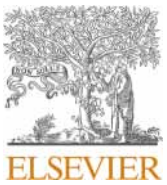
Infection, Genetics and Evolution 112 (2023) 105461



Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Combined polymorphisms involving the IgG heavy chain and Fc gamma receptors among Fulani and non-Fulani in Benin: implications for the natural protection of young Fulani against *Plasmodium falciparum* malaria infections

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ARTICLE INFO

Keywords:
G3m6 allotype
FcγRs
Malaria
Fulani
Benin

ABSTRACT

A decreased susceptibility of Fulani populations to malaria infections has been shown in Africa. A previous longitudinal cohort study conducted in the Atacora region of northern Benin showed a high merozoite-phagocytosis capacity in young Fulani. Here, we explored the combined polymorphisms in the constant region of the IgG3 heavy chain (presence/absence of the G3m6 allotype) and in Fc gamma receptors (FcγRs) as potentially involved in the natural protection against malaria of young Fulani in Benin. An active malaria follow-up was conducted among individuals from Fulani, Bariba, Otamari and Gando ethnic groups living in sympatry in Atacora, over the full malaria transmission season. FcγRIIA 131R/H (rs1801274), FcγRIIC C/T (rs3933769) and FcγRIIIA 176F/V (rs396991) were determined using the TaqMan method; FcγRIIIB NA1/NA2 was assessed by polymerase chain reaction (PCR) using allele-specific primers and G3m6 using allotype by PCR-RFLP. Individual carriage of G3m6 (+) was associated with an increased risk of *Pf* malaria infection (logistic multivariate regression model (lmrm), OR = 2.25, 95% CI = 1.06;4.74, $P = 0.034$). Combined haplotype G3m6 (+) – FcγRIIA 131H – FcγRIIC T – FcγRIIIA 176F – FcγRIIIB NA2 was also associated with an increased risk of *Pf* malaria infection (lmrm, OR = 13.01, 95% CI = 1.69;99.76, $P = 0.014$). G3m6 (−), FcγRIIA 131R and FcγRIIIB NA1 were more prevalent in young Fulani ($P = 0.002$, $P < 0.001$ and $P = 0.049$, respectively), while no Fulani presented the combined G3m6 (+) – FcγRIIA 131H – FcγRIIC T – FcγRIIIA 176F – FcγRIIIB NA2 haplotype that was carried by a majority of infected children. Our results highlight the combined factors G3m6 – FcγR as potentially involved in the merozoite-phagocytosis capacity and in the natural protection of young Fulani individuals against *P. falciparum* malaria in Benin.

1. Introduction

Immunoglobulin G (IgG) antibodies are major effectors of protection against *P. falciparum* (*Pf*) malaria by binding and opsonizing the parasite, initiating immune processes such as phagocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC) or antibody-dependent respiratory burst (ADRB) or by activating the classical pathway of the complement system (Cohen et al., 1961; Osier et al., 2014; Weaver et al., 2016). IgG can act on *Pf* directly by agglutinating the parasites and therefore preventing their reinvasion into the red blood cells (Beeson et al., 2016). They also can act indirectly by binding to Fc gamma receptors (FcγRs) expressed at the surface of immune cells, therefore triggering cell activation signals and immune response (Kim et al., 2003). The FcγRs are expressed on the majority of myeloid cells and play an important role in immune response, such as activation and modulation of the pro-inflammatory and cytotoxic pathways, and in the transport of circulating antibodies (Brooks et al., 1989; Kim et al., 2003; Hogarth and Pietersz, 2012).

In the family of Fc gamma receptors, FcγRIIA, FcγRIIC, FcγRIIIA and FcγRIIIB appear to play an important role in malaria susceptibility (Amiah et al., 2020). FcγRIIA initiates endocytosis, phagocytosis and the release of inflammatory mediators; it has two variants 131R (Arginine)

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https://doi.org/10.1016/j.meegid.2023.105461
Received 7 February 2023; Received in revised form 28 March 2023; Accepted 29 May 2023
Available online 1 June 2023

and 131H (Histidine) first described according to their IgG2 binding efficiency, with the 131H variant being more efficient (Parren et al., 1992; Vidarsson et al., 2014). FcγRIIC, derived from a cross-over between FcγRIIA and FcγRIIB, is an activating receptor expressed on natural killer (NK) cells. Indeed, *FCGR2C* is the product of an unequal crossover of the *FCGR2A* and *FCGR2B* genes encoding the activating FcγRIIA and inhibitory FcγRIIB (Amiah et al., 2020). FcγRIIC is involved in the cytotoxic response of natural killers (NK) cells triggered by FcγRIIIA (Warmerdam et al., 1993; Pincetic et al., 2014; Amiah et al., 2020). The intronic FcγRIIC variant is represented by a C/T transition (rs3933769) (Q13STP) resulting in the presence of a CAG codon (glutamine, Q) or a TAG codon (Stop codon) on the IgG Fc domain (Metes et al., 1998; Morel et al., 1999). Thus, one allele can be translated while the other cannot (null allele) (Ernst et al., 2002). It has been shown that the expression of FcγRIIC on NK cells was variable among individuals and correlated with the C/T allelic polymorphism (Morel et al., 1999; Ernst et al., 2002). Depending on this polymorphism, some individuals express only FcγRIIIA on the surface of their NK cells, whereas others co-express FcγRIIIA, with FcγRIIC potentially involved in the ADCC mechanism of NK cells (Metes et al., 1998; Morel et al., 1999; Ernst et al., 2002). The FcγRIIIA receptor is involved in phagocytosis and degranulation. There is a variant in FcγRIIIA consisting of the presence of F (Phenylalanine) or V (Valine) at position 176, with the 176 V variant offering a better affinity for IgG1 and IgG2 (Yoo and Morrison, 2010). FcγRIIIB is a C-terminus-linked glycosylphosphatidylinositol receptor expressed at the surface of neutrophils and eosinophils. Neutrophil antigen (NA) polymorphisms NA1/NA2 are located in the membrane-distal Ig-like domain of FcγRIIIB, which differs in amino acid positions 65 and 82 in two extra-glycosylation sites. The NA1 form is capable of better ingestion of IgG1 or IgG3 opsonized particles than the NA2 form (Huizinga et al., 1989).

The fixation of IgG on FcγR involves the constant regions (CH1, CH2 and CH3) of the heavy IgG chains with allelic variations found in these regions. These variations can lead to changes in the amino acid sequences of IgG subclasses, called allotypes, with consequences on their efficiency to bind FcγR. Allotypes correspond to serologically detected amino acid changes that characterize the polymorphism of a chain within a given isotype (Lefranc and Lefranc, 2012). The allotypes in the heavy IgG chain are designated as Gm (for gamma markers). There are 4 allotypes for IgG1 in the CH1 and CH3 constant domains called G1m [1, 2, 3, 17], 1 allotype for IgG2 in the CH2 constant domain called G2m23 and 13 allotypes for IgG3 in the CH2 and CH3 constant domains called G3m [5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28] (de Lange, 1989; Lefranc and Lefranc, 2012). Gm allotypes have been implicated in the individual susceptibility to malaria infections (Facer, 1980; Migot-Nabias et al., 2008; Fall et al., 2020).

