Limited genetic diversity of N-terminal of merozoite surface protein-1 (MSP-1) in Plasmodium ovale curtisi and P. ovale wallikeri imported from Africa to China

Ruilin Chu1†, Xinxin Zhang1†, Sui Xu2, Limei Chen1, Jianxia Tang2, Yuhong Li1, Jing Chen2, Yinhua Xuan1, Guoding Zhu2, Jun Cao1,2* and Yang Cheng1*

Abstract

Background: Plasmodium merozoite surface protein-1 (MSP-1) is released into the bloodstream during merozoite invasion, and thus represents a crucial malarial vaccine target. Although substantial research effort has been devoted to uncovering the genetic diversity of MSP-1 for P. falciparum and P. vivax; there is minimal information available regarding the genetic profiles and structure of P. ovale. Therefore, the aim of the present study was to determine the extent of genetic variation among two subspecies of P. ovale by characterizing the MSP-1 N-terminal sequence at the nucleotide and protein levels.

Methods: N-terminal of MSP-1 gene were amplified from 126 clinical samples collected from imported cases of malaria in migrant workers returning to Jiangsu Province from Africa using a conventional polymerase chain reaction (PCR) assay. The PCR products were then sequenced and analyzed using the GeneDoc, MegAlign, MEGA7 and DnaSP v.6 programs.

Results: The average pairwise nucleotide diversities (π) of P. ovale curtisi and P. ovale wallikeri MSP-1 genes (pomsp1) were 0.01043 and 0.01974, respectively, and the haplotype diversity (Hd) were 0.746 and 0.598, respectively. Most of the nucleotide substitutions detected were non-synonymous, indicating that the genetic variations of pomsp1 were maintained by positive diversifying selection, thereby suggesting their role as a potential target of a protective immune response. Amino acid substitutions of P. ovale curtisi and P. ovale wallikeri MSP-1 could be categorized into five and three unique amino acid variants, respectively.

Conclusions: Low mutational diversity was observed in pomsp1 from the Jiangsu Province imported malaria cases; further studies will be developed such as immunogenicity and functional analysis.

Keywords: Plasmodium ovale, MSP-1, Imported malaria cases

Background

Malaria is one of the most serious infectious diseases of humans worldwide. An estimated 216 million cases of malaria were reported in 2016 and the global tally of malaria-caused deaths reached 445,000 [1]. Five species in the genus Plasmodium (P. falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi) are known to cause human malaria under natural transmission [2]. In China, the majority of malaria cases are caused by P. vivax and P. falciparum, most of which are imported from malaria-endemic areas. Jiangsu Province, located in eastern China, was an unstable malaria transmission area and there has been no local malaria infection report since 2012. However, the number of imported malaria cases in Jiangsu ranked in the top three provinces in China, with 1799 imported malaria cases reported from 2005 to 2014 [3, 4].

As a neglected human parasite causing infection, P. ovale was first reported and named by Stephens in 1922 as one of the major Plasmodium species infecting humans [5].
Plasmodium ovale has a wide geographic distribution, including the Middle East, Indonesia, and Southeast Asia [6–8]. In Africa, only 0.7–10% of human malaria cases are caused by P. ovale infections; thus, the diagnosis of P. ovale is often overlooked due to the low levels of parasitemia and mixed-species malaria infections [7, 9]. Notably, approximately 300 malaria cases in China imported from Africa annually are caused by P. ovale. There are two subspecies of P. ovale, P. ovale curtisi (classical type) and P. ovale wallikeri (variant type) [10], which show dimorphism of multiple genetic loci [2].

