

High polymorphism of the *MBL2* gene in patients with atopic dermatitis

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Background: Low serum levels of mannose-binding lectin (MBL) are determined mainly by variant alleles of the *MBL2* gene and it has been suggested that MBL may play a role in the susceptibility to atopic dermatitis (AD).

Objective: The aim was to investigate the difference of the frequency of *MBL2* variant alleles in AD patients and in a group of individuals without AD, and associate the *MBL2* alleles with AD severity.

Methods: *MBL2* variant allele's frequency was investigated in 131 children with AD and 165 healthy children/adolescents matched by convenience. The severity of disease was graded according to the SCORing Atopic Dermatitis (SCORAD) index. The first exon variants were called "O" and the wild type "A". The variants in the promoter were H/L at -550 and X/Y at -221, determined by Real Time PCR.

Results: Children with AD had higher frequency of allele O and the genotypes related to low or deficient levels of MBL, when compared to the healthy group ($p = 0.0012$ and $p < 0.001$, respectively), but not with AD severity.

Conclusion: Low or deficient MBL serum levels determined genetically may contribute to the predisposition for AD, but not for disease severity.

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INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory disease, and patients with AD show a higher predisposition for cutaneous infection with microbes, particularly *Staphylococcus aureus*, which is also associated with disease exacerbation.^{1,2} An important characteristic of AD is intense pruritus, which predictably leads to extensive scratching, disturbing the skin innate immunity response barrier.¹ There has been an increase in studies focusing on AD or atopy in general and genetic polymorphisms of the proteins relevant for the innate immunity, including the pattern-recognition receptor molecules, such as the mannose-binding lectin (MBL).^{1,3–5} MBL is a C-type lectin able to bind highly conserved structures within bacteria, fungi, and viruses, which activate the complement system via the lectin pathway and facilitate phagocytosis.⁶

Polymorphisms in the promoter and first exon regions of the *MBL* gene (*MBL2*) cause deficient or low levels of serum MBL, which is a common immunodeficiency associated with

impaired phagocytosis by polymorphonuclear leukocytes and with an increased susceptibility to infections, mainly in immunocompromised individuals.⁶ MBL is an important molecule in the clearance of apoptotic cells.⁶ The delay in the removal of apoptotic cells could result in the cellular disintegration and release of intracellular components, which may facilitate an immune response to intracellular constituents.⁷ Thus, it has been suggested that MBL deficiency could lead to accumulation of cellular debris, thereby predisposing patients to autoimmunity.^{6,8}

The allelic variations of *MBL2* were identified as 3 different point mutations in codons 52 (allele D), 54 (allele B), and 57 (allele C) of the first exon and in positions -550 (allele H/L) and -221 (allele X/Y) of the promoter region.^{6,8,9} The variant alleles at the first exon are collectively called allele O, and the wild-type allele is A.⁹ Individuals with homozygous OO or heterozygous AO genotypes may be classified as low or deficient producers, and those carrying the homozygous wild-type AA genotype are considered higher producers. Regarding the promoter, the allele X has the major influence on reduction of the serum levels of MBL.^{6,9}

The first evidence of MBL involvement in AD was suggested by a family study that revealed that children with low MBL serum levels who were homozygous for allele B had associated pruritic skin disease and possibly AD, with or without recurring infections.³ However, Japanese patients diagnosed as having AD did not have an association with allele B of the *MBL2*.⁴ Recently, a study found an association between the variant alleles of the promoter and exon 1 regions of *MBL2* in a group of Brazilian patients with AD⁵; however, disease severity was not investigated.

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Table 1. Allelic and Genotypic Frequencies of the *MBL2* First Exon in Healthy Individuals and Children With Atopic Dermatitis (AD)

	Children with AD (n = 131)	Healthy individuals (n = 165)
Alleles ^a		
A	0.687 (n = 180)	0.803 (n = 265)
O	0.313 (n = 82)	0.197 (n = 65)
Genotypes ^b		
AA	0.534 (n = 70)	0.667 (n = 110)
AO	0.305 (n = 40)	0.273 (n = 45)
OO	0.160 (n = 21)	0.061 (n = 10)

^a $P = .002$ for alleles A vs O (χ^2 test for AD patients vs healthy individuals).

^b $P = .01$ for genotypes AA vs AO vs OO (χ^2 test for AD patients vs healthy individuals).

