

Update on Genetics of Leprosy

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Rec date: Jun 27, 2013, Acc date: Apr 08, 2014, Pub date: Apr 10, 2014

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Abstract

Leprosy is a chronic granulomatous infectious disease caused by *Mycobacterium leprae* that mainly infects skin macrophages and Schwann cells in peripheral nerves. Genetic and environmental factors play an important role in leprosy. It is estimated that approximately 90% of the population develop protective immunity in infection, and, therefore, do not get sick. Other individuals, however, show clinical susceptibility to a wide spectrum of pathogens associated to changes in immune response. Data observed in various populations show that some aspects related to disease progression are due to host genetic factors that influence control of the initial infection and the host immune response to that infection. Nevertheless, individual genetic factors have a strong influence on the acquisition of or protection against leprosy, since the limited genetic diversity observed among *M. leprae* strains is not a significant factor for the development and the clinical course of disease.

Keywords: Leprosy; Genetics

Introduction

Leprosy, a chronic granulomatous infectious disease caused by acid-bacilli (AFB) resistant *Mycobacterium leprae* (*M. leprae*) mainly infects skin macrophages and Schwann cells in nerves. Thus, leprosy can be seen as two combined disorders: one characterized by a chronic infection that depends on the ability of host immune response and the other being a peripheral neuropathy which starts during infection, but with consequences which may extend for many years after cure [1,2].

Genetic and environmental factors play an important role in leprosy. It is estimated that approximately 90% of the population develop protective immunity in infection, and, thus, do not get sick [3,4]. Others, however, show clinical susceptibility to a wide spectrum of pathogens associated to changes in immune response. Data observed in various populations show that some aspects related to disease progression are due to host genetic factors that influence control of the initial infection and the host immune response to that infection [5]. Nevertheless, individual genetic factors have a strong influence on the acquisition of or protection against leprosy, since the limited genetic diversity observed among *M. leprae* strains is not a significant factor for the development and the clinical course of disease [5].

Epidemiology

Leprosy remains a public health problem in the world and about 245,000 new leprosy patients were detected in 2009. In 2010, 211,903 leprosy cases were detected [6], mostly in underdeveloped countries [7]. Brazil ranks second in the number of new leprosy cases and is considered by the World Health Organization (WHO) as a country with high endemicity. With regard to prevalence, Brazil ranks first in the world with a rate of 22 cases /per 100,000 population [8].

Following the implementation of Multidrug Therapy (MDT) for leprosy, in 1981, there was a decrease in prevalence, i.e, reduction in the number of cases in a population, though not accompanied by a decrease in detection rate of new cases [7]. One of the problems encountered for reducing the incidence is late diagnosis and transferred active disease.

Etiological Agent - *Mycobacterium leprae*

M. leprae was the first causative agent of disease in humans to be identified by microscopy [9]. It has a rod-shaped appearance with a length ranging from 1.5 μm to 8 μm and a width ranging from 0.2 μm to 0.5 μm . *M. leprae* lasts up to 36 hours at room temperature and its multiplication in the host system takes 11-16 days, which is considered slow [10]. When examined by electron microscopy, it appears that *M. leprae* possesses a cell envelope made up of plasma membrane, cell wall, and an a lipid-rich outer layer. Survival of bacilli in the host cell depends on the structure of the cell wall. The phenolic glycolipid 1 (PGL-1) is the lipid that confers immunological specificity to *M. leprae*. In addition, the cell wall is responsible for low permeability and hence, contributes to resistance against antimicrobials [11].

Clinical Classification

Leprosy patients can be classified into one of the five groups of the clinical spectrum depending on the stage in disease progression and host cell-mediated immunity [12]. At one end of the spectrum is tuberculoid leprosy, paucibacillary or designated TT, characterized by a small number of bacilli, a few skin lesions with well-defined edges, asymmetric neural involvement, AFB (acid fast bacilli) absent or in small numbers in the skin and nerves. The local production of cytokines in this pole of the disease is type 1, including interferon-gamma (IFN- γ), interleukins (IL-2, IL-7, IL-12, IL-15 and IL-18) and specific cellular immune response characteristics are preserved. At the other end is the lepromatous pole, also known as LL or multibacillary

leprosy, characterized by large numbers of bacilli and nerves in the skin, many lesions extensively infiltrated with macrophages and mycobacteria spongy tissue easily detected within this and nerves. Therefore, important impairment of type-2 cytokine production cell mediated immunity (IL-4, IL-5, IL-10) occurs, so that the affected individuals are anergic to *M. leprae* and have high titers of circulating antibody. These patients have high potential to transmit the disease [13].

