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HIGHLIGHT

Association of Tumor Necrosis Factor-α and Interleukin-1 Gene Polymorphisms with Silicosis

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Silicosis, an interstitial lung disease prevalent among miners, sand blasters, and quarry workers, is manifested as a chronic inflammatory response leading to severe pulmonary fibrotic changes. Proinflammatory cytokines, such as TNFα and IL-1, produced in the lung by type II epithelial cells and alveolar macrophages, have been strongly implicated in the formation of these lesions. Recently, a number of single nucleotide polymorphisms (SNPs), which quantitatively affect mRNA synthesis, have been identified in the TNFα promoter and IL-1 gene cluster and their frequency is associated with certain chronic inflammatory diseases. To assess the role of these SNPs in silicosis, we examined their frequency in 325 ex-miners with moderate and severe silicosis and 164 miners with no lung disease. The odds ratio of disease for carriers of the minor variant, TNFα (−238), was markedly higher for severe silicosis (4.0) and significantly lower for moderate silicosis (0.52). Regardless of disease severity, the odds ratios of disease for carriers of the IL-1RA (+2018) or TNFα (−308) variants were elevated. There were no significant consistent differences in the distribution of the IL-1α (+4845) or IL-1β (+3953) variants with respect to disease status. In addition, several significant gene–gene and gene–gene–environment interactions were observed. Different associations between moderate cases and controls versus severe cases and controls were also observed in a number of these multigene comparisons. These studies suggest that gene–environment interactions involving cytokine polymorphisms play a significant role in silicosis by modifying the extent of and susceptibility to disease. © 2001 Academic Press

Key Words: **silicosis; tumor necrosis factor-α; interleukin-1; polymorphism; epidemiology; genetics.**

Silicosis, an interstitial lung disease resulting from inhalation of crystalline silica, is characterized by fibrotic nodules, thickening of the alveolar interstitium, and accumulation of inflammatory cells in the lung. The disease is prevalent among workers in the coal mining, manufacturing, and construction industries and contributed to 4882 deaths between 1979 and 1992 (Wagner, 1997). Silicotic lesions develop from the chronic secretion of inflammatory and fibrotic mediators. Experimental animal and clinical studies indicate that tumor necrosis factor-α (TNFα) and interleukin-1 (IL-1), derived primarily from alveolar macrophages and type II epithelial cells in the lung, are important in regulating these mediators in silicosis. In this respect, increased expression of inflammatory cytokines corresponds to pathological changes in lungs of silicotic rodents (Davis *et al.*, 1998; Driscoll *et al.*, 1990; Mohr *et al.*, 1991; Orfila *et al.*, 1998). A direct role has been demonstrated in TNFα-deficient mice, which are resistant to developing fibrosis from silica (Piguet *et al.*, 1990; Gossart *et al.*, 1996). Furthermore, the expression of the TNFα transgene in the murine lung causes spontaneous alveolitis, alveolar disruption, and a progressive fibrotic reaction (Miyazaki *et al.*, 1995). In humans, TNFα is released from peripheral blood monocytes in coal miners with coal workers' pneumoconiosis (CWP), while continuous release of TNFα and IL-1 from alveolar macrophages occurs in miners with progressive massive fibrosis and with simple pneumoconiosis (Borm *et al.*, 1988; Lasalle *et al.*, 1990). The local release of TNFα and IL-1 from humans has also been shown to coincide with pathogenesis of the disease (Savici *et al.*, 1994; Schmidt *et al.*, 1984).

It has been suggested that a number of genetic and epigenetic factors modify the severity of chronic inflammatory diseases. Susceptibility genes, including single nucleotide polymorphisms (SNPs), which quantitatively affect the production of inflammatory mediators fall into this category. In humans, the gene encoding for TNFα is located on chromosome 6 between HLA-B and DR within the class III region of the major histocompatibility complex (Carroll *et al.*, 1987). Two

