

HOST GENETICS AND PREDISPOSITION TO TUBERCULOSIS

Eileen G Hoal, PhD

Marlo Möller, BSc

Department of Medical Biochemistry and MRC Centre for Molecular and Cellular Biology, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa

SUMMARY

Host genetic factors play an important role in determining the susceptibility of the individual, and perhaps the population, to tuberculosis (TB). The techniques used to identify the genes involved in susceptibility range from animal studies and macrophage infection studies to the investigation of the underlying genetic deficiencies responsible for rare human susceptibility to normally non-pathogenic mycobacteria. Parametric and non-parametric linkage studies, and association studies of both the unrelated case-control and family-based type, have all contributed to provide evidence of the involvement of a number of genes in the development of TB. To a greater or lesser extent, HLA, *NRAMP1*, *MBL*, the IL-12/IFN- γ pathway and a number of newer candidates, have all been implicated in the susceptibility to TB seen at the general population level. Understanding the immune responses of individuals with more resistant genotypes, particularly where this can be replicated in a number of different populations, could suggest novel therapies to combat this highly successful pathogen.

INTRODUCTION

The role and importance of genetic background in disease is unequivocal, and the last decade has seen this principle extended to and accepted in infectious disease as well. Globally, tuberculosis (TB) is a major cause of morbidity and it is estimated to cause 2.6 million deaths annually with 98% of deaths occurring in developing countries,^{1,2} translating to a major health crisis.³ Parts of South Africa such as the Western Cape have among the highest incidences of TB ever recorded.⁴

Approximately one-third of the world's population is infected with *Mycobacterium tuberculosis*, the causative agent of TB. However, only 5-10% of these infected people (more if the situation is complicated by concomitant HIV infection) will progress to overt TB disease. The vast majority (up to 95%) will remain healthy, probably because of the mounting of an effective immune response in these individuals.⁵ In order to discriminate between those who succumb to *M. tuberculosis* and those who do not, a number of factors need to be considered. Socio-economic factors undeniably increase both the likelihood of infection and the chance that disease will result, but host genetic factors play a significant role as well.

Twin studies have shown greater concordance in TB disease between monozygotic than dizygotic twins,⁶ and adoption studies have indicated that the genetic

component in infectious disease is greater than that in cardiovascular disease or cancer. Experimentation in animals has confirmed that the distinction between certain strains of mice or rabbits that are resistant or susceptible to mycobacteria is based solely on minor genetic differences.⁷ In human populations, genes conferring susceptibility to one disease may provide the carrier with an advantage in another – the so-called heterozygote advantage as seen in malaria, which explains the persistence of apparently deleterious genes in a population. It is thought that exposure to TB has been a powerful genetic selective force in the western world over the last 300 years, which may in turn have led to an increase in susceptibility to rheumatoid arthritis⁸ and perhaps other auto-immune diseases.

Identification of the genes involved in complex disease such as infectious disease, and in particular, TB, has followed a number of different but normally complementary paths. Methods for the identification of human disease genes have progressed rapidly in the last decade due in part to the Human Genome Project. Genome projects have made resources such as maps, clones, sequences and data on expression and phenotype available and easily accessible. Advances in recombinant DNA technology allowed positional cloning, which involves the isolation and cloning of a gene with no prior knowledge of its function. The use of the polymerase chain reaction (PCR) in linkage studies and screening for mutations or polymorphisms has greatly simplified the identification of disease genes.

Complex disease, unlike monogenic conditions, can be influenced by several genes, with each gene making a small contribution to the overall susceptibility, but not direct causation of the disease.⁹ The methods for the identification of resistance and susceptibility genes to complex diseases include genome-wide scans, animal models of disease, studies of *in vitro* macrophage function and studies of patients with unusual immune deficiencies.¹⁰ In the light of the increase in drug-resistant strains of *M. tuberculosis* and the dearth of new antibiotics and vaccine candidates, it is possible that a deeper understanding of the successful immune response against this age-old pathogen will lead to novel therapies such as immunomodulation.

