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Plasmodium vivax infections of Duffy-negative erythrocytes: historically undetected or a recent adaptation?

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Abstract

Plasmodium vivax is the main cause of malarial disease in Asia and South America. *Plasmodium vivax* infection was thought to be absent in African populations who are Duffy blood group antigen negative (Duffy-negative). However, many cases of *P. vivax* infection have recently been observed in Duffy-negative Africans. This raises the question: were *P. vivax* infections in Duffy-negative populations previously missed or has *P. vivax* adapted to infect Duffy-negative populations? This review focuses on recent *P. vivax* findings in Africa and reports views on the parasite ligands that may play a role in Duffy-negative *P. vivax* infections. In addition, clues gained from studying *P. vivax* infection of reticulocytes are presented, which may provide possible avenues for establishing *P. vivax* culture *in vitro*.

Keywords

Plasmodium vivax; Duffy blood group antigen; Duffy-negative; ligands

Introduction

Plasmodium falciparum is the leading cause of death due to malaria around the world; *Plasmodium vivax* also causes severe disease in humans, predominantly in Asia and South America [1-3]. The high death rate around the Thames estuary centuries ago was presumed to be caused by *P. vivax* [4]. *Plasmodium vivax* was thought to be absent in African populations who were **Duffy blood group antigen (Duffy antigen)** (see Glossary) negative (**Duffy-negative**). Recently, however, many cases of *P. vivax* infection in Duffy-negative populations have been reported in Angola, Benin, Botswana, Cameroon, Ethiopia,

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Equatorial Guinea, Kenya, Madagascar, Mali, Mauritania, Senegal, Sudan and Uganda [5-20]. Among these new cases, infection has been observed to be less severe in Duffy-negative Africans than in Duffy blood group antigen positive (Duffy-positive) Africans. The concern is that mutations/evolution in *P. vivax* may put Duffy-negative Africans at risk of severe disease in the future.

The potential importance of *P. vivax* in Africa may be underestimated by leaders and policy makers. One reason for this could be the reporting of *P. vivax* infections, usually done on a case-by-case basis, rather than as a holistic map across Africa. In addition, because *P. vivax* may be asymptomatic, there is a need for PCR diagnostic tools to determine the true frequency of *P. vivax* throughout Africa. If *P. vivax* infections are not considered, *P. vivax* may hamper malaria control efforts and elimination in the future. Here, we summarize the existing evidence for *P. vivax* malaria in Duffy-negative Africans, highlight the *P. vivax* **ligands** and erythrocyte **receptors** involved in parasite-host interactions, and discuss possible avenues for culturing *P. vivax,* which will help in understanding parasite biology and support *P. vivax* elimination efforts.

Evidence for the inability of *P. vivax* to invade Duffy-negative erythrocytes

Malaria therapy for neurosyphilis was developed in 1919 by Julius Wagner-Jauregg. However, in 1932 *P. vivax* was found to be ineffective in treating African-Americans, who were thus deemed to be *P. vivax*-resistant [21]. Although a majority was resistant to *P. vivax* infection, resistance was not universal. Young *et al.* demonstrated that refractoriness was present after inoculation of *P. vivax*-infected blood, indicating that resistance was at the erythrocyte level [22]. Garnham described the absence of *P. vivax* in West Africa; however, 11 Europeans returning from West Africa were infected with *P. vivax* [23].

In 1975, to identify the erythrocyte receptors for *P. knowlesi* (a parasite that causes malaria in primates), *P. knowlesi* was tested for invasion using erythrocytes that lacked various blood group determinants. Of the tested erythrocytes, it was discovered that the erythrocytes that lacked the Duffy antigen were refractory to invasion by *P. knowlesi* [24]. It was known at the time that Duffy-negative was common in the parts of Africa where *P. vivax* did not occur, suggesting that the Duffy antigen was the receptor for *P. vivax.* Subsequently, this speculation was tested in *P. vivax* in humans, by demonstrating the resistance of Duffy-negative African Americans to *P. vivax* infection, induced through infected-mosquito bites [25]. All Duffy-positive African Americans were susceptible to *P. vivax* infection [25]. Further studies on naturally occurring *P. vivax* infections demonstrated that Duffy-negative African Americans in Honduras [26] and in the US Army in Vietnam [27] were resistant to infection with *P. vivax.*

Plasmodium vivax in Duffy-negative African populations: A recent change?

