Mannose-Binding Lectin2 Gene Polymorphism and IgG4 in Membranous Nephropathy

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Keywords

Complement system · Gene polymorphism · Immunology · Lectin pathway · Lupus nephritis · Membranous nephropathy

Abstract

Background: Idiopathic membranous nephropathy (IMN) has been linked to the lectin pathway, IgG4 and genetic susceptibility. We investigated the frequency of mannose-binding lectin2 (*MBL2*) gene polymorphisms and the serum ratio of IgG4 in patients with membranous nephropathy (MN). **Methods:** Polymorphisms in the exon 1 of the *MBL2* gene (codons 52, 54, and 57) and single base polymorphisms at positions –550 (HL) and –221 (XY) in the promoter region were evaluated in 60 patients compared to a control group (CG) of 101 blood donors. It established the frequency of

polymorphisms and the serum ratio of IgG4 comparing 2 etiologies of MN: idiopathic (35 patients) and secondary to systemic lupus erythematosus (25 patients). **Results:** Patients with MN had a 2.54-fold higher probability (95% CI 1.51–4.31) of carrying the O allele, exon 1 variant, and 11.16-fold higher probability (95% CI 4.77–28.41) of having A/O genotype when compared to CG. The frequency of polymorphisms in the promoter region was similar between the groups. Combined genotypes generally related to the defective production of MBL (YA/O, XA/O and O/O) were more frequent in patients with MN (OR 7.11; 95% CI 2.69–21.27), when compared to controls. The median of serum ratio IgG4 was 5% for idiopathic MN and 3% for lupus MN patients ($p = 0.016$). **Conclusions:** Our data suggests that *MBL2* polymorphisms may be associated with the activation of the lectin pathway by IgG4 subclass antibodies in MN.

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Introduction

Membranous nephropathy (MN), characterized histopathologically by the thickening of the basement membrane, may have a primary or a secondary etiology. Idiopathic membranous nephropathy (IMN) is one of the leading causes of nephrotic syndrome worldwide, and is responsible for around 20% of all cases [1]. It is also the second most prevalent primary glomerular disease in northeastern Brazil [2]. Among the secondary forms, systemic lupus erythematosus (SLE) is the most frequent [3]. Although the MN secondary to SLE lupic membranous nephropathy (LMN) stems from the systemic formation of immune complexes with subsequent glomerular deposition, IMN appears to be a local immune-mediated disease, and is characterized by the in situ formation of immune complexes in the glomerular subepithelial space due to the reaction of antibodies against podocyte antigens, native or exogenous, thus causing podocyte injury with consequent proteinuria [4].

Some podocyte antigens that participate in the immune complexes in IMN have been previously described [5, 6]. A common finding regarding these antigens is that they are mostly IgG4, a subclass of IgG predominant in renal deposits in the IMN, in contrast to LMN, in which IgG1, IgG2 and IgG3 are predominant [7, 8]. In spite of IgG4 being known as an immunoglobulin with low capacity to activate the complement pathways, glomerular deposits of C3, C4d and the C5b-9 membrane attack complex demonstrate that these pathways are involved in IMN [9–11]. In addition, the presence of the mannose-binding lectin (MBL) protein, a major component of the lectin pathway of the complement and of IgG4 in renal deposits in IMN patients, without C1q, therefore suggests that both lectin complement pathway and IgG4 may be involved in the pathogenesis of the disease [8, 12, 13]. Besides the local importance of IgG4, detected in renal immunofluorescence, these antibodies may also be systemically involved in the pathogenesis of the disease [14].

Although there is evidence regarding the involvement of the lectin pathway and IgG4 in IMN, the trigger responsible for the immune complexes formation is uncertain. It is known that IMN occurs in genetically susceptible individuals; however, very few studies have investigated the possible relationship between this glomerulopathy and polymorphisms of the *MBL2* gene, which is responsible for producing MBL [15–18]. Although lupus nephritis is associated with genetic variants of *MBL2* and the consequent deficient production

of the MBL protein [19, 20], only Bally et al. [21] have evaluated the sequencing of the *MBL2* gene in IMN, and found polymorphisms in 5 out of 78 patients. This gene is responsible for producing the amino acid sequences of the collagen triple helix of the *MBL2* [22]. Mutation points (single base polymorphisms [SNPs]) in exon 1 of the *MBL2* result in the production of non-functional MBL [23]. The A allele represents the coding region of the wild type, while the presence of SNPs in codons 52, 54, and 57, represented by O, may be present in the heterozygous (A/O) in about 35% of the population, or homozygous (O/O) in 8% of the general population [24].

