

# The Impact of *Plasmodium falciparum* Adenosine Triphosphatase-6 Gene (*PfATPase6*) Mutations in Artemisinin Resistance

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## Abstract

The World Health Organization (WHO) recorded an estimated 263 million malaria cases globally in 2023, leading to about 597,000 mortalities. Most of this burden occurred in the WHO African Region, which accounted for approximately 94% of cases and 95% of malaria-related deaths. Artemisinin-based combination therapies (ACTs) remain the mainstay of malaria treatment globally; however, the emergence of *Plasmodium falciparum* resistance compromises their sustained efficacy. Although mutations in the *Plasmodium falciparum* Kelch 13 (*Pfk13*) propeller domain are largely proven to be markers of partial artemisinin resistance, greater focus has turned to *Plasmodium falciparum* Adenosine Triphosphatase 6 (*PfATPase6*) as a potential supplementary determinant. This review compiled evidence from published articles between 2015 and 2025, sourced from Google Scholar, PubMed, ProQuest, and ScienceDirect, with a focus on *PfATPase6* polymorphisms, their distribution, functional role, detection techniques, and implications for malaria prevention. Notable nonsynonymous single-nucleotide polymorphisms (SNPs) such as E431K, S769N, A623E, S769M, and M699V have been reported spanning Asia, the Americas, and Africa. Several studies reveal a correlation with decreased *in vitro* susceptibility or enhanced artemether Half Maximal Inhibitory Concentration (IC<sub>50</sub>), although findings are inconsistent due to interrelated resistance markers, environmental differences, and deviations in methodology. Recent improvements in molecular monitoring techniques, like next-generation sequencing, high-resolution melting analysis, and advanced real-time polymerase chain reaction (PCR) techniques, have broadened the ability to detect uncommon variants and have reinforced surveillance systems. Despite inconsistency in findings, there is evidence that *PfATPase6* reduces sensitivity to artemisinin; therefore, it should be taken into consideration in resistance surveillance schemes. It is recommended to incorporate *PfATPase6* genotyping alongside *Pfk13* surveillance and treatment efficacy studies to offer more insights into the emergence of resistance. These approaches are vital to expound the underexplored role of the *PfATPase6* in resistance patterns and encourage the sustainability of antimalarial drugs.

**Keywords:** Malaria, Resistance, *PfATPase6*, Artemisinin, *Plasmodium falciparum*, Mutations

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## INTRODUCTION

Malaria is a significant public health threat, particularly in tropical and subtropical regions, caused by *Plasmodium falciparum*, which can be transmitted via the bite of an infected female *Anopheles* mosquito.<sup>1</sup> While the genus "Plasmodium" encompasses numerous species, five are associated with human malaria: *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium knowlesi*, and *Plasmodium falciparum*. The deadliest among the species is *Plasmodium falciparum*, which predominates in Sub-Saharan Africa.<sup>2</sup> According to the World Malaria Report 2024, an estimated 263 million malaria cases were recorded globally in 2023, leading to about 597,000 mortalities. Most of this burden occurred in the World Health Organization (WHO) African Region, which accounted for approximately 94% of cases and 95% of malaria-related deaths. Nigeria solely contributed approximately 68 million cases (26% of the worldwide total) and 30.9% of global mortalities, emphasizing the country's major role in the malaria landscape, globally.<sup>3</sup> This constant high burden reinforces the urgent demand for continual surveillance and significant advancement in effective control tactics.

The use of artemisinin-based combination therapies (ACTs) has revolutionized malaria treatment, providing effective alternatives against *Plasmodium falciparum* infections.<sup>3</sup> Artemisinin, obtained from *Artemisia annua* (a sweet wormwood plant), exhibits rapid action against *Plasmodium falciparum* and is often administered in combination with other partner drugs to enhance efficacy and reduce the risk of emerging resistance.<sup>4</sup> Given the rising incidence of resistance to older antimalarial medications, such as sulfadoxine-pyrimethamine and Chloroquine, reported from various regions, artemisinin and its derivatives have consistently gained prominence as an alternative drug.<sup>5,6</sup> Artemisinin combination therapy, which consists of artemisinin (ART) in combination with one or more other antimalarial medications known as partner therapies, is currently used as the first and second-line treatment for malaria in most endemic countries.<sup>7,8</sup> Six ACTs are currently recommended by the WHO for treating malaria cases worldwide: Artemether

+ Lumefantrine, Artesunate + Sulfadoxine-Pyrimethamine, Artesunate + Amodiaquine, Artesunate + Mefloquine, Dihydroartemisinin + Piperazine, and Artesunate + Pyronaridine.<sup>9</sup>

Although the increasing emergence of resistance to artemisinin and its partner drugs threatens these benefits.<sup>9</sup> Resistance has been documented majorly in Southeast Asia and, more recently, in numerous African nations, highlighting issues regarding the long-lasting sustainability of an apostrophe to the ACT potency.<sup>10</sup> To ensure treatment outcomes and develop efficient surveillance systems, it is pivotal to comprehend the molecular mechanisms driving artemisinin resistance.

