Association of HLA-G 3′UTR polymorphisms with response to malaria infection: A first insight

André García a,b,*, Jacqueline Milet c, David Courtin c, Audrey Sabbagh a,b, Juliana D. Massaro d, Erick C. Castelli e, Florence Migot-Nabias a,b, Benoit Favier f,g, Nathalie Rouas-Freiss f,g, Eduardo A. Donadi d, Philippe Moreau f,g

a Institut de Recherche pour le Développement, UMR 216, Mère et enfant face aux infections tropicales, Université Paris Descartes, 4, avenue de l’Observatoire, 75006 Paris, France
b Faculté de Pharmacie, Université Paris Descartes, Sorbonne Paris Cité, 4, avenue de l’Observatoire, 75006 Paris, France
c IRD, UMR 216, Centre d’Etude et de Recherche sur le Paludisme Associé à la Grossesse et à l’Enfance (CERPAGE), Faculté des Sciences de la Santé, BP 841, Cotonou, Benin
d Division of Clinical Immunology, School of Medicine of Ribeirão Preto, University of São Paulo, Brazil
e Biological Sciences Institute, Federal University of Goiás, Goiânia, Goiás, Brazil
f Commissariat à l’Energie Atomique et aux Energies Alternatives, Institut des Maladies Emergentes et des thérapies Innovantes, Service de Recherches en Hémato-Immunologie, Hôpital Saint-Louis, 1, avenue Claude Vellefaux, 75010 Paris, France
g Université Paris-Diderot, Sorbonne Paris-Cité, UMR-E5, Institut Universitaire d'hématologie, Hôpital Saint-Louis, 1, avenue Claude Vellefaux, 75010 Paris, France

A R T I C L E  I N F O

Article history:
Received 4 October 2012
Received in revised form 21 February 2013
Accepted 22 February 2013
Available online 14 March 2013

Keywords:
Malaria
HLA-G 3′UTR
Family-based association
Haplotype analysis
Immune development

A B S T R A C T

Malaria represents one of the most important causes of mortality and morbidity in Africa. Variability in clinical presentation is partly due to host genetic polymorphisms. Among them, human leukocyte antigen (HLA) class I and class II alleles may be responsible for malaria susceptibility; however less is known about the possible role of non classical HLA molecules. Among them, HLA-G is a tolerogenic molecule with immunomodulatory properties, which differs from classical HLA class I molecules by its lower genetic diversity, tissue expression and function. Although primarily associated with maternal-fetal tolerance, HLA-G is now known to be involved in a wide range of physiopathological conditions, such as tumor, autoimmunity, transplantation, inflammation and viral infection by suppressing the function of various immune cells. In this work, we present the first evidence of an association between HLA-G 3′UTR polymorphisms and malaria infection. More precisely, we showed that HLA-G polymorphisms are associated with asymptomatic infection through two parasitological phenotypes, the intensity of Plasmodium falciparum infection and the mean level of parasite density. The allele +3187G and its haplotype (UTR-1, 14 bp-Del/3001C/3003T/3010G/3035C/3052C/3142C/3187G/3196C) was associated with lower level of infection under a dominant model, and the haplotype UTR-3 (Del/3001C/3003T/3010C/3035C/3152C/3142G/3187A/3196C) was associated with high levels of infection under a recessive model. In conclusion, although further investigations are on the way to better address the possible involvement of the HLA-G molecule in the control of Pl. falciparum infection, this work presents the first evidence of an association between HLA-G polymorphisms and malaria infection. Further investigations are on the way to take into account the particularities of African populations.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Plasmodium falciparum (P. falciparum) malaria represents one of the most important causes of mortality and morbidity in tropical areas. Among the clinical presentations of malaria, asymptomatic infection (parasitemia without fever or any clinical sign) and mild malaria attack (association of fever and parasitemia) are the most frequent ones, and have an important economic burden to public health (Chima et al., 2003; Snow et al., 2005). Multiple factors are involved in the variability of host’s response to infection, such as environmental factors, parasite virulence and host characteristics like age or genetic background. The influence of host genetics to malaria development has been estimated to account for ~25% of the total variability in malaria incidence (Mackinnon et al., 2005), and has been extensively studied over the past twenty years (Driss et al., 2011; Kwiatkowski, 2005). If solid epidemiological evidence exists regarding the protective role of several polymorphisms affecting the structure or function of red blood cells
influence of both human leukocyte antigen (HLA) class I and class II alleles on malaria susceptibility, especially those involving the HLA-B, HLA-DRB1, and HLA-DQB1 loci (Hill et al., 1991, 1994). But much less is known about the possible role of non classical HLA molecules in determining the outcome of *P. falciparum* infection, despite a recent study showing that the combination of KIR2DL3 and its cognate HLA-C1 ligand is associated with the development of cerebral malaria (Hirayasu et al., 2012).

