Research article

Tumor Necrosis Factor-α +489G/A gene polymorphism is associated with chronic obstructive pulmonary disease

Mehmet Küçükaycan[1], Michiel Van Krugten[2], Herman-Jan Pennings[3], Tom WJ Huizinga[2], Wim A Buurman[4], Mieke A Dentener[1] and Emiel FM Wouters[1]

[1]Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Department of Pulmonology, Maastricht University, Maastricht, The Netherlands
[2]Department of Rheumatology, Leiden University Medical Centre, Leiden, The Netherlands
[3]Hornerheide, Pulmonary Rehabilitation Centre, Horn, The Netherlands
[4]Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Department of Surgery, Maastricht University, Maastricht, The Netherlands

Correspondence: Emiel FM Wouters - ewo@slon.azm.nl

Received: 1 November 2001
Revisions requested: 14 January 2002
Revisions received: 22 July 2002
Accepted: 13 August 2002
Published: 29 November 2002

Respir Res 2002, **3**:29

(Print ISSN 1465-9921; Online ISSN 1465-993X)

Abstract

Background: Chronic obstructive pulmonary disease (COPD) is characterized by a chronic inflammatory process, in which the pro-inflammatory cytokine Tumor Necrosis Factor (TNF)-α is considered to play a role. In the present study the putative involvement of *TNF*-α gene polymorphisms in pathogenesis of COPD was studied by analysis of four *TNF*-α gene polymorphisms in a Caucasian COPD population.
Methods: *TNF*-α gene polymorphisms at positions -376G/A, -308G/A, -238G/A, and +489G/A were examined in 169 Dutch COPD patients, who had a mean forced expiratory volume in one second (FEV1) of 37 ± 13%, and compared with a Dutch population control group of 358 subjects.
Results: The data showed that the *TNF*-α +489G/A genotype frequency tended to be different in COPD patients as compared to population controls, which was due to an enhanced frequency of the GA genotype. In line herewith, carriership of the minor allele was associated with enhanced risk of development of COPD (odds ratio = 1.9, p = 0.009). The other *TNF*-α gene polymorphisms studied revealed no discrimination between patients and controls. No differences in the examined four *TNF*-α polymorphisms were found between subtypes of COPD, which were stratified for the presence of radiological emphysema. However, comparison of the COPD subtypes with controls showed a significant difference in the *TNF*-α +489G/A genotype in patients without radiological emphysema (χ^2-test: $p < 0.025$ [Bonferroni adjusted]), while no differences between COPD patients with radiological emphysema and controls were observed.
Conclusion: Based on the reported data, it is concluded that COPD, and especially a subgroup of COPD patients without radiological emphysema, is associated with *TNF*-α +489G/A gene polymorphism.

Keywords: Caucasians, COPD, Gene polymorphism, Susceptibility, Tumor necrosis factor-α

Introduction

The pro-inflammatory cytokine Tumor Necrosis Factor (TNF)-α plays an important role in inflammatory processes. The gene coding for this cytokine is located on chromosome six in the class III region of the major histocompatibility complex. Several biallelic polymorphisms of this gene are known, including the *TNF*-α -308G/A gene polymorphism, which is the first discovered *TNF*-α gene polymor-

phism, the *TNF*-α -376G/A and the -238G/A gene polymorphisms. The latter gene polymorphisms are located in the promoter region of the gene, whereas another gene polymorphism found at position +489G/A, is located in the first intron of the gene [1–3].

Several studies have analyzed the relationship between *TNF*-α gene polymorphism and disease. Carriage of the *TNF*-α -308A allele was found to be associated with diseases such as melioidosis [4] mucocutaneous leishmaniasis [5] and cerebral malaria [6], although no association could be demonstrated with other disorders like meningococcal disease [7] and rheumatoid arthritis (RA) [8]. Contradictory results are reported on the relationship between *TNF*-α -308G/A polymorphism and asthma. Several studies found an association of this disease with the *TNF*-α -308 minor allele [9,10], whereas Albuquerque and colleagues reported an association of the *TNF*-α -308 common allele with childhood asthma [11]. Moreover, Louis et al failed to show any relationship between the *TNF*-α -308G/A polymorphism and asthma [12].