Among the implicated genetic factors in artemisinin resistance, the *Plasmodium falciparum* Adenosine Triphosphatase 6 (*PfATPase6*) gene has garnered attention because of its function in calcium homeostasis within the parasite.<sup>10</sup> It encodes a protein that is an ortholog of the sarco/endoplasmic reticulum  $Ca^{2+}$ -ATPase (SERCA) located in *Plasmodium falciparum*, the causative agent of the deadliest form of malaria.<sup>11</sup> This protein maintains calcium homeostasis, which depends on several cellular functions, such as muscle contraction and signal transduction. There is cognition that this *PfATPase6* is the target of artemisinin and its derivatives, which hamper calcium transport and eventually lead to the parasites' cell death.<sup>4</sup> Recent studies have shown that the interaction of artemisinin with *PfATPase6* is through hydrophobic interactions, specifically affecting protein conformation. Notably, mutations in this gene could alter its structure, resulting in target site alteration and decreasing drug binding affinity.<sup>12</sup> An *in silico* study revealed that specific mutations, including L263D, L263E, and L263K, could impact the *PfATPase6* structure and reduce the artemisinin's binding affinity.<sup>13</sup> Understanding the impact of mutations in the *PfATPase6* gene on treatment outcomes is pivotal for optimizing malaria control approaches and continually ensuring ACTs' effectiveness.

Although *Plasmodium falciparum* Kelch 13 (PfK13) mutations have been thoroughly investigated, the role of the *PfATPase6* gene mutations in artemisinin resistance is still negotiable and little understood. This study summarizes emerging information to determine

if *PfATPase6* mutations are a valid secondary molecular indicator of resistance, especially in African regions where traditional Pfk13 mutations are almost absent.

## MATERIALS AND METHODS

Published articles on malaria, antimalarial drug resistance, *PfATPase6* gene, and *Plasmodium falciparum* resistance to antimalarial drugs were obtained from Science Direct, ProQuest, PubMed, and Google Scholar within the range of 2015-2024. Relevant articles with keywords on Malaria, Resistance, *PfATPase6*, Artemisinin, and *Plasmodium falciparum* were retrieved for review. Papers featuring *Plasmodium falciparum* without significant data on the *PfATPase6* gene and resistance to artemisinin were excluded from the study.

## RESULTS AND DISCUSSION

### Life cycle of *Plasmodium falciparum*

Humans are the intermediate hosts where asexual reproduction occurs, but *Anopheles* mosquitoes are the definitive hosts that harbor the sexual reproduction stage.<sup>14</sup> In humans, parasitic infection starts following a bite from an infected female *Anopheles* mosquito.<sup>15</sup> Out of 460 *Anopheles* mosquito species, reports suggest that over 70 transmit falciparum malaria.<sup>16</sup> Among the most well-known and common vectors, *Anopheles gambiae* is predominant in Africa.<sup>17</sup> The mosquito uses its proboscis to pierce the skin during feeding, releasing the infectious stage (sporozoite) from its salivary glands.<sup>17</sup> The saliva of mosquitoes contains anti-inflammatory and anti-hemostatic enzymes that prevent blood clotting and suppress the pain response.<sup>16</sup> Usually, there are 20-200 sporozoites in each infected bite, but a few sporozoites invade the hepatocytes.<sup>18</sup>

The actin and myosin proteins under their plasma membrane work as a motor to propel the sporozoites as they glide through the bloodstream. After entering the hepatocytes, the parasite becomes a trophozoite, losing its surface coat and apical complex.<sup>19</sup>

Hepatocytes undergo 13-14 rounds of mitosis to produce schizonts, which are syncytial cells (coenocytes) that reside inside the

parasitophorous vacuole. This process is known as schizogony.<sup>16</sup> The surface of the schizont produces thousands of haploid (1n) daughter cells, called merozoites, from tens of schizont nuclei. In the liver stage, up to 90,000 merozoites can be produced, which are then released into the bloodstream as merozoites, which are parasite-filled vesicles.

The apicomplexan invasion organelles (pellicle, apical complex, and surface coat) are used by merozoites to recognize and enter the host erythrocyte (red blood cell).<sup>20</sup> Initially, the merozoites attach themselves randomly to the erythrocyte. After that, it reorients so that the erythrocyte membrane is close to the apical complex. The parasite can grow inside the erythrocyte by forming a parasitophorous vacuole.<sup>21</sup> Almost every parasite in the blood is in the same stage of growth during this infection cycle, which occurs in a highly coordinated manner.<sup>22</sup> The circadian cycle of the human host is necessary for this exact timing mechanism to function.

Haemoglobin digestion is essential for parasite metabolism within the erythrocyte.<sup>23</sup>

Anemia, fever, and neurological disorders are among the clinical presentations of malaria that manifest throughout the blood stage.<sup>24</sup> In addition, the parasite changes its erythrocytic shape by producing knobs on the membrane.<sup>25</sup> The liver, brain, and heart are only a few human tissues or organs where infected erythrocytes are frequently seen. This is because proteins on the erythrocyte membrane derived from parasites attach to receptors in human cells.<sup>22</sup>

Cerebral malaria is a highly critical form of disease that is caused by sequestration in the brain and raises the risk of mortality for the victim.<sup>22</sup>

Following erythrocyte invasion, the parasite differentiates into a spherical trophozoite inside a parasitophorous vacuole and loses its unique invasion organelles, surface coat, and apical complex.<sup>23</sup> By breaking down the proteins in the erythrocyte's hemoglobin, the trophozoite transforms the leftover heme into insoluble, chemically inert  $\beta$ -hematin crystals known as hemozoin.<sup>23</sup>

The parasite divides asynchronously into several mitotic divisions and replicates its Deoxyribonucleic Acid (DNA) several times

