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Research paper

Association of variants in *IL1B*, *TLR9*, *TREM1*, *IL10RA*, and *CD3G* and Native American ancestry on malaria susceptibility in Colombian populations

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ABSTRACT

Host genetics is an influencing factor in the manifestation of infectious diseases. In this study, the association of mild malaria with 28 variants in 16 genes previously reported in other populations and/or close to ancestry-informative markers (AIMs) selected was evaluated in an admixed 736 Colombian population sample. Additionally, the effect of genetic ancestry on phenotype expression was explored. For this purpose, the ancestral genetic composition of Turbo and El Bagre was determined. A higher Native American ancestry trend was found in the population with lower malaria susceptibility [odds ratio (OR) = 0.416, 95% confidence interval (95% CI) = 0.234–0.740, $P = 0.003$]. Three AIMs presented significant associations with the disease phenotype (MID1752, MID921, and MID1586). The first two were associated with greater malaria susceptibility (D/D, OR = 2.23, 95% CI = 1.06–4.69, $P = 0.032$ and I/D-I/I, OR = 2.14, 95% CI = 1.18–3.87, $P = 0.011$, respectively), and the latter has a protective effect on the appearance of malaria (I/I, OR = 0.18, 95% CI = 0.08–0.40, $P < 0.0001$). After adjustment by age, sex, municipality, and genetic ancestry, genotype association analysis showed evidence of association with malaria susceptibility for variants in or near *IL1B*, *TLR9*, *TREM1*, *IL10RA*, and *CD3G* genes: rs1143629-IL1B (G/A-A/A, OR = 0.41, 95% CI = 0.21–0.78, $P = 0.0051$), rs352139-TLR9 (T/T, OR = 0.28, 95% CI = 0.11–0.72, $P = 0.0053$), rs352140-TLR9 (C/C, OR = 0.41, 95% CI = 0.20–0.87, $P = 0.019$), rs2234237-TREM1 (T/A-A/A, OR = 0.43, 95% CI = 0.23–0.79, $P = 0.0056$), rs4252246-IL10RA (C/A-A/A, OR = 2.11, 95% CI = 1.18–3.75, $P = 0.01$), and rs1561966-CD3G (A/A, OR = 0.20, 95% CI = 0.06–0.69, $P = 0.0058$). The results showed the participation of genes involved in immunological processes and suggested an effect of ancestral genetic composition over the traits analyzed. Compared to the paisa population (Antioquia), Turbo and El Bagre showed a strong decrease in European ancestry and an increase in African and Native American ancestries. Also, a novel association of two single nucleotide polymorphisms with malaria susceptibility was identified in this study.

1. Introduction

Malaria is a parasitic disease with a huge impact on humans (Ashley et al., 2018; White et al., 2014). Transmission occurs in 91 countries, and half of the world's population is at risk of infection. In 2018, 228 million malaria cases and 405,000 deaths from the disease worldwide were reported (WHO, 2019). Human infection is caused by the five species of the genus *Plasmodium* and transmitted by mosquitoes of the genus *Anopheles* (Cohee and Laufer, 2017). Most malaria cases around the world are caused by *Plasmodium falciparum* or *Plasmodium vivax*; however, infections by *Plasmodium ovale* and *Plasmodium malariae* occur. In Southeast Asia, infection by *Plasmodium knowlesi* has been reported (Ashley et al., 2018).

Colombia is the third American country that reported the most cases (10%) after Venezuela (51%) and Brazil (23%). Approximately 20% of the country's population is at risk of acquiring malaria (WHO, 2019). In 2019, there were 78,512 reported cases (INS, 2019): 39,517 (50.3%) by *P. falciparum*, 38,125 (48.6%) by *P. vivax*, and 870 (1.1%) mixed

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infection (*P. falciparum* and *P. vivax*; INS, 2019). Antioquia registered 9.2% of the total reported cases in the country (INS, 2019).

Malaria is a complex disease, as the mosquito vector competence, parasite virulence, and human susceptibility are also complex from the genetic standpoint. This study focuses on human genetic susceptibility to the disease. Imprints of malaria on human evolution and its genome are well known. Disorders such as sickle cell anemia, thalassemia, glucose 6-phosphate dehydrogenase deficiency, and other erythrocytic deficiencies have remained to affect human populations despite deleterious effects (Hedrick, 2011; Kwiatkowski, 2005). However, genetic markers are not enough to explain the population's differential susceptibility. In Mali, the Fulani and Dogon ethnic groups, although living in close proximity, are isolated by their culture, showing substantial differences in malaria susceptibility (Dolo et al., 2005; Toure et al., 2012). Thus, the genetic structure of these populations supports the differences in human responses to the disease (Cherif et al., 2016; Maiga et al., 2013).

Genetic structure (or differentiation) implies differences in allele frequencies among human populations, leading to different combinations of gene variants of the response to infection as well as disease development (Campino et al., 2006; Hedrick, 2011). Thus, variation in malaria susceptibility depends mainly on the genetic makeup of the population (Klebanov, 2018; Verra et al., 2009). Studies on complex genetic traits must explore the structure and genetic composition of the studied population, as they can become important confounding factors that can lead to false associations during statistical analyses (Balding, 2006; Cardon and Palmer, 2003). The ancestral genetic composition of Latin American populations (e.g., Colombia and Brazil) has been shaped by recent complex processes of admixture among European, Native American, and African populations (Bedoya et al., 2006; Rojas et al., 2010; Vergara et al., 2013; Wang et al., 2008).

This study aimed to estimate the effect of the ancestral category (European, Native American, and African) and candidate genes on malaria in a cohort of individuals living in an endemic area for malaria. The effect of genes on malaria susceptibility and their relationship with environmental factors was analyzed. The potential associations of genes that might contribute to the malaria phenotype in the study area were also examined.

2. Materials and methods

2.1. Ethics statement

This research was approved by the Bioethics Committee of Universidad de Antioquia, Medellín, Colombia (Record 011 dated 28 July 2016). Verbal and written explanations were provided. Informed consent was obtained from all participants or their legal guardians before specimen collection following the principles of the Declaration of Helsinki. All DNA samples were anonymized.

2.2. Study area, population, and sample

A cross-sectional case-control study was conducted from November 2016 to June 2017, with 736 individuals from Turbo (n=351) and El Bagre (n=385) municipalities from Antioquia Department, Colombia.

Turbo (8°05′35″N, 76°43′42″W) is located in the Urabá region (Fig. 1), with an altitude of 2 m above sea level and an average temperature of 28°C (Alcaldía de Turbo - Antioquia, 2020). El Bagre (7°36′17′″N, 74°48′31″W) is located in the Bajo Cauca region (Fig. 1), with an altitude of 50 m above sea level and an average temperature of 37°C (Alcaldía de El Bagre - Antioquia, 2020). In 2018, the annual parasite index (parasite incidence per 1000 inhabitants) was 1.42 in Turbo and 26.4 in El Bagre. *P. vivax* was the predominant species in both municipalities (https://www.dssa.gov.co/index.php/estadisticas/eventos-en-salud-publica).

A total of 122 patients with febrile illness and any malaria parasite diagnosed by thick blood smear examination and confirmed by polymerase chain reaction (PCR) were included in the study. A total of 115 healthy individuals without self-reported malaria history and who tested negative by blood smear and confirmed by PCR for *Plasmodium* spp., at least three times tested negative by blood smear for parasites at follow-up (6 months) and with a residence time in the study area of at least 5 years, were included. Individuals who did not meet the inclusion criteria for malaria were considered in the population ancestry analysis (Fig. 2).

