

REVIEW

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# Understanding the population genetics of *Plasmodium vivax* is essential for malaria control and elimination

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## Abstract

Traditionally, infection with *Plasmodium vivax* was thought to be benign and self-limiting, however, recent evidence has demonstrated that infection with *P. vivax* can also result in severe illness and death. Research into *P. vivax* has been relatively neglected and much remains unknown regarding the biology, pathogenesis and epidemiology of this parasite. One of the fundamental factors governing transmission and immunity is parasite diversity. An understanding of parasite population genetic structure is necessary to understand the epidemiology, diversity, distribution and dynamics of natural *P. vivax* populations. In addition, studying the population structure of genes under immune selection also enables investigation of the dynamic interplay between transmission and immunity, which is crucial for vaccine development. A lack of knowledge regarding the transmission and spread of *P. vivax* has been particularly highlighted in areas where malaria control and elimination programmes have made progress in reducing the burden of *Plasmodium falciparum*, yet *P. vivax* remains as a substantial obstacle. With malaria elimination back on the global agenda, mapping of global and local *P. vivax* population structure is essential prior to establishing goals for elimination and the roll-out of interventions. A detailed knowledge of the spatial distribution, transmission and clinical burden of *P. vivax* is required to act as a benchmark against which control targets can be set and measured. This paper presents an overview of what is known and what is yet to be fully understood regarding *P. vivax* population genetics, as well as the importance and application of *P. vivax* population genetics studies.

**Keywords:** *Plasmodium vivax*, Malaria, Population genetics, Microsatellites, Genetic diversity, Elimination

## *Plasmodium vivax*: a global health threat

Presently, 2.85 billion people globally are at risk of *Plasmodium vivax* malaria infection [1]. Worldwide the regional incidence of *P. vivax* has been increasing despite a decrease in *Plasmodium falciparum* cases [2-5]. The most geographically widespread of the six *Plasmodium* species that infect humans, *P. vivax* is a major health threat to huge populations throughout Asia, the Middle East and the Pacific [1], where approximately 80 to 90% of the global *P. vivax* burden is concentrated [6]. A significant number of *P. vivax* cases also occur throughout Central and South America, and East and South Africa [6]. Despite this, research into *P. vivax* malaria has been relatively neglected and much detail of the biology,

pathogenesis and epidemiology of this parasite remains unknown. Traditionally, infection with *P. vivax* was thought to be benign and self-limiting and was not considered a research priority in comparison with the enormous burden of morbidity and mortality presented by *P. falciparum* [7,8]. Recent evidence is however challenging the long-held notion of the benign nature of *P. vivax* malaria, demonstrating that infection with *P. vivax* can also result in severe illness and death [9-14]. Indeed, the severe manifestations of *P. vivax* disease are very similar to those caused by *P. falciparum* and include cerebral malaria, acute respiratory distress, lung injury, renal failure, hepatic dysfunction, shock and death [12,14].

Lack of knowledge regarding the transmission and spread of *P. vivax* has been particularly highlighted in areas where malaria control and elimination programmes have made progress in reducing the burden of

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*P. falciparum*, yet *P. vivax* remains as a substantial obstacle [15]. The emergence and spread of drug resistant *P. vivax* is also of serious concern. Indeed, in the context of achieving malaria elimination targets, reports of primaquine resistance, the only available treatment to prevent relapse, is particularly worrisome [16,17]. One of the key problems undermining effective malaria control is a lack of understanding of the underlying *P. vivax* population structure and transmission dynamics. Population genetic studies are needed to define the diversity, distribution and dynamics of *P. vivax* populations, as parasite populations differ widely between locations, due to factors including prevalence, vector species, host genetics and a variety of environmental influences [18-21]. Mapping of global and local *P. vivax* population structure is essential prior to establishing goals for elimination and the rollout of interventions, and detailed knowledge of the spatial distribution, transmission and clinical burden of *P. vivax* is required to act as a benchmark against which control targets can be set and measured [1,19,22,23]. The Malaria Eradication Research Agenda (malERA) Consultative Group on Basic Science and Enabling Technologies recently reported that no campaign for malaria control or elimination can proceed without a comprehensive knowledge of disease epidemiology and host-parasite-vector interactions, and how these interactions are affected by intensified intervention measures [24].