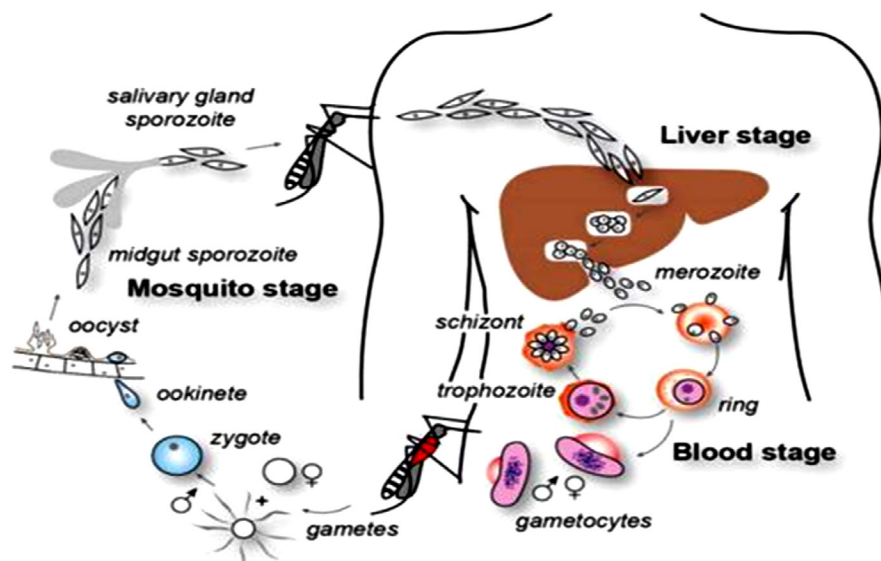
during the schizont stage.<sup>24</sup> The term erythrocytic schizogony refers to dividing and multiplying cells within the erythrocyte.<sup>25</sup> The schizonts generate 16-18 merozoites each, and the merozoites cause red blood cell rupture.<sup>26</sup> The released merozoites infiltrate newly formed erythrocytes, but before a free merozoite enters another erythrocyte, it spends around 60 seconds in the bloodstream. It takes about 48 hours to complete one erythrocytic schizogony. As a result, the infected erythrocytes rupture simultaneously, which leads to the typical clinical signs of falciparum malaria, including fever and chills.<sup>25</sup> Certain merozoites undergo sexual differentiation to become gametocytes, either male or female. Approximately 7 to 15 days are needed for these gametocytes to grow fully during gametocytogenesis, releasing sporozoites to complete the lifecycle.<sup>27</sup> A female *Anopheles* mosquito subsequently consumes these during a blood meal to begin another cycle. The life cycle of *Plasmodium falciparum* is presented in Figure 1.

### Molecular Basis of Antimalarial Drug Resistance in *Plasmodium falciparum*

Resistance to antimalarial drugs is a significant threat to controlling and eradicating malaria.<sup>28</sup> Malaria resistance is notably exhibited by *Plasmodium falciparum* and *Plasmodium vivax*, which have been reported by many

studies indicating their resistance to antimalarial medications.<sup>29</sup> The primary mechanism underlying the development of this phenotype is the mutation of drug resistant genes or an increase in the number of copies of those genes.<sup>30</sup> *Plasmodium falciparum*'s resistance to antimalarial medications has been demonstrated in most of the classes of antimalarials, including quinolines, antifolates, and ART derivatives.<sup>31</sup> According to the WHO, drug resistance refers to the ability of a parasite to survive and persist despite exposure to a drug and its toxic effects, even when the drug is present at adequate levels at the site of action. Antimalarial drug resistance emergence depends on the parasites' genetic makeup, with gene amplification or mutations that confer reduced susceptibility.<sup>32</sup> Changes in the quantity, structure, or composition of proteins cause resistance to drugs in organisms.<sup>33</sup> Gene amplification and SNPs are the two forms of genetic alteration that mediate the protein change.<sup>34</sup> Antimalarial resistance is currently a significant hindrance to disease control; hence, researchers are always looking for solutions. Since there is no guarantee that resistant parasite strains won't emerge, new compounds must constantly be developed; innovative drugs with unique modes of action are currently undergoing clinical trials.<sup>33</sup>

Resistance to currently available antimalarial drugs can arise for a few reasons. It



**Figure 1.** The life cycle of *Plasmodium falciparum*<sup>28</sup>

**Table 1.** Molecular markers that are commonly associated with antimalarial drug resistance

Genes	Notable Mutations	Chromosomal Locations	Class of Antimalarials associated with resistance	Phenotypic Effect	Ref.
<i>Pfprt</i>	K76T, C72SN326S, A220S1356T	Chromosome 7	Chloroquine and Amodiaquine	Resistance to Chloroquine, Reduction in drug accumulation in the food vacuole	35-37
<i>Pfmdr1</i>	N86Y, D1246YY184F, N1042D	Chromosome 5	Amodiaquine, Chloroquine, Mefloquine, Lumefantrine, Cycloguanil Pyrimethamine,	Regulation of the potency of ACT partner drugs Multiple drug transport modification	36,37
<i>Pfdhfr</i>	N51I, I164LC59R, S108N	Chromosome 4		Sulfadoxine-Pyrimethamine resistance Decreased binding to antifolate drugs	36,38
<i>Pfcytb</i>	Y268C, Y268SY268N	Mitochondrial genome	Atovaquone	Increase in atovaquone EC <sub>50</sub> /clinical therapeutic failure Altered binding of atovaquone	32,39
<i>Pfnhe-1</i>	Repeat polymorphisms (DNNND)	Chromosome 13	Quinine	Decreased susceptibility (higher IC <sub>50</sub> for quinine)	32,40
<i>PfATPase4</i>	P990R, G358SD1116G, D1116N398F, 917L, 211T, 415D	Chromosome 12	Pyrazoles, Dihydroisoquinolones	Decreased affinity for Naz, a High increase in IC <sub>50</sub> to medications targeting PfATP4	38,41
<i>PfATPase6</i>	L263E, A623E, 5769N, E431K	Chromosome 1	Artemisinin and its derivatives	Decreased susceptibility Potential altered Ca <sup>2+</sup> homeostasis	11
<i>PfK13</i>	C580Y, R539T1543T, Y493HF446I, P553LM476I, N458Y	Chromosome 13	Artemisinin and its derivatives	Ring stage tolerance Delayed parasite clearance	39,41,42
<i>Pfpm2</i>	Copy Number Variation (CNV); Increased copy number	Chromosome 14	Dihydroartemisinin-Piperaquine	Higher rates of treatment failures decreased vulnerability in piperaquine survival assays	40,43