MBL is particularly important in the early stages of infection, mainly, in the window of vulnerability while the child's immune system is immature.⁶ Moreover, the immunomodulatory role of MBL could be an important factor eliciting an adequate immune response to innocuous antigens, such as allergens or self-proteins.^{6,8}

Thus, the deficiency or low levels of MBL may contribute to susceptibility to the development of AD because MBL is an important molecule in the innate defense against bacteria and fungi, which represent the main class of microorganisms involved in AD.^{1,6} High frequency of the *MBL2* variant alleles in patients compared with healthy individuals could support the hypothesis that MBL is involved in AD susceptibility, thereby exacerbating the disease. Therefore, this article aimed to investigate the association of the frequency of the variant alleles of the first exon and promoter region of *MBL2* in children with AD compared with healthy individuals without AD and also to associate the polymorphism with the different degrees of disease severity.

METHODS

Patients

An exploratory study was conducted to compare the frequencies of the variant alleles of *MBL2* between 131 children with

Table 2. Disease Severity and *MBL2* First Exon Polymorphism Frequency in Children With Atopic Dermatitis (AD)

	Mild AD (n = 57)	Moderate or severe AD (n = 74)
Alleles ^a		
A	0.702 (n = 80)	0.676 (n = 100)
O	0.298 (n = 34)	0.324 (n = 48)
Genotypes ^b		
AA	0.561 (n = 32)	0.514 (n = 38)
AO	0.281 (n = 16)	0.324 (n = 24)
OO	0.158 (n = 9)	0.162 (n = 12)

^a $P = .75$ for A vs O (χ^2 test for mild vs moderate or severe AD).

^b $P = .84$ for AA vs AO vs OO (χ^2 test for mild vs moderate or severe AD).

Table 3. Frequency of Allele Variants of the First Exon and Promoter Regions of the *MBL2* in Healthy Individuals and Children With Atopic Dermatitis (AD)^a

<i>MBL2</i> expression	Children with AD (n = 131)	Healthy individuals (n = 165)
High ^b	0.48 (n = 64)	0.50 (n = 82)
Low ^c	0.26 (n = 33)	0.44 (n = 73)
Deficient ^d	0.26 (n = 34)	0.06 (n = 10)

^a $P < .001$ for AD vs healthy individuals (χ^2 test 3×2 analysis).

^b The high genotypes were HYA/HYA, HYA/LYA, HYA/LXA, LYA/LYA, and LYA/LXA.

^c The low genotypes were LXA/LXA, HYA/LYO, HYO/LYA, HYA/O, HXA/O, and LYA/O.

^d The deficient genotypes were LXA/O, HXA/O, HYO/LXA, and O/O.

AD and 165 healthy children or adolescents (no AD) from August 1, 2006 through July 31, 2008 and were referred for anamnesis and physical examination for the investigation of AD. To classify the extent and severity of signs and symptoms, the criteria of SCORing Atopic Dermatitis (SCORAD) were used. A score of less than 25 points was considered mild, 25 through 50 points was moderate, and more than 50 points was severe.¹⁰ After approval of the Ethics Committee for Research and the signing of the free informed consent form, a clinical evaluation of the child for disease severity was performed.

MBL2 Genotyping

The *MBL2* single-nucleotide polymorphisms -550, -221, and exon 1 were genotyped (Rotor Gene-3000 apparatus; Corbett Research, Sydney, Australia). The first exon polymorphism was performed with Sybr Green I chemistry (Applied Biosystems, Foster City, California) using a melting temperature assay with specific primers (forward 5'-GGCTTCCCAG-GCAAAGATG 3' and reverse 5'-AGCCCAACACGTAC-CTGGTT-3'). All 3 variant alleles of the first exon at *MBL2* were grouped as allele O, whereas the wild-type allele was called A. The identification of the curves was obtained by using the ABI 7900 HT program (Applied Biosystems). For H/L and X/Y alleles, 2 specific polymerase chain reactions (PCRs) were developed. For the H/L allele PCR, the reverse allele-specific primers 5'-TGCTTCCCCTTGGTGTITTTAC-3' and 5'-TGCTTCCCCTTGGTGTITTTAG-3' and the common forward primer 5'-GCCAGGGCCAACGTAGTAAG-3' were used. For the X/Y allele, 5'-CTGGAAGACTATAAACAT-GCTTTC-3' and 5'-CTGGAAGACTATAAACATGCTTTG-3'

Table 4. *MBL2* Gene Expression vs Atopic Dermatitis (AD) Severity^a

<i>MBL2</i> expression	Mild AD (n = 57)	Moderate or severe AD (n = 74)
High	0.49 (n = 28)	0.49 (n = 36)
Low	0.26 (n = 15)	0.24 (n = 18)
Deficient	0.25 (n = 14)	0.27 (n = 20)

^a $P = .94$ for *MBL2* expression vs AD severity (χ^2 test 3×2 analysis).

were used as the reverse allele-specific primers and 5'-CCGAAGAGGACATGGAGAGA-3' as the common forward primer.

