Introduction
Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, an obligate intracellular pathogen that principally infects macrophages and Schwann cells. The damage to skin and peripheral nerves results in the characteristic deformities and disabilities that contribute to the intense social stigma and discrimination of patients and their families. Leprosy is thought of as a disease of the past and it is true that there is impressive decline in the prevalence of leprosy worldwide. However, the incidence of leprosy has remained unchanged over the past 15 years, with approximately 700,000 new cases per year (WHO leprosy elimination project-status report 2003). Leprosy is still a major health problem in many developing countries including India, which carries the majority of the global leprosy burden. Although better case detection and multidrug therapy (MDT) has improved leprosy control, it appears that the use of MDT has had limited impact on the transmission cycle of *M. leprae* (Lockwood, 2002).

The complete sequencing of the *M. leprae* genome, an important landmark in the field, has paved way for understanding the *M. leprae* biology with a more holistic approach. Comparative genomics of *M. tuberculosis* and *M. leprae* have shown a strong gene decay in *M. leprae*, resulting in the loss of entire metabolic pathways present in *M. tuberculosis*. At 3.27 Mb, the genome of *M. leprae* is substantially smaller than that of *M. tuberculosis* and a mere 49.5% is occupied by the 1,605 protein-coding genes (Cole *et al*, 2001). This probably renders the leprosy bacillus dependent on host metabolic products and might explain its long generation time and inability to grow in culture. *M. leprae* has probably lost 2,000 genes during its evolution and the minimal gene set required by a pathogenic mycobacterium appears to have been defined naturally. The comparative genomic studies are being intensely pursued to identify novel specific drug targets for effective therapeutic intervention and new subunit vaccine candidates with higher efficacy. The molecular epidemiological studies using the *M. leprae* genome information have shown that different *M. leprae* isolates exhibit very limited genetic diversity (Monot *et al*, 2005). Though comparative genomics have provided better insights, the inability to culture *M. leprae* in *vitro* and the lack of a suitable animal model have hampered leprosy research such that the direct experimental evidences for understanding of interplay of host-*M. leprae* interaction and mechanisms of *M. leprae* survival within macrophages are still limited. The study of the disease pathology therefore has been investigated through an understanding of the host immune response to *M. leprae*.

Leprosy is a spectral disease in which pathology and immunology are inextricably related and it provides a unique critical model for investigating immunoregulatory mechanisms in
humans. Patients with tuberculoid leprosy have a few localized lesions with rare organisms and a strong cell-mediated immune response directed against Mycobacterium leprae antigens that ultimately kills and clears the bacilli, although often with concomitant injury to nerves. In contrast, lepromatous leprosy patients have numerous skin lesions containing extraordinarily high numbers of acid-fast bacilli and show specific immunological unresponsiveness to antigens of *M. leprae* in vivo and in vitro (Ridley and Jopling, 1966). The phenotyping of lymphocytes in leprosy lesions (Modlin et al, 1988, Yamamura et al, 1991; Salgame et al, 1991; Seiling et al, 1999; Sieling et al, 2000; Gansert et al, 2003; Sieling et al, 2005), selective mechanisms of accumulation of lymphocytes, their antigen specificity, and immunological functions of different lymphocyte subsets have shown striking dichotomy with Th1 type response in tuberculoid patients and Th2 type profile in lepromatous cases. The circulating lymphocyte responses to *M. leprae* antigens, however, do not show such distinct polarization across the spectrum, reflecting differential activation status of patients across the spectrum to chronic *M. leprae* infection (Howe et al, 1995; Misra et al, 1997). Recently, the gene expression profiles of tuberculoid and lepromatous cutaneous lesions were found to be consistent with the bias in type 1 and type 2 cytokine production according to leprosy subtype (Bleharski et al, 2003). However, the fundamental questions regarding the specific unresponsiveness of lepromatous patients to *M. leprae* antigens and the strategies employed by the pathogen to subvert immune response in immunocompromised leprosy patients still remain unanswered.

As there is no relevant animal model for human leprosy, forward genetics is the main method used to identify the genes, and consequently the immunological pathways, involved in the human response to *M. leprae* (Alcais et al, 2004) (Fig.1). Firstly, candidate gene studies can be carried out on genes of known functions that have a possible biological role in the control of infection or disease. A second approach utilizes a non-targeted genome wide linkage analysis, in which increased sharing of chromosomal regions by affected individuals leads to identification of positional candidates. There is substantial evidence from epidemiological, segregation and twin studies that host genetic factors contribute to susceptibility to leprosy (Haile et al, 1985; Wagener et al, 1988; Abel et al, 1988; Feitosa et al, 1995). Several genes that may modulate cell-mediated immunity have been investigated and some appear to have a role in either susceptibility to leprosy per se, or to leprosy type (Fitness et al, 2002). Genome wide scans for leprosy susceptibility loci have identified regions on chromosome 6q (Mira et al 2003; Mira et al 2004), 10p (Siddiqui et al. 2001) and 20p (Tosh et al, 2002), which may harbor genes affecting susceptibility to leprosy per
Fig. 1 The forward genetic approach for dissecting the complex basis of predisposition to leprosy.