Recently, *P. vivax* infection of Duffy-negative Africans has been observed in many parts of Africa (Figure 1 and Table 1) and in South America where Duffy-negative people of African origin live [5-11, 15, 19, 28-30]. *P. vivax* infections have also recently been observed in Senegal, Botswana and Uganda, but the Duffy antigen status in these cases was not

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determined [12, 16, 18, 31]. The first report of *P. vivax* infection in Duffy-negative Africans was from Kenya [5]. It is of significance that these infections were detected when the molecular tools became available to identify extremely low levels of *P. vivax* infection [5-13, 16, 17, 19, 28-30, 32] (Table 1). The work by Didier Menard and colleagues in Madagascar identified multiple cases of *P. vivax* in Duffy-negative Africans [6] (Figure 1 and Table 1). In addition, *P. vivax* infection in Duffy-negatives were found in Ethiopia [10, 14] and Sudan [17], where a high proportion of Duffy-positive people live [33]. Infections in Duffy-negative people in Madagascar and Ethiopia were less severe and had a lower parasitemia than in Duffy-positive people [6, 10, 14]. Since both Duffy-positive and -negative people live side-by-side in Madagascar, Ethiopia and Sudan (Figure 1) [33], it was suggested that infections were being passed back and forth between Duffy-positive and -negative people. It was presumed that selection would occur for Duffy-negative erythrocytes under circumstances where *P. vivax*-infected mosquitoes are constantly feeding on Duffy-positive people. This was found not to be the case, as *P. vivax* was reported in Duffy-negative populations in multiple places in Africa (Kenya, Mali and Cameroon) (Figure 1 and Table 1), where the predominant population is Duffy-negative [5, 11, 19, 20, 33].

In Bandiagara, Mali, a longitudinal study found around 2% of children were infected with *P. vivax,* all of whom were Duffy-negative [20]. All were asymptomatic, although anemia was seen in some children, which may have resulted from the *P. vivax* infection [20]. Twenty-seven *P. vivax*-infected Duffy-negative patients presented with fever in Dschang district hospital in Cameroon [19] (Table 1). Dschang is located 1400 meters above sea level with annual temperatures of 20.5 ± 6°C, which may not be the ideal temperature for *P. falciparum* development in mosquitoes, but appears to be suitable for *P. vivax* development. In Madagascar [6] and Ethiopia [10] some patients presented with malaria symptoms; however, a greater number of symptomatic cases were seen in Dschang. It is possible that *P. vivax* is more severe in Dschang because of mutations in the parasite there or is it because the patients were sampled from a large population of asymptomatic *P. vivax* in the community?

Merozoite-erythrocyte interactions

In 1995, the molecular basis for Duffy negativity was identified as a single point mutation in the binding site for GATA1, an erythroid transcription factor that binds upstream of the Duffy antigen coding region [34]. Because of the mutation, erythrocytes do not express the Duffy antigen on their surface and hence their refractoriness to *P. vivax* and *P. knowlesi* infection [24, 25]. The *P. vivax* ligands responsible for invasion of Duffy-negative erythrocytes are not known. Multiple ligands are found on *Plasmodium* **merozoites,** consisting of two families: Duffy binding proteins (DBP) and reticulocyte binding proteins (RBP) (Table 2). Duffy-binding proteins were first discovered in *P. falciparum* [35], later in *P. knowlesi* [36], and then in *P. vivax* [37], *Plasmodium knowlesi* has three DBP ligands (DBP α, β and γ). Only DBPα localized in the micronemes binds to human erythrocytes and requires the Duffy antigen for binding [36, 38, 39]. *Plasmodium knowlesi* merozoites attach to Duffy-negative erythrocytes and reorient apically, but cannot invade the erythrocytes [40]. Transmission electron microscopy has revealed that merozoites remain some distance from the Duffy-negative erythrocytes, with strands extending towards the erythrocyte, but **junction formation** does not occur [40]. The *P. vivax* DBP1 full length

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protein, as well as the recombinant Duffy binding domain (region II) of DBP1, binds strongly to Duffy antigen. This particular interaction is crucial for *P. vivax* merozoite entry and successful invasion into erythrocytes [14, 37, 41] (Table 2). However, DBP1 full length protein or the region II domain does not bind to Duffy-negative erythrocytes [14, 37, 41] and for this reason, *P. vivax* could not infect Duffy-negative Africans.

