TNF-α-308 promoter polymorphism in patients with chronic obstructive pulmonary disease and lung cancer

M. M. STANKOVIC1, A. R. NESTOROVIC1, A. M. TOMOVIC1, N. D. PETROVIC-STANOJEVIC1, M. S. ANDJELIC-JELIC1, V. B. DOPUDJA-PANTIC1, Lj. M. NAGORNI-OBRADOVIC1, M. M. MITIC-MILIKIC1, D. P. RADOJKOVIC1

1Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia, e-mail: aleka@imgge.bg.ac.rs; 2Modeling and Simulation, Novartis Pharma AG, Basel, Switzerland; 3Zvezdara University Medical Center, Belgrade, Serbia; 4Institute for Tuberculosis and Lung Disease, University Clinical Center of Serbia, Belgrade, Serbia

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Chronic obstructive pulmonary disease (COPD) and lung cancer (LC) are a major cause of morbidity and mortality worldwide. In both diseases airways inflammation plays an important role. Functional promoter polymorphism, at the position -308, of tumor necrosis factor (TNF)-α represents an attractive potential susceptibility marker for both diseases. In order to investigate the role of this polymorphism in COPD and LC, a case-control study was performed. The patient groups consisted of 97 subjects with COPD and 70 subjects with LC, while the control group encompassed 102 subjects. Results of our study showed significant decrease of heterozygote for TNF-α-308*1/*2 gene variant in COPD group in comparison to controls (p=0.043). According to our results heterozygous carriers of TNF-α-308*1/*2 polymorphism had a 2.3-fold decreased risk for COPD development (OR=0.44, 95%CI=0.20-0.97). In patients with lung cancer we also observed a trend of decreased distribution of TNF-α-308*1/*2 heterozygotes, but statistical significance was not achieved. To our knowledge, this is the first study implicating decreased frequency of TNF-α-308*1/*2 gene variant in patients with COPD and LC. Although these results need to be confirmed on larger cohort, they represent a new and interesting finding, not reported in other populations tested so far.

Key words: COPD, lung cancer, TNF-α-308 promoter polymorphism

Chronic obstructive pulmonary disease (COPD) and lung cancer (LC) are a major cause of morbidity and mortality worldwide. The etiology of both diseases is multifactorial, including genetic and environmental factors, and smoking is regarded as the most important causal factor. Nevertheless, only a minority of smokers develop COPD or LC, which indicates a difference in susceptibility to tobacco smoke injury might be related to genetic factors [1, 2]. Additionally, smokers who have COPD appear to be at increased risk for developing LC, suggesting the link between processes that induce COPD and LC [3]. It has been suggested that inflammation of airways plays an important role in COPD pathogenesis and that might contribute to the lung cancer development [4, 5].

Tumor necrosis factor (TNF)-α is a multifunctional proinflammatory cytokine produced mainly by macrophages in the response to injury and inflammation. It controls inflammatory cell populations and mediates other aspects of inflammatory process. The increased level of TNF-α in bronchoalveolar lavage fluid of patients with COPD and LC has been observed [6, 7]. A single nucleotide polymorphism in the promoter of TNF-α gene, at the position -308, resulting in a guanine (the common allele donated as TNF-α-308*1) to adenine (the rare allele donated as TNF-α-308*2) substitution, has been associated with higher baseline and induced expression of TNF-α [8]. Therefore, this functional polymorphism is of particular interest as it might contribute to COPD or LC by initiating or maintaining airway inflammation. An association of TNF-α-308*2 allele with COPD was observed in Taiwanese and Japanese, but not in a Caucasians [9, 10, 11, 12, 13]. Although extensively studied in many types of cancer, the association of TNF-α-308*1/*2 polymorphism with LC susceptibility has been reported only in two recent studies performed in different populations. The association of TNF-α-308*2 allele with LC susceptibility and severity in Taiwanese population has been reported, opposite from the findings in German LC patients, where no association has been observed [14, 15].
In the view of above mentioned controversial results reported in different populations, for both COPD and LC, we performed a case-control study to ascertain whether the TNF-α-308*1/*2 gene promoter polymorphism influences the risk for COPD and LC in Serbian population.

Materials and methods

**Subjects and study design.** The patient groups consisted of 97 subjects with COPD and 70 subjects with LC recruited from University Clinical Center of Serbia and Zvezdara University Medical Center. The diagnosis of COPD was established based on medical history, physical examination, pulmonary function tests, blood gas analyses and chest radiography, according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD). The inclusion criteria were as follows: forced expiratory volume in one second (FEV1)/forced vital capacity (FVC) of <70%, postbronchodilator FEV1 of <80% of the predicted value. The diagnosis of LC was established based on medical history, physical examination, chest radiography and bronchoscopy. The diagnosis was confirmed by histopathological examination of lung biopsy sample. The cases included squamous cell carcinomas (30), adenocarcinomas (18), small cell carcinomas (19), large cell carcinomas (2) and pleomorphic lung carcinoma (1). Control group encompassed 102 subjects who had no clinical evidence of COPD or LC and showed normal pulmonary function (FEV1/FVC >70% and FEV1 >80% of the predicted value). The study was approved by local Ethics Committee and informed consent was obtained from each participant.

