Parasite Epidemiology and Control 26 (2024) e00362

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi

Genetic diversity of the *Pv*MSP-3α gene in *Plasmodium vivax* isolates circulating in the National Capital Region (NCR) of India

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ARTICLE INFO

Keywords:
Genetic polymorphism
Genotyping
Malaria
Plasmodium vivax
*Pv*MSP-3α

ABSTRACT

Malaria is still a public health problem in tropical countries like India; major malaria parasite species are *Plasmodium falciparum* and *P. vivax*. Of which, *P. vivax* is responsible for ~40% of the malaria burden at least in the Indian scenario. Unfortunately, there is limited data on the population structure and genetic diversity of *P. vivax* parasites in India. In this study, we investigated the genetic diversity of *P. vivax* strains in the South-west district, Delhi and, Nuh district, Haryana [National Capital Region (NCR)], using a polymorphic marker- *P. vivax* merozoite surface protein-3α (*Pv*MSP-3α) gene. Dried blood spots from microscopically confirmed *P. vivax* patients were used for investigation of the *Pv*MSP-3α gene. PCR-RFLP was performed on the *Pv*MSP-3α gene to investigate the genotypes and allelic variability with *Hha*I and *Alu*I restriction enzymes. In total, 40 successfully PCR amplified *Pv*MSP-3α gene segments were subjected to RFLP analysis. Amplified products showed three different base pair size variations *viz.* genotype A in 31(77.5%), genotype B in 4(10%) and genotype C in 5(12.5%) *P. vivax* specimens. RFLP with *Hha*I and *Alu*I revealed 17 (H1-H17) and 25 (A1-A25) allelic variants, respectively. Interestingly, two similar sub-allelic variants, *ie.* H8 (with *Hha*I), and A4 (with *Alu*I) clustered within the rural area of Nuh district, Haryana in two samples. With this study, we propose to commission such type of genetic diversity analysis of *P. vivax* to investigate the circulating genotypes of the parasites from distinct geographical locations across India, that can have significant implications in understanding the population structures of *P. vivax*.

1. Background

The five different plasmodium species (*P. vivax, P. falciparum*, *P. ovale*, *P. malariae* and *P. knowlesi*) are known to cause human malaria. Amongst these, *P. vivax* malaria accounts for major malaria infections, particularly in South-East Asia and South America (Durante Mangoni et al., 2003; Leclerc et al., 2004). In India, *P. vivax* accounts for ~40% of the malaria burden (NVBDCP, 2024), and contrary to the notion, *P. vivax* also causes severe malaria and mortality in India (Joy et al., 2018; Mathews et al., 2019; Nadkar et al., 2012). It is therefore, crucial to investigate the *P. vivax* disease transmission and burden, which is inherently determined by the population structure and genetic diversity of the circulating *P. vivax* parasites in various geographical regions of India. Evaluation of the polymorphic gene *P. vivax* merozoite surface protein- 3α (*Pv*MSP-3α) at the regional level would provide valuable insights in

Abbreviations: NVBDCP, National Vector Borne Disease Control Programme; RDTs, Rapid Diagnostic Tests.

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https://doi.org/10.1016/j.parepi.2024.e00362
Received 12 August 2023; Received in revised form 3 May 2024; Accepted 8 June 2024
Available online 10 June 2024

deciphering the genetic diversity of *P. vivax* parasites (Arnott et al., 2012; Rice et al., 2014). The genetic variability of *P. vivax* had been investigated in some parts of India (Kaul et al., 2019; Prajapati et al., 2010; Upmanyu et al., 2020; Verma et al., 2016); yet, there are unexplored regions of the Indian subcontinent in context of the population structure of the *P. vivax* isolates. Importantly, there are geographical determinants to the genetic diversity and variability of the *P. vivax* population (Imwong et al., 2007). The presence of D allele of *Pv*MSP-3α gene in *P. vivax* population has been contended to have a possible association with vivax malaria severity (Upmanyu et al., 2020). Therefore, in order to assess the *P. vivax* variants predominantly circulating in the human populations at a regional level, understanding the genetic diversity of the *Pv*MSP-3α gene would be useful. Merozoite surface protein (MSP) has been routinely explored to investigate the diversity and phylogenetic structure of *P. vivax via* investigation of *Pv*MSP-3α gene using PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) (Kaul et al., 2019; Prajapati et al., 2010; Verma et al., 2016; Rayner et al., 2004; Zakeri et al., 2006); *Pv*MSP-1 (Putaporntip et al., 2002), *Pv*MSP-3, *Pv*MSP-1_b5 (Véron et al., 2009), *Pv*MSP-3β (Yang et al., 2006), and *Pv*MSP-5 *via* DNA sequencing (Rayner et al., 2004; Gomez et al., 2006). Genotyping of the *Pv*MSP-3α gene by sequencing analysis is a robust and sensitive technique to decipher the genetic diversity of *P. vivax* strains; but it is quite expensive and requires a state-of-art laboratory with trained personnel. However, PCR-RFLP is a well-established, inexpensive, accurate and robust alternative tool, which is quite adaptable in laboratories with modest infrastructure (Rungsihirunrat et al., 2011; Suphakhonchuwong et al., 2018; Ullah et al., 2021).

