Host susceptibility to malaria in human and mice: compatible approaches to identify potential resistant genes

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Hernandez-Valladares M, Rihet P, Iraqi FA. Host susceptibility to malaria in human and mice: compatible approaches to identify potential resistant genes. Physiol Genomics 46: 1–16, 2014. First published October 29, 2013; doi:10.1152/physiolgenomics.00044.2013.—There is growing evidence for human genetic factors controlling the outcome of malaria infection, while molecular basis of this genetic control is still poorly understood. Case-control and family-based studies have been carried out to identify genes underlying host susceptibility to malarial infection. Parasitemia and mild malaria have been genetically linked to human chromosomes 5q31-q33 and 6p21.3, and several immune genes located within those regions have been associated with malaria-related phenotypes. Association and linkage studies of resistance to malaria are not easy to carry out in human populations, because of the difficulty in surveying a significant number of families. Murine models have proven to be an excellent genetic tool for studying host response to malaria; their use allowed mapping 14 resistance loci, eight of them controlling parasitic levels and six controlling cerebral malaria. Once quantitative trait loci or genes have been identified, the human ortholog may then be identified. Comparative mapping studies showed that a couple of human and mouse might share similar genetically controlled mechanisms of resistance. In this way, char8, which controls parasitemia, was mapped on chromosome 11; char8 corresponds to human chromosome 5q31-q33 and contains immune genes, such as Il3, Il4, Il5, Il12b, Il13, Irf1, and Csf2. Nevertheless, part of the genetic factors controlling malaria traits might differ in both hosts because of specific host-pathogen interactions. Finally, novel genetic tools including animal models were recently developed and will offer new opportunities for identifying genetic factors underlying host phenotypic response to malaria, which will help in better therapeutic strategies including vaccine and drug development.

malaria; genetic resistance; human; mouse model; QT mapping

PLASMODIUM FALCIPARUM MALARIA remains a major cause of morbidity and mortality in many developing countries. According to the World Health Organization (WHO), the number of cases in 2012 reached 219 million [95% confidence interval (CI) 154–289 million], among which >660,000 (95% CI 490,000–836,000) were fatal (62, 181a). Drug-resistant parasite strains are spreading rapidly across the world, and, despite substantial efforts and encouraging results from recent trials, a fully protective malaria vaccine remains a distant prospect (154). Vaccine development faces major difficulties partly due to the genetic control of immunity to the parasite (155, 156, 163, 176) and/or to the parasitic antigenic variation (146).

The outcome of human malaria infection is thought to depend on both parasite and host genetic factors. P. falciparum genotypes have been associated with differences in disease outcome (41) and may be subject to selective pressure (34, 55). The influence of host genetic factors has been demonstrated in animal models (47, 49, 156), and there is accumulating evidence of human genetic control in malarial infection and disease. The development of efficient control measures calls for a better knowledge of human genes controlling the infection and the disease. Genetic epidemiology studies are aimed at estimating the heritability of phenotypes related to malaria and at localizing and identifying genes involved in susceptibility or resistance to malaria. Herein we will review the current knowledge in the field of the genetics of human malaria. We will particularly discuss the results of case-control studies and family-based and the animal model studies.

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Several malaria phenotypes have been considered in genetic epidemiology studies and are summarized in Table 1. The purpose of such studies is to identify genetic factors that control parasitemia and those that determine the risk of developing either mild malaria or severe malaria. Although the phenotypes are related, the study of each phenotype should give a particular insight into the understanding of malaria physiopathology.

Severe malaria is mainly composed of three subphenotypes: cerebral malaria, severe anemia, and respiratory distress (102). Separate or combined phenotypes have been considered in association studies. The studies of severe malaria phenotypes are based on in-hospital recruitment. For mild malaria, composed of parasitemia and fever-attack subphenotypes, the ascertainment is based on population living in an endemic area. A binary phenotype and quantitative phenotypes can be used for both severe and mild malaria: the presence or absence of malarial disease is a binary phenotype, while the risk of developing malarial disease is a quantitative phenotype. The calculation of the quantitative phenotype is generally based on a logistic regression model that takes into account significant covariates, such as age.

Asexual blood stages are responsible for mild and severe malaria. Parasitemia without clinical symptoms frequently occur in endemic area, but high parasitemia strongly increased the risk of developing mild malaria attacks. This has been clearly demonstrated in longitudinal studies in Africa (147). High parasitemia is often observed in case of severe malaria and is a WHO criterion for severe malaria diagnosis (182, 62, 113). However, in the case of severe malaria, infected red blood cells are sequestered at high levels, and low parasitemia can be observed (137, 138). In addition, it is conceivable that two individuals with the same parasitemia respond differently to the parasite infection, mounting different proinflammatory responses, and whereas one could develop severe malaria, the other could develop only mild malaria. Nevertheless, malaria pathogenesis is incompletely understood, and the study of parasitemia, which is a risk factor, should be helpful in understanding mechanisms involved in clinical malaria. Since parasitemia fluctuates considerably, using only one measurement is not sufficient to reflect the parasitic load of individuals. Therefore, cross-sectional studies (studies are based on data collected by measuring a parameter in many subjects at the same time point, or without regarding differences in time; thus, a
cross-sectional parasitemia survey is based on one parasitemia measurement) that are designed to test association of polymorphisms with severe malaria are generally not appropriate to the study of parasitemia. Conversely, longitudinal studies (in such studies, subjects are surveyed over time with continuous or repeating monitoring of a parameter; thus, a longitudinal parasitemia survey looks at parasitemia measured several times in the same subjects, examines how parasitemia changes over the course of time, and how subjects differ in parasitemia) that take into account >10 parasitemia measurements will be more appropriate for studying the influence of host genetic factors on parasitemia.