The cases designated as borderline (BL) share clinical, histological and immunological characteristics with the two polar forms of leprosy (TT and LL). This state can be unstable due to changes in the immune system during the progression of active disease. The initially infected individuals often have indeterminate immune response (II) and characteristic skin lesions or loss of sensation, though with minimal inflammatory response and low bacterial count. At this stage, in the absence of antibiotic therapy, there may be spontaneous remission or progression to any one of the poles [12]. In 1982, WHO established a simplified diagnosis and classification of leprosy based on the number of skin lesions, according to the following criteria: patients with bacterial index (BI) positive (subjects BB, BL and LL) were considered multibacillary (MB), whereas patients with negative BI (TT and BT) were classified as paucibacillary group (PB) [14].

Interaction *M. leprae* and Host

Host defense mechanisms include immunoregulatory cytokines with activities represented by specific populations of Th1 and Th2 lymphocytes and the generation of free radicals (Reactive Oxygen Species -ROS). T cells producing Th1-type cytokines IL-2, INF- γ and tumor necrosis factor- β (TNF- β) are responsible for cell-mediated immunity. INF- γ activates macrophages and IL-2 stimulates the growth of antigen-specific T-cells, mitigating or curing disease. On the other hand, Th2 type T cells produce IL-4, IL-5, IL-6, IL-8 and IL-10, which suppress macrophage activity, increasing humoral immune response and inhibiting macrophage activation and leading to progressive infection. In turn, the INF- γ enhances the production of reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI) by macrophages, stimulating macrophages to restrict growth of intracellular mycobacteria [15]. Mycobacteria elimination is associated with the macrophage respiratory "burst" that leads to the production of various molecules and Reactive Oxygen Species (ROS) [16]. The ROS, a major antimicrobial effector mechanism, has been implicated in host defense against *M. leprae* [17].

Genetic aspects in Leprosy

In order to investigate the genetic mechanisms of resistance and susceptibility to leprosy, different approaches are used: pedigree analysis, twin studies, genetic marker analysis and investigation of candidate genes. Twin studies have shown a higher incidence of leprosy in monozygotic twins when compared to dizygotic twins, indicating an increasingly important role of genetics in susceptibility to infectious diseases [18]. Investigation of genetic diseases can also be made through linkage studies or association. Linkage studies aim to carry out gene mapping experiments, ie to find particular genes responsible for the location of an effect positioned within the same chromosomal region. Association studies are based on comparison of allele frequencies of a particular gene between patients and healthy individuals. Such studies are commonly used in genetic epidemiology because of easy recruitment for healthy individuals (controls) or

patients (cases), and most are employed in leprosy investigation, using single-base polymorphisms (SNPs) as markers of candidate genes. The SNP is a single nucleotide change which can lead to alteration in the structure and / or function of a protein. Some studies suggest that healthy controls should have been previously exposed to *M. leprae* without getting ill, ie, they should have been put at risk of contracting the disease. In countries with high incidence of leprosy it is estimated that there is an exposure of 2-10% to *M. leprae*, and blood donors at a blood center located near an outpatient clinic attended by leprosy patients were the best option for healthy controls [19].

Candidate Genes

Studies have demonstrated host genetic predisposition to leprosy. Thus, many candidate genes have been identified, and the most consistent associations reported to date were observed among the subtypes of leprosy and certain alleles of genes that encode human leukocyte antigens (HLA) [20-22]. However, these genes probably represent only a small fraction of the many other genetic factors involved in the complex interaction of host gene regulation leading to disease [23].

