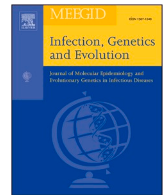
Infection, Genetics and Evolution 92 (2021) 104828

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid



Research paper

Human leukocyte antigen (*HLA*)-*F* and -*G* gene polymorphisms and haplotypes are associated with malaria susceptibility in the Beninese Toffin children

Paulin Sonon [a,b], Léonidas Tokplonou [c,d,e], Ibrahim Sadissou [a,f], Kuumaaté K.G. M'po [c,e], Sonya S.C. Glitho [c,e], Privat Agniwo [c,e], Moudachirou Ibikounlé [e], Andréia S. Souza [g], Juliana Doblas Massaro [a], Daniel Gonzalez [d], Théophile Tchégninougbo [h], Aurèle Ayitchédji [h], Achille Massougbodji [c], Philippe Moreau [i,j], André Garcia [d], Jacqueline Milet [d], Audrey Sabbagh [d], Celso T. Mendes-Junior [k], Kabirou A. Moutairou [l], Erick C. Castelli [g,m], David Courtin [d,1], Eduardo A. Donadi [a,*,1]

[a] *Post-graduate Program in Basic and Applied Immunology, Ribeirão Preto Medical School, University of São Paulo, Avenida Bandeirantes, 3900, Monte Alegre, 14049-900 Ribeirão Preto, SP, Brazil*
[b] *Immunogenetic Laboratory, Immunology Department, Aggeu Magalhães Institute, Oswaldo Cruz Foundation, Av. Moraes rego, s/n, Campus da UFPE, Cidade Universitária, 50670420 Recife, PE, Brazil*
[c] *Centre d'Etude et de Recherche sur le Paludisme Associé à la Grossesse et à l'Enfance (CERPAGE), Faculté des Sciences de la Santé, Cotonou, Bénin*
[d] *Université de Paris, UMR 261 MERIT, IRD, F-75006 Paris, France*
[e] *Département de Zoologie, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, Cotonou, Bénin*
[f] *Intertryp, IRD, Cirad, University of Montpellier, Avenue Agropolis, 34398 Montpellier Cedex 5, France*
[g] *São Paulo State University (UNESP), School of Medicine, Molecular Genetics and Bioinformatics Laboratory, Av. Prof. Dr. Walter Maurício Correa, s/n, 1861868, Botucatu, SP, Brazil*
[h] *Centre Médical Saint Joseph, Sô Ava, Benin*
[i] *CEA, DRF-Institut François Jacob, Service de Recherches en Hémato-Immunologie, Hopital Saint-Louis, 75010 Paris, France*
[j] *Université de Paris, CEA, U976 HIPI Unit (Human Immunology, Physiopathology, Immunotherapy), Institut de Recherche Saint-Louis, 75010 Paris, France*
[k] *Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, AV Bandeirantes, 3900, 14040901 Ribeirão Preto, SP, Brazil*
[l] *Laboratoire de Biologie et Physiologie Cellulaire, Université d'Abomey-Calavi, Cotonou, Bénin*
[m] *São Paulo State University (UNESP), Department of Pathology, School of Medicine, Botucatu, State of São Paulo, SP, Brazil*

ARTICLE INFO

Keywords:
HLA-Ib
Plasmodium falciparum
SNV
Haplotype
Antibody

ABSTRACT

Background: Little attention has been devoted to the role of the immunoregulatory *HLA-E/-F/−G* genes in malaria. We evaluated the entire *HLA-E/-F/−G* variability in Beninese children highly exposed to *Plasmodium falciparum (P.f.)* malaria.
Methods: 154 unrelated children were followed-up for six months and evaluated for the presence and number of malaria episodes. *HLA-E/-F/−G* genes were genotyped using massively parallel sequencing. Anti *P.f.* antibodies were evaluated using ELISA.
Results: Children carrying the G allele at *HLA-F* (−1499,rs183540921) showed increased *P.f.* asymptomatic/symptomatic ratio, suggesting that these children experienced more asymptomatic *P.f.* episodes than

Abbreviations: HLA-E/-F/−G, immunoregulatory non-classical class I human leukocytes antigens;; *P.f.*, *Plasmodium falciparum*; IgG, immunoglobulin-G; GLURP-R2, glutamate-rich protein; UTR, untranslated region; ILT, immunoglobulin-like transcript receptor; KIR2DL4, killer cell immunoglobulin-like receptor; NK, Natural Killer cells; CD94-NKG2A, inhibitory receptor expressed by NK cells; TBS, thick blood smear; RDT, rapid diagnostic test; *S.h*, *Schistosoma haematobium*; DNA, deoxyribonucleic acid; MSP3, merozoite surface protein; ELISA, enzyme-linked immunosorbent assay; HWE, Hardy-Weinberg Equilibrium; OR, odds ratio; 95%CI, 95% confidence interval; SNV, single nucleotide variation; INDEL, insertion/deletion; MAF, minor allele frequency; NFκB, nuclear factor Kappa B; IRF1, interferon regulatory factor-1; CIITA, class II transactivator.

* Corresponding author at: Ribeirão Preto Medical School, University of São Paulo, AV Bandeirantes, 3900, HC, Vila Monte Alegre, 14049900, Ribeirão Preto, SP, Brasil.
E-mail address: eadonadi@fmrp.usp.br (E.A. Donadi).

[1] These authors contributed equally to this study

https://doi.org/10.1016/j.meegid.2021.104828
Received 28 November 2020; Received in revised form 5 February 2021; Accepted 24 March 2021
Available online 27 March 2021
1567-1348/© 2021 Elsevier B.V. This article is made available under the Elsevier license (http://www.elsevier.com/open-access/userlicense/1.0/).

symptomatic one. Children carrying *HLA-G*-UTR-03 haplotype exhibited increased risk for symptomatic *P.f.* episodes and showed lower IgG2 response against *P.f.* GLURP-R2 when compared to the non-carriers. No associations were observed for the *HLA-E* gene.

Conclusion: HLA-F associations may be related to the differential expression profiles of the encoded immunomodulatory molecules, and the regulatory sites at the *HLA-G* 3'UTR may be associated to posttranscriptional regulation of HLA-G and to host humoral response against *P.f.*

1. Introduction

Plasmodium falciparum (*P.f.*) malaria remains a major infectious disease associated with high morbidity and mortality. The latest WHO report announced 228 million cases of malaria worldwide, and 94% of the deaths occurred in Africa. Children under the age of five years are the most vulnerable group, representing 67% of deaths attributable to malaria worldwide (www.who.int). *P.f.* malaria is endemic in Benin, particularly in the people living in wetlands, such as the Toffin population (etymologically known as "people of water"), who has been living in Benin for centuries, working as fishermen in the Sô-Ava district (Principaud, 1995).

