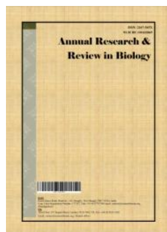


Annual Research & Review in Biology

37(1): 1-10, 2022; Article no.ARRB.77522
ISSN: 2347-565X, NLM ID: 101632869

Analysis of the Frequency of the Toll-like 2 Gene Polymorphism in Leprosy

Marcos Jessé Abrahão Silva [a], Everaldina Cordeiro dos Santos [a], Yan Corrêa Rodrigues [a], Karla Valéria Batista Lima [a] and Luana Nepomuceno Gondim Costa Lima [a*]

[a] *Evandro Chagas Institute (IEC) / Ministry of Health, Rodovia BR-316 km 7 s/n, Levilândia, Zip Code: 67030-000, Ananindeua / Pará, Brazil.*

Authors' contributions

This work was carried out in collaboration among all authors. Author MJAS was responsible for the conceptualization, formal analysis, investigation, methodology, statistical analysis, validation, visualization, writing, drafting and editing. Author ECS managed the analyses of the study. Author YCR performed the supervision, validation, visualization, writing, reviewing and editing. Author KVBL performed the supervision, validation, visualization, writing, reviewing and editing. Author LNGCL managed the conceptualization, investigation, methodology, protocol writing, project administration, supervision, visualization, writing, reviewing and editing. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2022/v37i130472

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/77522

Original Research Article

Received 15 October 2021
Accepted 20 December 2021
Published 05 January 2022

ABSTRACT

Polymorphisms in genes that are responsible for encoding cytokines and receptors involved in the immune response, such as Toll-like Receptor (TLR) 2 in leprosy, are of great interest for immunogenetic studies. This work aimed to analyze the possible association of single nucleotide polymorphism (SNP), synonymous, rs3804100 of the TLR2 gene with leprosy. The study was conducted in Bacteriology and Mycology section of Evandro Chagas Institute, Brazil between August 2020 and July 2021.The scope of the study consisted of 122 subjects from cities of Goianésia, Rondon, Curionópolis, Altamira, Parauapebas and Redenção of the State of Pará, Brazil. Genotyping was performed by conventional PCR and sequencing in the ABI 3130 Genetic Analyzer (Applied Biosystems®) using primer nucleotides designed by the Primer3Plus program from the genomic region "Homo sapiens toll like receptor 2 (TLR2) transcript variant X6, mRNA",

**Corresponding author: E-mail: luanalima@iec.gov.br;*

deposited in GenBank with reference XM_011532216.2. The analyzes were performed based on Fisher's exact test. It was managed in accordance with Helsinki Declaration and the Brazilian National Health Council and with approval of the ethics committee at Evandro Chagas Institute, under opinion number: 3.950.570. No associations between gender and leprosy were possible (P> 0.05). However, associations were observed between age groups, which were significant between those over 46 years old (P=0.004) and the 2nd dose of BCG as a more protective agent between the groups analyzed (P=0.004). For the subjects with the typed genotypes, 68 contacts had T/T genotype and only 4 T/C genotypes, while in multibacillary (MB) group only 1 T/C genotype was found and none in paucibacillary (PB) (P> 0.05). We conclude that there is no association between the TLR2 SNP rs3804100 and leprosy in the Pará population, which still indicates the need for new immunogenetic studies with other genes involved in the immune response and a greater number of polymorphisms.

Keywords: Epidemiology; leprosy; genetic polymorphism; immunogenetics.

1. INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, a bacillus-shaped mycobacterium that acts as an obligate intracellular parasite, mainly affecting the skin and nervous tissues [1]. Moreover, *M. leprae* infects predominantly macrophages, dendritic cells, and Schwann cells, which may lead to nerve damage and disability [2].

Epidemiological data position Brazil as the second in prevalence of cases globally, surpassed only by India [3]. According to data from the Brazilian Ministry of Health, high incidence of leprosy cases occurs in the Midwest, Northeast and North regions, and with the state of Pará as the 3rd in number of detected cases in 2020 within the Brazilian territory [4].

Leprosy progression is linked to immunogenetic factors that orientate the host's immune response against the pathogen. The tuberculoid form (TT) (paucibacillary) is understood by the high host resistance to infection, which is characterized by a T helper 1 (Th1) lymphocyte response. Oppositely, the lepromatous form (LL) (multibacillary) a reduced Th1 response is observed, causing infection spread, bacillary multiplication, and a Th2-based immune response. Finally, transitions clinical manifestations between the polar clinical (TT and LL) forms, correspond to the borderline group [5].

The innate immune response is the first line of defense against *M. leprae*, being a crucial step for the development of the response against the bacillus, as it has essential effector components in combating the pathogen, and is capable of directing adaptive immunity. Several cells of the immune system are activated through their receptors to generate an antigenic response, such as Natural Killer cells, lymphocytes, among others. Among the receptors expressed by Natural Killer cells, the Toll-Like receptors (TLR) on the cell surface and cytoplasm stand out [6].