Several mutations have been identified in *P. vivax* DBP1 region II binding domain from Duffy-positive erythrocytes in Madagascar and from Duffy-negative erythrocytes in Ethiopia [14, 29]. None of the mutated DBP1 sequences bound to Duffy-negative erythrocytes [14]. In the Madagascar study, the DBP1 duplication in *P. vivax* was identified in Duffy-positive erythrocytes for the first time and the duplications were identical and in tandem [29]. Later, the DBP1 duplication was also identified in western Thailand, western Cambodia and Papua, Indonesia [42]. Another DBP1 variant duplication was identified in Cambodia, Ethiopia and Brazil [43]. Similarly, DBPα duplication, the ligand for human erythrocytes, was observed in *P. knowlesi* when the parasites were adapted to grow in human erythrocytes [44]. Interestingly, the **DBP1 copy number expansion** was more pronounced in *P. vivax* samples (three and eight copies) from Duffy-negative erythrocytes from Ethiopia. However, the significance of this expansion in *P. vivax* for invasion of Duffy-negative erythrocytes has not yet been determined [14]. Based on this result, we speculated that there could be **leaky expression** of Duffy antigen in Duffy-negative erythrocytes that could have selected for more copies of DBP1. More data are needed to determine if *P. vivax* infection in Duffy-negative erythrocytes from various regions have more DBP1 copies than in Duffypositive erythrocyte infections in the same region.

Duffy antigen exposure to *P. vivax* on erythrocytes

Interestingly, DBP1 Region II, expressed on **COS cell** surfaces, has been shown to bind to all aged erythrocytes [41]. Significantly, Choe *et al.* found that tyrosine 41 sulfation in the N-terminal region of Duffy antigen is critical for the binding of DBP1 to erythrocytes [45]. The binding of COS cells expressing DBP-1 to erythrocytes was blocked by the wild type Duffy antigen sequence, but not by a mutated sequence (tyrosine 41 to phenylalanine) that lacked sulfation of tyrosine 41 [45]. Subsequently, higher binding affinity of recombinant soluble DBP1 region II to **reticulocytes** compared to erythrocytes was shown in at least three independent studies [46-48]. However, COS cells expressing DBP1 region II bind to all aged erythrocytes, suggesting that multimeric cell surface expression of DBP1 overcame the low affinity binding of the monomer to all aged erythrocytes. Thus, the ligand avidity would be stronger on reticulocytes than erythrocytes [46-48]? The binding of soluble DBP1 region II is monomeric, whereas DBP1 region II has multiple ligands on the COS cells and at the apical tip of the merozoites, and multiple receptors on the erythrocytes that can compensate for low affinity binding. The greater binding of DBP1 region II soluble proteins to reticulocytes [46-48] suggests that single copy ligands bind more strongly to reticulocytes. Another explanation may be the loss of expression (e.g., clipping by a sulfatase) or masking of sulfation at the tyrosine domain on mature erythrocytes by binding a positively charged molecule. In addition, it was found that the binding of monoclonal antibodies specific for the N-terminal portion of the Duffy antigen decreases as the erythrocytes mature, including Fya- and Fyb- specific antibodies [48]. This may reflect

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protease clipping or masking of the N-terminus portion of Duffy antigen. Furthermore, there was a drop in binding to Duffy antigen by Fy3-specific antibodies that recognize the loop between the transmembrane domains 6 and 7 [48]. It is possible that some Duffy antigens are lost in the maturation of reticulocytes to mature erythrocytes or masked by other erythrocyte molecules. Yet, Duffy antigens can be detected on mature erythrocytes using Fya- and Fyb- specific antibodies. Perhaps Duffy antigen exposure to the parasite at the immature stage contributes to the inability to culture *P. vivax in vitro* in mature erythrocytes.

Another ligand of the Duffy family

The de novo genome assembly of a *P. vivax* isolate from Cambodia led to the identification of erythrocyte binding protein (EBP/DBP2), which has a conserved Duffy binding-like domain and C-terminal cysteine-rich domain before the transmembrane domain [49]. The Duffy binding domain of EBP binds to both Duffy-positive and Duffy-negative erythrocytes at a very low level [14]. However, a study by Ntumngia *et al.* showed that EBP/DBP2 binds preferentially to young/immature CD71 high reticulocytes (Table 2). Sequence alignment shows EBP region II has 50% similarity with *P. vivax* DBP1 region II [50]. The antibodies against DBP1 region II, however, did not inhibit EBP binding to immature reticulocytes [50], indicating that immunity to DBP1 does not block binding of DBP2.

