Malaria Journal

BioMed Central
The Open Access Publisher

This Provisional PDF corresponds to the article as it appeared upon acceptance. Fully formatted PDF and full text (HTML) versions will be made available soon.

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

Malaria Journal 2012, **11**:14 doi:10.1186/1475-2875-11-14

Alicia Arnott (alicia@burnet.edu.au)
Alyssa E Barry (barry@wehi.edu.au)
John C Reeder (jreeder@burnet.edu.au)

ISSN	1475-2875
Article type	Review
Submission date	13 October 2011
Acceptance date	10 January 2012
Publication date	10 January 2012
Article URL	http://www.malariajournal.com/content/11/1/14

This peer-reviewed article was published immediately upon acceptance. It can be downloaded, printed and distributed freely for any purposes (see copyright notice below).

Articles in *Malaria Journal* are listed in PubMed and archived at PubMed Central.

For information about publishing your research in *Malaria Journal* or any BioMed Central journal, go to

http://www.malariajournal.com/authors/instructions/

For information about other BioMed Central publications go to

http://www.biomedcentral.com/

© 2012 Arnott *et al.* ; licensee BioMed Central Ltd.
This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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

ArticleCategory	:	Review
ArticleHistory	:	Received:13-Oct-2011; Accepted:07-Dec-2011
ArticleCopyright	:	© 2011 Arnott et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Alicia Arnott,[Aff1]
Email: alicia@burnet.edu.au

Alyssa E Barry,[Aff2 Aff3]
Email: barry@wehi.edu.au

John C Reeder,[Aff1 Aff4]
Corresponding Affiliation: Aff1
Email: jreeder@burnet.edu.au

[Aff1] Centre for Population Health, Burnet Institute, Melbourne, Australia

[Aff2] Division of Infection and Immunity, the Walter and Eliza Hall Institute for Medical Research, Parkville, Australia

[Aff3] Department of Medical Biology, University of Melbourne, Parkville, Australia

[Aff4] Department of Epidemiology and Preventative Medicine, Monash University, Melbourne, Australia

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 and 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 *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-3α), 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).

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
<u>Asia</u>						
Thailand	Imwong *et al.* 2007 [27]	1992–1993	92	Microsatellites	30 - >60	1.4

	Imwong *et al.* 2005 [30]	1995–1998	100	CSP + MSP1	26	1.29
	Cui *et al.* 2003 [31]	2001–2002	90	CSP	25.6	N/S
				MSP3 α	19.3	
				CSP + MSP3 α	35.6	
	Rungsihirunrat *et al.* 2011 [32]	2008–2009	100	MSP3 α + MSP3 β	21	N/S
Laos	Imwong *et al* 2007 [27]	2001–2003	81	Microsatellites	30 - >60	1.5
Vietnam	Van den Eede *et al.* 2010 [33]	1999–2000	69	Microsatellites	100	3.7
India	Prajapati *et al.* 2006 [34]	2000–2004	252	GAM-1	13.09	N/S
	Imwong *et al.* 2007 [27]	2003–2004	90	Microsatellites	10-40	1.2
	Kim *et al.* 2006 [35]	2003–2004	151	CSP, MSP1, MSP3 α	10.6	N/S
Sri Lanka	Wickramara-chchi *et al.* 2010 [36]	1998–2000	201	MSP3 α	13.8–20	N/S
	Karunaweera *et al.* 2008 [26]	2005	50	Microsatellites	9.1–60	N/S

	Gunawardena *et al.* 2010 [37]	2003–2008	140	Microsatellites	55	N/S
Pakistan	Khatoon *et al.* 2010 [38]	2007	50	MSP3 α	6	N/S
				MSP3 β	12	
				MSP3 α + MSP3 β	2	N/S
	Zakeri *et al.* 2010 [39]	2008	187	CSP, MSP-1, MSP3 α	30	
China	Yang *et al.* 2006 [40]	2004	54: Anhui Province	MSP3 β	5.6	N/S
		2005	31: Guizhou Province		0	
		2004	14: Guangxi Province		0	
	Zhong *et al.* 2011 [41]	2006–2008	140	MSP3 α	7.6–14.3	N/S
				MSP3 β	14.3–15	
Myanmar	Kim *et al.* 2010 [42]	2000	96	CSP	24.5	N/S
	Moon *et al.* 2009 [43]	2004	349	CSP	24.1	N/S
				MSP1	16.4	