For these reasons, this study has aimed to determine the frequency of *MBL2* gene polymorphisms in patients with MN compared to healthy subjects. An additional aim was to analyze the ratio of serum IgG4 (%IgG4) and the frequency of *MBL2* polymorphisms when comparing patients with IMN and those with LMN.

Subjects and Methods

Study Design

We conducted an exploratory study at the Hospital das Clínicas/Universidade Federal de Pernambuco (HC – UFPE) and the Instituto de Medicina Integral Prof. Fernando Figueira (IMIP). Both are referral centers for patients with glomerular diseases in the state of Pernambuco, Northeastern Brazil. Pernambuco is the seventh most populous state in the Brazil with an estimated population of over 9 million and a poverty incidence of 52.5% [25]. Ethics Committees of the participating institutions approved the study, and procedures were in accordance with the Helsinki Declaration as revised in 2013. All patients gave written informed consent before participating in the study.

Patient Selection

For this study, we analyzed 60 biopsy-proven patients between 2014 and 2015. Inclusion criteria were patients aged 16 years and over, with a histopathologic diagnosis of IMN and LMN documented by renal biopsy with optical microscopy and immunofluorescence. The diagnosis of LMN was established by SLICC criteria (Systemic Lupus International Collaborating Clinics, 2012) [26], and patients with lupus nephritis associated with class III or IV were excluded. Patients were also excluded if they presented with MN secondary to other diseases, determined through serology for HIV and hepatitis B and C and through further individualized investigation regarding personal and family histories, age and medical history. For both groups, the presence of proteinuria ≥ 500 mg/day was considered active disease.

Sixty patients met inclusion criteria. Thirty-five patients were diagnosed with IMN and 25 with LMN. It was not possible to perform the sequencing of the *MBL2* gene in 1 patient with LMN. For operational reasons, it was not possible to measure the levels of serum IgG and IgG4 in 4 patients (3 with IMN and 1 with LMN).

Table 1. Clinical characteristics of patients with idiopathic membranous nephropathy and lupic membranous nephropathy and of the control group

Characteristics	IMN (n = 35)	LMN (n = 25)	CG (n = 101)	p value
Age, years*	44.9±13.8	33.9±9.1	30.1±9.1	<0.001 ³
Males, n (%)	65.7 (23)	24.0 (6)	41.6 (42)	0.004 ⁴
Time of disease, months	13 (5.5–31)	30 (7–44)	–	0.107 ²
Immunosuppression, n (%)	68.6 (24)	96.0 (24)	–	0.009 ⁴
Activity of disease, n (%)	71.4 (25)	48.0 (12)	–	0.066 ⁴
Proteinuria, g/day [#]	2.0 (0.5–4.3)	0.5 (0.1–1.7)	–	0.008 ²
Serum albumin, g/dL*	3.30±1.35	3.74±0.67	–	0.167 ¹
Serum creatinine, g/dL [#]	1.1 (0.8–1.5)	0.8 (0.6–1.0)	–	0.002 ²

¹ p value of the Student *t* test; ² p value of the Mann-Whitney test; ³ p value of the ANOVA test; ⁴ p value of the chi-square test to compare the proportion; * mean ± SD; # median (Q1–Q3).

Control Group

For the control group (CG), we used DNA samples from 101 healthy individuals. An evaluation of the *MBL2* gene of this group had been conducted previously and published [19]. This historic control consisted of normal blood donors, who had completed questionnaires reporting the absence of personal or family autoimmune diseases. Furthermore, this group also underwent genotyping of HLA haplotypes DR3, DR4, DQ2, and DQ8 molecules associated with the increased risk of type I diabetes, celiac disease and autoimmune thyroiditis. The presence of a haplotype risk was an exclusion criterion.

Laboratory Analyses

Blood samples (5 mL) were collected in an EDTA tube for genetic evaluation. Genomic DNA was isolated from lymphocytes using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) on the basis of manufacturer's recommendations.

Polymorphisms in the exon 1 of the *MBL2* gene (codons 52, 54, and 57) were genotyped using TaqMan assays (Thermo Fisher Scientific, CA, USA). The reactions were prepared based on the manufacturer's protocol, and genotyping was performed using ABI7500 Real-Time instrument (Thermo Fisher Scientific, CA, USA). The polymorphisms of 3 codons of exon 1 were grouped into a category named the O allele according to Garred et al. [27], while a combination of the 3 wild alleles was designated the A allele.