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SNPs containing a G → A substitutions have been described in the promoter region at positions −308 and −238 (Wilson *et al.*, 1992; D'Alfonso and Richiardi, 1994). These transitions affect the level of TNFα expression in response to various stimuli and their presence has been associated with a variety of immune and inflammatory diseases, such as CWP, malaria, leishmaniasis, celiac disease, chronic bronchitis, psoriasis, and systemic lupus erythematosus (SLE) (Zhai *et al.*, 1998; McGuire *et al.*, 1994; Cabrera *et al.*, 1995; McManus *et al.*, 1996; Huang *et al.*, 1997; Arias *et al.*, 1997; Sullivan *et al.*, 1997). In humans, the IL-1 family consists of three genes located on the long arm of chromosome 2 that code for IL-1α, IL-1β, and the IL-1 receptor antagonist (RA) (Roux-Lombard, 1998). Each of these genes possesses exonic SNPs that affect their expression, by increasing either message stability or the rate of mRNA synthesis. The IL-1RA (VNTR) polymorphism is associated with SLE, lichen sclerosis, and alopecia areata (Blakemore *et al.*, 1994; Clay *et al.*, 1994; Tarlow *et al.*, 1994). SNPs in the IL-1RA exon 2 (T → C) are believed to be in linkage disequilibrium with intron 2 VNTR (Clay *et al.*, 1995). Two variants in the IL-1α gene, at sites −889 and +4845 (both C → T), are present at an increased frequency in juvenile rheumatoid arthritis and chronic polyarthritis (McDowell *et al.*, 1995; Jouvenne *et al.*, 1999), while a recently identified IL-1β polymorphism at position +3953 (C → T) was found to be more prevalent in individuals with severe periodontitis and psoriasis (Kornman *et al.*, 1997; di Giovine *et al.*, 1995).

Because not all individuals develop severe lung fibrosis despite similar work histories, it is assumed that genetic factors influence the extent of disease (moderate or severe). In view of this assumption and the paucity of information on the direct role of TNFα and IL-1 on progressive silicosis in humans, we investigated the relationship between genetic polymorphisms associated with these genes and the clinical severity of the disease. Specifically, the frequency of the TNFα (−308), TNFα (−238), IL-1α (+4845), IL-1β (+3953), and IL-1RA (+2018) variants in relationship to silicosis severity were examined.

MATERIALS AND METHODS

Study Population

Associations between TNFα and IL-1 polymorphisms and silicosis were examined in a case–control study. Lung tissue samples were obtained from the National Coal Workers Autopsy Study (NCWAS). Of the original 6580 samples collected for the NCWAS between 1972 and 1996, this study was limited to samples from Caucasian (males) underground miners. All samples were reviewed and graded for CWP, including silicosis and other disease status according to the criteria and scheme developed by a joint committee of the National Institute for Occupational Safety and Health and College of American Pathologists (Kleinerman *et al.*, 1979). Lesions, such as macules, nodules, progressive massive fibrosis (PMF), and silicosis, were graded subjectively for CWP into three groups: no disease, mild/moderate, and severe. Standard photographs for different types of lesions and their grades of severity were used for this classification. Disease severity and grading were evaluated on an average of five sections, with a range of 3–30 sections per case. All samples that met the criteria for a silicosis case and that could be successfully genotyped were included in the study. Thirty-five (12%) samples that did not show any evidence of pulmonary inflammation or disease were excluded from the study due either to early age at death (<55 years) or to short occupational exposure period (<16 years).

DNA Preparation and Genotyping

Five-micrometer lung tissue sections were prepared from formalin-fixed paraffin-embedded tissue blocks. Genomic DNA was extracted from lung tissue samples following deparaffinization in xylene and alcohol using a DNA isolation kit (QIAGEN, Chatsworth, CA) according to the manufacturer's instructions. Freshly obtained DNA from human skin samples was used as an internal quality control. Genotyping was performed using a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique. Briefly, 150 ng/50 μl genomic DNA was placed in 10 mM Tris–HCl, 50 mM KCl buffer containing 0.2 mM of each dNTP, 1.25 units of *Taq* polymerase, and 0.05% W-1 detergent. Amplifications of the TNFα (−308), TNFα (−238), IL-1α (+4845), IL-1α (+3953), and IL-1RA (+2018) polymorphisms were similar to those previously described (Wilson *et al.*, 1993; D'Alfonso and Richiardi, 1994; Jouvenne *et al.*, 1999; di Giovine *et al.*, 1995; Clay *et al.*, 1995) with minor modifications as described below.

TNFα (−308). Primers: 5′-AGGCAATAGGTTTTGAGGGCCAT-3′ (forward) and 5′-TCCTCCCTGCTCCGATTCCG-3′ (reverse) 0.2 mM; 1.5 mM $MgCl_2$; cycling: 35 cycles at 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, followed by 95°C for 1 min and 72°C for 5 min. PCR product was digested at 37°C for 2 h with 6 units of *Nco*I, resulting in allele 1 (87 + 20 bp) and allele 2 (107 bp) products.

