Review

Relationship between Duffy Genotype/Phenotype and Prevalence of Plasmodium vivax Infestation: A Systematic Review

Yelson Alejandro Picón-Jaimes , Ivan David Lozada-Martinez , Javier Esteban Orozco-Chinome , Jessica Molina-Franky , Domenica Acevedo-Lopez , Nicole Acevedo-Lopez , Maria Paz Bolaño-Romero , Fabriccio Visconti-Lopez , D. Katterine Bonilla-Aldana * , Alfonso J. Rodriguez-Morales

Posted Date: 14 July 2023

doi: 10.20944/preprints202307.1020.v1

Keywords: Plasmodium vivax; Duffy Blood-Group System; Vivax malaria; Plasmodium Duffy antigen binding protein; prevalence

Review

Relationship between Duffy Genotype/Phenotype and Prevalence of *Plasmodium vivax* Infestation: A Systematic Review

Yelson Alejandro Picón-Jaimes [1,†], Ivan David Lozada-Martinez [2,†], Javier Esteban Orozco-Chinome [3], Jessica Molina-Franky [4,5], Domenica Acevedo-Lopez [6], Nicole Acevedo-Lopez [3], Maria Paz Bolaño-Romero [3], Fabriccio Visconti-Lopez [7] and D. Katterine Bonilla-Aldana, [8,*] Alfonso J. Rodriguez-Morales [9,10]

1 Fac Ciències Salut Blanquerna, Univ Ramon Llul, Barcelona, Spain; ypicon@unab.edu.co
2 Epidemiology Program, Department of Graduate Studies in Health Sciences, Universidad Autónoma de Bucaramanga, Bucaramanga, Colombia; ilozada@unab.edu.co
3 Medical and Surgical Research Center, Future Surgeons Chapter, Colombian Surgery Association, Bogotá, Colombia; Efeso17@yahoo.es (J.E.O.-C.); nicol_1008@hotmail.com (N.A.L.); mbolanor1@unicartagena.edu.co (M.P.B.-R.)
4 Department of Inmunology and Theranostics, Arthur Riggs Diabetes and Metabolism Research Institute, Beckman Research Institute of the City of Hope, Duarte, CA, USA; Jessicamolinafranky@gmail.com
5 Molecular Biology and Inmunology Department, Fundación Instituto de Inmunología de Colombia (FIDIC), Bogotá, Colombia
6 School of Medicine, Fundación Universitaria Autónoma de las Américas-Institución Universitaria Visión de las Américas, Pereira, Risaralda, Colombia; domenica.acevedo@uam.edu.co
7 Sociedad Científica de Estudiantes de Medicina – UPC, Lima, Peru. E-mail: fabricciovisco@gmail.com
8 Research Unit, Universidad Continental, Huancayo, Peru
9 Clinical Epidemiology and Biostatistics Master Program, Universidad Cientifica del Sur, Lima, Peru; arodriguezmo@cientifica.edu.pe
10 Gilbert and Rose-Marie Chagoury School of Medicine, Lebanese American University, Beirut, P.O. Box 36, Lebanon; alphonso.morales@lau.edu.lb
* Correspondence: dbonilla@continental.edu.pe
† Equal first authors.

Abstract: The Duffy protein, a transmembrane molecule, functions as a receptor for various chemokines and facilitates attachment between the reticulocyte and the *Plasmodium* Duffy antigen-binding protein. Duffy expression correlates with the Duffy receptor gene for the chemokine, located on chromosome 1, and exhibits geographical variability worldwide. Traditionally, researchers have described the Duffy negative genotype as a protective factor against phenotypic malaria expression. However, recent studies suggest this microorganism's evolution potentially diminishes this protective effect. Nevertheless, there is currently insufficient global data to demonstrate this phenomenon. This study aimed to evaluate the relationship between the Duffy genotype/phenotype and the prevalence of *Plasmodium vivax* infestation. The protocol for the systematic review was registered in PROSPERO as CRD42022353427 and involved reviewing published studies from 2012 to 2022. Medline, Web of Science, Scopus, and Scielo databases were consulted. Assessments of study quality were conducted using the STROBE and GRADE tools. A total of 34 studies were included, with Africa accounting for most recorded studies. The results varied significantly regarding the relationship between the Duffy genotype/phenotype and Plasmodium vivax invasion. Some studies predominantly featured the negative Duffy genotype yet reported no malaria cases. Other studies identified minor percentages of infestations. Conversely, certain studies observed a higher prevalence (99%) of Duffy-negative individuals infected with *Plasmodium vivax*. In conclusion, no evidence of a gender-specific distribution of malaria between Duffy-negative men and women was found. However, evidence supports that the homozygous Duffy genotype positive for the A allele (FY*A/*A) is associated with a higher incidence of *Plasmodium* infestation. Furthermore, the negative Duffy genotype does not confer protection against this disease.

Keywords: *Plasmodium vivax*; vivax malaria; Duffy Blood-Group System; *Plasmodium* Duffy antigen binding protein; prevalence

1. Introduction

Malaria, one of the most prevalent potentially deadly infectious diseases worldwide, is estimated to have caused 229 million cases and 409,000 deaths in 2019, according to the World Health Organization (WHO) [1]. The Centers for Disease Control and Prevention (CDC) data indicate 247 million global cases and 619,000 deaths in 2021 [2]. In the United States, around 2,000 new cases are reported annually, predominantly due to imported episodes by travellers and immigrants from countries with high transmission rates, such as sub-Saharan Africa and Southeast Asia [3].

The disease results from parasites of the *Plasmodium* genus invading erythrocytes, leading to potentially fatal alterations. The invasion occurs when infected female Anopheles mosquitoes bite humans. It is known that five species, including *P. falciparum*, *P. vivax*, *P. ovale wallikeri*, *P. ovale curtisi*, *P. malariae*, and *P. knowlesi*, can cause pathology in humans. The most significant and widespread species are *P. falciparum* and *P. vivax*, with the former causing more severe disease and the latter having a global distribution [4].

The Duffy glycoprotein, a non-selective receptor, interacts with various chemokines such as IL-8 and melanoma growth-stimulating activity (MGSA) in the CXC group, as well as monocyte chemotactic protein-1 (MCP-1) and CCL5 in the CC group [5]. These interactions attract monocytes, memory CD4+ T lymphocytes, and eosinophils. The Duffy protein, encoded by the ACKR1 gene (also known as Duffy antigen receptor for chemokines [DARC]), is located on chromosome 1 at 1q23.2 [6,7]. The interaction with DARC is essential for the invasion of *Plasmodium vivax*. Consequently, the absence of this protein is considered a preventive factor against the invasion of these merozoites into red blood cells/reticulocytes. African populations exhibit lower expression of the Duffy antigen, which correlates with a lower proportion of *P. vivax* malaria in this region. Furthermore, heterozygous phenotypes Fy(a+b-) and Fy(a-b+) (Duffy positive heterozygotes) may exhibit a certain degree of resistance to parasite infestation compared to the Fy(a+b+) phenotype [7]. However, insufficient global data currently exists to support this phenomenon.

To achieve the WHO's goal of controlling and eradicating *P. vivax* malaria [1,8], it is imperative to design and conduct studies identifying the relationship between the Duffy genotype/phenotype and the prevalence of *P. vivax* infestation, given that more than one-third of the global population is exposed to it [9]. Recent publications have reported cases of *P. vivax* malaria in Duffy-negative patients, contradicting the long-established belief that the Duffy antigen is essential for entering this *Plasmodium* species into reticulocytes [10–12]. This finding holds significant implications for understanding the disease and designing new drugs. Thus, this study aims to evaluate the relationship between the Duffy genotype/phenotype and the prevalence of *P. vivax* infestation

2. Methods

2.1. Registration of the Systematic Review Protocol

The protocol of systematic review was registered at PROSPERO: CRD42022353427.

2.2. Guideline of Reporting Systematic Review

The reports of this systematic review followed the PRISMA statement [13].

2.3. Research Question

Is the Duffy-negative genotype/phenotype a protective factor in the population susceptible to *P. vivax* infestation compared to the expression of the Duffy-positive genotype/phenotype?

 doi:10.20944/preprints202307.1020.v1

2.4. Search Strategy

The search was conducted in Medline, Web of Science, Scopus, and Scielo. The selected terms were: Duffy Blood-Group System; ACKR1 protein, human; Duffy antigen binding protein, *Plasmodium*; *Plasmodium*; and Malaria. These terms were combined using the boolean operators "OR" and "AND". Due to the broadness of the search results, additional filters were applied to select only those manuscripts corresponding to case reports, clinical studies, clinical trials, controlled clinical trials, multicenter studies, observational studies, and randomised controlled trials. The searches were slightly modified according to each database.

2.5. Eligibility Criteria and Study Selection

The inclusion criteria for the studies were as follows: 1) Consideration was given to descriptive observational manuscripts, analytical observational studies, experimental studies, and quasi-experimental studies; 2) Texts in Spanish, English, and Portuguese that matched the search equation were included; 3) Texts referring to the Duffy genotype, Duffy binding protein, or DARC and their relationship with *P. vivax* or other *Plasmodium* infestation, within the specified time frame, were included; 4) Articles with a methodological quality score higher than 60% according to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) assessment tool were included; 5) Articles published within the last ten years were included; 6) Full-text articles were considered.

Conversely, articles meeting the following criteria were excluded: 1) Articles that did not provide data on events related to the Duffy genotype/phenotype and malaria or addressed infection or infestation by a microorganism other than *Plasmodium*; 2) Posters, abstracts, topic reviews, and informal texts were not included in the review; 3) Integrative reviews and systematic/meta-analyses were also excluded.

2.6. Quality of the Included Studies

The articles' quality was assessed using the STROBE tool [14], considering that the manuscripts were observational studies. The STROBE tool comprises a checklist of 22 items for reporting this type of manuscript. A minimum compliance threshold was established, requiring the fulfilment of at least 60% of the checklist items, equivalent to meeting at least 14 out of the 22 points. Furthermore, the study's risk of bias, certainty, and importance was evaluated using the GRADE tool [15].