### Genetic diversity of *Plasmodium vivax*

Within a malaria endemic area, multiple parasite clones can often co-infect the same host. To understand local population structure and genetic diversity, it is essential to be able to distinguish between distinct clones within the same infection as well as between infections. Not only useful in the context of molecular epidemiology studies, identifying and managing multiple clone infections may have additional public health implications, as increasing, or high levels of multiple clone *P. vivax* infections may drive increases in parasite virulence and fitness, as parasite clones compete for both resources within the infected host and survival against antimalarial interventions [25]. However, identifying, and distinguishing between clones can be difficult because the genetic diversity of *P. vivax* populations can vary significantly due to variations in malaria epidemiology in different regions [26,27]. Isolates obtained from distinct populations can either be genetically very diverse, so that multiple infections are easy to determine using a single molecular marker, or they can have quite low levels of diversity or even be clonal in the case of very low transmission or epidemics, which makes it more difficult to distinguish polyclonal from true monoclonal infections [28].

Genotyping to determine the multiplicity of infection (MOI) can be performed using either one or two markers that are extremely polymorphic, or a larger number of less polymorphic genome-wide markers. Currently, regions of the genome containing polymorphic repeat sequences, such as microsatellites or surface antigen genes, are the markers of choice for doing this. Microsatellite markers are short, tandem, one to six nucleotide repeats, found frequently throughout the genome and are typically, selectively neutral. Prior to the identification of microsatellites, earlier studies genotyped strains on the basis of polymorphic coding regions within parasite antigens such as the circumsporozoite protein (CSP) and the merozoite surface protein 3 alpha (MSP-3 $\alpha$ ), for which there are fewer alleles than the microsatellite markers [25,29]. Therefore, the number of clones per infection was most likely underestimated (Table 1).

The population genetic structure of *P. falciparum* is closely associated with transmission intensity, hence population structure and diversity varies greatly according to geographical location, at least on a global scale [65,66]. *Plasmodium falciparum* populations in regions with low levels of transmission generally have low proportions of polyclonal infection high levels of linkage disequilibrium (LD) suggesting significant inbreeding and infrequent recombination. The inverse is also true, with parasite populations in high transmission areas characterized by a high proportion of multiple infections low levels of LD suggesting significant outcrossing and frequent recombination [27,65]. Few studies have been performed amongst sympatric populations of *P. vivax* and *P. falciparum*, however from what is known, there appears to be a distinctly different model of population structure for *P. vivax* compared to that of *P. falciparum* [2,25,27,37]. Using samples collected from a low transmission setting in rural Amazonia, Ferreira and colleagues reported higher genetic diversity and frequency of polyclonal infections for *P. vivax* compared to *P. falciparum* [2]. Interestingly, strong LD, and frequent replacement of predominant microsatellite haplotypes over time was also observed amongst the same *P. vivax* population [2]. The unique biology of *P. vivax* is likely to be responsible for the apparent paradox of multiple clone infection in a low transmission setting. The latent hypnozoite stage of the *P. vivax* lifecycle increases the likelihood of superinfection with a second clone, potentially resulting in the reactivation of heterologous hypnozoites and an increased likelihood of meiotic recombination, ultimately increasing genetic diversity within the population [2,33].

