The lower susceptibility to *Plasmodium falciparum* malaria of Fulani of Burkina Faso (West Africa) is associated with low frequencies of classic malaria-resistance genes

David Modiano, Gaia Luoni, Bienvenu Sodionm Sirima, Alessandra Lanfranconi, Vincenzo Petracca, Fulvio Cruciani, Jacques Simpore, Bianca Maria Ciminalli, Enrica Foglietta, Paola Grisanti, Ida Bianco, Guido Modiano and Mario Coluzzi

**Abstract**

The gene frequencies in 1993-94 for haemoglobin S, haemoglobin C, alpha-thalassaemia, G6PDA, HLA B*5301 were estimated in Fulani, Mossi and Rimaibe ethnic groups of Burkina Faso, West Africa. The aim of the study was to verify whether the previously reported Fulani lower susceptibility to *Plasmodium falciparum* malaria was associated with any of these malaria-resistance genes. Similar frequencies for haemoglobin S were recorded in the 3 ethnic groups (0.024 ± 0.008, 0.030 ± 0.011, 0.022 ± 0.013; in Mossi, Rimaibe and Fulani, respectively). The Mossi and Rimaibe showed higher frequencies when compared to Fulani for haemoglobin C (0.117 ± 0.018, 0.127 ± 0.020, 0.059 ± 0.020), alpha-deletional thalassaemia (0.227 ± 0.040, 0.134 ± 0.032, 0.103 ± 0.028), G6PDA (0.196 ± 0.025, 0.187 ± 0.044, 0.069 ± 0.025) and HLA B*5301 (0.189 ± 0.038, 0.202 ± 0.041, 0.061 ± 0.024). Among Fulani the proportion of individuals not having any of these protective alleles was more than 3-fold greater than in the Mossi-Rimaibe group (56.8% vs 16.7%; P<0.001). These findings exclude the involvement of these genetic factors of resistance to *P. falciparum* in the lower susceptibility to malaria of Fulani. This evidence, in association with the previously reported higher immunity reactivity to malaria of Fulani, further supports the existence in this ethnic group of unknown genetic factor(s) of resistance to malaria probably involved in the regulation of humoral immune responses.

**Keywords:** malaria, *Plasmodium falciparum*, disease susceptibility, genes, ethnic differences, haemoglobinopathy, glucose 6-phosphate dehydrogenase deficiency, Burkina Faso

**Introduction**

Malaria is one of the most common causes of morbidity and mortality in sub-Saharan Africa. Every year, an estimated number of 1-2.8 million persons, mostly children, die from *Plasmodium falciparum* malaria (WHO, 1996). Approximately 2% of clinical attacks of malaria in African children are severe (GREENWOOD et al., 1991). Besides cultural reasons, resistance/susceptibility genetic factors of the host (HILL, 1996) and variation in virulence of parasite strains (GUPTA et al., 1994) may influence the clinical outcome of the disease. Several genes contributing to inter-individual variability in the susceptibility to severe malaria have been described (HILL, 1998). The further identification of factors related to susceptibility/resistance to mild and severe forms of *P. falciparum* malaria, especially those involved in the genetic regulation of the immune response, may improve the understanding of host-parasite interactions, eventually contributing to the development of new control tools.

One of the possible approaches in the study of human variation in the susceptibility to malaria consists in comparing malarialogical indicators between populations differing in their genetic background but living in the same epidemiological context, i.e., exposed to the same transmission level and to the same parasite strains. The possible observation of interethnic differences of susceptibility in such conditions may provide the opportunities to detect factors associated with protection. This interethnic comparative approach was recently applied in extensive studies performed in hyperendemic rural areas of Burkina Faso (formerly Upper Volta), West Africa. These studies showed striking interethic heterogenetics in the susceptibility to *P. falciparum* malaria among 3 sympatric ethnic groups, Fulani, Mossi and Rimaibe (MODIANO et al., 1995, 1996). Fulani (c. 13 million), also called Peuhl, or Fulbe, are nomadic pastoralists partly settled and characterized by non-negroid features of appearance (Peuhl, 1990; OLERUP et al., 1991; ALLSOPP et al., 1992). They are scattered in many parts of West Africa, from Lake Chad to the Atlantic coast, mostly in Cameroon, Nigeria, Niger, Mali, Burkina Faso, Guinea and Senegal (STENNING, 1965). The Mossi and Rimaibe are Sudanean negroid populations with a long tradition of sedentary farming in sub-Saharan savannahs. Mossi (c. 4 million) live in the central plateau of Burkina Faso (SKINNER, 1964). Closer to Mossi in terms of ethnic origin, the Rimaibe have adopted most of the socio-cultural habits of Fulani since they have been their slaves. Also, they live mostly in the same villages of Fulani as farmers and are marginally involved in cattle breeding.

