REVIEW

Genetics of host response in leprosy

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Summary In this review, we discuss recently accumulated data, analysing genetic influence on leprosy outcome. Most leprosy-related epidemiological studies are based on the comparison of frequencies of genetic markers in case-control designs using candidate genes, mainly on immunological pathways. Genomic scans using family-based designs also identified some chromosome regions to be tested for association with leprosy. The results have suggested that different genes are implicated in resistance/susceptibility to leprosy, such as tumour necrosis factor-alpha (TNFα), interleukin (IL)-10, vitamin D receptor (VDR), and parkin, although some of the results obtained in different populations are controversial. In spite of the recent advances in genomics and genetic epidemiology we have experienced, the results must be confirmed using better designed epidemiological studies to directly pinpoint the genes responsible for leprosy outcome. Furthermore, there is a clear requirement of functional/biological data in order to validate epidemiological findings. In this way, these genetic markers could be used to screen high-risk populations introducing gene testing as diagnostic and prognostic tools to interrupt the chain of transmission and prevent neurological damage.

Introduction

Leprosy outcome is influenced by the genetic background of the host and also environmental features like nutritional status, BCG vaccination and exposition rates to *Mycobacterium leprae* or other mycobacteria. Even though several clinical and epidemiological evidences suggest that leprosy has a genetic influence, some issues should be highlighted: (i) leprosy is an infectious disease in which the genetic diversity of the pathogen is very low[1] and could not

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account for the major clinical differences observed among patients; (ii) epidemiological studies have been reporting consistently that contacts exhibit the highest risk of developing the disease (household contacts with close consanguineous relationship to the index case are at the highest risk);[2] (iii) very few people, only 0·1–1% of the infected population, are susceptible to leprosy; (iv) twin studies showed that disease and disease type is more frequent among monozygotic twins when compared to dizygotic twins; (v) there are some reports suggesting that populations with different ethnic backgrounds living in the same endemic areas exhibit distinct prevalence rates;[3] (vi) clinical forms in leprosy are not interchangeable; (vii) BCG protection rates are highly variable among different populations; (viii) complex segregation analyses have suggested a predominant genetic rather than environmental model for associations in leprosy.

During the natural course of the disease some steps can be clearly identified. Thus, leprosy can be divided into: (i) leprosy *per se*; (ii) severity of clinical forms; and (iii) occurrence and severity of reactional states. The host responses in every one of those stages can originate a specific outcome (Figure 1). An infected person can either cure or develop the disease. In the latter case, it may evolve towards a mild (tuberculoid) or a severe (lepromatous) state of leprosy. Some patients can interrupt the natural course of the disease with inflammatory episodes of either type I (reversal reaction, RR) or type II (erythema nodosum leprosum, ENL). It is clear that immunological responses play a critical role in controlling each one of these stages, although response patterns can be modified along the course of the disease.

Resistance to intracellular pathogens such as mycobacteria is associated with the ability to mount T helper (Th)1 responses. The first line of interaction of *M. leprae* with humans is mediated by host receptors that recognize patterns of mycobacteria. Thus, Toll-like receptors (TLR) on phagocytic cells, especially TLR-2, are activated by *M. leprae* lipoproteins and the ability to start protective responses is directly associated to interleukin (IL)-12/23 secretion and differentiation of type-1 macrophages and dendritic cells.[4,5] This process can lead to expansion and differentiation of Th1 interferon (IFN)γ-producing cells that, in turn, induce *M. leprae* killing and control of disease spread. Furthermore, the dichotomy of Th1 versus Th2 as an explanation for clinical features observed among tuberculoid and lepromatous leprosy patients, respectively, also reflects the ability to mount an effective cellular immune response against the mycobacteria, consequently preventing the development of severe forms of the disease.
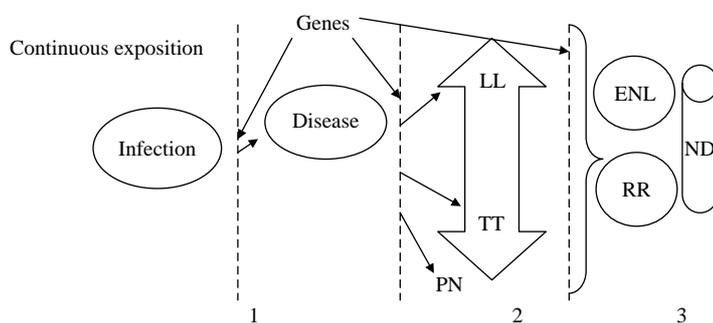

