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Review

The Human Genetics of Malaria

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Abstract

Human genetic resistance to malaria is the inherited changes changes in the human DNA that increase resistance to malaria and lead to increased survival of people with these genetic changes. The evolutionary pressure exerted by the malaria parasite is likely what led to the existence of these genotypes. The impact of host genetics on susceptibility to *Plasmodium falciparum* malaria has been widely studied over the past twenty years. It is now clear that the malaria *Plasmodium* parasites have imposed strong selective forces on the human genome in endemic regions. Different genes associated with different malaria-related phenotypes have been identified. Recent developments in human genome research technologies, like genome-wide association studies and genotyping tools, have enabled the discovery of several genetic polymorphisms and biomarkers. This review describes and analyzes the human gene polymorphisms that have been revealed to be associated with resistance to *P. falciparum* malaria. Although some polymorphisms play important roles in susceptibility to malaria, several discoveries are inconclusive and conflicting and should be carefully examined. The discovery of genetic polymorphisms associated with resistance to malaria will enable the development of interventions or cures for the malaria disease.

Keywords: *Plasmodium falciparum*, Human genetic factors, Red blood cell disorders, metabolic enzymes, Inflammatory response genes, Genetic polymorphism.

Резюме

Генетичната резистентност към маларията е свързана с унаследени промени в човешката ДНК, които повишават резистентността към това заболяване и водят до по-висока преживяемост при хора с тези генетични промени. Еволюционният натиск, упражняван от маларийния паразит, вероятно е довел до съществуването на подобни генотипове. Влиянието на генетиката на гостоприемника върху възприемчивостта към малария от *Plasmodium falciparum* е широко проучвана през последните двадесет години. Вече е ясно, че маларийните паразити от род *Plasmodium* са наложили силен селективен натиск върху човешкия геном в редица ендемични региони. Идентифицирани са гени, свързани с различни фенотипове за маларията. Неотдавнашното развитие на технологиите за изследване на човешкия геном, като например проучванията на асоциациите в целия геном и използването на съвременен инструментариум за генотипиране, позволиха откриването на няколко генетични полиморфизми и биомаркери. В този обзор се описват и анализират полиморфизмите на човешки гени, за които е установено, че са свързани с резистентността към малария от *P. falciparum*. Въпреки че някои полиморфизми играят важна роля за чувствителността към маларията, публикувани са неубедителни и противоречиви резултати, които трябва да бъдат внимателно проучени. Получаването на данни за генетичните полиморфизми, свързани с резистентността към маларията, ще даде възможност за разработване на интервенции или лекарства срещу това заболяване.

Introduction

Plasmodium falciparum malaria is a major cause of mortality and morbidity, particularly in endemic areas of sub-Saharan Africa. The etiology of the disease is variable and is attributable to environmental factors, host genetics, and parasite virulence (Mackinnon *et al.,* 2005). The several variations of *P. falciparum* infections manifesting different phenotypes include hyper or asymptomatic parasitemia, severe malaria anemia, and cerebral malaria. Host genetic factors contribute

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to the variability of malaria phenotypes (Weatherall *et al.,* 2002), and should therefore help to determine some of the mechanisms involved in susceptibility to *P. falciparum* infection. Knowledge gained since the 1980s using molecular genetics approaches has provided undisputed evidence about polymorphisms associated with malaria resistance and their complex interactions. Several genetic mutations and polymorphisms in human hosts confer a survival advantage and have increased in frequency by natural selection over generations. These include sickle cell trait (HbAS) and hemoglobinopathies such as thalassaemias and glucose-6-phosphate dehydrogenase (G6PD) deficiency. The development of molecular biology technologies and the completion of the Human Genome Project have discovered other loci that appear to directly or indirectly influence malaria susceptibility by modulating the immune response or interfering with host-parasite interactions. This has provided an understanding of a dual process of natural selection and co-adaptation of polymorphisms that occur in the malaria parasite and its human host, to maintain genetic diversity.

Hence, the purpose of this review is to describe and analyze the human gene polymorphisms that have been revealed to be associated with resistance to *P. falciparum* malaria. Although some polymorphisms play important roles in susceptibility to malaria, several discoveries are inconclusive and conflicting and should be carefully examined. The discovery of genetic polymorphisms associated with resistance to malaria will enable the development of interventions or cures to the malaria disease.

Human genetic factors associated with malaria

Red blood cells disorder

Sickle cell (HbS gene)

The HbS gene defect is a mutation of a single nucleotide (A to T) of the β-globin gene replacing the amino acid glutamic acid with the less polar amino acid valine at the sixth position of the β chain. Recent studies in West Africa suggest that the greatest impact of HbS appears to be to protect against either death or severe disease, that is, profound anemia or cerebral malaria while having little effect on infection. Children who are heterozygous for the sickle cell gene have only one-tenth of the risk of death from falciparum as compared to those who are homozygous for the normal hemoglobin gene. The binding of parasitized sickle erythrocytes to endothelial cells and blood monocytes is significantly reduced due to an altered display of *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), the parasite's major cytoadherence ligand and virulence factor on the erythrocyte surface (Cholera *et al.*, 2008). Protection also derives from the instability of sickle hemoglobin, which clusters the predominant integral red cell membrane protein (called band 3) and triggers accelerated removal by phagocytic cells.