Additional phenotypes have been used in genetic studies. Since immunoglobulins G (IgG) have been shown to inhibit the growth of parasites in cooperation with effector cells in vitro and to protect humans in vivo (24), IgG levels were considered as pertinent phenotypes. The levels of IgG subclasses were also assessed: the levels of cytophilic IgG that have been associated with protection from mild or severe malaria are the most interesting phenotypes (12, 13). IgG1 and IgG3, which bind to Fc receptors, and IgG2, which binds to FcRIIA-H131, which is frequent in Africa, likely kill the parasite in cooperation with cells such as monocytes, while IgG4 may block this antibody-dependent cell cytotoxicity mechanism (Fig. 1).

HERITABILITY OF PHENOTYPES RELATED TO MALARIAL INFECTION AND DISEASE

Comparisons of three sympatric West African ethnic groups show that the Fulani had lower blood infection levels and fewer clinical malaria attacks than the Mossi and the Rimaibe living in the same area in Burkina Faso (110). In addition, the Fulani had higher levels of IgG against malaria antigens (111). Similar ethnic differences in resistance to malaria have been observed in Mali (39) and in Nepal (160). Interestingly, the low frequency of protective globin variants in the Fulani group suggest that unknown genetic factors were involved in malaria resistance (112). Although such differences may be a result of differences in lifestyle, it is thought that the Fulani resistance to malaria has a genetic basis.

Furthermore, twin studies have shown that mild malaria and antibody responses to parasite antigens are more concordant within monozygotic twin pairs than in dizygotic pairs (72, 73, 153). The results of several family studies have shown conclusively a familial aggregation of blood infection levels (1, 52, 142), malaria attacks (72), severe malaria (141), and antimalarial immune responses (12, 155, 158). Segregation analyses of parasitemia yielded significant sibling correlation coefficients, which ranged from 0.16 to 0.24 (1, 52, 142). Similarly, several studies have found significant sibling correlation of IgG and IgG subclass responses to parasite antigens (12, 157, 158); sibling correlation coefficients ranged from 0.13 to 0.39. Although an initial study detected a major gene controlling parasitemia in Cameroon, segregation analyses of both parasitemia and antibody levels showed a complex genetic model in Cameroon, in Burkina Faso, and in Papua New Guinea (1, 52, 142, 157). The odds of having cerebral malaria were 2.49 times higher for a child whose sibling had a history of cerebral malaria than the odds for a child whose sibling had no history of cerebral malaria (34, 141). Similar results have been obtained for mild malaria and severe anemia (72, 141). In addition, Mackinnon et al. (97, 98) found that genetic factors explained ~25% of the variation in the incidence of mild malaria episodes. Interestingly, the studies attributed a small proportion of the variation of mild malaria incidence to hemoglobin S (HbS) and α-thalassemia and pointed out that other genes were involved in malaria resistance (97, 98).

CASE-CONTROL ASSOCIATION STUDIES HAVE MAINLY IDENTIFIED GENES ASSOCIATED WITH SEVERE MALARIA

Case-control studies have been carried out to investigate the role of candidate genes in clinical malaria and these studies are summarized in Table 1. Such studies test whether case and control groups differ in marker allelic frequencies. Case-control studies are based on candidate genes, which have been chosen on the basis of the high frequency of deleterious alleles at the homozygous stage, or their function. Fifty years ago, Haldane (59) and Allison (10) reported a similarity in geographical distribution of hemoglobinopathies (thalassemia and sickle disease) and P. falciparum malaria and proposed that thalassemia and HbS might confer protection against malaria (10, 59). Thus, genes encoding α-globin and β-globin have been considered as candidate genes. Other genes involved

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**Fig. 1.** Immunological mechanisms directed against asexual blood stages and genes located within chromosome 5q31-q33 (red) and chromosome 6p21.3 (green).
in erythrocyte surface, in immune responses that likely affect the outcome of infectious diseases, in the sequestration of *P. falciparum*-infected erythrocytes, or in erythrocyte metabolism, have also been analyzed in association studies.

**ERYTHROCYTE POLYMORPHISMS AND SEVERE MALARIA**

*Hbs* carriers are strongly protected against severe malaria (2, 4, 8, 67). This clearly supports that there is a significant impact of malaria in selecting *Hbs* as a protective allele, despite the deleterious effect of *Hbs* at the homozygous state. Another hemoglobin variant named *Hbc* was shown to be associated with protection against severe malaria (3, 107, 112). Strikingly, the *Hbc* and the *Hbs* mutations occur in the sixth position in the β-globin gene, and both mutations coexist in several populations in West Africa. Since *Hbc* is clinically less severe than *Hbs* in the homozygote state, it has been suggested that *Hbc* would replace *Hbs* in West Africa (161). Other β-globin and α-globin variants that cause the thalassemias have also been shown to protect against severe malaria (7, 108, 179). In addition, association studies have provided consistent evidence of a protective effect of glucose-6-phosphate dehydrogenase (*G6PD*) deficiencies against severe malaria (56, 149), and haplotype analyses support the hypothesis of recent positive selection (150, 164). Other red blood cell polymorphisms, such as the deletion of glycoporphin C (*GYPC*) exon 3 or a 27 bp deletion in band 3 gene (*SLC4A1*), have also been associated with protection from severe malaria (9, 54, 133, 134).

**CYTOADHERENCE GENES, IMMUNE GENES, AND SEVERE MALARIA**

Several studies have detected the association of CD36 antigen (*CD36*), complement receptor 1 (*CR1*), intercellular adhesion molecule 1 (*ICAM1*), and platelet/endothelial cell adhesion molecule 1 (*PECAM1*) variants with either resistance or susceptibility to severe malaria. However, the results have been highly variable in different geographical locations. For instance, *ICAM1*-Kilifi allele was found to be associated with protection from severe malaria in Gabon and with susceptibility in Kenya, while a large study in the Gambia failed to detect any association (18, 42, 81). In the same way, the *CD36*-1264G variant was associated with susceptibility to cerebral malaria in populations living in the Gambia and in Kenya (6) and with protection against severe malaria in another Kenyan population (132). Similar findings were obtained for the other candidate cytoadherence genes and variants. It has been suggested that parasite factors may profoundly influence the pattern of association, since the sequestration of the parasite depends on the constant switch between different forms of *P. falciparum* erythrocyte membrane protein 1 (*PfEMP-1*) (146).

