

## REVIEW

# Genetics of susceptibility to leprosy

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The ancient disease of leprosy can cause severe disability and disfigurement and is still a major health concern in many parts of the world. Only a subset of those individuals exposed to the pathogen will go on to develop clinical disease and there is a broad clinical spectrum amongst leprosy sufferers. The outcome of infection is in part due to host genes that influence control of the initial infection and the host's immune response to that infection. Identification of the host genes that influence host susceptibility/resistance will enable a greater understanding of disease pathogenesis. In turn, this should facilitate development of more effective therapeutics and vaccines. So far at least a dozen genes have been implicated in leprosy susceptibility and a genome-wide linkage study has led to the identification of at least one positional candidate. These findings are reviewed here. Genes and Immunity (2002) 3, 441–453. doi:10.1038/sj.gene.6363926

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## Introduction

Many think of leprosy as a disease of the past and it is true that the worldwide prevalence is declining, in part due to improved case detection and effective multi-drug therapy. Globally, however, there are still 700 000 newly detected cases each year, mainly in Africa, Asia and Latin America<sup>1</sup> and leprosy represents a major health problem in Brazil, India, Madagascar, Myanmar, Nepal and Mozambique. The causative agent of leprosy, *Mycobacterium leprae*, was identified by Armauer Hansen in 1873. It is an obligate intracellular pathogen that mainly infects macrophages and Schwann cells, though it also multiplies in muscles and vascular endothelium, and can infect other tissue such as the brain, eye and testis. Unlike tuberculosis, which also results from infection with a mycobacterium (*M. tuberculosis*), leprosy is not often a direct killer. Instead, due to the infective agent's predilection for skin and peripheral nerves, the common severe consequences of leprosy are deformity and disability. This has significant social and economic impact on both the patient and their community.

Leprosy is like tuberculosis in that in the majority of cases, infection does not lead to clinical disease and when disease does develop, much of the damage is not caused by the infecting organism, but rather by the host's immune responses to that organism. Host factors that influence control of the initial infection and the host's immune response play a significant role in the outcome of infection with either *M. tuberculosis* or *M. leprae*.

In leprosy the significance of the host response to infection is illustrated by the broad clinical spectrum observed amongst those that develop disease. At one pole is tuberculoid leprosy, characterized by strong cell-mediated immunity, a Th1 CD4+ cytokine profile (IL2, IFN- $\gamma$ ), very few bacteria and localized lesions. At the other pole is lepromatous leprosy, characterized by a lack

of cell-mediated immunity, Th2 CD4+ responses (IL4 and IL5), a strong humoral response, disseminated progressive disease and large numbers of bacteria. Thus tuberculoid patients can be thought of as those exhibiting the most resistance whereas lepromatous patients are those exhibiting the least. This is not to say that the pathogenesis associated with the more resistant pole is necessarily milder; strong Th1 responses that contain the bacterium can result in rapid and severe nerve damage.

Evidence that host genetic factors contribute to susceptibility to leprosy comes from epidemiological data, segregation and twin studies.<sup>2–6</sup> The goal of identification of host genetic factors that underlie susceptibility to leprosy can be approached in at least two ways: firstly, candidate gene studies can be carried out on genes of known function 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. How *M. leprae* targets Schwann cells, exactly how it brings about nerve damage and how it is killed in macrophages have not been fully elucidated and it is possible that host genes which influence these processes may influence the outcome of infection. It is clear, however, that the development of appropriate cell-mediated immunity is important in the control of mycobacterial disease. 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 (Table 1). These genes and the results of a genome-wide linkage analysis of leprosy susceptibility are discussed below.

## Major histocompatibility complex (MHC) region

Linkage and/or association studies have implicated several genes in the MHC region in susceptibility to leprosy. However the presence of strong linkage

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**Table 1** Summary of published non-HLA leprosy association studies

Gene(s)	Population	Polymorphisms typed	Phenotype	Allele/genotype/haplotype	Study size	Ref.
COL3A1	India	COL3A1	MB <sup>a</sup>	COL3A1 250 bp susc. <sup>b</sup>	16 PB <sup>d</sup>	132
CTLA4		CTLA4 size allomorphs		CTLA4 104 bp pro. <sup>c</sup>	9 MB 14 con. <sup>e</sup>	
SLC11A1	India	Promoter microsatellite, Exon 2, 469+14G/C, (TGTG) <sub>n</sub>	Leprosy	No association	124 MB 107 PB 166 con.	128
SLC11A1	Mali	Promoter microsatellite, 469+14G/C, (TGTG) <sub>n</sub>	Leprosy type	TGTG het. <sup>f</sup>	181 MB 92 PB 201 con.	129
TLR2	Korea	R 677W	MB	677W	45 MB 41 PB 45 con.	160
C4B	Brazil	C4B, C2, BF, C4A	ENL <sup>g</sup>	C4B*Q0	109 cases 46 ENL 172 con.	82
HSPA1A	India	HSPA1A	PB	A	49 PB 38 con.	88
MICA/HLA B haplotype	South China	HLA-DRB1,  HLA B, MICA-5A	MB	HLA-B46/MICA-5A5	50 MB  19 PB 112 con.	21
TAP2	North India	TAP1: I333V, N637G, TAP2: V379L, A565T, A665T	PB	TAP2-B	50 PB 40 con.	58
TNF $\alpha$	Bengali Indians	-308	MB	TNF*2	121 MB 107 PB 160 con.	42
TNF $\alpha$	Brazil	-308	Mitsuda response	TNF*2	74 PB	75
TNF $\alpha$	Brazil	-308	Reaction rate	TNF*2 het.	57 TNF*2 positive	79
TNF $\alpha$	Brazil	-308	MB	TNF*2	90 PB 210 MB 92 con.	74
TNF $\alpha$	Brazil	-308	Leprosy	TNF*1	T <sup>h</sup> 27 NT <sup>i</sup> 4	32
TNF $\alpha$ /LTA haplotype	Brazil	TNF -308, LTA Nco1	Leprosy	TNF*1/LTA*2 susc.  TNF*2/LTA*2 pro.	T 26 NT 11  T 2 NT 16	32
VDR	Bengali Indians	Taq1	Leprosy type	tt in PB TT in MB	124 MB 107 PB 166 con.	128

