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
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, Maharashtra (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 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% CI 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

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

<table>
<thead>
<tr>
<th>Area</th>
<th>Period of sample collection</th>
<th>No. of samples tested</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delhi 2003-04</td>
<td>29</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>Panna 2003</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Nadiad 2005</td>
<td>19</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Navi Mumbai 2004</td>
<td>11</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Goa 2003</td>
<td>30</td>
<td>1</td>
<td>29</td>
</tr>
<tr>
<td>Chennai 2003</td>
<td>27</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Car Nicobar 2003</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

*S117T rather than S117N (61M and 117T based on sequencing data).*
P. falciparum occur for a transient period only. However, in coastal regions (Navi Mumbai, Goa and Chennai) and island regions (Car Nicobar), perennial co-transmission with P. falciparum and P. vivax may have helped in the development and spread of mutant genotypes.

An earlier study [8], although carried out with a limited number of isolates, also showed a predominance of double mutants in coastal areas. The finding of predominantly wild-type P. vivax isolates in northern India agrees with the findings of Ahmed et al. [13], who reported a very low number of mutations in P. falciparum isolates from Delhi and the neighbouring state of Uttar Pradesh, but a high rate of mutations among P. falciparum isolates from Goa. Similarly, in Car Nicobar, both P. falciparum [14] and P. vivax (this study) isolates showed a predominance of quadruple mutants.

During chloroquine trials in India, 100% efficacy was observed in patients infected with P. vivax isolates showing the double mutant P. vivax dhfr genotype [7]. Tjitra et al. [15] have demonstrated the clinical efficacy of a sulphadoxine–pyrimethamine regimen against P. vivax patients infected with double mutant P. vivax dhfr genotypes in Indonesia. Based on these studies, it appears that P. vivax is still susceptible to sulphadoxine–pyrimethamine in India, and that, according to the model for evolution of drug resistance proposed by Hastings et al. [16], Indian P. vivax isolates are still in the first stage of the development and spread of resistance. The observed high prevalence of double mutants of the P. vivax dhfr gene among Indian field isolates in the areas of India with perennial co-transmission of malaria caused by P. falciparum and P. vivax suggests that the use of sulphadoxine–pyrimethamine as presumptive treatment for undiagnosed malaria should be avoided.

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**REFERENCES**


**RESEARCH NOTE**

**Preceding infections and anti-ganglioside antibodies in patients with Guillain–Barré syndrome: a single centre prospective case-control study**

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**ABSTRACT**

Preceding infections and anti-ganglioside antibodies were assessed among 80 Guillain–Barré syndrome (GBS) patients and 125 controls. Previous infections were more frequent among GBS patients than among controls (p <0.0001), and had a significant association with axonal subtype compared with acute inflammatory demyelinating polyneuropathy (AIDP) (29/46 vs. 10/34 patients; p <0.05). *Campylobacter jejuni* (26%) was the most common preceding infection among GBS patients, followed by *Mycoplasma pneumoniae* (15%). Anti-ganglioside antibodies were detected more frequently among GBS patients than among controls (65/80 vs. 13/125; p <0.001), and a higher proportion of axonal cases had these antibodies than did AIDP patients (43/46 vs. 22/34; p <0.01).

**Keywords** Anti-ganglioside antibodies, axonal subtype, *Campylobacter jejuni*, Guillain–Barré syndrome, preceding infections

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Guillain–Barré syndrome (GBS) is a common cause of acute flaccid paralysis, with an annual incidence of 1–2 cases/100 000 population [1]. The pathological spectrum of GBS includes acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN) and acute motor sensory axonal neuropathy (AMSAN). One-half to two-thirds of GBS patients usually report antecedent infections, and *Campylobacter jejuni*, cytomegalovirus (CMV), Epstein-Barr virus (EBV) and *Mycoplasma pneumoniae* are recognised triggering agents of GBS [2]. Anti-ganglioside antibodies are believed to play an important role in the pathogenesis of GBS because of molecular mimicry between some glycoconjugate epitopes of microbes and the nerve tissue of the host [3]. The present study investigated commonly described microbial infections and anti-ganglioside antibodies in patients grouped according to GBS subtype.

Eighty GBS patients admitted to the neurology ward, 80 healthy volunteers matched for age and gender, and without any history of apparent infectious illness at the time of sample collection, and 45 patients with neurological diseases other than GBS, were included in a prospective case-control study between February 2001 and March 2005. GBS patients were defined according to criteria described previously [4] and were further subdivided into GBS subtypes [5]. Single stool and serum samples were collected from all GBS patients and controls with other neurological diseases within 24–48 h of admission.