Figure 1. Natural history of leprosy: stages of infection and disease. Genetic factors can contribute to: outcome of the disease (1); severity of clinical forms (2); and occurrence of reactional episodes and nerve damage (3). LL = lepromatous; TT tuberculoid; PN = pure neural; ENL = erythema nodosum leprosum; RR = reversal reaction; ND = nerve damage.

The most categorical example towards resistance mediated by IL-12/23-IFNγ axis to mycobacterioses was obtained with rare hereditary diseases of patients exhibiting infection to low virulence intracellular pathogens, including *M. avium*, *M. bovis*-BCG, and *Salmonella spp.*[6] Genetic analyses in those patients revealed truncating mutations in IFNγ receptors or IL-12/23 common subunit (p40) and IL-12/23β1 receptors. Tumor necrosis factor-alpha (TNFα) is another very important cytokine involved in the regulation of mycobacterial diseases. The administration of anti-TNFα drugs for autoimmune diseases (rheumathoid arthritis and Crohn's disease) was capable of activating *Mycobacterium* latent infection and patients developed pulmonary tuberculosis or leprosy.[7,8] This example demonstrated the critical role of TNFα in granulogenesis and latency control of mycobacterium infection preventing dissemination.

Obviously, both situations described above are rare and very drastic. In common diseases like leprosy it is not expected to observe patients with Th1 responses completely knocked out, but it is possible that decreased expression of IL-12/23 reflects a down modulation of IFNγ secretion and an inability to mount strong Th1 protective responses resulting in disease. One hypothesis is that leprosy outcome is defined by the strength of Th1 responses once *M. leprae* challenge begins (Figure 2).

Thus, regulation of cytokine production can influence the strength of immune responses and it is expected that subtle differences in the levels of cytokines secreted could account for the establishment of different phenotypes (leprosy versus healthy or LL versus TT). Some
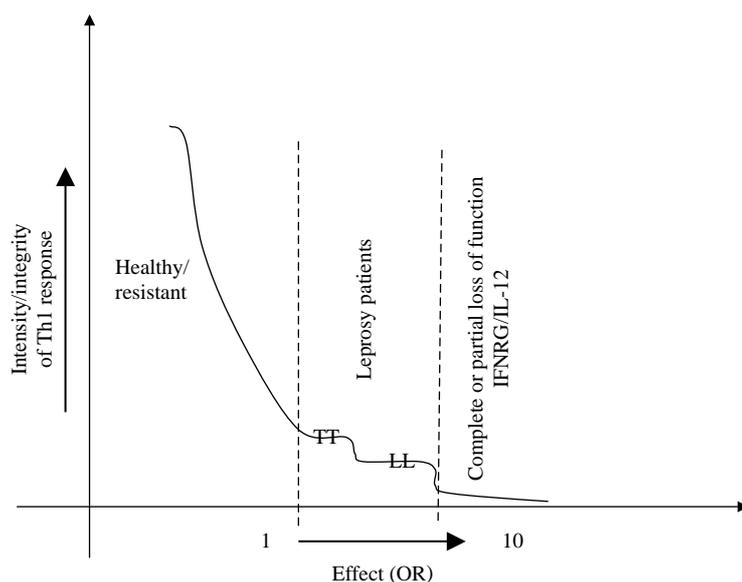

Figure 2. Genetic influence on immunological responses and resistance to intracellular pathogens, using leprosy as an example. According to the level of Th1 responses there are healthy individuals able to mount strong Th1 responses. At the other extreme, there are patients who develop infections caused by low virulent mycobacteria unable to activate IL-12/23-IFNγ axis. It is possible that leprosy patients are in the middle of these two extremes and within leprosy, the tuberculoid patients have stronger Th1 responses as compared to lepromatous ones, although during inflammatory reactions an upregulation of Th1 responses is observed. Since leprosy is a complex disease, it is expected that genetic effect evaluated as odds-ratios of different markers in leprosy patients varies from 1·3–4·0. Subtle genetic variations, such as SNPs, can modulate the levels of cytokines and consequently, immunological responses and could be responsible for the differences observed among individuals.

