Interleukin gene polymorphisms in pneumoconiosis

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Abstract. Inhaled asbestos fibres are known to cause inflammation processes with the result of lung or pleural fibrosis and malignancies. Interleukins (IL), such as IL-1β, IL-6 and IL-10, have various functions in the regulation of the inflammatory response and in proliferative processes after inhalation of silica dust and can, therefore, influence the pathogenesis of asbestos-induced fibrosis and carcinogenesis. Polymorphisms within these genes may be associated with susceptibility to silica and asbestos-induced lung diseases. Thus, IL-1β, IL-6 and IL-10 polymorphisms were examined to determine an association with asbestos or silica-induced fibrosis or malignancies. Association studies were performed in 1180 individuals, using control subjects (n=177), fibrosis patients (n=605), lung cancer (LC) patients (n=364) and malignant mesothelioma (MM) patients (n=34). IL-1β (C-511T; C+3954T), IL-6 (G-174C) as well as IL-10 (G-1082A) polymorphisms were investigated. Compared to a healthy (control) group, a higher risk was seen for malignant mesothelioma patients in all investigated polymorphisms. The IL-6 -174C allele showed a tendency towards a higher risk for fibrosis or asbestos-induced lung cancer ($OR_{asbestosis}$, 1.338; 95% CI, 0.71-2.53; $OR_{silicosis}$, 1.226; 95% CI, 0.54-2.81; $OR_{fibrosis\ other\ aetiology}$, 1.313; 95% CI, 0.58-2.98 and $OR_{LC\ asbestos}$, 2.112; 95% CI, 0.75-5.92). The IL-10 -1082A carrier seemed to be at higher risk for silicosis ($OR_{silicosis}$, 2.064; 95% CI, 0.78-5.49) but not for asbestosis. In summary, this study did not reveal sufficient evidence for a significant association of the investigated interleukin polymorphisms with asbestos or silica-induced diseases in the population studied.

Introduction

Inhaled asbestos fibres are known to cause progressive lung or pleural fibrosis and malignancies, such as lung cancer or diffuse malignant mesothelioma (1). Interindividual differences might play a crucial role in the outcome and severity of asbestos diseases (2). Inflammatory processes are of general importance in the pathogenesis of asbestosis and also in silicosis, as they are driven by chronic inflammation due to inhaled fibres and particles (3). The inhalation of asbestos or silica leads to interleukin-1β (IL-1β) secretion, which plays a key role in the pathogenesis of occupational-related pneumoconiosis (4,5). IL-1β is a pro-inflammatory cytokine with wide ranging effects on gene expression, such as upregulating cytokines and tissue remodelling enzymes (6,7). Chronic overexpression of IL-1β may also contribute to the growth, vascularisation and metastasis of malignant tumours (8-10).

The IL-6 multifunctional cytokine has the potential of initiating a fibrogenic response after asbestos exposure (11). As a result of the fibres, lung epithelial cells release IL-6, which in turn stimulates lung fibroblast DNA synthesis (12). Several studies have reported an involvement of IL-6 in the pathogenesis of inflammatory diseases such as coal workers' pneumoconiosis (CWP) (13) rheumatoid arthritis (14), chronic arthritis (15) and psoriasis (16).

Single-nucleotide polymorphisms (SNPs) in regulatory regions of cytokine genes have been associated with susceptibility to a number of complex disorders (17-19). The T alleles of the IL-1β C+3954T (rs1143634) and C-511T (rs16944) polymorphisms have been shown to correlate with higher IL-1β-protein levels *in vitro* (20,21). On the other hand, the C allele of the IL-6 G-174C polymorphism (rs1800795) was found to be associated with significant lower levels of plasma IL-6 (22).

IL-10 is an important immunosuppressor cytokine, controlling the balance between inflammatory and humoral response by inhibiting the release of pro-inflammatory mediators such as IL-1β and IL-6. Physiologically it is produced after pro-inflammatory mediators and, therefore, plays a role in limiting an excessive immune response and collateral damage (23,24). Interindividual variations of IL-10 levels (mRNA and serum protein levels) are genetically controlled by polymorphic variants present at the gene promoter. The G allele at position -1082 seems to be the most important genetic factor in the regulation of IL-10 levels (25).