The above mentioned studies (MODIANO et al., 1995, 1996) showed that, in spite of similar exposure to malaria and comparable use of protective measures, the Fulani were less parasitized and less affected by the disease when compared to Mossi and Rimaibe. Moreover, the analysis of the humoral immune response to several *P. falciparum* sporozoite and blood-stage antigens (circumsporozoite protein, thrombospondin-related adhesive protein, merozoite surface protein-1, Pf-355, merozoite surface protein-3) revealed higher seroprevalences and levels in Fulani as compared to Mossi and Rimaibe (MODIANO et al., 1995, 1996, 1998, 1999). Even though the immunoparasitological differences observed were probably not explainable in terms of known genetic factors of protection against malaria, since these factors confer neither an obvious parasitological resistance nor higher immune reactivity, nevertheless it was interesting to evaluate the possible
contribution of classic malaria-resistance genes to the interethnic differences observed. Thus, we evaluated the gene frequencies for haemoglobin S (HbS), haemoglobin C (Hbc), alpha-3.7 deletional thalassaemia (Alpha-3.7 Thal), glucose-6-phosphate dehydrogenase deficiency (G6PDA-) and HLA B*5301 in Fulani, Mossi and Rimaibe ethnic groups of Burkina Faso.

Material and Methods

Study area and subjects

The samples were collected in the villages of Watinoma, Barkoumbilen and Barkoundouba of the Oubritenga Province, Burkina Faso (Figure). Detailed descriptions of the study area have been reported elsewhere (MODIANO et al., 1995, 1996). Very intense P. falciparum transmission is recorded during the June—October rainy season, frequently reaching inoculation rates well above 1 infective bite/person/night (ESPOSTO et al., 1988). The main malaria vectors are Anopheles gambiae, An. arabiensis and An. funestus (PETRARCA et al., 1986). The study protocol was approved by the Centre National de Lutte contre le Paludisme of the Ministry of Health of Burkina Faso. Oral informed consent was obtained for multiple immunoparasitological, clinical, genetic and entomological surveys, from the Rimaibe communities living in the villages of Watinoma (1100 inhabitants), Barkoumboua (600 inhabitants) and Barkoundouba (1300 inhabitants). The samples analysed were collected from individuals aged > 10 years during malaria cross-sectional surveys performed in October 1993 in Watinoma and in August 1994 in Barkoumbilen and Barkoundouba. A total of 335 unrelated individuals (68 Fulani, 167 Mossi, 118 Rimaibe) were typed for Hbs and Hbc, 169 (58 Fulani, 55 Mossi, related individuals (68 Fulani, 167 Mossi, 118 Rimaibe) genetic tests were performed. DNA was extracted from transferred and kept at -20°C until serological and/or qualitative antibody response was already suggested by the higher humoral responses to P. falciparum sporozoite (CSP, TRAP) and blood-stage antigens.