A decreased susceptibility to malaria infections among Fulani has been documented by studies conducted in East (Sudan) and West Africa (Benin, Burkina Faso, Mali and Nigeria) through a lower number of uncomplicated and severe malaria attacks, a lower parasite density in asymptomatic carriers, a higher prevalence of splenomegaly and anemia, and higher IgG and IgM levels against *P. falciparum* antigens (Bryceson et al., 1976; Modiano et al., 1995; Modiano et al., 1996; Creasey et al., 2004; Maiga et al., 2014; Henry et al., 2022). A differential expression of FcγRIIA, FcγRIIB and FcγRIIC has been shown between Fulani and non-Fulani in Mali and Burkina Faso (Cherif et al., 2016; Combasseré-Cherif et al., 2021). Moreover, G3m6 has been shown to be less prevalent in Fulani in Sudan (Pandey et al., 2007) and associated with an increased risk of *Pf* malaria infection (Fall et al., 2020). Also, our previous results from a longitudinal cohort study conducted in Atacora in Benin among individuals living in sympatry (the BAObAB (Biocultural AdaptatiOn to malaria in Atacora, northern Benin) project) showed high IgG concentrations of the GLURP *Pf* antigen in Fulani as well as a greater capacity of young Fulani (compared to young Bariba, Otamari and Gando) to opsonize antibodies to *Pf* merozoites when using heat-inactivated plasma before the malaria transmission season (MTS) (Fall et al., 2023).

We previously demonstrated that combined polymorphisms G3m-FcγRIIA 131R/H – FcγRIIIA 176F/V – FcγRIIIB NA1/NA2 were involved in the susceptibility of malaria in Beninese children (Fall et al., 2020) and in the capacity of opsonic phagocytosis of *Pf* merozoites (Fall et al., 2023), while this capacity was associated with the control of *Pf* malaria asymptomatic infections (Fall et al., 2022a). Here, we investigated a differential carriage of combined polymorphisms G3m6 – FcγRIIA 131R/H – FcγRIIC C/T – FcγRIIIA 176F/V – FcγRIIIB NA1/NA2 between Fulani and non-Fulani in order to evaluate their influence on natural protection against *Pf* malaria infections and correlated results to antibody functionality measured by means of opsonic phagocytosis in Fall et al., 2023.

2. Methods

2.1. Ethical approval

The BAObAB project was reviewed and approved by the ethics committee of the Research Institute of Applied Biomedical Sciences (CER-ISBA/Institut des Sciences Biomédicales Appliquées) in Benin (no. 61/CER/ISBA/15). All study participants were informed before the beginning of the study. Individual written informed consent was obtained from adult participants or from the parents or guardians of participating children using a consent form translated into their native language.

2.2. Study population and design

The BAObAB project was implemented between 2015 and 2019 in the Atacora region to investigate the response to malaria infection in Fulani, Bariba, Otamari and Gando ethnic groups, living in sympatry. The Gando share a common language and way-of-life with the Fulani, while they share their genetic background with the Bariba, who are farmers (Sabbagh A, personal communication). For their part, the Otamari form a clan society of sedentary farmer breeders (Fall et al., 2023). The Fulani's nomadic lifestyle and pastoralism were often advanced as the main reasons explaining their natural protection against malaria. A recent study identified some genetic variants, appearing more frequently in the Fulani, that could explain their particular phenotype of lower susceptibility to malaria (Henry et al., 2022). Because the Fulani differ from the other groups both genetically and culturally, distinguishing between the two sources of explanation remains difficult, probably due to gene-environment interactions.

A clinical, epidemiological and parasitological follow-up was conducted in Tamandé, Kouboro, Gorgoba and Goufanrou, four rural villages located 3–10 km from each other, where these ethnic groups live in sympatry. The individuals under study were adults between 21 and 71 years old and children under 8 years old. Individuals were invited to the Birni or Goufanrou health centers for a medical consultation. An active malaria follow-up consisted of home visits every 15 days. A clinical examination was also performed in case of fever, whether related to malaria or not, at home or at the health center.

Cross-sectional visits were carried out before and after MTS during which a questionnaire was completed in addition to the clinical examination, temperature measurement and spleen palpation. A *Plasmodium* rapid diagnostic test (RDT) (SD BIOLINE Malaria Ag P.f©, Abbott) using immunochromatographic detection of histidine-rich protein two (HRP2) of *P. falciparum* and a thick blood smear (TBS) examined by optical microscopy were collected to confirm malaria infection (Fall et al., 2023). Saliva samples were collected for DNA extraction.

2.3. FcγRIIA, FcγRIIC and FcγRIIIA genotyping

Single nucleotide polymorphisms corresponding to FcγRIIA 131R/H (rs1801274), FcγRIIC C/T (rs3933769) and FcγRIIIA 176F/V

(rs396991) were genotyped by the Applied Biosystems TaqMan SNP Genotyping Assay using predesigned primer/probe sets (C_9077561_20, C_57480411_10 and C_25815666_10). PCR was performed using the ViiA™7 Real-time PCR System (Applied Biosystems) according to the following conditions: 1 cycle at 92 °C for 10 min and 40 cycles at 92 °C for 15 s and at 60 °C for 60s. The results were analyzed using the SDS software 14.

2.4. FcγRIIIB genotyping

Two different PCRs were performed using allele-specific oligonucleotides. For NA1, the forward 5'-CAG TGG TTT CAC AAT GTG AA-3′ and reverse 5' CAT GGA CTT CTA GCT GCA CCG 3′ primers were used; and 5'-CTC AAT GGT ACA GCG TGC TT-3′ and 5'-CTG TAC TCT CCA CTG TCG TT-3′ were the forward and reverse primers for NA2, respectively. The PCR reaction conditions for NA1 included 1 cycle at 95 °C for 5 min, followed by 30 cycles at 95 °C for 30s, 55 °C for 30s and 72 °C for 45 s in a mixture containing 100 µM $MgCl_2$, 1× buffer solution, 16 µM dNTPs, 2.8 µM sense and antisense primers and 0.5 unit of Taq polymerase. The PCR reaction condition for NA2 was 1 cycle at 95 °C for 5 min, followed by 30 cycles at 95 °C for 30s, 60 °C for 30s and 72 °C for 45 s in a mixture containing 45 µM $MgCl_2$, 1× buffer solution, 16 µM dNTPs, 1.39 µM sense and antisense primers and 0.5 unit of Taq polymerase. The products of 142 pb (NA1) and 169 pb (NA2) were revealed on a 2% agarose gel.

2.5. G3m6 genotyping

G3m6 was genotyped using a method adapted from a previously detailed protocol (Iriemenam et al., 2013). The gene region circumscribing the position determining G3m6 on the chromosome 14 (14q 32.3) was amplified using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Briefly, a PCR was conducted using the forward primer 5'-GCGGGCAGCCGGAGAA-CAACTACAAC-3′ and the reverse primer 5'-GCTTGCCGGCTATCG-CACTC-3′ in the mixture containing 1.5 µl genomic DNA, 3.6 µl buffer 5×, 1.26 µl MgCL2 25 mM, 1.44 µl dNTP 2.5 mM, 0.2 µl primer and reverse (each), and 0.5 unit of Taq polymerase.