3. Results

3.1. Search Results

At the end of the database search, along with reverse searching based on article references, 3,600 manuscripts were obtained. The distribution was as follows: Medline-Pubmed contained 3,101 texts, Web of Science had 263 texts, Scopus contained 230 texts, Scielo contained one text, and five texts were obtained through reverse searching. The review process involved discarding duplicates, screening titles and abstracts, and concluding with full-text reading. Ultimately, this systematic review included 34 articles [7,10–12,16–45] **(Figure 1)**.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 July 2023 doi:10.20944/preprints202307.1020.v1

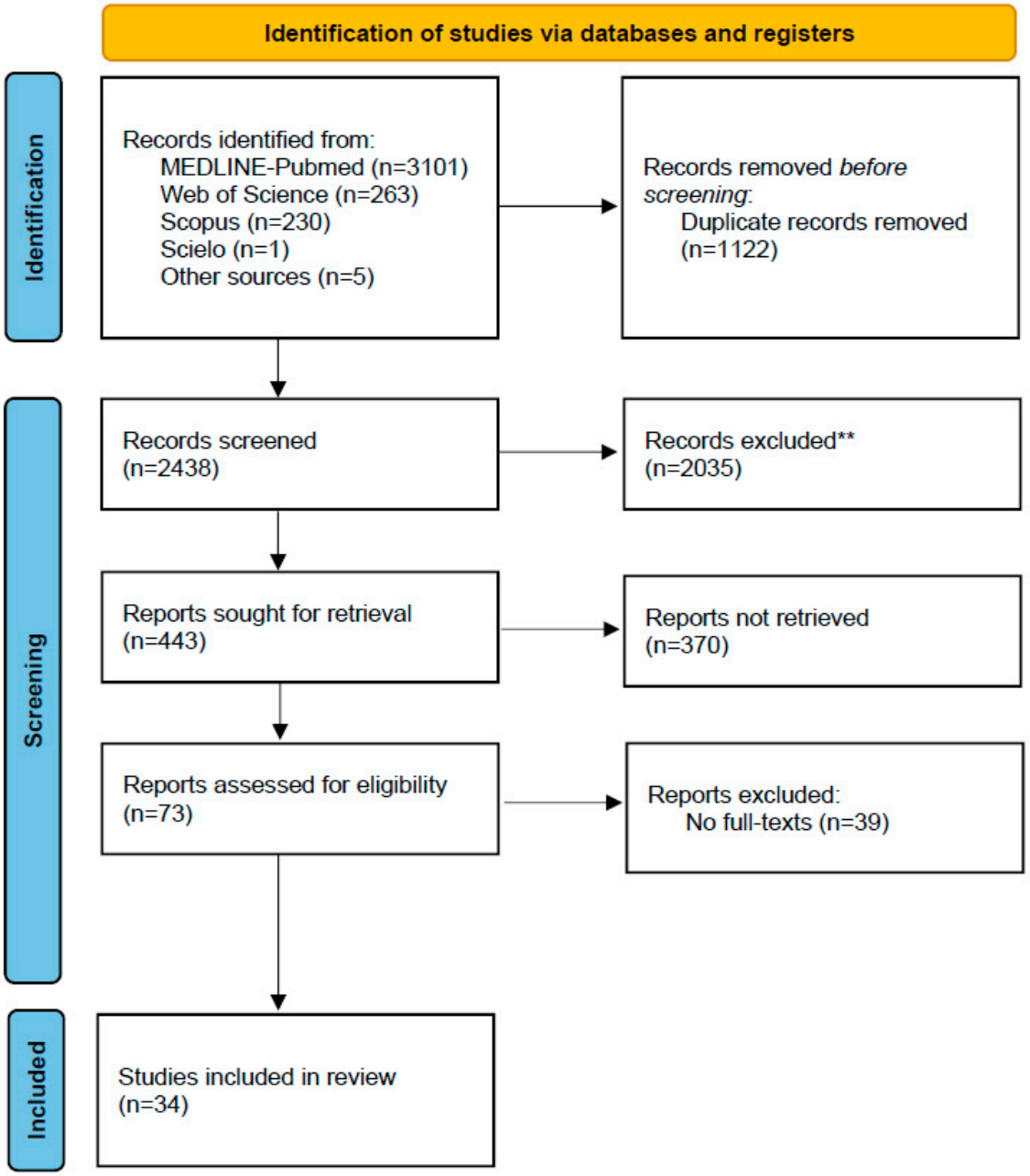

Figure 1. Study selection diagram.

3.2. Quality of the Included Studies

According to the STROBE criteria that assessed the study's quality with an overall score of 22 items, 19 studies achieved a score higher than 85% (n=19 items). In comparison, three studies obtained a lower score, with a compliance rate of 72.7% (n=16 items) of the items **(Figure 2)**. During the application of the GRADE tool, a risk of serious bias was found in 70.5% (n=24) of the studies, and the same percentage of studies exhibited moderate certainty of the evidence (70.5%; n=24) **(Table 1)**.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 July 2023 doi:10.20944/preprints202307.1020.v1

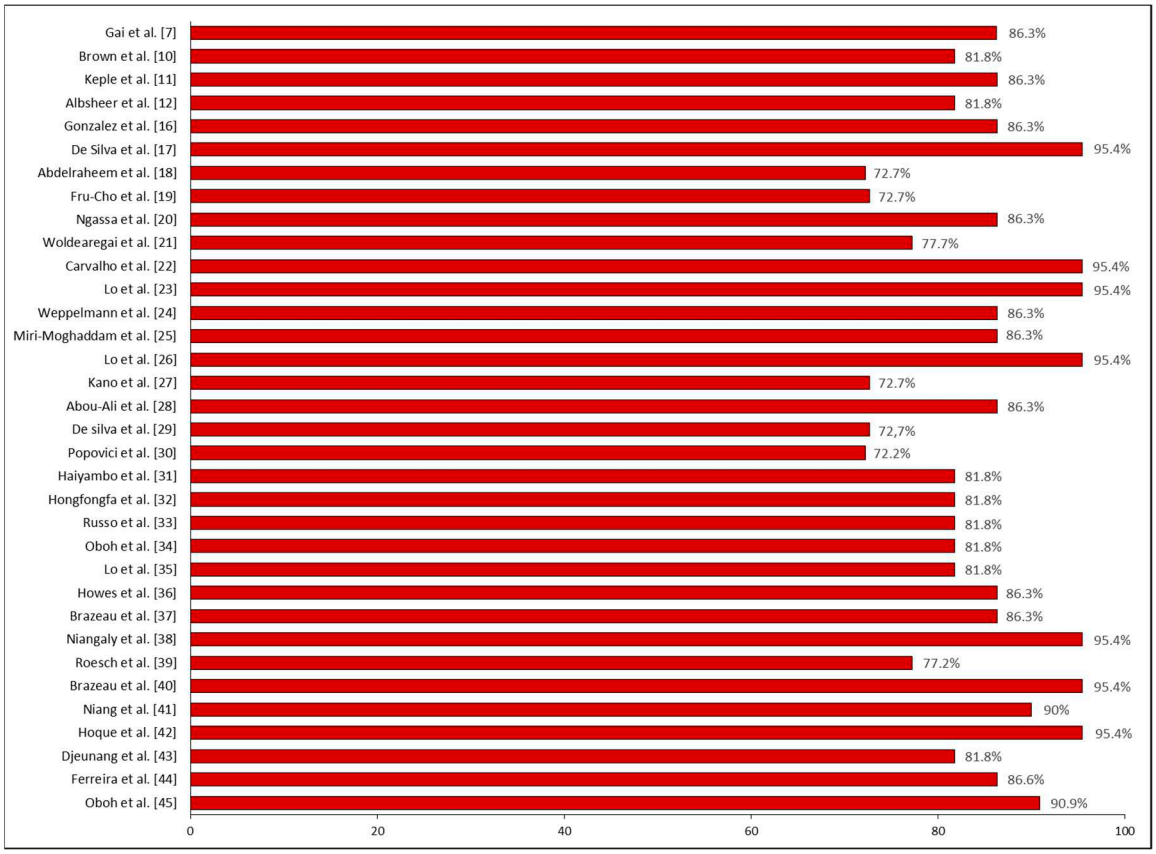

Figure 2. Percentage of compliance with reporting quality according to the STROBE tool.

3.3. General characteristics, frequency and distribution of Plasmodium infestation in the population studied

According to the distribution by continent, 21 studies were conducted in Africa. In Ghana, a study analysed 952 subjects, of whom 845 had malaria. Among these cases, 542 tested PCR-positive for *P. falciparum*, 1 for *P. malariae*, and 2 for *P. ovale* [10].

Sudan was the site of 5 studies. The first study analysed 412 blood samples from patients infected with *P. vivax*, including 155 subjects from Khartoum, the Nile River, and New Halfa. This study also included samples from Ethiopia, specifically from Jimma, Gojeb, and Arjo regions, where 150 subjects with *P. vivax* malaria were studied [11]. The second study, conducted in Khartoum, New Halfa, and the Nile River, recruited patients with symptomatic malaria from primary care centres. A total of 992 microscopy-positive samples were taken, confirming 186 cases as mono-infection by *P. vivax* and identifying 4 cases as mixed infections by *P. vivax*/*P. falciparum*, as determined by PCR [12]. The third study collected 63 blood samples, of which 42 demonstrated *P. vivax* infestation. These samples were obtained from patients in different areas of Sudan from 2014 to 2016 [42]. The fourth study in this country was conducted in Gezira, central Sudan, where 126 patients with suspected malaria were analysed from October to December 2009. *P. vivax* infestation was identified in 48 subjects, representing 38% of the samples, using PCR. Gezira state is characterised by a seasonal and unstable transmission of *P. vivax* malaria, with the rainy season starting in July and ending in October and an annual rainfall ranging between 140 and 225 mm. Regarding gender, 54.2% were women, and 79.2% were under ten years old [18]. The last study in this country included individuals from Botswana (regions of Kweneng East and Tutume) and Ethiopia (areas of Jimma and Bonga) and collected a total of 1215 febrile patients. Among the febrile patients in Botswana, 3% (n=9/301) from Kweneng East tested positive for *P. vivax*. In Tutume, 6.8% (n=12/176) of the febrile patients were detected with *P. vivax*, while in Ethiopia, out of 358 samples from Jimma, 37.4% (n=134/358) were diagnosed with *P. vivax* malaria. In Bonga, the malaria cases caused by this parasite were 30.3% (n=125/413) [45].