TNFα (−238). Primers: 5′-GAAGCCCCTCCCAGTTCTAGTTC-3′ and 5′-CACTCCCCATCCTCCCTGGTC-3′ 0.25 μM; 2 mM $MgCl_2$; cycling: 35 cycles at 94°C for 1 min, 61°C for 1 min, 72°C for 1 min, followed by 94°C for 3 min, and then a final 5 min at 72°C. Digestion with 5 units *Ava*II at 37°C for 2 h yielded allele 1 (63 + 49 + 21 bp) and allele 2 (70 + 63 bp) products.

IL-1α (+4845). Primers: 5′-ATGGTTTTAGAAATCATCAAGCCTAG-GGCA-3′ and 5′-AATGAAAGGAGGGGAGGATGACAGAAATGT-3′ 0.8 μM; 1 mM $MgCl_2$; cycling: 1 min denaturation at 95°C followed by 35 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 2 min, and a final 5-min extension at 72°C. The products were digested with 2.5 units of *Fnu*4H1 at 37°C for 2 h. This gave allele 1 (124 + 76 + 29 bp) and allele 2 (153 + 76 bp) products.

Il-1β (+3953). Primers: 5′-CTCAGGTGTCCTCGAAGAAATCAAA-3′ and 5′-GCTTTTTTGCTGTGAGTCCCG-3′ 1 μM, 2.5 mM $MgCl_2$; cycling: 95°C for 2 min followed by 35 cycles at 95°C for 1 min, 67.5°C for 1 min, 72°C for 1 min and then a final 5 min at 72°C. Digestion with 10 units of *Taq*I restriction enzyme at 65°C for 2 h yielded allele 1 (85 + 97 bp) and allele 2 (182 bp) products.

IL-1RA (+2018). Primers: 5′-CTATCTGAGGAACAACCAACTAG-TAGC-3′ and 5′-TAGGACATTGCACCTAGGGTTTGT-3′ 1 μM, 1.75 mM $MgCl_2$; cycling: 35 cycles at 94°C for 1 min, 57°C for 1 min, 70°C for 2 min, followed by 96°C for 1 min and then a final 5 min at 70°C. Digestion was performed at 37°C for 2 h with 5 units of *Alu*I and 5 units of *Msp*I. *Alu*I digestion gave allele 1 (128 + 26 bp) and allele 2 (154 bp) products. *Msp*I digestion yielded allele 1 (154 bp) and allele 2 (29 + 125 bp) products.

PCR products or restriction fragments were separated by electrophoresis on a 10% polyacrylamide–TBE gel (Bio-Rad, Richmond CA) at 150 V for 30 min and visualized by UV illumination after staining with ethidium bromide.

Statistical Methods

χ^2 tests (Rosner, 1990) were conducted to determine whether significant associations existed between inheritance of specific polymorphic variants and disease status. Odds ratios were also calculated using a logistic regression model for each SNP with separate regression models applied for moderate, severe, and overall silicosis, with and without adjustment for occupational exposure. The odds ratio represents the proportion among silicosis individuals

TABLE 1
Age, Smoking Status, and Years of Exposure by Disease Status

Population	Number of patients	Mean ± SD		
		Age	Years of smoking	Years of exposure
Controls	164	63.2 ± (50–87) 8.0	20.4 ± (0–50) 6.4	21.3 ± (1–58) 13.3
Moderate	140	66.9 ± (27–87) 9.2	20.5 ± (0–70) 19.1	34.4 ± (10–52) 10.1
Severe	185	68.7* ± (39–93) 8.8	17.9 ± (0–60) 18.4	34.2* ± (1–55) 11.3
Overall	489	66.3 ± (27–93) 9.0	19.5 ± (0–70) 18.0	29.9 ± (1–58) 13.2

Note. Values in parentheses are ranges.
* $P < 0.05$.

with the minor variant divided by the proportion of exposed individuals among controls without disease. An odds ratio of 2.0, for instance, would imply that the odds ratio of having severe disease are twice as high among individuals with the minor variant. The associations for two-way interactions between years of exposure and each polymorphism, each possible pair of polymorphisms, and three-way interactions between exposure and each gene–gene interaction were also assessed with logistic regression models. The *p* values were calculated using the likelihood ratio test between the model with all possible interactions and the model excluding only the interaction of interest.