The PCR reaction condition was 1 cycle at 95 °C for 5 min, followed by 30 cycles at 95 °C for 30s, 60 °C for 30s and 72 °C for 45 s. After digestion with *Fnu*4HI (SatI), the fragments were separated on 7% polyacrylamide gel. Three products corresponding to the three genotypes were detected: G3m6 +/+ (206 pb fragment), G3m6 +/− (206 pb, 110 pb and 96 pb fragment) and G3m6 −/− (110 pb and 96 pb fragment).

2.6. Statistical analysis

The statistical analyses were carried out using the Stata software version 14. The characteristics related to sex, age, presence of splenomegaly and RDT results before and after MTS were compared between groups using chi-square tests. The distribution of FcγRIIA, FcγRIIC, FcγRIIIA and FcγRIIIB genotypes and G3m6 genotypes [(+/+), (+/−), (−/−)] between groups (together and between children) were compared using chi-square independence tests. Also, the genotypic frequencies were tested for Hardy-Weinberg equilibrium (HWE).

Since a high level of opsonic phagocytosis of *Pf* merozoites has been described in young Fulani and related to fewer *Pf* malaria infections after MTS in this cohort (Fall et al., 2023), the correlation between individual carriage of G3m6 or FcγRIIA, FcγRIIC, FcγRIIIA and FcγRIIIB and the presence of *Pf* malaria infection before MTS was first studied through a logistic multivariate regression model using the following as covariates: splenomegaly, ethnic group, gender, rural village and age status (adults vs. children). Univariate analyzes on each of the covariates were made and only those with a *P* value <0.20 in the univariate model were included in the final multivariate model. Next the association between combined polymorphisms G3m6 – FcγRIIA, FcγRIIC, FcγRIIIA, FcγRIIIB and presence of *Pf* malaria infection before MTS was studied through the same regression model and covariates. Finally, the significant combined G3m6 – FcγRIIA, FcγRIIC, FcγRIIIA and FcγRIIIB genotypes and/or haplotypes were analyzed as a function of ethnic group, age status (adults vs. children) and presence of *Pf* malaria infection after MTS.

Table 1
Characteristics of the study group.

	Ethnic groups				
	Bariba [a] *N* = 85	Otamari [b] *N* = 93	Gando [c] *N* = 38	Fulani *N* = 61	P value
Children (n, %)	40 (47)	46 (49)	17 (45)	36 (59)	
Age (mean, ± SD)	5.26 (1.55)	5.19 (1.7)	5.23 (1.15)	4.94 (1.69)	
Boy (n, %)	20 (50)	29 (63)	12 (70)	22 (61)	
Girl (n, %)	20 (50)	17 (37)	5 (30)	14 (39)	
Adults (n, %)	45 (53)	47 (51)	21 (55)	25 (41)	
Age (mean, ± SD)	39.49 (8.69)	37.4 (9.2)	34.28 (8.53)	36.64 (12.89)	
Male (n, %)	26 (58)	21 (45)	11 (52)	8 (32)	**0.0002**[a]
Female (n, %)	19 (42)	26 (55)	10 (48)	17 (68)	0.059 [b] **0.004**[c]
Splenomegaly before MTS					
Children					
Yes (n, %)	7 (17)	11 (24)	3 (18)	12 (33)	**0.009**[a]
No (n, %)	33 (83)	35 (76)	14 (82)	24 (67)	**0.014**[c]
Adults					
Yes (n, %)	1 (2)	3 (6)	1 (5)	1 (4)	
No (n, %)	44 (98)	47 (94)	20 (95)	24 (96)	
RDT before MTS					
Children					
Positive (n, %)	23 (57)	17 (37)	7 (41)	16 (44)	0.065 [a]
Negative (n, %)	17 (43)	29 (63)	10 (59)	20 (56)	
Adults					
Positive (n, %)	5 (11)	5 (11)	3 (14)	2 (8)	
Negative (n, %)	40 (89)	42 (89)	18 (86)	23 (92)	
Splenomegaly after MTS					
Children					
Yes (n, %)	8 (20)	14 (30)	1 (6)	8 (22)	**0.001**[c]
No (n, %)	32 (80)	32 (70)	16 (94)	26 (78)	
Adults					
Yes (n, %)	0 (0)	3 (6)	1 (5)	2 (8)	**0.004**[a]
No (n, %)	45 (100)	44 (94)	20 (95)	23 (92)	
RDT after MTS					
Children					
Positive (n, %)	24 (60)	38 (83)	12 (70)	25 (69)	**0.020**[b]
Negative (n, %)	16 (40)	8 (17)	5 (30)	11 (31)	
Adults					
Positive (n, %)	13 (29)	17 (36)	4 (19)	8 (32)	**0.035**[c]
Negative (n, %)	32 (71)	30 (64)	17 (81)	17 (68)	
Rural villages					
Gorgoba	27	69	38	5	
Kouboro	76	38	0	6	
Goufanrou	0	0	0	34	
Tamande	19	0	0	16	

This table presents the characteristics of the study group. Statistical significance determined by Chi square analysis between Fulani and the other groups. ([a] comparison with Bariba; [b] comparison with Otamari and [c] comparison with Gando). MTS: malaria transmission season. RDT: rapid diagnostic test for malaria. Only significant (in bold) or nearly significant *P* values are shown as regards the variables age, splenomegaly and RDT.

3. Results

3.1. Characteristics of participants

No differences were found regarding gender ratio between the four groups studied (Fulani, Gando, Otamari and Bariba), while Fulani adult