The reports of the genetic diversity of *Pv*MSP genes from geographically and ecologically diverse regions of the Indian subcontinent have shed light on the genetic diversity of *P. vivax* in India (Kaul et al., 2019; Upmanyu et al., 2020; Verma et al., 2016). Importantly, it is worth noting that the *P. vivax* specimens investigated in these studies were collected in the first decade of twenty-first century (Kaul et al., 2019; Verma et al., 2016) or the latest reports about the circulating *P. vivax* parasites were from patients attending a tertiary care hospital in Delhi (Upmanyu et al., 2020). However, the current study presents the data on the genetic diversity of circulating *P. vivax* parasites collected from geographically distinct regional sites- South-west district, Delhi and Nuh district, Haryana [National Capital Region (NCR)] of India during 2014–2016. To date, this is the latest report on the genetic diversity of the *P. vivax* parasites circulating in the NCR of India. The results of this study would have implications in deciphering the circulating genotypes of *P. vivax* at a local level.

2. Materials and methods

2.1. Sample collection

A total of 45 confirmed *P. vivax* samples (29 from South-west district Delhi and 16 from Nuh district, Haryana were collected to study the genetic diversity of *P. vivax* population. The samples were collected from health care facilities from selected regions of NCR. Blood samples were collected on Whatman 3MM filter paper, from males and females between the age group of 1–50 years. The blood samples were tested by using Rapid Diagnostic Tests (RDTs)- SD BIOLINE Malaria Antigen P.f/P.v test (Standard Diagnostics Pvt. Ltd., Gurgaon, Haryana, India). The RDT positive *P. vivax* patients were further confirmed by microscopy (Barber et al., 2013). The confirmed *P. vivax* infected blood samples were subjected to genetic analysis using PCR-RFLP of the *Pv*MSP-3α gene.

2.2. DNA isolation and PCR amplification of *PvMSP-3α* gene

DNA was extracted by using QIAGEN kit (QIAamp® DNA Mini kit, Germany) as per the manufacturer's instructions and eluted in 100 µl of elution buffer and stored at −20 °C. Allelic diversity of *Pv*MSP-3α gene was assessed by using an established nested PCR method. The nested PCR reactions for *Pv*MSP-3α gene were performed for a total volume of 20 µl by using two sets of primers (P1-P2 & P3-P4) at a concentration of 0.2 µM, each in two rounds **(**Table 1**)**. The PCR reaction mix for the second round contained 1 µl of primary PCR product from first round, nested primers P3 and P4, 2.5 mM $MgCl_2$, 100 mM KCl, 20 mM Tris–HCl pH 8.0, total 10 µM of dNTPs, 1 unit of Taq DNA polymerase (Genei Laboratories Pvt. Ltd. India) (Bruce et al., 1999).

2.3. Genetic diversity of *PvMSP-3α* gene by PCR-RFLP

RFLP of *Pv*MSP-3α gene was performed by digesting the amplified product with restriction enzymes *Hha*I and *Alu*I, separately (Thermo Fisher Scientific, Inc., Vilnius, Lithuania). Briefly, the final reaction volume (20 µl) contained 5 µl of PCR product, 0.5 units of *Hha*I and *Alu*I restriction enzymes, and 1× reaction buffer. The reaction mixture was incubated at 37 °C for 3 h. and later at 65 °C for

Table 1
PCR primer sequences and thermal cycling conditions.