Merozoites surface proteins (MSPs) are released into the bloodstream of the host in extracellular forms, and are thus promising vaccine targets since they play a critical role in erythrocyte invasion [11]. As the predominant member of MSPs, MSP-1 has been detected in all examined Plasmodium species to date, and plays an important role during erythrocyte attachment [12]. Thus, naturally acquired antibodies to MSP-1 inhibit erythrocyte invasion and are associated with protection from clinical malaria in field studies [13]. However, MSP-1-based vaccines show low protective efficacy against clinical malaria, which may be attributed to the genetic diversity of MSP-1, leading to failure of anti-malaria parasite control measures. Moreover, antigenic diversity allows the parasite to evade natural immune responses, which may cause vaccines to lose efficacy [14]. The N-terminal fragments of the MSP-1 genes of P. falciparum and P. vivax (pfmsp1 and pvmsp1, respectively) show polymorphism due to selection pressure, which has hindered MSP-1-based vaccine development [15, 16]. Comparatively, the C-terminal of MSP-1 is a conserved sequence, which is carried into the infected erythrocytes during merozoite invasion [17]. A recent study demonstrated the low diversity of the pocmsp1 and powmsp1 gene in 10 P. ovale isolated from symptomatic malaria patients in Thailand, which may be related to a low transmission rate or repeated bottleneck effects [18]. However, there is limited evidence of the genetic diversity of pocmsp1. Therefore, characterization of pocmsp1 is necessary toward understanding the population genetic structure and finding a suitable candidate for vaccine development. Accordingly, in the present study, the pocmsp1 N-terminal sequence was analyzed from the both subspecies of P. ovale obtained from infected migrant workers returning to China from Africa. We determined the levels of polymorphisms and nucleotide divergence of msp1 sequences to validate the classification of P. ovale curtisi and P. ovale wallikeri as distinct species or subspecies, and trace signatures of selection.

Methods
Study areas and sample collection
The samples of P. ovale curtisi and P. ovale wallikeri were obtained from febrile patients at local hospitals of Jiangsu Province in China between 2012 and 2016, who had recently returned from working in tropical regions of sub-Saharan Africa endemic for malaria. A total of 126 P. ovale-infected blood samples were collected. Identification of the isolates was confirmed using polymerase chain reaction (PCR) of specific gene sequences. Parasite species were distinguished by PCR amplification using the real-time TaqMan PCR [3].

PCR amplification and sequencing of the pocmsp1 N-terminal
The N-terminal nucleotide sequences of MSP-1 from P. ovale curtisi and P. ovale wallikeri were amplified by PCR using the primers designed as pocmsp1-Forward (5'-GAA ACG CTC GTA AAC AGA CCT T-3') and pocmsp1-Reverse (5'-ACA GGA TCA GTA AAC AGA CCT T-3'), and powmsp1-Forward (5'-GAA ACG CTC GTA AAC AGA CCT T-3') and powmsp1-Reverse (5'-ATC GGT AAA CAG ACC TTC CAT-3'), respectively. The pocmsp1 (GenBank: KC137343) and powmsp1 (GenBank: KC137341) sequences from the GenBank database were used as reference gene sequences. The reactions were carried out in a volume of 20 μl including 1 μl genomic DNA, 7.4 μl double-distilled water, 0.8 μl of each primer, 0.5 units DNA polymerase, and 2 mM deoxynucleoside triphosphate within 10 μl premix (2× Phanta® Max Master Mix, Nanjing, China). The PCR amplification was performed in a Mastercycler (Eppendorf, Hamburg, Germany) under the following programme: denaturation at 95 °C for 3 min; followed by 35 cycles of 95 °C for 15 s, 51 °C for 15 s and 72 °C for 30 s; and a final extension at 72 °C for 5 min. The amplified products were analyzed by 1% agarose gel electrophoresis and visualized under an ultraviolet transilluminator (Bio-Rad ChemiDoc MP, Hercules, USA). The size of the PCR products was estimated based on the mobility relative to the standard DNA marker (TRANSGEN BIOTECH, Beijing, China). PCR products were cloned into pUC57 vector and the universal primers M13F (5'-TGT AAA ACG ACG GCC AGT-3') and M13R (5'-CAG GAA ACA GCT ATG AC-3') were used for sequencing, which was performed by GENEWIZ ( Suzhou, China) on an ABI 3730xl DNA Analyzer (Thermo Fisher Scientific, Waltham, USA).