Thick smear was prepared by Field's staining and examined by a certified expert in 200 microscope fields at 100× (INS, 2015). Two independent microscopists read each slide, and noncongruent readings were resolved by a third reader. Species infection was also confirmed by PCR according to published protocols (Singh et al., 1999). Symptomatic

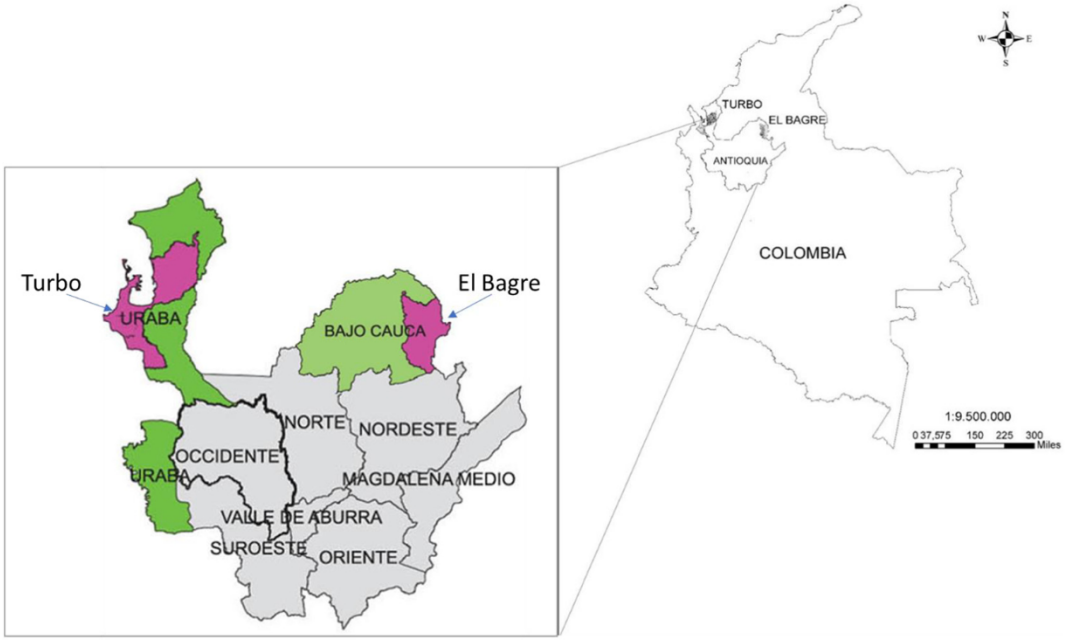

Fig. 1. Study sites.

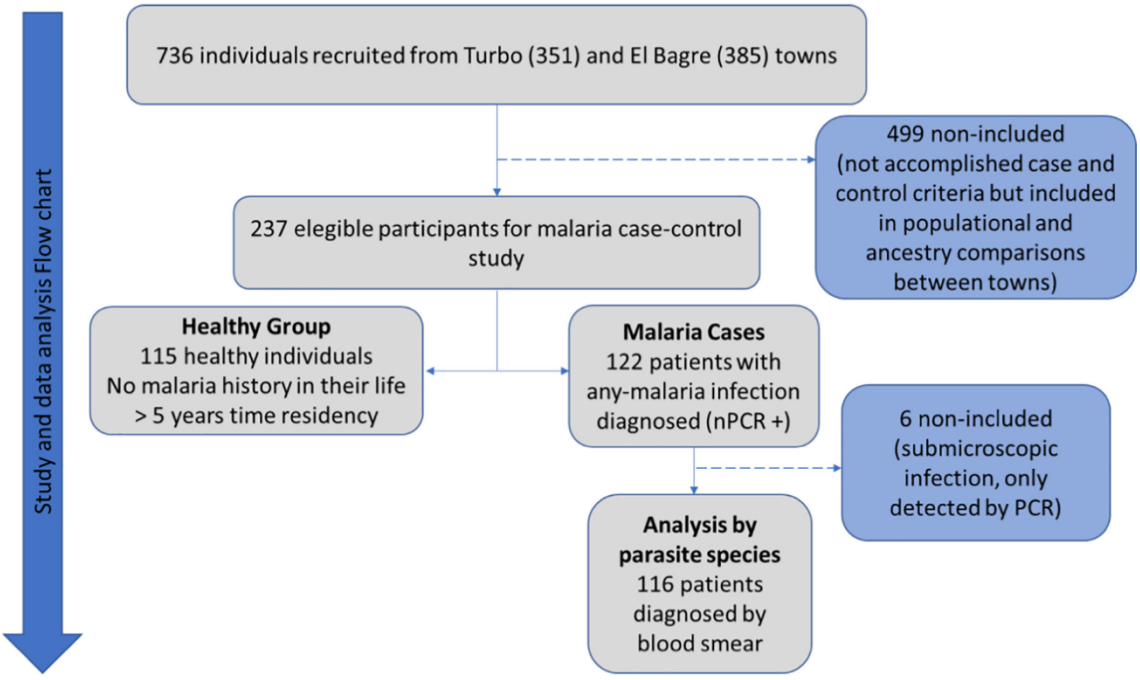

Fig. 2. Study and data analysis flow chart.

cases were diagnosed at the local health post and provided with antimalarial treatments according to national guidelines (INS, 2015).

2.3. Marker selection and genotyping

Peripheral blood samples were collected in 4 ml tubes with EDTA for DNA extraction from leukocytes by the phenol-chloroform method (Sambrook and Russell, 2006) or the salting-out protocol (Miller et al., 1988). European, Native American, and African ancestries were estimated using a panel of 30 autosomal ancestry-informative markers (AIMs; Table S1). These markers were selected, based on their discrimination power ($\delta > 45\%$) among parental populations, from Latino population panels reported by Parra et al. (2004, 1998), Shriver et al. (2003), and Molokhia et al. (2003) as well as from the Marshfield Diallelic Insertion/Deletion Polymorphisms database (Weber et al., 2002).

AIM genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism, capillary electrophoresis on an ABI-PRISM 310 genetic analyzer (Perkin-Elmer/Applied Biosystems, Waltham, MA, USA), and direct genotyping in agarose gel electrophoresis using previously described protocols (Parra et al., 1998; Shriver et al., 2003; Vergara et al., 2009). Genotype readings were performed by two independent researchers. The genotyping process was repeated in cases without a clear genotype or if there was no consensus in the reading.

Linkage disequilibrium (LD) was previously observed to persist in Latin American populations (Carvajal-Carmona et al., 2003; Hoggart et al., 2003; Parra et al., 1998; Service et al., 2006, 2001; Bedoya et al., 2006; Rojas et al., 2010). In this work, the selection of genetic variants has two criteria: (i) Variants of the immune response located 3 cM upstream and downstream of AIMs (Table S2). Tools such as Genomic View (dbSNP) and Genome Browser (UCSC and 1000Genomes) were used to determine distances (Altshuler et al., 2012; Kuhn et al., 2013; Sherry et al., 1999). (ii) Variants not related to AIMs with a previous association to malaria susceptibility in Africa and Brazil (TREM1, TREM2, IL18, IL10RA, PROCR, and EPB41L1); other variants of the immune response associated with response to parasites not previously reported, such as CD3 variants (Stelzer et al., 2016); and variants reported in genome-wide and other association studies (IL1B, TLR9, IL3, IL4, TNF, and STAT6; Table S2). All the gene variants had a MAF higher than 1% in the population CLM (1000Genomes database). Twenty-eight single nucleotide polymorphisms (SNPs) were genotyped using the high-throughput MassARRAY genotyping platform (Sequenom, Inc., San Diego, CA, USA) at the University of Minnesota Genomics Center (Minneapolis, MN, USA). Uniquely located negative controls were routinely included in each plate. These wells were used as controls for genotyping assays, and their unique locations served as a fingerprint to identify the plate and its orientation.

