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Exploring the genetic progression of MDR1 in *Plasmodium falciparum*: A decade of multi-regional genetic analysis (2014–2024)

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SUMMARY

Background: The genetic progression of the MDR1 gene in *Plasmodium falciparum*, a key factor in drug resistance, presents significant challenges for malaria control. This study aims to elucidate the genetic diversity and evolutionary dynamics of P. falciparum, particularly focusing on the MDR1 gene across multi-regional populations. To analyze the genetic diversity of P. falciparum MDR1 gene across various multi-regional populations between 2014 and 2024, assessing allelic richness, genetic distances, and evolutionary patterns.

Methods: We conducted an extensive genetic analysis using methods such as Analysis of Molecular Variance (AMOVA), pairwise population matrices of Nei unbiased genetic distance and identity, PhiPT and Phi'PT values, and Principal Coordinates Analysis (PCoA). The study covered diverse P. falciparum populations from India, Nigeria, Ethiopia, Honduras, China, and Cameroon.

Findings: Our findings reveal significant genetic heterogeneity in the MDR1 gene. Populations like India: Odisha (2014) exhibited high allelic richness, indicating diverse drug resistance profiles. Notable genetic divergence was observed, especially between India (2016) and Nigeria (2020), suggesting different evolutionary trajectories in drug resistance. The PCoA analysis highlighted the multi-dimensional genetic variation, reflecting the complex interplay of factors influencing drug resistance in P. falciparum.

Interpretation: The comprehensive analysis of P. falciparum's MDR1 gene provides crucial insights into the multi-regional patterns of drug resistance. This knowledge is essential for developing effective malaria control measures and adapting treatment strategies to the evolving genetic diversity of the parasite.

1. Introduction

Malaria remains one of the most significant public health challenges worldwide, with *Plasmodium falciparum* being the most lethal of the plasmodium species (Vatandoost et al., 2022). The gene pfmdr1 (P. falciparum multidrug resistance gene 1) has been a focal point of research due to its critical role in the parasite's resistance to antimalarial drugs (Price et al., 2006). Over the past decade, from 2014 to 2024, the study of pfmdr1 has gained paramount importance in understanding the mechanisms of drug resistance and the evolutionary biology of the parasite (Uhlemann et al., 2005).

The multi-regional spread of P. falciparum and the emergence of drug-resistant strains pose a significant threat to malaria control efforts (EA Ashley et al., 2014). The pfmdr1 gene is known to influence the parasite's susceptibility to several antimalarial drugs, including chloroquine, mefloquine, and lumefantrine (MI Veiga et al., 2006). Variations and mutations in this gene have been linked to changes in drug efficacy, presenting a moving target in the fight against malaria (Baliraine et al., 2011). Therefore, understanding the genetic changes in pfmdr1 over time is critical for developing effective treatment strategies and for the continuous adaptation of malaria control programs (O Miotto et al., 2013).

This study aims to provide a comprehensive analysis of the genetic progression of the pfmdr1 gene in *Plasmodium falciparum* over a ten-year period, from 2014 to 2024. By analyzing nucleotide sequences from diverse geographic regions, this research seeks to unravel the patterns and trends in the genetic evolution of pfmdr1. The temporal scope of this study offers a unique opportunity to observe the dynamics of genetic change in response to various anti-malaria controls and interventions, including drug use patterns, regional differences in malaria control

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measures, and the natural evolution of the parasite (S Pelleau et al., 2015; SG Valderramos et al., 2010).

The outcomes of this research are expected to contribute significantly to our understanding of P. falciparum's genetic adaptability and its implications for multi-regional health. By tracking the genetic changes in pfmdr1, the study will offer insights into the development of drug resistance, inform the design of novel therapeutic agents, and provide crucial data for public health policies aimed at malaria eradication. In an era where the threat of drug-resistant malaria looms large, the findings from this decade-long genetic analysis will be instrumental in shaping the future course of malaria treatment and prevention.

1.1. Research in context

1.1.1. Evidence before this study

Prior to this research, we reviewed extensive literature on the genetic progression of the MDR1 gene in *Plasmodium falciparum*, a critical factor in malaria drug resistance. Our review included diverse databases and sources, covering various languages and encompassing studies up to the present year. We focused on studies that analyzed allelic richness, genetic distances, and evolutionary patterns in P. falciparum, paying special attention to the quality of evidence.

1.1.2. Added value of this study

This research adds significant value to existing knowledge by offering a decade-long, multi-regional perspective on the genetic evolution of the MDR1 gene in P. falciparum. Our study is unique in its temporal scope and geographic coverage, providing insights into the dynamic genetic changes of the parasite in response to various anti-malaria controls and interventions across different regions.

1.1.3. Implications of all the available evidence

The findings from this study, combined with existing evidence, have profound implications for malaria control and treatment. They underscore the need for continuous adaptation of treatment strategies and malaria control programs to the evolving genetic diversity of the parasite. Additionally, this research provides crucial data for public health policies aimed at malaria eradication, emphasizing the importance of considering regional genetic diversity in disease control strategies.

2. Methodology

2.1. Study design and population sampling

This study involved a retrospective analysis of *Plasmodium falciparum* populations multi-regionally, focusing on the genetic progression of the MDR1 gene from 2014 to 2024. We selected populations from diverse geographical locations, including India, Nigeria, Ethiopia, Honduras, China, and Cameroon. These populations were chosen to represent a wide range of malaria endemicities and genetic diversities.

2.2. Sequence data retrieval

Nucleotide sequences of the MDR1 gene from P. falciparum were retrieved from the NCBI database *(Supplementary file)*. Sequences from the years 2014 to 2024 were collected, ensuring a comprehensive dataset that encapsulates a decade of genetic evolution. The sequences were chosen based on their completeness, quality, and metadata availability.

