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Mannose-binding lectin serum levels in patients with leprosy are influenced by age and *MBL2* genotypes

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SUMMARY

Background: Mannose-binding lectin (MBL) activates the complement system promoting opsonophagocytosis, which could represent an advantage for *Mycobacterium leprae*, an intracellular pathogen. Therefore, a single nucleotide polymorphism (SNP) in the *MBL2* gene associated with low levels of MBL could confer protection against the development of leprosy disease.
Methods: In this study, we investigated SNPs of the *MBL2* gene and MBL levels in 228 Brazilian leprosy patients and 232 controls.
Results: There were no differences in the frequencies of variant genotypes and haplotypes of *MBL2* between patients and controls, or between the different clinical forms of leprosy. In the group of patients with a genotype for high expression of *MBL2*, those aged > 40 years had decreased MBL levels compared to patients aged ≤40 years ($p = 0.037$).
Conclusion: Our results demonstrate that age could influence the phenotype of *MBL2*, but no evidence was found for an association of *MBL2* polymorphism with susceptibility to leprosy or its clinical forms.

1. Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, an intracellular pathogen that infects mainly macrophages and Schwann cells.[1] Most individuals do not develop clinical manifestations, even after prolonged exposure to *M. leprae*. Those who do develop clinical manifestations present four major forms: tuberculoid, lepromatous, borderline, and indeterminate. The high degree of clonality in the genome of *M. leprae* isolates from various parts of the world,[2] suggests that the variability in susceptibility to infection with the Mycobacterium is largely due to host genetic factors.[3] The single nucleotide polymorphism (SNP) of several genes that could influence the variability in susceptibility to leprosy has been extensively investigated, showing different results according to the population studied.[4–6]

Positive or negative associations between leprosy and polymorphisms of the immune system molecules have been described, such as the human leukocyte antigen (HLA),[4,7] interleukin 10 (IL-10),[8–10] Toll-like receptor 2 (TLR 2),[8] lymphotoxin alpha (LTA), tumor necrosis factor alpha (TNF-α), receptor for vitamin D (VDR), receptor for component of the complement system (CR1),[8] and mannose-binding lectin (MBL).[8,11,12] These studies have revealed a great genetic complexity involved in controlling susceptibility to leprosy and modulation of the clinical spectrum of leprosy in humans.

MBL is a lectin of the innate immune system that plays an important role as a pattern recognition molecule in the identification of pathogens; it is able to activate the complement system, promoting phagocytosis and modulation of inflammation.[13] SNPs in the MBL gene (*MBL2*) are responsible for reduced serum levels of the protein. Three SNPs in the promoter region modulate the expression of the molecule, of which the alleles are H/L (position – 550), X/Y (position – 221), and P/Q (position +4).[14] Exon-1 of *MBL2* presents allelic variants B (Gly54Asp), C (Gly57Glu), and D (Arg52Cys), collectively termed allele 'O', while the wild-type allele is called 'A'. The amino acid changes affect the oligomerization of variant MBL, which has a lower molecular weight and is dysfunctional compared to normal MBL; this influences the biological activity of the lectin and also reduces its serum concentration.[15]

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The exon-1 allelic variants that are associated with low serum levels of MBL predispose to infection by various pathogens,[14,16] as well as to the development of autoimmunity.[17] However, genotypes of low expression of MBL have been associated with protection against the development of visceral leishmaniasis,[18] tuberculosis,[19] and leprosy.[11] It is suggested that *MBL2* may have undergone natural selection pressures, which induce genetic polymorphisms, resulting in biological advantages. However, a selective neutrality test with respect to the complete sequencing of the gene showed significant results only for the population of individuals of European origin, but not for African-Americans or Hispanics.[20] Indeed, a larger study focusing on evolutionary insights into the high prevalence of *MBL2* allele deficiency worldwide, supports the idea that the role of MBL is redundant in human host defense.[21]

Studies of the association of *MBL2* polymorphisms with leprosy present conflicting results. Fitness et al.[8] found no association in a study of the first exon variant alleles in leprosy in a population of paucibacillary patients from the northeast of Africa. In contrast, de Messias-Reason et al.,[11] studying Brazilian patients with leprosy in the southern region, which has a large European colonization influence, showed a positive association between high-expression haplotypes of *MBL2* with susceptibility to leprosy per se, whereas those with low expression were associated with the tuberculoid form. In addition, in an Indian population from Nepal, the B variant allele was present in 4.3% of patients with the tuberculoid vs. 1.5% of patients with the lepromatous form, however the authors stated that the statistical significance was modest and that more studies were needed for conclusive validation.[12]

Due to the complex genetic regulation of susceptibility to leprosy, the influence of polymorphism in *MBL2* could vary according to the genetic background of the population studied. Boldt et al.[22] demonstrated that the frequencies of polymorphisms of exon-1 and the promoter of *MBL2* are different among Brazilians of different genetic backgrounds. Therefore, this study aimed to investigate the association of *MBL2* polymorphism and serum levels of MBL in a group of patients with different forms of leprosy from the northeast of Brazil.

