inside the propeller domain of *Pfk13* gene (amino acid from 441). Only 12.08% (Ahouidi *et al.*, 2021; Balikagala *et al.*, 2017; Castaneda-Mogollon *et al.*, 2019; Pacheco *et al.*, 2020) mutations were observed outside the propeller domain (amino acid below 440). The reported *Pfk13* non-synonymous SNPs occurring inside the propeller domain include A578S/D/V (88 isolates); R561H (20 isolates); R622G/K/I (20); N587K/I (16 isolates); V555A/L (9 isolates); S522C/M/N (9 isolates); T677A/K/R (9 isolates); Q613E/H (7 isolates), F509G (7 isolates) and V637I (6 isolates); N554H/K/D and A626S/T/V (5 isolates each); and N609D/L/S (5) (Ahouidi *et al.*, 2021; Ishengoma *et al.*, 2019; Kakolwa *et al.*, 2018; Kayiba *et al.*, 2021; Yobi *et al.*, 2021). The most frequently reported mutations outside the propeller domain include K189T/N (105 isolates), E208K (10 isolates), N142NN (9 isolates), T149S (6 isolates), E433D (6 isolates), and E401Q (5 isolates) (Guerra *et al.*, 2017; Lu *et al.*, 2017; Madamet *et al.*, 2017; Ocan *et al.*, 2016). Apart from D389H/N/Y (3 isolates), K378R (2 isolates) and D281V (2 isolates), other reported mutations outside the propeller domain (31/40) occurred singly (Bayih *et al.*, 2016; Boussaroque *et al.*, 2016b; Hussien *et al.*, 2020; Igbasi *et al.*, 2019). We also found that K189T/N mutation had a high prevalence in Senegal (Boussaroque *et al.*, 2016a; Gaye *et al.*, 2020; Torrentino-Madamet *et al.*, 2015).

Prevalence of *Pfk13* non-synonymous mutations across Africa

The articles reviewed in this study reported 331 unique non-synonymous mutations in *Pfk13* gene across Africa (Figure 6). An insertion was recorded in nine samples (N142NN). Of the nine validated *Pfk13* mutations (C580Y, R561H, F446I, N458Y, I543T, M476I, R539T, P553L, and Y493H) causing a delay

in artemisinin parasite clearance (Menard *et al.*, 2016), three (R561H, P553L, and M476I) were identified in Africa (Kakolwa *et al.*, 2018; Talundzic *et al.*, 2017; Uwimana *et al.*, 2020). R561H was identified in Rwanda and Tanzania (Uwimana *et al.*, 2020), P553L in Angola (Xu *et al.*, 2018), and M476I in Tanzania (Kakolwa *et al.*, 2018). In two isolates from Ghana (Tornyigah *et al.*, 2020), asparagine in position 458 (N458) was found to be replaced by aspartate (D) instead of tyrosine (Y). Though their link with delayed parasite clearance is yet to be established, other non-synonymous mutations reported are A481C in Ghana, A675V in Kenya (3 isolates), Rwanda (2 isolates) and Uganda (2 isolates); C469W/Y/F in Ghana, Kenya, Rwanda and Uganda; G538S in Democratic Republic of the Congo; G449S/C in Mali and Tanzania; N672I in Ghana (2 isolates); P574L in Uganda and Rwanda (1 and 3 isolates respectively); R539I/K in Kenya and Senegal as well as V568G in Kenya (3 isolates). The most frequent *Pfk13* mutations reported are K189T/N (105, 14.21%, A578S/D (88, 11.91%), R561H (20, 2.71%) which is a validated *Pfk13* linked with artemisinin resistance, and N587K/I (16, 2.17%). Furthermore, non-synonymous SNPs outside *Pfk13* propeller region (< 440) have been described. K189T variant is most frequently reported non-propeller *Pfk13* mutation (Gaye *et al.*, 2020; Torrentino-Madamet *et al.*, 2014). It was reported in 105 isolates from three countries (Equatorial Guinea, Eritrea, and Senegal). E208K was also reported in 10 isolates from Eritrea occurred at codon position. Non-propeller mutation at codon position 189 (K to T) has been associated with delayed ACTs *P. falciparum* parasite clearance (Gaye *et al.*, 2020; Torrentino-Madamet *et al.*, 2014).