Primer	Primer sequences (5′–3′)	Thermal cycling conditions
First-round		Initial denaturation at 94 °C for 4 min, 94 °C for 20 s; followed by the 35 cycles of 94 °C for 20 s, 54 °C for 30 s, 68 °C for 2.5 min and a final extension at 68 °C for 10 min.
P1	CAGCAGACACCATTTAAGG	
P2	CCGTTTGTTGATTAGTTGC	
Second round (nested)		
P3	GACCAGTGTGATACCATTAACC	
P4	ATACTGGTTCTTCGTCTTCAGG	

30 min. A 2% agarose gel was used to resolve the digested amplified DNA products.

3. Results

3.1. Investigation of PvMSP-3α genotypes

From a total of 45 samples, the specific fragment of *Pv*MSP-3α gene successfully amplified in 40 samples. Five samples could not be amplified owing to the low parasite load and large fragment size of *Pv*MSP-3α gene. Amplified products were observed in three size variants: Genotype A (1.8 kb) in 31(77.5%), genotype B (1.4 kb) in 4(10%), and genotype C (1.1 kb) in 5(12.5%) **(**Table 2**; Supplementary Fig. S1)**. Out of 31 *P. vivax* infected samples, genotype A was observed in 23 (57.5%) male subjects and 8 (25.80%) female subjects. The gender distribution in *P. vivax* infected subjects (male: female) was 3:1 for genotype B and 3:2 for genotype C.

Region-wise analysis of the PvMSP-3α genotypes: Out of the 29 samples collected from South-west district, Delhi, 3 samples were not amplified by PCR. Of the 26 samples, the *Pv*MSP-3α genotypes observed were as follows- genotype A 20(76.9%), genotype B 3(11.5%) and genotype C 3(11.5%). While from the Nuh district, Haryana, out of the collected 16 samples, 2 did not amplify with PCR and the genotype frequencies of the *Pv*MSP-3α in this region were observed to be- genotype A 11(78.5%), genotype B 1(7.1%) and genotype C 2 (14.2%). The results of the genotypes of *Pv*MSP-3α are summarized in **Supplementary Table 1**.

3.2. Allelic variations derived from PCR-RFLP using HhaI

RFLP with *Hha*I enzyme of 40 PCR amplified fragments of *Pv*MSP-3α gene revealed 17 allelic variants (H1-H17) **(**Fig. 1**; Supplementary Fig. S2)**. The H8 allelic variant of the *Pv*MSP-3α gene was predominant in 8(25.8%) followed by H3 in 6(19.4%) of *P. vivax* specimens of genotype A. The H16 variant was observed in 3(75%) *P. vivax* specimens of genotype B and 3(60%) of genotype C. It was interesting to observe that H8 allelic variant was observed predominantly in both the regions of the Delhi-NCR. Amongst the total of 17 allelic variants of *Pv*MSP-3α gene, genotype A alone carried 15 allelic variants from 31 *P. vivax* specimens. Other variants observed in the *Pv*MSP-3α genotype A were H2, H4, H7 and H9-H15 in 1(3.2%) each; H1 & H5 in 2(6.4%) and H6 in 3(9.6%). The allelic variants H15 in 1(25%) and H16 in 3(75%) were observed for the genotype B of *Pv*MSP-3α gene. Similarly, only two allelic variants H16 in 3(60%) and H17 in 2(40%) were found in genotype C of the *Pv*MSP-3α gene.

Region-wise analysis of the PvMSP-3α HhaI allelic variants: The study found 12 *Hha*I allelic variants from 26 samples from the South-west district, Delhi. Further, the allelic variants- H1, H2, H6, H9, H10, H11, H12, H15 were observed separately in 1(3.8%) sample each, while other *Hha*I variants were found as- H3 6(23%), H5 2(7.6%), H8 5(19.2%) and H16 5(19.2%). Similarly, from Nuh district,

Table 2
Genotypes and allelic frequencies after restriction digestion with *Hha*I and *Alu*I.

