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Review

Vitamin D receptor expression and hepcidin levels in the protection or severity of leprosy: a systematic review

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Abstract

Leprosy is a chronic infectious disease whose disequilibrium in the host's genetic, immunological and clinical mechanisms leads to distinct manifestations defining the type of immunological response. This review focuses its attention on the influence of the Vitamin D Receptor and hepcidin expressions that can suggest the protection or severity of leprosy.
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Keywords: Leprosy; Vitamin D receptor; Polymorphism; Hepcidin; Protection; Severity

1. Introduction

Leprosy is a neglected tropical disease (NTD) that affects populations whose association of inadequate conditions of life, work, and access to preventive and curative health services causes this illness, to continue to be the basis of the severe incapacity found in affected individuals, due mainly to the difficulty of early diagnosis during the evolution of the disease. *Mycobacterium leprae* is the etiological agent of leprosy that is slowly developing and contributes significantly to the difficulty of diagnosis in the initial phase of the disease [1,2].

The prevalence of leprosy has been declining in recent decades. Even so, in 2015, it was responsible for the infection of more than 210,000 individuals worldwide. Untreated patients were the main source of infection, influencing the diagnosis rate that, in Brazil, reported 26,395 new cases, representing 13% of all of the cases reported in the world, together with prevalence coefficients of higher than that defined by the WHO concerning the elimination of leprosy as a public health problem [3]. Early detection of cases of leprosy, their treatment, and the study of the genetic, immunological, and clinical characteristics due to infection caused by *M. leprae* are the core principles needed to control the disease [4].

Leprosy appears in different clinical forms, which are determined by the framework of the infected individual's immune response. The occurrence and severity of leprosy associated with the polymorphism of the vitamin D receptor (VDR) has been investigated in populations whose degree of endemicity for leprosy is still rather high, due to the direct action of the active form of the 1,25-dihydroxyvitamin D as an immunomodulatory factor in processes like an expressive reduction in the synthesis of cytokines [5], the production of immunoglobulins [6], the proliferation of lymphocytes, and the transcription of antimicrobial peptides [7−9]. The

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polymorphisms associated with the TaqI, FokI, BsmI, and ApaI sites in the VDR gene can affect the stability of the RNAm, leading to significant changes in protein levels and, consequently, to a disequilibrium in the Th1 and Th2 profiles of the immunological response when combatting the bacillus, which are essential in order to define the clinical manifestations and severity of leprosy [6,10].

On the other hand, hepcidin, a hormonal peptide produced by hepatocytes, has been considered a link between inflammation and anemia observed in chronic diseases like leprosy, which is characterized by the reduction in serum iron levels and the increase in its reserves in ferritin. Some pro-inflammatory cytokines, such as IL-6 and IL-1, link the inflammatory response to inflammatory anemia, much in the same way that bacterial infections mediated by macrophages can boost the production of hepcidin by activating Toll-like receptors (TLR- 4) [11−13]. Anemia has been described in patients with leprosy [14−17]; however, the exact mechanism that makes the link between the serum iron and the effect of reduced iron levels in leprosy have yet to be properly defined.

In an attempt to verify the studies published on the aforementioned themes, the present study sought to conduct a systematic review on the influence of VDR expression and hepcidin levels on the protection and severity of the clinical manifestations of leprosy.

2. Methods

2.1. Study strategy

The methodology of this systematic review is in accordance with the items set forth in PRISMA-P 2015 Protocol (Reporting Items for Systematic Reviews and Meta-Analyses) [18], which aims to answer the following core question: Is there a relationship between hepcidin levels or vitamin D receptors (VDR) and the severity of leprosy? This log can be found in the international database − *Prospective Register of Systematic Reviews* − which is available at: http://www.crd.york.ac.uk/PROSPERO/printPDF.php?RecordID=26792&UserID=13277, from the Centre for Reviews and Dissemination of the University of York.

The search for scientific studies was conducted in the following databases: OVID-Medline − International Literature in Health Sciences, Latin American and Caribbean Literature in Health Sciences (BVS-LILACS), the Scientific Electronic Library Online (SciELO), Google Scholar, and the Brazilian Digital Library of Theses and Dissertations (BDTD), between November 2015 and April 2016. No restrictions were placed on the year of publication of each study and all the studies that related to the central question were included. The definition of the key words was carried out by means of a search using Health Science descriptors (DeCS) and *Medical Subject Headings* (MeSH). The descriptors employed in the initial search and later crisscrossing in the English, Portuguese, and Spanish languages included: *leprosy, hepcidin, vitamin D receptor, infectious diseases (immunology, real-time polymerase chain reaction, macrophages, T-lymphocytes)*, together with filters to expand the study in question, such as *multibacillary, tuberculoid, lepromatous, borderline, calcitriol receptor, iron metabolism, severity,* and *progress,* using the search operators of quotation marks, parentheses, “AND”, “OR”, “exp”, and “mp”. The study protocol is available in annex 1.

For eligibility and inclusion in the scientific studies, two reviewers, ALGO and NSG, read the titles and abstracts. In the second stage, the complete text of each study was evaluated by ALGO, ATC, and CASM, and any disagreements were resolved by trying to answer the question that gave rise to this review.

2.2. Inclusion and exclusion criteria

The studies' inclusion criteria included: original articles and studies with individuals in the age range of 18−65 years. For exclusion from the study, the following criteria were used: narrative or systematic reviews or meta-analyses (G1); case studies, book chapters, editorials, and letters to the editor (G2); studies conducted with pregnant women and nursing mothers, children, adolescents, and elderly individuals of over 65 years of age (G3); experimental studies (G4); studies whose theme was not in accordance with the aims of this review (G5), and studies that evaluated VDR or hepcidin, but not in leprosy (G6).

2.3. Data collection

In an attempt to produce a better analysis and discussion of the results, the studies were grouped into two themes: **a)** expression of VDR and leprosy and **b)** hepcidin levels as regards their influence on the protection and severity of leprosy. Three stages were followed: (1) the identification of the studies through the pairing of descriptors; (2) exclusion after having read the titles, complying with pre-defined criteria; and (3) the reading of the abstract and exclusion of those that were inappropriate for this study. The selected studies were then read in full to define those that met the criteria of the present study in such a way that the content was placed in a chart under the following headings: author biodata, year of publication and country where the study was conducted, sample size, study design, comparative event (VDR expression or hepcidin levels), statistical analysis, and main results.