Concerning the *TNF*-α -238G/A polymorphism, a higher frequency of the A allele was shown in multiple sclerosis [13], and alcoholic liver disease [14], whereas others reported no association with Chagas' disease [15] and coal workers pneumoconiosis [16]. The *TNF*-α -376G/A gene polymorphism was reported to be an independent risk factor for the development of cerebral malaria [17], whereas this polymorphism was not associated with autoimmune diseases like RA [18]. Finally, a limited number of studies have examined the *TNF*-α +489G/A polymorphism, showing an association with prostate cancer, and with a subgroup of common variable immune deficiency patients with granulomata [19,20]. In contrast, a lower frequency of the minor allele was reported in RA patients, whereas in patients with idiopathic pulmonary fibrosis no association with *TNF*-α +489G/A polymorphism was observed [21,22].

In order to study the functionality of the *TNF*-α gene polymorphisms, in vitro studies have been performed. Some of those studies showed that cell lines with the minor *TNF*-α -308A allele produced more TNF-α compared with the cell lines carrying the -308G allele [23,24], whereas other authors studying the relation between TNF-α production and *TNF*-α gene polymorphisms at position -862C/A, -856C/T, -574G/A, -308G/A, -238G/A and +70(addition of C) failed to show an association [13,25,26]. Therefore, it appears that the relationship between *TNF*-α gene polymorphism and TNF-α production is yet unresolved.

Chronic inflammation is generally accepted as a characteristic finding in COPD patients, and is considered to contribute to pathology. Enhanced levels of TNF-α have been detected in sputum [27] and in circulation [28] of COPD patients, indicating that this cytokine is involved in both the local and systemic inflammation present in COPD. Analysis of possible *TNF*-α gene polymorphisms in COPD could be related to a higher susceptibility to develop this disabling pathological condition. Previously, a relationship between COPD and *TNF*-α -308G/A polymorphisms was reported in a Taiwanese [29] and a Japanese population [30]. Recent studies with Caucasian COPD populations did not support these data [31–33], although it has been suggested that homozygosity for the A allele predisposes for a worse prognosis in COPD [33]. In the present study the putative involvement of *TNF*-α gene polymorphisms in relation to COPD was extended by analysis of these polymorphisms at locations -376G/A, -308G/A, -238G/A and +489G/A, which are so far the most studied polymorphisms, and were reported, as described above, to be associated with certain diseases. The study was performed in a Caucasian COPD population, stratified for the presence of radiological emphysema based on high resolution computed tomography (HRCT) scanning.

Materials and Methods

Study Population and Control Population

The patient group consisted of 169 Caucasian Dutch COPD patients admitted to a pulmonary rehabilitation center (Horn, The Netherlands). The diagnosis of COPD was made according to the criteria of the American Thoracic Society [34]. The forced expiratory volume in one second (FEV1) had to be less than 70% of the reference value, and the increase in FEV1 after inhalation of a β_2-agonist less than 10% of the reference value. Patients with α1-antitrypsin deficiency, and patients with bronchial asthma were excluded from the study.

358 random healthy Dutch Caucasian donors from the Department of Immunohaematology and Blood Transfusion (Leiden, The Netherlands) were used as a population control group. Although recruited from different parts of The Netherlands, population stratification is unlikely to be an issue because of the small size of this country.

The local ethical review committee approved the research program, and informed consent was obtained.

DNA Isolation and TNF-α gene Polymorphism Typing

The four *TNF*-α gene polymorphisms located on positions -376G/A, -308G/A, -238G/A and +489G/A, relative to the transcriptional start site of the *TNF*-α gene, were detected in genomic DNA derived from peripheral blood leukocytes, as described previously [13,35]. In short, fragments of the *TNF*-α promoter gene were amplified by PCR using primer 'C', spanning -164/-144 (5'-TCTCGGTTTCTTCTCCATCG-3') and primer 'D' spanning -675/-655 (5'-GAGTCTCCGGGTCAGAATGA-3'). The amplified DNA segments were analysed by dot blot analysis using specific

biotin labelled probes to detect the G or the A variant of the three polymorphisms in the promoter region. To type for *TNF-α* +489G/A polymorphism a PCR product was generated using primer 'A' spanning +48/+67 (5'-GGAGAGAAGCAACTACAGAC-3') and primer 'B' spanning +598/+579 (5'-CACACTTAGTGAGCACCTTC-3'). PCR products were digested with *Tai I* (MBI Fermentas, St. Leon-Rot, Germany) and size separated on 1.2% agarose gel. In the PCR product derived from individuals carrying the normal allele two *Tai I* restriction sites are present, giving rise to fragments of 111, 159 and 281 bp. The +489 G to A substitution leads to loss of one *Tai I* restriction site resulting into the formation of two fragments of 159 and 392 bp after digestion.