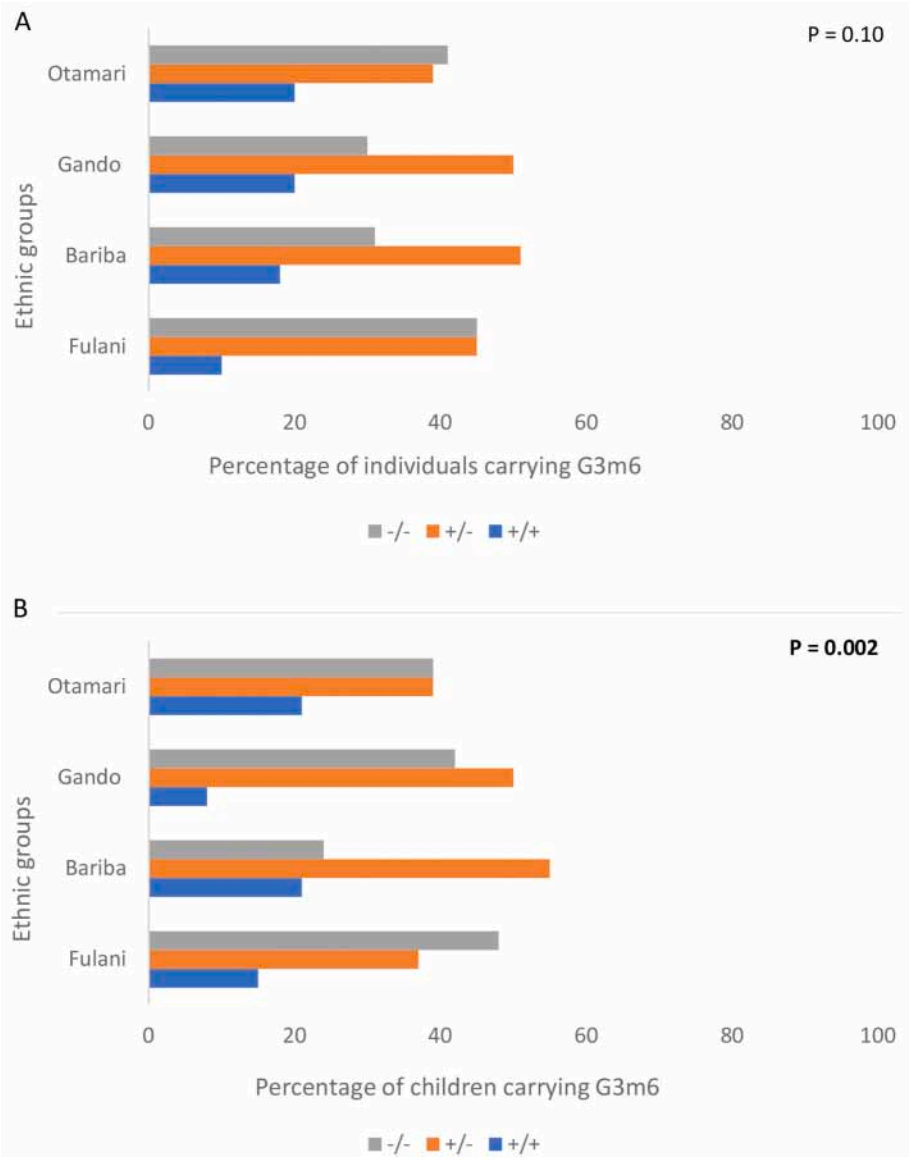

Fig. 1. Distribution of G3m6 genotypes in the study group. Fig. 1 represents the distribution of G3m6 genotypes in the whole study group (A) and in children (B) according to ethnic groups. The minor allele is G3m6(+) (40%) and the P-value of HWE test is $P = 0.25$. Chi square test was used to compare the distribution of genotypes between groups.

females were more numerous compared to Bariba ($P = 0.0002$), Gando ($P = 0.004$) and Otamari ($P = 0.059$). Regarding splenomegaly, more Fulani children presented splenomegaly before MTS than Bariba ($P = 0.009$) and Gando ($P = 0.014$) children, while no differences were found between adults. After MTS, more Fulani children had splenomegaly as compared to Gando children (22% vs. 6%, $P = 0.001$), and the same pattern was found for Fulani vs. Bariba adults (8% vs. 0%, P = 0.004). Before MTS, Bariba children had slightly more positive RDTs than Fulani children (57% vs. 44%, $P = 0.065$). After MTS, Otamari children had more positive RDTs than Fulani children (83% vs. 69%, $P = 0.020$), while for adults, Fulani had more positive RDTs than Gando (32% vs. 19%, $P = 0.035$) (Table 1).

3.2. Distribution of G3m6 allotype and association with malaria infection among ethnic groups

In the study population as a whole, the minor allele G3m6 (+) occurred at a frequency of 40%, and the observed genotype proportions conformed *to Hardy-Weinberg* equilibrium (HWE) expectations ($P =$ 0.25). Similar proportions of Fulani and Otamari individuals were characterized by G3m6 (+/−) and G3m6 (−/−), while among Gando and Bariba, a greater proportion of individuals carried G3m6 (+/−) (Fig. 1A). Among children, there were more Fulani with G3m6 (−/−) compared to other children ($P = 0.002$) (Fig. 1B).

Regarding malaria, there was a positive association between the

Table 2
Association between G3m6 carriage and *Pf* malaria infection.

Pf malaria infection	n	odds ratio	95%CI	P value
Genotypes				
G3m6 (− / −)	74			
G3m6 (+ / −)	95	2.45	1.12;5.36	**0.025**
G3m6 (+ / +)	36	1.74	0.62;4.93	0.294
Presence				
G3m6 (+)		2.25	1.06;4.74	**0.034**

Table 2 presents the logit model obtained through the control variables splenomegaly, age, rural village, and age status (children vs. adults). In bold significant *P*-value at $P \leq 0.05$.

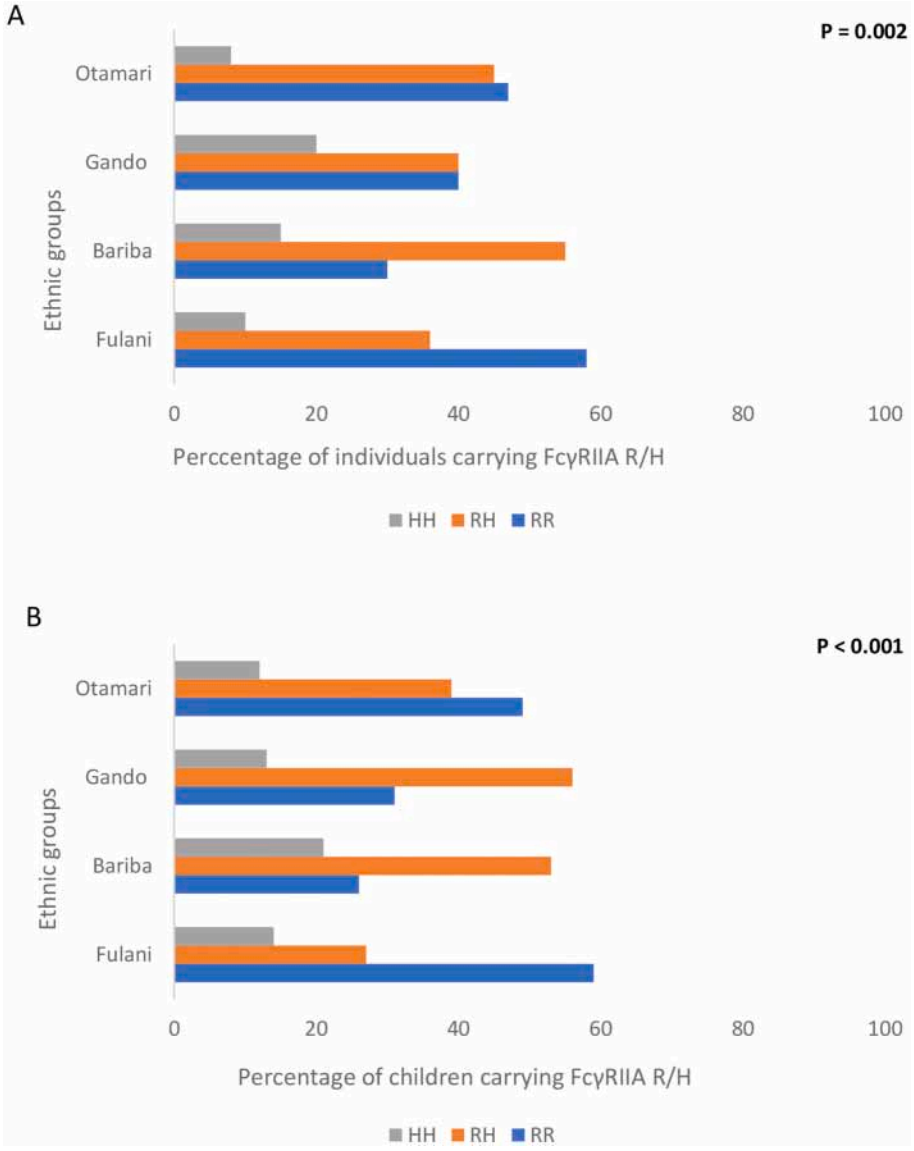

Fig. 2. : Distribution of FcγRIIA 131R/H genotypes in the study group.Fig. 2 shows the distribution of FcγRIIA 131R/H genotypes in the whole study group (A) and among children (B) according to ethnic groups. H is the minor allele (35%) and the P-value of HWE test is $P = 0.96$. Chi square test was used to compare distribution of genotypes between groups.

carriage of G3m6 (+/−) and the presence of a *Pf* malaria infection before MTS compared to the non-carriage of G3m6 (−/−) (OR = 2.45, 95%CI = 1.12;5.36, $P = 0.025$). Consistently, the presence of the G3m6 (+) allotype was associated with an increased risk of *Pf* malaria infection (OR = 2.25, 95%CI = 1.06;4.74, $P = 0.034$) (Table 2).