3. Results

Initially, the database search resulted in 406 studies, observing the pre-defined descriptors. By performing a search in the databases and crisscrossing the descriptors, the following were found: OVID-Medline = **94** studies, BVS-LILACS = **4**, SciELO = **46**, BDTD = **30** (theses and dissertations), and Google Scholar = **241**. With the exclusion of duplicate studies (24) and the application of inclusion and exclusion criteria, 382 articles were selected. After reading the titles and abstracts, 363 were excluded. With the reading of the full article, the relevant data for this systematic review

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included 10 studies (Fig. 1), which composed the chart with the following information: authors' biodata, year of publication, and country where the study was conducted, description of the sample or population, study design, comparative event, statistical analysis, and main results (Table 1).

Of the studies included in this systematic review, nine presented the main theme of VDR expression and leprosy, while one made reference to the levels of hepcidin in patients with multibacillary leprosy. The majority of studies, six (60%) were published as of 2010, nine (90%) were case control studies, and in the Master's thesis [19], two studies were presented, one of a population-based case control study and one of a replication study using families, using the Transmission Disequilibrium Test (TDT) to evaluate its association with leprosy.

As regards the VDR expression, the selected studies referred to the polymorphism of the specific regions of the VDR gene, analyzing the polymorphic variants of TaqI, followed by the remaining variants of ApaI [20,21], FokI [19,21,22], BsmI [20,22] and the genome region of rs4760658

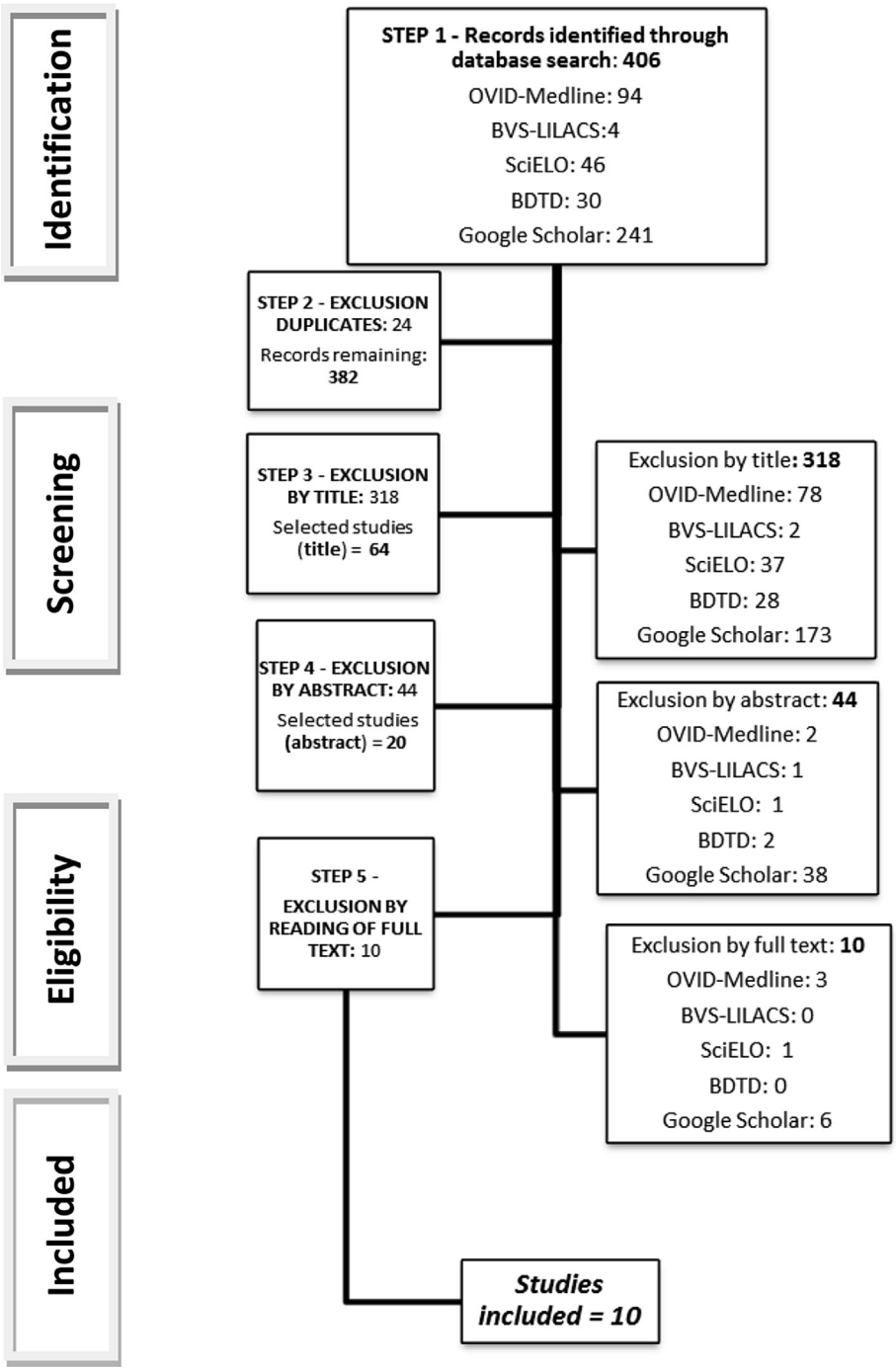

Fig. 1. Fluxogram of the study selection process for the systematic review.

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Table 1
Characteristics of the studies included in the systematic review regarding the VDR expression and the hepcidin levels in the protection against or severity of leprosy.