Reticulocyte binding proteins of *P. vivax*

A second family of proteins involved in *P. vivax* erythrocyte invasion consists of the reticulocyte binding proteins (RBP) (Table 2), that were first discovered in *P. yoelii [51]* Two proteins (PvRBP1a and PvRBP2c) were identified in a λgt11 *P. vivax* cDNA expression library that bound to reticulocytes [52]. The RBP family contains five reticulocyte homology (RH) genes in *P. falciparum* [53]. With the completed *P. vivax* genome sequences, 11 *P. vivax* RH genes have been identified [49, 54]. Unlike the DBP genes localized in the micronemes, RH genes in *P. falciparum* are primarily found in rhoptries [53]. Recently, Gupta *et al.* identified the binding domains of PvRBP1a and PvRBP2c that bind to reticulocytes to be in the N-terminal region and mapped the regions for both PvRBP1a and PvRBP2c, showing them to be a 749 residue rRBP1.1 region and a 413 residue rRBP2.2 region, respectively [55]. Both bind to different receptors based on the host receptor enzyme specificity; PvRBP1a binds to a receptor that is sensitive to trypsin and chymotrypsin and the PvRBP2c receptor is neuraminidase, trypsin and chymotrypsin resistant [55] (Table 2). Naturally acquired antibodies specific for PvRBP1a or PvRBP2c inhibited the binding of PvRBP1a and PvRBP 2c, respectively, to reticulocytes, suggesting they would make promising vaccine candidates [55]. A 30 kDa region of PfRH4 binding domain that shares homology with PvRBP1a and PvRBP1b was recombinantly synthesized and found to bind to reticulocytes [47]. Antibodies against the PvRBP1a and PvRBP1b binding domain localized the protein in the micronemes [47], unlike the *P. falciparum* RHs, which are localized to rhoptries [53]. Gruszczyk *et al.* identified that the PvRBP2a$_{160-1000}$ domain binds all aged erythrocytes and the erythrocyte binding domain was further narrowed to PvRBP2a$_{160-455}$ [56]. Furthermore, the critical amino acid residues required for binding were determined to be within the PvRBP2a$_{160-455}$ region [56]. However, a binding study by Franca *et al.* identified that PvRBP1a, PvRBP1b, PvRBP2a and PvRBP2-P2 bind to mature

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erythrocytes and only PvRBP2b binds to reticulocytes [57] (Table 2). The difference in binding of PvRBP1a and PvRBP1b to reticulocytes or all aged erythrocytes needs to be resolved [47, 55, 57]. Recently, the crucial interaction between the erythrocyte receptor transferrin receptor 1 (CD71) and the parasite ligand PvRBP2b was identified, which might be critical for initial reticulocyte recognition [58] (Table 2).

Other potential ligands of *P. vivax*

Other genes such as glycophosphatidylinositol anchored micronemal antigen (GAMA) and tryptophan-rich antigens may be responsible for the low-density *P. vivax* invasion of Duffy-negative erythrocytes (Table 2). Like *P. falciparum,* GAMA is also localized in the micronemes of the *P. vivax* merozoites and has been shown to bind to Duffy-negative erythrocytes, and more recently has been shown to bind more efficiently to $CD71^{hi}$ immature reticulocytes than to mature erythrocytes [59, 60]. The GAMA-binding receptor is chymotrypsin sensitive (Fig. 2) and the antibodies against GAMA inhibit binding to the receptor [60].

Thirty-six tryptophan-rich antigens are present in *P. vivax* [54], of which ten proteins (PvTRAg, PvTRAg26.3, PvTRAg33.5, PvTRAg34, PvTRAg35.2, PvTRAg36, PvTRAg36.6, PvTRAg38, PvTRAg69.4 and PvATRAg74) bind to **receptors** on erythrocytes [61,62] (Table 2). One member of this family, PvTRAg36.6, which is localized on the apical tip of the merozoites, has been shown to interact with *P. vivax* early transcribed membrane protein (PvETRAMP) [63]. Another member, PvTRAg56.2, which is expressed on the surface of the merozoites but does not bind to erythrocytes, has been shown to interact with the PvMSP7 protein [61,63]. The tryptophan-rich protein PvTRAg38 has two erythrocyte-binding regions, of which one interacts with erythrocyte receptor band 3 [64, 65] and the second binding domain interacts with basigin [66] (Table 2), which is the receptor for RH5 [67, 68] and rhoptry-associated protein 2 [69] in *P. falciparum.* Interestingly, some tryptophan-rich proteins are highly expressed in clinical isolates, suggesting that these proteins are important in *P. vivax* infection [70].