The clinical responses following a *P.f.* infection may be influenced by the genetic background of the host, which is associated with differential structure, function, or amount of molecules involved in the immune response (Mangano and Modiano, 2014). Gene variants of the HLA system seem to play a crucial role in malaria susceptibility. The *HLA-A*, *HLA—B, HLA—C, HLA-DRB1*, and *HLA-DQB1* loci have been associated with severe malaria, malaria-related anemia, and parasite density (Hill et al., 1991; Hirayasu et al., 2012; Wilson et al., 2008). Notwithstanding, little attention has been devoted to the role of the non-classical class I *HLA-E/-F/−G* genes (HLA-Ib) on malaria susceptibility. Although the peptide-binding cleft of the HLA-Ib molecules may present peptides, the primary role of these molecules has been attributed to the modulation of the immune response since they are expressed in the placenta protecting the semi-allogenic fetus against the mother immune response (Hunt et al., 2005). The HLA-Ib genes present much less polymorphism compared to the classical class Ia genes (*HLA-A/-B/-C*) (ipd/imgt/hla/stats.html; assessed 2020-09-17). Despite the number of variable sites already identified across HLA-Ib genes, only a few different protein versions (E*01:01 and E*01:03 for HLA-E, F*01:01 and F*01:03 for HLA—F, and seven versions of HLA-G) are frequently observed in worldwide populations (Castelli et al., 2014; Manvailer et al., 2014; Olieslagers et al., 2017; Pyo et al., 2006; Sonon et al., 2018), suggesting high conservation of the coding region of these genes, corroborating the role of these molecules on the control of the immune response.

HLA-G is the most studied Ib molecule, exhibiting a well-recognized immunomodulatory role. It inhibits the function of several cells of the immune system when interacting with immunoglobulin-like transcript receptor 2 (ILT-2) and 4 (ILT-4) and with the killer cell immunoglobulin-like receptor KIR2DL4 (Albayati et al., 2017) on T and Natural Killer (NK) cells. Some studies have reported a differential susceptibility to human African trypanosomiasis, American trypanosomiasis, and leishmaniosis regarding variable sites at the *HLA-G* 3′ untranslated region (3'UTR) (reviewed at (Dias et al., 2015)). Notwithstanding, all information regarding the role of *HLA-G* in malaria has focused only on the 3'UTR segment (Garcia et al., 2013). HLA-E may also exhibit immunomodulatory properties since it may bind to the inhibitory receptor CD94-NKG2A expressed by NK cells (Persson et al., 2017). Besides, HLA-E may interact with the alphabeta T-cell receptor expressed on CD8+ T cells (Pietra et al., 2010) and present non-self-peptides derived from viruses (Sullivan et al., 2008). HLA-F molecules have been detected on the surface of immune cells such as B, T, and NK cells, present peptides to T cells, and regulate the immune response through interactions with NK cell receptors (Dulberger et al., 2017).

Considering that: i) the molecules encoded by the HLA-Ib genes exhibit well-recognized tolerogenic properties; ii) the scanty literature findings regarding *P.f.* infection have focused only on a specific region of the *HLA-G* gene, iii) no studies have evaluated the role of *HLA-E/-F* genes on malaria susceptibility, we evaluated the coding and regulatory regions of the *HLA-E/-F/−G* genes in the Beninese Toffin population exhibiting symptomatic or asymptomatic *P.f.* malaria episodes along the 2013 malaria transmission season.

2. Materials and methods

2.1. Study population

One-hundred and fifty-four unrelated 4–8-year-old (average age of 6.70 ± 0.90 years) Beninese Toffin children (103 boys) were followed during the 2013 malaria transmission season (June to November). These school-aged children attended monthly scheduled visits as well as spontaneous consultations to evaluate *P.f.* infection episodes. At inclusion, a medical examination was performed, and blood samples were collected. Children were enrolled for scheduled monthly medical visits. The parents were invited to attend the St. Joseph Medical Health Center together with their child in case of any health problem (spontaneous consultations). Medical consultations and treatments were free of charge for all the participants. In the case of axillary temperature ≥ 37.5 °C (or a history of fever in the preceding 24 h), a thick blood smear (TBS) and rapid diagnostic test (RDT) were performed. Only symptomatic malaria cases were treated according to the recommendations of the National Malaria Control Programme.

To detect asymptomatic infections, TBS was performed at each monthly scheduled visit. In case of fever or history of fever in the preceding 24 h during the scheduled visit, TBS showing malaria infection was not considered as asymptomatic episode and was excluded from the analysis. Children attended an average of 5.21 ± 1.31 monthly visits and 2.00 ± 1.65 spontaneous consultations during the follow-up, and only those who attended at least 3 out of 6 monthly visits ($N = 146$) were included in the association study. Other clinical and laboratory information were collected, including: i) the use of mosquito net, ii) the practice of water-related activities (fishing, swimming, bathing, washing clothes, and washing dishes), iii) hemoglobin defects, and iv) the *Schistosoma haematobium* (*S.h*) co-infection status at the end of follow-up, which are considered the main co-factors that can also influence the host immune response against *P.f.* infection (Guthmann et al., 2002; Lyke et al., 2006; Mackinnon et al., 2005). The ethics committee of the Faculté des Sciences de la Santé de Cotonou, Benin (No.12/03/2012/CEIFSS/UAC) approved the project.

2.2. Malaria phenotypes

2.2.1. P.f. infections

The presence of symptomatic or asymptomatic *P.f.* infections was annotated during all follow-up, considering each monthly malaria event. The symptomatic infection was defined when the child exhibited positive TBS and/or RDT and axillary temperature ≥ 37.5 °C or fever history the day before the monthly visit or on the day of the spontaneous visit. Asymptomatic children exhibited no fever but presented positive TBS. Children who did not experience any symptomatic or asymptomatic malaria infections and presented negative TBS and/or RDT were considered non-infected by *P.f.*. Considering that: i) no drug resistance to *P.f.* malaria has been reported in Benin (Ogouyèmi-Hounto et al.,

2016), ii) new *P.f.* malaria infections have not been observed 13 days (95% CI 10.7–15.7) after the previous malaria treatment (Bretscher et al., 2020), we chose the 10 day time-interval to avoid counting an early reinfection episode. The participant characteristics and parasitological data are presented in Table 1.

2.2.2. Number of P.f. infections and ratio asymptomatic/symptomatic infections

During the follow-up, children might experience only asymptomatic infections (without fever), symptomatic infections, or both. We used the following phenotypes for each child: i) the total number of infections (both symptomatic or asymptomatic), ii) the number of symptomatic infections and iii) the ratio of asymptomatic/symptomatic infections. Therefore, patients exhibiting more asymptomatic than symptomatic *P. f.* infections during the whole follow-up showed ratio asymptomatic/ symptomatic >1, otherwise, they showed ratio < 1, and those who experienced an equal number of both phenotypes showed ratio = 1 (Table 1).

2.3. HLA-E/-F/−G typing

DNA extraction/quantification, DNA amplification, library preparation, and sequencing were performed as previously described (Castelli et al., 2017; Lima et al., 2016; Ramalho et al., 2017). Libraries were sequenced using the MiSeq system (Illumina, San Diego, CA) and the Illumina Reagent (V2 500 cycles) in a paired-end mode (2x250bp). The bioinformatics analyses of *HLA-E/-F/−G* massively parallel sequencing data, including alignment, variant calling, and haplotype calling, were performed as previously described (Castelli et al., 2018; Castelli et al., 2015; Lima et al., 2016).

