

# Association of TNF- $\alpha$ -308G/A, SP-B 1580 C/T, IL-13 -1055 C/T gene polymorphisms and latent adenoviral infection with chronic obstructive pulmonary disease in an Egyptian population

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## Abstract

**Introduction:** Chronic obstructive pulmonary disease (COPD) is a leading cause of disability and death. The most common cause of COPD is smoking. There is evidence suggesting that genetic factors influence COPD susceptibility and variants in several candidate genes have been significantly associated with COPD. In this study, we aimed to investigate the possible association of the TNF- $\alpha$  -308, SPB+1580, IL-13 -1055 gene polymorphisms and latent adenovirus C infection with COPD in an Egyptian population.

**Material and methods:** Our study included 115 subjects (75 smokers with COPD, 25 resistant smokers and 15 non-smokers) who were subjected to spirometric measurements, identification of adenovirus C and genotyping of TNF- $\alpha$  -308G/A, SP-B+1580 C/T and IL-13 -1055 C/T polymorphisms by real-time PCR.

**Results:** The adenovirus C gene was identified in all subjects. The distribution of TNF- $\alpha$  genotypes showed no significant differences between different groups. However, homozygous A genotype was associated with a significant decrease in FEV<sub>1</sub>, FEV<sub>1</sub>/FVC and FEF25/75% of predicted in COPD ( $p < 0.05$ ). As regards SP-B genotypes, resistant smokers had a significantly higher homozygous T genotype frequency compared to COPD and non smokers ( $p = 0.005$ ). Interleukin 13 genotypes showed no significant difference between different groups. There was a significant decrease in FEF25/75% of predicted in T allele carriers in COPD patients ( $p = 0.001$ ).

**Conclusions:** The COPD is a disease caused by the interaction of combined genes and environmental influences, in the presence of smoking and latent adenovirus C infection, TNF- $\alpha$  -308A, SPB +1580 T and IL-13 -1055 T polymorphisms predispose to the development of COPD.

**Key words:** single nucleotide polymorphism, smoking, adenovirus C, chronic obstructive pulmonary disease.

## Introduction

Chronic obstructive pulmonary disease (COPD) is a complex mix of signs and symptoms in patients with chronic bronchitis and emphysema that largely results from cigarette smoking [1]. Several lines of evidence strongly suggest that genetic factors influence COPD susceptibility as well; severe

$\alpha$ 1-antitrypsin deficiency is one of the proven genetic determinants of COPD [2]. Variants in several candidate genes have been significantly associated with COPD, suggesting an interaction between genetic and environmental influences [3].

Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) is a reasonable pathophysiological candidate gene which has been associated with COPD [4]. In a case control study, the distribution of TGF- $\beta$ 1 genotypes in an Egyptian population was demonstrated; the absence of the Pro-Pro alleles in all cases of the study denoted a high predisposition to COPD in Egyptian smokers. Moreover, the Pro allele of the TGF- $\beta$ 1 gene was expressed more commonly in resistant smokers than in smokers who developed COPD, demonstrating a protective role against COPD and an important effect in preserving further decline in forced expiratory volume in the 1 s (FEV<sub>1</sub>) [5].

Tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ), a proinflammatory cytokine, has many effects relevant to the pathogenesis of COPD, including neutrophil release from the bone marrow and neutrophil activation [6]. Enhanced levels of TNF- $\alpha$  have been detected in sputum [7] and in circulation [8] of COPD patients, indicating that this cytokine is involved in both the local and systemic inflammation present in COPD. Analysis of possible TNF- $\alpha$  gene polymorphisms in COPD could be related to a higher susceptibility to develop this disabling pathological condition. An association between the TNF- $\alpha$  -308G/A polymorphism and COPD was reported in Taiwanese [9] and Japanese populations [6].

Surfactant protein B (SP-B) polymorphisms have been associated with COPD susceptibility in the presence of a gene-environment interaction [10].

Interleukin 13 (IL-13) is strongly implicated in the development of asthma and COPD, overexpression of IL-13 in the adult murine lung caused emphysema, elevated mucus production, and inflammation reminiscent of human COPD [11]. An increased frequency of the -1055 T allele in COPD patients compared to healthy controls and smokers with normal lung functions was previously reported, implicating a functional role of IL-13 promoter polymorphism in enhanced risk of COPD development [12].

Adenovirus infection is a common cause of bronchiolitis in children and young adults [13] that could predispose to COPD in adulthood [14]. In particular, group C adenovirus is endemic, and exposure to infection during childhood could lead to latent infection that persists in the lung [15], tonsils [16], and peripheral blood lymphocytes [17]. The COPD is characterized by cough, sputum production, peripheral airways obstruction, and emphysematous destruction of the lung surface area. All these components have been related to an inflammatory process located in the conducting airways and lung parenchyma [18].

In this study we aimed to investigate the possible association of TNF- $\alpha$  -308G/A, SP-B+1580 C/T, IL-13 -1055 C/T gene polymorphisms and latent adenovirus infection with COPD in an Egyptian population.

## Material and methods

This was a cohort study; all subjects were selected randomly from the Chest Outpatient Clinic and Pulmonary Function Unit in the National Research Centre and Chest Department of Cairo University over 24 months.

Our study included 115 male subjects. Their age ranged between 40 and 78 years. They were divided into non-COPD groups, group 1 (non-smokers) which included 15 non-smoker healthy controls with normal spirometry and group 2 (resistant smokers) which included 25 smokers who did not develop COPD proved clinically and by normal spirometry, and a COPD group (group 3) which included 75 smokers with COPD.