is also important to take into consideration factors such as the total parasite load, the rate of drug effectiveness, drug compliance, and inadequate adherence to the malaria treatment protocol.<sup>33</sup> Misuse of medications, poor pharmacokinetic characteristics, and improper dosing result in insufficient drug exposure to parasites.<sup>35</sup> The danger of hyper-parasitemia, recrudescence, and hyper-gametocytaemia is enhanced by low-quality or fabricated antimalarials that lack active pharmaceutical ingredients (APIs), which can encourage resistance. The genes *Pfnhe1*, *Pfmdr1*, *Pfmrp*, *Pfdhps*, *Pfk13*, *Pfdhfr*, *Pfcrt*, and *PfATPase6* are associated with antimalarial drug resistance.<sup>36</sup> Identifying the molecular markers linked to resistance in the laboratory is essential to sustain the efficacy of the current antimalarial drugs. The molecular markers that are often implicated in antimalarial drug resistance are shown in Table 1.

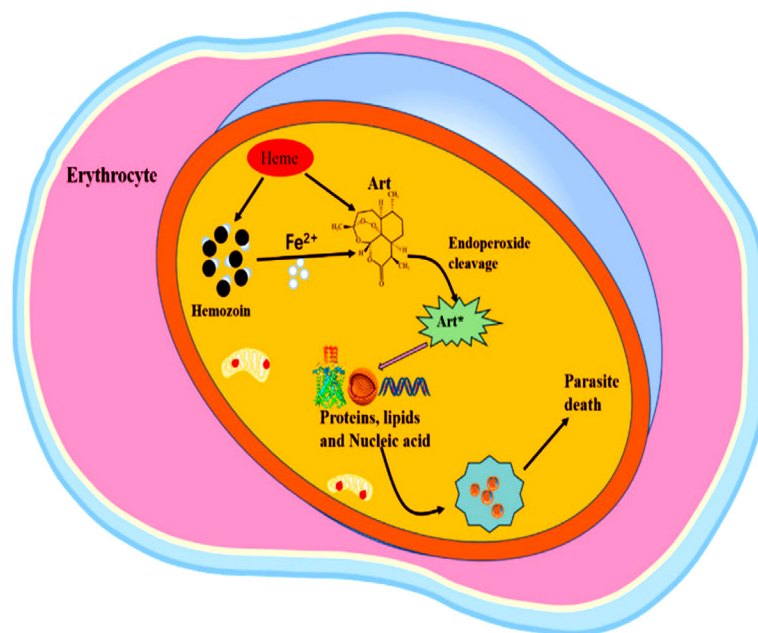
### Mechanisms of action of artemisinin

The mode of action of artemisinin against *Plasmodium falciparum* involves the destruction of proteins by carbon-centered radicals released upon activation of the drug. Artemisinin produces reactive oxygen species (ROS) when ferrous iron ( $Fe^{2+}$ ) is activated during hemoglobin digestion,

as shown in Figure 2. This activation results in the development of alkylating agents that disrupt vital biomolecules, lipids, and proteins, damaging cellular roles and causing the death of parasites.<sup>42</sup> This action radically differs from Other antimalarial medications, which usually target one enzyme or pathway.

Recent studies proposed a direct interaction between Artemisinin and *PfATPase6*, a calcium ATPase essential for maintaining calcium homeostasis in *Plasmodium falciparum*.<sup>32</sup> The binding of artemisinin to *PfATPase6* causes notable structural changes that impair its role, resulting in elevated levels of calcium intracellularly and, ultimately, cell death.<sup>43</sup> Emerging evidence shows that derivatives of Artemisinin could target Mitochondrial roles within the parasite, disrupting vital metabolic processes essential for survival, although this mechanism calls for more investigations.<sup>32</sup>

All drugs containing artemisinin are metabolized in the body to dihydroartemisinin, a sesquiterpene lactone endoperoxide.<sup>44</sup> This endoperoxide is responsible for the death of parasites by cleaving them and producing free radicals. To activate artemisinin, the reduced form of  $Fe^{2+}$  iron catalyzes reductive scission of



**Figure 2.** The Mode of Action of Artemisinin-based antimalarial drug<sup>43</sup>

the endoperoxide bond.<sup>44</sup> The main source of this iron is probably imported host hemoglobin, which, when proteolyzed, produces extremely reactive heme species. Although most of this heme is trapped in hemozoin crystals, a small amount would be left vacant and accessible for artemisinin activation. Other iron sources, including the low steady-state labile iron pool found in parasites, may be used for artemisinin activation.<sup>45</sup>