In Ethiopia, a study was conducted in Jimma by Lo et al., including 178 individuals, of whom 145 patients were symptomatic, and 33 were asymptomatic. However, detailed demographic data is

 doi:10.20944/preprints202307.1020.v1

unavailable [26]. Another study involved the collection of blood samples via finger prick from 416 febrile patients and 390 asymptomatic individuals visiting health centres. Among the symptomatic cases, *P. vivax* was detected in 164 samples, *P. falciparum* in 134 samples, and mixed P. vivax/P infections. *falciparum* in 33 samples [23]. In the same country, in 2009, Woldearegai et al. [21] analysed 1,304 and 627 febrile patients in Harar and Jimma, respectively. Of the samples analysed, 205 tested positive through microscopy and PCR, with 111 cases identified as *P. vivax* infestation and 94 cases as *P. falciparum* infestation. Among these subjects, 125 were male, accounting for 61% of the positive cases. *P. vivax* was detected in 74 out of 98 (75.5%) samples from Harar, while *P. falciparum* was more prevalent in Jimma (n=70/107; 65.4%).

In Senegal, a study on 48 schoolchildren aged between 8 and 11 years was conducted, with women comprising 58.3% of the participants. Four samples were collected at different time points, resulting in 192 samples. Among them, 38.5% (n=74/192) showed positive results for malaria through PCR. *P. falciparum* accounted for most cases and was a pure infestation in 79.7% (n=59/74) of the samples. No infestations by *P. malariae* or *P. ovale*, either single or mixed, were detected. However, 15 samples tested positive for pure infestations by *P. vivax*, corresponding to 5 children [41].

In the Democratic Republic of the Congo, 292 dried blood samples from children who participated in the Demographic and Health Survey of 2013-2014 were analysed. Fourteen cases of *P. vivax* malaria were identified through PCR, and nine were co-infected with *P. falciparum*. All *P. vivax* cases occurred in rural households, and only 5 out of the 14 cases were reported using long-lasting insecticidal nets. Regarding socio-economic status, 9 14 cases were classified within the poorest population [37]. Another study in the Congo assessed men and women aged 15 to 59 years and 15 to 49 years, respectively. Out of the 17,972 samples screened for *P. vivax* infestation, 579 tested positive through rapid PCR, and 534 were confirmed by nested PCR (92.2%), indicating strong agreement (Kappa = 0.80, $p < 0.05$). However, no further demographic data were available for this study [40].

In Nigeria, a study was conducted on blood samples from 242 subjects aged 25 years, of whom 55% were women. Malaria caused by *P. falciparum* was found in 133 individuals, while 6 cases showed mixed infestation of *P. falciparum*/*P. ovale*, 3 cases were attributed to *P. vivax*, and 1 case had co-infection of *P. falciparum*/*P. vivax*, and 1 case involved *P. malariae* and *P. ovale* [45]. Another study specifically targeted the states of Lagos (hypoendemic with a prevalence of 1.9%) and Edo (mesoendemic with a prevalence of 35%). It included patients over two years old presenting clinical symptoms of malaria. A total of 2,376 patients were enrolled, and malaria was confirmed in 436 samples. The mean age was 23 years, and 55% were women. *Plasmodium* RNA was amplifiable in 58.7% (n=256/436) of the subjects, with 110 from Edo and 146 from Lagos. The majority of cases were attributed to *P. falciparum* as a mono-infection (85.5%; n=219/256; 97 from Edo and 122 from Lagos) or mixed with *P. malariae* (6.3%; n=16/256), *P. vivax* (1.6%; n=4/256), or *P. ovale* (1.2%; n=3/256) [34].

In Cameroon, analysis was conducted on febrile outpatient patients of all ages who sought consultation at the Santchou, Dschang, and Kyéossi health centres. The individuals included were 400, 500, and 101, respectively. Two hundred eighty-seven cases of *P. falciparum* infestation were detected, along with 142 cases of *P. vivax*, 2 cases of *P. ovale*, and 3 cases of *P. malariae*. Additionally, there were 37 cases of co-infection with *P. falciparum*/*P. vivax*, 2 cases of *P. falciparum*/*P. ovale*, 4 cases of *P. falciparum*/*P. malariae*, and 2 cases of *P. vivax*/*P. malariae* [43]. In Dschang, a total of 484 samples were obtained from febrile outpatient patients by other researchers. Malaria parasite DNA was identified in 70 samples (14.5%), including 68 cases of mono-infection by *Plasmodium* (42 cases of *P. falciparum*, 25 cases of *P. vivax*, and 1 case of *P. malariae*), as well as 2 cases of co-infection with *P. falciparum*/*P. vivax*. Among the affected individuals, 57.1% were male, and the median age was 24. Specifically, 74.3% originated from an urban population area. In this case, a 2.3 times higher likelihood of testing positive for *Plasmodium* by PCR (95% CI: 1.39-3.89; $p = 0.0014$) was found to be associated with being male [33]. A third study involved 485 symptomatic patients who attended hospitals in five different areas in the country's southern region. PCR confirmed a total of 201 malaria cases, with 193 (96%) attributed to *P. falciparum*, six patients (3%) to *P. vivax*, and two cases (1%) to mixed infections of *P. falciparum*/*P. vivax*. Approximately 52% of the patients were male, and individuals up to 82 were included [20]. In another study conducted explicitly in Bolifamba, a rural

 doi:10.20944/preprints202307.1020.v1

multiethnic environment situated at an altitude of 530 meters on the eastern slope of Mount Cameroon, samples were collected from 269 individuals. The results revealed a *Plasmodium* prevalence of 32.3%. Exclusive or concomitant *P. vivax* infections accounted for 14.9% (n=13/87) of the cases, as established through PCR and microscopic examination [19].

In Mali, blood samples were collected from 300 children aged 0 to 6 years, revealing 25 cases of malaria caused by *P. vivax* and 109 cases caused by *P. falciparum*. However, no information was found regarding the population characteristics [38]. In Namibia, a study involved 952 individuals under 9, of whom 52.6% were females and 63.4% were afebrile. Most cases involved mono-infections by *P. falciparum* (n=23), and *P. vivax* infected three individuals. Additionally, there were four co-infections by *P. falciparum*/*P. vivax* and 3 by *P. falciparum*/*P. ovale* [31].

In Madagascar, a study focused on 129 individuals seeking antimalarial treatment between 2015 and 2017, all of whom had malaria caused by *P. vivax* [39]. Another study conducted in the western part of the country, specifically in Tsiroanomandidy, in the Bongolava region, analysed 2,143 subjects (53% females, average age of 19.6 years). This rural area is endemic to malaria caused by *P. falciparum* and *P. vivax*. Symptomatic malaria cases were sporadic, with only 11 individuals affected. *Plasmodium* invasions were generally submicroscopic, and 82.8% went undetected by microscopy (2.4% prevalence with microscopy-positive results (n=49) vs 13.8% prevalence with PCR-positive results (n=285)). Malaria cases caused by *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* were identified, although the last two were not detected by microscopy [36].

In India, on the Asian continent, 909 outpatient malaria patients and 2,478 healthy individuals were recruited between June and December 2015. The median age in the cases was 26 years, while in the controls, it was 30 years. Males constituted 92.8% of the cases and 57.5% of the controls. Only 4.2% of the cases reported using mosquito nets, compared to 42.4% in the control group. Among the patients, malaria caused by *P. vivax* (70%, n=633) was more prevalent than malaria caused by *P. falciparum* (9%, n=82) and the combination of *P. vivax*/*P. falciparum* (21%, n=194) [7]. Another study conducted in the borders between Thailand, Myanmar, and Malaysia collected samples from 1,100 malaria cases and 1,100 healthy subjects. Among them, 200 samples tested positive for *P. falciparum* and 900 tested positive for *P. vivax* [32].

In Latin America, specifically in Colombia, a study involving 320 volunteers was conducted, with women accounting for 59% of the participants. Among the volunteers, 73 individuals (23%) were Afro-Colombians, 74 (23%) were indigenous natives, and 173 (54%) were mestizos. Malaria was detected in 17% of the participants (52 out of 320) [16]. In Brazil, there have been reports of four studies. The first study diagnosed *P. vivax* malaria in 225 patients, of whom 52.4% were men. Among them, 97 had uncomplicated malaria, while 128 had severe malaria [44]. The second study evaluated 287 individuals (70% men) who were experiencing their initial diagnosis of *P. vivax* malaria without co-infection with other *Plasmodium* species or comorbidities [28]. The third study analysed blood samples from 690 individuals with a median age of 25. The aim was to assess the potential influence of DARC on susceptibility to clinical *P. vivax* malaria. The number of malaria episodes over seven years (2003-2009) was recorded. Although the median number of episodes was zero, a significant variation ranging from 0 to 24 was observed. The prevalence of malaria was 7% (n=35/498), with 89% of the cases being attributed to *P. vivax* [27]. The final study, conducted in the Marajó Archipelago situated east of the Amazon, involved the analysis of 678 individuals, and the presence of *Plasmodium* was detected in 137 samples, corresponding to 20.2% of the total samples analysed. The prevalence of *P. vivax* was determined to be 13.9% (n=94/678), while *P. falciparum* accounted for 5.8% (n=39/678) of the cases. Additionally, there were cases of co-infection involving *P. falciparum*/*P. vivax*, which represented 0.6% (n=4/678) of the cases [22].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 July 2023 doi:10.20944/preprints202307.1020.v1

Table 1. General characteristics of studies that assessed the relationship between Duffy genotype/phenotype and *P. vivax* prevalence.