Sequence alignment and data analysis
The geographical distribution map of P. ovale curtisi and P. ovale wallikeri was constructed using Arcgis10.2 software [19]. The primary structure of the PoMSP-1 protein was predicted with a bioinformatics tool (http://smart.embl-heidelberg.de/). To evaluate the diversity of the two subspecies, the pocmsp1 (KC137343) and powmsp1 (KC137341) sequences were used as templates and aligned using GeneDoc2.7.0 [20]. The nucleotide sequences of pocmsp1 were translated to deduced amino acid (aa)
sequences using MegAlign module of Lasergene 7 software package DNASTAR [21] and then aligned with reference aa sequences. The codon-based test of purifying selection was conducted using the MEGA7 program [22]. The rates of non-synonymous mutations \((dN)\) and synonymous mutations sites \((dS)\) were computed by \(Z\)-test using the Nei & Gojobori method [23] with the Jukes and Cantor correction and 100 bootstrap replications.

The average pairwise nucleotide diversity \((\pi)\), haplotype diversity \((Hd)\), and number of haplotypes \((H)\) were calculated by DnaSP v6 [24]. The nucleotide diversity was analyzed with a window length of 50 base pairs \((bp)\) and a step size of 3 bp using DnaSP v6. Tajima’s \(D\), Fu and Li’s \(D^*\), and Fu and Li’s \(F^*\) tests were used to measure the degree of deviation from neutrality [25, 26]. Phylogenetic trees of the N-terminal of MSP-1 were constructed using the neighbor-joining method according to the nucleotide sequences. The MSP-1 sequences of other Plasmodium species included the MSP-1 haplotypes of malaria parasites from humans \((P. falciparum, P. malariae, P. ovale\) and \(P. vivax)\), gorillas \((P. praefalciparum, P. alderi\) and \(P. billcollinsi)\), chimpanzees \((P. knowlesi\) and \(P. cynomolgi)\), and murine infections \((P. yoelii, P. chabaudi\) and \(P. berghei)\), which were collected from the NCBI and PlasmoDB databases. Evolutionary relationships of the aligned sequences were determined using neighbor-joining approaches in MEGA 7.0.

Results

Geographical origin of \(P. ovale curtisi\) and \(P. ovale wallikeri\)
The total of 126 \(P. ovale\) clinical isolates showed a geographical distribution across 15 countries of sub-Saharan Africa. The isolates were mainly derived from Equatorial Guinea \((n = 37, 29.7\%)\), Angola \((n = 27, 21.4\%)\), Nigeria \((n = 19, 15.1\%)\), and the Republic of Congo \((n = 13, 10.3\%)\) located on the west coast of Africa (Fig. 1). Comparatively, the \(P. ovale curtisi\) isolates spanned a wider range of countries \((15\) countries\) than \(P. ovale wallikeri\) isolates \((10\) countries\). Overall, 61 \((48.4\%)\) cases of \(P. ovale curtisi\) infection and 65 \((51.6\%)\) cases of \(P. ovale wallikeri\) infection were identified in this study (Table 1).

Characterization of PoMSP-1

The lengths of MSP-1 encoded by the full-length \(P. ovale curtisi\) (GenBank: KC137343) and \(P. ovale wallikeri\) (GenBank: KC137341) genes were 1727 and 1672 \((aa)\), respectively, each beginning with a predicted \(19\) aa signal peptide sequence \((aa 1–19)\). Similar to PvMSP-1 and PmMSP-1, some other specific regions were identified in the \(P. ovale curtisi\) predicted protein primary structure, such as a coiled-coil region \((aa 288–368\) and 450–495\), Pfam region \((aa 1011–1546)\), and EGF domains \((aa 1624–1660\) and \(1667–1705)\) (Fig. 2a). Similarly, the PowMSP-1 predicted protein primary structure contained a signal peptide \((aa 1–19)\), coiled-coil region \((aa 458–496)\), Pfam region \((aa 950–1491)\), and two EGF domains \((aa 1569–1605\) and \(1612–1650)\) (Fig. 2b).

