ORIGINAL ARTICLE

Association of tumor necrosis factor-α gene polymorphism (-308) and obstructive sleep apnea-hypopnea syndrome.

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Abstract

Background and Aim: Elevated serum tumor necrosis factor-α (TNF-α) concentration and a polymorphism of the TNF-α gene at the position –308 in the promoter region are associated with obstructive sleep apnea-hypopnea syndrome (OSAHS). We aimed to determine the association of this polymorphism with OSAHS in Greek patients.

Patients and Methods: A blood sample was obtained from 220 patients clinicaly diagnosed with OSAHS and 319 normal controls. TNF-α genotype was determined from nucleus-containing cells from whole blood using a PCR method.

Results: The results demonstrated that the distribution of alleles was significantly different when comparing the OSAHS patients group to the healthy controls. The appearance of AA ($p=0.04$) and AG ($p<0.001$) genotypes was significantly greater in OSAHS patients (8.6% and 32.7%, respectively) compared to the healthy control group (4.4% and 26.3%, respectively). Correspondingly, the appearance of the GG genotype was significantly lower in OSAHS patients compared to healthy controls (53.6% vs 69.3%). The A and G allele appeared at a frequency of 27.5% and 72.5% respectively in the OSAHS groups, and 17.6% and 82.4% in the control group respectively.

Conclusions: The distribution of genotypes and alleles of the single nucleotide polymorphism of TNF-α (-308) of OSAHS patients varies from healthy controls. Hippokratia 2012; 16 (3): 217-220

Key words: genetics, TNF-α, sleep apnea

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Obstructive sleep apnea-hypopnea syndrome (OSAHS) was first described in the scientific literature by Broadbent[1] in 1877 and since, the pathogenesis of the condition has been well described[2]. OSAHS affects 2-4% of middle-aged adults[3,4]. Anatomic predisposing factors to OSAHS include the upper airway anatomy[5], lung volume[6] and neck girth[7], whilst gains in abdominal circumference over adult life is closely associated with sleep disordered breathing severity[8]. Furthermore, the pathogenesis of OSAHS could be contributed to physiological traits such as upper airway motor control[9], ventilatory control stability and coordination[10,11] and respiratory arousal threshold[12].

Patients with OSAHS have been reported to have increased circulating TNF-α concentration in the morning[13] and immediately after the first episode of obstructive apnea[14]. Furthermore, *ex vivo* lipopolysaccharide stimulation of human whole blood cell culture resulted in a greater TNF-α production in the blood derived from individuals that were homozygous for TNF -308G compared to blood derived from individuals that were heterozygous[15]. Increased production of TNF-α has been reported to be associated with a TNF-α gene polymorphism, consisting of a guanine (G allele)– adenine (A allele) interchange at the –308 position in the promoter region[15,16]. The aim of the current study was to determine the association of the G→A TNF-α polymorphism with OSAHS in Greek patients.

Materials and Methods

Patients

Two hundred and twenty OSAHS patients and 319 healthy controls mathced for age, race and gender participated in this study. All patients were regional residents and had visited the Lung Clinic of the University Hospital of Larissa, during a 3-year period (2003-2006). A questionnaire reviewing sleep habits, symptoms, and medications was completed before the night of the polysomnography. Daytime sleepiness was evaluated using the Epworth Sleepiness Scale (ESS). Measurements of body weight and height were done the night of the sleep study. Their condition was confirmed clinicaly by overnight polysomnography at the Sleep Centre of University Hospital of Larissa according to the apnea-hypopnea index (AHI). Controls were individuals living in the same geographical area and had been referred to the Lung Clinic by their physician to undergo an overnight polysomnography but where asymptomatic of OSAHS. Subject characteristics and AHI are presented in Table 1.

A single blood sample was collected from all individuals at 8 a.m. and was analysed for the TNF-α (-308) gene polymorphism.

Table 1: Demographics and clinical characteristics of OSAHS patients and controls.