Authors	Country	Results	*P. vivax* prevalence	Risk of bias	Certainty	Significance
Brown et al. [10]	Ghana	-952 adults -Absence of FY*BES allele in 90.5% of the population -No cases of *P. vivax*	0% for negative Duffy	Serious	⊕⊕⊕◯ (Moderate)	Important
Oboh et al. [45]	Nigeria	-242 malaria cases -All were Duffy negative genotype	2.7% for negative Duffy	Serious	⊕⊕⊕◯ (Moderate)	Important
Ferreira et al. [44]	Brazil	-225 malaria cases -Fy(a+b-): 31.1% -Fy(a+b+): 42.7% -Fy(a-b+): 24.8% -Fy(a-b-): 0.44%	0.4% for Fy(a-b-)	Serious	⊕⊕⊕◯ (Moderate)	Important
Djeunang et al. [43]	Cameroon	-1001 malaria cases -181 caused by *P. vivax* with Duffy negative genotype	18% for negative Duffy	Serious	⊕⊕⊕◯ (Moderate)	Important
Hoque et al. [42]	Sudan	-42 malaria cases -83.3% Duffy positive (10 homozygous/25 heterozygous)	16.7% for negative Duffy	Serious	⊕⊕⊕◯ (Moderate)	Important
Niang et al. [41]	Senegal	-74 malaria cases -Pure infestation by *P. falciparum*: 79.7%	20.3% for negative Duffy	Non-serious	⊕⊕⊕⊕ (High)	Critical
Brazeau et al. [40]	Democratic Republic of Congo	-172 infestations by *P. vivax* -14 infestations in Duffy negative individuals	8.3% for negative Duffy	Serious	⊕⊕⊕◯ (Moderate)	Important
Roesch et al. [39]	Cambodia and Madagascar	-174 malaria cases -T/T substitution in 100% in Cambodia / 44% T/T - 56% T/C in Madagascar	100% for positive Duffy	Serious	⊕⊕⊕◯ (Moderate)	Important
Niangaly et at. [38]	Mali	-Screening of 300 children	-	Non-serious	⊕⊕⊕⊕ (High)	Critical

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 July 2023 doi:10.20944/preprints202307.1020.v1

		-1 to 3 cases per 25 Duffy-negative children				
Albsheer et al. [12]	Sudan	-992 samples -190 infestations by *P. vivax* (Fy(a-b+): 67.9% / Fy(a+b-): 14.2% / Fy(a-b-): 17.9%	67.9% Fy(a-b+) / 17.9% Fy(a-b-)	Non-serious	⊕⊕⊕⊕ (High)	Critical
Brazeau et al. [37]	Democratic Republic of Congo	-17,972 samples -579 infestations by *P. vivax* and 467 sequencings (n=464/467 for Duffy negative)	99.3% for negative Duffy	Serious	⊕⊕⊕◯ (Moderate)	Important
Howes et al. [36]	Madagascar	-1878 adults -48.7% Duffy negative -86 and 44 infestations by *P. vivax* with Duffy positive and negative, respectively	8.9% for negative Duffy / 4.8% for positive Duffy	Serious	⊕⊕⊕◯ (Moderate)	Important
Kepple et al. [11]	Sudan and Ethiopia	-107 and 305 individuals infected with *P. vivax* for Duffy negative and positive	-	Serious	⊕⊕⊕◯ (Moderate)	Important
Lo et al. [35]	Sudan and Ethiopia	-1963 samples -332 infestations by *P. vivax* (49 for Duffy negative)	9.2% – 86% for negative Duffy	Serious	⊕⊕⊕◯ (Moderate)	Important
Oboh et al. [34]	Nigeria	-436 samples and 256 cases -5 infestations by *P. vivax* (all Duffy negative homozygotes)	1.95% for negative Duffy	Serious	⊕⊕⊕◯ (Moderate)	Important
Russo et al. [33]	Cameroon	-484 samples -27 infestations by *P. vivax* (all Duffy negative)	5.6% for negative Duffy	Serious	⊕⊕⊕◯ (Moderate)	Important
Hongfongfa et al. [32]	Thailand and Myanmar	-900 cases of *P. vivax* -FY*A/*A: 83.5% of cases	0% for Fy(a–b–)	Non-serious	⊕⊕⊕⊕ (High)	Critical
Haiyambo et al. [31]	Namibia	-33 cases and 47 controls -3 infestations by *P. vivax* (all Duffy negative)	9% for negative Duffy	Non-serious	⊕⊕⊕⊕ (High)	Critical

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 July 2023 doi:10.20944/preprints202307.1020.v1

Popovici et al. [30]	**Cambodia**	**-22 Duffy positive cases (16 FY*A/*A homozygotes)**	**-**	Serious	⊕⊕⊕◯ (Moderate)	Important
Gai et al. [7]	**India**	**-909 malaria cases (43.9% FY*A/A vs 44.1% FYA/*B)** **-633 infestations by *P. vivax* (44.2% FY*A/A vs 43.7% FYA/*B)**	**0.3% for negative Duffy**	**Non-serious**	⊕⊕⊕⊕ (High)	**Critical**
De Silva et al. [29]	**Malaysia**	**-79 infestations by *P. knowlesi*** **-Equal distribution of FY*A/A and FYA/*B genotypes**	**-**	**Non-serious**	⊕⊕⊕⊕ (High)	**Critical**
Abou-Ali et al. [28]	**Brazil**	**-287 infected by *P. vivax*** **-23.7% FYA/FYA; 42.8% FYA/FYB; 3% FYB/FYB**	**-**	Serious	⊕⊕⊕◯ (Moderate)	Important
Kano et al. [27]	**Brazil**	**-Reduction in risk of clinical *P. vivax* malaria by 19% and 91% for FYA/B^{ES} and FYB^{ES}/B^{ES} genotypes, compared to FYA/*B**	**-**	Serious	⊕⊕⊕◯ (Moderate)	Important
Lo et al. [26]	**Ethiopia**	**-145 symptomatic individuals infected by *P. vivax*** **-69.7% FY*A/B^{ES} or FYB/*B^{ES}** **-1.4% FY*B^{ES}/*B^{ES} (Duffy negative homozygotes)**	**-**	Serious	⊕⊕⊕◯ (Moderate)	Important
Miri-Moghaddam et al. [25]	**Iran**	**-160 infestations by *Plasmodium*** **-FY*A/*B: 51.9%** **-FY*A/*A: 16.3%** **-FY*B/*B: 13.8%** **-FY*A/*B^{ES}: 10%**	**0.6% for negative Duffy**	**Non-serious**	⊕⊕⊕⊕ (High)	**Critical**
Weppelmann et al. [24]	**Haiti**	**-164 cases** **-99.4% FY^{ES} allele**	**-**	Serious	⊕⊕⊕◯ (Moderate)	Important
Lo et al. [23]	**Ethiopia**	**-416 samples and 94 cases for Duffy negative** **-3 cases of *P. vivax* in Duffy negative**	**3.1% for negative Duffy**	**Non-serious**	⊕⊕⊕⊕ (High)	**Critical**
Carvalho et al. [22]	**Brazil**	**-678 cases and 94 infestations by *P. vivax***	**6.9% for negative Duffy**	Serious	⊕⊕⊕◯ (Moderate)	Important

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 July 2023 doi:10.20944/preprints202307.1020.v1

		-29 Duffy negative individuals (2 cases of *P. vivax*)				
Woldearegai et al. [21]	**Ethiopia**	**-1931 adults** **-111 cases of *P. vivax***	**20% for negative Duffy**	Serious	⊕⊕⊕◯ (Moderate)	Important
Ngassa et al. [20]	**Cameroon**	**-201 symptomatic cases** **-8 cases of *P. vivax* infestation**	**3.9% for negative Duffy**	Serious	⊕⊕⊕◯ (Moderate)	Important
Fru-Cho et al. [19]	**Cameroon**	**-87 malaria cases** **-12 infestations by *P. vivax* (6 in Duffy negative individuals)**	**6.8% for negative Duffy**	Serious	⊕⊕⊕◯ (Moderate)	Important
Abdelraheem et al. [18]	**Sudan**	**-126 suspected cases** **-48 confirmed cases of *P. vivax* (4 in Duffy negative individuals)**	**8.3% for negative Duffy**	Serious	⊕⊕⊕◯ (Moderate)	Important
De Silva et al. [17]	**Malaysia**	**-111 samples** **-Fy(a+b−): 89.2%** **-FY*A/*A: 48 cases**	**0% for negative Duffy**	**Non-serious**	⊕⊕⊕⊕ (High)	**Critical**
Gonzalez et al. [16]	**Colombia**	**-52 individuals infected by *Plasmodium* (14 with *P. vivax*)** **-Amerindians and mestizos: T-46 allele in 90%–100% / Afro-Colombians 50%**	**-**	Serious	⊕⊕⊕◯ (Moderate)	Important

3.4. Relationship between Duffy genotype/phenotype and prevalence of Plasmodium vivax

Heterogeneous and interesting results were found in Africa, Asia, and Latin America in the relationship between being a carrier of the Duffy negative or positive genotype/phenotype and invasion by *P. vivax*.

In Ghana, among the 952 subjects studied (845 symptomatic and 107 healthy individuals), the FY*BES/BES genotype corresponding to the Duffy negative phenotype Fy(a−b−) was found in 862 subjects, while 53 individuals had the FYA/BES genotype with the Fy(a+b−) phenotype. Additionally, 22 individuals had the FYB/BES genotype with the Fy(a−b+) phenotype, and 15 had the FYA/B genotype with the Fy(a+b+) phenotype. No cases of *P. vivax* malaria were confirmed through PCR, and the limited evidence of *P. vivax* pathology was attributed to the high frequency of the FYES allele [10].

In Sudan, an analysis was conducted on 412 blood samples from patients infected with *P. vivax*. Of these samples, 305 were identified as Duffy positive and 107 as Duffy negative; from Ethiopia, 150 were Duffy positive and 83 were Duffy negative; while from Sudan, were 155 Duffy positive and 24 Duffy negative samples. Among individuals with Duffy negative status, the infestation rate was highest at 17.2%, while among Duffy positive individuals, the highest rate was 30.7% [11]. In another region of Sudan, in patients with symptomatic malaria, the following observations were made: out of 190 samples with *P. vivax* malaria and 67 healthy individuals, 129 cases (67.9%) were Fy(a-b+),

 doi:10.20944/preprints202307.1020.v1

14.2% were Fy(a+b-), and 17.9% were Fy(a-b-). The Fy(a+b+) phenotype was not detected. Among the healthy individuals, 45 (67.1%) were Fy(a-b-), 29.9% were Fy(a-b+), and only 3% had the Fy(a+b-) phenotype. The Fy(a-b+) and Fy(a+b-) phenotypes were significantly higher in *P. vivax*-infected patients than in healthy individuals ($p < 0.01$). Conversely, Duffy negative individuals (Fy(a-b-)) exhibited a significantly lower proportion of *P. vivax* infestation ($p < 0.01$). The most prevalent phenotype was Fy(a-b+). In New Halfa, 62.5% (n=25/40) of *P. vivax* samples were classified as Fy(a-b+). The prevalence of infestation in Khartoum and the Nile River showed a similar trend, with Fy(a-b+) accounting for 80.2% and 46.9%, respectively. Fy(a-b-) individuals had significantly lower levels of *P. vivax* parasites compared to Fy(a+b-) and Fy(a-b+) individuals ($p < 0.001$) [12].