A study by Archer *et al*. (2018) showed that impaired growth induced by HbS polymerization leads to a greater reduction in parasite proliferation than reduced cytoadherence, and proposed that HbS polymerization in infected erythrocytes sequestered in different human tissues with low oxygen levels is the primary protective mechanism against severe malaria in individuals with AS. iRBCs try to sequester themselves in hypoxic environments, including the bone marrow, brain, and liver, to prevent splenic clearance. The reduction of oxygen causes HbS to polymerize, resulting in inhibition of parasite growth since hemoglobin is inaccessible for protease digestion, a critical stage in parasite development during which essential amino acids are made available for protein expression, DNA replication, and parasite proliferation (Liu *et al.,* 2006).

Thalassemias (alpha and beta)

Thalassemia is an imbalance in the synthesis of the two polypeptide chains of hemoglobin. Hereditary hemoglobinopathy involves changes in the globin chains that make up the hemoglobin molecule. Four genes are needed (two from each parent) to make enough alpha globin protein chains while two genes are needed (one from each parent) to make enough beta globin protein chains. Alpha thalassemia occurs if one or more of the four genes are missing while beta thalassemia occurs if one or both genes are altered, thereby leading to an insufficient production of alpha and beta globin proteins respectively (NHLBI, 2022). The severity of alpha and beta thalassemia depends on how many of these genes are affected, the higher the number, the higher the symptom burden (NIH, 2018). The proposed mechanisms by which the alpha and beta hemoglobin mutations protect against malaria are classified as either immune-related or cellular. These include better immune clearance, decreased survival of malaria parasites inside red blood cells, and decreased parasite capacity for invading the red blood cells.

In 2008, the New York University School of Medicine and the University of Oxford, a joint team, working with children in Papua New Guinea who possess alpha thalassemia, found that their red blood cells were unusually small and more abun-

dant, leading to a mild form of anemia, compared with red blood cells of children without the genetic mutation that leads to the thalassemia. They then showed that alpha thalassemia had an advantage against malarial infection.

"There has never been a clinical study that has certainly shown that a beta thalassemia mutation is highly protective against malaria," Dr. Williams said, but researchers and clinicians do no doubt that these genes do protect against malaria, as RBC characteristics are the only traits that come up as positive in studies of malaria protection (Fowkes *et al.,* 2008).

HbC and HbEErythroids

Hemoglobin C (HbC) is an abnormal hemoglobin with the replacement of a glutamic acid residue for lysine residue of the β-globin chain, at the same β-6 position as that of the HbS mutation. People with this disease, particularly children, may have occurrences of abdominal and joint pain, an enlarged spleen, and mild jaundice, but they do not have severe crises, as happens in sickle cell disease. HbC polymorphism is most common in West Africa, with prevalence rates as high as 15% in parts of Burkina Faso (Piel *et al*., 2013). In the case of severe falciparum malaria, evidence of protection by the HbC variant is clear. Individuals with the homozygous HbCC genotype have an 80% reduced risk of severe malaria and a 30% reduced risk in individuals with heterozygous HbAC (Taylor *et al*., 2012). HbC modifies the quantity and distribution of the variant antigen *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) on the infected red blood cell surface and the modified display of malaria surface proteins reduces parasite adhesiveness (thereby avoiding clearance by the spleen) and can reduce the risk of severe disease (Fairhurst *et al.,* 2005). A further strong piece of evidence for overall protection comes from a genome-wide association study (GWAS), which showed that for each copy of the HbC allele, the risk of severe malaria was reduced by 29% (MalariaGEN, 2014). In the case of uncomplicated malaria, the picture is confusing, some studies show protection for HbAC and HbCC, but others don't. A meta-analysis of available studies concluded that protection against uncomplicated malaria by HbC genotype mutations is unknown (Taylor *et al*., 2012). Likewise, there is no conclusive evidence that HbAC or HbCC protects against asymptomatic malaria. The precise mechanisms by which HbCC and HbAC genotypes confer protection against malaria remain an open question (Goheen *et al.,* 2016).

Hemoglobin E is due to a single point mutation in the gene for the beta chain with glutamate to lysine substitution at position 26. It is one of the most prevalent hemoglobinopathies with 30 million people affected (carriers). It is most common in parts of Southeast Asia and India, reaching a prevalence of up to 60% in some areas (Williams and Weatherall, 2012). HbE erythrocytes have an unidentified membrane abnormality that renders the majority of the RBC population relatively resistant to invasion by *P. falciparum* (Chotivanich *et al.,* 2002).