Nucleotide polymorphism of pomsp1

The MSP-1 genes of the 126 \(P. ovale\) isolates were amplified corresponding to nucleotide positions 58–1125 (Fig. 2c). There were five genotypes of the \(pomsp1\) N-terminal, and 52 isolates \((85.2\%)\) showed a non-synonymous mutation compared with the reference GH01 strain, with \(44\) nucleotides \((67.7\%)\) showing a non-synonymous mutation in the \(pomsp1\) N-terminal. Interestingly, \(31\) \(P. ovale curtisi\) isolates had \(27\) serine residues, while only \(21\) serine residues existed in the others. There were \(6\) more non-synonymous aa changes detected in \(P. ovale wallikeri\) isolates (Fig. 3).

Overall, \(26\) single nucleotide polymorphisms \((SNPs)\) were found among \(61\) samples with an average \(\pi\) value of \(0.01043\) in \(pomsp1\), and \(42\) SNPs were detected among \(65\) samples with an average \(\pi\) value of \(0.01974\) in \(pomsp1\). A sliding method plot with a window length of \(50\) bp and a step size of \(3\) bp using DnaSP v6 revealed a \(\pi\) value in the range of \(0–0.09688\) and \(0–0.19221\) for \(pomsp1\) and \(powmsp1\), respectively. The conserved region was observed from \(0.2–0.7\) kb length in \(pomsp1\) and before \(0.6\) kb in \(powmsp1\) with approximate \(\pi\) values of \(0\) (Fig. 4). The haplotype \((gene)\) diversity of \(pomsp1\) could be categorized into five distinct haplotypes with an estimated \(Hd\) of \(0.746\) and three distinct haplotypes with an estimated \(Hd\) of \(0.598\) in \(powmsp1\) samples (Table 2). The average number of nucleotide differences \((k)\) for \(pomsp1\) and \(powmsp1\) was \(11.139\) and \(21.081\), respectively.

Genetic population structure based on the pomsp1 N-terminal

The population genetic structure of the \(P. ovale\) isolates was analyzed based on the MSP-1 N-terminal gene polymorphisms applied to the codon-based test of purifying selection according to average \(dS\) and \(dN\) values within each isolate. There was clear evidence of positive selection or diversifying selection in \(P. ovale\) MSP-1 \([Prob = 1.000, dS - dN = -0.06\ (pomsp1), -0.478 (powmsp1)]\). In addition, Tajima’s \(D\), Fu and Li’s \(D^*\) and \(F^*\) tests rejected a neutral model of polymorphism occurrence with values for \(pomsp1\) \((Tajima’s \(D = 3.22138, P < 0.01; Fu\) and \(Li’s \(D^* = 1.88498, P < 0.02; Fu\) and \(Li’s \(F^* = 2.77112, P < 0.02)\) and \(powmsp1\) \((Tajima’s \(D = 4.57287, P < 0.001; Fu\) and \(Li’s \(D^* = 2.00379, P < 0.02; Fu\) and \(Li’s \(F^* = 3.5575, P < 0.02)\)\).
Table 1 Origin of imported *P. ovale curtisi* and *P. ovale wallikeri* in 2012–2016

| Country                      | *P. ovale curtisi* |   | *P. ovale wallikeri* |   | Total Number |
|------------------------------|--------------------|---|----------------------|---|---------------|
|                              | Number             | Percent | Number             | Percent |               |
| Angola                       | 9                  | 14.8    | 18                  | 27.7    | 27            |
| Equatorial Guinea            | 16                 | 26.2    | 21                  | 32.3    | 37            |
| Republic of the Congo        | 5                  | 8.2     | 8                   | 12.3    | 13            |
| Democratic Republic of Congo | 4                  | 6.6     | 0                   | 0       | 4             |
| Guinea                       | 1                  | 1.6     | 2                   | 3.1     | 3             |
| Ghana                        | 1                  | 1.6     | 1                   | 1.5     | 2             |
| Gabon                        | 1                  | 1.6     | 0                   | 0       | 1             |
| Cameroon                     | 6                  | 9.8     | 4                   | 6.2     | 10            |
| Liberia                      | 2                  | 3.3     | 0                   | 0       | 2             |
| Mozambique                   | 1                  | 1.6     | 2                   | 3.1     | 3             |
| Niger                        | 1                  | 1.6     | 0                   | 0       | 1             |
| Nigeria                      | 12                 | 19.7    | 7                   | 10.8    | 19            |
| Sierra Leone                 | 1                  | 1.6     | 1                   | 1.5     | 2             |
| Zambia                       | 1                  | 1.6     | 0                   | 0       | 1             |
| Uganda                       | 0                  | 0       | 1                   | 1.5     | 1             |
| Total                        | 61                 | 100     | 65                  | 100     | 126           |
Phylogenetic analysis