Structure of *Pfcoronin* and *PfATPase6* proteins

Pfcoronin encodes 602 amino acids. The propeller domain of *Plasmodium falciparum* coronin gene has seven blades. This domain is made up of the WD40 repeats (tryptophan-aspartic acid 40) and a β -propeller in the N terminus region (Ashley *et al.*, 2014). The *Pfcoronin* gene is structurally similar to the six-bladed *PfKelch13* propeller domain. (Ashley *et al.*, 2014). *PfCoronin* is involved in organization of F-actin via its N-terminal propeller region and localizes to the parasite membrane (Olshina *et al.*, 2015). On the other hand, *Pfatpase6* contains 925 amino acids sequence. The function of *PfATPase 6* is mainly related to calcium metabolism (Jambou *et al.*, 2005). The amino acid sequence from 1-47 is the cation ATPase N-terminus (cation transporter), region 69-318 is the E1-E2 ATPase (a superfamily of ion-pumping ATPases), region 647-700 is the cation ATPase (P-type) while region 842-920 is the soluble P-type ATPase (conserved domain).

Prevalence of *Pfcoronin* and *PfATPase6* non-synonymous mutations in Africa

The two studies reporting *Pfcoronin* mutation collected a total number of 624 samples, the whole samples were PCR+. *Pfcoronin* gene was sequenced in 624 (100%) isolates (Delandre *et al.*, 2020; Velavan *et al.*, 2019). On the other hand, six studies reported *PfATPase6* polymorphisms. The six studies involved 1323 samples of which 752 *P. falciparum* isolates were PCR+, 644(85.63%) were successfully sequenced (Heuchert *et al.*, 2015; Koukouikila-Koussounda *et al.*, 2017; Kwansa-Bentum *et al.*, 2011; J. Li *et al.*, 2016). In Tanzania (Chilongola *et al.*, 2015), three (L263E, E431K, and S769N) of the four mutations (L263E, E431K, A623E, and S769N) in *PfATPase6* gene associated with delayed parasite

clearance in presence of artemisinin were reported (Table 1). *Pf*ATPase 6 E431K was reported in Congo and Ethiopia (Figure 8).

*Pf*coronin mutations were reported in five countries (Senegal, Gabon, Ghana, Kenya, and Democratic Republic of the Congo) P76S polymorphism was identified in four countries (Senegal, Gabon, Ghana, and Kenya) (Delandre *et al.*, 2020; Velavan *et al.*, 2019). The frequency of occurrence of P76S was higher in Senegal compared with the three other countries. Likewise, V62M was reported in Ghana and Gabon. (Table 2).

Table 1: The prevalence of *Pf*ATPase6 single nucleotide polymorphisms in Africa

Country	Mutation (no of isolates)	Reference
Congo	H243Y(2), I291V (1), L402V (3), E431K (3), N569K (7), A630S(3), G632E (1), G639D, F646F (1), H747Y (1)	(Koukouikila-Koussounda <i>et al.</i> , 2017)
Equatorial Guinea	N569K (11), A630S (2), I723V (1)	(J. Li <i>et al.</i> , 2016)
Ghana	D639G (34), E431K (7), D443E (5), M813Q (5), Q622H (3), L402V (2), F414L (2), D419N (2), C356Y (1), R377K, E384K (1), T403K(1), D405 (1), A425T(1), E432K (1), A630S (1), C645G (1), E696G (1), E710K (10, D734G (1)	(Kwansa-Bentum <i>et al.</i> , 2011)
Ethiopia	E431K (1)	(Heuchert <i>et al.</i> , 2015)
Tanzania	L263E (8), E431K (17), S769N (5)	(Chilongola <i>et al.</i> , 2015)
Ivory Coast	Y148F (1), D153E (1), S158N (1), Q254H (4), H638P (1), N663Y (1), N668Y (1), N669Y (6), S670C (5), W681G (10, D734Y (12)	(Koukouikila-Koussounda <i>et al.</i> , 2017)

