Article Review

Plasmodium falciparum Kelch 13-propeller gene mutation update in Nigeria – a systematic review

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Abstract

Background: Mutations in Plasmodium falciparum Kelch 13 propeller gene has been associated with artemisinin resistance. This review is a synthesis of evidence from research on Plasmodium falciparum Kelch 13 (Pfk13) propeller gene mutations conducted in Nigeria from 2011 to 2023 to determine the extent of spread of Pfk13 mutations in different states of Nigeria.

Methods: An electronic search of studies from 2011 to date was done in Medline via PubMed, database and Google Scholar using Mesh terms and Boolean operators. PRISMA guidelines were used to guide the screening of articles for inclusion. Data extraction form was developed in Excel 2016 and used for data extraction.

Results: A total of 84 articles were retrieved but only 12 were eligible for inclusion. Pfk13 gene mutations studies have been conducted in 11 out of 36 (30.6%) states of Nigeria. The total number of independent Pfk13 mutations identified across the different states was 44, 32 (72.7%) mutations were novels. None of the reported mutations in this review was among the validated k13 mutations associated with increased artemisinin resistance.

Conclusion: Number of Pfk13 gene mutations studies in Nigeria was scanty. States in the North-east, North-west, and South-south geopolitical regions of Nigeria have the poorest coverage. Pfk13 gene mutations including novel ones were identified, however, they were all non-validated k13 mutations. There is need to carry out further studies (in vitro and in vivo) to ascertain the role of these novel mutations in emergence and spread of ART resistance in Nigeria.

Keywords: K13-propeller gene polymorphism, Artemisinin resistance, Malaria, Plasmodium falciparum, Nigeria.

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Introduction

The use of efficacious antimalarial drugs for the treatment of malaria is critical to malaria control and elimination efforts. The World Health Organisation (WHO) recommends Artemisinin-based combination therapies (ACT) as the first- and second-line treatment regimen for uncomplicated *P. falciparum* and also for the treatment of chloroquine-resistant *P. vivax* malaria.[1] There has been validated reports of emergence and spread of parasites partially resistant to artemisinin in the Greater Mekong subregion (GMS) and Africa especially Rwanda, Eritrea, and Uganda.[1] There is the fear that

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these artemisinin-resistant parasites will behave the same way they did with chloroquine and later pyrimethamine in spreading westwards to reach Africa.[2] The identification of the artemisinin resistance biomarker, *Plasmodium falciparum* kelch 13 (Pfk13), six years after ACT was observed to record clinical failure in GMS changed the course of surveillance in South east Asia (SEA).[3] The use of Pfk13 as a molecular marker has provided the means of tracing the foci and origins of the emergence of, and spread of ART resistance in the GMS.[4] Studies (in vitro and in vivo) have shown that Pfk13 gene encodes a protein containing BTB/POZ domain and a 6-blade propeller domain at the C-terminal end.[5,6] The list of validated and molecular markers is continually being updated and all the markers are located within the Pfk13 BTB/POZ; and have been found to have varying effects on parasite clearance rate.[1] The current list include: F446I, N458Y, C469Y, M476I, Y493H, R539T, I543T, P553L, R561H, P574L, C580Y, R622I, A675V.[1,7] A number of possible molecular mechanisms by which the Pfk13 mutations confer artemisinin resistance has been a subject of intense research involving the use of in vitro and in vivo models. These research models agreeably propose the involvement of multiple cellular and metabolic processes including haemoglobin degradation, vesicular biogenesis, unfolded/proteotoxic protein stress response and oxidative stress response.[8-10]