In this review, the strategies used to identify susceptibility genes and some of the genes identified for mycobacterial pathogenesis are discussed.

STRATEGIES TO IDENTIFY SUSCEPTIBILITY GENES

The identification of susceptibility genes combines input from the clinic, the computer and the laboratory.¹¹ To identify the role of genetic factors in complex phenotypes, genetic epidemiology methods are used. Studies of human infectious disease can incorporate several elements: environmental factors influencing the risk of infection can be included in the analysis; the function of the gene and its role in response to the pathogen can be used to confirm its suitability as a candidate gene; and several quantitative traits such as clinical, biological and immunological parameters, can be measured to refine the phenotype under study.

Correspondence: Dr Eileen G Hoal, Department of Medical Biochemistry, University of Stellenbosch, PO Box 19063, Tygerberg 7505, South Africa. Tel (+27) 21-938-9412, fax (+27) 21-931-7841, e-mail egvh@sun.ac.za

Association studies

Association studies are usually case-control studies that follow the distribution of a certain marker allele between affected and unaffected subjects in the same population.¹² Accurate phenotyping, large sample size, and careful matching of cases and controls are all essential to avoid false-positive or false-negative results.¹³ Candidate genes must be tested individually to determine whether a polymorphism in the gene is associated with susceptibility to the disease. This allele could either be the disease-susceptibility allele, or be in linkage disequilibrium with the actual gene influencing disease development.

Population-based case-control studies of candidate genes

In case-control studies, allele frequencies for candidate genes are compared in unrelated individuals with the infection and in healthy controls. A significant difference indicates an association between a particular polymorphism in a gene and disease, and this is a statistical finding, not automatically a reflection of genetic linkage.

DNA from the cases and controls is genotyped for polymorphisms in or close to a gene that might play a role in the pathogenesis of the disease. Finding no association with an allele does not automatically mean that the candidate gene is excluded as a susceptibility gene, only that specific polymorphism. Different results may also be obtained in different populations, because of genetic causes (population history), statistical power (increased by larger sample size) and influences of the environment.¹⁴

Meta-analysis of over 300 genetic association studies covering 25 different associations has supported the notion of the contribution of common variants to susceptibility to common disease, with inconsistencies mainly due to underpowered studies.¹⁵ After a few years of scepticism, a measure of faith and confidence has been restored to the field of association studies, provided that minimum criteria are met.^{13,16}

Transmission disequilibrium test

In the transmission disequilibrium test (TDT), the transmission of alleles from heterozygous parents to their offspring is investigated, and markers are identified that are inherited more frequently than expected by affected individuals.^{17,18} These results are not affected by population stratification, which makes the TDT attractive and the theoretical method of choice in all but the most isolated of populations, although the effect of stratification on association studies is regarded as having been overestimated.¹⁹ As with any other association test, the associated allele may be a susceptibility factor, or may be in linkage disequilibrium with a susceptibility allele nearby. DNA samples from the parents and the affected children are needed, which is problematic with late-onset disease.¹⁸

Whole-genome association studies

Whole-genome association studies use a dense map of single nucleotide polymorphisms (SNP) markers and attempt to find an SNP that identifies a haplotype block. Haplotype blocks exist because of linkage disequilibrium, which appears to extend over large regions.^{20,21} It should be possible to select a minimum set of markers to efficiently cover the most common haplotypes.^{9,22,23} Whole-genome association studies are becoming feasible since the introduction of high-throughput genotyping systems. Limitations of such studies are the total cost of the genotyping, sample size and the statistical corrections that would have to be performed,²⁴

but this technology has good prospects for the future.^{25,26}

Linkage studies

Linkage studies are traditionally carried out in large affected families where the mode of inheritance is clear. This method has led to the discovery that *NRAMP1* markers were weakly linked to TB in 98 Brazilian families,²⁷ and strong linkage was found in a large aboriginal Canadian family when risk of disease was assigned to individuals in the family.²⁸

In complex disease, however, non-parametric linkage studies (or 'model-free', implying that the mode of inheritance is unknown) are usually used.

