



ORIGINAL ARTICLE

Is TNF- α gene polymorphism related to pulmonary functions and prognosis as determined by FEV₁, BMI, COPD exacerbation and hospitalization in patients with smoking-related COPD in a Turkish population?



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KEYWORDS

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Abstract

Introduction: Some conflicting results have been published about the relationship between TNF- α -308 gene polymorphism and chronic obstructive pulmonary disease (COPD). The aim of this study was to determine whether TNF- α -308 gene polymorphism was associated with smoking-related COPD and whether it was associated with pulmonary function parameters (PFTs), body mass index (BMI), and prognosis.

Methods: We studied the frequencies of TNF- α -308 gene polymorphism in 90 male subjects (60 subjects with COPD and 30 healthy smokers) in a Caucasian population.

Results: There was no significant difference in the frequency of G/G and G/A gene polymorphisms in the COPD group compared with control subjects ($p > 0.05$). We compared COPD patients as G/A gene polymorphism and G/G gene polymorphism; the PFTs and BMI before and after one year were not statistically significant ($p > 0.05$). Also, the exacerbation and hospitalization data of COPD patients were not significant between these groups.

Conclusion: In conclusion, there was no difference between smoking-related COPD and the control group according to TNF- α -308 gene polymorphism in a Caucasian population. In addition, it was shown that important determinants of prognosis of COPD such as FEV₁, BMI, COPD exacerbation and hospitalization were not associated with TNF- α -308 gene polymorphism.

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PALAVRAS-CHAVE

Doença pulmonar
obstrutiva crónica;
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Factor de necrose
tumoral alfa

Estará o polimorfismo do gene TNF- α relacionado com a função pulmonar e o prognóstico determinados pelo VEMS, IMC, exacerbações e hospitalizações em doentes com DPOC associados ao tabagismo numa população turca?

Resumo

Introdução: Foram publicados alguns resultados contraditórios sobre a relação entre o polimorfismo do gene TNF- α -308 e a doença pulmonar obstrutiva crónica (DPOC). O objectivo deste estudo foi determinar se o polimorfismo do gene TNF- α -308 estava associado à DPOC ligada ao tabagismo e se foi associado aos parâmetros de função pulmonar (PFTs), índice de massa corporal (IMC), e prognóstico.

Métodos: Estudámos as frequências do polimorfismo do gene TNF- α -308 em 90 indivíduos do sexo masculino (60 indivíduos com DPOC e 30 fumadores saudáveis) numa população caucasiana.

Resultados: Não houve uma diferença significativa na frequência de polimorfismos genéticos G/G e G/A no grupo de DPOC, em comparação com o grupo de controlo ($p > 0,05$). Comparámos os doentes com DPOC como polimorfismo genético G/A e polimorfismo genético G/G; os PFTs (parâmetros de função pulmonar) e o IMC (índice de massa corporal) antes e depois de um ano não eram estatisticamente significativos ($p > 0,05$). Da mesma forma, os dados de agravamento e hospitalização dos doentes com DPOC não eram significativos entre estes grupos.

Conclusão: Em conclusão, não existiu uma diferença entre o grupo com DPOC ligada ao tabagismo e o grupo de controlo, de acordo com o polimorfismo do gene TNF α -308, numa população caucasiana. Além disso, foi mostrado que determinantes importantes do prognóstico da DPOC, tal como VEMS, IMC, exacerbações da DPOC e hospitalização não estavam associados ao polimorfismo do gene TNF- α -308.

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Introduction

Cigarette smoking is the most common and important cause of chronic obstructive pulmonary disease (COPD). However, COPD develops in only 10–15% of smokers.¹ In addition, a familial predisposition for COPD and lung function is well known.^{2,3} These findings suggest that genetic factors are responsible for COPD. Alpha-1 antitrypsin deficiency is the best-known genetic risk factor for COPD, but such cases are estimated to account for only 1–2%.⁴

Tumor necrosis factor (TNF)- α is a cytokine primarily derived from macrophages. Increased concentrations of TNF- α have been found in induced sputum, bronchoalveolar lavage fluid, and bronchial biopsies from patients with COPD compared with healthy smokers.^{5–7} These findings have led researchers to further work on TNF- α . A genomic polymorphism resulting in the substitution of the nucleotide adenine (A) for guanine (G) at position-308 within a regulatory region of the TNF- α locus was discovered in 1992.⁸ This polymorphism at position-308 of the TNF- α gene showed elevated TNF- α secretion and blood levels in some studies.^{9,10}