Indeed, microsatellite genotyping has revealed that the level of genetic variability is highly variable among distinct *P. vivax* populations worldwide. Using the same panel of 17 microsatellites, 100% of *P. vivax* infections in southern

**Table 1 Global detection of multiple clone infections using molecular markers in *Plasmodium vivax* populations**

Study region	Study [reference]	Year samples collected	Number of samples analysed	Marker	Proportion infections with multiple clones (%)	Mean MOI
<b>Asia</b>						
Thailand	Imwong <i>et al.</i> 2007 [27]	1992-1993	92	Microsatellites	30 - > 60	1.4
	Imwong <i>et al.</i> 2005 [30]	1995-1998	100	CSP + MSP1	26	1.29
				MSP3 $\alpha$	19.3	
				CSP + MSP3 $\alpha$	35.6	
Laos	Imwong <i>et al.</i> 2007 [27]	2001-2003	81	Microsatellites	30 - > 60	1.5
Vietnam	Van den Eede <i>et al.</i> 2010 [33]	1999-2000	69	Microsatellites	100	3.7
India	Prajapati <i>et al.</i> 2006 [34]	2000-2004	252	GAM-1	13.09	N/S
	Imwong <i>et al.</i> 2007 [27]	2003-2004	90	Microsatellites	10-40	1.2
	Kim <i>et al.</i> 2006 [35]	2003-2004	151	CSP, MSP1, MSP3 $\alpha$	10.6	N/S
Sri Lanka	Wickramara-chchi <i>et al.</i> 2010 [36]	1998-2000	201	MSP3 $\alpha$	13.8-20	N/S
	Karunaweera <i>et al.</i> 2008 [26]	2005	50	Microsatellites	9.1-60	N/S
	Gunawardena <i>et al.</i> 2010 [37]	2003-2008	140	Microsatellites	55	N/S
Pakistan	Khattoon <i>et al.</i> 2010 [38]	[2007]	50	MSP3 $\alpha$	6	N/S
				MSP3 $\beta$	12	
	Zakeri <i>et al.</i> 2010 [39]	2008	187	MSP3 $\alpha$ + MSP3 $\beta$	2	N/S
				CSP, MSP-1, MSP3 $\alpha$	30	
China		2004	54: Anhui Province		5.6	N/S
	Yang <i>et al.</i> 2006	2005	31: Guizhou Province	MSP3 $\beta$	0	
		2004	14: Guangxi Province		0	
	Zhong <i>et al.</i> 2011 [41]	2006-2008	140	MSP3 $\alpha$	7.6-14.3	N/S
				MSP3 $\beta$	14.3-15	
Myanmar	Kim <i>et al.</i> 2010 [42]	2000	96	CSP	24.5	N/S
				CSP	24.1	N/S
	Moon <i>et al.</i> 2009 [43]	2004	349	MSP1	16.4	
	Zhong <i>et al.</i> 2011 [41]	2006-2008	72	MSP3 $\alpha$	21.6	
				MSP3 $\alpha$	10.2	N/S
				MSP3 $\beta$	16.4	
	Gunawardena <i>et al.</i> 2010 [37]	2007	167	Microsatellites	67.1	N/S
East Timor	Chen <i>et al.</i> 2007 [44]	2001	17	CSP, MSP-1, AMA-1	35	N/S
<b>Middle East</b>						
Iran	Zakeri <i>et al.</i> 2003 [45]	2000-2001	107	MSP-1	20	N/S
	Zakeri <i>et al.</i> 2006 [46]	2000-2003	374	CSP	12	N/S
	Zakeri <i>et al.</i> 2006 [47]	2000-2003	144	MSP3 $\alpha$	3.5	N/S
	Zakeri <i>et al.</i> 2010 [39]	2008	150	CSP, MSP-1, MSP3 $\alpha$	24.6	N/S
Uzbekistan	Severini <i>et al.</i> 2004 [48]	1999-2002	12 indigenous cases	MSP-1	8	N/S
			10 imported cases		10	
Afghanistan	Zakeri <i>et al.</i> 2010 [49]	2007	202	CSP	6.4	
				MSP-1	0	N/S
				MSP3 $\alpha$	3.5	