Concluding Remarks and Future Perspectives

Technical advancements in diagnostics have improved markedly in the past few decades. Such advancements may account for the recent detection of low-density *P. vivax* in Africans, which appears to have been originally missed. Although, *P. vivax* infection in Africa is low density, *P. vivax* can cause malarial disease in Duffy-negative populations. Some of the reported cases of *P. vivax* in Africa were symptomatic [6, 10], with some possibly inducing anemia [5, 20]. However, we cannot rule out the possibility that *P. vivax* evolved to infect Duffy-negative individuals and could evolve further to cause severe disease in these individuals. This could be a disaster for low-income countries where *P. falciparum* is already a huge public health problem. Molecular tools enable the detection of submicroscopic *P. vivax* infection. It is critical that the accessibility of diagnostic capacities in malaria endemic areas is increased, to identify the rising incidence of *P. vivax* infection. Going forward, current inadequate malaria control and elimination strategies will be affected by an increase in *P. vivax* infections. Adequate investments, development of research capacities and

political commitment are required to learn more about *P. vivax* infection in Duffy-negative individuals and to gain insights into the ligands responsible for the invasion of erythrocytes, transmission and epidemiology.

The leading vaccine candidate for *P. vivax* is DBP1 region II. It will be interesting to test the DBP1 vaccine in Duffy-negative populations. If the vaccine is effective, it suggests that there may be leaky expression of Duffy antigen on Duffy-negative erythrocytes. At the same time, the role of other invasion pathways is unclear. Hence, more focus should be on other ligands involved in the invasion of Duffy-positive and negative erythrocytes. Are RBPs and EBP/DBP2 involved in Duffy-negative invasion? If so, then can the RBP and EBP/DBP2 ligands replace DBP1 for close apposition to allow junction formation? Furthermore, what ligands lead to apical membrane antigen 1 (AMA1) binding to erythrocytes and junction formation?

At present, research is hampered by the limited availability of *P. vivax* parasites. While collaborations with teams in different field sites and monkey-based studies have helped to achieve what is currently known about *P. vivax,* it is important to have a functioning *P. vivax* culture system, as in *P. falciparum,* to enable more research on *P. vivax.* For the past 100 years, studies have been conducted on culturing *P. vivax in vitro,* with little success. But significant improvements have been made in understanding *P. vivax* culturing *in vitro.* It is now known that *P. vivax* prefers reticulocytes over mature erythrocytes and grows better in reticulocytes from cord blood and maybe from bone marrow [71-74]. However, it is still unclear why *P. vivax* prefers reticulocytes. However, recently, *P. vivax* isolates from Madagascar were maintained for more than 200 days in *in vitro* conditions in Saimiri monkey erythrocytes with AIM V medium at 10% CO_2 concentration. The parasites were maintained with blood that was not enriched with reticulocytes [75]. This phenomenon may be restricted only to Saimiri monkey erythrocytes. If *P. vivax* growth to elevated levels were possible in human erythrocytes, such a culture would be useful for research.

Is it possible that *P. vivax* requires metabolites that are lost as the erythrocytes mature (e.g. glucose-6-phosphate dehydrogenease to maintain reduced glutathione)? Metabolomics has been used to identify several differences between the reticulocytes and mature erythrocytes in both rats and humans [76]. Metabolomics is one way forward in studying the metabolites of reticulocytes, erythrocytes and the serum components that may provide clues to understand *P. vivax* biology and for culturing *P. vivax.*

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Glossary

COS cell

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A mammalian fibroblast-like cell line which express the parasite ligand on its surface for binding erythrocytes.

DBP1 copy number expansion

Duffy binding protein 1 (DBP1) copy number expansion may play a role in *P. vivax* invasion of Duffy-negative erythrocytes, although duplication also occurs in countries (e.g., South East Asia) where Duffy negativity does not occur.

Duffy blood group antigen (Duffy-antigen)

Duffy blood group antigen is a protein that is expressed on the surface of the erythrocytes in Duffy-positive populations. It is the erythrocyte receptor for *P. vivax* invasion.

Duffy-negative

The GATA 1 transcription factor binding site on Duffy-negative erythrocytes upstream of the start codon of Duffy antigen gene has a point mutation that resists GATA 1 binding. Hence, expression of the Duffy antigen is absent on the surface of the erythrocytes. This mutation occurs in Africa and in Papua New Guinea.

Junction formation

Tight binding of the parasite to the erythrocyte that depends on the AMA1 and the RON 2 proteins and leads to erythrocyte entrance into a vacuole formed by the erythrocyte membrane.

Leaky expression

The GATA1 transcription factor could not bind to the Duffy antigen gene due to a mutation at the promotor region and hence the Duffy antigen at the protein level is not detectable by antibodies against it. However, low expression of Duffy antigen could still be possible.