Patients were assigned to the study according to one or more of the following criteria: age above 40 years, smoking history above 20 pack years, clinical and radiological examination fulfilled the diagnostic criteria of COPD [19]. Exclusion criteria included diseases other than COPD and working in industrial areas. All patients gave written informed consent; the study was approved by the ethical committee of the National Research Centre.

All subjects of the study were subjected to medical history taking, clinical examination, plain chest X-ray postero-anterior view, and pre- and post-bronchodilator spirometric measurements of FEV<sub>1</sub>, FEV<sub>1</sub>/FVC (forced vital capacity) and forced expiratory flow (FEF) 25/75% of predicted. Normal pulmonary function test (PFT) was considered as FEV<sub>1</sub>% of predicted > 85%, FEV<sub>1</sub>/FVC % of predicted > 70%. Bronchodilators were stopped 24 h before PFT.

Thirteen of our COPD patients were admitted to the ICU for acute (on top of chronic) respiratory failure, so spirometric measurements were not performed on them.

## Laboratory methods

Identification of adenovirus C gene and genotyping of TNF- $\alpha$ , SP-B, and IL-13 gene single nucleotide polymorphisms (SNPs) were done by real-time polymerase chain reaction (real-time PCR).

## Genomic DNA extraction

Blood samples were collected using EDTA containing blood tubes and treated with sucrose lysis buffer and the nuclear cell pellets were stored at -80°C until DNA extraction. Genomic DNA was extracted from whole blood using the QIAamp DNA extraction kit (Qiagen Hilden, Germany, Cat

no. 51304) according to the manufacturer's instructions.

#### Identification of adenovirus C gene by real-time PCR

The sequence of the Adenovirus C (Adv5) gene (Bank Accession No: NC\_001406) was amplified by real-time PCR. Primers used were:

Forward primer 5'GCCATTACCTTTGACTCTTCTGT3',

Reverse primer 5' CCTGTTGGTAGTCCTTGATT-TAGTATC3'

and the probe sequence was as follows: AGAAACTCCAGCCCCATGAGCCG.

Real-time PCR was conducted using SYBR green nucleic acid dye in Light Cycler. The reactions were carried out in a capillary tube with 20 µl reaction volume containing 7 µl of H<sub>2</sub>O, 1 µl of forward primer, 1 µl of reverse primer, 9 µl of SYBR green master mix and 2 µl of extracted DNA. The following thermocycling profile was used for each reaction: 50°C for 2 min to activate uracil *N*-glycosylase in the master mix, 95°C for 10 min to activate enzyme followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Dissociation analysis was run at the end of each SYBR green real-time PCR reaction to verify a single PCR amplicon.

#### Genotyping of IL-13, SP-B and TNF-α SNPs by real-time PCR

The studied SNPs were IL-13 -1055 C/T (rs1800925 in the NCBI, SNP database), surfactant protein B +1580 C/T (rs2118177), and TNF-α -308 G/A (rs1800629). The surrounding sequences of these SNPs were: GGGTTCTGGAGGACTTCTAGGAAAA[C/T] GAGGGAAGAGCA (VIC/FAMGGAAAAGGCGACA) for IL-13-1055C/T, CTTTGTCAGAGGCGACTCCTAACCC[C/T] TTAGCAGGCTCTG (VIC/FAMCCCTAACTTACA) for SP-B+1580 C/T, and GAGGCAATAGGTTTTGAGGGGCATG [G/A] GGACGGGGTTC (VIC/FAMAGCCTCCAGGGTCC) for TNF-α-308 G/A, respectively. To amplify the regions containing polymorphisms, PCR was conducted for a 40-cycle amplification (10 min at 95°C, 15 s at 92°C and 1 min at 60°C) using 11.25 µl of genomic DNA diluted in dH<sub>2</sub>O, 12.5 µl of Taqman universal PCR Master Mix containing 2.5 mM dNTP, 2.5 mM MgCl<sub>2</sub> and 1 unit of Taq polymerase (Boehringer Mannheim), and 1.25 µl of genotyping assay mix 20× in each well.

After amplification, genotyping of polymorphisms was carried out using TaqMan SNP genotyping assay performed on an Applied Biosystems 7500 Real-Time PCR and ABI PRISM 7000 Sequence Detection System which is optimized to work with Taqman universal PCR master mix, and designed for the allelic discrimination of specific SNPs, enabling scoring of both alleles in a single well. Software automatically determined genotypes and gen-

erated an intuitive graphic representation of results in a cluster plot report.

#### Statistical analysis

Statistical Package for Social Sciences (SPSS) version 14.0 was used for statistical analysis. The quantitative results were expressed as means ± standard deviation (SD). For comparing quantitative results between two groups, independent *t*-test was used, and for more than two groups ANOVA and post hoc least significant test (LSD) were used. Qualitative results were expressed as number (No.) and percent (%). Pearson chi-square test ( $\chi^2$ ) was used for statistical analysis of the qualitative data. Differences were considered to be significant when the *p*-value was ≤ 0.05.

#### Results

The study included 115 male subjects. The age and the smoking history in the resistant smokers group and COPD patients group were matched without significant differences (Table I).