Among the genomic regions implicated in genetic susceptibility to leprosy is the association in 6q25, [24] containing the gene regulatory region associated with Parkinson's disease (PARK2) and co-regulator gene PACRG [25]. Other combinations include changes previously reported of a specific gene for a natural resistance associated macrophage protein (NRAMP1), [26,27] and also a polymorphism in the vitamin D receptor gene (VDR) [28]. Also, changes in the polymorphism in promoter regions of genes coding for cytokines were investigated, particularly interleukin-10 (IL-10) and IL-12, as well as tumor necrosis factor- α (TNF- α) [23,29-31] and its correlation with the differential susceptibility to leprosy, their shapes or the BI or the response to the Mitsuda test [29,32].

Major Histocompatibility Complex (MHC)

The MHC encodes highly polymorphic cell-surface glycoproteins. In man, it is called HLA, is located on chromosome region 6q21, acts directly on the immune response by presenting peptide derived from *M. leprae* to host T lymphocytes. They are divided into two groups: HLA class I (A, B and C) and class II (DR, DQ and DP). HLA class I molecules are found on all nucleated cells and present peptide fragments to CD8 + T lymphocytes, causing the death of cells infected with *M. leprae*. The class II molecules bind peptides of extracellular origin which are presented to CD4 + T lymphocytes, resulting in the production of cytokines and antibodies [33]. Case-control studies have shown an association of HLA gene regions with susceptibility or resistance to leprosy in different populations. Recently, in a study of genetic association of locus HLADRB1, Zhang et al. [34] provided strong evidence of the role of HLA-DRB1 * 15 allele in susceptibility to leprosy and of HLA-DRB1 * 09 allele in protection against leprosy in the Chinese population. In a family study, we found significant associations of HLA-DR2 (DRB1 * 16) and HLA-DQ alleles to susceptibility to leprosy in India, [35,36] Thailand [37] and Brazil [38]. Two studies have linked genes of the highly variable HLA class I (MICA) and B (MICB) and the occurrence of leprosy and in Chinese individuals, the HLA-B46 and MICA-A5 were associated with protection against the multibacillary form of the disease; [38] while in India an association of HLA-DRB1 and MICA * 5A5.1 with the disease was reported [39]. In fact, several studies have shown linkage and association between alleles and haplotypes of the HLA complex,

particularly HLA class II as an important genetic risk factor for susceptibility to leprosy per se and leprosy subtypes.

Tumor Necrosis Factor-Alpha (TNF- α) and Lymphotoxin Alpha (LTA)

TNF- α and lymphotoxin- α (LT- α) are proinflammatory cytokines, bind to the same receptor on target cells, and, thus, perform the same biological activities. In leprosy, TNF- α activates macrophages, contributing to stimulation of mycobactericidal activity and plays an important role in host defense. However, at high levels it contributes to tissue damage. TNF- α genes are located in the MHC class II region, on 6p21.3 closely related to LT- α gene. The SNP in the promoter region -308 (G / A) of the TNF gene has been widely investigated in the search for genetic determinants of leprosy susceptibility. Studies with Brazilian subjects showed significant association between polymorphism at position -308 in the TNF gene and leprosy [23,29,40,41]. In an analysis of several candidate genes for susceptibility to leprosy, Fitness et al. [42] found no association of the polymorphism -308 G / A in the TNF gene with the disease. The functional role of polymorphism in gene expression is still controversial. However, it is believed that the allele is associated with high levels of TNF- α secretion, although this region is located at the LTA gene, which plays a regulatory role in cell-mediated immunity and controls the activation of immune cells (adhesion molecules, cytokines and chemokines), recruitment and retention of lymphocytes [43]. The LTA gene has two alleles A and G. Dominance of an allele indicates propensity to leprosy. In a study of Brazilian families, Shaw and colleagues [21] found no correlation between SNP +252 (A / G) and leprosy. Analysis with haplotypes-308A / +252 G resulted in protection of leprosy. The role of LTA in host defense against leprosy is unknown, but some studies have detected the presence of ACL lesions in individuals with the tuberculoid leprosy with reversal reaction (type 1), indicating a putative role in the release of pro-inflammatory cytokines. The LTA +80 polymorphism encoding the promoter region of the LTA gene was considered an important risk factor for the development of leprosy per se, [44] this allele seems to be associated with lower levels of production of LTA, which would undermine the protective response to *M. leprae*.

