Int J Pharm Bio Sci 2016 Jan; 7(1): (B) 74 - 82

Review Article **Molecular biology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

CURRENT STATUS OF COMPARATIVE GENOMICS AND INHIBITORS OF PLASMODIUM SPECIES: A SYSTEMATIC REVIEW

MANOJ KUMAR YADAV [1]*, D SWATI [2] AND PK PATRA [1]

[1] Department of Biochemistry, Pt. J. N. M. Medical College, Raipur-492001, INDIA
[2] Departments of Bioinformatics and Physics, MMV, Banaras Hindu University, Varanasi- 221005, INDIA

ABSTRACT

Malaria is still one of the most infectious and potentially lethal diseases known to mankind and typified by a series of intermittent fever episodes. This disease is responsible for high cases of mortality and morbidity in humans. The global incidence of malaria and its fatal consequences continue to be one of the worst catastrophes ever faced by mankind. *P. falciparum*, a parasitic protozoan, is responsible for a majority of malaria deaths. Another apicomplexan species, *P. vivax*, is the most widely distributed human malaria parasite infecting millions of individuals annually. Recently, *P. knowlesi* that normally infects long-tailed macaques also starts infecting humans. So, in-depth study of the pathogen is essential for combating the disease efficiently. Moreover, rise of drug-resistant cases in many areas, not only in developing countries but industrialized countries as well, during the past decade will make this situation more alarming. These situations, particularly the global resurgence of malaria and the rapid emergence of disease resistance, underscore the importance of the development of new antimalarial drugs. Achieving the goal of malaria-elimination will depend on the existing knowledge and available new information of disease. Unfortunately, malaria is a disease of poverty, and despite a wealth of scientific knowledge there is insufficient market incentive to generate the competitive antimalarial drug research and development that is normally needed to deliver new products. In this review, we tried to show the comparative genomics-based status of malaria causing different *Plasmodium* pathogens and, effectiveness of available antimalarial drugs.

KEYWORDS: **Codon Usage Bias, Drug-Resistance, Genomics, Immune Evasion**

MANOJ KUMAR YADAV
Department of Biochemistry, Pt. J. N. M. Medical College, Raipur-492001, INDIA

***Corresponding author**

INTRODUCTION

Malaria is geographical distributed in tropical and subtropical regions of the world. The temperature, humidity and rainfall were the prominent climatic factors responsible for distribution of malaria disease. [1]. The life cycle of malaria parasite is completed in human and mosquitoes, so temperature is particularly a critical factor for survival and multiplication of parasite. For example, at temperatures below 20°C (68°F), *Plasmodium falciparum* (which causes severe malaria) cannot complete its growth cycle in the *Anopheles* mosquito, and thus cannot be transmitted. Its transmission is was more intense and occurs throughout the year in warmer regions closer to the equator like the Democratic Republic of the Congo and Nigeria of African continent [2]. The transmission will be less intense and more seasonal in cooler regions, and *P. vivax* is more prevalent in temperate regions of world since it is more tolerant of low ambient temperatures.

PRESENT SCENARIO OF PLASMODIUM PATHOGENS

All species of *Plasmodium* genus are parasitic. They complete their life cycle in vertebrate host and invertebrate host- usually a mosquito. The vertebrate host range of *Plasmodium* infection is wider and includes reptiles, birds, rodents and primates. Malaria in human is caused by *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Recent studies show that *P. knowlesi,* whose natural host is monkey, has also started infecting humans. *P. knowlesi* and *P. malariae* show similar morphological features and due to lack of species-specific diagnostic tests, *P. knowlesi* infections in human are often misdiagnosed with its counterpart [3]. Due to advent of new generation sequencing strategies, a lot of genome sequence information is now become available for *in silico* studies. *P. falciparum* parasite is responsible for majority of malaria related mortality. The *P. vivax* is also an important human parasite and is less virulent than *P. falciparum* but it is more widespread [4,5]. The infecting strategy of both the parasites is quite different, *P. falciparum* infects both the reticulocytes and mature RBC while *P.vivax* infection is restricted to reticulocytes only. The *P. vivax* species is less studied in comparison with its counterpart due to difficulties in continuous culturing of this parasite in laboratory. The genome sequences of *Plasmodium* parasites infecting rodents were also available [6,7]. These parasites are *P. chabaudi*, *P. berghei* and *P. yoelii*. The genome characteristics based codon usage study of six *Plasmodium* species shows that codon usage variation in the three rodent parasites, *P. berghei*, *P. chabaudii* and *P. yoelli* is strikingly similar to that of *P. falciparum*. The simian and human malarial parasite, *P. knowlesi* shows a variation similar to *P.vivax* [8]. The study of these parasites along with *P. falciparum* and *P. vivax* will explore new dimensions for combating against malaria.

