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Plasmodium vivax: C-terminal diversity in the blood stage SERA genes from Indian field isolates

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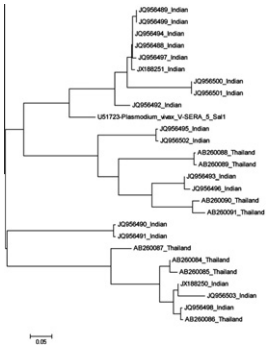
HIGHLIGHTS

- Study deals with genetic diversity of C-terminal region of PvSERA4 and PvSERA5.
- This is the first report in field isolates from India.
- Haplotypes reported in this study are unique and novel.
- Results show a very high extent of genetic diversity in PvSERA5.
- The C-terminal region of PvSERA4 is found to be highly conserved.

GRAPHICAL ABSTRACT



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ABSTRACT

The burden of *Plasmodium vivax* malaria is huge in India, affecting a large population annually. Recent reports of *P. vivax* contributing to severe illness and death, makes vaccine research on *P. vivax* malaria, a high priority. Extent of sequence variation in antigen coding genes is known to be a major hurdle in vaccine initiatives against malaria. Serine repeat antigens of *Plasmodium* are promising asexual blood stage vaccine candidates against malaria and have been implicated to have a key role in merozoite invasion and egress. Among the *P. vivax* SERA proteins, SERA4 and SERA5 are the major transcribed members in erythrocytic stages, making them encouraging candidates to be explored for their polymorphism and vaccine potential. Earlier reports suggest that diversity in these PvSERA antigens is localized to the C-terminal region of the proteins. Hence, genetic diversity study of this region seems prudent. Moreover, as there are no reports available from India, the present study aims to investigate the polymorphism in the C-terminal region of two highly transcribed members PvSERA4 and PvSERA5 in Indian field isolates. Our result of PvSERA5 demonstrates extensive genetic diversity, with major deletions, insertions and SNPs and signifies the gene to be under positive selection. On the other hand, high sequence conservation was seen in the PvSERA4 C-terminal region in Indian field isolates which was contrasting to earlier report from Thailand where they have shown diversity. Research data showcased in this study will greatly aid in gaining better understanding of antigenic variations, immune mediated selection mechanisms and the functional significance of these two *vivax* proteins. This study also makes a striking contribution towards understanding the antigenic repertoire of PvSERA genes in Indian isolates.

1. Introduction

Malaria remains a major global public health concern. The disease burden caused by *Plasmodium vivax* is most widely

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Table 1
Details of primers and thermal cycling profiles for amplification of PvSERA4 and PvSERA5 genes.

S. no.	Gene name	Primer details	Thermocycling profile
1	PvSERA4	Forward primer: CNRSERA4PV3F-5′GCGGGGATCCATCTTTGCCAACCTAACGA3′Reverse primer: CNRSERA4PV4R-5′GCGAAGCTTCTCTCTCCTACGGCAATGG 3′	3 min initial denaturation at 94 °C followed by 36 cycles of 1 min denaturation at 94 °C 1 min 50 s annealing at 58.8 °C 2 min 30 s extension at 72 °C andfinal extension at 72 °C for 10 min.
2	PvSERA5	Forward primer: CNRSERA5PV6F-5′GCGCGGGAAGAAGGTGCAAAG3′Reverse primer: CNRSERA5PV4R-5′GCGCCCGTCACACTCTTCCTAC3′	3 min initial denaturation at 94 °C followed by 36 cycles of 1 min denaturation at 94° C 1 min 30 s annealing at 58.8 °C 2 min 30 s extension at 72 °C andfinal extension at 72 °C for 10 min.

distributed geographically. Nearly 80 million clinical cases are caused every year and 2.5 billion people are at risk globally (Mueller et al., 2009). *P. vivax* constitutes nearly 65% of the malaria cases in the Indian subcontinent (Joshi et al., 2008). Re-emergence, increased transmission and drug resistance have further enhanced the complexities of *vivax* malaria. Diversity of *P. vivax* in terms of relapse patterns, drug response, clinical profiles, genetic variability of antigen genes, isoenzyme markers and microsatellites poses additional challenges in disease management (Hartl, 2004). Reports of severe malaria cases by *P. vivax* are also not uncommon from different parts of the world, including India (Kochar et al., 2009). However, *Plasmodium falciparum* has been the major focus of malaria research and comparatively *P. vivax* malaria has lagged disproportionately.

Slow progress in malaria vaccine development has been mainly due to complex parasite biology and extensive antigenic polymorphism (Thomas and Allan, 2002). Antigen encoding genes show greater diversity, whereas other protein coding genes show relatively low levels of polymorphism (Hartl, 2004). Knowledge about the vaccine antigens and the extent of its sequence variation is very crucial in vaccine efficacy trials (Takala and Plowe, 2009). Polymorphism studies are essential to build informed choices about alleles to be included in multivalent or chimeric vaccines, as allelic forms from different isolates differ in their immunogenic potential and their ability to abrogate host immune response (Palacpac et al., 2006). Very few vaccine candidate antigens have been characterized for *P. vivax* impeding vaccine initiatives (Sattabongkot et al., 2004; Sina, 2002). Among the various *Plasmodial* vaccine candidates, serine repeat antigens, expressed in the blood stages of the parasite life cycle, are encouraging prospects.