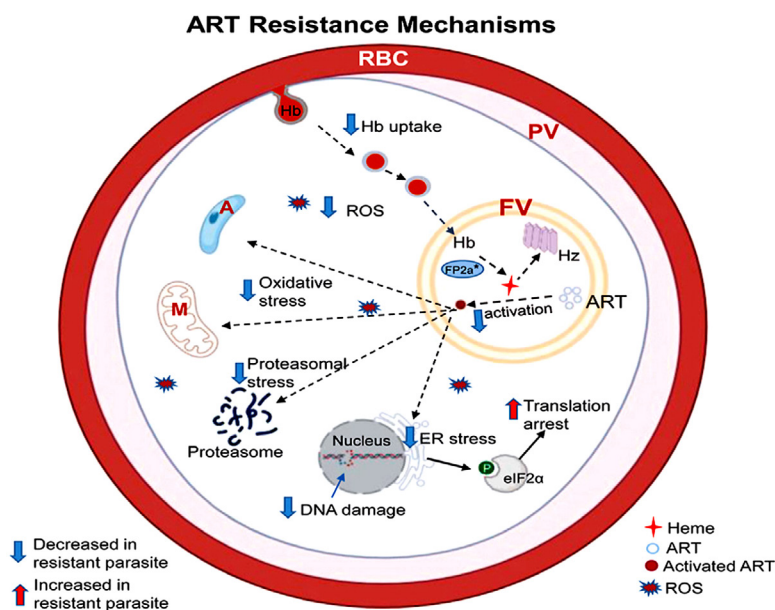
Lastly, there is evidence that the parasite's biosynthetic heme may also activate ART in early rings.<sup>46</sup> This reliance on free Fe<sup>2+</sup> for activation illustrates why ARTs are proactive against blood-stage parasites but ineffective against liver stages or mature gametocytes.<sup>47</sup>

### Mechanisms of artemisinin resistance

One vital component of antimalarial therapies is artemisinin, which is particularly effective against *Plasmodium falciparum*, the deadliest malaria parasite species.<sup>48</sup> However, artemisinin resistance has emerged, primarily in Southeast Asia and, more recently, in Africa,<sup>49</sup> emphasizing the demand for a deeper understanding of the underlying mechanisms. This complex resistance involves mutations in key genes, cellular stress response alterations, and hemoglobin uptake modification as shown in Figure 3.<sup>50</sup>

In clinical terms, artemisinin resistance is the inability of patients receiving artemisinin derivatives or artemisinin combination therapy to rapidly eliminate malaria parasites from their peripheral blood.<sup>43</sup> Slow parasite clearance and decreased early ring-stage parasite susceptibility are characteristics of ART resistance.<sup>51</sup> The relationship between ART resistance and polymorphisms in the *Plasmodium falciparum* Kelch 13 propeller protein has been shown by numerous studies, and parasites with different SNPs in the gene encoding it (Pfk13) exhibit different parasite clearance rate phenotypes.<sup>52</sup> The Pfk13 is now widely recognized as a reliable indicator of artemisinin resistance. However, about twenty more recorded polymorphisms need to be phenotypically described for their effects on parasite sensitivity to ARTs.<sup>53</sup> Currently, parasites with Pfk13 mutations, such as R561H, F446I, N458Y, M476I, Y493H, R539T, C580Y, I543T, and P553L, are linked to reduced *in vitro* drug sensitivity and delayed clearance in clinical trials.<sup>54</sup>

The most well-known genetic basis for artemisinin resistance is associated with alterations in the Pfk13 protein.<sup>55</sup> These variations have been linked with delayed parasite clearance after treatment, indicating decreased vulnerability to Artemisinin. A summary of *PfATPase6* gene



**Figure 3.** Mechanisms of ART Resistance in *Plasmodium falciparum*<sup>50</sup>

**Table 2.** Summary of *PfATPase6* gene mutations recorded from existing selected literature

Mutation	Country	Genotyping/Sequencing method	Prevalence %	Associated IC <sub>50</sub> Increase	Ref.
E431K	North-Eastern Tanzania	PCR-RFLP for <i>PfATPase6</i> gene mutations	16	Yes (Artemether)	56
	Nigeria	PCR + Sequencing	17	NA	11
	Gabon, Ghana, and Kenya	PCR, Sanger sequencing	41	NA	57
	Republic of Congo	Nested PCR	1.6	NA	58
L263E	North-Eastern Tanzania	PCR-RFLP for <i>PfATPase6</i> gene mutations	5.8	Yes	56
A623E, S769N	Gabon, Ghana, and Kenya	PCR, Sanger sequencing	3.1	NA	57
	North-Eastern Tanzania	PCR-RFLP for <i>PfATPase6</i> gene mutations	3.9	Yes	56
S769M	Southwest Nigeria	PCR, Sanger sequencing	3.6		59
M699V	Southwest Nigeria	PCR, Sanger sequencing	9.6		59
S679S	Southwest Nigeria	PCR, Sanger sequencing	8.4		59
R37K	Somalia (Afgol and Balad)	PCR-RFLP, Sanger sequencing	3.5	NA	60
G639D	Somalia (Afgol and Balad)	PCR-RFLP, Sanger sequencing	4.7	NA	60
I898I	Somalia (Afgol and Balad)	PCR-RFLP, Sanger sequencing	33.7	NA	60

NA = Not Applicable

mutations reported in previous studies, along with their prevalence, associated IC<sub>50</sub> increases, and genotyping methods, is presented in Table 2.

Research has proven that a specialized point mutation in the K13 propeller domain results in a notable reduction in drug potency, enabling the survival of parasites regardless of exposure to ART.<sup>56</sup> The rise of these mutations has been noticed as a selective response to the pressure brought about by Artemisinin-based therapies, resulting in drug-driven selection across several *Plasmodium falciparum* populations.