COMPARATIVE MALARIAL GENOMICS STUDY

The advent of Next-Generation Sequencing (NGS) gives a paradigm shift in the way researchers extract genetic information from any species by deciphering their DNA sequences [9]. Due to recent advancement in sequencing strategies, a number of genome sequences of *Plasmodium* species are available. The availability of these genome sequences facilitate the comparison of various *Plasmodium* species on the whole genome basis and can have deep insight into the disease mechanism. The comparison of *Plasmodium* genome with other species helps in identifying regions of similarity and differences between them. This information can enhance our understanding of structure and function of genes and thereby helpful in developing new strategies to combat malaria. Comparative genomics is not only helpful in identifying conserved genes among species, but also provides important knowledge for those genes that are responsible for unique characteristics of a particular *Plasmodium* species. The genome sequences of *Plasmodium* species reveal an apparent picture about evolutionary history of these pathogens. *P. falciparum* is the most lethal malaria causing species in human host and our knowledge of comparative genomics increases a step forward with the completion of its genome. The size of *P. falciparum* genome is 22.8 Megabase (Mb) and is divided into 14 chromosomes [5]. The size of all 14 chromosomes varies from approximately 0.643-3.29 Mb. All the chromosomes not only vary in length but they also show difference in their genomic composition. Most of these variations can be explained in terms of their AT content as their genome shows highest AT richness (around 80%) among all *Plasmodium* species sequenced till date. This AT richness also varies and depends on chromosome location and length of a particular chromosome. The chromosomal sub-telomeric regions are rich in AT content and show high level of sequence variation. Although sub-telomeric regions of chromosomes vary in length and nucleotide distribution, but they show high degree of conservation within the genome of different *Plasmodium* species. This conservation is most probably due to inter-chromosomal exchange of sub-telomeric regions [10]. The nuclear genome of *P. falciparum* encodes approximately 5,300 genes and these are distributed all over the genome. The genes that lie in the sub-telomeric and telomeric regions provide the basis for species specific antigenic variations. So, an in-depth analysis of structure and functional properties of sub-telomeric and telomeric regions is a prerequisite for understanding the underlying mechanism of antigenic diversity. *P. falciparum* contains three highly variable gene families at the telomeric regions which are known to be involved in evasion of the host immune system. The gene families include *var*, *rif* and *stevor* that encode for *P. falciparum* erythrocyte membrane protein 1(*Pf*EMP1), repetitive interspersed family (rifin) and sub-telomeric variable open reading frame (stevor) respectively [11]. The *var* genes encoded *Pf*EMP1 protein is exported, and expressed at the surface of an infected RBC and gets attached to the receptors of endothelial cells This phenomenon of attachment is known as cytoadherence and is observed only in *P. falciparum*. This cytoadherence property results in sequestration of infected RBC into deep tissues and thus help them to evade from splenic clearance. *Pf*EMP1 is an immunogenic protein but transcriptional switching between different *var* genes is responsible for antigenic variation which provides basis for

immune evasion. The stevor proteins are also expressed at the surface of host RBC and undergo antigenic variation. The stevor protein shows sequence similarity with rifin proteins but they are less polymorphic, and both proteins are involved in host-parasite interactions [12]. *P. vivax* is the most widely distributed and the second most lethal malaria causing species of the human host. *P. vivax* is a dominant malaria causing species outside Africa because of its restricted infection of Duffy-blood group positive reticulocytes, and causes relapses of fever in infected individuals of all age group. The hypnozoite, a dormant stage of *P. vivax*, can remain inactive for months or years in hepatocyte cells (liver cells) of infected individuals and after activation leads to relapses of fever. The *P. vivax* parasite is among the least studied malaria parasite because it kills infrequently and it is very difficult to make continuous culture in the laboratory [13]. The 26.8 Mb nuclear genome of *P. vivax* Sal-1 has been sequenced by whole-genome shotgun methods and contains 14 chromosomes [4]. *P. vivax* nuclear genome is responsible for coding approximately 5,433 protein coding genes. *P. vivax* chromosomes show a unique pattern of GC distribution in the form of isochore structures. The chromosomal internal regions have GC-rich isochores and a high AT content isochors are present in sub-telomeric regions. Till date, this is the most GC-rich (~42.3%) genome of *Plasmodium* parasite [4-6,14]. The sub-telomeric reigons of *P. vivax* contains a multigene family called *vir*. The copy number of *vir* genes per haploid genome is around 600-1,000 