Host genetics and tuberculosis susceptibility

P. Selvaraj

Department of Immunology, Tuberculosis Research Centre, Indian Council of Medical Research, Mayor V. R. Ramanathan Road, Chennai 600 031, India

Susceptibility to tuberculosis is multifactorial. The importance of host genetic factors on the susceptibility or resistance to tuberculosis has been emphasized by many workers. Host genetic factors such as human leucocyte antigens (HLA) and non-HLA genes that are associated with the susceptibility to tuberculosis will serve as genetic markers to predispose or predetermine the development of the disease. Such markers may be useful to understand the immune mechanism of susceptibility or resistance to tuberculosis. Association of various HLA and non-HLA genes with susceptibility to tuberculosis in various ethnic population has been established. HLA studies carried out in the Asian region, especially in India, revealed the association of HLA-DR2 and -DQ1 antigens with the susceptibility to pulmonary TB. Further, studies on DNA typing explored the association of DRB1 *1501 and *1502 (DR2 subtypes) in north Indian and DRB1 *1501, DRB1 *0601 (DQ1 subtype) and DPB1 *02 (DP2 subtype) in south Indian population. Various studies on non-classical major histocompatibility complex (MHC) genes and non-MHC/non-HLA gene polymorphisms such as transporter associated with antigen processing (TAP), tumour necrosis factor *a* and *b* (TNF *a* and *b*), mannose binding lectin (MBL), vitamin D receptor (VDR) (*Bsm*I, *Apa*I, *Taq*I and *Fok*I polymorphisms), Interleukin-1 receptor antagonist (IL-1RA) and natural resistance associated macrophage protein-1 (NRAMP-1) genes revealed the association of TAP2 gene variant along with HLA–DR2 and functional mutant homozygotes (FMHs) of MBL with the susceptibility to pulmonary TB. The polymorphic *Bsm*I, *Apa*I, *Taq*I and *Fok*I gene variants of VDR showed differential susceptibility and resistance with male and female subjects. These studies suggest that multicandidate genes are associated with the susceptibility to pulmonary tuberculosis in India.

Host genetics and susceptibility to disease

Host genetic factors explain, at least in part why some people resist infection more successfully than others. Rare gene disruptions cause fatal vulnerability to certain pathogens, but more subtle differences are common and arise from minor variations in many genes. To predict how much our genetic make up determines the different ways in which we respond to some infectious agents is a difficult task. This is especially difficult because of the many other contributory factors such as previous health status, acquired immunity and variability in the pathogen.

Analysis of the genetic basis of susceptibility to major infectious diseases is potentially a most complex area. Many immunogenetic loci influence susceptibility to several infectious pathogens. A genetic basis for interindividual variation in susceptibility to human infectious diseases has been indicated by twin, adoptee, pedigree and candidate gene studies[1].

HLA and non-HLA and disease association hypotheses

Several hypotheses were put forward to explain the mechanisms of major histocompatibility complex MHC and non-MHC gene association with the diseases. HLA-A, -B, -C (class-I) and -DR, -DQ and -DP (class-II) antigens could act directly as disease susceptibility agents. For this, three possible mechanisms have been suggested: (a) There could be antigenic cross-reactivity or mimicry between infectious organisms and a given HLA antigen. This phenomenon is termed as 'molecular mimicry'. Serological cross-reaction between HLA-B27 antigen and the bacterial strains *Klebsiella* and *Shigella* has been identified[2,3]. This means that common antigenic determinants are shared by HLA-B27 and the bacteria; (b) HLA antigens could act as receptors for microorganism; and (c) HLA antigens could influence particular immune responses, acting as immune response (*Ir*) genes. It has been shown that *Ir* genes regulate the immune response to any antigen or pathogen[4].

Immune response gene effects

Genetically controlled differences exist in the magnitude of immune responses. The genes, which are responsible for this variation, were called as immune response (*Ir*) genes initially, till it became clear that *Ir* genes were, in most cases, one and the same as *MHC* genes. Several HLA-linked examples of diseases are available and this provides a attractive mechanism to account for disease susceptibility. The three major mechanisms involved in *Ir* gene effects are:

Determinant selection

The individual MHC molecule selects the determinant of an antigen that is displayed to T-cells restricted by that MHC molecule.

*For correspondence. (e-mail: psraj21@hotmail.com)

Holes in the T-cell repertoire

Gaps or holes may be created in the exported repertoire of T-cells that manifest as a failure to recognize some intrinsic antigens.

