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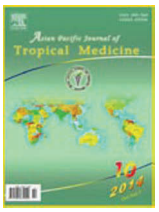

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Conserved regions of *Plasmodium vivax* potential vaccine candidate antigens in Sri Lanka: Conscious *in silico* analysis of prospective conformational epitope regions

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ABSTRACT

Objective: To do mapping and modeling of conformational B cell epitope regions of highly conserved and protective regions of three merozoitecandidate vaccine proteins of *Plasmodium vivax* (*P. vivax*) , *ie*. merozoite purface protein-1 (*Pv*MSP-1), apical membrane antigen -1 domain Ⅱ (*Pv*AMA1-D Ⅱ) and region Ⅱ of the Duffy binding protein (*Pv*DBP Ⅱ), and to analyze the immunogenic properties of these predicted epitopes. **Methods:** 3-D structures of amino acid haplotypes from Sri Lanka (available in GeneBank) of *Pv*MSP-1_{19} (n=27), *Pv*AMA1-D Ⅱ (n=21) and *Pv*DBP Ⅱ (n=33) were modeled. SEPPA, selected as the best online server was used for conformational epitope predictions, while prediction and modeling of protein structure and properties related to immunogenicity was carried out with Geno3D server, SCRATCH Protein Server, NetSurfP Server and standalonesoftware, Genious 5.4.4. **Results:** SEPPA revealed that regions of predicted conformational epitopes formed 4 clusters in *Pv*MSP-I_{19}, and 3 clusters each in *Pv*AMA1-D Ⅱ and *Pv*DBP Ⅱ, all of which displayed a high degree of hydrophilicity, contained solvent exposed residues, displayed high probability of antigenicity and showed positive antigenic propensity values, that indicated high degree of immunogenicity. **Conclusions:** Findings of this study revealed and confirmed that different parts of the sequences of each of the conserved regions of the three selected potential vaccine candidate antigens of *P. vivax* are important with regard to conformational epitope prediction that warrants further laboratory experimental investigations in in vivo animal models.

1. Introduction

The three main obstacles for eradication of malaria are the steady rise in drug resistant parasite strains, insecticide resistance of the vector mosquito and lack of successful vaccine(s)[1], which emphasize the importance of vaccination strategies for malaria. The multifaceted life cycle of the parasite, and widespread genetic diversity of the different parasite stages are adverse obstacles to successful malaria control[2].

Plasmodium vivax (*P. vivax*) is more important than conventionally thought for reasons that this parasite has a wider geographical range (Asia and America), potentially exposing more people to risk of infection, is less amendable to control, and most importantly, infections with *P. vivax* can cause severe clinical syndromes[3].Additionally, vivax malaria could be as fatal in a similar way to severe *Plasmodium falciparum (P. falciparum)* malaria and is malignant and common[4]. Unlike *P. falciparum*, difficulty of maintaining *P. vivax* in continuous in vitro culture with the exception of short term ex vivo cultures, to conduct laboratory research has lead *P. vivax* to be successfully tested in Non Human Primate models[5]. The occurrence of *P. vivax* relapses is detected in 20%-80% of patients which guarantee that up to 20% of an endemic population may undergo symptomatic infection each year, even in low-transmission areas, which in turn will collectively

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result in 10-30 episodes of malaria during the lifetime of an individual[6]. The failure of Primaquine treatment, the only therapeutic option against lethal relapses aggravates this situation[1].

Thus effective, stable malaria control may depend on developing reasonable, general defensive anti-malarial vaccines[7]. Anti-parasite vaccine strategies developed so far are in line with the parasite life cycle, and can be broadly categorized as pre-erythrocytic, blood stage and sexual stage vaccines[8]. An effective vaccine against the erythrocytic stages of the malaria parasite would aim to limit parasite multiplication, thereby reducing morbidity and mortality of drug resistant parasites[9].

Three merozoite proteins have been considered as prime targets for blood stage malaria vaccine(s) since they are exposed to effective immune mechanisms leading to an interruption of the parasite's erythrocytic cycle, namely the merozoite surface protein-1 (MSP-1), the apical membrane Antigen (AMA1) and the Duffy binding protein (DBP)[10].

