

Review

Parasite-host interaction in malaria: genetic clues and copy number variation

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Abstract

In humans, infections contribute highly to mortality and morbidity rates worldwide. Malaria tropica is one of the major infectious diseases globally and is caused by the protozoan parasite *Plasmodium falciparum*. Plasmodia have accompanied human beings since the emergence of humankind. Due to its pathogenicity, malaria is a powerful selective force on the human genome. Genetic epidemiology approaches such as family and twin studies, candidate gene studies, and disease-association studies have identified a number of genes that mediate relative protection against the severest forms of the disease. New molecular approaches, including genome-wide association studies, have recently been performed to expand our knowledge on the functional effect of human variation in malaria. For the future, a systematic determination of gene-dosage effects and expression profiles of protective genes might unveil the functional impact of structural alterations in these genes on either side of the host-parasite interaction.

Introduction

Malaria is a disease caused by Apicomplexa parasites of the genus *Plasmodium*, and is transmitted by the bite of an infected female *Anopheles* mosquito. Malaria has affected humans since their evolutionary emergence as a separate species. The divergence of man and chimpanzee is paralleled by the divergence of the two specific parasites *Plasmodium reichenowi* and *Plasmodium falciparum* [1].

The earliest references to malaria are descriptions of splenomegaly with fever from China in the Nei Ching Canon of Medicine in 1700 BC, and from Ancient Egypt in the Ebers Papyrus in 1570 BC. The parasite was identified by Alphonse Laveran in 1880; Giovanni B Grassi and Sir Roland Ross demonstrated that malaria parasites could be transmitted to infected vertebrate hosts (birds and humans) by mosquitoes.

Malaria is one of the main global causes of death from infectious diseases, which are one of the major public

health issues, especially in developing countries. In the year 2001, about 14.5 million deaths were attributed to the effects of infectious diseases. About 40% of the world population is at risk for malaria, causing around 1 million deaths each year, predominantly in infants [2].

Malaria is a devastating disease that not only has an effect on the health system, but also slows the rate of long-term economic growth and development. Global climate change and a possible further migration of parasites will put even more people at risk in the future.

Malaria has a broad distribution in both subtropical and tropical regions, with many areas of the tropics being endemic for the disease. The countries of sub-Saharan Africa carry the highest burden of all malaria cases. In many temperate and richer areas, such as Europe and the USA, public health measures, economic development and environmental changes have successfully eliminated the disease, apart from a minor number of cases imported by travelers.

The life cycle of all *Plasmodia* species is very similar and follows basically the same process (Figure 1). Development involves a host change from an arthropod vector to a vertebrate host (the female *Anopheles* mosquito and the human host for *Plasmodia*), with a mostly intracellular life style. Infection is initiated with the bite of an infected female *Anopheles* mosquito and the injection of sporozoites from the salivary glands into the host's bloodstream (Figure 1a). Within minutes, the sporozoites invade hepatocytes (Figure 1b) and undergo an asexual replication that results in the production of schizonts. The hepatocytes burst (Figure 1c) after five days at the earliest, and release merozoites into the bloodstream (Figure 1d). The released merozoites then invade red blood cells (RBCs) and initiate another asexual replication phase in the erythrocyte.

ADR, adrenoceptor; CNV, copy number variation; CQ, chloroquine; CR1, complement receptor 1; CYP2A6, cytochrome pigment 450; dhfr, dihydrofolate reductase; FcγR, receptor for the constant fragment of immunoglobulin G; FCGR, gene for the constant fragment of immunoglobulin G; FCGR2A/3B, receptor for the constant fragment of immunoglobulin G, low affinity IIa/IIIb; gch1, GTP-cyclohydrolase 1; GWAS, genome-wide association studies; GPC, glycoporphin C; HLA, human leukocyte antigen; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; MBL2, mannose/mannan-binding lectin2; Pfcr1, *Plasmodium falciparum* chloroquine resistance transporter; Pfmrd, *Plasmodium falciparum* multi-drug resistance; RBC, red blood cell; SNP, single nucleotide polymorphism.