It is necessary to clarify the effects of genetic polymorphisms of the TNF- α gene promoter region for the development of COPD and disease progression. Some conflicting results have been published about the relationship between TNF- α -308 gene polymorphism and COPD. A meta-analysis of twenty four studies showed that TNF- α -308 gene polymorphism is associated with an increased risk of COPD in the Asian population, but this relationship is not significant in the Caucasian population.¹¹ Although previous reports showed that the FEV1 and BMI values were

lower in COPD patients than in smoker controls, there was no significant difference among G/G, G/A and A/A gene polymorphisms in COPD patients.^{12–14} In a report that evaluates the prognosis of COPD and TNF- α gene polymorphism, COPD exacerbation was found to be the most common cause of death.¹² The aims of the present study were to determine whether TNF- α -308 gene polymorphism was associated with smoking-related COPD and whether it was associated with pulmonary function parameters (PFTs), body mass index (BMI), and prognosis in a Caucasian population.

Methods**Subjects**

The study was approved by the Research Ethics Committee of Erciyes University School of Medicine in Kayseri, Turkey and all subjects gave written informed consent. Ninety subjects were enrolled from August 2010 to December 2011. The patient group was recruited from the Pulmonary Outpatient Department. It consisted of 60 male adults with COPD. The diagnosis of COPD was made according to the Global Initiative for Chronic Obstructive Lung Disease standards.¹⁵ The criteria of enrollment were as follows: (1) >40 years old, (2) a smoking history of 20–80 pack-years, (3) available spirometry data for the previous year, (4) post-bronchodilator FEV₁/FVC <70%, and (5) patient's consent. Patients were excluded if they had had an exacerbation of COPD within the previous 6 weeks and significant reversibility (FEV₁ or FVC > 12% baseline and/or 200 ml) to 2.5 mg nebulized salbutamol.

Table 1 Demographic characteristics and pulmonary test parameters of the COPD patients and healthy control groups.

	COPD (<i>n</i> = 60)	Controls (<i>n</i> = 30)	<i>p</i>
Age (years)	60 \pm 10	54 \pm 7	0.002
Smoking (pack-years)	45 \pm 17	41 \pm 19	0.316
Current smoking, <i>n</i> (%)	5 (8%)	23 (77%)	<0.001
FEV ₁ (% predicted)	51 \pm 17	98 \pm 11	<0.001
FEV ₁ /FVC (% predicted)	52 \pm 10	78 \pm 4	<0.001
FEF 25–75 (% predicted)	20 \pm 10	82 \pm 25	<0.001
BMI (kg/m ²)	26 \pm 4	29 \pm 4	0.001

Values are expressed as mean \pm SD or as absolute numbers. COPD = Chronic obstructive pulmonary disease, FEV₁ = Forced expiratory volume in 1 s, FVC = Forced vital capacity, FEF25–75% = Forced expiratory flow 25–75%, BMI = Body mass index.

The previous year's PFTs and BMI (calculated as weight in kg divided by the square of height in meters) were recorded from files. The annual number of hospitalizations and COPD exacerbations were examined during the last year. Exacerbation of COPD was defined as "an event in the natural course of the disease characterized by a change in the patient's baseline dyspnea, cough and/or sputum that is beyond normal day-to-day variations, is acute in onset and may warrant a change in regular medication".¹⁵ New PFTs were performed by experienced technicians and BMI was calculated.

The control group consisted of 30 male chronic heavy smokers with normal lung function who were recruited randomly from the smoking cessation clinic. The inclusion criteria were as follows: (1) male older than 40 years of age, (2) heavy smoker with a smoking history of 20–80 pack-years, (3) post-bronchodilator FEV₁/FVC ratio > 70%, FEV₁% predicted > 80%, and FVC % predicted > 80%, (4) no other pulmonary disease.

Spirometer

All PFTs were performed by experienced lung function technicians with the same standard spirometer (Vmax20 system; SensorMedics, Yorba Linda, CA, USA) according to American Thoracic Society (ATS) recommendations.¹⁶ The best FVC measurement was recorded along with FEV₁ and the FEV₁/FVC calculations.