In the present study, we investigated the distribution of genotype frequencies of proinflammatory mediator polymorphisms (IL-1β C+3954T, IL-1β C-511T and IL-6 G-174C) and the anti-inflammatory cytokine IL-10 polymorphism (IL-10 G-1082A) in patients with asbestos- or silica-induced fibrosis or malignancies.

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Abbreviations: BKV, occupational disease regulation; PAH, polycyclic aromatic hydrocarbons; LC, lung cancer; LPS, lipopolysaccharide; OR, odds ratio; CI, confidence interval; PY, pack year; MM, malignant mesothelioma

Key words: interleukin gene polymorphism, asbestos, silica, lung fibrosis, lung cancer, single-nucleotide polymorphisms, cytokines

Table I. Discrimination between different kinds of exposure.

Diseases	N	Definition	Diagnosis criteria (Ref.)
Asbestosis	389	BKV 4103 according to the German list of occupational diseases	Caused by asbestos dust; diagnosis criteria (48)
Silicosis	161	BKV 4101 according to the German list of occupational diseases	Caused by silica dust; diagnosis criteria (29)
Lung interstitial fibrosis	55		No relevant exposure to asbestos
Asbetos-related lung cancer	49	BKV 4105 according to the German list of occupational diseases	Diagnosis criteria (49)
Diffuse malignant mesothelioma	34	BKV 4105 according to the German list of occupational diseases	Caused by asbestos dust
Lung cancer	315		No relevant exposure to asbestos

Several groups of patients suffering from lung fibrosis or lung cancer were created and compared to a non-exposed control group. BKV, German classification system of occupational diseases.

Materials and methods

Subjects. The study population consisted of a total of 1180 German individuals. 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, ex-smokers or never smokers. Individual smoking pack years (PY) were calculated. One PY was defined as smoking 20 cigarettes daily over one year. Written informed consent was obtained from all patients before inclusion in the study.

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

The lung fibrosis patients group contained 605 subjects. Only subjects with confirmed diagnosis of lung fibrosis, according to the WHO criteria (26,27) were included. The lung cancer patient group contained 364 and the malignant mesothelioma group contained 34 patients. Only subjects with histological confirmed diagnosis of primary lung cancer or diffuse malignant mesothelioma, according to the WHO criteria (28), were included.

Diagnosis of pulmonary diseases was based on physical examination, haematological, biochemical and immunological 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 (29).

To allow further discrimination between different kinds of exposure, several groups of patients suffering from fibrosis or lung cancer were created and compared to a non-exposed control group (Table I). 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 (29).

Chest X-ray findings. Chest X-rays were reviewed and graded according to the ILO 2000 classification. According to the ILO standard X-rays, three profusions (1, 2 and 3) and the six size and shapes of the opacities (p, q, r for rounded, s, t, u for irregular, and A, B and C for large opacities) were coded. Asbestos-related abnormalities were classified as asbestosis (parenchymal changes) or as asbestos-induced pleural diseases (Table II) (29). Diffuse pleural thickening without parenchymal bands were only observed in adipose patients and as such were attributed to subpleural fat. The Ethics Committee of the University Hospital, Giessen, Germany, approved the study (AZ:75/06).

Real-time PCR and polymorphism detection. Whole blood (3 ml) samples were collected by venipuncture in tubes containing EDTA (Sarstaedt, Nümbrecht, Germany). Genomic DNA was isolated from whole blood using the Versagene™ DNA purification kit (Gentra Systems, Minneapolis, MN, USA). Detection of the polymorphisms was performed by rapid capillary PCR, with melting curve analysis, using fluorescence-labelled hybridisation 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 III). The reaction mixture (20 μl) for IL-1β C+3954T comprised 2 μl of forward primer (10 μM), 1 μl of reverse primer (10 μM) and 0.4 μl of each probe (10 μM), 1.2 μl $MgCl_2$ (25 mM), 2 μl FastStart DNA Master Hybridization Probes (Roche Diagnostics) and 2 μl DNA. The reaction mixture (20 μl) for IL-6 and IL-10 comprised 0.6 μl of each primer (10 μM) and 0.4 μl of each probe (10 μM), 10 μl ABgene QPCR Capillary Master Mix (Thermo Fisher, Dreieich, Germany) and 2 μl DNA. Annealing temperatures were 60˚C for IL-1β C+3954T, 57˚C for IL-6 G-174C and 63˚C for IL-10 G-1082A polymorphism. The melting curves were generated to obtain melting temperatures. PCR contamination was checked by the inclusion of negative control, where cDNA was replaced by water.