The mean values of the post-bronchodilator FEV<sub>1</sub>, FEV<sub>1</sub>/FVC and FEF25/75% of predicted in COPD patients group were 47.3%, 59.0% and 29.2%, respectively. They were significantly lower than in non-smokers (86.6%, 85.4% and 81.8%, respectively) and resistant smokers (93.1%, 87.7% and 84.4%, respectively) with value of *p* < 0.0001 (Table II).

The mean values of the post-bronchodilator FEV<sub>1</sub>, FEV<sub>1</sub>/FVC and FEF25/75% of predicted negatively correlated with age in COPD patients (*p* < 0.05, *p* < 0.005 and *p* < 0.05, respectively). Additionally, the mean values of the post-bronchodilator FEV<sub>1</sub>, FEV<sub>1</sub>/FVC% of predicted negatively correlated with their smoking history (*p* < 0.01 and *p* < 0.005, respectively) (Table III).

As regards the adenovirus C gene, it was identified in all subjects of the study (Figure 1).

The genotype distribution of the TNF-α -308 G/A polymorphism was heterozygous in 13 cases and homozygous A in 102 cases. The frequency of the homozygous A genotype was 90.6% of COPD, 86.7% of non-smokers and 84% of resistant smokers, with no statistical significant difference between the 3 groups. Homozygous G genotype was not detected in any of our subjects (Table IV, Figure 2).

The genotype distribution of the SP-B +1580 C/T polymorphism showed that the resistant smokers had a significantly higher frequency of homozygous T than either COPD patients or non-smokers (*p* = 0.0050). However, homozygous C genotype frequency was higher in COPD patients than in either resistant smokers or non-smokers, *p* = 0.005 (Table IV, Figure 3).

Meanwhile, the genotype distribution of the IL-13 -1055 C/T polymorphism showed no signifi-

**Table I.** Statistical analysis of age (years) and smoking history (pack years) between the 3 groups

Parameter	Group	N <sup>o</sup>	Mean	± SD	ANOVA	
					F-ratio	Value of p
Age [years]	1	15	57.0	1.51	0.483	NS
	2	25	55.3	7.10		
	3	75	56.7	7.34		
Smoking history (pack years)	1	15	0.0	0.00	3.452*	NS
	2	25	41.6	13.09		
	3	75	50.1	21.51		

\*Independent t-test. Group 1 – non-smokers, group 2 – resistant smokers, group 3 – COPD

**Table II.** Statistical analysis of FEV<sub>1</sub>%, FEV<sub>1</sub>/FVC% and FEF<sub>25</sub>/75% of predicted between the 3 groups

Parameter	Group	N <sup>o</sup>	Mean	± SD	ANOVA		
					F-ratio	Value of p	LSD
FEV <sub>1</sub> %	1	15	86.6	22.43	81.128	< 0.0001	(2)
	2	25	93.1	10.12			(1,3)
	3	62	47.3	17.41			(2)
FEV <sub>1</sub> /FVC%	1	15	85.4	3.70	62.446	< 0.0001	(2)
	2	25	87.7	5.98			(1,3)
	3	62	59.0	15.10			(2)
FEF <sub>25</sub> /75%	1	15	81.8	14.25	109.09	< 0.0001	(2)
	2	25	84.4	21.22			(1,3)
	3	62	29.2	17.46			(2)

FEV<sub>1</sub> – forced expiratory volume in the 1 s, FVC – forced vital capacity, FEF – forced expiratory flow; group 1 – non-smokers, group 2 – resistant smokers, group 3 – COPD

**Table III.** Correlations between FEV<sub>1</sub>%, FEV<sub>1</sub>/FVC% and FEF<sub>25</sub>/75% of predicted with age (years) and smoking history (pack years)

Parameter	Group 1		Group 2		Group 3		
	Age [years]	Smoking history	Age [years]	Smoking history	Age [years]	Smoking history	
FEV <sub>1</sub> %	r	-0.3	(a)	-0.2	0.2	-0.3	-0.3
	Sig. (2-tailed)	NS		NS	NS	< 0.05	< 0.01
FEV <sub>1</sub> /FVC%	r	0.2		-0.2	0.3	-0.4	-0.4
	Sig. (2-tailed)	NS		NS	NS	< 0.005	< 0.005
FEF <sub>25</sub> /75%	r	0.05		-0.2	0.005	-0.3	-0.2
	Sig. (2-tailed)	NS		NS	NS	< 0.05	NS

(a) Cannot be calculated, smoking history is zero; group 1 – non-smokers, group 2 – resistant smokers, group 3 – COPD

cant difference between the studied groups although there was a high frequency of the homozygous T genotype in COPD patients (20%) compared to the resistant smokers (2.8%) and non-smokers (6.7%) (Table IV, Figure 4). Figure 5 shows the frequencies of different genotypes in different studied groups.

The post-bronchodilator FEV<sub>1</sub>/FVC% of predicted was significantly decreased in TNF- $\alpha$  homozygous A genotype (86.75) compared to heterozygous

G/A genotype (93.0%) in resistant smokers ( $p < 0.05$ ) (Table V).