3.3. Distribution of FcγR and association with malaria infection among ethnic groups

Apart from FcγRIIC and FcγRIIIB, which were not in accordance with HWE expectations ($P < 0.01$), the other FcγR SNPs studied were in conformity with HWE: FcγRIIA ($P = 0.96$) and FcγRIIIA ($P = 0.80$) in the whole study population. The minor allele for FcγRIIA was H, and there were more Fulani, including children, who carried the RR genotype as compared to others ($P = 0.002$ and $P < 0.001$) (Fig. 2A and B). T was the minor allele for FcγRIIC C/T, and the CT genotype occurred more in young Fulani ($P = 0.001$) (Fig. 3A and B). The V variant (minor allele) of FcγRIIIA 176F/V was absent under the homozygous VV genotype in the study group; and concerning children, only Fulani harbored the FV genotype (P = 0.001) (Fig. 4A and B).

For FcγRIIIB, Fulani presented similar proportions of NA1NA1 and NA2NA2 genotypes, while NA2NA2 predominated in other groups, although not significantly ($P = 0.21$) (Fig. 5A). Among children, the proportion of NA1NA1 was higher in Fulani and Gando compared to Bariba and Otamari ($P = 0.049$) (Fig. 5B).

Among FcγR, the carriage of the FcγRIIC CT genotype, the most prevalent genotype in young Fulani (Fig. 3B), was associated with an absence of *Pf* malaria infections before MTS (OR = 0.45, 95% CI = 0.21;0.96, $P = 0.040$) (Table 3).

3.4. Combined FcγR and G3m6 – FcγR polymorphisms and malaria infection

Only the FcγR genotype combination FcγRIIA RR – FcγRIIC CT – FcγRIIIA FF – FcγRIIIB NA2NA2 was associated with a decreased risk of *Pf* malaria infection (OR = 0.06, 95%CI = 0.004;1.22, $P = 0.050$). By contrast, the combined haplotypes FcγRIIA H – FcγRIIC T – FcγRIIIA F – FcγRIIIB NA2, FcγRIIA R – FcγRIIC C – FcγRIIIA F – FcγRIIIB NA2 and FcγRIIA H – FcγRIIC C – FcγRIIIA F – FcγRIIIB NA2 were associated with an increased risk of *Pf* malaria infection ($P = 0.007$, $P = 0.002$ and $P =$

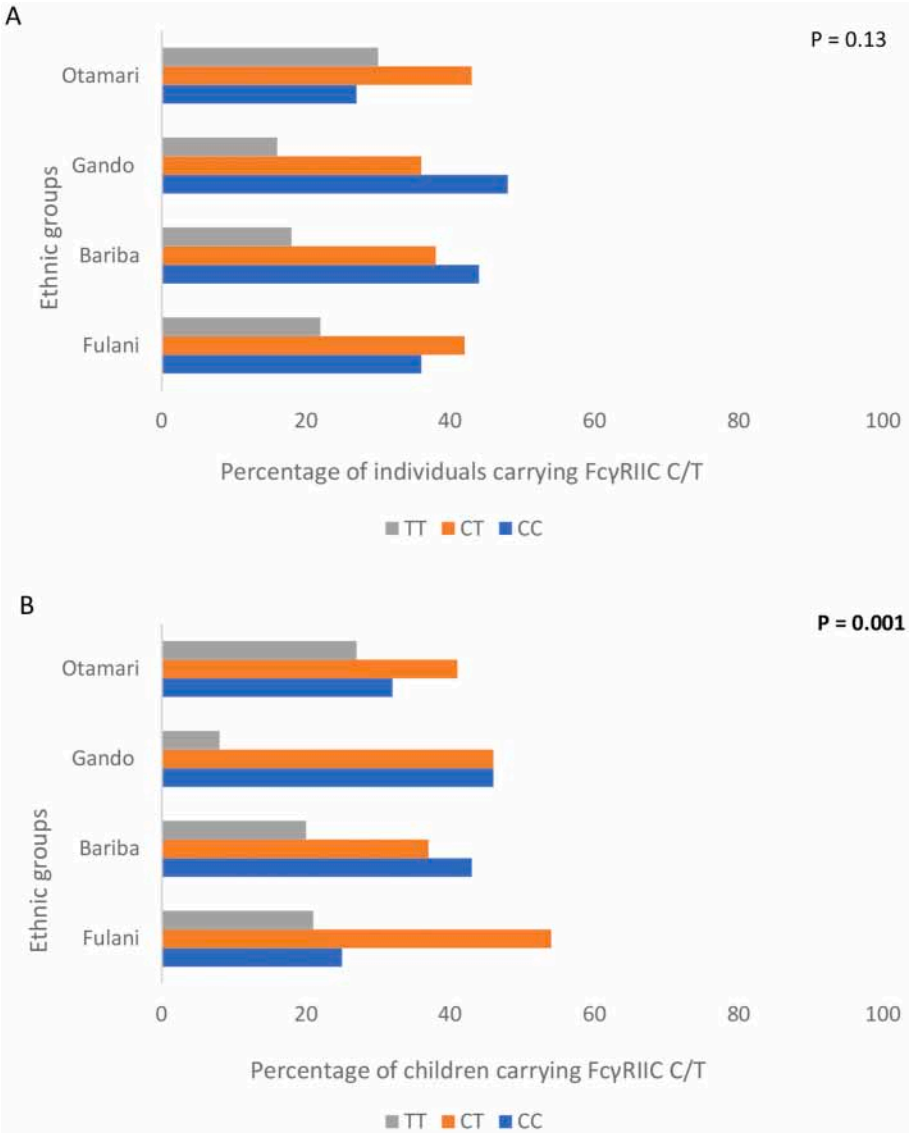

Fig. 3. Distribution of FcγRIIC C/T genotypes in the study group. Fig. 3 represents the distribution of FcγRIIC C/T genotypes in the whole study group (A) and in children (B) according to ethnic groups. The minor allele is T (43%) and the P-value of HWE test is $P = 0.007$. Chi square test was used to compare distribution of genotypes between groups.

0.021, respectively) (Table 4). The G3m6 (+) – FcγRIIA H – FcγRIIC T – FcγRIIIA F – FcγRIIIB NA2 polymorphisms combination was related to an increased risk of *Pf* malaria infection (OR = 13.01, 95%CI = 1.69;99.76, $P = 0.014$) at a higher level than the G3m6 (+) – FcγRIIA H – FcγRIIC T – FcγRIIIA F – FcγRIIIB NA1 combination (OR = 11.12, 95%CI = 1.10;111.85, $P = 0.041$) (Table 5).