As predicted based on the low level of genetic diversity and signature of positive selection described above, a close phylogenetic relationship was detected in pocmsp1 sequences between the subspecies based the branch lengths of pocmsp1 and powmsp1 with 100% bootstrap support (Fig. 5). Phylogenetic trees of 26 MSP-1 gene alleles from the 18 species of Plasmodium in human and non-human primates were constructed using the neighbor-joining method (Additional file 1: Figure S1).

Discussion

The life-cycle of the malaria parasite alternates between the human host and the mosquito vector, which is complex with extensive genetic and antigenic diversity across different stages of the parasite’s life [14]. The genetic diversity of P. ovale might have impacted malaria transmission and the success of malaria control strategies. Gaining a deeper understanding of the mechanisms and patterns of genetic recombination and sequence variation may help in designing a vaccine that could represent the worldwide repertoire of polymorphic malaria surface antigens [27]. The sequences of pocmsp1 and powmsp1 showed a low level of diversity in a limited number of Asian isolates [18]. Hence, we analyzed the N-terminus of pocmsp1 (61 isolates, 48.4%) and powmsp1 (65, 51.6%), and found that pocmsp1 was more conserved than powmsp1 with 26 (14 synonymous, 12 non-synonymous) and 42 (26 synonymous, 16 non-synonymous) sites of nucleotide diversity, respectively.

Neutrality tests were further performed to determine the signatures of natural selection on the MSP-1 N-terminal fragment of P. ovale. Significantly positive values for these...
statistics reflect an excess of intermediate frequency alleles, which can result from population bottlenecks or balancing selection. The sequences for subspecies *P. ovale curtisi* and *P. ovale wallikeri* were further divided into five and three branches that were all within the same evolutionary branches. The MSP-1 N-terminal sequences placed the two subspecies in a distinct bifurcating branch, and the split of *pocmsp1* and *powmsp1* seems to be relatively more recent. Therefore, the MSP-1 N-terminal sequences of *P. ovale curtisi* and *P. ovale wallikeri* support the ancient divergence times of the malaria parasite lineage [28].

The *Z*-test (*dS* - *dN* < 0) indicated that strong positive or purifying selection within the parasite population. These results were in agreement with previous studies which suggested that such mechanisms might be in favour of parasites to evade targeted host immune responses [29]. In addition, the genetic diversities at the *P. ovale* MSP-1 N-terminal [*π = 0.01043 ± SD 0.00061 (pocmsp1), π = 0.01974 ± SD 0.00055 (powmsp1)] were lower compared to that of *P. falciparum* and *P. vivax* [30], which may be related to the lower transmission rate of *P. ovale* from diverse geographical origins [8]. These findings were similar to previously published data demonstrating a low level of sequence diversity of the MSP-1 gene in *P. ovale* [18].

The intragenic recombination of MSP-1 gene is a major informative pattern at the level of population sequence diversity. The frequency of allelic recombination has important guiding significance for the population structure of parasites [31]. A previous study demonstrated that *P. falciparum* has a low level of genetic diversity in areas with low transmission rates and high level of sequence diversity in areas with high transmission rates [32]. High mutational diversity was observed in *pvmsp1* isolated from Thailand northwestern region [33]. The level of nucleotide diversity in both *pocmsp1* and *powmsp1* N-terminal sequences detected in this study showed lower magnitude than that reported for *pvmsp1* and *pfmsp1* [34, 35].