	Patients (n=220)	Controls (n=319)	p
Age (Y)	51.0 ± 12.4	50.6 ± 13.8	0.72*
Sex (M/F)	198 (90%)/22 (10%)	278 (87%) /41 (13%)	0.34**
AHI	42,6 ± 27, 5	3,3 ± 2,2	<0.001*
BMI	31.4 ± 5.24	29.6 ± 4.3	<0.001*
Diabetes mellitus (%)	16 (7.3)	22 (6.9)	0.87**
Smokers (%)	109 (49.8)	113 (35.4)	0.001**
Hypertensive (%)	88 (40.2)	45 (14.1)	<0.001**

M =male
F= female
AHI = apnea-hypopnea index (arbitrary units)
BMI = body mass index (kg/m^2)
* t-test
** χ^2

Sleep study

Overnight polysomnography was recorded in all patients by a computerized system (Alice 4 Diagnostic Device OBS/G7829, Respironics) The AHI score was calculated as the number of apneas recorded per hour of sleep. AHI was defined as the total number of obstructive apneas (cessation of airflow for at least 10 s) and hypopneas (decrease of the airflow signal amplitude by at least 50% accompanied by oxyhemoglobin desaturation of at least 4% or by an arousal) per hour of sleep. OSAS was defined as the combination of an AHI of 5 or more events/h with daytime sleepiness. Patients with an AHI score >5 were considered OSAHS.

Genotyping

DNA was extracted from whole blood using the method described by Sambrook[18], and the quality of DNA was confirmed by electrophoresis in agarose gel. The promoter region of the human TNF-α gene polymorphism (-308) was amplified using PCR with forward and fluorescent tagged reverse primers[19] 5'AGGCAATAGGTTTTGAOGGCCAT 3' and 5- TCCTCCCTGCTCCGATTCCG 3' in order to amplify a DNA fragment of 107 bp containing the variable nucleotide. This polymorphism consists of a guanine (G) to adenine (A) interchange. For restriction digestion, Ncol was added to samples. Digestion confirmed two alleles. G/G genotype gives two fragments of 87 bp and 20 bp, A/A genotype a single 107 bp fragment, and G/A genotype gives 107 bp, 87 bp and 20 bp.

Statistical analysis

Statistical analysis of the quantitative measures (age, BMI, and AHI) was conducted with a student's t-test. Statistical analysis of the qualitative measures (sex, hypertension, diabetes and number of smokers) was conducted by the use of the χ^2 statistical test. Any correlation between the genotype and alleles was investigated with the χ^2 statistical test, whereas the investigation of the presence of equilibrium was conducted with the exact χ^2 test. Regression analysis was used in order to control for various factors (eg sex, age, etc) to calculate the odds ratio. All results are presented as mean (%) unless otherwise stated.

Results

According to the present study, OSAHS was present predominantly in males, with of male to female ratio of 9:1 (Table 1). OSAHS patients have a BMI of 31.4 ± 5.24 kg·m^2 compared to 29.6 ± 4.2 kg·m^2 in the healthy controls (p<0.001), whereas almost 50% of the patient group were smokers compared to 35.5% of the healthy controls, a difference that was highly statistically significant (p<0.001, Table 1).

Approximately 40% of the patients were hypertensive in comparison to only 14% of the healthy controls (p<0.001, Table 1).

The distribution of the genotypes and the alleles of the TNF-α gene are presented in Table 2. The TNF-α (-308 A/G) genotype distribution varied significantly between the patient and control groups as 8.6% of the OSAHS patients were AA homozygote's compared to the 4.1% of the control group (p=0.04). In addition, more OSAHS patients were heterozygote's compared to the control group (37.7% vs 24.8% respectively, p< 0.001) (Table 2).

The AA genotype and the A allele appeared more frequently in OSAHS patients compared to the controls (27.5% vs 17.5%, respectively; p=0.002), whereas the G allele appeared less frequently in the OSAHS patient group compared to the control group (72.5% vs 82.5%, respectively, p=0.002).

Our results show that patients with the AA genotype had an OR of 2.5 compared to the AG/GG genotypes (p=0.0012), which decreased if only 1 A allele was present (OR=1.8, p=0.001) and when comparing the presence of the A allele to the G allele (OR=1.7, p=0.001, Table 3). The results persisted when our data was controlled for age, sex, diabetes mellitus, hypertension, smoking and BMI, with the exception of the presence of 2 AA alleles (p=0.109). When controlling for all parameters mentioned above, with the exception of hypertension, the presence of the A allele, either alone or when 2 alleles were carried, the OR ranged from 1.9 to 2.8 (Table 3).

Discussion

The demographic/clinical findings of this study sup-

Table 2: Polymorphism frequency in OSAHS patients and healthy controls.

	TNF-α (-308, A/G)						
	Genotypes				Alleles		
	AA	AG	GG	p	A	G	p
Patients (n = 220)	19	83	118	0.04[a]	121	319	0.002[c]
Controls (n = 319)	14	84	221	>0.001[b]	112	526	

[a] Pearson x^2 analysis, AA vs AG/GG

[b] Pearson x^2 analysis AA/AG vs GG

[c] controlled for age, sex, diabetes mellitus, hypertension, smoking BMI with the use of regression analysis

Table 3: Odds Ratio of the genotype effect in the OSAHS patients group for each allele and for the carriers of one or two alleles.