2. Patients and methods

2.1. Patients and controls

We recruited 232 healthy blood donors as the control group and 228 patients with leprosy from the city of Recife, northeast of Brazil, matched by place of origin. The control group had a mean age of 33 ± 8.3 years, and 58% of subjects were male. In the leprosy group, the clinical forms were distributed as follows: 26% lepromatous, 31% borderline, 16% tuberculoid, 7% indeterminate, and 20% were not classified. The average age of patients was 44.8 ± 16 years, with 59% male subjects. The classification of disease with regard to the operational class – paucibacillary (PB) and multibacillary (MB) – was based on the information provided by SINAN (Technical Information System for Notifiable Diseases/Ministry of Health, Brazil, 2008), which uses the Ridley and Jopling classification (1966).[23]

This study was approved by the Research Ethics Committee of Aggeu Magalhães (CEP/CPqAM/Fiocruz, record 45/06). All subjects in the study signed a consent form.

2.2. DNA extraction and genotyping

DNA samples were extracted from whole blood in anticoagulant solution (EDTA) using the QIAamp Mini Spin Columns Kit (Qiagen, Basel, Switzerland) following the manufacturer's instructions.

The promoter region of *MBL2* was genotyped by real-time PCR, using specific probes, performed by the TaqMan system. The probes and validated protocols for the regions − 550 and − 221 are available at http://snp500cancer.nci.nih.gov. For determination of alleles H/L, the following probes and primers were used: FAM-MGB-AGCCTGTGTAAAAC, VIC-MGB-CCTGTCTAAAACACC, CCAACGTAGTAAGAAATTTCCAGAGA-forward, and reverse-CAACC-CAGCCCAGAATTAACTG. For alleles X/Y, the following probes and primers were used: FAM-CATGCTTTCCGTGGCAG-MGB, VIC-MGB-CATGCTTTCGGTGGCAG, GCACGGTCCCATTTGTTCTCA-forward, and reverse-GCGTTGCTGCTGGAAGACTATAAA.

Genotyping of the structural region (exon-1) of *MBL2* was performed using the technique of real-time PCR with melting temperature assay (MTA), as described by Hladnik et al.,[24] using the following primers: forward primer 5′-AGGCAT-CAACGGCTTCCCA-3′, reverse primer 5′-CAGAACAGCCCAACACG-TACCT-3′. The three allelic variants of the *MBL2* gene in codon positions 52, 54, and 57 in exon-1 were designated 'O', and the wild-type allele designated 'A'.

2.3. MBL serum concentration

The serum concentrations of MBL were determined using a commercial capture enzyme-linked immunosorbent assay (ELISA; Antibody Shop, Copenhagen, Denmark). Sera were diluted 1:100 and added to ELISA plates coated with monoclonal antibody against the binding carbohydrate domain. The bound MBL was detected by a second biotin-labeled antibody and the streptavidin–peroxidase system. Tetramethylbenzidine was used as substrate. Reading of the reaction was performed at 450 nm using an ELISA plate reader (BioRad, CA, USA). According to the manufacturer, the concentrations of MBL in normal serum are classified as low at levels of < 100 ng/ml, intermediate at 100–1000 ng/ml, and high at >1000 ng/ml.