Genotyping protocol

Three milliliters of whole peripheral blood was obtained from each study participant by phlebotomy into a tube containing ethylene diamine tetra-acetic acid (EDTA). DNA was extracted promptly using a MagNA Pure LC DNA Isolation Kit I (Cat. No. 03003990001, Ver. 17.0) supplied by Roche (Mannheim, Germany).

TNF alpha-308 promoter gene polymorphism (rs1800629) was genotyped using the LightCycler real-time PCR system. The primers and hybridization probe for genotyping the -308 promoter polymorphism were designed by and obtained from Tib MolBiol GmbH, Berlin, Germany. The PCR reaction was carried out using the LightCycler real-time PCR machine 480 II (Roche Diagnostics GmbH, Mannheim, Germany), with software version 1.5.0. A reaction volume of 20 μ l (with 2 μ l of DNA Master Mix, 1 μ l of primers probe, 1.6 μ l of 25 mM

MgCl₂, 10.4 μ l H₂O and 5 μ l genomic DNA (10 ng)) was amplified in 96-well plates using the FastStart LC 480 II genotyping master kit (Roche Diagnostics GmbH). The PCR protocol for -308 promoter polymorphism consisted of initial denaturation at 95 °C for 10 min, followed by 45 cycles of denaturation (95 °C for 10 s, 4.4 °C/s), annealing (60 °C for 10 s, 2.2 °C/s) and elongation (72 °C for 10 s, 4.4 °C/s). This was followed by melting curve analysis consisting of 1 cycle at 95 °C for 30 s (4.4 °C/s), 40 °C for 2 min (1.5 °C/s) and a temperature rise to 75 °C at a slope of 0.19 °C/s with continuous measurement of fluorescence.

Statistical analysis

SPSS software (Statistical Package for the Social Sciences, version 15.0, SSPS Inc, Chicago, IL, USA) was used for statistical analyses. The distribution of continuous variables for normality was tested with the One-Sample Kolmogorov–Smirnov test and data are presented as mean \pm standard deviation (S.D.) or median and interquartile ranges, as appropriate. Demographic characteristics and parameters were compared using the Student *t*-test or Mann–Whitney *U* test for the continuous variables, where appropriate. Categorical variables were analyzed by the χ^2 -test. According to groups, the before and after values of the quantitative variables were assessed by the mean paired-sample *t*-test. The power of the study based on the gene polymorphism was 0.068 for (with alpha error of 0.05). Differences were considered statistically significant when *p* < 0.05.

Results

Comparison of COPD group and control group

The characteristics of the COPD (*n* = 60) and the control groups (*n* = 30) are summarized in Table 1. The COPD patients were older (60 \pm 10 vs. 54 \pm 7 years, *p* < 0.05) than the control group but had a similar pack-year history (45 \pm 17 vs. 41 \pm 19 pack-years, *p* > 0.05). PFTs (FEV₁%, FEV₁/FVC, FEF 25–75%) in the control group were near normal and significantly greater than those of the COPD group (*p* < 0.001). Mean BMI was also significantly lower in the COPD group (26 \pm 4 vs. 29 \pm 4 kg/m², *p* = 0.001).

The genotype frequencies of TNF- α -308 gene polymorphisms in the COPD group were compared with those in

Table 2 Genotype frequencies in patient and control groups.

TNF- α -308 Genotype	COPD (n = 60) (%)	Controls (n = 30) (%)	p
G/G	44 (73)	24 (80)	>0.05
G/A	16 (27)	6 (20)	
A/A	0 (0)	0 (0)	

control subjects. Forty-four (73%) patients had G/G and 16 (27%) patients had G/A gene polymorphisms in the COPD population; 24 (80%) patients had G/G and 6 (20%) patients had G/A gene polymorphisms in the control group. AA genotypes were not determined in any patient in the two groups. There was no significant difference in the frequency of G/G and G/A genotypes in the COPD population compared with control subjects ($p > 0.05$) (Table 2).

Comparison of COPD patients grouped by genotype

In COPD patients, the mean age and smoking status of patients were compared as groups of TNF- α -308 G/A gene polymorphism ($n = 16$) and G/G gene polymorphism ($n = 44$). The mean age [61 ± 11 (G/A)/ 60 ± 10 years (G/G)], the amount of smoking [45 ± 17 (G/A)/ 45 ± 17 pack-years (G/G)] and active smoking [0 (G/A)/5 (G/G)] was not statistically significant in these groups ($p > 0.05$). Also, a comparison of PFTs and BMI before and after one year were not statistically significant ($p > 0.05$). Table 3 shows the comparison of PFTs and BMI in both groups.