Blood samples and genetic analyses

A venous blood sample of 5 mL was obtained from each subject; blood samples were collected into sterile tubes containing tripotassium EDTA. Within 3-4 h from bleeding, plasma was separated and samples were kept at -20°C until serological and genetic tests were performed. DNA was extracted from peripheral blood leucocytes by standard methods (MILLER et al., 1988). Electrophoresis of haemoglobins was carried out in cellulose acetate with tri-EDTA-borate buffer, pH 8.6, as described by COLOMBO et al. (1993). Alpha-3.7 Thal was determined by polymerase chain reaction (PCR) according to FOGLIETTA et al. (1996). By the same technique the frequencies of alpha-4.2 and -MED variants of alpha thalassaemia were determined in a subsample of 15 Mossi, 12 Rimaibe and 15 Fulani. Moreover, in a total of 7 carriers of Alpha-3.7 (4 Mossi, 2 Rimaibe and 1 Fulani) the presence of Alpha-3.7 subtypes was evaluated. The 202 A-G mutation responsible for the G6PDA- deficiency, the most common African G6PD-deficiency variant, was determined by PCR-RFLP as described by BOUAHNA et al. (1998). HLA B*5301 was typed with the Amplification Refractory Mutation System—Polymerase Chain Reaction kits that were developed for the 1st and 11th International Histocompatibility Workshop and Conference (SADLER et al., 1994); for all individuals the HLA A, B and Cw class I haplotype was determined (MODIANO et al., 1997); only the B*5301 gene frequencies are presented in this paper.

Results

The frequencies and standard errors of the alleles studied are shown in the Table. Similar Hbs gene frequencies were recorded in the 3 ethnic groups; as for Hbc the Mossi and Rimaibe showed higher values than Fulani but the differences were barely significant. The frequency of Alpha-3.7 Thal was higher in Mossi when compared to Fulani, whereas no significant differences were observed in the Mossi vs Rimaibe or Rimaibe vs Fulani comparisons. None of the 42 individuals (15 Mossi, 12 Rimaibe and 15 Fulani) tested for alphae4.2 and -MED showed either of these 2 alpha thal variants. All further characterized 7 Alpha-3.7 Thal genes (4 Mossi, 2 Rimaibe and 1 Fulani) turned out to be of the alpha thal-7/-7 type. As regards G6PDA- and B*5301 the Mossi and Rimaibe showed similar values which were clearly higher than those of Fulani. A total of 91 individuals (37 Fulani [24 females, 13 males], 35 Mossi [28 females, 7 males] and 19 Rimaibe [14 females, 5 males]) were typed for all 4 genes. The 4 individuals with none of these 4 alleles known to be associated with protection against severe malaria was 56.8% in Fulani, 17.1% in Mossi and 15.8% in Rimaibe (Fulani vs Mossi-Rimaibe: P < 0.001).

Discussion

The aim of the present study was to ascertain whether the known or suspected genetic factors of resistance to malaria play a role in the lower susceptibility to the disease of Fulani of Burkina Faso as compared to sympatric Mossi and Rimaibe (MODIANO et al., 1995, 1996). As expected, all the classical erythrocytic malaria resistance genes exhibit high, or very high, frequencies in the 2 typical West African populations, Mossi and Rimaibe (Table). Similarly, very high frequencies were observed for HLA B*5301, suggesting selection also for this allele, by protection against severe malaria (HILL et al., 1991). The similar (Hbs) or higher (Hbc, Alpha-3.7 Thal, G6PDA- and HLA B*5301) gene frequencies recorded in the Mossi—Rimaibe group do not suggest the involvement of these malaria-resistance genes in the lower susceptibility to malaria of Fulani. This is perhaps not so surprising since the characteristics of the Fulani resistance do not fit with the typical protection conferred by classic malaria-resistance genes. In fact, the protection provided by those genes mainly concerns the severe complications of the disease, i.e., cerebral malaria and/or severe anaemia (HILL et al., 1991, RUWENDE et al., 1995, ALLEN et al., 1997), and not, or to a much lesser extent, the frequency of infection or the incidence of uncomplicated forms, which instead are unequivocally reduced among Fulani. The existence in this ethnic group of unknown malaria-resistance factors probably involved in the regulation of quantitative and/or qualitative antibody response was already suggested by the higher humoral responses to P. falciparum sporozoite (CSP, TRAP) and blood-stage antigens.
The present study reinforces, although indirectly, the immunological explanation. A further, distinct, question stems from the fact that the exclusion of classic malaria-resistance genes did not derive simply from a substantial similarity of the frequency of these genes in the 2 groups (Fulani vs Mossi–Rimaibe), but rather from the finding that all these genes, except \(b^3\), are less frequent among Fulani. The relatively low frequency of these genes in this ethnic group becomes striking if a combined comparison is made instead of a set of single comparisons computed one by one. In fact, the proportion of individuals with none of these genes, except \(b^3\) and \(\alpha^{-37}\), was 56.8% among Fulani and only 16.7% in the Mossi–Rimaibe group (\(P < 0.001\)). The lower frequency recorded in Fulani for \(\alpha^{-37}\), G6PD, and HLA B*5301, which are known to confer protection against severe malaria (HILL et al., 1991; RIJWENDE et al., 1995; ALLEN et al., 1997), could be explained as the consequence of the more recent entry of Fulani as compared to Mossi and Rimaibe in the highly selective malaria context of sub-Saharan West Africa. A further, non-alternative, hypothesis could be that the reduction of malaria selective pressure caused in the Fulani by other protective factor(s) has slowed down the accumulation of classic malaria-resistance genes some of which are, besides, associated with a heavy segregational load.