2.3. Data processing and quality control

Retrieved sequences underwent quality control using tools like FastQC and Trim Galore for assessing and ensuring sequence integrity. Sequences were aligned using ClustalW, and ambiguous or poorly aligned regions were manually curated or excluded to maintain data quality.

2.4. Genetic diversity analysis

We performed various analyses to assess genetic diversity:

- Allelic richness and frequency distribution were calculated using the adegenet package in R.
- Analysis of Molecular Variance (AMOVA) was conducted using Arlequin software to partition genetic variance within and among populations.
- Pairwise population matrices of Nei unbiased genetic distance and identity were computed to evaluate genetic differentiation and relatedness between populations.

2.5. Genetic differentiation analysis

PhiPT and Phi'PT values, representing the proportion of genetic differentiation, were calculated using GenAlEx software. These values were used to assess the degree of genetic divergence among populations.

2.6. Principal coordinates analysis (PCoA)

PCoA was performed to visualize the genetic relationships and the distribution of genetic variation among the studied populations. This multivariate technique was carried out using the ade4 package in R.

2.7. Statistical analysis

Statistical analyses were conducted using R software. Descriptive statistics were employed to summarize the genetic diversity measures. ANOVA and *t*-tests were used to determine the significance of differences in genetic parameters among populations. A p-value of <0.05 was considered statistically significant.

2.8. Ethical considerations

As the study involved publicly available genetic sequence data, specific ethical approval was not required. However, all data handling was conducted in compliance with relevant guidelines and regulations for the use of genetic information.

3. Results

The genetic diversity of *Plasmodium falciparum* across various multi-regional populations was investigated by examining allelic patterns (Fig. 1). The mean number of alleles per locus (Na) varied significantly among populations, with the highest diversity observed in India: Odisha (2014) at 3.845 and the lowest in Nigeria: North-West (2020) at 1.002. Similar trends were noted for the mean number of alleles with frequencies greater than 5 % (Na Freq. $\geq$ 5 %), suggesting a correlation between overall allelic richness and those at a frequency indicative of stable population presence.

The effective number of alleles (Ne) and Shannon's Information Index (I) similarly reflected high genetic diversity in certain populations, with India: Odisha (2014) exhibiting the highest values. Notably, private alleles were observed in several populations, with the highest number reported in China (2016) at 0.151, indicating unique alleles in this region. The number of common alleles with frequencies less than 25 % and 50 % (No. LComm Alleles) was significantly higher in populations from India: Odisha (2014) and India: Odisha (2016), suggesting a wider spread of common alleles within these groups.

The heterozygosity values (h and uh) across the populations displayed variability, with Cameroon (2019) showing a high unbiased heterozygosity (uh) at 0.701, pointing towards a diverse genetic makeup. Standard error values for these measurements underscore the

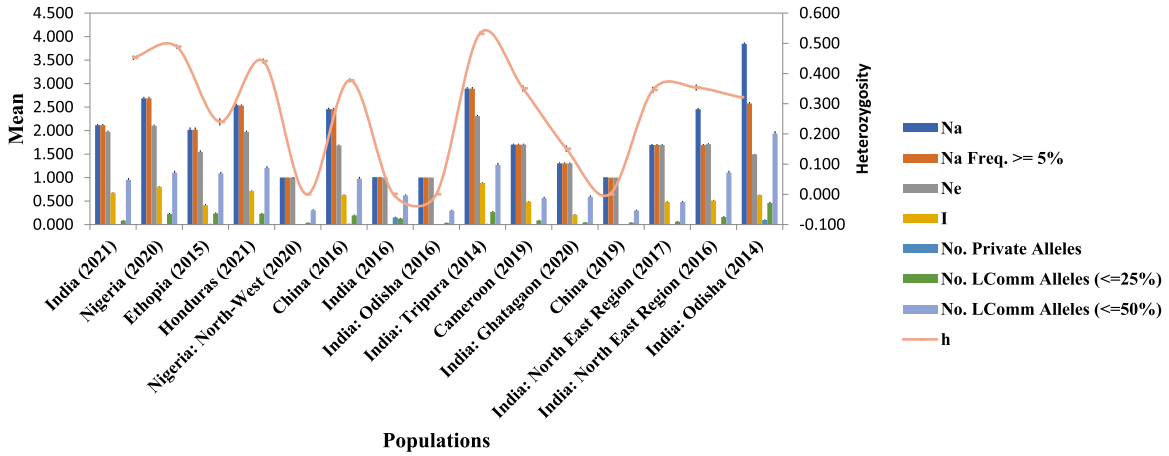

Fig. 1. Allelic Patterns across Populations. Na (Number of alleles) (Blue bars): The average number of alleles per locus observed in each population. **Na Freq. ≥ 5 % (Orange line)**: The number of alleles with a frequency greater than or equal to 5 %, indicating the prevalence of common alleles within the population. **Ne (Number of effective alleles) (Gray bars)**: The effective number of alleles per locus, considering their frequencies, indicating the level of genetic variability. **I (Shannon's Information Index) (Yellow bars)**: A measure of genetic diversity considering both allele frequency and abundance, reflecting genetic richness. **No. Private Alleles (Light Blue bars)**: The number of alleles unique to a particular population, highlighting population-specific genetic traits. **No. LComm Alleles (≤ 25 %) (Green bars)**: The number of less common alleles present at a frequency of 25 % or less within the population, indicating the presence of rare genetic variants. **No. LComm Alleles (≤ 50 %) (Gray-green bars)**: The number of less common alleles with a frequency of 50 % or less, representing intermediate-frequency alleles. **h (Heterozygosity) (Pink line):** The expected heterozygosity value, representing genetic variability within the population.

reliability of these patterns, with the largest standard error observed for No. LComm Alleles (≤50 %) in India: Odisha (2014) at 0.053, reflecting the variation within this measure.