In COPD patients, there was a statistically significant decrease in the mean values of the post-bronchodilator FEV<sub>1</sub>, FEV<sub>1</sub>/FVC and FEF<sub>25</sub>/75% of predicted in homozygous TNF- $\alpha$  A genotype (45.3%, 57.6% and 27.4%, respectively) compared to that in heterozygous G/A genotype (70.2%, 69.4% and 49.8%, respectively),  $p < 0.05$  (Table V). In addition, the mean values of the post-bronchodilator FEV<sub>1</sub>

and FEV<sub>1</sub>/FVC% of predicted were significantly decreased in SP-B homozygous T genotypes (40.9% and 54.1%, respectively) and homozygous C genotype (43.3% and 55.1%, respectively) compared to those in heterozygous C/T genotype (58.4% and 68.5%, respectively,  $p < 0.005$ ) (Table VI). Meanwhile, the mean values of the post-bronchodilator FEF<sub>25/75</sub>% of predicted was significantly decreased in IL-13 homozygous T and C/T genotypes (17.1% and 27.4%, respectively) compared to homozygous C genotype (37.6%),  $p = 0.001$  (Table VII).

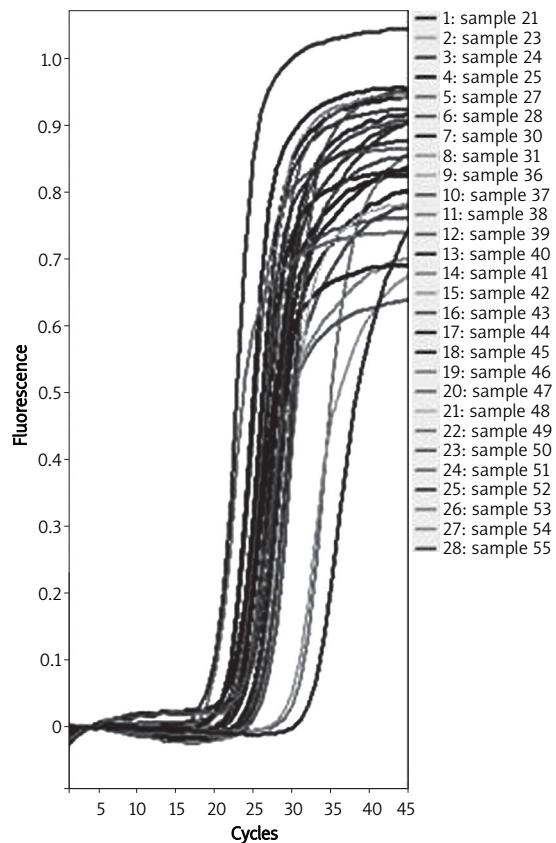
**Discussion**

This study selected randomized samples of subjects of the same sex and race. The mean age of the subjects and their smoking index were statistically matched. All subjects were of Caucasian origin; it was found that the frequency of an allele can vary in different racial groups [3].

The mean values of the post-bronchodilator FEV<sub>1</sub>, FEV<sub>1</sub>/FVC and FEF<sub>25/75</sub>% of predicted showed significant negative correlations with age and were significantly reduced in COPD patients compared to non-COPD groups ( $p < 0.0001$ ).

Our subjects had a heavy smoking history of more than 20 pack years. Mean values of the post-bronchodilator FEV<sub>1</sub>, FEV<sub>1</sub>/FVC% of predicted had significant negative correlations with their smoking history.

Adenovirus C infects more than 80% of the human population early in life [20]. Moreover, near-

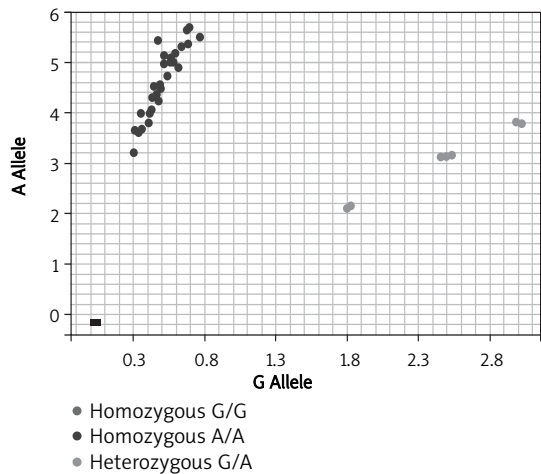


**Figure 1.** Amplification curve showing positive adenovirus C gene

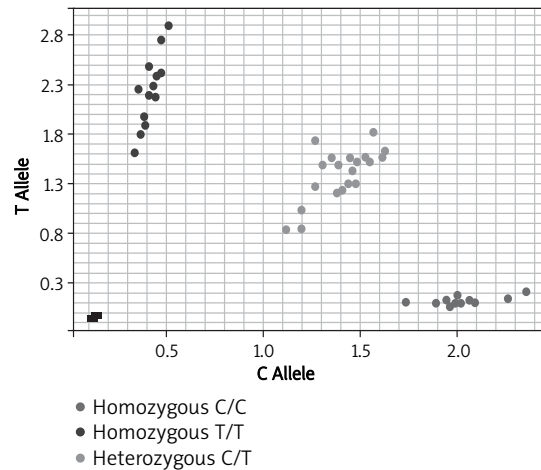
**Table IV.** Genotype frequencies of TNF- $\alpha$  -308, Surfactant Protein B+1580 and IL-13 -1055 polymorphisms in the 3 groups