	Authors/year of publication/ Place where study was conducted	Publication vehicle	Sample/population	Study design	Comparative event	Statistical analysis to obtain results	Results
1	Roy et al., 1999 India	The Journal of Infectious Diseases	N = 397 (C = 166; Tub = 107; LL = 124)	Case Control	VDR polymorphism (TaqI) with the clinical forms of leprosy (Tuberculoid and Leprosum)	Chi-square, Logistic Regression Analysis	**Genotype frequency for leprosy and controls** Tub-LL (p = 0.03); Tub-C (p = 0.005); LL-C (p = 0.04) Genotype tt (Tub-C p = 0.001; OR = 3.22) Genotype TT (LL-C p = 0.03; OR = 1.67) Genotype Tt LL-C (p = 0.008; OR = 0.58)
2	Fitness et al., 2004 Malawi	The American Journal of Tropical Medicine and Hygiene	N = 722 (LEPROSY = 270 (MB = 26); C = 452)	Case Control	VDR polymorphism (TaqI, ApaI, BsmI) Association with susceptibility to leprosy	Logistic regression	**Genotype frequency for leprosy and controls** **Apa I** a → A; Intron 8 G-T (t/t-t/g: OR = 0.7; p = 0.21; t/g-g/g: OR = 1.5; p = 0.43) **Bsm I** b → B; Intron 8 C-T (c/c-c/t: OR = 0.8; p = 0.35; c/t-t/t: OR = 2.1; p = 0.08) **TaqI** T → t; **Exon 9** T → C codon 352 (t/t-t/c: OR = 1.1; p = 0.54; t/c-c/c: OR = 4.3; p = 0.004)
3	Goulart et al., 2006 Brazil	FEMS Immunology and Medical Microbiology	N = 170 (C = 68; PB = 55; MB = 47)	Case Control	VDR Polymorphism (TaqI) and the relationship with the Mitsuda test and bacilloscopy index	Shapiro−Wilk and Kruskal−Wallis tests, Multiple Regression Analysis ($y = b_0 + b_1x_1 + b_2x_2 + e$), Chi-square, T Test	**High allele frequency t:** MB (p = 0.372), PB (p = 0.328), Control (p = 0.331) **Strong association of leprosy with Mitsuda test** Leprosy-Control (p < 0.0001) **Average tendency of reduction in Mitsuda test** Control-Leprosy (p = 0.0001) **Relative risk of Mitsuda response** Leprosy-Contact at home (OR = 3.0) 3 times greater **Association of bacilloscopy index and VDR genotype** (p = 0.2201) **High averages of BI for genotype tt** (4.3 ± 0.2) in relation to Tt (3.9 ± 1.2) and TT (3.8 ± 2.5) **Analysis of VDR genotypes and Mitsuda test** Leprosy-Contact at home, not blood related Tt (OR = 13.33) − 13 times greater probability Allele T − Leprosy: r = 1.412; p = 0.561 Genotype TT − LL patient: r = 0.549; p = 0.0417 Genotype Tt − Controls: r = 0.481; p = 0.0238
4	Velarde-Felix et al., 2009 Mexico	Public Health in México	N = 71 with LL and 144 controls N.A. Sample/Sample calculation	Case Control	VDR Polymorphism (TaqI) in Leprosum Leprosy patients	Pearson's correlation	**Genotype Frequency** **Association *borderline* with protection against leprosy** − Genotype CT of the SNP Fok (OR = 0.77; p = 0.05) - LEPROSY-Control: SNPs rs 4760658 (OR = 0.96; p = 0.85) and Taq (OR = 0.79; p = 0.35) **Study of the haplotype of the Fok/rs 4760658/ Taq combination** Haplotype C/C/C has association with protection against LEPROSY (OR = 0.46; p = 0.02) Haplotype T/T/T showed *borderline* protection (OR = 0.62; p = 0.04) **Study of Fok/Taq haplotype in the Transmission Disequilibrium Test** Haplotype T/T (p = 0.05) suggests protection against the disease
5	Marques, 2010 Brazil	Brazilian Digital Databank of Theses and Dissertations	1 - N = 1003 individuals (PB = 244, MB = 426 and 598 controls). 2−365 individuals (90 nuclear families)	1 − Case Control 2 − Replicative study	Allele and genotype frequencies of Fok, rs4760658, and Taq polymorphisms in the VDR gene	Chi-square test to evaluate the transmission of alleles and haplotypes (transmission disequilibrium test) Model of logistic regression for allele and genotype frequencies	

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6	Sapkota et al., 2010 Nepal	Human Immunology	N = 933 (Reversal Reaction type I = 240, ENL = 124, C = 101)	Case Control	VDR polymorphism (FokI, BsmI, TaqI) e association with type I reversal reaction and patients with erythema nodosum leprosum (ENL)	Chi-square, Distribution Frequency, Fisher Test	**Association of allele A with phenotype/ genotype frequency** Allele/genotype frequency **BsmI** G → A (C-LEPROSY OR = 1.03; p = 0.871/p = 0.976) (Tub-LL OR = 0.94; p = 0.578/p = 0.862) **FokI** C → T (C-LEPROSY = OR = 1.03; p = 0.832/p = 0.277) (Tub-LL = OR = 1.11; p = 0.334/p = 0.571) **TaqI** T → C (C-LEPROSY = OR = 0.84; p = 0.323/p = 0.438) (Tub-LL = OR = 0.97; p = 0.775/p = 0.782) **Association of polymorphism with Tuberculoid (TT) and Leprosum Leprosy (LL)** Genotype frequency (Recessive/Dominant) **BsmI** G → A (C-LEPROSY − Recessive OR = 1.00; p = 0.997/Dominant OR = 1.05; p = 0.836) (Tub-LL Recessive OR = 0.90; p = 0.673/ Dominant OR = 0.94; p = 0.641) **FokI** C → T (C-LEPROSY Recessive OR = 1.67; p = 0.203/ Dominant OR = 0.90; p = 0.612) (Tub-LL Recessive OR = 1.08; p = 0.729/ Dominant OR = 1.16; p = 0.290) **TaqI** T → C (C-LEPROSY Recessive OR = 0.61; p = 0.205/ Dominant OR = 0.88; p = 0.556) (Tub-LL Recessive OR = 1.11; p = 0.740/ Dominant OR = 0.93; p = 0.624) **Association of polymorphism with type I reversal reactions (RR)** Allele/genotype frequency **BsmI** G → A (NoRR-RR OR = 0.90; p = 0.443/p = 0.731) (NoENL-ENL OR = 0.89; p = 0.498/ p = 0.831) **FokI** C → T (NoRR-RR OR = 1.31; p = 0.032/ p = 0.111) (NoENL-ENL OR = 0.80; p = 0.160/ p = 0.223) **TaqI** T → C (NoRR-RR OR = 1.08; p = 0.585/ p = 0.432) (NoENL-ENL OR = 0.79; p = 0.207/ p = 0.481)
7	Souza et al., 2012 Brazil	Memories from Instituto Oswaldo Cruz	N = 63 (C = 25; MB = 38)	Case Control	RNAm expression of hepcidin and urinary hepcidin in multibacillary leprosy	**Spearman's Correlation** (Comparison between the hepcidin expression and bacilloscopy index)	**Urinary hepcidin levels** (Lep: p = 0.03) **Hepcidin expression in biopsies** (lesions MB: p = 0.0022) Positive correlation between **hepcidin levels and IL-1β** in Leprosy serum (r = 0.563; p= 0.04) Positive correlation between **hepcidin expression and Bacilloscopy index** from group 13 MB E 4 PB (r = 0.482; p = 0.025) **Hepcidin expression by immunofluorescence** was significantly higher in Leprosy than in controls (p < 0.001)
8	Teles et al., 2013. United States of America	Science	N = 23 skin lesions (LL = 10, Tub = 6, RR = 7)	Case Control	VDR expression in skin lesions from patients with Tuberculoid and Lepromatous leprosy and reverse reactions	Kruskal−Wallis test, Dunn's multiple comparison test	**RNAm expression of VDR in skin lesions of individuals with leprosy** **Higher scores** − Tub in relation to LL (p ≤ 0.01) and to RR in relation to LL (p ≤ 0.05).