T-cell mediated suppression

Active regulation of potentially reactive cells takes place by a population of cells whose function is to suppress an immune response[5].

The MHC genes may be physically close to the chromosome region that carries a gene conferring susceptibility or resistance to a particular disease. This hypothesis may explain the lack of complete association and geographical variation in the association, due to linkage disequilibrium.

Though classical genetic studies in humans and experimental models have clearly documented the primary contribution of the MHC genes, these genes themselves appear to be insufficient in conferring susceptibility or resistance to disease and suggest the association of non-HLA genes.

Host genetic factors and tuberculosis susceptibility/resistance

Mycobacterium tuberculosis is the causative pathogen for tuberculosis. Though environmental and socio-economic factors are primarily related, numerous studies have emphasized the importance of host resistance and hereditary susceptibility. It is estimated that one-third of the world's population is infected with *M. tuberculosis*. Among the infected only around 10% will ever develop clinical disease[6]. This raises the question 'What is different about those who succumb to tuberculosis?'. In 1926, accidental administration of live *M. tuberculosis* (in place of BCG) to babies in Lubeck, Germany left some babies unaffected whereas it led to severe disease and death in others[7]. This indicates that the majority of the population have effective innate resistance to tuberculosis.

Twin studies have supported a substantial role for host genetics in variable susceptibility to tuberculosis. These studies have compared the disease status among identical and non-identical twins, with the expectation that disease with genetically determined component. These twin studies have found higher concordance for tuberculosis among monozygotic twins compared to dizygotic twins[8,9].

The association of host genetic factors (HLA and non-HLA) with the susceptibility or resistance to tuberculosis has been studied using various methods such as case-control studies, candidate gene approach, family-based, genome-wide linkage studies.

Identifying HLA and non-HLA genes/gene products (antigens) which are associated with susceptibility or resistance to tuberculosis will serve to provide HLA genetic markers to predict the development or predispose tuberculosis. The protective association of HLA types will be useful for the development of new epitope-based vaccine. Studying the role of these markers in the immune mechanism underlying susceptibility or resistance to tuberculosis will be useful to understand the immunopathogenesis of the disease. Moreover, these studies may be useful for better management and control of the disease.

HLA studies in tuberculosis

Racial differences in susceptibility to tuberculosis are well known. Several studies revealed the association of various HLA antigens with the disease susceptibility in different ethnic populations[10–16]. For this type of geographic variation, possible explanations have been put forward. It seems likely that evolutionary selection pressures have given rise to frequent polymorphisms in genes involved in resisting infectious pathogens and contributed to marked allele frequency differences at the same loci. When geographic variation in pathogen polymorphism is superimposed on host genetic heterogeneity, considerable variation may occur in detectable allelic association. Gene–environmental interactions are likely to introduce another layer of complexity. The genes involved in defense against infectious pathogens evolve more rapidly than others and excessive polymorphism in the human genome may result from selection pressures exerted by infectious diseases. Similarly, the causative organism *M. tuberculosis* also has genetic variation. During evolution, all these polymorphic forms might have evolved due to the host–parasite interaction[17].

Studies in non-Asian countries

A large number of HLA association studies have been carried out in non-Asian countries. One of the first reports of an association between HLA and tuberculosis showed an increased frequency of HLA-B8 in Canada[10]. Other studies showed an increased frequency of HLA-B5, -B15 and -DR5 in the North American blacks[11,12], HLA-A2 and -B5 in the Egyptian population[14] and -B27 in the Greek population[15]. A negative association has been reported for -DR6 in American blacks[13].

Studies in Asian populations

Several studies of HLA association with pulmonary tuberculosis have been carried out in Chinese[16], Indonesian[18] and Russian patients[19]. A significantly increased frequency of HLA-DR2 was seen in the major studies which have revealed HLA-DR2 association with higher susceptibility

to tuberculosis. In a small study of tuberculosis in Vietnam, a susceptibility association with the rare HLA-DQB1 *0503 allele was reported[20]. Another study carried out in Thais revealed the association of HLA-DQB1 *0502 (ref. 21).