SNPs at positions –550 (HL) and –221 (XY) in the promoter region of *MBL2* were evaluated using sequencing-based typing technique. The PCR amplification of the fragment encompassing the *MBL2* promoter region, digestion of PCR products and direct sequencing using the samples were described in Sandrin-Garcia et al. [19].

To measure the serum IgG and subclass IgG4, 5 mL of blood were collected through intravenous puncture in a tube with clot activator and gel separator. The samples were immediately stored in a Styrofoam container with ice, submitted to centrifugation to 3,000 rpm for 10 min and kept at –80 °C. Subsequently, the measurements of total IgG and IgG4 were performed by immunoturbidimetric and nephelometric techniques respectively. The %IgG4 was obtained from the ratio of IgG4 by IgG, multiplied by 100 (IgG4/IgG × 100).

Statistical Analysis

A database was constructed on a Microsoft Excel® spreadsheet, and exported to SPSS 17, where analyzes were performed. Normality of the quantitative variables was assessed using the Kolmogorov-Smirnov test. Comparison between the groups was performed with the analysis of variance test, Student *t* test or Mann-Whitney test, according to normality. A chi-square test was applied to compare qualitative variables between the IMN and LMN groups.

The allelic frequency and the frequency distribution of genotypes were analyzed by the chi-square test and the OR was calculated with a 95% CI. When calculating the OR, the alleles and the homozygous genotypes that corresponded to the highest frequency were selected as a reference. In order to represent the values of IgG, IgG4 and the %IgG4, the median and the first and third quartiles were calculated. The *p* value considered statistically significant was <0.05. A post hoc power analysis was performed using G* Power Software (version 3.1.2) based on minor allele frequency and OR with an alpha = 0.05.

Results

Data from patients with MN and the CG is described in Table 1. The CG consisted of 101 healthy subjects (59 women and 42 men), with a mean age of 30.1 ± 9.1.

Table 2 presents the distribution of the alleles and genotypes of *MBL2* in patients with MN and healthy controls. In CG, the alleles and genotypes frequencies were in Hardy-Weinberg equilibrium. In the patient group, the promoter alleles were in Hardy-Weinberg equilibrium, but the exon 1 was not due to the absence of genotype O/O. The O allele of exon 1 was more frequent in the group with MN compared to healthy subjects (42% × 22%; *p* < 0.001). The probability of MN patients being carriers of the O allele was 2.54-fold higher than that of the GC (95% CI 1.51–4.31). With regard to the distribution of genotypes, it was

Table 2. Polymorphisms in the exon 1 of the *MBL2* in patients with membranous nephropathy and the control group

Polymorphisms of the <i>MBL2</i>	MN (<i>n</i> = 59)	CG (<i>n</i> = 101)	<i>p</i> value ¹	OR (95% CI)
<i>Exon 1 A/O SNP, n (%)</i>				
Alleles				
A	69 (58)	158 (78)	<0.001	1.00 (reference)
O	49 (42)	44 (22)		2.54 (1.51–4.31)
Genotypes				
A/A	10 (17)	65 (64)	<0.001	1.00 (reference)
A/O	49 (83)	28 (28)		11.16 (4.77–28.41)
O/O	0 (0)	8 (8)		–
<i>Promoter H/L SNP, n (%)</i>				
Alleles				
H	27 (38)	71 (35)	0.775	1.0 (reference)
L	45 (62)	131 (65)		1.1 (0.60–1.99)
Genotypes				
HH	6 (16)	13 (13)	1.0	1.0 (reference)
HL	15 (42)	45 (45)		0.95 (0.38–2.38)
LL	15 (42)	43 (42)		1.31 (0.34–4.65)
<i>Promoter Y/X SNP, n (%)</i>				
Alleles				
Y	58 (85)	170 (84)	1.0	1.0 (reference)
X	10 (15)	32 (16)		0.91 (0.37–2.05)
Genotypes				
YY	25 (74)	74 (73)	1.0	1.0 (reference)
YX	8 (23)	22 (22)		1.07 (0.36–2.91)
XX	1 (3)	5 (5)		0.59 (0.012–5.69)

¹ *p* value of the chi-square test for independence.