<sup>a</sup>multibacillary (includes patients at/near lepromatous pole).<sup>b</sup>susceptible.<sup>c</sup>protective.<sup>d</sup>paucibacillary (includes patients at/near tuberculoid pole).<sup>e</sup>controls.<sup>f</sup>heterozygotes.<sup>g</sup>Erythema Nodosum Leprosum.<sup>h</sup>Transmitted.<sup>i</sup>Not Transmitted.

disequilibrium in combination with a relatively large number of closely spaced, polymorphic genes encoding products involved in immune responses has made the task of determining true functional associations a difficult one. The most consistent results indicate that a locus in the class II region is involved in susceptibility to leprosy *per se* and/or modulation of disease.

### HLA genes

Located in the MHC region, HLA genes are highly polymorphic and present antigenic peptides to  $\alpha\beta$  T cells. The HLA genes were one of the first class of genes to be implicated in susceptibility to leprosy, and there have been numerous studies in many populations, probably partly because their gene products were such a likely biological candidate (the main hypothesis being that

presentation of certain antigens in a specific HLA context may influence the type of T-cell response that develops), and partly because serological typings could be performed in the pre-genotyping era.

A series of family studies in various populations have shown non-random segregation of parental HLA haplotypes amongst tuberculoid children<sup>7–11</sup> and lepromatous children.<sup>10,12</sup> As HLA haplotypes segregated randomly among healthy siblings<sup>8,10</sup> it was suggested early on that genes in the MHC region might influence leprosy type rather than susceptibility to leprosy *per se*.<sup>13</sup>

### Class I region

Several studies comparing HLA class I gene frequencies in leprosy cases and controls have found associations either with the polar forms of leprosy, or with leprosy itself, however these suggested associations have not been replicated.<sup>14–20</sup>

Recently an HLA-B46, MICA-5A5 haplotype was found less often amongst multibacillary leprosy patients compared to controls in a small south China study, indicating that this haplotype may carry a gene that is protective against multibacillary leprosy in this population.<sup>21</sup> MICA itself is a candidate susceptibility locus: located in the HLA class I region, MHC class I chain-related (MIC) genes encode membrane-bound polypeptides that do not bind peptides or associate with  $\beta$ 2-microglobulin, but instead act as a co-stimulatory signal by interaction with a receptor NKG2D to augment T-cell activation. MICA cell surface expression can be up-regulated not only by heat shock but also by infection with cytomegalovirus (CMV)<sup>22</sup> or *M. tuberculosis*.<sup>23</sup> The Chinese study found no association with leprosy and B46–/5A5+ or B46+/5A5– haplotypes indicating that neither the HLA B46 nor the MICA-5A5 allele was sufficient to confer protection. The MICA-5A5 polymorphism is located in exon 5 and is a nucleotide triplet repeat that encodes a series of alanine residues in the transmembrane domain. There are numerous other polymorphisms in the MICA gene, including many in exons 2–4, which encode the extracellular domains that bind to NKG2D<sup>24,25</sup> and it is possible that one that influences susceptibility to leprosy occurs on the B46/MICA-5A5 haplotype.

### Class II region

Numerous case–control studies have shown associations between the class II HLA genes and leprosy. DQ alleles, especially DQw1 have been shown to be associated with tuberculoid leprosy in India,<sup>26,27</sup> Korea,<sup>19</sup> Thailand<sup>28</sup> and Japan,<sup>29</sup> and with lepromatous leprosy in India<sup>15</sup> and Japan.<sup>18,30</sup> DQw1 was also implicated in a meta-analysis of pooled association studies<sup>31</sup> and in a Venezuelan lepromatous family study<sup>10</sup> whereas in Shaw's recent Brazilian family study significant evidence for linkage and association to tuberculoid leprosy and possibly leprosy *per se* was found with DQB1, DQA1 and DRB1 variants.<sup>32</sup> Unusual DQ restricted antigen-specific CD8 cells that predominantly produce IL4 were proposed to act as suppressor cells in lepromatous patients;<sup>33</sup> however, this does not explain the association with tuberculoid leprosy. Importantly, HLA-DQ1 is in strong linkage disequilibrium with HLA-DR2 in most populations and it has usually been difficult to discern whether the primary association is with the DR or the DQ variant.

