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ORIGINAL ARTICLE

Tumour necrosis factor-α gene polymorphisms in asbestos-induced diseases

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Abstract

Background: Tumour necrosis factor (TNF)-α influences the pathogenesis of lung fibrosis and carcinogenesis in normal cells. Polymorphisms of this gene have been suggested to be associated with susceptibility to lung diseases.

Methods: Association studies were performed in German subjects, using control subjects ($n = 177$), pulmonary fibrosis patients ($n = 612$) and bronchial carcinoma patients ($n = 374$).

Results: Compared with a healthy (control) group, a significant result could be obtained for the asbestosis (patient) group (crude odds ratio (OR_{crude}) = 1.57; 95% confidence interval (CI) 1.05–2.36; $p = 0.03$), especially with severe lung asbestosis ($OR_{crude} = 4.15$; 95% CI 1.06–16.16; $p = 0.04$). A significant association was revealed when comparing asbestosis patients ($OR_{crude} = 4.08$; 95% CI 1.53–10.54; $p = 0.004$ and $OR_{adjusted} = 3.89$; 95% CI 1.49–10.17; $p = 0.006$) with asbestos-induced lung cancer patients.

Conclusion: The results confirm the hypothesis that TNF-α polymorphisms are associated with asbestos-induced fibrotic or malignant lung diseases in Germans.

Keywords: *TNF-α gene polymorphism; asbestos; lung fibrosis; lung cancer; single-nucleotide polymorphisms; cytokines*

Introduction

Inhaled asbestos fibres are known to cause progressive lung or pleural fibrosis and malignancies such as lung cancer or diffuse malignant mesothelioma (for review see Kamp 2009). Interindividual differences might play a crucial role in outcome and severity of asbestos diseases (Schneider et al. 2006). Inflammatory processes are of general importance in the pathogenesis of asbestosis or silicosis, as they are driven by chronic inflammation of the airways due to inhaled fibres and particles (for review see Manning et al. 2002). Coal dust exposure, for example, stimulates an inflammatory response leading to increased release of cytokines such as tumour necrosis factor (TNF)-α and interleukin (IL)-1, which play a key role in pathogenesis of pneumoconiosis (Ates et al. 2009).

TNF-α, a multifunctional, proinflammatory cytokine, is produced primarily by monocytes, macrophages and lymphocytes. It exhibits many inflammatory effects (e.g. activating neutrophils and mononuclear cells, inducing expression of adhesion molecules, cytokines and chemokines) and is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation and apoptosis. TNF-α has been found to be significantly elevated in bleomycin and silica models of lung fibrosis (for review see Gharaee-Kermani & Phan 2005). Furthermore TNF-α is upregulated in macrophages, epithelial and mesenchymal cells after exposure to asbestos (Sullivan et al. 2008) and TNF-α receptor, knock-out mice fail to develop lung fibrosis after treatment with bleomycin (Liu & Brody 2001, Liu et al. 1998). TNF-α is also associated with *in vivo* and *in vitro* killing of tumour cells and causes cytolysis and

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cytostasis of many tumour cell lines (for review see Gharaee-Kermani & Phan 2005, Kuwano et al. 2001, Kolb & Schmidt 2003).

Among others, TNF-α induces the production of cytokines such as IL-6 (Sanceau et al. 1991) or transforming growth factor (TGF)-β (Sullivan et al. 2008) and downregulation of TNF-α is considered to be associated with an overproduction of IL-10 (Huaux et al. 1999).

Single-nucleotide polymorphisms (SNPs) in regulatory regions of cytokine genes have been associated with susceptibility to a number of complex disorders (for review see Bidwell et al. 1999, 2001, Hollegaard & Bidwell 2006). Variation in the TNF-α promoter region has been found to be associated with susceptibility to several lung diseases such as chronic bronchitis (Huang et al. 1997), fibrosing alveolitis (Whyte et al. 2000), asthma (Witte et al. 2002), silicosis (Yucesoy et al. 2001) and coal workers' pneumoconiosis (Ates et al. 2008), as well as non-small cell lung cancer (Shih et al. 2006).