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Table 1 (*continued*)

	Authors/year of publication/ Place where study was conducted	Publication vehicle	Sample/population	Study design	Comparative event	Statistical analysis to obtain results	Results
9	Neela et al., 2015 India	Human Immunology	N = 404 (PB = 87; MB = 135; C = 182)	Case Control	VDR polymorphism (TaqI, FokI, ApaI)	Chi-square, Logistic Regression Analysis	**Allele and genotype frequency of the polymorphism TaqI, FokI, and ApaI of VDR in individuals with leprosy (Lep) and healthy controls (C)** Taq I (Leprosy-C p = 0.12; MB-C p = 0.11; PB-C p = 0.35) Fok I (Leprosy-C p = 0.12; MB-C p = 0.12; PB-C p= 0.05) Apa I (Leprosy-C p = 0.001; MB-C p = 0.001; PB-C p = 0.08)
10	Mandal et al., 2015 India	New Microbes and New Infections	N = 45 (C = 15; LEPROSY = 15; RR = 15)	Case Control	VDR expression in PBMCs of patients with type I and type II leprosy reaction	Average and standard deviation (no statistics test was performed)	**mRNA levels in VDR in PBMCs normalized for GAPDH** Infected individuals N = 6 (Neuritis/ENL − reaction type 2) shows lower quantities of VDR expression in relation to control, of these, N= 2–4% and 5% compared to Control and N = 4–10 to 20% of reduction in VDR expression) N = 4 (lower levels of Vitamin D, N = 2 normal levels of Vitamin D)

N = number of study participants; C = health individuals (control group); Tub = Tuberculoid leprosy (including Tuberculoid - TT and Borderline Tuberculoid - BT); LL = Lepromatous Leprosy including Lepromatous − LL, Borderline Lepromatous − BL and Borderline Borderline − BB; PB= Paucibacillary; MB = Multibacillary; LEPROSY PER SE: individuals with leprosy; VDR = vitamin D receptor; TaqI, FokI, ApaI, BsmI = polymorphic variants of the VDR gene; RR = reverse reaction; ENL − erythema nodosum leprosum; PBMC= Peripheral Blood Mononuclear Cells.

[19], and then associated with the protection and susceptibility to the clinical forms of leprosy. The remaining studies presented the VDR expression in peripheral blood cells from patients with leprosy reaction [10] as well as in skin lesions from patients with leprosy [6]. As regards the hepcidin expression, the selected study analyzed the hepcidin levels in the urine and the RNAm expression of hepcidin in skin lesions [23].

Some studies have shown the association of the polymorphism of the VDR gene with the severity or protection against leprosy [6,10,19–21,24,25] and in two of them was not observed it [22,26].

Table 2 presents the results found regarding the expression and the polymorphism in specific variants of the VDR gene and the relationship with the protection or the susceptibility/ severity of the manifestations and clinical presentation of leprosy.

4. Discussion

4.1. Association of the VDR polymorphism and protection or severity of leprosy

Firstly, it was observed that the TaqI polymorphism in region 3′ of the VDR gene in Indian individuals, has shown that, when the groups of individuals with tuberculoid leprosy and those with lepromatous leprosy were compared with the controls, the overall distribution of the genotypes was significantly different ($p < 0.02$; $X^2 = 7.6$). In tuberculoid leprosy, genotype *tt* presented a significantly high frequency ($p < 0.001$, OR = 3.22) compared to the controls, followed by a slight increase in the genotype *TT* of lepromatous leprosy, when compared to the controls ($p = 0.03$; OR = 1.67). The heterozygous form of genotype *Tt* was less frequent in both clinical forms (*per se*) compared to the controls ($p < 0.01$; OR = 0.58) suggesting a kind of protection against leprosy. These values were corrected to adjust to the ethnic heterogeneity and the results demonstrated that the TaqI polymorphism in the VDR gene, specifically genotype *tt*, favors the susceptibility to leprosy, most likely because it affects the differentiation of the T cells and their maturation, thus hindering an efficient cell immune response [24].

These data were confirmed later, through studies of variants of TaqI, ApaI, and BsmI. Evidences of the association of the susceptibility to leprosy through the homozygous form (*tt*) for a simple change in **TaqI**, of T → C in the codon 352 of the VDR gene (OR 4.3, 95% CI 1.6–11.4, $p = 0.004$) in individuals in Malawi were confirmed. However, attention in the extrapolation of the interpretation of this genetic association, as it is based on a simple analysis of this population. For the other variants of ApaI and BsmI, no statistically significant association was observed between these and the protection or susceptibility to leprosy [20].

In a study conducted in Mexico, with leprosy patients, they observed a *borderline* expression in genotype *TT* for lepromatous leprosy when they compared with healthy individuals ($p = 0.0417$; OR = 0.549), while for genotype Tt, they found a

Table 2
Results from investigative studies of the association of the expression with the VDR gene polymorphism and leprosy.

Primary author and year of publication		Population/Continent	Expression and polymorphism of the VDR gene in leprosy			
			Analyzed variants	Method	Protection	Susceptibility/Severity
1	Roy et al., 1999	India/Asia	Taq I	PCR	**Taq I:** Heterozygous Tt: protection in the controls	**Taq I:** Genotype tt related to Tuberculoid Leprosy Genotype TT related to Virchowian Leprosy
2	Fitness et al., 2004	Malawi/Africa	Taq I Apa I BsmI	PCR-RFLP	Not found	**Taq I:** Genotype tt related to Leprosy *per se* (Multibacillary and Paucibacillary)
3	Goulart et al., 2006	Brazil/Latin America	Taq I	PCR-RFLP	**Taq I:** There was no statistical association of the polymorphism in these variants with the protection against or severity of leprosy. For genotype tt: Negative values for the Mitsuda test and positive values for the Bacilloscopy Index, when comparing Tt and TT, were associated to susceptibility to Virchowian Leprosy (without statistical significance).	Not found
4	Velarde-Felix et al., 2009	Mexico/Latin America	Taq I	PCR	**Taq I:** Heterozygous Tt: protection in the controls	**Taq I:** Allele T and genotype TT related to Virchowian Leprosy
5	Marques, 2010	Brazil/Latin America	Taq I Fok I rs4760658	PCR-RFLP	**Fok:** Genotype CT: protection in the controls **Fok/rs4760658/Taq:** Haplotypes C/C/C: protection against leprosy *per se* T/T/T: borderline protection against leprosy *per se* **Fok/Taq:** Haplotype T/T: borderline protection against leprosy *per se*	Not found