For any two-way interaction that exhibited statistical significance, the proportion of cases for those with and without the minor variant in the first gene was plotted against the proportion of cases with and without the minor variant in the second gene. The proportion of cases in each category was adjusted for differences in years of exposure (Wilcosky and Chambless, 1985). Any significant three-way interactions were similarly identified by plotting the proportion of cases in each possible gene–gene combination for two different categories of exposure (less than or equal to 30 years versus greater than 30 years). Years of exposure were coded as less than or equal to 20, 20 to 30, 30 to 40, or greater than 40 years of exposure for most regression analyses, where the main effect of exposure was included as a confounding covariate. For the three-way interactions, exposure was coded as less than or equal to 30 years or greater than 30 years to avoid numerical complexities associated with small numbers in some of the cells. Plots of significant interactions were performed separately for moderate and severe disease. All statistical analyses were conducted with SAS statistical software (SAS, 1999).

The proportion of cases and odds ratios for disease, as calculated in these analyses, are not interpretable as estimates of population risk or even relative risk, since the overall proportion of cases (approximately 0.66) was fixed by the study design. The odds ratio approximates the relative risk in the population only if the overall proportion of cases is sufficiently small. Although the analyses conducted can be interpreted with regard to which genes or factors are associated with increased risk of silicosis, the proportion of cases and odds ratios for disease should not be interpreted as population estimates of that risk.

RESULTS

Demographic data, including age, smoking, and exposure conditions, for 325 cases with severe or moderate silicosis and 164 controls with no apparent lung disease are presented in Table 1. Age at death and years of exposure were significantly increased in the population with severe silicosis, with a mean of 68 versus 63 years of age at death and 34 versus 21 years of underground exposure, respectively. Years of smoking were similar in the control and silicosis population. Age at death was slightly greater for severe cases, as opposed to moderate cases (68.7 versus 66.9), but years of occupational exposure were almost identical with 34 years each.

Table 2 summarizes the distribution of genotypes and allelic frequencies for TNFα (−308), TNFα (−238), IL-1RA, IL-1α, and IL-1β, by disease status and severity. For TNFα (−308) and TNFα (−238), differences in allelic frequency were associated with the extent of disease. For TNFα (−308), the allele 2 frequency was higher in moderate cases (0.37) and slightly less in severe cases (0.24) than in controls (0.27). A different relationship existed for TNFα (−238), as allelic frequency was increased for severe cases (0.40) over controls (0.24), but decreased for moderates (0.16). In contrast, the frequency of IL-1RA allele 2 was substantially increased in both moderate (0.35) and severe disease cases (0.22) compared to controls (0.16). Neither IL-1α nor IL-1β showed significant changes in allele 2 frequency, with less than a 0.05 difference between frequencies in moderate or severe cases versus controls. These relationships also held when the crude odds ratio (unadjusted for exposure) of disease for subjects with the minor variant was examined. Only the numbers of allele 2 carriers (1/2 or 2/2 allele states) are presented in the table and reflect current understanding that the heterozygote or homozygote state results in the same degree of phenotypic perturbation of cytokine posttranslational elaboration. The number of homozygote variants (2/2) can be calculated from the allelic frequency and total number with the variant.

For each polymorphism the odds ratio of disease and associated 95% confidence intervals for a subject with a minor variant are reported separately for moderate or severe disease, as well as total silicotic, using logistic regression models with and without adjustment for years of exposure. Confidence levels for the odds ratio not containing 1.0 can be considered equivalent to statistically significant results at $p < 0.05$. After controlling for years of exposure, the TNFα (−308), TNFα (−238), and IL-1RA were all significantly associated with moderate, severe, and overall disease ($p < 0.01$, $p = 0.04$, and $p < 0.01$, respectively). TNFα (−308) and IL-1RA showed an increased risk for both disease groups. However,