Since its original description (Ellis et al., 1986), considerable evidence supports a role for HLA-G in the suppression of immune responses and immune escape or tolerance. We postulate that the immunosuppressive properties of HLA-G might contribute to the susceptibility to and severity of malaria. Indeed, HLA-G is a tolerogenic molecule with immunomodulatory properties, which differs from classical HLA class I molecules by its lower genetic diversity, tissue expression and function. Although primarily associated with maternal-fetal tolerance, HLA-G is now known to be involved in a wide range of physiopathological conditions, such as tumor, autoimmunity, transplantation, inflammation and viral infection (Donadi et al., 2011). To facilitate their spread in the host, some viruses induce changes in the level and distribution of HLA-G, suppressing the function of various immune cells. This is particularly documented for human immunodeficiency virus (HIV-1), Herpes virus simplex, rabies virus (RABV), hepatitis C virus (HCV) and influenza A virus (IAV) infections (for a review see Fainardi et al., 2011).

Little information is available about the possible link between HLA-G and parasitic infections. It has been shown that elevated circulating levels of HLA-G molecules might help *Leishmania* parasites to evade cell-mediated immune responses (Donahgy et al., 2007). Other data suggest that over-expression of HLA-G would favor congenital transmission of *Toxoplasma* (Robert-Gangneux et al., 2011).

In the present study, we investigated for the first time the possible association of HLA-G genetic polymorphisms with malaria-related phenotypes in a Senegalese population of children. Since the magnitude of HLA-G expression is likely to be influenced by the 3′-untranslated region (3′UTR) variability through post-transcriptional mechanisms (Donadi et al., 2011), we particularly focused our study on the role of 3′UTR polymorphic sites and their relationship to the malaria-related phenotypes.

2. Materials and methods

2.1. Population, area and study design

The study took place in the Niakhar area, located 150 km South-East from Dakar, the capital of Senegal. The study was carried out in two villages of the area (Diohine and Toucar) because of the existence of a dispensary with which we collaborated for several years. Malaria is endemic in the area and its transmission is seasonal and it is estimated between 9 and 12 mosquito infective bites per person per year, occurring almost exclusively between September and December, following the rainy season. The study protocol was extensively described elsewhere (Milet et al., 2010). Briefly, in January 2001, 1202 individuals from 2 to 18 years old lived in these villages. Among them, a cohort of 504 subjects who permanently lived in the area together with their two parents was randomly constituted all from the Sereer ethnic group. They all agreed to take part in this study (informed consent signed by parents). Neither age nor sex differed significantly between children included and not included in the cohort (p-value > 0.18 and p-value > 0.08 respectively).

Thick blood smears (TBS) to measure parasite density (PD) were collected in June, September, November and December 2001, in January, June, September, October and November 2002 and in January, April, June, September, October and December 2003. TBS was stained with Giemsa and asexual parasites (*P. falciparum; Plasmodium malariae* and *Plasmodium ovale*) and leucocytes were counted, and PD was defined as the number of parasites per 100 leucocytes. A TBS was assigned negative when no parasite was detected in 200 microscopic fields. Between January 2002 and December 2003 all the children were closely followed-up by our team composed of nurses, health agents and technicians living in the villages and who get in touch with the families every day, including the week-end, in case of health problem. During this period an active clinical survey aiming to detect any malaria attack was performed during 2002 and 2003. The children were attended twice a week by trained primary health agents to check temperature and to ask for any clinical health problem that may have occurred. The parents were invited to bring the child with fever or history of fever to the dispensary. In case of presumptive malaria, a TBS was performed and a questionnaire concerning clinical signs and previous treatment was filled out. Children were considered to be suffering from a mild malaria attack when axillary temperature was greater than or equal to 37.5 °C (or reported history of fever between two systematic surveys) with a PD above 2500 trophozoites/µL. We only considered malaria attacks due to *P. falciparum*. Venous blood samples of 5 mL were obtained at the end of the follow-up from children and parents.