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Table 2 (*continued*)

Primary author and year of publication		Population/Continent	Expression and polymorphism of the VDR gene in leprosy			
			Analyzed variants	Method	Protection	Susceptibility/Severity
6	Sapkota et al., 2010	Nepal/Asia	Taq I Fok I BsmI	PCR	**FokI, BsmI e TaqI** There was no statistical association of the polymorphism in these variants with the protection against or the severity of leprosy.	Not found
7	Teles et al., 2013	Not specified	RNAm expression of VDR in skin lesions	qPCR	Not found	Statistical difference in the RNAm expression for VDR in skin lesions from patients with leprosy. Lower expression for Virchowian Leprosy patients as compared to those with Tuberculoid Leprosy and those with Reverse Reaction.
8	Neela et al., 2015	India/Asia	Taq I Fok I Apa I	PCR-RFLP	**Taq/Fok/Apa** Haplotype TFA related to the resistance to leprosy	**Apa:** Genotypes AA, Aa, aa related to the susceptibility to leprosy *per se*. **Fok:** Genotype ff and allele f related to the susceptibility to Tuberculoid Leprosy. **Taq/Fok/Apa** Haplotype TFa related to paucibacillary susceptibility Haplotype Tfa, related to the multibacillary susceptibility.
9	Mandal et al., 2015	India/Asia	RNAm expression of VDR in peripheral blood cells of patients with leprosy reaction	qPCR	Not found	Low VDR expression (about 5−10% lower) in mononuclear cells of the peripheral blood in patients with reverse reaction as compared to uninfected individuals.

VDR − Vitamin D Receptor; PCR − Polymerase Chain Reaction; PCR-RFLP − Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms; qPCR − quantitative Real Time PCR.

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greater significance in the controls than in the cases of leprosy ($p = 0.0238$; OR = 0.481). This result disagrees with other studies presented herein, but it does show the possibility of the participation of vitamin D in the immunological response against *M. leprae*. However, the authors suggested the need for further study using haplotypes formed by the polymorphisms BsmI, ApaI, and TaqI in individuals with tuberculoid and lepromatous leprosy in an attempt to shed light on questions that refer to the VDR polymorphism and its correlation with the specific clinical manifestations of the disease [25].

In the same line of investigation, the Master's thesis from Brazil aimed to reproduce the studies in the literature that reported the association of VDR with leprosy. To achieve this, the study selected the polymorphisms of TaqI (rs731236) and Fok (rs2228570), as well as the rs4760658 of VDR, characterized by the T → C exchange in intron 1. This case control study showed the association of the genotype C/T of the Single Nucleotide Polymorphism (SNP) Fok with the protection against leprosy ($p = 0.05$; OR = 0.77), whereas in the analysis of the haplotypes of the Fok/rs4760658/Taq combination, it was observed that the haplotype C/C/C presented the association with significant protection against leprosy ($p = 0.02$; OR = 0.62), while the haplotype T/T/T only presented a *borderline* protection ($p = 0.04$; OR = 0.62). The study of the Fok/Taq haplotype in the TDT, though it had indicated a borderline association of the T/T haplotype ($p = 0.05$), followed the same path as the case−control haplotype, suggesting protection against the disease [19].

On the other hand, in a study on the expression of VDR and CYP27B1 genes, which codify the 1α-hydroxylase, responsible for the conversion of 25(OH)VD in the bioactive form of 1,25(OH)2VD, in skin lesions from patients with leprosy, verified that there is a significant difference in the comparison of the skin lesions of individuals with tuberculoid leprosy and lepromatous leprosy ($p \leq 0.01$) with reverse reaction and lepromatous leprosy ($p \leq 0.05$); as well as when they compared CYP27B1, tuberculoid leprosy, and lepromatous leprosy ($p \leq 0.001$), with reverse reaction and lepromatous leprosy ($p \leq 0.01$) [6].

Furthermore, in a study analyzing the VDR expression in mononuclear cells of peripheral blood from patients with leprosy reaction, the authors observed that the majority presented a reduced level of vitamin D3 in the blood. Other studies correlated certain mutations in the VDR gene with the complexity of leprosy. However, in the present study, the authors found that for patients with neuritis or erythema nodosum leprosum (ENL), the VDR expression was from 5 to 10% lower than in uninfected individuals, and the bacilloscopy index values varied between 3+ and 5+. In light of the above findings, the authors have been suggesting that VDR therapy can aid in the treatment of individuals with leprosy [10].

In the next study, who analysed the three sites of TaqI, FokI, and ApaI, observed no association with leprosy *per se*, whether paucibacillary or multibacillary, due most likely to the different ethnic groups studied and to the complex etiology of the disease, but they did observe that genotype (Fok) *ff* and allele *f* were able to indicate susceptibility to paucibacillary leprosy ($p = 0.03$; OR = 1.57). For the Apa position, the results suggest that the homozygous *AA*, the heterozygous *Aa*, and the allele *A* can confer susceptibility to leprosy *per se* ($p \leq 0.001$) and multibacillary leprosy ($p \leq 0.001$). When the haplotypes of the variants were evaluated, it was observed that the haplotypes T-F-a ($p \leq 0.0002$) and T-f-a ($p \leq 0.01$) indicate susceptibility to the paucibacillary and multibacillary classifications, respectively, while the T-F-A can offer resistance to leprosy. This high frequency of the haplotypes T-F-a and T-f-a suggests that there is a direct relationship with leprosy in the Indian population, most likely due to the change in the levels and function of the RNAm of the VDR [21].

In addition, another study compared the VDR genotype expression, the Mitsuda test, and the bacilloscopy index, and observed a strong association of the clinical forms of leprosy with the results from the controls ($p < 0.0001$) when compared to Mitsuda tests. These authors also found that individuals with genotype *tt* showed lower levels than the average levels for genotypes *Tt* and *TT*. Although this difference proved to be insignificant, for the authors, the results conferred upon genotype *tt* a certain relationship with the development of the clinical form of lepromatous leprosy, probably because it affects the differentiation and the maturation of the T cells, thus compromising an effective cell immune response [26].