2.4. Grouping of genotypes according to serum MBL and haplotypes

Genotypes of the promoter region (−550 H/L, −221 X/Y) were grouped with genotypes of the exon-1 (A/O) and correlated to MBL serum concentrations in the leprosy patients to categorize groups of low, intermediate, and high expression.[14] The following genotypes were considered as high expression (total n = 87, median = 3190 ng/ml): HYA/HYA (n = 22, median = 3063 ng/ml), HYA/LYA (n = 32, median = 2658 ng/ml), LYA/LYA (n = 33, median = 3667 ng/ml); intermediate expression (total n = 105, median = 848 ng/ml): LYA/LXA (n = 19, median = 2121 ng/ml), HYA/LXA (n = 27, median = 2694 ng/ml), HYA/HYO (n = 6, median = 1805 ng/ml), HYA/LYO (n = 18, median = 564 ng/ml), LYA/LYO (n = 29, median = 338 ng/ml), LXA/LXA (n = 6, median = 946 ng/ml); and low expression (total n = 36, median = 30 ng/ml): HYO/LXA (n = 7, median = 190 ng/ml), HYO/LYO (n = 6, median = 16 ng/ml), LYO/LYO (n = 8, median = 20 ng/ml), LXA/LYO (n = 15, median = 59 ng/ml). The haplotypes were then divided into groups of low (LXA, HYO, LYO) and high (HYA, LYA) expression of the *MBL2* gene.[14]

2.5. Determination of serum C-reactive protein

C-reactive protein (CRP) levels were used as a marker of inflammation. The exam was performed using the Turbidimetric Ultra-sensitive CRP Kit (Biotech, Minas Gerais, Brazil), with sample collection and the test protocol as per the manufacturer's instructions, and the results were read on a TARGA 3000 (Rome, Italy). The reference concentration for CRP indicated by the kit manufacturer was <3 mg/l.

2.6. Statistical analysis

The Mann–Whitney or Kruskal–Wallis test was employed when appropriate for comparison of the variation in MBL serum

Table 1
Characterization of leprosy patients (all patients and by clinical form) and controls by gender, age, and serum concentrations of C-reactive protein and mannose-binding lectin

Group	Gender, M/F	Age (years), average ± SD	CRP (mg/l), median (min–max)[a]	MBL (ng/ml), median (min–max)
Control (n = 232)	135/97	33 ± 8.3	ND	ND
Lepromatous (n = 60)	43/17	45.2 ± 15.9	0.161 (0.003–4.08)	1.965 (9–10.203)
Borderline (n = 72)	46/26	41.9 ± 15.6	0.177 (0.001–8.88)	1.904 (10–14.713)
Indeterminate (n = 16)	7/9	46.1 ± 15.2	0.156 (0.004–9.24)	1.659 (19–8.478)
Tuberculoid (n = 36)	13/23	44.8 ± 16.0	0.177 (0.007–8.88)	1.675 (0–7.665)
Paucibacillary (n = 67)	25/42[b]	44.2 ± 16.3	0.180 (0.004–9.24)	1.413 (8–8.478)
Multibacillary (n = 161)	109/52	44.4 ± 16.0	0.182 (0.001–8.88)	1.817 (4–14.713)
All patients (n = 228)	134/94	44.8 ± 16.0	0.177 (0.001–9.24)	1.674 (4–14.674)

M, male; F, female; SD, standard deviation; CRP, C-reactive protein; MBL, mannose-binding lectin; ND, not determined.
[a] CRP kit reference value < 3.0 mg/l.
[b] Paucibacillary vs. multibacillary, gender male vs. female: p = 0.00004, odds ratio 3.52, 95% confidence interval 1.86–6.69.

concentration between the groups. The program GraphPad PRISM (version 5.0; San Diego, CA, USA) was used for these analyses. To verify the Hardy–Weinberg equilibrium and for the construction of haplotypes we used the program ARLEQUIN (version 3.11; University of Bern, Switzerland). To test for differences between cases and controls, the Chi-square test with Yates' correction was employed, and odds ratios (OR) with 95% confidence intervals (CI) were obtained. The estimated power was 92% to analyze the association of haplotypes. Epi Info (version 6.03; CDC, Atlanta, GA, USA) was used to calculate p-values and power.

3. Results

There were no differences between the control subjects and leprosy patients with regard to gender or mean age. There was also no difference in gender or age with regard to the clinical forms of leprosy (lepromatous, borderline, tuberculoid, and indeterminate). There was no difference in age between the operational classes (PB and MB), however the frequency of male patients was higher in the group with the multibacillary form compared to the paucibacillary form (OR 3.52, 95% CI 1.86–6.69; p = 0.00004). The median serum CRP level of all leprosy patients, as well as those grouped according to their clinical form or classified as PB or MB, was under 3 mg/l. The median MBL serum concentrations in all patients, as well as by clinical form and operational class, also did not differ (Table 1).

Table 2 shows the allelic and genotypic frequencies of *MBL2* in the control group and leprosy patients. The two groups were in Hardy–Weinberg equilibrium. There was no statistical difference between patients and controls for the allele frequencies of region − 550 (H and L) and its genotypes H/H, H/L, and L/L, or for the promoter region −221 (X and Y) and for genotypes Y/Y, X/Y, and X/X. Regarding the frequency of the alleles of exon-1 (A and O) and genotypes A/A, A/O, and O/O, there were also no significant differences.