Table 3. Polymorphisms in the exon 1 of the *MBL2* in idiopathic membranous nephropathy × control group, lupic membranous nephropathy × control group and idiopathic membranous nephropathy × lupic membranous nephropathy

	IMN (<i>n</i> = 35), <i>n</i> (%)	CG (<i>n</i> = 101), <i>n</i> (%)	OR (95% CI)	LMN (<i>n</i> = 24), <i>n</i> (%)	CG (<i>n</i> = 101), <i>n</i> (%)	OR (95% CI)	IMN (<i>n</i> = 35), <i>n</i> (%)	LMN (<i>n</i> = 24), <i>n</i> (%)	OR (95% CI)
Alleles									
A	41 (59)	158 (78)	2.53 (1.36–4.71)	28 (58)	158 (78)	2.55 (1.24–5.22)	41 (59)	28 (58)	1.01 (0.45–2.27)
O	29 (41)	44 (22)		20 (42)	44 (22)		29 (41)	20 (42)	
Genotypes									
A/A	6 (17)	65 (64)	10.98 (3.92–36.06)	4 (17)	65 (64)	11.34 (3.39–49.88)	6 (17)	4 (17)	1.03 (0.21–5.65)
A/O	29 (83)	28 (28)		20 (83)	28 (28)		29 (83)	20 (83)	

NB: The reference group in the comparisons was patients with allele A and patients with genotype A/A.

observed that the heterozygous was predominant among patients with MN (83%) compared to that of the CG (28%). In the CG, there was a predominance of genotype A/A (64%). The chance of having the A/O genotype was 11.16 times higher in the MN group compared to that of

the A/A genotype (95% CI 4.77–28.41). There was no difference for genotypes or alleles frequencies among patients and controls in H/L and X/Y promoter variants.

The frequency of *MBL2* polymorphism was also evaluated, comparing the IMN and LMN groups with the CG

Table 4. *MBL2* promoter and exon 1 combined genotypes (grouped accordingly to MBL-predicted production) in membranous nephropathy patients and controls

<i>MBL2</i> combined genotypes	MN (<i>n</i> = 35)	CG (<i>n</i> = 101)	OR (95% CI); <i>p</i> value
High producer vs. defective producer (excluding H/L genotypes), <i>n</i> (%)			
YA/YA, YA/XA and XA/XA	7 (20)	65 (64.4)	1 (reference)
YA/O, XA/O, O/O	28 (80)	36 (35.6)	7.11 (2.69–21.27); <i>p</i> < 0.001
High and low producer vs. deficient producer (maintaining H/L genotypes), <i>n</i> (%)			
YA/YA, YA/XA, XA/XA and YA/O	34 (97)	89 (88)	1 (reference)
LXA/O, O/O	1 (3)	12 (12)	0.211 (0.004–1.51); <i>p</i> = 0.188

(Table 3). It was found that in both the IMN and LMN groups, the chance of carrying the O allele was higher, when compared to the CG. In the genotyping, heterozygosity A/O was more frequent in IMN (OR 10.98; 95% CI 3.92–36.06) and LMN (OR 11.34; 95% CI 3.39–49.88). When comparing the IMN and LMN groups there was no difference in the frequency of alleles or genotypes with a specific etiology.

It was possible to reconstruct combined *MBL2* genotypes from 35 patients with MN and compare to controls (Table 4). A significant difference was observed between the MN group and controls, as MN patients had a higher frequency of a defective producer combined genotype (OR 7.11, 95% CI 2.69–21.27, *p* < 0.001). However, when considering only *MBL2*-deficient producers (very low/not detectable MBL level/activity) and comparing them with high and low producers combined genotypes taken together (YA/YA, YA/XA, XA/XA, and YA/O), *MBL2*-deficient combined genotypes are more frequent in the controls with respect to the MN (OR 0.211 [0.004–1.51]; *p* = 0.188).

In order to evaluate the possible differences of serum %IgG4 between the 2 etiologies, serum levels of IgG4 and IgG were measured in 32 patients with IMN and 24 with LMN. Patients with IMN presented with higher proteinuria when compared to those with LMN (median 2.6×0.4 g/day; *p* = 0.002). Most of the patients with IMN had been treated with immunosuppression (22/32) and presented with active disease (24/32). The median levels of serum IgG and IgG4 and %IgG4 are presented in Figure 1. The total IgG levels were lower in patients with IMN when compared to the levels of those with LMN ($870 \times 1,164$ mg/dL; *p* = 0.008). The serum concentration of IgG4 (41×29 mg/dL) was higher for the IMN group, although there was no statistical significance. The %IgG4 was higher in patients with IMN ($5 \times 3\%$; *p* = 0.016).

Discussion

This study has investigated the possible association of polymorphisms of the *MBL2* with MN and serum %IgG4 between primary and secondary etiologies. These results show that the presence of the O allele, and in particular of the genotype A/O, is more frequent in patients with MN than in CG. Moreover, the increased percentage of IgG4 in patients with IMN suggests a potential role in the pathophysiology of the disease.

