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Polymorphisms in *Plasmodium falciparum* Adenosine Triphosphatase 6 (*PfATPase6*) gene and their significance in finding the genetic marker for Artemisinin resistance

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ABSTRACT

Antimalarial drugs will continue to be a major control tool for controlling malaria, through treatment and prophylaxis, until an effective malaria vaccine is developed. Plasmodium falciparum, the major malaria parasite, has developed resistance to most available antimalarial drugs. The last decade has seen an increase in malaria burden due to drug resistant strains of P. falciparum. Currently combination therapy has been recommended by World Health Organization (WHO) for treating uncomplicated malaria, with formulations containing an artemisinin compound as policy standard. This is aimed at improving treatment as well as delaying resistance. Research has, however, reported in vitro resistance to artemisinins a few years after implementation of Artemisinin-based Combination Therapy (ACT) policy. The objective of this review is to provide comprehensive information on the Single nucleotide polymorphisms (SNPs) of the Plasmodium falciparum adenosine triphosphatase 6 (PfATPase6) gene and also to argue the significance of PfATPase6 SNPs in malaria treatment. Articles used for this review were searched from Hinari, Pubmed and JSTOR electronic databases. So far about forty-four (44) SNPs have been identified in the PfATPase6 gene in samples collected from thirty-five (35) countries. In vitro resistance tests have not been carried out on most of the identified SNPs. Again the E431K, A623E, S769N and L263E mutations that have been shown to confer resistance to artemisinins have not been found in most analyzed samples. The identified PfATPase6 SNPs have not been shown to have arisen as a result of drug selection pressure. The prevalence of SNPs which are associated with decrease in artemisinin susceptibility may increase under the new drug selection pressure and eventually impair ACT treatment.

INTRODUCTION

Malaria has been one of the most important human diseases in the world. It is caused by four different species of *Plasmodium*, namely *P. falciparum, P. ovale, P. vivax and P. malariae*. The most virulent of the four parasites is *P. falciparum*. It accounts for almost all severe malaria cases as well as malaria deaths.

The last decade has seen a renewed commitment to basic as well as clinical and operational research to prevent and treat malaria [1], resulting in a reported decrease in global incidence of disease and death [2]. Currently there are about three billion people are at risk of malaria in one hundred and six endemic countries and about 225 million episodes of the disease leading to 781,000 annually [2] primarily of young children and pregnant women [3]. *P. falciparum* has effectively developed resistance to most available antimalarial drugs, hence the increase in malarial burden in recent years. Anti-malarial drugs will continue to be the major control tool, through treatment and prophylaxis, until an effective antimalarial vaccine is developed [4].

Resistance to chloroquine has been long reported, in the late 1950s. Chloroquine resistance has spread throughout the malaria endemic countries of the world [5]. *P. falciparum*-resistance to sulphadoxine-pyrimethamine (Fancidar) is also rapidly increasing [6].

Resistance antimalarials arises from spontaneous point mutations in the genome or gene duplications which are independent of drug selection pressure. Once formed, the continuous use of parasite-resistant drug confers selective advantage to parasites that carry the resistant gene(s) [6]. This leads to transmission of the drug-resistant parasites through two mechanisms. First, the use of the parasite-resistant drug causes increase in the number of circulating gametocyte of the drug-resistant parasite [7, 8, 9]. Secondly, the gametocytes carrying resistant genes have been shown to be more infectious to mosquitoes, and infect a higher proportion of mosquitoes than those carrying sensitive genes [9].

Resistance of *P. falciparum* to cheaper and relatively safer antimalarial drugs provoked the search for better and long-lasting antimalarial therapy. In 2006 combination therapy was recommended as first-line treatment [6]. The policy demands that the combination therapy contains an artemisinin compound [10].

Artemether+lumefantrine, artesunate+amodiaquine, artesunate+mefloquine and artesunate+ sulphadoxine-pyrimetheamine (in alphabetical order) are the specific artemisinin combination therapies (ACTs) that have been recommended for use in malaria endemic regions for treating uncomplicated malaria. The basis for ACT is twin: the combination is often more effective than monotherapy; and in the event that a mutant parasite that is resistant to one of the drugs arises de novo during the course of the infection, the parasite will be killed by the other drug [6]. In ACTs the short half-life and fast-acting artemisinin compound rapidly reduces the parasite biomass, and the typically long half-life drug clears the remaining parasite population [11].

