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Mannose-binding lectin 2 gene polymorphisms and predisposition to allergic bronchial asthma in a western Romanian children population: an observational study

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Abstract

Objectives: To analyse: (1) the associations between different mannose-binding lectin 2 (*MBL2*) genotypes and susceptibility to bronchial asthma (BA) in Romanian children; and (2) the correlations between several patient sociodemographic variables and *MBL2* polymorphisms.

Methods: This prospective observational case–control study included paediatric patients with symptomatic BA and healthy controls. Participants were genotyped for two *MBL2* single-nucleotide polymorphisms (SNPs): exon 1 codon 54 A/B variant rs1800450, and -550 promoter H/L variant rs11003125 (GenBank accession). Associations between *MBL2* genotypes and

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susceptibility to BA were determined by calculated odds ratios, and Kendall Tau's correlations were used to investigate the associations between sociodemographic variables and SNPs.
Results: Among 59 patients with BA and 65 healthy controls, associations between *MBL2* polymorphisms and susceptibility to BA were not found to be statistically significant. Statistically significant weak positive correlations were found between age at diagnosis and A/B genotype, and between the smoking status of biologically male and female parents. A statistically significant weak inverse association was found between male parent smoking status and family history of BA.
Conclusion: These results may help guide future research into paediatric BA in Romania and Eastern Europe. Due to study limitations, the results require validation in future large-scale studies.

Keywords
Bronchial asthma, children, MBL2, polymorphism, rs11003125, rs1800450

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Introduction

Paediatric bronchial asthma (BA) is one of the top 10 most common chronic airway conditions of mid-childhood (ages 5–14 years), with increasing incidence in urban areas and a wide-ranging burden on families, society, and healthcare systems.[1] BA is a highly heterogeneous disease, characterized by genetically- and environmentally-induced bronchial hyperreactivity and inflammation. The various phenotypes of BA are habitually categorized as allergic and non-allergic, depending on the sensitization level to allergens and the existence of atopy, an immunoglobulin (Ig)E-mediated genetic tendency of developing striking immune responses against common allergens.[2–4]

Parts of the complement system from the innate immunity (the first line of host defence, with exceptional importance in the first years of life), are suggested to play crucial mediator roles in the pathogenesis of BA.[5] In particular, mannose-binding lectin (MBL), a protein secreted by the liver, serves as the first component of the complement system in the lectin pathway, being an acute phase reactant. Functionally, MBL is able to recognize and coat the polysaccharide structures on the surface of allergens and microorganisms, and activate the complement pathway by mediating opsonophagocytosis, granting protection against invasion by agents of exogenous origin.[6] In humans, MBL is encoded by a single functional gene (*MBL2*) located on chromosome 10q11.2-q21. MBL serum concentration is largely influenced by several *MBL2* gene polymorphisms (six of them being known for their functional effect). Common polymorphisms associated with MBL functional deficiency, which produce significantly lower levels of circulating MBL protein, include the single nucleotide polymorphism (SNP) rs1800450 (transition G > A, also known as 'B' allele, located on exon 1 region, codon 54, resulting in Gly54Asp substitution) and rs11003125 (located on promoter -550 region, transition C > G, also known as 'HL' variant, where L is the wild type allele).[7,8]

The *MBL2* SNPs are frequent and distributed in an uneven fashion across human

populations, e.g., rs1800450 A/B has a frequency of 0.14 in Caucasians, 0.25 in Asians, and is very rare in Africans, suggesting a somewhat evolutionary-induced biological advantage to certain populations.[9] However, data on the incidence of different *MBL2* genotypes in Eastern European patients with BA (Romania included) are relatively scarce. Of note, the Romanian Society of Pulmonology has estimated that in the past decade, the incidence of paediatric BA from urban areas has risen to 6.4% in children aged 14 years.[10]

Data on the associations between sociodemographic characteristics of patients with BA and *MBL2* gene polymorphisms are also currently lacking in Eastern European regions. Parental smoking has been shown to have a major impact on childhood asthma development, and thus, understanding the correlation between parental smoking and *MBL2* variants is of great importance, particularly in the Balkan region of Europe, where smoking rates are amongst the highest worldwide.[11,12] Taken together, these knowledge gaps impede the development of effective prevention programs and the implementation of personalized medicine approaches for the management and treatment of BA in primary care.