In another study conducted in the same country, it was revealed that among 42 patients with *P. vivax* malaria, 35 (83.3%) were identified as Duffy positive (10 homozygotes and 25 heterozygotes), while 7 (16.7%) were Duffy negative. This study detected 7 cases of P. vivax in Duffy-negative individuals, characterised by mutations in six PvRBP haplotypes. However, it was observed that 5 PvRBP haplotypes were shared between Duffy negative and Duffy positive individuals, except for one haplotype exclusive to Duffy negative individuals [42]. Lo et al. [35], who investigated this phenomenon in Sudan, Botswana, and Ethiopia, demonstrated that in Botswana, 83.5% (n=147/176) of febrile patients were Duffy negative. Among the febrile patients in Kweneng East, 3% (n=9/301) tested positive for *P. vivax*, comprising 8 Duffy negative homozygotes (CC) and one Duffy positive heterozygote (TC). In Tutume, 6.8% (n=12/176) of febrile patients were identified with *P. vivax*, with 10 of them being Duffy negative. Conversely, the proportion of febrile individuals from Ethiopia exhibited a Duffy negative pattern of 35.9% (n=235/655). In Bonga, 30.3% (n=125/413) of febrile patients were diagnosed with *P. vivax*, and 3.2% (n=4/125) were Duffy negative. In Sudan, 831 samples were collected, confirming 101 cases of *P. vivax* infestation, with seven occurrences in Duffy-negative individuals [35].

In Ethiopia, a study involving 178 individuals (145 with symptomatic *P. vivax* malaria and 33 asymptomatic) revealed that 101 (69.7%) of the symptomatic individuals had heterozygosity with a silenced Duffy allele (FY*A/BES or FYB/BES), two (1.4%) were homozygotes for Duffy negativity (FYBES/BES), 17 (11.7%) were homozygotes for Duffy positivity (FyA/A or FyB/B), and 25 (17.2%) were heterozygotes positive for Duffy (FyA/*B) [26]. Another study, which assessed 416 symptomatic malaria patients, identified that 94 (23%) samples exhibited homozygosity for the CC genotype at nucleotide position -33 (indicating Duffy negativity). The Duffy-positive samples numbered 322 (77%), with 108 (26%) being TT homozygotes and 214 (51%) being CT heterozygotes. Two of the 94 Duffy-negative samples tested positive for *P. vivax*, indicating mixed *P. vivax*/*P. falciparum* infections. Among samples from healthy individuals, 35.6% (n=139/390) were found to be Duffy negative, a proportion significantly higher compared to the case group ($p < 0.0001$). Overall, the prevalence of *P. falciparum* exceeded that of *P. vivax* in both Duffy-positive and Duffy-negative subjects [23]. In the same country, an analysis of 205 *Plasmodium*-positive samples revealed the presence of the FYA/A genotype in 1 individual (0.9%) in Jimma, FYA/B in 11 subjects (11.2%) in Harar and 14 (13.1%) in Jimma. The FYA/BES genotype was detected in 4 individuals (4.1%) in Harar and 18 (16.8%) in Jimma. Conversely, the most prevalent genotype was FYB/B, identified in 51 cases (52%) in Harar and 21 (19.6%) in Jimma. The FYB/*BES genotype was observed in 15 subjects (15.3%) in Harar and 29 (27.1%) in Jimma. Regarding the phenotype, Duffy's positive phenotype was identified in 82.7% and 77.6% of individuals in Harar and Jimma, respectively, while Duffy's negative phenotype prevalence in these locations was 17.3% and 22.4% [21].

In Senegal, a study was conducted on 48 children who were classified as Duffy negative (FYBES/BES), including five with *P. vivax* infection, which confirmed a surprisingly high proportion (20.3%) of *P. vivax* malaria among children with a negative phenotype [41]. In the Congo, the analysis of 292 samples from children by Brazeau et al. [37] confirmed that 14 *P. vivax*-positive children exhibited a negative phenotype. Another study conducted in the Congo, involving genotyping of 467 samples infected with *P. vivax*, enabled the calculation of a national prevalence of this parasite at 2.96% (95% CI: 2.28% - 3.65%). Almost all individuals affected by vivax malaria had a Duffy negative status (n=464/467; 99.36%) [40].

 doi:10.20944/preprints202307.1020.v1

In Nigeria, an analysis of 242 samples revealed that a single cytosine at nucleotide position 33 was present in four patients who had experienced *P. vivax* malaria. This finding confirmed the absence of Duffy gene expression in their cells, indicating a Duffy-negative genotype [45]. In Dschang, two homozygous Duffy positive genotypes (-33 TT), two heterozygotes (-33 TC), and 224 Duffy negative individuals (-33 CC) were identified by other researchers. All individuals with *P. vivax* demonstrated a Duffy-negative genotype. The overall frequency of the -33T allele was 1.3%, corresponding to a frequency of 1.7% (n=4/228) of positive Duffy phenotypes (homozygotes and heterozygotes) [33]. Another study in Cameroon revealed that among 201 malaria cases, including 8 cases of *P. vivax*, all eight patients exhibited the -33 CC mutation, indicating a Duffy-negative status in all eight native Cameroonians [20]. In Bolifamba (Cameroon), it was determined that 50% of individuals (n=6/12) affected by *P. vivax* malaria were also negative for the Duffy receptor [19].

In Mali, among 25 cases of *P. vivax* malaria in children, a Duffy negative genotype with the presence of the T to C mutation in the GATA1 5' binding site of the open reading frame was identified in all cases [38]. Namibia conducted a study on febrile children, of whom 7 had *P. vivax* malaria, either as mono-infection or in conjunction with *P. falciparum*. Five participants with *P. vivax* tested negative for the Duffy gene mutation. Among 9 out of 41 participants not infected with *Plasmodium* and 7 out of 28 participants with *P. falciparum*, the FYA genotype (Duffy positive) was present. There was a C136 G > A mutation in exon two that was present in all patients with *P. vivax* infection (n=5/7) [31].

In Madagascar, out of 129 individuals with *P. vivax* malaria, 55 exhibited a Duffy positive genotype, with 56% being heterozygotes and 44% being homozygotes for DARC gene expression [39]. Another study reported that out of 1878 individuals, the most frequent allele was the silent erythrocyte allele FYBES, followed by FYA and FYB. Approximately 48.7% of the subjects had a Duffy negative phenotype. Among Duffy-positive individuals (51.3% of the total population), Fy(a+b-) was the most common phenotype at 34.5%, followed by Fy(a-b+) at 11.6%, and Fy(a+b+) at 5.2%. The number of Duffy-positive individuals with *P. vivax* was 86 (8.9%), while Duffy-negative individuals were 44 (4.8%). Thus, it was determined that the risk of malaria for Duffy-negative hosts was half that of Duffy-positive hosts (prevalence of 4.8% vs 8.9%; OR 0.52; 95% CI: 0.35 - 0.75; p <0.001). There were no statistically significant differences in the likelihood of infection between homozygous Duffy positive and heterozygous Duffy individuals (p=0.429), although heterozygotes had a slightly lower infection prevalence (8.5% vs 10.2%). Finally, no association was found between Duffy's blood type and *P. falciparum* malaria [36].

In Asia, specifically in Cambodia, among 453 individuals with *P. vivax* malaria, the genotype was determined for 119 individuals who exhibited Duffy positivity with the T-33C substitution: T/T, indicating their homozygous Duffy-positive status [39]. In a separate study conducted by Popovici et al. in the same country, it was reported that all genotyped reticulocytes demonstrated Duffy positivity, with the majority of them (n=16/22) being homozygous FY*A/A. At the same time, the remaining (n=6/22) were heterozygous FYA/*B [30].

In India, a study genotyping all its cases and controls revealed the exclusive occurrence of DARC 298A when 125A was also present, specifically in FYB (p <0.0001) [7]. Within the study case sample, the most prevalent Duffy genotypes were FYA/A (43.9%) and FYA/B (44.1%), while the FYB/B genotype was present in 11.9% of cases. It is important to note that these genotypes showed no differences between cases and controls and thus were not independently associated with malaria odds, regardless of parasite species stratification. Genotypes associated with reduced expression of the FYB allele were more frequently observed in malaria patients (16.7%; n=152/909; p=0.19), particularly in those with *P. vivax* malaria (17.7%, n=112/633; p=0.09), compared to healthy controls (14.5%; n=132/909). When assessing hospitalisation rates among Duffy genotypes, it was determined that among cases, 3.5% (n=32/909) and 3.8% (n=35/909) of individuals were hospitalised with severe malaria, respectively. The proportion of hospitalised patients was higher in FYA/A individuals (5.0%; n=20/399), lower in FYA/B (3.5%; n=14/401, p=0.29), and significantly lower in FYB/B (0.9%; n=1/109, p=0.06). Duffy blood group negativity was observed in 0.3% of cases [7].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 July 2023 doi:10.20944/preprints202307.1020.v1

In Thailand and Myanmar, the FYA/A genotype was identified in 83.5% of patients and 75.0% of healthy individuals, while FYA/B was observed in 13% of patients with *P. vivax* and 24% of healthy individuals. FYB/B was detected in 3.5% of patients with *P. vivax* and 1% of healthy individuals. None of the study participants exhibited the FYAES/BES blood group, indicating Fy(a-b-) phenotype. The frequency of FYA/A was significantly higher in patients with *P. vivax* infection compared to healthy subjects (p=0.036). In contrast, the frequency of FYA/B was significantly higher in healthy subjects compared to those infected with *P. vivax* (p=0.005). Although FYB/B was more prevalent in patients with *P. vivax*, the difference was not statistically significant. The FY gene mutation at nucleotide position 265 could not be confirmed in 167 samples that tested negative for FYB [32]. In Iran, the analysis results revealed that the most common Duffy genotype in cases was FYA/B (n=83; 51.9%), followed by FYA/A (n=26; 16.3%), FYB/B (n=22; 13.8%), FYA/BES (n=16; 10%), FYB/BES (n=11; 6.9%), and FYBES/BES (n=2; 1.3%). The predominant phenotype in the cases group was Fy(a+b+) at 51.9%, similar to the controls but with a slightly lower percentage of 41.3% [25].