HLA-DR3 was associated with tuberculoid leprosy in Mexico<sup>34</sup> and in the South American populations of Surinam<sup>35</sup> and Venezuela,<sup>10</sup> whereas it was found at lower frequencies in lepromatous patients.<sup>35,36</sup> T-cell proliferation responses to certain epitopes of the mycobacterial hsp65 (p3–13) are DR3 restricted<sup>37</sup> and DR3 was associated with low T-cell responses to *M. leprae* in tuberculoid patients.<sup>38,39</sup> Increased HLA-DR2 frequencies have been reported in both tuberculoid and lepromatous patients compared to controls.<sup>15,18,19,28,29,31,40,41</sup> Association studies have also shown that DR2 is increased in leprosy patients of all subtypes compared to controls.<sup>42,43</sup> Family-based studies have shown a skewed distribution of DR2 alleles in Indian<sup>44</sup> and Egyptian tuberculoid siblings.<sup>11</sup> Molecular HLA typing has allowed DR2 to be refined into HLA-DRB1 subtypes. DRB1\*1501 has been associated with lepromatous leprosy<sup>26,30</sup> whereas DRB1\*1502 has been associated with tuberculoid leprosy.<sup>45</sup> The \*1501 and \*1502 alleles differ from each other by a single amino acid at codon 86. Class II molecules have polymorphic pockets that accommodate the side chains of bound peptides. The codon 86 residue lies in binding pocket 1. In another Indian study, both 1501 and 1502 were found to be associated with tuberculoid leprosy,<sup>46</sup> indicating that the residue in pocket 1 may not be involved in determining the outcome of leprosy infection. Instead it appears that certain residues that contribute to the net charge in the putative peptide-specific binding pocket 4 may be more important.<sup>47</sup> It is hypothesized that net negative or neutral charges in binding pocket 4 cause poor binding of the DRB1 molecule to *M. leprae* antigens. As HLA molecules with the highest affinity to peptide produce the greatest T-cell proliferation and IFN- $\gamma$  response,<sup>48</sup> peptide presentation by low affinity class II molecules may result in muted cell-mediated immunity.<sup>47</sup> Alternatively, peptide presentation by specific class II molecules may result in activation of suppressor/regulatory T-cells.<sup>49</sup>

### Transporter 2, ATP-binding cassette, sub-family B (TAP2).

TAP2 is critical for peptide translocation from the cytosol into the endoplasmic reticulum and for peptide binding to MHC class I molecules. Various combinations of polymorphisms at codons 379, 565 and 665 result in eight TAP2 alleles: (A–H). In the rat, products of different TAP2 alleles differ in permissiveness to transport peptides into the endoplasmic reticulum, thus modifying the spectrum of peptides presented by class I molecules on the cell surface.<sup>50</sup> Although some immune responses to infectious pathogens and autoimmune diseases are apparently independently associated with human TAP2 alleles,<sup>51–55</sup> it is unclear whether differences in human TAP2 alleles are functionally relevant.<sup>56,57</sup> Nevertheless, TAP2-B has been reported to be associated with tuberculoid leprosy and TAP2-A/F was increased in pulmonary tuberculosis patients in North India,<sup>58</sup> indicating that TAP2 alleles may influence mycobacterial susceptibility in this population.

### Class III region

*Tumor necrosis factor alpha (TNF $\alpha$ )*. The TNF $\alpha$  protein has pleiotropic effects which straddle the innate and adaptive immune responses. Produced mainly by macrophages, in mice it is involved in macrophage activation

and, possibly, killing of intracellular *M. leprae* as well as efficient antigen presentation through the class II molecules. It is also an important modulator of the cytokine production required for effective leukocyte localization and thus granuloma formation. One hypothesis for how TNF $\alpha$  may influence infectious disease in humans is that polymorphisms leading to low production could result in insufficient activation of macrophages, such that they are unable to kill mycobacteria. Alternatively, the important role TNF $\alpha$  plays in granuloma formation may mean that low TNF $\alpha$  levels result in a failure to contain infectious foci. On the other hand, TNF $\alpha$  overproduction may influence disease progression by inducing local tissue damage. Finally, a recent study has found that TNF $\alpha$  production supports virulent *M. tuberculosis* growth in human alveolar macrophages, leading the authors to suggest that mycobacteria may deliberately augment TNF $\alpha$  production as a method of immune invasion, for example by inducing apoptosis of infected cells and thus enabling their spread to uninfected macrophages.<sup>59</sup>

There are numerous polymorphisms in the TNF $\alpha$  promoter, several of which may be involved in regulation of TNF $\alpha$  expression. A single nucleotide polymorphism at -863 affects the binding of the NF kappa B p50-p50 dimer to an NF kappa B regulatory site.<sup>60</sup> The base change appears to inhibit p50-p50 binding, thereby reducing p50-p50 repression of reporter gene transcription. Another promoter polymorphism, -376, lies in a region of multiple DNA-protein interactions. The G to A change introduces a binding site for the transcription factor OCT-1. Recruitment of OCT-1 results in an increase in basal TNF $\alpha$  expression in human monocytes and the A allele has been associated with susceptibility to cerebral malaria.<sup>61</sup> The only TNF $\alpha$  polymorphism that has been investigated in relation to leprosy susceptibility is a G to A substitution located in the promoter, at position -308. The alleles have been designated TNF\*1 and TNF\*2, for G and A, respectively. Initial studies using TNF $\alpha$  promoter reporter constructs suggested the TNF\*2 allele is associated with increased TNF $\alpha$  levels<sup>62-64</sup> but this has been disputed by others.<sup>65-68</sup> Recently, it was shown that elevated TNF $\alpha$  levels are produced by TNF\*2 only when certain cell types and stimuli are used.<sup>64</sup> Consistent with the hypothesis that the -308 polymorphism is functional, the TNF\*2 allele has been associated with a variety of diseases in which excessive TNF $\alpha$  production has been postulated to be involved, including fatal meningococcal disease,<sup>69</sup> mucocutaneous leishmaniasis,<sup>70</sup> scarring trachoma,<sup>71</sup> cerebral malaria<sup>72</sup> and inflammatory bowel disease.<sup>73</sup>

