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Determination of *Plasmodium vivax* and *Plasmodium falciparum* Malaria Exposure in Two Ethiopian Communities and Its Relationship to Duffy Expression

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Abstract. Despite historical dogma that Duffy blood group negativity of human erythrocytes confers resistance to *Plasmodium vivax* blood stage infection, cases of *P. vivax* malaria and asymptomatic blood stage infection (subclinical malaria) have recently been well documented in Duffy-negative individuals throughout Africa. However, the impact of Duffy negativity on the development of naturally acquired immunity to *P. vivax* remains poorly understood. We examined antibody reactivity to *P. vivax* and *P. falciparum* antigens at two field sites in Ethiopia and assessed Duffy gene expression by polymerase chain reaction amplification and sequencing of the GATA-1 transcription factor–binding site of the Duffy antigen receptor for chemokines (*DARC*) gene promotor region that is associated with silencing of erythroid cell transcription and absent protein expression. Antibodies to three of the four *P. vivax* blood stage antigens examined, RBP2b, EBP2, and DBPIISal-1, were significantly lower ($P < 0.001$) in Duffy-negative individuals relative to Duffy-positive individuals. In stark contrast, no clear pattern was found across Duffy-negative and Duffy-positive genotypes for *P. falciparum* antibodies. We conclude that lack of erythroid Duffy expression is associated with reduced serologic responses, indicative of less naturally acquired immunity and less cumulative exposure to blood stage *P. vivax* parasites relative to Duffy positive individuals living in the same communities.

INTRODUCTION

Plasmodium vivax is globally the most widely distributed human malaria species. Blood stage infection and clinical vivax malaria infection occur throughout tropical, subtropical, and temperate regions of the world, and it is the predominant *Plasmodium* species in Asia and South America.[1] One reason for this high prevalence is that dormant liver stage *P. vivax* hypnozoites, maintained in host hepatocytes for months to years, can lead to relapses of blood stage infection without continuous exposure to infective mosquitos.[2,3] Another unique feature of *P. vivax* biology is that merozoites appear to require expression of the Duffy antigen receptor for chemokines (*DARC*), here referred to as the Duffy blood group, to invade reticulocytes and immature erythroid lineage cells in bone marrow. It has long been accepted that the lack of Duffy expression confers resistance to *P. vivax* blood stage infection.[4–6] Indeed, erythroid Duffy negativity is nearly fixed in populations indigenous to sub-Saharan Africa and has historically been credited with the lack of endemic *P. vivax* on the continent. Notably, *P. vivax* blood stage infection of Duffy-positive and Duffy-negative individuals also occurs in Madagascar, an Indian Ocean island country located 400 km from the southeastern coast of the African land mass.[7] However, recent observations indicate that *P. vivax* is endemic in many areas and regions of the continent, with the highest prevalence (approximately 8%) in the horn of Africa.[8–10] Because many areas of sub-Saharan Africa experience reduced malaria burden and transition toward elimination, traditional methods for surveillance of blood stage infection such as blood smear microscopy, polymerase chain reaction (PCR), and rapid diagnostic tests may be underestimating true malaria prevalence because they tend to miss low-density asymptomatic infections (subclinical malaria).[11,12] This is especially true for *P. vivax*; traditional testing primarily accounts for active blood stage infections, which can represent only a small portion of all *P. vivax* prevalence because they are unable to detect latent liver stage infections.[13]

Serological tools that detect antibodies to *P. vivax* antigens are a potentially robust and sensitive measure for assessing *P. vivax* exposure in low-transmission settings.[14–16] Serology has the ability to capture information not only about concurrent blood stage infections but also about past infections that occurred at different times regardless of antimalarial drug treatment.[17,18] There is currently little information on the relationship between erythroid Duffy blood group negativity and antibody responses to *P. vivax* in African populations.

We previously collected dried blood spots and whole blood for quantitative PCR (qPCR) diagnosis of *P. vivax* and *P. falciparum* infection from healthy community residents of two sites in southwestern Ethiopia. Plasma IgG antibodies to four *P. vivax* antigens and six *P. falciparum* antigens were quantified, and genomic DNA was isolated for Duffy blood group sequencing. The aims of the study were to answer the following questions: 1) What is the prevalence of *P. vivax* and *P. falciparum* blood stage infection according to Duffy expression? 2) Do antibody prevalence and level differ based on Duffy expression? 3) Is there spatial clustering of *P. vivax* and *P. falciparum* infection and serology according to Duffy expression? These questions are of key epidemiological importance and will offer valuable insight into the role Duffy expression plays immunologically.