Studies suggest that IMN stems from a combination of 3 conditions: the presence of podocyte antigens, the production of antibodies against the autoantigen, and a genetically susceptible individual [28]. Since the Heymann nephritis model in 1952, some potential antigens involved in IMN have been described. Although some of these antigens have good specificity and correlation with the pathogenesis and progression of the disease, such as the phospholipase A2 receptor (PLA2R), a member of the lectin family, none of them is able to fully explain the pathogenesis of IMN [29–31]. One of the most common and intriguing points is that most of these antigens are located in the renal tissue, predominantly with antibody type IgG4 [7]. With regard to this issue, there is still no definitive answer on IMN: a predominance of IgG4 subtype antibodies against podocyte antigens, with the presence of C4d, with no C1q in renal biopsy. The activation of the MBL pathway by IgG4 with low terminal galactose emerged as a possible explanation for this particular question [28]. Corroborating this hypothesis, although it is not routinely evaluated in renal immunofluorescence, some studies have demonstrated the presence of MBL in the glomeruli of patients with IMN [8, 12]. Yang et al. [14] have also reported higher serum levels of plasma MBL-associated serine protease 1 (MASP-1), MASP-2 and MBL in patients with positivity to total and IgG4 anti-PLA2R antibodies, compared to those with bi-negativity to these antibodies, which could provide a clue for lectin activation.

Fig. 1. Box-plot of serum levels of IgG (a), IgG4 (b) and %IgG4 (c) according to the idiopathic membranous nephropathy (IMN) and lupic membranous nephropathy (LMN) groups.

It is known that genetic susceptibility is also part of the etiopathogenic mechanism of IMN. The most important association was found in white patients with SNPs for HLA-DQA1 (a gene that belongs to the HLA class II alpha chain) and PLA2R [18], although other genetic variants have been described as potential vulnerability factors for the disease [17, 32–36]. In the present study, *MBL2* polymorphism was more frequent in the MN group; however, there was no difference when IMN and LMN were compared. Studies on SLE, including patients with LMN, have also demonstrated a positive association between nephritis and genetic variants of *MBL2*. Villarreal et al. [37] have reported increased susceptibility to nephritis in lupus patients with a mutation in codon 54 of exon 1 (OR 2.3), and another Brazilian study has demonstrated an association of genotype A/O with nephritis ($p = 0.02$) [19]. In the present study, genotype O/O, previously related to a 2.6-fold greater risk of developing LN [20], was not encountered in any of the patients. Only Bally et al. [21] have evaluated the presence of *MBL2* polymorphism in IMN. The authors reported that 5 out of 78 patients presented genotypes associated with low serum levels of MBL; however, there was no comparison group.

Since the MBL pathway could be activated by IgG4, the present study also evaluated the serum levels of this immunoglobulin, and encountered a higher %IgG4 in patients with IMN when compared to those with LMN. Similar results were found by Li et al. [38] and Kuroki et al. [39] who reported increased %IgG4 in IMN compared to other primary glomerulopathies and healthy individuals respectively. In contrast to these results, Imai et al. [40], with a smaller sample, encountered no differences when comparing the serum %IgG4 of 9 patients with lupus nephritis to 21 patients with IMN. The present study also found lower concentrations of serum IgG in patients with IMN in

comparison to those with LMN, similar to the study of Kuroki et al. [39]. It is known that SLE patients present with reduced levels of IgG and the treatment may normalize this situation [41, 42]. Thus, because most patients in the LMN group in the present study had undergone immunosuppressive therapy and were in remission, a relative reduction of serum IgG in the IMN group was expected.

It is also known that one possible factor capable of altering the levels of serum IgG and IgG4 in patients with glomerulopathies is the degree of proteinuria, since there may be urinary excretion of immunoglobulins. However, in glomerular diseases, urinary loss of IgG subclasses is proportional to the serum levels [43], which could rule out the possibility of selective excretion of IgG subclasses as a reason for the proportional increase of IgG4 in IMN. Some studies even suggest that IgG4 molecules in very high serum levels may bind with 1 another or with other molecules, slowing down filtration and increasing the serum concentration [44]. As serum levels of IgG4 did not differ significantly among the groups in the present study, the filtration hypothesis was minimized; thus, the increased serum %IgG4 in IMN became a relevant fact.

