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Research Article

STUDY OF THE IL-23 RECEPTOR AND THE IL-28B GENE POLYMORPHISM IN INDIVIDUALS FROM THE LEPROSY-ENDEMIC AREA IN THE BRAZILIAN AMAZON

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Abstract: Leprosy is an immunopathology caused by *Mycobacterium leprae* and its clinical evolution depends on the immunological aspects of the host. There is evidence that IL28B acts by activating TNK cells and IL-23 promotes the expansion of Th17 cells, both actions are fundamental in the response to mycobacteria. We verified the relationship between the rs12979860 polymorphism in the IL28B gene and the rs11209026 polymorphism in the IL23 receptor gene and leprosy. 240 individuals from the Brazilian Amazon were included in the research, from which blood samples were collected for DNA extraction, SNP typing and sequencing. Individuals between 32 and 46 years old had 7.6 and individuals over 46 years old were 12.4 times more likely to become patients. No associations were observed between the rs12979860 SNP in the IL28B gene and the rs11209026 SNP in the IL23R, their genotypes and alleles and leprosy.

Keywords: *Cytokines, Leprosy, Immunogenetics, Single Nucleotide Polymorphism, Disease Susceptibility*

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Introduction

In leprosy the immune response mechanism can be divided into innate and acquired response. In what concerns about the barrier created by the innate response against *M. leprae* infection, it is sustained by the integrity of the epithelium, production of secretion, action of immunoglobulin A (IgA), action of Natural Killer cells (NK) and activated macrophages, which combined with the low virulence of the bacillus, it can avoid the infection without the needing activation of adaptive immunity [1-3]. Thus, it can guarantee resistance to the host and preventing the development of the disease. Once the innate immune response mechanisms have been overcome, the regulation of inflammatory mediators in susceptible hosts may orientate the acquired immune response to the proliferation of opposite poles of T helper cells (Th), then promoting opposing responses to infection. The determinant factors for the direction of this response are still uncertain [4-8].

Due to this polarization of immune responses, the disease can present different clinical forms, classified according to their histopathological aspects and the host's immune status. There are two antagonistic presentations of the disease, tuberculoid leprosy and Virchowian leprosy. Tuberculoid leprosy is characterized by an intense cellular immune response, low bacillary proliferation, and lesion limitation, and virchowian leprosy is a form of high susceptibility of the host, characterized by a humoral immune response, high bacillary proliferation and dissemination of the infection to the viscera and nervous tissue. Between these two opposing forms are the dimorphic forms of the disease, which present varied clinical manifestations, alternating between the polar patterns of response [9-12].

Upon entering the body, the first line of contact between *M. leprae* and humans is mediated by Toll-like receptors (TLRs) present in host cells, which recognize molecular patterns of mycobacteria (PRR: pattern recognition receptors) and are activated up lipoproteins the *M. leprae*.

Their ability to initiate the protective response is linked to the secretion of the pro-inflammatory cytokine interleukin (IL) 12 and 23 and the differentiation of macrophages and dendritic cells, which present the antigen and activate T cells through the secretion of IL-12. This process differentiates naive T cells into IFN-γ and IL-2 producing cells, called Th1 [5,8].

IFN-γ acts by activating macrophages and IL-2 acts by stimulating the growth of antigen-specific T cells, which results in a microbicidal response, thus developing the mildest form of the disease or cure [4-6].

T cells that produce IL-4, IL-5 and IL-10, called Th2, enhance the humoral response. IL-4 acts by stimulating the production of IgE and both IL-4 and IL-10 act by stimulating B cells and inhibiting macrophage activation, which generates a suppressive response and results in a progressive infection, leading to the most severe forms of the disease [4-6].

Sadhu and Mitra reported that despite the influence of cytokines in the environment being the main determinant of regularization, there are also other factors that may be involved in the polarization of T cells, such as the presence of hormones of immunological activity, the dose and route of antigen administration, the type of antigen-presenting cell that stimulates T cells, and the "signal strength" of the T cell receptor for antigens of the Major Histocompatibility Complex (MHC) [6]. These authors excluded bacterial diversity as an essential factor of polarization due to the extreme similarity between the genomes of leprosy bacilli worldwide.

There are also other groups of T cells with important functions in the regulation of the leprosy immune response, such as NKT cells, Treg cells and Th17 cells. NKT cells initiate the rapid production of immunoregulatory cytokines (such as IL-4, IFN-γ and TNF-α), in addition to having a T cell receptor (TCR) that recognizes glycolipids linked to antigen molecules.

Treg cells, which are CD4+ CD25+ cells, perform suppression of the response by inhibitory cytokines, suppression by cytolysis, suppression by metabolic disruption and suppression by modulating the maturation of dendritic cells, seeking to balance the response. While Th17 cells mediate the pro-inflammatory function by recruiting neutrophils, activating macrophages and increasing Th1 effector cells. The function of Th17 and Treg cells in leprosy patients can be crucial in the immunopathogenesis of many disease states [9-15].