Detection of the IL-1β C-511T polymorphisms was performed by restriction fragment length analysis (RFLA). The reaction mixture (22 μl) for the PCR consisted of 1 μl

Table II. X-ray classification of asbestosis and pleural plaques.

Grade	Classification group
Classification of parenchymal changes	
<1/0	No definite lung fibrosis
1/1 and 1/2	Beginning lung fibrosis
2/1, 2/2 and 2/3	Moderate lung fibrosis
3/2, 3/3 and 3/+	Severe lung fibrosis
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; pleuro-parenchymal fibrous strand ('crow feet')

The groups were established using chest X-ray findings according to the International Labour Office Classification of Radiograhs of Pneumoconiosis (29).

of each primer (TIB Molbiol) (Table III), 1 μl Q-solution, 2 μl 10X buffer, 2 μl $MgCl_2$, 1 μl HotstarTaq® Plus (all from Qiagen, Hilden, Germany) 0.2 μl dNTPs (Fermentas, St. Leon-Rot, Germany) and 2 μl DNA. Annealing temperatures in 40 cycles was 58˚C. As a restriction enzyme *Ava*I (New England Biolabs, Ipswich, MA, USA) was used. Results were visualised by agarose gel electrophoresis.

Statistical analysis. The odds ratios (OR) and confidence intervals (CI) assessed the association between genotype distribution and patient status. The OR and 95% CI were used as an estimate of the risk in all cases and 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. Current smokers at the time of diagnosis were considered smokers. Ex-smokers were all the 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. PY were calculated for the cumulative cigarette smoking. The smokers were stratified by the PY values. All statistical analyses were performed using the statistical software package, SSPS 17.0 (SPSS Inc., Chicago, IL, USA). Allelic and genotype frequencies were obtained by direct counting. The 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 2x2, contingency table and a χ^2 test or the 2-tailed Fisher's exact test when the number of expected cases was too low. The level of significance was set at $P<0.06$.

Results

More smokers or ex-smokers were present in the patient (81%) compared to the control (42%) group. The lung cancer patient group (91%) but not the malignant mesothelioma group (42%) included more smokers when compared to the controls (Table IV). The mean age of the control group was 39.2 years (range 20-76) and 67.5 years (range 29-91) for all the cases.

Clinical manifestation of IL-6 G-174C polymorphisms. The frequency of the IL-6 (-174) C allele in the control group as well as in the patients group was 0.45. OR analyses were performed for the genotype G-174G vs. the genotypes carrying at least one C allele (G-174C or C-174C). Data was adjusted for PY, age (years) and gender.

Table III. Primer and probe sequences for interleukin polymorphisms.

Gene	SNP	Primer	Primer sequence 5'→3'
IL-1β	+3954 (C→T)	Forward	5'-CCTGCCCTTCTGATTTTATACC-3'
		Reverse	5'-CAGGATGTTTCCATTTACCTTG-3'
		Anchor	TCGTGCACATAAGCCCTCGTTATCCC-FL
		+3954(T)	640-TGTGTCAAAGAAGATAGGTTCTGAAA-p
	-511 (C→T)	Forward	5'-TGGCATTGATCTGGTTCATC-3'
		Reverse	5'-GTTTAGGAATCTTCCCACTT-3'
		Enzyme	*Ava*1
IL-6	-174 (G→C)	Forward	5'-TTACTCTTTGTCAAGACATGCCA-3'
		Reverse	5'-ATGAGCCTCAGACATCTCCAG-3'
		Anchor	AGCTGCACTTTTCCCCCTAGT-Fl
		-174(G)	640-GTGTCTTGC**G**ATGCTAAAGGA-p
IL-10	-1082 (G→A)	Forward	5'-CTCGCTGCAACCCAACTGGC-3'
		Reverse	5'-ATGGGGTGGAAGAAGTTGAA-3'
		Anchor	GGATAGGAGGTCCCTTACTTTCCTCTTACC-Fl
		+3954(G)	640-CCCTACTTCCCC**C**TCCCAAA-p

