Genetic diversity of *Plasmodium vivax* population in Anhui province of China

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**Abstract**

**Background:** Although the numbers of malaria cases in China have been declining in recent years, outbreaks of *P. vivax* malaria were still being reported in rural areas south of the Yellow River. To better understand the transmission dynamics of *P. vivax* parasites in China, the extent of genetic diversity of *P. vivax* populations circulating in Bozhou of Anhui province of China were investigated using three polymorphic genetic markers: merozoite surface proteins 1 and 3 (pvmsp-1 and pvmsp-3a) and circumsporozoite protein (pvcsp).

**Methods:** Forty-five *P. vivax* clinical isolates from Bouzhou of Anhui province were collected from 2009 to 2010 and were analysed using PCR/RFLP or DNA sequencing.

**Results:** Seven and six distinct allelic variants were identified using PCR/RFLP analysis of pvmsp-3\(\alpha\) with *Hha*I and *Alu*I, respectively. DNA sequence analysis of pvmsp-1 (variable block 5) revealed that there were SaI and recombinant types but not Belem type, and seven distinct allelic variants in pvmsp-1 were detected, with recombinant subtype 2 (R2) being predominant (66.7%). All the isolates carried pvcsp with VK210 type but not VK247 or *P. vivax*-like types in the samples. Sequence analysis of pvcsp gene revealed 12 distinct allelic variants, with VK210-1 being predominant (41.5%).

**Conclusions:** The present data indicate that there is some degree of genetic diversity among *P. vivax* populations in Anhui province of China. The genetic data obtained may assist in the surveillance of *P. vivax* infection in endemic areas or in tracking potential future disease outbreak.

**Keywords:** *Plasmodium vivax*, pvmsp-1, pvmsp-3\(\alpha\), pvcsp, Anhui, China

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**Background**

Malaria kills more than a million people a year, and approximately 40% of the world’s populations live in malarious countries [1]. *Plasmodium vivax* is the most widespread species of human malaria parasites in the world and is endemic in many countries of Asia, Central and South America, the Middle East, and parts of Africa, with an estimated burden of 70–80 million cases annually [2,3]. In China, the malaria eradication campaign initiated in the 1990s, using vector control and drug treatment of febrile individuals had been highly successful. However, episodes of *P. vivax* malaria infection were still being reported, and *vivax* malaria re-emerged in many counties of central China such as provinces of Anhui, Shandong, Hubei, Henan, and Jianguo in recent years [4]. Approximately 37% to 68% of malaria cases reported during 2003 to 2007 were from central China, where the predominant vector mosquito was *Anopheles sinensis*. Most importantly, the numbers of *vivax* malaria cases in Anhui province of China were the highest in the country during those years [5].

A countrywide malaria elimination policy was launched by the Ministry of Health of P.R. China in 2010, with the goal to eliminate malaria by 2015 in a majority of the regions with the exception of the border region in Yunnan province, and to completely eliminate malaria from China by 2020. To achieve this ambitious goal, studying population structure, genetic diversity, and transmission of
Plasmodium parasites in endemic areas will provide important information for disease control and management, including baseline data essential for monitoring drug resistance and for predicting the origin and spread of parasite variants within and between populations, and the performance of malaria vaccines under development [6,7]. Several genetic markers including microsatellites and the genes encoding circumsporozoite protein (csp), merozoite surface protein 1 (msp-1), and merozoite surface proteins 3α and 3β (msp-3α and msp-3β), have been used to study P. vivax population diversity [8-11]. However, so far the population structures of P. vivax are less well studied and understood and only a limited number of studies on the population genetics of P. vivax isolates in China have been conducted [7]. In this study, the population diversity of clinical P. vivax isolates from Anhui province of central China was evaluated using the above three polymorphic markers, and the molecular diversity data may provide important information for vivax malaria surveillance and for tracking parasites in future outbreaks in this area.