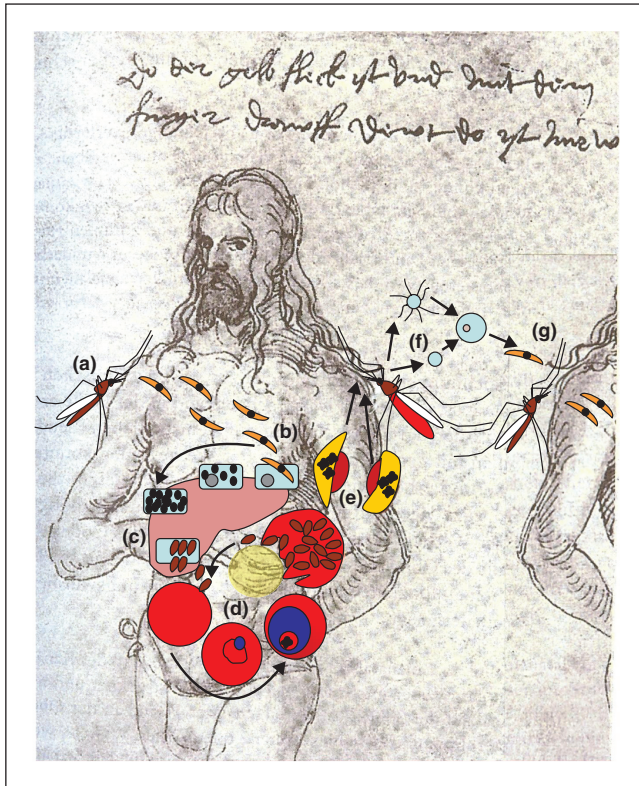


Figure 1

Life cycle of *Plasmodium falciparum* (a-g) projected onto a sketch drawn by the German Renaissance painter Albrecht Dürer (1471-1528). This sketch was drawn by Dürer for his doctor pointing to the site of nagging pain, presumably due to splenomegaly. Dürer supposedly contracted malaria on a trip to The Netherlands, never recovered completely and died of it 13 years later.

Invaded merozoites develop from the trophozoite to the schizont containing 16 to 32 new merozoites. These are released and re-invade new RBCs. The duration of the cycle is species dependent and can vary between 24 hours (*P. knowlesi*) and 72 hours (*P. malariae*). Alternatively, some parasites differentiate within the RBC into the sexual forms, the male and female gametocytes, which are taken up by a female mosquito (Figure 1e). Within the mosquito mid-gut, the male gametocyte undergoes a nuclear division, producing eight flagellated microgametes that will fertilize the female macrogamete (Figure 1f). At the end of the zygote development, hundreds of sporozoites are formed, which migrate to the mosquito salivary gland awaiting injection into the human host (Figure 1g).

The clinical spectrum of malaria is wide, spanning from asymptomatic infections, fever and mild anemia, to severe anemia and cerebral malaria and, subsequently, death [3]. Until recently, four distinct species were recognized as human pathogens: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*, with *P. falciparum* the most lethal, causing

the majority of deaths. Very few fatalities are caused by *P. vivax*, and no deaths have been reported by the latter two so far. A fifth parasite, *P. knowlesi*, known as a simian parasite, is now also considered to be pathogenic for humans [4].

The influence of host and parasite genetics in malaria

Malaria and single nucleotide polymorphisms

In humans, a genetic contribution to resistance against malaria has long been suspected, and epidemiological evidence exists for the protective sickle cell trait in malaria [5]. It is estimated that genetic factors account for approximately one-quarter of the total variability in malaria incidence of a study population, with the hemoglobin S gene explaining 2% [6]. This finding indicates the influence of many other unexplored protective genes, each individually resulting in small population effects.