Computed Tomography (CT) Emphysema Score

In all patients evaluation of the presence and severity of parenchymal destruction, the hallmark of emphysema, was performed by HRCT using a commercial scan (Somatom Plus; Siemens, Erlangen, Germany). For both the right and the left lung, five thin-section HRCT scans were obtained at full inspiration with the patient in supine position. Two scans of the upper zones were made at 3 cm and 6 cm above the carina, two scans of the lower zones at 3 cm and 6 cm below the carina, and one at the level of the carina. Scanning parameters were 1.0mm collimation, 137 kVp, 220 mA, 1.0 second scanning time, and high-resolution reconstruction algorithm (level B800 HU; width: 1600). The grade of radiological emphysema was assessed by visual CT emphysema score according to Sakai et al. [36], which is based on the assessment of two aspects of emphysema: severity and extent. Severity was graded on a 4-point scale: 0, no emphysema; 1, low attenuation areas < 5 mm in diameter; 2, circumscribed low attenuation areas > 5 mm in addition to those < 5 mm; 3, diffuse low attenuation areas without normal intervening lung. The extent of emphysema was on a 4-point scale: 1, $< 25\%$ of the lung parenchyma involved; 2, 25–50% involvement; 3, 50–75% involvement; 4, $> 75\%$ involvement. For each of the ten lung fields, the score for severity (4-point scale ranging from 0–3) was multiplied by that for extent (4-point scale ranging from 1–4) to give a degree of radiological emphysema score. The score for the ten lung fields were summed. The potential maximal score for each patient was 120. A CT emphysema score of < 30 is considered as having limited or no radiological emphysema [37]. All patients with radiological emphysema (CT score > 30) were taken together and they were compared with the group considered as having no radiological emphysema (CT score < 30).

Pulmonary Function

Lung function was measured by an experienced lung function technician using a pneumotachometer (Jaeger 7, Würzburg, Germany). All measurements were performed according to the European Respiratory Society recommendations [38]. The highest value from at least three technically acceptable spirometric manoeuvres was used.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). The Mann-Whitney U test was used for differences in age and FEV1 between groups. The χ^2-test was used to detect significant differences in genotype frequencies and sex. A Bonferroni correction was applied in multiple comparisons between study groups. Odds ratios (OR) with 95% confidence interval (CI) were calculated to estimate the potential risk for developing COPD among carriers (GA) versus non-carriers (GG) of the minor allele (uncorrected p; Fisher exact when needed). P-values smaller than 0.05 were taken as significantly different. In case of performing the Bonferroni correction, statistical significance was defined as $p < 0.05/k$, where k is the number of parameters for the Bonferroni correction in each set of comparisons. The data were analysed with the Statistical Package for the Social Sciences 10.0 computer program (SPSS Inc., Chicago, IL)

Results

Characteristics of the COPD populations

A Caucasian population of 169 Dutch COPD patients was studied (mean age 66 ± 8 years, range between 39–88 years). Male COPD patients constituted 65% of the COPD population. The spirometric data indicated that the patients suffered from severe airflow limitation (FEV1 predicted: mean 37 ± 13%, range 14–69%). Forty-nine patients (29%) were current smokers, 115 patients (68%) were ex-smokers and only 5 patients (3%) indicated that they had never smoked. The mean number of pack years of the current and ex-smokers was 33 ± 19 years (range 2–90). Maintenance treatment of the patients consisted in general of inhalation corticosteroids, β2-agonists and anticholinergics (ipratropium bromide). Oral corticosteroids were prescribed in 60% of the patients and oral theophylline in 55%. Continuous oxygen was used by 20% of the patients.

The population control group consisted of 358 random healthy Dutch Caucasian subjects derived from an anonymous panel of blood donors, with a mean age of 40 ± 17 years ($p < 0.025$ [Bonferroni adjusted] as compared to COPD population). Fifty seven percent of the controls were males, which was not different from the patient population ($p = 0.1$). No further characteristics of this population control group were available.