Methods

Study area

The study was conducted in Bozhou city of Anhui province, which borders six provinces: the Jiangsu province in the east, the Zhejiang province in the southeast, the Jiangxi province in the south, the Hubei province in the west, the Henan province in the northwest, and the Shandong province in the north (Figure 1), located in an area between latitudes 32°51’ and 35°05’, and longitudes 115°53’ and 116°49’. The population of this city was estimated to be 485,000 in 2010. The area has a distinct seasonal climate characterized by humid summer from June to September and dry winter from October to December, with temperature ranging from 14°C to 17°C and rainfall ranging from 500 mm to 750 mm per year [12]. Bozhou city was a vivax endemic area with seasonal transmission peaking from June to September. Climatic and ecological conditions of this area make the environment favorable for mosquito breeding, and the main malaria vector is An. sinensis and the secondary one is Anopheles anthropophagus.

Patients and sample collection

This study was reviewed and approved by the Ethics Committee of Sun Yat-sen University, China (IRB 2009A0101). Blood samples were collected from patients with symptomatic P. vivax malaria admitted to the local hospitals for antimalarial drug treatment in the city from June 2009 to October 2010. Inclusion criteria were patients infected with P. vivax, but not other human malaria species, as confirmed by peripheral smear examination. Preparation and staining of the blood slides were done following the procedures of WHO outlined in basic malaria microscopy. From patients who had the fever-chill cycle and visited a designated hospital for malaria diagnosis and treatment in Bozhou area, forty-five P. vivax-infected patients were diagnosed by Giemsa-stained thin and thick blood smears, in which only two out of the 45 P. vivax infected patients had a travel history outside of Anhui province, and none of the patients had a travel history abroad. After informed consent from all adults or legal guardians of children, 1.0 ml of whole blood sample was collected in EDTA and stored at −20°C until DNA extraction.

DNA extraction

Plasmodium vivax genomic DNA was extracted from 200 μl of each infected blood sample using DNA blood kit following the manufacturer’s instructions (Takara, Japan) with minor modifications. The DNA was dissolved in TE buffer (10 mM Tris–HCl, pH 8.0, 0.1 M EDTA) and stored at −20°C until use. The quality of total DNA was analysed by running 5 μL of each DNA sample on a 1.0% agarose gel stained with ethidium bromide and visualized with UV illumination.

PCR amplification of pvmsp-3α, pvmsp-1, and pvcspt genes

To amplify the pvmsp-3α, pvmsp-1, and pvcspt genes, a nested PCR amplification method was used following previously reported protocols with some minor modifications [10,13,14]. Oligonucleotide primers and cycling conditions are listed in Additional file 1. All amplification reactions were carried out in a total volume of 25 μL containing 17.0 μl of dH2O, 1.0 μl of each primer (10 pM), 1.5 μl of MgCl2 (25 mM), 0.5 μl of Taq polymerase (5 U/μl), 0.5 μl of dNTP mixture (10 mM each), and 2.5 μl of 10× PCR buffer, following the manufacturer’s instructions (BBI, Canada). Primary amplification

![Figure 1 Map of Anhui province in central China showing the location of Bozhou city where P. vivax isolates were collected. Scale bar = 100 kilometer.](http://www.malariajournal.com/content/13/1/13)
reactions were initiated with the addition of 1.0 μL of template genomic DNA prepared from the blood samples, and 1.0 μL of the primary reaction amplification was used as template in the secondary amplification reactions. The amplified PCR products were resolved on 1.0% agarose gel, and the sizes of the PCR products were determined using a 100 or 500 bp DNA ladder (NewProbe, China).

**PCR/RFLP analysis of pvmsp-3α gene**

For RFLP analysis of pvmsp-3α gene, the PCR products were digested individually with restriction enzymes HhaI and/or AluI in 20 μl reaction volumes at 37°C for 4 h as previously described [10]. Briefly, all digestion reactions were carried out in the presence of 10.0 μl PCR product, 7.0 μl of H2O, 1.0 μl of enzymes HhaI or AluI (5 U/μl), and 2.0 μl of buffer according to the manufacturer’s instructions (TaKaRa, Japan). After electrophoresis on 2.5% agarose gel, the enzyme-digested fragments were visualized under UV illumination. The sizes of the digested fragments were estimated using a 100 bp ladder of molecular weight markers. The results were recorded and analysed on a Gel Doc XR image analyzer using Quantity One software (Tanon, China).