The TNF\*2 allele has been associated with lepromatous leprosy in Bengali Indians<sup>42</sup> whereas in southern Brazil this allele appears to be protective against severe leprosy.<sup>74</sup> TNF\*2 also appears to be protective against lepromatous leprosy, tuberculoid leprosy and leprosy *per se* in northeastern Brazil, while TNF\*1 confers susceptibility.<sup>32</sup> Furthermore, Brazilian leprosy patients that carry the TNF\*2 allele have greater skin inflammatory responses to lepromin than those that do not;<sup>75</sup> thus, in Brazil it appears that increased production of TNF $\alpha$  may be important for inducing protective immune responses against leprosy.

TNF $\alpha$  levels have been shown to be increased during reaction responses in leprosy patients.<sup>76,77</sup> Reaction

responses are the cause of significant tissue damage and in combination with transforming growth factor  $\beta$ 1, TNF $\alpha$  causes significant Schwann cell death *in vitro*.<sup>78</sup> Thus it has been hypothesized that alleles that enhance TNF $\alpha$  production may be associated with the adverse effects of reaction responses. In an analysis of 57 TNF\*2-positive leprosy patients, reaction responses were found much more frequently amongst heterozygotes compared to homozygotes; however, TNF $\alpha$  levels were similar for a small series of both TNF\*1 and TNF\*2 patients during and in the absence of reaction, and both sets of patients showed similar increases in TNF $\alpha$  levels during reaction, indicating that these polymorphisms may not influence TNF $\alpha$  levels *in vivo*.<sup>79</sup> It would be of interest to reassess these associations in a larger data set.

*Lymphotoxin  $\alpha$  (LTA, formerly TNF $\beta$ )*. The LTA gene is located close to TNF $\alpha$  and encodes lymphotoxin  $\alpha$ , a chemokine secreted by lymphocytes and natural killer cells. Soluble lymphotoxin  $\alpha$  homotrimers can bind to the same receptors as TNF $\alpha$  whereas heterotrimers formed with membrane-bound lymphotoxin  $\beta$  interact with the lymphotoxin  $\beta$  receptor. Through these receptors, lymphotoxin  $\alpha$  exerts pleiotropic immunomodulatory effects. Recently, several loci in the HLA class II region including LTA were linked to susceptibility to leprosy in Brazil.<sup>32</sup> A G to A substitution abolishes an *Nco*I restriction site in intron 1 of LTA. This RFLP was not found to be associated to leprosy susceptibility on its own, however, two locus transmission disequilibrium testing indicated that the haplotype TNF\*1/LTA\*2 was associated with susceptibility while TNF\*2/LTA\*2 was associated with protection. As no transmission distortion was observed for the haplotype TNF\*1/LTA\*1, these data suggest that another polymorphism that occurs on a background of LTA\*2 may have a role in leprosy susceptibility. Polymorphisms that occur within the LTA gene and its regulatory regions<sup>80,81</sup> are potential candidates.

*Complement component 4B (C4B)*. Erythema Nodosum Leprosus (ENL) is an immune reaction which can occur in lepromatous leprosy patients and is postulated to be immune complex mediated. C4B is a class III gene which encodes the complement component 4B. The protein is involved in opsonization of pathogens and immune complex clearance. Non-expressed C4B alleles (C4B\*Q0) were found to be associated with lepromatous leprosy, and especially ENL in Brazil.<sup>82</sup> The possibility of linkage disequilibrium with other MHC loci was not examined.

*Heat shock 70kD protein 1A (HSPA1A)*. HSPA1A is also located in the class III region of the MHC, between the TNF and C2 genes. One of three 70kD heat shock proteins encoded in this region, HSPA1A is expressed constitutively at a low level and its expression is increased in response to thermal stress. Antigenic peptides can associate with HSP70s, leading to their uptake and presentation by antigen presenting cells.<sup>83</sup> Furthermore, HSP70 activates macrophages by induction of pro-inflammatory cytokine production via the Myd88/NF kappa B pathway.<sup>84</sup> This HSP70 response is mediated by TLR2 and TLR4, and indeed TLR2 and TLR4 may act synergistically, in a CD14-dependant fashion, to activate NF kappa B.<sup>85</sup> Another role of HSP70s

is to block decay of the AU-rich mRNA of cytokines and proto-oncogenes.<sup>86</sup> Three HSPA1A alleles, A–C, result from nucleotide substitutions at two sites (–110 and +120). The –110 position lies adjacent to a heat shock transcription factor binding site<sup>87</sup> and thus may affect the inducibility of HSPA1A. The HSPA1A-A allele was found to be associated with tuberculoid leprosy in a small North Indian study.<sup>88</sup> In Italians, the HSPA1A promoter polymorphisms were associated with specific HLA class II haplotypes<sup>87,89</sup> including those carrying HLA DR3; however, in North India the HSPA1A association appears to be independent of both class I or II loci, indicating that HSPA1A may play a role in susceptibility to leprosy in this population.