TNF-α gene polymorphism and susceptibility to develop COPD

The genotype frequencies of four *TNF-α* gene polymorphisms in the COPD population were compared with those in control subjects in order to assess differences between the two groups (Table 1). In both the patient and the control groups the alleles at the individual loci of the *TNF-α* gene

Table 1

Genotype frequencies of *TNF*-α -376G/A, -308G/A, -238G/A and +489G/A gene polymorphisms in COPD patients and population control group*

Genotype	COPD	Control	P-value †
TNF -376G/A			
GG	162 (98)	306 (99)	
GA	3 (2)	3 (1)	0.562
AA	0 (0)	1 (0)	
TNF -308G/A			
GG	113 (69)	237 (71)	
GA	49 (30)	91 (27)	0.397
AA	1 (1)	7 (2)	
TNF -238G/A			
GG	151 (92)	310 (94)	
GA	13 (8)	20 (6)	0.544
AA	0 (0)	1 (0)	
TNF +489G/A			
GG	118 (75)	264 (84)	
GA	38 (24)	45 (14)	0.019
AA	1 (1)	6 (2)	

* Values are expressed as absolute numbers; percentages are given in parentheses. For technical reasons, not all the samples were successfully genotyped. † The χ^2-test of the genotype frequencies of *TNF*-α -376G/A, -308G/A, -238G/A, and +489G/A gene polymorphisms. Statistical significance was defined as $p < 0.0125$ using Bonferroni correction

were in Hardy-Weinberg equilibrium, with non-significant χ^2-values (data not shown). For *TNF*-α +489G/A gene polymorphism, a tendency for a difference in genotype frequency between COPD patients and controls was observed ($p = 0.019$), which was due to enhanced GA frequency in the patient group. No differences were obtained for the three other polymorphisms tested at *TNF*-α gene locus position -376G/A, -308G/A and -238G/A. Since the control group was younger than the patient population, as reported above, the *TNF*-α +489G/A genotype frequency in the COPD patients was also compared with a subgroup of the control subjects of forty years and older ($n = 165$, age 53 ± 9 years), which revealed a significant difference ($p = 0.011$). Next the association between carriership of the *TNF*-α +489A allele and susceptibility to develop COPD was analysed using the whole population control group. This analysis revealed that carriers (GA) had twice the risk of developing COPD as compared to non-carriers (GG) (OR = 1.9 [95% CI = 1.2-3.1; $p = 0.009$]), which strengthens the above reported observations.

In order to analyse whether subtypes of COPD were related to *TNF*-α gene polymorphism, the COPD population was stratified for the presence of radiological emphysema based on HRCT scanning (Table 2). In 32 (20%) out of the 159 patients who underwent HRCT, no radiological emphysema could be detected, whereas in 80% of the patients radiological emphysema was present. Both subgroups of patients did not differ in age or sex. However, FEV1% predicted was significantly lower in the patients with radiological emphysema as compared to the subgroup without radiological emphysema. Strikingly, four out of the five patients who had never smoked were part of the subgroup without radiological emphysema, representing 12.5% of the patients. In contrast, only one of the 127 patients with radiological emphysema had never smoked (χ^2-test, $p < 0.01$ [Bonferroni adjusted]). In addition, the mean pack years tended to be higher in the subgroup of patients with emphysema.

No significant differences were observed between the subgroups of patients concerning *TNF*-α polymorphisms at position -376G/A ($p = 0.477$), -308G/A ($p = 0.334$), -238G/A ($p = 0.207$), and +489G/A ($p = 0.184$). However, as shown in Table 3, comparison of *TNF*-α +489G/A genotype between patients without radiological emphysema and population controls revealed a significant difference ($p = 0.004$, thus $p < 0.025$ [Bonferroni adjusted]), whereas no difference between control subjects and patients with radiological emphysema was observed ($p = 0.131$). In line herewith, analyses of the contribution of the *TNF*-α +489A allele (via comparison of GA genotype versus GG genotype) in the risk of the development of COPD revealed an OR of 3.6 (95% CI = 1.6 - 8.1; $p = 0.003$) in the subgroup of patients without radiological emphysema, while analysis of the COPD patients with radiological emphysema and controls revealed an OR of 1.7 (95% CI = 0.97 - 2.8; $p = 0.064$).

Table 2

Clinical characteristics of subgroups of COPD patients without and with radiological emphysema *

	Patients without radiological emphysema (n = 32)	Patients with radiological emphysema (n = 127)	P-value †
Age (year)	66 ± 8	66 ± 8	0.70
Sex (male/female)	16/16	86/41	0.062
FEV1% predicted	46 ± 15	35 ± 11	0.001
Smoking behavior			
-never smoked/(ex)-smoker	4/28	1/126	0.001
-pack years	26 ± 22	34 ± 18	0.037

* Values are expressed as mean ± SD or as absolute numbers. † The Mann-Whitney U test was used for differences in age and FEV1 between groups. The χ^2-test was used to detect significant differences in genotype frequencies and sex. Statistical significance was defined as $p < 0.01$ using Bonferroni correction.