Ligands

Parasite molecules that bind to erythrocyte receptors. DBP1 is an important ligand for *P. vivax*. DBP2 (EBP) is structurally similar to DPB1 but binds to a different erythrocyte receptor.

Merozoite

In blood-stage infections, merozoites are the form of the parasite that invade erythrocytes. After invasion, merozoite develop into rings, trophozoite and schizont stage parasite. DBP is a ligand expressed on the apical tip of merozoites and interact with Duffy antigens on the erythrocyte during invasion.

Receptors

Erythrocyte molecules to which parasite ligands bind for invasion. The Duffy blood group is the receptor for DBP1.

Reticulocytes

Reticulocytes are the immature stage of erythrocytes. Reticulocytes contain residual RNA but not the nucleus. *P. vivax* preferentially invades reticulocytes compared to mature erythrocytes.

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Highlights

- *Plasmodium vivax* infection is usually seen in Asia and South America but now is also observed in most parts of Africa.
- *P. vivax* is observed in Africa in places where Duffy-positive and Duffy-negatives live side-by-side and in places where Duffy-negativity is highly prevalent.
- Most *P. vivax* infections observed in Duffy-negatives have a low intensity parasitemia and are asymptomatic.
- Several invasion ligands other than DBP1 have been identified to bind to reticulocytes or mature erythrocytes. It will be interesting to identify the parasite ligands involved in *P. vivax* invasion of Duffy-negative erythrocytes.
- Unlike *Plasmodium falciparum, P. vi*vax preferentially invades immature reticulocytes that are mostly present in the bone marrow or cord blood. It is still unclear why *P. vivax* prefers young reticulocytes.

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Outstanding Questions

- When did *P. vivax* begin occurring in Duffy-negative Africans?
- Has *P. vivax* evolved to infect Duffy-negative Africans, or has technical advances in diagnostics helped identify *P. vivax* that previously was undetectable in blood films?
- If *P. vivax* parasites were missed in earlier studies in Africans, it is imperative to identify the parasite ligands that are involved in invasion of Duffy-negative erythrocytes. However, what if the parasites were not missed earlier but have evolved to infect Duffy-negative erythrocytes? This could be a major economic burden for countries that are still struggling to cope with *P. falciparum* infections. Is there a treatment regime for *P. vivax* in African countries?
- How does *P. vivax* infect Duffy-negative erythrocytes? What is the significance of the increase in the copy number of Duffy binding protein 1 (DBP1) in Duffy-negative infections? Is there leaky expression of Duffy blood group antigen in Duffy negatives or can the Duffy binding protein bind to different receptors with low affinity on Duffy-negative erythrocytes?
- Will the vaccine against DBP1 be effective when tested in Duffy-negative populations?
- Duffy binding protein 1 from Salvador I strain does not bind to Saimiri monkey erythrocytes but binds efficiently to Aotus monkeys and other *P. vivax* isolates bind to both. Can Salvador I strain in Saimiri monkeys serve as the model system to study Duffy-negative infections?
- Do *P. vivax*-like parasites infecting apes cause disease in humans?
- One major hurdle in *P. vivax* research is that there is no culture system to grow these parasites *in vitro*. Is the Duffy blood group antigen blocked or masked by other surface receptors or conformationally changed in mature erythrocytes compared to reticulocytes? If this is the case, is there a way to improve exposure of the Duffy blood group antigen in mature erythrocytes to improve in vitro culture?