2.2. Individual, behavioral and environmental risk factors

For each child the following information was collected: (1) age (in years); (2) sex; (3) ethnic group (all children were from the Sereer group); (4) village of residence; (5) genetic variants of Hemoglobin (Hb) S and of glucose-6-phosphate-dehydrogenase (G6PD) deficiency A- and (6) presence of *P. malariae* in the TBS since it has been described that co-infection with *P. malariae* could influence the level of *P. falciparum* infection (Molineaux et al., 1980). Malaria infection can be influenced by variation in exposition to mosquito’s bites as well as by prophylaxis intake. In the cohort, 29 children declared the use of bed net during the preceding night. Neither the measurements of *P. falciparum* asymptomatic infection nor mild malaria attack differed significantly (P > 0.10) between children declaring to use a bed net and other children. To deal with uncontrolled antimalaria medicine intake, urine samples were collected 12 times during the follow-up to control for the presence of chloroquine and its metabolites in urine. At each control, the presence of chloroquine metabolites in urine had no significant effect on the PD determined at the same moment (data not shown). Furthermore, if a child was treated, by our team, for malaria attack during the follow-up, his PD measurement was not included in the mean level of *P. falciparum* infection during the following 3 weeks. Both medicine intake and use of bed net were not included in further analyses.

This protocol was previously submitted to and accepted by the Ethic Committee of the Health Minister of Senegal (N 000526/MS/DERF/DER). We obtained informed consent from all participants involved in our study. This consent was written, translated to Sereer, and obtained from all the families included in the study.

2.3. Phenotypes of interest

In the present study, we focused on asymptomatic *P. falciparum* infection and mild malaria disease. The precise phenotypes definitions are fully described and summarized in Table 1 of Milet et al. [15].
Concerning asymptomatic infection, three phenotypes of interest were defined: (1) the mean level of \( P. falciparum \) density (MLPD) performed on log transformed PD (log PD + 1, to allow for 0 count) taking into account all the PD measurements performed during the follow-up, including negative TBS; (2) the intensity of asymptomatic \( P. falciparum \) infection (IntPl) that took into account only the positive TBS during the follow-up; (3) the prevalence of asymptomatic \( P. falciparum \) infection (PrevPl). MLPD can be considered as a reflection of the level of infection children suffered during the two seasons of transmission taking into account the fact that children can be uninfected (the mean rate of positive blood smears (SD) per child was 0.42 (0.18) during the follow-up). This phenotype is to date the more classical phenotype used in genetic epidemiology studies of malaria infection (Abel et al., 1992; Garcia et al., 1998; Rihet et al., 1998). As the comparison and the replication of results are essential in genetic epidemiology, we used this phenotype. However, to deal with the complexity of host response to malaria infection, we defined two other phenotypes of interest for infection. The phenotype IntPl reflects the mean level of PD during asymptomatic infections thus emphasizes the ability of an individual to tolerate parasite density without clinical disease. PrevPl reflects the acquisition of a non-sterilising immunity, occurring after repeated infections. As some children may have not been present at each visit, we considered only the children with at least 8 out of 15 measurements performed during the follow-up. Each phenotype related to \( P. falciparum \) infection was adjusted for the other risk factors (age, co-infection by \( P. malariae \) . . .) We also considered the total number of mild clinical malaria attacks (MMA), due to \( P. falciparum \), experienced per child during the active clinical survey.

The hemoglobin variant HbS and glucose-6-phosphate-dehydrogenase (G6PD) deficiency A- were determined and their effect on the phenotypes, as for other risk factors, were taken into account by including them as covariates in the statistical models used to define phenotypes.