The SNPs located at nucleotides -238 (rs361525) and -308 (rs1800629) of the TNF-α promoter region, are all substitutions of adenine for guanine (Herrmann et al. 1998). In the literature the allelic types of the -238 polymorphism are referred to as -238G and -238A (Ates et al. 2008), while the allelic types of the -308 polymorphism are referred to as -308G and -308A or TNF1 and TNF2, respectively (Witte et al. 2002, Mira et al. 1999). TNF2 is supposed to be a stronger transcriptional activator than the common allele (TNF1), as observed in a human B-cell line (Wilson et al. 1997).

In this study we investigated whether or not TNF-α promoter polymorphisms were associated with the outcome or progression of asbestos-induced fibrosis and related malignancies.

Methods

Subjects

The study population consisted of a total of 1196 German patients. All subjects included in this study were interviewed using a questionnaire to obtain information on lifestyle (including a lifetime history of tobacco use) and occupational history. According to their reported smoking habits, patients were classified into smokers, former smokers or never smokers. Individual pack-years (PY) were calculated. One PY was defined as smoking 20 cigarettes daily over 1 year. Written informed consent was obtained from all patients before inclusion in the study.

The control group comprised 177 unrelated, healthy subjects without known diseases and without any exposure to carcinogenic (or fibrogenic) agents at the workplace. Any subjects with diseases related to potential tissue fibrosis (e.g. diabetes, chronic lung disease, etc.) or any benign or malignant tumours were excluded.

The lung fibrosis patients group contained 612 subjects. Only subjects with a confirmed diagnosis of lung fibrosis, according to American Thoracic Society (ATS) criteria (2000, 2002) were included.

The lung cancer patients group contained 374 subjects and the malignant mesothelioma group contained 33 patients. Only subjects with a histologically confirmed diagnosis of primary lung cancer or diffuse malignant mesothelioma, according to the WHO criteria (Travis 2004), were included.

Diagnosis of pulmonary diseases was based on physical examination, haematological, biochemical and immunology laboratory analyses and pulmonary function tests. All patients underwent diagnostic procedures, including X-ray examination interpreted according to the International Labour Office (ILO) Classification of Radiographs of Pneumoconiosis from 2003 (Hering et al. 2003).

To allow further discrimination between different kinds of exposure, several groups of patients suffering from lung fibrosis or lung cancer were created and compared to a non-exposed control group (Table 1).

In a second evaluation step the asbestosis group was further subdivided into groups considering the expansion and severity code of fibrosis. These groups were established using chest X-ray findings according to the ILO Classification of Radiographs of Pneumoconiosis from 2003 (Hering et al. 2003).

Chest X-ray findings

Asbestos-related abnormalities were classified as asbestosis (parenchymal changes) or as asbestos-induced pleural diseases. Small irregular opacities (s, t, u) were considered and classification of severity was done according to the ILO (Table 2) (Hering et al. 2003). Diffuse pleural thickening without parenchymal bands was only observed in adipose patients and, as such, was attributed to subpleural fat.

The ethics committees of the university hospital, Giessen, Germany, approved the study (AZ:75/06).

Real-time polymerase chain reaction and polymorphism detection

Three millilitres of whole blood was collected by venipuncture in tubes containing EDTA (Sarstaedt, Nümbrecht, Gernmany). Genomic DNA was isolated from whole blood using the Versagene™ DNA Purification Kit (Gentra Systems, Big Lake, MN, USA). Detection of the polymorphisms was performed by rapid capillary polymerase chain reaction (PCR), with melting curve analysis, using fluorescence-labelled hybridization

Table 1. Discrimination between different kinds of exposure. Several groups of patients suffering from lung fibrosis or lung cancer were created and compared with a non-exposed control group.

Diseases	*n*	Criteria		Reference
Asbestosis	395	Defined in list of occupational disease No. 4103 BKV	Caused by asbestos dust See criteria for diagnosis	Parker, 1997
Silicosis	162	Defined in list of occupational disease No. 4101 BKV	Caused by silica dust Criteria for diagnosis see	Parker, 1997
Lung interstitial fibrosis	55		No relevant exposure to asbestos or silica	
Asbestos-related lung cancer	48	Defined in list of occupational disease No. 4104 BKV	asbestos or pleural plaques or cumulative asbestos exposure ≥ 25 fibres/ml × year	DeVuyst, 1997
Diffuse malignant mesothelioma	33	Defined in list of occupational disease No. 4105 BKV	Caused by asbestos dust	
Lung cancer	326		No relevant exposure to asbestos	

BKV, list of occupational diseases.