Parameter		Group 1 (n = 15)	Group 2 (n = 25)	Group 3 (n = 75)	$\chi^2$	Value of p	
TNF- $\alpha$ -308	G/A (n = 13)	Count	2	4	7	0.816*	NS
		% within groups	13.3	16	9.4		
	A/A (n = 102)	Count	13	21	68		
		% within groups	86.7	84	90.6		
SPB+1580	C/T (n = 41)	Count	6	8	27	14.74	0.005
		% within groups	40	32	36		
	T/T (n = 40)	Count	4	16	20		
		% within groups	26.7	64	26.7		
	C/C (n = 34)	Count	5	1	28		
		% within groups	33.3	4	37.3		
IL-13 -1055	C/T (n = 45)	Count	5	11	29	3.493	NS
		% within groups	33.3	44	38.7		
	T/T (n = 18)	Count	1	2	15		
		% within groups	6.7	8	20		
	C/C (n = 52)	Count	9	12	31		
		% within groups	60	48	41.3		

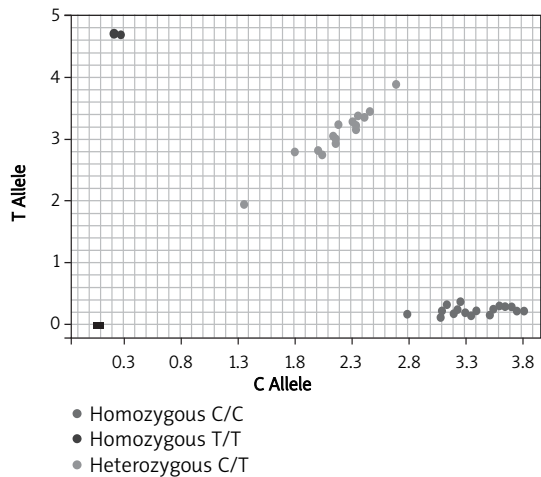
\*Likelihood ratio



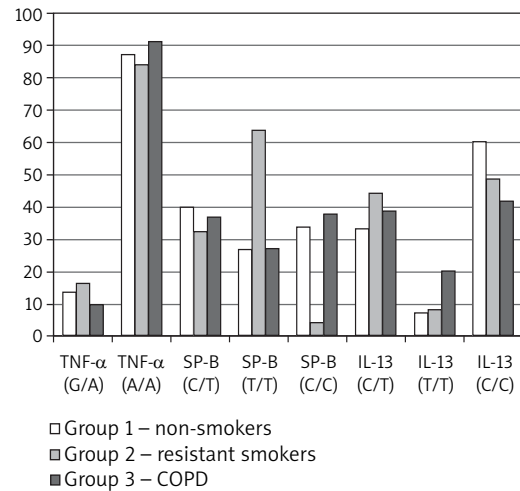
**Figure 2.** Allelic discrimination plot of TNF- $\alpha$  -308 genotypes



**Figure 3.** Allelic discrimination plot of surfactant protein B+1580 genotypes



**Figure 4.** Allelic discrimination plot of IL-13 -1055 genotypes



**Figure 5.** Genotypic frequencies of TNF- $\alpha$  -308, surfactant protein B -1580 and IL-13 -1055 genes single nucleotide polymorphisms in the 3 groups

ly 100% of adults have serum antibody to multiple serotypes – a finding indicating that infection is common in childhood [21].

In a study that included 60 children presenting with recurrent upper and/or lower respiratory tract infection done in the National Research Centre of Egypt, it was demonstrated that passive cigarette smoking remains an enormous health problem and is a principal cause of recurrence of respiratory infections in children, mainly adenovirus C infection. High prevalence and persistence of adenovirus C infection, exceeding 96% of cases, were demonstrated in children by positive serum IgG and documented by latent gene expression [22].

In our study, identification of adenovirus C in COPD and non-COPD subjects demonstrated the persistence of latent infection during childhood and

confirmed our previous study in children. Although cigarette smoking is the major risk factor for the development of COPD, only 15% to 20% of all smokers develop airway obstruction [23]. Therefore, adenovirus that remains from a previous infection of the lungs in a smoker may enhance the inflammatory process in their lungs [24]. The correlation between adenovirus C, COPD and deterioration of pulmonary function was not demonstrated in this study, because all included subjects were positive for adenovirus C and genotyping was not done.

Latent adenoviral infection contributes to chronic airway inflammation through E1A-dependent NF- $\kappa$ B (nuclear factor- $\kappa$ B) activation. It was found that E1A regulation of the lipopolysaccharide (LPS) response may play a role in acute exacerbations as a consequence of bacterial infections in COPD. Adenovirus E1A 13S (adenovirus early gene product)

**Table V.** Comparison of FEV<sub>1</sub>%, FEV<sub>1</sub>/FVC% and FEF25/75% of predicted between different TNF- $\alpha$  -308 genotypes in the 3 groups

Group	Genotype	N°	Mean	$\pm$ SD	T-test	Value of p	
1 (n = 15)	FEV <sub>1</sub> %	G/A	2	90.0	7.07	0.222	NS
		A/A	13	86.1	24.10		
	FEV <sub>1</sub> /FVC%	G/A	2	82.5	2.12	1.21	NS
		A/A	13	85.8	3.74		
	FEF25/75%	G/A	2	83.5	6.36	0.175	NS
		A/A	13	81.5	15.27		
2 (n = 25)	FEV <sub>1</sub> %	G/A	4	94.2	12.40	0.212	NS
		A/A	21	93.0	9.98		
	FEV <sub>1</sub> /FVC%	G/A	4	93.0	5.12	2.065	0.05
		A/A	21	86.7	5.68		
	FEF25/75%	G/A	4	103.3	22.08	2.06	NS
		A/A	21	80.9	19.57		
3 (n = 62)	FEV <sub>1</sub> %	G/A	5	70.2	6.30	3.312	< 0.005
		A/A	57	45.3	16.61		
	FEV <sub>1</sub> /FVC%	G/A	5	69.4	13.35	2.482	< 0.05
		A/A	57	57.6	14.57		
	FEF25/75%	G/A	5	49.8	7.69	2.917	0.005
		A/A	57	27.4	16.92		