### Other candidates

*Solute carrier family 11 member 1 (SLC11A1, formerly NRAMP1).* Located at 2q35, SLC11A1 is the human homologue of the mouse gene *Slc11a1*. Mice with a naturally occurring Gly169Asp mutation are susceptible to a range of intracellular pathogens including *Leishmania donovani*, *Samonella typhimurium*, some strains of *M. bovis*,<sup>90</sup> *M. lepraemurium*,<sup>91,92</sup> *M. intracellulare*,<sup>93</sup> *Toxoplasma gondii*,<sup>94</sup> *Candida albicans*<sup>95</sup> and *Leishmania infantum*,<sup>96</sup> but probably not *M. tuberculosis*.<sup>97–99</sup> This substitution of a charged amino acid in one of the putative transmembrane domains may cause misfolding of the *Slc11a1* protein, resulting in it being targeted for degradation at the endoplasmic reticulum. Null mutants have the same susceptibility phenotypes as those mice carrying the naturally occurring point mutation<sup>90</sup> indicating that the point mutation results in a completely non-functional gene product.

The biochemical function of the SLC11A1/*Slc11a1* gene product has not been completely elucidated and indeed is the subject of some controversy.<sup>100</sup> The mouse *Slc11a1* encodes an integral membrane protein expressed in late endosomal and lysosomal membranes of macrophages. On phagocytosis it is relocated to the phagosomal membrane where it is thought to act as a divalent cation pump. The controversy arises over the direction in which the cations are pumped. One theory is that resistance is mediated by pumping iron out of the phagosome, thus restricting the cations available to the pathogen.<sup>101</sup> The alternative theory is that the iron is pumped into the phagosome, where it is used as a catalyst in the Fenton/Haber Weiss reduction of superoxide anions to generate toxic hydroxyl radicals.<sup>102</sup> In either case, the *Slc11a1* gene product seems to have a direct antimicrobial effect, but it also has pleiotropic effects that include macrophage activation and regulation of the Th1:Th2 balance of the adaptive immune response to intracellular pathogens.

Macrophages containing the mutant *Slc11a1* have a defect in antigen processing for presentation to T-cells<sup>103</sup> possibly due to a metal ion requirement for metalloprotease activity and/or endosomal fusion events. In addition, *Slc11a1* appears to influence MHC class II molecules and cytokines which regulate antigen presentation, such as TNF $\alpha$  and IL1 $\beta$ .<sup>102</sup> Such effects may account for the polarity of Th1 vs Th2 responses observed in mice with wild-type *Slc11a1* compared to those with the mutant gene after infection with *L. donovani*<sup>104</sup> or exposure to typhoid toxin.<sup>105</sup>

Human susceptibility to infection may in part be determined by the direct influence of SLC11A1 on antimicrobial activity of macrophages; however, the reported pleiotropic effects in regulating Th1:Th2 balance in the immune response may also contribute. The mouse Gly169Asp mutation is unknown in the human homologue, but 11 other sequence variants have been described.<sup>106–108</sup> These include two missense coding changes and a promoter microsatellite reported to affect NRAMP1 expression levels.<sup>109</sup> Variants in SLC11A1, especially the higher expressing promoter microsatellite allele 3, have been reported to be associated with rheumatoid arthritis,<sup>110–112</sup> Crohn's disease,<sup>113,114</sup> type 1 diabetes<sup>115</sup> and sarcoidosis.<sup>116</sup> However, these studies have often been of limited size and some associations have not been replicated in other populations. The promoter microsatellite has also been associated with susceptibility to various infectious diseases including HIV in Colombia (allele 2),<sup>117</sup> visceral leishmaniasis, post kala-azar dermal leishmaniasis in the Sudan and severe meningococcal meningitis (allele 3).<sup>118</sup> Interestingly, although *Slc11a1* is not important in the control of *M. tuberculosis* infection in mice,<sup>97</sup> SLC11A1 has been associated with susceptibility to tuberculosis in several human populations including Japanese,<sup>119</sup> Brazilians,<sup>120</sup> Koreans<sup>121</sup> and West Africans.<sup>122,123</sup> In a large Canadian Aboriginal family<sup>124</sup> linkage was found to the chromosomal region containing SLC11A1 but linkage is not found in most studies.<sup>125</sup>

The relevance of SLC11A1 to leprosy susceptibility is less clear-cut. Non-random haplotype segregation in 20 South East Asian leprosy pedigrees implicated SLC11A1 in leprosy susceptibility; however, as haplotype sharing was more pronounced in 16 Vietnamese families compared to four Chinese families, the possibility of ethnic heterogeneity was suggested.<sup>126</sup> Such ethnic heterogeneity may explain why studies in other populations have failed to find SLC11A1 linkage or association with leprosy susceptibility *per se*.<sup>127–130</sup> An association with leprosy type was shown in Mali, where heterozygotes for a TGTG insertion/deletion in the 3' untranslated region were more common amongst multibacillary cases than in paucibacillary cases.<sup>129</sup> The putative functional microsatellite was also typed in the Mali study but no associations were found with its alleles. It is not known whether the TGTG polymorphism has a functional effect.

Recently, the SLC11A1 region was found to be in linkage with the Mitsuda response among both healthy and affected members of 20 South East Asian leprosy families.<sup>131</sup> The Mitsuda response is a measure of the size of the reaction obtained 28–30 days after intradermal injection of *M. leprae* antigen. It is regarded as a measure of the *M. leprae* induced granuloma-forming capacity. Thus, this finding is in agreement with the hypothesis that, in addition to, or instead of being important in control of mycobacterial infection at the macrophage level, SLC11A1 may play a role in the development of acquired antimycobacterial immune responses in humans.