Table 2: The prevalence of *Pf*coronin single nucleotide polymorphisms in Africa

Country	Mutation (no of isolates)	Reference
Senegal	P76S (53)	(Delandre <i>et al.</i> , 2020)
Gabon	V62M (1), P76S (11)	(Velavan <i>et al.</i> , 2019)
Ghana	I53I (1), V62M (1), K69I/R (3), P76S (9), N110Y (1)	(Velavan <i>et al.</i> , 2019)
Kenya	P76S (4)	(Velavan <i>et al.</i> , 2019)
Congo	K69I/R (11), P76S (8), N110Y/D (5), N112Y (10, K115E (1), L121F (1), K127E (1), K127I/R (5), N134Y/D (4), N137I/Y/D (12),	(Velavan <i>et al.</i> , 2019)

Discussion

The emergence of *P. falciparum* artemisinin-resistance in SEA is an imminent danger to the successful treatment and a potential public health burden. The large spectrum of mutations associated with artemisinin resistance reported so far in Africa raises concern about potential adaptation of *P. falciparum* to artemisinin. Consequently, African health workers and policymakers are reminded of the need for accurate and effective use of artemisinin derivatives for malaria treatment.

Compared to SEA, low prevalence of *Pfk13* SNPs were recorded across Africa. This could be associated with the late introduction of artemisinin in Africa (between 2000–2005), accompanied by a shorter period of artemisinin drug pressure unlike in SEA which experienced an early adoption of artemisinin in the 1970s (Li et al., 1994). Reports from SEA identified 31 major *Pfk13* SNPs nine of which were validated by the World Health Organization (WHO) as *Pf*-artemisinin resistance markers (Ishengoma et al., 2019; Talundzic et al., 2017). Seven of these polymorphisms (V568G, F446I, P553L, M476I, A675, R539I/K, and P574L) have shown a strong association in delayed *in vivo* parasite clearance (Ariey et al., 2014; Ashley et al., 2014; Bonnington et al., 2017; Cheeseman et al., 2015). Meanwhile, P533L, M476I and F446I have shown a decrease in *in-vitro* response to artemisinin (Ariey et al., 2014; Straimer et al., 2015; Wang et al., 2018). Some validated *Pfk13* markers have been identified in African isolates. For instance, P553L was identified in Ghana and Senegal (Talundzic et al., 2017). R561H was identified in Rwanda and Tanzania (Uwimana et al., 2020) and M476I was detected in Tanzania (Kakolwa et al., 2018). R539I was reported in Senegal, and in Kenya, lysine replaced isoleucine in R539 mutation (R539K) (de Laurent et al., 2018; Talundzic et al., 2017).

Furthermore, seven of the eleven unvalidated *Pfk13* mutations have been found in Africa. Though not listed as validated SNPs, some of these markers have been associated with reduced susceptibility to artemisinin *in vitro*. V568G and A481C were identified in Kenya and Ghana, respectively (de Laurent et al., 2018; Tornyigah et al., 2020) while A675V was reported in Kenya, Rwanda, and Uganda (Ikeda et al., 2018; Tacoli et al., 2016). The other associated markers identified were C469Y/W (Escobar et al., 2015; Talundzic et al., 2017; Uwimana et al., 2020), G538S (Doudou 2021), G449S/C (Ouattara et al., 2014), N672I (Tornyigah et al., 2020) and P574L (Rwanda and Uganda), (Tacoli et al., 2016; Uwimana et al., 2020). The other *Pfk13* mutations reported in African parasites such as C469Y, S522C, R575I/K, and P667R (Uwimana et al., 2020) were found at low frequencies and have not been substantially linked with artemisinin resistance in Africa (WHO, 2019) contrary to what was observed in SEA (WWARN, 2019). In the same vein, C469K mutation has been linked to low parasite clearance in SEA (WWARN, 2019) but not in Africa (Ikeda et al., 2018).

Therefore, it is possible that African parasites may have an artemisinin selection background that is different from SEA parasites. The suspected association of *Pf*ATPase6 and *Pf*coronin with artemisinin resistance suggests the potential emergence of other non-*Pfk13* mutations. *Pf*ATPase6 variants have been incriminated in delayed parasite clearance in Tanzania (Chilongola et al., 2015). *Pf*ATPase6 E431K was reported in Congo, Ethiopia and Ghana (Heuchert et al., 2015; Koukouikila-Koussounda et al., 2017; Kwansa-Bentum et al., 2011). The variant was also reported, in *in vitro* studies, to be associated with delayed artesunate-treated parasite clearance in Senegal (Jambou et al., 2005). However, a later study in

Iran suggested that the role of E431K variant in artemisinin resistance was suspect (Zakeri, 2012). *PfATPase6* E431K mutation often co-occurs with other *PfATPase6* gene polymorphisms, usually the S769N and L623E mutations (Tahar *et al.*, 2009).