Considering that only the coding region haplotypes have an officially recognized nomenclature defined by the IPD-IMGT/HLA database (Robinson et al., 2015), the nomenclature of the promoter and 3′UTR segments, as well as the nomenclature of the extended haplotypes encompassing these three major regions, were assigned as previously defined for *HLA-E* (Ramalho et al., 2017), *HLA-F* (Lima et al., 2016), and *HLA-G* (Castelli et al., 2017).

Table 1
Characteristics of the studied Toffin children ($N = 146$).

Age in years: groups (mean ± SD)	4–6 and 7–8 (6.70 ± 0.90)
Gender (Male/Female)	**(98/48)**
Hemoglobin defects	**38/146 (26%)**
Hemoglobin A and S (AS)	27/38 (71%)
Hemoglobin A and C (AC)	9/38 (24%)
Hemoglobin S and C (SC)	2/38 (5%)
Number of individuals using mosquito net	**133/146 (91%)**
Practice of water-related activities	**95/146 (65%)**
P.f.-non-infected children during the follow-up	12/146 (8%)
***P.f.*-infected children during the follow-up**	**134/146 (92%)**
Children registering both symptomatic or asymptomatic *P. f.* infection	63/134 (43%)
Children registering only symptomatic *P.f.* infection	65/134 (45%)
Children registering only asymptomatic *P.f.* infection	6/134 (4%)
Total number of *P.f.* infection during the follow-up	**134/134 (100%)**
Group 1 (1 or 2 infections; median 1)	49/134 (36%)
Group 2 (3 to 10 infections; median 4)	85/134 (64%)
Number of only symptomatic *P.f.*-infection during the follow-up	**65/134 (45%)**
Group 1 (1 or 2 infections; median 1)	35/65 (54%)
Group 2 (3 to 8 infections; median 4)	30/65 (46%)
Asymptomatic and symptomatic infection ratio	**128/134 (96%)**
P.f. asymptomatic infection/symptomatic ratio > 1	5/128 (4%)
P.f. asymptomatic infection/symptomatic ratio = 1	12/128 (9%)
P.f. asymptomatic infection/symptomatic ratio < 1	111/128 (87%)
***Schistosoma haematobium* (*S.h*) status at the end of follow-up**	**118/134 (88%)**
Positive for *S.h* (*S.h*+)	36/118 (31%)
Negative for *S.h* (*S.h*-)	82/118 (69%)

For multivariate analysis, the following categorical co-variables were used: i) Age was dichotomized in two age groups: 4–6 and 7–8 years old, ii) gender dichotomized into male and female, iii) hemoglobin defects: normal hemoglobin (AA) versus hemoglobin defect (AC, AS or SS), iv) the use of mosquito net was defined as: absence of using mosquito net during all the follow-up versus declaration of using mosquito net at least once during the follow-up, v) practice or not practice water-related activities (fishing, swimming, bathing, washing clothes, and washing dishes) and vi) presence or not of *S.h* infection at the end of the follow-up.

2.4. Quantification of antibodies levels against P.f. antigens at the end of the follow-up

The quantification of IgG1, IgG2, and IgG3 antibodies directed against the glutamate-rich protein (GLURP-R0: residues 25–514 and GLURP-R2: residues 706–1178), and merozoite surface protein (MSP3: residues 212–380) was performed using ELISA (Dechavanne et al., 2016).

2.5. Statistical analysis

The Hardy-Weinberg Equilibrium (HWE) was tested by the exact test of Guo and Thompson (Guo and Thompson, 1992), using the ARLEQUIN v3.5.2 software. Associations between polymorphisms or haplotypes (both exhibiting frequencies greater than 1%) and malaria phenotypes were performed using PLINK (Purcell et al., 2007) and R (R Core Team, 2017). In univariate analysis, allele and haplotype frequencies were compared between different phenotypes (total number of infections (both symptomatic or asymptomatic), number of symptomatic infections and ratio asymptomatic/symptomatic). The following phenotypes, the total number of infections and the number of symptomatic infections were analyzed using 2 × 2 contingency tables. For instance, regarding the "total number of *P.f.* infections", patients were distributed into two groups, group 1 (1–2 *P.f.* infections (reference) and group 2 (>2 *P.f.* infections) (Table 2). The same approach was used to analyze the "number of symptomatic infections" (Table 3). The group with lower number of *P.f.* infections (group 1) was compared to the group with more than 2 *P.f.* infections (group 2 (susceptible group)). The ratio asymptomatic/symptomatic was used as a quantitative variable (Table 4). Finally, only results showing *P*-values ≤0.05 after univariate analyses (Supplementary material 1) were considered eligible for multivariable analyses, which were adjusted using the following co-variables: age, gender, hemoglobin profiles, use of mosquito net, the practice of water-related activities, the helminth co-infection status. All co-variables were used as categorical variables (Table 1).

The strength of the association was evaluated by the odds ratio (OR) and its 95% confidence interval (CI), and *P*-values were obtained using the two-tailed Fisher's exact test. Logistic and linear multiple regression analyses were performed considering a dependent binary and quantitative variables, respectively. *P*-values ≤0.05 were considered to be significant.

The continuous variables (antibody levels) were analyzed for normality using the Shapiro-Wilk tests, followed by the Mann-Whitney (two groups) tests, considering $P \leq 0.05$ as significant.

3. Results

More than 95% of all DNA samples were successfully sequenced for *HLA-E* ($n = 150$), *HLA-F* ($n = 152$), and *HLA-G* ($n = 149$). We identified 37, 68, and 96 variable sites at *HLA-E/-F/−G* genes, respectively. From these, 37 (*HLA-E*), 63 (*HLA—F*), and 94 (*HLA-G*) variable sites were biallelic (single nucleotide variation (SNV) or insertion/deletion (INDEL), and eligible to be processed using Plink. From these variable sites, 100% (37/37 for *HLA-E*), 64% (40/63 for *HLA—F*), and 100% (94/ 94 for *HLA-G*) fitted the HWE, whereas 30/37, 31/63, and 87/94 variable sites, respectively, exhibited both minor allele frequency (MAF >

Table 2
Association between *HLA-E/-F/−G* (promoter, coding, 3′ untranslated region-3'UTR) variable sites and haplotypes encompassing these three regions, selected after univariate analysis and total number of *P. falciparum* infections. Comparisons were performed using the Plink (variable sites) and R software (haplotypes) using logistic multiple regression models, adjusted on the following co-variables: gender, age, use of mosquito net, practice of water-related activities, hemoglobin electrophoresis and the *Schistosoma haematobium* co-infection status. *P*-values ≤0.05 were considered significant.