and encodes proteins that were responsible for antigenic variation [15]. *P. knowlesi* is a primate malaria parasite whose natural host is long-tailed macaques (Macaca fascicularis) and recently it has been recognised as a human infecting pathogen, particularly in Southeast Asia. *P. knowlesi* is the first *Plasmodium* species in which the antigenic variation is responsible for the host immune evasion was studied [16]. The variant gene families of *P. knowlesi* like SICA*var* and *kir* are found at intra-chromosomal sites and are randomly distributed across all 14 chromosomes. The SICA*var* gene family is the largest variant gene family of *P. knowlesi* and encodes Variant SICA (schizont-infected cell agglutination) antigens that are expressed at the surface of infected erythrocytes and are responsible for its virulence property [17]. The *kir* represents the second largest variant gene family and their protein products are most probably expressed at the surface of infected erythrocytes and undergoes antigenic variation [18]. *P. knowlesi* shares common phylogenetic clade with its closely related human malaria parasite *P. vivax* [19]. The chromosomes of both the species are highly syntenic and their chromosome architecture show relatedness to ancestral *Plasmodium* genome [14]. Although *P. knowlesi* shows similarity with *P. vivax*, it shows key biological differences that include differences in their host range, duration of asexual cycle and preference to the host blood cell. The merozoites of *P. knowlesi* are more stable than other *Plasmodium* parasites, so it can serve as an excellent *in vivo* model for investigating the mechanism of parasite invasion in erythrocyte cells. *P. falciparum* and *P. vivax* are the most important human infecting malaria causing *Plasmodium* pathogen. The genome of both the species shows differences in their nucleotide content: *P. falciparum* having lowest GC content and *P.vivax* have highest GC-rich genome among all *Plasmodium* species. Although the ecological habitat of all *Plasmodium* species is different; but their genes show high degree of conservation according to their location on genome. The genes located near the centromere are somewhat more conserved than others. The genes present in the core regions of *P. falciparum* chromosomes are orthologous in all *Plasmodium* species including rodent parasites, *P. vivax* and *P. knowlesi* [20]. These highly conserved genes code for the *Plasmodium* metabolome including essential metabolic pathways, housekeeping functions and the repertoire of membrane transporters. Genes located near the telomeric and sub-telomeric regions of chromosomes are highly variable and shows divergence in their structure and functions in all the studied *Plasmodium* species. These non-orthologous genes are species specific and show variability because different *Plasmodium* species expresses different type of antigenic variation. The non-orthologous genes in *P. falciparum* are dominated by *var*, *stevor* and *rifin* gene families. The rodent malaria parasite *P. yoelii* harbours a largest multigene family called *yir* gene family at the sub-telomeric regions of chromosomes. This *yir* gene family is not only conserved in all rodent parasites but also in human parasite i.e. *P. vivax* except *P. falciparum* [21]. Malaria parasite encapsulates a relict plastid called 'apicoplast', which arises through a process of secondary endosymbiosis, and shows homology with the chloroplast of plants and algae. Apicoplast possesses its own circular genome and is semi-autonomous in nature. The apicoplast genome has lost its photosynthetic function, but is essential for parasite survival and known to function in synthesis of the fatty acids, isoprenoids and haem [22]. The apicoplast synthesizes limited amount of proteins and other essential proteins are supplemented to it by nuclear coded genes. These nuclear encoded proteins are post-transcriptionally targeted to apicoplast organelle. These transported proteins contain conserved bipartite signals having an amino-terminal secretory signal sequence followed by a plastid transit peptide [23]. Most of these transported proteins are conserved in both, *P. falciparum* and *P. vivax*, and provide a basis for identifying potential drug targets as this organelle is absent in human host. Different organism shows a wide range of variability in their genome size. There is a correlation between genome size and increase in complexity of genome in terms of number of genes for prokaryotic genomes. While in eukaryotes, the genome size considerably increases rapidly in comparison with number of genes. The large genome size of eukaryotic genome is mostly due to vast expansion of non-coding part along with the repetitive DNA sequences. The repetitive sequences can be divided into interspersed repeats and tandem repeats. Interspersed repeats are those repeat sequences that are ubiquitously and randomly distributed all over the genome. Interspersed repeats also known as mobile genetic elements (MGE's), move around the genome by multiplying via RNA or DNA intermediates. These mobile genetic elements are considered as a key role player in evolution of gene and genomes. Genome analysis of three *Plasmodium* species