No Fulani carried the G3m6 (+) – FcγRIIA H – FcγRIIC T – FcγRIIIA F – FcγRIIIB NA2 polymorphism combination associated with a high risk of *Pf* malaria infection (Fig. 6). Among Bariba, Otamari and Gando, there were more children with a positive *Pf* RDT carrying this combination than adults. Indeed, this group of 20 individuals was equally distributed among children and adults ($n = 10$). Only one adult had a positive *Pf* malaria test after MTS as opposed to eight children (Fig. 6). This corroborates the higher level of opsonic phagocytosis that was observed in Bariba, Otamari and Gando adults in this cohort (Fall et al., 2023). The five individuals with the G3m6 (+) – FcγRIIA H – FcγRIIC T – FcγRIIIA F – FcγRIIIB NA1 polymorphism combination were composed of three Fulani (two adults and one child) and two Otamari (one adult and one child). Among them, four were infected by *Pf* after MTS.

4. Discussion

This study investigated the diversity of combined polymorphisms G3m6 – FcγRIIA – FcγRIIC – FcγRIIIA – FcγRIIIB between Fulani and non-Fulani individuals as potentially involved in their natural protection against malaria infections and in their antibody functionality measured by means of opsonic phagocytosis of *Pf* merozoites.

Results showed that the absence of the G3m6 allotype (G3m6 (−)) was more frequent in young Fulani (48%) compared to other ethnic groups, while the carriage of G3m6 (+) was associated with an increased risk of *Pf* malaria infection. To our knowledge, only one study conducted in Sudan investigated the presence of G3m6 in Fulani and confirmed that it was less prevalent in this group and associated with a lower parasitemia, compared to another group of Masaleit living in sympatry (24% vs. 42%) (Pandey et al., 2007). The frequency of G3m6 is highly variable among populations, ranging from 8% to 62%, probably resulting from a differential selection caused by infectious diseases like malaria (Steinberg and Cook, 1981; Pandey et al., 2007). In a previous study, we showed an increased risk of *Pf* malaria infection associated with the G3m6 (+) carriage in Beninese infants (Fall et al., 2020). G3m6

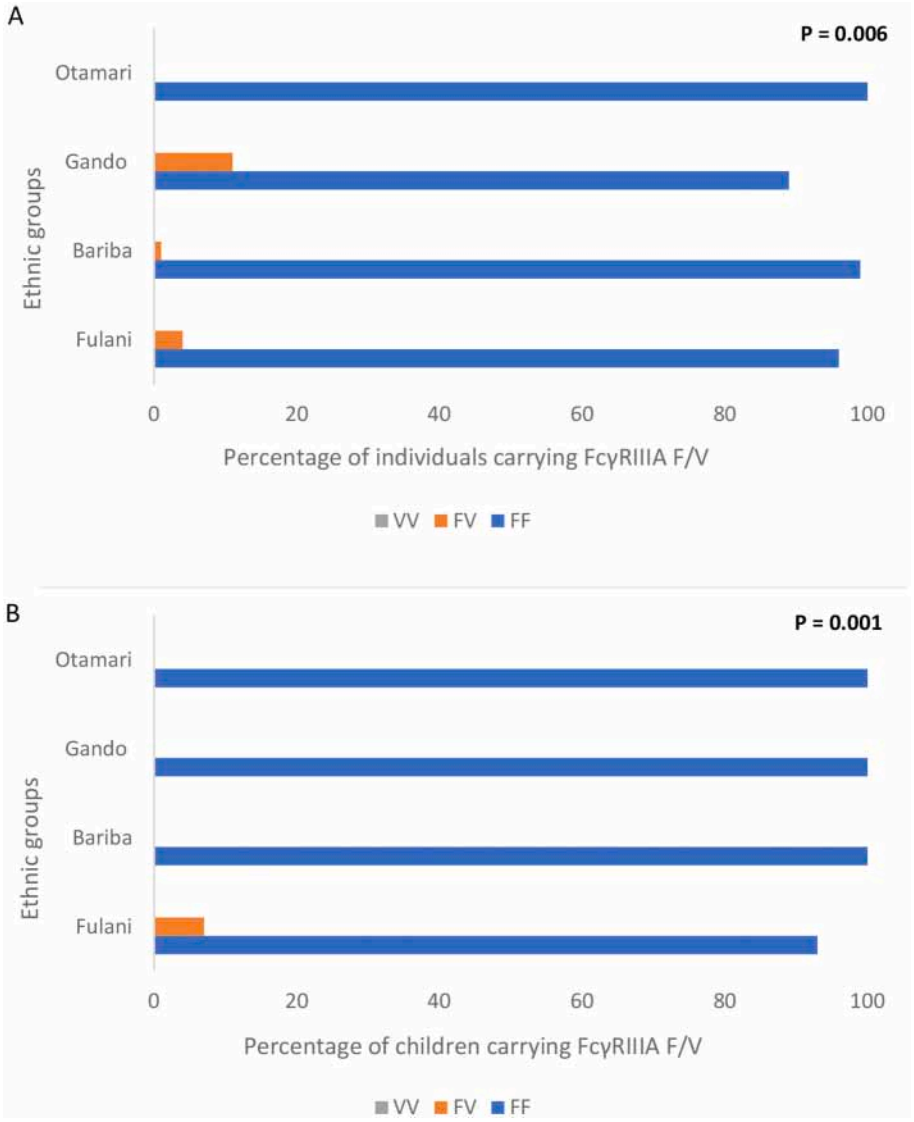

Fig. 4. : Distribution of FcγRIIIA 176F/V genotypes in the study group. Fig. 4 shows the distribution of FcγRIIIA 176F/V genotypes in the whole study group (A) and in children (B) according to ethnic groups. The minor allele is V (1.5%) and the P-value of HWE test is $P = 0.80$. Chi square test was used to compare distribution of genotypes between groups.

is characterized by the presence of a glutamic acid (Glu) at position 98 of the IgG Fc domain instead of glutamine (Gln). Glu 98 undergoes a post-translational modification, under the form of an ADP-ribosylation, which for its part has been associated with a decreased IgG affinity (Rissiek et al., 2017). Also, it has been shown that the IgG3 subclass is preferentially ribosylated (Knight and Barbieri, 1997). Further studies are needed to understand whether this post-translational modification would alter the functionality of IgG3 harboring G3m6 (+), which would be associated with a greater risk of *Pf* malaria infection.

In our study, the FcγRIIA 131RR genotype has been found to be mostly prevalent in Fulani (both in adults and children). The 131R allele has been identified as playing a major role in ADRB (Pleass et al., 2003; Shi et al., 2011) and conferring protection against high parasite densities and protection to malaria in contrast to the 131H allele (Cooke et al., 2003; Ntoumi et al., 2005; Ouma et al., 2006). Conversely, results in Mali and Sudan showed a higher prevalence of the 131H allele in Fulani compared to non-Fulani (Nasr et al., 2007; Maiga et al., 2014), and other studies have related the carriage of FcγRIIA 131H to malaria protection (Sinha et al., 2008; Zhao et al., 2014). It has been shown that the 131H allele contributes to an efficient binding to IgG2 and IgG3 as opposed to the 131R allele, which binds IgG1 efficiently (Vidarsson et al., 2014). Differences in sample size, ethnic origin and genetic diversity of the studied population groups, as well as differences in the clinical definition of malaria used or differences in gene-environment interactions and in patterns of malaria transmission may explain the inconsistent observations between studies of Fulani in Benin and in other African countries.