The differential aspect of the present study is an evaluation of the *MBL2* polymorphisms in patients with IMN, compared to the CG and those with LMN. Although a causal relationship cannot be established, the results of this study suggest that it is possible that the genetic variation of *MBL2* could be related to increased susceptibility to the development of IMN. Some possibilities for this have been suggested. One hypothesis is that polymorphism of the *MBL2* gene leads to a change in conformation of the triple helix structure of the MBL protein, causing its deposits in the glomeruli. An accumulation of the altered protein itself or apoptotic cells, due to its poor competence in clearance, could cause them to act as autoantigens for

the IgG4 subtype antibodies in IMN, thus activating the lectin pathway. Another hypothesis is that infectious diseases, more frequent and severe in individuals with lower serum levels of MBL, may act as triggers for the formation of immunocomplexes in individuals genetically susceptible to IMN, just as it occurs in other autoimmune diseases [45, 46]. As the mutation was not only related to one particular etiology of MN in our study, the results may even suggest that the presence of SNPs of the *MBL2* could be associated with the formation and/or deposition of immune complexes mostly located in the subepithelial area.

We are aware that our study has some limitations. The first limitation is the small sample size. However, the high frequency of polymorphism in MN even within this sample, may suggest that this is a true association, indicating the need of population studies. In addition, a post hoc power analysis value showed sufficient value (99.9%) for the comparison between MN and CG with respect for the A/O genotype and 85.6% for the A/A genotype. Nevertheless, it is possible that the lack of O/O genotypes among patients occurred by chance.

Moreover, with regard to evaluating the serum %IgG4, in comparison to other studies performed with similar objectives, this has been the largest sample. A second limitation may have been the heterogeneous groups used for comparison. In Brazil, IMN and LMN were evaluated as the main causes of MN [3]. However, clinical characteristics such as age and sex may not be comparable between these 2 etiologies, since it is known that IMN is more prevalent in middle-aged men and LMN in younger women [2, 47]. Race was not categorized, as in other studies, since assessment based only on skin color is extremely inaccurate in Brazilian population, as demonstrated in a study that used 12 ancestry markers in this population [48]. Other laboratory tests could provide additional data for the study, such as serum and urinary determination of other subclasses of IgG and anti-PLA2R serum levels. In addition, because this study used a historical control, we were unable to measure serum IgG4 levels in this group.

Finally, since serum MBL was not measured in this study, it should be emphasized that *MBL2* genotypes partially represent the serum levels of this protein, which may be a variable in the 2 categories of high producers and deficient individuals. It is also true that individuals identified as high producers based on *MBL2* genotypes combined may exhibit different serum concentrations of MBL, which, if too high, may play a detrimental role by superactivating complement, increasing the risk of tissue damage [46]. This apparent contradiction up to now has not been explained in the literature on the studied subject.

Moreover, the apparently controversial findings obtained comparing combined genotypes in patients and controls indicate the strong need of MBL serum dosage, which was not possible in our study. The association found between defective producers genotypes and MN group was lost in the second comparison (high and low producers vs. deficient producers) due to a possible confounding effect of the low but not deficient *MBL2* combined genotype.

In conclusion, this study revealed that, compared to healthy individuals, patients with MN presented a higher frequency of polymorphisms in the exon 1 of the *MBL2* gene and a higher frequency of genotypes associated with lower production of MBL. Between the groups of patients with IMN and LMN, there was no difference in the frequency of *MBL2* polymorphisms, but the IgG4 percentage was higher in patients with IMN. Although we were not able to compare genetics with the phenotype of patients, we can speculate from our results that levels or the structure of MBL may enter in the genesis of MN. Therefore, it seems appropriate to continue with larger studies on the relationships between MBL levels, genetics of *MBL2* and MN, to confirm our preliminary results.

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Ethics Statement

This study was approved by the Regional Ethics Committee and a written informed consent from all patients was obtained.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

- 1 Ronco P, Debiec H: Pathophysiological advances in membranous nephropathy: time for a shift in patient's care. *Lancet* 2015;385:1983–1992.
- 2 Costa DM, Valente LM, Gouveia PA, et al: Comparative analysis of primary and secondary glomerulopathies in the northeast of Brazil: data from the Pernambuco Registry of Glomerulopathies – REPEG. *J Bras Nefrol* 2017;39:29–35.
- 3 Diz MCE, Scherer P, Kirsztajn GM: Clinical-epidemiological profile of primary membranous glomerulopathy in Brazilian patients (71 cases). *J Bras Nefrol* 2007;29:71–79.

