Genetic Variations in Host Innate and Adaptive Immune Response in Tuberculosis: A Search for Risk Loci in North Indians

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Abstract

Infectious pathogens have long been recognized as potentially powerful agents impacting on the evolution of human genetic diversity. Analysis of case – control studies provides one of the most direct means of identifying human genetic variants that currently impact on susceptibility to particular infectious diseases. For over 50 years candidate gene studies have been used to identify loci for many major causes of human infectious mortality, including malaria, tuberculosis, human immunodeficiency virus/acquired immunodeficiency syndrome, bacterial pneumonia and hepatitis. There are several approaches to study the disease susceptibility with all the methods having their pros and cons. The goal of these studies is a better understanding of disease pathogenesis and resistance in the expectation that this will lead, in time, to improved interventions such as better drugs or vaccines to prevent or attenuate the great global burden of infectious disease morbidity and mortality.

A second application that is gaining increasing attention is the potential to stratify populations for risk of infectious disease based on genetic profiling. This has not been a priority until now as most preventive interventions such as childhood vaccines have been aimed at universal coverage. However, as more potentially useful vaccines are licensed and the costs of new vaccines escalate, targeted use is becoming a consideration.

The current thesis was designed to address the role and importance of genetic variations in susceptibility to tuberculosis in north Indians. The current times have seen a multitude of studies emerging on the subject from various parts of the world (Moller and Hoal., 2010). Even so, the current population was relatively underrepresented in such analysis. We have used a population based case-control approach for our study, which involves comparison of the allele prevalence in diseased versus non-related, non-diseased individuals.

This study contributes significantly to the TB host genetics field, since several candidate genes, never investigated before, were tested as susceptibility factors. In addition, we considered susceptibility genes previously identified in other populations. This is important as ethnic validation of commonly reported variants in different populations is desirable. In total, 112 polymorphisms in 25 genes (Table S1) were selected from both the innate and adaptive immune branches operating in tuberculosis, were genotyped in the north Indian population during this study. Allele frequencies were compared and linkage disequilibrium (LD) and haplotypes investigated. We also estimated the serum cytokine levels in tuberculosis to assess the profile in north Indians, and to identify serum biomarkers for this population. The serum cytokine levels were also correlated with the corresponding genotypes to search for possible correlation and demonstrate in the present synthesis that the serum cytokine levels vary with the respective genotypes for certain genetic variants.

We also, considered lymph node tuberculosis, an extra-pulmonary form of TB for analysis and could successfully identify the varying genetic and immune profile of this form from pulmonary tuberculosis, extending the idea that different clinical manifestations in tuberculosis can result due to variance in the genetic makeup of an individual in these immune response genes. Specifically, we identified a risk for rs1427294 of *SP110* polymorphism for LNTB but not pulmonary TB. This gene is important to control apoptosis of infected macrophages and any alteration of the function can have significant impact on

TB. Similarly a variant of the P_2X_7 gene showed higher risk for LNTB, which is also incidentally related to apoptosis, leading us to speculate that the apoptotic axis may be important in LNTB. As is recognized, after infection with *M. tuberculosis*, to generate an effective immune response the bacilli are carried to the draining lymph nodes for antigen presentation. During this time the apoptotic axis might play a crucial role. If the infected macrophage undergoes apoptosis, it would lead to better antigen presentation and any variations in the genes such as P_2X_7 and *SP110* regulating this axis affecting the normal functioning could lead to establishment of infection at these sites by the thriving bacilli.

The other genetic variants of importance were from the *CD209* gene and *LTA4H* gene which are important in innate immune response in tuberculosis. So, it emerges from the current study that variations in the innate immune variants have a closer relation to development of LNTB. Surprisingly, the cytokine genetic variants had no apparent effect on susceptibility to tuberculosis.

The cytokine axis and genetic variants in the cytokine genes were significant risk factors pulmonary tuberculosis as indicated in Table S1. We also show that there exists a significant gene – gene interaction among cytokine SNPs that may further accentuate the importance of the identified SNPs in governing the genetic susceptibility to tuberculosis in north Indians.

Some of the earlier investigated variants in other world populations were not replicated in the present study (Chapter 4). This could be due to i) the difference in ethnicity as it governs the outcome of tuberculosis susceptibility in tuberculosis (Stead et al., 1990), ii) some of the previously published associations considered during our study had extremely low sample numbers and were therefore underpowered.

Also, the human immune system is a complex network of interacting pathways which protect against infection. Some of these pathways are redundant and when a defect is presented they may compensate for each other. Within pathways, components may also be redundant. For this reason it is difficult to detect the effect of a single gene polymorphism, which could be one of the explanations as to why we did not find statistical associations with certain SNPs. A better idea would be to consider all the genes in a certain mechanistic pathway known to be involved in TB, which may mean that single SNP and candidate gene analysis might become of less importance individually, and require consolidation. But again, the analysis might become too complex to interpret.

Overall the work presented in this thesis contributes many markers, genetic and serological, towards understanding of the complex interaction behind the immunity in tuberculosis. The north Indian population was scanned thoroughly by a wide choice of markers. These markers identified in this thesis would aid in further investigations in a larger population in strengthening the role of genetic markers in tuberculosis.