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MATERIALS AND METHODS

Ethical approval. Ethical approval was obtained from the institutional review boards at the University of California at Irvine; Case Western Reserve University, Cleveland, OH; and the Tropical and Infectious Diseases Research Center at Jimma University, Ethiopia. All residents willing to participate in the study were included; adults provided signed consent for themselves and assent for minors under 18 years of age after explanation of the study objectives and methodologies.

Study site. Figure 1 shows the location of villages of study participants within the two study sites, Arjo in the eastern Wollega Zone of the Oromia Regional State and Gambella district in the Gambella Regional State. These sites were chosen because both locations were previously found to have high *DARC* gene polymorphisms and different *P. vivax* malaria endemicity.[19,20] Arjo is an irrigation site for a government owned commercial sugar plantation, and Gambella is an irrigation site for a commercially owned large-scale rice farm. Malaria endemicity in Arjo is low (infection prevalence $< 5\%$), and *P. vivax* is the predominant malaria species.[21] Residents from 15 villages were enrolled (Figure 1). Malaria endemicity in Gambella is moderate (infection prevalence 10%–20%), and *P. falciparum* is the predominant species.[22] Residents from six villages were studied.

Blood sample collection and processing. Dried blood spots containing 50 μL of capillary blood collected by finger prick were collected from healthy study participants during a house-to-house mass blood survey in October 2018. Age and sex of each participant were recorded at the time of collection. Dry blood spot samples were transported to the University of California, Irvine and stored at −20°C, and plasma was isolated from peripheral blood collected via venipuncture and stored at −80°C until processing.

DNA was extracted from dried blood spots using established protocols[23] following standardized saponin/chelex procedures. DNA was eluted into ~170 μL of molecular-grade water, and either stored at 4°C for short-term or at −20°C for long-term storage. *Plasmodium* species DNA determination and quantification of both *P. vivax* and *P. falciparum* were conducted using published protocols[24,25] with some modifications. qPCR amplification was conducted on a ThermoFisher (Waltham, MA) QuantStudio 3 at a total volume of 20 μL containing 6 μL TaqMan Fast Advanced Master Mix, 0.5 μL *P. vivax*-probe, 0.5 μL *P. falciparum*-probe, 0.4 μL forward and reverse primers and 2 μL genomic DNA. Primers were prepared at 10 μM and probes at 2 μM concentrations.

Determination of Duffy genotypes. Duffy expression for each sample was conducted using established protocols and primers.[9,26] An approximately ~1,100-bp region of the human *DARC* gene encompassing the 33rd nucleotide position was amplified and sequenced. Reactions contained 10 μL DreamTaq Green Master Mix (ThermoFisher), 0.3 μL forward and reverse primers and 2 μL genomic DNA. Thermocycling was performed with the conditions of 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 61°C for 30 seconds, and 65°C for 40 seconds, followed by a 2-min extension at 65°C. Six microliters of amplified DNA were run