SNPid or haplotype [a]	Total number of infections [c]					
	Phenotype	Estimate[b]	95%CI/SE	Z value	*P*-value	Model dispersion[d]
***HLA-E* haplotype**						
E-Promo-18	1 or 2 (ref) [e] vs >2	Estimate: 1.492	SE:1.509	0.989	0.323	1.170
E-Promo-18/*E**01:03:05/*HLA-E*-3UTR-01	1 or 2 (ref) [e] vs >2	Estimate: 1.492	SE:1.509	0.989	0.323	1.170
***HLA-G* haplotype**						
PROMO-*G*0104a	1 or 2 (ref) [e] vs >2	Estimate:0.790	SE:0.500	1.590	0.112	1.123
*G**01:04:01	1 or 2 (ref) [e] vs >2	Estimate:1.410	SE:0.810	1.740	0.082	1.117
PROMO-*G*0104a/*G**01:04:01/UTR-03	1 or 2 (ref) [e] vs >2	Estimate:1.410	SE:0.810	1.740	0.082	1.117

[a] SNPid, identification of the single nucleotide polymorphism regarding human genome19 (hg19) (rs - reference number) followed by IMGT/HLA, position at IPD-IMGT/HLA database and reference and alternative alleles. Variable sites and their frequency are shown in supplementary material 2. The list of all analyzed haplotypes is shown at supplementary material 3.
[b] OR: Odds ratio; Estimate: represents regression coefficients which can be positive or negative. Regression coefficients measure the increase (positive value) or decrease (negative value) of the dependent variable (asymptomatic/symptomatic infection ratio). Definition of phenotypes is described in Table 1.
[c] Total number of infections were analyzed as categorical variables: (1 or 2 versus > 2). Definition of phenotypes is described in Table 1.
[d] Model dispersion: summary(model.final)$deviance / summary(model.final)$df.residual. The model is dispersed when this value exceeds 1.5. The Overdispersion indicates that the model doesn't fit the data well.
[e] We defined two groups: group 1 (1 or 2 *P.f* malaria infections (reference group)) and group 2 (>2 *P.f* malaria infections).

Table 3
Association between haplotypes, selected after univariate analysis, and number of symptomatic *P. falciparum* episodes. Comparisons were performed with R software using the logistic multiple regression models, adjusted on the following co-variables: gender, age, use of mosquito net, practice of water-related activities, hemoglobin electrophoresis and the *Schistosoma haematobium* co-infection status. *P*-values ≤0.05 were considered significant.

SNPid or haplotype [a]	Number of symptomatic infections [c]					
	Phenotype	Estimate[b]	Standard Error	Z value	*P*-value	Model dispersion[d]
***HLA-G* haplotype**						
PROMO-*G*0104a	1 or 2 (ref) [e] vs >2	0.980	0.710	1.380	0.166	1.296
HLAG-UTR-03	1 or 2 (ref) [e] vs >2	1.490	0.730	2.040	**0.041**	1.231

[a] SNPid, identification of the single nucleotide polymorphism regarding human genome19 (hg19) (rs - reference number) followed by IMGT/HLA, position at IPD-IMGT/HLA database and reference and alternative alleles. Variable sites and their frequency are shown in supplementary material 2. The list of all analyzed haplotypes is shown at supplementary material 3.
[b] Estimate: represents regression coefficients which can be positive or negative. Regression coefficients measure the increase (positive value) or decrease (negative value) of the dependent variable (number of symptomatic infections). Definition of the phenotype is described in Table 1.
[c] Number of symptomatic infection episodes were analyzed as categorical variables: (1 or 2 versus > 2). Definition of the phenotypes is described in Table 1.
[d] Model dispersion: summary(model.final)$deviance / summary(model.final)$df.residual. The model is dispersed when this value exceeds 1.5. The Overdispersion indicates that the model doesn't fit the data well.
[e] We defined two groups: group 1 (1 or 2 *P.f* malaria infections (reference group)) and group 2 (>2 *P.f* malaria infections).

1%) and HWE *P*-value>0.01, and were included in the genetic association analysis (Supplementary material 2). Of note, the remaining *HLA-F* variable sites (23/63) that did not fit the HWE: i) were highly frequent (MAF > 20%), ii) were in complete LD between with each other ($r^2 = 1$) (Sonon et al., 2018), iii) had been already described either at 1000 Genomes phase 3 project (The 1000 Genomes Project Consortium, 2015), at IPDIMGT/HLA database (Robinson et al., 2015), or specific population (Lima et al., 2016). Moreover, only haplotypes presenting frequencies >1% were considered for association analyses. A total of 41, 42, and 55 haplotypes encompassing the *HLA-E/-F/−G* genes, respectively, were analyzed (Supplementary material 3). Since we evaluated the full *HLA-E/-F/−G* DNA region, we evaluated the associations in terms of i) single nucleotide variation (SNV) alleles, ii) specific gene region (promoter, coding, and 3'UTR) haplotypes.

3.1. HLA-E/-F/−G variable sites and haplotypes are associated with susceptibility/protection to P.f. malaria

After adjusting on the covariables above, the comparisons of individual variable sites and haplotypes regarding the total number of *P.f.* infections revealed the following results: no significant associations were observed for: *HLA-E/-F/−G* genes (SNPs and haplotypes) (Table 2).

Considering the same covariables in multivariable analysis, the comparisons of individual variable sites and haplotypes regarding the number of symptomatic *P.f.* infections revealed the following results: i) no associations were observed for the *HLA-E* and —*F* genes; however, ii) the presence of *HLA-G*-UTR-03 haplotype increased the risk for the high number of symptomatic *P.f.* episodes during the follow-up (*P* = 0.041, Estimate = 1.49, Standard Error = 0.73) (Table 3).

The comparisons of individual variable sites and haplotypes regarding the number of *P.f.* asymptomatic/symptomatic ratio (quantitative trait), after adjusting for the main covariables above revealed a positive association with the G allele at *HLA-F* (−1499, promoter, rs183540921) (*P* = 0.0004, Estimate = 0.861, 95%CI:0.41–1.32), suggesting that these children experienced more asymptomatic *P.f.* infections than symptomatic ones during the follow-up. No associations were observed for the *HLA-E* and *-G* genes (Table 4).

3.2. HLA-G UTR-03 haplotype and antibody levels

Considering that: i) the humoral immune response is vital for *P.f.* clearance (Adamou et al., 2019; Aucan et al., 2000; Courtin et al., 2009), ii) soluble HLA-G (sHLA-G) may inhibit the B cell response by the interaction with the ILT2/4 receptors (Naji et al., 2014), and iii) the presence of the *HLA-G*-UTR-03 haplotype increased the risk for the high number of symptomatic *P.f.* episodes, we associated the *HLA-G* UTR-03

Table 4
Association between *HLA-E/-F/−G* (promoter, coding, 3′ untranslated region-3'UTR) variable sites and haplotypes encompassing these three regions, selected after univariate analysis, and asymptomatic/symptomatic infection ratio. Comparisons were performed under Plink (variable sites) and R software (haplotypes) using logistic multiple regression models, adjusted on the following co-variables: gender, age, use of mosquito net, practice of water-related activities, hemoglobin electrophoresis and the *Schistosoma haematobium* co-infection status. *P*-values ≤0.05 were considered significant.