**Table 1 Global detection of multiple clone infections using molecular markers in *Plasmodium vivax* populations (Continued)**

Turkey	Zeyrek et al. 2010 [50]	2007-2008	31	MSP-1	3.2	N/S
<b>Africa</b>						
Ethiopia	Gunawardena et al. 2010 [37]	2006-2008	118	Microsatellites	73.70	N/S
<b>South &amp; Central America</b>						
Mexico	Joy et al. 2008 [20]	1997-2005	234	Microsatellites	15.8	1.01
Peru	Sutton et al. 2009 [51]	2003-2004	186	MSP3 $\alpha$	26.3	2-3 <sup>a</sup>
	Van den Eede et al. 2010 [28]	2006-2008	159	Microsatellites	11-70	1.1
Brazil	Ferreira et al. 2007 [2]	1999	74	Microsatellites	48 (1999)	N/S
		2004-2005			49 (2004-5)	
	Rezende et al. 2009 [52]	2003-2005	44	Tandem repeats	0-66	N/S
	Rezende et al. 2010 [53]	2003-2005	53	Microsatellites	32-57	N/S
	Storti- Melo et al. 2009 [54]	2003-2005	155	CSP	0-39.3	N/S
French Guiana	Orjuela-Sanchez et al. 2009 [55]	2005-2007	77	Microsatellites	10.1-42.4	N/S
		2009 [55]				
French Guiana	Veron et al. 2009 [56]	2005-2006	109	MSP-1 (57 samples)	12.3	N/S
				MSP3 $\alpha$ (109 samples)	13.8	
				MSP-1 + MSP3 $\alpha$ (57 samples)	21	
Guyana	Bonilla et al. 2006 [57]	2000	61	CSP	39.3	N/S
Colombia	Imwong et al. 2007 [27]	2001-2003	82	Microsatellites	10-40	1.1
	Cristiano et al. 2008 [58]	2006	55	MSP3 $\alpha$	36.4	N/S
Venezuela	Ord et al. 2005 [59]	1995-1997	58	MSP3 $\alpha$	10	N/S
	Leclerc et al. 2005 [60]	Not stated	39	MSP-1	0	N/A
<b>Oceania</b>						
Papua New Guinea	Henry-Halldin et al. 2011 [61]	2001-2003	703: Wosera region	CSP	36.8	N/S
			986: Mugil region			34.4
	Mueller et al. 2002 [62]	2002	11	MSP3 $\alpha$	18	N/S
	Koepfli et al. 2009 [63]	2004-2005	108	Microsatellites	81.4	1-8
	Gomez et al. 2003 [64]	Not stated	89	pvMS1	4.5	N/S

<sup>a</sup>Mean MOI was not reported, shown is the observed COI range

N/S: Mean MOI was not reported; N/A: none of the samples tested were polyclonal

Vietnam were found to be polyclonal [33], compared to 11-70% polyclonality observed following analysis of isolates collected in the Peruvian Amazon [28] (Table 1). Similarly, when using a panel of nine microsatellites Imwong and colleagues reported low genetic diversity, high levels of inbreeding and linkage disequilibrium in Colombia, compared to high levels of genetic diversity in India, Thailand and Laos [27] (Table 1). These results emphasize that it cannot be assumed that global parasite populations are equivalent and as a result, may not be impacted by intervention measures to the same extent.

In order to enable accurate comparisons of genetic diversity of global *P. vivax* populations, a standardized approach to microsatellite genotyping is required, similar to that implemented for investigation of *P. falciparum* [67]. However, there remain a number of challenges and limitations to developing such an approach. Selection of both the appropriate number and length of microsatellites to be used for genotyping is crucial to obtain accurate results [29]. Increasing the number of markers investigated increases the likelihood of detecting multiple clone infections. Havryliuk and colleagues reported that a

combination of nine markers was sufficient to identify 90% of multiple clone infections amongst samples collected in Acre, Brazil and that 11 markers enabled 100% of multiple clone infections to be identified [25]. In addition, it is known that repeat length can influence diversity associated with a microsatellite, with longer arrays of di-, tri- and tetra-nucleotide repeats more diverse than shorter sequences [2,53,68-71].