The intragenic recombination of MSP-1 gene is a major informative pattern at the level of population sequence diversity. The frequency of allelic recombination has important guiding significance for the population structure of parasites [31]. A previous study demonstrated that *P. falciparum* has a low level of genetic diversity in areas with low transmission rates and high level of sequence diversity in areas with high transmission rates [32]. High mutational diversity was observed in *pvmsp1* isolated from Thailand northwestern region [33]. The level of nucleotide diversity in both *pocmsp1* and *powmsp1* N-terminal sequences detected in this study showed lower magnitude than that reported for *pvmsp1* and *pfmsp1* [34, 35].

### Table 2

| Type          | No. samples | G + C content (%) | No. haplotypes | *Hd* | Diversity ± SD Nucleotide | Diversity ± SD Haplotype | Tajima’s *D* | Fu & Li’s *D*^*^ | Fu & Li’s *F*^*^ |
|---------------|-------------|------------------|----------------|-----|--------------------------|--------------------------|--------------|----------------|----------------|
| *P. ovale curtisi* | 61          | 34.1             | 5              | 0.746 | 0.01043 ± 0.00061       | 0.746 ± 0.035            | 3.22138      | 1.81498        | 2.77112        |
| *P. ovale wallikeri* | 65          | 35.2             | 3              | 0.598 | 0.01974 ± 0.00055       | 0.598 ± 0.036            | 4.57287      | 2.00379        | 3.5575         |

*Fig. 4* Sliding window plots of sequence diversity (*π*) and Tajima’s *D*. **a** Sequence diversity of *pocmsp1*. **b** Tajima’s *D* of *pocmsp1*. **c** Sequence diversity of *powmsp1*. **d** Tajima’s *D* of *powmsp1*.
Conclusions
This study provides valuable reference information on the genetic diversity of *P. ovale curtisi* and *P. ovale wallikeri* isolates imported from Africa to China based on analysis of the MSP-1 N-terminal sequence. To our knowledge, this is the first report of the genetic diversity, selection signature, and population structure of the N-terminal of *pomsp1* gene from an African population. The low level of genetic diversity indicated that these genes are under purifying selection. Therefore, these sequences have potential for vaccine development, which requires further investigation of the immunogenicity and antigenicity of *P. ovale* MSP-1.

Additional file

**Additional file 1:** Figure S1. Neighbor-joining tree of 26 unique alleles of the gene encoding *msp1* from 18 *Plasmodium* parasite species. (TIF 3756 kb)

Abbreviations
MSP-1: Merozoite surface protein-1; PCR: Polymerase chain reaction; aa: Amino acid; H: Haplotype diversity; SNP: Single nucleotide polymorphism; dN: Rates of non-synonymous substitutions; dS: Rates of synonymous substitutions; H: Haplotype; H: Haplotype diversity; k: Average number of pairwise nucleotide differences within the population; n: Nucleotide diversity

Acknowledgements
The authors thank all study participants, local health officials and doctors for their participation and support.

Funding
This study was funded by grants from National Natural Science Foundation of China (81601787), by Natural Science Foundation of Jiangsu Province (BK20160192, BK20150001), by the Jiangsu Provincial Key Research and Development Program (BE2016631), by the Fundamental Research Funds for the Central Universities funded by the Ministry of Education of China (JUSRP1710A), by Bill & Melinda Gates Foundation (OPP1161962), by National First-Class Discipline Program of Food Science and Technology (JUFSTR20180101), and by the Jiangsu Provincial Project of Invigorating Health Care through Science, Technology and Education.

Availability of data and materials
The data supporting the conclusions of this article are included within the article and its additional file.