In this context, the present study aimed to evaluate the associations between *MBL2* gene polymorphisms and the predisposition to allergic BA onset, in a paediatric (aged under 18 years) western Romanian population, and to analyse potential correlations between demographic characteristics (including parental smoking) and *MBL2* SNPs to reveal novel avenues in the field of paediatric asthma prevention.

Patients and methods

Study population

This prospective observational case–control study was conducted based on the STROBE statement guidelines.[13] Patients diagnosed with BA (aged <18 years), who attended a paediatric consultation at the Clinical Paediatric Unit of Emergency County Clinical Hospital (Arad, Western Romania) for respiratory symptoms and wheezing, plus age-, biological sex-, and ethnicity-matched healthy controls who attended the same clinic, were randomly enrolled upon admission to the clinic between September 2019 and January 2020, without any prearranged sequence or plan.

Patients were diagnosed with atopy by cutaneous prick-tests and plasma IgE quantification, determined by an electrochemiluminescence immunoassay method. Patients with BA were additionally diagnosed with asthma by performing ventilatory probes for pulmonary function (spirometric assessment).

Inclusion criteria for BA were: the existence of a forced expiratory volume variability in spirometric assessment, the presence of respiratory symptoms (i.e., wheezing, shortness of breath), and existing treatment with bronchodilators, inhaled corticosteroids, and antihistamine medication.

Data regarding age, biological sex, family history of BA and smoking status of both parents were retrieved from the medical files by the physician and self-reported by the patients (potential bias). All participant blood samples were deidentified prior to genomic DNA extraction.

This research was approved by the Ethics Committee of the participating institutions ('Vasile Goldis' Western University of Arad, Romania; [approval No. 2/19.07.2018] and the Emergency County Hospital of Arad, Romania [approval No. 128/07.12.2018]), and was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments. Written informed consent was provided by the parents/legal proxies of the included study participants for the use of the

biological specimen and for completing a sociodemographic questionnaire.

Sample processing and genomic DNA extraction

Whole blood was collected by venipuncture into ethylenediaminetetra-acetic acid-treated blood collection tubes and immediately centrifuged (15 min, 2000 ***g***). DNA was then extracted from 100 µl of the buffy coat containing white blood cells using a QIAmp DNA Mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions (using the spin column protocol). Genomic DNA was eluted in a total volume of 100 µl.

MBL2 genotyping

Participant samples were genotyped to discriminate *MBL2* alleles (-550 promoter and exon 1 codon 54 SNPs, GenBank accession: rs11003125 and rs1800450, respectively) using TaqMan® Custom SNP Genotyping Assays (Applied Biosystems, Waltham, MA, USA) and the compatible TaqMan® Genotyping Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), with previously published primer and probe sequences.[14] The wet DNA delivery method was used with a total reaction mix volume of 2.75 µl, according to the manufacturer's protocol, including the manufacturer-recommended thermal cycling conditions. Quantitative polymerase chain reaction (qPCR) was performed with a 7900HT Fast Real-Time PCR thermal cycler compatible with the kits (Applied Biosystems). The amplification plot was generated using SDS software, version 2.4, that was connected to the PCR instrument, according to the manufacturer's instructions. Genotyping experiments were performed in the Department of Biochemistry and Pharmacology, 'Victor Babes' University of Medicine and Pharmacy Timisoara, Romania.

Statistical analyses

Data are presented as *n* (%) prevalence or median (lower, upper quartile). The inter-group homogeneity of age between children with BA and healthy controls was assessed with Mann–Whitney *U*-test. To exclude the possibility of systematic genotyping errors and other biases (e.g., segmental duplication), the Hardy–Weinberg equilibrium for allele and genotype frequencies was verified using χ^2-tests. Odds ratios (ORs) were calculated with a 95% confidence interval (CI) to determine the association between SNP genotype and the risk of developing BA. The homozygous allele with the highest frequency among healthy controls was used as a reference group.