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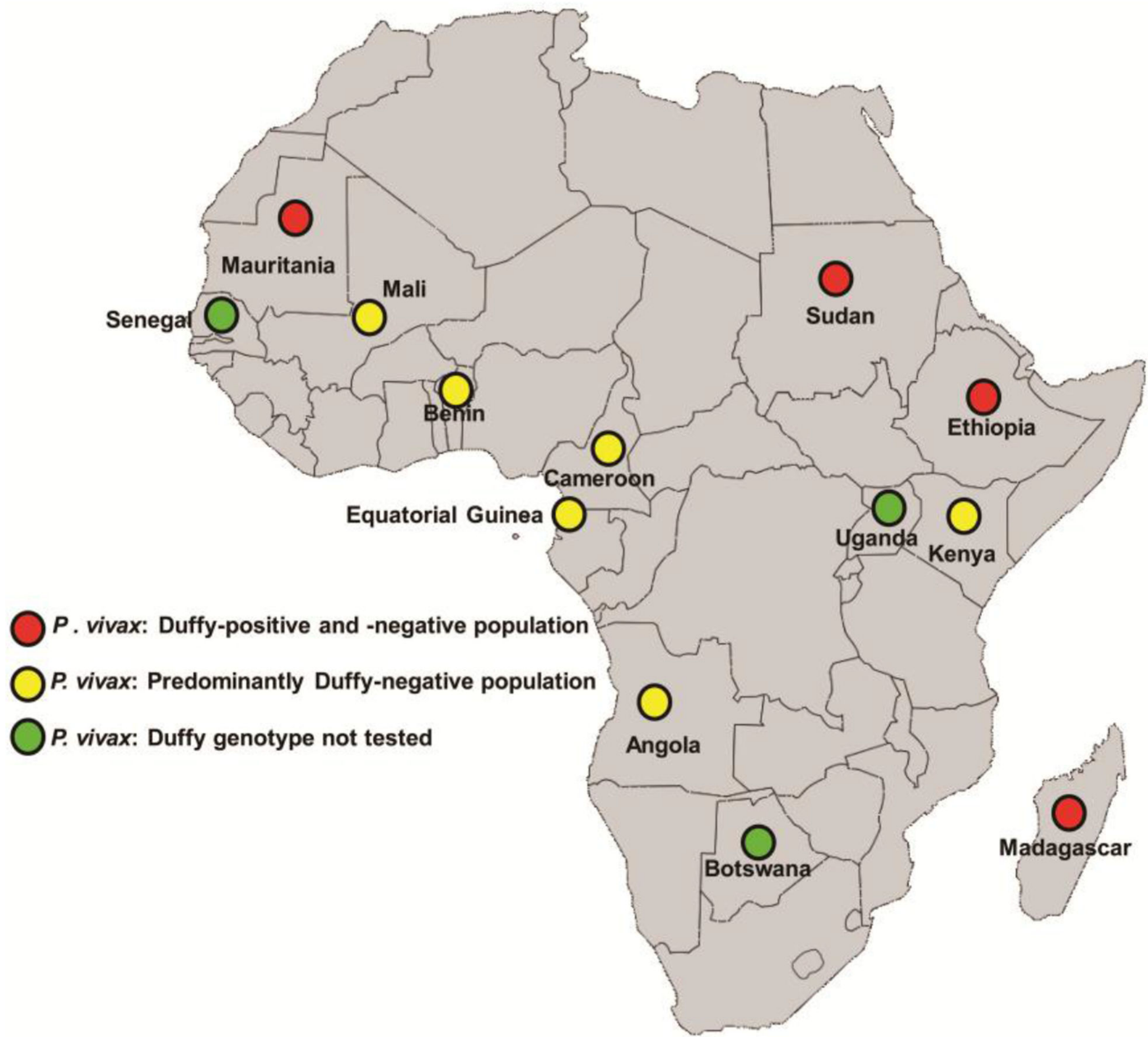

Figure 1. *Plasmodium vivax* infection in Duffy-negative populations in Africa

Map showing the regions on the African continent in which *P. vivax* infection in Duffy-negative people occurs. Red circles represent *P. vivax* infection in countries where both Duffy-positive and Duffy-negative people live side-by-side. Yellow circles represent molecular evidence of *P. vivax* infection in Duffy-negative individuals living in predominantly Duffy-negative populations in Sub-Saharan Africa. Green circles represent populations with *P. vivax* infection, but in which the Duffy status has not been checked. The map has been adapted from Wikimedia.

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Table 1.

Latest findings on Duffy-negative *Plasmodium vivax* infections in Africa and the molecular tools used.

Countries with *P. vivax* infection in Africa	Number of Duffy-negative infections	Diagnostic tools		Year	References
		P. vivax	Duffy genotyping		
Cameroon	27	Nested PCR[1], Sequencing	PCR – Melting curve analysis	2017	[19]
Mali	25	Microscopy, qPCR[2]	Nested PCR, Sequencing	2017	[20]
Uganda	4	Microscopy, RDT[3], Nested PCR	Not tested	2017	[18]
Ethiopia	2	Microscopy, Nested PCR, Sequencing	PCR, Sequencing	2016	[14]
Benin	13	Microscopy, Serology, Nested PCR, Sequencing	Nested PCR, Sequencing	2016	[15]
Sudan	4	Microscopy, RDT, PCR	PCR-RFLP[4]	2016	[17]
Botswana	169	Nested PCR	Not tested	2016	[16]
Cameroon	10	Nested PCR, Sequencing	PCR, Sequencing	2016	[11]
Ethiopia	4	Nested PCR, qPCR	PCR, Sequencing	2015	[10]
Senegal	4	Nested PCR, Sequencing	Not tested	2015	[12]
Cameroon	8	Nested PCR, Sequencing	PCR, Sequencing	2014	[9]
Cameroon	6	Microscopy, Nested PCR, Sequencing	PCR-RFLP, Sequencing	2014	[8]
Mauritania	1	qPCR	PCR, Sequencing	2011	[7]
Equatorial Guinea	8	Nested PCR, RFLP	PCR-RFLP, Sequencing	2011	[13]
Angola	7	Nested PCR, RFLP	PCR-RFLP, Sequencing	2011	[13]
Madagascar	42	Microscopy, RDT, PCR, qPCR, Sequencing	PCR, LDR-FMA[5], Sequencing, Flow cytometry	2010	[6]
Kenya	9	Microscopy, Nested PCR, Sequencinq	Flow cytometry	2006	[5]