Considering the role played by acquired immunity in malaria, it is noteworthy that, excluding HLA class I B*5301 allele, DRB1*1302-DQB1*0301 HLA class II haplotype and tumour necrosis factor (TNF)-2 promoter polymorphisms that were associated with protection (HILL et al., 1991) or increased susceptibility (MCGUIRE et al., 1994, 1999) to severe malaria in The Gambia, no further genes related to immune responses have so far been convincingly recognized to be implicated in variation of susceptibility to \(P. falciparum\) malaria. This may be explained by the more difficult identification of genes involved in immune mechanisms as compared to those mediated by erythrocytic factors which before being recognized as protective against malaria were known to be associated with blood diseases such as thalassaemia, sickle cell anaemia and acute haemolytic anaemia. The high frequency of severely harmful, or even lethal, alleles together with a strong positive correlation between their geographical distribution and that of \(P. falciparum\) malaria were the key elements for the proposal of Haldane's malaria hypothesis (HALDANE, 1949). Besides the fact that very little is known on the immunogenetics of human infectious diseases including malaria, it appears likely that none of these genes may have in any of its genotypic combinations so drastic a negative effect as those of sickle cell anaemia or Cooley's anaemia. Again, taking into account the role of acquired immunity in malaria, it is likely that susceptibility/resistance to this disease is influenced by a large number of polymorphic major histocompatibility complex (MHC) (HILL et al., 1991, 1992) and non MHC (STJBERG et al., 1992; JEPSON et al., 1997; GARCIA et al., 1998; RIHET et al., 1998) genes related to the immune response. The study, both at intraethnic (case-control, family, adoptee and twin studies) and interethnic level (sympatric populations differing in their genetic background and susceptibility to malaria) of genes involved in the modulation of immune responses, will probably allow the identification of immunogenetic factors of resistance to this disease. Unlike erythrocytic malaria-resistance genes, these factors may turn out to have important implications in the development of new control tools.

Acknowledgements

We are grateful to the villagers of Watinoma, Barkoundouba and Barkoummen for their kind participation throughout the investigation. We thank Dr S. Ouili, Director of the Centre National de Lutte contre le Paludisme (CNP) of the Ministry (MSA-1, Pf-155 RESA, Pf-332) (MODIANO et al., 1996, 1998, 1999). The present study reinforces, although indirectly, the immunological explanation.
of Health of Burkina Faso, and Dr F. Pagnoni of the Italian Co-
operation. We are deeply indebted to the personnel of the Immunoparasitology Laboratory of CNLP and particularly to Mr A. Yameogo for skilful technical assistance. The study was based at CNLP, supported by the Programma di Assistenza allo Sviluppo of the Italian Ministry of Foreign Affairs. The study was partially funded by the World Health Organization, by the Foundation Pasteur-Cenci Bolognetti of the University of Rome ‘La Sapienza’, and by the Italian Ministry for University and Research.

References


Received 26 May 2000; revised 15 August 2000; accepted for publication 16 August 2000.