Of the numerous Indian studies on HLA association with pulmonary tuberculosis, an increased frequency of HLA-DR2 and –DQ1 was shown to be associated with the susceptibility to pulmonary tuberculosis[22–24]. Molecular study has revealed that the allele DRB1 *1501 of HLA-DR2 was higher compared with DRB1 *1502 in north Indian patients[25]. Studies carried out in south Indian patients revealed that, HLA-DRB1 *1501, (refs 26, 27) HLA-DQB1 *0601 (a subtype of HLA-DQ1) and -DPB1 *02 were found to be positively associated with susceptibility to pulmonary tuberculosis while a negative association (preventive fractions associated with resistance) has also been identified (DRB1 *11(5), DRB1 *10, DQB1 *0501 and DRB1 *08). Haplotype analysis also supports the DRB1 *1501 -DQB1 *0601 association with susceptibility to pulmonary tuberculosis[26] (Table 1). Though HLA-DR2, DQ1 and their subtypes are significantly associated with the susceptibility to tuberculosis, they may not be the sole genetic markers to predispose tuberculosis (relative risk is around 2.5). This suggested to look for the association of various non-HLA gene polymorphic variants. Association of multi-candidate genes (HLA and non-HLA) has been suggested for various infectious diseases[17].

Non-HLA studies in tuberculosis

In north Indian pulmonary tuberculosis patients, compared with control subjects, the 'Transporter' associated with antigen processing gene 2 (TAP2) has been shown to be associated with the susceptibility to pulmonary tuberculosis along with HLA-DR2 (ref. 28). Definite association between tuberculosis and the haptoglobin 2–2 phenotype has been shown in Russian patients[29]. No such association is observed in Indonesians[30] and Indians[31].

Genome-wide linkage studies on sib-pairs of families affected with tuberculosis enable the identification of several candidate genes that are associated with the susceptibility to tuberculosis[32]. Some of the non-HLA candidate genes are discussed below.

Table 1. Association of important candidate gene variants of HLA and non-HLA genes with the susceptibility or resistance to pulmonary tuberculosis in Indian population

Candidate genes	Effect	Reference
HLA		
HLA-DR2	Susceptibility	22, 23, 24
Sub-type		
– DRB1 *1501, *1502	Susceptibility	25
– DRB1 *1501	Susceptibility	26, 27
HLA-DQ1	Susceptibility	24, 26
– DQB1 *0601	Susceptibility	26
HLA-DP		
– DPB1 *02	Susceptibility	26
Haplotype:		
DRB1 and 1501-DQB1 *0601	Susceptibility	26
DRB1 *11(5), DRB1 *10, DQB1 *0501	Resistance	26
Non-classical HLA		
Transporter Associated with Antigen Processing (TAP) gene TAP2 and DR2	Susceptibility	28
Non-HLA		
Functional Mutant Homozygotes of Mannose Binding Lectin (MBL) gene (codon 52, 54 and 57)	Susceptibility	36
– Heterozygotes of MBL codon 57	Resistance to bacteriological relapse	36
Vitamin D Receptor (VDR) gene variants (*Bsm*I, *Apa*I, *Taq*I and *Fok*I)	Differential susceptibility and resistance in males and females	45, 46
NRMAP1 [(CA)n, 823 C/T, TGTG+/del and D543N G/A]	No association with susceptibility or resistance	59
Cytokine gene		
TNF*a* – 238, – 308	No association	60
TNF*b*	No association	60
Haplotypes		
HLA-B17-TNF*a*-238/A HLA-B17-TNF*a*-308/2 HLA-B17-TNF*b*-2	Associated with bacteriological relapse	60

Mannose-binding protein

Mannose-binding protein (MBP), also known as mannose-binding lectin (MBL) is an acute phase protein secreted by the liver. It binds mannose and *N*-acetylglucosamine terminated glycoproteins and plays an important role in host defence against pathogens. Upon binding with certain carbohydrate moieties, such as terminal *N*-acetyl glucosamine or mannose, on various pathogens, MBP activates complement via specific protease and acts directly as an opsonin using the Clq receptor on macrophages. Mutations are found at the coding regions of the MBP genes, i.e. at codons 52, 54 and 57 that lead to low or near absent serum MBP levels in heterozygote and homozygotes respectively. Low serum level of MBP is associated with a common opsonic defect and is frequent in recurrent infections during infancy and possibly infections in adult life.

Several groups have studied MBL genotypes and tuberculosis, following a suggestion that MBL deficiency might have been maintained evolutionarily by a reduced capacity of mycobacteria to invade macrophages in the absence of MBL, leading to resistance to tuberculosis[33]. A study carried out in South Africa suggested that MBL-54 heterozygotes may be associated with protection against tuberculous meningitis[34] but a larger study in Gambia found no genotypic association[35]. Our study in south Indian population revealed an increased genotype frequency of MBP functional mutant homozygotes (including codons 52, 54 and 57) in pulmonary tuberculosis (10.9%) compared with control subjects (1.8%). Analysis of association of MBP genes and HLA-DR2 has showed that these genes are associated with susceptibility to pulmonary tuberculosis, independent of each other[36] (Table 1). Recently, a Mexican study of surfactant genes expressing collectins that are evolutionarily and functionally related to MBL genes has been suggested to influence tuberculosis susceptibility[37].

