Chapter 8

Genetic Variation in Pattern-Recognition Receptors and Association with Leprosy

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Additional information is available at the end of the chapter

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Abstract

Mycobacterium leprae is a highly infectious and low pathogenic microorganism that is the causal agent of leprosy. The differences in vulnerability to leprosy, the spectral immune response, and the clinical manifestations of this disease are related to different genetic backgrounds among individuals. In this sense, genetic variants, especially in genes related to mycobacteria recognition and host immune response, may be key factors to explain individual susceptibility and resistance to leprosy and their conditions. In this chapter, studies regarding association of genetic variants in pattern-recognition receptors (PRRs) and leprosy will be reviewed revealing the importance of molecules such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-containing protein 2 (NOD2) in leprosy initiation and maintenance.

Keywords: polymorphisms, pattern-recognition receptors, *mycobacterium leprae*, leprosy

1. Introduction

Leprosy is caused by *Mycobacterium leprae*, which is an intracellular bacterium with high infectivity and low pathogenicity. It means that there are a large number of people exposed to this pathogen; however, the majority of them are naturally resistant. On the other hand, some people develop leprosy once challenged with *M. leprae*. The people who develop disease may present different clinical forms of leprosy. Some of them develop a localized disease, named tuberculoid leprosy, with a strong host response, which does not avoid development of nerve injury and physical disabilities. Some patients develop a more severe form of leprosy, named lepromatous leprosy, whereas other patients present intermediate and instable clinical forms

(borderline tuberculoid, borderline borderline, and borderline lepromatous). Besides, there are different levels of susceptibility to leprosy reactions. The differences among *M. leprae* strains are not sufficient to explain this variable outcome of leprosy. There is a variable spectrum of host immune response that depends on the genetic characteristics of the infected individual. The immunological and genetic basis underlying *M. leprae* infection remains largely unknown.

In this sense, several studies have been conducted aiming to explore the molecular basis of leprosy, and they are mainly focused on aspects of host-pathogen interaction and modulation of host immune response against *M. leprae*.

Recognition of *M. leprae* by the host innate immune system is the first step in dealing with the invading bacteria and is crucial to initialize the adaptive immune response to infection. Microorganisms are recognized by host through germline-encoded pattern-recognition receptors (PRRs). The PRRs are able to sense highly conserved motifs from the invading microorganisms. These motifs are called pathogen-associated molecular patterns (PAMPs) [1].

One family of PRRs is the Toll-like receptors (TLRs) that are expressed on cell surface of different cells from innate immune system or endocytic vesicle membrane [2]. These receptors have an extracellular domain that recognizes different bacterial agonists. The activation of TLRs mediate host immune response regulating phagocytosis and antimicrobial activity or initiating signaling cascades (through activation of transcription factors NF-κB and IRF) culminating in modulation of cytokines and chemokines release [3].

There is another family of PRR, called nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) that are cytoplasmic receptors, and they are able to recognize bacterial agonists inside the host cells. Some members of this family activates NF-κB, interferon regulatory factors (IRFs), and mitogen-activated protein kinases (MAPKs), which control the expression of mediators of immune response, such as cytokines, chemokines, and type I interferons. On the other hand, other members lead to activation of caspase-1 acting through inflammasomes [4].

Genetic variants leading to functional changes in PRRs, such as TLRs and NLRs, may be involved with differences in host immune response modulation and consecutive susceptibility or resistance to leprosy and their clinical forms as well leprosy reactions.

In this chapter, studies about genetic association of PRRs, specifically TLRs and NOD2, with leprosy will be reviewed.

2. Genetic variations in toll-like receptors (TLRs)

TLRs were first described in the mid-1990s after genome sequencing of *Drosophila melanogaster*. Toll protein from *D. melanogaster* was identified as an important molecule in initiating innate immune response in this organism in response to fungal infection and Gram-positive bacteria. This molecule was also described as important in embryonic dorsoventral patterning [5]. After this first description, proteins similar to Toll protein from *D. melanogaster* were identified in mammalian cells, including humans, and they are referred as Toll-like receptors. TLRs are members of superfamily of interleukin receptors, and they act as PRRs recognizing PAMPs [6, 7].

