364 A COMPARATIVE STUDY OF *PLASMODIUM FALCIPARUM* AND ITS CLOSELY RELATED SPECIES, THE CHIMPANZEE PARASITE *P. REICHENOWI*. Escalante AA, Yang C, Shi YP*, Freeland D, and Lal AA. Division of Parasitic Diseases, NCID, CDC, U.S. Department of Health and Human Service, Atlanta, GA.

Because of its virulent nature, *Plasmodium falciparum* has been considered to be a recent human parasite. However, a recent phylogenetic analysis has shown that *P. falciparum* is not related to any human parasite, and the closest species is the chimpanzee parasite *P. reichenowi*. So far the only gene for which a comparative study has been conducted is the gene encoding the Circumsporozoite Protein. The study demonstrated that *P. reichenowi* is outside the observed polymorphism of *P. falciparum*. The present study involves a comparative analysis of several parasite genes expressed at different developmental stages in the parasite life cycle. The genes used in this study are, RAP-1, the N-terminal of LSA-1, the intron for the calmoduline gene, the C-terminal of the MSP-1, the Pfs48/45, Pfs25, and Pfs27. Our results show that *P. reichenowi* is outside the polymorphism for all the genes under study, except the MSP-1. *P. reichenowi* is clearly closely related to the Wellcome type allele of MSP-1. The polymorphism at this loci has been suggested to be 45 Myrs old, and our results agree with that hypothesis. We will discuss the relationship between gene and species trees especially in the case of closely related species that share the ancient polymorphism. We conclude that *P. reichenowi* is a different biological entity from *P. falciparum*, and that its existence suggests that the *P. falciparum*-human association could be as old as the separation of the Homo-Pan lineages

365 MEASURING GENETIC DIVERSITY IN *PLASMODIUM FALCIPARUM*: ASSESSING EVOLUTIONARY RATES AND NATURAL SELECTION. Escalante AA* and Lal AA. Division of Parasitic Diseases, NCID, CDC, U.S. Department of Health and Human Services, Atlanta, GA.

Understanding the nature and extent of genetic diversity in infectious agents has been a focus of research for molecular biologists working on *Plasmodium falciparum* and other infectious agents. With the availability of sequence data of genes encoding antigens it has become possible to quantitate genetic variability, and explore how the observed genetic diversity is maintained. We will describe several examples of genetic polymorphism and the problems faced in the interpretation of the observed pattern. We will focus mainly on the detection of positive natural selection and evolutionary rates. In the case of natural selection, the first aspect will be the widely used method of estimating Synonymous and Nonsynonymous substitutions as evidence of natural selection. We will show how unusual ratios of Synonymous-Nonsynonymous may be reflecting different processes unrelated with the effect of positive selection. In the specific case of *P. falciparum*, we will show how the characteristics of this parasite genome affect this ratio. We will show alternative approaches for detecting selection, including the Tajima' D test and McDonald-Kreitman test, and discuss their limitations. In terms of measuring evolutionary rates, we will focus in the sampling problem and several distance methods widely used in the literature. A comparison of different distance methods will be made and their appropriate use discussed.

366 MULTILOCUS GENE ANALYSIS OF KENYAN *PLASMODIUM FALCIPARUM* ISOLATES. Qari SH*, Benabderrazak S, Escalante AA, Shi YP, Tibayrenc M, and Lal AA. Division of Parasitic Diseases, NCID, CDC, U.S. Department of Health and Human Service, Atlanta, GA; Universite Montpellier, CNRS/ORSTOM, Montpellier, France.

The existence of the genetically defined strains in *Plasmodium falciparum* was tested by characterizing four loci located on three different chromosomes. These included three vaccine candidate antigens, CSP, MSP-1, and Pfs25, which are from different stages of the parasite life cycle, and one non-coding neutral marker, the second intron of β tubulin gene. Among the 21 parasite samples evaluated in this study, 19 originated from 5-21 month old children from a small locality in Kenya. All the samples appeared homogenous by isoenzyme analysis and had reproducible pattern of bands in RFLP and RAPD analysis. By nucleotide sequencing 14, 16, 14, and 9 different genotypes were identified at the CSP (261 bp C-terminal region), MSP-1 (1119 bp block 15, 16 and 17), Pfs25 (654 bp), and intron (~250 bp) loci, respectively. In the CSP gene 12 new genotypes were identified. Two novel alleles E-KSG, and E-KSR were identified in MSP-1 block 17, in addition to the three previously known alleles (E-TSR, E-KNG, and Q-KNG). Thirteen of the 14 genotypes of Pfs25 identified in this study have not been reported earlier. Polymorphism detected in the β tubulin intron resulted in different numbers (7 to 23) of AT repeats, generated possibly by unequal intragenic recombination. Phylogenetic analysis was done by UPGMA and Neighbor joining methods. The results of this study reveal that, unlike in some other medically important protozoans, there is no multilocus association in *P. falciparum* and the genes located on different chromosomes segregate independently during meiosis in the mosquito vector. Thus genetic/antigenic information of one antigen or a neutral marker can not be co-related with other antigens. No distinct genetic strains could be identified. Such studies will help in understanding the genetic makeup of *P. falciparum* in context of malaria intervention strategies.

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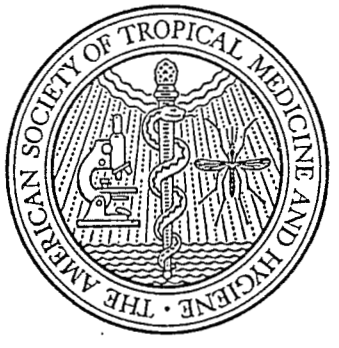

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