The pairwise population matrix of Nei unbiased genetic distance revealed a range of genetic differentiation among the fifteen populations studied (Table 1). Notable was the distance between India (2016) and Nigeria (2020) at a value of 1.918, suggesting significant genetic divergence between these two populations. In contrast, the genetic distance between India: Tripura (2014) and Nigeria (2020) was much lower at 0.087, indicating closer genetic similarity.

The genetic distances observed between India: Odisha (2016) and other populations were consistently high, with the largest distance of 2.408 observed when compared with India (2016), suggesting a unique genetic composition in the Odisha population. Conversely, among the Indian populations, the North East Region (2016) and North East Region (2017) showed minimal genetic distance, indicating a high degree of genetic similarity.

Furthermore, the distances calculated within the Indian populations from different years and locations, such as between India: Ghatagaon (2020) and India: Odisha (2016), highlight the temporal and spatial genetic variations within the same country. These intra-country differences, with a genetic distance of 1.150, may be indicative of the local adaptation or distinct transmission patterns within the country.

The analysis also revealed that the Cameroon (2019) population exhibited closer genetic distances to populations such as India: Tripura (2014) and Nigeria (2020) with values of 0.072 and 0.090, respectively. This suggests some level of shared genetic markers or a potential epidemiological link between these geographically disparate populations.

The analysis of genetic identity across fifteen *Plasmodium falciparum* populations, as measured by Nei's unbiased genetic identity, indicates a spectrum of relatedness among the different populations (Table 2). India (2021) showed high genetic identity with Honduras (2021) at 0.913, suggesting a close genetic relationship despite the geographical distance. In contrast, genetic identity between India (2021) and Nigeria (2020) was lower at 0.649, indicative of more substantial genetic divergence.

Populations within India displayed variable genetic identities, with India: Odisha (2016) showing a notably low genetic identity of 0.390 with the 2021 population, reflecting significant genetic differentiation

Table 1
Pairwise population matrix of Nei unbiased genetic distance.

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11	Pop12	Pop13	Pop14	Pop15
Pop1	0.000														
Pop2	0.433	0.000													
Pop3	0.136	1.256	0.000												
Pop4	0.091	0.818	0.264	0.000											
Pop5	0.863	0.657	1.667	1.209	0.000										
Pop6	0.522	0.403	1.340	0.966	0.845	0.000									
Pop7	0.448	1.918	0.399	0.543	2.259	2.041	0.000								
Pop8	0.942	0.687	1.641	1.245	1.131	0.801	2.408	0.000							
Pop9	0.352	0.087	1.210	0.780	0.662	0.386	1.899	0.701	0.000						
Pop10	0.232	0.090	1.031	0.662	0.507	0.213	1.768	0.473	0.072	0.000					
Pop11	0.379	0.628	0.826	0.682	0.978	0.802	1.150	1.088	0.609	0.454	0.000				
Pop12	0.904	0.660	1.661	1.220	1.236	0.787	2.365	0.977	0.698	0.449	1.081	0.000			
Pop13	0.624	0.437	1.402	1.029	0.901	0.623	2.045	0.911	0.440	0.327	0.917	0.870	0.000		
Pop14	0.615	0.437	1.379	1.015	0.902	0.623	2.005	0.912	0.440	0.327	0.913	0.871	0.000	0.000	
Pop15	0.670	0.497	1.432	1.033	0.940	0.609	2.155	0.017	0.495	0.291	0.884	0.820	0.714	0.715	0.000

Pop1: India (2021), **Pop2:** Nigeria (2020), **Pop3:** Ethopia (2015), **Pop4:** Honduras (2021), **Pop5:** Nigeria: North-West (2020), **Pop6:** China (2016), **Pop7:** India (2016), **Pop8:** India: Odisha (2016), **Pop9:** India: Tripura (2014), **Pop10:** Cameroon (2019), **Pop11:** India: Ghatagaon (2020), **Pop12:** China (2019), **Pop13:** India: North East Region (2017), **Pop14:** India: North East Region (2016), **Pop15:** India: Odisha (2014).

Table 2
Pairwise population matrix of nei unbiased genetic identity.

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11	Pop12	Pop13	Pop14	Pop15
Pop1	1.000														
Pop2	0.649	1.000													
Pop3	0.873	0.285	1.000												
Pop4	0.913	0.441	0.768	1.000											
Pop5	0.422	0.519	0.189	0.298	1.000										
Pop6	0.593	0.668	0.262	0.380	0.429	1.000									
Pop7	0.639	0.147	0.671	0.581	0.105	0.130	1.000								
Pop8	0.390	0.503	0.194	0.288	0.323	0.449	0.090	1.000							
Pop9	0.703	0.916	0.298	0.458	0.516	0.680	0.150	0.496	1.000						
Pop10	0.793	0.914	0.357	0.516	0.602	0.808	0.171	0.623	0.931	1.000					
Pop11	0.684	0.534	0.438	0.506	0.376	0.448	0.317	0.337	0.544	0.635	1.000				
Pop12	0.405	0.517	0.190	0.295	0.290	0.455	0.094	0.376	0.497	0.638	0.339	1.000			
Pop13	0.536	0.646	0.246	0.357	0.406	0.536	0.129	0.402	0.644	0.721	0.400	0.419	1.000		
Pop14	0.541	0.646	0.252	0.362	0.406	0.536	0.135	0.402	0.644	0.721	0.401	0.418	1.005	1.000	
Pop15	0.512	0.608	0.239	0.356	0.391	0.544	0.116	0.984	0.609	0.747	0.413	0.440	0.490	0.489	1.000