**Sequence analysis of pvmsp-1 and pvcsp genes**

The nested PCR products of pvmsp-1 and pvcsp genes were directly sequenced in both directions using an ABI PRISM3730 DNA sequencer by Sangon Biotech (Shanghai, China). Nucleotide and amino acid sequences were aligned and compared using CLUSTAL W of BioEdit 7.0 program with the following published sequences: VK210 (accession no. M28746) and VK247 (accession no. M28745) of pvcsp and Sal-I (accession no. M75674) and Belem (accession no. M60807) of pvmsp-1, respectively. Sequence relationship trees of the pvmsp-1 and pvcsp genes from the Anhui isolates and published sequences from isolates of different geographic locations of Asia were constructed using neighbour-joining method implemented in MEGA 4 program [15]. Bootstrap proportions were used to assess the robustness of the tree with 1,000 bootstrap replications.

**Results**

**PCR/RFLP analysis of the pvmsp-3α gene**

The pvmsp-3α gene was successfully amplified from all the 45 P. vivax isolates examined. All of the PCR products from P. vivax isolates were the same size of approximately 1.9 kb (type A); no type B (~1.5 kb) or type C (~1.1 kb) was detected. To further characterize variation in the gene, the PCR products were digested with restriction enzymes HhaI and AluI, and 7 (PH1 to PH7) and 6 (PA1 to PA6) distinct variants were detected, respectively, based on the restriction patterns of the PCR products (Figures 2A, B). In the HhaI digestion, allelic variants PH1, PH2, and PH3 were the most common patterns, with frequencies of 42.2%, 17.8%, and 17.8%, respectively (Figures 2C), representing 77.8% of the total samples tested. Similarly, 82.2% of the AluI digested samples were allele variants of PA1, PA2, and PA5, representing 40.0%, 20.0%, and 22.2%, respectively (Figures 2C).

**DNA sequence polymorphism and clustering of pvmsp-1**

All 45 isolates from Bozhou were successfully amplified for pvmsp-1 gene. The size of PCR products varied from 420 to 520 bp for the isolates examined, with the 420 bp
product being the most common fragment (80.2%). Sequence analyses of the PCR products showed that the isolates could be divided into two types: Sal-I (S) type (24.4%) or a recombination (R) type (75.6%) that was characterized by a combination of a Sal-I-like sequence at the 5′-end and a Belem-like sequence at the 3′-end, including a polymorphic poly Q segment [8,16]; no Belem type was found in these isolates. In total, seven distinct variants were found in the isolates, with five variant subtypes for the Sal-I type and two variant subtypes for the R-type, and with 13 and 10 poly Qs, respectively (Figure 3A). Of the seven sequence variants, R2-type was the most prevalent, accounting for 66.7% of all isolates examined. Sal-I type 5 variants were evenly distributed among the isolates, with frequencies of 6.7%, 4.4%, 4.4%, 4.4%, 4.4%, and 6.7% for Sal-I type S1, S2, S3, S4, S5, and R1 types, respectively (Figure 3B).

A dendrogram of the pvmsp-1 variable block 5 sequences was constructed using nucleotide sequences from the Anhui isolates and 57 published pvmsp-1 sequences obtained from the isolates of different geographic locations of China (seven from Anhui province, seven from Fujian province, eight from Hainan province, six from Liaoning province, and five from Yunnan province of China) and other Asian countries (three from Afghanistan, two from Bangladesh, three from India, three from Iran, two from Myanmar, one from North Korea, three from South Korea, four from Papua New Guinea, three from Thailand, and two including the Sal-I strain and Belem strain from Brazil). This analysis grouped the P. vivax Anhui population into two major clusters (Sal-I type and R-type) with different subtypes in each group (Figure 4). The Sal-I type strains clustered with isolates from Hainan, Fujian, and Anhui of China as well as those from Afghanistan, Myanmar, Bangladesh, India, Thailand, and Papua New Guinea, whereas the R-type parasites clustered with parasites from Liaoning of China (northern China), North Korea, Iran, Afghanistan, Myanmar, and Papua New Guinea. Some of the P. vivax isolates examined in this study showed 100% identity with strains from other regions of China or even from different Asian countries, including Anhui province [AY465398 with S3 (Sal-I type)], Liaoning province [JQ606831 with S3, and JQ606834 with R2], Fujian province [AY538672 with R2], Hainan province [AY465377 with S2], Yunnan province [AY465391 with R2], South Korea [HQ171940 with S3, and HQ171937 with R2], North Korea [AF153032 with R2], India [AY229866 with S1], and Iran [AY502161 with R1]. Sal-I type S4 and S5 allele variants were new alleles identified in this study.

