Summary and Conclusions
A minority (<5%) of individuals exposed to \textit{M. leprae} is known to develop clinically relevant disease leprosy, while the majority shows protective immunity (Schurr \textit{et al}, 1991). Factors reported to influence the risk of infection include: age, sex, crowding, socioeconomic conditions, urbanization and ethnic background (Naafs, 2001). The risk of developing disease after infection is strongly dependent on any condition that modifies the balance established in the body between the \textit{leprae} bacilli and host immune defenses. Thus, apart from age and sex, the factors that have been shown to influence this balance include, immunosuppressive treatment, malnutrition, and bacille Calmette-Gue`rin vaccination. In addition, there is increasing evidence that genetic factors, in part, determine differences in host susceptibility to the pattern of clinical disease due to \textit{M. leprae} infection (Wagener \textit{et al}, 1988; Abel \textit{et al}, 1988; Feitosa \textit{et al}, 1995). Genome wide linkage screens have identified variants in the common regulatory region of parkin, PARK2 and parkin coregulated, PACRG genes as major risk factors for susceptibility to leprosy per se (Mira \textit{et al}, 2004) and chromosomal region 10p (Siddiqui \textit{et al}, 2001) with susceptibility to paucibacillary leprosy in two ethnically distinct populations. Besides, candidate gene based case-control studies have found several immunogenetic polymorphisms with a moderate effect on the risk of susceptibility to leprosy and its polar clinical forms (Fitness \textit{et al}, 2002). Differential susceptibility to leprosy in all likelihood is determined by several genes and, their differential association in different populations necessitates the need to examine the role of polymorphisms in the candidate genes of innate and adaptive immune system which are continually selected by distinct environmental and natural selection forces.

The present study was designed as a case-control association study of functional polymorphisms in candidate genes involved in innate and adaptive immunity in leprosy in Indian population to investigate whether the variants in these immune regulatory genes, alone or in combination, influence intrinsic susceptibility to leprosy and/or its polar forms. A total of 286 clinically and histopathologically well defined unrelated leprosy patients and 350 unrelated healthy controls were included in the study. The patients and controls were well matched for their ethnicity and belonged to same geographical region. All the controls selected had no history of leprosy, tuberculosis or any other related infectious disease. Besides, the risk of population stratification, which limits the conclusions of case-control association study, was carefully addressed by analyzing our samples with a disease unrelated genomic control marker (whose mean heterozygosity was 48%). The allele and genotype frequency of the genomic control marker did not show any significant difference between leprosy patients and healthy controls.
Primers were designed to amplify the selected regions of candidate genes and the known functional polymorphisms within these genes were genotyped by direct PCR sequencing.

Toll like receptor 2 (TLR2) is a critical mediator of innate immune recognition of microbial pathogens including *M. leprae* and the regulated expression and activation of TLR2 at the site of disease contributes to the host defense against *M. leprae* infection (Krutzik et al, 2003). The significance of mutations in TLR2 in influencing leprosy pathogenesis has been suggested because of association of a nonfunctional variant in the conserved intracellular signaling domain of TLR2 gene with lepromatous form of the disease (Kang et al, 2001; Bochud et al, 2003). Our investigation of TLR2 Arg677Trp polymorphism by an *in-silico* analysis and its experimental validation showed that this variant, associated with lepromatous leprosy (Kang et al, 2001), in all likelihood, could have resulted from the variation present in the duplicated region present approximately 23 kb upstream to TLR2 gene with 93% homology with the authentic exon 3 of TLR2 gene. Due to present preoccupation of many researchers with innate immunity genes, it can be safely assumed that the initial genetic association study (Kang et al, 2001) and the following mutagenesis study (Bochud et al, 2003) had stimulated many genotyping experiments and our study advocates the need to study TLR2 polymorphism with caution in future because of the presence of variations in the duplicated (pseudogene) region representing exon 3 of TLR2 gene. The absence of any variant in the conserved promoter and intracellular signaling regions of the TLR2 gene in our study indicates a need to investigate other regulatory mechanisms, which could control TLR2 function.

PARK2, a ubiquination E3 ligase, probably involved in the delivery of polyubiquinated proteins to the proteasomal complex, has as yet an undefined role in leprosy pathogenesis. The function of another gene, PACRG, in leprosy pathogenesis is also unknown although linked again to the ubiquitin–proteasome system. The variants in the common regulatory region of parkin (PARK2) and parkin coregulated (PACRG) genes have been identified as major risk factors for susceptibility to leprosy per se in two ethnically distinct populations (Brazilian and Vietnamese) (Mira et al, 2004). The LD pattern clearly indicated that an 80-kb block, overlapping the 5’ regulatory regions of the PARK2 and PACRG genes, harbored 9 SNPs which showed significant associations with leprosy in both Brazilian and Vietnamese population (Mira et al, 2004). Among these, PARK2_e01 (-2599) and rs1040079, were the two most significantly associated SNPs. Independently these SNPs conferred relatively lower risk in a dominant manner whereas these SNPs in cis as a haplotype showed significant dominant effect in susceptibility to leprosy per se (Mira et al,
The risk allele T of SNP 28kb target_2_1 showed differential associations with susceptibility to leprosy between Brazilian and Vietnamese population (Mira et al., 2004). It was, however, interesting to find in our study that the susceptibility to leprosy per se is not spread over the whole 80 kb block region in Indian population, instead it is confined to one major risk SNP PARK2_e01 (-2599) which showed significant recessive effect with susceptibility to leprosy, though with a modest effect (OR = 1.42), when compared to the observed dominant effects of the risk haplotype in the previous study (O.R = 5.0) (Mira et al., 2004). There was a significant difference in the prevalence of the risk allele T of PARK2_e01 (-2599) in Indian population and the two populations (Brazilian and Vietnamese). We did not find a significant association with the other risk SNPs or haplotypes with leprosy per se or with different clinical forms of leprosy in the studied Indian population group, suggesting a heterogeneity in association of these SNPs with the susceptibility to leprosy in different populations and the differences in the relative importance of these SNPs as susceptibility loci in Indian population.