Table 2. X-ray classification of asbestosis and pleural plaques. The groups were established using chest X-ray findings according to the International Labor Office Classification of Radiographs of Pneumoconiosis from 2000 (Hering et al. 2003).

Classification of parenchymal changes	
<1/0	No definite lung fibrosis
1/1 and 1/2	Beginning lung fibrosis (asbestosis)
2/1, 2/2 and 2/3	Moderate lung fibrosis (asbestosis)
3/2, 3/3 and 3/+	Severe lung fibrosis (asbestosis)
Classification of pleural changes	
0	No definite pleural plaques
1	<1/4 of lateral thoracic wall
2	1/4 - 1/2 of lateral thoracic wall
3	>1/2 of lateral thoracic wall
Hyalinosis complicata	Costophrenic obliteration, diffuse pleural thickening and pleuroparenchymal fibrous strand ('crow feet')

probes in a LightCycler System (Roche Diagnostics, Mannheim, Germany). The PCR primers as well as the fluorescent-labelled detection probes were synthesized by TIB MOLBIOL (Berlin, Germany) (Table 3). A duplex assay was used to analyse the TNF-α promoter polymorphisms (Dietmaier et al. 2002). The reaction mixture comprised 1 µl of each primer (10 µM) and 0.4 µl of each probe (10 µM), 0.8 µl $MgCl_2$ (25 mM), 2 µl FastStart DNA Master Hybridization Probes (Roche Diagnostics, Mannheim, Germany) and 2 µl DNA. After an initial denaturation step of 95°C for 10 min, the thermocycling conditions were 50 cycles of 95°C for 5 s, 55°C for 10 s and 72°C for 15 s. The melting curves were generated to obtain melting temperatures, which were visualized in canal F2 for the TNF-α (-238) and in canal F3 for the TNF-α (-308), respectively, of the LightCycler instrument.

Therefore, the melting curve of DNA homozygous for allele A contained a single peak at a melting temperature of 64.5°C (-238 and -308), samples homozygous for the allele G showed a single peak at 69.5°C (-238) and 67°C (-308), respectively, while heterozygous probes gave rise to biphasic melting curves. PCR contamination was checked by the inclusion of a negative control, where cDNA was replaced by water.

Statistical analysis

The odds ratio (OR) and confidence interval (CI) assessed the association between genotype distribution and patient status. OR and 95% CI were used as an estimate of the risk in all cases. The OR and CI were calculated by unconditional logistic regression. Adjustments for age, gender and tobacco smoking (PY) were computed to estimate the association between certain genotypes and diseases. Smokers were considered current smokers at the time of diagnosis. Ex-smokers were all people who had ever smoked. Information was collected on the usual number of cigarettes smoked per day, the age at which the subject started smoking and, if the person was an ex-smoker, the age at which the subject stopped smoking. Pack-years were calculated for the cumulative cigarette smoking. The smokers were stratified by the pack-year values. All statistical analyses were performed using the statistical software package, SSPS 15.0 (SPSS Inc., Chicago, IL, USA). Allelic and genotype frequencies were obtained by direct counting. Hardy–Weinberg equilibrium was assessed by a χ^2 test with 2 degrees of freedom. Allelic and genotype frequencies in patient and control groups were compared using a 2 × 2, contingency table and χ^2 test or the two-tailed Fisher's exact test when the number of expected cases was too low. The level of significance was set at $p < 0.05$.

Results

More smokers were present in the case groups (80.6%) when compared with the controls (58.2%). The lung cancer patient group (90.4%) but not the malignant mesothelioma patient group (42.4%) included more smokers when compared with the control group (Table 4). The mean age of the control group was 39.1 years (range 20–76) and of all the cases was 67.4 years (range 29–91).

Genotype and allele frequencies of TNF-α promoter polymorphisms

The A-allele frequency in the control group of TNF-α (-308) polymorphism (0.124) was higher than the A-allele frequency of TNF-α (-238) polymorphism (0.042) (Table 5). Compared with the control group, higher allele frequencies for the A-allele of TNF-α (-238) were revealed in the asbestosis No 4103 BKV group (BKV – occupational disease regulation) (0.098) and the hyaline pleural plaques extend 3 group (0.094). Lower allele frequencies were revealed in the asbestosis-induced lung cancer No 4104 BKV group (0.021).