Vitamin D receptor

It has long been suspected that vitamin D may be important in immunity to *M. tuberculosis*. Prior to the availability of antituberculous drugs, vitamin D was used in the treatment of patients with cutaneous tuberculosis and was reported to have dramatic effects[38]. The prevalence of both vitamin-D deficiency and tuberculosis is high among Asian immigrants in the UK, suggesting that vegetarian diet is a risk factor for tuberculosis[39]. 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is an important immuno-modulatory hormone which activates monocytes and suppresses lymphocyte proliferation, immunoglobulin production and cytokine synthesis[40,41]. *In vitro*, 1,25 $(OH)_2D_3$ enhances the ability of human monocytes to restrict the growth of *M. tuberculosis*[40,42]. The effects of vitamin D are exerted by interaction through vitamin D receptor (VDR). Various diallelic polymorphisms have been identified in the vitamin D receptor gene and these polymorphic variants have been shown to be associated with the susceptibility or resistance to tuberculosis.

In a study carried out in the Gambian (West Africa) pulmonary TB patients, the *tt* genotype of *Taq*I polymorphism of *VDR* gene was found less frequently in cases of pulmonary TB, suggesting that this genotype may be associated with resistance to pulmonary TB whereas *Apa*I polymorphism showed no association[43]. The variant *ff* genotype (homozygote) of *Fok*I polymorphism of *VDR* gene and 25-hydroxylcholecalciferol deficiency have been shown to be strongly associated with pulmonary tuberculosis in Gujarati Indians living in London[44]. Our preliminary studies in south Indian pulmonary TB patients on *Bsm*I, *Apa*I, *Taq*I and *Fok*I polymorphisms of *VDR* gene showed an increased frequency of the genotypes Bb (heterozygote) of *Bsm*I, TT (homozygote) of *Taq*I and FF (homozygote) of *Fok*I polymorphism, in males and *tt* genotype (homozygote) of *Taq*I polymorphism in female patients suggesting the association with the susceptibility to TB[45,46]. Whereas genotypes BB (homozygotes) of *Bsm*I and AA (homozygous) of *Apa*I polymorphism are associated with resistance to pulmonary tuberculosis in male subjects[45,46]. The variant genotypes of *Bsm*I, *Apa*I, *Taq*I and *Fok*I sites of *VDR* gene either alone or in combination with each other as haplotype may be associated with susceptibility or resistance to pulmonary tuberculosis in males or females (Table 1). This type of differential susceptibility with variant genotypes of *VDR* gene in male and female subjects may be due to the circulating level of vitamin D_3, dietary intake of vitamin D_3, level of vitamin D receptor expression and other host factors. Further, studies on the level of circulating vitamin D_3, vitamin D receptor expression and the variant genotypes of vitamin D receptor will explore the mechanism of tuberculosis susceptibility in males and females. It is well established that the prevalence of tuberculosis is more in males[47]. Recently, an X chromosome susceptibility gene has been suggested which may contribute to the excess of males with tuberculosis observed in many populations[48].

Natural resistance associated macrophage protein 1 (NRAMP1)

NRAMP1 (recently renamed as SLC11A1-solute carrier family 11, member 1) was identified by several groups working on a mouse locus that confers susceptibility to intracellular infections, such as *Leishmania*, *Salmonella* and the BCG strain of *Mycobacterium bovis*[49]. NRAMP1, like the related NRAMP2 (SLC11A2), is probably a divalent cation transporter and is found in the membrane of the phagolysosomes[50]. In mouse models, NRAMP1 is important in resistance to several intracellular infections.

The human *NRAMP1* gene has several polymorphisms[51]. The effects of *NRAMP1* gene variants seem more modest, association has been found between tuberculosis susceptibility and NRAMP1 in populations as diverse as West Africans[52,53], Japanese and Koreans[54]. A study carried out in Taiwanese population revealed no association of *NRAMP1* gene variants with the susceptibility to tuberculosis[55]. Linkage between tuberculosis and the *NRAMP1* locus has been shown in a large Canadian pedigree[56], but linkage was not seen in Brazilian, West African or South African populations[57,58].

