Mannose-binding Lectin Gene Polymorphisms in Brazilian Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Rheumatoid arthritis (RA) is a disease with unknown etiology but it is probably multifactorial. RA susceptibility is related to genetic, hormonal, immunologic, and environmental factors. Mannose-binding lectin (MBL) is an important protein of the human innate immune system, encoded by the *MBL2* gene. Polymorphisms in *MBL2* were associated with several diseases, and may be an important factor in RA susceptibility. We analyzed 3 *MBL2* gene polymorphisms in 322 Brazilian patients with RA and 345 ethnically matched healthy controls.

Methods. *MBL2* gene variants were analyzed through polymerase chain reaction sequencing.

Results. Considering *MBL2* B, C, and D alleles separately, a significant difference in both genotypic and allelic frequencies, particularly concerning frequency of the C allele, was observed comparing European-derived and African-derived individuals (European-derived patients 0.022 vs African-derived patients 0.205; European-derived controls 0.029 vs African-derived controls 0.144; both $p < 0.001$). We also analyzed *MBL2* genotype in relation to extraarticular manifestations. Considering *MBL2* variants together, we found an increased frequency of the OO genotype among patients with rheumatoid nodules ($p = 0.031$), although this association lost significance after Bonferroni correction.

Conclusion. Our findings suggest an association of *MBL2* genotypes with some clinical manifestations of RA, but more studies are needed to clarify the actual role of MBL in RA. (J Rheumatol First Release Oct 15 2011; doi:10.3899/jrheum.110052)

Key Indexing Terms:
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The etiology of rheumatoid arthritis (RA) is unknown, but it is probably related to genetic, immunological, and environmental factors. Considering its genetic component, a combination of genes, instead of a single gene, predisposes to an immunological disorder that leads to defective mechanisms of immunological tolerance, leading to autoantibody production and immune complex formation and deposition.

Since mannose-binding lectin (MBL) is an important protein of the human innate immune system, it is possible to hypothesize that this molecule is linked to RA susceptibility. MBL acts as an activator of the complement system through the lectin pathway, inducing opsonization of microorganisms and phagocytosis of apoptotic cells by macrophages. Three functional single-nucleotide polymorphisms (SNP) have been described in codons 54 (allele B), 57 (allele C), and 52 (allele D) and these were associated with changes in the structure and functional deficiency of this protein. In codon 54, an A to G substitution alters an aspartic acid to a glycine at protein level. In codon 57 there is a G to A substitution (glycine to glutamic acid), and in codon 52 a C to T substitution leads to a change from arginine to cystein. Altogether, the presence of any of the variant alleles has been collectively labeled O, while the simultaneous absence of variants at the 3 positions has been called allele A, the wild-type allele.

We analyzed *MBL2* polymorphisms in patients with RA and healthy individuals of different ethnic origins. We also evaluated different clinical symptoms in order to identify possible associations between the *MBL2* polymorphic variants and RA.

MATERIALS AND METHODS

The RA group was composed of 322 patients. Of these, 300 were identified as European-derived and 22 as African-derived individuals. This classification was based on physical appearance and on data about the ethnicity of parents/grandparents reported by the participants, a classification supported by

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recent studies using a 48-insertion-deletion ancestry-informative marker panel[1]. Patients received followup care at the Division of Rheumatology of the Hospital de Clínicas de Porto Alegre (HCPA), meeting the American College of Rheumatology criteria[2]. The Disease Activity Score (DAS28) and the Health Assessment Questionnaire (HAQ) were applied to each patient as a measure of disease activity and physical ability and clinical manifestations were evaluated. The control group was formed by 345 individuals from the urban population of Porto Alegre, the same geographic area as for the patients. Genotyping was performed using polymerase chain reaction amplification as described[3] and the amplified fragments were sequenced using MegaBACE 1000 (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). The chromatograms were observed using the FinchTV Version 1.4.0 software.

Our study protocol was approved by the Ethics Committee of the HCPA and informed consent was obtained from all patients.