Elliptocytosis (Ovalocytosis)

Elliptocytosis is a blood disorder in which an abnormally high number of the patient's RBCs are elliptical. Ovalocytosis is a subtype of elliptocytosis and is an inherited condition in which RBCs are oval rather than round. Ovalocytosis is rare in most populations, but Southeast Asian Ovalocytosis (SAO) occurs in up to 15% of the indigenous populations in Malaysia and Papua New Guinea. Several abnormalities of SAO erythrocytes have been reported, including increased red cell rigidity and reduced expression of some red cell antigens. SAO is caused by a mutation in the gene encoding the erythrocyte band 3 protein. There is a deletion of the 27 nucleotides encoding the amino acids 400 to 408 of band 3, leading to a deletion of 9 amino acids at the boundary between the cytoplasmic and transmembrane domains of band 3 protein (Liu *et al.*, 1990).

The presence of mutated band 3 results in significant alterations to the mechanical and antigenic properties of the red blood cell membrane. Consequently, the resistance of SAO RBCs to invasion by many parasite lineages cannot be directly attributed to a faulty interaction of merozoite ligands with band 3 because it could also be explained by the increased rigidity of SAO RBCs (Dluzewski *et al*., 1992) or by the reduced mobility of band 3 in SAO that may prevent the formation of a smooth protein-depleted membrane patch which may be necessary for invasion. In support of the latter possibility, antibodies against the cytoplasmic domain of band 3 that limit its mobility also inhibited invasion. Another possible explanation for the resistance to invasion of SAO RBCs is a defective interaction of the merozoite with RBC receptor(s) whose distribution is altered by the presence of mutated band 3. Importantly, band 3 could be part of a large membrane complex that includes glycophorin A and glycophorin B (which are used by *P. falciparum* as receptors for invasion), and the Rh pro-

teins (Beckmann *et al.,* 2001).

Duffy antigen receptor negativity

Plasmodium vivax is widespread in tropical countries but absent or rare in a large region of West and Central Africa, as recently confirmed by PCR species typing (Culleton *et al.,* 2008). This distribution gap has been attributed to the absence of expression of the Duffy antigen receptor for chemokines (DARC) in the red blood cells of many sub-Saharan Africans. Duffy-negative individuals are homozygous for a DARC allele and carry a single nucleotide mutation (DARC 46 T → C) that affects promoter activity by disrupting a binding site for the GATA1 transcription factor of the erythroid lineage. In broadly cited in vitro and in vivo studies, Miller *et al.* (1976) reported that the Duffy blood group is the receptor for *P. vivax* and that the absence of the Duffy blood group on red blood cells is the resistance factor to *P. vivax* in people of African lineage. This has become a well-known example of innate resistance to an infectious agent due to the lack of a receptor for the agent on the target cells.

However, accumulated observations show that Miller's original report needs qualification. In human studies of *P. vivax* transmission, there is evidence of *P. vivax* transmission between Duffy-negative populations in western Kenya, the region of the Brazilian Amazon, and Madagascar. The Malagasy population of Madagascar has a mix of Duffy-positive and Duffy-negative people from different ethnic backgrounds (Pierron *et al.,* 2018) and 72% of the island's population was Duffy-negative. *P. vivax* positivity was found in 8.8% of 476 asymptomatic Duffy-negative individuals, and clinical *P. vivax* malaria was found in 17 of these individuals. Genotyping showed that numerous *P. vivax* strains invaded the red blood cells of Duffy-negative people. The authors suggest that there are enough Duffy-positive people in the Malagasy population to maintain mosquito-borne transmission and liver infection. More recently, Duffy-negative individuals infected with two different strains of *P. vivax* have been found in Angola and Equatorial Guinea. In addition, *P. vivax* infections have been found in both humans and mosquitoes, indicating that active transmission is taking place. The frequency of such transmission is yet unknown. Based on these various reports from different parts of the world, it is clear that some *P. vivax* variants are transmitted to humans who do not express DARC on their red blood cells. The same phenomenon has been noticed in New World monkeys. Nevertheless, DARC still appears to be an important receptor for the transmission of *P. vivax* in humans. The spread of Duffy negativity in Africa does not correlate exactly with the transmission of *P. vivax* (Culleton *et al.,* 2008). Duffy negativity prevalence is as high in East Africa (over 80%), where the parasite is transmitted, as in West Africa, where it is not. The potency of *P. vivax* as an agent of natural selection is unknown and may differ from one location to another. DARC negativity is a good example of innate resistance to an infection, but it produces relative and not absolute resistance to *P. vivax* transmission.