Parkin (PARK2) and PACRG

Parkin (PARK2) gene and parkin co-regulated gene (PACRG) were first identified in a recent study aimed to find a locus within these genes linked to Parkinson's disease. Classically PARK2 PACRG and E3-ubiquitin ligase are involved in ubiquitin-dependent protein degradation play an important role in various immune responses, and the impaired function of these proteins leads to cell death [45]. A study of genomic scans for leprosy to find locus within the genes apparently not associated with the disease, using the technique of positional cloning in the candidate region 6q25, identified a set of SNPs located in the promoter region shared by two genes associated with PARK2 and PACRG with leprosy per se. Haplotypes composed of these two SNPs (-2599 T allele in the region of PARK2 and C allele in SNP rs1040079) were responsible for the phenotype of susceptibility to leprosy in Vietnamese families, as much as in a Brazilian population [25]. Thus, these results indicate overall risk factors for leprosy.

Interleukin 10

The IL10 gene is located at chromosome 1q31-q32. It provides an anti-inflammatory and immunoregulatory Th1 response by suppressing IFN- γ , TNF- α and IL-1. In leprosy, high levels of IL-10 have been correlated to better prognosis among healthy household contacts (contacts) [46]. The promoter region of this gene is composed of polymorphic microsatellite loci and SNPs at positions -592, -819, -1082 (proximal) and -2763, -2849, -3575 (distal). The functional effect of these haplotype SNPs located in the proximal portion has been associated with a low production of IL-10. Santos and colleagues [23] studied SNP located in the position -819 on the IL-10 gene found in Brazilian individuals and a phenotype of susceptibility allele in the presence of T. In a recent study, sequencing of six SNPs was performed in Indian subjects and associated haplotypes 592C,-819C,-1082A,-2763C,-2849G,-3575T resistance to leprosy per se and to the development of multibacillary leprosy [47]. Franceschi et al. [46] found no significant differences of genotypes IL-10 in leprosy patients compared with healthy controls. These differences in results probably result from the genetic diversity of these populations.

Vitamin D Receptor (VDR)

The vitamin D receptor gene located at chromosome 12q12-q14 and responsible for conversion of vitamin D into its active form (1.25 dihydroxyvitamin D₃) was originally studied as a candidate gene in infectious tuberculosis in a study conducted in Gambia [48]. In 1994, Morrison et al. [49] found an association between a polymorphism in the 3' region of the VDR gene and osteoporosis. Subsequently, gene polymorphisms are associated with various diseases such as hepatitis B, HIV and leprosy [28,48,50].

The first evidence of association of the VDR gene in leprosy was reported by a case-control study in an Indian population. Analysis of the TaqI polymorphism (T to C substitution) at codon 352 of the VDR gene found a higher frequency of TT genotype in individuals with the lepromatous form, since the opposite CC genotype was associated with tuberculoid disease [28]. In the district of Karonga in Malawi, a case-control study, reported susceptibility to leprosy in the presence of CC genotype [42]. Goulart et al. [51] contrary to previous results, found no statistically significant differences in the VDR gene compared to the subtypes of leprosy and the Mitsuda test.

Toll-like Receptors (TLRS)

Toll-like receptors (TLRs) are a family of receptors that play a central role in the skin immune defense system. Activation with TLR ligands leads to production of pro-inflammatory stimuli. These stimuli activate macrophages inducing IL-12 production, thus establishing a Th1-type immune response [52]. A study on TLRs showed an association between the TLR2 Arg677Trp polymorphism and susceptibility to lepromatous leprosy, indicating a probable role in determining the type of leprosy [53]. Recently, Bochud et al. [54] two SNPs associated TLR4 gene (896G / A and 1196C / T) with a protective effect against the disease. The above data indicate the TLRs as important candidate genes for association with leprosy.