TABLE 2
Distribution of Genotypes and Allele Frequencies

	Disease status					
	Normal 1/1 Alleles	Carrier 1/2 or 2/2	Total (% Carrier)	Allele 2 frequency	Crude OR (CI)[a]	Adjusted OR (CI)[b]
TNFα (−308)[c]						
Controls	75	79	154 (51.3)	0.27	1.00	1.00
Moderate	40	97	137 (70.8)	0.37	2.30 (1.4–3.7)	3.59 (2.0–6.4)
Severe	83	74	157 (47.1)	0.24	0.85 (0.5–1.3)	1.61 (0.9–2.8)
All silicotic[d]	123	171	294 (58.2)	0.30	1.32 (0.9–2.0)	2.25 (1.4–3.6)
TNFα (−238)[c]						
Controls	87	73	160 (45.6)	0.24	1.00	1.00
Moderate	91	41	132 (31.1)	0.16	0.54 (0.3–0.9)	0.52 (0.3–0.9)
Severe	42	141	183 (77.0)	0.40	4.00 (2.5–6.4)	4.00 (2.4–6.8)
All silicotic	133	182	315 (57.8)	0.30	1.63 (1.1–2.4)	1.59 (1.0–2.5)
IL-1RA (+2018)[c]						
Controls	113	44	157 (28.0)	0.16	1.00	1.00
Moderate	54	60	114 (52.6)	0.35	2.85 (1.7–4.7)	2.54 (1.4–4.5)
Severe	95	65	160 (40.6)	0.22	1.76 (1.1–2.8)	2.01 (1.2–3.4)
All silicotic	149	125	274 (45.6)	0.27	2.16 (1.4–3.3)	2.15 (1.3–3.5)
IL-1α (+4845)						
Controls	125	31	156 (19.9)	0.10	1.00	1.00
Moderate	111	21	132 (15.9)	0.08	0.76 (0.4–1.4)	0.47 (0.2–0.9)
Severe	113	42	155 (27.1)	0.15	1.50 (0.9–2.5)	0.90 (0.5–1.6)
All silicotic	224	63	287 (22.0)	0.12	1.13 (0.7–1.8)	0.76 (0.4–1.3)
IL-1β (+3953)						
Controls	43	95	138 (72.4)	0.36	1.00	1.00
Moderate	35	75	110 (68.2)	0.40	0.97 (0.6–1.7)	0.8 (0.5–1.6)
Severe	55	88	143 (61.5)	0.36	0.72 (0.4–1.2)	0.72 (0.4–1.3)
All silicotic	90	163	253 (64.4)	0.38	0.82 (0.5–1.3)	0.75 (0.4–1.2)

[a] Odds ratio (95% confidence limits).
[b] Odds ratio (95% confidence limits) adjusted for exposure with logistic regression.
[c] Significantly associated with moderate, severe, and overall disease ($P < 0.05$).
[d] Represents total population studied with silicosis.

subjects with the TNFα minor (−238) variant were at four times greater odds for developing severe silicosis, but half as likely to develop moderate disease. The odds ratio for disease in miners with the IL-1RA variant was increased for moderate (2.54) and severe (2.01) silicosis. The odds ratio of the TNFα (−308) variant was increased in individuals with moderate disease. However, the association with severe disease was confounded by occupational exposure, as indicated by the differing results of logistic regression with and without adjusting for exposure. Neither the IL-1α nor IL-1β variants showed a significant association with overall silicosis ($p > 0.25$ for both SNPs). However, IL-1α was slightly, but significantly, associated with a decreased risk of moderate silicosis, as indicated by an adjusted odds ratio of 0.47 ($p = 0.04$). Although consistently less than one, the adjusted odds ratios between IL-1β and disease were not significant for any disease category.

Two gene–gene interactions, IL-1α (+4845) by TNFα (−238) and IL-1RA (+2018) by TNFα (−308), were associated with altered risk for combined disease ($p = 0.04$ for both). The presence of both IL-1α and TNFα (−238) variants was required to decrease the odds of moderate silicosis, while the presence of only one variant led to a result similar to those with the common genotype (Fig. 1A). With regard to severe disease, TNFα (−238) was associated with an increased odds of silicosis, but the magnitude of this effect was greater in those subjects without the IL-1α variant (Fig. 1B). For the joint distribution of IL-1RA and TNFα (−308), the proportion of moderate cases increased independently with the presence of either minor variant (Fig. 1C). For severe disease, the interaction between IL-1RA and TNFα (−308) was driven by subjects with both variants (Fig. 1D).