The TLRs are a group of transmembrane receptors present from *Drosophila* to mammals. In humans, the group of these receptors consists of 10 protein subtypes. These proteins are specific cells receptors for virus, bacterial and fungal components, and to initiate the activation of the cytokine production and adaptive immune response [7].

Each member of the TLR family is activated by specific antigens that lead to different transcriptional activation profiles, initiating an adequate immune response to the pathogen. These polymorphic receptors recognize lipoproteins present in the bacillus and promote the differentiation of monocytes into macrophages and CD1b+ dendritic cells, which in turn activate lymphocytes that release cytokines such as TNF-α and IL-12, promoting an antimicrobial activity. Of all mammalian TLRs, TLR2 detects the broadest repertoire of molecular patterns from a wide variety of pathogens, including mycobacteria, making it of particular clinical importance [8]. Just because *M. leprae*, in the host, initiates cell signaling through activation of the TLR1/TLR2 heterodimer, single nucleotide polymorphisms (SNP) in these genes may be able to confer susceptibility or protection to leprosy [9].

Kang and Chae, 2001 studied the SNP rs121917864 (C to T substitution) at nucleotide 2029 from the start codon of the TLR2. The mutation would substitute Arg to Trp at amino acid residue 677, one of the conserved regions of TLR2. They only found the mutated base in lepromatous leprosy individuals [10].

Another SNP in the TLR2 gene, the rs3804100 is a synonymous mutation that does not lead to a substitution of amino acid serine (Ser) at residue 450 and was predicted to have a functional effect in decreasing the number of exonic splicing enhancing motifs [11]. This SNP has been extremely associated with several infectious diseases [12–17], including tuberculosis, in which the infectious agent is of the same genus as leprosy and has a host cellular immune response similar to leprosy [18]. However, the only work in the literature that studied the relationship of the SNP rs3804100 with leprosy was by Bochud P-Y et al., 2008, with samples from a population from Africa. However, SNPs change from one population to another (genetic makeup) and so do results on disease associations [19].

Then, the present study aimed to verify the relationship between the SNP rs3804100 of the TLR2 gene and leprosy by comparing the genotypic and allelic frequencies among leprosy cases and their healthy contacts from an endemic area of Brazil.

2. MATERIALS AND METHODS

2.1 Sampling and Study Location

This study involved patients in health care centers in the cities of Altamira, Goianésia, Rondon, Parauapebas, Redenção and Curionópolis of the State of Pará, Brazil. The sample consisted 50 patients, including 25 TT and 25 LL, which were diagnosed according to the Brazilian Ministry of Health criteria [20]. Additionally, the patients' household contacts were invited to participate in the study, forming a group of 72 subjects, which have no genetic link with patients and did not present clinical symptoms of leprosy. This group was called control. Blood samples were collected from patients and contacts by venipuncture in 5ml tubes and stored at -20°C for laboratory procedures at the Bacteriology and Mycology section, Evandro Chagas Institute. Household contacts with a history or suspicion of leprosy were excluded.

2.2 SNP rs3804100 Typing of the TLR2 Gene

The SNP under analysis is rs3804100 from Thymine to Cytosine (T>C) at position 450 of the extracellular domain of TLR2 at nucleotide 1350 (1350T/C). The typing was based on two PCR primer nucleotides: Primer 1 - F: 5'ATTCAGCCTGTGAGGATGCC 3' and R: 5' TTGCTGCTCCTGAGTGAAGG 3' generating a 512 bp fragment and Primer 2 – F: 5' ACCGGAGAGACTTTGCTCAC 3' and R: 5' GCTTGCTGCTCCTGAGTGAA 3' generating fragment of 437 bp. Primers used in PCR essays were generated on the Primer3Plus program based on the genomic region "Homo sapiens toll like receptor 2 (TLR2) transcript variant X6, mRNA", deposited in GenBank with the reference XM_011532216. 2.

DNA extractions were performed using the DNeasy Blood & Tissue kit (QIAGEN, Hilden, Germany), following the manufacturer's guidelines. Conventional PCR essays for TLR2 gene amplification were performed using PlatinumTaq (Invitrogen, Carlsbad, CA, U.S.A) on the Veriti Thermocycler (Applied Biosystems, Foster City, CA, U.S.A.), under the following conditions: initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 1 minute, followed by a final extension at 72°C for 10 minutes. Amplified products were submitted to electrophoresis in 3,6% agarose gel stained with 3,0 µL of SybrSafe for visualization of the amplified DNA fragments. Reaction products were purified and bidirectionally sequenced using Big Dye Terminator v3.1 chemistry on ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Obtained sequences were analyzed and compared to those available at BLAST on the National Center for Biotechnology Information (NCBI) website.

2.3 Data Analysis

The results were organized in a database of the Epi info^{tm} 7 program. The observed proportions of polymorphism presence within each studied group were analyzed using Fisher's exact test. The analysis for SNPs prospection considering the dominant and variant alleles of the searched nucleotide base was performed by means of a bioinformatics program, Bioedit^{tm} v7.2.5.