The TLRs are also type 1 transmembrane receptors and may be expressed at cell surface or endocytic vesicle membrane. The receptors TLR1, TLR2, TLR4, TLR5, and TLR6 are expressed at cell surface, whereas TLR7, TLR8, and TLR9 are expressed at endosomes and lysosomes. TLR3 may be expressed either at cell surface and endosomes or lysosomes [2]. The intracellular TLRs recognize viral and bacterial nucleic acids [8].

There are ten human TLRs already described (TLR1–TLR10), and they recognize different kinds of ligands [2, 9]. TLR1, TLR2, and TLR6 are mainly stimulated by lipoproteins from Gram-positive bacteria; TLR2 also recognizes lipoteichoic acid (Gram-positive bacteria), zymosan, β-glucan from fungi, and GPI anchors; TLR3 recognizes dsRNA from viruses; TLR4 is activated by lipopolysaccharides (Gram-negative bacteria) and GPI anchors; TLR5 recognizes bacterial flagellin; TLR7 and TLR8 recognize ssRNA from viruses; TLR9 is activated by DNA; and ligands for TLR10 are still not known [10]. TLRs are also able to sense and signal tissue damage through recognition of damage-associated molecular pattern molecules (DAMPs) [11].

After recognition of ligands by the specific TLR, a dimerization of TLRs as homodimers or heterodimers may occur. Ligation of TLR and a ligand may also lead to conformational changes in dimers. The differential association in heterodimers causes a diversification in ligand recognition. For example, the heterodimer TLR1-TLR2 recognizes triacylated bacterial lipopeptides, whereas TLR2-TLR6 heterodimer recognizes diacylated lipopeptides [12, 13].

Dimerization and conformational changes will activate intracellular signaling cascade and innate immune response against the pathogen, produce acute inflammation, lead to modulation of adaptive immune response, and induce antimicrobial pathways [3, 12, 14].

The important roles played by TLR in recognizing PAMPs, especially PAMPs from *M. leprae* and initiation of immune response, highlight its potential involvement with leprosy susceptibility and development. In the following sections, studies about association between polymorphisms in TLR1, TLR2, and TLR4 leprosy will be presented. The genetic association studies between TLRs and leprosy are summarized in **Table 1**. The other TLRs do not interact with *M. leprae* or do not have genetic association with leprosy.

2.1. Genetic variations in TLR1

The heterodimer formed by TLR1 and TLR2 recognizes killed *M. leprae* through triacylated lipoproteins leading to cell activation. These two receptors are expressed in a higher level in localized tuberculoid form than in disseminated lepromatous form, evidencing the role of TLRs in host defense. Type-1 cytokines IFN-γ, GM-CSF, IL-12, and IL-18 stimulate TLR1 and TLR2 activation, while type-2 cytokines IL-4 and IL-10 impair its activation. Although TLR1 response is important to fight infection, exacerbated activation of TLR can lead to immunopathology, tissue damage, and, in the case of leprosy, nerve damage [15].

The involvement of TLR1 in leprosy has been highlighted by several association studies.

Some polymorphisms in TLR1 were studied in different populations revealing their association with conditions related to leprosy. One of them is the SNP rs5743618 (T/G I602S) which presents a variable frequency depending on the population. Hawn et al. (2007) showed that