SNPid or haplotype [a]	Asymptomatic/symptomatic ratio[c]					
	Estimate[b]	95%CI/Standard error	Z value	Model	*P*-value	Tested allele
HLA-E SNP						
rs17875364: −1159 (A > G)	−0.066	95%CI:-0.20 to 0.07	–	Additive	0.343	G
	0.026	95%CI:-0.25 to 0.30	–	Recessive	0.856	G
	−0.131	95%CI:-0.31 to 0.05	–	Dominant	0.161	G
rs146647219: −1079 (G > T)	NA	95%CI:NA	–	NA	NA	T
***HLA-E* haplotype**						
E-Promo-01/*E**01:01:01:01/*HLA-E*-3UTR-01	0.140	SE:0.09	1.700	–	0.092	–
HLA-F SNP						
rs183540921: −1499 (T > G)	0.861	95%CI:0.41–1.32	–	Additive	**0.0004**	G
	NA	95%CI:NA	–	Recessive	NA	G
	0.861	95%CI:0.41–1.32	–	Dominant	**0.0004**	G
rs17184813: +1943 (G > A)	0.071	95%CI:-0.22 to 0.36	–	Additive	0.634	A
	NA	95%CI:NA	–	Recessive	NA	A
	0.071	95%CI:-0.22 to 0.36	–	Dominant	0.634	A

NA: not available.
[a] SNPid, identification of the single nucleotide polymorphism regarding human genome19 (hg19) (rs - reference number) followed by IMGT/HLA, position at IPD-IMGT/HLA database and reference and alternative alleles. Variable sites and their frequency are shown in supplementary material 2. The list of all analyzed haplotypes is shown at supplementary material 3.
[b] Estimate: represents regression coefficients which can be positive or negative. Regression coefficients measure the increase (positive value) or decrease (negative value) of the dependent variable (asymptomatic/symptomatic infection ratio). Definition of the phenotype is described in Table 1.
[c] asymptomatic/symptomatic infection ratio was analyzed as continuous variable. Definition of the phenotype is described in Table 1.

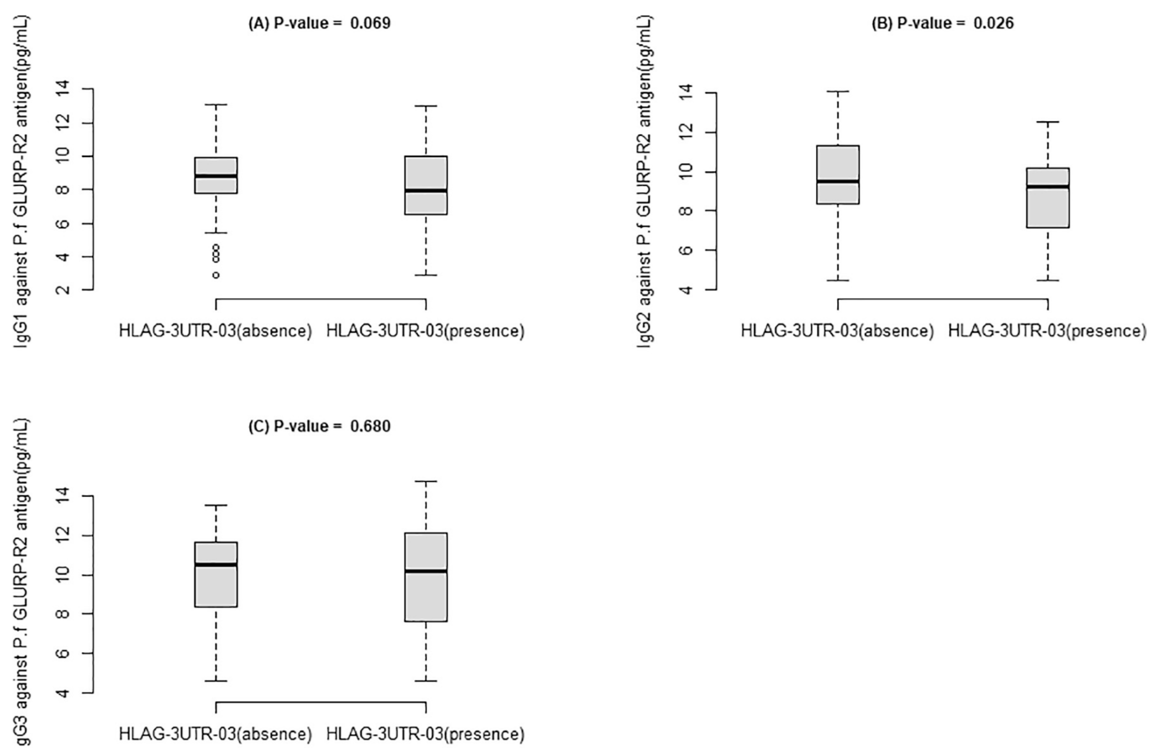


Fig. 1. IgG1 (A), IgG2 (B) and IgG3 (C) antibody levels against *Plasmodium falciparum* (*P.f.*) glutamate-rich protein (GLURP-R2, amino acids 706–1178), stratified according to the presence ($n = 54$) or absence ($n = 67$) of the *HLA-G* 3'untranslated region (*HLA-G*-UTR-03) haplotype in children exhibiting symptomatic and/or asymptomatic *P.f.* infection. All associations between different groups were performed on R software v3.4.2. The Mann-Whitney Test was used for comparisons.

haplotype (Table 3) with the antibody levels in infected groups. Indeed, children exhibiting the *HLA-G* 3'UTR-03 haplotype showed significantly lower IgG2 (but not significant for IgG1 and IgG3) response against the *P.f.* GLURP-R2 antigen when compared to other 3'UTR haplotypes ($P = 0.026$, Fig. 1B). In contrast, no differences were observed regarding the IgG levels against the *P.f.* MSP-3 (Supplementary Fig. 1) or GLURP-R0 antigens (Supplementary Fig. 2), independently on the presence or absence of 3'UTR-03 haplotype.

4. Discussion

The role of the non-classical HLA-Ib molecules has been associated with the regulation of the immune response rather than the antigen presentation (Sabbagh et al., 2018). Similar to classical class I

molecules, HLA-Ib molecules can accommodate peptides in their grooves (Diehl et al., 1996); however, convincing evidence of antigen presentation is lacking for HLA-G. On the other hand, HLA-E and HLA-F may present peptides and may interact with inhibitory and activating receptors (Dulberger et al., 2017; Pietra et al., 2010). Although the expression of these HLA-Ib molecules in chronically infected virus cells has been associated with the spread of the virus, worsening the infection outcome (Carosella et al., 2015), little attention has been devoted to the role of these molecules on parasite infections, particularly on malaria. As an initial approach to malaria pathogenesis, we evaluated the full gene sequence of the *HLA-E/-F/−G* genes in the Beninese Toffin children who are highly exposed to similar microenvironment factors and pertain to the same ethnic background (Bourgoignie, 1972). Since all children are highly exposed to *P.f.* malaria (Djènontin et al., 2017), we evaluated disease susceptibility in terms of symptomatic and asymptomatic infection and by the total number of malaria infections.

This is the first report evaluating the full *HLA-F* gene sequence and malaria. Children who experienced more *P.f.* asymptomatic infections than symptomatic ones during the follow-up were those that carried the G allele at *HLA-F* (−1499, promoter, rs183540921). Since the NFκB, interferon regulatory factor-1 (IRF1), IFN-γ, and class II transactivator (CIITA) (Gobin and van den Elsen, 2000; Howcroft and Singer, 2003) can regulate the *HLA-F* gene, one may hypothesize that the −1499 G allele, at promoter region may be targeted by the transcription factors, and may quantitatively impact the HLA-F protein production, and consequently the immunomodulatory role of this molecule. However, a further study will be necessary to functionally prove the role of this variable site on HLA-F expression.

