Association of the wild-type A/A genotype of MBL2 codon 54 with asthma in a North Indian population

Niti Birbian\textsuperscript{a}, Jagtar Singh\textsuperscript{a,∗}, Surinder Kumar Jindal\textsuperscript{b}, Amit Joshi\textsuperscript{c}, Navneet Batra\textsuperscript{d} and Neha Singla\textsuperscript{a}

\textsuperscript{a}Department of Biotechnology, Panjab University, Chandigarh, India
\textsuperscript{b}Department of Pulmonary Medicine, PGIMER, Chandigarh, India
\textsuperscript{c}Department of Biotechnology, S.G.G.S. College, Chandigarh, India
\textsuperscript{d}Department of Biotechnology, G.G.D.S.D. College, Chandigarh, India

Abstract. Background: High serum MBL level as well as polymorphisms in the mannose-binding lectin 2 (MBL2) gene resulting in MBL deficiency are involved in the mechanism of a number of non-infectious diseases such as asthma, conferring either risk or protection in different population studies. MBL being the first reactant of the MBL pathway is also a major determinant of the fate of the anaphylatoxins such as C3a and C5a, which are also pro-inflammatory mediators. The MBL2 gene polymorphisms thus control the serum levels of MBL as well as C3a and C5a.

Objective: This is the first case-control study conducted in India, investigating the role of MBL2 codon 54 A/B polymorphism in asthma pathogenesis.

Methods: A case-control study was performed with a total of 992 adult subjects, including 410 adult asthmatics and 582 healthy controls from regions of North India. The MBL2 codon 54 A/B polymorphism was genotyped by PCR-RFLP.

Results: Statistical analysis for the codon 54 polymorphism revealed that the wild (A) allele was significantly associated with asthma with \( OR = 1.9, 95\% \ CI (1.4–2.4), \) and \( p<0.001. \)

Conclusion: The MBL2 codon 54 A/B polymorphism is significantly associated with asthma and its phenotypic traits as the wild (A/A) genotype confers a significant risk towards the disease in the studied North Indian population.

Keywords: Asthma, mannose-binding lectin 2 (MBL2), anaphylatoxins, C3a, C5a, MBL2 codon 54 A/B polymorphism, North Indian population

1. Introduction

Asthma is a complex genetic inflammatory disorder of the lungs, characterized by acute bronchial hyper responsiveness (BHR), shortness of breath (SOB), chest tightness, cough and sputum production in response to a variety of external stimuli [36], with or without atopy [26].

Mannose-binding lectin (MBL), an active component of the innate immune system, is a Ca-dependent serum protein synthesized by the hepatocytes in the liver [6]. MBL is a “pattern recognition molecule” encoded by the MBL2 gene located at 10q11.2-q21 [17,23] and is a trimer of three identical polypeptide chains, each possessing a cysteine rich region, a collagen like region, a neck and a ‘carbohydrate recognition domain’ (CRD), which enables the lectin protein to recognize the mannose and N-acetyl glucosamine moieties on the surface of a variety of pathogens including viruses, bacteria, fungi as well as protozoans [21].

While the MBL may be implicated in the microorganism destruction by the formation of a ‘membrane attack complex’ (MAC), it more importantly displays an opsonin effect by tagging the pathogen surface for recognition and ingestion by phagocytes via the
mucous diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, Type-I diabetes, Crohn’s disease, cystic fibrosis, sepsis, lung injury, myocardial ischemia-reperfusion as well as asthma [8,16,20,27,28,35]. Increased MBL levels have also been associated with infectious diseases such as tuberculosis (TB) and leprosy [2,29], and it has been suggested that low MBL levels confer protection against TB [12].

Moreover, it has been observed that post allergen challenge, the anaphylatoxin C3a, which is otherwise produced as a byproduct of the MBL pathway, is present in elevated levels in the broncholavolar lavage (BAL) fluid of asthma patients [1,10,25,38] as well as in the plasma [38], highlighting the role of C3a in MBL pathway. In two separate studies conducted in the USA, it has been observed that the deletion of C3a receptor in murine models of asthma protects against lung damage observed during allergen challenge during asthma attack, thereby signifying the role of C3a in asthma [1,33]. Another study has suggested that the complement system not only plays an important role in innate immunity, but if uncontrolled, contributes to amplified inflammation [19].