The mechanism of action of Artemisinin is directly associated with the breakdown of hemoglobin within the parasite's food vacuole.<sup>57</sup> The process starts with endocytosis of hemoglobin from the host red blood cells (RBC) through cytostomes.<sup>58</sup> This uptake is essential because the breakdown of hemoglobin generates heme, which is hazardous to the parasite but also necessary to activate Artemisinin.<sup>59</sup> Decreased breakdown and hemoglobin uptake have been linked to changes in molecules involved in hemoglobin transport (AP-2  $\mu$ , Rab GTPases, and Coronin). By implication, this results in lesser production of heme and reduced activation of Artemisinin, ultimately decreasing oxidative stress within the parasite.<sup>60</sup>

Recent studies have emphasized the

function of Transfer Ribonucleic Acid (tRNA) alteration in conferring resistance to Artemisinin.<sup>61</sup> Adjustments in tRNA molecules enable *Plasmodium falciparum* to swiftly fit the expression of its protein profile under drug-induced conditions. This acclimatization allows the survival of resistance strains regardless of reduced levels of heme and artemisinin activation.<sup>61</sup> The ability to modify tRNA insinuates a complicated level of regulation that improves the parasite's resilience against therapeutic strategies.<sup>62</sup>

Artemisinin employs its impact partially via the production of reactive oxygen species after heme activation as shown in Figure 2.<sup>42</sup> There is proof that reactive oxygen species are modified either via alterations in metabolic pathways that combat oxidative stress response or by a reduction in the availability of heme.<sup>63</sup> This modification enables resistant strains to tolerate the cellular injury induced by Artemisinin. Figure 3 presents mechanisms of ART resistance in *Plasmodium falciparum*.

### Impact of *Pfatpase6* mutations on the effectiveness of artemisinin-based therapies

The *PfATPase6* gene is a significant focus point in *Plasmodium falciparum* regarding malaria treatment outcomes, especially in relation to



artemisinin-based combination therapies. Studies have pointed out specific mutations in this gene that correspond with decreased susceptibility to treatments, making close investigation of their effects on malaria management necessary.

Research has linked decreased susceptibility to artemisinin-based treatments to several single-nucleotide polymorphisms (SNPs).<sup>64</sup> Studies have reported that decreased sensitivity to artemisinin is associated with specific mutations. Notably, the E431K mutation occurs in approximately 17% of samples from affected regions.

Furthermore, the S769N mutation is linked to an increased Half Maximal Inhibitory Concentration (IC<sub>50</sub>) value for artemether, implying a correlation with artemisinin resistance. However, the prevalence depends on the region.<sup>65,66</sup> Mutation such as A623E is usually studied alongside E431K, which may contribute to decreased drug efficacy with other SNPs.<sup>65</sup> Other forms like L263E and R37K have been reported but infrequently. These mutations reveal the genetic diversity and adaptive abilities of *Plasmodium falciparum* in response to drug pressure from artemisinin-based combination therapies.<sup>12,65</sup>

A study in Nigeria specifically investigated the molecular profile of the *PfATPase6* gene among *Plasmodium falciparum* isolates and ascertained a minimal prevalence of already established SNPs associated with resistance, indicating that other factors may be part of treatment failures.<sup>11</sup> Conversely, broader studies have investigated numerous genes (*pfmdr1*, *pfcr1*, and others, including *PfATPase6*).<sup>66</sup> These investigations often show complex interactions between various genetic factors affecting antimalarial drug resistance, revealing that while *PfATPase6* is important, it is among a broader network of genes that collectively impact treatment outcomes.

A recent finding emphasizes the significance of continuous molecular surveillance, aiding an improved comprehension of the dynamics of these mutations and their implications in malaria treatment options.<sup>67</sup> Constant surveillance would be imperative as new mutations may emerge due to selective pressure from the broad use of ACTs.

Research utilizing field isolates and eukaryotic models has suggested that changes in

*in vitro* sensitivity to Artemisinin or its derivatives are caused by SNPs in the *PfATPase6* gene.<sup>68</sup> Furthermore, it has been demonstrated in many studies that *PfATPase6* has many SNPs, four of which (L263E, E431K, A623E, and S769N) have been linked to a significant rise in artemether IC<sub>50</sub>.<sup>69</sup>

However, previous research with *P. falciparum* revealed that the main target of these medications may be a sarcoplasmic and endoplasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA)-type protein encoded by the gene *pfatp6*, and mutations in this gene could alter *P. falciparum*'s susceptibility to artemisinin.<sup>70</sup> The idea that *PfATPase6* is a target for artemisinin emphasizes the necessity of a continuous fight against malaria to mitigate the emergence of resistance to these therapies.

After heterologous expression of *PfATPase6*, inhibitor profiles for antimalarial activity *in vitro* showed a positive association.<sup>70</sup> Parasites transfected with the *PfATPase6* mutant L263E-*PfATPase6* exhibited reduced sensitivity to artemisinin and dihydroartemisinin in both *ex vivo* and *in vivo* tests and greater variability in sensitivity assays.<sup>70</sup>

### Comparative synthesis of studies reporting *PfATPase6* mutations

Although SNPs like S769N and E431K have been observed in regions such as Tanzania, Brazil, and Nigeria, research on their direct impact on phenotypic resistance remains uncertain.<sup>71</sup> Nguetse et al.<sup>57</sup> recorded a high frequency of the E431K mutation (118 cases in 287 samples), which could suggest a possible hotspot for the mutation, probably because of high antimalarial pressure among the studied population. The E431K may be adaptive in response to artemisinin-based combination therapies, improving parasite survival because the *PfATPase6* gene is implicated in calcium transport and ATPase functions. Suradji et al.<sup>56</sup> and Adedire et al.<sup>11</sup> observed a moderate occurrence of the E431K mutation (16.2% and 17%), respectively. These differences may be due to variations in the partner drug combinations employed in ACTs, sampling times, and parasite genetic backgrounds, which may suggest that, regardless of the E431K mutation, the selection pressure in these regions may not be high compared to Nguetse et al.<sup>57</sup> It will be important

to investigate if these two studies were carried out in regions with low usage of ACT, contrary to Nguetse et al.<sup>57</sup>

Standardized methods, such as *ex vivo* IC<sub>50</sub> measurements and ring-stage survival assays, must be employed in future studies to investigate the functional importance of mutations in the *PfATPase6* gene. Koukouikila-Koussounda et al.<sup>58</sup> observed a very low frequency of 2 cases in 120 samples (1.7%). This obvious variation from other studies with the same mutation indicates that the mutation is not widespread yet in the population, or that artemisinin drug pressure is notably very low in this region. Moreover, variations in the genetic backgrounds of parasites in this region could impact the presence of the mutation.