Gene	Polymorphism	Associated Condition	Reference
TLR1	Rs5743618 (T/G – I602S)	Leprosy susceptibility TLR1 trafficking to the cell surface	[17]
		Leprosy susceptibility	[18]
		NF-κB activation Reduced cytokines response Reversal reaction protection	[19]
	Rs4833095 C/T (N248S)	Leprosy susceptibility Leprosy reactions	[21]
		Leprosy susceptibility TNF/IL-10 ratio	[22]
		IL-12p40 and IL-17 levels	[23]
TLR2	Rs121917864 (C/T – R677W)	IL-12 level	[26]
		Lepromatous leprosy susceptibility	[28]
		NF-κB activation	[29]
		IL-2, IL-12, IFN-γ, TNF-α and IL-10 levels	[30]
	Rs3804099 (C/T – N199N)	Leprosy susceptibility IL-17 and IL-6 levels	[23]
		Leprosy reversal reaction	[33]
	Rs7656411 (G/T)	CXCL-10 level	[23]
	VNTR CT and TG Upstream to TLR2 start codon	Leprosy reversal reaction	[33]
	VNTR GT Intron 2 TLR2	(13 repeats) Leprosy protection (longer repeats) increased *TLR2* expression and decreased IL-10 expression; leprosy susceptibility	[34]
TLR4	Rs1927914 (A/G)	IL-17 and IL-1β levels	[23]
	Rs4986790 (G/A – D299G)	Leprosy protection	[39]
		Leprosy susceptibility	[42]
	Rs4986791 (C/T – T399I)	Leprosy protection	[39]
TOLLIP	Rs3793964 (C/T)	Leprosy susceptibility TOLLIP and IL1-Ra levels	[47]

Table 1. Association studies of genetic polymorphisms in TLRs and leprosy.

rs5743618 in *TLR1* is involved with the regulation of innate immune response to triacylated lipopeptides (ligands of TLR1/TLR2 heterodimer), and it is also related to the differential response to *M. tuberculosis* extract. The 602I variant was shown as able to induce an increased level of NF-κB signaling after induction by lipopeptides in a Vietnamese population. As expected, there is also an association with the cytokine profile as the 602I genotype presents a higher production of IL-6 in whole blood stimulated with lipopeptides supporting the role of TLR receptors in activating innate immune response against *M. leprae* [16]. In the same year, Johnson and coll. (2007) noted an association of this same SNP rs5743618 with surface trafficking of TLR1 and response of blood monocytes to bacterial ligands. More specifically, the variant 602S is associated with an impairment of TLR1 expression in cell surface leading to a loss of cellular responses. Corroborating these evidences, the variant 602S was shown to be a protective factor for leprosy [17]. Wong and coll. (2010) have analyzed the data from more than 1500 individuals from different studies and regions and identified *TLR1* and *HLA-DRB1DQA1* as the main genes related to leprosy susceptibility. Besides that, the protective variant 602S is rare in Africa but is the most frequent among European descendants, which suggests the selection pressure over this locus from mycobacteria [18].

Investigating the possible involvement of TLR1 with adaptive immune response affecting the clinical manifestations of leprosy, Misch and coll. (2008) evaluated rs5743618 polymorphism demonstrating a decrease of NF-κB activity related to the 1805G allele. In a Nepalese sample, the 1805G allele was protective against reversal reaction, which is characterized by an exacerbated Th1 cytokine response [19]. Another polymorphism able to interfere with TLR1 activity is rs4833095 (C/T N248S) as the 248 N variant impairs TLR1 functioning and sensing of microbial cell wall components [20]. In a Bangladeshi study, the homozygous 248SS genotype was associated with leprosy, but 248 N is homogeneously distributed among subjects. The 248 N allele is associated with erythema nodosum leprosum, while patients with reversal reaction are more likely to have the 248S allele [21]. These results were corroborated in a Brazilian sample, in which an association effect of 248S allele with leprosy susceptibility was found. In this same study, no association was identified between rs5743618 and leprosy diverging from previous results. However, rs4833095 was shown to be in a moderate linkage disequilibrium with rs5743618, suggesting a higher effect of the last one among Brazilians. The susceptibility allele 248S leads to a lower tumor necrosis factor (TNF)/IL-10 ratio after stimulation with *M. leprae*, and this same allele influences on TLR1 structure which may explain the functional alterations [22]. The association of 248S allele was not confirmed by Santana and coll. (2017) in another study with a Brazilian sample. However, they found association of rs4833095 with differential production of IL-17 and IL-12p40 [23]. These variations in TLR1 highlight the functional role of these genetic determinants in modulating the immune response during *M. leprae* infection.