The present study hypothesizes that the functional MBL2 codon 54 wild A allele results in high serum MBL level that leads to a greater availability of MBL for participation in the lectin pathway and hence result in an enhanced complement activation which cause asthma. On the other hand, the MBL2 exon 1 codon 54 A/B polymorphism results in low MBL production, resulting in a lesser participation in the MBL pathway by lowering the production of the first metabolite of the cascade itself, thereby resulting in a reduced production of anaphylatoxins such as C3a and C5a, which are otherwise mediators of inflammation and asthma.

2. Methods

Ethical Clearance for conducting the study on human blood samples was granted by the “Ethics Committee, PGIMER, Chandigarh”. The study was conducted strictly in accordance with the ethical guidelines for bio-medical research on human subjects proposed by the “Central Ethics Committee on Human Research (CECHR) ICMR–2000” and of those contained in the “Declaration of Helsinki”. The selection of asthma patients was based on physician’s diagnosis. However, only the patients fulfilling the criteria of GINA (Global Initiative for Asthma) guidelines for diagnosis of bronchial asthma were recruited in the study.
This is the first case-control study conducted in India evaluating the role of MBL2 exon1 codon 54 A/B polymorphism in asthma pathogenesis by recruiting a total of 992 adult subjects. The patients were recruited from different states of North India such as Punjab, Haryana, Chandigarh, Uttar Pradesh, Himachal Pradesh, Uttarakhand, Jammu and Kashmir, Rajasthan and New Delhi. A total of 410 asthma patients visiting the Out Patient Department (OPD), Pulmonary Medicine, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, were enrolled in the study on the basis of physicians’ diagnosis and spirometry test results. Out of the total, 323 subjects were asthma patients with allergic rhinitis. No ABPA (Allergic Bronchopulmonary Aspergillosis) patients were taken in the study. Informed Consent was duly obtained in written from the asthma patients participating in the study, and a detailed proforma of the asthma patients with a complete questionnaire regarding the clinical symptoms of the disease, i.e. wheeze/wilhersing, cough, shortness of breath (SOB), allergy, early morning or night symptoms, along with spirometry tests, etc., was assessed. Complete information of the patient regarding name, age, sex, history of the disease, occupation, etc., was taken into account (Table 1). Asthma patients with history of any other pulmonary ailment such as tuberculosis, Chronic Obstructive Pulmonary Disease (COPD), bronchitis, etc., were excluded from the study.

A total of 582 normal, healthy controls, with no history of asthma or allergic diseases or any other co-morbid illness were inducted in the study. Some of the healthy volunteers were blood donors at various blood donation camps, educational institutes, employee groups. Care was taken that the control subjects did not have any of the patient conditions in the past. Any subject having a first degree relative with asthma or allergy has not been recruited as a control in the present study. Not only the respiratory or allergic skin disorders, any subject with other diseases such as diabetes, high blood pressure, etc., or with drinking and smoking habits have also not been included as controls in the study. Each control was first enquired for all of the above conditions at the time of taking their written informed consent and before the collection of blood samples.

Blood samples were collected in EDTA coated vials, and stored at −80°C until genomic DNA extraction was done. Genomic DNA was isolated from the thawed blood samples by the Sodium Saline Citrate Buffer Method, and checked for DNA on 0.8% agarose gel by electrophoresis.

| Table 1 | Characteristics of the study population |
|---------|----------------------------------------|
|          | Asthma patients | Controls |
| Sex      | 410 (%)         | 582 (%)  |
| Males    | 183 (44.6)      | 351 (60.3) |
| Females  | 227 (55.4)      | 231 (39.7) |
| Age      |                |           |
|          | 38.1 ± 16.2     | 41.9 ± 16.6 |
| Allergic rhinitis | 323 (78.8) | 0 |
| No rhinitis | 87 (21.2)  | 582 |
| Allergic to at least |          |           |
| 2 provoking factors | 366 (89.3) | 0 |
| Non-allergic | 44 (10.7) | 582 |
| Non-Smoker | 345 (84.1) | 582 |
| Ever-Smoker | 65 (15.9) | 0 |
| Spirometry data* | (n = 190) |          |
| FVC observed | 2.56 ± 0.96 | 0 |
| FVC predicted | 3.19 ± 0.73 | 0 |
| FEV1 observed | 1.94 ± 0.82 | 0 |
| FEV1 Predicted | 2.68 ± 0.77 | 0 |
| FEV1/FVC observed | 75.00 ± 13.71 | 0 |
| FEV1/FVC predicted | 83.12 ± 5.84 | 0 |

FVC, Forced Vital Capacity; FEV1, Forced Expiratory Volume in 1 second.