The comparisons regarding the *HLA-G* SNVs among children exhibiting symptomatic or asymptomatic *P.f.* episodes or regarding the total number of malaria infections did not reach the more strict significance values; however, *HLA-G*-UTR-03 haplotype was associated with the susceptibility to *P.f.* symptomatic episodes. Considering that *HLA-G* polymorphisms may: i) be associated with sHLA-G levels (Martelli-Palomino et al., 2013), ii) inhibit the humoral immune response (Naji et al., 2014), and iii) interfere with the cytokine polarization (Carosella et al., 2001), to further understand the role of *HLA-G* gene polymorphisms on *P.f.* malaria, we evaluated the humoral immune response against *P.f.* antigens, and associated *HLA-G* gene variants with these soluble mediators. We observed relevant results associating *HLA-G* haplotypes with *P.f.* antibody levels, particularly at the *HLA-G* 3'UTR segment. The 14 bp INDEL polymorphism (carried by *HLA-G* 3'UTR haplotypes) has been extensively studied, and numerous genetic associations have been reported between this variant and disease morbidity or susceptibility (de Almeida et al., 2018). Functional studies have reported that the presence of the 14 bp sequence (INS) is associated with a lower mRNA expression compared to the deletion (DEL) allele (Rousseau et al., 2003); however, at the protein level, homozygosity for the INS allele has been associated with lower sHLA-G levels, while genotypes carrying at least one copy of the DEL allele have been associated with the higher levels (Chen et al., 2008). In this series, it was possible to observe the effect of the INDEL polymorphism in two circumstances: i) children carrying the *HLA-G* 3'UTR-03 haplotype (containing the DEL allele) exhibited a high number of symptomatic *P.f.* malaria episodes, and ii) a lower IgG2 against *P.f.* GLURP-R2 antigen. These findings corroborate the influence of HLA-G on the humoral immune response against *P.f.* infection, also supporting previous evidence that IgGs seem necessary for *P.f.* clearance (Adamou et al., 2019; Aucan et al., 2000; Courtin et al., 2009).

5. Conclusion

This study is the first to focus on the genetic contribution of immunomodulatory genes on the susceptibility to the most dreadful tropical infectious disease (*P.f.* malaria) in African children, using in-depth sequencing analysis for the evaluation of the *HLA-E/-F/−G* genes. We identified variation sites at regulatory regions of the *HLA-F* and *HLA-G* genes, associated with susceptibility/protection to malaria. *HLA-F* associations may be related to the differential expression profiles of the encoded immunomodulatory molecules, and the regulatory sites at the *HLA-G* 3'UTR may be associated to the posttranscriptional regulation of the *HLA-G* gene and to the host humoral response against *P.f.*.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2021.104828.

Author contributions

Achille Massougbodji, Moudachirou Ibikounlé, Eduardo A. Donadi, and **David Courtin:** Conceptualization, design and grant application. **Léonidas Tokplonou, Kuumaaté K. G. M'po, Sonya S. C. Glitho, Privat Agniwo, Daniel Gonzalez, Théophile Tchégninougbo** and **Aurèle Ayitchédji:** sample collection and provided clinical and laboratory information. **Paulin Sonon, Ibrahim Sadissou** and **Juliana Doblas Massaro:** *HLA-E/-F/−G* gene typing. **Paulin Sonon, Andréia S. Souza** and **Erick C. Castelli:** data processing. **Paulin Sonon:** writing and methodology. **Paulin Sonon, Jacqueline Milet, Audrey Sabbagh, Celso T. Mendes-Junior, Kabirou A. Moutairou, Erick C. Castelli, André Garcia, Philippe Moreau, David Courtin,** and **Eduardo A. Donadi:** data curation.

Declaration of Competing Interest

none.

Acknowledgments

This work was supported by grants from the Institut de Recherche pour le Développement (IRD-France, JEAI PALUCO); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil #406594/2013-9, #304931/2014-1, #466036/2013-5 and 302060/2019-07) and USP/COFECUB (Uc/Me#169-17). P·S was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–CAPES, Brazil (Finance Code 001, and PROCAD#88881-068436/2014-1) and L.T. was sponsored by an IRD scholarship (ARTS Program).

The authors thank the school children and parents for their free consent and the teachers of the Sô-Ava district for their extraordinary collaboration.

References

Adamou, R., Dechavanne, C., Sadissou, I., d'Almeida, T., Bouraima, A., Sonon, P., Amoussa, R., Cottrell, G., Le Port, A., Theisen, M., Remarque, E.J., Longacre, S., Moutairou, K., Massougbodji, A., Luty, A.J.F., Nuel, G., Migot-Nabias, F., Sanni, A., Garcia, A., Milet, J., Courtin, D., 2019. Plasmodium falciparum merozoite surface antigen-specific cytophilic IgG and control of malaria infection in a Beninese birth cohort. Malar. J. 18, 194. https://doi.org/10.1186/s12936-019-2831-x.

Albayati, Z., Alyami, A., Alomar, S., Middleton, D., Bonnett, L., Aleem, S., Flanagan, B.F., Christmas, S.E., 2017. The influence of cytomegalovirus on expression of HLA-G and its ligand KIR2DL4 by human peripheral blood leucocyte subsets. Scand. J. Immunol. 86, 396–407. https://doi.org/10.1111/sji.12594.

Aucan, C., Traoré, Y., Tall, F., Nacro, B., Traoré-Leroux, T., Fumoux, F., Rihet, P., 2000. High immunoglobulin G2 (IgG2) and low IgG4 levels are associated with human resistance to plasmodium falciparum malaria. Infect. Immun. 68, 1252–1258. https://doi.org/10.1128/iai.68.3.1252-1258.2000.

Bourgoignie, G.E., 1972. Ethno-écologie d'une collectivité régionale: Les cités lacustres du Dahomey. Can. J. Afr. Stud. / Revue canadienne des études africaines 6, 403–431. https://doi.org/10.1080/00083968.1972.10803680.

Bretscher, M.T., Dahal, P., Griffin, J., Stepniewska, K., Bassat, Q., Baudin, E., D'Alessandro, U., Djimde, A.A., Dorsey, G., Espié, E., Fofana, B., González, R., Juma, E., Karema, C., Lasry, E., Lell, B., Lima, N., Menéndez, C., Mombo-Ngoma, G., Moreira, C., Nikiema, F., Ouédraogo, J.B., Staedke, S.G., Tinto, H., Valea, I., Yeka, A., Ghani, A.C., Guerin, P.J., Okell, L.C., 2020. The duration of chemoprophylaxis against malaria after treatment with artesunate-amodiaquine and artemether-lumefantrine and the effects of pfmdr1 86Y and pfcrt 76T: a meta-analysis of individual patient data. BMC Med. 18, 47. https://doi.org/10.1186/s12916-020-1494-3.

Carosella, E.D., Moreau, P., Aractingi, S., Rouas-Freiss, N., 2001. HLA-G: a shield against inflammatory aggression. Trends Immunol. 22, 553–555. https://doi.org/10.1016/S1471-4906(01)02007-5.

Carosella, E.D., Rouas-Freiss, N., Roux, D.T.-L., Moreau, P., LeMaoult, J., 2015. HLA-G. In: Advances in Immunology. Elsevier, pp. 33–144. https://doi.org/10.1016/bs.ai.2015.04.001.