Discussion

The present study describes the relation between COPD and four TNF-α gene polymorphisms in a Dutch Caucasian population. The data showed that the TNF-α +489G/A genotype frequency tended to be different in COPD patients compared with a population control group, while no significant differences could be demonstrated in the TNF-α gene at position -376G/A, -308G/A, and -238G/A. Since the population control group was younger than the COPD population, a subgroup of control subjects of forty years and older was selected, and analysis revealed significant differences in TNF-α +489G/A genotype frequency as compared with COPD patients. Moreover, carriership of the TNF-α +489A allele was associated with enhanced risk of developing COPD (OR = 1.9). The higher frequency of TNF-α +489G/A polymorphism in the COPD population could be related to an increased percentage of individuals with TNF-α +489 GA genotype, compared with healthy controls, whereas frequency of the +489 AA was too low (1% in patients versus 2% in controls) to allow meaningful comparisons. Based upon HRCT score, a technique that has generally been accepted to detect emphysema accurately at relatively early stage, and to allow objective measurements of the degree of abnormality [37,39], patients were divided in those with and without radiological emphysema. Since HRCT does not detect microscopic emphysema, it cannot be excluded that patients classified as having no radiological emphysema, could have microscopic emphysema. This indicates that in this study patients with more severe emphysema (diagnosed by the presence of radiological emphysema) were compared with patients with less severe or no emphysema (diagnosed by the absence of radiological emphysema), which is also supported by the observed differences in FEV1 in both subgroups. The differences in TNF-α +489G/A polymorphism seem to be related to a higher prevalence of this polymorphism in the subgroup of patients without radiological emphysema. Interestingly, four out of the 32 (12.5%) patients from this subgroup had never smoked. Of those four patients three exhibited the heterozygote genotype (data not shown). These data strongly suggest TNF-α +489G/A polymorphism as a risk factor for COPD. Further studies have to be performed to confirm this observation, analysing large panels of patients with smoking and non-smoking related COPD. In addition, the use of a control population of more comparable age and for whom smoking data was available would further enhance the strength of these kinds of studies.

The gene polymorphism of *TNF*-α +489G/A was described for the first time in 1996 and is located in the first intron of the gene [3]. Till now, limited numbers of studies have examined a possible relationship between this polymorphism and pathologic conditions. In patients with prostate cancer an increased incidence of *TNF*-α +489 GA genotype was reported, implicating that changes at *TNF*-α +489G/A may be involved in oncogenesis of prostate cancer [19]. Furthermore, the minor *TNF* +489A allele was associated with a subgroup of common variable immune deficiency patients with granulomata [20]. Contrary to these studies, and to the present findings in COPD, a significantly lower frequency of the *TNF*-α +489 GA and *TNF*-α +489 AA genotypes was reported in RA patients as compared with controls. Those RA patients carrying the minor +489A allele also showed a less severe course [21]. Furthermore, in patients with idiopathic pulmonary fibrosis no association with *TNF*-α +489G/A polymorphism was observed [22]. Therefore, further research is needed to elucidate the impact of this *TNF*-α gene polymorphism on susceptibility and severity of inflammatory diseases.

Recently, Kaijzel et al investigated the functional consequence of the +489G/A polymorphism, by analysing the contribution of distinct *TNF*-α alleles in TNF pre-mRNA production. No difference could be detected between the different alleles in TNF-α pre-mRNA yield upon in vitro and

Table 3

Genotype frequencies of *TNF*-α +489 G/A gene polymorphism in subgroups of COPD patients versus population control group*

	GG	GA	AA	P-value † Patients versus controls
Patients without radiological emphysema	18 (62)	11 (38)	0	0.004
Patients with radiological emphysema	92 (77)	26 (22)	1 (1)	0.131
Population controls	264 (84)	45 (14)	6 (2)	

* Values are expressed as absolute numbers; percentages are given in parentheses. For technical reasons, not all the samples were successfully genotyped. † χ^2-test of the genotype frequencies of *TNF*-α +489G/A gene polymorphism. Statistical significance was defined as $p < 0.025$ using Bonferroni correction.

