Interaction of the cytokine gene variants (gene-gene interaction): Influence on susceptibility to tuberculosis
8. Introduction

Gene-gene interaction is an alternate new approach to study the genetic susceptibility to tuberculosis. In a multifactorial disease such as tuberculosis it is evident that gene-gene interactions of various relevant genes will have a larger role to play as compared to influence of a single polymorphism. These interactions can either have an enhancer or suppressor effect on the expression of one another. In order to assess the possibility of gene-gene interaction among cytokine genes studied in chapter 5 we analysed the SNPs to explore the possibility of gene-gene interaction among these cytokine genetic variants in order to identify some additional variants of importance which may have an interacting effect but were not evident in a single locus analysis.

When we think about factors that cause disease, we often think about specific mutations in individual genes or the environmental factors that contribute to a disease phenotype. Yet, disease-causing mutations may not cause disease in all individuals. One possible important reason for this is that the effect of a mutation can depend upon other genetic variants in a genome. These epistatic interactions between mutations occur both within and between molecules, and studies in model organisms show that they are extremely prevalent. However, epistatic interactions are still poorly understood at the molecular level, and consequently difficult to predict de novo. A more complete understanding of epistasis will be vital for making accurate predictions about the phenotypes of individuals. Gene – gene interaction also known as epistasis was defined by Bateson et al., (1905) to describe the suppression of an allelic phenotype by an allele at another allele at another locus. Epistasis results from the way in which genetic elements interact with each other in CAUSATION of a phenotype. Epistatic interactions can be both alleviating (i.e. suppressive, with a better than expected outcome, alternatively referred to as positive or antagonistic epistasis) or aggravating (i.e. increasing severity, with a worse than expected outcome: negative or synergistic epistasis). Interactions can also occur between sequence variants in the same gene (‘intramolecular epistasis’) and between variants in different genes (‘intermolecular epistasis’) (Lehner, 2011). In fact, understanding epistatic interactions may be the key to understanding complex diseases, such as Alzheimer’s disease, diabetes, cardiovascular disease, tuberculosis and cancer.

Due to multifactorial nature of tuberculosis and also complex nature of interaction of the immune system, the role of gene-gene interaction influencing the outcome of disease is increasingly supported by many workers (Williams et al., 2000; Ritchie et al., 2001; Tsai et al., 2003).
Epistatic gene-gene interactions are perhaps more common than we think. Indeed, some scientists believe that epistasis is ubiquitous in biology and has been ignored for too long in studies of complex traits (Moore, 2003; Carlborg & Haley, 2004). Research has shown that genes don’t function alone; rather, they constantly interact with one another. These biological interactions are critical for gene regulation, signal transduction, biochemical networks, and numerous other physiological and developmental pathways (Moore, 2003; Greenspan, 2001).

This old concept has found a major renaissance lately and the major diseases addressed have been the non-infectious diseases such as asthma (Kim et al., 2006; Lee et al., 2004), Parkinson’s disease (Pankratz et al., 2003; Deng et al., 2004), diabetes (Koeleman et al., 2004; Bergholdt et al., 2005) and rheumatoid arthritis (Martinez et al., 2006).

Very little literature exists regarding these interactions in infectious disease such as TB, until recently (Wit et al., 2011; Velvez et al., 2009). This study would thus contribute to the importance of such studies.

We applied semi-exhaustive testing for pairwise interaction using PLINK v 4.0. The -fast epistasis along with -case-only option was used for this purpose. This has been hailed as a powerful approach by some workers. It is also considered advantageous over the multi-dimensional scaling method (Gao and Stamer, 2007; Gao and Martin, 2009). It provides a logistic regression test for interaction. This analysis exploits the fact that under certain conditions an interaction term in logistic regression equation corresponds to dependency or correlation between relative predictor variables within the population of cases. It uses an allelic model for both main effects and interactions and genotypes are not correlated.

### 8.1 Results

A significantly 13 interactions were identified and are enlisted below in table 8.1.
8.3 Discussion

The present analysis could identify as high as 13 interactions among SNPs of the cytokine genes. We identified some SNPs which did not show any association in the single locus analysis. \textit{IL1RA} emerged as the gene having a significant interaction with \textit{IL12}, \textit{IL4} and \textit{TNFB} genetic variants. Most of the \textit{IL1RA} interactions were protective with odds ratio <1. Only one interaction between its own SNP (intra-molecular epistasis) was showing an eight fold risk (p = 3.066E-05). The presence of this interaction (individual polymorphic for both SNPs) in an individual could be important in defining his/her genetic susceptibility to tuberculosis.