For the A-allele of TNF-α (-308) higher allele frequencies compared with the control group were revealed in the asbestosis No 4103 BKV group (0.180), especially in the 2/1, 2/2 and 2/3 ILO groups (0.278). Additionally, the hyaline pleural plaques group (0.244) (extend 1 group (0.375), extend 2 group (0.219) and extend 3 group (0.188)) revealed higher allele frequencies. Lower allele frequencies were seen in the asbestosis-induced lung cancer No 4104 BKV group (0.063) and in the malignant mesothelioma patient group (0.091).

The studied population, including controls, did not deviate from Hardy–Weinberg equilibrium ($\chi^2 = 2.395$ for TNF-α (-238) and $\chi^2 = 0.49$ for TNF-α (-308) polymorphism).

TNF-α (-308) promoter polymorphisms and clinical manifestation

OR analyses were performed for homozygous wild-type (G-308G) genotype versus the genotypes carrying at least

Table 3. Primer and probes sequences for the -238 and -308 tumour necrosis factor (TNF)-α promoter polymorphisms.

Gene	SNP	Primer	Primer sequence 5'–3'
TNF-α	-238	TNF-396	TTCCTGCATCCTGTCTGGAA
	(G→A)	TNF-069	CAGCGGAAAACTTCCTTGGT
		anchor	Fl-TTCAGCCTCCAGGGTCCTACACACAAATCAGTCAGTGGCCCAGAAGA
		-238(G)	705-CCTCGGAATCGGAGCAGGGAGGA
	-308	TNF-396	TTCCTGCATCCTGTCTGGAA
	(G→A)	TNF-069	CAGCGGAAAACTTCCTTGGT
		anchor	Fl-TTCAGCCTCCAGGGTCCTACACACAAATCAGTCAGTGGCCCAGAAGA
		-308(G)	AATAGGTTTTGAGGGGCATGGGGACT-LC640

SNP, single-nucleotide polymorphism

Table 4. Demographic and disease parameters of controls and patients.

		Age (years)			Smoking habit (%),
	n	Mean	Range	Median	smoker/ex-smoker
Control group	177	39.1	20–76	34.9	58.2
Patient group	1019	67.4	29–91	68.7	80.6
Lung fibrosis	612	68.1	29–91	69.3	76.6
Silicosis (BK 4101)	162	70.5	40–89	71.3	80.2
Asbestosis (BK 4103)	395	67.8	45–91	68.5	76.7
Lung fibrosis of other genesis	55	64.7	29–82		65.5
Malignant mesothelioma (BK 4105)	33	65.1	46–75	67.0	42.4
Lung cancers	374	66.4	34–83	68.1	90.4
Asbestos-induced Lung cancers (BK 4104)	48	67.3	52–81	67.5	97.9
Lung cancer of other genesis	326	66.3	34–84	68.1	89.3

Table 5. Tumour necrosis factor (TNF)-α genotype distribution and allele frequencies in Caucasian healthy controls and patients.

	TNF-α -238				TNF-α -308			
	G/G	G/A	A/A	q(A) =	G/G	G/A	A/A	q(A) =
Controls	162	15	0	0.042	136	38	3	0.124
Fibrosis	550	61	1	0.051	424	168	20	0.169
Asbestosis (4103 BKV)	356	39	0	0.098	268	112	15	0.180
Silicosis (4101 BKV)	144	17	1	0.059	117	41	4	0.151
Fibrosis of other genesis	50	5	0	0.045	39	15	1	0.155
Lung asbestosis	110	12	0	0.049	84	37	1	0.160
1/1 and 1 /2 ILO	58	5	0	0.039	44	19	0	0.151
2/1, 2/2 and 2/3 ILO	41	5	0	0.054	32	13	1	0.167
3/2, 3/3 and 3/+ ILO	8	1	0	0.055	4	5	0	0.278
Pleural asbestosis	188	22	0	0.052	144	56	10	0.181
Calcified pleural plaques								
Overall	108	12	0	0.050	86	30	4	0.158
Extend 1	30	5	0	0.071	24	11	0	0.157
Extend 2	34	1	0	0.014	27	7	1	0.129
Extend 3	40	6	0	0.065	31	12	3	0.196
Hyaline pleural plaques								
Overall	40	5	0	0.055	27	14	4	0.244
Extend 1	11	1	0	0.042	5	5	2	0.375
Extend 2	15	1	0	0.031	10	5	1	0.219
Extend 3	13	3	0	0.094	11	4	1	0.188
Hyalinosis complicata	40	5	0	0.055	31	12	2	0.177
Lung cancers	338	36	0	0.048	290	79	5	0.119
Asbestos-induced lung cancers (4104 BKV)	46	2	0	0.021	43	4	1	0.063
Lung cancer of other genesis	292	34	0	0.052	247	75	4	0.127
Mesothelioma (4105 BKV)	30	3	0	0.045	27	6	0	0.091