physiological stimulation conditions, in healthy individuals and RA patients [35]. Therefore, no indications for a functional significance for this particular *TNF*-α gene variant have been demonstrated so far. In general, an association between a specific disease and an allele can be interpreted either as a causal relation or as a linkage disequilibrium between the allele and a nearby gene or a set of genes involved in the pathophysiology of that disease. The question of whether the minor *TNF*-α +489A allele itself is related to a higher susceptibility for developing COPD or that the allele has a linkage disequilibrium with a nearby causal gene also remains to be determined. The *TNF* gene is located in the class III region of the MHC, on chromosome 6p. In this region several genes are present that encode for proteins involved in immune and inflammatory responses, which could therefore be potential candidates to be studied. These genes include the complement system proteins C2, C4 and Factor B, lymphotoxin (LT) α and β, as well as members of the 70 kDa heat shock protein (hsp70) family [40]. As well as studying single nucleotide polymorphisms, extended haplotypes of genes in this region should also be analysed, since it is unlikely that only one gene locus is responsible for predisposition to and clinical outcome of the disease. The only *TNF*-α gene polymorphism that has been examined in COPD is the *TNF*-α gene polymorphism on location -308G/A. In line with our data, studies in Caucasian populations showed no association between COPD and *TNF*-α -308G/A polymorphism [31–33]. However, Keatings et al, showed that homozygosity for the A allele predisposes to a worse prognosis for COPD, implicating that an increase in A allele in the COPD group may have been missed due to survival bias in the GA and GG groups [33]. In contrast to these studies on white subjects, two studies on Asian subjects showed an association at *TNF*-α -308G/A polymorphism between COPD patients and population controls [29,30]. The possible cause for this discrepancy may be an ethnic difference affecting prevalence. The *TNF*-α-308A allele frequency ranged from 10% [32] to 17% [31] in the white population, and from 5% [29] to 8% [30] in the Asian population. Alternatively, the allele could be in linkage disequilibria with another gene that increases susceptibility to COPD in smokers only in the Asian population.

The allele frequencies as reported in our study of *TNF*-α -376A (1% in both study groups), *TNF*-α -238A (3% and 4% in controls and COPD respectively), and *TNF*-α +489A (12% and 9% respectively in controls and COPD) are in agreement with other studies analyzing Caucasian populations [3,13,18,22]. However, due to the fact that the uncommon allele at gene loci -376 and -238 is very rare, we cannot exclude the possibility of a type II error (the study population was too small to detect a difference).

Conclusion

In conclusion, from the present study it is established that the *TNF*-α +489G/A gene polymorphism is associated with COPD, especially in a subgroup of patients without radiological emphysema. In contrast, the genotype frequencies of *TNF*-α -376G/A, -308G/A and -238G/A gene polymorphisms in the COPD population were not different from a population control group. The impact of this *TNF*-α +489G/A gene polymorphism in relation to susceptibility of COPD and impact on the inflammatory processes in COPD needs to be explored.

Abbreviations

A = Adenine, bp = base pairs, CI = confidence interval, COPD = chronic obstructive pulmonary disease, FEV1 = forced expiratory volume in one second, G = Guanine, HRCT = high resolution computed tomography, OR = odds ratio, RA = Rheumatoid arthritis, SD = standard deviation, TNF = Tumor Necrosis Factor

Acknowledgements

We wish to thank M. Nio for isolation of the DNA samples, and Dr. A.M.W.J. Schols for her statistical help.

This study was financially supported by a grant from GlaxoSmithKline, The Netherlands.

References

1. Wilson AG, di Giovine FS, Blakemore AI, Duff GW: **Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product.** *Hum Mol Genet* 1992, **1**:353