2.2. Genetic variations in TLR2

TLR2 can form heterodimer with TLR1, as mentioned before, and with TLR6. These different forms of presentation allow TLR2 to recognize different cell wall components, such as lipopeptides, peptidoglycan, glycosylphosphatidylinositol-linked proteins, and zymosan [24].

TLR2 is responsible by recognition of cell wall fractions from *M. leprae* and mediate proinflammatory signaling by stimulating TNF-α production in macrophages in a Toll-like receptor-dependent manner [25]. Moreover, TLR2 is able to stimulate NF-κB signaling and subsequent induction of inflammatory cytokines [26]. As described to TLR1, type-1 cytokines IFN-γ, GM-CSF, IL-12, and IL-18 lead to enhancement of TLR2 activation, and type-2 cytokines IL-4 and IL-10 inhibit activation of TLR2 [15]. TLR2 is also expressed at surface of Schwann cells. Recognition of lipoproteins from *M. leprae* by TLR2 is required to stimulate apoptosis of these cells and may be related to nerve injury in leprosy [27].

In this way, variations in *TLR2* could be associated with a differential response against mycobacteria and be involved with individual susceptibility to leprosy. Looking for genetic variants in TLR2 in an association study about leprosy, Kang and coll. (2001) performed a screening in intracellular domain in leprosy patients. They detected the polymorphism rs121917864 (C/T R677W) in a conserved region of TLR2 present only in lepromatous patients, which suggests a role in susceptibility to this form of the disease [28]. After this, the same research group published a study demonstrating a role of rs121917864 in innate immune response activation, specifically the response by monocytes. Patients with the variant 677 W present a decrease in serum levels of IL-12 after stimulation with cell lysate of *M. leprae* compared to the patients with no amino acid substitution confirming that TLR2 is involved with immune response against *M. leprae* [26]. Another study also showed the relation of TLR2 with innate immune response studying the polymorphism rs121917864. In this study, the *M. leprae*-dependent activation of NK-κB signaling was impaired with 677 W variant in response to cell wall fractions of *M. leprae* and *M. tuberculosis*. These results allow to hypothesize that this variation in *TLR2* may be involved with the poor cellular immune response in leprosy patients [29]. The profiles of different cytokines are also affected by rs121917864 substitution. Peripheral blood mononuclear cells (PBMC) from lepromatous leprosy patients carrying this transition showed a lower level of expression of IL-2, IL-12, IFN-γ, and TNF-α after stimulation with *M. leprae*. On the other hand, levels of IL-10 increased, while there is no change in IL-4 production. This alteration in cytokines profiles in lepromatous patients can also be involved with a low level of cellular immune response in these patients during *M. leprae* infection [30]. Despite of what was shown in Korean population by Kang and coll. (2001), Malhotra and coll. (2005) did not identified the rs121917864 substitution in an Indian population. According to authors, rs121917864 is in fact a variation in a duplicated region 93% homologous located in exon 3 of *TLR2*. This duplicated region is a pseudogene [28, 31]. The rs121917864 polymorphism was also not identified in Japanese leprosy patients [32]. However, although such conflicting results may be related to the fact occurring in a duplicated region, these different results may also be explained by different genetic backgrounds among different populations.

Another important SNP in *TLR2* in the context of leprosy is the silent mutation rs3804099 (C/T N199 N). Santana and coll. (2017) found that the T allele is associated with an increased risk to leprosy and patients carrying this allele produce a high level of IL-17 and IL-6. In this same study, an intronic transition of SNP in *TLR2* rs7656411 (G3724 T) presented no association with leprosy in Brazilian population. However, individuals carrying G allele presented high levels of CXCL10 production [23]. Although no association of rs3804099 (C/T N199 N) with leprosy

was found, Bochud and coll. (2008) have already demonstrated that T allele has a protective effect against reversal reaction [33].