Three-way interaction analysis between each gene–gene interaction and exposure led to several marginal associations ($0.05 < p < 0.11$). The general pattern demonstrated in each of these interactions is exemplified by the IL-1α and TNFα (−308) association ($p = 0.05$) (Figs. 1E and 1F). The prevalence of silicosis increases with increasing exposure, except in the case where both minor variants are present. For the group in which subjects are allele 2 carriers

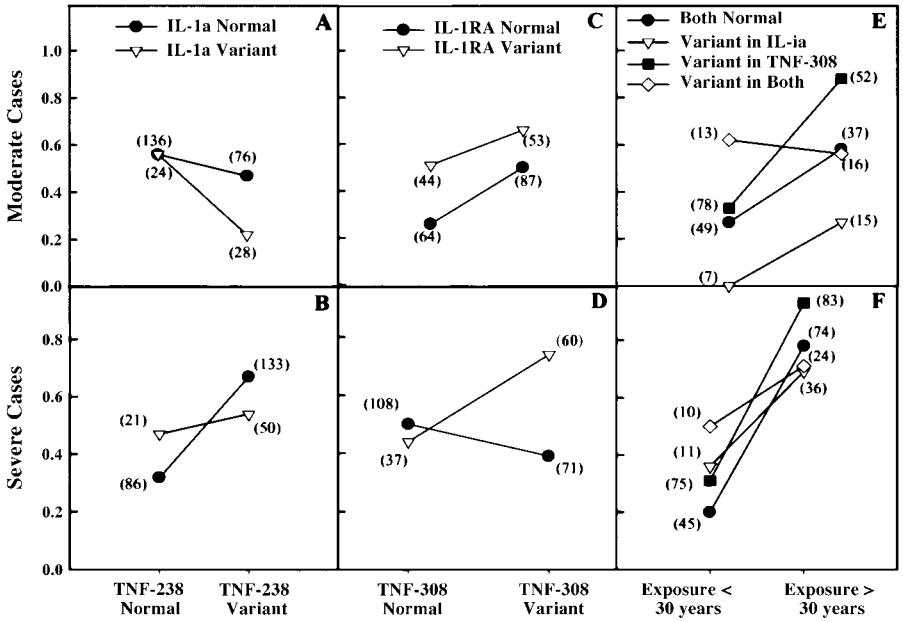

FIG. 1. The (exposure-adjusted) proportion of cases depends on the joint combination of IL-1α and TNF (-308) (A and B) and of IL-1RA and TNF (-238) (C and D) with sample sizes for each combination given in parentheses. (A) The odds of moderate disease decreases with the presence of either gene, but also decreases significantly through an interactive effect for those subjects with variants in both genes. (B) Regardless of IL-1α, the odds of severe disease increases with the presence of TNF (-308), although the increase is greater without the variant in IL-1α. (C) The odds of moderate disease increases independently with minor variants in either gene. (D) For severe cases, the odds of disease increases substantially with the presence of TNF (-238), but only in those subjects who also have the IL-1RA variant. The proportion of cases depends on the joint combination of IL-1α, TNF (-308), and years exposure (E and F). The odds of either moderate (E) or severe (F) disease increases significantly with increasing exposure, except when both minor variants are present. In this case the odds of disease are either slightly decreased (moderate disease) or increased less significantly (severe disease), reflecting the observation that the group with both variants has the highest proportion of moderate and severe cases for those exposed less than 30 years.

in both polymorphisms, there is little or no effect of increasing exposure and this group has the highest proportion of moderate and severe cases for those exposed less than 30 years.

DISCUSSION

Chronic inflammatory diseases are multifactorial, determined by both environmental and genetic factors. While the interaction of genes with environmental and host factors initiates the disease process, other genetic factors, such as polymorphisms, can modify the extent or severity of the disease. In this respect, SNPs, which influence the quantity of inflammatory cytokines produced, have been associated with the severity of several chronic inflammatory disorders, such as systemic lupus erythrematosus, alopecia areata, and periodontitis (Pociot *et al.*, 1992; Blakemore *et al.*, 1994; Mandrup-Poulsen *et al.*, 1994; Cork *et al.*, 1996; Kornman and di Giovine, 1998; Gore *et al.*, 1998). Silicosis, resulting from the interaction of silica particles and lung cells, is manifested by persistent overproduction of inflammatory mediators, leading to alveolar damage, fibroblast proliferation, collagen deposition, and granuloma formation. Animal models have suggested that IL-1 and, particularly, TNF-α play significant roles in silica-induced lung damage (Driscoll *et al.*, 1990; Davis *et al.*, 1998; Orfila *et al.*, 1998). For example, treatment with neutralizing antibodies to TNFα causes a significant reduction of the fibrotic response in silica-exposed mice (Piguet *et al.*, 1990). Consistent with these observations, we observed a strong association between silicosis and the TNFα (-238) variant, as the allele carriers were significantly reduced in moderate disease and significantly predictive of severe disease (adjusted odds ratio of 0.5 and 4.0, respectively). This implies that individuals with the TNFα (-238) variant are predisposed to more rapid development of severe silicosis, which would account for the apparently protective effect on moderate outcomes, since those individuals are progressing past moderate status with a higher probability. After adjusting for years of occupational exposure, TNFα (-308) also showed a strong relationship with disease, as the presence of the variant was associated with an increased odds for both moderate and severe disease (adjusted odds ratios of 3.6 and 1.6, respectively).