For the bacilloscopy index, no significant differences were observed among the genotypes, though higher values were observed with genotype *tt* when compared to the others. The authors considered that the lack of association among the genotypes, the bacilloscopy index, and the Mitsuda test suggest that they should be considered as separate events, favoring individually in the development of leprosy [26].

When comparing individuals with leprosy and non-blood-related contact, when combining the VDR genotype and the Mitsuda test, it was observed that the patients with genotype *tt* and a negative Mitsuda value presented a 13 times greater probability of having this combination than did the controls. This indicates a 4.5 times higher average of disease occurrence for *tt* in relation to the individuals with *TT* and *Tt*, when the Mitsuda value was negative, correlating the genotype with the development of leprosy [26].

The T allele showed a lower odds ratio than those observed for the negative Mitsuda test *per se*, with no genotype classification (all of the genotypes included, OR = 3.0), suggesting that genotypes *TT* and *Tt* are not risk factors for the occurrence of leprosy, and that the main predisposed factor for leprosy, in this case, is the negative response obtained from the Mitsuda test. One high frequency of genotype *tt* in patients with negative Mitsuda and positive bacilloscopy index was observed, suggesting that this combination can be partially involved in the susceptibility to the disease, reflecting a specific deficiency of the immunity mediated by cells. Though each of these two factors can contribute autonomously to the development of leprosy, the synergetic occurrence of these unfavorable factors, the negative Mitsuda test, and genotype *tt* predispose the individuals to a higher chance of developing leprosy [26].

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As presented in Table 1, in the analyses performed in the Nepalese individuals, it was impossible to confirm the association of the polymorphism of the variants of FokI, BsmI, and Taq I with leprosy, a fact attributed by the authors to the difference regarding the ethnic origin of the population under study, the sample size, and the study design [22].

As indicated above, in the studies that comprised this systematic review, the distribution of the allele and genotype frequencies in the VDR gene polymorphism do not follow a single response pattern for protection or susceptibility to leprosy. Studies suggest that genotype *tt* is related to a strong cell immune response and genotype *TT* to a strong humoral immune response, thus favoring tuberculoid and lepromatous leprosy, respectively. Genotype Tt would be related to the protection against the Leprosy *per se*. This diversity of responses is presented by the authors as the result of certain factors, such as ethnic heterogeneity, the sample size and each study's design, other regions of polymorphism that have yet to be analyzed and the possibility of difference in the virulence of *M. leprae* in the geographic regions studied herein.

Regarding factors related to protection against leprosy, the studies selected in this paper suggest that a particular genotype is directly involved in protection against disease in healthy individuals (Tt) and that the combination of allelic frequencies of a genetic marker (C/C/C or T/T/T) of affected individuals compared to unaffected individuals could generate protection responses against leprosy *per se* (Table 2).

Association studies in the genetics of infectious diseases such as leprosy have been important in elucidating the molecular bases of host protection and susceptibility control in *M. leprae*. Nowadays, the notion that different sets of genes modify the susceptibility to leprosy in the control of the infection *per se*, e.g., the disease independently of its form of clinical manifestation; and once the infected individual, in defining the different clinical forms of the disease.

4.2. Hepcidin levels in the protection or severity of leprosy

Hepcidin is a peptide hormone of 25 amino acids, known mainly as the key regulator of iron homeostasis. This peptide is linked to ferroportin (FPN), leading to its internalization and degradation in hepatocytes, enterocytes, and macrophages, in turn hindering the carrying of iron to the plasma and causing cells to retain iron [11,27]. Hepcidin is also a peptide with antimicrobial activity similar to defensins [28].

The increase in hepcidin expression occurs in response to the iron deposits and inflammation, and is inhibited in anemia, erythropoiesis, hypoxia, and oxidative stress [29]. In chronic inflammatory diseases, studies have verified that, although the iron deposit is adequate in individuals, it is not available for erythropoiesis, thus blocking the normal hemoglobinization of the erythrocytes [30,31].

Cytokines are generated in response to infections by pathogenic agents that are dependent on iron and that stimulate the immune response [32]. Moreover, upon studying hepcidin in cell cultures of human and mouse livers, the authors reported that IL-6 stimulates the production of this peptide during inflammation and that this cytokine is responsible for the hypoferremia of the inflammation, working as a negative regulator of iron absorption and capturing the iron within the macrophages [11]. Other cytokines, including IL-1 [12], IL-22 [33], and interferon-γ [34], also present a positive association with the hepcidin expression.

In leprosy, iron retention within the host cells tends to perform an important role in inflammation, providing an ideal environment for the growth of the bacillus. A recent study observed increased indexes of hepcidin in the urine of patients with multibacillary leprosy ($p = 0.03$), a high hepcidin expression. In lesions from patients with lepromatous leprosy ($\text{hepcidina}_{\log}$ $p = 0.04$), a moderate positive correlation can be observed between the urinary hepcidin and serum IL-1β ($p = 0.04$), as well as between hepcidin expression and the bacilloscopy index ($p = 0.025$) [23].

As regards the hematological parameters, low levels of hemoglobina ($p = 0.0015$), hematocrit ($p = 0.0005$), red blood cell count ($p = 0.0064$), and average corpuscular volume ($p = 0.025$) were observed in multibacillary patients. As regards the iron parameters, the total capacity of connection to iron ($p = 0.017$) was less, while the levels of ferritin were higher in patients with lepromatous leprosy ($p = 0.0465$) than in the controls. For serum iron, a marginal significance level was observed ($p = 0.054$), while for serum ferritin ($p = 0.0465$), the soluble transferrin receptor ($p = 0.0002$) and the soluble $\text{transferrin}_{\log}$ receptor ($p = 0.0054$) of the observed levels were higher for patients with lepromatous leprosy when compared to healthy individuals. The authors suggest that these changes indicate a change in metabolism altered by iron and seem to result in a mixture of anemia caused by inflammation and iron deficiency [23].

Some studies have suggested that the reduction in serum iron may well contribute to the defense of the host against invader pathogens, which can influence the clinical manifestations of the infectious diseases [35,36], and that hepcidin inhibitors could be used as a strategy to treat the anemia of chronic diseases. It is not well-defined whether or not hepcidin exerts independent effects of iron upon the host's defense system; therefore, other studies concerning hepcidin regulation mechanisms are warranted [37–39]. Although some studies have consistently demonstrated an association between hepcidin and inflammatory anemia, the evidence in leprosy has been inconsistent and it needs to be more understood.