Our studies on *NRAMP1* gene polymorphism [$(CA)_n$, 823 C/T, TGTG+/del and D543N G/A] in south Indian pulmonary and spinal tuberculosis patients revealed no association with the susceptibility to pulmonary and spinal TB in Indian population. It was suggested that MHC and other non-MHC gene polymorphic variants may be associated[59] (Table 1).

Cytokine genes and receptors

An analysis of the course of infection in gene-knock-out mice has provided examples of the potential relevance of polymorphism in cytokine and cytokine receptor genes to infectious disease susceptibility in humans.

Tumour necrosis factor-a and b: Increased production of inflammatory cytokines, such as tumour necrosis factor-*a* (TNF-*a*) has been found in tuberculosis and various other infectious diseases. TNF-*a*, is mainly produced by monocytes and macrophages and TNF-*b* by T-lymphcoytes. Variant genotypes of TNF-*a* are associated with increased production of TNF-*a*. Association studies have been carried out on polymorphisms in and near the tumour necrosis factor (TNF) gene located in class III region of MHC. Our studies on TNF-*a* (– 238 and – 308) and TNF-*b* gene polymorphisms in Indian pulmonary tuberculosis patients revealed no association either with susceptibility or resistance[60] (Table 1). A study carried out in Cambodian tuberculosis patients also revealed no association with TNF-*a*[61].

Interleukin-1(IL-1): Interleukin-1 (*a* and *b*), another inflammatory cytokine, gene polymorphism has been studied in Gambians[62], Gujarati Indians[63] and Cambodians[61]. These studies revealed no association with the susceptibility to tuberculosis.

IL-1 receptor antagonist (IL-1RA): Interleukin-1 receptor antagonist (IL-1RA) is another cytokine factor which competes for the IL-1 binding site. The association of *IL-1RA* gene variants in various diseases has been studied. Macrophages from carriers of IL-1RA alleles have been shown to produce more IL-1RA and less IL-1*a* than other genotypes. *IL-1RA* gene variants are not associated with the susceptibility to pulmonary tuberculosis. However, association of the haplotype IL-1 Ra $A2^-$/IL-1*b* (+ 3953) $A1^+$ with the susceptibility has been reported with tuberculous pleurisy[63]. Our study on *IL-1RA* gene polymorphism in Indian pulmonary tuberculosis patients revealed no association with any of the genotypes but spinal tuberculosis patients showed a trend towards an increased frequency of genotype 22 compared with the control subjects[45,64].

Interleukin-10: This is a macrophage-deactivating cytokine. *NRAMP1* gene has been suggested to influence tuberculosis susceptibility by regulation[65] of interleukin-10. In Cambodian patients, association of heterozygosity for the -1082 polymorphism of the IL-10 promoter with TB susceptibility has been reported[61].

Interleukin-12 receptor (IL-12R): Interleukin-12, a cytokine associated with increased production of Th1 type of cytokines, binds to interleukin-12 receptor. A case control study carried out in Japanese tuberculosis patients revealed the association of homozygosity for R214-T365-R378 allele (genotype 2/2) with the susceptibility to tuberculosis. This genetic variation has been suggested to predispose individuals to tuberculosis infection by diminishing receptor responsiveness to IL-12 and to IL-23, leading to partial dysfunction of interferon-gamma-mediated immunity[66].

Interferon-g receptor (IFN-gR): Interferon-*g* receptor (IFN- *g*R)/gene variants has been shown to be associated with the susceptibility to atypical mycobacterial infection with *M. fortuitum*, *M. cheloni* and *M. avium*[67]. A different mutation, IFN-*g*R1, was identified in a child with fatal disseminated BCG infection[68].

Conclusions

Developments in modern genetics and genomics have contributed to our understanding of the pathogenic processes that underlie major infectious diseases by allowing a more systematic study of the genetic influences. The number of candidate susceptibility genes is expanding rapidly. Moreover, genome-wide linkage analysis is also beginning to provide insights into complex disease. Advances in single nucleotide polymorphism (SNP) typing, microarray technology and bioinformatics will be helpful in the study of infectious diseases.

The development of tuberculosis or other mycobacterial diseases is the result of a complex interaction between the host and pathogen influenced by environmental factors. Numerous host genes are likely to be involved in this process. Using a variety of study methods, substantial progress has already been made in advancing our understanding of genetic susceptibility to tuberculosis. However, only a small part of the total familiar clustering observed in tuberculosis can be explained by the host

genes identified to date. There is much work still to be done as there are likely to be many more tuberculosis-susceptibility genes to be identified[69].

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