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RESEARCH NOTE

Allelic polymorphism in the *Plasmodium vivax* dihydrofolate reductase gene among Indian field isolates

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ABSTRACT

In total, 129 *Plasmodium vivax* isolates from different geographical areas in India were analysed for point mutations in the *P. vivax* dihydrofolate reductase gene that were associated with pyrimethamine resistance. A gradual increase in the frequency of mutant genotypes was observed from north to south ($p < 0.0001$). In the northern region (Delhi, Panna and Nadiad), the wild-type genotype was most prevalent, while the mutant genotype predominated in the coastal regions of southern India (Navi Mumbai, Goa and Chennai). Isolates from the Car-Nicobar islands showed only mutant genotypes. The differential geographical pattern of mutations may be associated with the transmission pattern.

Keywords Allelic polymorphism, dihydrofolate reductase gene, genotypes, geographical distribution, malaria, *Plasmodium vivax*

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The worldwide spread of chloroquine-resistant strains of *Plasmodium falciparum* has led to the use of sulphadoxine–pyrimethamine as the first-line anti-malarial agent in south-east Asian countries. Sulphadoxine and pyrimethamine sequentially inhibit the dihydropteroate synthase (Dhps) and dihydrofolate reductase (Dhfr) enzymes, respectively, in the folate biosynthesis pathway to give a synergic anti-malarial effect [1]. However, *P. falciparum* has overcome the effect of sulphadoxine–pyrimethamine by evolving point mutations in the genes encoding the Dhps and Dhfr enzymes that reduce their drug-binding affinity [2,3]. In India, chloroquine remains the first-line anti-malarial agent for treatment of both *P. falciparum* and *Plasmodium vivax*. Chloroquine resistance in *P. falciparum* has been reported in India [4,5], and areas with a chloroquine resistance level of >25% have switched to the use of sulphadoxine–pyrimethamine as the first-line anti-malarial agent. Although *P. vivax* is still susceptible to chloroquine in India [6,7], the use of sulphadoxine–pyrimethamine to treat chloroquine-resistant *P. falciparum* is creating selection pressure in the *P. vivax* population. Therefore, the aim of the present study was to obtain information concerning mutations related to pyrimethamine resistance in the *P. vivax dhfr* gene of Indian field isolates.

A previous study [8] identified six new mutations in the *P. vivax dhfr* gene by sequencing, but none was located in the active sites [9]. Therefore, in order to screen field isolates for *P. vivax dhfr*

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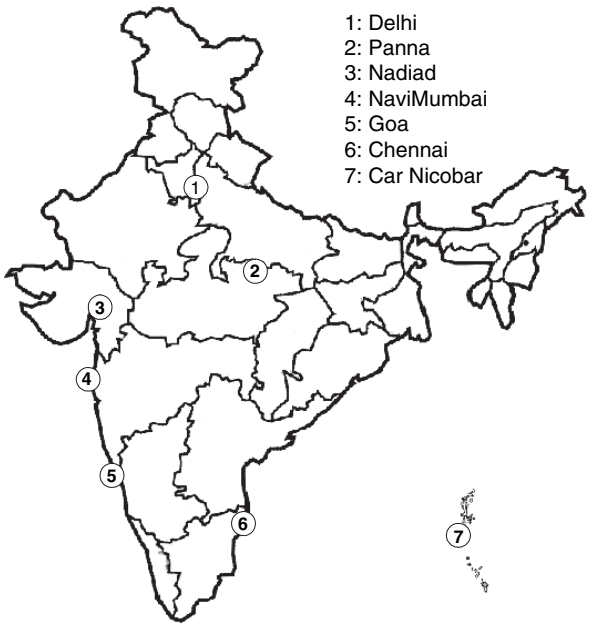

Fig. 1. Location of the study sites in India.

mutations, the present study used the simple method of PCR–restriction fragment length polymorphism analysis. Blood samples were collected by conducting spot surveys in different geographical regions of India, including coastal and mainland areas (Fig. 1). Blood from *P. vivax*-positive patients (diagnosed microscopically) was spotted on autoclaved 3-mm filter paper (Whatman, Mumbai, India), dried and stored at 4°C. The study was approved by the Ethics Committee of the National Institute of Malaria Research (Delhi, India) and all bloodspots were collected with the consent of the patients. Genomic DNA was extracted from bloodspots using a QIAamp mini DNA kit (Qiagen, Hilden, Germany), and this was followed by PCR using the primers and protocols described by Imwong *et al*. [10]. Sequencing of the amplicons from 11 samples yielded results identical to the restriction fragment length polymorphism analysis, and no new mutations were detected.

In total, 129 *P. vivax* isolates from Delhi (n = 29), Panna, Madhya Pradesh (6), Nadiad, Gujarat (19), Navi Mumbai, Maharastra (11), Goa (27), Chennai, Tamil Nadu (30) and the Car Nicobar, Andaman and Nicobar islands (7) were analysed for mutations at codons 33, 57, 58, 61, 117 and 173 of the *P. vivax dhfr* gene. Mutations were observed only at codons 57, 58, 61 and 117 (Table 1). The data revealed a gradual increase in

Table 1. Mutations occurring in the *Plasmodium vivax* dihydrofolate reductase gene of Indian isolates

Area	Period of sample collection	No. of samples tested	No. of isolates		Substitutions at *P. vivax dhfr* codons			
			Wild-type	Mutant	F57L	S58R	T61M	S117N/T
Delhi	2003–04	29	26	3	0	2	0	2
Panna	2003	6	6	0	0	0	0	0
Nadiad	2005	19	14	5	0	5	0	5
Navi Mumbai	2004	11	2	9	0	7	0	9
Goa	2003	30	1	29	1	27	1	28/1[a]
Chennai	2003	27	2	25	0	25	0	24
Car Nicobar	2003	7	0	7	5	7	4	3/4[a]

[a]S117T rather than S117N (61M and 117T based on sequencing data).

the frequency of mutant genotypes from north to south, with predominance of the wild-type genotype in northern regions (Delhi, Panna and Nadiad), and mutant genotypes in southern regions (Navi Mumbai, Goa and Chennai). A statistically significant difference was observed between the datasets from the northern and southern regions (chi-square 81.0, df 1, p <0.0001; OR 80.5, 95% Cl 24.8–261.4). In Delhi, 26 of 29 isolates had the wild-type allele, with two single mutations at codons 58R and 117N, and one double mutation at 58R/117N. In Panna, all six isolates were wild-type. In Nadiad, 14 of 19 isolates were wild-type and five were double mutants (58R/117N). In Navi-Mumbai, seven of 11 isolates were double mutants (58R/117N), two were wild-type and two had single mutations at codon 117N. In Goa, 26 of 30 isolates were double mutants (58R/117N), two were single mutants (117N), one was a quadruple mutant (57L/58R/61M/117T), and one was wild-type. In Chennai, 24 of 27 isolates were double mutants (58R/117N), one was a single mutant (codon 117N) and two were wild-type. Of the seven Car Nicobar isolates, four showed quadruple mutations (57L/58R/61M/117T), one was a triple mutant (57L/58R/117N), and two were double mutants (58R/117N). In total, six different haplotypes, FSTS (n=51), FRTS (2), FSTN (5), FRTN (65), LRTN (1) and LRMT (5), were observed among the study isolates (Fig. 2).

P. vivax is the predominant malarial species on the plains of northern India. Transmission occurs from March to November, with an interruption in the hot summer month of June; only during the late post-monsoon months (September–November) does the number of cases of *P. falciparum* malaria start to rise [11,12]. Thus, in Delhi, Panna and Nadiad, co-infections with *P. vivax* and