Genotypes $N = 40$ n(%)	Allelic variants (*Hha*I)	Allele frequencies n(%)	Allelic variants (*Alu*I)	Allele frequencies n(%)
A (1.8 kb) 31(77.5%)	H1	2(6.5)	A1	1(3.2)
	H2	1(3.2)	A2	1(3.2)
	H3	6(19.4)	A3	1(3.2)
	H4	1(3.2)	A4	6(19.5)
	H5	2(6.5)	A5	1(3.2)
	H6	3(9.7)	A6	2(6.5)
	H7	1(3.2)	A7	1(3.2)
	H8	8(25.8)	A8	1(3.2)
	H9	1(3.2)	A9	2(6.5)
	H10	1(3.2)	A10	2(6.5)
	H11	1(3.2)	A11	1(3.2)
	H12	1(3.2)	A12	1(3.2)
	H13	1(3.2)	A13	1(3.2)
	H14	1(3.2)	A14	2(6.5)
	H15	1(3.2)	A15	1(3.2)
	H17	1(3.2)	A16	2(6.5)
			A17	1(3.2)
			A18	1(3.2)
			A19	1(3.2)
			A20	1(3.2)
			A21	1(3.2)
B (1.4 kb) 4(10%)	H15	1(25)	A4	1(25)
	H16	3(75)	A20	1(25)
			A22	1(25)
			A23	1(25)
C (1.1 kb) 5(12.5%)	H16	3(60)	A4	2(40)
	H17	2(40)	A20	1(20)
			A24	1(20)
			A25	1(20)

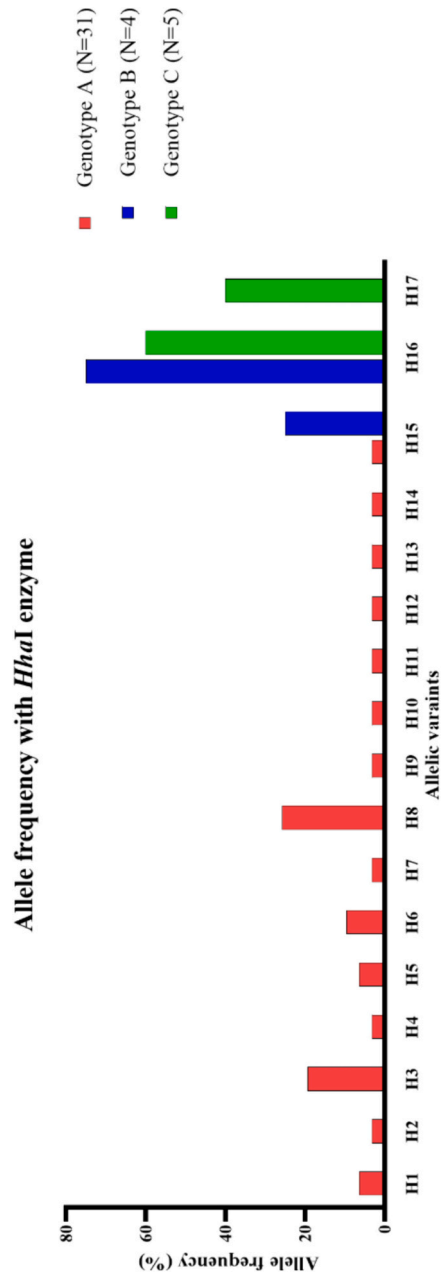

Fig. 1. Allelic frequency of *PvMSP-3α* gene with restriction enzyme *Hha*I.