Exacerbation and hospitalization data of COPD patients

The exacerbation and hospitalization data of COPD patients are summarized in Table 4. Eleven (69%) patients who had G/A gene polymorphism and 27 (61%) patients who had G/G gene polymorphism in the COPD group had acute COPD exacerbation in the past year. Six (38%) patients with G/A gene polymorphism and 14 (32%) patients with G/G gene polymorphism were hospitalized because of COPD exacerbation. Neither of these differences were statistically significant ($p > 0.05$).

Table 4 The exacerbation and hospitalization of COPD patients by grouped as gene polymorphism.

	G/A (n = 16)	G/G (n = 44)	p
Exacerbation	11 (69%)	27 (61%)	>0.05
Hospitalization	6 (38%)	14 (32%)	>0.05

Discussion

The present study demonstrates that there is no difference in the frequency of G/A and G/G gene polymorphism in patients with COPD compared to healthy, smoking control subjects in a Caucasian population. Many studies have shown an association between COPD and TNF- α -308 gene polymorphism in an Asian population.¹⁷⁻²⁰ However, in a Caucasian population, although studies have been conducted in different countries, most of them did not show any association; this was similar to our results.^{12,21-30} A large family study from a Boston early-onset COPD study and another American study are the only two studies to show an association between COPD and TNF- α -308 polymorphism in a Caucasian population.^{13,31} In addition, a study from our country recently showed a significant difference among COPD stages in terms of TNF- α 308 G/A polymorphism.³² However, a meta-analysis of data for 6118 subjects also showed that TNF-308 gene polymorphisms were significantly associated with an increased risk of COPD among an Asian population but not among a Caucasian population.¹¹ In this situation, it seems that polymorphisms in the TNF- α -308 gene that stimulate the production of cytokines are not major genetic determinants for the development of COPD in most of the Caucasian population.

The major etiologic factor for the development of COPD is cigarette smoking. The risk of COPD development among people with a similar history of smoking may be different due to genetic predisposition and/or lifestyles. In a study investigating the association between antioxidant genes and COPD, the definition of "resistant smoker" was used for smokers who did not develop COPD.³³ Therefore, we identified individuals with a smoking history of 20-80 pack-years and created similar patient and control groups. TNF- α -308 gene polymorphism was found to be ineffective in the development of COPD in these groups which had a similar smoking history.

The second major finding in the present study is that COPD patients with G/A and G/G gene polymorphism had similar PFTs (FEV₁, FEV₁%, FEV₁/FVC) before and after one

Table 3 Comparison of PFTs and BMI before and after one year grouped by gene polymorphism in patients with COPD.

	G/A (n = 16)			G/G (n = 44)			p1*	p2**
	Before	After	p	Before	After	p		
FEV ₁ (L)	1.45 \pm 0.50	1.45 \pm 0.58	0.947	1.59 \pm 0.57	1.64 \pm 0.64	0.309	0.376	0.298
FEV ₁ (%)	47.7 \pm 15.4	47.3 \pm 15.7	0.866	52.4 \pm 17.5	54.8 \pm 20.1	0.154	0.345	0.185
FEV ₁ /FVC (%)	50.9 \pm 10.8	51.4 \pm 10.6	0.835	52.2 \pm 9.8	53.0 \pm 10.4	0.364	0.661	0.605
BMI (kg/m ²)	24.9 \pm 3.6	25.1 \pm 3.8	0.721	26.1 \pm 4.6	25.9 \pm 4.9	0.428	0.375	0.548

* Comparison of before values of G/A and G/G group.

** Comparison of after values of G/A and G/G group.

year evaluation. The FEV₁ value was the most commonly used parameter for monitoring disease progression.¹⁵ Only one report exists that evaluates the prognosis of TNF- α -308 gene polymorphism in COPD patients.¹² Keatings et al. showed that AA homozygous patients had less reversible air-flow obstruction and a significantly greater mortality (both all-cause and respiratory deaths) on follow-up despite a shorter cigarette smoking history. In another study, the homogenous A genotype in COPD patients was accompanied by a significant reduction in the mean values of the post-bronchodilator FEV₁, FEV₁/FVC and FEF₂₅/75% of predicted compared to heterozygous G/A genotypes in COPD.³⁰ However, in the present study, there were no patients with A/A polymorphism for comparison purposes.