genetic polymorphisms are able to regulate cytokine levels. The most common and studied of those polymorphisms are microsatellites and single nucleotide polymorphisms (SNPs). The latter are biallelic point substitutions (G-A, C-T, C-A and so on) lying on intragenic non-coding regions, like promoters and introns, but also on coding as well as intergenic regions. As a result, SNPs can alter binding affinity to transcriptional factors at promoter regions or modify splice sites. When SNPs are located at coding regions they can change an amino acid leading to variations in protein structure and/or function. Use of SNPs also provides a chance to link biological phenomena to epidemiological evidences, since SNPs can also be used as genetic markers in disease association and linkage studies.

Recently, extended haplotypes have increasingly been used as genetic markers. Haplotypes are combinations of two or more polymorphisms (SNPs in this case) within a single chromosome in an individual. It has become a very popular approach to study the association of haplotypes with diseases when two or more SNPs are in linkage disequilibrium (LD) in the population, i.e. the SNPs are inherited together more frequently than it would be expected by chance alone. The advantages of using haplotypes instead of individual SNPs are that: (i) less information is contained in a SNP alone than in a combination of SNPs that are in LD; (ii) correction for multiple comparisons (e.g. Bonferroni) would have to be employed if the information gathered separately from several SNPs were combined, considerably lowering the power to detect associations; iii) there would be an increased chance of gathering epidemiological along with biological evidences since a larger region of a chromosome is analysed.

Genetic case-control studies for infectious diseases

There are two basic approaches in genetic epidemiology to study the association of genetic markers, such as SNPs, with a disease: (i) family studies; and (ii) population-based candidate gene association studies. Both approaches have advantages and disadvantages, some of which were recently discussed elsewhere.[9]

Case-control studies are the most popular in genetic epidemiology because of the simplicity of recruiting either healthy individuals (controls) or patients (cases). This type of design has been largely employed in leprosy studies; generally SNPs are used as markers in candidate genes, like TNFα and IL-10, and comparisons are made with unrelated (independent) controls. The design of these studies are just like any classical epidemiological study, i.e. cases are identified in the source population assessed for exposure to a risk or protection factor and other covariates, and controls are chosen randomly from the source population, where we expect, on average, that controls differ from cases only by their outcome.

Case-control studies can be challenging to design and extreme care should be taken to choose suitable controls, in order to avoid bias and control for confounding variables.[10] It is not uncommon to find articles in the literature that use inadequate controls in case-control studies of infectious diseases. It is likely that some of the results obtained are spurious associations due to poor selection of the controls. Moreover, the different results obtained for the same SNPs/genes in studies conducted in different populations also suggest some sort of bias in few of these designs. For example, most of the published papers compare leprosy cases with healthy blood donors. It should be considered that controls have to be exposed to *M. leprae* and not develop infection in order to guarantee one of the basic concepts about controls: they need to have some probability of getting the disease. In endemic countries like

Brazil and India, depending on the city, and specifically the area being studied, a variation of 20–100% exposure to *M. leprae* is estimated, as evaluated using PGL-I antibody detection (X. Illarramendi, personal communication). Thus, if blood donors are the best choice to obtain healthy controls, it is mandatory to choose a hospital/clinic located nearby the area where the leprosy out-patient is and if possible run a PGL-I ELISA to test rates of latent infection. Even though, it would be impossible to tell for sure if a person was exposed or not to a certain infectious agent, there are some ways to at least increase this probability, which would be to work with household contacts.