* Spirometry test was conducted for 190 asthma patients.

The amplification of the MBL2 codon 54 was done with forward 5’—AGTGACCAAGATTGTAGGACA GAG–3’ and reverse 5’—AGGATCCAGGCAGTTTCC TCTGGAAGG–3’ primers [14]. PCR was carried out in a thermal cycler, in a total volume of 25 µl containing: 10X PCR Buffer, 3 mM MgCl2, 1 mg/ml nuclease free BSA, 50 pmol of each primer, 10 mM of each dNTP, 0.125 U Taq polymerase and 2 µl genomic DNA. The PCR conditions were: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 60°C for 1 min, 72°C for 1 min, and final extension step at 72°C for 10 min. A 349-bp PCR product was observed as a parent band by electrophoresis on 2% agarose gels stained with ethidium bromide and visualized by UV transillumination. Alleles A and B were detected using 5U Ban I restriction digestion of the 349-bp fragment. Ban I cleaves the wild allele A into two fragments of 260 bp and 89 bp while leaving the mutant allele B uncut as 349 bp, while heterozygous A/B genotype is observed as 349, 260 and 89 bp bands in a single lane (Fig. 1).

The European Molecular Genetics Quality Network (EMQN) good practice guidelines have been followed. A few PCR vials with all the PCR contents except the DNA, were also included per PCR batch as “negative controls”. No contamination was observed and there were no “false positives”. To minimize the risk of contamination, sterilized and autoclaved solutions and equipment were used during DNA isolation. The ingredients for PCR were well stored at −20°C and were
Table 2

| Genotype Frequencies | Asthma patients (410) | Controls (582) | OR (95% CI) | p-value |
|----------------------|-----------------------|----------------|-------------|---------|
| Genotype             | 410 (%)               | 582 (%)        |             |         |
| B/B                  | 5 (1.2)               | 25 (4.3)       | Ref (1.0)   |         |
| A/A                  | 308 (75.1)            | 353 (60.7)     | 2.0 (1.5–2.6) | 0.0001 |
| A/B                  | 97 (23.7)             | 204 (35.1)     | 0.6 (0.4–0.8) | 0.0001 |
| A/A+A/B              | 405 (98.8)            | 557 (95.8)     | 3.6 (1.3–10.9) | 0.0053 |

| Allele Frequencies   | 410 (%)               | 582 (%)        |             |         |
|----------------------|-----------------------|----------------|-------------|---------|
| B                    | 107 (13.0)            | 254 (21.8)     | Ref (1.0)   |         |
| A                    | 713 (87.0)            | 910 (78.2)     | 1.9 (1.4–2.4) | 0.0001 |

A/A, Homozygous Wild; A/B, Heterozygous; B/B, Homozygous Mutant; OR, Odds Ratio.

Fig. 1. PCR-RFLP products of MBL2 codon 54 A/B polymorphism on 2% Agarose gel. Lanes 1, 2, 3, 4, 5, 8, 9, 10, 12, 13, 15, 16: Homozygous wild A/A genotype (260 and 89 bp); Lanes 6, 11, 14: Heterozygous A/B genotype (349, 260 and 89 bp); Lane 7: Homozygous mutant B/B genotype (349 bp); Lane 17: 100 bp Ladder.

The genotypic as well as the allelic distribution of the MBL2 codon 54 A/B polymorphism, between the asthmatics and control subjects, were analyzed statistically using Fisher’s exact test. The data was analyzed with SPSS 17.0 software and Epi Info version 3.4.3. Statistical significance was assumed for \( p < 0.05 \).

3. Results

In the present study, a total of 992 subjects, including 410 adult asthma patients and 582 adult healthy controls were genotyped for the MBL2 codon 54 A/B polymorphism. Very interesting results were observed for the polymorphism in the current study.