namely *P. falciparum*, *P. yoelii* and *P. vivax* shows that number of MGE's is lowest in case of *P. falciparum* in comparison to other two species [24]. This is due to presence of inherent resistance to MGE invasion in *P. falciparum*. Consequently, it can be speculated that MGE's may be considered as an important driving force for shaping high AT-compositional bias of *P. falciparum* genome. Tandem repeats are contiguous repeats present all over the genome and can be of macrosatellites, minisatelites and microsatellites types. Tandem repeats arise due to unequal recombination rates and slippage-strand replication [25]. The overall number of tandem repeats is high in case of *P. falciparum* compared to *P. vivax* and shows significant positive correlation with their respective chromosome length. The detailed study shows that number of tandem repeats per unit length of chromosome is high in *P. falciparum* to *P. vivax* [26]. The distribution of tandem repeats in human infecting *Plasmodium* pathogen may be related with disease severity and is one of the important subjects of study.

CURRENT STATUS AND NEW DEVELOPMENTS OF ANTIMALARIAL DRUGS:

Malaria remains one of the greatest threats for mankind. Continued and sustainable improvements in antimalarial medicines through focused research and development are essential for the world's future ability to treat and control malaria. Traditionally, the drugs against malaria is identified and developed empirically and more often by serendipity like quinine which is the earliest drug used effectively since 16th century. Quinine is obtained from the bark of Cinchona tree. Nowadays, advancement of biological sciences and availability of genome sequences of host and parasite have made drug discovery and development process more rational and systematic. Chloroquine is the most successful drug for the treatment and prophylaxis of malaria [27]. It is a safe and affordable drug, and it was effective before resistant strains began to emerge in the 1960s. Due to emergence of resistant strains of parasites against most commonly used drugs like chloroquine has made it ineffective. The ability of parasite strain to develop and multiply in presence of a drug that normally kills the parasite is known as drug resistance. Chloroquine resistance in *P. falciparum* is responsible for increase in cases of mortality and morbidity. Antimalarial drugs can be basically classified in to seven classes namely: 4-Aminoquinolines, Arylaminoalcohols, 8-Aminoquinolines, Artemisinines, Antifolates, Inhibitors of the respiratory chain, and Antibiotics. 4-Aminoquinolines classes of drugs possess amino group at fourth position of quinoline. Derivatives of 4-Aminoquinolines like chloroquine, amodiaquine (Fig 1) shows antimalarial activity and interact with the ferriprotoporphyrine IX (FPPIX) and prevent its polymerization into non-toxic haemozoin. FPPIX is an intermediate compound, toxic to parasite, formed during digestion of haemoglobin by parasite [28]. Resistance against 4-aminoquinolines develops by point mutations in *Pf*CRT (chloroquine resistance transporter), including the mutation of Lysine to Threonine (K76T) in the transmembrane domain and results in the removal of 4-aminoquinolines from the digestive vacuole [29]. Chloroquine and Amodiaquine are well-established antimalarial drugs that are included in this class. The massive use of chloroquine drug results in the development of resistant malaria in different malaria endemic regions. The use of chloroquine in treating *P. falciparum* has declined in most of the malaria-endemic countries, although it remains effective against *P. ovale*, *P. malariae*, and *P. vivax* [30]. Amodiaquine, the chloroquine analogue with aromatic moieties in the side chain, is effective against low-level chloroquine-resistant *P. falciparum* but not against highly chloroquine-resistant parasites [31]. Arylaminoalcohol class of drugs include Quinine, Mefloquine, Halofantrine and Lumefantrine (Fig 1). The mode of action of arylaminoalcohols is still not clear but they are supposed to interfere with the haem digestion. The strong association between single nucleotide polymorphism of *Pfmdr*1 genes and arylaminoalcohol drug response may be responsible for drug resistance [32]. *Pfmdr*1 gene codes a transporter protein, called *Pf*MDR1 (*P. falciparum* multi-drug resistance 1), located in the membrane of digestive vacuole. This protein may be responsible for efflux of arylaminoalcohol drugs from the digestive vacuole. 8-Aminoquinolines is a class of drug that acts on different targets as compared with drugs of aminoquinolines and arylaminoalcohols class (Fig 1). It is active against liver and the sexual blood stages of different *Plasmodia*. Primaquine is the only 8-aminoquinoline class of drug that is licensed for the radical cure of *P. vivax* and *P. ovale* infections. At this point, how primaquine acts against the erythrocytic form of the malaria parasite is not well understood. If primaquine is given alone, the cure rate is only 20%. So primaquine dosage is given along with the quinine or chloroquine for getting radical cure [33]. Primaquine also shows serious side effects in humans with glucose-6-phosphate-dehydrogenase (G6PD) deficiency and leads to a potentially life-threatening haemolysis [34]. The 8-amino substituent has been the main cause of antiplasmodial activity but also in the formation of methaemoglobin. Artemisinins are extracted from sweet wormwood herb (*Artemisia annua*) of China (Fig 1). The most widely used artemisinin derivatives, artemether and artesunate, are hemisynthetic derivatives of artemisinin (the active ingredient of the herb, sweet wormwood). The endoperoxide of artemisinin may be cleaved by intraparasital iron-II sources to yield carbon-centered radicals. The exact mechanism of these radicals is still in debate but there is a possibility that these radicals are involved in modifying the multiple targets like proteins and haem in the digestive vacuole unspecifically [35] or may specifically inhibit an endoplasmic reticulum-located calcium pump (PfATP6) [36]. Artemesinin drugs are potent antimalarials that can act against both, the late ring stages as well as the small ring stages present in the erythrocytes [37]. Artemisinins are highly active drugs and can decrease the parasite biomass up to 10000-fold in a single asexual cycle [38]. This makes artemisinins the most active and rapid-acting antimalarial drugs known today. Artemisinin-derived drugs are semi-synthetic and their production mainly relies on limited supply of artemisinin extracted from plants, natively cultivated in China and Vietnam. Due