	2009 [43]			MSP3 α	21.6	
	Zhong *et a.l* 2011 [41]	2006–2008	72	MSP3 α	10.2	N/S
				MSP3 β	16.4	
	Gunawardena *et al.* 2010 [37]	2007	167	Microsatellites	67.1	N/S
East Timor	Chen *et al.* 2007 [44]	2001	17	CSP, MSP-1, AMA-1	35	N/S
Middle East						
Iran	Zakeri *et al.* 2003 [45]	2000–2001	107	MSP-1	20	N/S
	Zakeri *et al.* 2006 [46]	2000–2003	374	CSP	12	N/S
	Zakeri *et al.* 2006 [47]	2000–2003	144	MSP3 α	3.5	N/S
	Zakeri *et al.* 2010 [39]	2008	150	CSP, MSP-1, MSP3 α	24.6	N/S
Uzbekistan	Severini *et al.* 2004 [48]	1999–2002	12 indigenous cases	MSP-1	8	N/S
			10 imported cases		10	
				CSP	6.4	

Afghanistan	Zakeri *et al.* 2010 [49]	2007	202	MSP-1	0	N/S
				MSP3 α	3.5	
Turkey	Zeyrek *et al.* 2010 [50]	2007–2008	31	MSP-1	3.2	N/S
Africa						N/S
Ethiopia	Gunawardena *et al.* 2010 [37]	2006–2008	118	Microsatellites	73.70	N/S
South & Central America						
Mexico	Joy *et al.* 2008 [20]	1997–2005	234	Microsatellites	15.8	1.01
Peru	Sutton *et al.* 2009 [51]	2003–2004	186	MSP3 α	26.3	2-3[a]
	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.*	2003–2005	155	CSP	0–39.3	N/S

	2009 [54]					
	Orjuela-Sanchez *et al.* 2009 [55]	2005–2007	77	Microsatellites	10.1–42.4	N/S
French Guiana	Veron *et al.* 2009 [56]	2005–2006	109	MSP-1 (57 samples)	12.3	N/S
				MSP3 α (109 samples)	13.8	
				MSP-1 + MSP3 α (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 α	36.4	N/S
Venezuela	Ord *et al.* 2005 [59]	1995–1997	58	MSP3 α	10	N/S
	Leclerc *et al.* 2005 [60]	Not stated	39	MSP-1	0	N/A
<u>Oceania</u>						
Papua New Guinea	Henry-Halldin *et al.*	2001–2003	703: Wosera region	CSP	36.8	N/S

	2011 [61]		986: Mugil region		34.4	
	Mueller *et al.* 2002 [62]	2002	11	MSP3 α	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

[a]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

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 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 also 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 through 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*, into 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 lifecycle 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 25kD 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.

Competing interests

The authors declare that they have no competing interests.

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.

Acknowledgements

This work was supported by Project Grant 1003825 from the National Health and Medical Research Council (NHMRC) of Australia. JCR is supported by a NHMRC Principal Research Fellowship. This work was made possible through Victorian State Government Operational Infrastructure Support and Australian Government NHMRC IRIISS.

References

1. Guerra CA, Howes RE, Patil AP, Gething PW, Van Boeckel TP, Temperley WH, Kabaria CW, Tatem AJ, Manh BH, Elyazar IR, Baird JK, Snow RW, Hay SI: **The international limits and population at risk of *Plasmodium vivax* transmission in 2009.** *PLoS Negl Trop Dis* 2010, **4:**e774.

2. Ferreira MU, Karunaweera ND, da Silva-Nunes M, da Silva NS, Wirth DF, Hartl DL: **Population structure and transmission dynamics of *Plasmodium vivax* in rural Amazonia.** *J Infect Dis* 2007, **195:**1218–1226.

3. Kasehagen LJ, Mueller I, McNamara DT, Bockarie MJ, Kiniboro B, Rare L, Lorry K, Kastens W, Reeder JC, Kazura JW, Zimmerman PA: **Changing patterns of Plasmodium blood-stage infections in the Wosera region of Papua New Guinea monitored by light microscopy and high throughput PCR diagnosis.** *Am J Trop Med Hyg* 2006, **75:**588–596.

4. Jun G, Yeom JS, Hong JY, Shin EH, Chang KS, Yu JR, Oh S, Chung H, Park JW: **Resurgence of *Plasmodium vivax* malaria in the Republic of Korea during 2006–2007.** *Am J Trop Med Hyg* 2009, **81:**605–610.

5. Carme B, Ardillon V, Girod R, Grenier C, Joubert M, Djossou F, Ravachol F: **Update on the epidemiology of malaria in French Guiana.** *Med Trop (Mars)* 2009, **69:**19–25.

6. Mendis K, Sina BJ, Marchesini P, Carter R: **The neglected burden of *Plasmodium vivax* malaria.** *Am J Trop Med Hyg* 2001, **64:**97–106.

7. Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, Anstey NM: **Vivax malaria: neglected and not benign.** *Am J Trop Med Hyg* 2007, **77:**79–87.

8. Mueller I, Galinski MR, Baird JK, Carlton JM, Kochar DK, Alonso PL, del Portillo HA: **Key gaps in the knowledge of *Plasmodium vivax*, a neglected human malaria parasite.** *Lancet Infect Dis* 2009, **9:**555–566.

9. Genton B, D'Acremont V, Rare L, Baea K, Reeder JC, Alpers MP, Muller I: ***Plasmodium vivax* and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea.** *PLoS Med* 2008, **5:**e127.

10. Tjitra E, Anstey NM, Sugiarto P, Warikar N, Kenangalem E, Karyana M, Lampah DA, Price RN: **Multidrug-resistant *Plasmodium vivax* associated with severe and fatal malaria: a prospective study in Papua, Indonesia.** *PLoS Med* 2008, **5:**e128.

11. Alexandre MA, Ferreira CO, Siqueira AM, Magalhaes BL, Mourao MP, Lacerda MV, Alecrim MG: **Severe *Plasmodium vivax* malaria, Brazilian Amazon.** *Emerg Infect Dis* 2010, **16:**1611–1614.

12. Barcus MJ, Basri H, Picarima H, Manyakori C, Sekartuti, Elyazar I, Bangs MJ, Maguire JD, Baird JK: **Demographic risk factors for severe and fatal vivax and falciparum malaria among hospital admissions in northeastern Indonesian Papua.** *Am J Trop Med Hyg* 2007, **77:**984–991.

13. Fernandez-Becerra C, Pinazo MJ, Gonzalez A, Alonso PL, del Portillo HA, Gascon J: **Increased expression levels of the pvcrt-o and pvmdr1 genes in a patient with severe *Plasmodium vivax* malaria.** *Malar J* 2009, **8:**55.

14. Kochar DK, Das A, Kochar SK, Saxena V, Sirohi P, Garg S, Kochar A, Khatri MP, Gupta V: **Severe *Plasmodium vivax* malaria: a report on serial cases from Bikaner in northwestern India.** *Am J Trop Med Hyg* 2009, **80:**194–198.

15. Feachem RGA, with A.A. Phillips and G.A. Targett (eds): **Shrinking the Malaria Map: A Prospectus on Malaria Elimination**. First edn. San Francisco: The Global Health Group, Global Health Sciences, University of California, San Francisco; 2009.

16. The malERA Consultative Group on Drugs: **A research agenda for malaria eradication: drugs.** *PLoS Med* 2011, **8:**e1000402.

17. Baird JK, Hoffman SL: **Primaquine therapy for malaria.** *Clin Infect Dis* 2004, **39:**1336–1345.

18. Joshi H, Prajapati SK, Verma A, Kang'a S, Carlton JM: ***Plasmodium vivax* in India.** *Trends Parasitol* 2008, **24:**228–235.

19. Schultz L, Wapling J, Mueller I, Ntsuke PO, Senn N, Nale J, Kiniboro B, Buckee CO, Tavul L, Siba PM, Reeder JC, Barry AE: **Multilocus haplotypes reveal variable levels of diversity and population structure of *Plasmodium falciparum* in Papua New Guinea, a region of intense perennial transmission.** *Malar J* 2010, **9:**336.

20. Joy DA, Gonzalez-Ceron L, Carlton JM, Gueye A, Fay M, McCutchan TF, Su XZ: **Local adaptation and vector-mediated population structure in *Plasmodium vivax* malaria.** *Mol Biol Evol* 2008, **25:**1245–1252.

21. Reid H, Vallely A, Taleo G, Tatem AJ, Kelly G, Riley I, Harris I, Henri I, Iamaher S, Clements AC: **Baseline spatial distribution of malaria prior to an elimination programme in Vanuatu.** *Malar J* 2010, **9:**150.

22. Grynberg P, Fontes CJ, Hughes AL, Braga EM: **Polymorphism at the apical membrane antigen 1 locus reflects the world population history of *Plasmodium vivax*.** *BMC Evol Biol* 2008, **8:**123.

23. Cui L, Escalante AA, Imwong M, Snounou G: **The genetic diversity of *Plasmodium vivax* populations.** *Trends Parasitol* 2003, **19:**220–226.

24. Baum J, Billker O, Bousema T, Dinglasan R, McGovern V, Mota MM, Mueller I, Sinden R: **A research agenda for malaria eradication: basic science and enabling technologies.** *PLoS Med* 2011, **8:**e1000399.