Genome-wide scans

A non-parametric genome-wide scan can identify areas of linkage to the disease. Affected sibling pairs and their parents are screened at a large number of markers spread throughout the genome for genetic markers that segregate with disease more often than would be expected by chance. Once evidence for linkage has been found, fine genetic and physical maps are constructed to narrow down the interval on the chromosome and allow gene identification by candidate gene selection (when the function of the gene is known) or by positional cloning (when the function is unknown).¹²

The first genome-wide scan for TB was conducted in affected sib-pairs and their families from The Gambia and South Africa²⁹ and suggested that regions on chromosome 15q and Xq were linked to TB. Subsequent analysis of the 15q11-q13 region identified the gene *UBE3A*, which is expressed in macrophages and encodes a ubiquitin ligase. This gene, which functions in the ubiquitination and degradation of specific proteins, was associated with TB.³⁰

A recent genome-wide scan for TB and leprosy, conducted in Brazil, found a cluster of susceptibility genes across chromosome 17q11.²³¹ and indicated that four separate candidate genes, *NOS2A*, *CCL18*, *CCL4* and *STAT5B* may contribute to this region of linkage.

Other strategies

Animal models and *in vitro* studies of macrophage infection by mycobacteria can provide many clues to genes responsible for susceptibility.⁵ Often, however, Nature's experiments as seen in rare instances of human susceptibility to usually non-pathogenic organisms, can indicate entire pathways to investigate. A case in point is the Maltese kindred and other rare patients, which led to the identification of the interleukin-12/interferon-gamma (IL-12/IFN- γ) pathway³² where a number of abnormalities in these genes have been characterised (see below).

MYCOBACTERIA

Knowledge of the bacterium and its methods of subverting the host defences is important particularly in the selection of candidate genes. More than 70 species of mycobacteria exist. The *Mycobacterium tuberculosis* complex (*M. tuberculosis*, *M. africanum*, *M. bovis*, *M. bovis BCG* and *M. microti*) and *M. leprae* are responsible for TB and leprosy respectively. The remaining mycobacteria are mostly environmental bacteria, known as Mycobacteria Other Than Tuberculosis (MOTTs) and cause opportunistic infections. *M. tuberculosis* is an intracellular bacterium, and its preferred host cell is the macrophage, where it has evolved mechanisms that enable it to escape the normal defence mechanisms and replicate in susceptible individuals. The host defences against intracellular bacteria

are mainly cell-mediated but also humoral and therefore any genetic deficiencies in components that play a role in these systems can lead to susceptibility.³³ Infectious disease genetics is complicated by the fact that two genomes, one prokaryotic and one eukaryotic, are interacting in an age-old contest.

SUCCESSSES IN IDENTIFYING SUSCEPTIBILITY GENES

A number of susceptibility or resistance genes have been identified for TB, primarily in case-control studies, and a few have been replicated in ethnically diverse populations. This is an important concern if and when any gene pathway is considered for its potential to suggest new therapies.³⁴

Human leukocyte antigen

The human leukocyte antigen (HLA) genes are the MHC I and MHC II genes and all can present peptides to T cells, but each protein binds a different range of peptides.³³

HLA genes have been examined in several susceptibility studies³⁵ and were some of the first genes to be associated with TB. In Indian, Mexican and Brazilian populations, susceptibility to TB has been associated with the class II antigen HLA-DR2.^{10,36,37} Association with HLA-DQ has also been reported in Cambodia³⁸ and Iran.³⁹