to the growing need resulted from the increased adaptation of Artemisinin derivatives by more and more countries, the raw material is already in short supply, so we have to decrease our dependency and in parallel, search for other antimalarial alternatives. Antifolates class of drugs act against two enzymes of the biosynthesis of tetrahydrofolate, the dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR) of *Plasmodia* (Fig 1). The combination of the sulfonamide sulfadoxine with the DHFR inhibitor pyrimethamine, known under its brand name Fansidarm, replaced chloroquine as first line antimalarial therapy [39]. Several years ago, the prophylactic use of this combination was avoided in most countries because its long term use can increase the risk of agranulocytosis and toxic epidermal necrolysis. The spread of resistance strains with mutated *dhfr* and *dhps* genes is responsible for discarding it in many regions. Despite its limited efficacy, it is still widely used in Africa in combination with chloroquine, amodiaquine, or artesunate because of its low price. The combination of dapsone (DDS) and chlorproguanil is also antifolate class of drugs that is introduced in the market under the trade name LapDap [40]. The chloroguanil drug is similar to proguanil and both metabolize in to the active component chlorcycloguanil (CCG) by an oxidative ring closure. DDS/CCG drug is active against the triple mutant DHFR (Dihydrofolate reductase) *Plasmodia* strains, which are primarily seen in Africa so far, but it is inactive against strains harbouring the quadruple mutant abundant in Asia and South America [41]. But this critical I164L mutation has already been recorded from different locations of Africa and extensive use of DDS/CCG combination may further spread the quadruple mutations. Inhibitors of the respiratory chain include atovaquone, buparvaquone, derivatives of 3-methoxyacrylic acid and other compounds (Fig 1). The antimalarial drug atovaquone is responsible for disrupting the membrane potential across mitochondrial membrane and thus, results in inhibition of mitochondrial electron transport chain. This drug blocks the movement of an iron-sulfur cluster containing a protein domain by binding on ubiquinone binding site of the cytochrome bc-1 complex [42]. The use of single drug atovaquone results in the development of resistant strains. In such resistant strains the ubiquinine binding site is altered and the sensitivity of the cytochrome bc1 complex towards atovaquone is reduced upto 1000 folds [43]. Due to the rapid development of resistance, the use of mono-drug atovaquone is unsuitable for the therapy and prophylaxis of malaria. Fortunately, the non-metabolized proguanil is responsible for the synergism with atovaquone. Proguanil itself has no measurable effect on the mitochondrial membrane potential, but it decreases the concentration of atovaquone, necessary to dismantle the membrane potential. The combination of atovaquone and proguanil under the trade name malarone is used for prophylaxis and therapy of uncomplicated malaria [44]. However, the selection of resistant strains against this combination therapy is reduced, but once a strain becomes resistant to atovaquone, it also become resistant to its combination with proguanil. To protect the atovaquone/ proguanil combination from emerging resistance, it has been successfully combined with artesunate [45] for the treatment of uncomplicated malaria. Antibiotics, the anti-bacterial agents, are known to specifically target prokaryotic structures but some agents also display antimalarial activity (Fig 1). This can be explained by the presence of two prokaryotic origin organelles, the mitochondrion and the apicoplast. Typically, most of the antibiotics do not exert any noticeable effect in the first intracellular cycle, but the parasites are killed after invading the new host cell during the second cycle. This phenomenon is known as “delayed death phenotype” or “delayed kill effect” [46]. The antibiotics like clindamycin, chloramphenicol, and tetracycline, inhibitors of prokaryotic protein synthesis, invoke the delayed death phenotype in *P. falciparum*. If antibiotics are given as a mono-drug then due to delayed kill effect, fever and parasite clearance times become significantly longer than they are with classical anti-malarials (approximately 4 versus 2 days). This delay may be fatal in non-immune patients, so antibiotics are used only in combination with a faster-acting drug (quinine, artesunate, or fosmidomycin) for the therapy of acute malaria. The classical faster acting antimalarials reduce the parasite burden quickly while the antibiotic deals with that parasite strain that shows less sensitivity against classical antimalarials. Till date, no clinically relevant resistance of malaria parasites against antibiotics has been reported. Doxycyclin, the most widely used antibiotic of the tetracycline class, is used in combination with quinine or artesunate for treating uncomplicated as well as severe malaria [47]. This doxycyclin combination cannot be given to children under the age of eight or pregnant women due to its incorporation in developing bones and teeth. The clindamycin drug is used as an alternative to doxycyclin, so combination of clindamycin and faster acting antimalarial is given to children and pregnant women [48]. Artemisinin-based combination therapy (ACT) has been the main source of treatment for *falciparum* malaria in Southeast Asia for more than 10 years and is now increasingly recommended as first-line treatment throughout the rest of the world. The main concern for widespread deployment of ACT is its high cost and development of resistant strains. Since the current therapy mainly relies on ACT, the emergence and subsequent spread of resistance against artemisinins would result in a disaster. Nowadays, cases of malaria infections are on the rise and have reached record numbers. The problem of drug resistance is global for malaria, so in order to combat this problem a repertoire of new antimalarials with novel mechanism of actions is in urgent need.