Another parameter that was shown to have prognostic importance in patients with COPD was BMI.³⁴ In a study by Wilson et al., BMI was shown to be an important prognostic factor after FEV₁ in male patients with COPD.³⁵ Furthermore, low BMI was shown to be independently associated with reduced survival in patients with severe COPD.³⁶ In the present study, BMI was significantly lower in patients with COPD than in the healthy smoker control group. When we examined the effect of the TNF- α -308 gene on BMI, neither the current BMI nor the BMI of the previous year were statistically significant between the patients with G/A and G/G gene polymorphisms.

The most important limitation of this study was the low number of patients. In addition, mortality was not examined in the present study. No patients had A/A gene polymorphism, so we have no data about A/A gene polymorphism in our population.

Conclusion

In conclusion, there was no difference between smoking-related COPD and control group according to TNF- α -308 gene polymorphism in a Caucasian population. In addition, it was shown that important determinants of prognosis of COPD such as FEV₁, BMI, COPD exacerbation and hospitalization were not associated with TNF- α -308 gene polymorphism.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data and that all the patients included in the study received sufficient information and gave their written informed consent to participate in the study.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Conflicts of interest

The authors have no conflicts of interest to declare.

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References

- Mannino DM, Homa DM, Akinbami LJ, Ford ES, Redd SC. Chronic obstructive pulmonary disease surveillance-United States, 1971-2000. *MMWR Surveill Summ.* 2002;51:1-16.
- Silverman EK. Genetic epidemiology of COPD. *Chest.* 2002;121 3 Suppl.:1-6.
- Sandford AJ, Silverman EK. Chronic obstructive pulmonary disease: susceptibility factors for COPD the genotype environment interaction. *Thorax.* 2002;57:736-41.
- Koyama H, Geddes DM. Genes oxidative stress, and the risk of chronic obstructive pulmonary disease. *Thorax.* 1998;53 2 Suppl.:10-4.
- Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor- α in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med.* 1996;153:530-4.
- Churg A, Wang RD, Tai H, et al. Macrophage metalloelastase mediates acute cigarette smoke-induced inflammation via tumor necrosis factor- α release. *Am J Respir Crit Care Med.* 2003;167:1083-9.
- Mueller R, Chanez P, Campbell AM, Bousquet J, Heusser C, Bullock GR. Different cytokine patterns in bronchial biopsies in asthma and chronic bronchitis. *Respir Med.* 1996;90:79-85.
- Wilson AG, Di Giovine FS, Blakemore AIF, Duff GW. Single base polymorphism in the human TNF- α gene detectable by Nco1 restriction of PCR product. *Hum Mol Genet.* 1992;1:353.
- Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor- α promoter polymorphism effects transcription. *Mol Immunol.* 1997;34:391-9.
- Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor- α promoter on transcriptional activation. *Proc Natl Acad Sci USA.* 1997;94:3195-9.
- Zhan P, Wang J, Wei SZ, et al. TNF-308 gene polymorphism is associated with COPD risk among Asians: meta-analysis of data for 6,118 subjects. *Mol Biol Rep.* 2011;38:219-27.
- Keatings VM, Cave SJ, Henry MJ, Morgan K, O'Connor CM, FitzGerald MX. A polymorphism in the tumor necrosis factor- α gene promoter region may predispose to a poor prognosis in COPD. *Chest.* 2000;118:971-5.
- Gingo MR, Silveira LJ, Miller YE, et al. Tumor necrosis factor gene polymorphisms are associated with COPD. *Eur Respir J.* 2008;31:1005-12.
- Chen YC, Liu SF, Chin CH, et al. Association of tumor necrosis factor- α -863C/A gene polymorphism with chronic obstructive pulmonary disease. *Lung.* 2010;188:339-47.
- Rabe KF, Hurd S, Anzueto A, et al., Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med.* 2007;176:532-55.
- American Thoracic Society. Standardization of spirometry 1994 update. *Am J Respir Crit Care Med.* 1995;152:1107-36.
- Sakao S, Tatsumi K, Igari H, Shino Y, Shirasawa H, Kuriyama T. Association of tumor necrosis factor α gene promoter polymorphism with the presence of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2001;163:420-2.
- Sakao S, Tatsumi K, Igari H, Watanabe R, Shino Y, Shirasawa H. Association of tumor necrosis factor- α gene promoter

