

Marker/Control/Samples loaded:

Lane 1:	Blank
Lane 2:	Marker
Lane 3:	Gold Tequila Tr 100 μ g
Lane 4:	Gold Tequila Tr 50 μ g
Lane 5:	Gold Tequila Tr 25 μ g
Lane 6:	Gold Tequila Tr 12.5 μ g
Lane 7:	Marker
Lane 8:	Gold Tequila UnTr 100 μ g
Lane 9:	Gold Tequila UnTr 50 μ g
Lane 10:	Gold Tequila UnTr 25 μ g
Lane 11:	Gold Tequila UnTr 12.5 μ g
Lane 12:	Marker

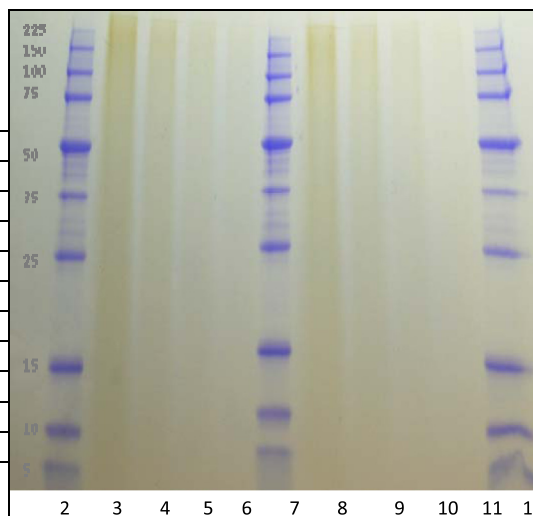


Figure 1. Gold tequila test polyacrylamide gel electrophoresis: Gold tequila was loaded in serial dilutions and run at 125 volts for 2 hours, then the gel was stained with Coomassie blue and observed for bands. Lanes 3–6 are dialyzed and lyophilized gold tequila treated with ammonium sulfate to attempt a protein precipitation. Lanes 8–11 are untreated gold tequila that was dialyzed and lyophilized. Unfortunately, no bands were observed.

This patient experienced recurrent and progressive allergic reactions to gold tequila ingestion. Her skin testing to a lyophilized extract was positive and provoked anaphylaxis with objective signs in clinic. Our conclusion is that this was likely an immunoglobulin E–mediated reaction to gold tequila. An anaphylactoid reaction would not have resulted in a positive skin test. There are several documented cases of anaphylaxis to beer and wine.^{2–5} However, an exhaustive literature review did not reveal any previously reported cases of anaphylaxis to distilled liquor. The most interesting aspect of this case is that this patient's reactions were confined to gold tequila. Because this patient also has oak pollenosis, we propose that during the aging process in oak barrels, perhaps a reaction takes place while imparting the tequila color and mellowing of flavor that generated reactivity in this patient. The blanco tequila does not go through this barrel aging process. Because of the patient's recurrent anaphylaxis to gold tequila, it was recommended that the patient continue to avoid gold tequila and any distilled liquors aged in oak barrels.

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Brett D. Coons, MD
Kevin White, MD

Wilford Hall Ambulatory Surgical Center
San Antonio, Texas
brett.coons@us.af.mil

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Mannose-binding lectin 2 gene polymorphisms affect serum mannose-binding lectin levels in adult asthmatics

Asthma is a chronic inflammatory disease of the airway characterized by airway hyperresponsiveness, tissue remodeling, and infiltration of immune cells. Although the pathophysiological mechanisms of airway inflammation are not fully understood, significant roles have been described for the complement activation byproducts C3a and C5a anaphylatoxins. It was suggested that generation of C3/C3a contributes to the development of common allergic pathways that enhance airway hyperresponsiveness, eosinophil infiltration, and Th2 cytokine production.¹ Conversely, ablation of the C5a and its receptor signaling results in the loss of dendritic cell modulation, which induces dysregulation of Th2-biased immune responses.¹

Mannose-binding lectin (MBL) is a C-type lectin that initiates the lectin complement pathway by binding to MBL-associated serine proteases 1 and 2. The *MBL2* gene is located on chromosome 10q11.2–q21, and genetic polymorphisms in the promoter and exon 1 regions of the *MBL2* gene were identified in adults with asthma in different ethnic groups.^{2–5} In particular, the C allele at –221 G>C is associated with decreased lung function.³ Moreover, polymorphisms in the *MBL2* gene could affect serum MBL levels in various populations.^{2,6} We investigated the genetic association of *MBL2* gene polymorphisms in Korean adults with asthma.