DNA sequence polymorphism and clustering of pvcs
To analyse polymorphisms in the pvcs gene, the central repeat and its flanking regions of the gene from the samples examined were amplified and sequenced. All of the 45 isolates were successfully amplified for the pvcs gene

![Figure 3](alignment_of_amino_acid_sequences_of_7_pvmsp-1_distinct_allelic_variants_and_frequencies_of_pvmsp-1_allelic_variants_identified_from_45_P_vivax_Anhui_isolates.png)

Figure 3 Alignment of amino acid sequences of 7 pvmsp-1 distinct allelic variants and frequencies of pvmsp-1 allelic variants identified from 45 P. vivax Anhui isolates. (A) Sequences were constructed to the published amino acid sequences of Belem (M60807) and Sal-I (M75674). Dots and dashes represent identical residues and deletions, respectively. Amino acid changes resulting from nucleotide substitutions are shown in bold. S: Sal-I type; R: Recombination type. (B) Allelic frequencies of 7 pvmsp-1 allelic variants obtained from P. vivax Anhui isolates. S: Sal-I type; R: Recombination type of pvmsp-1.
with PCR products having sizes ranging from 540 to 720 bp, with a 600 bp fragment being the predominant product among these isolates (58.5%). All of the DNA sequences belonged to VK210 type with 12 distinct variants; no VK247 and \(P.\) vivax-like types were detected within these isolates (Figure 5A) [17-19]. All variants started with the same pre-repeat sequence (KLKQP Region). The isolates displayed variations in the central peptide repeat motifs GDRA (A/D) GQPA with alternations of the repeating units, ranging from 7 to 17 repeats among these isolates. Of the 12 variants, the most prevalent sequence variant was VK210-1 subtype (42.2%, 19/45), followed by subtypes VK210-6 (15.6%, 7/45) and VK210-7 (13.3%, 6/45). The remaining nine variants of \(pvcsp\) gene were evenly distributed among the isolates (Figure 5B).

A dendrogram of the \(pvcsp\) sequences was constructed using neighbor-joining method based on the nucleotide sequences from Anhui isolates in this study and 22 published \(pvcsp\) sequences from the isolates of different geographic locations of Asia (seven from China, one from Indonesia, two from India, two from Iran, two from Myanmar, two from North Korea, two from Papua New Guinea, two from the Solomon Islands, and two from South Korea) (Figure 6). The sequences clustered into two distinct groups for the VK210 and VK247 type isolates. And the analysis clearly showed that all subtypes of \(pvcsp\) from Anhui isolates clustered with the VK210 type in the tree. Some of the \(P.\) vivax Anhui isolates in this study showed 100% identity with other strains from China and Asian countries previously published, such as China’s Tibet (FJ601725
with VK210-6), North Korea (M20670 with VK210-6), and South Korea (DQ859770 with VK210-10); whereas the remaining VK210 subtypes were new alleles identified in this study.