The nitric oxide synthase type 2A (*NOS2A*)-954C variant has been associated with protection against severe malaria in Gabon, while no association was detected in the Gambia and in Tanzania (26, 80, 90). Similarly, the human leukocyte antigen *HLA-B33* was associated with protection against severe malaria, but not in Kenya (67, 68). In contrast, several tumor necrosis factor (*TNF*) alleles have been associated with severe malaria in different studies: these include *TNF*-308A and *TNF*-238A (5, 104, 105, 175). The Fc gamma receptor Ila (*FCGR2A*)-H131 variant was also found to be associated with susceptibility to severe malaria in two different studies in Thailand and the Gambia (35, 129).

The association of erythrocyte genetic variants with severe malaria seems to be well established, while the pattern of associations of cytoadherence and immune responses with severe malaria is less clear and remains a subject of ongoing research. Strikingly, most of erythrocyte genetic variants that protect against severe malaria have been associated also with protection against mild malaria, erythrocyte resistance to invasion by the parasite, or a reduced parasitemia, while a few genes involved in immune responses, such as *TNF* and *NOS2A*, have been found to be associated with mild malaria or parasitemia (Table 1). The absence of association with parasitemia may be partly due to the cross-sectional study design, which is less appropriate than the longitudinal study design. Candidate immune genes associated with severe malaria may also weakly influence parasitemia and/or mild malaria, and other immune genes may be more important for these phenotypes. Nevertheless, it remains possible that the association of some genes with severe malaria arise from statistical artifacts, such as biases due to population stratification or population admixture.

**Family-Based Association and Linkage**

Family-based association and linkage studies test whether a particular allele of the marker is transmitted from parents to affected offspring in families more often than expected by chance. To date, most of the candidate genes have not been analyzed by using family-based studies. Nevertheless, such studies recently confirmed the association of *HbAS* and interferon gamma receptor 1 (*IFNGR1*)+2200C with protection against severe malaria (2, 77) and revealed the association of *IFNG*-183T with reduced risk of cerebral malaria (76, 29). In addition, *Hbc*, which protects from severe malaria, has also been shown to protect also against mild malaria (144).

Linkage analyses do not focus on a particular allele, and alleles co-inherited with disease may vary from one family to another, leading to the localization of genes controlling parasitemia and mild malaria on chromosome 5q31-q33 (44, 53, 143) and chromosome 6p21-p23 (45, 74). Chromosome 5q31-q33 contains several candidate genes implicated in the regulation of immune responses (Fig. 1). These include genes involved in the Th1 and Th2 balance, such as interleukin 4 (*IL4*) and *IL12B*, and genes involved in the activation of effector cells, such as *IL3*, *IL5*, colony stimulating factor 2 (*CSF2*), and colony stimulating factor 1 receptor (*CSF1R*), and genes involved in the production of antibodies, such as *IL4* and *IL13*. Chromosome 6p21-p23 corresponds to the MHC region, and the peak of linkage was found to be close to *TNF* (45). A systematic polymorphism screening revealed a family-based association of *TNF*-308, *TNF*-238, *TNF*-851, and *TNF*-1304 polymorphisms with mild malaria or parasitemia (46). Nevertheless, *TNF* genetic variation did not completely explain the linkage of the MHC region with mild malaria. More recently, results have shown that the natural cytotoxicity triggering receptor 3 (*NCR3*) is close to *TNF*, and we showed that a *NCR3* polymorphism located within the promoter was associated with mild malaria and that this association was not due to the association of *TNF* with mild malaria (36).

Interestingly, recent studies have shown mouse resistance to *Plasmodium chabaudi* to be linked to mouse chromosome 11.
and mouse chromosome 17 (63, 65), which carry regions homologous to chromosome 5q31-q33 and the MHC region, respectively. These findings might help to dissect the human loci and to demonstrate the effect of candidate genes in vivo. So far, only the effect of Tnf on resistance to P. chabaudi has been demonstrated in Tnf-deficient mice (66), which were more susceptible to the infection with higher parasitic levels and mortality rates.

CURRENT LESSONS FROM HUMAN GENETIC STUDIES OF RESISTANCE TO MALARIA

Case-control studies have clearly shown the protective effect of red blood cell polymorphisms on malaria (14, 40, 43, 89, 123, 140). A number of case-control studies have also detected association of genes involved in cytoadherence or immune responses. However, most of these studies yielded conflicting results, suggesting a possible role for hitherto unknown traits associated but not examined in these studies. Family-based studies avoid biases due to population stratification. This refers to a situation in which the study population includes two subpopulations genetically different; in this case, the two subpopulations differ in allele frequencies at a number of genetic markers located within different chromosomal regions (18, 25, 69, 76, 82, 95, 96, 105, 108, 124, 125, 130, 165, 180). Since these differences can be due to different population histories, family-based studies should be useful to clarify this point. To date, very few family-based association studies have been conducted, and they have confirmed the protective effect of hemoglobin variant and some immune gene variants.

Linkage and association analyses can be viewed as a more general approach to localize and to identify genes involved in malaria resistance (15, 23, 78, 114, 115, 151, 169). The first studies have identified two chromosomal regions that contain malaria resistance genes (Fig. 1), and these results were confirmed in linkage replication studies in humans. Two genes, TNF and NCR3, which are located on chromosome 6p21.3, have been shown to be independently associated with mild malaria, indicating that there are at least two genes located on the central region of HMC involved in the genetic control of human malaria. Preliminary analysis suggests that chromosome 5q31-q33 also contains several genes controlling parasitemia (P. Rihet, unpublished data). To date, those malaria resistance genes have not been identified. It is well known that the identification of genes underlying complex traits is a challenging task, and strategies combining mouse and human studies may be helpful in identifying malaria resistance genes. In this way, two mouse loci have been mapped to chromosomal regions that correspond to human chromosomes 5q31-q33 and 6p21.3 (see the section of mouse genetic studies of malaria resistance in this chapter). This suggests that mouse models might help to identify malaria resistance genes in humans.