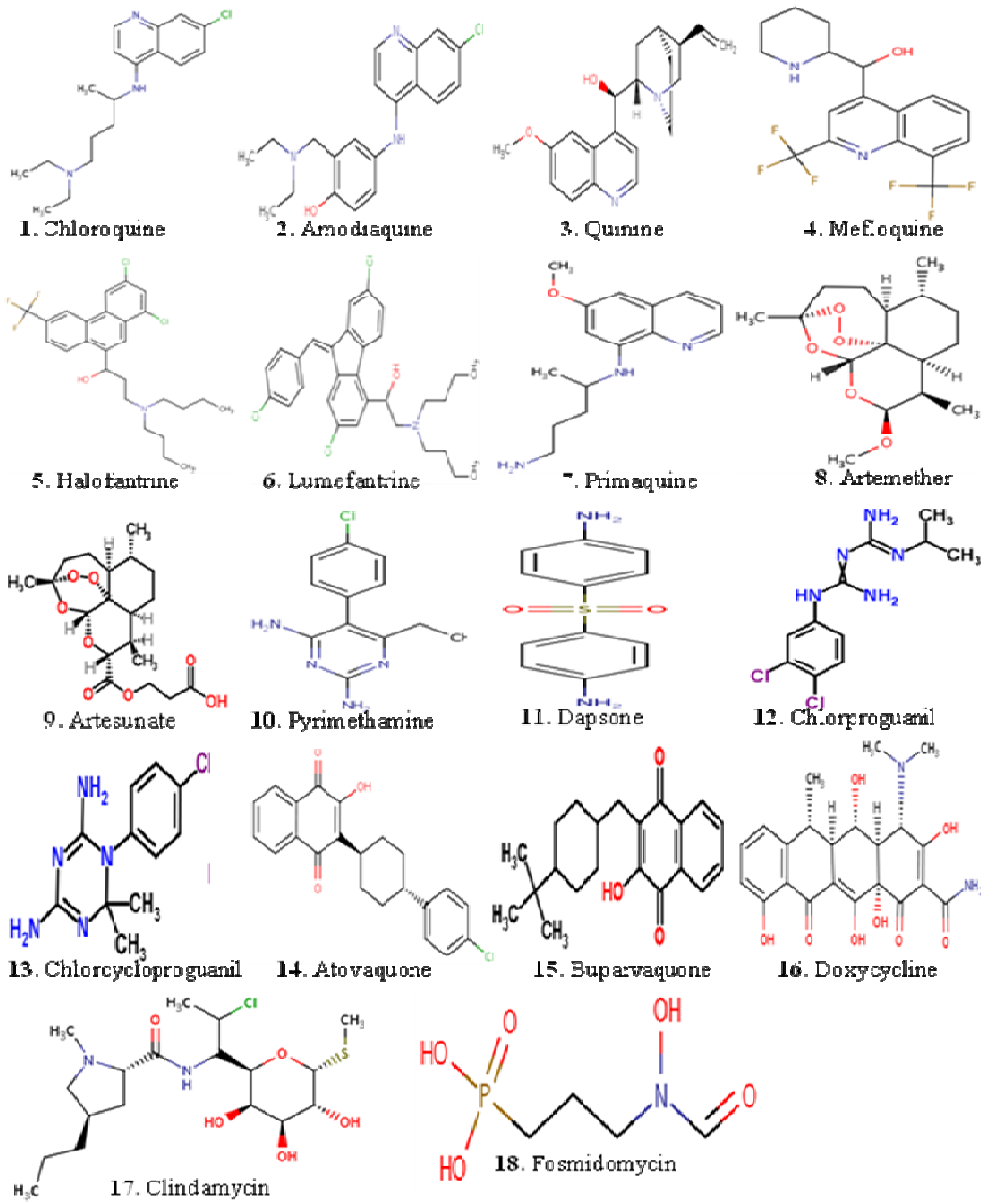

Figure 1

Structure of different classes of antimalarials; 4-Aminoquinolines (1-2), Arylaminoalcohols (3-6), 8-Aminoquinolines (7-9), Antifolates (10-13), Inhibitors of the respiratory chain (14-15) and Antibiotics (16-18).

Nowadays drugs used against traditional drug targets were showing resistance against the disease, so one have to identify new drug targets of *Plasmodium* species using available genome information. Recently ferroquine, a derivative of chloroquine, shows antimalarial properties. Recent work shows high efficacy of ferroquine and its derivatives like hydroxyferroquines, trioxaferroquines, chloroquine-bridged ferrocenophanes, thiosemicarbazone derivatives, ferrocene dual conjugates, 4-N-substituted derivatives as antimalarial compounds[49]. Identification of new drug targets and drugs will surely enhance our capability to combat the malaria.

CONCLUSION

This review will enhance the individual knowledge and develop deep understanding about the current challenges for disease eradication. Worldwide, basically in tropical and sub tropical regions, malaria remains the most frequent and important infectious disease causing morbidity and death. World Health organization has taken multidimensional strategy to curb the morbidity, mortality, and disability due to malaria. A lot of drugs were available in the market to inhibit disease, but majority of them become less effective in present or become ineffective due to development of resistance. So, we