Genetic markers such as single nucleotide polymorphisms (SNPs) are a valuable tool to study risk assessment and progression of infectious diseases. SNPs are highly frequent and are abundant in the human genome [7]. SNPs that lead to amino acid changes are of particular importance, providing an indication of how the protection is mediated.

It is obvious that parasitic and human receptor-ligand interaction is involved in the parasite's various invasion processes into erythrocytes or liver cells. This interplay of two organisms has to be balanced carefully to ensure the survival of both. This balance is reflected in genetic alterations in both the host and the parasites.

Genetic variation in response to malaria

Epidemiological studies of malaria in humans have demonstrated that malaria phenotypes, including severity, disease incidence and parasitemia, can significantly vary amongst individuals [8]. In 1997, a longitudinal study of malaria in sibling pairs was designed to investigate the extent of the genetic determinants of predisposition to clinical malaria in rural Gambia [9]. In this study, a strong association of human leukocyte antigen (HLA) genes was found to contribute to the risk of contracting uncomplicated malaria. In 2000, a quantifying analysis of the implication of genetic and non-genetic factors in malarial infection was conducted in a rural Sri Lankan population [10]; the heritability was estimated to be around 10% for the intensity of the clinical signs and about 15% for the incidence of asymptomatic and symptomatic *P. falciparum* infections. A strong association of age with potential genetic determinants was also detected, whereas the extent of the genetic effect was higher in infants in comparison with adults [11]. Genetic epidemiological studies using segregation analyses in a sib-pair linkage analysis identified genetic factors that were linked to the level of malarial infection in Burkina Faso [12]. This analysis

focused on the chromosomal region 5q31-33 and suggested that the genes coding for β_2 -adrenergic receptor (ADR) and the interleukin genes (IL)-9 and -13 are involved in the control of the infection. Many immunological studies have investigated the genetic regulation of the variation of human immune responses to malaria. In the early 1990s, one of the leading studies was conducted in twin pairs from Liberia and Madagascar, measuring antibody levels to malaria antigens [13]. A high analogy in antibody levels was detected in monozygotic twins compared with dizygotic twins or sex- and age-matched siblings, and unrelated individuals under similar exposure to malaria transmission. In the following sections, we will describe some of the most important genetic alterations that have an impact on malaria.

Single nucleotide polymorphisms and malaria

Countless association studies have been performed with numerous SNPs in cohorts of different geographic origin looking at differing disease presentations. Many of these studies have reported conflicting associations or genetic alterations that cannot be explained by malaria exposure alone. For example, a SNP termed ICAM-1^{Kilifi} located in the gene for the intercellular adhesion molecule 1 (ICAM-1) was found to result in an amino acid change at position 29 (Lys to Met). This highly frequent alteration has been associated with a predisposition to cerebral malaria in Kenya [14]. On the contrary, in a case-control study of Gabonese infants, ICAM-1^{Kilifi} was associated with protection against severe malaria [15]. The largest study conducted to analyze ICAM-1^{Kilifi} investigated more than 4,000 individuals, and no association with any malaria phenotype was observed [16].

An interesting phenotype that has been associated with severity of plasmodial infections is so-called rosetting, by which a parasitized erythrocyte surrounds itself with uninfected red blood cells with the involvement of complement receptor 1 (CR1) [17-19]. Other studies have, however, failed to support the association of disease severity with rosetting [20,21]; on the other hand, results from a study in a Papua New Guinean population have shown that polymorphisms leading to CR1 deficiency mediate protection against severe malaria [22].

Interestingly, reduced rosetting may also play a role in the weakly protective role of blood group o. In African regions with a strong overlap of malaria endemicity and high prevalence of blood group o, it has been reported that this blood group forms rosettes very inefficiently compared to other blood groups [23]. Evidently, in indigenous South American populations with 100% of the people being o positive, other mechanisms must be in action.

In Papua New Guinea, the erythrocytes of a high proportion of the population do not carry glycoprotein C (GPC),

the component that forms the Gerbich blood type [24]. Malaria parasites use GPC to invade erythrocytes [25]; whether this deficiency has positive effects on malaria outcomes remains to be elucidated.