\*Likelihood ratio

stimulates the TNF gene in inflammatory cell lines. Moreover, it could contribute to airway remodeling in COPD by the viral E1A gene, inducing transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) and connective tissue growth factor (CTGF) expression and shifting cells to a more mesenchymal phenotype [25].

The TNF- $\alpha$  mediated inflammation is thought to play a key role in both the respiratory and systemic features of COPD. Single nucleotide polymorphism (SNP) in the promoter region of the TNF- $\alpha$  gene (G $\rightarrow$ A at position -308) directly affects gene regulation, and is associated with high TNF- $\alpha$  production [26].

In the present study, the genotype distribution of TNF- $\alpha$  -308 polymorphism in Caucasian populations was heterozygous G/A in 13 cases (11.3%) and homozygous A in 102 cases (88.7%). Homozygous alleles G/G were not detected in the studied groups. The TNF- $\alpha$  -308A allele frequency ranged from 10% [27] to 17% [28] in the white population, and from 5% [9] to 8% [6] in the Asian population. The possible cause of this discrepancy may be an ethnic difference affecting prevalence.

The study demonstrated no statistically significant difference between the 3 groups in the distribution of homozygous A genotype. The heterozygous G/A was present in a higher percentage in the resistant smoker group compared to the other groups, with no statistically significant difference

between them. These results were in accordance with several studies that fail to find an association between COPD and TNF- $\alpha$  -308G/A polymorphism in Caucasians [27-32], which might be explained by variation in genotype frequencies between races, or by linkage disequilibrium with HLA alleles, seen previously in the Caucasian population [33].

In contrast to these studies on white subjects, two studies on Asian subjects showed an association of TNF- $\alpha$  -308G/A polymorphism and COPD [6, 9]. Alternatively, the allele could be in linkage disequilibrium with another gene that increases susceptibility to COPD in smokers only in the Asian population [34].

On the other hand, the homogenous A genotype in COPD patients in the current study was accompanied by a statistically significant reduction in the mean values of the post-bronchodilator FEV<sub>1</sub>, FEV<sub>1</sub>/FVC and FEF25/75% of predicted compared to heterozygous G/A genotypes in COPD ( $p < 0.05$ ). Our findings agreed with those of Keatings *et al.* [29], who demonstrated that homozygosity for the A allele predisposes to a worse prognosis for COPD.

The surfactant proteins are hydrophobic proteins that contribute to regulation of surface tension in the alveoli. Components of surfactant also have a role in host defense and control of inflammation. Alterations of surfactant might therefore be a factor in COPD [26].

**Table VI.** Comparison of FEV<sub>1</sub>%, FEV<sub>1</sub>/FVC% and FEF25/75% of predicted between different Surfactant Protein B+1580 genotypes in the 3 groups

Group		Genotype	N°	Mean	± SD	F-ratio	Value of p
1 (n = 15)	FEV <sub>1</sub> %	C/C	5	93.8	7.95	1.098	NS
		C/T	6	76.2	33.51		
		T/T	4	93.3	5.38		
	FEV <sub>1</sub> /FVC%	C/C	5	85.0	3.32	0.331	NS
		C/T	6	84.8	3.31		
		T/T	4	86.8	5.25		
	FEF25/75%	C/C	5	87.4	18.27	0.987	NS
		C/T	6	82.3	14.01		
		T/T	4	74.0	6.48		
2 (n = 25)	FEV <sub>1</sub> %	C/C	1	74.6		1.913	NS
		C/T	8	93.2	12.34		
		T/T	16	94.3	8.29		
	FEV <sub>1</sub> /FVC%	C/C	1	87.2		0.007	NS
		C/T	8	87.9	5.11		
		T/T	16	87.6	6.71		
	FEF25/75%	C/C	1	69.0		1.602	NS
		C/T	8	94.8	30.27		
		T/T	16	80.3	14.19		
3 (n = 62)	FEV <sub>1</sub> %	C/C	25	43.3	13.92	6.844	< 0.005
		C/T	19	58.4	16.13		
		T/T	18	40.9	18.10		
	FEV <sub>1</sub> /FVC%	C/C	25	55.1	13.25	6.429	< 0.005
		C/T	19	68.5	14.13		
		T/T	18	54.1	14.48		
	FEF25/75%	C/C	25	24.3	15.37	2.648	NS
		C/T	19	36.3	20.14		
		T/T	18	28.3	15.39		

The genotype distribution of the SP-B +1580 C/T gene polymorphism showed that the non-smokers had a higher frequency of the heterozygous C/T genotype compared to either resistant smokers or COPD patients. Meanwhile, resistant smokers had a significantly higher frequency of T/T genotype, and C/C genotype was higher in COPD patients ( $p = 0.005$ ). The allelic variation of SP-B +1580 between smokers (C/C and T/T) and non-smokers (C/T) indicates that these alleles appeared to be "smoking-dependent". Moreover, the homozygous alleles (C/C and T/T) were associated with significant impairment of pulmonary function in the COPD group compared to that in the heterozygous alleles (C/T).