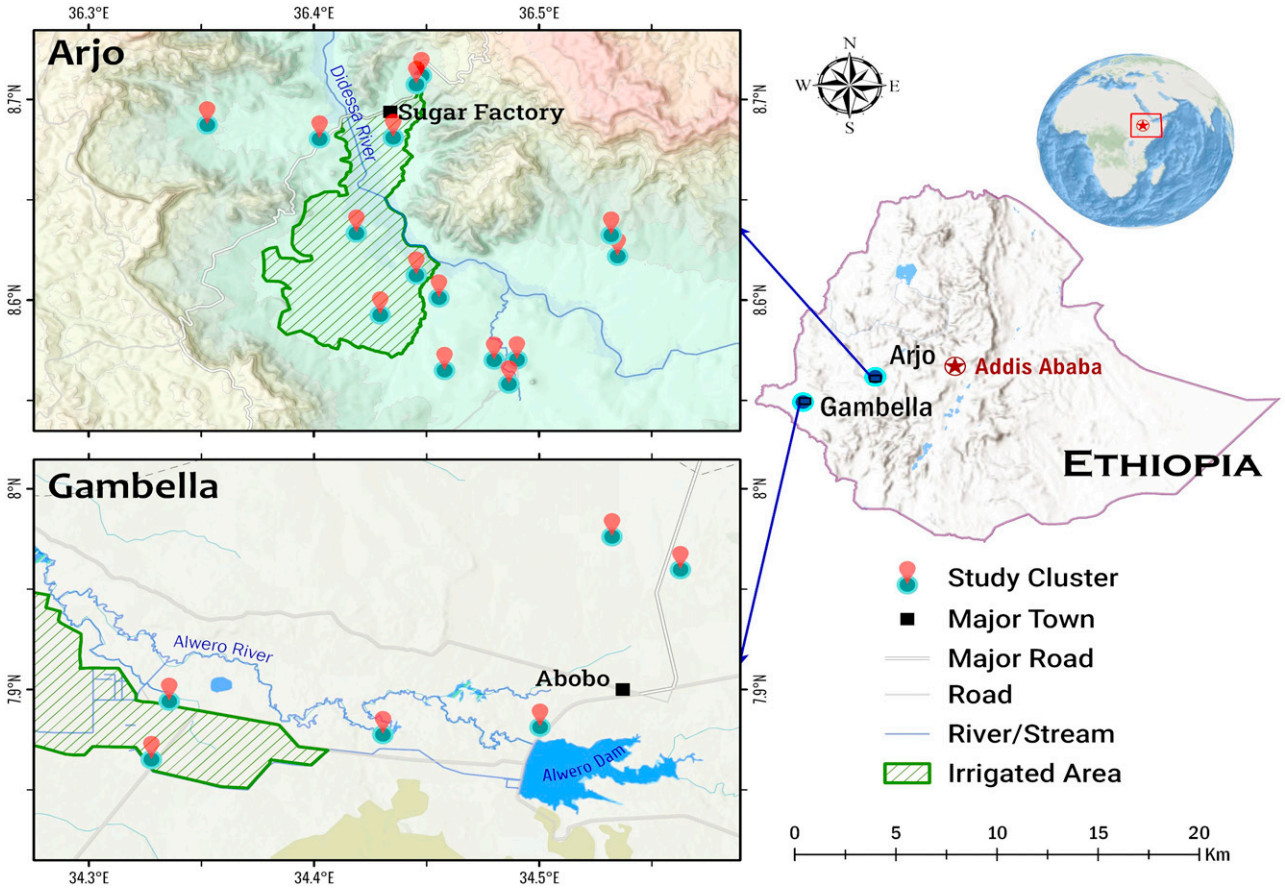

FIGURE 1. Map of the two selected Ethiopian study sites showing study resident locations and irrigation boundaries.

TABLE 1
Selected immune biomarkers used to test participant plasma and assess malaria exposure in Ethiopian communities

Species	Abbreviation	Antigen	Parasite stage
Plasmodium vivax	PvMSP1-19	Merozoite surface protein	Merozoite
	PvRBP2b	Reticulocyte binding protein	Merozoite
	PvEBP2	Erythrocyte binding protein	Merozoite
	PvDBPsal1	Duffy binding protein	Merozoite
Plasmodium falciparum	PfRh2a.b	Reticulocyte binding protein homolog	Merozoite
	PfMSP1-42	Merozoite surface protein	Merozoite
	PfAMA1	Apical membrane antigen1	Merozoite
	PfCSP	Circumsporozoite protein	Pre-erythrocytic
	PfEBA175	Erythrocyte binding antigen	Merozoite
	PfMSP3	Merozoite surface protein	Merozoite

on a 2.0% agarose gel to check for amplification. Successfully amplified samples were submitted to Retrogen Inc. (San Diego, CA) for Sanger sequencing. Sequencing result chromatograms were visually analyzed for five single neucleotide polymorphisms (SNPs) informing Duffy Fy genotypes; T33C, G125A, G145A, C265T, and G298A (see Duffy blood group nomenclature[26]). Genotype information from sequence data was used to infer Duffy expression and red blood cell (RBS) phenotype (Supplemental Table 1). Dual peaks were determined to be heterozygous.

Plasma collection and quantification of IgG antibodies to Pf and Pv proteins. MAGPIX (Luminex, Austin, TX) carboxylated microspheres were coupled to target four *P. vivax* and six *P. falciparum* recombinant protein antigens (Table 1) according to manufacturer's protocols with previously described modifications.[27] To determine an optimal antibody signal, varying concentrations of each target protein were coupled to beads and tested with plasma pooled from 20 healthy Kenyan adult residents of Kisumu County, Kenya, who donated blood samples in 2000. Quantification of IgG antibodies followed previously published methods.[27,28] Plasma pools were 4-fold serially diluted from ratios of 1:50 to 1:51,200, yielding a 6-point standard curve. Standard curves were run on each plate and used to determine the linear range of antibody binding for each bead. We quantified antibody magnitude using the dilution that fell within the linear range of the positive control pool. R-Phycoerythrin AfniPure F(ab′)2 fragment goat anti-human IgG F(ab′)2 (Jackson ImmunoResearch Laboratories, West Grove, PA) was used as the secondary antibody, and mean fluorescent intensity (MFI) values obtained during the assay were divided by the average MFI of malaria-naive negative controls on the same plate to normalize results and obviate variations across plates. Results are presented as the fold-increase of participant sample MFI over malaria-naive negative controls. Samples with an MFI greater than the mean plus 3 standard deviations of negative controls were considered positive; MFI values below this cutoff were set equivalent to malaria-naive controls at 1.