*COL3A1 and CTLA4.* In a very small study, polymorphisms in the COL3A1 (procollagen III alpha I) and CTLA4 (cytotoxic T lymphocyte associated antigen) genes located at 2q31–33 were found to be associated with

leprosy.<sup>132</sup> The dinucleotide repeat examined in CTLA4 occurs in the 3'UTR, and at first alleles were postulated to effect mRNA stability but recently strong linkage disequilibrium has been shown between the dinucleotide repeat alleles and known functional polymorphism located in the promoter and exon 1.<sup>133</sup> Interestingly, the allele associated with protection against leprosy in India also displayed increased transmission to IDDM siblings in Russian families.<sup>134</sup> Indeed markers in this region have been linked and associated with several autoimmune diseases.<sup>135</sup> As CTLA4 is an important negative regulator of T-cell activation it is a plausible biological candidate for leprosy susceptibility; however, both COL3A and CTLA4 lie close to the genes encoding CD28 and ICOS, two other T-cell costimulatory receptors which are also likely candidates. To date, no other reports of associations between leprosy and any of these genes have been reported. Furthermore, it is just possible that the associations found could reflect an underlying linkage disequilibrium with alleles of the SLC11A1 gene at 2q35.

**Vitamin D receptor (VDR).** The vitamin D receptor is a nuclear hormone receptor encoded by the VDR gene (located at 12q12–q14). It is the mediator of the effects of the active form of vitamin D,  $1\alpha$  25(OH)<sub>2</sub>D<sub>3</sub>, which acts as a hormone. VDR contains several domains including a DNA-binding domain (DBD) consisting of two zinc fingers and a ligand-binding domain. On ligand binding, VDR is translocated to the nucleus and a conformational change occurs, allowing interaction with various coactivators. The DBD binds specifically to vitamin D response elements (VDRE) in the regulatory region of vitamin D responsive genes. A stable protein–DNA complex requires a homo- or hetero-dimeric complex with a second partner receptor such as the retinoid X receptor (RXR). This complex of nuclear receptors, vitamin D and coactivators can then act to modulate transcriptional activities. Genes with VDRE in their promoters include osteocalcin, hence the association of VDR polymorphisms with vitamin D resistant rickets, bone density and height in some studies.<sup>136–138</sup>

In addition to its role in calcium metabolism regulation,  $1\alpha$  25(OH)<sub>2</sub>D<sub>3</sub>, acting through VDR, is a potent immunomodulator involved especially in suppression of inflammation. Some of its immunomodulatory effects may be mediated via VDRE; however, it appears that other complex cross-talk interactions are also involved. Recently, it has been shown that the DBD of VDR interacts with Stat1, the nuclear transcription factor of IFN- $\gamma$ .<sup>139</sup> In addition to antagonizing the transcriptional activation of a specific VDRE-containing gene by preventing interaction between VDR and RXR, this interaction is proposed to prolong Stat1-mediated induction of IFN- $\gamma$ -regulated genes in macrophages by protecting Stat1 from inactivation by tyrosine dephosphorylation.

Several cytokine genes including IL2,<sup>140</sup> GM-CSF<sup>141</sup> and IFN- $\gamma$ <sup>142</sup> are direct targets of  $1\alpha$  25(OH)<sub>2</sub>D<sub>3</sub>/VDR-mediated repression in activated T-cells. VDR/RXR also represses IL12 p40 expression in activated macrophages and dendritic cells.<sup>143</sup> IL12 is important in the development of a Th1 response and initiation of a cell-mediated response to pathogens. Through VDR,  $1\alpha$  25(OH)<sub>2</sub>D<sub>3</sub> inhibits fasL (CD95) mRNA production.<sup>144</sup> FasL mediates

activation-induced programmed cell death in activated T lymphocytes, but it also induces maturation of dendritic cells, resulting in upregulation of MHC class II molecule expression and secretion of proinflammatory cytokines.<sup>145</sup> Thus through its receptor, the hormone  $1\alpha$  25(OH)<sub>2</sub>D<sub>3</sub> has pleiotropic anti-inflammatory effects that may inhibit the development of a protective Th1 response.

Several polymorphisms have been described in the VDR gene, including three neighboring RFLPs: *BsmI* and *ApaI*, located in intron 8, and a silent T to C polymorphism in codon 352 that creates a *TaqI* RFLP in exon 9. Exon 9 encodes part of the ligand-binding domain of VDR. The polymorphisms are designated Aa (*ApaI*), Bb (*BsmI*) and Tt (*TaqI*), where the uppercase letter signifies absence of the restriction site and lowercase signifies the presence of the restriction site. The AA and BB genotypes have been associated with increased serum osteocalcin levels.<sup>146</sup> The B and t RFLPs were in strong linkage disequilibrium in Australian Caucasians, and the BB genotype correlated with bone mineral density in the same population.<sup>146</sup> In a luciferase reporter gene assay, haplotypes bearing BAT produced more activity than those bearing baT,<sup>147</sup> although the functional significance of these variants, if any, is unclear.<sup>147,148</sup>