In *P. vivax*, SERA belong to a multigene family, with 12 homologues. Among the PvSERA homologues, earlier reports indicate that only PvSERA5 gene transcript was detected in *P. vivax* infected monkey erythrocytes based on RNA transcript analysis of Sal I (PvSERA1–5) VSERA genes (Kiefer et al., 1996). Subsequently, PvSERA4 was reported to be the most strongly transcribed member followed by PvSERA2, 5, 10, and 11 (Palacpac et al., 2006). These characteristic high expression profiles of PvSERA4 and PvSERA5 in erythrocytic stages (Kiefer et al., 1996; Palacpac et al., 2006) crafts them as encouraging candidates to be explored further for

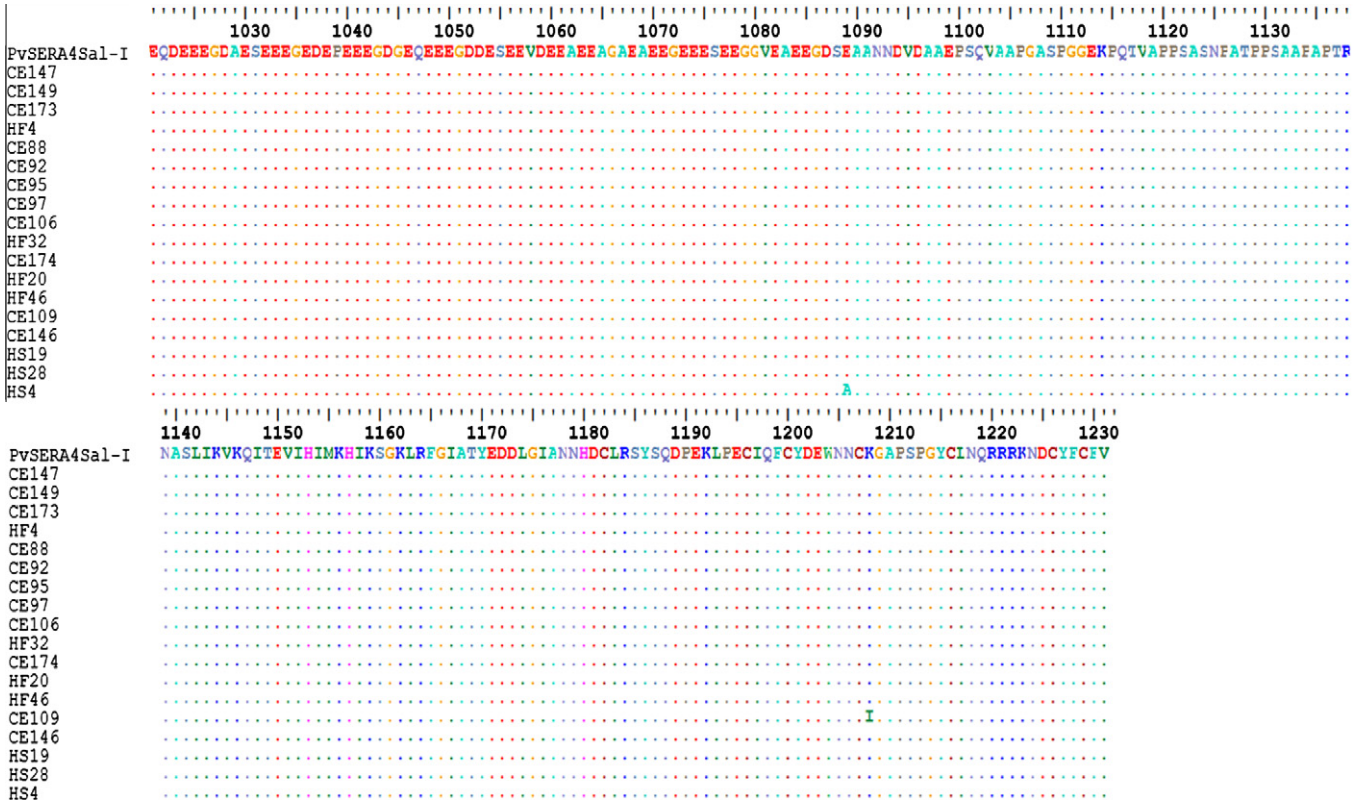

Fig. 1. Multiple sequence alignment (MSA) of translated protein sequences of 18 Indian isolates against Sal I strain in the C-terminal region of the PvSERA4 gene. The MSA was generated using the ClustalW application in Bio Edit software.