Red blood cell invasion by *P. vivax* merozoites appears to be strongly dependent on the interaction between *P. vivax* Duffy Binding Protein (PvDBP) and DARC (Fang *et al*., 1991). Whole genome sequencing of *P. vivax* clinical isolates collected from Duffy-negative individuals indicates the presence of two or more copies of the PvDBP gene (Hosetler *et al*., 2016).

Assuming a high adaptability of the *Plasmodium* species, it is likely that the duplications arose in the parasites in response to human Duffy blood group variation in malaria-endemic environments. Recent studies have shown that amplification of the PvDBP gene not only facilitates binding to an alternative lower-affinity receptor in Duffy-negative reticulocytes (Gunalan *et al.,* 2016) but also allows *P. vivax* to evade host humoral immunity against PvDBP (Popovici *et al*., 2020), making the PvDBPII region a promising candidate for a blood-stage vaccine against *P. vivax* (Miller *et al*., 1976). The PvDBP polymorphic nature certainly allows *P. vivax* to colonize a variety of ecological niches and evade the host's immune system (Golassa *et al.,* 2020).

In the invasion of Duffy-negative individuals, the involvement of putative parasite ligands has been reported in several studies, but which specific receptor on human erythrocytes helps *P. vivax* invade Duffy-negative reticulocytes is unclear. (Popovici *et al.,* 2020).

Glycophorin C deficiency (Gerbich antigen receptor negativity)

The Gerbich antigen system is an essential membrane protein of erythrocytes and plays an important role in maintaining the shape of red blood cells. It also acts as the receptor for the *P. falciparum* merozoite erythrocyte-binding antigen-140 ligand (EBA-140). Gerbich-negative blood group (Ge−), resulting from the deletion of exon 3 in the glycophorin C-encoding gene (GYPCΔex3), has long been involved in malaria resistance because of its very high frequencies in malaria-endemic re-

gions of Papua New Guinea (Booth and McLoughlin, 1972).

GYPCΔex3 has now been shown to protect *in vitro* against a subset of parasites that can invade red blood cells via a pathway involving *P. falciparum* EBA-140. Further work indicates that GYPCΔex3 is also a major cause of ovalocytosis. Extensive cross-sectional surveys reveal that GYPCΔex3 affects neither the prevalence nor the density of asymptomatic parasitemia (Patel *et al.,* 2004).

The reduced binding of EBA-140 observed in Gerbich-negative erythrocytes suggests that the peptide encoded by exon 3 of the GYPC (amino acids 36-63) is of particular importance. However, what appears to support the hypothesis that amino acids 36-63 of Glycophorin C are important in merozoite invasion is that the frequency of the allele underlying Gerbich negativity has increased to almost 50% malaria-endemic populations, such as New Guinea (Jaskiewicz *et al.,* 2019).

Human leucocyte antigen (HLA) polymorphisms

The most polymorphic human genes are encoded by the HLA complex, and its diversity is believed to be driven by pathogen resistance. The HLA complex consists of genes on chromosome 6 that encode molecules that mediate antigen recognition and presentation and immunity against infectious pathogens, including *P. falciparum* (Mosaad, 2015).

HLA class I molecules are the major ligands for killer-cell immunoglobulin-like receptors (KIRs) and as such play a role in regulating Natural Killer (NK) cell activity during malaria infection. HLA class I molecules present malaria antigens to T cells therefore making them important in adaptive immunity to malaria, which is crucial during liver-stage *P. falciparum* malaria infection. HLA class II molecules mediate the clearance of RBCs infected with *P. falciparum* parasites through the stimulation of helper T cells (Belachew, 2018). Studies have clearly revealed that HLA molecules affect antibody concentrations against malaria antigens, including glutamate-rich protein and merozoite surface antigens (Ghosh, 2008).

Fetal hemoglobin (HbF)

HbF is the main oxygen-carrying protein in the human fetus. HbF is found in fetal RBCs and is involved in the transport of oxygen from the mother's bloodstream to the fetal organs and tissues. It is produced around 6 weeks of gestation and remains high after birth until the baby is around 2 to 4 months old. Levels of hemoglobin F gradually decrease in the newborn, and reach adult levels (less than 1% of entire hemoglobin) usually within the first year, as adult forms of hemoglobin start to be produced (Wild, 2017). Hereditary persistence of fetal hemoglobin (HPFH) is a benign state in which increased production of fetal hemoglobin i.e. hemoglobin F (HbF) persists well into adulthood, disregarding the normal endpoint after which only adult-type hemoglobin should be produced (Thein and Menzel, 2009).