Table 2
Genotype allele, and haplotype frequencies of *MBL2* gene polymorphism in patients with leprosy and the control group[a]

	Control (n = 232; 464)	Leprosy (n = 228; 456)	MB (n = 161; 322)	PB (n = 67; 134)	Lepromatous (n = 60; 120)	Borderline (n = 72; 144)	Tuberculoid (n = 36; 72)	Indeterminate (n = 16; 32)
Promoter (−550)								
Allele								
L	0.67 (310)	0.68 (310)	0.69 (223)	0.65 (87)	0.67 (81)	0.67 (96)	0.63 (45)	0.59 (19)
H	0.33 (154)	0.32 (146)	0.31 (99)	0.35 (47)	0.33 (39)	0.33 (48)	0.37 (27)	0.41 (13)
Genotype								
L/L	0.46 (106)	0.48 (110)	0.50 (81)	0.43 (29)	0.47 (28)	0.46 (33)	0.42 (15)	0.37 (6)
H/L	0.42 (98)	0.40 (90)	0.38 (61)	0.43 (29)	0.42 (25)	0.42 (30)	0.42 (15)	0.44 (7)
H/H	0.12 (28)	0.12 (28)	0.12 (19)	0.14 (9)	0.11 (7)	0.12 (9)	0.16 (6)	0.19 (3)
Promoter (−221)								
Allele								
Y	0.83 (387)	0.82 (376)	0.83 (267)	0.81 (109)	0.82 (99)	0.83 (120)	0.85 (61)	0.81 (26)
X	0.17 (77)	0.18 (80)	0.17 (55)	0.19 (25)	0.18 (21)	0.17 (24)	0.15 (11)	0.19 (6)
Genotype								
Y/Y	0.71 (164)	0.67 (154)	0.68 (109)	0.67 (45)	0.68 (41)	0.68 (49)	0.69 (25)	0.69 (11)
Y/X	0.25 (59)	0.30 (68)	0.30 (49)	0.28 (19)	0.28 (17)	0.31 (22)	0.31 (11)	0.25 (4)
X/X	0.04 (9)	0.03 (6)	0.02 (3)	0.05 (3)	0.03 (2)	0.01 (1)	0 (0)	0.06 (1)
Exon-1 (52,54,57)								
Allele								
A	0.80 (372)	0.77 (352)	0.76 (246)	0.80 (107)	0.77 (93)	0.76 (110)	0.78 (56)	0.78 (25)
O	0.20 (92)	0.23 (104)	0.24 (76)	0.20 (27)	0.23 (27)	0.24 (34)	0.22 (16)	0.22 (7)
Genotype								
A/A	0.63 (147)	0.61 (139)	0.60 (96)	0.64 (43)	0.62 (37)	0.60 (43)	0.58 (21)	0.63 (10)
A/O	0.34 (78)	0.33 (75)	0.33 (54)	0.31 (21)	0.32 (19)	0.33 (24)	0.39 (14)	0.31 (5)
O/O	0.03 (7)	0.06 (14)	0.07 (11)	0.05 (3)	0.06 (4)	0.07 (5)	0.03 (1)	0.06 (1)
Haplotype								
High expression								
HYA	0.31 (142)	0.28 (127)	0.27 (86)	0.31 (41)	0.27 (33)	0.30 (43)	0.35 (25)	0.25 (8)
LYA	0.33 (153)	0.32 (146)	0.33 (105)	0.31 (41)	0.32 (39)	0.30 (43)	0.28 (20)	0.34 (11)
Low expression								
LXA	0.17 (77)	0.18 (80)	0.17 (55)	0.19 (25)	0.18 (21)	0.17 (24)	0.15 (11)	0.19 (6)
HYO	0.03 (12)	0.04 (19)	0.04 (13)	0.04 (6)	0.05 (6)	0.03 (5)	0.03 (2)	0.16 (5)
LYO	0.17 (80)	0.18 (84)	0.20 (63)	0.16 (21)	0.18 (21)	0.20 (29)	0.19 (14)	0.06 (2)

MB, multibacillary; PB, paucibacillary.
[a] All comparisons were not significant; O = B + C + D alleles.