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

		Age (years)			Smoking habit (%)
	n	Mean	Range	Median	Smoker/ex-smoker
Control total	177	39.19	20.0-75.5	34.9	58.2
Patient total	1003	67.46	29.2-91.2	68.8	81
Lung fibrosis	605	68.18	29.2-91.2	69.3	77
Silicosis (BKV 4101)	161	70.47	40.3-88.9	71.5	80.7
Asbestosis (BKV 4103)	389	67.89	45.7-91.2	68.6	77.1
Lung fibrosis of other aetiology	55	63.5	29.2-82.1	63.8	65.5
Malignant mesothelioma (BKV 4105)	34	66.6	34.3-83.9	68.2	44.1
Lung cancer	364	66.6	34.3-83.9	68.2	90.9
Asbestos induced lung cancer (BKV 4104)	49	66.9	50.4-80.9	67.4	98
Lung cancer of other aetiology	315	66.5	34.3-83.9	68.3	89.8

Table V. Adjusted odds ratio of IL-6 G-174C polymorphisms.

		Genotype, n (%)		Multivariate analysis		
Diagnosis	n	GG	GC or CC	OR	95% CI	p-value
Lung fibrosis	605	213 (35.2)	392 (64.8)	1.307	0.725-2.356	0.373
Silicosis (BKV 4101)	161	56 (34.8)	105 (65.2)	1.226	0.535-2.809	0.63
Asbestosis (BKV 4103)	389	138 (35.5)	251 (64.5)	1.338	0.708-2.532	0.37
Lung asbestosis (BKV 4103)	122	37 (30.3)	85 (69.7)	1.966	0.823-4.698	0.128
1/1 and 1/2 ILO	66	19 (28.8)	47 (71.2)	2.288	0.798-6.564	0.124
2/1, 2/2 and 2/3 ILO	46	15 (32.6)	31 (67.4)	1.45	0.524-4.013	0.475
3/2, 3/3 and 3/+ ILO	9	3 (33.3)	6 (66.7)	1.97	0.19-20.45	0.57
Lung fibrosis of other aetiology	55	19 (34.5)	36 (65.5)	1.313	0.579-2.978	0.514
Malignant mesothelioma	34	9 (26.5)	25 (73.5)	1.918	0.675-5.450	0.222
Lung cancer (LC)	364	129 (35.4)	235 (64.6)	1.091	0.574-2.074	0.79
Asbestos induced LC (BKV 4104)	49	16 (32.7)	33 (67.3)	2.112	0.754-5.916	0.155
LC of other aetiology	315	113 (35.9)	202 (64.1)	1.002	0.521-1.926	0.995

Data adjusted for the pack years (PY), gender and age (years) were used to calculate the odds ratios (OR) for the IL-6 G-174C polymorphism in patients of the investigated groups compared to the healthy controls. OR analyses were performed for the more frequent genotypes (G-174G) vs. the genotypes carrying at least one of the less frequent (C) allele (G-174C or C-174C).

This analysis revealed that patients carrying at least one C allele of the IL-6 -174 polymorphism were at higher risk for fibrotic lung diseases ($OR_{asbestosis}$, 1.338; 95% CI, 0.71-2.53; $OR_{silicosis}$, 1.226; 95% CI, 0.54-2.81 and $OR_{fibrosis\ other\ aetiology}$, 1.313; 95% CI, 0.58-2.98). Additionally higher risk were revealed for malignant mesothelioma (OR_{MM}, 1.918; 95% CI, 0.68-5.45) and asbestos-induced lung cancer ($OR_{LC\ asbestos}$, 2.112; 95% CI, 0.75-5.92) but not for lung cancer of other aetiology ($OR_{LC\ other\ aetiology}$, 1.00; 95% CI, 0.52-1.93) (Table V).