Natural-resistance-associated macrophage protein

The natural-resistance-associated macrophage protein 1 (*NRAMP1* or *SLC11A1*) gene is a major determinant of natural resistance to intracellular infections.⁴⁰ The gene consists of 15 exons spanning 11.5 kb and codes for a 90-100 kDa protein.¹⁰ It is an integral membrane protein expressed only in the lysosome of macrophages and monocytes. After phagocytosis of bacteria, *NRAMP1* is targeted to the membrane of the phagosome containing the bacterium, where it may modify the environment to affect the replication of the bacterium, perhaps by removing iron or other divalent cations from the phagosome.⁴⁰

The gene, first named *Bcg*, was identified in mice by the use of positional cloning, where it controlled resistance to infection with mycobacteria, *S. typhimurium* and *Leishmania*,⁴¹ although susceptibility to *M. tuberculosis* in mice is not controlled by this gene alone.⁴² Evidence from linkage studies has been presented above. In a large case-control study in The Gambia, polymorphisms in the gene were examined and four were found to be associated with susceptibility to TB.⁴³ This demonstrated the use of mouse models to identify susceptibility genes in humans. Associations of *NRAMP1* with TB have also been found in Japan, Canada, Korea, Guinea-Conakry, Vietnam¹⁰ and South Africa,⁴⁴ and in most instances the allele over-represented in controls is thought to drive the highest rate of transcription of the protein.⁴⁵ Stepwise logistic regression analysis of the South African results indicated that the 5' and 3' polymorphisms contribute separate main effects.⁴⁴

Vitamin D receptor

The active form of vitamin D (1.25 dihydroxyvitamin D₃) has an important role in the regulation of calcium metabolism, but it is also a hormone that modulates the immune system.³⁵ It can stimulate cell-mediated immunity and activate monocytes, which in turn inhibits the growth of intracellular bacteria like *M. tuberculosis*.⁴⁶ These effects are controlled by the vitamin D

receptor (VDR).³⁴ Vitamin D deficiency is linked to TB by epidemiological evidence. It was found that the prevalence of both vitamin D deficiency and TB was high in Asians because of their vegetarian diet.¹⁰

In several populations the *Taq1* polymorphism in VDR was found to be associated with TB, but in The Gambia an allele was found in codon 352 that conferred resistance against TB.⁴⁷ However, the same allele was associated with female patients in an Indian population,⁴⁸ and was found to increase susceptibility to pulmonary TB in the Gujerati population, but only in patients with a lack of serum vitamin D.⁴⁹ This polymorphism has also been associated with leprosy in India.⁵⁰ Recently, a study in Peru found an association between the *Fok1* and *Taq1* polymorphisms and sputum culture conversion in patients, but did not find a significant association with susceptibility to TB disease,⁵¹ although the *Fok1* polymorphism was associated with TB in the Chinese Han population.⁵² A large study in West Africa indicated in both case controls and families that the VDR haplotype rather than individual polymorphisms more accurately reflects associations with TB.⁵³ This dependence on the haplotype, if a common occurrence, could explain many of the divergent findings on this and other genes.

Mannose-binding lectin

Mannose-binding lectin (MBL) is a calcium-dependant serum lectin produced by the liver.⁵⁴ It acts as an opsonin to promote phagocytosis by binding to microbial surface carbohydrates and can activate the complement system. Intracellular micro-organisms may increase their infectivity by using this system, because it promotes the uptake of bacteria into macrophages, in which intracellular bacteria can survive.⁵⁴

Low functional MBL-serum levels can occur because of the presence of three variant alleles in exon 1, namely allele B, allele C and allele D, which lead to an unstable protein. MBL concentrations can also be influenced by nucleotide substitutions in the promoter region of the *mb12* gene. Low MBL levels can protect against infection with *M. tuberculosis*. This was found in case-control studies where heterozygosity for the MBL variant alleles was associated with protection against the disease^{55,56} and the B allele has also been associated with protection against TB and particularly tuberculous meningitis in South Africa.⁵⁷ Conversely, however, an increased susceptibility to pulmonary TB was found in homozygous carriers of the variant alleles in India⁵⁸ and a study in Texas gave equivocal results.⁵⁹