3. RESULTS

From a total of 122 individuals with the defined genotypes, 39 were from Altamira (31,97%), followed by Redenção (33 – 27,05%), Goianésia (19 - 15,57%), Parauapebas (12-9,84%), Rondon (10 - 8,20%), and Curionópolis (9 - 7,38%). Regarding household contacts individuals, 32 were from Altamira

(44,44%), followed by Redenção (21 - 29,17%) and Goianésia (8 - 11,11%). Most of the patients group came from Redenção with 12 (24,00%), then from Goianésia with 11 (22,00%) and Parauapebas with 10 (20,00%). Distribution of individuals according to location and clinical manifestation is presented in Table 1.

Socio-epidemiological data revealed that most study subjects were women (78 - 63,93%), over 46 years of age (41,80%), and having taken only 1 dose of BCG (bacillus *Calmetté-Guerin*) vaccine (63 - 51,64%). Among the group of household contacts, 49 were females (68,08%), over 46 years of age (27 - 37,50%), and having taken only 1 dose of BCG vaccine (40 - 55,56%). The group of patients was also mostly female, 29 (58%), with a predominance of 24 of them older than 46 years (48%), and 25 of them had not had a BCG vaccination (50%) (Table 2).

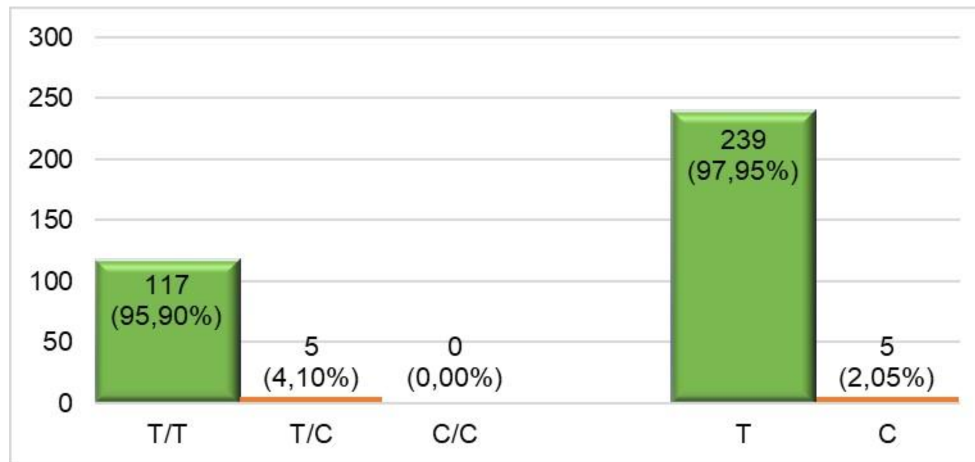
Table 1. Distribution of number and frequency of sampling of contacts, LL, TT, and patients according to municipalities

Municipalities	Contacts N (%)	LL N (%)	TT N (%)	Patients N (%)	Total N (%)
Altamira	32 (44,44%)	6 (24,00%)	1 (4,00%)	7 (14,00%)	39 (31,97%)
Redenção	21 (29,17%)	6 (24,00%)	6 (24,00%)	12 (24,00%)	33 (27,05%)
Goianésia	8 (11,11%)	1 (4,00%)	10 (40,00%)	11 (22,00%)	19 (15,57%)
Parauapebas	2 (2,78%)	9 (36,00%)	1 (4,00%)	10 (20,00%)	12 (9,84%)
Rondon	2 (2,78%)	3 (12,00%)	5 (20,00%)	8 (16,00%)	10 (8,20%)
Curionópolis	7 (9,72%)	0 (0,00%)	2 (8,00%)	2 (4,00%)	9 (7,38%)
Total	72 (100,00%)	25 (100,00%)	25 (100,00%)	50 (100,00%)	122 (100,00%)

Table 2. Demographics of the subjects as to sex, age, and BCG

	CONTACTS N (%)	LL N (%)	TT N (%)	PATIENTS N (%)	TOTAL N (%)
Sex					
Male	23 (31,94%)	12 (48,00%)	9 (36,00%)	21 (42,00%)	44 (36,07%)
Female	49 (68,06%)	13 (52,00%)	16 (64,00%)	29 (58,00%)	78 (63,93%)
Age					
0-15	15(20,83%)[a,b,c,d]	1 (4,00%)[a,c,e]	1 (4,00%)[a,d,e]	2 (4,00%)[b]	17 (13,93%)
16-31	16 (22,22%)[a,b,c,d]	2 (8,00%)[a,c,e]	3 (12,00%)[a,d,e]	5 (10,00%)[b]	21 (17,21%)
32-46	14 (19,44%)[a,b,c,d]	6 (24,00%)[a,c,e]	13 (52,00%)[a,d,e]	19 (38,00%)[b]	33 (27,05%)
>46	27 (37,50%)[a,b,c,d]	16 (64,00%)[a,c,e]	8 (32,00%)[a,d,e]	24 (48,00%)[b]	51 (41,80%)
BCG					
0	18 (25,00%)[f,g,h,i]	12 (48,00%)[f,h,j]	13 (52,00%)[f,i,j]	25 (50,00%)[g]	43 (35,25%)
1	40 (55,56%)[f,g,h,i]	12 (48,00%)[f,h,j]	11 (44,00%)[f,i,j]	23 (46,00%)[g]	63 (51,64%)
≥2	14 (19,44%)[f,g,h,i]	1 (4,00%)[f,h,j]	1 (4,00%)[f,i,j]	2 (4,00%)[g]	16 (13,11%)