ILO, International Labor Office; BKV, BKV, occupational disease regulation.

one mutant (A) allele (G-308A and A-308A). Data for crude and for pack-years (PY), age (years) and gender-adjusted analyses of TNF-α gene polymorphism (-308) in patients, are shown in Table 6.

This analysis revealed that patients with at least one mutant (A) allele of TNF-α (-308) were at higher risk for fibrotic lung diseases (OR_{crude} = 1.472; 95% CI 0.99–2.17; p = 0.05). Significant results could be obtained for the asbestosis patient group (OR_{crude} = 1.57; 95% CI 1.05–2.36; p = 0.03). In particular, the group with severe lung asbestosis (3/2, 3/3 and 3+), revealed a higher risk (OR_{crude} = 4.15; 95% CI 1.06–16.16; p = 0.04). Additionally, a significantly increased risk for (A) allele carriers was seen for patients with hyaline pleural plaques (OR_{crude} = 2.21; 95% CI 1.11–4.1; p = 0.02 and $OR_{adjusted}$ = 2.90; 95% CI 1.08–7.78; p = 0.03). This result was evident in a subgroup of patients with hyaline pleural plaques (OR_{crude} = 4.64; 95% CI 1.40–15.41; p = 0.01 and $OR_{adjusted}$ = 5.32; 95% CI 1.22–23.34; p = 0.03).

Only the crude OR revealed a decreased risk for individuals with asbestos-induced bronchial carcinoma, when carrying at least one mutant (A) allele at -308 of the TNF-α promoter (OR_{crude} = 0.39; 95% CI 0.14–1.04). After adjusting for PY, age and gender no association was detected.

When OR analyses were performed for lung fibrosis patients, compared with lung cancer patients the (A) allele at -308 of the TNF-α promoter was significantly associated with a higher risk for fibrotic lung diseases (OR_{crude} = 1.53; 95% CI 1.14–2.06; p=0.050 and $OR_{adjusted}$ = 1.75; 95% CI 1.25–2.45; p = 0.001). A significant association for the (A) allele was also revealed when comparing asbestosis patients (OR_{crude}= 4.08; 95% CI 1.53–10.54; p = 0.004 and $OR_{adjusted}$ = 3.89; 95% CI 1.49–10.17; p = 0.006), with asbestos-induced lung cancer patients. Additionally, a significant result was obtained when comparing non-asbestos fibrosis with non-asbestos lung cancer ($OR_{adjusted}$ = 1.58; 95% CI 1.00–2.5; p = 0.049) (see Table 8).

TNF-α (-238) promoter polymorphisms and clinical manifestation

OR analyses were performed for the homozygous wild-type (G-238G) genotype versus the genotypes carrying at least one mutant allele (G-238A or A-238A). Data for crude and to PY-, age- and gender-adjusted analyses of TNF-α gene polymorphism -238 in patients are shown in Table 7.

This analysis revealed that patients with at least one mutant (A) allele in the promoter of TNF-α were at higher

Table 6. Crude and adjusted odds ratio (OR) for tumour necrosis factor (TNF)-α promoter polymorphism -308 in patients.