2. Hamann A, Mantzoros C, Vidal-Puig A, Flier JS: **Genetic variability in the TNF-alpha promoter is not associated with type II diabetes mellitus (NIDDM).** *Biochem Biophys Res Commun* 1995, **211:**833-839
3. D'Alfonso S, Richiardi PM: **An intragenic polymorphism in the human tumor necrosis factor alpha (TNFA) chain-encoding gene.** *Immunogenetics* 1996, **44:**321-322
4. Nuntayanuwat S, Dharakul T, Chaowagul W, Songsivilai S: **Polymorphism in the promoter region of tumor necrosis factor-alpha gene is associated with severe meliodosis.** *Hum Immunol* 1999, **60:**979-983
5. Cabrera M, Shaw MA, Sharples C, Williams H, Castes M, Convit J, Blackwell JM: **Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis.** *J Exp Med* 1995, **182:**1259-1264
6. McGuire W, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D: **Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria.** *Nature* 1994, **371:**508-510
7. Westendorp RGJ, Langermans JAM, Huizinga TWJ, Verweij CL, Sturk A: **Genetic influence on cytokine production in meningococcal disease.** *Lancet* 1997, **349:**1912-1913
8. Brinkman BM, Huizinga TW, Breedveld FC, Verweij CL: **Allele-specific quantification of TNFA transcripts in rheumatoid arthritis.** *Hum Genet* 1996, **97:**813-818
9. Moffatt MF, Cookson WOCM: **Tumour necrosis factor haplotypes and asthma.** *Hum Mol Gen* 1997, **6:**551-554
10. Chagani T, Paré PD, Zhu S, Weir TD, Bai TR, Behbehani NA, Fitzgerald JM, Sandford AJ: **Prevalence of tumor necrosis factor-α and angiotensin converting enzyme polymorphisms in mild/moderate and fatal/near fatal asthma.** *Am J Respir Crit Care Med* 1999, **160:**278-282
11. Albuquerque RV, Hayden CM, Palmer LJ, Laing IA, Rye PJ, Gibson NA, Burton PR, Goldblat J, Lesouëf : **Association of polymorphisms within the tumour necrosis factor (TNF) genes and childhood asthma.** *Clin Exp Allergy* 1998, **28:**578-584
12. Louis R, Leyder E, Malaise M, Bartsch P, Louis E: **Lack of association between adult asthma and the tumour necrosis factor α-308 polymorphism gene.** *Eur Respir J* 2000, **16:**604-608
13. Huizinga TW, Westendorp RG, Bollen EL, Keijsers V, Brinkman BM, Langermans JA, Breedveld FC, Verweij CL, van de Gaer L, Dams L, Crusius JB, Garcia-Gonzalez A, van Oosten BW, Polman CH, Pena AS: **TNF-alpha promoter polymorphisms, production and susceptibility to multiple sclerosis in different groups of patients.** *J Neuroimmunol* 1997, **72:**149-153
14. Grove J, Daly AK, Bassendine MF, Day CP: **Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis.** *Hepatology* 1997, **26:**143-146
15. Beraun Y, Nieto A, Collado MD, Gonzalez A, Martin J: **Polymorphisms at tumor necrosis factor (TNF) loci are not associated with Chagas' disease.** *Tissue Antigens* 1998, **52:**81-83
16. Zhai R, Jetten M, Schins RP, Franssen H, Borm PJ: **Polymorphisms in the promoter of the tumor necrosis factor-alpha gene in coal miners.** *Am J Ind Med* 1998, **34:**318-324
17. Knight JC, Udalova I, Hill AV, Greenwood BM, Peshu N, Marsh K, Kwiatkowski D: **A polymorphism that affects OCT-1 binding to the TNF promoter region is associated with severe malaria.** *Nature Genet* 1999, **22:**145-150
18. Kaijzel EL, van Krugten MV, Brinkman BM, Huizinga TW, van der Straaten T, Hazes JM, Ziegler-Heitbrock HW, Nedospasov SA, Breedveld FC, Verweij CL: **Functional analysis of a human tumor necrosis factor alpha (TNF-alpha) promoter polymorphism related to joint damage in rheumatoid arthritis.** *Mol Med* 1998, **4:**724-733
19. Oh BR, Sasaki M, Perinchery G, Ryu SB, Park YI, Caroll P, Dahiya R: **Frequent genotype changes at -308, and 488 regions of the tumor necrosis factor-α (TNF-α) gene in patients with prostate cancer** *J. Urol* 2000, **163:**1584-1587
20. Mullighan CG, Fanning GC, Chapel HM, Welsh KI: **TNF and lymphotoxin-alpha polymorphisms associated with common variable immunodeficiency: role in the pathogenesis of granulomatous disease.** *J Immunol* 1997, **159:**6236-6241
21. Van Krugten M, Huizinga T, Kaijzel E, Zanelli E, Drossaers-Bakker K, van de Linde P, Hazes J, Zwinderman A, Breedveld F, Verweij C: **Association of the TNF +489 polymorphism with susceptibilty and radiographic damage in rheumatoid arthritis patients.** *Genes Immun* 1999, **1:**91-96