The other important player was \textit{IL4} which showed interaction with variants of \textit{IL12}, \textit{IL1RA} and \textit{TNFB}. Interestingly, all the interactions of \textit{IL4}, an anti-inflammatory cytokine with other proinflammatory cytokines such as \textit{IL12} and \textit{TNFB} were showing a very high risk (18 –fold risk) with highly significant p-values.

These genetic interactions enabled us to test the hypothesis that the disease outcome in tuberculosis can be due to interaction of the cytokine genes polymorphisms. The risk indicated by these interactions was far more pronounced than reported in chapter 5 (Most of the risk ratios obtained were 3 fold but here it is 18 fold). The 13 observed interactions strengthen the fact that epistasis has a clear role in genetic susceptibility to tuberculosis. Also, many of the loci identified here were not significant in
The cytokine genes have been known to modulate each other’s levels and thereby influence the pathogenesis of disease. IL-1, a proinflammatory cytokine with two main forms, IL-1\(\alpha\) and IL-1\(\beta\), is produced by several cell types (Li et al., 1996). IL-1 receptor antagonist (IL-1ra) is a naturally occurring inhibitor which competes with IL-1 for occupancy of IL-1 cell surface receptors. IL-1 is an important mediator of inflammation and tissue damage in multiple organs, both in experimental animal models of disease and in human diseases. The balance between IL-1 and IL-1Ra in local tissues plays an important role in the susceptibility to and severity of many diseases. IL-1 plays an important role in host resistance against microorganisms that divide inside cells, such as mycobacteria or listeria (Arend, 2002).

A report has indicated that IL-4 modulates the IL-1Ra levels in-vitro by downregulating its levels (Perrier et al., 2002). It should also be kept in mind that IL-4 and IL-12 are the players under whose influence distinctive Th1 and Th2 lineages develop (Abbas et al., 1996). Also, it has been reported that IL-1\(\beta\) helps in proliferation of the mycobacteria by leading to macrophage deactivation. IL-1Ra being the receptor antagonist of IL-1\(\beta\) would compete with the later and bind to the receptor thereby reducing the IL-1\(\beta\) level and improving the immunity to tuberculosis.

It is therefore not surprising that an interaction between \(IL1RA\) and pro-inflammatory cytokine gens \(IL12\) and \(TNFB\) improves chances of survival and show significant protective association for this population.

IL-4, an anti-inflammatory cytokine has been implicated to down-regulate IFN-\(\gamma\), and thus have a deleterious effect on TB patients (Powrie and Coffman, 1993; Lucey et al., 1996). It also promotes the induction of Th2 cells (Abbas et al., 1996). In the interaction of the anti and proinflammatory axis as in case of \(IL4\) and \(IL12\) and \(TNFB\) could result in down regulation of protective immune response and hence these interactions pose a serious risk in this population.

The results obtained here are very encouraging. We have accounted for population stratification in our samples thereby eliminating any chances of confounding associations due to population stratification, a serious limitation in genetic studies. A multiple correction testing ought to be applied in our analyses but we had about 300 interactions tested in these results and applying a bonferroni correction would be too conservative. But the \(p\) values obtained are in the range of \(10^{-6}\) and \(10^{-8}\) which is very significant and convincing. Taken together, the results presented here are the first on interaction of cytokine SNP
and tuberculosis. Although cytokine gene interactions have been implicated in periodontitis (Marullo et al., 2011) but not many reports are available in tuberculosis. Also, it is the first report on an Indian population reporting on gene-gene interaction in tuberculosis.

Having discussed all the probabilities, to understand the effect of these interactions better we ought to consider the importance of these cytokines and their network in tuberculosis. It is possible that these genes and their products in tuberculosis constantly interact to govern the outcome of the disease. The benefit of such studies could be multiple. The information generated would help us to identify the genetic markers to screen people of being “resistant” or “prone” to the disease. Identifying the alleles that impart a risk of developing the disease in the population under study and the identified markers could help in providing appropriate and targeted therapy to the prone individuals (Bose, 2012). Further, identification of the gene-gene interaction could add to the repertoire of important connotations that can be used for targeted therapy or personalised medicine.