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A comparative study of the genetic diversity of the 42 kDa fragment of the merozoite surface protein 1 in *Plasmodium falciparum* and *P. vivax*

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Abstract

We investigated the genetic diversity of the 42 kDa fragment of the merozoite surface protein 1 (MSP-1) antigen in *Plasmodium falciparum* and *P. vivax*, as well as in non-human primate malarial parasites. This fragment undergoes a proteolytic cleavage generating two fragments of 19 kDa (MSP-1_{19}) and 33 kDa (MSP-1_{33}) that are critical in erythrocyte invasion. We found that overall the MSP-1_{33} fragment exhibits greater genetic diversity than the MSP-1_{19} regardless of the species. We have found evidence for positive natural selection only in the human malaria parasites by comparing the rate of non-synonymous versus synonymous substitutions. In addition, we found clear differences between the two major human malaria parasites. In the case of *P. falciparum*, positive natural selection is acting on the MSP-1_{19} region while the MSP-1_{33} is neutral or under purifying selection. The opposite pattern was observed in *P. vivax*. Our results suggest different roles of this antigen in the host–parasite immune interaction in each of the major human malarial parasites.
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1. Introduction

The malaria burden is particularly high in sub-Saharan Africa where *Plasmodium falciparum* is predominant. However, malaria ‘‘out of Africa’’ is characterized by the presence of *P. vivax*, the second most important malaria parasite in terms of its morbidity. Although there are clear biological and genetic differences between these two parasites (Coatney et al., 1971), they overlap in their geographic distribution and there is increasing evidence for their interaction (Snounou and White, 2004).

Among the antigens currently under consideration in malaria vaccine formulations, one of the most promising candidates is the major merozoite surface protein 1 (MSP-1) (Good et al., 2004). The MSP-1 antigen is expressed as a large protein of 190–200 kDa on the parasite surface (Holder and Freeman, 1982). This precursor undergoes two steps of proteolytic cleavage during the merozoite maturation. First, it is cleaved into four major fragments of 83, 30, 38 and 42 kDa (further referred to as MSP-1_{83}, MSP-1_{30}, MSP-1_{38}, and MSP-1_{42}) then, before erythrocyte invasion the MSP-1_{42} fragment undergoes a second cleavage resulting in the generation of 33 and 19 kDa (MSP-1_{33} and MSP-1_{19}) fragments where the latter remain on the merozoite surface during invasion.

Plasmodium spp. MSP-1 exhibits extensive genetic polymorphism (Tanabe et al., 1987; Putaporntip et al., 2002) that appears to be maintained by positive natural selection in *P. falciparum* (Hughes, 1992; Escalante et al., 1998; Conway et al.,

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2000) and *P. vivax* (Putaporntip et al., 2006). Similar observations have been made about other malarial vaccine antigens (see Escalante et al., 2004) on which the host immune system is considered the driving selective force that allows for the accumulation and frequent switch of suitable mutations in the parasite population. Under this scenario, mutations are maintained longer in the parasite population than expected if genetic drift were the sole process acting on the genetic polymorphism.

The conclusion that positive selection maintains the genetic diversity of genes encoding malarial antigens is supported, among others lines of evidence, by the observation in *P. falciparum* that non-synonymous nucleotide substitutions (those that change the amino acid) are more common than synonymous substitutions (mutations that do not change the amino acid) (Hughes and Hughes, 1995; Escalante et al., 1998, 2004). Since natural selection acts on phenotypic differences, an excess of non-synonymous substitutions over synonymous is considered evidence that natural selection is favoring the maintenance of genetic polymorphism.

In the case of *Plasmodium* spp. MSP-1, most of the genetic diversity analyses have subdivided the gene into blocks (segments) based on their level of genetic diversity but not using any other biological criteria (Tanabe et al., 1987; Putaporntip et al., 2002, 2006); however, few studies have been done considering the proteolytic fragments as functional units (Escalante et al., 1998).

The MSP-1_{42} and MSP-1_{19} fragments have received special attention in *P. falciparum* as part of vaccine formulations given that they are relatively conserved and antibodies against these fragments inhibit the parasite invasion into the red blood cells (Yang et al., 1999; Stanisic et al., 2004). In addition, the critical role of the MSP-1_{19} fragment in the erythrocyte invasion is conserved even among distantly related species (O'Donnell et al., 2000).