P>0.05; [a]P=0.003; [b]P=0.004; [c]P=0.001; [d]P=0.003; [e]P=0.002; [f]P=0.024; [g]P=0.004; [h]P=0.025; [i]P=0.015; [j]P=0.036, Fisher's Exact Test



Graph 1. Distribution of the T/T, T/C, C/C genotypes and of the T and C alleles for the SNP in TLR2

Table 3. Distribution of genotypes in the contact, LL, TT and patient groups for SNP rs3804100

Genotype	Contact N (%)	LL N (%)	TT N (%)	Patient N (%)
T/C	4 (5,56%)	1 (4,00%)	0 (0,00%)	1 (2,00%)
T/T	68 (94,44%)	24 (96,00%)	25 (100,00%)	49 (98,00%)
Total	72 (100,00%)	25 (100,00%)	25 (100,00%)	50 (100,00%)

P>0.05, Fisher's Exact Test

Table 4. Allele distribution for the contact, LL, TT and patient groups for SNP rs3804100

ALLELE	Contact N (%)	LL N (%)	TT N (%)	Patient N (%)
T	140 (97,22%)	49 (98,00%)	50 (100,00%)	99 (99,00%)
C	4 (2,78%)	1 (2,00%)	0 (0,00%)	1 (1,00%)
Total	144 (100,00%)	50 (100,00%)	50 (100,00%)	100 (100,00%)

P>0.05, Fisher's Exact Test

The genotypic frequencies in the study population agreed with the Hardy-Weinberg equilibrium (*P*=0.053). The genotype distribution for the SNP in TLR2 revealed that 117 individuals presented the T/T genotype (95,90%), followed by 5 presenting the T/C genotype. (4,10%). As for the alleles, 239 were T (97,95%) and 5 were C (2,05%) (Graph 1).

Considering the distribution of SNP rs3804100 genotypes of the TLR-2 gene among the evaluated groups, the majority of household contacts presented T/T genotype (68 – 94,4%) and only four the T/C genotype (5,56%). Among the LL group, one individual presented the T/C genotype (4,00%), while the T/T genotype was detected among all TT individuals (Table 3).

Allele distribution for SNP rs3804100 among the groups demonstrated that all TT individuals had only the T allele (100%), while in LL there was only 1 C allele, mutant (2%). In the contact group there were only 4 C alleles (2,78%). In the patient group, the only C allele found represented 1% (Table 4).

4. DISCUSSION

Even though not fatal, and despite the advances in therapy, diagnosis and research, leprosy remains as a global public health problem, being one of the most frequent causes of non-traumatic peripheral neuropathy worldwide. Leprosy reactions, as a result of acute episodes of clinical inflammation during the chronic course of the disease are a challenging issue as nerve damage increases morbidity even after treatment. In addition, its endemicity in tropical and developing countries is delimited by

epidemiological, immunogenetic, socioeconomic and environmental risk factors [21].

Immunogenetic studies in relation with leprosy are essential to identify new markers (genes) in the genetic profile which may be associated with susceptibility and/or protection against the disease [22]. The innate immune response mediated by PRRs, such as TLR2, is the first line of host defense, which takes into account pathogen recognition. TLR2 is also required for the induction of IL-12, as the IL-12-dependent IFN-γ pathway is of paramount importance in cellular immunity, promoting Th1 response. The human TLR2 gene is located on chromosome 4 (4q32), consists of 3 exons, two coding and one non-coding [23]. TLR2 SNP rs3804100 has been described to affect and reduce exonic splicing sites, but still with uncertain role in TLR2 function [11].

Mycobacterium tuberculosis and *M. leprae* are mycobacteria that have common surface elements detected by the host. Krutzik et al., 2003 demonstrated that TLR1/2 heterodimers mediate monocyte activation promoted by *M. leprae*, indicating the presence of mycobacterial lipoproteins in addition to lipoarabinomannan (LAM) and phosphatidylinositol mannoside (PIM), characterizing examples of TLR2 binding to mycobacterial components [24,25]. Thus, SNPs in the TLR2 gene may impair binding of the TLR1/2 heterodimer to mycobacterial lipoproteins, as in the case of SNP rs121917864, which promoted susceptibility to tuberculosis and leprosy in studies by Ben-Ali et al., 2004 and Kang and Chae, 2001 [10,18], respectively. The 19 kDa adhesion lipoprotein from *M. tuberculosis* shares 47% amino acid sequence similarity with the lipoprotein from M. leprae [26].