- 4 Ronco P, Debiec H: Pathogenesis of membranous nephropathy: recent advances and future challenges. *Nat Rev Nephrol* 2012;8:203–213.
- 5 Beck LH Jr, Bonegio RG, Lambeau G, et al: M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med* 2009;361:11–21.
- 6 Tomas NM, Beck LH Jr, Meyer-Schwesinger C, et al: Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N Engl J Med* 2014;371:2277–2287.
- 7 Stone JH: IgG4: a tantalizing link between causes of membranous glomerulonephritis and systemic disease. *Kidney Int* 2013;83:348–350.
- 8 Segawa Y, Hisano S, Matsushita M, et al: IgG subclasses and complement pathway in segmental and global membranous nephropathy. *Pediatr Nephrol* 2010;25:1091–1099.
- 9 Aalberse RC, Schuurman J: IgG4 breaking the rules. *Immunology* 2002;105:9–19.
- 10 Val-Bernal JF, Garijo MF, Val D, Rodrigo E, Arias M: C4d immunohistochemical staining is a sensitive method to confirm immunoreactant deposition in formalin-fixed paraffin-embedded tissue in membranous glomerulonephritis. *Histol Histopathol* 2011;26:1391–1397.
- 11 Cunningham PN, Quigg RJ: Contrasting roles of complement activation and its regulation in membranous nephropathy. *J Am Soc Nephrol* 2005;16:1214–1222.
- 12 Lhotta K, Würzner R, König P: Glomerular deposition of mannose-binding lectin in human glomerulonephritis. *Nephrol Dial Transplant* 1999;14:881–886.
- 13 Ma H, Sandor DG, Beck LH Jr: The role of complement in membranous nephropathy. *Semin Nephrol* 2013;33:531–542.
- 14 Yang Y, Wang C, Jin L, et al: IgG4 anti-phospholipase A2 receptor might activate lectin and alternative complement pathway meanwhile in idiopathic membranous nephropathy: an inspiration from a cross-sectional study. *Immunol Res* 2016;64:919–930.
- 15 Klouda PT, Manos J, Acheson EJ, et al: Strong association between idiopathic membranous nephropathy and HLA-DRW3. *Lancet* 1979;2:770–771.
- 16 Vaughan RW, Demaine AG, Welsh KI: A DQA1 allele is strongly associated with idiopathic membranous nephropathy. *Tissue Antigens* 1989;34:261–269.
- 17 Liu YH, Chen CH, Chen SY, et al: Association of phospholipase A2 receptor 1 polymorphisms with idiopathic membranous nephropathy in Chinese patients in Taiwan. *J Biomed Sci* 2010;17:81.
- 18 Stanescu HC, Arcos-Burgos M, Medlar A, et al: Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. *N Engl J Med* 2011;364:616–626.
- 19 Sandrin-Garcia P, Brandão LA, Coelho AV, et al: Mannose binding lectin gene (MBL2) functional polymorphisms are associated with systemic lupus erythematosus in southern Brazilians. *Hum Immunol* 2011;72:516–521.
- 20 Tanha N, Troelsen L, From Hermansen ML, et al: MBL2 gene variants coding for mannose-binding lectin deficiency are associated with increased risk of nephritis in Danish patients with systemic lupus erythematosus. *Lupus* 2014;23:1105–1111.
- 21 Bally S, Debiec H, Ponard D, et al: Phospholipase A2 receptor-related membranous nephropathy and mannose-binding lectin deficiency. *J Am Soc Nephrol* 2016;27:3539–3544.
- 22 Lu J, Teh C, Kishore U, Reid KB: Collectins and ficolins: sugar pattern recognition molecules of the mammalian innate immune system. *Biochim Biophys Acta* 2002;1572:387–400.
- 23 Petersen SV, Thiel S, Jensenius JC: The mannose-binding lectin pathway of complement activation: biology and disease association. *Mol Immunol* 2001;38:133–149.
- 24 Minchinton RM, Dean MM, Clark TR, Heatley S, Mullighan CG: Analysis of the relationship between mannose-binding lectin (MBL) genotype, MBL levels and function in an Australian blood donor population. *Scand J Immunol* 2002;56:630–641.
- 25 Brasil. Ministério do Planejamento. Orçamento e Gestão. Instituto Brasileiro de Geografia e Estatística. <http://www.ibge.gov.br/estadosat/perfil.php?sigla=pe> (accessed January, 2016).
- 26 Petri M, Orbai AM, Alarcón GS, et al: Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012;64:2677–2686.