Nevertheless, linkage analysis identified VDR as a positional candidate for inflammatory bowel disease and the tt genotype was subsequently found to be associated with Crohn's disease in Caucasians.<sup>149</sup> In Bengali Indians, the tt genotype was associated with the more resistant form of leprosy, while TT was associated with lepromatous leprosy.<sup>128</sup> Likewise, in The Gambia, tt was found to be associated with resistance to tuberculosis.<sup>150</sup> Consistent with these findings, both  $1\alpha$  25(OH)<sub>2</sub>D<sub>3</sub> deficiency and the combination of the presence of a T allele and  $1\alpha$  25(OH)<sub>2</sub>D<sub>3</sub> deficiency were associated with tuberculosis susceptibility in Gujarati Indians living in London.<sup>151</sup> In contrast, susceptibility to pulmonary disease caused by infection with the opportunist *M. malmonese* was associated with increased frequencies of the t allele, the A allele and the At haplotype<sup>152</sup> and in Southern Indian females the tt genotype was associated with susceptibility to pulmonary tuberculosis.<sup>153</sup>

These opposing results are difficult to resolve if the silent *TaqI* polymorphism does have a direct impact on the immunomodulatory function of VDR; therefore, it seems more likely that other polymorphisms (or combinations thereof) in linkage disequilibrium with the *TaqI* RFLP may account for the associations with mycobacterial resistance and susceptibility described above. Ethnic differences in the linkage disequilibrium of some VDR polymorphisms have been described.<sup>154</sup> In addition to polymorphisms in the 3'UTR, an RFLP that results in structurally and functionally distinct VDR isoforms occurs in the first ATG start codon of VDR. This T to C substitution abolishes a *FokI* restriction site, resulting in translation initiation at an in-frame start site three codons downstream. Allele designation follows that for the 3' RFLPs; the f allele encodes 427 amino acids while the F allele encodes 424 amino acids. The F allele product interacts more efficiently with the human basal transcription factor IIB (TFIIB) and exhibits enhanced transcriptional activity.<sup>155</sup> In addition, this allele is associated with increased bone mineral density

whereas the *f* allele may be associated with protection against pulmonary disease caused by *M. malonesi*.<sup>152</sup> As yet no studies investigating association between the *FokI* RFLP and leprosy susceptibility have been reported.

**Toll-like receptor 2 (TLR2).** Toll-like receptors (TLRs) are pattern recognition receptors able to activate direct antimicrobial effector mechanisms in response to various microbial components. Also, activation of mammalian TLRs facilitates transcription of genes that regulate the adaptive response, including cytokines and co-stimulatory molecules. TLR2 is able to respond to many microbial components, including mycobacterial lipoproteins and lipoarabinomannan<sup>156,157</sup> and appears to be very important in generating a pro-inflammatory, protective immune response against mycobacteria in mice.<sup>158</sup> In both mice and humans, TLR2 activation by *M. tuberculosis* lipoprotein leads to killing of intracellular *M. tuberculosis*. Killing is nitric oxide (NO) dependant in mice, but in humans this process is NO independent.<sup>159</sup> Recently, 86 Korean leprosy patients were screened for polymorphisms in a highly conserved part of the TLR2 intracellular domain. A previously undescribed C to T substitution that results in an arginine to tryptophan change at the highly conserved amino acid 677 was found in 22% of the 45 lepromatous leprosy patients.<sup>160</sup> No other polymorphisms were found in the region and it was hypothesized that the substitution may affect the intracellular signaling of TLR2. Another polymorphism of a conserved C-terminal arginine in TLR2 reduces NF kappa B activation in response to Gram-positive bacterial peptides and may be associated with susceptibility to staphylococcal infection,<sup>161</sup> consistent with the hypothesis that TLR2 has an important role in the protection against bacterial infections in humans.

**Mannose-binding lectin (protein C) 2 (MBL2).** Located at 10q11.2–q21, MBL2 encodes a calcium-dependant mannose-binding lectin (MBL, also called mannose-binding protein (MBP)) found in serum. MBL binds to arrays of terminal mannose groups on a variety of bacteria. Binding can initiate complement activation, and promote opsonophagocytosis independent of antibodies and Clq. Any one of three exon 1 point mutations (in codons 52, 54 and 57) in MBL2 reduces MBL serum concentrations, probably by interfering with the oligomerization of the protein. Low levels of MBL may be associated with recurrent infections in young children, yet low producing alleles are found with reasonable frequency in all populations, leading to the hypothesis that the disadvantage of increased infection susceptibility in infancy may be counterbalanced by unknown advantages of low MBL concentration.<sup>162</sup> As mycobacteria utilize phagocytosis to gain entry into host cells, it has been hypothesized that low MBL levels may be protective against mycobacterial infection by limiting this entry route.<sup>163</sup> In agreement with this hypothesis, increased MBL levels have been observed in active and fully recovered South African Cape Coloured tuberculosis patients,<sup>164</sup> Tanzanian tuberculosis patients<sup>165</sup> and in a small number of Ethiopian leprosy patients.<sup>163</sup> Exon 1 variants underlying serum MBL deficiency were found less commonly in

Cape Coloured meningeal tuberculosis,<sup>164</sup> but not in Gambian pulmonary tuberculosis patients.<sup>166</sup> Intriguingly, it appears that pathogenic mycobacteria may encourage opsonization by production of a molecule that interacts with C2a to form a C3 convertase that results in deposition of C3b on the bacterial wall.<sup>167</sup> However, no associations have been reported between leprosy susceptibility and MBL2 variants, and in contradiction to the above reports, MBL2 variants were found with increased frequency in a limited Indian pulmonary tuberculosis study.<sup>168</sup>

## Genome-wide linkage analysis

The development of high throughput genotyping technologies and the identification of thousands of polymorphic microsatellite markers have made genome-wide linkage studies possible. This approach has the advantage that no disease model or prior knowledge of the structure, function or location of the disease gene is required. The chromosomal regions identified by this approach will initially be much larger than that of an association study (several megabases compared to a few hundred kilobases); however, fine mapping and subsequent identification of the genes involved has been aided greatly by the release of the draft human genome sequence. The genome-wide approach also has the advantage that genes of unknown function and those not previously suspected as possible candidates can be identified.