25. Havryliuk T, Ferreira MU: **A closer look at multiple-clone *Plasmodium vivax* infections: detection methods, prevalence and consequences.** *Mem Inst Oswaldo Cruz* 2009, **104:**67–73.

26. Karunaweera ND, Ferreira MU, Munasinghe A, Barnwell JW, Collins WE, King CL, Kawamoto F, Hartl DL, Wirth DF: **Extensive microsatellite diversity in the human malaria parasite *Plasmodium vivax*.** *Gene* 2008, **410:**105–112.

27. Imwong M, Nair S, Pukrittayakamee S, Sudimack D, Williams JT, Mayxay M, Newton PN, Kim JR, Nandy A, Osorio L, Carlton JM, White NJ, Day NP, Anderson TJ: **Contrasting genetic structure in *Plasmodium vivax* populations from Asia and South America.** *Int J Parasitol* 2007, **37:**1013–1022.

28. Van den Eede P, Van der Auwera G, Delgado C, Huyse T, Soto-Calle VE, Gamboa D, Grande T, Rodriguez H, Llanos A, Anne J, Erhart A, D'Alessandro U: **Multilocus genotyping reveals high heterogeneity and strong local population structure of the *Plasmodium vivax* population in the Peruvian Amazon.** *Malar J* 2010, **9:**151.

29. Brito CF, Ferreira MU: **Molecular markers and genetic diversity of *Plasmodium vivax*.** *Mem Inst Oswaldo Cruz* 2011, **106**(Suppl 1):12–26.

30. Imwong M, Pukrittayakamee S, Gruner AC, Renia L, Letourneur F, Looareesuwan S, White NJ, Snounou G: **Practical PCR genotyping protocols for *Plasmodium vivax* using Pvcs and Pvmsp1.** *Malar J* 2005, **4:**20.

31. Cui L, Mascorro CN, Fan Q, Rzomp KA, Khuntirat B, Zhou G, Chen H, Yan G, Sattabongkot J: **Genetic diversity and multiple infections of *Plasmodium vivax* malaria in Western Thailand.** *Am J Trop Med Hyg* 2003, **68:**613–619.

32. Rungsihirunrat K, Chaijaroenkul W, Siripoon N, Seugorn A, Na-Bangchang K: **Genotyping of polymorphic marker (MSP3alpha and MSP3beta) genes of *Plasmodium vivax* field isolates from malaria endemic of Thailand**. *Trop Med Int Health* 2011, **16**:794-801.

33. Van den Eede P, Erhart A, Van der Auwera G, Van Overmeir C, Thang ND, Hung le X, Anne J, D'Alessandro U: **High complexity of *Plasmodium vivax* infections in symptomatic patients from a rural community in central Vietnam detected by microsatellite genotyping.** *Am J Trop Med Hyg* 2010, **82:**223–227.

34. Prajapati SK, Verma A, Adak T, Yadav RS, Kumar A, Eapen A, Das MK, Singh N, Sharma SK, Rizvi MA, Dash AP, Joshi H: **Allelic dimorphism of *Plasmodium vivax* gam-1 in the Indian subcontinent.** *Malar J* 2006, **5:**90

35. Kim JR, Imwong M, Nandy A, Chotivanich K, Nontprasert A, Tonomsing N, Maji A, Addy M, Day NP, White NJ, Pukrittayakamee S: **Genetic diversity of *Plasmodium vivax* in Kolkata, India.** *Malar J* 2006, **5:**71.

36. Wickramarachchi T, Premaratne PH, Dias S, Handunnetti SM, Udagama-Randeniya PV: **Genetic complexity of *Plasmodium vivax* infections in Sri Lanka, as reflected at the merozoite-surface-protein-3alpha locus.** *Ann Trop Med Parasitol* 2010, **104:**95–108.

37. Gunawardena S, Karunaweera ND, Ferreira MU, Phone-Kyaw M, Pollack RJ, Alifrangis M, Rajakaruna RS, Konradsen F, Amerasinghe PH, Schousboe ML, Galappaththy GN, Abeyasinghe RR, Hartl DL, Wirth DF: **Geographic structure of *Plasmodium vivax*: microsatellite analysis of parasite populations from Sri Lanka, Myanmar, and Ethiopia.** *Am J Trop Med Hyg* 2010, **82:**235–242.

38. Khatoon L, Baliraine FN, Bonizzoni M, Malik SA, Yan G: **Genetic structure of *Plasmodium vivax* and *Plasmodium falciparum* in the Bannu district of Pakistan.** *Malar J* 2010, **9:**112.