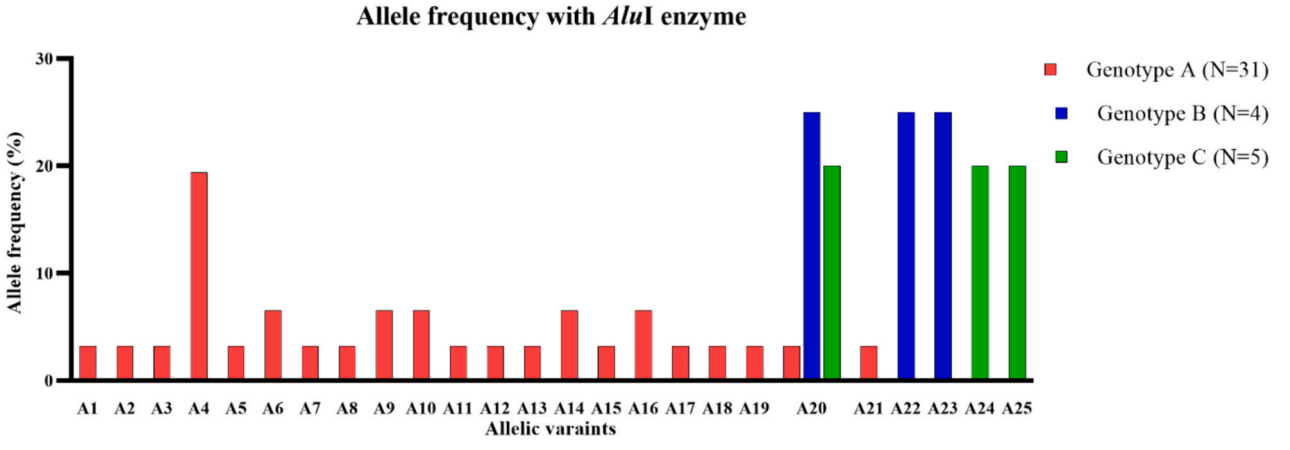

Fig. 2. Allelic frequency of *PvMSP-3α* gene with restriction enzyme *Alu*I.

Haryana, 10 allelic variants of *Hha*I were observed from 14 samples. The allelic variants H1, H4, H7, H13, H14, H15, H16 observed separately in 1(7.1%) sample each, while other variants observed were as- H6 2(14.2%), H8 3(21.4%) and H17 2(14.2%) **(Supplementary Table 1**).

3.3. Allelic variations derived from PCR-RFLP using AluI

The digestion of 40 PCR amplified fragments with restriction enzyme *Alu*I revealed 25 allelic variants (A1-A25) **(**Fig. 2**; Supplementary Fig. S3)**. Amongst the 31 *P. vivax* specimens of genotype A of *Pv*MSP-3α gene, the most predominant allelic variant A4 was observed in 6(19.5%). The allelic variants A6, A9, A10, A14, A16 were observed in 2(6.5%) samples each of the *P. vivax* specimens of genotype A. Other allelic variants in the genotype A were A1, A2, A3, A5, A7, A8, A11, A12, A13, A15, A17, A18, A19, A20, A21 in 1 (3.2%) of the *P. vivax* samples amplified. In the genotype B of the *Pv*MSP-3α gene 4 allelic variants A4, A20, A22, A23 were observed in 1(25%) each of the *P. vivax* specimens amplified. Similarly, the genotype C also depicted 4 allelic variants- A4 in 2(50%) and A20, A24 and A25 in 3(20%) each of the samples investigated. In the context of genotypic and allelic variations, we observed the alleles to be geographically distributed.

Region-wise analysis of the PvMSP-3α AluI allelic variants: Similarly, with *Alu*I, a total of 17 allelic variants were observed from 26 samples of South-west district, Delhi. The allelic variants were observed as- 1(3.8%) sample of each of the following variants (A1, A2, A3, A7, A8, A11, A12, A13, A14, A15, A16, A24) and rest of the variants were found as- A4 4(15.3%), A6, A9, A10 and A20 in 2(7.6%) samples each. While from Nuh district, Haryana, 10 allelic variants were observed from 14 samples. The allelic variants from 1(7.1%) sample each were as- A14, A16, A17, A18, A19, A20, A21, A23, A25; while A4 variant was observed in 5(35.7%) samples. An interesting observation was made, where two samples (H301 and H88) from same area of Nuh district, Haryana found to have same genotype A of the *Pv*MSP-3α and same allelic variants with *Hha*I (H8) and *Alu*I (A4) **(Supplementary Table 1)**.