In addition to SNPs, microsatellites are important polymorphisms that can be markers of disease susceptibility, and there are studies trying to identify microsatellites in *TLR2* and investigating their association with leprosy conditions. In an case-control study with leprosy patients from Ethiopia, a microsatellite upstream to *TLR2* start codon containing two variable nucleotide tandem repeats (VNTR) (CT and TG) is associated with leprosy reversal reaction which is mainly characterized by a Th1 immune profile [33]. TLRs are able to modulate the immune response inducing a Th1 or Th2 profile; in this way, changes in these receptors may lead to a differentiation in immune profile from patients and involve with development of leprosy reactions. The microsatellite characterized by GT repeats on intron II of TLR2 is associated with protection against leprosy with 13 repeats being related to resistance. On the other hand, longer GT repeats are associated with a decrease in *TLR2* expression and increase in IL-10 production being associated with leprosy susceptibility [34].

2.3. Genetic variations in TLR4

TLR4 has as main ligand LPS from Gram-negative bacteria. However, studies have already demonstrated that TLR4 also recognizes ligands from *M. leprae* [35, 36]. TLR4 acts through MyD88-dependent and MyD88-independent (TRIF) signaling [37, 38].

The association of polymorphisms in *TLR4* with leprosy susceptibility was investigated in some studies reinforcing the involvement of this gene in response against leprosy. Bochud and coll. (2009) identified two polymorphisms rs4986790 (G/A D299G) and rs4986791 (C/T T399I) with the minor alleles presenting a protective effect against leprosy in an Ethiopian population. Eventually, these variations may have a role in *TLR4*-induced effects in immune response against leprosy [39]. Polymorphism rs4986790 was also investigated in a population from Malawi revealing borderline association with leprosy. There is a increased frequence of heterozygosity in control group which is contradictory with another study that demonstrated that heterozygosity of rs4986790 was associated with hyporesponsiveness after LPS inhalation [40, 41]. However, the Malawi study was corroborated by a family-based and case-control study, in which the AA genotype was associated with leprosy susceptibility and GA genotype associated with leprosy protection in an Indian population [42]. Aiming to understand the effect of polymorphisms in TLR4 signaling, Figueroa and coll. (2012) compared cells of human embryonic kidney 293/CD14/MD2 complemented with wild-type *TLR4* and mutant variants. No differences in *TLR4* expression were identified after induction by LPS. However, the *TLR4* signaling was altered since MyD88 and TRIF recruitment were impaired with *TLR4* carrying the rs4986790 polymorphism. These results were also observed with polymorphism rs4986791, however, in a lesser extent [43].

Changes in non-translated regions of *TLR4* gene may also be able to induce changes in immune profile. The A > G substitution in an intergenic upstream region (rs1927914) and A > G substitution in an intronic region (rs1927911) were associated with both leprosy per se and leprosy reactions. Although rs1927911 induces no differences in cytokines and chemokines level of production, the

allele A of rs1927914 is associated with a higher expression of IL-17 and IL-1β, which is highly produced in multibacillary leprosy patients [23].

2.4. Genetic variations in toll-interacting protein (TOLLIP)

The activity of TLRs must be under tight regulation to avoid an exacerbated response that would be harmful to the host. Toll-interacting protein (TOLLIP) is an adaptor protein which impairs the NF-κB and JNK signaling induced by TLR2 and TLR4, among other signaling pathways, playing a role in immune response regulation. TOLLIP is able to suppress IRAK-1 activity facilitating IL-1R/TLR-induced cell signaling during inflammation [44, 45]. After TLR2 stimulation, TOLLIP regulates the pro-inflammatory and anti-inflammatory balance by inhibiting IL-6 and TNF and stimulating IL-10 [46].