39. Zakeri S, Raeisi A, Afsharpad M, Kakar Q, Ghasemi F, Atta H, Zamani G, Memon MS, Salehi M, Djadid ND: **Molecular characterization of *Plasmodium vivax* clinical isolates in Pakistan and Iran using pvmsp-1, pvmsp-3alpha and pvcsp genes as molecular markers.** *Parasitol Int* 2010, **59:**15–21.

40. Yang Z, Miao J, Huang Y, Li X, Putaporntip C, Jongwutiwes S, Gao Q, Udomsangpetch R, Sattabongkot J, Cui L: **Genetic structures of geographically distinct *Plasmodium vivax* populations assessed by PCR/RFLP analysis of the merozoite surface protein 3beta gene.** *Acta Trop* 2006, **100:**205–212.

41. Zhong D, Bonizzoni M, Zhou G, Wang G, Chen B, Vardo-Zalik A, Cui L, Yan G, Zheng B: **Genetic diversity of *Plasmodium vivax* malaria in China and Myanmar.** *Infect Genet Evol* 2011, **11**:1419-1425.

42. Kim TS, Kim HH, Lee SS, Na BK, Lin K, Cho SH, Kang YJ, Kim DK, Sohn Y, Kim H, Lee HW: **Prevalence of *Plasmodium vivax* VK210 and VK247 subtype in Myanmar.** *Malar J* 2010, **9:**195.

43. Moon SU, Lee HW, Kim JY, Na BK, Cho SH, Lin K, Sohn WM, Kim TS: **High frequency of genetic diversity of *Plasmodium vivax* field isolates in Myanmar.** *Acta Trop* 2009, **109:**30–36.

44. Chen N, Auliff A, Rieckmann K, Gatton M, Cheng Q: **Relapses of *Plasmodium vivax* infection result from clonal hypnozoites activated at predetermined intervals.** *J Infect Dis* 2007, **195:**934–941.

45. Zakeri S, Dinparast Djadid N, Zeinali S: **Sequence heterogeneity of the merozoite surface protein-1 gene (MSP-1) of *Plasmodium vivax* wild isolates in southeastern Iran.** *Acta Trop* 2003, **88:**91–97.

46. Zakeri S, Abouie Mehrizi A, Djadid ND, Snounou G: **Circumsporozoite protein gene diversity among temperate and tropical *Plasmodium vivax* isolates from Iran.** *Trop Med Int Health* 2006, **11:**729–737.

47. Zakeri S, Barjesteh H, Djadid ND: **Merozoite surface protein-3alpha is a reliable marker for population genetic analysis of *Plasmodium vivax*.** *Malar J* 2006, **5:**53.

48. Severini C, Menegon M, Di Luca M, Abdullaev I, Majori G, Razakov SA, Gradoni L: **Risk of *Plasmodium vivax* malaria reintroduction in Uzbekistan: genetic characterization of parasites and status of potential malaria vectors in the Surkhandarya region.** *Trans R Soc Trop Med Hyg* 2004, **98:**585–592.

49. Zakeri S, Safi N, Afsharpad M, Butt W, Ghasemi F, Mehrizi AA, Atta H, Zamani G, Djadid ND: **Genetic structure of *Plasmodium vivax* isolates from two malaria endemic areas in Afghanistan.** *Acta Trop* 2010, **113:**12–19.

50. Zeyrek FY, Tachibana S, Yuksel F, Doni N, Palacpac N, Arisue N, Horii T, Coban C, Tanabe K: **Limited polymorphism of the *Plasmodium vivax* merozoite surface protein 1 gene in isolates from Turkey.** *Am J Trop Med Hyg* 2010, **83:**1230–1237.

51. Sutton PL, Neyra V, Hernandez JN, Branch OH: ***Plasmodium falciparum* and *Plasmodium vivax* infections in the Peruvian Amazon: propagation of complex, multiple allele-type infections without super-infection.** *Am J Trop Med Hyg* 2009, **81:**950–960.

52. Rezende AM, Tarazona-Santos E, Couto AD, Fontes CJ, De Souza JM, Carvalho LH, Brito CF: **Analysis of genetic variability of *Plasmodium vivax* isolates from different Brazilian Amazon areas using tandem repeats.** *Am J Trop Med Hyg* 2009, **80:**729–733.

53. Rezende AM, Tarazona-Santos E, Fontes CJ, Souza JM, Couto AD, Carvalho LH, Brito CF: **Microsatellite loci: determining the genetic variability of *Plasmodium vivax*.** *Trop Med Int Health* 2010, **15:**718–726.