Molecular marker prevalence from infected patients, treatment efficacy studies, in-vitro or ex-vivo drug studies are key methods of tracking the emergence and spread of artemisinin drug resistance.[11] The use of molecular markers for real-time surveillance for artemisinin resistance is progressively becoming a common practice, although only treatment efficacy studies can provide direct information on clinical drug failure.[11-12] The recent emergence of artemisinin resistance in Africa calls for a concerted efforts towards ensuring that efficacious treatments remain available.[13-15] Improving the phenotypic and genotypic surveillance to better map the extent of resistance spread per time and space should be one of the immediate priorities of malaria control and elimination efforts of any endemic country. The World Health Organisation (WHO) recommends a change of first-line therapy when the clinical artemisinin resistance (clinical failure with treatment with ACT first line) exceeds 10%.[16] It therefore behoves the national malaria community to continually monitor the prevalence of the Pfk13 molecular marker which serves as a good indicator for detecting clinical resistance. We therefore aim in this review to give a summary update of the coverage and the findings of the previous work on Pfk13 gene mutations carried out in different states of Nigeria between 2011 to date. This systematic review provides an overview of coverage of molecular research that investigated human samples for the Pfk13 biomarker and its distribution in different geopolitical regions of Nigeria.

Methods

Selection Criteria

A search of MEDLINE database via PubMed was performed to identify studies that investigated Pfk13 gene polymorphisms among *P. falciparum* parasites in Nigeria. The search covered a period from 2011 to date. Mesh and search terms were combined with Boolean operators ("AND", "OR") in the article search. The terms used include: "artemisinin resistance", "kelch-13", "pfk13", "pfk13-propeller gene", "k13 marker", "plasmodium falciparum k13 gene", "Nigeria". Google Scholar was also searched using the above search terms. The searches, using the stated search terms were restricted to titles and abstracts. Specific terms such as those that focus on study design were not included to maximize outcomes of the search. All searches were done in English.

Data management

Mendeley desktop (version 1.19.8, 2008-2020) and Rayyan software (web/mobile app for systemic reviews) were used in the data management of the review articles. All identified articles were imported into Rayyan software where duplicates were removed. Following a priori criteria, screening of the articles was carried out and grouped into two categories – included and excluded. Mendeley desktop was used majorly as a reference manager.

Eligibility criteria for article selection

Study articles that investigated prevalence of Pfk13 mutations in any state of Nigeria were included. Studies that assessed for molecular markers of artemisinin-resistance in Pfk13 gene, or reported both molecular and phenotypic artemisinin-resistance were included. Also, studies that analysed pfk13 polymorphisms using sequencing techniques were included. Studies of either clinical trial or cross-sectional designs were included.

Exclusion criteria for ineligible articles

Unrelated studies that focused on other plasmodium resistant genes, malaria epidemiology, mathematical modelling of resistance were excluded. Excluded also were studies outside Nigeria, case reports, reviews, pre-

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prints and studies for which we could not retrieve their full articles were excluded.

Ethics statement

Since this study only reviewed published, publicly available articles and did not require the collection of original data or interaction with human participants in any form, therefore ethical clearance was not sought. Also, since human participants were not involved in the study, consent was neither required nor feasible.

Data extraction

Data extraction form was developed using Microsoft Excel 2016 (Microsoft Corp, Washington, USA) and the following information were extracted: authors and their affiliations, states in Nigeria where study was conducted, the year of study, duration of study, study design, study population, sample size, sequencer type deployed, and analysis software. Also extracted from the included articles were Pfk13 mutation, type of mutation, reference alleles, mutant alleles, and pfk13 mutation frequencies. The k13 mutations were grouped into novel and already reported mutations.