**Haplotypes from combined pvmsp-3α, pvmsp-1, and pvcsp allelic variants**

By analysing variants of the three genetic markers (pvmsp-3α, pvmsp-1, and pvcsp) in combination, a total of 16 *P. vivax* haplotypes were identified in the 45 Anhui isolates with haplotype A of PH1 and PA1 of pvmsp-3α, R-2 of pvmsp-1, and VK210-1 of pvcsp being the most common haplotypes (37.8%), followed by haplotype D of PH2 and PA2 of pvmsp-3α, R-2 of pvmsp-1, and VK210-6 of pvcsp (Table 1). These haplotypes also contained the most common allelic variants of the individual markers. PH1 and PA1 of pvmsp-3α (42.2% and 40.0%,

**Figure 5** Sequence alignments of amino acid sequences of 12 pvcsp distinct allelic variants and frequencies of pvcsp allelic variants identified from 45 *P. vivax* Anhui isolates. (A) Sequences were aligned to the amino acid sequences of the reference strain VK210 (M28746). Dots and dashes represent identical residues and deletions, respectively. Amino acid changes resulting from nucleotide substitutions are shown in bold. (B) Allelic frequencies of 12 pvcsp allelic variants obtained from *P. vivax* Anhui isolates.
respectively), R-2 of pvmsp-1 (66.7%), and VK210-1 of pvcsp (42.2%).

**Discussion**

Malaria is one of the major parasitic diseases having a wide distribution in China with the prevalence gradually decreasing from south to north. Compared with southern (Hainan province) and southwestern (Yunnan province) regions, central China (including Anhui province) has a low endemicity of malaria, with *P. vivax* being the only species after the year 2000. Anhui province is the most seriously affected area in central China, with the largest number of malaria cases reported during the years 2005–2010. Different parasite genotypes are circulating in each...
Table 1 Combined haplotypes and frequencies of pvmsp-3α, pvmsp-1, and pvcsps from 45 vivax isolates of Anhui province

| Allelic variants | Genes          | Number (%) |
|------------------|----------------|------------|
|                  | pvmsp-3α | pvmsp-1 | pvcsps |
| A                 | PH1     | PA1     | R-2    | VK210-1 | 17 (37.8) |
| B                 | PH1     | PA1     | R-2    | VK210-11| 1 (2.2)   |
| C                 | PH1     | PA5     | R-2    | VK210-1 | 1 (2.2)   |
| D                 | PH2     | PA2     | R-2    | VK210-6 | 7 (15.6)  |
| E                 | PH2     | PA2     | R-2    | VK210-1 | 1 (2.2)   |
| F                 | PH3     | PA5     | S-3    | VK210-7 | 2 (4.4)   |
| G                 | PH3     | PA5     | R-1    | VK210-5 | 1 (2.2)   |
| H                 | PH3     | PA5     | R-1    | VK210-3 | 1 (2.2)   |
| I                 | PH3     | PA5     | R-2    | VK210-8 | 3 (6.6)   |
| J                 | PH3     | PA5     | R-2    | VK210-9 | 1 (2.2)   |
| K                 | PH4     | PA4     | S-2    | VK210-7 | 2 (4.4)   |
| L                 | PH4     | PA4     | S-4    | VK210-7 | 2 (4.4)   |
| M                 | PH5     | PA6     | S-1    | VK210-12| 1 (2.2)   |
| N                 | PH5     | PA5     | S-1    | VK210-4 | 2 (4.4)   |
| O                 | PH6     | PA2     | S-5    | VK210-10| 1 (2.2)   |
| P                 | PH6     | PA3     | S-5    | VK210-10| 1 (2.2)   |
| Q                 | PH7     | PA5     | R-1    | VK210-2 | 1 (2.2)   |

PH: The allelic type of pvmsp-3α was based on the fragment sizes of HhaI-digested PCR products; PA: The allelic type of pvmsp-3α was based on the fragment sizes of AluI-digested PCR products.
S: SalI type; R: Recombination type of pvmsp-1.