	Crude OR			OR adjusted for PY, age, gender		
TNF-α -308	OR	95% CI	*p*-Value	OR	95% CI	*p*-Value
Controls	1.00			1.00		
Fibrosis	1.47	0.99–2.17	0.05	1.42	0.76–2.64	0.27
Asbestosis (4103 BKV)	1.57	1.05–2.36	0.03	1.62	0.82–3.21	0.17
Silicosis (4101 BKV)	1.28	0.78–2.08	0.33	1.23	0.53–2.88	0.63
Fibrosis of other genesis	1.36	0.68–2.68	0.37	1.25	0.51–3.05	0.63
Lung asbestosis	1.50	0.89–2.52	0.13	1.60	0.66–3.87	0.30
1/1 and 1 /2 ILO	1.43	0.75–2.72	0.27	1.64	0.61–4.43	0.33
2/1, 2/2 and 2/3 ILO	1.45	0.71–2.98	0.31	1.65	0.58–4.68	0.35
3/2, 3/3 and 3/+ ILO	4.15	1.06–16.16	0.04	5.55	0.91–33.72	0.06
Pleural asbestosis	1.52	0.97–2.39	0.07	1.52	0.72–3.20	0.72
Calcified pleural plaques						
Overall	1.31	0.77–2.23	0.32	1.19	0.52–2.74	0.68
Extend 1	1.52	0.69–3.37	0.3	1.56	0.59–4.38	0.40
Extend 2	0.98	0.42–2.33	0.97	1.06	0.34–3.33	0.92
Extend 3	1.61	0.79–3.26	0.19	1.56	0.57–4.27	0.39
Hyaline pleural plaques						
Overall	2.21	1.11–4.41	0.02	2.90	1.08–7.78	0.03
Extend 1	4.64	1.40–15.41	0.01	5.32	1.22–23.34	0.03
Extend 2	1.99	0.68–5.81	0.21	0.49	0.69–9.04	0.17
Extend 3	1.51	0.50–4.59	0.47	1.77	0.46–6.68	0.41
Hyalinosis complicata	1.50	0.73–3.08	0.27	1.68	0.63–4.49	0.30
Lung cancers	0.96	0.63–1.47	0.85	1.19	0.59–2.46	0.63
Asbestos-induced lung cancers (4104 BKV)	0.39	0.14–1.04	0.06	1.19	0.59–2.42	0.63
Lung cancer of other genesis	1.06	0.69–1.63	0.79	1.372	0.67 -2. 81	0.39
Mesothelioma (4105 BKV)	0.74	0.29–1.91	0.53	0.61	1.89–1.89	0.41

CI, confidence interval; PY, pack year; ILO, International Labor Office; BKV, BKV, occupational disease regulation.

risk for fibrotic lung diseases (OR_{crude} = 1.22; 95% CI 0.67–2.20 and $OR_{adjusted}$ = 1.77; 95% CI 0.67–4.98). The asbestosis and the silicosis patients groups revealed higher risks (OR_{crude} = 1.18; 95% CI 0.63–2.21 and $OR_{adjusted}$ = 2.12; 95% CI 0.64–6.96 for asbestosis and OR_{crude} = 1.35; 95% CI 0.66–2.78 and $OR_{adjusted}$ = 1.95; 95% CI 0.46–8.23 for silicosis).

In contrast to the TNF-α (-308) findings above, individuals carrying at least one mutant (A) allele in the promoter of TNF-α (-238) had a decreased risk for asbestos-induced bronchial carcinoma (OR_{crude} = 0.47; 95% CI 0.10–2.13 and $OR_{adjusted}$ = 0.72; 95% CI 0.09–5.56). The lung cancer of other genesis shows the same trend towards a lower risk ($OR_{adjusted}$ = 0.75; 95% CI 0.26–2.18).

These results did not gain any significance, even when OR was performed for lung fibrosis patients versus lung cancer patients. The lung fibrosis patients were at a higher risk when carrying the (A) allele but significance remained to be achieved (Table 8).

Discussion

In this study we demonstrated a significant association for the (A) allele at position -308 of the TNF-α with a higher risk for fibrotic lung diseases. Simultaneously, we showed a significant association of the (A) allele with higher risk for fibrotic lung diseases and a lower risk for lung cancers, when both groups were compared with each other.

TNF-α, as a proinflammatory cytokine, plays an important role in particle-induced inflammation of the lung, through induction of adhesion molecules and stimulation of other proinflammatory molecules (Driscoll 2000).

In cancer TNF-α has rather conflicting roles. TNF-α has an antitumour effect by inducing apoptosis, and at high doses, it is cytotoxic. On the other hand, chronic low-dose production of TNF-α is supposed to promote cancer growth (for review see Balkwill 2006, Szlosarek et al. 2006). Nuclear factor κB activated by TNF-α leads to survival and resistance against the cytotoxic effects of asbestos in human mesothelial cells and therefore, increases the risk of malignant transformation (Yang et al. 2006). Just as in other multifactorial diseases, there is a large interindividual variability of susceptibility to asbestosis.