Genotyping studies have allowed associations between SNPs in the TLR gene and infectious diseases, such as those caused by mycobacteria. A study by Chen Y-C et al., 2010 in a Taiwanese population correlated the SNP rs3804100 and susceptibility to latent tuberculosis conferred by the CC genotype (in 4,3% of patients and in 4,3% of controls), which triggered elevation in the NK cell counts in the blood and, consequently, lead to disease progression [27]. Human NK cells have been reported to directly recognize mycobacteria through TLR2 and release TNF-α and IFN-γ [28]. However, these results are divergent in different populations due to distinction in the genetic makeup [29,30]. This genetic makeup corresponds to the genetic basis of the individual, which undergoes variations over time and due to environmental exposure [31].

There were no statistically significant associations between sex and leprosy, which was also observed among a Indian population in study by De Vries and Perry, 1985 [32]. However, most of the recent studies portray a discrepancy between the sexes: in Asian countries, leprosy affects men more than women, likewise in Brazilian studies, while in African countries women are more affected. Interestingly, among LL groups, African and Brazilian men were more affected [33–37]. The detection of leprosy among African women is probably explained by an aesthetics (body care) compared to men, as well as easier access to health services such as prenatal care [33]. The predominance of leprosy cases among men globally is probably due to the higher environmental exposure to *M. leprae* and/or the lack of women undergoing a complete physical examination in some cultures, leading to delayed diagnosis and the generation of physical disabilities [38].

In Brazil, studies by Rocha et al., 2020 and Romão and Mazzoni, 2013 revealed that higher detection of new cases are observed among those older than 46 years, likewise in the present study [39,40], which is probably linked e to the lower exposure of younger people to the bacillus and longer incubation period for LL cases [38]. Analysis regarding age highlight the involvement of the economically active population and low transmission of the disease, in addition to contributing factors related to age-related immunological changes and higher susceptibility to infectious diseases among elderly people. Despite this concerning fact, most of cases among elderly are excluded from the Global Health Strategies for leprosy, making it difficult to conduct surveillance and control programs for the disease [41,42].

Since the 1960s, studies conducted in six different countries support the results obtained in the present study regarding the protection conferred by the second dose of BCG vaccine against leprosy among the groups, as 12,5% of the group of patients who took the 2nd dose in our study population [43,44]. In the present study, within the group of patients, about 50% had no BCG vaccination scar, revealing the lack of protective factor against the disease. The presence of a vaccination scar was the most

common finding among all the groups analyzed. The calculation of BCG vaccine efficacy ranged from 0 to 50%, respectively, in Brazil and Malawi [45,46]. In India, the protection afforded by the first dose of BCG vaccine was 14% in the general population and 80% in contacts with leprosy. Several case-control studies have been conducted to evaluate the protective effect of BCG vaccine in leprosy, 12 of which included individuals with the first dose [44,47–50]. Most of these studies were conducted with people in tuberculosis control programs. The overall protection observed in these studies was between 36% and 90%, which is in line with the present study [48,50–53].

As similar in study by Bochud P-Y et al., 2008, the TLR2 SNPs rs3804099; rs3804100 were associated with leprosy reverse reaction, involving Ethiopian population, and for rs3804100 no significant associations were observed (P= 0.73), with CC genotype present in 0,2% of patients and 0% among controls, with C allele being found rarely [19], corroborating the information in the present study about the lack of association between the studied SNP and leprosy. The T/C genotype was also more detected in both studies in the LL group compared to the TT group, which means similarity between contacts and TT due to lack of mutation in these groups, which could indicate that the T allele is related to protection from the disease and/or its more severe form.

Furthermore, in vivo recognition of mycobacteria seems to depend on multiple TLRs, since mice lacking a single type of receptor (such as TLR2) are only slightly more susceptible when exposed to aerosolized *M. tuberculosis* [54]. Another study by Bafica et al., 2005 demonstrated that concomitant recognition of mycobacteria through TLR2 and TLR9 produced most or all of the stimuli required to induce protective antimycobacterial immune responses that depend on TLRs [55].

The results of the present study have not shown associations between SNP rs3804100 and susceptibility or protection to leprosy, also demonstrating that this genomic region is well preserved in the Brazilian Amazon population. Thus, due to the low frequency of SNPs evaluated in the population investigated, further in-depth studies are required, in addition to the investigation of other polymorphisms, in order to elucidate the relationship between SNP rs3804100 in the TLRs gene and the immune response developed by the pathogen-host interaction.