The presence of HbF in three types of red blood cells (cord blood (CB), infant, and adult hereditary persistence of fetal hemoglobin (HPFH)) was thought to limit parasite growth. HbF was found to impair the binding of parasitized red blood cells to human microvascular endothelial cells (MVECs), monocytes, and non-parasitized red blood cells (cytoadherence interactions that contribute to the development of high parasite densities and malaria symptoms). Abnormal expression of the *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), the parasite's cytoadherence antigen on HbF red blood cells corresponds with these findings and is close to that on HbC and HbS red blood cells (Cholera *et al.,* 2008). It was also discovered that IgG purified from the plasma of immune adults from Mali notably reduced the attachment of parasitized CB erythrocytes to MVECs. These data suggest a malaria-protective model in which HbF and maternal PfEMP-1-specific IgG act cooperatively to alter the cytoadherence of parasitized red blood cells in the first few months of life (Amaratunga *et al.*, 2011).

Whether HbF protects against malaria infection with *P. falciparum* is an important controversy that needs to be resolved. A study by Archer *et al*. (2019) revealed that *P. falciparum* growth is not inhibited in either CB or heterozygote HPFH erythrocytes. It was found that the growth in cord blood erythrocytes was not significantly delayed compared with growth in adult (HbAA) erythrocytes and that the growth of *P. falciparum* is oxygen-independent in fetal hemoglobin erythrocytes. However, it was also noted that if HbF is protective against malaria infection, it may rely on other protective mechanisms such as cytoadherence or the enhancement of antibody influence, but growth inhibition shouldn't be considered as a factor (Archer *et al.,* 2019).

Metabolic enzymes

Glucose-6-phosphate dehydrogenase deficiency (G6PD)

X-linked glucose-6-phosphate dehydrogenase (G6PD) deficiency, the most common human

enzymopathy, was first established as a protective candidate against malaria due to its widespread distribution in malaria-endemic populations and its varying genetic origins (Thomas, 2006). The exact mechanism of this protection is yet unknown, but there are two postulated explanations. According to the first proposal, it was found that the parasites that cause malaria can only survive under conditions of low oxygen. This shows that these parasites are very susceptible to oxidative stress. It is known that in the pentose phosphate pathway of red blood cells, the enzyme glucose-6-phosphate dehydrogenase (G6PD) plays an important role in the production of NADPH and GSH. This is the only survival mechanism for red blood cells. The GSH produced by the reduction of NADP+ reacts with H_2O_2 and reduces it to H_2O. This prevents the development of oxidative stress in the red blood cells. However, G6PD activity is significantly reduced in G6PD-deficient erythrocytes. Hence, oxidative stress can be induced in red blood cells whose G6PD enzymes are deficient. So, it is thought that since malaria parasites are susceptible to oxidative stress, they don't live in the red blood cells where their maturation takes place. In addition, during oxidative stress, the loss of potassium from the cell and the parasite can lead to the parasite's death. According to the second proposal, *Plasmodium* parasites oxidize NADPH and decrease the level of reduced glutathione (GSH) in red blood cells. In the state of G6PD deficiency, this effect becomes increasingly severe and induces oxidative-induced damage within the red blood cells. Moreover, *Plasmodium* parasites break down hemoglobin and release toxic components such as iron, and these substances cause hemolysis. Hence, the rates of development of *Plasmodium* parasites are diminished (Allahverdiyev, 2012).

Also, red blood cells affected by oxidative stress and are damaged, are eliminated by the immune system through phagocytosis. This elimination reduces the growth of parasites much more as it occurs during an early ring stage of the maturation of the parasites. Therefore, all these show that G6PD deficiency can protect against infections of malaria (Allahverdiyev, 2012).

According to the research carried out by Mbanefo *et al*. (2017), they observed no negative (protective) association between G6PD deficiency and uncomplicated falciparum malaria, also severe malaria. Likewise, no significant association was found between G6PD deficiency and other malaria species, including *P. vivax* and *P. malariae*, or a combination of two or all three types. Therefore, further research into the association between these species and G6PD deficiency is strongly encouraged. A negative association was found in studies conducted in Africa compared to Asia and other continents but with the presence of publication bias. This suggests that ethnicity other than endemicity could modify the effect of G6PD deficiency in the study (Mbanefo *et al*., 2017).

While the homozygous female and hemizygous male genotypes showed no association with protection against uncomplicated malaria, the heterozygous female genotype showed a highly significant association with malaria protection. This association with heterozygous females is strongly supported by a study by Uyoga *et al*. (2015), which also showed that hemizygous males are not only unprotected against malaria but may be at a high risk of severe malaria. This is because, G6PD-deficient red blood cells are susceptible to early destruction by oxygen free radicals (Luzzatto, 2015). Usanga and Luzzatto postulated a prestigious mechanism that explains the association with heterozygous females (Usanga and Luzzatto, 1985). They observed that the parasite undergoes habitual changes when passing through successive red blood cells that are deficient in G6PD, to be more adaptive. These changes led to the production of the parasite's own enzyme, which in turn led to the survival and replication of the parasite (Yoshida and Roth, 1987). Since the heterozygous females are genetically mosaic, the parasite did not survive (Usanga and Luzzatto, 1985). More studies are necessary to investigate the mechanism of association with heterozygous females.