Solute Carrier Family 11 Member 1 (SLC11A1A)

SLC11A1 protein, also known as NRAMP1 (natural resistance-associated macrophage protein) is located in the 2q35 region. Its gene product is expressed in macrophages and encodes a protein found on

the phagosomal membrane, which acts as a carrier of ions (essential for survival of pathogens in macrophages). Removal of these ions limits intracellular pathogen multiplication [55]. The first association of NRAMP1 gene with leprosy was found in a study where the deletion of four nucleotides (TGTG) in the 3' region was more frequent among people with multibacillary (MB) leprosy [27].

Other Genomic Regions

Interleukin 12 (IL-12) is a cytokine secreted by macrophages and dendritic cells in response to intracellular pathogens. Several polymorphisms have been described in the region of IL-12 gene. Nevertheless, Alvarado-Navarro [56] found no significant association of polymorphisms (3'UTR 1188 A / C gene IL-12p40) and subtypes of leprosy. Other studies have shown a substantial difference between the genotypes of individuals with leprosy compared to control samples [31,57].

The MRC1 gene encodes C-type lectin receptor, a cell surface protein that binds to specific pathogen-associated molecular patterns (PAMPs). It has been associated with susceptibility to tuberculoid leprosy [58]. A case-control study in a Brazilian population showed significant association of the polymorphism of the gene MRC1 G396 with leprosy per se [59].

The Ninjurin was first identified as a co-regulatory molecule of Schwann cells and neurons after peripheral nerve injury. Further analysis revealed that the Ninjurin is a cell surface molecule that promotes cell aggregation and stimulates axonal growth, suggesting an important role in nerve regeneration [60]. NINJ1- The gene is located on chromosome 9q22, has polymorphism: A -> C transversion at the nucleotide sequence, being responsible for replacing Asparagine with Alanine at position 110 of the protein [61,62]. Two studies of this gene in leprosy suggested an association between allele ala110 and development of nerve damage in leprosy patients [62,63].

Methodology

This is a literature review of research, descriptive in nature, aimed to identify updated information on clinical forms of leprosy, immune responses, genetic studies, candidate genes, histocompatibility, tumor necrosis factor alpha (TNF- α) and Lymphotoxin alpha (LTA), Parkin (PARK2) and PACRG, Interleukin 10, Vitamin D Receptor (VDR), Toll-like receptors (TLRs), Solute carrier family 11 member 1 (SLC11A1A), among other genomic regions.

Conclusion

Mycobacteria do not produce virulence factors that explain the clinical manifestations of infections, which are due to host response to infection. In fact, there is no confirmation of host immune response in susceptibility to leprosy. However, contradictory results about genetic variations indicate that these associations are moderate. There are few studies on the relevance of genetic variants associated with the evolution of leprosy, which makes it difficult to clearly define the role of these genes in susceptibility. Additionally, not all of these associations have been replicated in different populations, suggesting that each one has a different genetic profile of susceptibility. The high prevalence of leprosy in Brazil suggests that the inter-racial mixture may have led to a genetic profile that contributes to the persistent occurrence of the disease. The conflicting results may point out possible misunderstandings regarding the study design, such as

inappropriate choice of statistical tools, poor selection of control subjects and a reduced number of samples in the case study of polymorphisms.

The explosion of studies using genetic epidemiology, (case controls studies) with candidate genes, of infections have risen after common belief that genes greatly influence susceptibility to infectious diseases.

Finally, twin and familial studies, especially in leprosy, provide the idea of genetic inheritance that has been consistently depicted in genome scans of families. Among all of the genes that participate in immune response against infectious disease it is likely that cytokines and other genes associated with inflammatory and immune response play a crucial role. Indeed, efficient activation of a cellular immune response is very important in triggering a protective response against, *M. leprae*.

Regarding genetic factors, the genes involved and their biological functions are still largely unknown. We note that all articles conclude that leprosy is a genetic disease, i.e. depend on the genetic susceptibility of the individual to develop the disease or not, there are many genes involved in this susceptibility, however, no study has developed a diagnostic test or preventing leprosy. Further studies on infectious diseases could help developing new strategies for diagnosis and contribute to eradicate leprosy. Despite advances in basic science in the field of leprosy still need to make considerable progress that their findings may have practical implications for public health, particularly in the transmission control and prediction of disease.

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