MATERIALS AND METHODS

Relevant articles were searched in Hinari, Pubmed and JSTOR electronic databases. Search items included '*Plasmodium falciparum*', '*Plasmodium falciparum* Adenosine Triphosphatase 6', 'Artemisinin', 'Artemisinin-based combination therapy', 'Malaria', 'Diversity', 'Resistance', and 'Polymorphism' used in combinations like 'Artemisinin + Resistance', '*Plasmodium falciparum* Adenosine Triphosphatase 6 + Polymorphism', etc.

Papers were considered for inclusion in this review if;

i. They were originally published in English
ii. They were published between 1990 and 2009
iii. The *plasmodium* sp being considered was *Plasmodium falciparum* or *Plasmodium falciparum* together with another *Plasmodium* sp
iv. The study involved a clinical trial with an Artemisinin compound, sequencing of *PfATPase*6 gene and/or *in vitro* artemisinin susceptibility test.

The following information was sought from the selected papers, where applicable;

i. Country(ies) where study was undertaken
ii. Number of samples analysed
iii. Polymorphisms of *PfATPase*6 gene found
iv. Resistant polymorphs of the *PfATPase*6 gene

RESULTS AND DISCUSSION

About two thousand six hundred and fifty-two (2652) human blood samples had been collected from thirty four (35) countries for *PfATPase*6 gene analysis (Table 1) between 1990 and 2009. Out of these thirty five countries, twenty five (25) are geographically located in Sub-Saharan Africa, five (5) in Asia, three (3) in America, and two (2) in Oceania.

So far about forty one (41) polymorphs have been recorded in the *PfATPase*6 gene (Table 2).

Four (L263E, E431K, S769N and A623E) of the forty-four SNPs have been shown to inhibit artemisinin action on the *PfATPase*.

Table 1: countries from which human blood samples have been collected for *PfATPase*6 analysis

	Country	Number of samples collected
1.	Angola	10
2.	Benin	1
3.	Burkina Faso	1
4.	Cambodia	150
5.	Cameroon	67
6.	China	1
7.	Colombia	9
8.	Comoros Islands	34
9.	Congo Dem. Republic	5
10.	Cote D'Ivoire	68

11.	Ecuador	1
12.	French Guiana	289
13.	Gambia	5
14.	Ghana	14
15.	Guinea Conakry	1
16.	Indonesia	1
17.	Kenya	4
18.	Liberia	1
19.	Madagascar	2
20.	Malawi	1
21.	Mali	49
22.	Mozambique	2
23.	Niger	93
24.	Nigeria	11
25.	Papua New Guinea	3
26.	Sao Tome and Pricipe	70
27.	Senegal	149
28.	Sri Lanka	1
29.	Sudan	1
30.	Tanzania	1244
31.	Thailand	1
32.	Uganda	4
33.	Vanuatu	3
34.	Zanzibar	355
35.	Zimbabwe	1
TOTAL		**2652**

Table 2: Country(ies) where SNPs were sampled from

SNPs		**Countries sampled from**
1.	L263E	*
2.	A623E	Senegal, Zanzibar
3.	E431K	Senegal, Angola, Ghana, Cote D'Ivoire, Nigeria, Kenya, Congo Dem Rep., Madagascar, Niger, China, Zanzibar, Tanzania, Gambia, Liberia, Malawi, Sudan, Uganda,
4.	S769N	French Guiana.
5.	D537D	Niger,
6.	K561N	Niger,
7.	N569I	Zanzibar
8.	N569K	Niger, Zanzibar, Tanzania, Gambia, Malawi, Sudan, Papua New Guinea
9.	A630S	Niger, Angola, Zanzibar, Tanzania,
10.	G639D	Niger,
11.	L402V	Senegal, Ghana, Nigeria, Kenya, Cameroon, Congo Dem Rep., Niger,
12.	N683K	Senegal, Cambodia
13.	K771E	Angola, Ghana, Niger,
14.	K776N	China,
15.	H243Y	Angola, Zanzibar
16.	V229I	Zanzibar
17.	252SYN	Zanzibar
18.	G585D	Zanzibar
19.	T602I	Zanzibar
20.	L610I	Zanzibar
21.	K611N	Zanzibar
22.	N612Y	Zanzibar
23.	A621D	Zanzibar