Interferon- γ pathway

IFN- γ production by T cells and natural killer cells is stimulated by IL-12.⁶⁰ Detailed investigation of patients with rare infections by normally non-pathogenic mycobacteria has led to the identification of mutations in five autosomal genes in the IFN- γ pathway that can increase susceptibility to these atypical mycobacterial infections.³² These patients suffer repeated infections with mycobacteria such as *M. bovis* Bacille Calmette-Guerin (BCG), *M. avium*, *M. kansasii*, *M. smegmatis*, *M. fortuitum* and *M. chelonae*. Mutations in these genes may lead to repeated *Salmonella* infections,¹⁰ and *Listeria monocytogenes* has also been detected in a patient.⁶¹

The first defect identified in a gene in the IFN- γ pathway was the autosomal-recessive IFN- γ receptor ligand binding (IFN- γ R1) deficiency.^{62,63} This rare mutation occurs mainly in families, but in one study a frameshift mutation was detected in three unrelated families in the identical region of the *IFN- γ R1* gene. This resulted in an overexpression of a dominant form of the IFN- γ R1,

which binds IFN- γ , but lacks the intracellular domain needed for signalling. This area in the gene could therefore be a mutational hot spot.^{60,64}

Other cases were also described where patients had complete IFN- γ receptor signal transduction chain (IFN- γ R2) deficiency,⁶⁵ autosomal-dominant partial deficiency of the signal transducer and activator of transcription⁶⁶ and autosomal-dominant partial deficiency of the IFN- γ R1.⁶⁷ Differing results have been found regarding IFN- γ R1-deficiency in the general population. In a Croatian population an allele of a polymorphic microsatellite was associated with protection against pulmonary TB,⁶⁸ but in a Gambian population no association was found.⁶⁹

A promoter polymorphism (+874 A \rightarrow T) in the IFN- γ gene itself, which appears to result in lower NF- κ B binding and lower transcription levels of IFN- γ , was demonstrated in case-control studies to be associated with susceptibility to TB in Sicily,⁷⁰ Spain⁷¹ and South Africa.⁷² The finding in the South African population was replicated in an independent TDT-study,⁷² confirming the importance of this gene in TB at the population level.

Interleukin-12

IL-12 stimulates IFN- γ production by lymphocytes, induces type 1 helper T-cell responses and is essential for resistance against infection with intracellular bacteria. It is produced by macrophages particularly when infection with intracellular micro-organisms occurs.⁷³ IL-12 is a cytokine composed of a heavy chain of 40 kDa (p40) (IL-12B) and a light chain of 35 kD (p35) (IL-12A).

The functional response of lymphocytes to IL-12 is dependent on the expression of the IL-12 receptor.⁷⁴ Any deficiency in these genes will cause a decrease in IFN- γ production, as discussed above.⁷³ In one study an autosomal-recessive IL-12 deficiency, caused by a large gene deletion in two coding exons of *IL12P40*, was associated with BCG and *S. enteritidis* infection.⁷⁵ In patients with IL-12 receptor deficiencies, susceptibility to the same bacteria was found.⁷³

At the population level, association with TB was found with a haplotype of *IL12RB1* in Japan,⁷⁶ with 2 promoter polymorphisms in a family-based study in Morocco,⁷⁷ and with an intron 2 allele and a specific haplotype of 4 polymorphisms covering the whole *IL12B* gene⁷⁸ in a large study in Hong Kong.

WAITING IN THE WINGS

A number of theoretically logical candidates have given tantalising glimpses of their potential to be implicated in TB, but have not yet been replicated in a number of studies. These include:

Interleukin-10: IL-10 is primarily a regulatory cytokine and its chief role is to limit the inflammatory response. A promoter polymorphism which alters the rate of cytokine production was not associated with TB susceptibility in the Spanish population,⁷¹ but showed marginal association in Cambodia.⁷⁹ Awomoyi *et al.*⁸⁰ have suggested that *NRAMP1* influences TB susceptibility by regulation of IL-10.