2.4. Genetic ancestry estimation

Proportions were estimated by modeling the sample with the prior distribution of allele frequencies of AIMs in parental populations: African (Yoruba in Ibadan, Nigeria), European (Utah residents with northern and western European ancestry, CEPH), and Native American (Han Chinese in Beijing, Maya, and Pima from Mexico); no dispersion of allele frequencies was considered to get a posterior distribution of the ancestral contribution in each sample (Hoggart et al., 2003). Individual and average ancestry proportions were estimated using the software ADMIXMAP for Windows (McKeigue et al., 2000). Ancestry estimates were recorded as percentages, with the three ancestry components adding up to 100% for any participant.

2.5. Genetic and statistical analyses

Allele frequencies, genotype frequencies, and Hardy-Weinberg equilibrium (HWE) were calculated using GENEPOP (Rousset, 2008). Ancestry estimation and association analyses were applied for markers with a call rate of >95% and without significant deviations from HWE after Bonferroni correction and a minimum allele frequency of >1%.

Comparisons between groups (towns and cases/controls) were estimated using the Mann-Whitney U test for continuous variables and χ^2 test for categorical variables. Frequencies were expressed as numbers, qualitative variables were expressed as percentages, and quantitative variables were expressed as medians followed by the interquartile range (IQR).

The effect of genetic ancestry on malaria status (cases/controls) was estimated by the individual genetic ancestry and used as a covariate in the genetic association analysis to control the effect of admixture stratification. Variables (age, sex, and town) in the models were based on the statistical definition and/or by association with every variable and with an outcome at $P \leq 0.05$.

The effect of every ancestral genetic component (as a categorical variable based on the 50th percentile of its distribution) on malaria status adjusted by covariates was assessed by binary logistic regression.

The genetic association of AIMs and candidate variants was performed using the "SNPassoc" R package (González et al., 2007). For association analysis of binary traits (malaria status), variables such as age, sex, town, and genetic admixture were included in regression models as covariates.

Using a two-sided significance threshold (α level) of 5% and a power of 80%, the required sample size for the European ancestry to be associated with clinical malaria outcome is 200 cases with a 1:1 case/control ratio, assuming an odds ratio (OR) of 1.5 and a proportion of the European ancestry in controls according to the previously reported population samples from Peque municipality (Rojas et al., 2010; Wang et al., 2008). This calculation was performed using Power Analysis in Multi-ancestry Admixture Mapping (or PAMAM), a free web tool developed to calculate power and sample size in admixed population studies (Gautam et al., 2019; https://research.cchmc.org/mershalab/PAMAM/login.html).

Mann-Whitney *U* test, χ^2 test, and logistic regression models were performed using Prism 7 (GraphPad Software, San Diego, CA, USA) and SPSS 18.0 (SPSS, Inc., Chicago, IL, USA). Other analyses were conducted using the R statistical package 3.5.0 (http://www.r-project.org; R Core Team, 2018). *P* values were two-tailed, and significance was defined as $P < 0.05$ unless otherwise indicated.

3. Results

3.1. Characteristics and sample description

There were 736 volunteers enrolled in the study: 351 from Turbo and 385 from El Bagre (Fig. 1). After excluding the participants who did not fulfill the inclusion criteria, a total of 237 individuals (122 cases and 115 controls) were analyzed (Fig. 2). The demographic data by malaria status are shown in Table 1. None of the quantitative variables presented a normal distribution. No differences were found between residence time and proportions of individuals by origin. Differences were found in age and sex ratios between cases and controls (Table 1). These characteristics were used as confounding variables for association analyses. The percentages of infection by species in cases (Table 1) were consistent with previous reports of the epidemiological surveillance system for the Antioquia Department (DSSA, 2019).

Table 1
Characteristics of the study population by case-control status

		Cases	Controls	Total	*P*[a]
Sample individuals		n = 122	n = 115	237	
Age (years)		21 [13–31]	12 [8–19]		**<0.0001**
Sex	Male	75 (61.5)	50 (43.5)	125 (52.7)	**0.006**
	Female	47 (38.5)	65 (56.5)	112 (47.3)	
Residence time (years)		9 [3–17]	9 [6–14]		0.0722
Town	El Bagre	63 (51.6)	53 (46.1)	116 (48.9)	0.393
	Turbo	59 (48.4)	62 (53.9)	121 (51.1)	
Infection by *Plasmodium* species	*P. vivax*	85 (73.3)	—		
	P. falciparum	29 (25.0)	—		
	Mixed	2 (1.7)	—		
Parasitemia total		6080 [2805–9840]	—		

Data are expressed as n (%) or median [IQR].
[a] Mann-Whitney *U* test for quantitative variables and χ^2 test for qualitative variables.

3.2. AIMs and estimates of admixture proportions

A high genotype rate of >99.5% for AIM was obtained (21,980 of 22,080 genotypes). The HWE for every AIM for the whole population was determined. No deviations from HWE were observed in the population groups for AIMs after Bonferroni correction for multiple comparisons. The supplemental materials show the allele frequencies and HWE for AIMs from the sample populations (El Bagre vs. Turbo and cases vs. controls; Tables S3 and S4).

The individual admixture estimates for each parental population in the total sample are shown in the supplemental materials (Fig. S1). For the total population, the estimated percentage for individual admixture showed high variability among individuals: 0.1 to 0.505 for the African ancestry, 0.157 to 0.529 for the European ancestry, and 0.228 to 0.71 for the Native American ancestry. For the case-control cohort, the estimated percentage did not show differences to those of the total population.

The estimated average proportions of genetic ancestry for the combined, Turbo, and El Bagre populations are shown in Fig. 3 and Table 2. The total sample showed a predominant contribution of the Native American ancestry (46.9%) followed by the European ancestry (29.5%) and a lower proportion of the African ancestry (21%). In the comparison of each component among populations, significant differences were found in the average proportions of the Native American and European ancestries, the first highest in the Turbo population (49.3% vs. 45.8%, $P = 0.0005$) and the second highest in the El Bagre population (30.7% vs. 28.2%, $P < 0.0001$). No difference was observed in the average proportions of the African ancestry among these populations (Table 2). Due to this structure, the municipality was also considered as a covariate.

Ancestry proportions for the three ancestral components differed significantly among cases and controls (Table 3; Fig. 4). The average Native American ancestry was significantly higher in controls than in cases (50.3% vs. 43.1%, $P < 0.0001$). In contrast, the average European and African ancestries were higher in cases than in controls (30.2% vs. 27.7% and 21.5% vs. 19.8%, $P = 0.006$ and 0.0279, respectively).

3.3. Effect of the human genetic component on malaria

These results showed a possible effect of ancestry on malaria outcomes (Table 3). To detect the magnitude of the association, logistic regression models were carried out as described using the previously mentioned confounding variables (Table 1).

Individuals with the Native American ancestry above 46.9% (the median value in Table 3) showed lower malaria susceptibility [Table 4; OR = 0.416, 95% confidence interval (95% CI) = 0.234–0.74, $P = 0.003$]. Although none of the other genetic ancestral components had a significant effect on malaria status, the European ancestry showed a strong tendency toward greater susceptibility to disease ($P = 0.05$). In contrast, the African ancestry oscillates between risk and protection (Table 4). The previous trend was maintained even when the analysis was carried out for each municipality, but only El Bagre showed a significant association (Table S5). The analysis discriminated by parasite species showed the same trend (Table 5). The Native American ancestry had a possible protective effect on infection by any type of *Plasmodium* ($P < 0.05$), the European ancestry conferred susceptibility to infection by any species ($P > 0.05$), and the African ancestry behaved as a possible risk factor for *P. falciparum* ($P < 0.05$) but conferred a protective effect for *P. vivax* ($P > 0.05$).