Castelli, E.C., Ramalho, J., Porto, I.O.P., Lima, T.H.A., Felício, L.P., Sabbagh, A., Donadi, E.A., Mendes-Junior, C.T., 2014. Insights into HLA-G genetics provided by worldwide haplotype diversity. Front. Immunol. 5, 476. https://doi.org/10.3389/fimmu.2014.00476.

Castelli, E.C., Mendes-Junior, C.T., Sabbagh, A., Porto, I.O.P., Garcia, A., Ramalho, J., Lima, T.H.A., Massaro, J.D., Dias, F.C., Collares, C.V.A., Jamonneau, V., Bucheton, B., Camara, M., Donadi, E.A., 2015. HLA-E coding and 3′ untranslated region variability determined by next-generation sequencing in two West-African population samples. Hum. Immunol. 76, 945–953. https://doi.org/10.1016/j.humimm.2015.06.016.

Castelli, E.C., Gerasimou, P., Paz, M.A., Ramalho, J., Porto, I.O.P., Lima, T.H.A., Souza, A.S., Veiga-Castelli, L.C., Collares, C.V.A., Donadi, E.A., Mendes-Junior, C.T., Costeas, P., 2017. HLA-G variability and haplotypes detected by massively parallel sequencing procedures in the geographicaly distinct population samples of Brazil and Cyprus. Mol. Immunol. 83, 115–126. https://doi.org/10.1016/j.molimm.2017.01.020.

Castelli, E.C., Paz, M.A., Souza, A.S., Ramalho, J., Mendes-Junior, C.T., 2018. Hla-mapper: an application to optimize the mapping of HLA sequences produced by massively parallel sequencing procedures. Hum. Immunol. 79, 678–684. https://doi.org/10.1016/j.humimm.2018.06.010.

Chen, X.-Y., Yan, W.-H., Lin, A., Xu, H.-H., Zhang, J.-G., Wang, X.-X., 2008. The 14 bp deletion polymorphisms in HLA-G gene play an important role in the expression of soluble HLA-G in plasma. Tissue Antigens 72, 335–341. https://doi.org/10.1111/j.1399-0039.2008.01107.x.

Courtin, D., Oesterholt, M., Huismans, H., Kusi, K., Milet, J., Badaut, C., Gaye, O., Roeffen, W., Remarque, E.J., Sauerwein, R., Garcia, A., Luty, A.J.F., 2009. The quantity and quality of African children's IgG responses to merozoite surface antigens reflect protection against Plasmodium falciparum malaria. PLoS One 4, e7590. https://doi.org/10.1371/journal.pone.0007590.

de Almeida, B.S., Muniz, Y.C.N., Prompt, A.H., Castelli, E.C., Mendes-Junior, C.T., Donadi, E.A., 2018. Genetic association between HLA-G 14-bp polymorphism and diseases: a systematic review and meta-analysis. Hum. Immunol. 79, 724–735. https://doi.org/10.1016/j.humimm.2018.08.003.

Dechavanne, C., Sadissou, I., Bouraima, A., Ahouangninou, C., Amoussa, R., Milet, J., Moutairou, K., Massougbodji, A., Theisen, M., Remarque, E.J., Courtin, D., Nuel, G., Migot-Nabias, F., Garcia, A., 2016. Acquisition of natural humoral immunity to P. falciparum in early life in Benin: impact of clinical, environmental and host factors. Sci. Rep. 6, 33961. https://doi.org/10.1038/srep33961.

Dias, F.C., Castelli, E.C., Collares, C.V.A., Moreau, P., Donadi, E.A., 2015. The role of HLA-G molecule and HLA-G gene polymorphisms in tumors, viral hepatitis, and parasitic diseases. Front. Immunol. 6 https://doi.org/10.3389/fimmu.2015.00009.

Diehl, M., Münz, C., Keilholz, W., Stevanović, S., Holmes, N., Loke, Y.W., Rammensee, H.-G., 1996. Nonclassical HLA-G molecules are classical peptide presenters. Curr. Biol. 6, 305–314. https://doi.org/10.1016/S0960-9822(02)00481-5.

Djènontin, A., Zogo, B., Ahlonsou, J., Bouraima, A., Ibikounle, M., Courtin, D., Pennetier, C., 2017. Mosquitoes fauna diversity, Plasmodium falciparum infection and insecticide resistance status in malaria vectors in a lagoon area in Southern Benin, West Africa. IJMCR 5. https://doi.org/10.14741/ijmcr/v.5.1.23.

Dulberger, C.L., McMurtrey, C.P., Hölzemer, A., Neu, K.E., Liu, V., Steinbach, A.M., Garcia-Beltran, W.F., Sulak, M., Jabri, B., Lynch, V.J., Altfeld, M., Hildebrand, W.H., Adams, E.J., 2017. Human leukocyte antigen F presents peptides and regulates immunity through interactions with NK cell receptors. Immunity 46. https://doi.org/10.1016/j.immuni.2017.06.002, 1018-1029.e7.

Garcia, A., Milet, J., Courtin, D., Sabbagh, A., Massaro, J.D., Castelli, E.C., Migot-Nabias, F., Favier, B., Rouas-Freiss, N., Donadi, E.A., Moreau, P., 2013. Association of HLA-G 3′UTR polymorphisms with response to malaria infection: a first insight. Infect. Genet. Evol. 16, 263–269. https://doi.org/10.1016/j.meegid.2013.02.021.

Gobin, S.J.P., van den Elsen, P.J., 2000. Transcriptional regulation of the MHC class Ib genes HLA-E, HLA-F, and HLA-G. Hum. Immunol. 61, 1102–1107. https://doi.org/10.1016/S0198-8859(00)00198-1.

Guo, S.W., Thompson, E.A., 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48, 361–372.

Guthmann, J.P., Llanos-Cuentas, A., Palacios, A., Hall, A.J., 2002. Environmental factors as determinants of malaria risk. A descriptive study on the northern coast of Peru. Tropical Med. Int. Health 7, 518–525. https://doi.org/10.1046/j.1365-3156.2002.00883.x.

Hill, A.V.S., Allsopp, C.E.M., Kwiatkowski, D., Anstey, N.M., Twumasi, P., Rowet, P.A., Bennettt, S., Brewster, D., Greenwood, B.M., 1991. Common West African HLA antigens are associated with protection from severe malaria, 352, p. 6.

Hirayasu, K., Ohashi, J., Kashiwase, K., Hananantachai, H., Naka, I., Ogawa, A., Takanashi, M., Satake, M., Nakajima, K., Parham, P., Arase, H., Tokunaga, K., Patarapotikul, J., Yabe, T., 2012. Significant association of KIR2DL3-HLA-C1 combination with cerebral malaria and implications for co-evolution of KIR and HLA. PLoS Pathog. 8, e1002565 https://doi.org/10.1371/journal.ppat.1002565.

Howcroft, T.K., Singer, D.S., 2003. Expression of nonclassical MHC class Ib genes: comparison of regulatory elements. IR 27, 1–30. https://doi.org/10.1385/IR:27:1:1.

Hunt, J.S., Petroff, M.G., McIntire, R.H., Ober, C., 2005. HLA-G and immune tolerance in pregnancy. FASEB J. 19, 681–693. https://doi.org/10.1096/fj.04-2078rev.