must have to develop deep understanding about genomics and proteomics of malaria pathogen before going to develop any drug or vaccine.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Goswami P., Murty U.S., Mutheneni S.R., Krishnan S.T. Relative roles of weather variables and change in human population in malaria: comparison over different states of India. PLoS One, 9 (6): e99867, (2014).
2. Paaijmans K., Blanford J., Crane R., Mann M.E., Ning L. Schreiber K.B., Thomas M.B., Downscaling reveals diverse effects of anthropogenic climate warming on the potential for local environments to support malaria transmission. Climatic Change, 125 (3-4), 479-488, (2014).
3. Antinori S., Galimberti L., Milazzo L. Corbellino M., *Plasmodium knowlesi*: the emerging zoonotic malaria parasite. Acta Trop, 125 (2): 191-201, (2013).
4. Carlton J.M., Adams J.H., Silva J.C., Bidwell S.L., Lorenzi H., Caler E., Crabtree J., Angiuoli S.V., Merino E.F., Amedeo P., Cheng Q., Coulson R.M.R., Crabb B.S., del Portillo H.A., Essien K., Feldblyum T.V., Fernandez-Becerra C., Gilson P.R., Gueye A.H., Guo X., Kang/'a S., Kooij T.W.A., Korsinczky M., Meyer E.V.S., Nene V., Paulsen I., White O., Ralph S.A., Ren Q., Sargeant T.J., Salzberg S.L., Stoeckert C.J., Sullivan S.A., Yamamoto M.M., Hoffman S.L., Wortman J.R., Gardner M.J., Galinski M.R., Barnwell J.W., Fraser-Liggett C.M. Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. Nature, 455 (7214):757-763, (2008).
5. Gardner M.J., Hall N., Fung E., White O., Berriman M., Hyman R.W., Carlton J.M., Pain A., Nelson K.E., Bowman S., Paulsen I.T., James K., Eisen J.A., Rutherford K., Salzberg S.L., Craig A., Kyes S., Chan M.-S., Nene V., Shallom S.J., Suh B., Peterson J., Angiuoli S., Pertea M., Allen J., Selengut J., Haft D., Mather M.W., Vaidya A.B., Martin D.M.A., Fairlamb A.H., Fraunholz M.J., Roos D.S., Ralph S.A., McFadden G.I. Cummings L.M., Subramanian G.M., Mungall C., Venter J.C., Carucci D.J., Hoffman S.L., Newbold C., Davis R.W. Fraser C.M., Barrell B., Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature, 419 (6906): 498-511, (2002).
6. Carlton J.M., Angiuoli S.V., Suh B.B., Kooij T.W., Pertea M., Silva J.C., Ermolaeva M.D., Allen J.E., Selengut J.D., Koo H.L., Peterson J.D., Pop M., Kosack D.S., Shumway M.F., Bidwell S.L., Shallom S.J., van Aken S.E., Riedmuller S.B., Feldblyum T.V., Cho J.K., Quackenbush J., Sedegah M., Shoaibi A., Cummings L.M., Florens L., Yates J.R., Raine J.D., Sinden R.E., Harris M.A., Cunningham D.A., Preiser P.R., Bergman L.W., Vaidya A.B., van Lin L.H., Janse C.J., Waters A.P., Smith H.O., White O.R., Salzberg S.L., Venter J.C., Fraser C.M., Hoffman S.L., Gardner M.J., Carucci D.J. Genome sequence and comparative analysis of the model rodent malaria parasite *Plasmodium yoelii yoelii*. Nature, 419 (6906): 512-519, (2002).
7. Hall N., Karras M., Raine J.D., Carlton J.M., Kooij T.W.A., Berriman M., Florens L., Janssen C.S., Pain A., Christophides G.K., James K., Rutherford K., Harris B., Harris D., Churcher C., Quail M.A., Ormond D., Doggett J., Trueman H.E., Mendoza J., Bidwell S.L., Rajandream M.-A., Carucci D.J., Yates J.R., Kafatos F.C., Janse C.J., Barrell B., Turner C.M.R., Waters A.P., Sinden R.E. A Comprehensive survey of the *Plasmodium* life cycle by genomic, transcriptomic, and proteomic analyses. Science, 307 (5706): 82-86, (2005).
8. Yadav M.K., Swati D. Comparative genome analysis of six malarial parasites using codon usage bias based tools. Bioinformation, 8 (24): 1230-1239, (2012).
9. Zhang J., Chiodini R., Badr A., Zhang G. The impact of next-generation sequencing on genomics. J Genet Genomics, 38 (3):95-109, (2011).
10. Figueiredo L.M., Freitas-Junior L.H., Bottius E., Olivo-Marin J.C., Scherf A. A central role for *Plasmodium falciparum* subtelomeric regions in spatial positioning and telomere length regulation. EMBO J, 21 (4): 815-824, (2002).
11. Flick K., Chen Q. var genes, PfEMP1 and the human host. Mol Biocheml Parasit, 134 (1):3-9, (2004).
12. Kyes S., Horrocks P., Newbold C. Antigenic variation at the infected red cell surface in malaria. Annu Rev Microbiol, 55 (1):673-707, (2001).
13. Luo Z., Sullivan S.A., Carlton J.M. The biology of *Plasmodium vivax* explored through genomics. Ann N Y Acad Sci, 1342: 53-61, (2015).
14. Pain A., Bohme U., Berry A.E., Mungall K. Finn R.D., Jackson A.P., Mourier T., Mistry J., Pasini E.M., Aslett M.A., Balasubrammaniam S., Borgwardt K., Brooks K., Carret C., Carver T.J., Cherevach I., Chillingworth T., Clark T.G., Galinski M.R., Hall N., Harper D., Harris D., Hauser H., Ivens A., Janssen C.S., Keane T., Larke N., Lapp S., Marti M., Moule S., Meyer I.M., Ormond D., Peters N., Sanders M., Sanders S., Sargeant T.J., Simmonds M., Smith F., Squares R., Thurston S., Tivey A.R., Walker D., White B., Zuiderwijk E., Churcher C., Quail M.A., Cowman A.F., Turner C.M.R., Rajandream M.A., Kocken C.H.M., Thomas A.W., Newbold C.I., Barrell B.G., Berriman M., The genome of the simian and human malaria parasite *Plasmodium knowlesi*. Nature, 455 (7214): 799-803, (2008).