Data analysis. Malaria prevalence was calculated for Duffy-negative and Duffy-positive individuals for *P. vivax* and *P. falciparum* separately. Statistical significance between the two Duffy groups was tested using the Fisher's exact test. Mean fluorescent intensity was compared across Duffy phenotypes by Kruskall–Wallis test. Significance was adjusted using the Bonferroni correction for multiple comparisons for four *P. vivax* antigens and six *P. falciparum* antigens. Raw MFI data were converted to binary data to assess overall seroprevalence. Given the proportional nature of the converted data, seroprevalence data was arcsine transformed and compared across Duffy expression and phenotypes for all *P. vivax* and *P. falciparum* antigens. Statistical significance was assessed via two-sample *t* test for Duffy expression and Kruskall–Wallis test for Duffy phenotypes. Clustering of antibody profiles was assessed via principal component analysis (PCA) for *P. vivax* and *P. falciparum* antigens. Nonoverlapping confidence ellipses representing a 95% CI indicate significant variation between Duffy negative and Duffy positive individuals.

RESULTS

Malaria burden and Duffy expression. A total of 527 dried blood spots were collected from 15 villages in Arjo and six villages in Gambella, and genotyped for T-33C mutation of the DARC gene. The prevalence of *P. falciparum* blood stage infection was approximately twice as high as *P. vivax* at both study sites. In total, 17 (3.2%) samples were positive for *P. falciparum*; *P. falciparum* prevalence was 0.7% in Duffy positives and 6.2% in Duffy negative. Overall, eight (1.5%) *P. vivax* infections were identified, with 2.1% prevalence among Duffy positives and 0.8% prevalence among Duffy negatives. Analyses of prevalence and Duffy expression showed a significant difference in *P. vivax* and *P. falciparum* infection distribution between Duffy negatives and Duffy positives ($P < 0.01$, Table 2), where *P. vivax* infections occurred more rarely in Duffy negative individuals. By qPCR, none of the malaria positive samples exhibited a mixed infection with both *P. falciparum* and *P. vivax*.

Among the 527 samples genotyped for T-33C mutation, 395 randomly selected samples were successfully genotyped at four additional *DARC* SNPs (G125A, G145A, C265T, and G298A). A detailed breakdown of number of samples in each Duffy phenotype, relative to the 13 observed genotypes, is presented in Supplemental Table 1. Overall, 179 (45.3%) were homozygous Duffy negative Fy(a−b−), whereas the remaining 216 samples were Duffy positive. Duffy-positive individuals comprised four distinct RBS phenotypes inferred from

TABLE 2
Prevalence of *Plasmodium vivax* and *Plasmodium falciparum* in Duffy-negative and Duffy-positive individuals from pooled Arjo and Gambella dried blood spots

Duffy expression	*n*	*Plasmodium vivax*	*Plasmodium falciparum*
Positive	284	6 (2.11%)	2 (0.70%)
Negative	243	2 (0.82%)	15 (6.17%)
Total	527	8 (1.52%)	17 (3.23%)

TABLE 3
Distribution Duffy phenotypes in adult plasma samples collected from Arjo and Gambella, Ethiopia

Duffy expression*	Duffy phenotype	Arjo	Gambella	Total
Positive	a+b+	12 (6.7%)	18 (9.8%)	30 (8.3%)
	a+b−	36 (20.1%)	21 (11.5%)	57 (15.7%)
	a+b^{weak}	0	1 (0.5%)	1 (0.3%)
	a−b+	52 (29.1%)	60 (32.8%)	112 (30.9%)
Negative	a−b−	79 (44.1%)	83 (45.4%)	162 (44.8%)
Total		179	183	362

*Duffy expression is determined based on T-33C mutation in the promote region of the *DARC* gene.

genotype information; 30 (7.6%) Fy(a+b+), 63 (15.9%) Fy(a+b−), 1 (0.3%) Fy(a+b^{weak}), and 122 (30.9%) Fy(a+b−). Distribution of Duffy expression was similar in both study sites. In Arjo, 93 or 204 individuals (45.6%) were homozygous Duffy negative Fy(a−b−). In Gambella distribution was similar, with 86 of 191 (45.0%) of individuals exhibiting homozygous negativity Fy(a−b−).