MOUSE MODELS FOR STUDYING MALARIA

The mouse is a leading model for studying biological processes in mammals and provides models of particular importance in the study of the host response to infectious disease and understanding why some naturally occurring genotypes are dramatically more sensitive to disease than others. This clearly applies to the malaria disease. Human and mouse genomes are very similar. Mouse strains show different degrees of susceptibility and resistance to malaria as observed in human cases. There are a number of different parasites infecting the laboratory mouse, and while none of these behaves like P. falciparum, the deadly strain of human malaria, they do model parts of the disease. There are also significant interstrain variations, which have been exploited to study host responses. Therefore, the mouse model offers a powerful tool for dissecting and understanding the host response mechanism to malaria, and through comparative genomics it may be possible to identify the orthologous genes in humans.

Mouse Populations for Gene Mapping Studies

In crosses between genetically well-defined strains of mice, chromosomal regions responsible for the genetic variance of quantitative traits (i.e., parasitemia) can be mapped to a large genetic interval as quantitative trait loci (QTL) in experimental resource populations [F2 or backcross (BC) generation] (87, 135). A QTL is defined as a region of DNA that is associated with a particular phenotypic trait. Knowing the number of QTL that explains variation in the phenotypic trait tells us about the genetic complexity of a trait. QTL mapping is the statistical study of the alleles that occur in a locus and the phenotypes that they produce. Due to the limit of recombination events in F2 and BC populations, QTL usually maps within 20–30 cM genomic regions, which may consist hundreds if not thousands of genes; 1 cM represents ~2,000 Kb of DNA in the mouse genome.

Subsequently, fine mapping of the QTL can be achieved by a number of approaches including advanced intercross lines (AIL), recombinant inbred lines (RIL), congenic strains (CS), using single nucleotide polymorphisms (SNPs) (181), haplotype mapping (58), and Collaborative Cross (CC) (33, 162, 166). They are unquestionably providing an opportunity to identify host genetic factors that influence disease progression.

Once QTL or genes have been identified, genetic analysis may then be extended successfully to humans (167). For example, the genetic basis of total and HDL cholesterol concentrations was examined in experimental crosses involving various pairs of inbred mouse strains maintained on regular chow and high-fat diet (71, 79, 99, 172). Although these experiments discovered >27 QTL, they could explain only a fraction of the total genetic variance in cholesterol metabolism. Importantly, however, comparative mapping showed that the majority of murine QTL and candidate genes have known correspondents in the human genome, emphasizing the relevance of QTL analysis in the mouse model for understanding complex disease in humans (173).

Murine Malaria Parasites

For more than 20 years, laboratory mice have been exposed to a wide range of health-threatening conditions, infected with all kind of microorganisms, phenotyped, cured with testing drugs, and immunized with potential vaccines. The availability of mouse genetic markers (e.g., microsatellites and SNPs), new mouse genomes resources, which are available at the following websites: http://www.ncbi.nlm.nih.gov/genome/guide/mouse/, http://wwwensembl.org/Mus_musculus/, http://genome.ucsc.edu/, http://www.informatics.jax.org/, and the published initial sequencing of the mouse C57BL/6J genome by the Mouse Genome Sequencing Consortium (117) have highly encour-
aged the study of the genetic bases of resistance to diseases in mouse. Four mouse *Plasmodium* species (*P. chabaudi*, *P. berghei*, *P. yoelii*, and *P. vinckei*) were found to infect rodents and mosquitoes from several Central African regions several decades ago (31, 32, 84, 85). Thereafter, numerous studies described the morphological and developmental characteristics and isoenzyme patterns from the different species (86, 170). The malaria mouse models became popular laboratory tools with which to study the drug (mainly pyrimethamine, cloroquine, and sulphonamide) resistance issues that were alarming worrying in the affected human communities (131, 152, quinine, and sulphonamide) resistance issues that were alarming worrying in the affected human communities (131, 152, 174, 184).

Among the four species, *P. chabaudi* has provided a remarkable experimental tool with many similarities to human *P. falciparum*, such as analogous blood-stage antigens, invasion of red blood cells, suppression of cell-mediated immune responses, and parasite sequestration in target organs (159). Two subspecies of *P. chabaudi* have been described: *P. chabaudi chabaudi* and *P. chabaudi adami*. The latter is known to induce a severe form of the disease characterized with more pronounced pathologies compared with the infections by *P. chabaudi*. *P. yoelii* was a common experiment model of mouse cerebral malaria some decades ago. However, it has been extensively used in the development and characterization of vaccine candidates in the last decade (119). Three subspecies have been defined: *P. yoelii yoelii*, *P. yoelii killicki*, and *P. yoelii nigeriensis*. *P. berghei* is currently the preferred mouse parasite for the study of cerebral malaria and its lethal pathologies (93). Researchers have available five different isolates: k173, SP11, LUKA, NK65, and ANKA, although most of the mouse cerebral malaria investigations have been carried out with *P. berghei* ANKA. Four subspecies of *P. vinckei* are recognized: *P. vinckei vinckei*, *P. vinckei petteri*, *P. vinckei lentum*, and *P. vinckei brucechewi*. Despite being the most distributed mouse parasite in nature, *P. vinckei* is the least characterized mouse parasite. *P. vinckei petteri* has been used in the identification of new antimalarial drugs (21).

**Common Laboratory Mouse Strains Used In Malaria Studies**

In the last few decades, up to 13 different inbred mouse strains have been infected with several *Plasmodium* species, and their susceptibility to malaria (and to other infectious diseases) has been characterized (71a, 83, 126). Most of the mouse strains are susceptible to the infections by the four murine parasites. However, C57BL/6J and DBA/2J mice are resistant to infections by *P. chabaudi* and *P. berghei* parasites, respectively, and are commonly used in investigations of the molecular basis of mouse resistance to malaria. Susceptible and resistant mice to *P. chabaudi* infections suffer from severe anemia 2 wk postinfection. Susceptible animals die with high parasitic loads and low red blood cell concentrations, whereas resistant animals clear parasites from the bloodstream and recover the preinfection red blood cell values. Moreover, the outcomes from susceptible and resistant mice after infection with mouse parasites can differ substantially. While *P. berghei*-susceptible mice die from cerebral malaria few days after infection, resistant animals die from severe anemia after several weeks postinfection. Table 2 summarizes the current information about the susceptibility of laboratory mice to malaria induced by the most common murine parasites in mouse studies, *P. chabaudi*, *P. berghei*, and *P. yoelii*. Since 1997, a remarkable number of publications have described more than a dozen genetic loci (Table 3) linked to murine malaria resistance (47, 48, 92, 100).