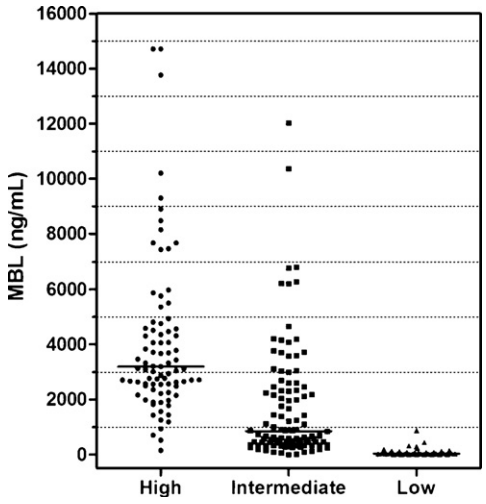

Figure 1. Levels of serum mannose-binding lectin (MBL) in patients with leprosy according to the genotypes of the promoter and exon-1 of the *MBL2* gene; Kruskal–Wallis $p < 0.0001$. The following genotypes were considered as high expression (total $n = 87$, median = 3190 ng/ml): HYA/HYA ($n = 22$, median = 3063 ng/ml), HYA/LYA ($n = 32$, median = 2658 ng/ml), LYA/LYA ($n = 33$, median = 3667 ng/ml); intermediate expression (total $n = 105$, median = 848 ng/ml): LYA/LXA ($n = 19$, median = 2121 ng/ml), HYA/LXA ($n = 27$, median = 2694 ng/ml), HYA/HYO ($n = 6$, median = 1805 ng/ml), HYA/LYO ($n = 18$, median = 564 ng/ml), LYA/LYO ($n = 29$, median = 338 ng/ml), LXA/LXA ($n = 6$, median = 946 ng/ml); and low expression (total $n = 36$, median = 30 ng/ml): HYO/LXA ($n = 7$, median = 190 ng/ml), HYO/LYO ($n = 6$, median = 16 ng/ml), LYO/LYO ($n = 8$, median = 20 ng/ml), LXA/LYO ($n = 15$, median = 59 ng/ml). The haplotypes were divided into low (LXA, HYO, LYO) and high (HYA, LYA) expression of *MBL2* gene.[14]

Figure 1 shows the correlation between the genotypes and the serum concentration of MBL, which was statistically significant ($p < 0.001$). The three categories of genotype, defined as high, intermediate, and low, were associated with leprosy and its clinical forms (Table 3). The frequencies of the haplotypes related to high (HYA, LYA) and low (LXA, HYO, LYO) expression of *MBL2* showed no significant associations with the clinical forms of leprosy.

In addition, leprosy patients were divided into levels categorized by the three genotypes of the −221 promoter region and exon-1, due to their strong influence on the expression and oligomerization, respectively, of the protein.[14] The genotypes comprising these groups were associated with the operating classes PB and MB. The distribution of patients with PB and MB by genotypes was: YA/YA, $n = 26$ PB/59 MB; YA/XA, $n = 14$ PB/33 MB; XA/XA, $n = 4$ PB/4 MB; YA/YO, $n = 15$ PB/38 MB; YO/XA, $n = 6$ PB/14 MB; YO/YO, $n = 2$ PB/12 MB. No statistical difference was observed for any association between PB vs. MB (Figure 2).

Nevertheless, with regard to the serum levels of MBL, a significant difference was observed when patients were divided into two groups by age: ≤40 years and >40 years ($p = 0.02$) (Figure 3A). This difference was also evident for ages 4–20 years (median = 3717 ng/ml) and 21–30 years (median = 2615 ng/ml) vs. 41–50 years (median = 433 ng/ml); $p = 0.0247$. The frequency of genotypes of low, intermediate, and high *MBL2* gene expression did not vary according to age. The median levels of serum MBL, grouped according to genotypes of low, intermediate, and high expression of *MBL2* demonstrate that patients with high-expression genotypes aged >40 years had lower MBL serum levels compared to those aged ≤40 years (Figure 3B). There was no difference between gender and serum levels.

4. Discussion

The hypothesis that high serum levels of MBL could facilitate the uptake of *M. leprae* by macrophages was proposed for the first time by Garred et al. in 1994.[25] In a study conducted in the south region of Brazil, no difference was found in the median MBL serum levels between patients with leprosy ($n = 191$) and a control group ($n = 110$), or between the individual clinical forms. However, the authors showed that the frequency of serum MBL deficiency (<100 ng/ml) was higher in patients with the tuberculoid form compared with the lepromatous one, suggesting that low levels of MBL could be protective against progression to the lepromatous form.[26] The authors reported that CRP levels were elevated in all the patients. Increased CRP levels could alter the MBL levels, since the production of interleukin 6 (IL-6) and growth hormone (GH) during the inflammatory response have been reported to positively regulate MBL production, even though the increase has been shown to be discrete.[27,28] Therefore, the higher frequency of MBL deficiency in the tuberculoid group of patients in spite of the high CRP levels, was probably due to genetic restriction.