- polymorphism with low attenuation areas on high-resolution CT in patients with COPD. *Chest*. 2002;122:416–20.
19. Huang SL, Su CH, Chang SC. Tumor necrosis factor- α gene polymorphism in chronic bronchitis. *Am J Respir Crit Care Med*. 1997;156:1436–9.
 20. Jiang L, He B, Zhao MW, Ning LD, Li XY, Yao WZ. Association of gene polymorphisms of tumour necrosis factor- α and interleukin-13 with chronic obstructive pulmonary disease in Han nationality in Beijing. *Chin Med J*. 2005;118:541–7.
 21. Kucukaycan M, Van Krugten M, Pennings HJ, et al. Tumor necrosis factor- α +489G/A gene polymorphism is associated with chronic obstructive pulmonary disease. *Respir Res*. 2002;3:29.
 22. Brøgger J, Steen VM, Eiken HG, Gulsvik A, Bakke P. Genetic association between COPD and polymorphisms in TNF ADRB2 and EPHX1. *Eur Respir J*. 2006;27:682–8.
 23. Ruse CE, Hill MC, Tobin M, et al. Tumour necrosis factor gene complex polymorphisms in chronic obstructive pulmonary disease. *Respir Med*. 2007;101:340–4.
 24. Patuzzo C, Gilè LS, Zorzetto M, et al. Tumor necrosis factor gene complex in COPD and disseminated bronchiectasis. *Chest*. 2000;117:1353–8.
 25. Seifart C, Dempfle A, Plagens A, et al. TNF α , TNF β IL-6-, and IL-10-promoter polymorphisms in patients with chronic obstructive pulmonary disease. *Tissue Antigens*. 2005;65:93–100.
 26. Higham MA, Pride NB, Alikhan A, Morrell NW. Tumour necrosis factor- α gene promoter polymorphism in chronic obstructive pulmonary disease. *Eur Respir J*. 2000;15:281–4.
 27. Sandford AJ, Chagani T, Weir TD, Connett JE, Anthonisen NR, Paré PD. Susceptibility genes for rapid decline of lung function in the lung health study. *Am J Respir Crit Care Med*. 2001;163:469–73.
 28. Tanaka G, Sandford AJ, Burkett K, et al. Tumour necrosis factor and lymphotoxin α polymorphisms and lung function in smokers. *Eur Respir J*. 2007;29:34–41.
 29. Ferrarotti I, Zorzetto M, Beccaria M, et al. Tumour necrosis factor family genes in a phenotype of COPD associated with emphysema. *Eur Respir J*. 2003;21:444–9.
 30. Ezzeldin N, Shalaby A, Saad-Hussein A, et al. Association of TNF- α -308G/A, SP-B 1580C/T IL-13-1055C/T gene polymorphisms and latent adenoviral infection with chronic obstructive pulmonary disease in an Egyptian population. *Arch Med Sci*. 2012;8:286–95.
 31. Hersh CP, Demeo DL, Lange C, et al. Attempted replication of reported chronic obstructive pulmonary disease candidate gene associations. *Am J Respir Cell Mol Biol*. 2005;33:71–8.
 32. Melek K, Ulubay G, Sarınc Ulaşlı S, Verdi H, Ataç B, Öner Eyüboğlu F. Associations between TGF- β 1 G/A and TNF- α 308 G/A gene polymorphisms with airway resistance in chronic obstructive pulmonary disease. *Tüberk Toraks*. 2013;61:1–11 [Article in Turkish].
 33. Young RP, Hopkins R, Black PN, et al. Functional variants of antioxidant genes in smokers with COPD and in those with normal lung function. *Thorax*. 2006;61:394–9.
 34. Vestbo J, Prescott E, Almdal T, et al. Body mass, fat-free body mass, and prognosis in patients with chronic obstructive pulmonary disease from a random population sample: findings from the Copenhagen City Heart Study. *Am J Respir Crit Care Med*. 2006;173:79–83.
 35. Wilson DO, Rogers MR, Wright EC, Anthonisen NR. Body weight in chronic obstructive pulmonary disease The National Institutes of Health Intermittent Positive-Pressure Breathing Trial. *Am Rev Respir Dis*. 1989;139:1435–8.
 36. Gray-Donald K, Gibbons L, Shapiro SH, Macklem PT, Martin JG. Nutritional status and mortality in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 1996;153:961–6.