Statistical analysis. The total numbers of patients and controls with each given genotype were compared in order to confirm Hardy-Weinberg equilibrium. Independence tests between patients and controls were performed using chi-square tests or Fisher's exact test. For comparison of clinical and laboratory variables with the frequencies of polymorphic variants, we used the chi-square test to compare qualitative variables and Student's t test (or Mann-Whitney U test) for quantitative variables. Bonferroni correction for multiple comparisons was applied to confirm whether the p values were significant. Mean DAS28 and HAQ values were analyzed using 1-way ANOVA and Kruskal–Wallis test, respectively. The significance level was set at $\alpha = 0.05$ (2-tailed) and all statistical analyses were performed with SPSS 13.0 (SPSS Inc., Chicago, IL, USA) and WINPEPI.

RESULTS

This study analyzed a possible genetic association between *MBL2* polymorphisms and susceptibility to RA as well as clinical features of this disease in Brazilian patients. Table 1 summarizes the clinical and laboratory features for patients.

The frequencies of *MBL2* gene polymorphisms were studied in patients with RA and healthy controls. All groups were in Hardy-Weinberg equilibrium. When alleles B, C, and D were considered together as allele O (since all different alleles result in the same phenotype, i.e., reduced MBL function), no differences were observed between the groups classified by ethnic origin and therefore we grouped all patients together (Table 2). Nevertheless, when we compared the allelic and genotypic frequencies between European-derived and African-derived patients, considering the *MBL2* A, B, C, and D variants isolated, significant differences were seen, with a high frequency of allele C among the African-derived patients. Garred, *et al*[4,5] showed the same high frequency of allele C in African-derived patients with RA. Therefore, this result reflects the differences of ethnic origin rather than individual differences due to RA.

Table 1. Demographic, clinical, and laboratory features of patients with rheumatoid arthritis (RA).

Features	n (%)	Mean (SD)
Female	259 (80.4)	
European-derived	300 (93.2)	
African-derived	22 (6.8)	
Age, yrs		60.5 (12.3)
Age at diagnosis, yrs		45.6 (13.6)
Mean DAS score		3.9 (1.3)
Mean HAQ score		1.25 (0.89)
Rheumatoid factor-positive	270 (83.9)	
Erosions	281 (87.3)	
Extraarticular manifestations	84 (26.1)	
Rheumatoid nodules	65 (20.7)	
Vasculitic	8 (2.5)	
Felty syndrome	1 (0.3)	
Amyloidosis	3 (1)	
Episcleritis	11 (3.5)	
Pneumonitis	3 (1)	
Pericarditis	0 (0)	
Subluxations	32 (10.2)	

DAS: Disease Activity Score; HAQ: Health Assessment Questionnaire.

We analyzed the *MBL2* variants in relation to clinical and laboratory features and considered disease severity measured by DAS28 and HAQ. No significant differences were observed when alleles were analyzed individually, suggesting that these polymorphisms are not related to the disease physiopathology. Considering homozygous OO individuals, female patients presented lower, although not statistically significant, mean DAS28 scores, genotype AA or AO = 4.11 ± 1.28 (n = 203), and genotype OO = 3.49 ± 1.16 (n = 11; p = 0.10); and mean HAQ scores, AA or AO genotype = 1.31 ± 0.76, and OO genotype = 0.86 ± 0.62 (p = 0.06), compared to controls.

DISCUSSION

Several studies have shown that *MBL2* gene polymorphisms can be associated with clinical and laboratory features in situations such as cardiovascular diseases, proinflammatory conditions, and autoimmune diseases such as systemic lupus erythematosus[3,6,7]. Specifically concerning RA, different clinical features were associated with polymorphic variants of the *MBL2* gene in different populations (Table 3). We analyzed the *MBL2* genotype in relation to extraarticular manifestations. Considering the alleles individually, no significant differences were observed. When considering *MBL2* variants together, however, an increased frequency of the OO genotype was observed in patients with rheumatoid nodules [AA or AO genotype = 0.96 (n = 240) and OO genotype = 0.04 (n = 10); p = 0.031], although this association lost significance after Bonferroni correction.