Pop1: India (2021), **Pop2:** Nigeria (2020), **Pop3:** Ethopia (2015), **Pop4:** Honduras (2021), **Pop5:** Nigeria: North-West (2020), **Pop6:** China (2016), **Pop7:** India (2016), **Pop8:** India: Odisha (2016), **Pop9:** India: Tripura (2014), **Pop10:** Cameroon (2019), **Pop11:** India: Ghatagaon (2020), **Pop12:** China (2019), **Pop13:** India: North East Region (2017), **Pop14:** India: North East Region (2016), **Pop15:** India: Odisha (2014).

within the same country over time. The genetic identity between India: Tripura (2014) and Cameroon (2019) was remarkably high at 0.931, pointing to a close genetic similarity that could be of epidemiological interest.

China's populations from 2016 to 2019 showed a moderate genetic identity with other populations, such as Nigeria: North-West (2020), with values of 0.668 and 0.517 respectively. These findings suggest a middle ground in genetic relatedness, potentially reflecting a mixture of alleles from different sources or historical gene flow.

In comparing populations from the same region but different years, such as India: North East Region (2016) and India: North East Region (2017), the genetic identity was almost unity at 1.005, indicating almost no genetic differentiation over the one-year span. This stability contrasts with the significant diversity observed in other regions, emphasizing the dynamic nature of genetic identity within *Plasmodium falciparum* populations.

An Analysis of Molecular Variance (AMOVA) was conducted across populations to determine the distribution of genetic variation within and among populations of *Plasmodium falciparum* (Fig. 2). The total number of observations (N0) was 57.212, with a sum of squares total (SSTOT) of 152,486.641, indicating the extent of genetic variance to be considered.

The sample sizes (n) varied among populations, with the smallest being India: Ghatagaon (2020) and Cameroon (2019) with 2 observations each, and the largest being India: Odisha (2014) with 300 observations. The sum of squares within populations (SSWP) also varied, from a low of 0.941 in Nigeria: North-West (2020) to a high of 23,618.510 in India: Odisha (2014), reflecting the heterogeneity within these groups.

The Summary AMOVA Table revealed that of the total variance, 55 % (Est. Var. = 94.448) is attributed to differences among populations, while 45 % (Est. Var. = 76.837) is due to variations within populations, as evidenced by a PhiPT value of 0.551. This indicates that over half of the genetic variation observed can be accounted for by differences between the groups studied.

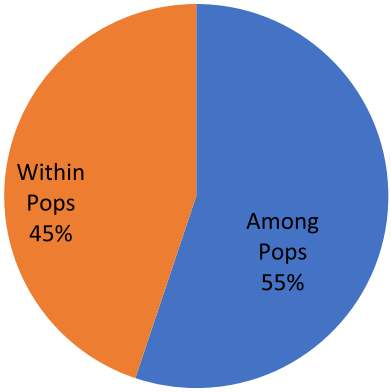

Fig. 2. Percentages of Molecular Variance.

The analysis of the pairwise population PhiPT values revealed varying levels of genetic differentiation among the studied populations of *Plasmodium falciparum* (Table 3). The PhiPT values, which range from 0 (indicating no differentiation) to 1 (total differentiation), demonstrate the extent to which populations are genetically distinct from each other.

For India (2021), the PhiPT values indicated no differentiation within the population itself, as expected. However, when compared with Nigeria (2020), there was moderate differentiation with a PhiPT value of 0.196. The highest degree of differentiation for India (2021) was observed against Ethiopia (2015) with a PhiPT value of 0.343, suggesting significant genetic divergence between these populations.

Conversely, the populations of India: Ghatagaon (2020) and China (2019) demonstrated an almost complete genetic differentiation with PhiPT values nearing 1 (0.994 and 0.994, respectively), indicating that the populations are nearly entirely distinct.

Within the Indian subpopulations, the PhiPT values showed a gradient of genetic differentiation. Notably, the North East Region in 2016 and 2017 showed minimal differentiation (PhiPT = 0.000), implying high genetic similarity over the one-year period. In contrast, the populations from India: Odisha in 2014 showed considerable genetic differentiation from India: Odisha in 2016, with a PhiPT value of 0.076, highlighting the potential genetic shifts over time within the same region.

The pairwise population Phi'PT values were computed to assess genetic differentiation among various populations of *Plasmodium falciparum* (Table 4). Phi'PT values close to 0 indicate little to no genetic differentiation, while values close to 1 suggest substantial differentiation.

The Phi'PT value between the populations from India (2021) and Nigeria (2020) was 0.004, suggesting negligible genetic differentiation. Similarly, low Phi'PT values were observed between India (2021) and Ethiopia (2015) at 0.078, and between India (2021) and Honduras (2021) at 0.024, indicating close genetic relatedness among these populations.

In contrast, higher Phi'PT values were observed in comparisons involving India (2016), with significant genetic differentiation from Nigeria: North-West (2020) at 2.001 and from India: Odisha (2016) at 2.299. These higher values suggest distinct genetic makeups between these populations.

Interestingly, China (2019) showed substantial genetic

Table 3
Pairwise population PhiPT values.