Seroprevalence, serologic reactivity, and clustering effects of Duffy expression on serology. Because of the limited number of children younger than 5 years ($n = 1$) and aged 5 to 15 ($n = 32$), only plasma samples from individuals older than 15 years ($n = 362$) were included in serologic reactivity analyses (Table 3). We observed distinctly different trends in *P. vivax* and *P. falciparum* antibodies among the different Duffy phenotypes. *P. vivax* antibodies exhibited significantly reduced seroprevalence in Duffy-negative compared with Duffy positive people ($P < 0.001$; Figure 2). Conversely, *P. falciparum* antibodies showed no significant variation between Duffy phenotypes ($P > 0.05$). Assessment of seroprevalence across Duffy phenotypes was also analyzed for all phenotypes with $N > 10$. We found greater significance between Duffy phenotypes for *P. vivax* antibodies than for *P. falciparum* antibodies (Supplemental Figure 1).

Serologic responses of *P. vivax* antigens were distinctly different in most cases depending on Duffy phenotype. Homozygous Duffy-negative phenotype generally exhibited significantly lower MFI than Duffy-positive phenotypes against PvRBP2b, PvEBP2, and PvDBPSal1 antigens but not for PvMSP1-19 (Figure 3). Conversely, serologic responses did not vary significantly for six *P. falciparum* antigens across Duffy phenotypes. No significant variation was found between Duffy negatives Fy(a−b−) and any Duffy positives Fy(a−b+), Fy(a+b−) and Fy(a+b+) for PfCSP, PfEBA175, and PfMSP3. Interestingly, homozygous negative Fy(a−b−) phenotype was found to show a significantly higher MFI than Fy(a+b−) phenotype for PfRh2a.b, PfMSP1, and PfAMA1 ($P < 0.05$).

The PCA showed distinct difference in clustering patterns between Duffy phenotype for *P. vivax* and *P. falciparum* (Figure 4). For *P. vivax*, serologic response nonoverlapping confidence ellipses provide evidence that antibody profiles

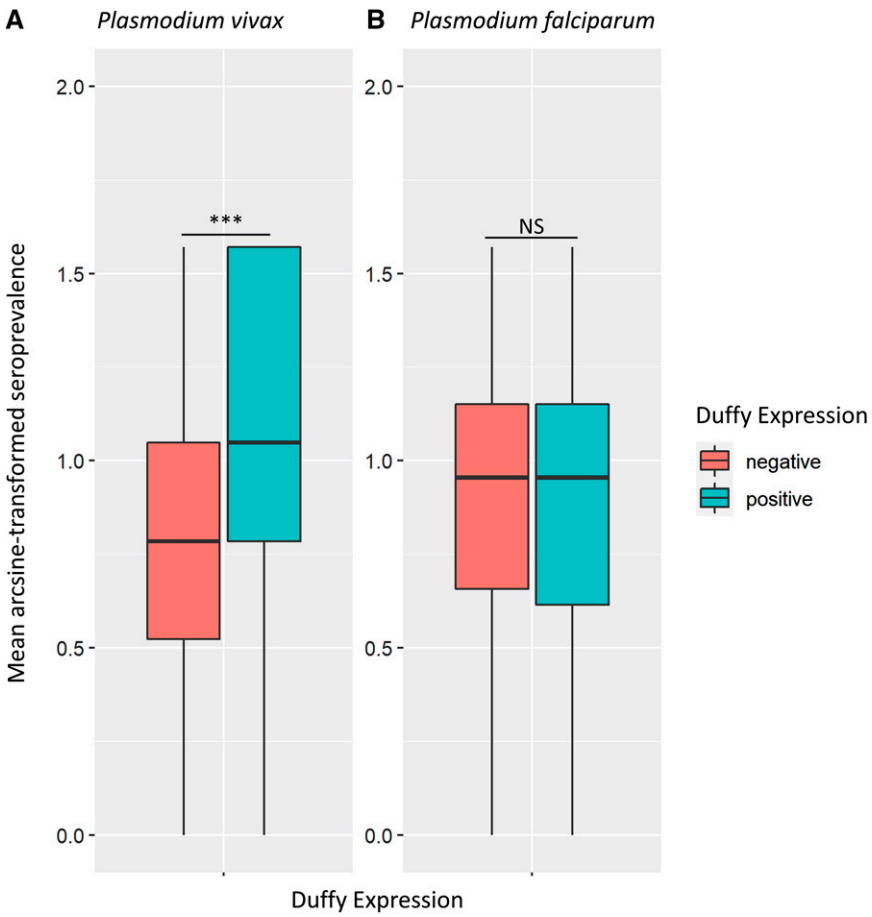

FIGURE 2. Box plots of seroprevalence for Duffy-negative and Duffy-positive individuals across four *Plasmodium vivax* antigens (**A**) and six *Plasmodium falciparum* antigens (**B**). Significance was obtained via two-sample *t* test. NS = nonsignificant; ***$P < 0.001$.