The diversity of a given microsatellite, and the number of microsatellites required to accurately genotype *P. vivax* strains will differ in different epidemiological settings and geographic regions [27,33,63]. Gunawardena and colleagues reported diversity of the MS16 microsatellite was far higher amongst Sri Lankan *P. vivax* strains compared to strains collected in Ethiopia, despite a higher rate of polyclonal infections detected amongst the Ethiopian samples tested [37] (Table 1). A similarly high level of MS16 diversity was observed by Koepfli and colleagues, reporting that more clones were detected using MS16, compared with the MSP1-f3 marker amongst strains from Papua New Guinea (PNG), due to a greater likelihood of clones sharing the same MSP1-f3 allele [72]. Furthermore, the same authors also demonstrated that in the context of a multiple clone infection, clones representing a minority of the population may be missed by PCR, and that detectability of specific *P. vivax* clones in a particular individual varied depending on the day that sample was collected [72]. Taken together, these results suggest that prior to development of a standardized strategy for *P. vivax* genotyping, the suitability of candidate markers must be widely assessed in distinct populations. In addition to identifying suitable markers, the criteria for assigning minor/multiple alleles must also be standardized to further limit discrepancies reported between studies.

### **The importance of *Plasmodium vivax* population genetics studies**

#### **Identifying routes of transmission and gene flow**

Investigating the genetic diversity of *Plasmodium vivax* populations is essential from both a public health perspective, and

to help achieve malaria control and elimination. Detection and analysis of individual clones within populations has not only provided a greater understanding of genetic diversity, but also of *P. vivax* prevalence and incidence [31,46,73,74]. Prevalence is one indicator of transmission and the main malariometric measurement in investigations including the global Malaria Atlas Project (MAP) [75]. For control and elimination strategies to succeed, the origin and movement of *P. vivax* populations must be identified, which can be achieved by genotyping isolates from several different regions. Using a panel of 12 microsatellite markers, Gunawardena and

colleagues reported that isolates collected in Sri Lanka, Myanmar and Ethiopia all clustered according to their geographic origins, demonstrating that these microsatellites may be used to map the origin of *P. vivax* isolates, at least on a broad continental scale [37]. Genome-wide single nucleotide polymorphisms (SNPs) may provide a higher resolution for geographic positioning on a local scale, however such SNPs have not yet been identified for *P. vivax*.

The construction of population structure networks and maps demonstrating genetic relatedness of parasites in specific regions can enable not only the identification of parasite origins but also routes of population movement, and therefore the likelihood of successful elimination within a given region with respect to parasite diversity, population movement and the burden of imported cases [76]. The Pacific Malaria Initiative (PacMI) recently reported the results of an epidemiological survey performed in order to investigate the likelihood of success of potential malaria elimination campaigns in two isolated island provinces of Vanuatu and the Solomon Islands, Tafea and Temotu provinces, respectively [77]. The results of prior studies conducted in other provinces of both nations reported a predominance of *P. falciparum*, with high parasite prevalence (up to 35%) and transmission rates [77-80]. Although extensive genetic characterization of the parasite populations was not performed, the results of the recent PacMI survey revealed that compared to other provinces within each country, the malaria epidemiology in the isolated Tafea and Temotu provinces was hypoendemic, with low parasite prevalence and a predominance of *P. vivax* [77]. Coupled with restricted travel and screening of incoming passengers for infection with malaria parasites, both island provinces were flagged as candidates for future malaria elimination campaigns [77].