Statistical analysis of the results indicated that the mutant B allele was more prevalent among the controls (21.8%) than in asthma patients (13.0%) while the overall genotypic distribution of the wild allele (A) was higher among asthma patients (87.0%) as compared to the controls (78.2%), conferring a significant risk towards asthma as there was a positive association between asthma and the A allele with \( OR = 1.9, 95\% CI (1.4–2.4)\) and \( p < 0.001 \) (Table 2).

The genotypic frequencies revealed that the homzygous mutant genotype (B/B) was more prevalent in control subjects (4.3%) than in asthma patients (1.2%). The heterozygous genotype (A/B) was more prevalent among the controls (35.1%) as compared to the asthma patients (23.7%) with \( OR = 0.6, 95\% CI (0.4–0.8)\) and \( p < 0.001 \). However, it was observed that the homozygous wild genotype (A/A) was more prevalent in asthma patients (75.1%) than in the controls (60.7%) with \( OR = 2.0, 95\% CI (1.5–2.6)\) and \( p < 0.001 \). Furthermore, it was observed that asthma patients with homozygous wild genotype A/A or at least one copy of the wild allele (A/A+A/B), were predisposed to the disease with \( OR = 3.6, 95\% CI (1.3–10.9)\) and \( p = 0.005 \) (Table 2).
Table 3

Phenotypic characteristics of the study population and MBL2 codon 54 A/B polymorphism

| Phenotypic traits            | n    | A/A | A/B | B/B | A  | B  | OR (95% CI) | p-value |
|------------------------------|------|-----|-----|-----|----|----|-------------|---------|
| **Controls**                 |      |     |     |     |    |    |             |         |
| Males                        | 351  | 201 | 134 | 16  | 536| 166| Ref (1.00)  |         |
| Females                      | 231  | 152 | 70  | 9   | 374| 88 | Ref (1.00)  |         |
| **Asthmatics**               |      |     |     |     | A  | B  |             |         |
| Sex                          |      |     |     |     |    |    |             |         |
| Males                        | 183  | 135 | 46  | 2   | 316| 50 | 2.1 (1.4–3.2) | 0.001   |
| Females                      | 227  | 173 | 51  | 3   | 397| 57 | 1.7 (1.1–2.6) | 0.018   |
| **Occurrence**               |      |     |     |     |    |    |             |         |
| Seasonal                     | 282  | 216 | 64  | 2   | 316| 50 | Ref (1.00)  |         |
| Throughout                    | 128  | 92  | 33  | 3   | 217| 39 | 0.8 (0.5–1.3) | 0.367   |
| **Severity**                 |      |     |     |     |    |    |             |         |
| Wheeze on exertion           | 216  | 155 | 58  | 3   | 368| 64 | Ref (1.00)  |         |
| Wheeze at rest               | 194  | 153 | 39  | 2   | 345| 43 | 1.5 (0.9–2.4) | 0.121   |
| **Family History**           |      |     |     |     |    |    |             |         |
| Family History (Nil)         | 285  | 219 | 62  | 4   | 500| 70 | Ref (1.00)  |         |
| Family History (+ve)         | 125  | 89  | 35  | 1   | 213| 37 | 0.8 (0.5–1.2) | 0.274   |
| **Smoking Status**           |      |     |     |     |    |    |             |         |
| Non Smoker                   | 345  | 253 | 88  | 4   | 594| 96 | Ref (1.00)  |         |
| Ever Smoker                  | 65   | 55  | 9   | 1   | 119| 11 | 2.0 (0.9–4.4) | 0.076   |
| **Cough**                    |      |     |     |     |    |    |             |         |
| Cough (Nil)                  | 74   | 57  | 16  | 1   | 130| 18 | Ref (1.00)  |         |
| Cough (+ve)                  | 336  | 251 | 81  | 4   | 583| 89 | 0.9 (0.5–1.7) | 0.786   |
| **Sputum Production**        |      |     |     |     |    |    |             |         |
| Sputum (Nil)                 | 95   | 69  | 26  | 0   | 164| 26 | Ref (1.00)  |         |
| Sputum (+ve)                 | 315  | 239 | 71  | 5   | 549| 81 | 1.2 (0.7–2.1) | 0.613   |
| **Pattern of Daily Symptoms**|      |     |     |     |    |    |             |         |
| Morning/Night SOB            | 312  | 229 | 79  | 4   | 537| 87 | Ref (1.00)  |         |
| Anytime SOB                  | 98   | 79  | 18  | 1   | 176| 20 | 1.5 (0.8–2.8) | 0.191   |
| **Rhinitis**                 |      |     |     |     |    |    |             |         |
| Rhinitis (Nil)               | 87   | 62  | 22  | 3   | 146| 28 | Ref (1.00)  |         |
| Males                        | 36   | 27  | 8   | 1   | 62 | 10 | Ref (1.00)  |         |
| Females                      | 51   | 35  | 14  | 2   | 84 | 18 | Ref (1.00)  |         |
| Rhinitis (+ve)               | 323  | 246 | 75  | 2   | 567| 79 | 1.3 (0.7–2.3) | 0.424   |
| Males                        | 147  | 108 | 38  | 1   | 254| 40 | 0.9 (0.4–2.3) | 0.980   |
| Females                      | 176  | 138 | 37  | 1   | 313| 39 | 1.7 (0.8–3.5) | 0.208   |
| **Allergy**                  |      |     |     |     |    |    |             |         |
| Allergy (Nil)                | 44   | 30  | 14  | 0   | 74 | 14 | Ref (1.00)  |         |
| Males                        | 19   | 15  | 4   | 0   | 34 | 4  | Ref (1.00)  |         |
| Females                      | 25   | 15  | 10  | 0   | 40 | 10 | Ref (1.00)  |         |
| Allergic (+ve)               | 366  | 278 | 83  | 5   | 639| 93 | 1.5 (0.7–3.0) | 0.345   |
| Males                        | 164  | 120 | 42  | 2   | 282| 46 | 0.7 (0.2–2.5) | 0.408   |
| Females                      | 202  | 158 | 41  | 3   | 357| 47 | 2.4 (0.9–6.2) | 0.076   |