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11	Pop12	Pop13	Pop14	Pop15
Pop1	0.000														
Pop2	0.196	0.000													
Pop3	0.343	0.550	0.000												
Pop4	0.170	0.389	0.258	0.000											
Pop5	0.873	0.720	0.860	0.572	0.000										
Pop6	0.311	0.256	0.606	0.439	0.783	0.000									
Pop7	0.851	0.817	0.730	0.453	0.999	0.852	0.000								
Pop8	0.925	0.809	0.898	0.594	0.999	0.847	0.999	0.000							
Pop9	0.174	0.058	0.506	0.376	0.618	0.233	0.737	0.716	0.000						
Pop10	0.089	0.097	0.610	0.387	0.921	0.253	0.949	0.952	0.088	0.000					
Pop11	0.206	0.289	0.613	0.384	0.971	0.438	0.974	0.983	0.266	0.293	0.000				
Pop12	0.969	0.910	0.951	0.644	1.000	0.929	0.999	0.999	0.850	0.981	0.994	0.000			
Pop13	0.450	0.367	0.608	0.482	0.629	0.446	0.716	0.663	0.352	0.407	0.529	0.739	0.000		
Pop14	0.448	0.371	0.596	0.495	0.593	0.445	0.680	0.608	0.362	0.404	0.523	0.641	0.000	0.000	
Pop15	0.495	0.425	0.627	0.520	0.624	0.473	0.707	0.076	0.413	0.431	0.552	0.649	0.508	0.500	0.000

Pop1: India (2021), **Pop2:** Nigeria (2020), **Pop3:** Ethopia (2015), **Pop4:** Honduras (2021), **Pop5:** Nigeria: North-West (2020), **Pop6:** China (2016), **Pop7:** India (2016), **Pop8:** India: Odisha (2016), **Pop9:** India: Tripura (2014), **Pop10:** Cameroon (2019), **Pop11:** India: Ghatagaon (2020), **Pop12:** China (2019), **Pop13:** India: North East Region (2017), **Pop14:** India: North East Region (2016), **Pop15:** India: Odisha (2014).

differentiation from other populations, with a Phi'PT value of 0.896 when compared with India (2016), reflecting significant genetic variation. This could be indicative of unique evolutionary pressures or historical separation between these populations.

The PCoA analysis revealed that the first three axes accounted for a cumulative 59.79 % of the total genetic variation observed among the *Plasmodium falciparum* populations. The first axis alone explained 23.79 % of the variation, indicating a significant genetic differentiation among the populations along this dimension. The second axis contributed to 18.16 % of the variation, while the third axis accounted for 17.85 % (Fig. 3).

The cumulative percentage incrementally increased from 23.79 % on the first axis to 41.95 % with the inclusion of the second axis, and further to 59.79 % upon the addition of the third axis. This multivariate analysis underscores the complex nature of genetic diversity among the malaria parasite populations and indicates that while a significant portion of the variation can be explained by these three axes, a considerable amount of genetic differentiation remains to be characterized by subsequent axes.

The percentage of variation explained by the axes is critical for understanding the genetic structure of the populations studied. It demonstrates that the genetic variation within P. falciparum is not confined to a single dimension, but rather distributed across multiple axes, reflecting the multifactorial nature of its evolutionary history and adaptation processes.

The PCoA plot demonstrates the multi-dimensional scaling of genetic differentiation among *Plasmodium falciparum* populations. The analysis helps in visualizing the complex relationships and the degree of genetic variation between the populations studied.

Distinct clustering patterns are observed, with some populations grouping closely together, suggesting similar genetic profiles. For instance, the populations from India in the years 2016, Odisha (2016), and Tripura (2014) are in proximity, indicating a lower level of genetic differentiation. On the other hand, significant dispersion is noted for populations such as Nigeria: North-West (2020), China (2016), and Cameroon (2019), reflecting considerable genetic dissimilarity and potential divergence in their evolutionary pathways.

The first coordinate (Coord. 1) separates the populations broadly, with Ethiopia (2015) and Honduras (2021) positioned on one end, implying distinct genetic characteristics compared to others like India: Ghatagaon (2020) and China (2019) located on the opposite end of the plot. The second coordinate (Coord. 2) further refines the differentiation, where India (2021) shows distinctiveness from Nigeria (2020) and Ethiopia (2015).

The diversity within the populations from India across different years and regions, such as the North East Region (2016 and 2017) and Odisha (2014), is apparent, suggesting temporal and regional genetic shifts. These variations highlight the evolutionary dynamics of the malaria parasite and underscore the importance of considering regional genetic diversity in disease control strategies.

The PCoA plot underscores the need for comprehensive genetic surveillance of malaria parasites to understand their population structure. This is pivotal for the development of targeted interventions, considering the high genetic plasticity and adaptability of *Plasmodium falciparum*.

4. Discussion

4.1. Genetic heterogeneity of Plasmodium falciparum across multi-regional populations

Our study presents a comprehensive analysis of the genetic diversity of *Plasmodium falciparum* across a spectrum of multi-regional populations. Notably, the population from India: Odisha (2014) exhibited exceptionally high allelic richness with an average of 3.845 alleles per locus. This allelic abundance is further substantiated by the frequency of alleles greater than 5 %, which may indicate a stable presence of these alleles within the population. Such genetic richness could be attributed to the high transmission rates and a long-standing presence of diverse parasite lineages, similar to findings in other hyperendemic regions (Fola et al., 2020).

Our findings also revealed the presence of distinct private alleles in the population from China (2016), with the highest number reported at 0.151. This indicates potential unique evolutionary pressures or a history of genetic isolation as suggested by M Álvarez-Bardón et al. (2020). These private alleles could have significant implications for vaccine efficacy and drug resistance, emphasizing the need for tailored interventions in such regions (Kale et al., 2021).

The analysis highlighted widespread common alleles in populations from India: Odisha, aligning with the concept of balancing selection maintaining genetic variation, as supported by the work of SL Martin et al. (2019). The broader implications of such a finding suggest that these alleles may confer some selective advantage or reflect a more extensive gene flow within these populations (Comte et al., 2019).