For patients with BA, the association between age at diagnosis, biological sex, family history of asthma, smoking status of the biologically female and male parent, respectively, and genotype (rs11003125 and rs1800450 polymorphisms) were investigated using Kendall's Tau rank correlation coefficient. Binary variables (stratified into two categories) are recommended for this type of correlation calculation. Thus, patients were stratified into those aged <5 years and those aged ≥ 5 years; the rest of the variables being implicitly dichotomous: biological sex (male versus female); family history of asthma and smoking status of biologically female or male parent (Yes versus No); and genotype of the marker SNPs rs11003125 and rs1800450 (homozygous versus heterozygous). All statistical analyses were conducted using Statistica software, version 7 (StatSoft Inc, Tulsa, OK, USA), except for the OR calculations, which were performed using SciStat online statistics software (https://www.medcalc.net/statisticaltests/odds_ratio.php). In all

cases, a P value <0.05 was considered statistically significant.

Results

A total of 59 patients with BA and 65 healthy controls were included in the study. Sociodemographic data are summarized for 58 patients with BA (due to incomplete data) and 65 controls (Table 1). Among 58 patients with BA, the majority were aged over 5 years (49 [84.48%]), with slightly more female (31 [53.45%]) than male patients. A quarter of patients with BA presented a family history of BA (16 [27.59%]), and proportionally more male than female parents were smokers (23 [39.66%] versus 16 [27.59%], respectively). Median (lower quartile, upper quartile) age in patients with BA and healthy controls was 8 (5, 11) years and 7 (4, 13) years, respectively. Mann–Whitney U-test for the homogeneity of age in patients with BA and healthy controls revealed a Z value of –0.39 ($P = 0.609$).

Table 1. Sociodemographic characteristics amongst paediatric patients with bronchial asthma (BA) and age-, sex-, and ethnicity-matched healthy controls.

Characteristic	Patients with BA ($n = 58$)	Healthy controls ($n = 65$)
Age		
<5 years	9 (15.52)	22 (33.84)
≥5 years	49 (84.48)	43 (66.16)
Biological sex		
Male	27 (46.55)	32 (49.23)
Female	31 (53.45)	33 (50.77)
Family history of BA		
Yes	16 (27.59)	0 (0.00)
No	42 (72.41)	65 (100.00)
Female parent smoking status		
Yes	16 (27.59)	0 (0.00)
No	42 (72.41)	65 (100.00)
Male parent smoking status		
Yes	23 (39.66)	0 (0.00)
No	35 (60.34)	65 (100.00)

Data presented as n (%) prevalence.

Frequencies of promoter -550 H/L (rs11003125) polymorphism were 32 (54.24%) and 27 (45.76%) for homozygous (GG and CC together) and heterozygous (GC), respectively, among 59 patients with BA, and 29 (44.62%) and 36 (55.38%), respectively, among 65 healthy controls. Regarding exon 1 codon 54 A/B (rs1800450) polymorphism, genotype frequencies were 50 (84.75%) and 9 (15.25%) for homozygous (GG and AA together) and heterozygous (GA), respectively, among 59 patients with BA, and 56 (86.15%) and 9 (13.85%), respectively, among 65 healthy controls (Table 2). No statistically significant differences were found in allele or genotype frequencies between patients with BA and healthy controls, suggesting no significant association between the *MBL2* polymorphisms and BA (Table 2).

Frequencies of the *MBL2* rs11003125 polymorphism were found to be consistent with Hardy–Weinberg equilibrium in all participants (healthy controls: $\chi^2 = 0.73$, $P = 0.39$; patients with BA: $\chi^2 = 0.40$, $P = 0.53$). Similar results were found for rs1800450 (healthy controls: $\chi^2 = 1.54$, $P = 0.22$; patients with BA: $\chi^2 = 0.41$, $P = 0.52$).

Analysis of the associations between various sociodemographic factors and *MBL2* polymorphisms using Kendall's Tau correlation coefficient revealed that most of the analysed associations were not statistically significant ($P > 0.05$; Table 3). However, statistically significant weak positive associations were identified between age at diagnosis and the rs1800450 polymorphism, and between smoking status of the female parent and smoking status of the male parent. In addition, a statistically significant moderate inverse association was identified between smoking status of the male parent and family history of BA.

Table 2. Mannose-binding lectin 2 (*MBL2*) allele and genotype frequencies for rs11003125 and rs1800450 among paediatric patients with bronchial asthma (BA) and healthy controls.