A/A, Homozygous Wild; A/B, Heterozygous; B/B, Homozygous Mutant; OR, Odds Ratio; SOB, Shortness of Breath.

However, categorizing the asthma patients on the basis of the phenotypic characteristics of the disease (Table 3), as obtained from their detailed proforma, no significant association was observed between the MBL2 codon 54 A/B polymorphism and asthma phenotypes (all p > 0.05).

4. Discussion

The present study is the first one to investigate the role of MBL2 codon 54 A/B polymorphism in asthma propensity in a North Indian population and this research has revealed a highly protective effect of the polymorphism.

The results obtained from the current study supported the above hypothesis with the observations that both the allelic as well as the genotypic frequencies revealed a major role of the functional MBL2 gene in conferring risk towards asthma. In the overall scenario, the wild A allele was more prevalent in the asthma patients (87.0%) than in the controls (78.2%) in contrast to the mutant B allele which was more prevalent among the control subjects (21.8%) than in the asthma patients.
has been observed that the deletion of C3a receptor in murine models of asthma protects against lung damage observed during allergen challenge during asthma attack, thereby signifying the role of C3a in asthma [1, 33]. Another study has suggested that the complement system not only plays an important role in the innate immunity, but if uncontrolled, contributes to amplified inflammation [19].

Thus, the findings of the present study reveal a protective role of the MBL exon 1 codon 54 A/B polymorphism in asthma by offering checkpoints at the MBL as well as the anaphylatoxin production steps in the complement pathway and highlight the major role of the MBL codon 54 gene with the functional wild A allele in asthma pathogenesis and suggest that the enhanced serum MBL levels may predispose an individual to asthma by resulting in an increase in the production of pro-inflammatory anaphylatoxins such as C3a and C5a during the lectin complement pathway.

The role of MBL2 gene polymorphisms in asthma is a much debated scenario. However, the reason for the contrasting results of the studies on MBL2 gene polymorphisms association with asthma obtained in different populations globally can be attributed to the differences in the ethnicities as well as to the complex interplay of the multiple genes and environmental factors involved in the mechanism of asthma pathogenesis.