In this way, changes in TOLLIP activity may be related to leprosy susceptibility. In a Mexican population, the possible association of TOLLIP polymorphisms with leprosy susceptibility was analyzed. The following four polymorphisms were investigated in lepromatous leprosy patients and control individuals: promoter −526 C > G (rs5743854), silent mutation rs3750920 (C/T P139P), rs5744015 (C/A A222S), and 3'UTR rs3750919 (G/A). However, no association was identified between the cited polymorphisms and lepromatous leprosy, but a trend of protective association to lepromatous leprosy was identified in homozygous CC genotype of rs3750920 [44].

Six haplotype-tagging SNPs were also analyzed regarding association with leprosy in a population from Nepal. Among the tested SNPs, the intronic rs3793964 (C/T) exhibited association with leprosy being the TT genotype associated with leprosy susceptibility. Moreover, TT genotype and T allele were associated with increased expression of TOLLIP and IL-1Ra in the skin tissue. The study also demonstrates that TOLLIP induces IL-1Ra in monocytes that inhibit IL-1R activating helping *M. leprae* to evade from host immune response in early stages of leprosy development [47].

3. Genetic variations in nucleotide-binding oligomerization domain-containing protein 2 (NOD2)

Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) is a family of intracellular PRR that is able to recognize bacterial molecules. Humans have 23 different NLRs that present two characteristic domains: NOD and leucine-rich repeats (LRRs). The first one is required for oligomerization, while the second one is required for ligand binding. There are different subfamilies of NLRs [48]. Some NLRs activate the protease caspase-1, so they act through inflammasomes leading to pyroptosis. The inflammasome-related NLRs are NLRP1, NLRP3, NLRP6, NLRP7, NLRP12, NLRC4, and NAIP. However, other components of NLR family (NOD1, NOD2, NLRP10, NLRX1, NLRC5, and CIITA) act through activation of NF-κB, mitogen-activated protein kinases (MAPKs), and interferon regulatory factors (IRFs) leading to innate immune response stimulation [4].

There are some PAMPs exclusively recognized by NLRs, such as muramyl dipeptide (MDP) that is a constituent of peptidoglycan cell wall present in Gram-positive and Gram-negative bacteria [49]. *M. leprae*, in turn, presents a distinct MDP structure, and some studies have evidenced the role of NOD2 in recognizing *M. leprae* through MDP [50]. NOD2-mediated recognition of MDP from *M. leprae* induces bacterial killing activating NF-κB signaling cascade, production of IL-32, differentiation of dendritic cells, and autophagy [51–53].

Due to its role in immune response modulation, variations in *NOD2* gene is involved with different inflammatory diseases, such as Crohn's disease [54], Blau's syndrome [55], arthritis [56], and others. In this sense, several studies have investigated the association of *NOD2* genetic variants with leprosy susceptibility and leprosy reaction.

In a genome-wide association study from Zhang and coll. (2009) about susceptibility to leprosy in a Chinese population, variants in six genes of innate immune response were identified as associated with the disease (*CCDC122*, *C13orf31*, *NOD2*, *TNFSF15*, *HLA-DR*, and *RIPK2*). Specifically, in *NOD2* gene, two variants were identified in association with susceptibility to the disease: rs9302752 (A/G) and rs7194886 (G/A), being the first one with a stronger association with multibacillary leprosy. The two other evaluated SNPs in *NOD2*, rs8057341 (A/G) and rs3135499 (A/C), exhibited no association with leprosy in this study [57]. After this genome-wide association study, Grant and coll. (2012), in a family-based study, tried to validate in a Vietnamese population the genetic variants previously associated with leprosy in the Chinese study. They analyzed SNPs in *HLA-DR-DQ*, *RIPK2*, *CCDC122-LACC1*, and *NOD2* in leprosy patients and control individuals. Four polymorphisms were evaluated in *NOD2*: rs9302752, rs7194886, rs8057341, and rs3135499. The G risk allele for the variant rs9302752 was associated with leprosy, as identified in Chinese population. According to this Vietnamese study, some variants identified by Zhang and coll. may not directly associated with leprosy risk, but they are in linkage disequilibrium with another variants, which are the causal ones. This possibility could explain the divergent results found in different studies [57, 58].