Pyruvate kinase deficiency (PKD)

Also known as erythrocyte pyruvate kinase deficiency, is an inherited metabolic disorder of the pyruvate kinase enzyme. In this condition, the lack of pyruvate kinase slows down the glycolysis process. This effect is especially devastating in cells lacking mitochondria. In the absence of mitochondria (which are lacking in mature erythrocytes), the enzyme is essential for energy production (Kodjo *et al.,* 2008). The mechanisms responsible for the resistance of PK-deficient red blood cells to malaria are unknown but may be a result of ATP deficiency, which impairs parasite invasion in vitro. Decreased ATP results in cross-linking of membrane proteins, which may affect parasite invasion, growth, or egress. Low ATP concentration also increases 2,3-diphosphoglycerate (2,3-DPG), which i) disrupts spectrin, actin, and protein interactions, lead-

ing to membrane instability and having an impact on the survival of the intracellular parasite, and ii) inhibits G6PD, leading to an increase in oxidative stress (Min-Oo and Gros, 2005).

According to research conducted by Morias *et al*. (2022) in investigating how the glycolytic metabolite 2,3-DPG may be involved in the protective effect against malaria showed that, when 2,3-DPG 8 mM was added daily into a culture medium, there was a sharp reduction in parasite densities and parasite progeny at the end of the intraerythrocytic development cycle. Change in the cytoskeleton of RBCs was also noticed which led to the experiment of Carvalho *et al*. (2023) to determine the correlation of these changes in the cytoskeleton of RBCs upon treatment with 2,3-DPG and to determine if these changes interfere with *P. falciparum* invasion.

Increased levels of 2,3-DPG are also found in other red blood cell disorders such as beta-thalassemia, G6PD deficiency, or sickle cell anemia, as a result of the activation of glycolysis upstream to the pyruvate kinase. These disorders have already been associated with protection against malaria infection and it cannot be ruled out that this metabolite might also involved in the mechanisms underlying this protective effect. 2,3-DPG is an endogenous host metabolite that could become a new antimalarial tool with few adverse effects on uninfected cells in the future (Carvalho *et al*., 2023).

Inflammatory response genes

Tumor Necrosis Factor (TNF)

It is an essential pro-inflammatory cytokine of the immune system. Tumor necrosis factor (TNF) plays a very important role in host defense during infection, showing a double role in human *P. falciparum* infection. TNF is produced to reduce parasitemia and destruction of intraerythrocytic parasites and its level is elevated in the serum of infected individuals (Penha-Gonçalves, 2019). Though high TNF levels are correlated with more rapid clearance of parasites and resolution of malaria attacks, however, serum TNF levels are continually elevated in children with severe malaria, suggesting a complicated role in malaria pathogenesis. Therefore, both beneficial and adverse roles for TNF in the outcome of *P. falciparum* malaria infection have been suggested (Nguyen *et al.,* 2017). A high level of TNF upregulates the expression of adhesion molecules that interact with the parasite factor, such as the *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1). As a result, cytoadherence, a pathological condition in cerebral malaria, increases (Sinha *et al.,* 2008).

Nitric oxide synthase 2 (NOS2)

The NOS2 gene encodes inducible nitric oxide synthase (iNOS) (Penha-Gonçalves, 2019). Nitric oxide is a beneficial component of the innate immune system, and NO produced by iNOS plays a role in both the pathogenesis and control of viral, bacterial, and parasitic infections (Legorreta-Herrera *et al.,* 2011). NO production is mainly controlled by pro-inflammatory cytokines via transcription mechanisms. The Increased expression of endogenous NO during blood-stage malaria infection demonstrated protection against *P. falciparum*. In an in vitro study, it was suggested that NO could be both cytotoxic and cytostatic to *P. falciparum*. A study on rodent malaria models showed the protective effects of Nitric Oxide (Dzodzomenyo *et al*., 2018). NO reduces the growth of malaria parasites, cytoadherence, and TNF toxicity. In addition, NO modulates the immune response by regulating apoptosis and upregulating cytokine expression. In severe malaria, NO level decreases, which in turn increases the expression of adhesion molecules such as ICAM1, resulting in increased cytoadherence of parasitized red blood cells.

How the bioavailability of NO contributes to the status of severe malaria is still controversial with most reports affirming disease severity with low levels of NO (Anstey *et al*., 1996). Low levels of NO have been associated with host factors during malaria, such as free hemoglobin from infected erythrocyte bursts or intravascular hemolysis, which scavenges NO, arginase activation, which catalyzes the synthesis of ornithine and urea from L-arginine (L-Arg) in infected or uninfected RBCs; the presence of endogenous iNOS inhibitors like methylated arginines (asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA)); and the decline in tetrahydrobiopterin levels, which uncouples iNO. However, the availability of L-Arg, iNOS substrate, would inevitably be the limiting factor in nitric oxide production (Corbett *et al*., 2018).