**Determination of genotype.** Genomic DNA was extracted from whole blood using GFX Genomic Blood DNA Purification Kit (Amersham Biosciences). The TNF-α-308*1/*2 polymorphism was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as previously described [10]. The DNA products were separated on a 3% agarose gel (3:1 NuSieve-Seakem, FMC) and visualized by ethidium bromide staining.

**Statistical methods.** Age, cumulative cigarette consumption (expressed as pack-years) and pulmonary function data were expressed as mean±SD. Clinical data of patient and control group were compared using Fisher’s exact test or Student’s t test, as appropriate. Deviations of genotypes’ distributions from Hardy-Weinberg equilibrium were assessed by χ²-test for each cohort. The distribution of TNF-α-308*2 allele variant among COPD and LC patients and control group was compared using Fisher’s exact test. Odds Ratios (ORs) and 95% Confidence Intervals (95%CI) for associations between genotypes and COPD or LC status were calculated by binary logistic regression model. The outcome variable was adjusted for age, sex and cumulative cigarette consumption. A p-value of less than 0.05 was considered significant. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences). In situation when significant differences were not observed, retrospective statistical power was calculated. Although it is not completely appropriate to analyze statistical power retrospectively, calculated post hoc power might be useful for planning future studies or for meta-analyses [16]. The Java Applets for Power and Sample Size was used for power calculation with significance level of 0.05 [17].

Results and Discussion

Today COPD and LC loom as two of the greatest challenges in pulmonary medicine. Both diseases cluster in families and worsen with age, and are often related to smoking and/or various occupational exposures. Although cumulative smoking history increases the risk for developing both diseases, it is important to recognize that the majority of smokers develop neither COPD nor LC. Smoking and occupational toxins, as well as community air pollution, may impose a series of accumulated and damaging mutations that ultimately inflame and destroy airways alveoli and also induce dysplastic and ultimately neoplastic changes in the lungs. TNF-α as a potent inflammatory cytokine that might influence LC and COPD through modulation of non-specific inflammation, has been chosen to be analyzed, on the genetic level, in Serbian population.

In this study 97 subjects with COPD, 70 subjects with LC and 102 controls were analyzed. The main characteristics and clinical parameters of patients and controls are shown in Table 1. The distribution of mean age, males and cumulative cigarette consumption was significantly higher in patient groups than in controls.

The obtained results indicated statistical significant difference (p=0.043) of TNF-α-308*1/*2 genotype distribution among COPD patients and controls. The frequency of TNF-α-308*1/*2 genotype was lower in patients with COPD (17.5%) compared with controls (27.4%), suggesting a protective role of this genotype in COPD development. According to our results COPD carriers of TNF-α-308*1/*2 genotype have 2.3-fold decreased risk for development of this disease (OR=0.44, 95%CI=0.20-0.97) (Table 2).

The association of the TNF-α-308*2 allele with a higher level of TNF gene expression in vitro has been shown and

| Table 1. The main characteristics of patients and control group. |
|-----------------|--------|--------|--------|
| Subjects, n     | 97     | 70     | 102    |
| Age, yr         | 59.3±14.8 | 58.7±7.5 | 50.5±13.8 |
| Sex, male/female| 65/32  | 57/13  | 37/65  |
| Smokers, %      | 79.1   | 91.4   | 51.0   |
| Smoking history, pack-years | 35.7±16.6 | 43.8±20.2 | 25.5±16.2 |
| FEV1 % pred.    | 44.4±21.2 | -      | 110.3±16.5 |
| FEV1/FVC        | 48.4±16.1 | -      | 81.7±8.0 |

Data are presented as mean±SD. FEV1: forced expiratory volume in one second; FVC: forced vital capacity; % pred: percentage of the predicted value
Table 2. The TNF-α-308*1/*2 genotype and allelic distribution in patients with COPD and LC and controls.

<table>
<thead>
<tr>
<th>Genotypes and alleles</th>
<th>COPD n=97 (%)</th>
<th>LC n=70 (%)</th>
<th>controls n=102 (%)</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>COPD vs. control group</td>
</tr>
<tr>
<td>*1/*1</td>
<td>79 (81.5)</td>
<td>57 (81.4)</td>
<td>71 (69.6)</td>
<td>0.44†</td>
</tr>
<tr>
<td>*1/*2</td>
<td>17 (17.5)</td>
<td>13 (18.6)</td>
<td>28 (27.4)</td>
<td>(0.20-0.97)</td>
</tr>
<tr>
<td>*2/*2</td>
<td>1 (1.0)</td>
<td>-</td>
<td>3 (3.0)</td>
<td>0.52</td>
</tr>
<tr>
<td>*2</td>
<td>19 (19.8)</td>
<td>13 (19.3)</td>
<td>34 (33.7)</td>
<td>0.54</td>
</tr>
</tbody>
</table>

The distribution of TNF-α-308*1/*2 polymorphism was in Hardy-Weinberg equilibrium for each group. † Adjusted for age, sex and cumulative cigarette consumption; §p=0.043; ‡p=0.054; £p=0.056.