Clinical manifestation of IL-10 G-1082A polymorphisms. The frequency of the IL-10 (-1082) G-allele was 0.47 in the control and in the patient group. The OR analyses were performed for the genotype G-1082G vs. the genotypes carrying at least one A allele (G-1082A or A-1082A). Data was adjusted for PY, age (years) and gender. This analysis revealed that patients carrying at least one A allele of the IL-10 -1082 polymorphism were at higher risk for silicosis ($OR_{silicosis}$, 2.064; 95% CI, 0.78-5.49) but not for asbestosis ($OR_{asbestosis}$, 0.986; 95% CI, 0.48-2.03) or fibrosis of other aetiology ($OR_{fibrosis\ other\ aetiology}$, 0.903; 95% CI, 0.37-2.23). A higher risk was revealed for malignant mesothelioma (OR_{MM}, 1.726; 95% CI, 0.54-5.51) (Table VI).

Clinical manifestation of IL-1β C+3954T and IL-1β C-511T polymorphisms. The frequency of the less frequent IL-1β (+3954) T allele in the control group was 0.26 and in the patient group 0.25. The frequency of the less frequent IL-1β (-511) T allele was 0.31 in the control as well as in the patient group.

Table VI. Adjusted odds ratio of IL-10 G-1082A polymorphisms.

		Genotype, n (%)		Multivariate analysis		
Diagnosis	n	GG	GA or AA	OR	95% CI	p-value
Lung fibrosis	605	126 (20.8)	479 (79.2)	1.144	0.586-2.235	0.693
Silicosis (BKV 4101)	161	28 (17.4)	133 (82.6)	2.064	0.776-5.492	0.147
Asbestosis (BKV 4103)	389	84 (21.6)	305 (78.4)	0.986	0.480-2.027	0.97
Lung asbestosis (BKV 4103)	122	24 (19.7)	98 (80.3)	0.922	0.357-2.381	0.867
1/1 and 1/2 ILO	66	16 (24.2)	50 (75.8)	0.761	0.260-2.223	0.617
2/1, 2/2 and 2/3 ILO	46	8 (17.4)	38 (82.6)	1.133	0.342-3.757	0.838
3/2, 3/3 and 3/+ ILO	9	0 (0.0)	9 (100.0)	-	-	-
Lung fibrosis of other aetiology	55	14 (25.5)	41 (74.5)	0.903	0.366-2.227	0.825
Malignant mesothelioma	34	6 (17.6)	27 (79.4)	1.726	0.541-5.508	0.356
Lung cancer (LC)	364	84 (23.1)	280 (76.9)	1.165	0.565-2.399	0.679
Asbestos induced LC (BKV 4104)	49	13 (26.5)	36 (73.5)	1.239	0.389-3.946	0.717
LC of other aetiology	315	71 (22.5)	244 (77.5)	1.167	0.56-2.434	0.68

Data adjusted for the pack years (PY), gender and age (years) were used to calculate the odds ratios (OR) for the IL-10 G-1082A polymorphism in patients of the investigated groups compared to the healthy controls. OR analyses were performed for the more frequent genotypes (G-1082G) vs. the genotypes carrying at least one of the less frequent (A) allele (G-1082A or A-1082A).

Table VII. Adjusted odds ratio of IL-1β C-511T polymorphisms.

		Genotype, n (%)		Multivariate analysis		
Diagnosis	n	CC	CT or TT	OR	95% CI	p-value
Lung fibrosis	605	281 (46.4)	324 (53.6)	0.818	0.454-1.472	0.502
Silicosis (BKV 4101)	161	81 (50.3)	80 (49.7)	0.669	0.297-1.508	0.332
Asbestosis (BKV 4103)	389	173 (44.5)	216 (55.5)	0.946	0.502-1.786	0.865
Lung asbestosis (BKV 4103)	122	53 (43.4)	69 (56.6)	0.861	0.376-1.971	0.723
1/1 and 1/2 ILO	66	35 (53.0)	31 (47.0)	0.694	0.268-1.8	0.453
2/1, 2/2 and 2/3 ILO	46	15 (32.6)	31 (67.4)	1.293	0.462-3.615	0.624
3/2, 3/3 and 3/+ ILO	9	3 (33.3)	6 (66.7)	3.583	0.271-47.47	0.333
Lung fibrosis of other aetiology	55	27 (49.1)	28 (50.9)	0.854	0.382-1.911	0.701
Malignant mesothelioma	34	14 (41.2)	20 (58.8)	1.28	0.491-3.334	0.614
Lung cancer (LC)	364	185 (50.8)	179 (49.2)	0.597	0.317-1.125	0.11
Asbestos induced LC (BKV 4104)	49	23 (46.9)	26 (53.1)	0.975	0.357-2.664	0.961
LC of other aetiology	315	162 (51.4)	153 (48.6)	0.534	0.279-1.024	0.059