The E431K in the *PfATPase6* gene is suspected to impact artemisinin resistance through numerous mechanisms. It could impair the function of ATPase, possibly impacting the transport of calcium ions within the parasite.<sup>72,73</sup> Furthermore, it may be associated with compensatory mutations that allow parasites to survive regardless of exposure to ACTs.<sup>70</sup> While this mutation could be linked to decreased sensitivity to artemisinin, more research is required to define its specific role in resistance.<sup>74</sup> This corresponds with the findings of Chilongola et al.<sup>71</sup> who proposed that the *PfATPase6* E431K variant has been linked with enhanced artemether IC<sub>50</sub> when found in alongside mutation A623E in *P. falciparum* isolates. In their study, this mutation (E431K) was the second most prevalent (16.4%) due to its occurrence at every site sampled and was present in three different haplotypes.

Tola et al.<sup>75</sup> examined 83 samples and observed three distinct mutations (M699V, S679S, and S769M). Among the three mutations, M699V had a relatively high prevalence (9.6%), although its specific impact is still uncertain. S769M was found among 3 samples out of the 83 samples analysed (3.6%). This mutation has been pointed out in recent studies as a candidate marker implicated in artemisinin resistance because of its effect on the role of ATPase. A synonymous mutation such as S679S (8.4%) usually has no functional role but might be associated with nearby resistance-conferring variations.

Out of the 86 samples examined by Jalei et al.,<sup>60</sup> four distinct mutations were identified (R37K, I898I, S769N, and G639), having mutation frequencies of 3/86, 29/86, 0/86, and 4/86, respectively. The R37K and G639D have not been broadly studied concerning resistance, which makes their significance uncertain. The synonymous mutation (I898I) does not incur any changes in the amino acid sequence, but its high frequency suggests that it could be a polymorphism that occurs frequently among *Plasmodium falciparum* populations. Remarkably, the S769N was absent among the 86 samples analysed, even though a similar mutation (S769M) was identified in a study by Mwaiswelo et al.<sup>76</sup> This implies that while S769M may be significant in resistance, S769N is not implicated. In a similar study by Hassan et al.,<sup>77</sup> all the 1000 (100%) isolates carried the wild-type allele, which shows that there are no S769N mutations for the *PfATPase6* gene in that region at present. Since there was no artemisinin-resistant gene detected in the study, it was concluded that the drug remains effective against malaria in that area.

#### Demographic factors for *PfATPase6* mutations

Recent research has highlighted the notable influence of age and gender on malaria rate and resistance to antimalarials, especially mutations in the *PfATPase6* gene.<sup>72</sup> The prevalence of malaria in many sub-Saharan regions is high in children below the age of five (5) years due to their underdeveloped immune systems.<sup>73</sup> This demographic trend implies that young people are more vulnerable to severe malaria, which may impact the selection pressure for drug resistant strains of malaria. Gender disparities are significant in the epidemiology of malaria.<sup>74</sup>

Research has revealed that males could be at higher risk of malaria infection compared to females because of their frequent outdoor activities and the risk of their jobs.<sup>65</sup> Additionally, biological factors associated with gender could influence immunological responses, which may affect the emergence of resistance to antimalarial drugs.<sup>78</sup> Studies have ascertained that the prevalence of certain mutations in the *PfATPase6* could differ among genders, although there is insufficient data to support this notion.<sup>75</sup>

### Prevalence of *PfATPase6* mutations in Nigeria

*Plasmodium falciparum*, which causes malaria, possesses a gene known as *PfATPase6*. The gene (*PfATPase6*) is associated with resistance to antimalarial drugs, specifically artemisinin-based combination therapies.<sup>32</sup> Efforts to control malaria are hindered by the presence of mutations in this gene, especially in endemic regions or rural areas with limited access to healthcare.

Recent research has highlighted the prevalence of *Plasmodium falciparum* in the Southwestern part of Nigeria. Wicht et al.<sup>32</sup> observed several antimalarial drug resistance markers alongside mutations in *PfATPase6* and other key resistance genes, such as *pfprt* and *pfmdr1*.<sup>32</sup> Non-synonymous mutations in the *PfATPase6* were observed in this research from samples of symptomatic malaria patients, indicating a possible correlation to reduced drug efficacy. In another research on the molecular detection of drug-resistant polymorphisms, 3.6% of the tested cases exhibited ACT-resistant-related single-nucleotide polymorphisms (S769) in *PfATPase6*, highlighting the significance of continuous surveillance.<sup>76</sup>

### Molecular detection methods for *PfATPase6* mutations

The *PfATPase6* gene in *Plasmodium falciparum* is an essential marker in evaluating artemisinin resistance.<sup>76</sup> Numerous detection methods have been the target of recent research to identify mutations in this gene, which is necessary for understanding the mechanisms underlying antimalarial drug resistance. Several molecular methods have been utilized to identify mutations in the *PfATPase6* gene. The Polymerase chain reaction is the primary method of detecting mutations, followed by Sanger sequencing.<sup>77</sup>

In a study conducted in Brazil, the scientists used PCR to amplify specific regions of the genome to detect polymorphisms at codons 37, 630, and 898, with a significant prevalence of mutation, including R37K at 16% and A630S at 32% observed in the isolates.<sup>78</sup> The Mutation Surveyor enhanced the analysis of these sequences, emphasizing the effectiveness of automated techniques in identifying genetic mutations.