Therefore studied in various inflammatory, autoimmune and malignant diseases [8]. However, correlation between TNF-α-308*2 gene variant and ex vivo TNF-α production in white blood cells in cystic fibrosis patients as well as in vivo TNF-α release from sarcoid bronchoalveolar lavage cells was not confirmed [18, 19].

So far, the role of TNF-α-308*1/*2 polymorphism in COPD development was analyzed in different populations, but obtained results were controversial. An association of TNF-α-308*2 allele was found in Taiwanese chronic bronchitis patients and Japanese COPD patients [9, 10]. However, another study in Japanese population did not confirm association of this polymorphism and COPD status [20]. Investigations of this polymorphism in Caucasian population revealed that it was not associated with susceptibility and severity of COPD [11, 12, 13, 21, 22].

The frequency of TNF-α-308*2 allele was higher in control group (16.7%) than in patients with COPD and LC (9.8% and 9.3% respectively), but statistical significance was not achieved (Table 2). The TNF-α-308*1/*2 genotype and allelic frequencies in our control group were similar as those found in English and German control populations, while Italian control population had decreased frequency of this variation [12, 13, 21]. However, the TNF-α-308*2 allelic frequency in our control group was lower than in English and German COPD patients. Since the etiology of COPD is affected with multiple genetic factors and their interaction with environment, the relationship between genetic polymorphism and COPD development might be ethnic dependent. Although COPD represents a complex disease, with variety of phenotypes, it is unlikely that our result is a product of population stratification due to the same ethnic background. Another possible explanation of our results is that the TNF-α-308*2 allele may be in linkage disequilibrium with another variant responsible for protective role of COPD development. This relates to the location of TNF-α gene within a highly polymorphic major histocompatibility complex Class III region on chromosome 6p21.3.

In patients with lung cancer, we observed a decreased distribution of TNF-α-308*1/*2 polymorphism in comparison to control group, but statistical significance was not achieved (Table 2). However, small sample size and low statistical power of 0.21 of LC group might be a reason for the lack of significance, even if genotype and allelic frequencies of TNF-α-308*1/*2 polymorphism in LC patients corresponded to those observed in COPD group.

TNF-α is expressed in a range of human tumors and its presence is generally associated with poor prognosis [23]. An association of TNF-α-308*2 allele and different malignant tumors was observed [24]. This polymorphism was associated with higher TNF-α mRNA and serum level, as well as with tumor grade in patients with bladder tumor [25]. On the other hand, TNF-α is crucial in killing of some tumor cells and TNF high dose therapeutic administration can induce apoptosis and necrosis of tumor cells, which partially might explain decreased frequency of TNF-α-308*2 allele in LC group [23].

The role of TNF-α-308*1/*2 polymorphism has not been extensively studied in patients with LC. The study in German population found similar genotypes’ distributions among LC patient and controls [15]. On the contrary, an investigation in Taiwan population revealed the TNF-α-308*2 allele as a risk factor for LC susceptibility and severity [14].

Our results are in consistency with several studies conducted on different malignant diseases. Significant increase of the TNF-α-308*1 allele in a group of patients with chronic lymphocytic leukaemia was reported as well as an association with susceptibility to oral squamous cell carcinoma [26, 27]. It was suggested that TNF-α-308*2 allele had a protective effect against oral squamous cell carcinoma, possibly by increasing of TNF-α production [27]. In addition, the high producing allele TNF-α-308*2 was significantly underrepresented among patients diagnosed with specific pre-cancerous condition of oral cancer [28]. On the contrary, patients with hepatocellular carcinoma had significantly higher frequency of TNF-α-308*1/*2 genotype [29].

To our knowledge, this is the first study of TNF-α-308*1/*2 gene variant in COPD, LC patients and control group with the same ethnic background. Both groups of patients showed...
decreased frequency of TNF-α-308*1/*2 polymorphism than observed in controls, and this decrease was significant in COPD patients (OR=0.44, 95%CI=0.20-0.97). Poor statistical power is a major limitation of our study, and caution in interpretation of our findings is recommended. On the other hand, small scale studies, such as the one presented here, may be useful for planning future investigations providing information for large scale studies concerning proper study design, sample size and relevant clinical information or for meta-analyses. The results of our study, although conducted on small sample size, might be useful for new research directions concerning better understanding of TNF-α role in both diseases. Based on our findings, we propose replication in large series of COPD and LC patients with the same ethnic background in well-organized and orchestrated collaborative study with enough statistical power to detect the association. Although our results need to be confirmed on larger cohort, they represent a new and interesting finding, not reported in other populations tested so far.

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References