In Latin America, particularly in Haiti, the presence of the FYBES allele was demonstrated in 99.4% (n=163/164) of *P. vivax* cases [24]. In Colombia, among Amerindian and mestizo populations, the T-46 allele frequency ranged from 90% to 100%, while among Afro-Colombians, it was 50%. At the 131 loci, the maximum frequency of the G allele was 30% in Amerindians, and the maximum frequency of the A allele was 69% in Afro-Colombians. The results revealed the absence of Duffy-negative individuals infected with *P. vivax* [16].

In Brazil, among several studies conducted, the initial study included 225 patients with *P. vivax* malaria. The distribution of the Duffy genotype/phenotype was as follows: 96 individuals had FYA/FYB, Fy(a+b+), 36 patients had FYA/FYBES, Fy(a+b-), 36 individuals had FYB/FYB, Fy(a-b+), 34 had FYA/FYA, Fy(a+b-), 20 individuals had FYB/FYBES, Fy(a-b+), 2 had FYA/FYAW, Fy(a+w), and 1 had FYBES/FYBES, Fy(a-b-) [44]. Another study involved 287 individuals diagnosed with *P. vivax* malaria, with FYA/FYA, observed in 63 subjects (23.7%), FYA/FYB in 114 individuals (42.8%), FYA/FYBES in 23 individuals (8.6%), FYA/FYBW in 01 individual (0.4%), FYB/FYB in 55 individuals (20.7%), FYB/FYBES in 08 individuals (3%), FYB/FYBW in 01 individual (0.4%), and FYBES/FYBW in 01 individual (0.4%) [28]. Similarly, researchers found in the Northeast region of the Brazilian Amazonas state that the Duffy genotype FYA/FYB was present in 29.6% of the population, FYA/FYA in 23.2%, and FYA/FYBES in 20.1%. Conversely, the FYBES/FYBES genotype was observed in only 3% of cases. Overall, the study population showed a predominance of the functional DARC alleles FYA (48%) and FYB (33.6%). An adjusted Poisson regression analysis, considering the place of residence and duration of residence in the endemic area, revealed a 19% risk reduction (95% CI: 2% - 32%; p=0.029) for clinical *P. vivax* malaria in individuals with FYA/FYBES genotype and a 91% risk reduction (95% CI: 67% - 97%; p=0.0003) in those with FYBES/FYBES genotype compared to individuals with FYA/FYB genotype. Conversely, individuals with FYB/FYBES genotype had a higher risk (26%; 95% CI: 3% - 53%; p=0.023) of clinical malaria compared to individuals with the reference genotype FYA/FYB. Furthermore, susceptibility to malaria decreased among DARC genotypes with longer duration of residence in the endemic area. Each additional year of residence in the endemic area resulted in a 3% reduction (95% CI: 2.5% - 3.4%; p<0.0001) in the risk of *P. vivax* malaria [27]. The latest study conducted in this country revealed that 4.3% (n=29/678) of the patients included in the study were categorised as Duffy-negative (FYBES/FYBES), whereas 95.7% (n=649/678) were classified as Duffy-positive. Among the individuals with Duffy-negative status, 6.9% (n=2/29) presented *P. vivax* malaria, whereas the prevalence was 14.7% among those with Duffy-positive status. The risk of *P. vivax* malaria occurrence in Duffy-negative individuals was lower, although not statistically significant, compared to Duffy-positive individuals (OR 0.4460; 95% CI: 0.1044 - 1.9060; p=0.3983) [22].

4. Discussion

Malaria remains the most important vector-borne parasitic disease in the world, with *P. vivax* being one the most important species according to its prevalence in some areas of the globe. The evolution of changes in genotype and phenotype expression trends over time in various regions of

continents with endemic areas, including Africa, Asia, and Latin America, should be emphasised. Notably, an association was observed between the Duffy-positive genotype and a higher incidence of *Plasmodium* infection. Nevertheless, despite the heterogeneous nature of the results, a clear trend indicating the lack of protective effect of the Duffy-negative genotype against malaria was evident. However, thoroughly analysing all the variables is necessary for a more comprehensive conclusion.

In terms of sex, findings indicate that in countries such as Sudan, Senegal, Nigeria, Namibia, and Madagascar, women represented the majority of malaria cases, with percentages ranging from 52.6% to 58.3% [18,31,34,36,41,45]. Conversely, Ethiopia, Cameroon, India, and Brazil exhibited a higher percentage of *Plasmodium* infestation in men, ranging from 50.3% to 92.8%. One study even reported an association between being male and a positive PCR result for *Plasmodium* (OR 2.3; 95% CI: 1.39 - 3.89; p = 0.0014) [7,20,21,28,33,44]. The data from WHO and CDC do not emphasise gender differences in the presentation of this disease. However, it is essential to note that pregnant women constitute a significant portion of the at-risk population, and malaria can have detrimental outcomes for both the mother and the newborn.

Regarding age, the analysed studies displayed significant variation. Some studies reported infestation in children under 11 years old, as observed in Sudan, Nigeria, Namibia, Mali, and Senegal [18,31,34,38,41]. Others, such as those conducted in Ethiopia and Madagascar, assessed individuals who were approximately 18 years old [23,36]. Studies conducted in Nigeria, Cameroon, India, and Brazil included subjects with average ages between 25 and 30 years [27,33]. In contrast, a study in Congo evaluated men and women up to 59 years old, while another study in Cameroon included individuals up to 82 years old [20,40]. These findings align with other analyses documenting high malaria prevalences in individuals under 20, particularly in countries with very low incomes [46–48]. An ecological analysis examining *Plasmodium* infestation and the risks of severe disease in Africa revealed that severe and potentially fatal malaria is predominantly concentrated among the youngest children (3 to 59 months of age). However, the risk of severe malaria changes throughout life, as the analysis by these authors suggests that even a few early-life infections may provide some functional immunity to severe malaria in all endemic settings [49]. Regardless, children under five remain the primary focus for malaria prevention and control in Africa.

Racial and demographic characteristics exerted influence on the disease's behaviour. Data from Haiti reveal that this infestation primarily affected the Afro-descendant population, while Colombia exhibited a more diverse ethnic distribution, with 23% Afro-Colombians, 23% indigenous natives, and 54% mestizo races [16,24]. In India, a study found that 77.8% of the evaluated cases were migrants, and only 33% had formal education, with a mere 4.2% utilising bed nets for sleeping [7]. In Congo, rural households with inadequate usage of bed nets and long-lasting insecticides experienced all cases of *P. vivax* malaria. Additionally, 64.3% of the affected individuals belonged to the poorest population segment in the region [37]. The use of bed nets treated with long-lasting insecticides has been established as an effective measure for reducing the risk of contracting malaria. For instance, Tsegaye et al. [50] reported that consistent use of insecticide-impregnated bed nets can reduce the probability of *Plasmodium* infestation by up to 92% compared to non-users (95% CI: 0.08 - 0.09) [50]. Unfortunately, religion is one of the variables influencing bed net usage, with individuals adhering to traditional beliefs exhibiting a reduced probability of net utilisation by up to 27% compared to Christianity [51,52]. Thus, access to insecticides and health education for vector-borne disease prevention remains a global health priority.

Shifting focus to the Duffy genotype/phenotype relationship and *P. vivax* invasion, studies have reported a predominance of the Duffy negative genotype FY*BES/*BES. Yet, no cases of *P. vivax* malaria were documented [10,24]. In contrast to reported cases in Ethiopia, Sudan, Cameroon, Madagascar, and Brazil, where Duffy-negative subjects had a range of *P. vivax* infestation proportions from 0.8% to 6.9% [21,22,32,36,38,42], the risk of malaria for Duffy-negative hosts in Madagascar was half that of Duffy-positive individuals (OR 0.52) [36]. Similarly, in Brazil, Duffy-negative subjects had a lower but not significantly different risk of presenting malaria caused by *P. vivax* compared to Duffy-positive individuals (OR 0.44; p=0.3983) [22]. Additional studies by Hoque et al. [42] and Keple et al. [11] demonstrated a higher prevalence of *P. vivax* malaria in Duffy-negative subjects, with rates

of 16.7% and 14%, respectively. Three notable studies conducted in vulnerable areas with minimal resources drew attention due to their high prevalence, ranging from 86% to 99% [33,35,40].

Only one systematic review has investigated the prevalence of *P. vivax* malaria in Duffy-negative individuals [53]. This review revealed that 100% of Duffy-negative subjects had reported cases of *P. vivax* malaria in 11 studies. These studies were conducted in various regions of Africa, including West Africa (Nigeria, Senegal, Mali, and Benin), Central Africa (Cameroon, Angola, and Equatorial Guinea), North Africa (Sudan), and East Africa (Kenya). Additionally, a meta-analysis of 14 studies showed that the combined prevalence of *P. vivax* infestation in Duffy-negative subjects was 25%. Furthermore, a meta-regression analysis was performed to determine the significance of the African continent as a covariate and a source of heterogeneity, which was found to be significant [53]. Notably, both this meta-analysis [53] and our systematic review found that the reported prevalences are higher than those described in high-risk populations, such as pregnant women, who have been observed to have figures up to 11.1% using a random-effects model [54], resulting in a combined prevalence of 4.5%. Similarly, the prevalence of congenital malaria is only 6.9% [55].