Polymorphism		OR (95%CI)		
		Not Controlled	Controlled for all parameters*	Controlled for all parameters' except for hypertension
TNF-α (-308 A/G)	Carrier of 2 A alleles (AA vs AG/GG)	2.5 (1.2-5.3), p=0.012	1.9 (0.9-4.2), p=0.109	2.8 (1.3-6.1), p=0.006
	Carrier of 1 A allele (AA/AG vs GG)	1.8 (1.3-2.7), p=0.001	1.7 (1.2-2.8), p=0.003	2.0 (1.3-2.9), p<0.001
	A vs G	1.7 (1.3-2.4), p<0.001	2.1 (1.4-2.9), p<0.001	1.9 (1.1-2.7), p=0.001

* controlled for age, sex, diabetes mellitus, hypertension, smoking BMI with the use of regression analysis

port the evidence of previous studies in that OSAHS patients have a statistically significant greater BMI when compared to healthy controls[13,20], and furthermore, OSAHS appears to be more frequent among smokers compared to non-smokers[21]. In addition, the results presented the positive correlation between the incidence of OSAHS and hypertension. Malhorta and White[22] reported that males had a 2-3 times increased risk of developing OSAHS compared to females. In the current study the greater percentage of patients was male. Indeed, out of 220 patients only 10% (n=22) were female, indicating that males have a 9 times greater risk of presenting with OSAHS compared to females.

In the literature there is no clear consensus regarding whether OSAHS is hereditary[23], however it has been shown that OSAHS may be caused by an inherited respiratory instability in sleep[24].

Riha et al[25] reported that the genotypes AA, AG and GG appeared in 7%, 43% and 51% respectively in OSAHS patients, whereas in the control group the distribution was 4%, 27% and 69% for the AA, AG and GG genotypes respectively. In the present study, the frequency of the A allele presentation was 27.5 %, almost identical to the findings of Riha et al (28%), and was by virtue mirrored in the frequency of presentation of the G allele (72.5% in the present study vs 72% in the study published by Riha et al[25]).

Regression analysis of our results confirmed the correlation between the TNF-α gene polymorphism (-308) and the presence of OSAHS in our patient group (Table 2).

Our findings suggest that OSAHS patients with an AA homozygote genotype have a 2.5 times greater chance of presenting with OSAHS compared to individuals with an AG genotype. In addition, the chance of developing OSAHS appears to be independent from other factors such as age, sex, diabetes mellitus, hypertension, smoking and BMI.

Regression analysis of our results showed that the (-308) TNF-α gene polymorphism affected mostly those who were not hypertensive (Table 3), indicating that hypertension in the presence of the (-308) TNF-α polymorphism may have a positive effect on the appearance of OSAHS. This result is in contradiction to the available literature regarding the correlation of hypertension with OSAHS[26]. Therefore further studies are required to confirm if there is an inverse correlation between OSAHS and hypertension in the presence of the (-308) TNF-α gene polymorphism.

The single nucleotide polymorphism G/A -308 of the TNF-α gene appears to correlate with other pathologies as well, i.e. the age of onset of Alzheimer's[27], Crohn's disease[28], pulmonary cancer[29], T-cell large granular lymphocyte leukemia[30], and the time of intensive unit stay after repair from an aortic aneurism[31]. In contrast, no correlation was observed between the increased presence of the A allele of the TNF-α (-308) gene and pathologies such as rheumatoid arthritis[32], malignant melanoma[33], chronic immune thrombocytopenic purpura[34], bronchiolitis obliterans syndrome or survival after lung transplant[35] or chronic obstructive pulmonary disease[36].

A potential limitation of the present study was the fact that we did not take into consideration whether relatives existed within or between the patient and control groups. Due to the above, we were unable to investigate whether

our results could be influenced by familial factors. The strengths of the present study are the number of people included in the patient and control groups and that all participants underwent a sleep study.

In conclusion, the present study has investigated for the first time a single nucleotide polymorphism of pre-inflammatory cytokines in a Greek population TNF-α (-308G/A). Furthermore, we demonstrated that the A allele correlates directly and independently to the incidence of OSAHS in Caucasians.

Conflict of interest:
The authors declare no conflicts of interest.

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