To date, very few genome-wide linkage studies of infection or infectious diseases have been published. The first pathogen to be investigated in this way was *Schistosoma mansoni*. The parasite burden in 11 extended families from Brazil was examined and a region on chromosome 5 (5q31–33) was identified.<sup>169</sup> Further studies of *S. mansoni* and malaria parasite density also showed linkage to this region, which contains a cluster of genes encoding several Th2 cytokines.<sup>170,171</sup> Linkage to this same region of chromosome 5 has also been identified in a study of candidate regions for atopy and asthma.<sup>172–174</sup>

Bellamy *et al* carried out a genome screen for tuberculosis susceptibility loci in 136 African families containing 173 independent affected sib pairs.<sup>125</sup> Two chromosomal regions showed suggestive evidence of linkage (Xq27 and 15q11). Although the evidence of linkage did not reach the criteria for genome-wide statistical significance,<sup>175</sup> further support for the presence of the loci was obtained using the method of common ancestry mapping. Very recently, genome-wide linkage scans have also identified new susceptibility loci for persistent hepatitis B virus infection (Frodsham *et al*, unpublished).

Leprosy is a disease that is well suited to genome-wide linkage studies in that it has a clear phenotype and the disease progression is slow. The disease has a very low mortality rate that not only enables the collection of older family members, but also suggests that if a major susceptibility gene does exist, it might be able to persist in the population. Segregation studies have suggested the presence of a major gene controlling susceptibility to leprosy; however, the gene associations previously detected in candidate gene studies appeared only to

contribute a small proportion to the overall genetic component.

Only one genome screen for leprosy susceptibility has been published to date. A two-stage genome scan was carried out in South India using a total of 224 families (245 independent sib pairs) with mainly the tuberculoid form of leprosy.<sup>176</sup> A region of strongly significant linkage was identified on chromosome 10p13 ( $P < 2 \times 10^{-5}$ ). A gene that lies at the peak of this linkage is the macrophage mannose receptor (MRC1). This receptor mediates phagocytosis and endocytosis of pathogens by recognition of the mannose and fucose structures present on their surface.<sup>177</sup> The terminal mannosyl units of lipoarabinomannan (LAM) from the surface of *M. tuberculosis* were found to be ligands for MRC1 allowing phagocytosis of the organism.<sup>178</sup> The receptor also appears to have a role in the processing of glycolipids derived from mycobacteria.<sup>179</sup>

It is therefore possible that MRC1 could also have a role in the immune response to *M. leprae* infection. To fully define the relevance of this positional candidate gene in leprosy, an extensive analysis of numerous variants in this gene in the South Indian families used for the genome-wide scan is in progress (Tosh *et al*, unpublished).

Although the region of linkage on chromosome 10p13 is thought to contribute substantially to the total genetic component of leprosy susceptibility in this population, a follow-up of markers showing weaker evidence of linkage identified a second region of linkage on chromosome 20p12 ( $P = 0.00003$ ).<sup>180</sup> This linkage is not as strong as that identified on chromosome 10; however, it is interesting that this same region of chromosome 20 (p12) has been found to determine susceptibility to atopic dermatitis and psoriasis.<sup>172,174</sup> These are all diseases caused by inappropriate immune responses to an environmental stimulus. This suggests the presence of a gene involved in the regulation of immune responses, although no strong positional candidates have been identified as yet.

Even though there have been many studies showing linkage and association of the MHC to leprosy, no strong linkage was detected in South India, suggesting heterogeneity in genetic susceptibility. Furthermore, Tosh *et al*, showed that the strength of the chromosome 20 linkage differs in the two geographic regions of India studied. Also, as the sibpairs studied were mainly paucibacillary, it will also be interesting to see if the two regions identified so far contain susceptibility loci for leprosy *per se* or to tuberculoid leprosy.

It is encouraging that regions of linkage, reaching genome-wide statistical significance, have been identified as it seemed possible that infectious diseases were too polygenic to have enough power to detect linkage using the genome-wide approach; however, further genome-wide linkage studies in different populations will be required to determine the extent of genetic heterogeneity that exists for leprosy susceptibility.

## Conclusions

Thus far all the genes suggested to have a role in the susceptibility to leprosy either act to directly modulate development of the adaptive response (HLA, MICA,

TAP2, CTLA4, VDR), or may bridge the innate and adaptive responses (NRAMP1, TLR2, HSP70, TNF $\alpha$ , MRC1). This is consistent with the idea that an appropriate cell-mediated response is critical in the control of mycobacterial infection. Many of the associations, have only been found in a small series of patients, or in a single population (Table 1), and should be repeated in larger studies. Lack of correlation in results between populations should not necessarily be regarded as a negation of initial associations but may instead reflect heterogeneity in the genetic susceptibility to this enigmatic disease.

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