Allele and genotype	Patients with BA ($n = 59$)	Healthy controls ($n = 65$)	Statistical significance	OR (95% CI)
Promoter -550 'H/L' variant				
Allele				
H (G allele)	61 (52)	78 (60)	Ref	Ref
L (C allele)	57 (48)	52 (40)	$P = 0.189$	1.41 (0.85, 2.32)
Genotype				
H/H (GG)	17	21	Ref	Ref
H/L (GC)	27	36	$P = 0.854$	0.93 (0.41, 2.09)
L/L (CC)	15	8	$P = 0.124$	2.32 (0.79, 6.75)
H/L + L/L (GC + CC)	42	44	$P = 0.674$	1.18 (0.55, 2.54)
Exon 1 codon 54 'A/B' variant				
Allele				
A (G allele)	109 (92.37)	119 (91.53)	Ref	Ref
B (A allele)	9 (7.62)	11 (8.46)	$P = 0.809$	1.11 (0.44, 2.80)
Genotype				
A/A (GG)	50	55	Ref	Ref
A/B (GA)	9	9	$P = 0.851$	1.10 (0.40, 2.99)
B/B (AA)	0	1	$P = 0.468$	3.29 (0.13, 82.78)
A/B + B/B (GA + AA)	9	10	$P = 0.687$	1.22 (0.45, 3.25)

Data presented as *n* (%) prevalence.
OR, odds ratio; CI, confidence interval; Ref, the homozygous allele with the highest frequency among healthy patients.

Table 3. Analysis of the correlation between mannose-binding lectin 2 (*MBL2*) rs11003125 and rs1800450 polymorphisms, age at diagnosis, biological sex, family history of bronchial asthma (BA) and smoking status of both parents in paediatric patients with BA.

Variable	rs11003125	rs1800450	Age at diagnosis	Biological sex	BA family history	Smoking status (female parent)
rs11003125						
rs1800450	–0.14					
Age at diagnosis	–0.03	0.19*				
Biological sex	0.07	0.05	–0.09			
BA family history	–0.04	0.13	0.11	–0.06		
Smoking status (female parent)	0.08	–0.09	–0.09	0.04	–0.01	
Smoking status (male parent)	–0.01	–0.08	–0.09	0.00	–0.24*	0.17*

*Statistically significant correlation ($P < 0.05$; Kendall's Tau correlation coefficient).

Discussion

To the best of our knowledge, this is the first investigation in Romania of the association between two *MBL2* gene polymorphisms (promoter -550 H/L rs11003125 and exon 1 codon 54 A/B rs1800450) and the susceptibility of developing allergic BA in a population encompassing 59 children with diagnosed BA and

65 age-, sex-, and ethnicity-matched controls. For the promoter H/L variant, the heterozygous (H/L or GC) genotype was the most frequent in patients and controls, and for the exon 1 codon 54 A/B variant, the homozygous (A/A or GG) genotype was the most frequent. The ORs indicated no statistically significant association between *MBL2* polymorphisms and predisposition to BA development, irrespective of the analysed SNPs.

An investigation of the association between six *MBL2* polymorphisms and the susceptibility to respiratory tract infections in a population of 111 young male patients with BA (military recruits) and 362 healthy controls revealed a significant association for the promoter -221 Y/Y genotype (rs7096206) and a marginal-significant association for exon 1 variant alleles following BA status adjustments.[14] In addition, a Chinese observational study investigating the -550, -221 promoter and codon-54 *MBL2* SNPs in 317 children aged 5–18 years with allergic BA and 140 healthy controls found no significant association between *MBL2* polymorphisms and asthma, atopy, sensitization to individual aeroallergens, or spirometric variables.[15] The same conclusion (no significant association) was found by a large German observational study, investigating codons 52, 54, 57, and promoter c.1-290 *MBL2* polymorphisms in 749 children with BA (aged up to 11 years).[16] Furthermore, a meta-analysis of nine studies (with 2066 patients with BA and 2183 healthy volunteers in total), performed due to inconsistent results across individual studies, showed that codon 54 A/B, -550 and -221 promoters (H/L and X/Y, respectively) were not significantly associated with a predisposition to develop BA in Asians, not even in ethnicity subgroup analysis.[17]

The aforementioned studies suggest that *MBL2* polymorphism-induced MBL deficiency does not constitute a predisposing element for BA in children. Nonetheless, inconsistencies across reports may arise due to technical and methodological differences regarding study designs, cohort sizes, method of *MBL2* genotyping, and choice of statistical analysis. Currently, there are no established validated guidelines for conducting a robust *MBL2* genotyping analysis, and research study designs often include in-house methodologies.