Lima, T.H.A., Buttura, R.V., Donadi, E.A., Veiga-Castelli, L.C., Mendes-Junior, C.T., Castelli, E.C., 2016. HLA-F coding and regulatory segments variability determined by massively parallel sequencing procedures in a Brazilian population sample. Hum. Immunol. 77, 841–853. https://doi.org/10.1016/j.humimm.2016.07.231.

Lyke, K.E., Dabo, A., Sangare, L., Arama, C., Daou, M., Diarra, I., Plowe, C.V., Doumbo, O.K., Sztein, M.B., 2006. Effects of concomitant Schistosoma haematobium infection on the serum cytokine levels elicited by acute plasmodium falciparum malaria infection in Malian children. Infect. Immun. 74, 5718–5724. https://doi.org/10.1128/IAI.01822-05.

Mackinnon, M.J., Mwangi, T.W., Snow, R.W., Marsh, K., Williams, T.N., 2005. Heritability of malaria in Africa. PLoS Med. 2, e340 https://doi.org/10.1371/journal.pmed.0020340.

Mangano, V.D., Modiano, D., 2014. Host genetics and parasitic infections. Clin. Microbiol. Infect. 20, 1265–1275. https://doi.org/10.1111/1469-0691.12793.

Manvailer, L.F.S., Wowk, P.F., Mattar, S.B., da Siva, J.S., da Graça Bicalho, M., Roxo, V.M.M.S., 2014. HLA-F polymorphisms in a Euro-Brazilian population from Southern Brazil: HLA-F polymorphisms in a Brazilian population. Tissue Antigens 84, 554–559. https://doi.org/10.1111/tan.12444.

Martelli-Palomino, G., Pancotto, J.A., Muniz, Y.C., Mendes-Junior, C.T., Castelli, E.C., Massaro, J.D., Krawice-Radanne, I., Poras, I., Rebmann, V., Carosella, E.D., Rouas-Freiss, N., Moreau, P., Donadi, E.A., 2013. Polymorphic sites at the 3' untranslated region of the HLA-G gene are associated with differential hla-g soluble levels in the Brazilian and French population. PLoS One 8, e71742. https://doi.org/10.1371/journal.pone.0071742.

Naji, A., Menier, C., Morandi, F., Agaugué, S., Maki, G., Ferretti, E., Bruel, S., Pistoia, V., Carosella, E.D., Rouas-Freiss, N., 2014. Binding of HLA-G to ITIM-bearing Ig-like transcript 2 receptor suppresses B cell responses. J. Immunol. 192, 1536–1546. https://doi.org/10.4049/jimmunol.1300438.

Ogouyèmi-Hounto, A., Azandossessi, C., Lawani, S., Damien, G., de Tove, Y.S.S., Remoue, F., Kinde Gazard, D., 2016. Therapeutic efficacy of artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in northwest Benin. Malar. J. 15, 37. https://doi.org/10.1186/s12936-016-1091-2.

Olieslagers, T.I., Voorter, C.E.M., Groeneweg, M., Xu, Y., Wieten, L., Tilanus, M.G.J., 2017. New insights in HLA-E polymorphism by refined analysis of the full-length gene: OLIESLAGERS ET AL. HLA 89, 143–149. https://doi.org/10.1111/tan.12965.

Persson, G., Melsted, W.N., Nilsson, L.L., Hviid, T.V.F., 2017. HLA class Ib in pregnancy and pregnancy-related disorders. Immunogenetics 69, 581–595. https://doi.org/10.1007/s00251-017-0988-4.

Pietra, G., Romagnani, C., Manzini, C., Moretta, L., Mingari, M.C., 2010. The emerging role of HLA-E-restricted CD8+ T lymphocytes in the adaptive immune response to pathogens and tumors. J. Biomed. Biotechnol. 2010, 907092. https://doi.org/10.1155/2010/907092.

Principaud, J.-M., 1995. La pêche en milieu lagunaire dans le sud-est du Bénin. L'exemple de l'exploitation des acadjas (en danger) sur le lac Nokoué et la basse Sô. caoum 48, 519–546. https://doi.org/10.3406/caoum.1995.3582.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575. https://doi.org/10.1086/519795.

Pyo, C.-W., Williams, L.M., Moore, Y., Hyodo, H., Li, S.S., Zhao, L.P., Sageshima, N., Ishitani, A., Geraghty, D.E., 2006. HLA-E, HLA-F, and HLA-G polymorphism: genomic sequence defines haplotype structure and variation spanning the nonclassical class I genes. Immunogenetics 58, 241–251. https://doi.org/10.1007/s00251-005-0076-z.

R Core Team, 2017. R Core Team (2017). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. http://www.R-project.org/. R Foundation for Statistical Computing.

Ramalho, J., Veiga-Castelli, L.C., Donadi, E.A., Mendes-Junior, C.T., Castelli, E.C., 2017. HLA-E regulatory and coding region variability and haplotypes in a Brazilian population sample. Mol. Immunol. 91, 173–184. https://doi.org/10.1016/j.molimm.2017.09.007.

Robinson, J., Halliwell, J.A., Hayhurst, J.D., Flicek, P., Parham, P., Marsh, S.G.E., 2015. The IPD and IMGT/HLA database: allele variant databases. Nucleic Acids Res. 43, D423–D431. https://doi.org/10.1093/nar/gku1161.

Rousseau, P., Le Discorde, M., Mouillot, G., Marcou, C., Carosella, E.D., Moreau, P., 2003. The 14 bp deletion-insertion polymorphism in the 3′ UT region of the HLA-G gene influences HLA-G mRNA stability. Hum. Immunol. 64, 1005–1010. https://doi.org/10.1016/j.humimm.2003.08.347.

Sabbagh, A., Sonon, P., Sadissou, I., Mendes-Junior, C.T., Garcia, A., Donadi, E.A., Courtin, D., 2018. The role of HLA-G in parasitic diseases. HLA 91, 255–270. https://doi.org/10.1111/tan.13196.

Sonon, P., Sadissou, I., Tokplonou, L., M'po, K.K.G., Glitho, S.S.C., Agniwo, P., Ibikounlé, M., Massaro, J.D., Massougbodji, A., Moreau, P., Sabbagh, A., Mendes-Junior, C.T., Moutairou, K.A., Castelli, E.C., Courtin, D., Donadi, E.A., 2018. HLA-G, −E and -F regulatory and coding region variability and haplotypes in the Beninese Toffin population sample. Mol. Immunol. 104, 108–127. https://doi.org/10.1016/j.molimm.2018.08.016.

Sullivan, L.C., Clements, C.S., Rossjohn, J., Brooks, A.G., 2008. The major histocompatibility complex class Ib molecule HLA-E at the interface between innate and adaptive immunity. Tissue Antigens 72, 415–424. https://doi.org/10.1111/j.1399-0039.2008.01138.x.

The 1000 Genomes Project Consortium, 2015. A global reference for human genetic variation. Nature 526, 68–74. https://doi.org/10.1038/nature15393.

Wilson, M., Oduro, A.R., Rogers, W.O., Koram, K.A., Hodgson, A., Osafo-Addo, A.D., 2008. HLA-DRB1*04 allele is associated with severe malaria in northern Ghana. Am. J. Trop. Med. Hyg. 78, 251–255. https://doi.org/10.4269/ajtmh.2008.78.251.