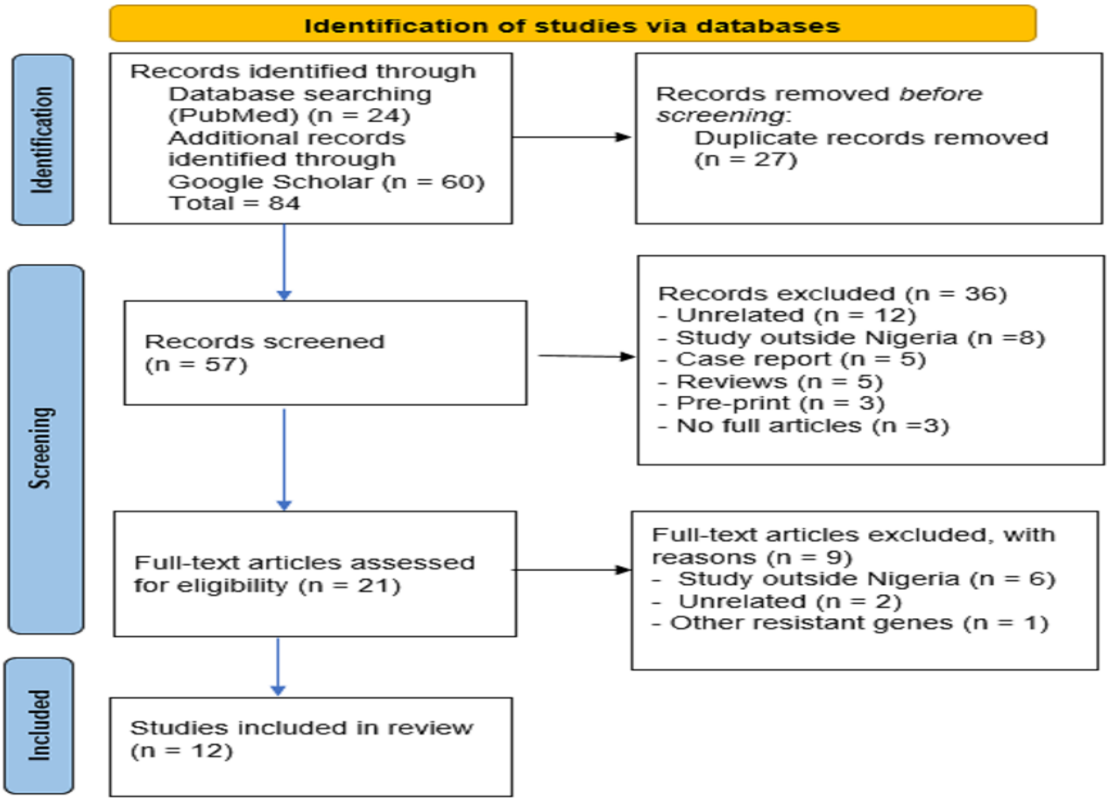

Fig. 1 PRISMA flow diagram showing article selection for the review

Results

Study identification and selection

A total of 84 articles were found. A total of 27 articles were excluded because of duplication, while 45 others either had unrelated titles and abstracts, were not conducted in Nigeria, were case reports, reviews, or un-published articles, and as such were not included in the review (Fig. 1). Also, articles whose full texts could not be retrieved were excluded. Only a total of 12 articles were deemed eligible to be included in the review after meeting the eligibility criteria.

Table 1: Characteristics of studies included in the systematic review

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Author (Year)	State	Study period	Design	Study population	Sample size sequenced	Sequencer	Sequence Data analysis
Kamau et al.(17) (2015)	Lagos	2013-2014	CS	≥6 months	89	Sanger	GAP 4
Oboh et al.(18) (2018)	Lagos		CS	All ages	25 (Lagos)	Sanger	Bio Edit v7
	Edo				25 (Edo)	Sanger	Bio Edit v7
Igbasi et al. (19) (2019)	Lagos	2010-2011 & 2013-2014	CS	All ages	195	ABI 3130xl	BLAST search tool
Idowu et al. (20) (2019)	Lagos	2015-2016	CS	2 – 73 years	26	Sanger & NGS	Geneious R10
Olasehinde et al. (21) (2019)	Ogun	2016-2018	CS	All ages	27	---	---
Tola et al.(22) (2020)	Lagos	Aug., 2014	CS	All ages	83	Sanger with BigDye terminator	CLC Main Workbench v6.7.1 & MEGA 7.0.4
	Osun						
Abubakar et al. (23) (2020)	Kano	Aug., 2018	CS	18 -56 years	50	pJET1.2-F & pJET1.2-R	BioEdt v7
Ikegbunam et al. (24) (2021)	Anambra	2015	CS	1-72 years	180	BigDye terminator v2.0	Geneious v9.1.5
	Osun	2004, 2015		1-12 years			
Muhammad et al. (25) (2022)	Gombe	NR	CS	All ages	57	NR	BLAST search tool
Ajogbasile et al. (26) (2022)	Enugu	2018	TES	6-96 months	332	Sanger with BigDye terminator	Geneious v2020.0.4
	Kano						
	Plateau						
Afolabi et al. (27) (2022)	Ondo	NR	CS	≤5 months	200	NR	NR
Adulugba et al. (28) (2022)	Benue	No mutation	CS	All ages	60	RFLP	