The Th1/Th2/Th17 immune response mechanism is described in leprosy, but it does not cover all aspects observed in this complex disease, leaving questions to be answered about the susceptibility observed in a minor part of the population exposed to the bacillus, which would probably be related to genetic polymorphisms associated with innate mucosal immunity or cellular response in the skin [6,16].

Cytokines are a critical component of the acute inflammatory response and are essential for the survival of lymphocytes. In this context, to realize their influence it is necessary to interpret the genetic basis of the host, since the variation of gene expression that occurs naturally between the genomes of different individuals and different populations may explain its phenotypic variation, thus clarifying the evolution of the disease process. The IL28 and IL23 cytokines, as well as polymorphisms in the genes responsible for the synthesis of these proteins, are widely studied and their functions have already been described in the development of several pathologies [17–27].

The IL28B acting by increasing the expression of MHC I, and activating γ T lymphocytes, CD8+ T lymphocytes and NK cells, it is associated with the generation of CD8+ T cells in non-human primates and is capable of increasing long-term responses in CD8+ T cells, driving the memory generation of these cells. It is also responsible of activating the Janus kinase (JAK) pathway - signal transducer and activator of transcription (STAT) pathway, through specific cellular receptors, carrying cellular signals to carry out previously established commands. Hence presenting a prominent role in the inflammatory response [28–33].

The IL23 is a pro-inflammatory cytokine, produced by several types of cells, such as macrophages, dendritic cells, NK cells, in response to infection by intracellular pathogens. Through interaction with its IL23R receptor, it promotes the proliferation of memory T cells and Th17 cell-mediated IL-17 secretion [34].

In this way, the analysis of polymorphisms rs12979860 in the IL-28B gene, and rs11209026 in the IL23R gene, may contribute to a better insight of immunogenetics in leprosy [17–27].

The IL28B gene is found on chromosome 19q13.13, which is responsible for encoding interleukin 28B (IL28B), known as interferon Lambda type III (IFN-λ3). There are different SNPs in the IL28-B gene. Among them, there are those that have a greater association with HCV infection, with SPNs rs12979860 in the IL-28B gene associated with the evolution to spontaneous resolution in cases of acute hepatitis [35,36].

The IL-23 receptor gene (IL23R) is located on chromosome 1 (1p31) and the formation of interleukin (IL)-23 occurs through the binding of a shared IL12p40 subunit to a p19 protein [37,38].

Studies have shown that the SNP rs11209026 (or Arg381Gln or R381Q, switch from G to A) encodes an amino acid change in the IL2-3 receptor gene (IL-23R) in exon 9, causing a decrease in IL-17 production, which is dependent on IL-23 [37]. This polymorphism is rare, with the G allele being found in 96% of the global population and the A allele in 4%. In the literature, it has been shown to provide a protective effect against the development of Crohn's disease [39]. The A allele has also been shown to protect against inflammatory bowel disease, ankylosing spondylitis, associated with susceptibility to psoriasis and severity in pulmonary tuberculosis [40–43].

Materials and Methods

Sampling and ethical considerations

This is a cross-sectional, quantitative study, in which individuals residing in the state of Pará, belonging to the Amazon region of Brazil, were included. Individuals who agreed to participate in this study signed a consent form in accordance with Resolution N°466 of the National Health Council. The study was approved by the Municipal Health Department, authorities and by the Research Ethics Committee of the Evandro Chagas Institute (Ministry of Health).

Individuals with a clinical diagnosis of leprosy, undergoing treatment or who finished treatment, were called a group of patients, which at the time of diagnosis were classified according to the Ministry of Health as paucibacillary (PB) or multibacillary (MB) [1]. Thus, were included 70 MB and 35 PB. Additionally, 135 individuals who did not present clinical symptoms of leprosy, who live or have lived with a patient with leprosy in the last five years, before the patient started treatment, were included.

DNA extraction and genotyping

Blood samples were collected from patients and contacts, from which DNA extractions were performed, using the kit DNeasy Blood & Tissue (QIAGEN), following the manufacturer's guidelines.

To genotype the polymorphisms, the Veriti Thermocycler device (Applied Biosystems, Foster City, CA, USA) was used, which amplified a 736 bp fragment (base pairs) for the rs11209026 SNP in the IL23R gene and a 300 bp fragment for the SNP rs12979860 of the IL-28B gene, through primers for PCR, designed by the Primer3Plus program from the corresponding genomic region, deposited in GenBank.

Amplification of the rs11209026 SNP (IL23R gene) was performed using the following conditions: initial denaturation at 95°C for 15 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 65°C for 30 seconds, extension at 72°C for 1 minute and extension final at 72°C for 10 minutes.