Another key feature that should be carefully handled is the calculation of a suitable sample size. On one hand, the sample size should not be so small that it will not allow tests with enough power to detect a clinically significant difference; on the other hand, given scarce resources, it should not be bigger than necessary to provide adequate power to test the phenomenon being studied, to avoid unnecessary waste of time and money. Sample size depends on several factors: desired power, probability of type I error, the magnitude of the difference to be tested and the variance of the variable studied in the source-population. Simulations would help find a suitable sample size, which is done by the construction of power curves and evaluation of different scenarios, given some guesstimation for prior parameters, which describe a very simple situation, when comparing two proportions in a balanced setting (they could be allelic proportions, genotype proportions in a dominant model, etc). Note that for very common situations in our setting, where we are dealing with low baseline proportions, huge sample sizes would be required to detect low differences, suggesting that many of the studies are underpowered (please check below "candidate genes" and Table 1).

For case-control studies that use independent controls, the analytical tools available for classical epidemiology have also been used in genetic case-control studies. Logistic and log-linear regression models are suitable for many analyses that are carried out in those studies, when we are trying to infer the association between SNPs genotypes or alleles with a certain disease. Recently, a method that combines generalized linear models (GLM) with the expectation-maximization (EM) algorithm have been proposed,[11] in which a score statistic is used to infer the association of haplotypes of unknown phase and diseases, and with the possibility to include covariates in the model.

Candidate genes

As discussed above, due to the importance of immune response in leprosy, the most common candidate genes are those of cytokines and other important molecules in immunological pathways. One of the first studies reporting candidate gene experimental design was performed with the transporter associated antigen processing molecule 2 (TAP2), which is located on chromosome 6p21, and plays an important role in peptide translocation inside the cell and their binding to major histocompatibility complex (MHC) molecules. A study performed among Indians reported association of the TAP2-B allele with susceptibility to tuberculoid leprosy.[12]

HUMAN LEUKOCYTE ANTIGEN COMPLEX

The human leukocyte antigens (HLA) complex, located on chromosome 6p21, is the most polymorphic genetic system in mammalians. The well known function of the HLA gene

Table 1. Leprosy association studies using the candidate gene approach and SNPs as markers. Susceptibility or protection phenotypes are shown with odds ratio (OR) and statistical significance (*P*-value)

Gene	Population origin	SNP/Haplotype	Phenotype	Reference
VDR	India	Codon 352 (TT)	Susceptibility to lepromatous form, (OR = 1·67; *P* = 0·03)	42
		Codon 352 (CC)	Susceptibility to tuberculoid form (OR = 3·22; *P* = 0·001)	
		Codon 352 (TC)	Protection (OR = 0·58; *P* < 0·01)	
	Malawi	Codon 352 (CC)	Susceptibility (OR = 4·3; *P* = 0·004)	31
CR1	Malawi	K1590E (GG)	Protection (OR = 0·3; *P* = 0·02)	31
TNFA	India	−308A	Susceptibility to lepromatous form (OR = 3·0; *P* = 0·02)	27
	Brazil	−308A	Protection against leprosy *per se* (OR = 0·62; *P* = 0·05) and multibacillary forms (OR = 0·52; *P* = 0·02)	28−29
	Malawi	−238/−308/−376/−863	No association	31
IL10	Brazil	−819T	Susceptibility (OR = 2·28; *P* < 0·01)	29
	Brazil	−3575A/−2849G/−2763C	Protection against leprosy *per se* (OR = 0·35; *P* = 0·0005) and multibacillary forms (OR = 0·32; *P* = 0·006)	38
		−3575T/−2849A/−2763C	Susceptibility (OR = 2·37; *P* = 0·027)	
	India	−3575T/−2849G/−2763C/ −1082A/−819C/−592C5	Protection (OR = 0·58; *P* = 0·01)	39
	Malawi	−592/−819/−1082	No association	31
IL12RB2	Japan	−1035G	Susceptibility (OR = 3·97; *P* < 0·001)	47
		−1023G	Susceptibility (OR = 2·95; *P* < 0·01)	
		−464G	Susceptibility (OR = 3·64; *P* < 0·01)	