**Table 2. Susceptibility of inbred mouse strains to malaria after infection with 3 Plasmodium subspecies (83, 136)**

<table>
<thead>
<tr>
<th>Inbred Mouse Strain</th>
<th><em>P. chabaudi</em></th>
<th><em>P. berghei</em></th>
<th><em>P. yoelii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>A/HeJ</td>
<td>susceptible</td>
<td>susceptible</td>
<td>ND</td>
</tr>
<tr>
<td>A/J</td>
<td>susceptible</td>
<td>susceptible</td>
<td>ND</td>
</tr>
<tr>
<td>AKR/J</td>
<td>resistant</td>
<td>susceptible</td>
<td>ND</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>susceptible</td>
<td>susceptible</td>
<td>ND</td>
</tr>
<tr>
<td>CBA/J</td>
<td>resistant</td>
<td>susceptible</td>
<td>ND</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>susceptible</td>
<td>resistant</td>
<td>ND</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>resistant</td>
<td>susceptible</td>
<td>ND</td>
</tr>
<tr>
<td>C58/6J</td>
<td>ND</td>
<td>resistant</td>
<td>ND</td>
</tr>
<tr>
<td>DBA/1J</td>
<td>susceptible</td>
<td>resistant</td>
<td>ND</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>resistant</td>
<td>resistant</td>
<td>ND</td>
</tr>
<tr>
<td>SJL/J</td>
<td>susceptible</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ST/J</td>
<td>ND</td>
<td>susceptible</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not done.
intercrosses of lines at the N 7 or N8 generation. Genetic different recombination outputs around background genome. A total of 10 were selected for subsequent BCs to the parental mice to fix the protein (Cmkbr4) and chemokine receptor 4 (human region 3q24 –26, which contains two genes involved by different species. The locus position is homologous to the parasitemia; CM, cerebral malaria; CS approach was used to generate locytes and mature red blood cells, pymr (char1) might represent a relevant common locus controlling malaria traits induced by different species. The locus position is homologous to the human region 3q24 –26, which contains two genes involved in immune responses, cytokine-inducible SH-2-containing protein (Cish), and chemokine receptor 4 (Cmkbr4).

Further studies with the promising loci, char1 and char2, could aim at identifying the key gene. In fact, 5 years later, a CS approach was used to generate char2-CS using C3H/HeJ and C57BL/6J as parental inbred lines (28). At each generation, mice carrying the genotype of interest at the char2 locus were selected for subsequent BCs to the parental mice to fix the background genome. A total of 10 char2-CS, each carrying different recombinant outputs around char2, originated from intercrosses of lines at the N7 or N8 generation. Genetic analysis of P. chabaudi adami DS-infected animals from each line suggested a large congenic interval on chromosome 8, which conferred susceptibility on background of resistant parental mice (the CS was named Char2–7). The interval most likely comprised several genes that could interact to control survival and parasitic levels. One of them was mapped to the distal region of the chromosome when compared congenic intervals from nonsusceptible animals of resistant background. Because char2 was localized in the central region on chromosome 8 in previous studies (47, 49), these congenic studies strongly supported the existence of several genes at this locus, sufficient to decrease the survival to malaria. To refine the map positions of the char2 genes, a series of recombinant lines from the Char2–7 CS has been recently derived (91) by crossing back to the parental line C57BL/6J. Nine Char2–7 recombinant CS, targeting the distal region of chromosome 8, were inbred, infected with P. chabaudi adami DS, and tested for susceptibility to malaria. Because of changes of the parasite lethality and the difficulties to discriminate phenotypes among the recombinant CS, mortality rates from each strain were normalized and a statistical model was used to predict the position of a single locus or multiple loci. Results from the different models were compared, and two critical regions showed the highest likelihood of containing the char2 genes.

One, on the proximal portion of chromosome 8 (35–42.6 cM), was mapped on the same position as in the original studies (47, 49). The other, on the distal portion of the chromosome (68.9 –75.4 cM), was suggested in the first congenic study (28), and it could be now confirmed as the second char2 locus controlling susceptibility to malaria.

A different approach to refine the position of char2 in the proximal region of chromosome 8 was carried out using an F11 AIL from an F1 cross of A/J and C57BL/6J mice (63). The power of the AIL approach in fine mapping resistance loci has been reported in murine trypanosomiasis and pulmonary adenoma studies (70, 171), reducing 95% CIs of loci positions up to sevenfold compared with those observed in F2 studies. However, the 95% CI of char2 location (between 13 and 16 cM) could not be narrowed down as expected from the AIL approach. This was most likely due to the little phenotypic differences found in the parental mouse strains infected by P. chabaudi chabaudi 54X compared with the well-distinguished phenotypes observed in infections by P. chabaudi chabaudi AS (156). As it has been shown in later studies, environmental factors as well as parasite polymorphisms among isolates might influence the severity of malaria between susceptible and resistant mice (64). Moreover, the AIL study did not detect the presence of linked loci on the proximal region of chromosome 8 as suggested by the first congenic line study (28) and supporting the results from the second congenic line study (91), which showed the location of the second char2 locus on the distal region of the chromosome. Two more candidate genes were presented to control parasitemia in the AIL study: chondroitin sulfate proteoglycan 3 (Csgp3), a major constituent in blood vessels, and interleukin 12 receptor beta 1 (il12rb1)

While many efforts toward the positional cloning of char2 genes have been performed, refinement studies on the map position of char1 have not been performed, despite the relevance of the locus found in two separate studies with different mouse and parasite species.