The genetic variant at position G-308A is supposed to have functional effects on gene transcription activity (Wilson et al. 1992, 1997). Higher constitutive and inducible transcriptional levels were presented in carriers of the rare TNF2 allele (-308A) (Wilson et al. 1997). Coal

Table 7. Crude and adjusted odds ratio (OR) for tumour necrosis factor (TNF)-α promoter polymorphism -238 in patients.

	Crude OR			OR adjusted for PY, age, gender		
TNF-α -238	OR	95% CI	*p* value	OR	95% CI	*p* value
Controls	1			1.00		
Fibrosis	1.22	0.67–2.2	0.51	1.77	0.67–4.98	0.28
Asbestosis (4103 BKV)	1.18	0.63–2.21	0.59	2.12	0.64–6.96	0.22
Silicosis (4101 BKV)	1.35	0.66–2.78	0.42	1.95	0.46–8.23	0.37
Fibrosis of other genesis	1.08	0.37–3.12	0.98	1.26	0.30–5.20	0.75
Lung asbestosis	1.18	0.53–2.61	0.69	2.15	0.46–9.93	0.33
1/1 and 1 /2 ILO	0.93	0.32–2.68	0.89	1.50	0.26–8.87	0.65
2/1. 2/2 and 2/3 ILO	1.32	0.45–3.83	0.61	1.72	0.33–8.98	0.52
3/2. 3/3 and 3/+ ILO	1.35	0.16–11.53	0.78	1.61	0.12–22.66	0.72
Pleural asbestosis	1.26	0.63–2.52	0.51	2.03	0.56–7.46	0.28
Calcified pleural plaques						
Overall	1.20	0.54–2.66	0.65	2.24	0.48–10.38	0.30
Extend 1	1.80	0.61–5.32	2.88	2.97	0.58–15.21	0.19
Extend 2	0.32	0.04–2.49	0.28	0.38	0.03–4.37	0.44
Extend 3	1.62	0.59–4.44	0.35	2.10	0.38–11.55	0.40
Hyaline pleural plaques						
Overall	1.35	0.46–3.93	0.58	1.83	0.39–8.62	0.45
Extend 1	0.98	0.12–8.13	0.99	1.58	0.14–17.91	0.71
Extend 2	0.72	0.09–5.83	0.76	0.94	0.09–10.48	0.96
Extend 3	2.49	0.64–9.73	0.19	5.72	0.81–40.3	0.08
Hyalinosis complicata	1.35	0.46–3.93	0.59	1.87	0.38–9.17	0.44
Lung cancers	1.15	0.61–2.16	0.66	0.71	0.25–2.05	0.53
Asbestos-induced lung cancers (4104 BKV)	0.47	0.10–2.13	0.33	0.72	0.09–5.56	0.75
Lung cancer of other genesis	1.26	0.67–2.38	0.48	0.75	0.26–2.18	0.59
Mesothelioma (4105 BKV)	1.08	0.29–3.96	0.91	1.98	0.34–11.54	0.45

CI, confidence interval; PY, pack year; ILO, International Labor Office; BKV, occupational disease regulation.

Table 8. Crude and adjusted odds ratio (OR) for tumour necrosis factor (TNF)-α promoter polymorphism in fibrosis patients compared with bronchial carcinoma patients.

	Crude OR			OR adjusted		
	OR	95% CI	*p*-Value	OR	95% CI	*p*-Value
TNF-α -238						
Fibrosis vs lung cancers	1.058	0.687–1.631	0.797	1.056	0.650–1.716	0.826
Asbestosis (4103 BKV) vs asbestos-induced lung cancers (BK 4104)	1.018	0.582–1.782	0.950	1.157	0.607–2.208	0.657
Fibrosis of other genesis vs lung cancer of other genesis	2.520	0.589–10.782	0.213	2.003	0.457–8.792	0.357
TNF-α -308						
Fibrosis vs lung cancers	1.531	1.137–2.061	0.050	1.753	1.252–2.454	0.001
Asbestosis (4103 BKV) vs asbestos-induced lung cancers (BK 4104)	4.075	1.526–10.536	0.004	3.891	1.488–10.172	0.006
Fibrosis of other genesis vs lung cancer of other genesis	1.223	0.828–1.805	0.312	1.583	1.002–2.500	0.049

CI, confidence interval; BKV, occupational disease regulation.

dust-stimulated release of TNF-α, was significantly higher in TNF-α (-238) (A) allele carriers compared with TNF-α (-238) (G) allele carriers (Ates et al. 2009).