products is the processing and presentation of peptide antigens to T cells.[13] Thus, proper activation of T cell subsets, in the context of T cell receptors and major histocompatibility complex, might be influenced by HLA haplotypes. This can reflect the amount of IFNγ production upon *M. leprae* stimulation and it is historically accepted that HLA is involved in differential development of leprosy clinical forms.[14] HLA molecules are cell surface glycoproteins divided into two groups: HLA class I (A, B and C) and class II (DR, DQ and DP) antigens. Case-control studies on HLA loci as candidate genes have described different haplotypes of either class I or class II regions associated with susceptibility or resistance in different populations. In Chinese, a negative association of HLA-B46 antigen was observed in leprosy patients, suggesting that there might be an HLA-B-linked disease resistance.[15] Recently, the existence of positive association of the HLA A*0206, A*1102, B*4016, B*5110, Cw*0407, and Cw*0703 alleles with the disease, and also a highly significant association of the HLA-A*1102-B*4006-Cw*1502 haplotype[16] with leprosy susceptibility was reported in Mumbai.

However, a greater number of studies analyzing HLA class II were conducted showing more consistent associations. In a family study, using co-segregation analyses in Latin American people have shown the presence of HLA-DQw1 associated with LL patients.[14] On the HLA-DRB1 locus, several studies have provided evidence for the association of the previously assigned HLA-DR2 (now DRB1*15 and DRB1*16) with susceptibility to leprosy

(both lepromatous and tuberculoid) in India,[17,18] Thailand[19] and Brazil.[20] Some other alleles were also implicated in leprosy susceptibility, especially HLA-DRB1*03 in Surinam[21] and Mexico.[22]

In 1998, Rani and coworkers,[23] described in India a new subset of DR2 (HLA-DRB1*1506) that differs from DRB1*1501 only at codon 50 in the second exon, where the nucleotide sequence has changed from GTG to GCG, resulting in one amino acid substitution from valine to alanine in DRB1*1506. This codon (50) has always been considered non-polymorphic and it becomes necessary to study which DRB1*15 carriers are truly susceptible to leprosy.

NON-HLA GENES

There are increasing numbers of epidemiological case-control studies in leprosy. Some association studies using non-HLA candidate genes approach using SNPs as markers are highlighted in Table 1 and some others polymorphisms are discussed along the text. Only data for LTα/TNFα, IL-10, and vitamin D receptor (VDR) have been assessed in more than one population. Some of the studies confirm the previous findings, but not always the same alleles are associated with leprosy *per se* or some specific form of the disease. Moreover, very few papers report biological relevance of the epidemiological findings, which is a huge gap in genetic studies of leprosy.

Tumour necrosis factor alpha (TNFA) and lymphotoxin alpha (LTA)

TNF-α and lymphotoxin-α (LT-α) are structural and functionally related proteins. They are pro-inflammatory cytokines with some redundant functions as they share the same receptor. The TNF genes are located on class III MHC region at 6p21·3. TNFα is involved in inflammatory response in the skin[24] and in granuloma formation after mycobacterial infection.[25] The TNFA gene is the most common target of association studies in leprosy, especially the G-A promoter SNP at position -308. The functional role of this polymorphism in gene expression has been widely tested. However, differences between protocols, including stimuli used and time of exposure, has led to opposite results.[26] Nevertheless, it is believed that -308A is associated with higher levels of TNFα secretion. The first study with TNFα SNPs in leprosy reported susceptibility to lepromatous leprosy among carriers of the -308A variant.[27] Other studies, in a Southern Brazilian population, reported opposite results where the protective effect of the same allele against leprosy *per se* was observed.[28,29] Another group performed both linkage and association studies with Northern Brazilian families. At first, the importance of the TNF locus in linkage studies was reported, after which association studies were developed using transmission disequilibrium tests and replication of the results showed protection against leprosy *per se* for the -308A allele.[30] Even though, Fitness and coworkers (2004)[31] did not find associations of -308 TNFα promoter SNP with leprosy.

LTα gene was also studied where a SNP located in the first intron, at the $+252$ position (A-G) was investigated. No association between $+252$ and leprosy was found, although when analysis was performed with haplotypes, a protective association with the -308A/$+252$G haplotype resulted in protection.[30] Functional characterization using gene reporter assays demonstrated an increase in LTA gene expression in the presence of allele G.[32] Taken together, it seems that, at least in Brazilians, the -308A carriers (and -308A/$+252$G

carriers) are able to restrict *M. leprae* replication and progression of disease possibly by production of higher TNFα and LTα levels.