While lack of availability of an effective vaccine improves the success of continuing the parasite burden, an important step in the fundamental design of a novel vaccine is the meticulous determination of conformational epitopes of neutralizing antibodies. The polymorphism associated with conformational B cell epitopes is important to understand the mechanism of antibody recognition and the reaction of the host immune system, which in turn will obstruct the parasite's ability to elude the host immune response[11].

An accurate immune response could be induced by a correctly chosen mixture of pertinent epitopes focused on conserved and highly immunogenic regions of an antigen or several antigens. This selection can be based on aspects such as immunogenicityand conserved nature of the antigens. Furthermore, epitope vaccines have attracted considerable importance as these present with multiple advantages together with their validity in personalized medicine[12-14].

A research programme conducted by the Department of Zoology, University of Colombo, Sri Lanka, led to the identification of locally and globally conserved regions of three potential *P. vivax* vaccine candidate antigens, *ie*. MSP-1 (*Pv*MSP-1)[15], AMA1-Ⅰ (*Pv*AMA1-DⅠ)[16], and DBPⅡ (*Pv*DBPⅡ)[17]. The respective T cell and linear B cell epitope predictions for these molecules were reported therein.

Though a vast amount of data exist on linear epitopes, as discovery of conformational B-cell epitopes is a challenge, the information on the latter are scarce[11]. X ray crystallography structures and methods applying NMR cross-saturation with TROSY detection have revealed several epitopes on malaria vaccine candidates with respect to *P. falciparum*[18-21]. Conversely, such information for *P. vivax* is lacking.

Epitopes can be discriminated from non-epitope regions of a protein with characteristics related to structural and functional aspects[22], and certain physicochemical properties of amino acids have shown relationship to the locations of linear epitopes within protein sequences[12]. Based on these observations, some of the epitope related characteristics proposed to be studied were hydrophilicity, hydrophobicity, surface accessibility, and antigenicity[23].

The main focus of the current study was the in silico prediction and mapping of conformational B cell epitopes of the highly conserved and protective regions of MSP-1(PvMSP-1_{19}), AMA-1 (PvAMA1-DⅠ) and DBP (PvDBPⅡ)[10], both in local and global *P. vivax* parasite isolates, using bioinformatics tools. Furthermore, the molecular immunogenicity of the predicted epitopes was evaluated in order to augment the abovefindings. The outcome of this study would assist in the rational design of avivax malaria vaccine construct based on these 3 potential asexual vaccine candidate antigens to be tested in immunization trials.

2. Materials and methods

2.1. Haplotype retrieval and extracting conserved regions for the 3 proteins

The different Sri Lankan haplotype sequences uploaded to the GeneBank (http://www.ncbi.nlm.nih.gov/genbank/) were retrieved for PvMSP-1_{19} (n=27), PvAMA1-DⅠ (n=21) and PvDBPⅡ (n=33) via accession numbers reported previously and the conserved regions were identified according to previous studies[15-17].

2.1.1. Modeling 3D structures of the P. vivax haplotypes

Due to lack of 3D structures of given sequences, except for PvMSP-1_{19} which is conserved globally[15]and of which the crystal structure is available on the Protein Data Bank (PDB) under PDB ID 2NPR[24], the homology modeling technique was used to generate the 3D structures of the local isolates of PvAMA1-DⅠ and PvDBPⅡ using the PDB structures 1W81 and 2C6J respectively[25,26], which were required as the inputs to the conformational epitope predicting server Geno3D[27]. Geno3D is an automatic web server (http://geno3d-pbil.ibcp.fr/cgi-bin/geno3d_automat.pl?page=/GENO3D/geno3d_home.html) for protein molecular modeling. Starting with a query of protein sequence, the server identifies a homologous protein through a sequence similarity search using PSI-BLAST for protein sharing less that 95% pair wise identity. The template can be chosen from a special interface provided by the server, which leads to the point where modeling could be initiated. The built 3D protein model using distance geometry, simulated annealing and energy minimization algorithms, was made available with atomic coordinates of each model with best satisfying