In this systematic review, the selected studies indicate an association between specific genotypes and the VDR expression for protection or susceptibility to leprosy, but there are still questions that favor the intensification of studies and the investigation of other genome regions that aim to shed light on the involvement of VDR in leprosy. The results further our understanding of genetic and immunological factors that influence the evolution of leprosy, but these studies still remain inconclusive and sometimes contradictory due to the high degree of heterogeneity between different populations and to the complex genetic model of influence the susceptibility to the disease.

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As regards hepcidin expression, further studies are needed to clarify the influence of altered iron metabolism in inflammatory anemia of chronic infectious diseases, given that the growth of *M. leprae* is favored, by oxygenation levels that lead to the growth and development of this microbacteria. We, therefore, suggest that other studies involving risk markers and genetic susceptibility be conducted, since there is neither substantial evidence nor agreement on the genetic factors linked to VDR or to the metabolic role of hepcidin in a patient's susceptibility to leprosy.

Conflicts of interest

We declare no conflict of interest in writing or content of this systematic review.

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Annex 1. Systematic review protocol

VDR and hepcidin regarding the severity of leprosy

To compose this systematic review, the following guiding question was formulated: Is there an relationship between hepcidin levels or vitamin D receptor (VDR) expression and the severity of leprosy?

The search strategy was conducted by two separate reviewers with the cooperation of a librarian specialized in electronic searches in the Health Library of the UFMG School of Medicine. Each descriptor was translated to the vocabulary established for each databank (DeCS for Lilacs and MeSH for OVID-Medline) and into words of free text in titles and/or abstracts, using a systematized process of optimization for the other databanks (SciELO, Google Scholar, Brazilian Digital Library of Theses and Dissertations – BDTD).

The first selection, based on the titles of the studies and their abstracts, was performed by ALGO, who discussed any doubts with NSG, who then discarded or added to the already selected studies. In the second stage, the complete text of each work was evaluated by ALGO, ATC, and CSAM, who made the decision to include or exclude the work from the previously selected studies. The exclusion criteria were the following: narrative or systematic reviews or meta-analyses; case control studies; book chapters; editorials and letters to the editor; research with pregnant women and nursing mothers, children, adolescents, and elderly individuals of over 65 years of age; experimental studies; studies whose theme was not in accordance with the aim of this review; and studies that evaluated VDR and hepcidin, but not in leprosy.

Search strategies

PubMed

1. Search strategies for Vitamin D, Vitamin D Receptors, Real Time Polymerase Chain Reaction, Immunology, Leprosy, combined with filters to expand as multibacillary, paucibacillary, tuberculoid, lepromatous, borderline and the search operators: quotation marks, parentheses, “AND”, “OR”, “exp”, “mp”.

(((Vitamin D OR Vitamina D) AND (“Cholecalciferol receivers” OR “Vitamin D receptors” OR “Vitamin D 3 receptors” OR “Receptores de colecalciferol” OR “Receptores de vitamina D” OR “La vitamina D 3 receptores” OR “Receptores de Colecalciferol” OR “Receptores de Vitamina D” OR “Receptores de Vitamina D 3” OR “Reaction Polymerase Chain in Real” OR “Time Real-Time PCR” OR “Reação da Polimerase em Cadeia em Tempo Real” OR “PCR em Tempo Real” OR “Real Time PCR” OR “Real-Time Polymerase Chain Reaction” OR “Reacción en Cadena en Tiempo Real de la Polimerasa” OR “Reação em Cadeia da Polimerase em Tempo Real” OR “Receptors, Calcitriol” OR “Receptores de Calcitriol” OR “Receptores de Calcitriol”)) AND (Alergoimunologia OR Allergology OR “Allergology and Immunology” OR Immunology OR “Immunology and Allergology” OR Immunoallergology OR Alergoimunologia OR Alergología OR “Alergología e Inmunología” OR inmunología OR “Inmunología y Alergología” OR immunoallergology OR Alergoimunologia OR Alergologia OR “Alergologia e Imunologia” OR Imunologia OR “Imunologia e Alergologia” OR Imunoalergologia OR “Allergy and Immunology” OR “Alergia e Inmunología” OR “Alergia e Imunologia” OR Macrophages OR Macrófagos OR Macrófagos OR “T-Lymphocytes” OR “Linfocitos T” OR “Linfócitos T”)) AND (Leprosy OR Lepra OR Hanseníase)

2. Search Strategy for Leprosy and Hepcidin Levels

(Leprosy OR Lepra OR Hanseníase) AND (Hepcidins OR Hepcidinas OR Hepcidinas) AND (Severity OR Severidade OR Gravidade)

Scielo (Scielo.org)/Google Scholar (googlescholar.com)/ Lilacs – Virtual Health Library (bvs.org)

1. (Hanseníase OR Leprosy) AND (vitamin D) AND (Vitamin D receptors OR “Calcitriol receptor”) AND (gravidade OR Severity)

2. (Hanseníase OR Leprosy) AND Hepcidins OR Hepcidinas) AND (Severity OR Severidade OR Gravidade)

BDBTD – Brazilian Digital Library of Theses and Dissertations (bdtd.ibict.br)

1. Hanseníase|Leprosy|VDR|
2. Hanseníase|Leprosy|Hepcidina

References

[1] OMS. Trabalhando para superar o impacto global de doenças tropicais negligenciadas: Primeiro relatório da OMS sobre doenças tropicais negligenciadas. OMS 2010. Available from: http://www.who.int/eportuguese/publications/pt/.

[2] Houweling TAJ, Karim-Kos HE, Kulik MC, Stolk WA, Haagsma JA, Lenk EJ, et al. Socioeconomic inequalities in neglected tropical diseases: a systematic review. PLoS Negl Trop Dis 2016;10:e0004546.

[3] WHO – World Health Organization. Weekly epidemiological record. Available from: http://www.who.int/wer/en/; 2016. 35, 405–420.

[4] BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância das Doenças Transmissíveis. Diretrizes para vigilância, atenção e eliminação da Hanseníase como problema de saúde pública: manual técnico-operacional [recurso eletrônico]/Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância das Doenças Transmissíveis. – Brasília. Ministério da Saúde; 2016. p. 58. il.

[5] Yamamura M, Uyemura K, Deans RJ, Weinberg K, Rea TH, Bloom BR, et al. Defining protective responses to pathogens: cytokine profiles in leprosy lesions. Science 1991;254:277–9.

[6] Teles RMB, Graeber TG, Krutzik SR, Montoya D, Schenk M, Lee DJ, et al. Type I interferon suppresses type II interferon-triggered human anti-mycobacterial responses. Science 2013;339:1448–53.

[7] Bhalla AK, Amento EP, Krane SM. Differential effects of 1,25-dihydroxyvitamin D3 on human lymphocytes and monocyte/macrophages: inhibition of interleukin-2 and augmentation of interleukin-1 production. Cell Immunol 1986;98:311–22.