4. Discussion

While there are several factors that can influence the genetic diversity of *P. vivax* parasites, *viz.* interactions between multiple clones of *P. vivax*, parasite-human host interactions, parasite-mosquito vector interactions and geographical variations (Suphakhonchuwong et al., 2018); it becomes more and more important to keep track about the advent of new or circulating genotypes at regional level. As new variants evolve, there might be changes in the disease transmission dynamics with crucial implications in epidemiology of *P. vivax* parasites circulating population (Upmanyu et al., 2020; Pacheco et al., 2016). The present study intended to investigate the genetic diversity of *P. vivax* parasites from two regions of the NCR with known *P. vivax* incidence (South-west district, Delhi and Nuh district, Haryana; although there are previous reports on the genotyping of the *Pv*MSP-3α gene from Delhi region, there were no reports of circulating *P. vivax* isolates from the Nuh district, Haryana (Kaul et al., 2019; Upmanyu et al., 2020; Verma et al., 2016). We employed PCR-RFLP techniques to investigate the genetic diversity of 40 PCR amplified samples of the *Pv*MSP-3α gene using restriction enzymes *Hha*I and *Alu*I. Genotyping of *P. vivax* by conventional PCR-RFLP technique is simple, inexpensive, and eliminates the need of state-of-art infrastructure, and it has been proficiently used in previous studies (Rungsihirunrat et al., 2011; Suphakhonchuwong et al., 2018). In the present study, three different genotypes *i.e.*, 1.8 kb (genotype A), 1.4 kb (genotype B), and 1.1 kb (genotype C) were observed from 40 *P. vivax* specimens. A total of three *Pv*MSP-3α genotypic variants were also reported from other geographical regions of Asia, including Pakistan (Khan et al., 2014), Afghanistan (Zakeri et al., 2010), Thailand (Rungsihirunrat et al., 2011; Gupta et al., 2015) and Sri Lanka (Schousboe et al., 2011). Collectively, these studies revealed genotype A to be the most prominent circulating genotype in the Asian region. In our study, we also observed genotype A to be most prevalent even from geographically distant sites.

The results indicated the presence of highly variable polymorphic nature of *Pv*MSP-3α in *P. vivax* strains circulating at local level in Delhi-NCR of India. Previous studies have also reported and corroborated the infection of *P. vivax* with multiple genotypes in the Asian region such as Thailand, Myanmar, Pakistan and China (Suphakhonchuwong et al., 2018; Khan et al., 2014; Li et al., 2015; Moon et al., 2009). The multiplicity of infection is often associated with the severity of *P. vivax* malaria and has been previously reported by Suphakhonchuwong et al. and Pacheco et al. (Suphakhonchuwong et al., 2018; Pacheco et al., 2016). A study from south India has also reported 14 sub-allelic variants with *Hha*I and 17 with *Alu*I in *P. vivax* specimens (Prajapati et al., 2010). The RFLP patterns of the *Pv*MSP-3α gene with restriction enzyme *Alu*I revealed 22 allelic variants from Pakistan and 29 from Thailand (Suphakhonchuwong et al., 2018; Khan et al., 2014). The high genetic diversity in *Pv*MSP3-α gene was also reported from India, Pakistan, Bangladesh, and Thailand in previous studies (Moon et al., 2009; Kibria et al., 2015; Kim et al., 2006; Raza et al., 2013). The major factors that might play role in the emergence of new variants of *P. vivax* might be drug pressure, existence in different ecological and geographical conditions (Suphakhonchuwong et al., 2018). The high genetic polymorphic nature of the *Pv*MSP-3α gene makes it a suitable marker for molecular, epidemiological and evolutionary studies of *P. vivax* parasites. Thus, our findings are in accordance with the previous studies and supports the idea that *Pv*MSP-3α gene should be used as a molecular marker for surveillance of *P. vivax* strains circulating at a local level (Kaul et al., 2019; Khan et al., 2014; Kibria et al., 2015; Kim et al., 2006; Adhikari et al., 2012).

Interestingly, the allelic variants from genotype A: H8 (with restriction enzyme *Hha*I) and A4 (with restriction enzyme *Alu*I) were observed in two samples within the same area of Nuh district, Haryana. In our previous study, we observed the transmission of *P. vivax* strain within the family using a minisatellite marker that can differentiate between relapse and re-infection (Das et al., 2016). However, further studies on a larger sample size will provide more insights on the genetic polymorphisms in the Indian scenario.

5. Conclusion

The study has revealed the genetic diversity, and allelic polymorphism in *Pv*MSP-3α gene of *P. vivax* isolates from South-west district, Delhi, and Nuh district, Haryana form NCR of India. Our findings demonstrated that the allelic variants H8 and A4 formed a cluster within the locality of Nuh district, Haryana. These allelic variants could serve as potential molecular markers to identify the geographical origins of the *P. vivax* strain in malaria-endemic areas. Further research would be helpful to determine the geographical distribution of H8 and A4 allelic variants in *P. vivax* population across the country.