5. CONCLUSION

In conclusion, no significant association between sex and leprosy was observed in this study. An association between age group and leprosy was observed, which was significant among those older than 46 years. Correlation of 2nd dose of BCG was found to be more protective among the groups analyzed. The allelic and genotypic frequencies of the SNPs found were undifferentiated among the three study groups. No association was observed between the SNP rs3804100 of TLR2 and leprosy. A predominance of T/T genotype and T alleles was observed in all PB group individuals. The mutant genotype, T/C was observed only in contacts and TT.

CONSENT

All authors declare that ‘written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

The present study was conducted in accordance with Helsinki Declaration and the Brazilian National Health Council and with approval of the ethics committee at Evandro Chagas Institute, under opinion number: 3.950.570.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Han XY, Seo Y-H, Sizer KC, Schoberle T, May GS, Spencer JS, et al. A new Mycobacterium species causing diffuse lepromatous leprosy. Am J Clin Pathol. 2008;130:856–864. doi:10.1309/AJCPP72FJZZRRVMM
2. Weddell G, Palmer E. The pathogenesis of leprosy. An experimental approach. Lepr Rev. 1963;34: 57–61. doi:10.5935/0305-7518.19630010

3. World Health Organization = Organisation mondiale de la Santé. (2020). Global leprosy (Hansen disease) update, 2019: time to step-up prevention initiatives – Situation de la lèpre (maladie de Hansen) dans le monde, 2019: le moment est venu d'intensifier les initiatives de prévention. Weekly Epidemiological Record = Relevé épidémiologique hebdomadaire. 2019; 95 (36):417- 438.
4. Brazil, Ministry of Health. Secretariat of Health Surveillance. Department of Epidemiological Surveillance. Leprosy Epidemiological Bulletin: 2020. Hansen Epidemiological Bull. 2020; Jan: 52.
5. Araújo MG. Leprosy in Brazil. Rev Soc Bras Med Trop. 2003;36:373–382. doi:10.1590/S0037-86822003000300010
6. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010;11: 373–384. doi:10.1038/ni.1863
7. Reis LM, Rodrigues FW, Silva RE da, Taleb AC, Ávila MP de. Analysis of the relationship of the occurrence of single nucleotide polymorphism of the DOCK9 gene in keratoconus. Rev Bras Ophthalmol. 2016;75: 223–227. doi: 10.5935/0034-7280.20160046
8. Santana N de L, Rêgo JL, Oliveira JM, Almeida LF de, Braz M, Machado LMM, et al. Polymorphisms in genes TLR1, 2 and 4 are associated with differential cytokine and chemokine serum production in patients with leprosy. Mem Inst Oswaldo Cruz. 2017/03/02 ed. 2017;112: 260–268. doi:10.1590/0074-02760160366
9. Rea TH, Levan NE. Current Concepts in the Immunology of Leprosy. Archives of Dermatology. 1977;113: 345–352. doi:10.1001/archderm.1977.01640030091017
10. Kang TJ, Chae GT. Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. FEMS Immunol Med Microbiol. 2001;31: 53–58. doi:10.1111/j.1574-695X.2001.tb01586.x
11. Yuan H-Y, Chiou J-J, Tseng W-H, Liu C-H, Liu C-K, Lin Y-J, et al. FASTSNP: an always up-to-date and extendable service for SNP function analysis and prioritization. Nucleic Acids Res. 2006;34: W635–W641. doi:10.1093/nar/gkl236
12. Thuong NTT, Hawn TR, Thwaites GE, Chau TTH, Lan NTN, Quy HT, et al. A polymorphism in human TLR2 is associated with increased susceptibility to tuberculous meningitis. Genes Immun. 2007;8: 422–428. doi:10.1038/sj.gene.6364405
13. Jabłońska A, Paradowska E, Studzińska M, Suski P, Nowakowska D, Wiśniewska-Ligier M, et al. Relationship between toll-like receptor 2 Arg677Trp and Arg753Gln and toll-like receptor 4 Asp299Gly polymorphisms and cytomegalovirus infection. Int J Infect Dis. 2014;25: 11–15. doi:10.1016/j.ijid.2014.04.001
14. Jabłońska A, Studzińska M, Szenborn L, Wiśniewska-Ligier M, Karlikowska-Skwarnik M, Gęsicki T, et al. TLR4 896A/G and TLR9 1174G/A polymorphisms are associated with the risk of infectious mononucleosis. Sci Rep. 2020;10: 13154–13154. doi:10.1038/s41598-020-70129-4
15. Golovkin AS, Ponasenko AV, Yuzhalin AE, Salakhov RR, Khutornaya MV, Kutikhin AG, et al. An association between single nucleotide polymorphisms within TLR and TREM-1 genes and infective endocarditis. Cytokine. 2015;71: 16–21. doi:10.1016/j.cyto.2014.08.001
16. Bochud P-Y, Magaret AS, Koelle DM, Aderem A, Wald A. Polymorphisms in TLR2 Are Associated with Increased Viral Shedding and Lesional Rate in Patients with Genital Herpes Simplex Virus Type 2 Infection. The Journal of Infectious Diseases. 2007;196: 505–509. doi:10.1086/519693
17. Royse KE, Kempf M-C, McGwin G Jr, Wilson CM, Tang J, Shrestha S. Toll-like receptor gene variants associated with bacterial vaginosis among HIV-1 infected adolescents. J Reprod Immunol. 2012/09/28 ed. 2012;96: 84–89. doi:10.1016/j.jri.2012.08.002
18. Ben-Ali M, Barbouche M-R, Bousnina S, Chabbou A, Dellagi K. Toll-like receptor 2 Arg677Trp polymorphism is associated with susceptibility to tuberculosis in Tunisian patients. Clin Diagn Lab Immunol. 2004;11: 625–626. doi:10.1128/CDLI.11.3.625-626.2004
19. Bochud P-Y, Hawn TR, Siddiqui MR, Saunderson P, Britton S, Abraham I, et al. Toll-like receptor 2 (TLR2) polymorphisms are associated with reversal reaction in leprosy. J Infect Dis. 2008;197: 253–261. doi:10.1086/524688
20. Brazil, Ministry of Health. Database of the Unified Health System-DATASUS. [cited 22 Jul 2021].