Mannose-binding lectin (MBL) is a collagen-like serum protein that acts as an unspecific antibody binding to the carbohydrate moieties of pathogens in order to enable macrophages to opsonize them [26]. Common genetic variants located in the human *MBL2* gene locus impact the stability and the serum level of the resulting protein, which influences the predisposition and clinical outcome of various infectious diseases of bacterial and parasitic origin [27,28]. A comparison of MBL plasma levels in young Gabonese patients suffering severe and mild malaria suggested a protective effect for MBL [29]. Genetic variants causing lower concentrations of the protein were associated with different malaria phenotypes [30,31]. The effect of MBL on malaria may be explained by a direct binding of MBL to glycoproteins of the merozoites or the infected erythrocyte, as shown by enrichment of parasite proteins on MBL affinity columns [28,32]. Other studies, however, have not shown any association with malaria [33].

Moving from candidate gene studies to genome-wide association studies

The sequencing of the human genome has generated large-scale genomic data, and the HapMap project has identified millions of SNPs, allowing the performance of genome-wide association studies (GWASs) [34]. The first investigations of multifactorial infectious diseases utilizing a genome-wide technology were conducted in African tuberculosis patients [35] and in Brazilian patients suffering from schistosomiasis [36]. In 2007, a GWAS approach was used to identify the major determinants for host control of HIV [37]. In malaria research, two studies have been performed so far: the first on mild malaria in Ghanaian children [38] and a second on a Gambian cohort with severe malaria [39]. Both studies failed to detect known protective traits, probably due to technical reasons. On the other hand, it is likely that there is not just one protective trait; malaria-affected humans may have many ways of coping with infection. The mechanisms by which one population battles disease may not necessarily be the same as those in a different population. It is probably a feature of complex diseases that they provoke complex weaponry.

Copy number variations in malaria

One approach that has not been tried so far in malaria research is the determination of copy number variation (CNV) and its possible association with the disease. These structural variations are defined as two-fold or more multiplications of DNA segments larger than 1 kb [40]. It has become widely accepted that genomic structural alterations, rather than DNA single nucleotide substitutions, account for a significant amount of human genetic

variation [41]. CNVs between unrelated people can reach 0.4% of the genome [42]. In an initial CNV study in the human genome, a large number of CNVs was found in immunorelevant genes, some of which are also candidate genes for malaria protection, such as the genes for the receptor for the constant fragment of immunoglobulin G (*FCGR*) - important regulators of the immune response - and the human leukocyte antigens (*HLA*) [43]. The association of genetic polymorphisms in the low-affinity receptors IIa and IIIb (*FCGR2A*, *FCGR3B*), which encode the FcγR molecules, with susceptibility to cerebral malaria is well known [44].

Various studies link the human genetic variations of the *HLA* genes, whose protein products are responsible for antigen presentation to the immune system, to disease progression and outcome. The first evidence for an association between *HLA* genetic variants and predisposition to malaria was identified in Sardinians about 25 years ago, when the frequency of *HLA* alleles was observed to be variable in villages located at different altitudes of the island, indicating an influence of malaria transmission intensity as selective pressure [45]. Independent protective effects against severe malaria in the *HLA* locus were found in a West African population [46]. Protective genetic variants of *HLA* were highly prevalent in Africans but rare in other populations, which points to malaria as a creating force. As a result, the functionality of the HLA immune component has been the focus of various studies aiming to develop an HLA subunit vaccine.

Copy number variation in malaria treatment

Interestingly, treatment of malaria may also be influenced by CNV [47,48]. The cytochrome pigment 450 (*CYP* 2A6 of the P450 family) is involved in the metabolism of the newly recommended drug artesunate and present in the genome as multiple copies. Increase in *CYP2A6* copy number is also associated with a higher plasma level of the nicotine detoxification product cotinine. Whether individuals with multiple copies also metabolize artesunate more quickly will be the subject of future investigation.