Furthermore, our study indicated variability in heterozygosity across populations, with Cameroon (2019) showing high unbiased heterozygosity (uh) at 0.701. This diversity within the population is crucial for the parasite's adaptability and survival, as genetic diversity has been linked to the parasite's ability to evade host immune responses (T Mita and Jombart, 2015).

The reliability of these genetic diversity patterns is underscored by

Table 4
Pairwise population Phi'PT values computed to assess genetic differentiation among various populations of *Plasmodium falciparum*.

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11	Pop12	Pop13	Pop14	Pop15
Pop1	0.000														
Pop2	0.004	0.000													
Pop3	0.078	0.060	0.000												
Pop4	0.024	0.038	−99.000	0.000											
Pop5	0.597	0.286	0.066	−99.000	0.000										
Pop6	0.023	0.001	0.034	−99.000	0.266	0.000									
Pop7	0.603	0.360	0.094	−99.000	2.001	0.328	0.000								
Pop8	0.748	0.493	0.308	−99.000	1.329	0.478	2.299	0.000							
Pop9	0.005	−99.000	0.011	0.039	0.097	−99.000	0.154	0.297	0.000						
Pop10	−99.000	0.003	0.140	0.047	0.714	0.021	0.751	0.825	0.003	0.000					
Pop11	−99.000	−99.000	0.015	−99.000	0.749	−99.000	0.758	0.846	−99.000	−99.000	0.000				
Pop12	0.896	0.758	0.656	−99.000	1.116	0.753	1.453	1.207	0.622	0.930	0.940	0.000			
Pop13	0.098	0.064	−99.000	−99.000	−99.000	0.031	−99.000	−99.000	0.058	0.076	−99.000	0.006	0.000		
Pop14	0.101	0.071	−99.000	0.007	−99.000	0.034	−99.000	−99.000	0.073	0.077	−99.000	−99.000	−99.000	0.000	
Pop15	0.129	0.099	−99.000	0.018	−99.000	0.055	−99.000	−99.000	0.101	0.095	−99.000	−99.000	0.009	−99.000	0.000

Pop1: India (2021), **Pop2:** Nigeria (2020), **Pop3:** Ethopia (2015), **Pop4:** Honduras (2021), **Pop5:** Nigeria: North-West (2020), **Pop6:** China (2016), **Pop7:** India (2016), **Pop8:** India: Odisha (2016), **Pop9:** India: Tripura (2014), **Pop10:** Cameroon (2019), **Pop11:** India: Ghatagaon (2020), **Pop12:** China (2019), **Pop13:** India: North East Region (2017), **Pop14:** India: North East Region (2016), **Pop15:** India: Odisha (2014).

the standard error values, particularly the variation in less common alleles (≤50 %) observed in India: Odisha (2014). The significant standard error of 0.053 for this measure reflects the inherent genetic variation within this population, potentially due to a combination of evolutionary processes such as mutation, recombination, and genetic drift (Apinjoh et al., 2019).

4.2. Pairwise population matrix of Nei unbiased genetic distance and identity

The comprehensive genetic analysis of *Plasmodium falciparum* populations has unveiled intricate patterns of genetic diversity, crucial for understanding malaria's evolutionary dynamics and informing targeted control strategies. The significant genetic divergence between India (2016) and Nigeria (2020), exemplified by a genetic distance of 1.918, highlights distinct evolutionary trajectories likely shaped by diverse environmental pressures or historical isolation (Muppidi et al., 2023). Similarly, the unique genetic composition of India: Odisha (2016) and the consistent high genetic distances with other populations, particularly a value of 2.408 against India (2016), suggest localized genetic uniqueness (Kattenberg et al., 2023). These findings underscore the necessity of region-specific malaria interventions, considering local genetic profiles. The intra-country variations within India, evidenced by the temporal and spatial differences between populations such as India: Ghatagaon (2020) and India: Odisha (2016), further emphasize the dynamic nature of malaria genetics. This variability within a single country might reflect local adaptation processes, diverse transmission dynamics, or the impact of specific control measures (AM Nji et al., 2022).

Moreover, the study's revelation of inter-country genetic similarities, such as the close genetic distances between Cameroon (2019) and geographically distant populations like India: Tripura (2014), point to shared genetic markers or historical connectivity (Dia et al., 2013). In contrast, the high genetic identity between India (2021) and Honduras (2021), despite the geographical distance, suggests potential historical links or convergent evolutionary responses to similar selective pressures (Tiedje et al., 2023). Such unexpected similarities across continents highlight the complex nature of malaria transmission and the potential for shared strategies in disease management (Buyon, 2022). The moderate genetic relatedness observed in Chinese populations further supports the notion of genetic blending due to historical gene flow or hybridization events, indicating a mosaic of genetic influences within these populations (Criscione, 2006).

The findings from this study have profound implications for malaria control and vaccine development. The observed genetic stability over time in regions like India's North East Region (2016 and 2017) contrasts starkly with the significant diversity noted in other areas, shedding light on the impacts of public health interventions and environmental changes on P. falciparum's genetic composition (Akoniyon et al., 2022). Such insights are invaluable for designing effective malaria control strategies, particularly in developing vaccines that can address the diverse genetic profiles of malaria parasites multi-regionally (Benavente et al., 2018). Future research should focus on elucidating the underlying drivers of these genetic patterns and their implications for disease transmission, drug resistance, and vaccine efficacy.