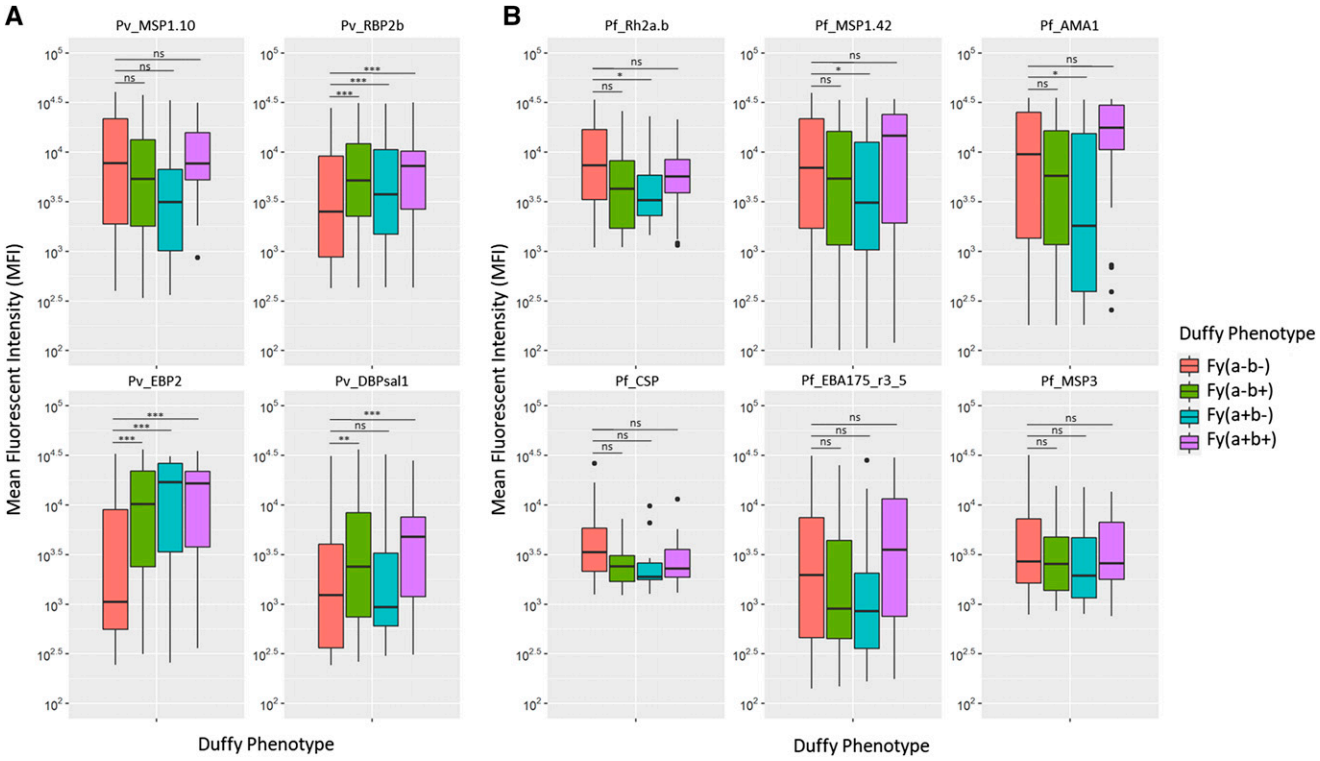

FIGURE 3. Mean fluorescent intensity box plot of *Plasmodium vivax* (**A**) and *Plasmodium falciparum* antigens (**B**) across different Duffy phenotypes. Phenotypes are classified as homozygous negative Fy(a−b−), heterozygous positive Fy(a−b+) and Fy(a+b−), and homozygous positive Fy(a+b+). Central box plot represents interquartile range with a median center line. *P* values were obtained by Kruskall–Wallis test with Bonferroni correction for multiple comparisons. NS = nonsignificant; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

differ distinctly between Duffy-negative and Duffy-positive individuals, whereas *P. falciparum* antibody profiles did not show significant overlap.

DISCUSSION

Overall, our study sites exhibited high levels of Duffy heterogeneity, with approximately 45.3% (179/395) individuals being homozygous negative for the Duffy antigen. These levels are notably lower than other areas of the continent, with West and Central Africa generally having nearly fixed allele levels of Duffy negativity (> 97%),[29] yet they are higher than previous studies on Duffy expression in Ethiopia, which recorded levels of Duffy negativity to range from 20% to 30% in areas around Harar and Jimma.[9,30] Cases of *P. vivax* were found predominantly in Duffy-positive individuals yet did not exhibit significant variation from Duffy-negative infection

