

- 448 MOLECULAR POPULATION GENETICS OF *PLASMODIUM FALCIPARUM*: A STUDY OF THE GENE ENCODING THE CIRCUMSPOROZOITE PROTEIN. Escalante AA\*, Qari SH, Goldman IF, and Lal AA. Division of Parasitic Diseases, NCID, CDC, Atlanta, GA.

Considerable polymorphism has been reported in *Plasmodium falciparum* vaccine antigen genes. The working hypothesis is that intragenic recombination and natural selection are the forces driving this extensive polymorphism. Previous studies suggested that the synonymous substitutions are seriously constrained by the *P. falciparum* codon bias. Given the constraints on the synonymous substitutions in *P. falciparum*, the ratio of synonymous and nonsynonymous substitutions used to detect positive natural selection should be taken as preliminary evidence. The lack of synonymous substitutions also compromise our capacity to differentiate between recombination events from convergence due to selection. In the case of the CS protein gene, for instance, there is clear immunologic data suggesting that selection is acting on the observed pattern of point mutations. However, conventional tests have failed in detecting departure from neutrality. A heuristic approach is needed that incorporates the known information about the gene. We studied the regions encoding the Th2R and Th3R epitopes in the CS protein and found evidence of linkage disequilibrium and intragenic recombination/gene conversion. We conclude that the most parsimonious hypothesis to explain the observed pattern of genetic diversity is that it is maintained by the action of natural selection. A geographic level study of the genetic variation at these epitopes revealed that African populations are more variable than those from South America, Asia, and the Pacific.

- 449 FIBRONECTIN BINDING ACTIVITY OF *PLASMODIUM FALCIPARUM* GRP 78 PROTEIN. Ware LA\*, Lanar DE, Hansen B, and Stoute JA. Department of Immunology, Walter Reed Army Institute of Research, Washington DC.

Fibronectin is a heterodimeric protein involved in cell adhesion, opsonization and anchoring. We observed that *Plasmodium falciparum* infected red cells exhibit fibronectin binding activity. To identify the parasite antigen(s) involved in this interaction, we screened a blood stage lambda gt11 cDNA library with human plasma fibronectin. The amino acid sequence of a positive clone, 721, was identical to amino acids 408-655 of *P. falciparum* Glucose-Regulated Protein 78 (GRP 78), a heat shock protein. Clone 721 was expressed in pET32 as a c-terminal thioredoxin fusion protein. Biotinylated fibronectin bound to 721 in ELISA. Binding was inhibited by unlabelled fibronectin and by a 30kDa heparin binding fragment of fibronectin, but not by thioredoxin. Rabbits were immunized with thioredoxin, 721, or a c-terminal peptide (721 peptide). Immunoblotting of parasite lysates with anti-peptide and anti-721 antibodies recognized a protein of ~80kDa. The same protein was also identified when lysates were probed with fibronectin. Anti-721 and anti-thioredoxin antibodies inhibited the binding of fibronectin to 721 in ELISA but anti-peptide antibodies did not. This suggests that the binding motif is present in the N-terminal portion of 721. Studies to further identify the binding epitope(s) and to define the role of fibronectin with respect to the parasite will be conducted.

- 450 A NEW FAMILY OF ERYTHROCYTE BINDING PROTEINS OF MALARIA PARASITES . Kappe SH\*, Noe AR, Fraser TS, Blair PL, and Adams JH. Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.

Malaria merozoites express proteins that mediate the molecular interactions between merozoites with erythrocyte surface receptors. One family of proteins involved in host cell recognition by merozoites is the erythrocyte binding protein (EBP) family. The EBP family includes the Duffy binding protein of *Plasmodium vivax* and *P. knowlesi* and the EBA-175 of *P. falciparum*. In a PCR-based search for *ebps* in rodent malaria parasites *P. yoelii* and *P. berghei* novel genes were isolated containing sequence elements highly similar to the carboxyl cysteine-rich region typical for the EBP family. In addition, the exon/intron structure at the 3'end and the conservation of the exon splice junctions identified these genes as EBP homologues. However, the deduced amino cysteine-rich domains encoded by the 5' region of these genes showed no sequence similarity to the EBP family, but significant shared sequence similarity with the apical membrane antigen-1 (AMA-1) family of *Plasmodium*. Expression of the locus was shown by reverse transcriptase-PCR (RT-PCR). Differential splicing of transcripts was observed in the virulent YM clone of *P. yoelii*. Two transcripts were detected by Northern blot analysis. This unusual splicing might be correlated to the expression of a secreted form of the protein. The amino cysteine-rich domain of this new erythrocyte binding protein family represents the putative adhesion domain that binds to determinants on the host erythrocyte.

- 451 PREDICTED AND OBSERVED ALLELES OF *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN-1 (MSP-1), A POTENTIAL MALARIA VACCINE ANTIGEN. Qari S\*, Shi YP, Goldman I, Nahnen BL, Tibayrenc M, and Lal AA. Division of Parasitic Diseases, NCID, CDC, Atlanta GA; Vector Biology and Control Research Center, Kenya Medical Research Institute, Kisumu, Kenya; and Laboratory for Genetics for Parasites and Vectors, UMR CNRS/ORSTOM, 34032 Montpellier, Cedex 01, France.