### 2.4. Genotyping

Nucleotide sequence variation of the \( HLA-G \) 3’UTR region was evaluated by direct sequencing of a 314-bp fragment (between the 3′ ends of the primers) encompassing the genomic positions +2945 and +3259, by using a methodology described elsewhere (Castelli et al., 2010). This fragment encompasses all the genetic variants known to be relevant for \( HLA-G \) post-transcriptional control (Donadi et al., 2011). Rigorous quality control was performed before statistical analyses to ensure reliable genotyping data. Pedstats (Wigginton and Abecasis, 2005) was used to detect genotypes with Mendelian inconsistency and genotypes at the marker were deleted in members of the respective families. Non-Mendelian errors, i.e. unlikely genotypes, were also investigated with MERLIN (Abecasis et al., 2002) and automatically removed when detected. Finally, analyses were performed on 564 individuals, including 334 children and 230 parents belonging to 128 families.

### 2.5. Statistical analyses

We tested the association of \( HLA-G \) 3’UTR polymorphisms, both individually and combined into haplotypes, with the four malaria-related phenotypes, by using the FBAT v2.0.2c software package (Horvath et al., 2001; Laird et al., 2000), which implements a generalized version of the original transmission disequilibrium test (TDT) (Spielman et al., 1993). The TDT measures the association of genetic markers in nuclear families by analyzing the allele transmission from parent to affected offspring. If an allele increases the risk of having a disease (or being infected) then this allele is expected to be transmitted from parent to affected offspring more often. Moreover TDT approach has been extended to handle quantitative phenotype such as parasite density, several children per family and compute tests of different genetics model. The four quantitative traits were analyzed using the mean score as offset, under additive, dominant and recessive genetic models. The minimum number of informative families necessary to perform the analyses was set to 10. In family-based association studies an informative family is a family which contributes to the statistic test computation for a considered SNP. As an example under an additive model a family is informative if at least one parent is heterozygous for the considered SNP. The level of significance was determined at \( P < 0.05 \). As the study was exploratory, no correction for multiple testing was applied.

### 3. Results

#### 3.1. Description of the population

At the beginning of the study mean age (SD) was 8.97 years (3.57) with a sex ratio (male:female) of 1.15. The great majority of the children (91.5%) are strictly younger than 14 years. Each child was not present at each visit and the average number of TBS during the entire follow-up was 14. Four children with less than eight thick blood smears (TBS) during the follow-up were excluded from analyses. The mean rate of positive blood smears (SD) per child was 0.42 (0.18) during the follow-up. The number of mild malaria attacks ranged from zero to six and 52% of children experienced at least one malaria attack. Due to the very close survey of the cohort there was no severe malaria attack.

During the whole follow-up of this cohort \( P. ovale \) was encountered only twice. \( Plasmodium vivax \) was not present in the area. \( P. malariae \) was encountered at an average prevalence of 2.1% during the entire study, peaking at 6.3% in June 2002.

\( HLA-G \) 3’UTR analysis was performed on 618 individuals belonging to 133 families, i.e., 246 parents and 372 children out of the 504 ones included in the follow-up. Among the 132 children for whom no genotyping data was available, 48 were absent or refused to collect blood samples and, for the 84 remaining children, technical problems occurred at the time of DNA extraction or DNA quality control. There was no difference concerning the four phenotypes under study between children for whom DNA was available or not.

#### 3.2. Sequencing analyses

The sequencing analysis of \( HLA-G \) 3’UTR revealed the presence of nine polymorphic sites in this region, including the 14-bp insertion/deletion (indel) and eight single-nucleotide polymorphisms (SNPs) at the genomic positions +3001 C/T (unreported), +3003 T/C (r1707), +3010 C/G (r1710), +3035 C/T (r17179108), +3052 C/T (unreported), +3142 G/C (r1063320), +3187 A/G (r9380142), and +3196 C/G (r1610696). All these polymorphisms have been described in previous studies (Castelli et al., 2010; Lucena-Silva et al., 2012), except the +3052 C/T SNP, which was identified for the first time. It is noteworthy that the +3052 T allele was also identified by our group in three Yansi individuals from the Democratic Republic of Congo (unpublished data). None of the variants deviated significantly from Hardy–Weinberg equilibrium when considering the unrelated founders of the sample (all \( P \)-values > 0.15). Apart from the uncommon +3001 C/T and +3052 SNPs, all genetic variants presented allele frequencies above 0.05 in this Senegalese population (Table 1).
3.3. Association analyses

In the single-marker analyses carried out by FBAT, a statistically significant association was observed between the +3187 SNP and the intensity of asymptomatic \textit{P. falciparum} infection (IntPI) under a dominant model, with preferential transmission of the +3187 G allele to children with a lower level of PD during asymptomatic infection \((Z = -2.154, P = 0.0312, \text{Table 3})\). This result is highly consistent with the univariate FBAT results since this haplotype is the only one among the six common haplotypes carrying a G allele at position +3187.

