5. Summary

- The alleles from genes that may have important role to play during *P.falciparum* malaria exhibit population-specific distribution across India. On the basis of SNPs that were analyzed, some tribal populations inhabiting falciparum malaria endemic areas show significant genetic differentiation from those residing non-endemic regions of India.

- The pan-India SNP frequency information and the observed differential distribution of minor alleles with respect to malaria endemicity, was used to plan a case-control study in a malaria endemic (Orissa and Chattisgarh) and non-endemic (Uttar Pradesh) region of India with *P.falciparum* malaria patients.

- The *TNF* enhancer was found to be highly polymorphic exhibiting six single base substitutions which included one novel polymorphism at -76 position. Through EMSA we showed that -76 SNP may increase (4-to 5-fold) affinity of NFATp binding to the *TNF* enhancer in activated human T-cells. Mutant genotypes of two enhancer SNPs at -1031 and -863 position and haplotypes homozygous for both these mutations, correlated with elevated plasma TNF levels. When these SNPs were validated with *P.falciparum* malaria patients in the case-control study, it was found that -1031C allele strongly correlated with malaria disease severity while a similar trend was observed with the -863A allele.

- Populations inhabiting regions of varying disease intensity differ in the relative measures of cytokines they produce in response to *P.falciparum* malaria infection. Multivariate cluster and step-wise discriminant analysis revealed that patients from both regions show discrete patterns of cytokine profiles suggesting that endemic and non-endemic individuals differ in their clinical immunity to malaria.

- Among the regulatory region polymorphisms analyzed in cytokine encoding genes (*TNF, IL4, IL12* and *IL13*), except two of the *TNF* SNPs (-1031 and -863) none of mutations in any of the genes correlated with altered plasma cytokine levels suggesting their alternative regulatory roles. However,
individual SNP-disease association analysis revealed that homozygous mutants for two polymorphisms, the \textit{IL4} -590 (C/T) and the ins/del polymorphism in \textit{IL12} promoter was associated with protection from \textit{P.falciparum} malaria in endemic and non-endemic region respectively.

- CR1 involved in the pathogenesis of cerebral malaria (at low levels due to rosetting) and severe malarial anemia (at high levels contributing to enhanced phagocytes of iRBCs) varies in RBC surface levels among populations inhabiting areas of differential endemicity. The endemic region controls exhibit very low RBC CR1 levels (<200 molecules/cell) as compared to non-endemic individuals (60-950 molecules/cell). RBC CR1 levels are genetically determined. The mutant genotypes of exon 22 (GG) and intron 27 (TT) SNPs correlate with low RBC CR1 levels either independently or as a diplotype in both endemic and non-endemic regions. Also, the minor allele of exon 22 (A/G) SNP was strongly associated with severe malaria in the non-endemic region.

- RBC CR1 levels associate differentially with disease state in areas of varying endemicity. Low CR1 levels correlate with disease severity in non-endemic region while high CR1 levels were associated with disease manifestation in patients of endemic areas. This differential association of CR1 levels indicates the varying effects of CR1 on disease pathogenesis in areas of high or low disease transmission which may influence the susceptibility of an individual to severe malarial anemia or cerebral malaria.

- Four genetic variants in three adhesion molecule genes exhibit correlation with falciparum malaria. The mutant homozygotes for the \textit{ICAM1} exon 6 (A/G, Gln/Lys) SNP has been identified as a risk factor for severe falciparum malaria in both endemic and non-endemic region. The -53G/T mutation positioned at the downstream promoter of the \textit{CD36} gene correlated with protection from severe malaria. Another \textit{CD36} SNP in exon 1a (T/A) of the gene was found to be associated with disease severity in both endemic and non-endemic region.
The exon 3 (C/G, Leu/Val) PECAM1 SNP correlated differentially with falciparum malaria in regions of varying endemicity. The mutant G allele is risk factor for severe malaria in the endemic region, while an inverse correlation exists in the non-endemic region where the G allele associates with protection to falciparum malaria.

Taken together, our results indicate that endemic and non-endemic populations substantially differ in their relative immune responses and allelic association profiles to *P. falciparum* malaria disease severity and highlight the importance of comparative analysis of association studies in regions of varying endemicity.