Finally, studies have reported a significantly higher frequency of the FYA/A genotype in *P. vivax*-infected patients than in healthy individuals. In comparison, healthy individuals showed a significantly higher frequency of FYA/B than those infected with *P. vivax* [28,32], indicating a highly heterogeneous distribution of Duffy genotypes and phenotypes. However, in Iran, malaria cases predominantly exhibited the FYA/B genotype (51.9%), followed by FYA/A (16.3%) [25]. In Brazil, the main genotypes/phenotypes identified among the cases were FYA/B, Fy(a+b+), FYA/BES Fy(a+b), and to a lesser extent FYBES/BES Fy(a-b-) [27,44]. When comparing these results with the evidence, the meta-analysis by Wilairatana et al. [53] revealed that Duffy genotype negativity acted as a protective factor against *P. vivax* infestation in studies conducted in Sudan, Madagascar, Ethiopia, and Mauritania. Only one study conducted in Brazil showed a higher risk of infestation among Duffy-negative individuals. However, four studies conducted in Cameroon, Ethiopia, Sudan, and Iran identified no differences in infestation risk. The combined analysis demonstrated decreased odds of *P. vivax* malaria among Duffy-negative individuals (OR 0.46; 95% CI: 0.26 - 0.82, p=0.009) [53]. These findings correlate with differences in the evolution pattern of *Plasmodium* and humans, as observed in the global variation of genotypes and phenotypes. For instance, in Arabia, FYA and FYB antigens frequencies were 12.58% and 11.18%, respectively. The Fy phenotypes were distributed as follows: Fy(a+b-), 15 (10.48%); Fy(a-b+), 13 (9.10%); Fy(a+b+), 3 (2.10%); and Fy(a-b-), 112 (78.32%) [56]. On the other hand, in Colombia, the Fy(a-b-) phenotype had the highest prevalence (48%), followed by the Fy(a-b+) phenotype, the Fy(a+b-) phenotype, and to a lesser extent, the Fy(a+b+) phenotype [57]. Continuous research is necessary to assess risks and propose strategies from a translational approach that enables the development of drugs or effective and sustainable interventions over time. This research should focus on studying disease distribution and understanding the expression of Duffy genotype and phenotype in humans.

Regarding limitations, we must acknowledge that we identified a limited number of studies that report *P. vivax* infestation among Duffy-negative individuals. Additionally, there is insufficient scientific evidence concerning the relationship between the Duffy genotype/phenotype and infestation by other types of *Plasmodium*. Secondly, the studies exhibit high heterogeneity, making it challenging to organise the information and potentially causing confusion due to the topic's density and complexity. Thirdly, many studies employ a descriptive observational methodology, which hinders the extrapolation of associations that could enhance our understanding of the relationship between the parasites' Duffy binding protein and erythroid cell Duffy antigens. Consequently, it is not easy to establish causal principles. Then, the obtained information could not be meta-analyzed due to the high heterogeneity in the reporting of genotypes and phenotypes in the included studies, as well as significant variations in the methodology utilized in across these studies. Nonetheless, this systematic review represents one of the first comprehensive evaluations providing valuable and novel evidence on the relationship between Duffy genotype/phenotype and *P. vivax* prevalence.

5. Conclusions

No evidence of a gender-specific distribution of malaria between Duffy-negative men and women was found. However, evidence supports that the homozygous Duffy genotype positive for the A allele (FY*A/*A) is associated with a higher incidence of *Plasmodium* infestation. Furthermore, the negative Duffy genotype does not confer protection against this disease.

Author Contributions: YAPJ: Conceptualisation, methodology, formal analysis, investigation, writing—original draft preparation, and writing review and editing. IDLM: Conceptualisation, formal analysis, investigation, writing—original draft preparation, and writing review and editing. JEOC: Conceptualisation, formal analysis, investigation, writing—original draft preparation, and writing review and editing. JMF: Conceptualisation, investigation, writing—original draft preparation, and writing review and editing. DAL: Conceptualisation, methodology, investigation, writing—original draft preparation, and writing review and editing. NAL: investigation, writing—original draft preparation, and writing review and editing. MPBR: investigation, writing—original draft preparation, and writing review and editing. DKBA: investigation, writing—original draft preparation, and writing review and editing. AJRM: investigation, writing—original draft preparation, and writing review and editing.

Funding: APCs for this publication were covered.

Data Availability Statement: Not applicable

Ethics statement: This study did not involve human participants

Conflicts of Interest: The authors declare no conflict of interest.

References

1. World Health Organization. WHO Guidelines for malaria [Internet]. Geneva: World Health Organization; 2022 [cited 10 May 2023]. Available from: https://www.who.int/publications/i/item/guidelines-for-malaria
2. Centers for Disease Control and Prevention. Malaria [Internet]. [cited 10 May 2023]. Available from: https://www.cdc.gov/parasites/malaria/index.html
3. Boualam, M.A.; Pradines, B.; Drancourt, M.; Barbieri, R. Malaria in Europe: A Historical Perspective. Front. Med. 2021, 8, 691095.
4. Douglas, N.M.; Burkot, T.R.; Price, RN Malaria eradication revisited. Int. J. Epidemiol. 2022, 51, 382-392.
5. Kaur, H.; Sehgal, R.; Rani, S. Duffy antigen receptor for chemokines (DARC) and susceptibility to *Plasmodium vivax* malaria. Parasitol. Int. 2019, 71, 73-75.
6. Höher, G.; Fiegenbaum, M.; Almeida, S. Molecular basis of the Duffy blood group system. Blood Transfus. 2018, 16, 93-100.
7. Gai, P.P.; van Loon, W.; Siegert, K.; Wedam, J.; Kulkarni, S.S.; Rasalkar, R.; et al. Duffy antigen receptor for chemokines gene polymorphisms and malaria in Mangaluru, India. Malar. J. 2019, 18, 328.
8. Centers for Disease Control and Prevention. CDC Global Health Strategy [Internet]. [cited 10 May 2023]. Available from: https://www.cdc.gov/globalhealth/strategy/default.htm
9. Malleret, B.; El Sahili, A.; Tay, M.Z.; Carissimo, G.; Ong, A.S.M.; Novera, W.; et al. *Plasmodium vivax* binds host CD98hc (SLC3A2) to enter immature red blood cells. Nat. Microbiol. 2021, 6, 991-999.
10. Brown, C.A.; Pappoe-Ashong, P.J.; Duah, N.; Ghansah, A.; Asmah, H.; Afari, E.; et al. High frequency of the Duffy-negative genotype and absence of *Plasmodium vivax* infections in Ghana. Malar. J. 2021, 20, 99.
11. Kepple, D.; Hubbard, A.; Ali, M.M.; Abargero, B.R.; Lopez, K.; Pestana, K.; et al. *Plasmodium vivax* From Duffy-Negative and Duffy-Positive Individuals Share Similar Gene Pools in East Africa. J. Infect. Dis. 2021, 224, 1422-1431.
12. Albsheer, M.M.A.; Pestana, K.; Ahmed, S.; Elfaki, M.; Gamil, E.; Ahmed, S.M.; et al. Distribution of Duffy Phenotypes among *Plasmodium vivax* Infections in Sudan. Genes (Basel) 2019, 10, 437.
13. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021, 372, n71.
14. von Elm, E.; Altman, D.G.; Egger, M.; Pocock, S.J.; Gøtzsche, P.C.; Vandenbroucke, J.P.; et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J. Clin. Epidemiol. 2008, 61, 344-9.