Acknowledgements

N. Birbian is highly grateful to Dr. Jagdeep Kaur, Chairperson, Department of Biotechnology, Panjab University, Chandigarh, for her guidance and kind support during the study. The study was supported by research grant from DST, New Delhi (SR/FT/LS-018/2008).

References

[1] A.A. Humble, B. Lu, C.A. Nilsson, C. Lilly, E. Israel, Y. Fujiwara, N.P. Gerard and C. Gerard, A role for the C3a anaphylatoxin receptor in the effector phase of asthma, Nature 406 (2000), 998–1001.
[2] A. Bonar, M. Chmiela and B. Rozalska, Level of mannose-binding lectin (MBL) in patients with tuberculosis, Pneumonology Alergology Polska 72 (2004), 201–205.
[3] A. Nagy, G.T. Koza, M. Keszeli, A. Tesz, A. Falus and C. Szalai, The development of asthma in children infected with Chlamydia pneumonia is dependent on the modifying effect of mannose-binding lectin, Journal of Allergy and Clinical Immunology 112 (2003), 729–734.
[4] A. Uguz, Z. Berber, M.Coskun, S. Halide Akbas and O. Yegin, Mannose-binding lectin levels in children with asthma, *Pediatric Allergy and Immunology* 16 (2005), 231–235.

[5] C.M. Hogaboam, K. Takahashi, R.A. Ezekowitz, S.L. Kunkel and J.M. Schulh, Mannose-binding lectin deficiency alters the development of fungal asthma: effects on airway response, inflammation, and cytokine profile, *Journal of Leukocyte Biology* 75 (2004), 805–814.

[6] D.C. Kilpatrick, Mannan-binding lectin: clinical significance and applications, *Biochimica et biophysica acta* 1572 (2002), 401–413.

[7] D.L. Jack, N.J. Klein and M.W. Turner, Mannose-binding lectin: targeting the microbial world for complement attack and opsonophagocytosis, *Immunological Reviews* 180 (2001), 86–99.

[8] E. Zimmermann-Nielsen, H. Gronbaek, J.F. Dahlerrup, G. Baarup and O. Thorlacius-Ussing, Complement activation capacity in plasma before and during high-dose prednisolone treatment and tapering in exacerbations of Crohn’s disease and ulcerative colitis, *BMC Gastroenterology* 5 (2005), 31.

[9] F. Larsen, H.O. Madsen, R.B. Sim, C. Koch and P. Garred, Disease-associated mutations in human mannose-binding lectin compromise oligomerization and activity of the final protein, *Journal of Biological Chemistry* 279 (2004), 21302–21311.

[10] F.F. Castro, M. Schmitz-Schumann, U. Rother and M. Kirchink, Complement activation by house dust: reduced reactivity of serum complement in patients with bronchiial asthma, *International Archives of Allergy and Applied Immunology* 96 (1991), 305–310.

[11] G. Sunan Latha, V. Vijayalakshmi, B. Anuradha, V. Hari Sai Priya, S. Kaur, M. Vaid, T. Madan, P.U. Sarma and K.J.R. Murthy, Screening of MBL and SP-D Genes in Indian Population for SNPs and Their Association with Atopic Asthma, *Murthy*, Screening of MBL and SP-D Genes in Indian Population for SNPs and Their Association with Atopic Asthma, *Indian Journal of Allergy and Immunology* 2 (2003), 305–322.

[12] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[13] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[14] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[15] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[16] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[17] M.W. Turner and R.M. Hamvas, Mannose-binding lectin: structure, function, genetics and disease associations, *Reviews in Immunogenetics* 2 (2000), 305–322.

[18] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[19] N. Krug, T. Tschernig, V.J. Erpenbeck, J.M. Hofhfield and J. Kohl, Complement factors C3a and C5a are increased in bronchial lavage fluid after segmental allergen provocation in subjects with asthma, *American Journal of Respiratory and Critical Care Medicine* 164 (2001), 1841–1843.

[20] N. Novak and T. Bieber, Allergic and nonallergic forms of atopic diseases, *Journal of Allergy and Clinical Immunology* 112 (2003), 252–262.

[21] N.P. Gerard and C. Gerard, Complement in allergy and asthma, *Current Opinion in Immunology* 14 (2002), 705–708.