The first evidence of H2 locus involved in the control of survival to malaria infections in mouse appeared two decades ago (183). However, the H2 locus was not significantly linked

### Table 3. Mouse genetic loci controlling P and CM

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Locus</th>
<th>Chromosome</th>
<th>Murine Parasite</th>
<th>Candidate Genes</th>
<th>Gene Identified</th>
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<tr>
<td>P</td>
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<td>Tmf, Rbp</td>
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<tr>
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<tr>
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<td>char3</td>
<td>17</td>
<td>P. c. adami DS</td>
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<tr>
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<td>3</td>
<td>P. c.c. AS</td>
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<td>P. c.c. 54X</td>
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</tr>
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<sup>c</sup>"Candidate Genes" column shows genes initially proposed as candidates. "Genes Identified" column shows the variation that alters the phenotype. P. parasitemia; CM, cerebral malaria; P.c., Plasmodium chabaudi; P.c.c., Plasmodium chabaudi chabaudi; P.b., Plasmodium berghei.
to either survival or peak parasitemia in BC and BC-F2 combined studies (49). A new study using an F3 generation of C3H/HeJ and C57BL/6J mice was carried out to detect loci responsible for the control of daily parasitemia levels (27). A significant locus at the proximal end of chromosome 17 (a region harboring the H2 complex), named char3, was identified to control parasite clearance from the blood stream after peak parasitemia, whereas char1 and char2 controlled peak parasitemia levels, confirming previous results (47, 49). Because char3 did not play any role in the control of peak parasitemia but was critical in the eradication of parasites, this locus might represent the complex H2 locus that could modulate T-cell responses required for the clearance of primary and secondary parasitemias (Fig. 2) (88). Interestingly, human MHC alleles have been involved in the protection of West African children from severe malaria (67), and TNF, a gene harbored within the H2 loci, was implicated in the immune response to cerebral malaria (46). The char3 region has also been involved in the control of TRBV4 CD8+ T cells during mononucleosis induced by γ-herpes virus (60).

The AIL study with an (A1xC57BL/6J) F11 population infected with P. chabaudi chabaudi 54X resolved this locus into two loci in coupling phase, one of them was mapped near the H2 region (char3), whereas the other, named in this study char7, mapped within a region containing interesting candidate genes, i.e., complement component 3 (C3), interleukin 25 (Il25), and immune response 5 (Ir5). Further linkage studies of both loci using daily parasitemia traits revealed that char3 and char7 acted in early stages of the infection before peak parasitemia, in disagreement with previous mapping studies using P. chabaudi parasites. These controversial results showed that different P. chabaudi subspecies and mouse-specific parasitemia development might influence the mode of action of the resistant loci.

Another remarkable effort to narrow down the positions of char1 and char2 loci and map new malaria resistance loci was carried out using a set of 37 recombinant CS derived from A/J and C57BL/6J parental mice (50). The individual AcB strains, created from (F1xA/J)xA/J double backcrosses, contained on average 13% of the C57BL/6J genome, and the total AcB set contained almost 80% of the resistant genome. Susceptibility phenotypes to P. chabaudi chabaudi AS infection and genotypes at char1 and char2 loci were studied in all the AcB strains (51). In particular, the AcB55 strain was found to be resistant to infection (low parasitic levels and 0% mortality), despite carrying susceptibility alleles at char1 and char2 loci, indicating the presence of a novel resistance locus transferred by a small C57BL/6J chromosomal region into the A/J genomic background. An (AcB55xA/J)F2 population was monitored for parasitic levels and mortality and genotyped at a maximum spacing of 10 cM covering all murine chromosomes containing C57BL/6J genomic segments. While genetic linkage was not detected when mortality was used as phenotypic trait, a significant locus was detected on chromosome 3 (char4) when peak parasitemia was used as phenotype. A second, less significant, locus (not named in this study) was mapped on chromosome 10. Both loci explained 18% of the variance of the peak parasitemia. The size of char4 locus was estimated between 6 and 10 cM, a region narrow enough to transfer into A/J genomic background and create congenic lines for further dissection of the traits. The char4 region, syntenic with the human 4q21-q25, contained interesting candidate genes: lymphoid enhancer binding factor 1 (Lef1), a transcription factor that regulates gene expression in T lymphocytes, complement component factor I (Cfi), and the p105 subunit of NF-kB1, an essential regulator for B cell functions.

Further clinical examinations of two malaria-resistant AcB congenic lines, AcB55 and AcB61 (the latter carried susceptibility alleles at char1 and char2 but did not carry resistance allele at char4), were carried out to detect possible biochemical mechanisms that could have conferred resistance to the infection (106). In fact, mice from both CS showed significant splenomegaly caused by the expansion of the red pulp at the time of necropsy, a higher expression of erythroid-specific (globin, transferrin receptor and solute carrier family 11 member 2) and cell cycle genes, and abnormally higher reticulocyte concentrations. Genetic analysis of (AcB55xA/J)xF2 and (AcB55xDBA/2J)xF2 populations using reticulocytosis as the quantitative trait mapped a new A/J-derived locus proximal to char4 region. Liver- and cell-specific pyruvate kinase (pklr) gene was selected as a strong candidate in the new locus for playing essential roles in the synthesis of ATP in red blood cells and cause hemolytic anemia in humans when it is mutated. DNA sequence analysis detected an isoleucine-to-asparagine substitution at amino acid 90 of the pklr protein in both AcB55 and AcB61 congenic mice. Moreover, association studies demonstrated that homozygous pklr mutants had reduced peak parasitemia and mortality rates when compared with heterozygous mice. These spectacular results showed that inactivating pklr mutations were protected against P. chabaudi chabaudi AS malaria and that splenomegaly and an enhanced erythropoiesis and reticulocytosis worked as compensatory mechanisms to overcome the infection. These investigations proved that the recombinant CS approach can successfully be used for genetic dissection of complex malaria traits. The Pklr269A mutation controlling hemolytic anemia in mice affected the related and more complex parasitic level trait, which might be controlled by additional, not-yet detected genetic factors. Epidemiological studies have not detected a selective pressure on pyruvate kinase deficiency by the malaria parasites, as in the cases of sickle cell trait and thalassemias, but a possible protective role of this deficiency against the different malarias in humans cannot be ruled out. Currently, the impact of pyruvate kinase deficiency in humans infected with malaria...
parasites is being studied. So far, the finding of the \( {\text{Pklr}}^{260A} \) mutant as a resistant gene in mice represents the most remarkable achievement from genetic studies of malaria resistance in mouse models and shows the optimal strategy that it should be carried out toward a successful identification of more candidate genes from preliminary \( F_2 \) or BC studies.