Secondly, the haplotype analysis also revealed novel associations involving the UTR-3 haplotype and: (i) a higher mean level of PD during the follow-up (MLDP) \((Z = 2.248, P = 0.0246)\) as well as (ii) a higher intensity of asymptomatic \textit{P. falciparum} infection \((Z = 1.990, P = 0.0465)\), both under a recessive model (\text{Table 3}). It’s important to notice that the minimum number of informative families under a recessive model was achieved only for the UTR-2 and UTR-3 haplotypes.

Single-marker analyses and haplotypic analyses did not reveal any evidence of association for HLA-G 3'UTR polymorphisms with MMA and PrevPI phenotypes.

4. Discussion

Our data are consistent with the involvement of HLA-G in the variability of response to \textit{P. falciparum} infection. More precisely, we showed that HLA-G polymorphisms are associated with asymptomatic infection through two parasitological phenotypes, the intensity of \textit{P. falciparum} infection (IntPI) and the mean level of parasite density (MLDP).

---

**Table 1**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Allele</th>
<th>Frequency</th>
<th>Families</th>
<th>(Z)</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>+3187</td>
<td>C</td>
<td>0.122</td>
<td>49</td>
<td>0.806</td>
<td>0.420</td>
</tr>
<tr>
<td>+3196</td>
<td>G</td>
<td>0.652</td>
<td>36</td>
<td>0.019</td>
<td>0.846</td>
</tr>
</tbody>
</table>

---

**Table 2**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>HLA-G 3'UTR polymorphisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTR-2</td>
<td>Ins C T C C C G A G</td>
</tr>
<tr>
<td>UTR-3</td>
<td>Del C T C C C G A C</td>
</tr>
<tr>
<td>UTR-1</td>
<td>Del C T C C C C G C</td>
</tr>
<tr>
<td>UTR-5</td>
<td>Ins C T C C C G A C</td>
</tr>
<tr>
<td>UTR-6</td>
<td>Del C T C C C C A C</td>
</tr>
<tr>
<td>UTR-4</td>
<td>Del C T C C C C C A C</td>
</tr>
</tbody>
</table>

---

\(^a\) 3'UTR haplotypes with a frequency > 0.05.
The importance of polymorphisms within the Major Histocompatibility Complex (MHC) region at chromosome 6p21.3, encoding HLA antigens, has been underlined since the 70s (Piazza et al., 1972), and several additional studies, reviewed by Ghosh (2008), have explored an association between specific HLA antigens and malaria. The findings of these studies, most often inconsistent, have focused on the potential role of the encoded antigens in the HLA restricted immune response to malaria (Hill et al., 1992). Most studies did not focus on the control of immune response to malaria (Hill et al., 1991). However, this association is also supported by the potential role of the encoded antigens in the HLA restricted immune response to malaria (Hill et al., 1992). Most studies did not focus on the control of disease severity. The phenotypes for which associations have been detected in the present study are consistent with the fact that HLA polymorphisms are certainly involved in the control of the interactions between host immune system and infection.

The HLA-G gene displays several particularities that are distinct from those of classical HLA class I genes, and is unequivocally involved in the control of immune response. Among these particularities one may cite the limited protein variability, the restricted tissue expression and its strong effect on the modulation of immune response. HLA-G indeed exerts several immunomodulatory effects, being beneficially implicated in embryo implantation and fetal survival but, conversely, being potentially detrimental in tumors and infections (Donadi et al., 2011). Although HLA-G expression has been evaluated in several pathological conditions, the influence of HLA-G gene polymorphism has not been studied to the same extent, in particular in the context of infections. A few examples concerned chronic viral infections, such as HIV-1 (Alkhiunbarea et al., 2006; Fabris et al., 2009; Segat and Crowella, 2012), HBV (Souto et al., 2011) and HCV (de Oliveira Crispim et al., 2012; Martinetti et al., 2006), human cytomegalovirus (Zheng et al., 2009) and human papillomavirus (Ferguson et al., 2011). All these studies suggested that the expression of HLA-G might contribute to an immunological environment affecting the outcome of viral infection.