Statistical Analysis

The linkage disequilibrium was estimated using downloadable Arlequin software, version 3.01 (<http://cmpg.unibe.ch/software/arlequin3/>; hosted by University of Geneva, Geneva, Switzerland). The differences of frequencies were analyzed by the χ^2 test for pairwise comparisons using the contingency tables of 2×2 or 3×2 , and the significance level was set at $P < .05$.

RESULTS

The age of the group with AD ranged from 1 through 12 years, with a mean age of 2 years; 39.7% were male; and 78.6% were younger than 6 years. The healthy comparison group was composed of 165 children or adolescents without AD (determined by clinical questionnaire); 48.5% were male, ranging in age from 4 through 18 years of age, with a mean age of 10 years. The first exon and promoter allelic variations were in linkage disequilibrium in the AD and comparison groups.

Table 1 indicates the frequencies of the first exon variants of *MBL2* in Brazilian children with AD and the healthy group without AD. Both the allele and genotypes of the first exon related to low MBL production were associated with the AD patient group. No association was found between the frequency of variants of the polymorphism of the first exon of the *MBL2* and severity of AD (Table 2). In Tables 3 and 4, the genotypes are compared, considering the variations of the promoter region H/L and X/Y combined with the first exon region and classifying them into 3 categories according to the producing serum MBL level: high, low, or deficient.⁹ Among the patients with AD, there was a predominance of variant alleles, which code for deficient production of the *MBL2*, compared with the healthy group ($P < .001$). However, AD severity was not associated with variant alleles of *MBL2*, either at the first exon or promoter region.

DISCUSSION

The increased predisposition of patients with AD to infection in part is a consequence of the mechanical trauma caused by itching, which disrupts the protection role of the extract corneum barrier, lowering lipid levels and consequently leading to abnormal keratinization.¹ Studies show that impaired expression of antimicrobial peptides of the innate immune system occurs in patients with AD, who have an increased risk to *Staphylococcus aureus* and *Candida* sp. infection.¹ These microbes are recognized by MBL, which elicits a protective immune response against them.^{6,11} Hence, the immunodeficiency caused by the *MBL2* polymorphism may contribute to development of the skin disease. However, the MBL recognition of glycan structures is important not only for controlling microbes or activation of adaptive immunity but also for immune cell homeostasis.^{6,12}

It has been demonstrated that UV irradiation can recruit MBL to irradiated skin, which in turn binds to apoptotic keratinocytes.¹² In the dermatomyositis model, MBL binds to keratinocytes, increasing the uptake of these cells by dendritic cells and allowing the noninflammatory clearance of apoptotic debris.^{12,13} This mechanism indicates that self-structures aberrantly produced by the host may lead to unnecessary inflammation and breakdown of homeostasis.^{1,6,8} Therefore, the deficiency of MBL in AD physiopathology may be relevant because significant apoptotic keratinocytes and T lymphocytes are present in patients with AD.^{2,14}

Our results show that a group of children with AD had high frequency of allele O and genotype AO and OO when compared with children and adolescents without AD. These results suggest that MBL deficiency may be a risk factor contributing to the development of AD. Two other studies have addressed the association of MBL polymorphism and AD. One study⁴ investigated the association of the frequency of allele B in Japanese patients with AD but did not reveal an association. In the other study of Brazilian patients,⁵ we showed that either structural or promoter polymorphisms were associated with AD, although we studied adults as the comparison group and did not investigate the severity of the disease. The application of the clinical questionnaire to adults may lead to imprecise information.

MBL may represent an important innate factor before the adaptive immune response with participation of antibodies and T cells and also an immunomodulatory molecule of inflammation in the absence of infection. The probable increased association with AD development in MBL-deficient children may involve other environmental factors, such as bacterial, fungi, or virus infections. A study with a larger number of patients, as well as a better characterization regarding the presence of infection, is needed to establish the definitive influence of the *MBL2* polymorphism in AD severity.

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