Gene duplications also help the parasite to cope with the unfriendly environment of a chemotherapy-treated patient. Studies in the mechanisms of artemisinin resistance show that drug resistance is conferred by an increased number of gene copies of the multi-drug resistance (*pfmdr*) gene 1 [49-51]. A decrease of copy numbers results in susceptibility to drugs like mefloquine, lumefantrine, halofantrine, quinine and artemisinin [52].

A systematic analysis of the parasite's genome revealed a number of genes in multiple copies [53]. One of them, GTP-cyclohydrolase I (*gch1*), is situated in a pathway targeted by drugs, but had not previously been identified as mediating resistance to antifolate drugs. Amplification

of *gch1* was also detected in a separate study looking at geographically distinct parasites with known drug resistance profiles [54]. The genomic amplification in *gch1* resulted in an increased expression level of the corresponding mRNA. It was also shown that the presence of multiple copies of the gene was associated with mutations in the gene for dihydrofolate reductase (*dhfr*), which had been identified as the only cause of antifolate resistance. Amplification of *gch1* may be vital to compensate for the putatively fitness-reducing mutations in *dhfr*.

The long-suspected role of *pfmdr* amplification in chloroquine (CQ) resistance may also be a result of compensation [55]. Later on, it became obvious that *pfmdr* could not be held accountable for all the phenomena of CQ resistance. Finally, the *P. falciparum* chloroquine resistance transporter gene (*pfert*) was identified, and mutations mediating resistance were characterized and confirmed clinically [56,57]. Several years later, it was shown that parasites with point mutations in *pfert* also multiply the *pfmdr* gene *in vitro*, in comparison to their isogenic 'sister' parasites [58].

Duplications exist in other genes responsible for cell division, cell-cycle regulation and sexual differentiation; others remain un-annotated [59]. A very interesting duplication affects the surfins, molecules possibly involved in invasion [60]. The *surfins* represent a family of ten members; the relevant gene product of the duplicated surfin is localized on the surface of merozoites [60,61]. Multiplication of this gene may lead to the birth of new family members awaiting selectable mutations to evade immune responses or to explore novel invasion pathways. CNV could be the starting point of a new round in the arms race between parasites and the human immune system.

Conclusions

CNVs are a feature of the parasite-host interaction in malaria. Whereas the parasite perspective of malaria has been under intense investigation, especially regarding drug response and resistance, the host side has so far received less attention. The study of CNVs in relation to malaria could explain some of the discrepancies between genetic association studies, since some of the candidate genes identified do occur as multiple copies.

Knowledge of the role of CNV in malaria could have three major impacts: the most important feature is to monitor changes in CNV in the parasite population to recognize emerging drug resistance quickly and early. Drug resistance is one of the biggest problems in battling malaria, and even the newest therapies with artemisinin derivatives are under threat by resistant parasites [62,63]. Investigating CNVs of drug-metabolizing P450 may lead to personalized adjustment of drug dosage to compensate for increased degradation of drugs if a surplus of copies is present.

On the other hand, if a reduced copy number or a SNP with a strong influence on malaria suppresses expression of a protective gene product, one could consider replacement therapy. As has been done for hemophilia patients with factor VIII replacement for decades, infectious diseases like malaria may in the future be treated using protective serum factors such as MBL2 [64] or components of cytokine pathways that are not sufficiently expressed [65].

A joint analysis of CNVs, SNPs and transcriptomics may shed light on the genetics of host-parasite interaction during malaria pathogenesis. Combined with the individual genome sequences [66], 'personalized' genetic analysis (personomics) will give a clear answer to the question of which SNP or CNV influences gene expression in a particular person and how this particular person deals with malaria.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

IF wrote the sections on SNPs and malaria and the GWAS studies and human CNV studies; EGC wrote the historic remarks and the description of the parasite cycle, and JK contributed the parasite-host relationship and parasite CNV studies, conclusions and final editing.

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