Regarding parasitic infections, all available studies have focused on the levels of soluble HLA-G (sHLA-G) rather than on gene polymorphisms. Elevated levels of sHLA-G were found in amniotic fluid from women with acquired toxoplasmosis during pregnancy, with maximal concentrations when the fetus was congenitally infected (Robert-Gangneux et al., 2011). In HIV–Leishmania co-infected patients, sHLA-G secretion has been shown to contribute to the tolerogenic environment and to Leishmania immune evasion (Donaghy et al., 2007).

To our knowledge, this is the first study to investigate the possible relationship of HLA-G polymorphisms with malaria. Because variations in HLA-G 3’UTR have been proposed to be associated with HLA-G gene expression levels, this regulatory region may play a role in the control of response to malaria. Our results are consistent with the preferential transmission of the +3187 G allele and of the UTR-1 haplotype, which uniquely carries +3187 G to children with lower intensity of infection. Interestingly, an adenine at position +3187 has been associated with decreased mRNA stability in vitro due to an expansion of an AU-rich motif leading to a less stable mRNA, what is associated with decreased HLA-G expression (Yie et al., 2008). Consequently, the transmission of +3187G is theoretically consistent with an increased HLA-G expression in children. Our haplotype analysis also revealed associations involving the UTR-3 haplotype and a higher level of intensity of infection together with a higher mean level of P. falciparum density. The transmission of the +3187G is theoretically consistent with an increased HLA-G expression in children. Our haplotype analysis also revealed associations involving the UTR-3 haplotype and a higher level of intensity of infection together with a higher mean level of P. falciparum density.

Regarding the distribution of the 3’UTR polymorphisms. Elevated levels of sHLA-G were found in amniotic fluid from women with acquired toxoplasmosis during pregnancy, with maximal concentrations when the fetus was congenitally infected (Robert-Gangneux et al., 2011). In HIV–Leishmania co-infected patients, sHLA-G secretion has been shown to contribute to the tolerogenic environment and to Leishmania immune evasion (Donaghy et al., 2007).

To our knowledge, this is the first study to investigate the possible relationship of HLA-G polymorphisms with malaria. Because variations in HLA-G 3’UTR have been proposed to be associated with HLA-G gene expression levels, this regulatory region may play a role in the control of response to malaria. Our results are consistent with the preferential transmission of the +3187 G allele and of the UTR-1 haplotype, which uniquely carries +3187 G to children with lower intensity of infection. Interestingly, an adenine at position +3187 has been associated with decreased mRNA stability in vitro due to an expansion of an AU-rich motif leading to a less stable mRNA, what is associated with decreased HLA-G expression (Yie et al., 2008). Consequently, the transmission of +3187G is theoretically consistent with an increased HLA-G expression in children. Our haplotype analysis also revealed associations involving the UTR-3 haplotype and a higher level of intensity of infection together with a higher mean level of P. falciparum density.

Regarding the distribution of the 3’UTR polymorphisms. Elevated levels of sHLA-G were found in amniotic fluid from women with acquired toxoplasmosis during pregnancy, with maximal concentrations when the fetus was congenitally infected (Robert-Gangneux et al., 2011). In HIV–Leishmania co-infected patients, sHLA-G secretion has been shown to contribute to the tolerogenic environment and to Leishmania immune evasion (Donaghy et al., 2007).

To our knowledge, this is the first study to investigate the possible relationship of HLA-G polymorphisms with malaria. Because variations in HLA-G 3’UTR have been proposed to be associated with HLA-G gene expression levels, this regulatory region may play a role in the control of response to malaria. Our results are consistent with the preferential transmission of the +3187 G allele and of the UTR-1 haplotype, which uniquely carries +3187 G to children with lower intensity of infection. Interestingly, an adenine at position +3187 has been associated with decreased mRNA stability in vitro due to an expansion of an AU-rich motif leading to a less stable mRNA, what is associated with decreased HLA-G expression (Yie et al., 2008). Consequently, the transmission of +3187G is theoretically consistent with an increased HLA-G expression in children. Our haplotype analysis also revealed associations involving the UTR-3 haplotype and a higher level of intensity of infection together with a higher mean level of P. falciparum density.