In this study, 313 patients with asthma and 168 controls were recruited consecutively from Ajou University Hospital, Suwon, South Korea. Diagnosis of asthma was conducted in accordance with the guidelines of the International Global Initiative for Asthma (GINA).⁷ All patients were in well-controlled states based on GINA guidelines.⁷ Using questionnaire data, controls were chosen from healthy individuals with no history of respiratory symptoms and

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normal findings on simple chest radiograms and spirometry. Clinical parameters such as sex and smoking history of the control group were well matched with the asthma group, but their mean age was younger. Informed consent was obtained from all subjects, and the Institutional Review Board of Ajou University Hospital approved the study. Atopy was defined as 1 or more positive reactions on a skin prick test using 55 common aeroallergens (Bencard Co., West Sussex, United Kingdom). Serum total immunoglobulin E (IgE) was measured using the UniCAP system (Phadia, Valinge, Sweden). The methacholine challenge test was performed in patients with asthma, and the concentration of inhaled methacholine that causes a 20% decrease in forced expiratory volume in 1 second (PC₂₀) was measured. Human serum MBL levels were measured using a DuoSet ELISA Development kit (R&D systems, Minneapolis, Minnesota).

For single-nucleotide polymorphism (SNP) identification, genomic DNAs from 40 healthy Korean volunteers were amplified by polymerase chain reaction and sequenced directly using the forward primer 5'-GCTAGGCTGCTGAGGTTTCTT-3' and the reverse primer 5'-GGGCTGGCAAGACAATTAG-3'. We selected 4 SNPs of the *MBL2* gene, 3 in the promoter (–554 G>C, rs11003125; –431 A>C, rs11003124; and –225 G>C, rs7096206) and 1 in the exon 1 (226 G>A, rs1800450). Samples were genotyped for the 4 SNPs by allelic discrimination TaqMan assays using TaqMan probes (Applied Biosystems, Foster City, California). The primer information for *MBL2* SNP genotyping is summarized in Table 1.

The genotype frequency was examined using the χ^2 test, and differences in genotype and haplotype frequencies were examined using logistic regression analysis with codominant, dominant, and recessive models after accounting for age and sex as covariables. A multiple comparison test was applied to compare serum MBL

Table 1
Primers and probes for SNP genotyping

Gene	Locus (SNP ID)	Primer (5' → 3')
<i>MBL2</i>	–554 G>C (rs11003125)	Forward
		Reverse
		VIC probe
		FAM probe
	–431 A>C (rs11003124)	Forward
		Reverse
		VIC probe
		FAM probe
	–225 G>C (rs7096206)	Forward
		Reverse
		VIC probe
		FAM probe
	226 G>A (rs1800450)	Forward
		Reverse
		VIC probe
		FAM probe

Abbreviations: MBL2, mannose-binding lectin 2; SNP, single-nucleotide polymorphism.

level according to 2 genotypes, –554 G>C and 226 G>A. Clinical phenotypes according to genotype and haplotype were compared using the χ^2 test for categorical variables and the independent *t*-test for continuous variables. The haplotype block pattern was constructed using Haploview ver. 2.4 software. *P* values of less than or equal to .05 were considered to indicate statistical significance.

The prevalence of atopy and serum total IgE level were significantly higher in patients with asthma than in controls (*P* < .001, respectively). The genotype and haplotype frequencies of the *MBL2* gene polymorphisms were not significantly different between the

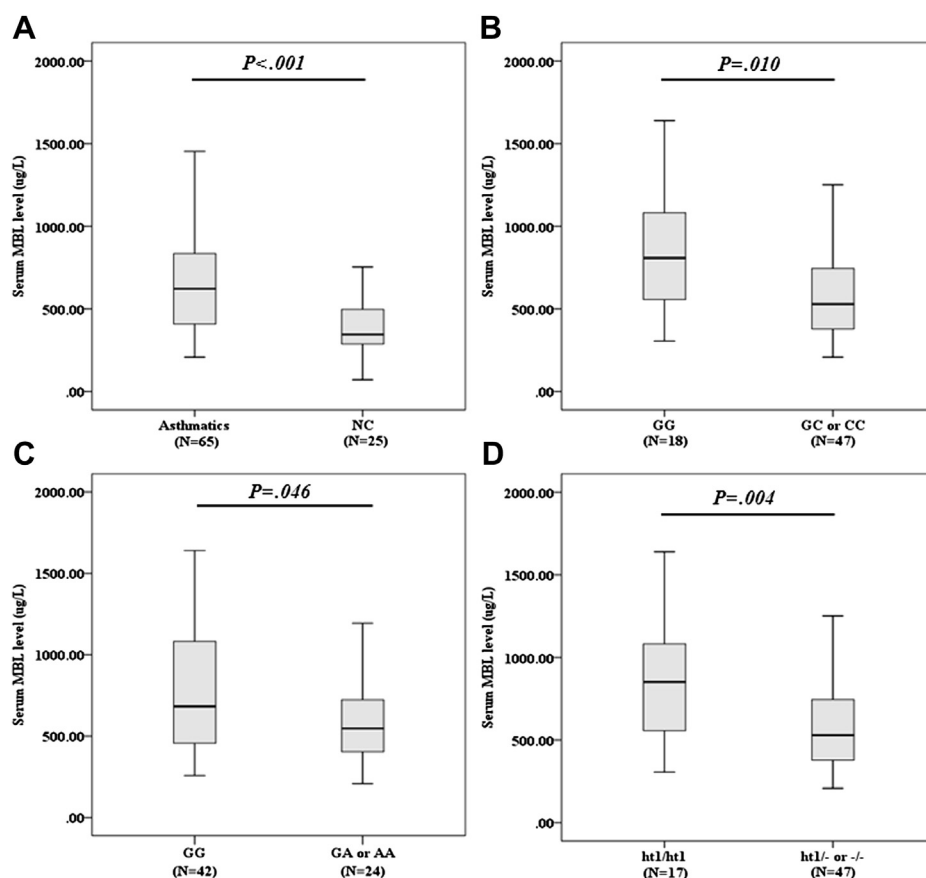


Figure 1. Serum MBL level between patients with asthma and normal controls (A) and according to genotypes of the *MBL2* gene, –554 G>C (B), 226 G>A (C), and haplotype 1 [GAGG] (D).

asthma and control groups. Furthermore, no significant associations were found between the 4 SNPs and asthma-related clinical parameters, including atopy, serum total IgE, the level of PC₂₀ methacholine, and forced expiratory volume in 1 second. No significant association was seen in the genotype frequencies between severe and nonsevere asthma groups, where the American Thoracic Society definition of severe asthma was applied.⁸ Serum MBL level was significantly higher in the asthma group than in the control group (Fig 1; 686.73 ± 339.16 vs 376.63 ± 169.1 $\mu\text{g/L}$; $P < .001$). Patients with asthma carrying the GG genotype at $-554 \text{ G} > \text{C}$ or the GG genotype at $226 \text{ G} > \text{A}$ of *MBL2* showed significantly higher serum MBL levels compared with those with other genotypes (GG vs GC/CC, 854.33 ± 365.88 $\mu\text{g/L}$ vs 606.23 ± 287.51 $\mu\text{g/L}$, $P = .010$; GG vs GA/AA, 750.16 ± 376.00 $\mu\text{g/L}$ vs 575.73 ± 229.94 $\mu\text{g/L}$, $P = .046$; respectively). Moreover, the patients with asthma having haplotype 1 [GAGG] of the *MBL2* gene showed a significantly higher serum MBL level compared with those with other haplotypes (870.51 ± 370.45 $\mu\text{g/L}$ vs 606.23 ± 287.51 $\mu\text{g/L}$, $P = .004$). However, the serum MBL level was not significantly different between the severe and non-severe asthma groups.

Mannose-binding lectin, a key molecule in innate immunity, acts as an acute phase protein, but it also involves in chronic airway inflammation.^{9,10} Previous studies suggested that a lower serum MBL level may represent an immune deficiency state by predisposing a patient to severe infections, including upper respiratory infections,² whereas study in pediatric patients with asthma demonstrated that an increased serum MBL level is correlated with blood eosinophil count.¹⁰ In the present study, nevertheless, no significant associations were found among the 4 SNPs of *MBL2* and the phenotypes of adult asthma in a Korean cohort; the serum MBL level was significantly higher in the adult asthma group. Although the mechanism of increased MBL production in patients with asthma is unclear, chronic airway inflammation in patients with asthma may stimulate MBL production by hepatocytes, which induces complement activation and potentiates Th2-derived airway inflammation. These findings may differ according to *MBL2* genotype.

In conclusion, adult patients with asthma have higher serum MBL levels. Two genetic polymorphisms at *MBL* $-554 \text{ G} > \text{C}$ and $226 \text{ G} > \text{A}$, could affect serum MBL levels.

Su-Jin Bae, BS

Seung-Hyun Kim, PhD

Purevsuren Losol, MD

Eun-Mi Yang, MS

Hae-Sim Park, MD, PhD

Department of Allergy and Clinical Immunology

Ajou University School of Medicine

Suwon, South Korea

hspark@ajou.ac.kr

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Rapid desensitization to doxycycline

A 48-year-old woman had a well-documented history of an anaphylactic reaction to doxycycline prescribed for the treatment of a febrile illness associated with headache, myalgia, facial pain, and cervical lymphadenopathy. She presented to the Emergency Department 15 minutes after taking her first dose, with generalized urticaria, facial angioedema, throat constriction, hoarseness, shortness of breath, and expiratory wheeze. The reaction was promptly reversed with intramuscular administration of epinephrine and inhalation of high-flow oxygen and nebulized salbutamol. She was able to tolerate doxycycline previously. Her prolonged febrile illness was eventually diagnosed as Q fever, and 4 months after her anaphylaxis and failure to respond to an empirical trial of other antimicrobial agents a request for desensitization was made by the treating physician. However, no such protocol was available in the literature.

There is no standardized skin testing protocol or specific immunoglobulin E assay for doxycycline. An oral challenge was deemed an excessive risk. Although serum tryptase levels were not measured, her presentation to the Emergency Department with objective signs of anaphylaxis and no other agents likely to have caused the reaction was compelling enough evidence that the patient had anaphylaxis to doxycycline. An oral desensitization

regimen was not feasible, because the oral formulations of doxycycline were not sufficiently soluble to prepare very low concentrations of the drug. Therefore, a protocol was formulated using an intravenous preparation (Table 1) with the administration of slow intravenous pushes, and the patient was desensitized in the intensive care unit, which allowed for continuous monitoring and supervision. For simplicity in our unit, we preferred to use 4 different escalating concentrations of the intravenous preparation to administer as bolus injections, but other methods such as the 12-step, 3-bag desensitization protocol from the Brigham and Women's Hospital Rapid Drug Desensitization Program also could have been a feasible option.¹

The intravenous component of the rapid desensitization protocol was successful and occurred without an adverse event, and a cumulative dose of 98.888 mg was attained. This dose approximated half of the daily dose prescribed for the patient. The remainder of the daily dose was administered in oral form to ensure that the patient was able to tolerate the formulation that she was prescribed for the treatment of her infection. The patient was subsequently able to tolerate several weeks of doxycycline 100 mg twice per day orally.

Anaphylaxis to tetracyclines is rare, and there is only previous report of an adverse reaction to doxycycline, which involved the intravenous administration of the drug.² The mechanism of anaphylaxis to tetracyclines is unclear, and in only 3 case reports