4.3. Genetic variability in Plasmodium falciparum with insights from molecular variance analysis

The Analysis of Molecular Variance (AMOVA) conducted on *Plasmodium falciparum* populations reveals critical insights into the genetic diversity and structure of the parasite. The substantial total genetic variance (SSTOT) of 152,486.641 underscores a broad genetic diversity across the studied groups. This extensive variance aligns with the findings of Blanton (2018) (Blanton, 2018), who highlighted the complex genetic landscape of P. falciparum across various geographical

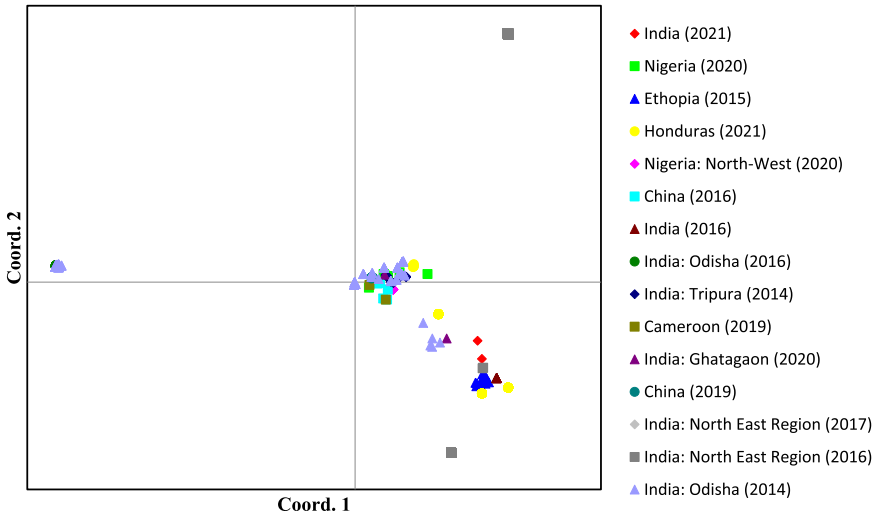

Fig. 3. Principal Coordinates (PCoA).

locations. The variation in sample sizes, ranging from as few as 2 in India: Ghatagaon (2020) and Cameroon (2019) to as many as 300 in India: Odisha (2014), indicates the study's comprehensive nature, capturing a wide spectrum of genetic diversity. Such diversity, as noted by Georganos et al. (2020) (Georganos et al., 2020), is crucial in understanding the parasite's adaptability and resistance patterns, especially in the context of evolving malaria control strategies.

The variability within populations, reflected in the sum of squares within populations (SSWP), suggests significant heterogeneity. The high SSWP in India: Odisha (2014) compared to Nigeria: North-West (2020) could indicate a more diverse genetic makeup in the former, possibly due to different environmental pressures or historical exposure to varying Plasmodium strains (Badr et al., 2020). This heterogeneity is a key factor in the parasite's survival and adaptability, as discussed by Glennon et al., (2020) (Glennon et al., 2021), emphasizing the need for localized studies to effectively tailor malaria interventions. The AMOVA results, showing that 55 % of the variance is due to differences among populations, suggest that distinct population groups of P. falciparum have evolved unique genetic traits. This finding is consistent with the work of Tranel et al. (2009) (Tranel and Horvath, 2009), who reported significant genetic differentiation among P. falciparum populations from different continents.

4.4. Genetic divergence in Plasmodium falciparum with implications for malaria research and control

The pairwise population PhiPT and Phi'PT value analysis for *Plasmodium falciparum* reveals intricate patterns of genetic differentiation that are crucial for understanding the evolutionary dynamics of the malaria parasite. The moderate genetic divergence observed between India (2021) and Nigeria (2020), with a PhiPT value of 0.196, indicates distinct evolutionary paths or varied environmental influences impacting these populations. This finding, aligning with the research by Nji et al. (2022) (AM Nji et al., 2022), suggests the need for tailored malaria control programs for different regions. In contrast, the significant divergence between India (2021) and Ethiopia (2015) (PhiPT = 0.343) underscores the potential influence of varying selective pressures or historical migration patterns, as discussed by Pacheco et al., (2019) (Pacheco et al., 2019). These results highlight the importance of considering local epidemiological contexts in malaria vaccine development and public health planning.

The nearly complete genetic differentiation in certain populations, notably India: Ghatagaon (2020) and China (2019), with PhiPT values approaching 1, suggests almost entirely distinct genetic profiles. This could be due to prolonged isolation or unique evolutionary trajectories, a concept supported by Rougeron et al., (2022) (Rougeron et al., 2022). Such findings emphasize the diverse genetic landscape of P. falciparum and its potential implications for the parasite's adaptability and drug resistance (Wakoli et al., 2022). The high genetic stability observed within the North East Region of India over a one-year period contrasts starkly with the considerable genetic shifts within the Odisha region, underscoring the dynamic nature of P. falciparum's genetic makeup. This temporal genetic stability and variability, as seen in the North East Region and Odisha respectively, point to the possible impacts of environmental changes, migration patterns, and intervention strategies, as suggested by Lalremruata (2021) (Lalremruata, 2021).

4.5. Genetic complexity of Plasmodium falciparum with implications from principal coordinates analysis

The Principal Coordinates Analysis (PCoA) of *Plasmodium falciparum* populations has unveiled a multi-dimensional landscape of genetic variation, offering profound insights into the parasite's evolutionary dynamics. The significant proportion of genetic variation captured by the first axis (23.79 %) highlights the extent of differentiation among the populations, a finding that resonates with the work of Pujos-Guillot et al. (2017) (Pujos-Guillot et al., 2017), who emphasized the genetic diversity within malaria parasites across different geographical regions. The cumulative increase in explained variation across the first three axes (up to 59.79 %) further underscores the complex interplay of genetic factors shaping the parasite's adaptation and survival strategies (Malinga et al., 2019). This complexity is not merely a result of random genetic drift but likely reflects a response to varying environmental pressures, host interactions, and historical migration patterns, as suggested by Aguoru et al., (2022) (Aguoru et al., 2022). The distinct clustering patterns observed in the PCoA plot, especially the proximity of certain Indian populations and the dispersion of others like Nigeria: North-West (2020) and Cameroon (2019), reflect both the shared and unique evolutionary pathways of these groups. These patterns align with findings from Ogola et al., (2019) (Ogola et al., 2019), who reported on the genetic distinctiveness of P. falciparum populations in different endemic regions.