An additional pair of loci of opposite additive effects, \( \text{char5} \) and \( \text{char6} \), controlling parasitemia curves was mapped on chromosome 5 in the AIL \( F_{11} \) study (63). A previous study with a BC population of A/J and C57BL/6J as parental mice detected \( \text{char6} \) (49), although \( F_2 \) and combined BC and \( F_2 \) studies could not confirm the presence of the locus. The lack of confirmation in the latter studies could have been due to the use of software to perform genetic analysis to detect single loci, possibly missing the detection of linked loci, as in this case, on chromosome 5. As in the case of linked \( \text{char3} \) and \( \text{char7} \) loci on chromosome 17, the 95% CI of \( \text{char5} \) and \( \text{char6} \) positions could not be determined. Analysis of \( \text{char5} \) and \( \text{char6} \) for daily parasitemia traits showed that both loci seemed to act in the days of highest parasitemia, in agreement with the results from the suggestive linkage to peak parasitemia in the BC study. Linked \( \text{char5} \) and \( \text{char6} \) regions contained a substantial number of potential candidate genes: blood antigen acetylcholinesterase (\( \text{Ache} \)), erythropoietin (\( \text{Epo} \)), heat shock 27-kDa protein 1 (\( \text{Hspb1} \)), correlation in cytokine production 1 (\( \text{Cora1} \)), NADPH oxidase subunit (\( \text{Ncf1} \)), and actin-related gene 1 (\( \text{Act1} \)).

**COMPARATIVE APPROACH WITH HUMAN AND MOUSE GENOMES**

Human homologous genes may be detected by comparative mapping of human and mouse genomic regions (22, 177). However, it is also possible to perform an inverted strategy and study the role of homologous human loci of resistance to malaria in mouse models. The identification of genetic factors involved in the malarial disease in both humans and mice could reveal common genes controlling similar mechanisms of infection from different mammalian malaria (101).

Several association and linkage analysis of \( \text{Plasmodium falciparum} \) parasitemia mapped \( \text{pfbi} \) (blood infection) locus on the 5q31-q33 chromosome region (see Table 1), flanked by DNA microsatellite D5S642 and interleukin 12 gene (\( \text{IL12B} \)) (143). The segment contained several candidate genes involved in the regulation of the immune TH1 and TH2 responses to the infection such as interleukin 4 (\( \text{IL4} \)), \( \text{IL12B} \), and interferon regulatory factor 1 (\( \text{IRF1} \)). Interestingly, the 5q31-q33 region has also been identified as controlling \( \text{Schistosoma mansoni} \) infections (161) and regulating immunoglobulin E levels (101). In an attempt to find novel murine loci for control of parasitemia on regions homologous to human chromosome 5q31-q33 (Fig. 3), the (A/JxC57BL/6J)\( F_{11} \) population of mice was genotyped on chromosomes 11 and 18, but not on chromosome 13 (65). A new locus, named \( \text{char8} \), was found on chromosome 11 controlling parasitic levels. The locus was more significant for the trait with parasitemia scores up to \( \text{day 11} \) postinfection than for the trait with parasitemia scores up to \( \text{day 7} \) postinfection, indicating that \( \text{char8} \) might act from early to late stages of the parasitemia curve. Although >300 genes were found within the 95% CI of \( \text{char8} \), \( \text{IL3} \), \( \text{IL4} \), \( \text{IL5} \), \( \text{IL12b} \), \( \text{IL13} \), \( \text{Irfl} \), granulocyte-macrophage colony-stimulating factor 2 (\( \text{Cs2} \)), growth differentiation factor 9 (\( \text{Gdf9} \)), heat shock protein 4 (\( \text{Hspa4} \)), T-cell phenotype modifier (\( \text{Tcmp1} \)), T-cell-specific transcription factor 7 (\( \text{Tcf7} \)), il2-inducible T-cell kinase (\( \text{ltk} \)), and T-cell immunoglobulin and mucin domain containing genes (\( \text{Tim1} \) and \( \text{Tim3} \)) were presented as interesting candidate genes with homologs in human chromosome 5q31-q33.

Moreover, one of them, \( \text{Cs2} \), has been involved in the development of splenomegaly, leukocytosis, and granulocyte-macrophage hematopoiesis, controlling the resistance to \( \text{P. chabaudi} \) infections (145). Because any genetic factor controlling the malaria infection was not found on mouse chromosome 18, this locus comparative approach might lead to further dissection studies to map \( \text{pfbi} \) gene(s) on chromosome 5q31-q33.

**GENETIC LOCI LINKED TO MURINE CEREBRAL MALARIA RESISTANCE**

\( \text{P. berghei} \) ANKA induces the symptoms that can mimic some of the aspects of human cerebral malaria in susceptible mouse strains. These aspects include respiratory distress syndrome, low body temperature, ataxia, paralysis, and coma, followed by death. Histopathological studies showed that sequestration of parasitized red blood cells in brain vessels and damage of endothelial tissues were involved in the irreversible effects of the infection (120, 121, 136). Based on the percentage of mortality from cerebral malaria, laboratory strains of mouse can be divided into highly susceptible (e.g., C57BL/6J, \( \text{CBA/J} \), and 129/SvJ), weakly susceptible (e.g., BALB/cJ and C3H), and resistant (e.g., DBA/2J) (16). Interestingly, suscep-
tible mice died from neurological damages with low parasitemia, whereas resistant mice died with severe anemia and high parasitic levels.