An important characteristic of *P. vivax* is that it invades reticulocytes, a process that is mediated by specific proteins such as the reticulocyte binding proteins and Duffy receptor (Galinski et al., 1992; Chitnis and Miller, 1994). However, MSP-1 in *P. vivax* also appears to play an important role in this process (Rodríguez et al., 2002; Espinosa et al., 2003; Han et al., 2004; Sachdeva et al., 2004). Indeed, peptides with high specific binding activity (HSBA) to reticulocytes have been found in the MSP133 (Espinosa et al., 2003; Rodríguez et al., 2002).

This investigation aims to compare the genetic diversity of the MSP-1_{42} in *Plasmodium* spp. focusing on *P. falciparum* and *P. vivax.* We have analyzed 120 sequences of the MSP-1_{42} of *P. falciparum* and 75 sequences of the homologous region in *P. vivax*, and we have explored the genetic diversity of the MSP-1_{33} and MSP-1_{19} fragments. In the case of *P. vivax*, we further explored its genetic diversity by comparing it with the homologous regions in primate malarial parasites that are closely related to *P. vivax* (Escalante et al., 2005). Although we find evidence that positive natural selection is acting on the observed polymorphism in MSP-1_{42}, it operates differently in each of the two major human malarial parasites. We conclude that inferences made about *P. falciparum* MSP-1 cannot simply be ''translated'' into *P. vivax.*

2. Materials and methods

The gene encoding the 42 kDa fragment of MSP-1 or MSP-1_{42} was amplified by polymerase chain reaction (PCR). The primers forward-GAA TGA TAT TCC TAA GAA GTT AGA GG and reverse-GAT AGA TTA TTT AAT AAG AAA AAA GAA CTT GGC CAA GAC AAA ATG C were used to amplify the partial *P. falciparum* 3′ sequences. The PCR conditions for amplifying the MSP-1_{42} from *P. falciparum* were: a partial denaturation at 94 °C for 1 min and 30 cycles with 1 min at 94 °C, 1 min at 50 °C and 3 min extension at 72 °C. A final extension of 3 min was added in the last cycle. The primers forward-GAC CAA GTA ACA ACG GGA G and reverse-CAA AGA GTG GCT CAG AAC C were used for *P. vivax*, *P. cynomolgi*, *P. inui*, and *P. knowlesi*. In the case of *P. fragile*, we used the forward primer GAC CAA GTA ACA ACG GG. The PCR conditions for amplifying the MSP-1_{42} from *P. vivax* and non-human primate malarias were: a partial denaturation at 94 °C for 3 min and 35 cycles with 1 min at 94 °C, 45′ at 50–58 °C and 2 min extension at 72 °C, a final extension of 10 min was added in the last cycle.

The amplified product was purified, cloned using the pGEM-TEasy Vector System I from Promega (USA), and sequenced. Both strands were sequenced from at least two clones. The alignment was performed using ClustalW Version 1.7 with manual editing using the alignment reported by Miller et al. (1993) in the case of *P. falciparum* and those reported by Putaporntip et al. (2002, 2006) in the case of *P. vivax* and related species.

In the case of *P. falciparum*, we sequenced the MSP-1 42 kDa in 34 isolates from Asembo Bay, western Kenya in this investigation. In addition, a total of 20 isolates (5 from India, 9 from Venezuela, and 6 from Thailand) were sequenced for the 3′ end. We used in our investigation prior published sequences (Chang et al., 1988; Qari et al., 1998; Jongwutiwes et al., 1992, 1993; Tanabe et al., 2004) and unpublished sequences under the accession numbers U20726–U20733 and U20653–U20656. A total of 120 MSP-1_{42} sequences were considered in our analyses. In addition, we included 55 sequences of the MSP-1_{19} reported in the literature (Kaneko et al., 1997; Kumar et al., 2005) and unpublished sequences under the accession numbers AF29507–AF29537 in order to obtain a complete picture of the MSP-1_{19} alleles that have been reported.

In the case of *P. vivax*, we report five sequences from laboratory isolates (Rio Meta, Sumatra I, Indonesia I, Mauritania I, and Vietnam II) and used the sequences reported in literature (Putaporntip et al., 2000, 2002) for a total of 75 sequences. In addition, we analyzed 10 sequences from different isolates of *P. cynomolgi* (the sequence AY869723 from the GenBank together with new sequences from the strains B strain, Berok, Cambodian, Ceylonensis, Gombok, Mulligan, PT1, PT2, and RO), 15 sequences from isolates of *P. inui* (Celebes I and II, Hackeri, Hawking, Leaf Monkey I and II, Leucosphyrus, Mulligan, N-34, OS, Perak, Perlis, Philippine, Taiwan I and II), a sequence of *P. knowlesi* (Hackery strain), *P. hylobati* (parasite from gibbons), and *P. fragile* (Nilgiri strain). Information about the biology of these species and the origin of

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