[22] P. Garred, H.O. Madsen, H. Marquart, T.M. Hansen, S.F. Sorensen, J. Petersen, B. Volck, A. Svejgaard, N.A. Graudal, P.M. Rudd, R.A. Dwek, R.B. Sim and V. Andersson, Two- edged role of mannose binding lectin in rheumatoid arthritis: a cross sectional study, *Journal of Rheumatology* 51 (2000), 26–34.

[23] P. Garred, M. Harboe, T. Oettinger, C. Koch and A. Svejgaard, Dual role of mannose-binding protein in infections: another case of heterosis? *European Journal of Immunogenetics* 21 (1994), 125–131.

[24] R.F. Guo and P.A. Ward, Role of C5a in in *Annual Review of Immunology* 3 (2005), 821–852.

[25] R.J. Lipscombe, M. Svejgaard, A.V. Hill, Y.L. Lau, R.J. Levin sky, J.A. Summerfield and M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[26] S. Kaur, V.K. Gupta, A. Shah, S. Thiel, P.U. Sarma and T. Madan, Elevated levels of mannanbinding lectin (MBL) and cosinophila in patients of bronchial asthma with allergic rhinitis and asthma, *Indian Journal of Allergy and Immunology* 2 (2003), 305–310.

[27] J. Aittoniemi, H. Soranummi, A.T. Rovio, M. Hurme, T. Pessi, M. Nieminen and J. Karjalainen, Mannose-binding lectin 2 (MBL2) gene polymorphism in asthma and atopy among adults, *Clinical and Experimental Immunology* 142 (2005), 120–124.

[28] J.E. Jordan, M.C. Montalto and G. Stahl, Inhibition of man nosebinding lectin reduces postischemic myocardial reperfu sion injury, *Circulation* 104 (2001), 1413–1418.

[29] K. Sastry, G.A. Herman, L. Day, E. Deignan, G. Bruns, C.C. Morton and R.A. Ezekowitz, The human mannose-binding protein gene. Exon structure reveals its evolutionary relationship to a human pulmonary surfactant gene and localization to chromosome 10, *Journal of Experimental Medicine* 170 (1989), 1175–1789.

[30] L. Sompayrac, How the immune system works, Blackwell Science: Malden, 1999, pp. 17-19.

[31] M. Abe, Complement activation and inflammation, *Rinsho Byori* 54 (2006), 744–756.

[32] M.A. Seelen, A. Roos and M.R. Daha, Role of complement in innate and autoimmune, *Journal of Nephrology* 18 (2005), 642–653.

[33] M. Matsushita and T. Fujita, Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease, *Journal of Experimental Medicine* 176 (1992), 1497–1502.

[34] M. Sumiya, M. Super, P. Tabona, R.J. Levi nsky, T. Arai, M.W. Turner and J.A. Summerfield, Molecular basis of opsonic defect in immunodeficient children, *Lancet* 337 (1991), 1569–1570.

[35] M.W. Turner and R.M. Hamvas, Mannose-binding lectin: structure, function, genetics and disease associations, *Reviews in Immunogenetics* 2 (2000), 305–322.

[36] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[37] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[38] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[39] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[40] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[41] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[42] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[43] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.

[44] M.W. Turner, High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene, *Human Molecular Genetics* 1 (1992), 709–715.
[35] T.K. Hansen, L. Tarnow, S. Thiel, R. Steffensen, C.D. Stehouwer, C.G. Schalkwijk, H.H. Parving and A. Flyvbjerg, Association between mannose-binding lectin and vascular complications in type 1 diabetes, *Diabetes* **53** (2004), 1570–1576.

[36] W. Cookson, The alliance of genes and environment in asthma and allergy, *Nature* **402** (1999), 5–11.

[37] X. Wang, J. Saito, Y. Tanino, T. Ishida, T. Fujitawa and M. Munakata, Mannose binding lectin gene polymorphisms and asthma, *Clinical and Experimental Allergy* **37** (2007), 1334–1339.

[38] Y. Nakano, S. Morita, A. Kawamoto, T. Suda, K. Chida and H. Nakamura, Elevated complement C3a in plasma from patients with severe acute asthma, *Journal of Allergy and Clinical Immunology* **112** (2003), 525–530.