The first genetic study of resistance to cerebral malaria in mouse used an F2 population of susceptible C57BL/6J and resistant DBA/2J mice (118). Animals surviving to day 14 postinfection were phenotyped as resistant, whereas those dying before that day were phenotyped as susceptible. The analyses showed a significant locus (unnamed in this study) in the middle region of chromosome 18 controlling mortality to cerebral malaria. Less significant loci were found on chromosomes 5, 8, and 14. Strikingly, the position of the locus on chromosome 8 mapped within the char2 region, suggesting a common segment involved in two different malaria phenotypes, parasitemia and cerebral malaria. Several candidate genes on chromosome 18 were suggested to control cerebral malaria: colony-stimulating factor 1 receptor (Csf1r), the platelet-derived growth factor receptor, beta peptide (Pdgfrb), and the CD14 antigen (Cd14). Interestingly, the expression of the latter appeared to increase in acute respiratory distress syndrome, one of the features associated with the fatalities from cerebral malaria (94).

To find out new strains of mouse resistant to cerebral malaria and make use of more genetic polymorphisms, several wild-derived mouse strains from Asia, Western and Eastern Europe, and Northern Africa were challenged with P. berghei ANKA. Six of the 12 strains tested were resistant to cerebral malaria (16). WLA, a Mus musculus domesticus strain, has been used as the resistant mouse line in subsequent genetic resistance studies. In fact, a (WLAxC57BL/6J)F1 population was backcrossed to C57BL/6J. The resulting animals were infected with P. berghei ANKA, genotyped covering the entire genome, and phenotyped as susceptible when they died with typical neurological symptoms of cerebral malaria resistance. It was noted that transforming growth factor β2 (Tgfb2), a gene known to control the severity of mouse infections by P. chabaudi and P. berghei (38, 128), is located 0.5 cM apart from the marker with the highest significance for genetic linkage on chromosome 1. A new linkage analysis genotyping for Tgfb2 showed equal association strength, suggesting this gene as a potential candidate for cerebral malaria control. A parallel study with a series of recombinant inbred mice from susceptible BALB/cJ (non-mammary tumor virus 7, Mtv-7, gene carriers) and resistant DBA/2J (Mtv-7 carriers), showed that animals with the Mtv-7 gene inserted in their genomes were resistant where those without the insert were susceptible to cerebral malaria (57). However, because Mtv-7 was not mapped within the most significant linked segment on chromosome 1 to the cerebral malaria phenotypes, this gene was not presented as a potential candidate.

A new cerebral malaria susceptibility locus, cmsc (cerebral malaria susceptibility), within the H2 region on chromosome 17 was found in a BC population bred from susceptible CBA and resistant DBA/2J mice infected with P. berghei ANKA (127). This study did not find previously reported loci such as berr1, berr2, or the unnamed locus on chromosome 18, raising the possibility of the existence of different susceptible loci that contribute to the cerebral malignancies of CBA and C57BL/6 mice. In agreement with a previous study on susceptibility of different mouse strains to P. berghei ANKA-induced cerebral malaria (146), which showed that H2 haplotypes were not highly involved in the control of the pathogenesis, cmsc could not be identified as an H2 susceptible haplotype since resistant mice such as C3H/HeJ and AKR share the same CBA-susceptible haplotype. However, as char3, cmsc mapped near attractive candidate genes such as Tnf and lymphotixin a (Ltxa), which are critical mediators of the pathogenesis induced by cerebral malaria. Recently, a gene knockout study has shown that Tnf-deficient C57BL/6J mice had higher peak parasitemias and mortality rates than proficient animals (66). The multiple roles of Tnf in the different malaria pathologies as its different modes of action during the parasitic infection still remain to be fully understood. Current sequence analysis of both Tnf and Ltxa genes from CBA and DBA/2 mice might suggest the identity of the causative genetic variant within the cmsc locus in the near future.

Previous genetic studies to find novel loci associated with resistance to cerebral malaria used the reported (WLAxC57BL/6)F2 population of mice (30). Using survival time and cure as cerebral malaria phenotypes, investigators found a resistant locus on distal chromosome 1, mapping within the previously reported berr1. Two more loci, berr3 and berr4, were mapped on chromosome 9 and 4, respectively. Interestingly, berr3 mapped near to previously reported char1 and pymr loci, involved in the resistance to infections by P. chabaudi and P. yoelii. This region has gained a special interest since it might harbor a common resistant gene to the infections of several murine Plasmodium species, and it might represent a universal mechanism of defense against different parasites. Recently, a deficiency in chemokine receptor Ccr5 (encoding gene is located within the berr3 region) has been associated to reduced susceptibility of P. berghei cerebral malaria (19). The attractive genes in the berr4 segment are a toll-like receptor (Tlr4) and the complement component 8 beta subunit.

CONCLUSIONS AND FUTURE DIRECTIONS

A mapping experiment using the human parasite requires the use of mosquitoes to infect chimpanzees with P. falciparum gametocytes. Murine malaria makes it possible to work entirely in the mouse, a far more tractable and cost-effective model. Such studies have focused on increasing our understanding of the parasite, including the development of drug resistance, antigenic variation, and erythrocytic invasion pathways. Mouse models have also been extensively used to dissect the host response to disease including the innate and adaptive immune responses. This review has shown the use of mouse models to map genetic regions controlling malaria traits that need to be further dissected to find the resistant genes. In the immediate future, many research groups will generate congenic lines for the known QTLs, and the results from examinations of the biological features of these lines will be presented. These could give some interesting insights into associated phenotypes ahead of the identification of the underlying genes.

The development of microarray technology with the spotting of mouse genes on chips is presently being used to follow changes in expression of all mouse genes as a response to infection. In particular we now are able to contrast the response of resistant and susceptible genotypes. Recently, Delahaye et al. (37) were successful in using this technology to identify
GENETIC RESISTANCE TO MALARIA IN HUMAN AND MURINE

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.H.-V. and F.A.I. prepared figures; M.H.-V., P.R., and F.A.I. drafted manuscript; M.H.-V., P.R., and F.A.I. approved final version of manuscript.

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