A high genotype rate of >99% for SNP candidate variant genotypes was obtained (6597 of 6636 genotypes). All SNPs were at HWE in both

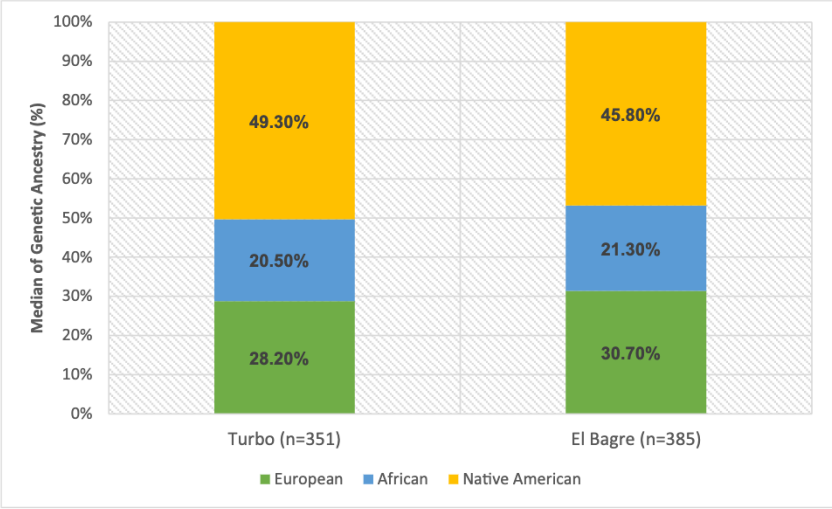

Fig. 3. Median of genetic ancestry percentages by town.

Table 2
Estimated percentages of genetic ancestry by town

	Total (n = 736)	El Bagre (n = 385)	Turbo (n = 351)	P^a
	Ancestry, median [IQR]			
European (%)	29.5 [25.4–34.3]	30.7 [26.5–35.4]	28.2 [24.5–33.1]	**<0.0001**
African (%)	21.0 [16.7–26.4]	21.3 [17.1–26.1]	20.5 [16.1–26.9]	0.2338
Native American (%)	46.9 [39.8–54.8]	45.8 [39.5–53.1]	49.3 [40.3–56.8]	**0.0005**

P values in **boldface** are significant at <0.05.
[a] *P* for Mann-Whitney *U* test.

Table 3
Estimated admixture proportions by case-control status

	Total (n = 237)	Cases (n = 122)	Controls (n = 115)	P^a
	Ancestry, median [IQR]			
European (%)	29.4 [24.4–33.8]	30.2 [25.4–37.2]	27.7 [23.9–32.2]	**0.0060**
African (%)	20.5 [15.8–25.6]	21.5 [16.6–27.4]	19.8 [14.9–24.9]	**0.0279**
Native American (%)	46.9 [40.5–56.2]	43.1 [38.1–53.1]	50.3 [43.3–58.4]	**<0.0001**

P values in **boldface** are significant at <0.05.
[a] *P* for Mann-Whitney *U* test.

groups after Bonferroni correction, but only rs3024944 in STAT6 had MAF < 1% and was excluded from the analysis (Table S6).

Genotype associations of each AIM and SNP on malaria phenotype (response variable) were performed using logistic regression analysis without adjustment and adjusting for the municipality, age, sex, and ancestral genetic composition as confounding variables. Table 6 shows the significant genotype associations of the markers with the phenotype, where the effect of the genotype model is estimated using OR. Only markers that persisted after adjustment with the genetic ancestry estimates and the confounding variables are shown.

Three AIMs presented significant associations with the disease phenotype: MID1752, MID921, and MID1586. The first two AIMs were associated with greater malaria susceptibility (D/D, recessive model, OR = 2.23, 95% CI = 1.06–4.69, $P = 0.032$ and I/D-I/I, dominant model, OR = 2.14, 95% CI = 1.18–3.87, $P = 0.011$, respectively). The last one had a protective effect on the appearance of malaria (I/I, recessive model, OR = 0.18, 95% CI = 0.08–0.40, $P < 0.0001$; Table 6).

After adjustment by age, sex, municipality, and genetic ancestry, genotype association analysis showed evidence of association with malaria susceptibility for variants in or near *IL1B*, *TLR9*, *TREM1*, *IL10RA*, and *CD3G* genes (Table 6). There were six significant results for individuals with malaria versus the no-malaria group: rs1143629-IL1B (G/A-A/A, dominant model, OR = 0.41, 95% CI = 0.21–0.78, $P = 0.0051$), rs352139-TLR9 (T/T, recessive model, OR = 0.28, 95% CI = 0.11–0.72, $P = 0.0053$), rs352140-TLR9 (C/C, recessive model, OR = 0.41, 95% CI = 0.20–0.87, $P = 0.019$), rs2234237-TREM1 (T/A-A/A, dominant model, OR = 0.43, 95% CI = 0.23–0.79, $P = 0.0056$), rs4252246-IL10RA (C/A-A/A, dominant model, OR = 2.11, 95% CI = 1.18–3.75, $P = 0.01$), and rs1561966-CD3G (A/A, recessive model, OR = 0.20, 95% CI = 0.06–0.69, $P = 0.0058$).

4. Discussion

The association of mild malaria with 28 variants in 16 genes previously reported in other populations and/or close to AIMs selected was evaluated in an admixed Colombian population sample. The effect of ancestral genetic composition on phenotype expression was also explored. The results showed the participation of genes involved in diverse physiological processes and suggested an effect of ancestral genetic composition over the traits analyzed.

4.1. Ancestry analysis of sample populations

Although there were observed differences in the percentages of ancestral components, with a greater contribution of the Native American ancestry for Turbo and a greater proportion of the European ancestry for El Bagre, the relative proportions of the admixture in these two populations were similar (Table 2; Fig. 3). The predominance of the ancestral components in descending order is Native American ancestry (47%) followed by European ancestry (30%) and African ancestry (21%). This agreed with the evaluation of the overall genetic divergence (Weir and Cockerham, 1984) between these two populations where there was no difference between them at a general level (Table S7), which did not preclude the possibility of the underlying structure (Sethuraman, 2013). This result was supported by previous studies regarding ecology, geography, epidemiology, and population migration processes in the Urabá and Bajo Cauca regions (Carmona-Fonseca, 2017;

