

LETTER TO THE EDITOR

PREVALENCE OF ATOPIC SYMPTOMS AMONG BLOOD DONOR CARRIERS OF
MANNOSE-BINDING LECTIN VARIANT ALLELES

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Mannose-binding lectin (MBL) is a C-type soluble collectin involved in the innate immune response. Carriers of MBL gene variant alleles (MBLva) have decreased plasma concentrations of MBL and increased susceptibility to bacterial and viral infections. The aim of the present study is to test the hypothesis that carriers of MBLva could have a different frequency of atopic symptoms as compared to wild-type carriers. A total of 385 consecutively enrolled Caucasian blood donors were studied. Blood specimens underwent genomic analysis and genotyping for MBLva by polymerase chain reaction (PCR). MBLva carrier status was associated with a reduced frequency of allergic rhinitis (OR 0.41 [95% CI 0.2 to 0.8], $\chi^2=6.98$, $p=.008$). No relationship was found between MBLva carrier status and asthma or atopic skin symptoms. MBLva might be one of the host-related genetic factors involved in atopic disorders, namely allergic rhinitis.

Mannose-binding lectin (MBL) is a C-type soluble collectin playing a key role in the innate immune response (1). It recognizes carbohydrate moieties on the surface of a variety of pathogens (including bacteria, viruses, and fungi), thereby activating the lectin pathway of complement and promoting phagocytosis (1). A single gene, MBL2, located on chromosome 10 at 10q11.2-q21, encodes for MBL. Three variant alleles, due to single-base pair substitutions at codon 54 (allele B), codon 57 (allele C), and codon 52 (allele D), have been identified in exon 1 of the MBL2 gene, causing low plasma

MBL levels (1). These three codon variants are very common, with estimated frequencies of 30% and 5% for heterozygosity and homozygosity (or compound heterozygosity), respectively, in most populations (1-3). Three regulatory mutations have also been identified in the MBL2 promoter and 5' untranslated regions (at position 550, -221, and +4), affecting the amount of MBL production (1). Carriers of MBL gene variant alleles (MBLva) have decreased plasma concentrations of MBL and increased susceptibility to bacterial and viral infections, mostly in the first two years of life (1, 4-5). An increased prevalence

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of wheezing in carriers of MBLva with Chlamydia pneumoniae infection has also been demonstrated (6). Exposure to some infectious agents or microbial products in association with other non-infective and lifestyle factors is believed to drive the process of immune deviation from a T_H2 to a T_H1 response throughout life (7).

The aim of the present study is to test the hypothesis that carriers of MBLva could have a different frequency of atopic symptoms as compared to wild-type carriers.

MATERIALS AND METHODS

A total of 425 consecutively enrolled Caucasian blood donors (aged 18 to 65 years, M/F ratio 2.2) were studied between April 1st and April 29th, 2006, at a University Hospital in Southern Italy. A standardized questionnaire was given to all recruited individuals. Items regarding a past or present physician-diagnosed asthma, allergic rhinitis or atopic eczema were taken from the European Community Respiratory Health Survey questionnaire (8). Further questions regarding allergic sensitisation and lung function measurement, when appropriate, were included. When doubtful, data concerning the results of any previous diagnostic work-up, were confirmed by telephone.

Of the 425 subjects recruited, 27 (6.3%) refused to fill out the questionnaire. They did not differ with regard to age, gender, level of education, occupational or smoking habits from the participating subjects studied. Soon after collection, blood samples were stored at -20°C until analysis. However, at the time of genotyping, 13 blood samples were found to be unsuitable for analysis (broken test tube, insufficient blood sample, etc.). Hence, 385 blood specimens underwent genomic analysis and were genotyped for MBL2 structural gene mutations. Informed written consent was obtained from all participants and the study was approved by the local Ethics Committee.

Genotyping for MBLva was performed by a polymerase chain reaction (PCR) to amplify a 119-bp region of exon 1 of the MBL2 gene, containing codons 52, 54 and 57. The upstream primer was a mutated oligonucleotide (5'-CATCAACGGCTTCCAGGCA AAGACGCG-3') which introduced a new restriction enzyme cut site in the presence of the codon 52 mutation, as described by Madsen et al. (9). The downstream primer was the same as that described by Lipscombe et al. (5'-AGGCAGTTTCCTCTGGAAGG-3') (3). PCR was performed in a reaction of 50 μl , containing 0.1 μg genomic DNA, 25 pmol of the specific primers and standard reaction buffer with 200 μM dNTPs, 1.5 mM MgCl_2 and 1 U Taq DNA Polymerase (MBI Fermentas

Inc., Burlington, Canada). PCR reactions were as follows: 95°C hold for 5'; 35 cycles of 94°C for 30", 60°C for 30" and 72°C for 30"; 72°C hold for 5'. PCR products were digested with BshN I, Mbo II and Mlu I (MBI Fermentas Inc., Burlington, Canada), followed by separation in a 4% agarose gel and ethidium-bromide staining.