Regarding the distribution of the 3’UTR polymorphisms. Elevated levels of sHLA-G were found in amniotic fluid from women with acquired toxoplasmosis during pregnancy, with maximal concentrations when the fetus was congenitally infected (Robert-Gangneux et al., 2011). In HIV–Leishmania co-infected patients, sHLA-G secretion has been shown to contribute to the tolerogenic environment and to Leishmania immune evasion (Donaghy et al., 2007).

To our knowledge, this is the first study to investigate the possible relationship of HLA-G polymorphisms with malaria. Because variations in HLA-G 3’UTR have been proposed to be associated with HLA-G gene expression levels, this regulatory region may play a role in the control of response to malaria. Our results are consistent with the preferential transmission of the +3187 G allele and of the UTR-1 haplotype, which uniquely carries +3187 G to children with lower intensity of infection. Interestingly, an adenine at position +3187 has been associated with decreased mRNA stability in vitro due to an expansion of an AU-rich motif leading to a less stable mRNA, what is associated with decreased HLA-G expression (Yie et al., 2008). Consequently, the transmission of +3187G is theoretically consistent with an increased HLA-G expression in children. Our haplotype analysis also revealed associations involving the UTR-3 haplotype and a higher level of intensity of infection together with a higher mean level of P. falciparum density.

Regarding the distribution of the 3’UTR polymorphisms. Elevated levels of sHLA-G were found in amniotic fluid from women with acquired toxoplasmosis during pregnancy, with maximal concentrations when the fetus was congenitally infected (Robert-Gangneux et al., 2011). In HIV–Leishmania co-infected patients, sHLA-G secretion has been shown to contribute to the tolerogenic environment and to Leishmania immune evasion (Donaghy et al., 2007).

To our knowledge, this is the first study to investigate the possible relationship of HLA-G polymorphisms with malaria. Because variations in HLA-G 3’UTR have been proposed to be associated with HLA-G gene expression levels, this regulatory region may play a role in the control of response to malaria. Our results are consistent with the preferential transmission of the +3187 G allele and of the UTR-1 haplotype, which uniquely carries +3187 G to children with lower intensity of infection. Interestingly, an adenine at position +3187 has been associated with decreased mRNA stability in vitro due to an expansion of an AU-rich motif leading to a less stable mRNA, what is associated with decreased HLA-G expression (Yie et al., 2008). Consequently, the transmission of +3187G is theoretically consistent with an increased HLA-G expression in children. Our haplotype analysis also revealed associations involving the UTR-3 haplotype and a higher level of intensity of infection together with a higher mean level of P. falciparum density.
Nevertheless it is important to consider that African populations usually present higher genetic diversity and different patterns of linkage disequilibrium than non African populations. These differences must be taken into account when interpreting our results that seem to go counter the classic expected role of HLA-G. Indeed, in our Sereer population from Senegal, the linkage disequilibrium between the 3187, 3142 SNPs and 14pb indel is much less frequent, around 0.30, than that reported in Caucasian populations and reported associations between this haplotype and its effect on the level of soluble HLA-G must be confirmed. Furthermore, we have shown very recently (data not shown, manuscript in preparation) in the same Sereer population that very strong linkage disequilibrium exists between the 14 bp indel and a variant located in the promoter region of HLA-G which may be the true causal variant. This result that has never been described before is of the utmost importance since the functionality of the 14 bp indel is unknown until today.

Moreover, in several populations studied so far reported, UTR-3 is associated with the coding allele group HLA-G:01:04 (Castelli et al., 2011), which is very frequent in African populations (Donadi et al., 2011), and has been previously associated with a high SHLA-G production (Rebmann et al., 2001). Therefore, the predictive allele influence on HLA-G expression is not the only criterion for the magnitude of HLA-G production. This phenomenon is certainly very complex and also depends on specific microenvironment, including the presence of specific cytokines, microRNAs and transcription factors. All these factors must be taken into account in specific studies. Finally, in the present study we postulated that the diversity of genetic variations of HLA-G in African populations together with their unknown functionality and the role of other effectors could explain our results. Complementary studies, concerning malaria but also other parasitic tropical diseases, conducted by our team are already ongoing in other Western and Central African populations to elucidate this complexity.