In the present study the median CRP in the group of patients with leprosy was 0.177 mg/l, suggesting that the majority of patients were producing serum MBL at baseline level. Although the serum levels provide evidence for a genetic deficiency of MBL, studies on the frequencies of polymorphisms in the promoter and exon-1 regions of *MBL2* are important, because the assays for MBL determination preferentially recognize MBL high oligomers associated with wild-type MBL alleles, while the low oligomers found in the serum of individuals with variant alleles are poorly detected.[14,29] Thus, the results of MBL ELISA detection could be difficult to interpret, mainly because MBL is an opsonin in addition to its role in complement activation, and the effects of the MBL variant alleles on this and other functions is poorly understood.[30]

In this context, our results suggest that oligomerization of MBL in the serum of patients with different clinical forms of leprosy do not differ significantly. However it has previously been reported that variants B and D are able to form high oligomers and activate the complement system with different effectiveness.[29] A study of levels and activity of MBL in patients with leprosy was performed by Gomes et al., who demonstrated that high levels of MBL and also complement activity were more frequent in the lepromatous form.[31]

In the present study the genotypes associated with high, medium, and low expression of *MBL2* did not show different frequencies between patients and controls or between the clinical

Table 3
Genotype distribution of the *MBL2* gene grouped according to the expression of MBL levels in high, medium, and low concentration in patients with leprosy

Expression with respect to *MBL2* genotypes	Leprosy $N = 228$ (%)	Clinical forms			
		Lepromatous $n = 60$ (%)	Borderline[a] $n = 72$ (%)	Indeterminate $n = 16$ (%)	Tuberculoid $n = 36$ (%)
High	87 (38)	23 (38)	25 (35)	6 (37)	15 (42)
Intermediate	105 (46)	28 (47)	37 (51)	8 (50)	15 (42)
Low	36 (16)	9 (15)	10 (14)	2 (13)	6 (17)

MBL, mannose-binding lectin.
[a] Borderline tuberculoid leprosy, borderline borderline leprosy, borderline lepromatous leprosy. No association was significant for all possible combinations when the Chi-square test with Yates' correction was used for statistical analysis.

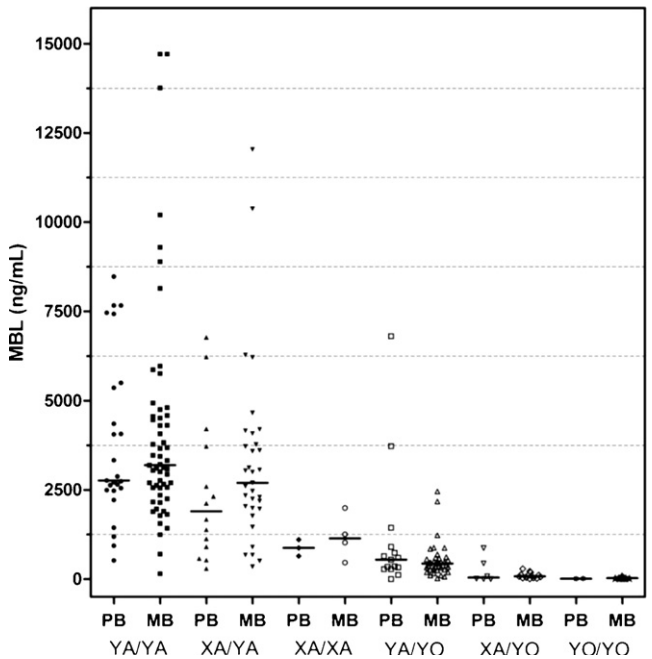

Figure 2. Mannose-binding lectin (MBL) levels according to genotype at the −221 promoter region and exon-1 of the *MBL2* gene in patients with leprosy, according to operational class: paucibacillary (PB) or multibacillary (MB). Y and A are wild-type alleles, X and O are mutant alleles.

forms. Our results are in agreement with those of Fitness et al.,[8] who studied 270 paucibacillary leprosy patients vs. 452 controls in the Republic of Malawi, a country in the northeast of Africa, showing no association between the genotypes of exon-1 of *MBL2* with susceptibility to the disease.