An χ^2 -test for categorical variables was used for statistical analysis. All calculations were performed using SPSS 11.5 software (SPSS, Inc, Chicago, Ill). A p-value <0.05 was considered significant.

RESULTS

Overall, 179 (46.4%) out of 385 individuals were found to be carriers of MBLva, of which 8 (2.1%) were homozygous, 16 (4.1%) compound heterozygous, and 155 (40.2%) heterozygous for MBLva. Furthermore, 125 (32.4%) out of 385 subjects were carriers of substitutions at codon 54, 61 (15.8%) out of 385 at codon 52, 17 (4.4%) out of 385 at codon 57 (Table I).

Of the 385 blood donors, 46 (11.9%) reported suffering from current or past nasal allergies, including hay-fever. MBLva carrier status was associated with a reduced frequency of allergic rhinitis (OR 0.41 [95% CI 0.2 to 0.8], $\chi^2 = 6.98$, $p = .008$); further, 20 (5.1%) out of the 385 blood donors reported suffering from current or past asthma and 41 (10.6%) from atopic eczema or skin allergy. No relationship was found between MBLva carrier status and asthma (OR 2.22, [95% CI 0.7 to 6.4], $\chi^2 = 2.9$, $p = .08$) or atopic skin symptoms (OR 1.23 [95% CI 0.5 to 2.9], $\chi^2 = 0.4$, $p = .52$) (Table II).

DISCUSSION

Our data suggest that the MBL2 gene, or a gene close to it, may be one of the host-related genetic factors involved in some atopic disorders, namely allergic rhinitis. One possible explanation might be that individuals carrying one or both MBLva develop a prominent T_H1 polarization due to an increased burden of respiratory or gastrointestinal infections in early childhood or later (4-5). However, other possible explanations may also exist. Kaur et al. have shown that patients with allergic bronchopulmonary aspergillosis or bronchial asthma with allergic rhinitis have higher plasma MBL levels as compared with non-allergic control subjects (10).

Table I. Proportion (No., %) of individual carriers of MBLva* and type of mutations detected in the population studied (No. 385 subjects).

Homozigous	Compound heterozygous	Heterozygous	Codon 54 MBLva	Codon 52 MBLva	Codon 17 MBLva
Total 179			Total 203		
8/385 (2.1%)	16/385 (4.1%)	155/385 (40.2%)	125/385 (32.4%)	61/385 (15.8%)	17/385 (4.4%)

*MBLva = MBL2 gene variant alleles

Table II. Prevalence of MBLva* (at least one mutated allele) among subjects reporting atopic symptoms.

	Total	MBLva*	MBLwt**	OR (95% CI)	<i>p</i>
Asthma	20/385	13/20	7/20	2.22 (0.7-6.4)	0.08
Allergic rhinitis	46/385	13/46	33/46	0.41 (0.2-0.8)	0.008
Eczema / skin allergy	41/385	21/41	20/41	1.23 (0.5-2.9)	0.52

* MBLva=MBL2 gene variant alleles

**MBLwt = MBL2 gene wild-type

They also found a significant correlation of MBL levels and complement activity with peripheral blood eosinophils in both groups of patients. The authors hypothesized that high MBL levels might contribute to the recruitment of eosinophils in airways through the lectin-pathway of the complement cascade and the release of chemotactic factors, such as C3a and C5a. Moreover, recently Staley et al. found higher MBL levels in children with moderate wheezing receiving ICSs compared with children affected by mild wheeze receiving no treatment and healthy children (11). They hypothesized that MBL might play a modulatory role in the progression of asthma and thus be a marker for a distinctive asthma phenotype

(11). We found that a carrier status of MBLva, known to be related to decreased MBL levels in blood, is also associated with a lower prevalence of allergic rhinitis but not of bronchial asthma. This latter finding is intriguing. In our opinion, the lack of association between the MBLva carrier status and asthma might be due to a counterbalancing effect in patients with mild disease from the increased susceptibility to some wheezing-related pathogens, such as *Chlamydia pneumoniae* (6).

However, because our study was carried out on blood donors, our findings need to be confirmed in larger and unselected populations of patients with asthma and allergic rhinitis.

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