Ethical approval

ICMR-National Institute of Malaria Research Delhi Institutional ethics committee was approved the study (ECR/NIMR/2013/102).

Consent

We obtained signed consent forms from the participants and guardians (Institutional ethics committee approval no. ECR/NIMR/2013/102).

CRediT authorship contribution statement

Ram Das: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kapil Vashisht:** Writing – review & editing, Validation, Investigation. **Deepali Savargaonkar:** Writing – review & editing, Methodology, Investigation. **L.L. Mercy Aparna:** Methodology, Investigation. **Ajay Nayak:** Writing – review & editing, Methodology. **Kailash C. Pandey:** Writing – review & editing, Validation, Supervision.

Declaration of competing interest

It is claimed that the authors have no conflict of interest.

Data availability

On request, the corresponding author can provide the data used to support the findings of this study.

Acknowledgements

The authors acknowledge the support of the ICMR–National Institute of Malaria Research, New Delhi for providing the laboratory facilities. The authors are grateful to the Indian Council of Medical Research, Department of Health Research, Ministry of Health and Family Welfare, Government of India for funding this study (Grant No. ICMR/REC NO. ECD/*ad-hoc*/38).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parepi.2024.e00362.

References

Adhikari, M., et al., 2012. Genetic diversity of plasmodium vivax merozoite surface protein-3alpha (Pvmsp-3alpha) gene in Jhapa District of Nepal. Southeast Asian J. Trop. Med. Public Health 43 (2), 280–286.
Arnott, A., Barry, A.E., Reeder, J.C., 2012. Understanding the population genetics of plasmodium vivax is essential for malaria control and elimination. Malar. J. 11, 14.
Barber, B.E., et al., 2013. Limitations of microscopy to differentiate plasmodium species in a region co-endemic for plasmodium falciparum, plasmodium vivax and plasmodium knowlesi. Malar. J. 12, 1–6.
Bruce, M.C., et al., 1999. Polymorphism at the merozoite surface protein-3alpha locus of plasmodium vivax: global and local diversity. Am. J. Trop. Med. Hyg. 61 (4), 518–525.
Das, R., et al., 2016. Genotyping of plasmodium vivax by minisatellite marker and its application in differentiating relapse and new infection. Malar. J. 15, 115.
Durante Mangoni, E., et al., 2003. Case report: an unusual late relapse of Plasmodium vivax malaria. Am. J. Trop. Med. Hyg. 68 (2), 159–160.
Gomez, A., et al., 2006. High polymorphism in plasmodium vivax merozoite surface protein-5 (MSP5). Parasitology 133 (Pt 6), 661–672.
Gupta, B., et al., 2015. Molecular evolution of PvMSP3α block II in plasmodium vivax from diverse geographic origins. PLoS One 10 (8), e0135396.
Imwong, M., et al., 2007. Relapses of plasmodium vivax infection usually result from activation of heterologous hypnozoites. J. Infect. Dis. 195 (7), 927–933.
Joy, S., et al., 2018. Presence of novel triple mutations in the pvdhfr from plasmodium vivax in Mangaluru city area in the southwestern coastal region of India. Malar. J. 17 (1), 167.
Kaul, A., et al., 2019. Genetic diversity and allelic variation in MSP3α gene of paired clinical plasmodium vivax isolates from Delhi, India. J. Infect. Public Health. 12 (4), 576–584.
Khan, S.N., et al., 2014. PCR/RFLP-based analysis of genetically distinct plasmodium vivax population of Pvmsp-3α and Pvmsp-3β genes in Pakistan. Malar. J. 13, 355.