54. Storti-Melo LM, de Souza-Neiras WC, Cassiano GC, Joazeiro AC, Fontes CJ, Bonini-Domingos CR, Couto AA, Povoa MM, de Mattos LC, Cavasini CE, Rossit AR, Machado RL: ***Plasmodium vivax* circumsporozoite variants and Duffy blood group genotypes in the Brazilian Amazon region.** *Trans R Soc Trop Med Hyg* 2009, **103:**672–678.

55. Orjuela-Sanchez P, da Silva NS, da Silva-Nunes M, Ferreira MU: **Recurrent parasitemias and population dynamics of *Plasmodium vivax* polymorphisms in rural Amazonia.** *Am J Trop Med Hyg* 2009, **81:**961–968.

56. Veron V, Legrand E, Yrinesi J, Volney B, Simon S, Carme B: **Genetic diversity of msp3alpha and msp1_b5 markers of *Plasmodium vivax* in French Guiana.** *Malar J* 2009, **8:**40.

57. Bonilla JA, Validum L, Cummings R, Palmer CJ: **Genetic diversity of *Plasmodium vivax* Pvcsp and Pvmsp1 in Guyana, South America.** *Am J Trop Med Hyg* 2006, **75:**830–835.

58. Cristiano FA, Perez MA, Nicholls RS, Guerra AP: **Polymorphism in the *Plasmodium vivax* msp 3: gene in field samples from Tierralta, Colombia.** *Mem Inst Oswaldo Cruz* 2008, **103:**493–496.

59. Ord R, Polley S, Tami A, Sutherland CJ: **High sequence diversity and evidence of balancing selection in the Pvmsp3alpha gene of *Plasmodium vivax* in the Venezuelan Amazon.** *Mol Biochem Parasitol* 2005, **144:**86–93.

60. Leclerc MC, Gauthier C, Villegas L, Urdaneta L: **Genetic diversity of merozoite surface protein-1 gene of *Plasmodium vivax* isolates in mining villages of Venezuela (Bolivar State).** *Acta Trop* 2005, **95:**26–32.

61. Henry-Halldin CN, Sepe D, Susapu M, McNamara DT, Bockarie M, King CL, Zimmerman PA: **High-throughput molecular diagnosis of circumsporozoite variants VK210 and VK247 detects complex *Plasmodium vivax* infections in malaria endemic populations in Papua New Guinea.** *Infect Genet Evol* 2011, **11:**391–398.

62. Mueller I, Kaiok J, Reeder JC, Cortes A: **The population structure of *Plasmodium falciparum* and *Plasmodium vivax* during an epidemic of malaria in the Eastern Highlands of Papua New Guinea.** *Am J Trop Med Hyg* 2002, **67:**459–464.

63. Koepfli C, Mueller I, Marfurt J, Goroti M, Sie A, Oa O, Genton B, Beck HP, Felger I: **Evaluation of *Plasmodium vivax* genotyping markers for molecular monitoring in clinical trials.** *J Infect Dis* 2009, **199:**1074–1080.

64. Gomez JC, McNamara DT, Bockarie MJ, Baird JK, Carlton JM, Zimmerman PA: **Identification of a polymorphic *Plasmodium vivax* microsatellite marker.** *Am J Trop Med Hyg* 2003, **69:**377–379.

65. Anderson TJ, Haubold B, Williams JT, Estrada-Franco JG, Richardson L, Mollinedo R, Bockarie M, Mokili J, Mharakurwa S, French N, Whitworth J, Velez ID, Brockman AH, Nosten F, Ferreira MU, Day KP: **Microsatellite markers reveal a spectrum of population structures in the malaria parasite *Plasmodium falciparum*.** *Mol Biol Evol* 2000, **17:**1467–1482.

66. Mu J, Awadalla P, Duan J, McGee KM, Joy DA, McVean GA, Su XZ: **Recombination hotspots and population structure in *Plasmodium falciparum*.** *PLoS Biol* 2005, **3:**e335.

67. Anderson TJ, Su XZ, Bockarie M, Lagog M, Day KP: **Twelve microsatellite markers for characterization of *Plasmodium falciparum* from finger-prick blood samples.** *Parasitology* 1999, **119**(Pt 2):113–125.

68. Imwong M, Sudimack D, Pukrittayakamee S, Osorio L, Carlton JM, Day NP, White NJ, Anderson TJ: **Microsatellite variation, repeat array length, and population history of *Plasmodium vivax*.** *Mol Biol Evol* 2006, **23:**1016–1018.

69. Russell B, Suwanarusk R, Lek-Uthai U: ***Plasmodium vivax* genetic diversity: microsatellite length matters.** *Trends Parasitol* 2006, **22:**399–401.