IL-10 is as a potent anti-inflammatory and immunosuppressive cytokine, regulating protective immunity in leprosy (Misra et al., 1995; Lima et al., 2000). The analysis of the entire region encompassing -4 to 0.5 kb of IL-10 promoter region, using genotyping data of the six IL-10 promoter SNPs in the present study suggested that the extended haplotype, -3575T, -2849G, -2763C, -1082A, -819C, -592C, was protective and was associated with resistance to leprosy per se and to the development of severe types of leprosy and the haplotype -3575T, -2849G, -2763C, -1082A, -819T, -592A was significantly associated with risk of developing MB leprosy. The frequency of the haplotypes, -3575A, -2849G, -2763C, and -3575T, -2849A, -2763C defined as a marker of resistance and susceptibility to leprosy, respectively in Brazilian population (Moraes et al. 2004) were observed in a very low frequency both in controls and leprosy patients in our population. Nevertheless, the involvement of different promoter haplotypes of IL-10 in two ethnically distinct populations suggests the important role of IL-10 in early and late phases of immunity to *M. leprae* and underscores the need to study the interaction of IL-10 with other genes involved in controlling innate and acquired immunity to leprosy.

We did not find significant interaction between IL-10 (-819C>A) and PARK2_e01 (-2599) T>C polymorphisms. However, the individuals with IL-10 -819T/T and PARK2_e01 (-2599)T/T genotype showed significantly increased frequency among patients with leprosy than among controls. When the effect of one of the polymorphisms was adjusted for the
effect of the other, each of them remained associated with leprosy suggesting that the two polymorphisms have independent effects.

A protective immune response to *M. lepra* infection is mainly mediated by the balanced activity of cell mediated immune response. The altered profile is always likely to shift the protective immunity to detrimental response by shifting Th1-Th2 response. Since this shift mainly depends on the altered levels of IFN-γ, IL-4, TNF-α, TGF-β and IL-10 secreted by T lymphocytes in response to antigenic stimuli, an attempt was made in the present study to determine the genotype status of regulatory polymorphisms in these genes to correlate with susceptibility to leprosy. The interindividual variability in production of cytokines is partly determined by genetic background and for most of the cytokines this heritability lies between 35-50%. However, in this study, the results for IFN-gamma -288A>T promoter polymorphism and IL-4 5’UTR -33C>T polymorphism did not show any significant association with disease susceptibility.

The investigation of TNF-alpha -418G>A and -488G>A polymorphism showed for the first time a possible role of TNF-α-418 G/A allele in susceptibility/resistance to leprosy. The TNF-α -488,-418 G-G haplotype was associated with susceptibility whereas the TNF-α -488,-418 A-G and TNF-α -488,-418 G-A haplotypes were associated with resistance to leprosy in our study. The effect of -418A allele was more pronounced in providing resistance to leprosy per se whereas the -488 A allele was significantly associated with providing resistance to MB but not PB leprosy. Since TNF-α gene lies in MHC locus and is in tight linkage disequilibrium with MHC class I and class II molecules, identifying the true functional effect of -418 polymorphism becomes difficult and necessitates the investigation of involvement of DR2 (class II) and DQ1(class I) molecules and the extended haplotype associated with disease susceptibility.

The investigation of the role of functional polymorphisms in the promoter and signal peptide region of TGF-β1 showed for the first time a suggestive evidence of TGF-β1 promoter polymorphism in susceptibility to leprosy type. The association of TGF-β1 -1349 T allele with susceptibility to multibacillary form of the disease and not with paucibacillary leprosy suggests that this polymorphic allele could be one of the genetic markers in development of disseminated disease. We did not find any significant interaction between TGF-β1 -1349 C>T and TNF-α -418G>A polymorphisms.

An improved understanding of the pathogenesis of leprosy and effective treatment for it are significantly influenced by our ability to untie the effects of host genetic factors in leprosy. On the basis of our study, it may be concluded that susceptibility to leprosy is critically
influenced by IL-10 promoter polymorphisms. The non involvement of the major risk alleles in regulatory region of PARK2 and PACRG with leprosy susceptibility in Indian population and population specific allele frequencies of SLC11A1, IL-12p40, IL-10, PARK2 and PACRG gene SNPs suggests that the Indian population has its own spectrum of variations in genes which confer risk or protection to leprosy and necessitates the need to investigate genetic predisposition to leprosy at the genome wide level to dissect common and population specific predisposing gene(s) associated with leprosy susceptibility.