In this study, we did not find any association between FcγRIIA 131R/H and the occurrence of *Pf* malaria infections in this population group from northern Benin, in line with our previous investigations of Beninese populations from southern Benin in the Tori Bossito (Fall et al., 2020) and Allada study sites (Fall et al., 2022b).

We found a low frequency of the FcγRIIIA 176 V allele, in agreement with observations made in other populations from sub-Saharan Africa (RefSNP Report: https://www.ncbi.nlm.nih.gov/snp/rs396991); only Fulani children carried it through the 176FV genotype. The 176 V variant has been reported to improve the FcγRIIIA affinity for IgG1 and IgG2 (Bruhns et al., 2009). Moreover, there were more Fulani children carrying the NA1 allele despite equal frequencies of NA1 and NA2 in the whole Fulani group. The FcγRIIIB NA1 form is capable of better ingestion of IgG1 or IgG3 opsonized particles than the NA2 form (Huizinga et al., 1989). In comparison to NA1, the carriage of NA2 has been related

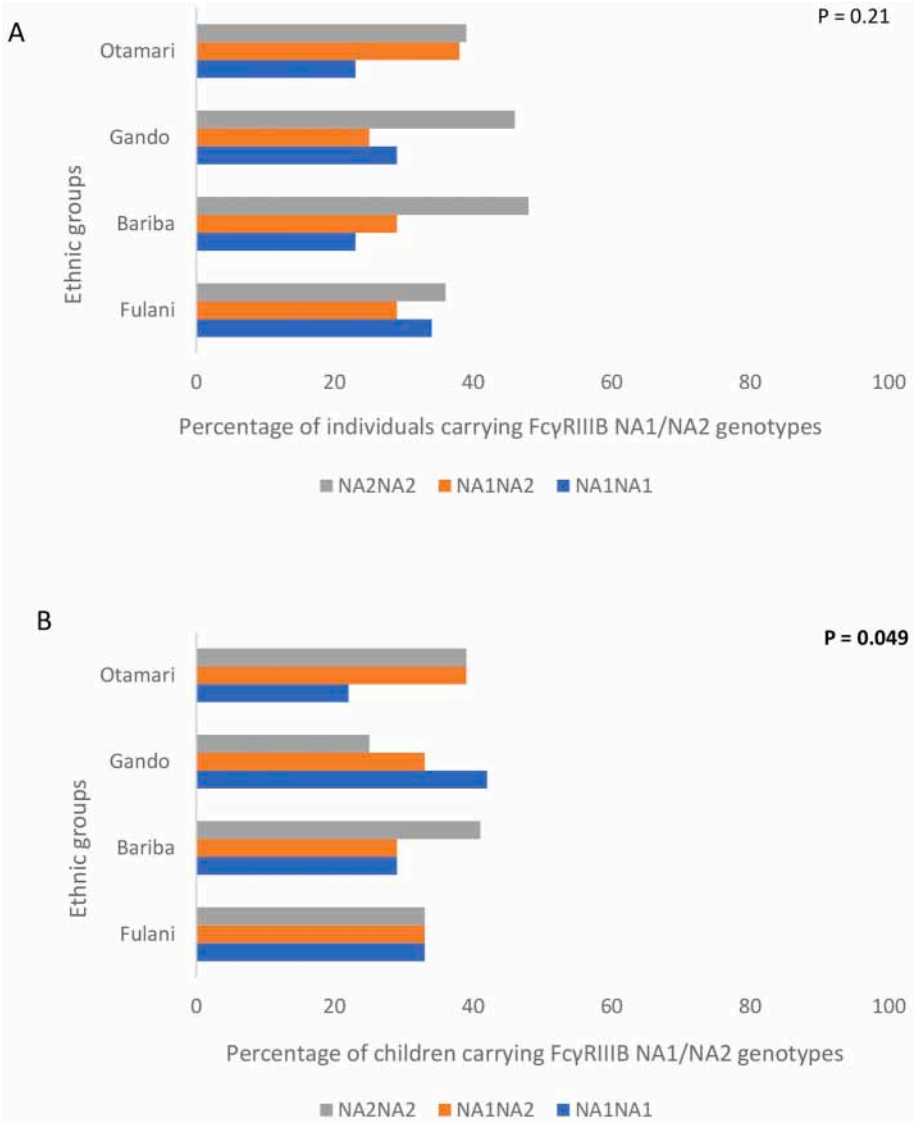

Fig. 5. : Distribution of FcγRIIIB NA1/NA2 genotypes in the study group. Fig. 5 represents the distribution of FcγRIIIB NA1/NA2 genotypes in the whole study group (A) and in children (B) according to ethnic groups. The minor allele is NA1 (42%) and the P-value of HWE test is <0.001. Chi square test was used to compare distribution of genotypes between groups.

Table 3
Significant associations between FcγR and *Pf* malaria infection.

Pf malaria infection	n	odds ratio	95%CI	P value
FcγRIIC C/T Genotypes				
CC	84			
CT	93	0.45	0.21;0.96	**0.040**
TT	53	0.78	0.33;1.85	0.585
Presence				
T		0.55	0.28;1.08	0.089

Table 3 presents the logit model obtained through the control variables splenomegaly, age and age status (children vs. adults). In bold significant *P*-value at $P \leq 0.05$.

to a decreased phagocytosis (Salmon et al., 1990) as well as to a risk for clinical malaria (Adu et al., 2012) or cerebral malaria (Omi et al., 2002). This observation may help explain the natural protection of Fulani against malaria infection. Moreover, we also observed more Gando children carrying the NA1NA1 genotype. This result could explain the small difference in values of opsonic phagocytosis found in Gando

Table 4
FcγRIIA – FcγRIIC – FcγRIIIA – FcγRIIIB combinations and *Pf* malaria infection.

Pf malaria infection	n	odds ratio	95%CI	P value
FcγRIIA – FcγRIIC – FcγRIIIA – FcγRIIIB combinations				
Genotype combinations				
FcγRIIA RR – FcγRIIC CT – FcγRIIIA FF – FcγRIIIB NA2NA2	12	0.06	0.004;1.22	**0.050**
Haplotype combinations				
FcγRIIA H – FcγRIIC T – FcγRIIIA F – FcγRIIIB NA2	32	10.80	1.92;60.48	**0.007**
FcγRIIA R – FcγRIIC C – FcγRIIIA F – FcγRIIIB NA2	75	6.75	2.00;22.74	**0.002**
FcγRIIA H – FcγRIIC C – FcγRIIIA F – FcγRIIIB NA2	56	8.76	1.39;55.15	**0.021**

Table 4 presents the logit model obtained through the control variables splenomegaly, and age status (children vs. adults). In bold significant *P*-value at $P \leq 0.05$. The references were respectively FcγRIIA RR – FcγRIIC CC – FcγRIIIA FF – FcγRIIIB NA2NA2 and FcγRIIA H – FcγRIIC T – FcγRIIIA F – FcγRIIIB NA1 for genotype and haplotype combinations.

Table 5
G3m6 – FcγRIIA – FcγRIIC – FcγRIIIA – FcγRIIIB combinations and *Pf* malaria infection.