Authors’ contributions
YC and JC conceived and designed the study. SX, JXT, JC and GDZ collected the samples. RLC, XXZ and LMC performed the acquisition of data and data analysis. RLC, XXZ, YHL and YHX conducted the laboratory work, data handling and analysis and reviewed the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate
This study was approved by the Ethics Committee, Jiangsu Provincial Key Laboratory on Parasite and Vector Control Technology, Jiangsu Institute of Parasitic Diseases (JIPD) (IRB00004221), Wuxi, China. Written informed consent was obtained from all of the participants.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
Author details
1Laboratory of Pathogen Infection and Immunity, Department of Public Health and Preventive Medicine, Wuxi School of Medicine, Jiangnan University, Wuxi, Jiangsu, People’s Republic of China. 2Key Laboratory of National Health and Family Planning Commission on Parasitic Disease Control and Prevention, Jiangsu Provincial Key Laboratory on Parasite and Vector Control Technology, Jiangsu Institute of Parasite Diseases, Wuxi 214064, Jiangsu, People’s Republic of China.

References
1. WHO. World Malaria Report (2008–2017). Geneva: World Health Organization; 2017. http://www.who.int/malaria/publications/world_malaria_report/en/

2. Sutherland CJ, Polley SD. Two nonrecombining sympatric forms of the human malaria parasite Plasmodium ovale occur globally. J Infect Dis. 2010;201:1544.

3. Cao Y, Wang W, Liu Y, Cotter C, Zhou H, Zhu G, et al. The increasing importance of Plasmodium ovale and Plasmodium malariae in a malaria elimination setting: an observational study of imported cases in Jiangsu Province, China, 2011–2014. Malar J. 2016;15:459.

4. Luo EP, Wang WM, Liu YB, Cao YY, Zhou HY, Xu T. Analysis of epidemic situation of malaria in Jiangsu Province from 2005 to 2014. Chin J Schisto Control. 2015;27:251–4 (In Chinese).

5. Stephens JWW. A new malaria parasite of man. Ann Trop Med Parasitol. 1922;16:383–8.

6. Holder AA, Blackman MJ, Burghaus PA, Chappel JA, Ling IT, McCollumdeighton N, et al. Malaria merozoite surface protein (MSP1)–1922;16:383–8.

7. Collins WE, Jeffery GM. Plasmodium ovale: parasite and disease. Clin Microbiol Rev. 2005;18:570–81.

8. Win TT, Lin K, Mizuno S, Zhou M, Liu Q, Ferreira MU, et al. Wide distribution of Plasmodium ovale in Myanmar. Trop Med Int Health. 2010;27:31–9.

9. Kawamoto FO, Liu Q, Ferreira MU, Tantular IS. How prevalent are Plasmodium ovale and P. malariae in East Asia? Parasitol Today. 1999;15:422–6.

10. Bichara C, Flahaut P, Costa D, Bienvenu AL, Picot S, Gargala G. Cryptic Plasmodium ovale concurrent with mixed Plasmodium falciparum and Plasmodium malariae infection in two children from Central African Republic. Malar J. 2017;16:339.

11. Collins WE, Jeffery GM. Plasmodium ovale: parasite and disease. Clin Microbiol Rev. 2005;18:570–81.

12. Holder AA, Blackman MJ, Burghaus PA, Chappel JA, Ling IT, McCollumdeighton N, et al. Malaria merozoite surface protein (MSP1)–1922;16:383–8.

13. Egan AF, Barnish G, Allen S, Greenwood BM, Kaslow DC, et al. Parasites & Vectors 2011;124:755–6.

14. Egan AF, Barnish G, Allen S, Greenwood BM, Kaslow DC, et al. Parasites & Vectors 2011;124:755–6.

15. Mayengue PI, Ndounga M, Malonga FV, Bitemo M, Ntoumi F. Genetic polymorphism of merozoite surface protein-1 and merozoite surface protein-2 in Plasmodium falciparum isolates from Brazzaville, Republic of Congo. Malar J. 2011;10:276.

16. Carmen FB, Sergi S, Matina B, Staniscil DJ, Alves FP, Camargo EP, et al. Naturally-acquired humoral immune responses against the N- and C-termini of the Plasmodium vivax, MSP1 protein in endemic regions of Brazil and Papua New Guinea using a multiplex assay. Malar J. 2010;9:29.