Indeed, the functionality of the −308A TNFα promoter SNP can be measured indirectly using clinical and biological parameters. For example, the −308A allele was found to be associated with a stronger response to heat-killed *M. leprae* in borderline tuberculoid leprosy patients.[33] Slit skin smears can also be used as an alternative for *in vivo* determination of the bacterial load. Our recent data[34] demonstrate that the TNFα −308A SNP has an impact on the amount of bacilli that a patient is carrying, since −308A carriers had lower bacteriological index (BI) than non-carriers. Thus, augmented TNF production seems to be associated with the −308A allele based on this indirect evidence.

Interleukin 10

The IL-10 gene is located at 1q31-q32 and its product is classically considered an anti-inflammatory molecule that counterbalances the effect of proinflammatory cytokines such as TNFα and IL-1β. Also, IL-10 is an immunoregulatory cytokine involved in IL-4-producing T cell expansion,[35] and B cell proliferation and differentiation.[36] High TNF-α/IL-10 levels have been correlated with better prognosis in leprosy outcome among household contacts.[37] The first study of this gene in leprosy was provided by genotyping the promoter SNPs located at position −819 in Brazilians.[29] This study reported a susceptibility phenotype in the presence of allele T. Moreover, IL-10 promoter SNPs were arranged in extended haplotypes[38] and a combination of distal SNPs in a haplotype −3575A/−2849G/−2763C revealed an increased frequency among controls when compared to patients. Furthermore, the −3575T/ −2849A/−2763C haplotype, which is in linkage with allele −819T was associated to the disease. First, the Brazilian study was replicated in Malawians and −1082, −819 and −592 promoter SNPs were not associated with leprosy. In India the data suggested association of the locus with protection, although a different haplotype −3575T/−2849G/−2763C/ −1082A/−819C/−592C was responsible for resistance.[39] This work also found the association of allele −819T with leprosy susceptibility.[39] Therefore, there is confirmatory epidemiological evidence that IL-10 is participating in resistance/susceptibility to leprosy. There is no biological evidence linking those genotypes/haplotypes and IL-10 production in leprosy. Nevertheless, the role of IL-10 in differentiation of type-2 macrophages and Th2 maintenance could suggest that lower levels were expected among resistant individuals. The data obtained with blood cells from healthy donors, stimulated with LPS suggest that the −3575T/−2849G/−2763C/−1082A/−819C/−592C haplotype leads to low IL-10 production.[40] Nevertheless, this needs confirmation for *M. leprae* stimulation.

Vitamin D receptor (VDR)

The VDR gene is located at 12q12-q14 and is responsible for the biological effects of vitamin D in its active form, being produced by monocytes and T and B lymphocytes. The active form of vitamin D, 1a,25-dihydroxyvitamin D_3 (1a,25(OH)2D3), induces monocyte differentiation, while macrophage activation through VDR, in combination with TNFα and IFNγ is responsible for killing of *Mycobacterium tuberculosi*s.[41] The evidence for association of the VDR gene in leprosy was firstly reported in a study in an Indian population (Bengalis), testing for a T-C substitution at codon 352, located at the 3′ gene region.[42] This work detected susceptibility to lepromatous and tuberculoid leprosy in the presence of genotypes TT and

CC, respectively. Recently, a larger case-control study conducted in Malawians reported susceptibility to leprosy *per se* in the presence of the CC genotype.[31] There is no further information about the influence of this SNP on VDR function and the possible consequences in leprosy.

Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1 (SLC11A1)

The SLC11A1 protein, also known as NRAMP1 (natural resistance-associated macrophage protein-1) is located at 2q35 and has a role in ion transportation. NRAMP was described as the gene involved in resistance/susceptibility to intracellular pathogens in mice where a single coding SNP was responsible for susceptibility in Balb/C. In humans, it is more complicated and this direct correlation was not found in patients with infectious diseases caused by intracellular pathogens, even though several reports have tested NRAMP1 for leprosy association. A study using Vietnamese families reported linkage between this locus and leprosy.[43] They also performed association studies with a haplotype composed of six polymorphisms and obtained borderline results ($P = 0.06$). The work by Roy and colleagues (1999)[42] could not find association in a microsatellite in the promoter region. The first association evidence for this gene in leprosy was found in a study where a 4 nucleotide deletion in the 3′ untranslated region was found more frequently among multibacillary patients.[44]