Amplification of the SNP rs12979860 (IL-28B gene) was performed using the following conditions: initial denaturation at 95°C for 15 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 1 minute and final extension at 72°C for 10 minutes.

The amplified products were subjected to electrophoresis in a 2.0% agarose gel containing 3.0 µL of Sybr Safe to visualize the amplified DNA fragments. The amplified product was subjected to purification with the kit EasyPure PCR Purification, according to the manufacturer's recommendations, and after being purified, it was subjected to a sequencing reaction.

The sequencing reaction for the two SNPs was performed using the BigDye® Terminator v3.1Cycle Sequencing kit reagents, from Applied Biosystems. The reaction consisted of 10.5µl of nuclease-free water, 2µl of buffer, 0.75µl of Big Dye, 0.75µl of primer (5 pmols) and 2µl of DNA and submitted to the following conditions in the thermocycler: initial denaturation at 96°C for 1 minute, followed by 25 cycles of denaturation at 96°C for 10 seconds, annealing at 50°C for 05 seconds and extension at 60°C for 4 minutes. The resulting product was subjected to sequencing plate mounting.

The amplified and purified products, after plate assembly, using 10ul of sample, plus 10ul of X-terminator, plus 45ul of Sam Solution, were submitted to the ABI 3130 Genetic Analyzer sequencer (Applied Biosystems®) with subsequent BLAST performed on the website of National Center for Biotechnology Information (NCBI).

Results

The SNP sequencing of 240 individuals was performed, where 108 individuals were sequenced for both SNPs (IL23R rs12979860 and IL28B rs11209026), 78 individuals sequenced only for rs11209026 in IL23R and 54 individuals sequenced only for rs12979860 in IL28B, totaling 348 sequences tests [Table-1].

Table-1 *Quantitative analyzed corresponding to each SNP and haplotype.*

SNP*	Total number of analysis performed
rs11209026**	186
rs12979860***	162

**SNP - Single nucleotide polymorphism. **rs11209026 - polymorphism in the IL23 receptor gene. ***rs12979860 - IL28B gene polymorphism.*

The characterization of the age group of the participants is shown in [Table-2], according to the group. Individuals aged 32 to 46 years were 7.63 times more likely (OR= 7.63; 95%CI= 2.8081 – 20.7360) to become patients and individuals over 46 years of age were 12.4 times more likely to become patients more likely (OR= 12.4; 95%CI= 2.961 - 51.811).

Table-2 *Characterization of the age group of the study group*.

Age group	Patient		Contact		OR* (p)	IC95%*	p-value	
	n	%	n	%			Test G**	x2#
0-15	5	4,8	31	23	1	-	-	
16-31	10	9,5	34	25,2	1,82	0,5610 - 5,9269	0,313	p<0,001†
32-46	80	76,2	65	48,1	7,63	2,8081 - 20,7360	p<0,001†	
>46	10	9,5	5	3,7	12,4	2,961 - 51,811	p<0,001†	
Age group	**MB**		**PB**		**OR***	**IC95%***	**p-value**	
	n	**%**	**n**	**%**			**Test G****	**x2#**
0-15	2	2,9	3	8,6	1	-	-	
16-31	4	5,7	6	17,1	1	0,111 - 8,947	1	
32-46	55	78,6	25	71,4	3,30	0,5186 - 21,0007	0,184	p=0,04†
>46	9	12,8	1	2,9	13,5	0,8777 - 207,6345	0,038	

**Odds Ratio, with a 95% confidence interval. **G test of independence. x2# - Chi-Square Test. †Statistically significant. MB: multibacillary. PB: paucibacillary*.

Considering only the patients, there was a higher frequency of individuals aged 32 to 46 years among the multibacillary and paucibacillary. There were more women among the contacts (66.7%) and more men among the patients (51.4%) (p=0.004). Considering the classification of the disease, there was a higher frequency of male patients among the MB (57.1%) and a higher frequency of female patients among the PB (60.0%). Men were 0.47 times more likely (OR=0.47; 95%CI=0.2796-07974) to become patients [Table-3].

Table-3 *Characterization of the groups regarding the gender of the participants*.

Gender	Patient (MB+PB)		Contact		OR* (p)	IC95%*	p-value**
	n	%	n	%			
Feminine	51	48,6	90	66,7	0,47	0,2796 - 07974	0,004†
Masculine	54	51,4	45	33,3			
Gender	**MB**		**PB**		**OR* (p)**	**IC95%***	**p-value****
	n	**%**	**n**	**%**			
Feminine	30	42,9	21	60	0,50	0,2190 -1415	0,097
Masculine	40	57,1	14	40			

**Odds Ratio, with a 95% confidence interval. **G test of independence. †Statistically significant. MB: multibacillary. PB: paucibacillary.*

For the analysis of SNPs rs11209026 in the IL23R, 33 PB, 56 MB and 97 contacts were sequenced, totaling 186 individuals. The general distribution of genotypes in the study population showed the G/G genotype more frequently among all individuals (89.7%, N= 167/186), followed by the G/A genotype (9.6%, N= 18/186) and finally, with a much rarer frequency, the A/A genotype (0.7%, N= 1/186).