Due to chromosomal location and biological effects, it has been speculated that a polymorphism within the TNFα gene may play a role in the genetic association of MHC haplotypes with autoimmune and inflammatory diseases (Wilson *et al.*, 1993). Thus, TNFα expression may depend on polymorphisms in the TNFα promoter region or a linkage association with the HLA genotype (D'Alfonso and Richiardi, 1994; Pociot *et al.*,

1993). In this respect, the −308 allele is associated with the HLA A1, B8, DR3, DR4, and the DQ2 haplotypes; the DR2 is associated with low TNFα responses; and the DR3 and DR4 genotypes are associated with high TNFα production (Jacob *et al.,* 1990; Wilson *et al.,* 1993). Therefore, the increased production of TNFα could contribute to the increased incidence of autoimmune diseases observed in individuals with HLA A1, B8, and DR3 haplotypes (Kroeger *et al.,* 1997). In Caucasian patients with silicosis, the highest risk of developing severe fibrosis was found to be associated with the HLA-Aw19-B18 haplotype (Koskinen *et al.,* 1983). Immunogenetic analysis in Japanese populations revealed that susceptibility to silicosis is associated with HLA-Bw54, suggesting that a TNFα allele, in linkage disequilibrium with this haplotype, might help determine predisposition to silicosis (Honda *et al.,* 1993). Our results cannot exclude the possibility of a direct linkage disequilibrium with the HLA alleles implicated in susceptibility to silicosis. It should be noted that the estimated contribution of genetic factors to variability in TNFα levels between individuals is approximately 60% (Westendorp *et al.,* 1997), suggesting the variability in TNFα levels may only be partly explained by the −238 and −308 polymorphisms.

The frequency of the IL-1RA allele 2, as well as IL-1RA protein, is increased in inflammatory diseases such as alopecia areota and ulcerative colitis. (Tarlow *et al.,* 1994; Mansfield *et al.,* 1994; Danis *et al.,* 1995). Likewise, it has been reported that an increase in the frequency of the IL-1RA allele 2 is often more prevalent in severe forms of the disease (Blakemore *et al.,* 1994; Tarlow *et al.,* 1994). In line with these observations, we noted that allele 2 of the IL-1RA exon 2 polymorphism was significantly increased in miners with both moderate and severe silicosis. The higher frequency of allele 2 suggests that this variant affects susceptibility to silicosis rather than extent of disease. Although there was no association with the IL-1β variant, an allelic association between IL-1RA and IL-1α was found ($p = 0.04$). This may also represent a susceptibility factor for silicosis, as the IL-1/IL-1RA ratio is important in the regulation of inflammatory processes (Casini-Raggi *et al.,* 1995). It is possible that other polymorphisms in the IL-1 gene family are associated with severity of silicosis.

Although some inconsistencies were observed, the two-way gene–gene interactions also provided some insight into the contributions of these SNPs and silicosis. For example, the IL-1RA and TNFα (−308) interaction showed a strong independent association between each SNP and moderate disease, which is consistent with the individual results in Table 2, after adjusting for exposure. For severe disease, however, the analyses indicated that the presence of both variants leads to much higher odds of disease (see Figs. 1C and 1D), thus driving the significant increase in disease for either gene when examined independently. This would be consistent with the overlapping functions of inflammatory cytokines in chronic inflammatory diseases as shown in the case of periodontitis (Gore *et al.,* 1998).

In conclusion, these results show both independent and interrelated effects of IL-1 and TNFα polymorphisms on susceptibility and extent of silicosis in underground miners. These findings also indicate that occupational exposures must be considered in interpreting results, as both the magnitude and the direction of associations can be influenced by years of exposure. The assessment of such interactions and of extent of disease severity provides insight into the underlying mechanisms relating silicosis to genetic factors, thus leading toward better estimation of susceptible populations and individual human risk assessments.

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