The 19-kDa antigenic domain of *Plasmodium falciparum* merozoite surface protein (MSP-1) is a potential malaria vaccine candidate. Based on the amino acid substitution, four known alleles, E-TSR (PNG-MAD20 type), E-KNG (Uganda-PA type), Q-KNG (Wellcome type), and Q-TSR (Indo type) of this domain have been identified. Due to the identification of an additional allele-determining-residue (L/F) in this and one previous study, the description of alleles have been redefined. Using single or double crossover recombinational events, we predicted the existence of a total of 10 alleles, of which four were previously reported. The presence of the predicted alleles was determined in parasite samples from western Kenya, undertaking a cross-sectional and a longitudinal study. The study lead to the identification of 3 new alleles: E-KSG-L (Kenya-1 type); E-KSR-L (Kenya-2 type); and E-KNG-F (Kenya-3 type). The results of this study suggest that it may be possible to predict complexity of the genetic makeup of natural parasite populations, especially in the context of vaccine antigen genes.

452 INTER- AND INTRASPECIES VARIABILITY IN MEROZOITE SURFACE PROTEIN-1 FROM *PLASMODIUM BERGHEI*. Wiser MF\* and Jennings GJ. Department of Tropical Medicine, Tulane University School of Public Health, New Orleans, LA.

Merozoite surface protein-1 (MSP-1) is a leading candidate for a bloodstage vaccine. In this study the complete gene for MSP-1 from *Plasmodium berghei* was cloned and sequenced. Comparison of the PbMSP-1 sequence with MSP-1 from other rodent parasites reveals 5 highly conserved domains interrupted by 4 variable blocks. These variable blocks exhibit no sequence homology, but do exhibit similar amino acid compositions with the predominant amino acids being aliphatic and of intermediate hydrophobicity. Interestingly, the primary proteolytic processing sites are located at the boundaries between conserved and variable domains. We propose that the primary processing which occurs during merozoite maturation results in an extension and increased exposure of the variable domains. The intermediate hydrophobicity and extended conformation suggest that the variable domains play a role in the initial nonspecific interactions between the merozoite and erythrocyte. PCR primers flanking the variable blocks were used to examine intraspecies variation among *P. berghei* strains. The predominant intraspecies difference is in the number and arrangement of tandem repeats within the variable blocks. The inter-and intraspecies differences suggest that the variable blocks are regions of high recombination activity such as slipped-strand mispairing and/or unequal crossing-over events. This high level of intragenic recombination provides a mechanism by which the parasite can generate antigenic diversity while preserving function as dictated by the amino acid composition. A better understanding of mechanisms by which the malaria parasite can generate variability will assist in vaccine development.

453 GENETIC POLYMORPHISMS OF *PLASMODIUM VIVAX* IN THE BRAZILIAN AMAZON. Laserson KF\*, Fontes CJ, Duarte EC, Pang L, delCorral H, Zalis M, Wirth DF, and Maguire J. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Universidade Federal de Mato Grosso-Faculdade de Ciencias Medicas, Cuiaba, MT, Brazil; and Fundacao Nacional de Saude-Coordenadoria Regional de Mato Grosso, Cuiaba, MT, Brazil; Fundacao Nacional de Saude-Coordenadoria Regional de Mato Grosso, Cuiaba, MT, Brazil; Walter Reed Research Institute, Rio de Janeiro, RJ, Brazil; and Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Malaria is one of the principal health problems in the Brazilian Amazon. Epidemiological information indicates a high number of secondary cases of *P. vivax* infection. Primaquine resistance may be developing in this region as it has elsewhere in the world, or the observed failures of treatment may be the result of low rates of compliance with treatment. Accordingly, we began an *in vivo* study of the sensitivity of *P. vivax* to primaquine. As part of the protocol, we applied PCR-based molecular techniques to evaluate isolates from this area and have identified multiple genotypes of the gene for the MSP-1 protein. MSP-1 PCR products from these patients range between 500 and 800 basepairs. This finding suggests that it will be possible to identify the strain or strains of *P. vivax* present in primary and recurrent infections as a step towards defining sensitivity of *P. vivax* to primaquine.

454 THE SECOND AMINO ACID (Glu) IN LUCIFERASE IS ESSENTIAL FOR ENZYME ACTIVITY. Tsai YL\* and Krogstad DJ. Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

In order to optimize electroporation conditions for transfection in *Plasmodium falciparum*, simple markers that can be detected rapidly may be extremely useful, to avoid waiting the several weeks required for positive selection *in vitro*. Luciferase is such a marker and has been used for this purpose previously as a fusion protein with *Pgs28* in a construct which deleted the first amino acid of luciferase and was expressed in *P. gallinaceum*, as reported previously. In recent experiments, we used a modified luciferase construct, pGL3-Promoter (Promega, E1761) to transfet CHO cells and found readily detectable luciferase activity within 24 hours. The coding region of this construct was then amplified by PCR using altered primers to create an *Nsi* I site at the 5' end of the coding region.

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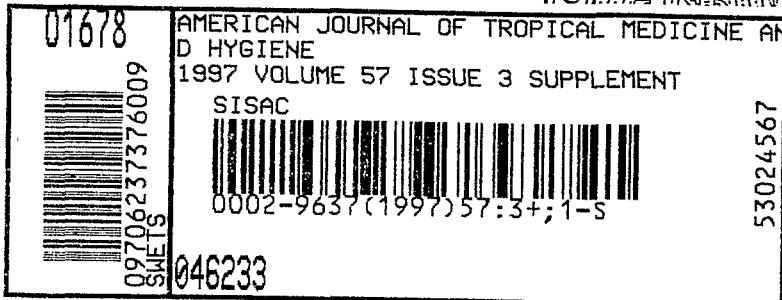
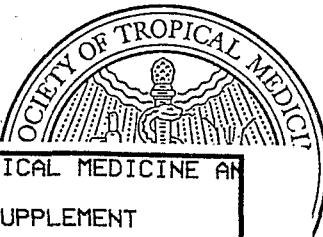
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