Available:http://www2.datasus.gov.br/DATASUS/index.php
21. Fine PEM. Global leprosy statistics: a cause for pride, or frustration? Lepr Rev. 2006;77: 295–297.
22. Prevedello FC, Mira MT. Leprosy: a genetic disease? AnBras Dermatol. 2007; 82:451–459. DOI:10.1590/S0365-05962007000500009
23. Nemazee D, Gavin A, Hoebe K, Beutler B. Toll-like receptors and antibody responses. Nature. 2006;441:E4. DOI:10.1038/nature04875
24. Krutzik SR, Ochoa MT, Sieling PA, Uematsu S, Ng YW, Legaspi A, et al. Activation and regulation of Toll-like receptors 2 and 1 in human leprosy. Nature Medicine. 2003;9:525–532. DOI:10.1038/nm864
25. Krutzik SR, Modlin RL. The role of Toll-like receptors in combating mycobacteria. Seminars in Immunology. 2004;16: 35–41. DOI:10.1016/j.smim.2003.10.005
26. Takeuchi O, Sato S, Horiuchi T, Hoshino K, Takeda K, Dong Z, et al. Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. J Immunol. 2002;169:10–14. DOI:10.4049/jimmunol.169.1.10
27. Chen Y-C, Hsiao C-C, Chen C-J, Chin C-H, Liu S-F, Wu C-C, et al. Toll-like receptor 2 gene polymorphisms, pulmonary tuberculosis, and natural killer cell counts. BMC Med Genet. 2010;11:17–17. DOI:10.1186/1471-2350-11-17
28. Marcenaro E, Ferranti B, Falco M, Moretta L, Moretta A. Human NK cells directly recognize Mycobacterium bovis via TLR2 and acquire the ability to kill monocyte-derived DC. Int Immunol. 2008;20:1155–1167. DOI: 10.1093/intimm/dxn073
29. Sánchez D, Lefebvre C, Rioux J, García LF, Barrera LF. Evaluation of Toll-like receptor and adaptor molecule polymorphisms for susceptibility to tuberculosis in a Colombian population. International Journal of Immunogenetics. 2012;39:216–223. DOI:10.1111/j.1744-313X.2011.01077.x
30. Ma M, Xie L, Wu S, Tang F, Li H, Zhang Z, et al. Toll-like receptors, tumor necrosis factor-α, and interleukin-10 gene polymorphisms in risk of pulmonary tuberculosis and disease severity. Hum Immunol. 2010;71:1005–1010. DOI:10.1016/j.humimm.2010.07.009
31. Johnson KR, Zheng QY, Noben-Trauth K. Strain background effects and genetic modifiers of hearing in mice. Brain Res. 2006/03/31 ed. 2006;1091:79–88. DOI:10.1016/j.brainres.2006.02.021
32. de Vries JL, Perry BH. Leprosy case detection rates by age, sex, and polar type under leprosy control conditions. Am J Epidemiol. 1985;121: 403–413. doi:10.1093/oxfordjournals.aje.a114012
33. Varkevisser CM, Lever P, Alubo O, Burathoki K, Idawani C, Moreira TMA, et al. Gender and leprosy: case studies in Indonesia, Nigeria, Nepal and Brazil. Lepr Rev. 2009;80:65–76.
34. Veloso DS. Clinical-epidemiological profile of leprosy in the state of Piauí, from 2009 to 2016. 2018 [cited 24 Jul 2021]. Available:https://www.arca.fiocruz.br/handle/icict/31715
35. Oliveira AEVM de, Araújo KM da FA, Queiroga RPF de, Bezerra LLO, Chaves AEP. Epidemiological analysis of leprosy by sex in Paraíba. Res Soc Dev. 2020;9: e755985778–e755985778. DOI:10.33448/rsd-v9i8.5778
36. Barbosa DRM, Almeida MG, Santos AG dos. Epidemiological and spatial characteristics of leprosy in the State of Maranhão, Brazil, 2001-2012. Med Ribeirão Preto. 2014;47:347–356. DOI: 10.11606/issn.2176-7262.v47i4p347-356
37. Vieira G de D, Aragoso I, Carvalho RMB, Sousa CM de. Leprosy in Rondônia: Epidemiol E Serviços Saúde. 2014;23: 269–275. DOI:10.5123/S1679-49742014000200008
38. Nobre ML, Illarramendi X, Dupnik KM, Hacker M de A, Nery JA da C, Jerônimo SMB, et al. Multibacillary leprosy by population groups in Brazil: Lessons from an observational study. PLoS Negl Trop Dis. 2017;11:e0005364. DOI:10.1371/journal.pntd.0005364
39. Rocha MCN, Nobre ML, Garcia LP. Epidemiological characteristics of leprosy in the elderly and comparison with other age groups, Brazil (2016-2018). Public Health Cad. 2020;36. doi: 10.1590/0102/311X00048019
40. Romão ER, Mazzoni AM. Epidemiological profile of leprosy in the city of Guarulhos, SP. RevEpidemiol and Infection Control. 2013;3:22–27. DOI:10.17058/reci.v3i1.3344