Clinical parameters such as induration of Mitsuda reaction were also used in these studies. Alcais and coworkers (2000) showed that NRAMP1 was linked to lepromin reaction,[45] whereas another group worked with the microsatellite of the promoter region and found that a combination between some genotypes and the lepromin negative reaction could contribute to susceptibility.[46]

OTHER GENES

There are other studies testing for IL-12Rβ1,[47] laminin,[48] and TLR-2[49] which are good candidates for leprosy susceptibility. Indeed, the coding polymorphism at the +7809 position in the laminin gene were associated to genotypes TT and TC and the development of lepromatous and tuberculoid leprosy, respectively, in a study with a small population from Indonesia.[48] A study with TLR2 reported association between a coding polymorphism Arg677trp and susceptibility to the lepromatous form of leprosy.[49] Then, this SNP was not found in several populations analysed even among Brazilians (M. O. Moraes, unpublished data). Recently, Malhotra and colleagues (2005)[50] demonstrated that this alleged polymorphism was in fact the detection of an exon duplication. The IL-12β1 receptor promoter SNPs, associated with lower transcriptional activity, have a significantly increased frequency among lepromatous patients.[47]

Nevertheless, it is important to bear in mind that negative results in association studies can be found more often than positive ones. SNPs in IL12/IL23/IFN-γ genes were screened in a population from Korea and results showed no evidence for association in any of the polymorphisms typed.[51] Fitness and colleagues (2004)[31] reported association for two markers (located at VDR and CR1 genes) among 38, distributed in 13 candidate genes.

Genomic scan studies

The use of genomic scans in leprosy has the ability to uncover a region/gene not apparently associated with disease to be tested as candidate genes. This is a great advantage in the study of complex diseases, especially leprosy, in which low mortality rates enable the collection of samples from several generations, which is necessary in this kind of test.[52] Genetic linkage studies performed for leprosy are summarized in Table 2.

The first study in Indians detected linkage in the 10p13 region, which contains the macrophage mannose receptor, important in lipoarabinomanan recognition.[52] Another study in Indians reported a peak at the 20p12 region.[53] The study developed by Mira and colleagues found linkage in the 6q25 region[54] and also replicated the results in region 10p13 but only for tuberculoid leprosy. The analysis of families used in the first study indicated that most of the families used in the Siddiqui work were composed by paucibacillary patients. Thus, it seems that the 10p13 region is important to leprosy susceptibility, although no further information on the potential genes involved have been provided.

Later, Mira and colleagues (2004)[55] used positional cloning techniques to pinpoint the gene in chromosome 6q25 and scan the region with increasing resolution, i.e. augmenting the number of genetic markers. This methodology identified a promoter region shared by *PARK2* also denoted as parkin and *PACRG* (parkin co-regulated gene). A panel of SNPs depicted as few as two tag SNPs representing a block of approximately 80 kb, located in the *PARK2* promoter region. The haplotype composed by allele T at region -2599 and C at SNP rs1040079 was responsible for the susceptibility phenotype both in Vietnamese families and a Brazilian population.[55]

Parkin (PARK2) and parkin co-regulated gene (PACRG) were first identified during genome-wide studies to find genes and regions linked to early onset Parkinson's disease,[57] and then characterized structurally and functionally as a RING-type E3 ubiquitin ligase.[58] These proteins are very important in a series of functions involving immunological responses, where parkin loss-of-function leads to apoptosis, for example. The specific role of parkin in leprosy susceptibility remains to be defined, but the genetic uncovering in leprosy of a gene involved in neurodegeneration in Parkinson's disease provides new insights of immune response control in leprosy.