In a subsequent study, Marcinek and coll. (2013) genotyped the variant rs9302752 but now in an Indian population. However, this variant was not in Hardy-Weinberg equilibrium in this population. Some other variants in genes involved in signaling pathways mediated by NOD2 were shown to be associated with leprosy in this study. One of them is the haplotype formed by rs40457 G and rs42490 A in *RIPK2* gene that together with *NOD2* is responsible for activate NF-κB signaling. This haplotype gives an increased risk to leprosy in individuals, whereas the haplotype AA has a protective role. In addition, the minor A allele of rs1873613 polymorphism in *LRRK2* is associated with paucibacillary leprosy progression [59].

Other conflicting results about genetic variants in *NOD2* and their relation with leprosy emerged in a Brazilian study from Marques and coll. (2014) [60]. In this study, authors aimed to validate the results found in the previous Chinese study regarding SNPs in four genes: *CCDC122-LACC1*, *NOD2*, *TNFSF15*, and *RIPK2* [57]. The A allele of variant rs8057341 in *NOD2* was associated with leprosy resistance in Brazilian population. This result is also different from that identified in Vietnamese study, in which rs8057341 has no association with leprosy. Other variants from *NOD2*, besides rs8057341 A, were related to leprosy protection being under-transmitted to the affected offspring in Brazilian population: rs2111234 G and

rs3135499 C [58, 60]. These conflicting results may be, at least partially, related to different genetic backgrounds among different populations.

The previous study conducted by Zhang and coll. was realized in a Han Chinese population, which is the main ethnic group from China [57, 61]. A second study was realized in a Chinese population evaluating the genetic association of *NOD2* (*C13orf31* and *CCDC122*) with leprosy but now in a population from Yi ethnic group that represents a minority group. The following polymorphisms in *NOD2* were investigated: rs9302752, rs7194886, rs8057341, and rs3135499. Only the SNP rs3135499 was differentially distributed between leprosy patients and healthy control individuals [62]. This result is different from that found for rs9302752 by Zhang and coll., Marcinek and coll., and Grant and coll. and rs7194886 and rs8057431 by Zhang and coll. [57–59].

Even with some discrepant results, the cited studies agree in associate *NOD2* with leprosy, however, with different specific variants. These differences may be due to the influence of different ethnicities or linkage disequilibrium between different SNPs.

In addition to association with susceptibility to leprosy, variation in *NOD2* may be related to susceptibility to development of leprosy reactions. Leprosy reactions are acute inflammatory reactions that may occur before leprosy diagnosis, during the treatment or after the treatment. Some factors are able to initiate these reactions, like intercurrent infections, pregnancy, and physical and emotional stress, among others [63]. Leprosy reactions are classified into two main types: type 1—reversal reaction (RR) and type 2—erythema nodosum leprosum (ENL). RR occurs mainly in earlier stages of treatment but can also occur after the treatment, in borderline clinical forms. It is characterized by an exacerbated Th1 response. The patient with RR presents an exacerbation of preexisting lesions, which became edematous and erythematous and may develop ulceration. RR is the leading cause of nerve damage in leprosy and consequent physical disability [63–65]. ENL, in turn, mainly occurs in lepromatous and borderline lepromatous leprosy and may happen during treatment but is more frequent after the treatment. Patients present subcutaneous nodules that are painful and erythematous and also may be ulcerative. In this kind of reaction, immune complexes are related to their initiation, but cell-mediated immunity also plays a role in ENL. There is an increase in $CD4^+$ T cells, TNF-α, and IFN-γ [63, 66, 67]. Several studies have already evidenced the possible functional mechanisms by which NOD2 plays a role during development of leprosy reactions by pro-inflammatory activities. The stimulation of Th1 and Th2 is one of these mechanisms [68, 69], as well as negative regulation of TLR2-mediated Th1 response [33, 70, 71].