In contrast to our findings, Guo *et al.* [35] in a Mexican population with COPD reported that the frequency of the homozygous alleles C/C represented 49% of cases and the heterozygous alleles

C/T represented 76% of cases. When both marker alleles were present, the odds ratio (OR) for COPD was considerably increased (OR = 24.3,  $p < 0.001$ ) compared to when both markers were absent. No significant differences were observed when one marker was present and the other was absent (OR for T = 0.73,  $p = 0.81$ ; OR for C = 1.9,  $p = 0.31$ ).

In a study done in a Chinese Han population, it was concluded that the SP-B 1580 T allele was probably associated with increased susceptibility to COPD [36].

In spite of the difference in the distribution of allelic markers between populations, probably due to ethnic variation, all studies agreed that SP-B+1580 C/T is a smoking-dependent gene which plays a role in COPD and that the T allele is associated with deterioration of pulmonary functions. But in the present study, SP-B homozygous alleles



**Table VII.** Comparison of FEV<sub>1</sub>%, FEV<sub>1</sub>/FVC% and FEF25/75% of predicted between the different IL-13-1055 genotypes in the 3 groups

Group	Genotype	N°	Mean	± SD	F-ratio	Value of p	
1 (n = 15)	FEV <sub>1</sub> %	C/C	9	84.2	28.99	0.135	NS
		C/T	5	89.2	6.38		
		T/T	1	95.0			
	FEV <sub>1</sub> /FVC%	C/C	9	86.0	2.78	0.275	NS
		C/T	5	84.6	5.50		
		T/T	1	84.0			
	FEF25/75%	C/C	9	82.8	15.99	0.050	NS
		C/T	5	80.6	13.92		
		T/T	1	79.0			
2 (n = 25)	FEV <sub>1</sub> %	C/C	12	91.8	9.80	0.868	NS
		C/T	11	93.1	10.11		
		T/T	2	102.0	14.14		
	FEV <sub>1</sub> /FVC%	C/C	12	89.8	6.11	1.471	NS
		C/T	11	85.7	5.88		
		T/T	2	85.9	1.20		
	FEF25/75%	C/C	12	78.0	13.46	1.072	NS
		C/T	11	90.4	26.49		
		T/T	2	90.5	28.99		
3 (n = 62)	FEV <sub>1</sub> %	C/C	25	43.4	17.58	1.486	NS
		C/T	22	52.1	17.45		
		T/T	15	46.7	16.37		
	FEV <sub>1</sub> /FVC%	C/C	25	61.8	19.38	1.487	NS
		C/T	22	59.5	9.20		
		T/T	15	53.2	13.07		
	FEF25/75%	C/C	25	37.6	19.48	7.754	0.001
		C/T	22	27.4	15.17		
		T/T	15	17.1	6.11		

(either C/C or T/T) were found to be associated with worse prognosis of COPD.

Studies in transgenic mice have shown that the targeted expression of IL-13 in the adult lung causes emphysema, mucus metaplasia, and inflammation that mirror, in many ways, the lesions seen in human COPD. The IL-13 may play an important role in the pathogenesis of COPD, particularly in patients with asthma-like features [11].

The distribution of the genotype of IL-13 -1055 C/T polymorphism showed no significant difference between the studied groups although there was a higher frequency of homozygous T genotype in the COPD patient group compared to other groups. Also, the homozygous T and heterozygous C/T genotypes were associated with a significant reduction in the mean values of the post-bronchodilator

FEF25/75% of predicted compared to that in the case of homozygous C genotype in the COPD group ( $p = 0.001$ ). These results indicate that the T allele is associated with COPD and deterioration of pulmonary functions.

Our results are in accordance with those of van der Pouw Kraan *et al.*, who demonstrated that IL-13 promoter region polymorphism -1055 C→T, associated with increased IL-13 production, is more common in COPD patients [12].

In conclusion, from our results we concluded that COPD is a disease caused by the interaction of combined genes and environmental influences. In the presence of smoking and latent adenovirus C infection, TNF- $\alpha$  -308G/A, SP-B+1580 C/T and IL-13 -1055 C/T gene polymorphisms predispose to the development of COPD and decline of lung functions.