Keys: CS = cross-sectional study, NR = not reported, TES = Therapeutic Efficacy Study, RFLP = restriction fragment length polymorphism

Characteristics of the papers included in this review

The included articles comprised of primary studies that investigated pfk13 molecular marker from across Nigeria with 1349 participants, up till February, 2023. Studies have been conducted in 11 out of 36 States of Nigeria (30.6%) (Table 1). The primary studies were mostly done in the southwest region of Nigeria. South-west (Lagos, Ogun, Ondo, and Ogun, n=4/6 (66.7%)); South-east (Anambra and Enugu, n=2/5 (40%)); South-south (Edo, n=1/6 (16.7%)); North-central (Benue and Plateau, n=2/6 (33.3%)); North-east (Gombe, n=1/7 (14.3%)); and North-west (Kano, n=1/7 (14.3%)). Eleven out of twelve (91.7%) of the studies were cross-sectional studies, one was randomized control trial. The largest sample size was 332 from a multi-state study carried out in 3 states – Enugu, Kano, and Plateau, while

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the least was 25 from study conducted in Edo and Lagos (Table 1). Half of the studies (50.0%) enrolled participants of all ages (children and adults) in their study.

Prevalence and distribution of Pfk13 polymorphisms across Nigeria

Table 2 shows a summary of the distribution of K13 molecular marker reported from the 12 published studies successfully reviewed and analysed. A total of 44 independent single nucleotide polymorphisms (SNPs) were recorded from the articles reviewed. Of the 44 SNPs, 31 (70.5%) were non-synonymous, and 32 (72.7%) were novels. Only 12 (27.2%) had been reported in SEA and in other African countries.[29] The overall prevalence of non-synonymous k13 mutations in Nigeria is 2.3% (31/1349). Also, of the 44 reported non-synonymous SNPs in this review, 23 (74.2%) were found in single parasite infections. All the k13 mutations reported in Nigeria are of low frequency (<5%) except K189T with a frequency of 65.4%, occurring in 17 infections in a single study.[20] None of the k13 mutation found in Nigeria has been validated by WHO to cause delayed ART *P. falciparum* parasite clearance ($t_{1/2}$>5 h). Lagos had the largest number of samples analysed as well as highest single nucleotide polymorphisms (SNPs). A study by Abubakar et al. in Kano in 2020 was the only one-state study with the highest number of SNPs,[23] whereas one by Ajogbasile et al. in 2022 was the multi-state (Enugu, Plateau, and Kano) study with the highest number of SNPs.[26] Three (6.8%) mutations (A557S, A578S, and Q613H) were found in more than one state-A557S reported in Enugu and Ondo States; A578S reported in Enugu and Lagos, while Q613H had been reported in Enugu, Lagos, and Plateau States. This makes Q613H mutation the SNP with the widest spread in Nigeria based on the reviewed articles.

Codon positions where SNPs occur in pfkelch13 gene

Whereas majority of the mutations reported in this review are located in the propeller domain of the pfk13 gene, few are non-propeller mutations. K189T and H136N mutations reported by Idowu *et al.*[20] in Lagos in 2019 are non-propeller mutations in the pfk13 gene. Of the observed non-synonymous SNPs in this review, 96.8% (30/31) of them occurred in the propeller domain of pfk13 gene, while only one mutation K189T (3.2%) occurred outside of the propeller region. Few double mutations in the same codon were observed at codon positions A578S/K,[19,22,26] I684N/T,[23] F434I/S,[23] and Q613H/Q.[19,20,24,25] (Table 2)