41. Lepra org. International day for older people. [cited 24 Jul 2021]. Available:https://www.lepra.org.uk/News/international-day-for-older-people
42. Diniz LM, Maciel LB. Leprosy: clinical and epidemiological study in patients above 60 years in Espírito Santo State - Brazil*. An Bras Dermatol. 2018;93: 824–828. DOI: 10.1590/abd1806-4841.20187092
43. Merle CS, Cunha SS, Rodrigues LC. BCG vaccination and leprosy protection: review of current evidence and status of BCG in leprosy control. null. 2010;9: 209–222. DOI:10.1586/erv.09.161
44. Fine PEM, Carneiro IAM, Milstien JB, Clements CJ, World Health Organization. Issues relating to the use of BCG in immunization programmes: A discussion document; 1999. Available:https://apps.who.int/iris/handle/10665/66120
45. Cunha SS, Rodrigues LC, Pedrosa V, Dourado IM, Barreto ML, Pereira SM. Neonatal BCG protection against leprosy: a study in Manaus, Brazilian Amazon. Lepr Rev. 2004;75: 357–366.
46. Barreto ML, Pereira SM, Ferreira AA. BCG vaccine: efficacy and indications for vaccination and revaccination. J Pediatr (Rio J). 2006;82: s45–s54. DOI:10.1590/S0021-75572006000400006
47. Abel L, Cua VV, Oberti J, Lap VD, Due LK, Grosset J, et al. Leprosy and BCG in southern Vietnam. Lancet. 1990;335: 1536. doi:10.1016/0140-6736(90)93086-5
48. Lombardi C, Pedrazzani ES, Pedrazzani JC, Filho PF, Zicker F. Protective efficacy of BCG against leprosy in São Paulo. Bull Pan Am Health Organ. 1996;30: 24–30.
49. Zodpey SP, Bansod BS, Shrikhande SN, Maldhure BR, Kulkarni SW. Protective effect of Bacillus Calmette Guerin (BCG) against leprosy: a population-based case-control study in Nagpur, India. Lepr Rev. 1999;70:287–294. DOI:10.5935/0305-7518.19990032
50. Zodpey SP, Ambadekar NN, Thakur A. Effectiveness of Bacillus Calmette Guerin (BCG) vaccination in the prevention of leprosy: a population-based case-control study in Yavatmal District, India. Public Health. 2005;119:209–216. doi:10.1016/j.puhe.2004.04.007
51. Fine PE, Ponnighaus JM, Maine N, Clarkson JA, Bliss L. Protective efficacy of BCG against leprosy in Northern Malawi. Lancet. 1986;2: 499–502. doi:10.1016/s0140-6736(86)90367-3
52. Convit J, Smith PG, Zuniga M, Sampson C, Ulrich M, Plata JA, et al. BCG vaccination protects against leprosy in Venezuela: A case-control study. Int J Lepr Other Mycobact Dis. 1993;61: 185–191.
53. Muliyil J, Nelson KE, Diamond EL. Effect of BCG on the risk of leprosy in an endemic area: A case control study. Int J Lepr Other Mycobact Dis. 1991;59: 229–236.
54. Drennan MB, Nicolle D, Quesniaux VJF, Jacobs M, Allie N, Mpagi J, et al. Toll-Like Receptor 2-Deficient Mice Succumb to Mycobacterium tuberculosis Infection. Am J Pathol. 2004;164: 49–57.
55. Bafica A, Scanga CA, Feng CG, Leifer C, Cheever A, Sher A. TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to Mycobacterium tuberculosis. J Exp Med. 2005;202: 1715–1724. DOI:10.1084/jem.20051782

© 2022 Silva et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/77522