70. Leclerc MC, Durand P, Gauthier C, Patot S, Billotte N, Menegon M, Severini C, Ayala FJ, Renaud F: **Meager genetic variability of the human malaria agent *Plasmodium vivax*.** *Proc Natl Acad Sci U S A* 2004, **101:**14455–14460.

71. de Souza-Neiras WC, de Melo LM, Machado RL: **The genetic diversity of *Plasmodium vivax*--a review.** *Mem Inst Oswaldo Cruz* 2007, **102:**245–254.

72. Koepfli C, Schoepflin S, Bretscher M, Lin E, Kiniboro B, Zimmerman PA, Siba P, Smith TA, Mueller I, Felger I: **How much remains undetected? Probability of molecular detection of human plasmodia in the field**. *PLoS One* 2011, **6:**e19010.

73. Bruce MC, Galinski MR, Barnwell JW, Donnelly CA, Walmsley M, Alpers MP, Walliker D, Day KP: **Genetic diversity and dynamics of *plasmodium falciparum* and *P. vivax* populations in multiply infected children with asymptomatic malaria infections in Papua New Guinea.** *Parasitology* 2000, **121**(Pt 3):257–272.

74. Daubersies P, Sallenave-Sales S, Magne S, Trape JF, Contamin H, Fandeur T, Rogier C, Mercereau-Puijalon O, Druilhe P: **Rapid turnover of *Plasmodium falciparum* populations in asymptomatic individuals living in a high transmission area.** *Am J Trop Med Hyg* 1996, **54:**18–26.

75. Guerra CA, Hay SI, Lucioparedes LS, Gikandi PW, Tatem AJ, Noor AM, Snow RW: **Assembling a global database of malaria parasite prevalence for the Malaria Atlas Project.** *Malar J* 2007, **6:**17.

76. Moonen B, Cohen JM, Snow RW, Slutsker L, Drakeley C, Smith DL, Abeyasinghe RR, Rodriguez MH, Maharaj R, Tanner M, Targett G: **Operational strategies to achieve and maintain malaria elimination.** *Lancet* 2010, **376:**1592–1603.

77. The Pacific Malaria Initiative Survey Group (PMISG) on behalf of the Ministries of Health of Vanuatu and Solomon Islands: **Malaria on isolated Melanesian islands prior to the initiation of malaria elimination activities.** *Malar J* 2010, **9:**218.

78. Maguire JD, Bangs MJ, Brennan L, Rieckmann K, Taleo G: **Cross-sectional characterization of malaria in Sanma and Shefa Provinces, Republic of Vanuatu: malaria control implications.** *P N G Med J* 2006, **49:**22–31.

79. Maitland K, Williams TN, Peto TE, Day KP, Clegg JB, Weatherall DJ, Bowden DK: **Absence of malaria-specific mortality in children in an area of hyperendemic malaria.** *Trans R Soc Trop Med Hyg* 1997, **91:**562–566.

80. Hii JL, Kanai L, Foligela A, Kan SK, Burkot TR, Wirtz RA: **Impact of permethrin-impregnated mosquito nets compared with DDT house-spraying against malaria**

transmission by Anopheles farauti and An.punctulatus in the Solomon Islands. *Med Vet Entomol* 1993, **7:**333–338.

81. Feachem R, Sabot O: **A new global malaria eradication strategy.** *Lancet* 2008, **371:**1633–1635.

82. Rodriguez-Morales AJ, Delgado L, Martinez N, Franco-Paredes C: **Impact of imported malaria on the burden of disease in northeastern Venezuela.** *J Travel Med* 2006, **13:**15–20.

83. Khan FY, Lutof AK, Yassin MA, Khattab MA, Saleh M, Rezeq HY, Almaslamani M: **Imported malaria in Qatar: a 1 year hospital-based study in 2005.** *Travel Med Infect Dis* 2009, **7:**111–117.

84. Choi KM, Choi YK, Kang YA, Seo SY, Lee HW, Cho SH, Lee WJ, Rhie HG, Lee HS, Kim JY: **Study of the genetic discrimination between imported and autochthonous cases of malaria in South Korea.** *J Travel Med* 2011, **18:**63–66.

85. Rodriguez-Morales AJ, Ferrer MV, Barrera MA, Pacheco M, Daza V, Franco-Paredes C: **Imported cases of malaria admitted to two hospitals of Margarita Island, Venezuela, 1998–2005.** *Travel Med Infect Dis* 2009, **7:**44–48.