[8] Mora JR, Iwata M, Von-Adrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. Nat Rev Immunol 2008;8:685–98.

[9] Matzner M, Al Samie AR, Winkler HM, Nemeth J, Graznek A, Indra A, et al. Low serum levels of cathelicidin LL-37 in leprosy. Acta Trop 2011;117:56–9.

[10] Mandal D, Reja AHH, Biswas N, Bhattacharyya P, Patra PK, Bhattacharyya B. Vitamin D receptor expression levels determine the severity and complexity of disease progression among leprosy reaction patients. New Microbes New Infect 2015;6:35–9.

[11] Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen B, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Investig 2004;113:1271–6.

[12] Lee P, Peng H, Gelbart T, Wang L, Beutler E. Regulation of hepcidin transcription by interleukin-1 and interleukin-6. Proc Natl Acad Sci US.A 2005;102:1906–10.

[13] Peyssonnaux C, Zinkernagel AS, Datta V, Lauth X, Johnson RS, Nizet V. TLR4-dependent hepcidin expression by myeloid cells in response to bacterial pathogens. Blood 2006;107:3727–32.

[14] Sher R, Shulman G, Baily P, Politzer WM. Serum trace elements and vitamin A in leprosy subtypes. Am J Clin Nutr 1981;34:1918–24.

[15] Lapinsky SE, Baynes RD, Schulz EJ, MacPhail AP, Mendelow B, Lewis D, et al. Anaemia, iron-related measurements and erythropoietin levels in untreated patients with active leprosy. J Intern Med 1992;232:273–8.

[16] Jain A, Mukherjee A, Chattopadhya D, Saha K. Biometals in skin and sera of leprosy patients and their correlation to trace element contents of *M. leprae* and histological types of the disease – a comparative study with cutaneous tuberculosis. Int J Lepr Other Mycobact Dis 1995;63:249–58.

[17] Rea TH. Decreases in mean hemoglobin and serum albumin values in erythema nodosum leprosum and lepromatous leprosy. Int J Lepr Other Mycobact Dis 2001;69:318–27.

[18] Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic reviews and meta-analyses protocols (PRISMA-P) 2015: elaboration and explication. BMJ 2015:1–25.

[19] Marques CS. Estudo de associação entre o gene VDR e a hanseníase [Dissertação de Mestrado]. Rio de Janeiro: Instituto Oswaldo Cruz, Pós Graduação em Biologia Celular e Molecular; 2010. p. 86. Available from: http://arca.icict.fiocruz.br/handle/icict/5723.

[20] Fitness J, Floyd S, Warndorff DK, Sichali L, Mwaungulu L, Crampin AC. Large-scale candidate gene study of leprosy susceptibility in the Karonga district of northern Malawi. Am J Trop Med Hyg 2004;71:330–40.

[21] Neela VSK, Suryadevara NC, Shinde VG, Pydi SS, Jain S, Jonnalagada S, et al. Association of Taq I, Fok I and Apa I polymorphisms in vitamin D receptor (VDR) gene with leprosy. Hum Immunol 2015;76:402–5.

[22] Sapkota BR, Macdonald M, Berrington WR, Ann Misch E, Ranjit C, Siddiqui MR, et al. Association of *TNF*, *MBL*, and *VDR p*olymorphisms with leprosy phenotypes. Hum Immunol 2010;71:992–8.

[23] Souza VNB, Malaspina TSS, Campanelli AP, Ghidella C, Ura S, Dalpino D, et al. Increased hepcidin expression in multibacillary leprosy. Mem Inst Oswaldo Cruz 2012;107:183–9.

[24] Roy S, Frodsham A, Saha B, Hazra SK, Mascie-Taylor CG, Hill AVS. Association of vitamin D receptor genotype with leprosy type. J Infect Dis 1999;179:187–91.

[25] Velarde-Félix JS, Cázarez-Salazar SG, Castro-Velázquez R, Rendón-Maldonado JG, Rangel-Villalobos H. Relación del polymorphism *Taq*I del gen del receptor de la vitamina D con la lepra lepromatosa en población mexicana. Salud Publica Mex 2009;51:59–61.

[26] Goulart LR, Ferreira FR, Goulart IMB. Interaction of Taq I polymorphism at exon9 of the vitamin D receptor gene with the negative lepromin response may favor the occurrence of leprosy. FEMS Immunol Med Microbiol 2006;48:91–8.

[27] De Domenico I, Mc Vey Ward D, Kaplan J. Regulation of iron acquisition and storage: consequences for iron-linked disorders. Nat Rev Mol Cell Biol 2008;9:72–81.

[28] Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem 2001;276:7806–10.

[29] Viatte L, Vaulont S. Hepcidin, the iron watcher. Biochimie 2009;91(10):1223–8.

[30] Thomas C, Thomas L. Biochemical markers and hematologic indices in the diagnosis of functional iron deficiency. Clin Chem 2002;48:1066–76.

[31] Weiss G. Anemia of chronic disorders: new diagnostic tools and new treatment strategies. Semin Hematol 2015;52:313–20.

[32] Andrews NC. Anemia of inflammation: the cytokine-hepcidin link. J Clin Investig 2004;113:1251–3.

[33] Armitage AE, Eddowes LA, Gileadi U, Cole S, Spottiswoode N, Selvakumar TA, et al. Hepcidin regulation by innate immune and infectious stimuli. Blood 2011;118:4129–39.

[34] Ryan JD, Altamura S, Devitt E, Mullins S, Lawless MW, Muckenthaler MU, et al. Pegylated interferon-γ induced hypoferremia is associated with the immediate response to treatment in hepatitis C. Hepatology 2012;56:492–500.

[35] Weinberg ED. Iron, infection and neoplasia. Clin Physiol Biochem 1986;4:50–60.

[36] Schaible EU, Kaufmann SHE. Iron and microbial infection. Nature 2004;2:946–54.

[37] Michels K, Nemeth E, Ganz T, Mehrad B. Hepcidin and host defense against infectious diseases. PLoS Pathol 2015;11:e1004998.

[38] Schmidt PJ. Regulation of iron metabolism by hepcidin under conditions of inflammation. J Biol Chem 2015;290(31):18975–83.

[39] Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med 2005;352:1011–23.

Please cite this article in press as: Oliveira ALGd, et al., Vitamin D receptor expression and hepcidin levels in the protection or severity of leprosy: a systematic review, Microbes and Infection (2017), http://dx.doi.org/10.1016/j.micinf.2017.03.001