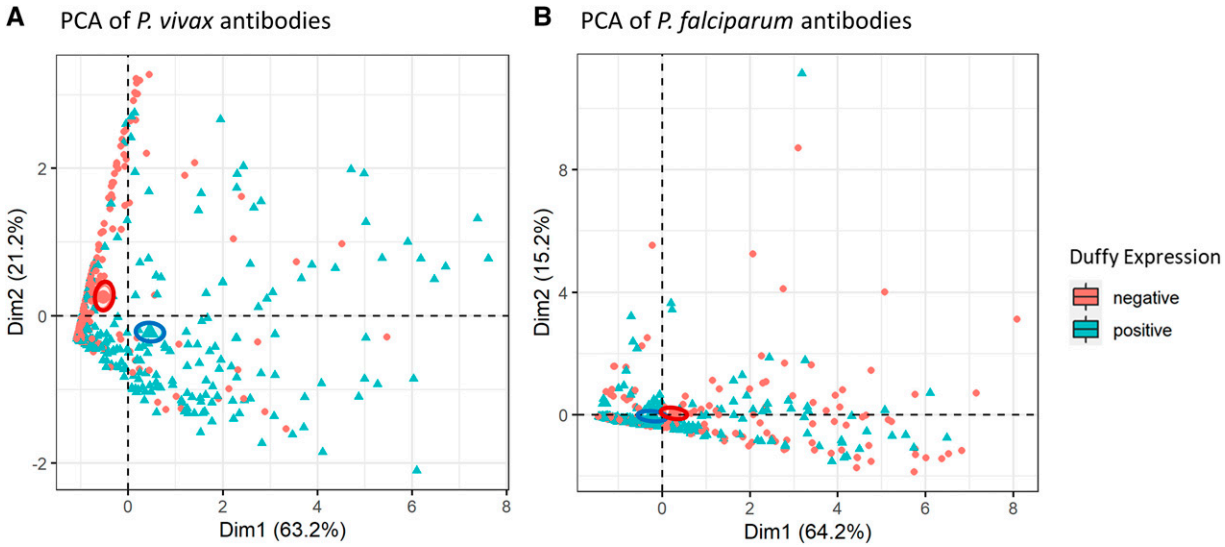

FIGURE 4. Principal component analysis on antibody profiles of Duffy-negative and Duffy-positive individuals for combined *Plasmodium vivax* and *Plasmodium falciparum* serologic responses.

rates. Conversely and interestingly, *P. falciparum* infections were mainly found in Duffy-negative individuals and exhibited significant variations in infection levels across Duffy phenotypes. Given that these results are derived from small sample sizes of positive malaria infections, caution should be exercised in their interpretation. Antibody levels were generally significantly reduced in homozygous Duffy negative peoples Fy(a−b−) compared with the three Duffy-positive phenotypes for all *P. vivax* target antigens except PvMSP1-19. No significant difference in antibody levels was observed between Duffy negatives and positives for *P. falciparum* antigens. This phenomenon was more clearly observed in seroprevalence analyses where *P. vivax* seroprevalence was significantly lower in Duffy-negative individuals, whereas *P. falciparum* seroprevalence did not differ in Duffy expression. Additionally, PCA visualizations depict clear clustering of Duffy expression for *P. vivax* antigens yet yield no observable pattern for *P. falciparum*, corroborating the pattern that Duffy-negative people experience greatly reduced development of *P. vivax* antibodies.

Despite historical evidence that Duffy negativity inhibits infection by *P. vivax*,[4–6,31] our study adds to the growing body of research showing that cases of active *P. vivax* infection in Duffy-negative people occur in Africa.[28,32–35] We also observed *P. falciparum* infections occurred more frequently in Duffy-negative individuals. This pattern could simply be resultant of a small number of *P. falciparum* infections in a low-transmission zone. Some studies, however, have linked Duffy binding–like (DBL) domains to the susceptibility of *P. falciparum*–infected red blood cell (RBCs) by natural killer (NK) cells.[36–38] Killing of *P. falciparum* infected RBCs by NK cells requires specific contact between NK natural cytotoxicity receptors and DBL-1α domain expressed on the *P. falciparum* erythrocyte membrane protein.[38] It is possible that an interaction exists between phenotypic Duffy positivity and NK innate recognition of DBL domains on *P. falciparum*–infected RBCs. The pattern observed in our study is highly novel and interesting, warranting further study and investigation. However, we acknowledge that it arises from small sample sizes, and thus caution in interpretation is necessary and represents a limitation in this aspect of our study. Any such conclusions will require further large-scale studies of *P. falciparum* infections across a wide range of Duffy-positive and Duffy-negative individuals. Future studies should seek to determine whether there is a relationship between Duffy phenotype and immune response by NK cells to *P. falciparum* infection.