Some of the previously mentioned polymorphisms in *NOD2* (rs7194886, rs9302752, and rs8057341), besides the additional rs751271 (G/T) and rs2066843 (C/T), were investigated for their association with leprosy inflammatory reactions in a Brazilian population. The variant rs751271 is associated with leprosy, being the genotype TT related to faster reaction development, whereas the genotype GT and G allele carriers are protection factors against leprosy reactions. The authors also suggest that rs751271-GT genotype produces lower levels of IL-6 in patients without reaction, so the hypothesis is that individuals with this genotype present a better balance in cytokine production which could be related to protection against leprosy reactions [72].

Polymorphism rs751271 were already investigated in a Nepal population regarding its association with reactive states of leprosy showing no association with type 1 reversal reaction (RR) neither with erythema nodosum leprosum (ENL). In this study, 32 polymorphisms in *NOD2* gene were evaluated. Four out of 32 polymorphisms were associated to leprosy susceptibility when the allele frequencies were compared between leprosy patients and healthy control: rs12448797, rs2287195, rs8044354, and rs1477176. When the genotype frequencies were compared, eight SNPs were associated with leprosy: rs2287185, rs8044354, rs8043770, rs13339578,

Gene	Polymorphism	Associated Condition	Reference
NOD2	Rs9302752 (A/G)	Leprosy susceptibility Multibacillary leprosy	[57]
		Leprosy susceptibility	[58]
	Rs8057341 (A/G)	Leprosy protection	[60]
	Rs7194886 (G/A)	Leprosy susceptibility	[57]
	Rs2111234 (A/G)	Leprosy protection	[60]
	Rs3135499 (A/C)	Leprosy protection	[60]
	Rs751271 (G/T)	Leprosy reactions	[72]
	Rs12448797 (C/T) Rs1477176 (C/T) Rs13339578 (A/G)	Leprosy susceptibility	[73]
	Rs8043770 (C/G) Rs4785225 (A/C/G) Rs751271 (G/T)	Leprosy susceptibility, RR	
	Rs2287195 (A/G) Rs8044354 (A/G)	Leprosy susceptibility, ENL, RR	
	Rs7194886 (C/T) Rs1861759 (G/T)	ENL, RR	
	Rs17312836 (A/C) Rs6500328 (A/G) Rs18611758	ENL	

*ENL = erythema nodosum leprosum, RR = reversal reaction

Table 2. Association studies of genetic polymorphisms in NOD2 and leprosy.

rs4785225, rs751271, rs12448797, and rs1477176. The variants rs12448797 and rs1477176 are located in genes adjacent to *NOD2*, *SL1C1*, and *CYLD*, which are regions in linkage disequilibrium with *NOD2* that may influence the expression of *SL1C1* or *CYLD* and *NOD2*. Regarding to RR, five SNPs showed association of their allele frequencies (rs2287195, rs8044354, rs8043770, rs7194886, and rs1861759), and seven SNPs exhibited association of their genetic frequencies in a dominant model (rs2287195, rs8044354, rs8043770, rs7194886, rs1861759, rs4785225, and rs751271). Four SNPs were associated with ENL in an allelic level (rs8044354, rs17312836, rs1861758 and rs1861759), while seven SNPs at the genotypic level (rs2287195, rs8044354, rs7194886, rs6500328, rs17312836, rs1861759, and rs18611758) [73].

The association of polymorphisms in *NOD2* gene with leprosy reactions highlights the potential role of this gene in susceptibility and development of RR and ENL. The genetic association studies between NOD2 and leprosy are summarized in **Table 2**.

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