Table-4 *Distribution of rs11209026 genotypes the IL23R in the groups studied*.

Groups	G/G		G/A		A/A		p-value *
	n	%	n	%	n	%	
MB	52	92,9	4	7,1	0	0	0,652
PB	29	87,9	4	12,1	0	0	
MB	52	92,7	4	7,3	0	0	0,839
PB+Contacts	115	88,4	14	10,7	1	0,9	
Patients (MB+PB)	81	91	8	9	0	0	0,955
Contacts	86	88,7	10	10,3	1	1	
PB	29	87,9	4	12,1	0	0	0,859
Contacts	86	88,7	10	10,3	1	1	
MB	52	92,9	4	7,1	0	0	0,866
Contacts	86	88,7	10	10,3	1	1	

**Chi-Square Test or G test of independence. MB: multibacillary. PB: paucibacillary. A: adenine. G: guanine.*

Table-5 *Risk associations between genotypes of rs11209026 in the IL23R between multibacillary and paucibacillary and contact union; and between multibacillary and contacts*.

Genotype rs11209026	Patient		Contact		OR*	IC95%*	p-value**
	n	%	n	%			
G/A	8	9	10	10,3	0,846	0,319 - 2,258	0,807
A/A mutant	0	0	1	1	0,53	0,047 - 5,962	1
A/A e G/A	8	9	11	11,3	0,772	0,319 - 2,258	0,807
G/G wild	81	91	86	88,7	-	-	-
Genotype rs11209026	**MB**		**PB**		**OR***	**IC95%***	**p-value****
	n	**%**	**n**	**%**			
G/A	4	12,1	4	7,1	1,793	0,417 - 7,709	0,462
A/A mutant	0	0	0	0	-	-	-
G/G wild	29	87,9	52	92,9	-	-	-

**Odds Ratio, with a 95% confidence interval. ***Fisher's Exact Test. MB: multibacillary. PB: paucibacillary. A: adenine. G: guanine.*

The genotypic distribution of rs11209026 in the IL23R according to the groups is shown in [Table-4]. The G/A genotype was rarely found in all groups, being more frequent among PB group. The A/A genotype is rare, it was found only in the contact group. No associations were observed between groups and genotypes [Table-5].

The allele distribution of the rs11209026 polymorphism, it is observed that the G allele stands out in the general population of the study, with 94.6%, compared to the A allele, with 5.4%. When comparing the presence of allele frequencies of rs11209026 between the studied groups, no significant difference was observed, with the proportions of A and G alleles being similar between the groups [Table-6].

Table-6 *Distribution of the frequency of alleles in the rs11209026 polymorphism by group of investigated individuals*.

Allele IL23	Contact		Patient		OR*	IC95%*	p-value**
	n	%	n	%			
A	12	6,3	8	4,5	1,433	0,572 – 3,592	0,496
G	180	93,7	172	95,5			
Allele IL23	**MB**		**PB**		**OR***	**IC95%***	**p-value****
	n	**%**	**n**	**%**			
A	4	3,5	4	6,1	0,574	0,138 – 2,376	0,438
G	108	96,5	62	93,9			

**Odds Ratio, with a 95% confidence interval. **Fisher's Exact Test. MB: multibacillary. PB: paucibacillary. A: adenine. G: guanine*.

As for the analysis of rs12979860 in IL28B, 42 multibacillary patients, 26 paucibacillary patients and 94 contacts were sequenced, totalling 162 individuals. The general distribution of genotypes in the study population showed the C/C genotype more frequently among all individuals (46.2%, N= 75/162), followed by the C/T genotype (40.7% N= 66/162), and finally, with a rarer frequency, the T/T genotype (13.1%, N= 21/162).

The result of the genotypic analysis obtained through sequencing is shown in [Table-7]. When comparing the studied groups, a greater presence of the C/C genotype was observed in all groups, being more frequent in paucibacillary patients (53.8%). The presence of the C/T genotype was more frequent in the group of contacts (46.8%) and the T/T genotype was rarely found, being more frequent in multibacillary patients (16.7%). No risk associations were observed between groups and genotypes [Table-8].

Table-7 *Distribution of rs12979860 genotypes in IL28B in the groups studied*.