## References

1. Molfino NA. Genetics of COPD. *Chest* 2004; 125: 1929-40.
2. Silverman EK. Progress in chronic obstructive pulmonary disease genetics. *The Proceedings of the American Thoracic Society* 2006; 3: 405-8.
3. McCloskey SC, Patel BD, Hinchliffe SJ, et al. Siblings of patients with severe obstruction pulmonary disease have a significant risk of airflow obstruction. *Am J Resp Crit Care Med* 2001; 164: 1419-24.
4. Wu L, Chau J, Young RP, et al. Transforming growth factor-beta1 genotype and susceptibility to chronic obstructive pulmonary disease. *Thorax* 2004; 59: 126-9.
5. El Bohy M, Ahmed M, Ezzeldin N, et al. Estimation of TGF-beta1 genotypes in Egyptian smokers: association with FEV1 in COPD patients. *Arch Med Sci* 2010; 6: 71-6.
6. Sakao S, Tatsumi K, Igari H, et al. Association of tumor necrosis factor alpha gene promoter polymorphism with the presence of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001; 163: 420-2.
7. Keatings VM, Collins PD, Scott DM, et al. 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: 530-4.
8. Takabatake N, Nakamura H, Abe S, et al. 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: 1179-84.
9. Huang SL, Su CH, Chang SC. Tumor necrosis factor-alpha gene polymorphism in chronic bronchitis. *Am J Respir Crit Care Med* 1997; 156: 1436-9.
10. 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.
11. Zheng T, Zhu Z, Wang Z, et al. Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J Clin Invest* 2000; 106: 1081-93.
12. van der Pouw Kraan TC, Kucukaycan M, Bakker AM, et al. Chronic obstructive pulmonary disease is associated with the -1055 IL-13 promoter polymorphism. *Genes Immun* 2002; 3: 436-9.
13. Edwards KM, Thompson J, Paolini J. Adenovirus infections in young children. *Pediatrics* 1985; 76: 420-4.
14. Samet JM, Tager IB, Speizer FE. The relationship between respiratory illness in childhood and chronic air-flow obstruction in adulthood. *Am Rev Respir Dis* 1983; 127: 508-23.
15. Matsuse T, Hayashi S, Kuwano K, et al. Latent adenoviral infection in the pathogenesis of chronic airways obstruction. *Am Rev Respir Dis* 1992; 146: 177-84.
16. Neumann R, Genersch E, Eggers HJ. Detection of adenovirus nucleic acid sequences in human tonsils in the absence of infectious virus. *Virus Res* 1987; 7: 93-7.
17. Horvath J, Palkonyay L, Weber J. Group C adenovirus DNA sequences in human lymphoid cells. *J Virol* 1987; 59: 189-92.
18. Bosken CH, Wiggs BR, Pare PD, et al. Small airway dimensions in smokers with obstruction to airflow. *Am Rev Respir Dis* 1990; 142: 563-70.
19. American Thoracic Society. COPD criteria of diagnosis. *Am J Resp Crit Care Med* 1995; 152-77.
20. Garnett CT, Erdman D, Xu W, et al. Prevalence and quantization of species C adenovirus DNA in human mucosal lymphocytes. *J Virol* 2002; 76: 10608-16.
21. Dolin R. Common viral respiratory infections and severe acute respiratory syndrome (SARS). In: Harrison's internal medicine. 17<sup>th</sup> ed. McGraw (ed.). Hill Professional 2008; Chapter 179.
22. Ezzeldin N, Motawah A, Mohsen M, et al. Detection of a latent adenovirus C gene in children with recurrent respiratory tract infection. Passive smoking and infection. *Egypt J Chest* 2010; 59: 1-7.
23. Fletcher C, Peto RA. Natural history of chronic airflow obstruction. *BMJ* 1977; 1: 1645-8.
24. Meshi B, Vitalis T, Ionescu D. Emphysematous lung destruction by cigarette smoke: the effects of latent adenoviral infection on the lung inflammatory response. *Am J Respir Cell Mol Biol* 2002; 26: 52-7.
25. Ogawa E, Mark Elliott W, Hughes F, et al. Latent adenoviral infection induces production of growth factors relevant to airway remodeling in COPD. *Am J Physiol Lung Cell Mol Physiol* 2004; 286: 189-97.
26. Wood AM, Stockley RA. The genetics of chronic obstructive pulmonary disease. *Respir Res* 2006; 7: 130.
27. Patuzzo C, Gile LS, Zorzetto M, et al. Tumor necrosis factor gene complex in COPD and disseminated bronchiectasis. *Chest* 2000; 117: 1353-8.
28. Higham M, Pride N, Alikhan A, et al. Tumor necrosis factor-alpha gene promoter polymorphism in chronic obstructive pulmonary disease. *Eur Respir J* 2000; 15: 281-4.
29. Keatings VM, Cave SJ, Henry MJ, et al. A polymorphism in the tumor necrosis factor-alpha gene promoter region may predispose to a poor prognosis in COPD. *Chest* 2000; 118: 971-5.
30. The International HapMap Consortium. A haplotype map of the human genome. *Nature* 2005; 437: 1299-320.
31. Sandford AJ, Chagani T, Weir TD, et al. Susceptibility genes for rapid decline of lung function in the Lung Health Study. *Am J Respir Crit Care Med* 2001; 163: 469-73.
32. Lanus EC, De-Torres JP, Baz-Dávila R, et al. Siblings of patients with severe obstruction pulmonary disease have a significant risk of airflow obstruction. *Am J Resp Crit Care Med* 2001; 164: 1419-24.
33. Wilson AG, De Vries N, Pociot F, et al. An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA A1, B8, and DR3 alleles. *J Exp Med* 1993; 177: 557-60.
34. Küçükaycan M, Van Krugten M, Pennings H, et al. Tumor necrosis factor-alpha +489G/A gene polymorphism is associated with chronic obstructive pulmonary disease. *Respir Res* 2002; 3: 29.
35. Guo X, Lin HM, Lin Z, et al. Surfactant protein gene A, B, and D marker alleles in chronic obstructive pulmonary disease of a Mexican population. *Eur Respir J* 2001; 18: 482-90.
36. Hu R, Xu Y, Zhang Z. Surfactant protein B 1580 polymorphism is associated with susceptibility to chronic obstructive pulmonary disease in Chinese Han population. *J Huazhong Univ Sci Technol Med Sci* 2004; 24: 216-38.