endemic area of malaria-hypoendemic regions of Southeast Asia, and that geographic isolation may exist [7]. Additionally, with increasing movement of human populations, the P. vivax populations and transmission pattern in the region are also changing constantly [20]. However, the genetic diversity of P. vivax parasites circulating in the area of central China is poorly understood. To better understand the P. vivax populations and transmission dynamics in Anhui of central China, the extent of genetic diversity of P. vivax parasites circulating in Bozhou of Anhui province were investigated using three polymorphic genetic marker pvmsp-3α, pvmsp-1, and pvcsps genes, and their relationships with parasites from other regions of China and Asian countries were analysed in this study. The data in the present study indicate that there was some degree of genetic diversity of P. vivax in Anhui province, and there appeared to be signs of genetic exchange and genetic recombination among vivax parasites in the region. Although a relatively small number of samples (45) could be obtained, the patients came from many parts of the city. Additionally, the diverse genetic backgrounds from the three genetic markers suggested that the parasites were not from outbreaks of 1–2 parasite strains and should be able to represent the genetic backgrounds of the parasite populations in the region.

It has been proposed that pvmsp-3α is a reliable molecular marker for analysis of P. vivax populations [11]. Based on the length of PCR products, four different allelic types of pvmsp-3α have been characterized around the world: type A (~1.9 Kb), type B (~1.5 Kb), type C (~1.1 Kb), and type D (~0.3 Kb) [6,21]. In the present study, only type A (~1.9 Kb) was identified among the 45 Anhui isolates tested. The observation was different from a previous report from several locations in China [7], in which type A, B, and C were detected in P. vivax isolates from Suzhou city of Anhui province, Sanya city of Hainan province, and Dehong prefecture of Yunnan province. The lack of other pvmsp-3α allele types in Bozhou city of Anhui province could simply be due to the relatively small sample size of this study. The results in some degree are consistent with the reports of type A being the most prevalent type around the world, with a frequency 70% to 100% (average ~80%) in many regions of China and countries in Asia and South America [7,21-27]. Seven patterns were detected in the pvmsp-3α gene after digestion of the PCR products with HhaI, with the most frequent allele variant being PH1 subtype. The results were consistent with those reported from other regions of the world, including those from Myanmar (n = 14) [7], Iran (n = 6) [14], Colombia (n = 9) [22], French Guiana (n = 11) [23], Thailand (n = 6) [28], Venezuela (n = 9) [29], and Peru (n = 17) [30]. However, outside Anhui province, the allele PH3, the second most common one in the present study, was the most prevalent in other areas of China including Hainan and Yunnan provinces, and also in Myanmar [7]. All of the HhaI allelic types found in the present study have been reported in other regions of the world, including Myanmar (PH1, PH3, PH4, PH5, and PH6) [7], India Chennai (PH1, PH4, PH5, and PH6) [11,21], Colombia (PH1, PH2, PH3, PH4, PH5, and PH6) [22], Brazil (PH3 and PH4) [26], and Thailand (PH1, PH3, PH4, PH6, and PH7) [28]. In China, five of the seven alleles were reported previously, e.g., PH1, PH3, PH4, PH5, and PH6 from Suzhou city of Anhui province; PH3, PH4, PH5, and PH6 from Hainan province; and PH1, PH3, and PH4 from Yunnan province [7].

Six patterns were detected in the pvmsp-3α gene after digestion of the PCR products with AluI in the present study, with the most predominated PA1 pattern in Bozhou city. This observation has not been reported in other areas of the world. Some allele types of AluI digestion were comparable to those reported in other areas of the world, such as Colombia (PA2, PA4, PA5, and PA6), India (PA1, PA2, PA4, PA5, and PA6), and Iran (PA1 and PA2), suggesting that these allele types of P. vivax may have a global distribution [14,21,22]. The allele variant PA3 was a new allele identified in this study. The analyses from pvmsp-3α suggest that P. vivax populations in Bozhou city shared the majority of allelic variants with
other parts of China and the world; however, some new allelic types were emerging.