22. Pantelidis P, Fanning F, Wels AU, Welsh KI, Du Bois RM: **Analysis of tumor necrosis factor-α, lymphotoxin-α, tumor necrosis factor receptor II, and interleukin-6 polymorphisms in patients with idiopathic pulmonary fibrosis.** *Am J Respir Crit Care Med* 2001, **163:**1432-1436
23. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW: **Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation.** *Proc Natl Acad Sci U S A* 1997, **94:**3195-3199
24. Kroeger K, Carville K, Abraham L: **The -308 Tumor Necrosis Factor-a promotor polymorphism effects transcription.** *Mol Immunol* 1997, **34:**391-399
25. Brinkman BM, Zuijdeest D, Kaijzel EL, Breedveld FC, Verweij CL: **Relevance of the tumor necrosis factor alpha (TNF alpha) -308 promoter polymorphism in TNF alpha gene regulation** *J Inflamm* 1995, **46:**32-41
26. Uglialoro AM, Turbay D, Pesavento PA, Delgado JC, McKenzie FE, Gribben JG, Hartl D, Yunis EJ, Goldfeld AE: **Identification of three new single nucleotide polymorphisms in the human tumor necrosis factor-α gene promoter.** *Tissue Antigens* 1998, **52:**359-367
27. Keatings VM, Collins PD, Scott DM, Barnes PJ: **Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma.** *Am J Respir Crit Care Med* 1996, **153:**530-534
28. Takabatake N, Nakamura H, Abe S, Inoue S, Hino T, Saito H, Yuki H, Kato S, Tomoike H: **The relationship between chronic hypoxemia and activation of the tumor necrosis factor-alpha system in patients with chronic obstructive pulmonary disease.** *Am J Respir Crit Care Med* 2000, **161:**1179-1184
29. Huang SL, Su CH, Chang SC: **Tumor necrosis factor-alpha gene polymorphism in chronic bronchitis.** *Am J Respir Crit Care Med* 1997, **156:**1436-1439
30. Sakao S, Tatsumi K, Igari H, Shino Y, Shirasawa H, Kuriyama T: **Association of tumor necrosis factorα gene promoter polymorphism with the presence of chronic obstructive pulmonary disease.** *Am J Respir Crit Care Med* 2001, **163:**420-422
31. Higham M, Pride N, Alikhan A, Morrell M: **Tumor necrosis factor-a gene promoter polymorphism in chronic obstructive pulmonary disease.** *Eur Respir J* 2000, **15:**281-284
32. Patuzzo C, Gile LS, Zorzetto M, Trabetti E, Malerba G, Pignatti PF, Luisetti M: **Tumor necrosis factor gene complex in COPD and disseminated bronchiectasis.** *Chest* 2000, **117:**1353-1358
33. Keatings VM, Cave SJ, Henry MJ, Morgan K, O'Connor CM, FitzGerald MX, Kalsheker N: **A polymorphism in the tumor necrosis factor-alpha gene promoter region may predispose to a poor prognosis in COPD.** *Chest* 2000, **118:**971-975
34. **Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. American Thoracic Society.** *Am J Respir Crit Care Med* 1995, **152:**S77-S121
35. Kaijzel E, Bayley J-P, Van Krugten M, Smith L, Van de Linde P, Bakker A, Breedveld F, Huizinga T, Verweij C: **Allele-specific quantification of tumor necrosis factor (TNF) α transcription and the role of promoter polymorphisms in rheumatoid arthritis patients and healthy individuals.** *Genes Immun* 2001, **2:**135-144
36. Sakai F, Gamsu G, Im JG, Ray CS: **Pulmonary function abnormalities in patients with CT-determined emphysema.** *J Comput Assist Tomogr* 1987, **11:**963-968
37. Lamers RJ, Thelissen GR, Kessels A, Wouters EFM, Van Engelshoven JM: **Chronic obstructive pulmonary disease: evaluation with spirometrically controlled CT lung densitometry.** *Radiology* 1994, **193:**109-113
38. Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo JL: **Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults.** *Eur Respir J* 1993, **16:**53-83
39. Gould GA, MacNee W, McLean A, Warren PM, Redpath A, Best JJ, Lamb D, Fenley DC: **Measurements of lung density in life can quantitate distal airspace enlargement – an essential defining feature of human emphysema.** *Am Rev Respir Dis* 1988, **37:**380-392
40. Aguado B, Milner CM, Campbell RD: **Genes of the MHC class III region and the functions of the proteins they encode.** *In HLA and MHC: Genes, Molecules and Function (Edited by: Browning M, McMichael A) Oxford: BIOS Scientific Publishers* 1996, 39-75