Data adjusted for the pack years (PY), gender and age (years) were used to calculate the odds ratios (OR) for the IL-1β C-511T polymorphism in patients of the investigated groups compared to the healthy controls. OR analyses were performed for the more frequent genotypes (C-511C) vs. the genotypes carrying at least one of the less frequent (T) allele (T-511C or T-511T).

The OR analyses were performed for the more frequent genotypes (C+3954C and C-511C) vs. the genotypes carrying at least one of the less frequent (T) allele (C+3954T or T+3954T and C-511T or T-511T). Data was adjusted for PY, age (years) and gender.

Patients carrying at least one less frequent (T) allele of the IL-1β -511 polymorphism showed decreased risk for fibrotic and malignant lung diseases ($OR_{asbestosis}$, 0.946; 95% CI, 0.50-1.79; $OR_{silicosis}$, 0.67; 95% CI, 0.30-1.51 and OR_{LC}, 0.597; 95% CI, 0.32-1.13) but not for malignant mesothelioma (OR_{MM}, 1.28; 95% CI, 0.49-3.33). Rather an increasing risk was seen for severe lung asbestosis (2/1, 2/2 and 2/3 ILO: OR, 1.29; 95% CI, 0.46-3.62; 3/1, 3/2 and 3/+ ILO: OR, 3.583; 95% CI, 0.27-47.47) (Table VII).

Table VIII. Adjusted odds ratio of IL-1β C+3954T polymorphisms.

		Genotype, n (%)		Multivariate analysis		
Diagnosis	n	CC	CT or TT	OR	95% CI	p-value
Lung fibrosis	605	343 (56.7)	262 (43.3)	0.915	0.508-1.647	0.766
Silicosis (BKV 4101)	161	82 (50.9)	79 (49.1)	1.175	0.525-2.63	0.694
Asbestosis (BKV 4103)	389	228 (58.6)	161 (41.4)	0.836	0.443-1.577	0.58
Lung asbestosis (BKV 4103)	122	66 (54.1)	56 (45.9)	1.113	0.489-2.533	0.798
1/1 and 1/2 ILO	66	29 (43.9)	37 (56.1)	1.789	0.69-4.641	0.232
2/1. 2/2 and 2/3 ILO	46	32 (69.6)	14 (30.4)	0.594	0.21-1.681	0.326
3/2. 3/3 and 3/+ ILO	9	4 (44.4)	5 (55.6)	0.973	0.087-10.92	0.982
Lung fibrosis of other aetiology	55	33 (60.0)	22 (40.0)	0.828	0.37-1.857	0.648
Malignant mesothelioma	34	19 (55.9)	15 (44.1)	1.17	0.454-3.012	0.746
Lung cancer (LC)	364	207 (56.9)	157 (43.1)	1.108	0.588-2.087	0.751
Asbestos induced LC (BKV 4104)	49	27 (55.1)	22 (44.9)	1.272	0.467-3.466	0.638
LC of other aetiology	315	180 (57.1)	135 (42.9)	1.055	0.553-2.013	0.871

Data adjusted for the pack years (PY), gender and age (years) were used to calculate the odds ratios (OR) for the IL-1β C+3954T polymorphism in patients of the investigated groups compared to the healthy controls. OR analyses were performed for the more frequent genotypes (C+3954C) vs. the genotypes carrying at least one of the less frequent (T) allele (C+3954T or T+3954T).