The association between parasite density and immunity is complex and, even if genetic polymorphisms played a role, they certainly interact with other parameters such as parasite virulence, co-infection, nutrition status etc. These parameters have not been taken into account in the present study, but more than 65% of this Senegalese population harbors helminth infections that can modify immunity and interact with *P. falciparum* parasite density (Briand et al., 2005). Hence, the evaluation of the role of HLA-G polymorphisms on the control of host response to *P. falciparum* using parasite density derived phenotypes may not be the only suitable variable. It can be argued that HLA-G plays a role in the control of *P. falciparum* infection very early in life, during the development of immunity, or even during the *in utero* life by creating a tolerogenic environment. Indeed, it has been shown that children, born of mothers with *P. falciparum* placental infection, are more susceptible to infection during their first months of life (Le Port et al., 2012; Mutabingwa et al., 2005; Schwarz et al., 2008). It should be pointed out that our population is composed of children and young adults from 2 to 18 years old and, therefore, it might present a specific pattern of HLA-G expression.

The associations we found in this study concern non clinical, or asymptomatic, infections. Nowadays, malaria transmission and morbidity have been reported to have declined in areas of Africa, which is assumed to be at least partly a result of the up-scaling of interventions (Karema et al., 2012). In this particular period, the epidemiology of asymptomatic malaria in different transmission settings is attracting increasing attention despite its apparent absence of consequence in terms of morbidity. Indeed, asymptomatic individuals are still able to produce gametocytes and therefore provide the reservoir for transmission. Considering that in some endemic areas more than 85% of the parasite-positive patients can be asymptomatic (Geiger et al., 2013), this question must be considered quite obviously as important. Asymptomatic infection phenomenon is certainly associated with the age-specific role of immune mechanisms developing in populations exposed to malaria. The prevalence of parasitemia and the risk of clinical, even severe, disease caused by infection decrease markedly with age beyond early childhood. Young children exhibit an “anti disease immunity”, rapidly acquired, which affects the risk and extent of morbidity associated with a given parasite density. In contrast, an “antiparasite immunity”, slowly acquired, confers protection against high-density parasitemia and the attendant risk of severe disease (Marsh and Snow, 1997). Sterilizing immunity against infection is never fully achieved, and an asymptomatic carrier status is the rule among adults. In the present population we recently concluded that naturally-acquired immunity relies on the presence of cytophilic IgG antibodies, displaying differing specificities and probably having dispersive functional attributes (Courtin et al., 2009). These antibodies certainly interact with other effectors of immunity (e.g., responses that diminish proinflammatory cytokines) to suppress parasitaemia to levels below those causing disease (Courtin et al., 2009). We also showed in this population that asymptomatic carriers during the dry season have a significant lower risk to develop clinical malaria during the following rainy season (Males et al., 2008). Put together all these arguments underlined the interest of studying the immune response and its potential genetic control on asymptomatic carriers. In that sense our results consistent with a potential role HLA-G, have to be considered as a first insight and need to be pursued.

In conclusion, further investigations in different populations are crucial to confirm our results given the high genetic heterogeneity of African populations which display variable patterns of linkage disequilibrium across locations. Moreover, the contrasting patterns of linkage disequilibrium between the African population under study and the other non African populations surveyed to date may partly explain why the present results seem to go counter to the current theory of the role of HLA-G established in non African populations. Although replication studies are on the way to better address the possible involvement of the HLA-G molecule in the control of *P. falciparum* infection, this work presents the first evidence of an association between HLA-G polymorphisms and malaria infection, which may have a predictive value for parasite infection outcome.

**Funding sources**

This work was supported by the French Research Ministry Programme PAL+ (2001), the Institut de Recherche pour le Développement and the Binational Institutional Research Program CAPES/COFECUB (#653/09).

**Conflict of interest**

The authors have declared no conflicting interests.

**Acknowledgments**

We are deeply grateful to the Niakhar population for participating to this follow-up. We would like to acknowledge the IRD teams in Dakar and Niakhar.

**References**


A. García et al. / Infection, Genetics and Evolution 16 (2013) 263–269