24.	A623T	Zanzibar
25.	A623E	Zanzibar
26.	G632E	Zanzibar
27.	T635A	Zanzibar
28.	T635K	Zanzibar
29.	E637G	Zanzibar
30.	G639D	Zanzibar
31.	S641G	Zanzibar
32.	E643Q	Zanzibar
33.	N644I	Zanzibar
34.	K649E	Zanzibar
35.	N683K	Burkina Faso
36.	I723V	Zanzibar
37.	H747Y	Zanzibar
38.	H747R	Zanzibar
39.	756SYN	Vanuatu
40.	758SYN	Zanzibar
41.	K783	Zanzibar
42.	801SYN	Zanzibar
43.	R809G	Zanzibar
44.	898SYN	Sao Tome and Principe

** SNP detected by laboratory manipulation*

Table 3: Geographical occurrence of SNPs of the *PfATPase6*

Region	SNPs	% (N = 44SNPS)
Sub-Saharan Africa	A623E, E431K, D537D, K561N, N569I, N569K, A630S, G639D, L402V, N683K, K771E, K776N, H243Y, V229I, 252SYN, G585D, T602I, L610I, K611N, N612Y, A621D, A623T, A623E, G632E, T635A, T635K, E637G, G639D, S641G, E643Q, N644I, N683K, K649E, I723V, H747Y, H747R, 758SYN, K783, 801SYN, R809G, 898SYN	95.5
Asia	E431K, K776N	4.5
South America	S769N	2.3
Oceania	756 SYN, N569K	4.5

These results indicate that the *PfATPase6* is very much diverse, considering the number of SNPs that have been found in the gene. Much of these SNPs (95.5%), are found in Sub-Sahara African samples. Only one (2.3%) SNP has been found in South American samples, whereas two (4.5%) SNPs have been found in Oceanic (Vanuatu and Papua New Guinea) samples, and four (9.1%) in Asian samples.

The high prevalence of the *PfATPase6* SNPs in Sub-Saharan Africa might be due to the high endemicity of *falciparum* malaria in this region. It is worthy of note that most of the SNPs found in samples coming from Sub-Saharan Africa were recorded at a time when the use artemisinin compounds was rare in the region. The parasites were therefore not exposed to these drugs [12] during the period of the various studies. Thus, the occurrence of these SNPs cannot be said to be as a result of drug pressure. It therefore remains to be observed whether the ACTs, as have been introduced in Sub-Saharan Africa, will cause development of new SNPs and/or select artemisinin-resistant SNPs of the *PfATPase6*.

In vitro susceptibility tests have not been carried out on most of the identified SNPs. Only four of the forty-four *PfATPase6* SNPs have been shown to confer some level of resistance to artemisinins. The L263E mutation has been recorded only by laboratory engineering [13] and not yet in any wild parasite/field isolate. The question still remains to be answered whether the L263E mutation can ever develop *in vivo*, since laboratory culture settings arguably cannot be completely projected for internal mammalian conditions. The S769N, A623E and E431K mutations, on the other hand, have been found in field isolates [10, 12]. Their occurrence however is very rare with the exception of the E431K mutant, which has been found in samples collected from sixteen Sub-Sahara African countries and China. The S769N mutation has only been found in French Guianan samples and the A623E mutation, in Senegalese and Zanzibari samples. It is just in one case that the A623E and E431K mutations have been found to be associated with reduced *P. falciparum* susceptibility to artemisinins [10], and in this case they occurred together as a double mutant (even though their occurrence together could not be explained). It is still, thus, unclear if the E431K and A623E mutants can independently reduce *P. falciparum* susceptibility to artemisinins.

The prevalence of these resistant SNPs, so far, seems relatively rare possibly because they may confer a loss of fitness to the parasite. This gives an assurance that no stable resistance has, hitherto, developed against the artemisinin compounds.

Despite the enormous use of the *PfATPase6* gene to find artemisinin resistance, we still cannot confidently claim that the *PfATPase6* gene is the most appropriate genetic marker for artemisinin resistance. Stable resistance to artemisinins has been developed without any mutations in the *PfATPase6* gene [4]. Again the synthetic peroxide RBX11160 (OZ 277), which has similar pharmacodynamic properties to the artemisinins, is only a weak inhibitor of *PfATPase6* [14]. These facts bring to fore an assertion that other mechanisms of action may be involved, together with that of the *PfATPase*6, in development of artemisinin resistance.

CONCLUSION

Single nucleotide polymorphisms of *PfATPase*6, which have been shown to reduce susceptibility to artemisinins are rarely observed. Their prevalence, however, may increase under the new selective pressure of ACTs and artemisinin monotherapies. Therefore, there is a risk that some of the herein identified SNPs may be associated with resistance to artemisinins and can hence be selected under the present drug pressure and eventually impair ACT treatment.

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