This analysis revealed that patients carrying at least one T allele of the IL-1β +3954 polymorphism were at higher risk for moderate lung asbestosis (1/1 and 1/2 ILO: OR, 1.79; 95% CI, 0.69-4.64) as well as for hyaline pleural plaques (OR, 1.44; 95% CI, 0.6-3.5). Additionally, a higher risk was seen in the asbestos-induced lung cancer patients ($OR_{LC\ asbestos}$, 1.27; 95% CI, 0.47-3.47) rather than in lung cancer of other aetiology ($OR_{LC\ other\ aetiology}$, 1.055; 95% CI, 0.55-2.01) (Table VIII). Significance could not be gained in the described results.

Discussion

Pneumoconiosis, such as asbestosis or silicosis are occupational lung diseases caused by inhaled dust particles. Among others, cytokines such as TNF-α, TGF-β, IL-1β, IL-6 or IL-10 play an important role in the pathogenesis of pneumoconiosis (1,30-33). Coal dust exposure stimulates inflammatory response leading to increased release of IL-1 (5). In previous studies we described an association of TNF-α (34) and TGF-β (35) polymorphisms with asbestos induced diseases. Since interleukins play an important role in inflammatory processes, genetic factors of these genes could also modify the susceptibility of asbestos or silica-related diseases.

Our results did not show any association with the IL-1β (+3954) or IL-1β (-511) polymorphism. Also Yucesoy *et al* (36) did not find any association of the IL-1β (+3954) polymorphism with silicosis. In contrast a higher risk of lung cancer (OR, 5.45; 95% CI, 2.75-4.42) was reported for T-allele carriers in the Japanese population (37).

Inflammatory cytokines TNF-α, IL-1 and IL-6 are released during inflammation. IL-6 then shows anti-inflammatory effects by inhibiting the secretion of TNF-α and IL-1. Therefore IL-6 is a pro- and anti-inflammatory cytokine (38). The -174C allele of IL-6 was associated with a lower basal expression, which persisted also after stimulation with LPS or IL-1. Significant lower plasma level of IL-6 in healthy subjects was associated with the -174C allele (22). This study did not detect any significant effect of the IL-6 (-174G/C) polymorphism in a cohort of 1180 subjects. A tendency for a higher risk of C-allele carriers for fibrotic lung diseases, malignant mesothelioma or asbestos-induced lung cancer was noted. A significant association of the GG homozygous genotype with an improved survival in sepsis has been previously described (39). In contrast, a protective effect of the IL-6 variant was found on the development and severity of coal workers' pneumoconiosis in Turkey (40).

IL-10 as an anti-inflammatory cytokine inhibits the release of proinflammatory mediators, such as TNF-α, IL-1β and IL-6 (23). Genotypes carrying the IL-10 -1082A allele are associated with a lower IL-10 production (41-43). Our study revealed, that patients carrying at least one A allele are at lower risk for asbestosis or fibrotic lung diseases of other aetiology, but at higher risk for malignant mesothelioma. Significant higher risk for chronic obstructive pulmonary disease (COPD) (OR, 1.66; 95% CI, 1.01-2.75; P=0.0046) and small-cell lung cancer (SCLC) (OR, 3.01; 95% CI, 1.21-7.48; P=0.0006) was detected in German -1082G-allele carriers (44). Higher ORs (OR, 5.26; 95% CI, 2.65-10.4; P<0.0001) were observed for non-small cell lung cancer (NSCLC) in Chinese patients carrying the IL-10 -1082G allele (45). A higher frequency of the IL-10 -1082G allele was reported in Chinese patients with cardia gastric cancer (46). Worth noting is the fact that the -174C allele of IL-6 and the -1082A allele of IL-10 have opposite effects regarding fibrotic lung diseases.

This study did not reveal sufficient evidence for an association of the investigated interleukin polymorphisms with asbestos or silica-induced diseases. Since the system of carcinogenesis/fibrogenesis is very complex, the changes caused by one SNP may not have enough impact to reveal significance.

Therefore, it appears to be important and appropriate to undertake more studies with new methods, to analyse combination effects and the multifaceted interactions between genetic and environmental factors (47).

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