Pf malaria infection	n	odds ratio	95%CI	P value
G3m6 – FcγRIIA – FcγRIIC – FcγRIIIA – FcγRIIIB combinations				
Genotype combinations				
G3m6 (−/−) – FcγRIIA RR – FcγRIIC CT – FcγRIIIA FF – FcγRIIIB NA2NA2	7	0.07	0.0003;1.78	0.072
Haplotype combinations				
G3m6 (+) – FcγRIIA H – FcγRIIC T – FcγRIIIA F – FcγRIIIB NA2	20	13.01	1.69;99.76	**0.014**
G3m6 (+) – FcγRIIA H – FcγRIIC T – FcγRIIIA F – FcγRIIIB NA1	5	11.12	1.10;111.85	**0.041**

Table 5 presents the logit model obtained through the control variables splenomegaly, and age status (children vs. adults). In bold significant P-value at $P \leq 0.05$. The references were respectively G3m6 **(+/+) –** FcγRIIA RR – FcγRIIC CC – FcγRIIIA FF – FcγRIIIB NA2NA2 and G3m6 **(+) –** FcγRIIA R – FcγRIIC T – FcγRIIIA F – FcγRIIIB NA2 for genotype and haplotype combinations.

between adults and children compared to Bariba and Otamari (Fall et al., 2023). To our knowledge, no study has explored the carriage of FcγRIIIB NA1/NA2 variants among Fulani yet.

We observed a deviation from HWE expectations for FcγRIIC C/T and FcγRIIIB NA1/NA2 in our study group. Regarding FcγRIIIB NA1/NA2, this result is consistent with our previous observations in two other Beninese populations (Fall et al., 2020; Fall et al., 2022b). The HWE deviation observed for *FCGR3B* could be due to either consanguinity, or natural selection with a disease-related evolutionary selection pressure exerted by *P. falciparum* and/or other past infectious diseases occurring in the population. The proportion of Fulani children carrying the FcγRIIC CT genotype was higher compared to other children, and CT has been associated with a reduced malaria risk compared to the carriage of FcγRIIC CC. The T allele of FcγRIIC has also been found to be prevalent in Fulani from Mali and related to low parasite burden (Cherif et al., 2016). FcγRIIC, expressed on NK cells, is an activating IgG receptor capable of inducing ADCC. Differences in receptor expression levels on the cell surface can alter the balance between activating and inhibitory signals and therefore change the cellular response toward IgG (Van der Heijden et al., 2012). From our results, we can hypothesize that the FcγRIIC CT genotype is associated with a better balance between activating and inhibitory cellular signals leading to a better response of IgG through ADCC.

Lastly, we found that the G3m6 (+) – FcγRIIA 131H – FcγRIIC T – FcγRIIIA 176F – FcγRIIIB NA2 polymorphism combination was associated with an increased risk of *Pf* malaria infection before and after MTS. Interestingly, no Fulani carried this haplotype, consistent with their already described greater resistance to malaria infections combined with a high level of opsonizing antibodies to *Pf* merozoites (Fall et al., 2023). In parallel, this combination was more prevalent in young Bariba, Gando and Otamari who were more likely to present malaria infections than adults from the same groups. A higher level of opsonizing antibodies was shown in adults compared to children in these groups (Fall et al., 2023). Among the five individuals with the G3m6 (+) – FcγRIIA 131H – FcγRIIC T – FcγRIIIA 176F – FcγRIIIB NA1 polymorphism combination, also associated with an increased risk, four presented a *Pf* malaria infection after MTS including only one young Fulani. Both of these combinations may be associated with a poorer ingestion of IgG opsonized particles or a poorer ADCC mechanism (Huizinga et al., 1989; Van der Heijden et al., 2012).

In conclusion, these results showed potential involvement of combined G3m6 – FcγR polymorphisms in the natural protection of Fulani against *Pf* malaria infections through a better capacity of opsonizing antibodies. However, it would be interesting to replicate this study on a larger scale and in other countries from a longitudinal study cohort with more data related to symptomatic and asymptomatic *Pf* malaria infections.

Contributions

AS and AG designed and conducted the field study. AKDJF, CD, DC and FMN conceptualized this research. AKDJF carried out the laboratory experiments as well as analyses and wrote the first draft of the manuscript. AKDJF, CD, DC and FMN verified data and carried out critical review of the manuscript. All authors read and approved the submission of the manuscript.

Funding

This research was supported by a grant from Labex GR-Ex and from the National Geographic Society Committee for Research and Exploration (Grant 9804–15). The Labex GR-Ex, reference no. ANR-11-LABX-0051, is funded by the Programme d'Investissements d'Avenir (PIA) of the French National Research Agency, reference no. ANR-11-IDEX-0005-02. The Université Paris Cité awarded a PhD scholarship to AKDJF.

Ethical statement

The BAObAB project was reviewed and approved by the ethics committee of the Research Institute of Applied Biomedical Sciences (CER-ISBA/Institut des Sciences Biomédicales Appliquées) in Benin (no. 61/CER/ISBA/15). All study participants were informed before the

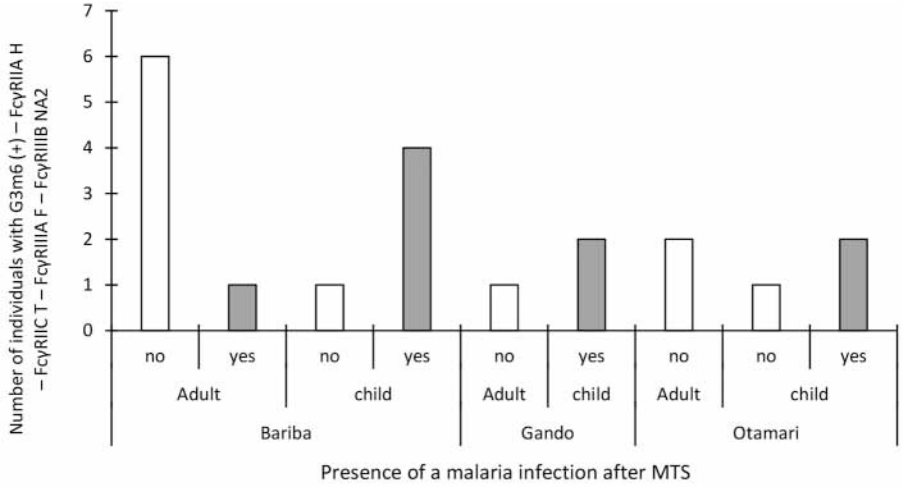

Fig. 6. : Distribution of defined G3m6 – FcγR polymorphisms combinations according to ethnic groups and presence of a malaria infection after MTS. Fig. 6 shows the distribution of the G3m6 (+) – FcγRIIA H – FcγRIIC T – FcγRIIIA F – FcγRIIIB NA2 polymorphism combination according to ethnic groups (Bariba, Gando and Otamari), the age status (adult or child) and the presence or not of a malaria infection after the malaria transmission season (MTS): "yes" in grey color and "no" in white color.

beginning of the study. Individual written informed consent was obtained from adult participants or from the parents or guardians of participating children using a consent form translated into their native language.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

Acknowledgements

The authors are grateful to all the women, men and children who agreed to participate in this project; to field supervisors and community health workers; and to health auxiliaries of the health centers.

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