The role that Duffy expression plays in the development of natural immunity is not well understood, and little work has been done exploring the relationship between Duffy negativity and immune response development given exposure to *P. vivax* infection.[39,40] Our data show distinct trends of immune response across Duffy expression for *P. vivax* antibodies but not for *P. falciparum* antibodies. Duffy-negative individuals had notably and significantly lower immune responses to *P. vivax* antigens than did Duffy-positive participants, suggesting that successful blood stage infection plays a role in broad immune response, despite the fact that only one of our target antigens was directly related to the Duffy antigen system (PvDBPSal1). The notable exception to the observed pattern in *P. vivax* antigens was the Pv-merozoite surface protein (PvMSP1-19), which did not vary significantly between Duffy phenotypes but was also the only class of antigen to be analyzed for both *P. falciparum* and *P. vivax* (PvMSP1-19/PfMSP1-42/PfMSP3). Kano et al.[41] found that Duffy-negative individuals had a greater chance of acquiring high levels of anti-PvMSP1-19 antibodies than Fy(a+/b+) individuals in the northeast of Amazonas State, Brazil, but we did not find such a phenomenon. The finding on the relationship between Duffy phenotypes and antibody response to *P. vivax* antigens should be interpreted with caution because both the frequency and levels of the antibody response to PvMSP1-19 was low. Previous work has shown that certain immunities established against one *Plasmodium* species are able to cross react against other *Plasmodium* species through commonly shared epitopes and do indeed exhibit high levels of cross reactivity between *P. vivax* and *P. falciparum* antigens.[42] It is possible that the lack of antibody variation for PvMSP1-19 between Duffy phenotypes could be attributed to cross-species reactivity of the MSP antigen class; however, several studies have also shown MSP antibodies to be highly species specific[40,43] and therefore further study is required to determine why PvMSP1-19 development does not appear to be influenced by Duffy expression.[44,45]

Plasmodium vivax malaria, although far less pervasive and causal of much less mortality than *P. falciparum* in Africa, presents a notable problem for management and elimination and is increasingly attributed to significant morbidity in endemic areas.[46] Vaccination development efforts for *P. vivax* have been hampered by a multitude of species-specific challenges. These include lifecycle complexities such as relapses and latent-stage hypnozoites, technical challenges in in vitro culturing for preclinical experimentation, and difficulties in both understanding and establishing functionally conserved epitopes from antigens at various life stages of infection.[47] Our work clearly shows that Duffy expression is now an additional complexity to be considered in *P. vivax* vaccine development. Areas of high Duffy negativity and indeed nearly all sub-Saharan Africa may not respond to *P. vivax* vaccine efforts as predicted if Duffy-negative individuals experience significantly reduced development of *P. vivax* antibodies. This information is vitally important to further understanding the epidemiology of *P. vivax* on the continent and establishing future elimination and management strategies.

Due to the rarity of *P. vivax* infection in Duffy-negative people, our study was limited in that few active cases of *P. vivax* were found and that even fewer of these occurred in Duffy-negative people. Our study also primarily examined merozoite blood-stage antigens for *P. vivax* and therefore could be presenting an incomplete picture of antibody profile and development across different lifecycle stages. Future studies would do well to examine antibody profiles closely for individuals both with and without active *P. vivax* infection and to include more pre-erythrocytic serologic markers to assess whether reduction in antibody response is occurring only at the erythrocytic stage where Duffy negativity inhibits infection. Inclusion of salivary gland antibodies[48] to known local vectors would also be highly beneficial because they could potentially control for exposure differences and ensure that variations in antibody profiles are not simply resultant of reduced exposure.

Our study adds to the growing body of literature showing that Duffy expression potentially plays a significant role in *P. vivax* naturally acquired immunity and that Duffy-negative people appear to develop a greatly reduced immune response to *P. vivax* compared with Duffy positive individuals. In addition to vaccine developments and implementation, this work is of vital importance as studies and projects continue to use serological tools as a surveillance method. The use of serologic tools to inform malaria transmission is often highly beneficial and can give a higher resolution of transmission dynamics.[14–18] They are able to account for asymptomatic and subpatent infections (subclinical malaria) missed by traditional microscopy, RDT, and qPCR; they are also able to provide insight into the timeline of both infections and prevalences by accounting for individual antibody half-lives. Thus, studies aiming to use serologic assays as a surveillance tool in areas of high Duffy admixture should strongly consider the effect of Duffy expression levels in the area of study on their results. Finally, our work adds to the growing evidence that Duffy negativity may no longer offer complete resistance to infection by *P. vivax*.[28,31–35,49] Together these findings lead to concern that because *P. vivax* endemicity in Duffy-negative populations continues, these people could not only be more susceptible to infection but also lack naturally acquired immunity, leaving them more susceptible to greater levels of infection prevalence and morbidity.

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