We observed a significant difference between European-derived and African-derived patients, considering the C allele, reflecting a higher prevalence of this allele among African-derived subjects. Moreover, a tendency was observed suggesting a possible association of homozygous OO individuals with rheumatoid nodules. These findings suggest an association of the *MBL2* gene and clinical manifestations in RA. However, more studies are needed to clarify the true role of *MBL2* in RA.

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Table 2. *MBL2* genotype and allelic frequencies in patients with RA and healthy controls.

	European-derived		African-derived		All	All
	Patients,	Controls,	Patients,	Controls,	Patients,	Controls,
	Genotype (considering only A and O alleles)					
	n = 300	n = 244	n = 22	n = 101	n = 322	n = 345
AA	160 (0.53)	148 (0.61)	11 (0.50)	59 (0.58)	171 (0.53)	207 (0.60)
AO	123 (0.41)	83 (0.34)	8 (0.36)	37 (0.37)	131 (0.41)	120 (0.35)
OO	17 (0.06)	13 (0.05)	3 (0.14)	5 (0.05)	20 (0.06)	18 (0.05)
p[a]	0.22		0.30		0.20	
Allele	n = 600	n = 488	n = 44	n = 202	n = 644	n = 690
A	443 (0.74)	379 (0.78)	30 (0.68)	155 (0.77)	473 (0.73)	534 (0.77)
B	112 (0.19)	79 (0.16)	5 (0.11)	13 (0.06)	117 (0.18)[b]	92 (0.13)[b]
C	13 (0.02)	14 (0.03)	9 (0.20)	29 (0.14)	22 (0.03)[c]	43 (0.06)[c]
D	32 (0.05)	16 (0.03)	0 (0.00)	5 (0.02)	32 (0.05)	21 (0.03)
p[a]	0.20		0.34		0.003	
Genotype (considering A, B, C, and D alleles)						
	n = 300	n = 244	n = 22	n = 101		
AA	160 (0.53)	148 (0.61)	11 (0.50)	59 (0.58)	171 (0.53)	207 (0.60)
AB	91 (0.30)	60 (0.25)	4 (0.18)	10 (0.10)	95 (0.30)[d]	70 (0.20)[d]
AC	8 (0.03)	9 (0.04)	4 (0.18)	22 (0.22)	12 (0.04)[e]	31 (0.09)[e]
AD	24 (0.08)	14 (0.06)	0 (0.00)	5 (0.05)	24 (0.07)	19 (0.05)
BB	7 (0.02)	6 (0.02)	0 (0.00)	1 (0.01)	7 (0.02)	7 (0.02)
BC	3 (0.01)	5 (0.02)	1 (0.05)	1 (0.01)	4 (0.01)	6 (0.02)
BD	4 (0.01)	2 (0.01)	0 (0.00)	0 (0.00)	4 (0.01)	2 (< 0.01)
CC	1 (0.00)	0 (0.00)	2 (0.09)	3 (0.03)	3 (< 0.01)	3 (< 0.01)
DD	2 (0.01)	0 (0.00)	0 (0.00)	0 (0.00)	2 (< 0.01)	0 (0.00)
p[a]	0.41		0.37		0.018	

Data in parentheses represent frequencies. [a] Chi-square test; [b] residual p = 0.015; [c] residual p = 0.017; [d] residual p = 0.006; [e] residual p = 0.006 (uncorrected values). RA: rheumatoid arthritis; MBL: mannose-binding lectin.

Table 3. Characteristics of studies on the *MBL2* gene and RA.

Population	Alleles/genotypes Studied	Clinical Features
Danish[7]	AA, AO, and OO	Joint destruction
White Danish[8]	AA, AO, and OO	Early erosive arthritis
Finnish[9]	AA, AO, and OO	Systemic amyloidosis
Danish[10,11]	AA, AO, and OO	Atherosclerosis and cardiovascular disease
Southern Chinese[12]	Allele B	Associated with RA
Japanese[13]	Allele B	Associated with autoimmune disorders
Dutch[14]	AA, AO, and OO	Not associated with RA
Indian Whites[15]	Allele B	Protection against RA

MBL: mannose-binding lectin; RA: rheumatoid arthritis.

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