86. Gatton ML, Cheng Q: **Can estimates of antimalarial efficacy from field studies be improved?** *Trends Parasitol* 2008, **24:**68–73.

87. Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, Guthmann JP, Nosten F, Carlton J, Looareesuwan S, Nair S, Sudimack D, Day NP, Anderson TJ, White NJ: **Relapses of *Plasmodium vivax* infection usually result from activation of heterologous hypnozoites.** *J Infect Dis* 2007, **195:**927–933.

88. Teka H, Petros B, Yamuah L, Tesfaye G, Elhassan I, Muchohi S, Kokwaro G, Aseffa A, Engers H: **Chloroquine-resistant *Plasmodium vivax* malaria in Debre Zeit, Ethiopia.** *Malar J* 2008, **7:**220.

89. Havryliuk T, Orjuela-Sanchez P, Ferreira MU: ***Plasmodium vivax*: microsatellite analysis of multiple-clone infections.** *Exp Parasitol* 2008, **120:**330–336.

90. Jongwutiwes S, Putaporntip C, Hughes AL: **Bottleneck effects on vaccine-candidate antigen diversity of malaria parasites in Thailand.** *Vaccine* 2010, **28:**3112–3117.

91. Galinski MR, Barnwell JW: ***Plasmodium vivax*: who cares?** *Malar J* 2008, **7**(Suppl 1):S9.

92. Golenda CF, Li J, Rosenberg R: **Continuous in vitro propagation of the malaria parasite *Plasmodium vivax*.** *Proc Natl Acad Sci U S A* 1997, **94:**6786–6791.

93. Mons B, Collins WE, Skinner JC, van der Star W, Croon JJ, van der Kaay HJ: ***Plasmodium vivax*: in vitro growth and reinvasion in red blood cells of Aotus nancymai.** *Exp Parasitol* 1988, **66:**183–188.

94. Chotivanich K, Silamut K, Udomsangpetch R, Stepniewska KA, Pukrittayakamee S, Looareesuwan S, White NJ: **Ex-vivo short-term culture and developmental assessment of *Plasmodium vivax*.** *Trans R Soc Trop Med Hyg* 2001, **95:**677–680.

95. Gentil F, Bargieri DY, Leite JA, Francoso KS, Patricio MB, Espindola NM, Vaz AJ, Palatnik-de-Sousa CB, Rodrigues MM, Costa FT, Soares IS: **A recombinant vaccine based on domain II of *Plasmodium vivax* Apical Membrane Antigen 1 induces high antibody titres in mice.** *Vaccine* 2010, **28:**6183–6190.

96. Weedall GD, Conway DJ: **Detecting signatures of balancing selection to identify targets of anti-parasite immunity.** *Trends Parasitol* 2010, **26:**363–369.

97. Gunasekera AM, Wickramarachchi T, Neafsey DE, Ganguli I, Perera L, Premaratne PH, Hartl D, Handunnetti SM, Udagama-Randeniya PV, Wirth DF: **Genetic diversity and selection at the *Plasmodium vivax* apical membrane antigen-1 (PvAMA-1) locus in a Sri Lankan population.** *Mol Biol Evol* 2007, **24:**939–947.

98. Richards JS, Beeson JG: **The future for blood-stage vaccines against malaria.** *Immunol Cell Biol* 2009, **87:**377–390.

99. Dias S, Longacre S, Escalante AA, Udagama-Randeniya PV: **Genetic diversity and recombination at the C-terminal fragment of the merozoite surface protein-1 of *Plasmodium vivax* (PvMSP-1) in Sri Lanka.** *Infect Genet Evol* 2011, **11:**145–156.

100. Ferreira MU, da Silva Nunes M, Wunderlich G: **Antigenic diversity and immune evasion by malaria parasites.** *Clin Diagn Lab Immunol* 2004, **11:**987–995.

101. Arevalo-Herrera M, Chitnis C, Herrera S: **Current status of *Plasmodium vivax* vaccine.** *Hum Vaccin* 2010, **6:**124–132.

102. Remarque EJ, Faber BW, Kocken CH, Thomas AW: **Apical membrane antigen 1: a malaria vaccine candidate in review.** *Trends Parasitol* 2008, **24:**74–84.

103. Ord RL, Tami A, Sutherland CJ: **ama1 genes of sympatric *Plasmodium vivax* and *P. falciparum* from Venezuela differ significantly in genetic diversity and recombination frequency.** *PLoS One* 2008, **3:**e3366.

104. Polley SD, Chokejindachai W, Conway DJ: **Allele frequency-based analyses robustly map sequence sites under balancing selection in a malaria vaccine candidate antigen.** *Genetics* 2003, **165:**555–561.