Interferon-alpha receptor-1 (IFNAR1)

It is one of the most important defense proteins of the human host, its role in the pathogenesis of malaria is being researched in many ethnic groups. Almost each type of cell has IFNAR1 (Prinz *et al.,* 2008).

Genetic variants in the IFNAR1 are associated with severe disease in African populations. The protective Uncomplicated malaria-associated vari-

ant, rs914142, is associated with decreased IFNAR1 expression, while cerebral malaria (CM)-associated variants, rs12626750 and rs1041867, are associated with increased IFNAR1 expression (Feintuch *et al.* 2018). Overexpression of pro-inflammatory cytokines such as interferons (IFNs) leads to severe disease outcomes. Interferons bind to their receptors to activate the interferon-stimulated genes (ISGs). It may be proposed that induction of the type I interferon (IFN-I) pathway might contribute to the CM pathogenesis. This activation is achieved with the help of IFNAR1 and its overexpression contributes to the pathogenesis and outcome of malaria. IFNAR1 polymorphisms have been shown to affect IFNAR1 gene expression. Some genetic variants are associated with overexpression and thus activation of the ISGs. Such activation may cause inflammation and pathogenesis of malaria. The binding of Type I interferons (IFN-Is) to IFNAR induces a cascade of downstream signaling events to initiate the transcription of hundreds of ISGs. ISGs include antimicrobial proteins, chemokines/cytokines and inflammation-inducing mediators which target critical molecules and pathways of a pathogen directly. Other ISGs encode for proapoptotic proteins, leading to cell death under certain conditions (Lukhele *et al*., 2019).

Heme oxygenase-1 (HO-1)

Heme oxygenase-1 (HO-1) is a catabolic enzyme that cleaves to heme to produce biliverdin, carbon monoxide, and also ferrous iron. At the liver stage, HO-1 and/or its byproducts CO and biliverdin have been reported to protect infected hepatocytes from cell death, thereby significantly increasing the number of parasites reaching the erythrocytic stage of the parasite life cycle. Thus, the cytoprotective effects of HO-1 are associated with decreased disease resistance and increased parasite load during the liver stage, revealing a putative mechanism of parasite evasion that's associated with the induction of HO-1 expression. During the erythrocyte phase, HO-1 is essential for preventing heme-induced inflammation through its catabolic activity (Silva *et al.*, 2020). Its byproduct CO has several effects that result in a beneficial outcome of the disease, including its ability to bind to heme, thereby reducing its release from hemoglobin, inhibiting pro-inflammatory cytokines, leukocyte recruitment, and tissue damage, without affecting the parasite load. These fundamental findings were confirmed with a CO-releasing molecule (CO-RM) that is capable of protecting against ECM (Experimental Cerebral Malaria) by inducing HO-1 without affecting the transport of oxygen by hemoglobin (Pena *et al.,* 2012).

Toll-like receptors (TLRs)

Toll-like receptors (TLRs) are components of the immune system that recognize pathogen-associated molecular patterns (PAMPs) and are infection sensors at the start of innate immune responses. Possibly, TLR signaling could be involved in the resolution of early infection as well as increased inflammation and immunopathology. It has been revealed that binding to infected erythrocytes (IE) and recognition of *Plasmodium* moieties such as glycosylphosphatidylinositol (GPI) anchors or hemozoin-DNA complexes can be mediated by different TLRs, providing sensor redundancy in the immuno-detection of the blood stage parasites. Several reports suggest that variants in different TLRs and their adaptor molecules are involved in the control of parasite load (TLR9, rs187084), severe disease susceptibility (TLR1, rs4833095), fatal disease resilience (TIRAP S180L), and severe disease susceptibility including poor pregnancy outcomes (TLR4, rs4986790) (Costa *et al.,* 2017). Altogether, these studies convey the idea that recognition of *Plasmodium*-derived molecules by innate receptors contributes to the host response at various stages of the natural course of malaria, likely through an association with pro-inflammatory responses, such as the production of interferon (Yu *et al.,* 2016).

Downstream effectors of Toll-like receptor signaling include variations of pro-inflammatory cytokines and chemokines such as IFN-γ, IL-6, TNF, IL-12, IFN-1, MCP-1, and IL-8 involved in the amplification of antiparasitic responses during acute blood-stage infection (Erdman *et al*., 2008). The association of different TLRs with clinical outcomes in malaria suggests that *Plasmodium* components of differing nature trigger innate responses that are genetically controlled, which in turn determine the course of infection.