The PCR-RFLP (Restriction Fragment Length Polymorphism) is another broad technique to detect SNPs linked with artemisinin resistance.<sup>79</sup> A study in Tanzania employed PCR-RFLP to evaluate mutations such as L263E and E431K and discovered the prevalence rate of local isolates.<sup>80</sup> This approach allows rapid analysis of multiple samples and is essential for epidemiological research.

Real-time quantitative PCR (RT-qPCR) is also an effective technique in molecularly characterizing the *PfATPase6* mutations.<sup>65</sup> This method quantifies gene copy number and can be employed in Allelic discrimination, making it possible for researchers to distinguish between wild and mutant alleles efficiently. A study in Nigeria utilized RT-qPCR to evaluate the prevalence of mutations linked with resistance to antimalarial drugs, revealing its usefulness in high-throughput settings.<sup>76</sup>

Genotyping techniques are essential in understanding the prevalence of the *PfATPase6* gene mutations in various geographic regions and their interaction with artemisinin resistance.<sup>72</sup> One such technique that has gained attention is High-Resolution Melting analysis (HRM), which can differentiate between closely related genotypes without broad post-PCR processing. This method was efficiently utilized in research that evaluated *Plasmodium falciparum* isolates from Iran, and mutations were linked to treatment outcomes.<sup>78</sup>

Next-generation sequencing (NGS) techniques are revolutionizing research, making it possible to conduct a detailed analysis of genetic diversity in the *PfATPase6* gene.<sup>68</sup> The NGS can provide an understanding of several mutations simultaneously and their possible interactions, providing more holistic insights into the impact of mutations on resistance mechanisms.<sup>81</sup> Studies utilizing NGS have evaluated new mutations that may not be feasible with conventional methods, highlighting the significance of adopting advanced genomic technologies in malaria research.

To evaluate how specific *PfATPase6* mutations affect drug binding or calcium transport, subsequent studies must go beyond conventional genotypic cataloguing and introduce functional genomics methods, like Clustered Regularly

Interspaced Short Palindromic Repeats CRISPR associated protein 9 (CRISPR-Cas9)-based gene editing alongside heterologous expression platforms. Treatment outcome models could be enhanced by incorporating *PfATPase6* mutation data into real-time resistance monitoring frameworks like those for PfK13

### Limitations

This review is limited by its reliance on previously published articles, which differ in study design, study population, and geographic distribution. Data from several African regions remains insufficient, making it hard to make continent-wide inferences. Furthermore, the biological role of several *PfATPase6* mutations has not been completely verified in a clinical or experimental context, and differences in the use of partner drugs may further complicate interpretation. These limitations emphasize the demand for more detailed, region-specific studies and functional genomic investigations to spell out the contribution of *PfATPase6* mutations to artemisinin resistance. Bridging these gaps will enhance global initiatives to sustain the potency of ACTs and educate future malaria therapeutic approaches.

### Prospect for future research and treatment strategies

The obvious evidence of the impact of *PfATPase6* mutations highlights the necessity for ongoing surveillance to monitor dynamics in resistance patterns. Evaluating genetic mutations within *Plasmodium falciparum* is vital for comprehending resistance mechanisms and ensuring efficient treatment protocols.<sup>33</sup> Although certain mutations have been linked to altered vulnerability to artemisinin treatments, the total effect on treatment efficacy diverges notably across different areas.<sup>12</sup> Looking forward, certain areas need more exploration. Functional genomic research is essential to understand the biological importance of mutations in the *PfATPase6* gene and their interplay with other determinants of resistance.

Broadening research in marginalized regions of Africa will help bridge knowledge gaps and improve the completeness of surveillance

data. Furthermore, utilizing NGS and other advanced molecular methods will enhance the sensitivity for detecting minor forms that can act as early signals of emerging resistance.

Maintaining the potency of artemisinin and its partner medications will therefore need a cohesive approach that combines clinical surveillance, molecular epidemiology, and improved health systems. Further research should focus on clarifying these interplays and delving more deeply into other molecular markers that could be implicated in resistance, thereby facilitating efforts to mitigate malaria efficiently.

### CONCLUSION

The emergence of artemisinin resistance in *P. falciparum* presents a notable challenge in efforts to mitigate malaria worldwide. This article examined the genetic basis of resistance, with a major focus on mutations in the *PfATPase6* gene. Findings propose that certain mutations (A623E, L263E, and E431K) could impair drug efficacy, highlighting the necessity for continuous molecular surveillance of these genetic mutations; however, there are variations in the frequency of occurrence and clinical impact across regions. The study points out that although mutations in the *PfATPase6* gene have been linked to altered treatment potency, their prevalence and effect differ across diverse regions, including Nigeria's populace. Additionally, demographic factors affecting the frequency of the *PfATPase6* gene mutations were evaluated, providing valuable information on how local populations may respond to artemisinin-based combination therapies. The molecular methods of detection explored provided vital tools to identify these genetic mutations in medical settings, allowing early interventions; hence, the impact of demographics and the parasite's genetic basis further affects treatment outcomes. Sustained genetic monitoring alongside advanced detection techniques will be vital to maintain the efficiency of ACTs.

Genetic-based knowledge of ART resistance will be critical in developing efficient protocols, enhancing global health policies aimed at malaria eradication. Continuous research on *PfATPase6* gene mutations and other resistant

genes is imperative to guarantee the effectiveness of treatment regimens against emerging strains of *Plasmodium falciparum*.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

#### AUTHORS' CONTRIBUTION

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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