Table 2: Nature of detected polymorphism (Pfk13 mutation)

Author (Year)	State	Pfk13 mutation	Type of mutation	Reference allele	Mutant allele	New Pfk13 codon mutation	Pfk13 mutation already detected else-where	PfK13 mutation prevalence %
Kamau et al.[17] (2015)	Lagos	No SNPs	---	---	---	---	---	---
Oboh et al.[18] (2018)	Edo	G665C	nsp	G	T	G665C		4.0
	Edo	V666V	sp	A	C	V666V		4.0
	Edo	P553P	sp	G	A	P553P		4.0
	Lagos	V510V	sp	G	A	V510V		4.0
Igbasi et al.[19] (2019)	Lagos	A578S	nsp	NR	NR		A578S	0.5
		D464N	sp	NR	NR		D464N	0.5
		Q613H	sp	NR	NR		Q613H	1.6
Idowu et al.[20] (2019)	Lagos	Q613H	nsp	NR	NR		Q613H	3.8
		K189T	nsp	NR	NR		K189T	65.4
		H136N	sp	NR	NR	H136N		3.8
Olasehinde et al.[21] (2019)	Ogun	No SNPs	---	---	---	---	---	---
Tola et al.[22] (2020)	Lagos & Osun	G496S	nsp	---	---	G496S		1.2
		R539F	nsp			R539F		1.2
		I543V	nsp			I543V		1.2
		A557?	nsp			A557?		3.6

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		V566K	nsp			V566K		1.2
		A578K	nsp			A578K		2.4
		D584I	nsp			D584I		1.2
		C580Y*	nsp				C580Y*	1.2
Abubakar et al.[23] (2020)	Kano	E433G	nsp	A	G	E433G		2.0
		F434I	nsp	T	A	F434I		4.0
		F434S	nsp	T	C	F434S		2.0
		F442F	sp	T	C	F442F		2.0
		F492F	sp	T	C	F492F		2.0
		I684N	nsp	T	A	I684N		2.0
		I684T	nsp	T	A	I684T		2.0
		E688K	nsp	G	A	E688K		2.0
Ikegbunam et al.[24] (2021)	Anambra	V510V	sp	NR	NR	V510V		2.8
	Anambra	R515R	sp	NR	NR	R515R		2.8
	Anambra	D547G	nsp	NR	NR	D547G		2.8
	Anambra	Q613Q	sp	NR	NR	Q613Q		2.8
	Anambra	E688E	sp	NR	NR	E688E		2.8
	Anambra	N458N	sp	NR	NR	N458N		2.8
	Osun	V520A	sp	C	T		V520A	1.4
	Osun	V581I	sp	G	A	V581I		1.4
Muhammad et al.[25] (2022)	Gombe	No SNPs	---	---	---	---	---	---
Ajogbasile et al.[26] (2022)	Kano	K438N	nsp	A	T		K438N	0.3
	Kano	G449S	nsp	G	A		G449S	0.6
	Kano	F451L	nsp	T	---	F451L		0.3
	Enugu	C469C	sp	C	T		C469C	1.2
	Enugu	V487E	nsp	T	A	V487E		0.3
	Enugu	A557S	nsp	C	T		A557S	0.3
	Enugu	A578S	nsp	G	T		A578S	0.3
	Enugu, Plat	Q613H	nsp	A	T		Q613H	1.5
	Enugu	A621A	sp	T	A	A621A		0.3
	Enugu	Q661H	nsp	A	---	Q661H		0.3
	Plat	N664I	nsp	A	T	N664I		0.3
	Enugu, Plat	V692G	nsp	T	A	V692G		0.6
	Kano	N694K	nsp	T	A		N694K	0.3
Afolabi et al.[27] (2022)	Ondo	A557S	nsp	C	T		A557S	0.5
Adulugba et al.[28] (2022)	Benue	No SNPs	--	--	--	--	--	--

Keys: *Mixed alleles (580Y & 580C). ? = not an amino acid, NR = not reported, Plat = Plateau State, TES = Therapeutic efficacy study