Ramirez *et al*. (2022) performed a meta-analysis to examine the relationship between the studied Toll-like receptors and the three essential aspects of malaria, namely susceptibility, parasitemia, and severity. TLRs were found not correlated with susceptibility. However, differing results exist on the effect of TLRs on the severity of malaria, with a correlation not found between TLRs and severity on a large scale but subgroup analysis of each TLRs shows varying correlation with severity. Regarding severity, TLR6 increases severity while TLR2 was observed to be protective against severe malaria, and regarding parasitemia, an association between

TLR1 and TLR9 receptors was shown to be associated with higher parasitemia, while TLR4 was protective against parasitemia. (Ramirez *et al*., 2022).

CD36 and adhesion molecules

Several evidences suggest that adhesion molecules, including Intercellular Adhesion Molecule-1 (ICAM-1), Platelet endothelial cell adhesion molecule-1 (PECAM-1) and Endothelial Protein C Receptor (EPCR), expressed on endothelial cells are involved in the pathogenesis of severe malaria by promoting cytoadhesion and possibly the sequestration of infected red blood cells in microvessels (Cabrera *et al*., 2014). CD36 is a multifunctional class B scavenger receptor that also functions as a pattern recognition receptor (PRR) on innate immune cells. CD36 not only interacts with differing ligands but also binds and mediates pathogen uptake and apoptotic cells. CD36 plays several essential roles in malaria, including the sequestration of parasites in the microvascular capillaries of several organs, in phagocytic parasites clearance by uptake of infected red blood cells, and immune responses. Of all these, it is best known for its function as a receptor for various members of the *P. falciparum* erythrocyte membrane protein-1 (PfEMP1) family of variant proteins expressed on the surface of infected red blood cells (Hsieh *et al.,* 2016). This interaction mediates the adherence of infected red blood cells to the microvascular endothelia, thereby allowing the sequestration of parasites in host organs and preventing them from being cleared from circulation. When immunity to an adherent PfEMP1 is present in the host, parasites that express other PfEMP1 variants that have a different adherent specificity are sequestered in host organs. The parasites multiply and accumulate in great numbers in the organs and thus contribute to the pathogenesis of severe malaria (Bernabeu *et al.,* 2016).

Studies showed that between 75-85% of var genes encode PfEMP1s which has a CIDRα2-6 domain for binding to CD36. Since most parasites in infected individuals express CD36-binding PfEMP1, CD36 likely plays an important role in controlling parasitemia, although sequestration may promote parasite growth to some extent (Fonager *et al*., 2012). The dynamics between parasitemia control and the promotion of parasite growth may depend on host factors like immune status and receptor polymorphisms. When parasites are sequestered in organs, locally enhanced pro-inflammatory responses due to increased parasite biomass may contribute to severe pathology and organ dysfunction. Conversely, the CD36-dependent enhancement of immune responses and its contribution to increased clearance of parasites are likely to reduce the risk of severe disease. Thus, the clinical outcomes of malaria infection depend on the balance between these double roles of CD36 (Thylur *et al*., 2017). It appears that CD36 more often promotes pathology in sequestering parasite infection (Lagassé *et al*., 2016), although it can be protective in some situations (Aitman *et al*., 2000). However, in situations without parasitic sequestration, such as was observed in non-fatal mouse malaria, CD36-dependent interaction is likely to be beneficial. More detailed studies of CD36-mediated signaling events in immune responses to malaria will likely increase our understanding of the role of CD36 in malaria immunity and pathogenesis (Thylur *et al*., 2017).

CD40 ligand (CD40L) and adaptive immunity genes

CD40L is a type II membrane glycoprotein that is expressed by activated CD4+ T cells. The CD40 protein is a member of the tumor necrosis factor (TNF) receptor family, that is expressed on the surface of a broad variety of cells, which include B cells (Cassiano *et al*., 2016). The binding of CD40 to its ligand CD40L, which is expressed on the surface of activated T cells, provides the major co-stimulatory signal for B cells to mount a humoral response. The interaction mediated by this signaling pathway is responsible for the proliferation and differentiation of B cells, activation of antigen-presenting cells, immunoglobulin isotype switching, germinal center formation, prevention of B cell apoptosis, and antibody secretion (Clark, 2014).

Recent research and advances indicated that CD40-CD40L binding could initiate the activation of TNF receptor-associated factor 2/3 (TRAF2/3) mediated nuclear factor kappa B (NF-kB) pathways and the production of interferon regulatory factor 1 (IRF1) to eventually induce the expression of interferon-β (IFN-β) (Moschonas *et al*., 2012), which is a cytokine that regulates the adaptive immune response to infections and tumors. Its effect is highly dependent on its level and timing of expression (Assouvie *et al.,* 2020). CD40 has been reported to be involved in the elimination of malaria parasites, and thereby reduce the severity of malaria (Parmar *et al*., 2018).

Conclusion

Genetically based mutations bringing about protective polymorphisms have been seen to confer resistance to malaria thereby increasing the abili-

ty of the populace that possess these traits to adapt in various malarious areas. A better understanding of how these alterations confer protection can be useful in the development of novel therapeutics, thereby increasing the well being of the populace affected by this disease.

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