Furthermore, the etiopathogenesis differs between atopic (allergic) and non-atopic BA, with the latter being more severe, and appearing to be related to bacterial infections, e.g., with *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.[18] The present study focused on atopic BA and found no significant associations between the susceptibility to this form of BA and the SNPs analysed. These findings might provide empirical evidence on the lack of association between *MBL2* genotype and atopy risk in the population studied.

In the present study, statistically significant positive correlations were revealed between age at diagnosis and exon 1 codon 54 A/B rs1800450 and between the smoking status of the female parent and smoking status of the male parent, while BA family history was inversely correlated with the smoking status of the male parent. The present results may reveal hypothetical evidence for earlier BA onset in patients with homozygous genotypes. Interestingly, the most significant correlation found in the present study was the inverse association between paternal smoking and BA family history, but no relationship between maternal smoking and BA family history. These findings may indicate that, at least for the population investigated, paternal smoking might be more associated with BA family history than maternal smoking. Nevertheless, data on smoking and BA family history, as well as other variables from the present study were self-reported and vaguely described, therefore potential

bias is likely to have occurred. There was no clear smoking definition and timeline, nor were there details regarding BA family history, or other parental behavioural factors. A similar previously published analysis showed that the risk of BA was significantly elevated if both parents smoked, but was higher in only paternal-smoking families, relative to only maternal-smoking families.[19] By contrast, a study that investigated the relationship between childhood BA, atopy, and smoking in 8585 Tasmanian children, found that a history of asthma in 7-year-olds was associated with maternal, but not paternal, smoking.[20] In addition, a meta-analysis of asthma prevalence from 14 case–control studies indicated that the risk of developing BA appears to be primarily driven by maternal smoking, but the risk was even higher when both parents were smokers.[21] Thus, from the overall data, it is becoming increasingly clear that raising awareness of parental smoking cessation should be firmly implemented, particularly in countries where smoking rates are alarmingly high, including the Balkan region of East Europe.[12]

In this regard, the present small-scale observational study represents a research stepping-stone into BA prevention in this geographical area, and should be viewed in the larger context of BA risk-factor discoveries, since it encompasses several limitations. First, the sample size was relatively small, which considerably decreased the statistical power and may have contributed to the lack of significant results. Secondly, the study lacked relevant information on environmental risk factors for allergic BA, such as pollution, diet, and socio-economic markers, and detailed data on family background (i.e., history of allergic BA and smoking). Thirdly, the choice of correlation analyses with dichotomous categorical variables might have yielded poor significant associations between different variables related to children with BA. Lastly, the study was observational, thus definite conclusions cannot be drawn. Future studies with increased statistical power that include more continuous variables and larger sample sizes might produce different and more relevant results for the selected genotypes.

Conclusion

The present study revealed, for the first time in Romania, that there were no significant associations between *MBL2* polymorphisms and susceptibility to paediatric BA development. The data may represent an important tool for guiding future research studies on BA in Eastern European countries, and may also open novel avenues for BA prevention in children.

Author Contributions

Study conceptualization, SB, MP and CM; methodology, SB and IDM; software, IDM and DN; validation, SB, MP and CM; formal analysis, IDM and DVN; investigation, SB, AC and RP; data curation, SB, AC and RP; original draft manuscript preparation, SB, DN, DVN and IDM; manuscript review and editing, SB, DN, IDM and CM; visualization, SB, CM and DVN.; supervision, CM; project administration, SB. All authors have read and agreed to the final version of the manuscript.

Data accessibility

All data is published in the present manuscript. Additional information regarding the study population is available from the corresponding author upon request.

Declaration of conflicting interest

The Authors declare that there is no conflict of interest.

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