A study in the south region of Brazil,[11] investigating the association of *MBL2* polymorphisms at three positions in the promoter region (H/L, X/Y, and P/Q) and the variant alleles of the structural region (B, C, and D) in patients with leprosy (n = 264) vs. controls (n = 214), showed a higher frequency for LYPA (high MBL expression) in the leprosy patients and in patients with the lepromatous forms. These authors also showed that the haplotypes and genotypes of low MBL expression were associated with protection from the lepromatous form of leprosy. The lack of association of the lepromatous form with other MBL high producer haplotypes such as HYPA in the study by de Messias-Reason et al.,[11] could be explained by the fact that in Amerindians of South America this haplotype may be associated with lower serum MBL than is expected.[32] This study was complementary to one that determined MBL levels, which observed a higher frequency of MBL deficiency for the tuberculoid form in the same group of patients,[26] supporting the association of MBL and progression to the lepromatous form in Brazilians from the south region.

One explanation for the differences between the results observed by de Messias-Reason et al. [11] and those of the present study may be the variation in composition of haplotypes and genotypes, since in the present study the genotyping of untranslated position +4 of the *MBL2* gene (alleles P/Q) was not performed. However, since the influence of the Q variant on MBL serum levels is quite small and overlapping,[14] the information possibly obtained from genotyping this allele may not influence the association results concerning the frequency of haplotypes and genotypes with the circulating high oligomers of serum MBL. On the other hand, the haplotypes and genotypes constructed with the Q variant may provide additional genetic insights other than the influence of this allele on MBL serum levels.

Furthermore, the variant alleles of the structural region of *MBL2* in the present study were not individually determined, but were considered collectively as allele 'O', giving a secure approach to the estimate of MBL levels as suggested by Garred et al.[14] Alternatively, important information regarding the allele specific genotype could be missing, since the variant alleles seem to present different biological features besides the simple variation in high oligomer formation. Studies of the different expression systems with regard to the features of human recombinant MBL variants have shown that the D variant allele has relatively normal carbohydrate binding and complement activation function and that the allele B is weakly active, while the C variant allele is inactive in activating the complement.[29,33,34] Interestingly, a study