To obtain more information of genetic polymorphism of the vivax malaria samples, the DNA sequences encoding the pvmsp-1 and pvcsp genes were analysed. Three alleletypes, Belem, Sal-I, and recombinant types of the variable block 5 have been described from P. vivax isolates worldwide [8,16]. Genetic studies of P. vivax malaria parasites using the pvmsp-1 marker have been reported from many countries, including India, Myanmar, Pakistan, and Korea [15,25,31,32]. In the present study, only Sal-I and R-types were detected among the Anhui isolates with R-type being the predominant allele types (75.6%). It has been reported that the R-type result from intragenic recombination of Belem and Sal-I types in mosquito vector [33,34]. The results indicate that inter-allelic recombination can be an important source of pvmsp-1 genetic diversity among Anhui province locations. The high level of inter-allelic recombinant types in this isolate was in concordance with previous reports in Pakistan [25], India [32], Colombia [32], Thailand [35], and Brazil [36]. Although the Belem type is the predominant allele type in other P. vivax malaria endemic regions in the world, including northern Iran [14], south-eastern and south-western parts of Afghanistan [37], and Azerbaijan [38], Belem type was not found among the Anhui isolates in this study. The absence of Belem type is intriguing, considering the presence of the recombinant types, which could be related to population changes of mosquito species or sampling bias. It has been reported that the distributions of different P. vivax populations in southern Mexico were largely determined by their infectivity to two species of anopheline vectors in the areas [39]. A similar situation could occur in China; changes in mosquito strains or species could favor a subpopulation of vivax parasites, leading to changes in parasite population structure.

For pvcsp gene, three sequence types of VK210, VK247, and P. vivax-like have been detected based on a central repeat domain that varies in sequences and number of repeat units [17-19,40,41]. VK210 being the predominant type has been observed in Myanmar (98.3%) [15], Iran (69.3%) [24,42], Thailand (77%) [28], Afghanistan (86.6%) [37], Pakistan (95.7%) [37], Azerbaijan (100%) [38], Brazil (86%) [40], Guyana (92%) [41], and India (99.3%) [43], although VK247 was also reported to be the predominant type previously [44-46]. The VK247 and P. vivax-like types were not detected in the Anhui isolates in the present study. It has been proposed that the geographical differences in distribution of different parasite types may be related to the susceptibility of different species of Anopheline vectors to the infections by different malaria parasites in endemic areas [47,48]. The main malaria vectors in Anhui province are An. sinensis and An. anthropophagus, but the density of An. anthropophagus is very low in recent years, and the re-emergence of P. vivax malaria was mainly caused by An. sinensis [49]. Therefore, the prevalence of VK210 type may be correlated with the increase of An. sinensis malaria vector in the region. A total of 12 VK210-1 subtypes were identified in 45 blood samples from Anhui province, two subtypes of which, VK210-6 and VK210-10, were also detected in other endemic areas, including China’s Tibet, North Korea, and South Korea through phylogenetic and Blast analysis.

In summary, P. vivax populations in Bozhou city of Anhui province have some degree of genetic diversity in three highly polymorphic markers, with 16 unique haplotypes observed among 45 samples (~36%). However, the present results also showed that P. vivax populations in Bozhou city of Anhui province had relative even major genotypes, pvmsp-3α type A (100%), pvmsp-1 R2 (75.6%), and pvcsp VK210 subtype (100%), suggesting its relative uniform parasite populations. It is interesting that pvmsp-1 recombinant type was detected, which may be related to the recent changes in vector population (increase in An. sinensis mosquito) in the region, or may have been simply sampled some subpopulations from recent outbreaks. The data in this study showed shared genotypes with those from Korea and other countries; there is a possibility that these parasites have the same origin and were disseminated by travelers. Although limited samples were included in this study, and the data may not represent the whole picture of genome diversity of the parasite populations in the region, the results suggest parasite populations with relatively diverse genetic backgrounds in the region.

The genetic diversity information obtained may provide valuable information for tracking and monitoring future malaria infections in this region.

### Additional file

**Additional file 1:** Sequences of the primers and cycling conditions used to amplify the pvmsp-3α, pvmsp-1, and pvcsp genes of P. vivax isolates from Anhui province. The data provided oligonucleotide primers and cycling conditions of pvmsp-3α, pvmsp-1, and pvcsp genes.

### Competing interests

The authors declare that they have no competing interests.

### Authors’ contributions

XZS and FL designed and supervised the study, analysed the data, and approved the final manuscript. SW participated in field work and preliminary data analysis. All authors read and approved the final manuscript.

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