A recent study using a panel of 350 healthy controls and 286 leprosy patients reported significant association for allele T at -2599 region. Nevertheless, this significant result disappeared after correcting results for multiple comparisons. Also, haplotype analysis did not replicate the previously reported association.[56] The inability to replicate the associations

Table 2. Genetic linkage studies in leprosy using genomic scan approach

Main associated region	Population analysed	Phenotype observed*	Reference
10p13	Indian	Susceptibility	52
20p12	Indian	Susceptibility	53
6q25	Vietnamese	Susceptibility	54
10p13		Severity (paucibacillary form)	
17q11	Brazilian	Susceptibility	59, 60

*Unless indicated, all susceptibility phenotypes are related to leprosy *per se*.

as observed in parkin haplotypes should be carefully analysed in the light of sample size, allele frequencies and power as suggested above.

The most recent genome-wide study implicated chromosome region 17q11 in susceptibility to leprosy. This region contains many genes involved in immune regulation, such as *NOS2A* and *CCL2* (MCP-1), but none of these regions has been depicted with greater resolution and there is no confirmation of the association of these genes with leprosy.[59,60]

Conclusions

Genetic studies have shed some light on pathways leading to leprosy outcome. Indeed, there is confirmation of the role of the immunological response in protection/susceptibility of the disease. Nevertheless, conflicting results were observed and there are few papers exploring the physiological relevance of the associated genetic variants with leprosy outcome, which imposes difficulties in clearly defining the role of these genes in resistance/susceptibility. The conflicting results primarily reflect biases of design such as bad choice of the statistical tools, poor selection of controls and recruitment of a low number of individuals since most of the studies are underpowered. But, for TNFα, IL-10, VDR and parkin there is confirmation of epidemiological associations in different populations. TNFα $-$308A was associated with lepromatous leprosy in Indians, whereas in Brazilians it was associated with resistance. These results may also be ascribed to an association of other polymorphisms in *TNFA* loci, that could be in linkage disequilibrium to the $-$308A allele, due to differences in haplotypic structures in both Indians and Brazilians.

We cannot rule out some artefacts in genotyping to explain the discrepancies observed among the studies. The Hardy–Weinberg test is generally used to evaluate departures from equilibrium in observed genotypic frequencies. Sometimes these deviations are observed due to genotypic errors and if this is the case it introduces a huge bias in statistical analysis. Indeed, the evaluation of some of the published associated findings demonstrate that either control or patient populations are deviating from the Hardy–Weinberg equilibrium (HWE) in some of the studies, which may indicate some sort of bias.[27,29,31] Thus extreme care should be taken in the interpretation of these data with departure from HWE. Recently, Shin and co-workers (2005)[61] have raised this point towards the $-$1082G/A IL-10 promoter SNP in association studies in tuberculosis. Alternatively, the discrepancies in different studies can be influenced by ethnic-specificity of susceptibility/resistant variants that are being selected by different environmental pressures. Distinct populations have been exposed to very different selective environments in past centuries. Thus, it is possible that they mounted distinct strategies to cope with infectious pathogens. Canadian aborigines are much more susceptible to tuberculosis than Caucasian Canadians due to the recent history of exposure.[62] This ethnic specificity of association may also suggest that leprosy is a result of the genetic heterogeneity, where variations in different genes are responsible for the outcome of the same phenotype, leprosy.

Although we are experiencing great achievements towards genetics of leprosy, there is no published data investigating the genetic basis of inflammatory reactional states and neurological damage. Genetic testing is very expensive, especially to screen high-risk populations like household and peri-domiciliary contacts, but the definition of genetic severity markers, specifically to nerve impairment could have a great prognostic value. Genetic testing could improve predictability of other tests, like PGL-1 antibody detection.[63] This is a very important issue since it could reduce the morbidity upon patient testing for

markers that may indicate the odds of developing a reaction and/or neuritis at the time of diagnosis, thus preventing disabilities.

High throughput techniques to genotype large numbers of SNPs and statistical tools to organize and test this information are now available. The recruitment of large samples and correct choice of controls and patients is critical to better design and produce reliable results. The replication in different populations is also expected and the ultimate validation will only be achieved when the functional significance of epidemiologically associated SNPs/genes are discovered, which is essential to understanding leprosy immunopathogenesis and, finally control the disease. It is possible that in the near future these genetic tools can be used to better diagnose and treat patients as well as to help eradicate leprosy.

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