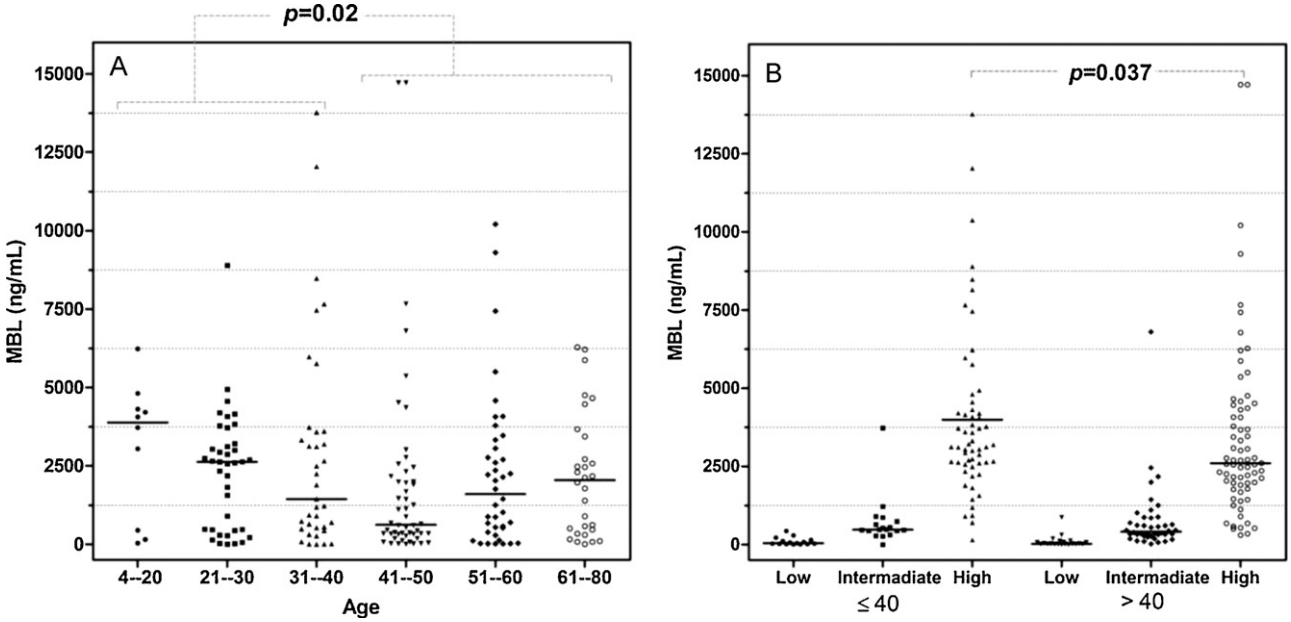

Figure 3. Serum levels of mannose-binding lectin (MBL) in patients with leprosy and their relationship with age. (A) Serum levels of MBL by age in years 4–20 (n = 12), 21–30 (n = 41), 31–40 (n = 40), 41–50, (n = 56), 51–60 (n = 44), 61–80 (n = 35). Considering a cut-off of age ≤40 years and >40 years, there was a difference in the levels of MBL; Mann–Whitney U-test p = 0.02. (B) Distribution of serum MBL concentration range low, intermediate, and high, according to the *MBL2* genotypes and age ≤40 and >40 years. The high-expression genotype showed a significant difference between age groups; Mann–Whitney U-test p = 0.037.

with a larger number of patients (n = 933) with leprosy from Nepal showed a weak association only between the genotype BB (G161A) and the tuberculoid form. However, no association with any disease form was found for the allelic frequency of the B variant.[12]

A limitation of our study was the lack of a racial/color methodology to estimate ancestry. However, this criterion applied to the Brazilian population brings with it the problem of regional subjective differences in color perception, and thus could not be applied with confidence.[35] Indeed, a study with a panel of 40 validated ancestry-informative insertion–deletion DNA polymorphisms individually estimated the European, African, and Amerindian ancestry components of 934 self-categorized White, Brown, or Black Brazilians from the four most populous regions of the country. This study showed that in all regions investigated, the European ancestry was predominant, with proportions ranging from 60.6% in the northeast to 77.7% in the south.[35] Based on these data we could assume that there was most probably a similar proportion of genetic backgrounds in the studied groups. However, differences in ethnicity in the groups cannot be discounted.

Therefore, it could be inferred that the differences in findings between our study and that of de Messias-Reason et al.[11] may be related to differences in the background populations. While the present study included a population of different proportions of those of Black descent, Amerindians, and White Europeans, which is characteristic of the northeast of Brazil,[35] de Messias-Reason et al. studied a population of predominantly European composition. In fact, Boldt et al.[22] showed a significant difference in the distribution of haplotypes of the *MBL2* gene among populations of African-Brazilians, Euro-Brazilians, and intermingled Brazilians. This may have implications for the interaction of the *MBL2* polymorphism with other genes in the development of the disease.

Ip et al.,[36] in their study of 689 Chinese adults, showed a decline in serum levels of MBL among healthy individuals aged over 40 years. These authors used the same monoclonal antibody that we used in the present study to detect MBL in the ELISA. In contrast, Ytting et al.,[37] in their study of 348 healthy individuals from Denmark, employed an immunofluorometric assay using mannan as ligand for MBL, and found no difference in MBL levels with age. The decline in MBL levels in our study was significant in MBL higher producers and could be related to changes in the levels of hormones triiodothyronine/thyroxine (T3/T4) and GH that occur with age, which seem to have a strong influence on the production of MBL.[27,28,38]

Therefore, age should be considered as an important variable in studies of MBL deficiency determined by MBL immune recognition ELISA. In previous studies on patients with leprosy, the association of genotype, serum MBL, and age has not been investigated, hence it is difficult to make any direct comparisons with our results. Regarding the association between MBL levels or genotypes with gender, no difference was found in patients with the different forms of leprosy, in contrast to the results of Dornelles et al., [26] who showed a significant increase in MBL levels in male over female patients with leprosy.

A significant association between the MB form and male gender was observed in our study, and may be because women seek more health services for esthetic reasons, or greater health care, than men.[39] Also, the median MBL levels in MB patients according to genotyping for −221 (Y/X) and exon-1 (A/O) was slightly higher compared to PB patients, though this was not significant (Figure 2).

This study shows that the *MBL2* polymorphism associated with the detection of high oligomer MBL does not represent a risk factor for leprosy or for protection against the lepromatous form of the disease in patients from the northeast of Brazil. Moreover, we observed that levels of MBL vary with age, particularly after 40 years, in individuals with leprosy who have a high-expression genotype for MBL, suggesting that this variable should be taken into account in future studies regarding MBL immunogenetics. Finally, the findings of our study when considered together with the published reports, stress the importance of genotyping the structural *MBL2* variant alleles individually and the development of new detection systems for variant MBL.

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