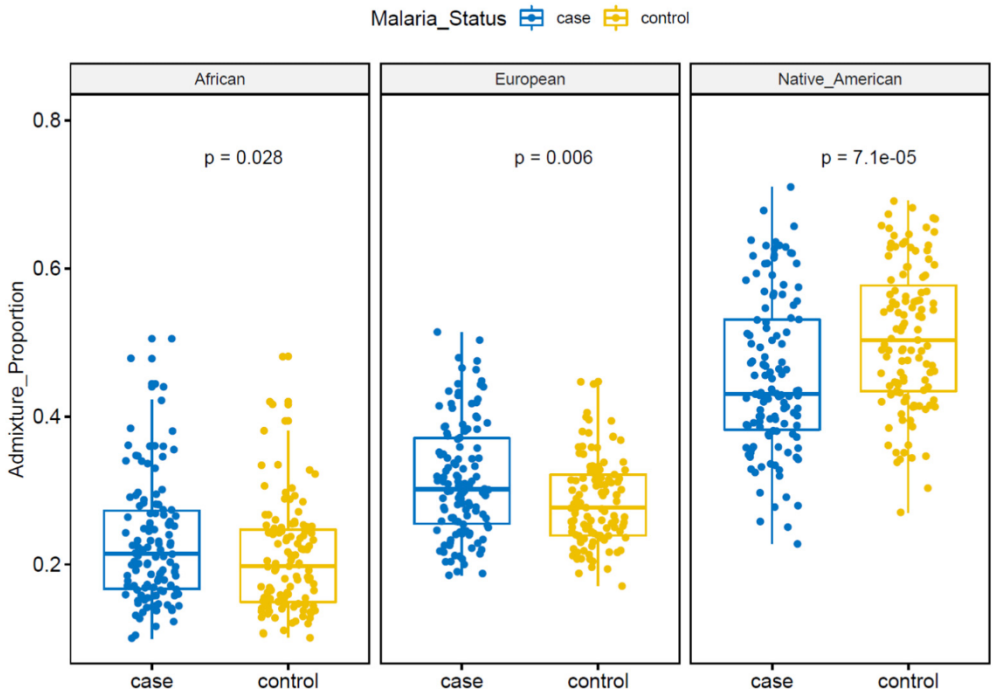

Fig. 4. Distribution and comparison of genetic ancestries in cases and controls. The *P* of the Mann-Whitney *U* test to compare medians between the two groups is shown.

Table 4Data in strikethrough have been retained. Please check if this is appropriate
Association analysis of genetic ancestry and malaria status

Ancestry	Category[a]	OR (95% CI)[b]	*P*
European	Low	1.00	—
	High	1.719 (1.001–2.953)	0.050
African	Low	1.00	—
	High	1.227 (0.704–2.139)	0.470
Native American	Low	1.00	—
	High	0.416 (0.234–0.740)	**0.003**

P values in **boldface** are significant at <0.05.
[a] Each category (high/low) of the ancestral genetic component is defined with respect to the 50th percentile of its distribution.
[b] Adjusted by town, age, and sex.

Carmona-Fonseca, 2004; Gómez Cardona et al., 2008).

The percentages of ancestry estimated in this work were different from previous studies on Antioquia populations, specifically Medellín in the Valle de Aburrá region (at the center of Antioquia; Fig. 1), where components with the greatest contribution to the admixture of these individuals were the European and Native American ancestries. Unlike this work, those studies reported at least two times greater contribution of the European ancestry than the Native American ancestry. The African ancestry had the lowest contribution in the admixture (Bedoya et al., 2006; Carvajal-Carmona et al., 2003; Carvajal-Carmona et al., 2000; Price et al., 2007; Rishishwar et al., 2015; Rojas et al., 2010; Wang et al., 2008). Peque, another Antioquia population located in the Occidente region (to the central west of the department; Fig. 1), closer to the Turbo and Urabá regions than Valle de Aburrá, presented proportions of ancestry more similar to this study's sample. Unlike that of Medellín, Peque's Native American contribution doubled the European ancestry, and the African ancestry again had the lowest percentage in the admixture (Rojas et al., 2010; Wang et al., 2008).

These differences can be attributed to the different demographic patterns of each population, such as the density of the Native American population at the time the colonizers arrived and the number of Europeans and Africans who migrated to each region (Adhikari et al., 2017; Wang et al., 2008). Specifically, it can be attributed to the greater availability of matings with the native indigenous population during the first few generations after its settlement by White individuals (Adhikari et al., 2016; Jordan et al., 2019; Ruiz-Linarés, 2015). Another possible explanation is the high gene flow of Urabá and Bajo Cauca (Gómez Cardona et al., 2008) and the high density of indigenous reserves, where Zenú, Emberá-Chamí, and Emberá-Katío ethnic groups predominate (Gerencia Indígena, 2016). In El Bagre and Turbo, with the first known

Table 5
Association analysis of genetic ancestry and malaria by *P. falciparum* and *P. vivax*

Ancestry	Category[a]	*P. falciparum*		*P. vivax*	
		OR (95% CI)[b]	*P*	OR (95% CI)[b]	*P*
European	Low	1.00	—	1.00	—
	High	1.948 (0.772–4.917)	0.158	1.722 (0.957–3.097)	0.070
African	Low	1.00	—	1.00	—
	High	5.150 (1.72–15.418)	**0.003**	0.842 (0.453–1.563)	0.585
Native American	Low	1.00	—	1.00	—
	High	0.177 (0.060–0.522)	**0.002**	0.447 (0.236–0.845)	**0.013**

P values in **boldface** are significant at <0.05.
[a] Each category (high/low) of the ancestral genetic component is defined with respect to the 50th percentile of its distribution.
[b] Adjusted by town, age, and sex.

Table 6
Significant genotype associations with malaria outcome

Gene/marker	Variant ID	Model[a]	Genotype	Controls, n (%)	Cases, n (%)	OR[b] (95% CI)	*P*
MID1752	rs2307948	Recessive	I/I-I/D	94 (83.9)	91 (77.1)	1.00	—
			D/D	18 (16.1)	27 (22.9)	2.23 (1.06–4.69)	0.032
MID921	rs1611004	Dominant	D/D	70 (62.5)	54 (45.8)	1.00	—
			I/D-I/I	42 (37.5)	64 (54.2)	2.14 (1.18–3.87)	0.011
MID1586	rs2307782	Recessive	D/D-I/D	75 (67)	101 (85.6)	1.00	—
			I/I	37 (33)	17 (14.4)	0.18 (0.08–0.40)	<0.0001
IL1B	rs1143629	Dominant	G/G	49 (43)	58 (48.3)	1.00	—
			G/A-A/A	65 (57)	62 (51.7)	0.41 (0.21–0.78)	0.0051
TLR9	rs352139	Recessive	C/C-T/C	93 (82.3)	109 (93.2)	1.00	—
			T/T	20 (17.7)	8 (6.8)	0.28 (0.11–0.72)	0.0053
TLR9	rs352140	Recessive	T/T-C/T	87 (77)	105 (86.8)	1.00	—
			C/C	26 (23)	16 (13.2)	0.41 (0.20–0.87)	0.019
TREM1	rs2234237	Dominant	T/T	64 (56.1)	93 (76.9)	1.00	—
			T/A-A/A	50 (43.9)	28 (23.1)	0.43 (0.23–0.79)	0.0056
IL10RA	rs4252246	Dominant	C/C	63 (55.3)	52 (43)	1.00	—
			C/A-A/A	51 (44.7)	69 (57)	2.11 (1.18–3.75)	0.01
CD3G	rs1561966	Recessive	G/G-A/G	101 (88.6)	117 (96.7)	1.00	—
			A/A	13 (11.4)	4 (3.3)	0.20 (0.06–0.69)	0.0058

[a] Best genetic model according to the Akaike information criterion.
[b] Adjusted by town, age, sex, and genetic admixture.

settlements dating back to 1675 and 1840, respectively, the main driver of their population dynamics is the migration of populations from the interior of Antioquia to the lowlands, which is due to processes that occurred in the twentieth century, associated with the origin of economic interests in the region — the creation of extended banana cultivation in Urabá and the consolidation of the cattle ranch, mining, and timber activities in Bajo Cauca (Alcaldía de El Bagre - Antioquia, 2020; Alcaldía de Turbo - Antioquia, 2020; Ocampo, 1988).