15. del Portillo H.A., Fernandez-Becerra C., Bowman S., Oliver K., Preuss M., Sanchez C.P., Schneider N.K., Villalobos J.M., Rajandream M.-A., Harris D., da Silva L.H.P., Barrell B., Lanzer M. A superfamily of variant genes encoded in the subtelomeric region of *Plasmodium vivax*. Nature, 410 (6830):839-842, (2001).
16. Ramasamy R. Zoonotic malaria–global overview and research and policy needs. Front public health, 2:123, (2014).
17. Frech C., Chen N. Variant surface antigens of malaria parasites: functional and evolutionary insights from comparative gene family classification and analysis. BMC Genomics, 14: 427, (2013).
18. Janssen C.S., Phillips R.S., Turner C.M., Barrett M.P. *Plasmodium* interspersed repeats: the major multigene superfamily of malaria parasites. Nucleic Acids Res, 32 (19): 5712-5720, (2004).
19. Perkins S.L., Schall J. A molecular phylogeny of malarial parasites recovered from cytochrome b gene sequences. J Parasitol, 88 (5): 972-978, (2002).
20. Liew K., Hu G., Bozdech Z., Peter P. Defining species specific genome differences in malaria parasites. BMC Genomics, 11 (1): 1-14, (2010).
21. Janssen C.S., Barrett M.P., Turner C.M., Phillips R.S. A large gene family for putative variant antigens shared by human and rodent malaria parasites. Proc Biol Sci, 269 (1489): 431-436, (2002).
22. Lim L., McFadden G.I. The evolution, metabolism and functions of the apicoplast. Philos Trans R Soc Lond B Biol Sci, 365 (1541):749-763, (2010).
23. Tomavo S., Slomianny C., Meissner M., Carruthers V.B. Protein trafficking through the endosomal system prepares intracellular parasites for a home invasion. PLoS Pathog, 9 (10): e1003629, (2013).
24. Durand P., Oelofse A., Coetzer T. An analysis of mobile genetic elements in three *Plasmodium* species and their potential impact on the nucleotide composition of the *P. falciparum* genome. BMC Genomics, 7 (1):282, (2006).
25. Levinson G., Gutman G.A. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol Biol Evol, 4 (3), 203-221 (1987).
26. Yadav M.K., Mishra A., Swati D. Comparative genomics and phylogenetic study of *Plasmodium falciparum* and *Plasmodium vivax*. Int J BioSc Technol, 3 (4): 46-54, (2010).
27. Ridley R.G. Medical need, scientific opportunity and the drive for antimalarial drugs. Nature, 415 (6872): 686-693, (2002).
28. Manohar S., Tripathi M., Rawat D.S. 4-Aminoquinoline Based Molecular Hybrids as Antimalarials: An Overview. Curr TopMed Chem, 14 (14): 1706-1733, (2014).
29. Ley B., Wernsdorfer G., Frank C. Sirichaisinthop J., Congpuong K., Wernsdorfer W.H., Pharmacodynamic interaction between 4-aminoquinolines and retinol in *Plasmodium falciparum* in vitro. Wien Klin Wochenschr, 120 : 74-79, (2008).
30. White N.J. The Treatment of Malaria. N Engl J Med, 335 (11): 800-806, (1996).
31. O'Neill P.M., Ward S.A., Berry N.G. Jeyadevan J.P., Biagini G.A., Asadollaly E., Park B.K., Bray P.G., A medicinal chemistry perspective on 4-aminoquinoline antimalarial drugs. Curr Top Med Chem, 6 (5):479-507, (2006).
32. Duraisingh M., Refour P. Multiple drug resistance genes in malaria – from epistasis to epidemiology. Mol Microbiol, 57 (4): 1365-2958 (2005).
33. Álvarez G., Pineros J.G., Tobon A., Rios A., Maestre A., Blair S. Carmona-Fonseca J., Efficacy of three chloroquine-primaquine regimens fro treatment of *Plasmodium vivax* malaria in Colombia.Am J Trop Med Hyg, 75 (4):605-609, (2006).
34. AlKadi H.O. Antimalarial Drug Toxicity: A Review. Chemotherapy, 53 (6): 385-391, (2007).
35. Haynes R.K., Krishna S. Artemisinins: activities and actions. Microbes Infect, 6 (14): 1339-1346, (2004).
36. Eckstein-Ludwig U., Webb R.J., Van Goethem I.D., East J.M., Lee A.G., Kimura M., O'Neill P.M., Bray P.G., Ward S.A., Krishna S. Artemisinins target the SERCA of *Plasmodium falciparum*. Nature, 424 (6951): 957-961, (2003).
37. Chotivanich K., Sattabongkot J., Udomsangpetch R., Looareesuwan S., Day N.P., Coleman R.E., White N.J. Transmission-blocking activities of quinine, primaquine, and artesunate. Antimicrob Agents Chemother, 50 (6): 1927-1930, (2006).
38. White N.J. Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. Antimicrob Agents Chemother, 41 (7): 1413-1422, (1997).
39. Baird J.K. Effectiveness of Antimalarial Drugs. N Engl J Med., 352 (15): 1565-1577, (2005).
40. Lang T., Greenwood B. The development of Lapdap, an affordable new treatment for malaria. Lancet Infect Dis, 3 (3): 162-167, (2003).
41. McCollum A.M., Poe A.C., Hamel M., Huber C., Zhou Z., Shi Y.P., Ouma P., Vulule J. Bloland P., Slutsker L., Barnwell J.W., Udhayakumar V., Escalante A.A., Antifolate Resistance in *Plasmodium falciparum*: Multiple Origins and Identification of Novel dhfr Alleles. J Infect Dis, 194 (2):189-197, (2006).
42. Vaidya A.B. Mitochondrial and plastid functions as antimalarial drug targets. Curr Drug Targets Infect Disord, 4 (1): 11-23, (2004).
43. Nixon G.L., Moss D.M., Shone A.E., Lalloo D.G., Fisher N., O'Neill P.M., Ward S.A., Biagini G.A. Antimalarial pharmacology and therapeutics of atovaquone. J Antimicrob Chemother, 68 (5): 977-985, (2013).
44. Patel S.N., Kain K.C. Atovaquone/proguanil for the prophylaxis and treatment of malaria. Expert Rev Anti Infect Ther 3 (6): 849-861, (2005).
45. Van Vugt M., Leonardi E., Phaipun L., Slight T., Thway K.L., McGready R., Brockman A., Villegas L., Looareesuwan S., White N.J., Nosten F.

Treatment of uncomplicated multidrug-resistant *falciparum* malaria with artesunate-atovaquone-proguanil. Clin Infect Dis, 35 (12): 1498-1504, (2002).

46. Ramya T.N., Mishra S., Karmodiya K., Surolia N., Surolia A. Inhibitors of nonhousekeeping functions of the apicoplast defy delayed death in *Plasmodium falciparum*. Antimicrob Agents Chemother, 51 (1):307-316, (2007).
47. Ashley E.A., White N.J. Artemisinin-based combinations. Curr Opin Infect Dis, 18 (6): 531-536, (2005).
48. Lalloo D.G., Shingadia D., Pasvol G., Chiodini P.L., Whitty C.J., Beeching N.J., Hill D.R., Warrell D.A., Bannister B.A. UK malaria treatment guidelines. J Infect, 54 (2): 111-121, (2007).
49. Wani W.A., Jameel E., Baig U. Mumtazuddin S., Hun L.T., Ferroquine and its derivatives: New generation of antimalarial agents. Eur J Med Chem, 101:534-551, (2015).