Groups	C/C		C/T		T/T		p-value*
	n	%	n	%	n	%	
MB	21	50	14	33,3	7	16,7	0,953
PB	14	53,8	8	30,8	4	15,4	
MB	21	50	14	33,3	7	16,7	0,464
PB+Contacts	54	45	52	43,3	14	11,7	
Patients (MB+PB)	35	51,5	22	32,4	11	16,1	0,162
Contacts	40	42,6	44	46,8	10	10,6	
PB	14	53,8	8	30,8	4	15,4	0,336
Contacts	40	42,6	44	46,8	10	10,6	
MB	21	50	14	33,3	7	16,7	0,295
Contacts	40	42,6	44	46,8	10	10,6	

**Chi-Square Test or G test of independence (chi-Square residual analysis). MB: multibacillary. PB: paucibacillary. C: Cytosine. T: Thymine.*

Table-8 *Risk associations between the genotypes of rs12979860 in IL28B between multibacillary and the union of paucibacillary and contacts; and between multibacillary and contacts*.

Genotype rs12979860	Patient		Contact		OR*	IC95%*	p-value**
	n	%	n	%			
C/T	22	32,4	44	46,8	0,571	0,288 - 1,1132	0,123
T/T mutant	11	16,4	10	10,6	1,257	0,477 - 3,313	0,805
C/T e T/T	33	48,6	54	57,4	0,698	0,373 - 1,307	0,269
C/C wild	35	51,4	40	42,6	-	-	-
Genotype rs12979860	**MB**		**PB**		**OR***	**IC95%***	**p-value****
	n	**%**	**n**	**%**			
C/T	14	33,3	8	30,8	1,166	0,388 - 3,507	1
T/T mutant	7	16,7	4	15,4	1,166	0,287 - 4,742	1
C/T e T/T	21	50	16	46,2	0,875	0,342 - 2,236	0,81
C/C wild	21	50	14	53,8	-	-	-

**Odds Ratio, with a 95% confidence interval. **Chi-Square Test or G test of independence (chi-Square residual analysis). MB: multibacillary. PB: paucibacillary. C: Cytosine. T: Thymine*.

Regarding the distribution of rs12989860 alleles in the population studied, the C allele is the more frequent in the general population (66,6%).

When comparing the presence of allelic frequencies of rs12989860 between the groups studied, no significant difference was observed and there was no association of the different alleles with possible clinical outcomes, with the proportions of C and T alleles being similar between them [Table-9].

Table-9 *Distribution of rs12989860 allele frequency by group of investigated individuals.*

Allele IL28	Contact		Patient		OR*	IC95%*	p-value**
	n	%	n	%			
C	124	66	92	67,6	0,926	0,579 - 1,481	0,75
T	64	34	44	32,4			
Allele IL28	**MB**		**PB**		**OR***	**IC95%***	**p-value****
	n	%	n	%			
C	56	66,6	36	69,2	0,888	0,422 - 1,869	0,756
T	28	33,4	16	30,8			

**Odds Ratio, with a 95% confidence interval. **Fisher's Exact Test. MB: multibacillary. PB: paucibacillary. A: Adenine. C: Cytosine.*

Discussion

The long incubation period of the bacillus (average of 2 to 10 years) is described in the literature, which reduces the incidence of leprosy in children and makes the incidence in this age group an indicator of active transmission of the disease in the general population. Thus, a higher incidence of the disease is expected in adult individuals, which was observed in the results of this research, in which a higher frequency of patients in the age group from 32 to 46 years and individuals over 32 years old were more likely to develop the disease and/or the most severe form (MB). These results are in agreement with the study by Reis *et al*., in which the authors indicated a higher incidence of the disease in individuals aged between 41 and 60 years [44]; are also in agreement with the study carried out by Barbosa *et al*., in which the authors identified that the mean age of the patients was 46 years and pointed out that 88.4% of the patients had some occupation before starting treatment, a percentage that dropped to 75% during treatment and to 73% post-discharge from treatment [45]. It's noted, therefore, that the disease mainly affected the economically active population, sometimes causing impacts on the development of the individual's functions in the society in which he lives [46,47].

Regarding gender, in this study it was possible to observe a higher frequency of female individuals among the contacts (66.7%) and a higher frequency of male individuals among the patients (51.4%). Male subjects had a 0.47 chance of becoming patients. Among the patients, there was a higher frequency of males among the MB (57.1%) and a higher frequency of female patients among the PB (60.0%). Data that agree with the study carried out by Oliveira *et al*., in which he pointed out that males represent the majority of multibacillary cases (66.79%) and females as the majority of paucibacillary cases (59.52%) [48]. And they agree with the authors Silva *et al*. who also highlight the predominance of males (68%), with multibacillary operational classification (85%) [49]. It is possible that this fact is associated with the lower concern of men with health and body aesthetics, seeking care only with curative intent, in more severe cases, with the disease installed and causing damage to the development of their routine [48–50].