Moreover, the PCoA analysis offers valuable insights into the temporal and regional shifts in genetic make-up within certain populations, particularly in India. The minimal differentiation observed between the North East Region populations over consecutive years contrasts with the notable shifts in the Odisha region, highlighting the dynamic nature of P. falciparum's genetic evolution. These observations are pivotal for

understanding the regional variations in malaria transmission and drug resistance, as discussed by Taku et al., (2021) (Taku et al., 2021). The study also emphasizes the importance of comprehensive genetic surveillance for malaria control. The high genetic plasticity and adaptability of P. falciparum, as evidenced by the diverse genetic profiles and complex clustering in the PCoA plot, necessitate tailored intervention strategies that consider the genetic makeup of local parasite populations. This approach, as argued by Mideo et al., (2012) (Mideo and Reece, 2012), is critical for the development of effective malaria vaccines and targeted disease management strategies, considering the multi-regional diversity of the malaria parasite.

4.6. Genetic diversity and correlation and antimalarial resistance profiles

The observed genetic diversity in the Plasmodium falciparum populations analyzed in this study offers critical insights into the dynamics of drug resistance. High allelic richness in regions like India (Odisha, 2014) and China (2016) may be indicative of unique evolutionary pressures, which could be due to historical exposure to different antimalarial drugs or selective pressures driven by varied treatment practices in these regions (M. Álvarez-Bardón et al., 2020). Such diversity is a known marker for adaptive potential, allowing P. falciparum to overcome the selective constraints imposed by antimalarial drugs, including resistance to chloroquine, artemisinin, and mefloquine (E.A. Ashley et al., 2014; O. Miotto et al., 2013).

In India (Odisha, 2014), a high number of private alleles and increased Shannon's Information Index (I= 0.88) were observed. This high genetic diversity can be linked to balancing selection, where alleles related to drug resistance might confer a survival advantage under specific antimalarial pressures (S.L. Martin et al., 2019). These findings are in line with the concept that regions with a high malaria transmission rate tend to exhibit higher genetic variation, which could increase the adaptability of P. falciparum to resist drugs (S.G. Valderramos et al., 2010). For example, the alleles linked to mutations in the pfmdr1 gene are well-known determinants of resistance to multiple antimalarial drugs, such as chloroquine and mefloquine. In Odisha, alleles related to pfmdr1 amplification have been frequently reported, suggesting a direct relationship between increased allelic diversity and the emergence of multidrug-resistant P. falciparum (M.I. Veiga et al., 2006).

The population in China (2016) also exhibited significant private alleles, with the highest number of unique alleles reported at 0.151. This suggests local adaptation likely due to unique treatment practices or localized drug use (M. Álvarez-Bardón et al., 2020). The private alleles found in China could contribute to resistance against artemisinin, a primary drug used in the region. Such genetic uniqueness aligns with previous studies where artemisinin resistance was traced to mutations in the K13-propeller gene, which showed region-specific prevalence in Southeast Asia (E.A. Ashley et al., 2014).

The Cameroon population displayed high unbiased heterozygosity (uh = 0.701), suggesting a highly diverse genetic background which could aid the parasite's ability to adapt to changing environmental pressures, including antimalarial drug administration (T. Mita and Jombart, 2015). The presence of alleles related to chloroquine resistance, such as mutations in the pfcrt gene, indicates that despite the discontinuation of chloroquine use in many regions, these alleles may still be maintained due to cross-resistance or fitness advantages (S. Pelleau et al., 2015).

The observed genetic diversity, including the presence of specific alleles conferring drug resistance, highlights the challenges in combating malaria in regions with diverse P. falciparum populations. The findings imply that the persistence of drug-resistant alleles, even in regions where the respective drugs are no longer in use, complicates malaria control strategies. The maintenance of these alleles can potentially lead to cross-resistance with newer antimalarials (E.A. Ashley et al., 2014). Therefore, region-specific interventions are crucial, particularly where unique or private alleles are prevalent, to ensure the effectiveness of current and future antimalarial therapies.

5. Conclusion

Our decade-long study (2014–2024) on *Plasmodium falciparum*'s MDR1 gene reveals significant insights into the genetic evolution of malaria resistance. The varied genetic profiles across multi-regional populations underscore the dynamic nature of the parasite's adaptation. High genetic diversity in regions like Odisha, India, contrasts with relative stability in others, highlighting differing evolutionary pressures. These findings emphasize the critical need for region-specific malaria control interventions and the importance of genetic surveillance in informing vaccine development, novel antimalaria drugs and drug resistance management. Our study contributes significantly to understanding malaria's genetic landscape, offering a foundation for targeted, effective multi-regional malaria interventions.

Credit author statement

OAM, GOA and MBA conceived and designed the study. GOA coordinated data collection GOA and SI developed methods, and analysed and interpreted data. OAM, MBA, GOA, TM, IIF and SI wrote and revised the manuscript. All authors provided critical revision of the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Declaration of competing interest

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George Oche Ambrose reports was provided by University of Ilorin Teaching Hospital. George Oche Ambrose reports a relationship with University of Ilorin Teaching Hospital that includes: employment. No conflict of Interest If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2024.100304.

Data availability

Data will be made available on request.

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