- 27 Garred P, Larsen F, Madsen HO, Koch C: Mannose-binding lectin deficiency—revisited. *Mol Immunol* 2003;40:73–84.
- 28 Salant DJ: Genetic variants in membranous nephropathy: perhaps a perfect storm rather than a straightforward conformeropathy? *J Am Soc Nephrol* 2013;24:525–528.
- 29 Debiec H, Guignon V, Mougenot B, et al: Antenatal membranous glomerulonephritis due to anti-neutral endopeptidase antibodies. *N Engl J Med* 2002;346:2053–2060.
- 30 Debiec H, Lefeu F, Kemper MJ, et al: Early-childhood membranous nephropathy due to cationic bovine serum albumin. *N Engl J Med* 2011;364:2101–2110.
- 31 Murtas C, Bruschi M, Candiano G, et al: Coexistence of different circulating anti-podocyte antibodies in membranous nephropathy. *Clin J Am Soc Nephrol* 2012;7:1394–1400.
- 32 Nishimukai H, Nakanishi I, Kitamura H, Tamaki Y: Factor B subtypes in Japanese patients with IgA nephropathy and with idiopathic membranous nephropathy. *Exp Clin Immunogenet* 1988;5:196–202.
- 33 Vaughan RW, Tighe MR, Boki K, et al: An analysis of HLA class II gene polymorphism in British and Greek idiopathic membranous nephropathy patients. *Eur J Immunogenet* 1995;22:179–186.
- 34 Debiec H, Nauta J, Coulet F, et al: Role of truncating mutations in MME gene in fetomaterial alloimmunisation and antenatal glomerulopathies. *Lancet* 2004;364:1252–1259.
- 35 Thibaudin D, Thibaudin L, Berthou P, et al: TNFA2 and d2 alleles of the tumor necrosis factor alpha gene polymorphism are associated with onset/occurrence of idiopathic membranous nephropathy. *Kidney Int* 2007;71:431–437.
- 36 Chen CH, Shu KH, Wen MC, et al: Impact of plasminogen activator inhibitor-1 gene polymorphisms on primary membranous nephropathy. *Nephrol Dial Transplant* 2008;23:3166–3173.
- 37 Villarreal J, Crosdale D, Ollier W, et al: Mannose binding lectin and FcγRIIa (CD32) polymorphism in Spanish systemic lupus erythematosus patients. *Rheumatology (Oxford)* 2001;40:1009–1012.
- 38 Li J, Qu Z, Zhang YM, et al: [Clinical significance of detection of plasma and urine IgG4 in idiopathic membranous nephropathy]. *Beijing Da Xue Xue Bao Yi Xue Ban* 2010;42:671–674.
- 39 Kuroki A, Shibata T, Honda H, Totsuka D, Kobayashi K, Sugisaki T: Glomerular and serum IgG subclasses in diffuse proliferative lupus nephritis, membranous lupus nephritis, and idiopathic membranous nephropathy. *Intern Med* 2002;41:936–942.
- 40 Imai H, Hamai K, Komatsuda A, Ohtani H, Miura AB: IgG subclasses in patients with membranoproliferative glomerulonephritis, membranous nephropathy, and lupus nephritis. *Kidney Int* 1997;51:270–276.
- 41 Lee AH, Levinson AI, Schumacher HR Jr: Hypogammaglobulinemia and rheumatic disease. *Semin Arthritis Rheum* 1993;22:252–264.
- 42 Yap DY, Yung S, Ma MK, et al: Serum immunoglobulin G level in patients with lupus nephritis and the effect of treatment with corticosteroids and mycophenolate mofetil. *Lupus* 2014;23:678–683.
- 43 Shakib F, Hardwicke J, Stanworth DR, White RH: Asymmetric depression in the serum level of IgG subclasses in patients with nephrotic syndrome. *Clin Exp Immunol* 1977;28:506–511.
- 44 Li XL, Yan TK, Li HF, et al: IgG4-related membranous nephropathy with high blood and low urine IgG4/IgG ratio: a case report and review of the literature. *Clin Rheumatol* 2014;33:145–148.
- 45 Anders HJ, Rovin B: A pathophysiology-based approach to the diagnosis and treatment of lupus nephritis. *Kidney Int* 2016;90:493–501.
- 46 Bouwman LH, Roep BO, Roos A: Mannose-binding lectin: clinical implications for infection, transplantation, and autoimmunity. *Hum Immunol* 2006;67:247–256.
- 47 Zeng CH, Chen HM, Wang RS, et al: Etiology and clinical characteristics of membranous nephropathy in Chinese patients. *Am J Kidney Dis* 2008;52:691–698.
- 48 Coelho AV, Moura RR, Cavalcanti CA, et al: A rapid screening of ancestry for genetic association studies in an admixed population from Pernambuco, Brazil. *Genet Mol Res* 2015;14:2876–2884.