Research on *Pfcoronin* as a potential marker of artemisinin resistance in African parasites is relatively recent and still evolving. *Pfcoronin* mutations reported so far include I53I, V62M, K69K/I/R, P76S, N110Y/D, N112Y/D, K115E, L121F, K127E, K127I/R, N134Y/D, N137Y/D, N137I/S (Delandre *et al.*, 2020; Velavan *et al.*, 2019). In five countries where *Pfcoronin* was genotyped, P76S variant was observed in all the populations at varying frequencies: 15% in Senegal, 11% in Gabon, 16% in Ghana, 4% in Kenya and 17% in DR Congo. None of the variants suspected to be associated with delayed parasite clearance in the presence of artemisinin pressure (E107V, G50E, and R100K) in laboratory isolates was reported in natural African populations. Even though *Pfcoronin* polymorphisms (Delandre *et al.*, 2020; Velavan *et al.*, 2019) have not yet been validated in clinical isolates as markers of delayed parasite clearance, their structural similarity with *Pfkelch13* suggests the possibility of a common mechanism of resistance emergence (Delandre *et al.*, 2020; Henrici and Sutherland, 2018). Detailed analysis of the phenotypic effects of these mutations can provide information on the continued efficacy or otherwise of artemisinin-based treatment of malaria in Africa.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are included in this published article and its supplementary information file. All codes generated and/or analysed during the current study are available in Github (<https://github.com/CeGRIB/pfk13.coronin.atpase6>).

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Authors' contributions

A.O. and K.M.O. conducted the literature review and drafted the manuscript. A.O., M.J.O., E.T.I. and K.M.O. reviewed the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Year of publication of research articles reporting Pfk13, Pfcoronin and PfATPase6 (KCA) gene mutations. The coloured dots represent the number of publications reporting polymorphisms in the KCA genes.

Figure 2

Year of molecular surveillance for Pfk13, Pfcoronin and PfATPase6 (KCA) gene mutations. The coloured dots represent the number of sites included in the KCA studies.

Figure 3

Demography of research articles reporting Pfk13, Pfcoronin and PfATPase6 gene mutations. (a) First author affiliation, (b) Last author affiliation (c) Where research funding came from (d) Sampling technique (e) Clinical status (f) Molecular assay (PCR = polymerase chain reaction; RT-qPCR = real-time quantitative PCR; PET = photo-induced electron transfer)

Figure 4

Identified journals reporting Pfk13, Pfcoronin and PfATPase6 gene mutations in Africa between 2014 to 2021.

Figure 5

PfK13 propeller annotation. The Apicomplexa-specific N-terminal coiled-coil-containing (CCC), bric-a-brac (BTB), and Kelch-repeat propeller (KREP). FASTA sequence of the protein was downloaded from uniprot (<https://www.uniprot.org/uniprot/Q8IDQ2.fasta>) and modelled in <https://swissmodel.expasy.org/>. The legend (right) describes the positions of Kelch-13 variants.

Figure 6

6a: Distribution of unique non-synonymous *P. falciparum* Kelch-13 single nucleotide polymorphisms (SNPs) in African countries. 6b: Distribution of total *P. falciparum* Kelch-13 single nucleotide polymorphisms in African countries. The pie charts describe the frequencies of validated and associated Kelch-13 polymorphisms per country.

Figure 7

(a) 3D structure of Pfcoronin protein, (b) PfATPase6 protein. (FASTA sequence of the Pfcoronin and PfATPase6 protein was downloaded from ncbi (<https://www.ncbi.nlm.nih.gov/protein/KOB62717.1?report=fasta>, Pfcoronin; <https://www.ncbi.nlm.nih.gov/protein/ADC29779.1?report=fasta>, PfATPase 6), modelled in <https://swissmodel.expasy.org/>). The coloured dots indicate the positions of important variants within the proteins.

Figure 8

The prevalence of PfATPase6 mutations in Africa

Figure 9

Supplementary Files

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