Kibria, M.G., et al., 2015. Genetic diversity of plasmodium vivax in clinical isolates from Bangladesh. Malar. J. 14, 267.
Kim, J.R., et al., 2006. Genetic diversity of plasmodium vivax in Kolkata, India. Malar. J. 5, 71.
Leclerc, M.C., et al., 2004. Genetic diversity of plasmodium vivax isolates from Azerbaijan. Malar. J. 3, 40.
Li, Y.C., et al., 2015. Genetic diversity of plasmodium vivax population before elimination of malaria in Hainan Province, China. Malar. J. 14, 78.
Mathews, S.E., Bhagwati, M.M., Agnihotri, V., 2019. Clinical spectrum of plasmodium vivax infection, from benign to severe malaria: a tertiary care prospective study in adults from Delhi, India. Trop. Parasitol. 9 (2), 88–92.
Moon, S.U., et al., 2009. High frequency of genetic diversity of plasmodium vivax field isolates in Myanmar. Acta Trop. 109 (1), 30–36.
Nadkar, M.Y., et al., 2012. Clinical profile of severe plasmodium vivax malaria in a tertiary care Centre in Mumbai from June 2010-January 2011. J. Assoc. Physicians India 60, 11–13.
NVBDCP, 2024. National Vector Borne Diseases Control Program- Malaria (Magnitude of the problem) [cited 2024; Available from: https://ncvbdc.mohfw.gov.in/index4.php?lang=1&level=0&linkid=420&lid=3699.
Pacheco, M.A., et al., 2016. Multiplicity of infection and disease severity in plasmodium vivax. PLoS Negl. Trop. Dis. 10 (1), e0004355.
Prajapati, S.K., Joshi, H., Valecha, N., 2010. Plasmodium vivax merozoite surface protein-3 alpha: a high-resolution marker for genetic diversity studies. J. Vector Borne Dis. 47 (2), 85–90.
Putaporntip, C., et al., 2002. Mosaic organization and heterogeneity in frequency of allelic recombination of the plasmodium vivax merozoite surface protein-1 locus. Proc. Natl. Acad. Sci. USA 99 (25), 16348–16353.
Rayner, J.C., et al., 2004. Plasmodium vivax merozoite surface protein PvMSP-3 beta is radically polymorphic through mutation and large insertions and deletions. Infect. Genet. Evol. 4 (4), 309–319.
Raza, A., et al., 2013. Genetic diversity of plasmodium vivax clinical isolates from southern Pakistan using pvcsp and pvmsp1 genetic markers. Malar. J. 12, 1–5.
Rice, B.L., et al., 2014. The origin and diversification of the merozoite surface protein 3 (msp3) multi-gene family in plasmodium vivax and related parasites. Mol. Phylogenet. Evol. 78, 172–184.
Rungsihirunrat, K., et al., 2011. Genotyping of polymorphic marker (MSP3α and MSP3β) genes of plasmodium vivax field isolates from malaria endemic of Thailand. Trop. Med. Int. Health 16 (7), 794–801.
Schousboe, M.L., et al., 2011. Analysis of polymorphisms in the merozoite surface protein-3α gene and two microsatellite loci in Sri Lankan plasmodium vivax: evidence of population substructure in Sri Lanka. Am. J. Trop. Med. Hyg. 85 (6), 994–1001.
Suphakhonchuwong, N., et al., 2018. Evaluation of plasmodium vivax isolates in Thailand using polymorphic markers plasmodium merozoite surface protein (PvMSP) 1 and PvMSP3. Parasitol. Res. 117 (12), 3965–3978.
Ullah, I., et al., 2021. PCR-RFLP based genetic diversity of plasmodium vivax genotypes in district Mardan, Pakistan. Braz. J. Biol. 82, e241110.
Upmanyu, K., et al., 2020. Allelic variation of msp-3α gene in plasmodium vivax isolates and its correlation with the severity of disease in vivax malaria. Infect. Genet. Evol. 85, 104530.
Verma, A., et al., 2016. Plasmodium vivax msp-3α polymorphisms: analysis in the Indian subcontinent. Malar. J. 15 (1), 492.
Véron, V., et al., 2009. Genetic diversity of msp3alpha and msp1_b5 markers of plasmodium vivax in French Guiana. Malar. J. 8, 40.
Yang, Z., et al., 2006. Genetic structures of geographically distinct plasmodium vivax populations assessed by PCR/RFLP analysis of the merozoite surface protein 3beta gene. Acta Trop. 100 (3), 205–212.
Zakeri, S., Barjesteh, H., Djadid, N.D., 2006. Merozoite surface protein-3alpha is a reliable marker for population genetic analysis of plasmodium vivax. Malar. J. 5, 53.
Zakeri, S., et al., 2010. Genetic structure of plasmodium vivax isolates from two malaria endemic areas in Afghanistan. Acta Trop. 113 (1), 12–19.