Discussion

In 2020, there were an estimated 241 million cases of malaria in 85 countries that were endemic for malaria, an increase from 227 million cases in 2019 and majority of the countries are from the WHO African Region.[30] Of the six countries that accounted for over half of all malaria deaths in 2020, Nigeria's malaria deaths were the greatest, contributing 27% of the total death toll. In the same year, malaria mortality rate rose from 56 per 100,000 population at risk to 62 per 100,000 [30]. This rise in malaria mortality might be sustained or even worsen in the near future by the threat posed by the possible widespread emergence of *P. falciparum* parasites' resistance to ACT agents in malaria endemic countries. Surveillance of pfk13 gene mutations associated with artemisinin resistance is now being carried out throughout the WHO African Region.[31] There is increasing reports of clonal expansion of pfk13

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mutations in Rwanda and Uganda.[13,32] Rwanda was the first country in Africa in which artemisinin resistance was confirmed. In 2014, the k13 R561H mutation was first identified in Rwanda and further studies carried out in 2018 and 2019 observed clonal expansion of R561H mutation in over 15% of samples.[33]

This review aimed to provide an updated summary of research articles that investigated K13 polymorphisms in Nigeria. The review tried to profile the prevalence and distribution of pfk13 gene mutations in Nigeria. Nigeria is divided into six geo-political regions, with the northern and the southern hemisphere each made up of three geo-political zones. Lagos, one of the six states of south-south geo-political zone of Nigeria, recorded the highest number of studies investigating K13 mutation in Nigeria. Interestingly, the first K13 mutation study that yielded no K13 mutation was carried out in Lagos.[17] About half of the total studies reviewed were carried out in Lagos giving rise to three-quarter of the total reported K13 mutations. Osun (another south-south region State) and Kano (north-west) State had two studies each according to this review. The remaining States only had one study each. The reason for more studies in Lagos than any other States could be due to the metropolitan advantage Lagos has over other States in Nigeria. Lagos State is highly populated with a lot of social amenities and tertiary/research institutions located within and around it, thus attracting people from different backgrounds to the State. Geo-political zones with the least number of studies in the review are south-south with only one study conducted in Edo State, and north-east that also had only one study that was carried out in Gombe State. More studies will need to be conducted in these States and regions with poor surveillance coverage to determine the extent of the spread of pfk13 mutations and the relationships between identified mutations and artemisinin resistance.

In this review, the overall prevalence of non-synonymous SNPs mutations of pfk13 gene of 2.3%. There was no confirmed (WHO-validated) K13 mutation found in this review, however, two non-synonymous non-validated SNPs in pfk13 gene associated with delayed ART clearance of *P. falciparum* in Africa: K189T, previously reported in Uganda and Senegal (34,35), and Q613H, first detected in Senegal[34] were observed. Twelve k13 polymorphisms reported in this review were entirely novel, and their role in artemisinin resistance is not yet well studied.

Kamau et al.[17] conducted a study in 12 sub-Saharan African countries including Nigeria from 2013-2014 in which 89 of the 1212 samples collected were from Lagos, Nigeria. They identified 22 unique k13 mutations, however, none was from Nigeria. That study provided the baseline prevalence of k13 mutations in sub-Saharan Africa. Two of the non-synonymous SNPs – A578S and A557S reported in that study were later found in Nigeria.[19,26,27] Igbasi et al.[19] and Ajogbasile et al.[26] in their studies in Lagos and Enugu respectively, reported A578S also as one of the detected pfk13 mutations. A578S was one of the eighteen pfk13 mutations described in SEA and also in Ghana, Kenya, DRC, and Gabon.[3,36] A578S has been reported as the most common K13 mutation in Africa,[29] however, in this review, it is the second to the most common K13 mutation Q613H (in terms of spread across the country), while KI89T is the most prevalent (detected in17 infections only in one study/state).