Porous land borders between malaria endemic countries, specifically throughout Southeast Asia and the Middle East, are potential barriers to the successful implementation of malaria elimination campaigns [77,81]. Migration of infected individuals resulting in importation of *Plasmodium* spp. and subsequent transmission by local vectors has contributed to the global spread of malaria [82]. Migration of infected individuals may seriously confound containment of *P. vivax*, as individuals can be unaware of their infection status due to long incubation periods. Hence, the potential for reintroduction of endemic malaria into non-endemic countries, is high. Khan and colleagues recently reported both detection of competent malaria vectors and an increase in malaria cases, mostly due to *P. vivax*, in Qatar, due to a massive influx of migrant workers from India and Pakistan [83]. Imported cases also have the potential to increase genetic diversity of existing *P.*

*vivax* populations in malaria endemic countries [82]. Following reports of increasing *P. vivax* genetic diversity in Korea [84], Choi and colleagues used genotyping to successfully distinguish between autochthonous and imported *P. vivax* cases, identifying that the imported infections originated in neighbouring Asian countries. Imported infections also pose a risk in non-endemic regions of endemic countries. Many South American countries are geographically heterogeneous with regard to malaria transmission, and imported cases can result in serious illness and death amongst non-immune populations [85].

Genotyping imported infections is also essential to monitor the introduction and spread of drug resistance into sensitive populations. Population genetic surveys in Venezuela enabled identification of the introduction and spread of chloroquine resistant *P. falciparum*, resulting in changes to the national malaria treatment guidelines and rollout of more effective anti-malarials to combat the spread of drug resistance [82].

Combined, the results of these studies demonstrate the importance of population surveillance; to enable identification of routes of *P. vivax* introduction to, and transmission within, a given population. To achieve malaria elimination targets, the movement of *P. vivax* populations must be controlled to limit not only reintroduction of the disease into non-endemic countries, but also to restrict global genetic diversity and the spread of drug resistance.

### **Impact assessment of intervention and vector control strategies**

Investigation of genetic diversity within *P. vivax* populations is a useful gauge of both the likelihood of success and subsequently, the impact of intervention methods. Interventions such as anti-malarials and candidate vaccines would be anticipated to be more successful in a population with low genetic diversity and any reduction in genetic diversity following the introduction of intervention measures may be regarded as an indicator of success.

In the absence of a continuous *in vitro* culture system and defined markers to identify drug resistance, genotyping is currently used to monitor treatment efficacy, and the emergence of resistant *P. vivax* strains. An understanding of haplotype frequency within a given population is therefore essential [86]. With respect to *P. vivax* parasites detected following drug treatment, there are three possible sources: re-infection with a new clone, recrudescence of a drug resistant clone, or relapse as a result of reactivation of liver hypnozoites [87]. As biomarkers do not currently exist to determine whether recurrent *P. vivax* parasitaemia is due to reactivation of liver hypnozoites, genotyping is used to identify whether

recurrent episodes of *P. vivax* infection are the result of re-infection with a new clone or recrudescence/relapse of an existing drug-resistant clone [87,88]. The basis for this approach is that genetic diversity is sufficient to be able to distinguish between strains using a panel of diverse markers [86]. Confounding the distinction between relapse and re-infection, Imwong and colleagues reported that contrary to the long-held belief that reactivated hypnozoites were genetically identical to the strain responsible for primary infection, reactivated hypnozoites might indeed be heterologous [87]. Hence, it may not always be possible to distinguish re-infection from relapse, especially in regions with reduced parasite diversity. To maximize the ability to distinguish between strains, a panel of diverse markers should be used [63,86,89]. The markers used must be sufficiently diverse and located in distinct genomic positions, however markers may be more or less suited for use dependent upon genetic diversity within a given population [27,63,86]. As a result, community-based investigations of parasite diversity and allele frequencies are vital to enable accurate analysis of anti-malarial interventions [27,86].