Other Colombian populations with greater reported Native American ancestry than European ancestry are Bolívar, Peque, Norte de Santander, Casanare, Nariño, Cauca, and Huila. A high Native American ancestry (60%) was observed in individuals from Casanare, Peque, Nariño, Cauca, and Cundinamarca (Rojas et al., 2010). These, together with this study's results, confirmed that there was an extensive geographic population structure in Colombia, with populations from different regions in the country having very distinct ancestry profiles (Adhikari et al., 2017; Ossa et al., 2016; Rojas et al., 2010; Wang et al., 2008), even within its administrative divisions, such as Antioquia (Bedoya et al., 2006; Carvajal-Carmona et al., 2003; Chacón-Duque et al., 2014; Rojas et al., 2010). Therefore, the sample of the Medellín population (CLM) from the 1000Genomes database (Altshuler et al., 2012) cannot be taken as representative of the country but only as one of its subpopulations (Caicedo et al., 2019; Medina Rivas et al., 2016). Accordingly, an analysis of more populations within and between the country's regions is required for a further understanding of the relationship between genetic ancestry and health determinants in Colombia (Chacón-Duque et al., 2014; Chande et al., 2017; Conley et al., 2017; Nagar et al., 2019).

4.2. Ancestry analysis for the case-control cohort

Differences in ancestral genetic makeup were observed between cases and controls (Table 3). Native American ancestry was significantly higher in controls than in cases, suggesting a protective effect from the disease. The magnitude of this association was measured, indicating that individuals with no malaria history had higher Native American ancestry than those with malaria phenotype (Table 4; OR = 0.416, 95% CI = 0.234–0.740, $P = 0.003$), suggesting a decrease in the risk of disease onset by 58% in individuals with a percentage of Native American ancestry greater than the median of the observed distribution. Considering the municipality, age, and sex, an increase in the sample size would be useful to validate this finding. This trend remained when each municipality was analyzed (Table S5).

In European and African ancestries (Table 4), no effect was detected despite the observed differences between the phenotype categories (Table 3). When these analyses were done without categorizing ancestry (Table S8), the proportion of European obtained a significant risk association ($P = 0.003$), but with a wide range in 95% CI, which may be due to the small number of individuals in the contingency table to make comparisons. To confirm the possible effect of this component, it is necessary to increase the sample size. This trend remained when each municipality was analyzed and the ancestry was categorized (Table S5). In the African percentage, there was no association in the uncategorized analysis after adjustment for covariates (Table S8), although it was slightly greater in cases than in controls. This trend toward a possible risk effect remained after analyzing each municipality without categorizing the ancestry (Tables S9 and S10). Just as the European component presented very wide 95% CIs, this uncertainty was most likely due to the sample size. It is the genetic component with the lowest contribution in the admixture of the studied sample.

The ancestral genetic makeup also appeared to confer infection susceptibility depending on the parasite species (Table 5). In this study, the European component increase was associated with a single infection by *P. falciparum* or *P. vivax*, the high percentage of African component increase, susceptibility to infection by *P. falciparum*, and decrease by *P. vivax*. The Native American contribution conferred a protective effect on *P. falciparum* infection and, to a lesser extent, on *P. vivax* infection. The results for the European and African ancestries were consistent with previous reports (Battle et al., 2015; Gething et al., 2012; Hay et al., 2009).

Regarding the signals found for Native American component, it was documented that when *P. falciparum* arrived in America it had to adapt to new hosts, both humans and mosquitoes. The low genetic diversity in these parasites is due to a reduction in the population and the selection exerted mainly by the immune system of New World mosquitoes (Molina-Cruz and Barillas-Mury, 2014), affecting the virulence and morbidity of these parasites with respect to those in Africa. However, more recently, the widespread use of the antimalarial drug chloroquine may also have greatly reduced the genetic diversity of *P. falciparum* and its fitness and relation to the immune response of the human host (Wootton et al., 2002). The arrival of *P. vivax* in America or its exposure to Native American populations might have occurred before the colonial period, so there would have been a history of adaptation (Rodrigues et al., 2018; Wiscovitch-Russo et al., 2019).

Fossil evidence indicated that Anophelinae could have originated (~100–110 Mya) in Gondwana and colonized the interconnected lands before the continents separated (Poinar et al., 2019). It also established a minimum age for the genus *Plasmodium* and placed avian malaria in the Americas by the mid-Tertiary. With the presence of *Plasmodium*

dominicana (Poinar, 2005a), the first fossil record of *Plasmodium* malaria, it was demonstrated that this genus was established in the New World at least 15 million years ago (Poinar, 2005b), supporting the premise that *Plasmodium* malaria was carried from birds to mammals (including simians and hominoids) and eventually to humans when they arrived in South America (Poinar, 2016). It was specifically proposed that *P. dominicana* could have evolved into *Plasmodium brasilianum*, a simian malaria that does not occur anywhere else in the world and is closely related to the human parasite *P. malariae*, which infects both simians and humans (Huffman and Chapman, 2009; Poinar, 2016).

The observed malaria susceptibility in the Native American ancestry agreed with previous observations (Poinar, 2016; Rodrigues et al., 2018; Wiscovitch-Russo et al., 2019). Another plausible and non-discordant hypothesis suggested that the current Native American ancestry showed an emergent response due to the strong selection by the parasites, with an observed trend to protection (Deng et al., 2016; O'Fallon and Fehren-Schmitz, 2011).

4.3. Host genetics to malaria response

This work supported the idea that some human genes and variants have evolved due to malaria (Hedrick, 2011; Kwiatkowski, 2005), similar to chromosomal fragments in LD inherited from the parental to recent origin populations. Upon contact, pressured genes may have modulated the interaction between the parasite and the host, which depends on the intensity of transmission and the exposure time. These may or may not make the parasite thrive in blood tissue. Therefore, the host's response according to its genetic architecture will depend on whether there is a manifestation of clinical symptoms and the appearance of the disease (Marquet, 2018; Rishishwar et al., 2015). In other words, host genetics plays two roles in the presentation of disease: (1) not to establish the infection and (2) to modulate the parasite response, which can lead to outcomes ranging from asymptomatic infection to severe forms of the disease.

A clear example of the aforementioned is shown in a recent study in Juquitiba (southeastern Brazil), which reported that the European genetic component in the studied population does not prevent plasmodial infection. However, as its contribution to the genome increases, it affects the distribution of polymorphisms in Toll-like receptors (TLRs), increasing the probability of carrying a haplotype that is associated with a decreased response of the immune system to infection, which explains the high frequency of asymptomatic infection in the study population (Guimarães et al., 2018a). Although in that study the genetics of the circulating parasite in the region was not considered, it also played a role in the severity of the disease presentation. These same authors then used the results of the admixture in this Brazilian population to correct the statistical analyses of a study of association to candidate genes, where variants in the TLR genes were associated with malaria susceptibility (Guimarães et al., 2018b). This highlighted the importance of knowing the ancestral genetic composition of populations and using AIMs in association studies to avoid obtaining biased results.