The two other K13 mutation reported by Igbasi et al.[19] were D464N and Q613H. Evidently, Q613H was the most widespread pfk13 mutation in this review. Igbasi et al.[19] from Lagos, Idowu et al.[20] also from Lagos, and Ajogbasile et al.[26] from Enugu and Plateau States all reported Q613H in their studies. Despite the low frequencies at which Q613H occurred in those studies, and its yet to be validated artemisinin resistance status, its detection confirms possibility of independent development of k13 resistance genotype.[17,36,37] Idowu et al.[20] also described K189T mutation in their study at a very high prevalence of 65.4%. K189T is a yet-to-be validated non-propeller pfk13 gene mutation that has been associated previously with artemisinin resistance.[34,36,38] This finding poses a threat to Nigeria's malaria elimination and control programme, though it requires multiple mutations to functionally confer drug resistance.[38] Two research teams led by Ajogbasile et al.[26] in Enugu, and Afolabi et al.[27] in Ondo detected A557S pfk13 mutation. A557S has also been reported in SEA and other sub-Saharan African countries.[3,38,39,40]

The findings from a study conducted by Oboh et al.[18] in two States – Lagos and Edo States of Nigeria revealed four novel K13 mutations outside of those conferring resistance to ART.[1] Furthermore, the DNA sequencing and sequence analyses they performed showed neither evolutionary selection pressure nor association of mutations in pfk13 gene with mutations previously reported three genes conferring resistance to CQ and SP. Based on these findings, they deduced that malaria public health is not under immediate ART resistance

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threat in southwestern Nigeria. Though, the four K13 mutations were synonymous, non-validated mutations, the codon position of one of them P553P detected in Edo State has borne a non-synonymous mutation at the same position, with phenylalanine changing to leucine to give rise to P553L, a recently validated pfk13 mutation associated with delayed parasite clearance.[1] This observation calls for a more committed malaria parasite surveillance in Nigeria.

Conclusion

It is now a well-known fact that pfk13 gene mutations associated with artemisinin resistance has emerged in Africa, with evidence of clonal expansion in Uganda and Rwanda. Some surveys have revealed that C469Y and A675V mutations have even been detected in more than 15% of samples in some study sites.[13,32] This finding calls for a response that will ensure that efficacious malaria treatments are still available especially as there are no new anti-malarial drugs currently in sight or in the development pipeline. One of the immediate responses should be to try to understand the likely factors that could have led to the selection of the pfk13 gene mutations and try to educate the health professionals and the general public to refrain from such practices. Next line of action should be to put mechanisms in place to track the spread of artemisinin resistance.

This review was therefore carried out to provide a Nigeria-wide perspective on the current status of the prevalence and distribution of pfk13 mutations. Mostly low prevalence mutant Pfk13 genes that are not yet validated in their role to cause artemisinin resistance were described from different studies and diversities of population across Nigeria. Of utmost concern is the finding in a mono-state study of a highly prevalent, non-propeller domain mutant pfk13 gene, though not yet validated, but has been associated with delayed *P. falciparum* clearance previously.

Currently, only a few research projects have investigated or conducted surveillance studies on pfk13 gene mutations in Nigeria. Even the few research projects that investigated pfk13 mutations concentrated more on a particular geo-political region than on the others, thus showing skewed distribution. Also, much of the projects are from independent research projects rather than coming from the national public health ministry or the countries' malaria elimination and control programme. There is need for a working collaboration between National Malaria Elimination Programme (NMEP) and institutional and individual researchers to streamline and standardize antimalarial drug resistance surveillance, and harness the findings of such surveillances for future planning and policy making. Molecular surveillance for pfk13 gene mutations would be more useful if combined with well-planned therapeutic efficacy study. A continuous large-scale genomic surveillance of pfk13 gene mutations and a continuous validation of newly detected pfk13 gene mutations is advised in Nigeria.

Declarations

Contributions of authors: AAI and GIO conceptualized this study. AAI and PAO, and DEE conducted the literature searches, collated articles, and drafted this review. UEE, PCE, LNN reviewed, edited, and contributed to the final version of the manual script.

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Conflict of interest: The authors declare no conflict of interest.

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