The IL-23 / Th17 pathway is of great importance in microbial defenses, and its deregulation can lead to inflammation. This is the reason because IL23 is able to modulate responses in multiple cell populations, including differentiation and maintenance of CD4+ Th17 lymphocytes and CD8+ Tc17 lymphocytes, through its binding to the IL23R complex and signaling by the JAK-STAT and via nuclear factor kappa B (NF-κB). Therefore, the genetic variation of the host in relation to rs11209026 in the IL23 receptor can result in a loss of function, leading to a decrease in the production of cytokines of the IL-23/Th17 pathway (such as IL-17A, IL17F, IL-2) or a gain of function, leading to an increase in microbial clearance [51,52].

Zhang *et al*. consider IL23R as a new susceptibility gene involved in innate immunity and in the development of leprosy and point out autophagocytosis as a crucial action of host defense against *M. leprae* infection [27].

In this study, there was no association between the genotypes and alleles of rs11209026 in IL23R and the development of leprosy. However, Ben-Selma and Boukadida associated rs11209026 in IL23R to the severity of pulmonary tuberculosis (TB), which is a disease caused by a bacterium of the same genus as *M. leprae* and has some similar immune response characteristics [40]. Further on, Jiang *et al* analyzed a sequence of SNPs in the IL23R axis and its association with the severity of pulmonary tuberculosis in a Chilean population with a high rate of TB, excluded rs11209026 due to its rare presentation, but concluded that IL23R polymorphisms may be considered risk factors for active pulmonary TB and its severe clinical forms, in addition to associating these SNPs with TB susceptibility, drug resistance, and pulmonary TB cavity formation [53].

Leturiondo *et al*., when studying the relationship of another IL23R SNP, rs76418789, in the Amazonian population with leprosy, did not find an association of this SNP with the disease and found the rare frequency of the mutant allele in the studied population, as in our study [54]. We studied another SNP, however, although leprosy also presents an inflammatory response, and this SNP is very promising in the development of these pathologies, there was no association of rs11209026 with the disease in the population of this study. Thus, IL23R polymorphisms may not play an important role in leprosy. Especially in our population, which of the 186 genotyped individuals, only 1 presented the A/A genotype for rs11209026 and the A allele had a low frequency both in the patient group (4.5%) and in the contact group (6, 3%). Results that agree with the literature, in which it is reported that the A allele is present in only 4% of the population, and the wild G allele is present in 96% of the population [39].

Deveci *et al*. studying ankylosing spondylitis (AS), characterized by inflammation of the joints of the spine and large joints such as the hips, shoulders and other regions, described the frequency of the A allele of rs11209026 as very low (~3%), reported that there were no patients homozygous for the A allele and very few were heterozygous [55]. With the A allele presenting a rare frequency, similar to that presented in our study. Xia *et al*. in their meta-analysis pointed out nine polymorphisms in the IL23R axis associated with susceptibility to AS, among them is the SNP rs11209026. They analyzed different populations; Europeans, Americans and Asians; and when separating them by ethnicity, they identified a significant association between seven SNPs of the IL-23R axis and susceptibility to AS in Europeans and Americans, but there was no association in Asians [56]. Overall, these studies emphasize the importance of immunogenetic studies in different populations. Polymorphisms of the IL23R gene have also been studied in order to see how they can influence the development of psoriasis, which is a non-contagious, chronic skin disease characterized by the presence of pink or reddish patches, covered by whitish scales, and psoriatic arthritis (PsA), which is a chronic condition that affects the skin and joints [57,58]. Loures *et al*., among other polymorphisms, suggested that rs11209026 in IL23R influences the immune response of PsA and, therefore, its development [58].

Hamdy *et al*., when studying rheumatoid arthritis (RA), a systemic, inflammatory, chronic and progressive disease that affects the joints resulting in loss of function and disability, suggested that the A/A genotype of rs11209026 in IL23R influences the etiology of RA; consequently, it may be a genetic marker for RA [59]. Zou *et al*., in their meta-analysis, disagreed with this result, describing the absence of association between the rs11209026 polymorphism of the IL-23R gene and RA in all the genetic models they studied [60]. In agreement with the research by Zou *et al*. and by Paradowska-Gorycka *et al*, when analyzing the polish population and the association of rs11209026 of the IL-23R gene, they described that this SNP did not influence RA susceptibility in this population [60,61].

Recently, Li *et al*. published a study describing the influence of the rs11209026 SNP of the IL-23R gene on the development of infection by Candida albicans, an opportunistic fungus that causes Vulvovaginal candidiasis (VVC), a common vaginal infection in women, second only to vaginosis bacterial. They found the risk relationship of the presence of the A/A, G/A genotypes or allele of the A allele with the development of VVC and that this risk increases if the carrier of the G/A genotype is a smoker. They also found that carriers of the G/A genotype of the rs11209026 polymorphism of the IL-23R gene had higher serum levels of IL23 compared to carriers of the G/G genotype among patients with VVC [62]. In our study, we obtained a higher frequency of the G/G genotype (89.7%) among all groups of the population studied, the G/A genotype was rarely found, being more frequent in paucibacillary patients (12.1%).