In agreement with our findings for asbestosis, an increased risk was also seen for silicosis patients with the TNF-α (-308) (A) allele independently of the severity in Americans (Yucesoy et al. 2002, 2001) and among the Han population of southwest China (Wang et al. 2005a). The TNF-α (-238) (A) allele frequency was significantly higher in severe forms of silicosis and reduced in moderate silicosis, indicating that the TNF-α (-238) (A) allele predisposes for a more rapid

development of the severe form (Yucesoy et al. 2001, 2002). These findings were confirmed in a population of black South African miners (Corbett et al. 2002). We did not find any significant association with the TNF-α (-238) (A) allele, in 162 investigated silicosis patients. This may be because we did not discriminate between the severities of silicosis. However we did observe similar results for the lung asbestosis and the hyaline pleural plaque patients groups. Also we revealed lower ORs for the moderate forms, while higher ORs were seen in severe disease forms. Maybe through the low TNF-α (-238) (A) allele frequency, we did not detect significant results but still we can show a trend towards a higher risk of TNF-α (-238) (A) allele carriers towards fibrotic lung diseases

Confirming our results, higher TNF-α (-308) (A) allele frequencies were seen in Japanese, as well as Europeans, with coal workers' pneumoconiosis (Wang et al. 2005b, Zhai et al. 1998). Carriage of the TNF-α (-308) (A) allele was also associated with increased risk of fibrosing alveolitis in English and Italian populations (Whyte et al. 2000) and with idiopathic pulmonary fibrosis in an Australian cohort (Riha et al. 2004). An association with a favourable prognosis was noted for the TNF-α (-308) (A) allele of pulmonary sarcoidosis patients in the Netherlands (Abraham & Kroeger 1999).

Mirroring its complexity in different types of cancer and differences within populations, there are varying results in the literature concerning the role of TNF-α (-308) polymorphism in tumour diseases. In our study we could not prove a definite effect of this polymorphism in the context of lung cancer. An association between the TNF-α (-308) (A) allele and an increased susceptibility to non-Hodgkin's lymphoma, uterine endometrial cancer and prostate cancer has been described (Skibola et al. 2010, Sasaki et al. 2000, Oh et al. 2000). A promoter effect was also shown, for lung cancer development and progression, in Chinese individuals with non-small cell lung cancer (Shih et al. 2006). A contrasting result has been reported in Taiwanese patients with oral squamous cell carcinoma. Compared with healthy controls a higher frequency of TNF-α (-308) G/G genotype was detected (Liu et al. 2005). A study in North China also connects the TNF-α (-308) (A) allele with a lower risk and the TNF-α (-308) (G) allele with a higher risk for of developing oesophageal squamous cell carcinoma and gastric cardiac adenocarcinoma (Guo et al. 2005). No associations were seen with different types of lung cancer in a German (Seifart et al. 2005) and a Croatian (Flego et al. 2009) population.

The TNF-α -238A allele was significantly lower in Korean patients with gastric cancer, uterine cervical cancer, colorectal cancer or renal cell carcinoma compared with healthy controls, indicating a protective effect against cancers (Jang et al. 2001). Additionally, lower ORs were revealed for non-small cell lung cancer in Chinese individuals carrying the at least one TNF-α (-238) (A) allele (Shih et al. 2006). In Taiwanese patients with oral squamous cell carcinoma the TNF-α (-238) G/A genotype frequency was lower compared with healthy controls, offering a protective effect against oral squamous cell carcinoma (Liu et al. 2005).

These findings confirm our results. Even though we could not present significant data, we demonstrated a lower risk for asbestos-induced lung cancer, as well as lung cancers of other genesis when carrying the TNF-α (-238) (A) allele. The protective effect of the TNF-α (-238) (A) allele against cancer may be provided by its function of decreasing TNF-α production, which supports the proposed role of TNF-α as an endogenous tumour promoter (Balkwill 2006).

The most relevant finding of the present study was the significant association for the (A) allele at position -308 of the TNF-α with a higher risk for fibrotic lung diseases. Additionally a significant association of the (A) allele with higher risk for fibrotic lung diseases and a lower risk for lung cancers, when both groups were compared with each other was demonstrated.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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