Interleukin-8: IL-8, a chemokine, functions as a chemo-attractant recruiting leukocytes to the inflammatory site. Association with TB was shown with the -251 promoter polymorphism in a study in Texas⁸¹ but not in The Gambia.⁸²

Inducible nitric oxide synthase: In humans, nitric oxide (NO) is produced by three different nitric oxide synthases (NOS).⁸³ Nitric oxide synthase type 2 (*iNOS*, *NOS2A*) is induced in response to infections and cytokines and the enzyme produced generates NO by

using L-arginine as a substrate.⁸⁴ In the murine model, NO plays an important role in the killing of *M. tuberculosis* by mononuclear phagocytes, and the -1026 G \rightarrow T polymorphism in *NOS2A* was associated with susceptibility to TB in a case-pseudo-control analysis in a Brazilian population.³¹

Toll-like receptors: The toll-like receptor (TLR) family recognises specific molecular patterns in pathogens and has an important role in signalling in the induced responses resulting in local inflammation, recruitment of new effector cells, containment of local infection, and the initiation of an adaptive immune response. A small study in Morocco found that the Arg677Trp (previously associated with lepromatous leprosy)⁸⁵ may be associated with TB,⁸⁶ and evidence from Turkey indicates the same for the Arg753Gln polymorphism.⁸⁷

Parkin 2 and PARK2 co-regulated gene: Susceptibility to leprosy has been convincingly associated with polymorphisms in the upstream regulatory regions of the Parkinson disease gene *Parkin 2* (*PARK2*) and the *PARK2* co-regulated gene (*PACRG*). *PARK2* encodes an ubiquitin E3 ligase. The function of *PACRG* is not known, but it may be involved with the delivery of polyubiquitinated proteins to the proteasome. These genes are expressed in immune tissues, especially nerve cells (Schwann cells) and macrophages, which are the primary host cells for *M. leprae*. A combination of techniques was used in this elegant study. Firstly, a locus was mapped to chromosome 6q25-q26.⁸⁸ The region was examined further by sequencing and database searches which led to the identification of 64 SNPs. The SNPs were used as markers in a genome wide association scan and a total of 197 Vietnamese families with leprosy-sufferers were genotyped. Seventeen significantly associated SNPs were found in a block of about 80 kb in the regulatory region shared by *PARK2* and *PACRG*. Having only two of these 17 alleles may greatly increase the risk of developing leprosy. These results were confirmed in a case-control study with 975 Brazilians. This finding has led to speculations that ubiquitin-mediated proteolysis might be important in controlling leprosy.⁸⁹ It remains to be seen whether these genes have any relevance in TB, caused by the very closely related *M. tuberculosis*.

CONCLUSION

Infection with TB is responsible for millions of deaths annually, even with the use of antibiotics and intensive treatment. From the results obtained in several studies, it is clear that it is not only the pathogen which plays an important role in the development of infectious disease, but also the genome of the host.

Genetic studies in infectious disease are usually complicated because of the presence of two different genomes and the influence their interaction can have on the disease.³⁴ Although several genes have been identified as susceptibility genes for a number of intracellular bacteria, it is necessary to bear in mind that other genes and the environment can have an influence on the development of the disease,^{90,91} which is the reason that no single major susceptibility gene has been identified in any infectious human disease.⁹² The results from strategies used to identify candidate genes or to associate the candidate genes with infectious disease are not the final word on the subject of susceptibility, but provide important evidence on the pathways involved. A greater understanding of the immune response to TB could provide insights into novel treatments that target genetically based susceptibility, such as aerosolised IFN- γ , TNF-modulation, or even simple supplementation of, for example, vitamin D. These therapies could specifically target the more vulnerable

individuals in a population and lead to improved health in the entire community.

ACKNOWLEDGEMENTS

The financial support of the Centre of Excellence for Biomedical Tuberculosis Research, the South African National Research Foundation, and The Wellcome Trust is acknowledged.

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