Zhu *et al*., in their meta-analysis, evidenced the role of this polymorphism as a protective factor against the development of inflammatory bowel disease (IBD), which is a pathology characterized by the presence of a chronic inflammation of unknown etiology in the intestine [63]. Data that agree and give further evidence to the findings of Li *et al*. who support in their meta-analysis the protective role of rs11209026 on the IL23 receptor in the development of IBD [64]. This makes rs11209026 in the IL23 receptor one of the most significant human genetic polymorphisms in autoimmunity, with evidence of its contribution in protecting against inflammatory diseases [40,59,62,63,65].

Additionally, other polymorphisms in the IL23 receptor have been associated with the development of infectious diseases by other authors. Jiang *et al*., in a study with tuberculosis, demonstrated the association of rs7518660 with susceptibility to pulmonary tuberculosis, rs11465802 with drug resistance and rs1884444 with cavity injury [53]. Zhao *et al*. associated the G allele and the GG genotype of rs11209032 with the occurrence, severity and outcome of immunosuppressive therapy in patients with aplastic anemia, which is a rare disease that affects the formation and development of blood cells [66]. Then, these researches emphasize the importance of understanding the immunogenetic collaboration of IL23R in different pathologies.

The IL28B gene is a strong human genetic determinant for spontaneous viral clearance as it is associated with the innate immune response of the host through the coding of Interferons (IFNs), which are directly connected to viral control (prevents viral replication in a non-specific way). Therefore, genetic variations of the host such as rs 12979860 in the IL28B gene can influence the development immune response, making it crucial for the development of pathologies whose course is defined by the immune response, such as leprosy [28–33].

The rs12979860 SPNs in the IL28-B gene was first described in individuals with chronic hepatitis C (HCV) in 2009, when Ge *et al*. identified that those with the genotype homozygous and heterozygous for the T allele were less likely to be successful in therapy [29]. This result was confirmed by Pineda *et al*., adding that even patients co-infected with HIV can achieve successful therapy if the responder genotype (homozygous for the C allele) is identified [67]. Other studies have also shown that this genotype is present at higher frequencies in individuals infected with hepatitis C who cleared the infection spontaneously compared to those who developed HCV [68,69].

In this study, it was not possible to observe associations between leprosy and the genotypes and alleles of rs12979860 in the IL28B gene, although the presence of the T/T genotype and the T allele are already associated with a poor prognosis of cirrhosis, hepatic fibrosis and hepatocellular carcinoma and to the development of the pathogenesis associated with the Andes virus [19,70–72].

Silva *et al*. warned of the involvement of IL28B SNPs related to the immune system and the development of severe Dengue virus (DENV) infection, which can cause, through unclear mechanisms, symptoms of classic dengue, with mild fever/flu, dengue hemorrhagic fever and dengue shock syndrome, leading to death [49]. In this study, the authors associated the T allele of rs12979860 in the IL28B gene to a higher risk of developing severe forms of the infection, thus bringing an immunogenetic bias to a possible explanation of the different clinical outcomes in DENV infections. Which corroborate the findings of Vargas-Castillo *et al*. who, despite not finding an association between rs12979860 and the development of severe dengue in the Mexican population, reported the mild form as being the most frequent in the population studied, suggesting that the genetic background can modify directly the role of these molecules related to the immune system [73]. In our study, the T allele was more frequent in the MB group of patients (33.4%), but not statistically significant.

When analyzing HIV (Human immunodeficiency virus) positive patients and co-infection with human papillomavirus (HPV), Ouladlahsen *et al*. showed that the rs12979860 SNP is not the main determinant of susceptibility to HPV infection and its progression to abnormal cervical lesions in women who living with HIV [74]. Machmach *et al*. associated the presence of the C/C genotype to the spontaneous control of HIV [21] and Zaidane *et al*. reported that rs12979860 did not influence the susceptibility to HIV-1 and the development of AIDS, but it is able to affect the patient's response to treatment, measured by CD4+ T cell count [75]. In our study, we observed a greater presence of the C/C genotype in all groups of the population studied, being more frequent in paucibacillary patients (53.8%), but there was no statistically significant association between this genotype and leprosy. The influence of this polymorphism has also been studied in cases of congenital infection by human cytomegalovirus (cCMV), which is transmitted through the placenta to the immature fetus and can lead to severe damage to the central nervous system. There is already evidence that the rs12979860 SNP is associated with an increased risk of thrombocytopenia in neonates with cCMV, and neonates carrying the C/T genotype of rs12979860 in IL28B had a three to fourfold increased risk of cystic lesions on ultrasound or magnetic resonance, and a two-fold increased risk of ventricular dilatation diagnosed by magnetic resonance imaging [76,77]. In adults, Bravo *et al*. and Chmelova *et al*. pointed to a protective effect of the T allele against cytomegalovirus infection [17,78]. In this study, the presence of the C/T genotype was more frequent in the contact group (46.8%) and the T/T genotype was rarely found, being more frequent in multibacillary patients (16.7%), both did not present a statistically significant association with leprosy in the population studied.