[1]PCR, Polymerase chain reaction;

[2]qPCR, Quantitative PCR;

[3]RDT, Rapid diagnostic test;

[4]RFLP, Restriction fragment length polymorphism;

[5]LDR-FMA, Ligase detection reaction-fluorescent microsphere assay.

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Table 2.

Plasmodium vivax invasion ligands and their host receptor specificities.

P. vivax ligands	PlasmoD B ID	Localization	Binding to reticulocytes/erythrocytes	Host Receptor specificities based on enzyme treatment	Interaction Partner: Host Receptor/Parasite ligand	References
DBP1	PvX_110810	Microneme	Erythrocytes, higher affinity for reticulocytes	Sensitive to chymotrypsin	Duffy blood group antigen	[37, 39, 41]
DBP2/EBP	PVP01_0102300	Unknown	Reticulocytes	Unknown	Unknown	[50]
RBP1a[1]	PVX_098585	Microneme	Reticulocytes or all aged erythrocytes?	Sensitive to trypsin and chymotrypsin	Unknown	[47, 55, 57]
RBP1b[1]	PVX_098582	Microneme	Reticulocytes or all aged erythrocytes?	Partially sensitive to trypsin and chymotrypsin	Unknown	[47, 57]
RBP2a	PVX_121920	Unknown	Erythrocytes	Sensitive to trypsin	Unknown	[56, 57]
RBP2b	PVX_094255	Unknown	Reticulocytes	Sensitive to trypsin and chymotrypsin	Transferri n receptor	[57, 58]
RBP2c	PVX_090325	Merozoite apical end	Reticulocytes	Resistant to trypsin, chymotrypsin and neuraminidase	Unknown	[55]
RBP2-P2	PVX_101590	Unknown	Erythrocytes	Unknown	Unknown	[57]
GAMA	PVX_088910	Microneme	Reticulocyte	Sensitive to chymotrypsin, partially resistant to trypsin	Unknown	[59, 60]
PvTRAg	PVX_090265	Unknown	Erythrocytes	Resistant to trypsin, chymotrypsin and neuraminidase	Unknown	[62]
PvTRAg26.3	PVX_112660	Unknown	Erythrocytes	Resistant to trypsin, chymotrypsin and neuraminidase	Unknown	[61]
PvTRA g33.5	PVX_121897	Unknown	Erythrocytes	Resistant to trypsin, chymotrypsin and neuraminidase	Unknown	[62]
PvTRA g34	PVX_112685	Unknown	Erythrocytes	Resistant to trypsin, chymotrypsin and neuraminidase	Unknown	[61]
PvTRA g35.2	PVX_109280	Unknown	Erythrocytes	Resistant to trypsin, chymotrypsin and neuraminidase	Unknown	[62]
PvTRA g36	PVX_112675	Unknown	Erythrocytes	Sensitive to chymotrypsin	Band 3	[61]
PvTRA G36.6	PVX_112690	Merozoite apical end	Erythrocytes	Resistant to trypsin, chymotrypsin and neuraminidase	ETRAMP	[61, 63]
PvTRA g38	PVX_088820	Unknown	Erythrocytes	Partially sensitive to chymotrypsin	Band 3, Basigin	[61, 62, 64, 66]
PvTRA g69.4	PVX_115465	Unknown	Erythrocytes	Resistant to trypsin, chymotrypsin and neuraminidase	Unknown	[62]
PvATR Ag74	PVX_101510	Unknown	Erythrocytes	Partially sensitive to chymotrypsin	Band 3	[61, 62]

[1]The specificities of RBP1a and RB1b for reticulocytes are controversial [47,55,57].