17. Kaslow DC, Hui G, Kumar S. Expression and antigenicity of Plasmodium falciparum major merozoite surface protein (MSP) variants secreted from Saccharomyces cerevisiae. Mol Biochem Parasitol. 1994;63:283.

18. Potapovt C, Hughes AL, Jongwutiwes S. Low level of sequence diversity at merozoite surface protein-1 locus of Plasmodium ovale curtisi and P. ovale waltikeri from Thai isolates. PLoS One. 2013;8:e58962.

19. ESRI. ArcGIS Desktop: Release 10. Redlands: Environmental Systems Research Institute. 2011.

20. Nicholas K, Nicholas H. GeneDoc: A tool for editing and annotating multiple sequence alignments. Ver. 2.7.000. 1996. Distributed by the author. 1997. http://kubic.bio.indiana.edu/soft/molbio/dmnp/genedoc-readme.html.

21. Burland TG, DnaStar’s lasergene sequence analysis software. Methods Mol Biol. 2000;132:71–91.

22. Kumar S, Stecher G, Tamura K. MEGA7. Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–4.

23. Nei M, Gojobori T. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol Biol Evol. 1986;3:418–26.

24. Rozas J, Ferencmata A, Sánchezdezbarrio JC, Guairancosio S, Librado P, Ramosonionsi SE, et al. DnaSP 6: DNA sequence polymorphism analysis of large datasets. Mol Biol Evol. 2017;34:3299–302.

25. Tajima F. Simple methods for testing the molecular evolutionary clock hypothesis. Genetics. 1993;135:599–607.

26. Fu YX, Li WH. Statistical tests of neutrality of mutations. Genetics. 1993;133: 693–709.

27. Bhatti PK. Genetic diversity in the block 2 region of the merozoite surface protein-1 of Plasmodium falciparum in central India. Malar J. 2012;11:78.

28. Silva JC, Egan A, Friedman R, Munro JB, Carlton JM, Hughes AL. Genome sequences reveal divergence times of malaria parasite lineages. Parasitology. 2011;138:1737–49.

29. Chenet SM, Branch OH, Escalante AA, Lucas CM, Bacon DJ. Genetic diversity of vaccine candidate antigens in Plasmodium falciparum isolates from the Amazon basin of Peru. Malar J. 2008;7:29.

30. Atroshch WM, Almekhlafi HM, Mahdy MA, Safsafi R, Almekhlafi AM, Surin J. Genetic diversity of Plasmodium falciparum isolates from Pahang, Malaysia based on MSP-1 and MSP-2 genes. Parasit Vectors. 2011;4:233.

31. Putaporntip C, Jongiwutsivgetsan, Sakihana, Ferreira MU, Kho WG, Kaneko A, et al. Mosaic organization and heterogeneity in frequency of allelic recombination of the Plasmodium vivax merozoite surface protein-1 locus. Proc Natl Acad Sci USA. 2002;99:16348–53.

32. Bannister LH, Hopkins JM, Fowler RE, Krishna S, Mitchell GH. Microsatellite markers reveal a spectrum of population structures in the malaria parasite Plasmodium falciparum. Mol Biol Evol. 2000;17:1467.

33. Putaporntip C, Hongsrimuang T, Seethamchai S, Kobasa T, Limkittikul K, Cui L, et al. Differential prevalence of Plasmodium infections and cryptic Plasmodium knowlesi malaria in humans in Thailand. J Infect Dis. 2009;199: 1143–50.

34. Versiani FG, Almeida ME, Mariuba LA, Orlandi PP, Nogueira PA. N-Terminal N-Terminal region of Plasmodium vivax merozoite surface protein-1, a potential subunit for malaria vivax vaccine. Clin Dev Immunol. 2013;2013:695841.

35. Tanabe K, Sakihana N, Rooth I, Björkman A, Färnert A. High frequency of intragenic recombination in central India. Malar J. 2012;11:78.

36. Golden Access which fosters wider collaboration and increased citations.

37. maximum visibility for your research: over 100M website views per year.

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions