

# Association Between Tuberculosis and Atopy: Role of the CD14-159C/T Polymorphism

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## ■ Abstract

*Background:* The development of allergic hypersensitivity depends on both genetic and environmental factors. Different amounts of microbial products could affect patients with atopy and different genotypes.

*Objective:* We aimed to evaluate the role of varying degrees of exposure to infection by *Mycobacterium tuberculosis* (tuberculosis) in atopic patients and analyze the association with genetic factors.

*Methods:* We performed CD14-159C/T genotyping in atopic patients (n=118) and healthy individuals (n=62) and recorded the following variables: rural lifestyle, exposure to persons with tuberculosis, bacille Calmette-Guérin (BCG) vaccination, tuberculin skin test (TST), skin prick test, and phenotypes of atopy. Blood samples were analyzed for soluble-CD14 (sCD14), interferon (IFN)  $\gamma$ , total immunoglobulin (Ig) E, and eosinophil levels. A score was used to identify the likelihood of exposure to tuberculosis.

*Results:* Almost all the study participants had had a BCG vaccination, and half had a positive TST result. No differences were observed between atopic patients with high/low tuberculosis scores and CD14 genotypes in terms of atopic phenotypes, allergen sensitization, and levels of total IgE, sCD14, and IFN- $\gamma$ . However, the frequency of asthma was higher in atopic patients with a high tuberculosis score and was not associated with CD14 genotypes. Eosinophil counts in blood were higher in atopic patients with a high tuberculosis score and CC+CT genotypes.

*Conclusions:* These results suggest that the C allele of the CD14-159C/T polymorphism has a marked effect on eosinophil levels in atopic patients with increased exposure to tuberculosis. In addition, the degree of exposure to tuberculosis in atopic patients may modify the development of asthma.

**Key words:** Atopy. BCG. CD14 polymorphism. Rural life. Tuberculosis

## ■ Resumen

*Antecedentes:* El desarrollo de hipersensibilidad alérgica depende de factores tanto genéticos como ambientales. Diferentes cantidades de productos microbianos pueden afectar a los pacientes con atopia y diferentes genotipos.

*Objetivo:* El objetivo fue evaluar el papel de distintos grados de exposición a la infección por *Mycobacterium tuberculosis* (tuberculosis) en pacientes atópicos y analizar la asociación con factores genéticos.

*Métodos:* Se realizó el genotipado de CD14-159C/T en pacientes atópicos (n = 118) e individuos sanos (n = 62) y se registraron las variables siguientes: estilo de vida rural, exposición a personas con tuberculosis, vacuna del bacilo Calmette-Guérin (BCG), prueba cutánea de la tuberculina (PCT), prueba de punción cutánea y fenotipos de atopia. Se analizaron muestras de sangre para la detección de CD14 soluble (sCD14), interferón (IFN)  $\gamma$ , inmunoglobulina (Ig) E total y niveles de eosinófilos. Se utilizó una puntuación para identificar la probabilidad de exposición a la tuberculosis.

*Resultados:* Casi todos los participantes en el estudio habían recibido una vacuna de BCG, y la mitad presentó un resultado positivo en la PCT. No se observaron diferencias entre los pacientes atópicos con puntuaciones elevadas/bajas de tuberculosis y los genotipos CD14 en términos de fenotipos atópicos, sensibilización frente a alérgenos y niveles de IgE total, sCD14 e IFN- $\gamma$ . No obstante, la frecuencia de asma fue más elevada en los pacientes atópicos con una puntuación elevada de tuberculosis y sin asociación con genotipos CD14. Los recuentos de eosinófilos en sangre fueron más elevados en pacientes atópicos con una puntuación elevada de tuberculosis y genotipos CC+CT.

*Conclusiones:* Estos resultados indican que el alelo C del polimorfismo CD14-159C/T tiene un efecto marcado sobre los niveles de eosinófilos en pacientes atópicos con una mayor exposición a la tuberculosis. Asimismo, el grado de exposición a la tuberculosis en pacientes atópicos puede modificar el desarrollo de asma.

**Palabras clave:** Atopia. BCG. Polimorfismo CD14. Vida rural. Tuberculosis.

## Introduction

Atopy is generally considered to be caused by the interaction of genetic and environmental factors. Exposure to microbial products such as lipopolysaccharide (LPS) or other endotoxins during childhood appears to stimulate CD14 (an endotoxin coreceptor, along with toll-like receptor [TLR] 4) and could activate maturation of type 1 helper T cells ( $T_H1$ ), thereby suppressing the  $T_H2$  response and leading to atopy [1,2]. CD14 is a component of the innate immune response. It is expressed as a soluble component in serum and on the membrane surface of monocytes, macrophages, and neutrophils [3]. Although LPS is the main ligand of CD14, the coreceptor also recognizes other pathogen-associated molecular patterns such as those of *Mycobacterium species*. Even though TLR2 mediates the control of *Mycobacterium* infection (tuberculosis), mycobacterial TLR4 agonists also increase the sensitivity of the TLR2 response by approximately 100-fold [4]. Therefore, CD14 seems to be the component of the immune system that is common to both atopy and tuberculosis.

In 1999, a single-nucleotide polymorphism (SNP) in the CD14-159C/T genome was reported to be associated with decreased  $T_H1$  function and increased intensity of atopy [5]. However, studies of CD14 polymorphisms and atopy have shown remarkable heterogeneity: some indicate both the T allele [5] and the C allele as a risk factor [6-8], while others found no such association [9-11]. These discrepancies can be explained in part by recent data, which suggest that the LPS concentration might affect atopic phenotypes in alternative genotypes by altering cytokine production [12,13].

*Mycobacterium* species has been shown to stimulate cellular and innate immunity by CD14<sup>+</sup> monocytes [14], and generation of a  $T_H1$ -polarized memory during infancy was suggested to have a protective effect on atopic sensitization [15,16]. Nevertheless, findings are contradictory [17-19]. Therefore, in addition to the timing and dose of endotoxin exposure [7-10], we hypothesized that the *Mycobacterium* concentration in the endotoxin signaling pathway might affect atopy phenotypes and genotypes in a way that has not been studied to date.

The effect of CD14 polymorphisms on tuberculosis has been investigated elsewhere [20,21]; however, previously unstudied genetic aspects of the association between atopy and bacterial infection could provide us with a more detailed picture. Our primary objective was to evaluate the role of different levels of exposure to tuberculosis in atopy with respect to the CD14-159C/T polymorphism in a group of atopic patients from central Anatolia in Turkey.

## Materials and Methods

We performed an observational study at the Allergy Department of the University Hospital in Kirikkale, Turkey. The study was approved by the local ethics committee (2008/061), and written informed consent was obtained from the study participants.

## Study Population

The sample included patients (age, >15 years) with a positive skin prick test (SPT) result. The age-matched control group was composed of volunteers of both sexes from a random general population in Kirikkale with negative SPT results and known diseases. The exclusion criteria were diagnosis of systemic disease, infection within the previous 6 weeks, pregnancy, smoking, and current therapy with corticosteroids or antihistamines.

Atopy was defined as the presence of relevant atopic symptoms with positive SPT results. Rhinitis, asthma, food allergy, and atopic dermatitis were diagnosed according to guidelines [22-25]. The participants completed a questionnaire to obtain personal information and data on risk factors for atopy, history of therapy for tuberculosis, and exposure to a tuberculosis index case. Rural lifestyle was defined as having lived in the countryside for at least 1 year.

## Skin Prick Test and Pulmonary Function Test

Participants underwent SPTs with a common aeroallergen panel (ALK-Abelló) and pulmonary function tests. Those with airway obstruction were tested for airway reversibility; a difference between postbronchodilator and prebronchodilator forced expiratory volume in the first second of expiration [FEV<sub>1</sub>] >200 mL was considered a positive response (MIR Spirolab-II) [23].

## Tuberculin Skin Test

All participants underwent a tuberculin skin test (TST) with 5 tuberculin units (0.1 mL each) of purified protein derivatives (PPD). The reactions were read after 72 hours by measuring the horizontal and vertical diameters of the induration. Those with no reaction to PPD were retested at least 2 weeks later to exclude false negativity. Recent live-virus vaccination, viral disease, and severe tuberculosis that could lead to false negativity were investigated in detail. The TST response was scored as positive if the diameter of the induration was  $\geq 10$  mm, irrespective of bacillus Calmette-Guérin (BCG) vaccination status [26]. Participants with a positive TST result were screened for active disease using chest radiography, sputum acid-fast bacillus smear, and sputum culture.

## Tuberculosis Score

In order to create a simple diagnostic tool and identify the likelihood of clinical exposure to tuberculosis, a predictive score was developed from the significant variables previously identified in our population [27]. We assigned scores for 5 variables: 1) total number of BCG scars, 0-3; 2) TST response scored as 0 (negative) or 1 (positive); 3) history of tuberculosis therapy scored as 0 (no) or 1 (yes); 4) history of contact with a tuberculosis index case scored as 0 (no) or 1 (yes); and 5) results confirming tuberculosis in tests scored as 0 (no) or 1 (yes). We used receiver operating characteristic (ROC) curve analysis to determine whether the tuberculosis score could identify different degrees of exposure to tuberculosis. The area under the ROC curve for the differentiation between a low and high tuberculosis score was 0.83 (95% CI, 0.76-0.89;  $P < .001$ ).

A total score of 0-3 represented low exposure to tuberculosis and 4-7 represented high exposure. A cut-off value of 4 yielded the least error of classification in differentiating patients with low exposure from those with high exposure at a sensitivity of 54%, specificity of 87%, positive likelihood ratio of 4.2, and negative likelihood ratio of 0.53.

### Laboratory Assessment

Blood eosinophil counts were recorded using a hemocytometer (Beckman Coulter). Serum samples were tested for total IgE (electrochemiluminescence immunoassay), sCD14 (enzyme-linked immunosorbent assay [ELISA], R&D Systems Inc), and human interferon (IFN)  $\gamma$  (ELISA, Biosource International Inc) from serum samples stored at  $-80^{\circ}\text{C}$ .

Polymerase chain reaction (PCR) was performed as described elsewhere [13]. The primary pairs used for genotyping were 5'-GTGCCAACAGATGAGGTTTCAC-3'/5'-CCTCTGTGAACCCTGATCAC-3' and 5'-CCTGAAACATCCTTCATTGC-3'/5'-CGCAGCGAAATCTTCATC-3'. Molecular analysis was performed in the Laboratory of the Paediatric Allergy Unit of Hacettepe University (Ankara, Turkey).

### Statistical Analysis

The atopic and control groups and the subgroups of genotypes (CC/CT and TT) and tuberculosis scores (low and high) were compared using both a parametric approach (*t* test) and nonparametric approach (Mann-Whitney test) depending on the normality of the distribution of the variables. Categorical variables (frequencies) were analyzed using the  $\chi^2$  test, whereas small sample sizes were analyzed using the Fisher exact test. Hardy-Weinberg equilibrium was tested using the  $\chi^2$  statistic. Results were considered significant at  $P < .05$  (2-tailed). Calculations were performed using SPSS.

### Results

The study sample comprised 180 participants (118 atopic patients and 62 controls) aged between 16 and 58 years; 60% were female (Table 1). A family history of atopy was prevalent in the atopic patients, and smoking was frequent in the control group ( $P < .001$ ). Mean time living in the countryside was longer in the control group (17.8 [2.8] vs 6.5 [0.8] years;  $P < .001$ ).

Table 1. Demographic and Functional Characteristics of the Study Group

Characteristics	Before	After	Before	After
Mean (SD) age, y	32.2 (10.3)	32.5 (10.6)	31.7±9.6	.47
Female/Male, %	60/40	66.1/33.9	48.4/51.6	.05
Family history of atopy, No. %	76 (42.2)	75 (63.6)	1(1.6)	<.001
Smoking habit, No. %	45 (25)	17 (14.4)	28 (45.2)	<.001
Mean (SD) time living in the countryside, y	11.3 (2.5)	6.5 (0.8)	17.8±2.8	<.001
BCG vaccination, No. (%)	175 (97.8)	115 (97.4)	61 (98.4)	.68
TST positivity, No. (%)	83 (46.1)	58 (49.2)	25 (40.3)	.25
Exposure to index TB case, No. (%)	25 (13.9)	20 (16.9)	5 (8.1)	.41
TB score, No. (%)				.51
Low	59 (33)	39 (33.3)	20 (32.3)	
High	120 (67)	78 (66.7)	42 (67.7)	

Abbreviations: BCG, bacillus Calmette-Guérin; TB, tuberculosis; TST, tuberculin skin test.

Table 2. CD14 Genotyping, Cytokine, and Blood Analysis Results

Characteristics	Total (N=180)	Atopic (n=118)	Control (n=62)	P Value
CD14-159C/T genotype, No. (%)				.35
CC + CT	120 (66.6)	84 (71.2)	37 (59.7)	
TT	60 (33.4)	34 (28.8)	25 (40.3)	
Mean (SD) sCD14, ng/mL	6.5 (5.5)	7.5 (7.7)	4.7 (2.1)	.89
Mean (SD) IFN- $\gamma$ , pg/mL	38.9 (27.8)	31.3 (24.9)	46.5 (30.8)	.19
Mean (SD) total IgE, 0-240 IU/mL	239.8 (59.3)	303 (35.7)	117.1 (44.6)	<.001
Mean (SD) eosinophil count, cells /mm <sup>3</sup>	241 (20)	289 (79)	149 (14)	<.001

Abbreviations: Ig, immunoglobulin E; IFN, interferon; sCD14, soluble CD14.

Table 3. Atopy Data and Blood Analysis Results

	Low TB Score			High TB Score			Overall <sup>a</sup>
	CC+CT (n=29)	TT (n=10)	P Value	CC+CT (n=53)	TT (n=25)	P Value	P Value
Atopy data							
Allergic rhinitis, No. (%)	23 (79.3)	9 (90)	.44	39 (73.6)	21 (84)	.30	.52
Food allergy, No. (%)	4 (13.8)	2 (20)	.49	12 (22.6)	3 (12)	.21	.60
Atopic dermatitis, No. (%)	10 (34.5)	24 (45.3)	.23	2 (20)	11 (44)	.17	.14
Allergen sensitivity, No. (%)							
House Dust Mite	8 (27.6)	5 (50)	.19	23 (43.4)	10 (40)	.77	.34
Pollens and others	23 (79.3)	6 (60)	.22	35 (66)	18 (72)	.59	.47
Blood analysis							
Mean (SD) sCD14, ng/mL	4.7 (0.9)	5.4 (3.6)	.96	6 (1.3)	8.7 (2.2)	.22	.17
Mean (SD) IFN- $\gamma$ , pg/mL	41.7 (13)	31.6 (4.6)	.41	37.6 (3.1)	29.7 (16)	.65	.13
Mean (SD) total IgE, IU/mL	525 (165)	351 (163)	.71	300 (85)	260 (105)	.38	.49

Abbreviations: IgE, Immunoglobulin E; IFN, interferon; TB, tuberculosis; sCD14, soluble CD14.

<sup>a</sup>P Value between the groups of low and high exposure TB composed of the sum of both genotypes (not given in the table).

Almost all the participants had received a BCG vaccination (Table 1). The ratio of TST positivity, frequency of exposure to a tuberculosis index case, and high/low tuberculosis score were similar in both groups. One score was missing in the atopic group. The total number of BCG scars was close to 2 in both groups, and 2 healthy and 1 atopic participant had a history of tuberculosis therapy (not shown in the table). Three atopic patients received prophylaxis after screening for active disease.

The distribution of each genetic variant was in Hardy-Weinberg equilibrium. The frequency of CD14 genotypes was similar between atopic participants (CC+CT, 71.2%; TT, 28.8%) and healthy participants (CC+CT, 59.7%; TT, 40.3%),

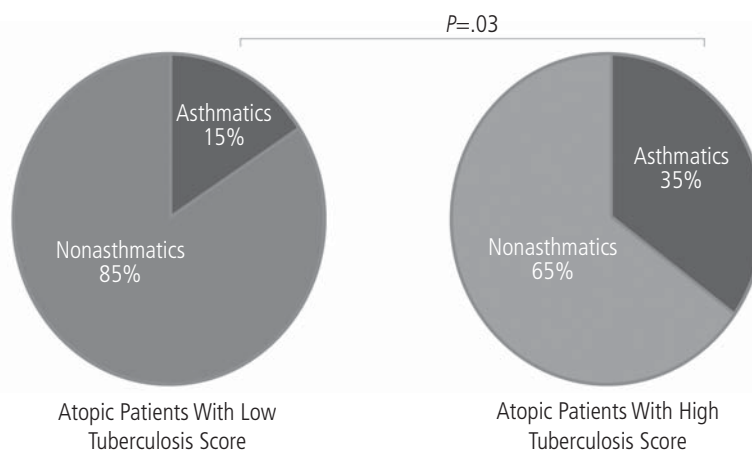


Figure 1. The frequency of asthma between atopic patients with high and low tuberculosis scores indicating the likelihood of exposure to tuberculosis infection.

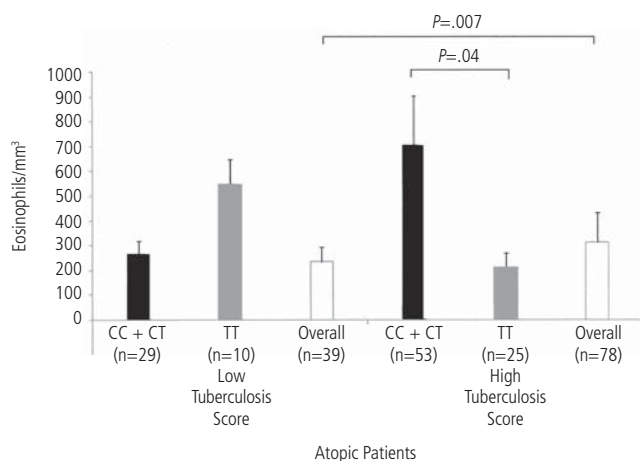


Figure 2. Comparison of blood eosinophil counts in atopic patients with low and high tuberculosis scores in relation to CC+CT genotypes at position of -159 of the CD14 gene.

as were levels of sCD14 and IFN- $\gamma$  (Table 2). The atopic group had a higher mean total IgE level and eosinophil count than the controls ( $P < .001$ ).

The frequency of atopic phenotypes—except asthma, allergen sensitization, levels of sCD14, IFN- $\gamma$ , and total IgE—was similar between the groups of tuberculosis scores and CD14 genotypes in atopic patients (Table 3).

The frequency of asthma was higher in atopic patients with a high score (Figure 1) and no association with CD14 genotypes (not shown), whereas the eosinophil count in blood was higher in atopic patients with a high tuberculosis score and CC+CT genotypes (Figure 2).

## Discussion

The present study showed that, in a group of Turkish adults, neither the CD14-159C/T polymorphism nor different levels of exposure to tuberculosis infection were associated with atopy, except for the protective effect of a longer time living in the countryside, the higher prevalence of asthma, and higher levels of eosinophil counts in atopic CC/CT carriers with high exposure to tuberculosis infection.

We compared atopic and healthy individuals with similar demographic characteristics. A family history of atopy was prevalent in the atopic patients, who smoked less than the controls, possibly because of asthma. Time living in the countryside was longer in healthy participants than in atopic patients, thus reflecting the protective effect of exposure to endotoxin against atopy [28,16]. This result is valuable, as it demonstrates that the field study was well conducted and that individual outcomes are reliable.

Although BCG protects against *Mycobacterium bovis*, it is at least 80% effective in preventing tuberculosis by *Mycobacterium tuberculosis* through activation of CD4<sup>+</sup> T<sub>H</sub>1 cells [29]. Therefore, even though it was not a marker for disease, we evaluated BCG vaccination in relation to tuberculosis, since BCG vaccination and tuberculosis commonly affected immunity. Additionally, the TST indicates tuberculosis infection, previous BCG vaccination, and environmental exposure to mycobacteria [26]. We developed a score to measure the degree of exposure to tuberculosis. Nevertheless, it was impossible to assess the effect of BCG vaccination on development of atopy, since almost all study participants had received the BCG vaccination. Other components of the score—TST positivity, number of BCG scars, history of tuberculosis therapy, contact with an index case, and screening results—were found to be similar in both groups. In some studies, BCG vaccination and tuberculosis seemed to reduce the prevalence of asthma and other atopic diseases [14-16], whereas in others, no association was reported between development of atopic phenotypes and history of tuberculosis, TST response, or BCG vaccination [17-19]. Repeated BCG vaccination in asthmatic patients had no effect on the outcome of asthma [30]. Even though neonatal BCG vaccination was found to have a long-term effect on reduced airway hyperresponsiveness in TST-positive humans and mice by increasing immunity based on IFN- $\gamma$  and T<sub>H</sub>1 [14,15], no significant association was found between TST reactivity and atopic sensitization in BCG-vaccinated Turkish children [31], except for a negative correlation between BCG vaccination and serum IgE levels during the first year of life [32]. The inverse association between tuberculosis and atopy could exist because tuberculosis increases the T<sub>H</sub>1/T<sub>H</sub>2 ratio; however, this hypothesis may not fit all populations. We can attribute the lack of an association to the multifactorial structure of atopy, which includes genetic factors, infections other than those by *Mycobacterium*, timing, and levels of exposure to infection [12-14].

We observed higher total IgE levels and eosinophil counts in the atopic patients, probably as a result of IgE-mediated inflammation. Although *Mycobacterium* induces release of sCD14 and IFN- $\gamma$  [33], an sCD14 to total IgE ratio was reported

to be high in TT carriers [5,34] and low in CC carriers [9,10]. In the present study, levels of sCD14 and IFN- $\gamma$  were found to be similar between atopic patients and healthy controls, between individuals with high tuberculosis scores and individuals with low scores, and in CD14-159C/T subgroups. These results could be due to the fact that, as BCG is a childhood vaccine, exposure to *Mycobacterium* in childhood might not have a long-term effect in adulthood, and cellular immunity might be stronger than innate immunity in the defence against tuberculosis.

The distribution of CD14-159C/T alleles in our adult population was consistent with the results for Turkish children [34]. The distribution of CC and TT alleles was similar between the atopic patients and healthy individuals and between atopic patients with high and low tuberculosis scores in means of atopic phenotypes—except asthma—and allergen sensitization. Even though some reports confirm the correlation between the CC allele and SPT positivity (including mite and mold allergy) [7,10] and the increased or decreased risk of allergic rhinitis in TT carriers [6,8], others did not find the alleles of -159C/T to be associated with asthma and allergic rhinitis in children and adults, as was the case in our study [7,9-11]. We suggest that the association between CD14-159C/T and allergen sensitization that could be present in childhood may no longer be apparent in adulthood [35], or even in some children [5].

In contrast to the reported protective effect of tuberculosis in patients with asthma [14-16], we found that atopic patients with a high tuberculosis score indicating an increased likelihood of infection were more likely to have asthma and increased eosinophil levels in CC and CT carriers. Eosinophils are reported to play an important role in the pathogenesis of allergic inflammation and immunity to tuberculosis, since they are granular antimycobacterial cells with cytotoxic action [36], and this interaction between *Mycobacterium* and eosinophils has also been reported in a patient with tuberculous pleurisy [37]. Recruitment of *Mycobacterium*-activated eosinophils, B cells, and granulocytes in the lung may play a key role in susceptibility to asthma by worsening lung disease [38]. Even though the TT allele was more susceptible to tuberculosis [20] and the CC allele was more susceptible to atopy [7], we only observed high eosinophil counts in those atopic CC/CT carriers with high exposure to tuberculosis. Earlier studies reported nonsignificantly higher eosinophil counts in patients with the CC genotype than in patients with the TT allele at position 159 of the *CD14* gene [13] and in patients with moderate to severe atopic asthma [35], whereas the CD14/159 genotype was not associated with asthma or peripheral blood eosinophilia in other studies [39,40]. Our results may explain these conflicting findings by highlighting the presence of the CD14 genotype in atopic patients with high exposure to tuberculosis, since, to our knowledge, this is the first study to assess the interaction between tuberculosis, atopy, and genetic factors.

In summary, besides the common finding of time living in the countryside as a protective factor in the development of atopy, we report a new finding, namely, that increased exposure to tuberculosis infection in atopic patients was associated with increased prevalence of asthma and higher eosinophil levels in CC and CT carriers. Contrary to the prevailing theory that tuberculosis protects against atopy, this finding has important

implications for our understanding of how the impact of degree of exposure to tuberculosis in atopic patients can modify development of asthma and the CD14-159C/T polymorphism.

## Acknowledgments

We thank all the laboratory employees who took part in the study in the Laboratory of Paediatric Allergy Unit-Hacettepe University, Ankara, Turkey. We are particularly grateful to Professor Omer Kalayci for assistance with enrollment and laboratory support.

This work was supported by a grant from Kirikkale University Projects of Scientific Research (Grant No. 2008/5) awarded to Drs Baccioglu Kavut, Kalpaklioglu, and Ayaslioglu. None of the authors have any other financial disclosures to make.

*Previous presentation:* The abstract was presented at the 29<sup>th</sup> congress of European Academy of Allergy and Clinical Immunology in 2010, London, UK.

*Conflict of interest:* None

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- *Manuscript received September 30, 2011; accepted for publication January 9, 2012.*
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