15. Schumemann, H.; Brożek, J.; Guyatt, G.; Oxman, A. GRADE Handbook. Grading of Recommendations Assessment, Development and Evaluation, Grade Working Group. 2013. Available from: https://gdt.gradepro.org/app/handbook/handbook.html
16. Gonzalez, L.; Vega, J.; Ramirez, J.L.; Bedoya, G.; Carmona-Fonseca, J.; Maestre, A. Relationship between genotypes of the Duffy blood groups and malarial infection in different ethnic groups of Choco, Colombia. Colomb. Med. (Cali) 2012, 43, 189-95.
17. De Silva, J.R.; Lau, Y.L.; Fong, M.Y. Genotyping of the Duffy blood group among *Plasmodium* knowlesi-infected patients in Malaysia. PLoS One 2014, 9, e10895.
18. Abdelraheem, M.H.; Albsheer, M.M.A.; Mohamed, H.S.; Amin, M.; Mahdi Abdel Hamid, M. Transmission of *Plasmodium vivax* in Duffy-negative individuals in central Sudan. Trans. R. Soc. Trop. Med. Hyg. 2016, 110, 258-60.
19. Fru-Cho, J.; Bumah, V.V.; Safeukui, I.; Nkuo-Akenji, T.; Titanji, V.P.; Haldar, K. Molecular typing reveals substantial *Plasmodium vivax* infection in asymptomatic adults in a rural area of Cameroon. Malar. J. 2014, 13, 170.
20. Ngassa Mbenda, H.G.; Das, A. Molecular evidence of *Plasmodium vivax* mono and mixed malaria parasite infections in Duffy-negative native Cameroonians. PLoS One 2014, 9, e103262.
21. Woldearegai, T.G.; Kremsner, P.G.; Kun, J.F.J.; Mordmüller, B. *Plasmodium vivax* malaria in Duffy-negative individuals from Ethiopia. Trans. R. Soc. Trop. Med. Hyg. 2013, 107, 328-331.
22. Carvalho, T.A.; Queiroz, M.G.; Cardoso, G.L.; Diniz, I.G.; Silva, A.N.; Pinto, A.Y.; et al. *Plasmodium vivax* infection in Anajás, State of Pará: no differential resistance profile among Duffy-negative and Duffy-positive individuals. Malar. J. 2012, 11, 430.
23. Lo, E.; Yewhalaw, D.; Zhong, D.; Zemene, E.; Degefa, T.; Tushune, K.; et al. Molecular epidemiology of *Plasmodium vivax* and *Plasmodium* falciparum malaria among Duffy-positive and Duffy-negative populations in Ethiopia. Malar. J. 2015, 14, 84.
24. Weppelmann, T.A.; Carter, T.E.; Chen, Z.; von Fricken, M.E.; Victor, Y.S.; Existe, A.; et al. High frequency of the erythroid silent Duffy antigen genotype and lack of *Plasmodium vivax* infections in Haiti. Malar. J. 2013, 12, 30.
25. Miri-Moghaddam, E.; Bameri, Z.; Mohamadi, M. Duffy blood group genotypes among malaria *Plasmodium vivax* patients of Baoulch population in southeastern Iran. Asian Pac. J. Trop. Med. 2014, 7, 206-207.
26. Lo, E.; Hostetler, J.B.; Yewhalaw, D.; Pearson, R.D.; Hamid, M.M.A.; Gunalan, K.; et al. Frequent expansion of *Plasmodium vivax* Duffy Binding Protein in Ethiopia and its epidemiological significance. PLoS Negl. Trop. Dis. 2019, 13, e0007222.
27. Kano, F.S.; de Souza, A.M.; de Menezes Torres, L.; Costa, M.A.; Souza-Silva, F.A.; Sanchez, B.A.M.; et al. Susceptibility to *Plasmodium vivax* malaria associated with DARC (Duffy antigen) polymorphisms is influenced by the time of exposure to malaria. Sci. Rep. 2018, 8, 13851.
28. Abou-Ali, R.K.; Dhyani, A.; Terço, A.L.; Toro, D.M.; Gomes, K.S.; Tezza, L.C.; et al. Impact of Duffy polymorphisms on parasite density in Brazilian Amazonian patients infected by *Plasmodium vivax*. Malar. J. 2019, 18.
29. De Silva, J.R.; Amir, A.; Lau, Y.L.; Ooi, C.H.; Fong, M.Y. Distribution of the Duffy genotypes in Malaysian Borneo and its relation to *Plasmodium* knowlesi malaria susceptibility. PLoS ONE 2019, 14, e0222681.
30. Popovici, J.; Roesch, C.; Carias, L.L.; Khim, N.; Kim, S.; Vantaux, A.; et al. Amplification of Duffy binding protein-encoding gene allows *Plasmodium vivax* to evade host anti-DBP humoral immunity. Nat. Commun. 2020, 11, 953.
31. Haiyambo, D.H.; Aleksenko, L.; Mumbengegwi, D.; Bock, R.; Uusiku, P.; Malleret, B.; et al. Children with *Plasmodium vivax* infection previously observed in Namibia were Duffy negative and carried a c.136G > A mutation. BMC Infect. Dis. 2021, 21(1).
32. Hongfongfa, P.; Kuesap, J. Genotyping of ABO and Duffy blood groups among malaria patients in Thailand. J. Parasit. Dis. 2022, 46(1), 178-185.
33. Russo, G.; Faggioni, G.; Paganotti, G.M.; Djeunang Dongho, G.B.; Pomponi, A.; De Santis, R.; et al. Molecular evidence of *Plasmodium vivax* infection in Duffy negative symptomatic individuals from Dschang, West Cameroon. Malar. J. 2017, 16, 74.
34. Oboh, M.A.; Badiane, A.S.; Ntadom, G.; Ndiaye, Y.D.; Diongue, K.; Diallo, M.A.; et al. Molecular identification of *Plasmodium* species responsible for malaria reveals *Plasmodium vivax* isolates in Duffy negative individuals from southwestern Nigeria. Malar. J. 2018, 17, 439.
35. Lo, E.; Russo, G.; Pestana, K.; Kepple, D.; Abargero, B.R.; Dongho, G.B.D.; et al. Contrasting Epidemiology and Genetic Variation of *Plasmodium vivax* Infecting Duffy Negatives across Africa. Int. J. Infect. Dis. 2021, 108, 63-71.
36. Howes, R.E.; Franchard, T.; Rakotomanga, T.A.; Ramiranirina, B.; Zikursh, M.; Cramer, E.Y.; et al. Risk Factors for Malaria Infection in Central Madagascar: Insights from a Cross-Sectional Population Survey. Am. J. Trop. Med. Hyg. 2018, 99(4), 995.

37. Brazeau, N.F.; Whitesell, A.N.; Doctor, S.M.; Keeler, C.; Mwandagalirwa, M.K.; Tshefu, A.K.; et al. *Plasmodium vivax* Infections in Duffy-Negative Individuals in the Democratic Republic of the Congo. Am. J. Trop. Med. Hyg. 2018, 99(5), 1128-1133.
38. Niangaly, A.; Karthigayan Gunalan; Amed Ouattara; Coulibaly, D.; Sá, J.M.; Adams, M.; et al. *Plasmodium vivax* Infections over 3 Years in Duffy Blood Group Negative Malians in Bandiagara, Mali. Am. J. Trop. Med. Hyg. 2017, 97(3), 744-752.
39. Roesch, C.; Popovici, J.; Bin, S.; Run, V.; Kim, S.; Ramboarina, S.; et al. Genetic diversity in two *Plasmodium vivax* protein ligands for reticulocyte invasion. PLoS Negl. Trop. Dis. 2018, 12, e0006555.
40. Brazeau, N.F.; Mitchell, C.L.; Morgan, A.P.; Deutsch-Feldman, M.; Watson, O.J.; Thwai, K.L.; et al. The epidemiology of *Plasmodium vivax* among adults in the Democratic Republic of the Congo. Nat. Commun. 2021, 12, 4169.
41. Niang, M.; Sane, R.; Sow, A.; Sadio, B.D.; Chy, S.; Legrand, E.; et al. Asymptomatic *Plasmodium vivax* infections among Duffy-negative population in Kedougou, Senegal. Trop. Med. Health 2018, 46, 45.
42. Hoque, M.R.; Elfaki, M.M.A.; Ahmed, M.A.; Lee, S.K.; Muh, F.; Ali Albsheer, M.M.; et al. Diversity pattern of Duffy binding protein sequence among Duffy-negatives and Duffy-positives in Sudan. Malar. J. 2018, 17, 297.
43. Djeunang Dongho, G.B.; Gunalan, K.; L'Episcopia, M.; Paganotti, G.M.; Menegon, M.; Efeutmecheh Sangong, R.; et al. *Plasmodium vivax* Infections Detected in a Large Number of Febrile Duffy-Negative Africans in Dschang, Cameroon. Am. J. Trop. Med. Hyg. 2021, 104(3), 987-992.
44. Ferreira, N.S.; Mathias, J.L.S.; Albuquerque, S.R.L.; Almeida, A.C.G.; Dantas, A.C.; Anselmo, F.C.; et al. Duffy blood system and G6PD genetic variants in vivax malaria patients from Manaus, Amazonas, Brazil. Malar. J. 2022, 21, 144.
45. Oboh, M.A.; Singh, U.S.; Ndiaye, D.; Badiane, A.S.; Ali, N.A.; Bharti, P.K.; et al. Presence of additional *Plasmodium vivax* malaria in Duffy negative individuals from Southwestern Nigeria. Malar. J. 2020, 19, 229.
46. Onyiah, A.P.; Ajayi, I.O.; Dada-Adegbola, H.O.; Adedokun, B.O.; Balogun, M.S.; Nguku, P.M.; et al. Long-lasting insecticidal net use and asymptomatic malaria parasitaemia among household members of laboratory-confirmed malaria patients attending selected health facilities in Abuja, Nigeria, 2016: A cross-sectional survey. PLoS ONE 2018, 13(9), e0203686.
47. Umunnakwe, F.A.; Idowu, E.T.; Ajibaye, O.; Etoketim, B.; Akindele, S.; Shokunbi, A.O.; et al. High cases of submicroscopic *Plasmodium* falciparum infections in a suburban population of Lagos, Nigeria. Malar. J. 2019, 18(1), 433.
48. Swana, E.K.; Yav, T.I.; Ngwej, L.M.; Mupemba, B.N.; Suprianto; Mukeng, C.K.; et al. School-based malaria prevalence: informative systematic surveillance measure to assess epidemiological impact of malaria control interventions in the Democratic Republic of the Congo. Malar. J. 2018, 17(1), 141.
49. Paton, R.S.; Kamau, A.; Akech, S.; Agweyu, A.; Ogero, M.; Mwandawiro, C.; et al. Malaria infection and severe disease risks in Africa. Science 2021, 373(6557), 926-931.
50. Tsegaye, A.T.; Ayele, A.; Birhanu, S. Prevalence and associated factors of malaria in children under the age of five years in Wogera district, northwest Ethiopia: A cross-sectional study. PLoS ONE 2021, 16(10), e0257944.
51. Bawuah, A.; Ampaw, S. Ownership and use of insecticide-treated nets under Ghana's National Malaria Control Program: What are the correlates? Trop. Med. Int. Health 2021, 26(12), 1593-1608.
52. Kanmiki, E.W.; Awoonor-Williams, J.K.; Phillips, J.F.; Kachur, S.P.; Achana, S.F.; Akazili, J.; et al. Socio-economic and demographic disparities in ownership and use of insecticide-treated bed nets for preventing malaria among rural reproductive-aged women in northern Ghana. PLoS ONE 2019, 14(1), e0211365.
53. Wilairatana, P.; Masangkay, F.R.; Kotepui, K.U.; De Jesus Milanez, G.; Kotepui, M. Prevalence and risk of *Plasmodium vivax* infection among Duffy-negative individuals: a systematic review and meta-analysis. Sci. Rep. 2022, 12(1), 1-13.
54. Cardona-Arias, J.A.; Carmona-Fonseca, J. Meta-analysis of the prevalence of malaria associated with pregnancy in Colombia 2000–2020. PLoS ONE 2021, 16(7), e0255028.
55. Bilal, J.A.; Malik, E.E.; Al-Nafeesah, A.; Adam, I. Global prevalence of congenital malaria: A systematic review and meta-analysis. Eur. J. Obstet. Gynecol. Reprod. Biol. 2020, 252, 534-542.
56. Halawani, A.J.; Saboor, M.; Abu-Tawil, H.I.; Mahzari, A.A.; Mansor, A.S.; Bantun, F. Prevalence of Duffy Blood Group Antigens and Phenotypes among Saudi Blood Donors in Southwestern Saudi Arabia. Clin. Lab. 2021, 67(1).
57. Quintero-Santacruz, M.; Flórez Elvira, L.; Mejía Hurtado, A.F.; Macia Mejía, C. Estimated prevalence of the Duffy null phenotype Fy (a-b-) among black blood donors in Southwestern Colombia. Transfus. Apher. Sci. Off. J. World Apher. Assoc. Off. J. Eur. Soc. Haemapheresis. 2020, 59(6), 102884.