Source: Alcais et al, Curr. Opin. Immunol, 2004
se or to paucibacillary (PB) leprosy in particular. The susceptibility to leprosy and to different clinical phenotypes of the disease suggests that the nature of leprosy pathogenesis is apparently under a two stage genetic control whereby some genes affect intrinsic susceptibility to leprosy (i.e. advancement from innocuous infection or carrier status to clinically evident disease) and other loci modify the clinical form of the disease (Mira et al, 2003). The variants in the common regulatory regions of parkin (PARK2) and parkin coregulated gene (PACRG) have been identified as major determinants in controlling susceptibility to leprosy per se in two ethnically distinct populations (Brazilian and Vietnamese). Another gene Nramp1 (natural resistance associated macrophage protein—currently designated as SLC11A1) is also implicated in controlling susceptibility to leprosy per se in the Vietnamese families (Miller et al, 2004). It is interesting, however, that the similar method of linkage analysis has identified different regions in susceptibility to leprosy. The role of HLA linked loci in controlling leprosy type is consistently supported by several studies (Mira et al, 2003; Fitness et al, 2002). Besides, the role of chromosome 10p region in determining susceptibility to PB leprosy has also been shown in two ethnically distinct populations (Siddiqui et al. 2001; Mira et al 2003). Although it is encouraging that the regions of linkage reaching genome wide statistical significance have been identified for the multifactorial disease leprosy, however, the linkage studies and many of the association studies have not been replicated in different populations either due to underpowered nature of study design or due to genetic heterogeneity in susceptibility to leprosy between populations (Fitness et al, 2002; Wallace et al, 2004). Further genome wide linkage studies and association studies in different populations are required to examine the extent of genetic heterogeneity that exists for leprosy susceptibility.

India carries the majority of the global leprosy burden but there are very few reports that have investigated the role of non-HLA genes in association with leprosy susceptibility. The candidate genes known to play an important role in leprosy pathogenesis at innate and adaptive level were selected in the present study. The innate immune system has dual roles in host defense, providing a direct and immediate response against microbial invaders and an instructive role, influencing the nature of adaptive immunity (Fig.2). This instructive role of the innate immune system is served in part by antigen presenting cells (macrophages, dendritic cells) which, upon encounter with microbial inflammatory stimuli recognize the pattern associated molecular patterns (PAMPs) through their pathogen recognition receptors (PRRs) and release cytokines that can direct the adaptive T cell response toward either a Th1 or Th2 pattern. The varying immunological responses to M.leprae determine the
Fig. 2 The role of innate immune system in a) direct recognition and killing of pathogen and b) instructing adaptive immune response.
clinical manifestations of the infection and the outcome in the host. Nowhere is this more
evident than in leprosy, where a clear dichotomy exists between the T cell cytokine pattern
and the clinical form of the disease. The development of the T cell cytokine patterns is
known to be influenced by the balance between pro and anti-inflammatory monocyte-
derived cytokines (Sieling et al., 1994; Modlin, 1994; Libraty et al., 1997; Bleharski et al.,
2003). Among the factors that skew the development of innate and adaptive immune
response towards Th1 or Th2 type, the functional polymorphisms in the pro and anti-
inflammatory cytokine genes and genes known to play an important role in recognition of
*M. leprae*, might play an important role. We carried out a case control association study of
functional polymorphisms in candidate genes involved in innate (TLR2, SLC11A1, PARK2
and PACRG, IL-12p40, IL-10) and adaptive (TNF-α, IL-4, IFN-γ, TGF-β1) immunity to
leprosy with a twin objective of genetically dissecting the basic elements of interplay
between *M. leprae* and its human host and to examine the extent of genetic heterogeneity in
susceptibility to leprosy. The main problem of low power and cryptic population
stratification, which limits the conclusions of the case-control studies, was carefully
addressed by selecting an adequate sample size of 286 patients and 350 controls and
analyzing our samples with genomic controls, respectively. The rationale of our study was
consistent with the proposed hypothesis of a two-step nature of leprosy pathogenesis
(Fig.3).
Schwann cells

Some genetic loci affecting intrinsic susceptibility to infection per se.

Other genetic factors modifying the clinical form of the disease

Primary Targets of M. leprae

Macrophages

infection

no infection

MHC class II downregulation

Immuno-suppression

Pro-Inflammatory cytokines

MHC class II

Microbiocidal Tissues damage

Cellular Immunity

DTH

NO and respiratory burst

MB

PB

Fig 3. M Leprae infection and the two stage genetic control of susceptibility to infection.
With this background, the present work entitled “Genetic Predisposition to Leprosy: A study of functional polymorphisms in candidate genes involved in innate and adaptive immunity in leprosy” was undertaken laying down the following objectives.

I. To identify the susceptibility/resistance alleles of candidate genes involved in innate and adaptive immune response to *M. leprae* infection and in controlling leprosy pathogenesis in a case-control association study of clinically defined leprosy patients and unrelated healthy controls.

II. To measure the level of significance and the strength of associations of individual single nucleotide polymorphisms, haplotypes or interacting loci among the selected candidate genes, with susceptibility/resistance to leprosy.

III. To examine the extent of genetic heterogeneity associated with leprosy susceptibility of the candidate gene polymorphisms between Indian population and other populations studied.