In the development of human T lymphotropic virus (HTLV) infection, a virus that has a tropism for lymphocytes and is associated with rheumatic diseases, adult T-cell leukemia/lymphoma, HTLV-1-associated myelopathy/tropical spastic paraparesis, Vallinoto *et al*. did not found no association between the rs12979860 SNP and the outcome of the development of HTLV-1-associated myelopathy/tropical spastic paraparesis [79]. De Sá *et al*., when analyzing the rs12979860 SNP and the development of HTLV-associated arthropathy (HAA) pointed out that patients with HAA who presented the C/C genotype for rs12979860 expressed high levels of TNF-β and IFN-γ, and patients with the genotype C/T and T/T for rs12979860 showed high levels of IL-10 [80]. These cases accentuate the relevance of studying the influence of this SNP on the development of the immune response.

The association of the rs12979860 SNP and the development of hepatitis C virus (HCV) infection has been extensively studied and the detection of the rs12979860 SNP is already used as a clinical aid for the management of patients with HCV, since it is evident that individuals who have the T allele are less likely to be successful in therapy, while individuals with a genotype homozygous for the C allele are more likely to achieve spontaneous virus clearance [29,67–69]. Attallah *et al*. described the T/T genotype as more prevalent in patients with advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC), associating the T/T genotype of this SNP with poor outcomes in chronic HCV patients and an increased risk of developing HCC [71].

Additionally, other polymorphisms in the IL28B gene have been associated with the development of infectious diseases by other authors. Grebely *et al*., when evaluating the role of IL28B in treatment-induced spontaneous clearance after recent HCV infection, found that patients with the G/G and G/T genotype for rs8099917 in IL28B had a low probability of spontaneous viral clearance, therefore treatment should be initiated close to the time of clinical presentation [30]. Pérez-García *et al*., when analyzing the SNP rs12980275 and patients undergoing major surgery, associated the rs12980275 polymorphism with death related to septic shock in patients undergoing these procedures. They concluded that the A allele is associated with protection and the G allele is associated with an increased risk of death [81]. These instances highlight the extent to which it is still possible to advance in immunogenetic research to elucidate the different obstacles to clinical success.

Conclusion

When analyzing the population of Pará, in the Amazon region, Brazil, we observed that the genotype A/A of rs11209026 in IL23R is rare (0.7%) in this population, that the G/G genotype is the one with the highest frequency among all individuals (89.7%) and that the G/A, despite being less frequent in the entire population studied (9.6%), when analyzing the groups, it was more frequent in the group of paucibacillary patients (12.1%). No associations were observed between the rs11209026 SNP in IL23R, its genotypes and alleles and leprosy.

As for the analysis of rs12979860 in IL28B, the C/C genotype was more frequent in the entire population (46.2%), however, with a very balanced frequency as the C/T genotype (40.7%).

The T/T genotype had a low frequency in the general population (13.1%) and in the analysis of the groups it was more frequent in the group of multibacillary patients (16.7%). No associations were observed between the rs12979860 SNP in the IL28B gene, its genotypes and alleles and leprosy.

Application of research: It is important to understand the role of the IL-23 receptor and IL-28B SNPs in leprosy from an endemic area of Brazil.

Research Category: Immunogenetics, Cytokines

Abbreviations: Ig: Immunoglobulin; NK: Natural Killer; Th: T helper; TLR: Toll-like receptor; PRR: pattern recognition receptor; IL: interleukin; IFN: interferon; NKT: natural killer T; Treg: regulatory T; MHC: Major Histocompatibility Complex; TNF: tumoral necrosis factor; M: microbicidal factor of macrophages; TGF: tumoral growth factor; TCR: T cell receptor; JAK: Janus kinase; STAT: signal transducer and activator of transcription; IFN-λ3: interferon Lambda type III; PB: paucibacillary; MB: multibacillary; AS: ankylosing spondylitis; RA: rheumatoid arthritis; VVC: vulvovaginal candidiasis; IBD: inflammatory bowel disease; DENV: Dengue virus; HIV: human immunodeficiency virus; HPV: human papillomavirus; cCMV: human cytomegalovirus; HTLV: human T lymphotropic virus; HCV: hepatitis C virus; HCC: hepatocellular carcinoma.

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Authors agree to submit ethical clearance certificate from the concerned ethical committee, Institutional Animal Ethics Committee (IAEC) or institutional biosafety committee, if the project involves field trails/experiments/exchange of specimens, human & animal materials.

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