In another study, local ancestry patterns were evaluated to search for regions in the genome with higher percentages of one of the three ancestral components that contributed to the recent admixture of the Colombian population (Rishishwar et al., 2015). Genes and pathways related to the adaptive and innate immune system were particularly over-represented in segments with high percentages of specific parental ancestry, including genes (*CD226*, *HLAB*, *MICA*, and *MAPK10*) that were previously characterized as the object of positive selection, and these were involved in defense against endemic pathogens, such as malaria (Rishishwar et al., 2015). This same group in a subsequent study associated various immune system pathways with SNPs with a high percentage of African or European ancestry, including cytokine receptor interaction pathways, T-cell receptor (TCR) signaling, and pathways of presentation and processing of antigens (Norris et al., 2018). Specifically, they proposed that the higher percentage of African alleles for the *HLAB* locus may have contributed in the Colombian population a more effective defense against malaria and/or other pathogens (Rishishwar et al., 2015). *MAPK10*, another gene related to immunity, was found in a segment with a high percentage of Asian ancestry in the Colombian population (Rishishwar et al., 2015) and whose alleles have shown signs of selection for resistance to malaria in Malaysia (Liu et al., 2015). This gene is in the region of the expected LD of the MID1586 marker, which would explain why this AIM was associated with the phenotype in this study (and outside of HWE in controls), probably due to a selective sweep by *MAPK10*.

Significant associations regarding AIMs used in this study were found (Table 6). These were considered true (remained after adjustment by each component of the admixture) but indirect associations with the studied phenotype. MID1752 (rs2307948) D/D genotype, associated with a risk of malaria, is an intron variant in DHCR24 located at 1p32.3. The most probable origin for the D allele in the sample is the European population. The MID921 (rs1611004) marker with insertion (I) under a dominant model is associated with the risk of the disease. This variant is in the intergenic region (LINC01266) at 3p26.3. The African origin is the most probable origin for the I allele. The MID1586 (rs2307782) I/I genotype, associated with a protective effect against malaria, is an intronic variant in AFF1 located at 4q21.3-q22.1. The most probable origin of the I allele is Africa (Table S1). The above-mentioned associations suggest that the genetic component that affects the variability in malaria susceptibility is partially composed of gene variants at surrounding regions of the associated AIMs. Considering the knowledge on the persistent LD in Latin American populations (Service et al., 2006; Bedoya et al., 2006; Rojas et al., 2010; Smith and O'Brien, 2005), further work is required to explore adjacent regions in the associated AIMs in this study to search for genes physically linked to the AIMs and that could explain the genetic etiology of the disease in populations such as the one studied through its subsequent evaluation in studies of candidate genes. Some genes in these regions were *MAPK10* (close to MID1586), as discussed above, and *IL5RA* (close to MID921), interleukin (IL)-5 receptor subunit α, which is involved in biological processes such as mitogen-activated protein kinase cascade, inflammatory response to an antigenic stimulus, signal transduction, and cytokine-mediated signaling pathway, among others (Stelzer et al., 2016).

This study has given an account of the association between malaria and the variants in SNPs rs1143629 for *IL1B*, rs352139, and rs325140 for *TLR9*, rs2234237 for *TREM1*, and two SNPs of novel association with malaria, rs4252246 for *IL10RA* and rs1561966 for *CD3G* (Table 6). Among them, only *IL10RA* was associated with the risk of malaria, and the rest were associated with a protective effect on the disease onset.

The IL-1 family of cytokines, produced mainly by macrophages, are important mediators of the inflammatory response to infection and fever (de Mendonça et al., 2012). In a Gambian case-control study, an SNP in *IL1B* (rs1143634) showed a marginal association with malaria susceptibility (Walley et al., 2004). This same variant was significantly associated with parasitemia in a sample of Brazilian patients with *P. vivax* malaria (Santos et al., 2016). The *IL1B* variant tested here (rs1143629) was associated with malaria susceptibility similar to that reported for a Brazilian population adjusted by ancestry (Sortica et al., 2012). In a more recent study, locus-specific patterns of ancestry were evaluated to search for genomic regions that are enriched across the population for particular ancestry contributions in Colombian genomes from Medellín (Rishishwar et al., 2015). A significant over-representation of ancestry-enriched genes was found among components of the immune system, including genes that map to pathways involved in both innate and adaptive immune responses; among them, *IL1B* showed patterns of high Native American ancestry (Rishishwar et al., 2015). It indicates a possible role for *IL1B* on malaria onset, and the results found in the population of Medellín are consistent with the current results.

TLRs are components of the immune system that recognize pathogen-associated molecular patterns through extracellular receptor modules and are infection sensors at the onset of an innate immune

response. *TLR9* has been reported to recognize *Plasmodium* DNA or the hemozoin pigment (Eriksson et al., 2013). The four most commonly studied SNPs in the *TLR9* gene are rs187084 (-1486C>T), rs5743836 (-1237C>T), rs352139 (1174G>A), and rs352140 (2848G>A). First, rs187084 was associated with an increased risk of low birth weight and maternal anemia in primigravidae women from Ghana (Mockenhaupt et al., 2006). This same SNP showed a strong association with high parasitemia in adults with mild malaria in Brazil (Leoratti et al., 2008). This SNP was also associated with severe malaria in Cameroonians (Apinjoh et al., 2013). A metanalysis, including six studies, associated the -1486C allele with the risk of severe malaria (Dhangadamajhi et al., 2017). However, in a large family and population-based association study, in Malawi and Gambia, no association was found with the above-mentioned polymorphisms in *TLR9* for malaria severity (Campino et al., 2009). Alleles C and G of -1237 and 1174 variants, respectively, were associated with higher levels of interferon-γ (IFN-γ) among children with cerebral malaria from Uganda (Sam-Agudu et al., 2010). Also, TLR9 -1237 showed association with mild malaria in Burundian children (Esposito et al., 2012). rs352139 showed an association with increased risk of mild malaria and higher parasitemia. Conversely, rs352140 showed a reduced risk of symptomatic malaria in Ghanian children. Furthermore, TTAG and CTGA TLR9 haplotypes were associated with reduced and increased risk of mild malaria, respectively (Omar et al., 2012). More recently, -1486C/T was associated with susceptibility to *P. vivax* malaria, and the variant -1237C/C was correlated with high parasitemia in Amazon Brazilians (Costa et al., 2017). Also, the 1237C allele showed an increased risk of malaria susceptibility in Atlantic Forest Brazilians (Guimarães et al., 2018b), and -1486 seemed to influence the levels of circulating cytokines IL-6, IFN-γ, IL-2, IL-10, and IL-4 during *P. vivax* malaria (Costa et al., 2018).

In previous studies, polymorphisms in TLR9 play a role on malaria outcome. Despite the differences and controversies between some studies, in most cases, the variants in this gene were associated with the mild form of the disease. These variants could be regulating parasitemia. It seemed that they can influence or alter the levels of circulating cytokines during parasitic infection and play a modulating role in the transition from mild to severe forms in interaction with other factors. Besides, when considering the genotypes of the variants evaluated as a haplotype, they agreed with that reported for reduced risk of mild malaria. This observation was supported by the Colombian population of 1000Genomes. These two variants (rs352139 and rs352140) showed high LD ($r^2 = 0.918319$, D' = 0.999998 via 1000Genomes).

TREM1 is a cell-surface activating receptor belonging to the immunoglobulin superfamily involved in monocytic activation and regulates innate immune responses by modulation of an inflammatory response (Bleharski et al., 2003; Bouchon et al., 2001; Bouchon et al., 2000; Netea et al., 2006; Radsak et al., 2004; Wu et al., 2011). In the malaria context, TREM1 has been characterized as a predictor biomarker of mortality among Ugandan children with severe malaria (Erdman et al., 2011). Also, TREM1 signaling pathways have been implicated in the pathogenesis of human brain endothelial cells in an in vitro model of platelet-induced cerebral malaria (Barbier et al., 2011). A more recent study on TREM1 modulation using specific antibodies showed that TREM1 was positively correlated with parasitemia development during severe malaria infection in *Plasmodium berghei*-infected mice model, suggesting a positive involvement of TREM1 in severe malaria development (Chin et al., 2019). Adukpo et al. (2016) also reported that the TREM1 rs2234237T variant, which was associated with higher TREM-1 plasma levels, was significantly more frequent among children with severe malaria than those with uncomplicated malaria from Ghana, consistent with the results that the carriage of the TREM1 rs2234237T allele appeared to be a risk factor for the development of severe malaria in Ghana children. In this study, the carriage of the rs2234237A allele conferred a protective effect on malaria outcome. The results, in conjunction with the previously mentioned research, confirmed that the role of TREM1 in malaria paralleled its role in inflammation-associated disorders, where the carriage of the rs2234237T allele was associated with the risk of malaria and development of severe forms.