The impact of vector control strategies, such as insecticide spraying, is also measurable using population genetics methods. Jongwutiwes and colleagues reported differences in the diversity of *P. vivax* amongst populations in the north-west and south of Thailand [90]. Limited diversity, suggestive of a recent population bottleneck was observed in the south, where anti-malarial insecticide spraying had been implemented and was ongoing. In the north-west, a region bordering Myanmar, anti-malarial control measures have not been implemented to the same extent as in the south, and as a result, diversity of the *P. vivax* population investigated was high [90].

### **Identification of immunogenic targets and potential vaccine candidates**

Development of a vaccine targeting *P. vivax* lags far behind efforts to design a vaccine against *P. falciparum* [91]. This is an inevitable reflection of the reduced research focus on *P. vivax*. The main obstacle impeding *P. vivax* research is the lack of available parasite material, since *P. vivax* cannot be continuously cultured *in vitro* and infected individuals typically present with low parasitaemia [92-95]. As a result, the majority of clinical immunology studies rely on using recombinant *P. vivax* proteins, typically expressed from reference strains [95]. However, an understanding of population genetic structure can also give insight into the development of host immune responses. Population genetics studies can identify signatures of balancing selection within parasite surface antigen genes, enabling identification of domains

targeted by strong host immune pressure and thus identification of potential vaccine candidates, as has been done for *P. falciparum* [23,96,97]. The utility of diversity data is enhanced when additional information is known, such as the allelic frequency within a given population [96]. For example, in a population with low microsatellite diversity, low diversity amongst genes encoding antigens would also be expected. As strain-specific immunity is thought to be a major reason for the failure of malaria vaccine trials to date [98], reduced diversity amongst antigen-encoding genes would encode less diverse surface antigens, increasing the breadth of vaccine efficacy, and the generation of effective immune responses [99,100].

There are three phases of the malaria parasite life-cycle that could be effectively targeted by host immune responses: inhibition of hepatocyte invasion at the pre-erythrocytic stage (e.g. vaccines targeting antigens such as CSP), inhibition of erythrocyte invasion during the asexual blood stage (merozoite surface protein 1, MSP1; apical membrane antigen 1, AMA1), and inhibition of parasite fertilization and development in the mosquito midgut (oocyst/ookinete 25 kD surface protein, Pvs25) [91,101]. The majority of the vaccine candidates currently under investigation for *P. vivax* are orthologues of *P. falciparum* vaccine candidate antigens [91,101]. However, due to biological differences, and differences in the extent and distribution of genetic diversity, it is not always possible to draw conclusions for *P. vivax* on the basis of what is known for *P. falciparum*. Few studies have been performed to investigate the diversity of vaccine candidate antigens in sympatric populations. This is despite the fact that many believe a globally effective malaria vaccine must contain not only multiple antigens, but also a combination of *P. falciparum* and *P. vivax* antigens due to sympatric circulation of both species in many endemic regions [101]. Indeed, differences may exist between regions of the same antigen under immune pressure in *P. falciparum* and *P. vivax* parasites, as has been reported for AMA1 [97,102-104]. Assessment of genetic diversity, and therefore suitability of candidate antigens is therefore essential to design an effective multi-species and/or a *P. vivax* vaccine.

## Conclusions

Partly as a consequence of the common misconception that *P. vivax* infection is benign, research funding and focus directed towards this neglected malaria parasite has been limited relative to *P. falciparum*. As a result, much remains unknown regarding *P. vivax* biology, epidemiology and pathogenesis. However, it is now understood that globally, *P. vivax* populations are highly genetically diverse, and that this diversity varies greatly

according to geographic region. In order to achieve malaria control and elimination targets, population genetic surveys are vital to map the diversity and structure of local populations and to estimate the likelihood of success and measure the outcome of malaria intervention methods. To assist vaccine development, genetic structure and diversity of candidate antigens needs to be assessed in sympatric *P. vivax* and *P. falciparum* populations worldwide.

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## Authors' contributions

AA drafted the paper. AEB and JCR provided input into scope and content and assisted in drafting the paper. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

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