IL10RA is a cell-surface receptor for cytokine IL-10, an anti-inflammatory cytokine. This cytokine is a principal regulator of immunity to infection. IL10RA participates in IL10-mediated anti-inflammatory functions, thus mediating the inhibition of proinflammatory cytokines, which without regulation may induce immune pathology during acute infections (Stelzer et al., 2016; Sung et al., 2006). Several studies that used *Plasmodium*-infected mice models have underscored the importance of IL10RA signaling in preventing T-cell- and cytokine-mediated pathology during potentially lethal malaria infections. IL10RA blockade directly and differentially affected the gametocyte load of distinct parasite genotypes, facilitated the development of acute immune pathology, increased the risk of host death independent of the total parasite burden, and induced cerebral malaria in normally resistant mice. Also, these studies have shown that IL10RA drives natural killer cell production of IL10. This pathway is critical for regulating immune responses and host resistance during *Plasmodium* infections. Thus, host resistance to *Plasmodium*-induced acute immune pathology is regulated by IL10RA signaling in mice (Clark et al., 2019; Claser et al., 2017; Long et al., 2008; Wang et al., 2013). In humans, the promoter variants rs56356146 (-185) and rs7925112 (-116) of IL10RA were evaluated in Gabonese children. None of these SNPs had any significant association neither in children with mild or severe malaria, but the TT -185/-116 haplotype in combination with the AC -754/-750 haplotype of ILRB was associated with the mild form of the disease (Velavan et al., 2012). Those same variants, -185 and -116, and -163T/G (rs199832262), were tested in Congolese children, but only the -163G allele was significantly more frequent among asymptomatic children than those with uncomplicated malaria. Conversely, the -163TT genotype was associated with the risk of developing symptoms. The CTG (rs56356146/rs7925112/rs199832262) haplotype was associated with protection against the disease (Koukouikila-Koussounda et al., 2013). IL10RA variants presented in these previous studies were, to the authors' best knowledge, the only ones that have been associated with any form of malaria in humans. Here, an SNP with a novel association on malaria susceptibility, rs4252246 in IL10RA, was reported, which was associated with a higher risk of mild malaria. This is an intron variation without reports of the related phenotype. Until now, rs4252246, together with previously reported variants, seemed to shed light on the possible role of IL10RA on malaria in humans, with tendencies toward the development of the mild form and protection from the severe form. Further research is necessary to clarify the possible regulatory role of these variations in IL10RA expression.

CD3G is the protein-coding gene for CD3-γ polypeptide, which, together with another heterodimer, forms the TCR-CD3 complex. TCR plays an important role in coupling antigen recognition to several intracellular signal transduction pathways. Defects in CD3G are associated with T-cell immunodeficiency (Stelzer et al., 2016). A few studies have evaluated the possible role of this gene on the pathogenesis of malaria. CD3G haploinsufficient mice showed reduced TCR expression and resistance to cerebral malaria (Muñoz-Ruiz et al., 2016). Human gene expression studies have shown that increased expression of *CD3G* and genes related to the Th1 response was associated with better health and higher resistance to malaria (Boldt et al., 2019; Hu, 2013; Torcia et al., 2008). Another variant of novel association with malaria found in this study was rs1561966, a stop gain polymorphism at the CD3G intronic region, which was significantly associated with a reduced risk of malaria (OR = 0.20, P = 0.0058). The A/A genotype was higher in controls than in cases (11.4% vs. 3.3%), The A allele is the minor allele frequency in several populations all around the globe, but Asian populations have a higher frequency than any else (~60%), suggesting the possible origin of this variant in the study population. This is consistent with the association analysis of genetic ancestry (Table 4).

4.4. Study limitations

As this is a cross-sectional study, the association with malaria status should be interpreted with caution, as it does not necessarily imply causality. Although in the Native American component the power of this sample is particularly high (>90%) to detect significant associations, that is not the case for the other ancestry contributions. For the European component, as shown in Materials and Methods, a sample of 200 cases and 200 controls was required to reach a power of 80%, which actually had a power close to 55% to detect differences in this ancestral component. Finally, the power of the sample was 16.2%. For the detection of significant associations, based on the differences of the African ancestry (the lowest ancestral component) between cases and controls, it was calculated that approximately 1034 cases in a 1:1 ratio with controls were required to reach a power of 80% ($\alpha = 0.05$). Therefore, some results should be considered trends or signs that must be confirmed.

All variants here used are not TagSNPs they include AIMs with high discriminatory power to the Colombian population (Carvajal-Carmona et al., 2000; Bedoya et al., 2006; Vergara et al., 2009; Rojas et al., 2010; Hoyos-Giraldo et al., 2013; Chacón-Duque et al., 2014) and variants of the host immune response to malaria (Table S2).

The sample in this study was convenient, not random, so it was not considered a representative sample. The small sample size might be explained by the low incidence of malaria during sampling and follow-up. In addition, there were other possible sampling biases, as an active search was made for the controls on historically endemic sidewalks and close to the urban area due to access limitations by the field staff and even to public order problems.

Diagnostic PCR for parasite gender and species was not used at follow-up of the controls. Only microscopic diagnosis (thin and thick smear) was used. Considering the differences in the sensitivity to low parasitemia infections, submicroscopic infections may have been underestimated and/or non-fever participants due to the detection limit by thick smear were possibly classified as controls.

Variables that investigated the quality of life, such as socioeconomic status (SES), were not analyzed. In previous studies of Latino populations, ancestry was associated with complex features, such as type 2 diabetes, but the result was confused by the correlation between SES and ancestry (Campbell et al., 2012; Florez et al., 2009), requiring a more careful analysis.

Despite the aforementioned limitations, interesting signs are evident that may lack statistical robustness but not biological relevance. Despite the limited sample size, the results showed a significant association between immune response genes and clinical presentation of malaria, suggesting that polymorphisms in immunity genes could affect one or another aspect of malaria pathogenesis.

5. Conclusions

The host's genetic factors play an important role in determining susceptibility to pathogenic agents. This work indicates novel associations and replicates of known associations between genetic variants and malaria. A number of these associations have important roles in the immune system. The relationship between genetic ancestry and malaria status in two Latino populations from Antioquia, Colombia, was analyzed. Both populations inherited European, Native American, and African ancestries. The Native American ancestry showed a protective effect on malaria outcomes. Genetic ancestry seems to confer malaria susceptibility depending on the parasite species. Genetic admixture is a powerful tool for the detection of previously reported variants in other populations and the discovery of new loci associated with complex entities, such as malaria, despite the small sample size. The results showed a significant association between immune response genes and the clinical presentation of malaria, suggesting that polymorphisms in immunity genes could affect one or another aspect of malaria pathogenesis. Variants in *IL1B*, *TLR9*, *TREM1*, *IL10RA*, and *CD3G* genes are part of the genetic component that determines malaria susceptibility. These genetic data may contribute to the understanding of molecular mechanisms that regulate immune response to *Plasmodium* infections.

Declaration of Competing Interest

None

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Appendix A. Supplementary data

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