The role of genetics in occupational respiratory diseases

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Introduction

Silicosis and Progressive Massive Fibrosis (PMF) are dust induced inflammatory/fibrotic lung diseases characterized by severe scarring that leads to obliteration of normal lung structures. The pulmonary response following dust exposure is characterized by inflammation, epithelial cell injury, proliferation of interstitial cells and collagen production. Cytokines released by activated macrophages are involved in the recruitment of inflammatory cells into the alveolar walls and spaces. These mediators also play a role in the remodeling process through stimulation of fibroblast proliferation and collagen synthesis. Therefore, the early and persistent expression of pro-inflammatory cytokines and subsequent presence of growth factors and cell-surface adhesion molecules are intimately involved in pulmonary fibrosis. As a multifactorial disease, pulmonary fibrosis is influenced by a number of genetic and environmental factors. These studies were undertaken to examine individual and combinatorial effects of multiple single nucleotide polymorphisms (SNPs) in interleukin-1 (IL-1), IL-6, tumor necrosis factor-alpha (TNFα), transforming growth factor beta-1 (TGFβ1), vascular endothelial growth factor (VEGF), intercellular cell adhesion molecule-1 (ICAM-1) and matrix metalloproteinase-2 (MMP-2) genes in a large group of coal workers. Selected polymorphisms in these genes known to be associated with differential expression of their respective gene products and have been previously associated with inflammatory/fibrotic diseases were examined.

Methods and Materials

Study populations

All the underground coal miners included in the silicosis and PMF studies were Caucasian, males and selected from a total of 6580 National Coal Workers' Autopsy Study (NCWAS) cases from 1972 to 1996. All samples were reviewed and graded for silicosis, coal worker's pneumoconiosis (CWP), PMF and other disease status according to the criteria and schema developed by a joint committee of the National Institute for Occupational Safety and Health College of American Pathologists. Before the selection of the study population the subjects with silicosis were reviewed and graded into three grades of severity; no disease, moderate, and severe, based on absence of disease or its profusion and size in histological sections. The histological criteria used to establish PMF cases were the presence of discrete highly coal-dust laden fibrotic lesions measuring greater than one centimeter in size with irregular deposition of collagen fibers in a minimum of six lung sections from each case. Miners with similar exposure histories, but without any clinical or histological evidence of pulmonary disease served as controls. The study groups were from different mines but similar geographic coal mining areas.

DNA preparation and genotyping

Genomic DNA was prepared from formalin-fixed, paraffin-embedded lung tissue blocks using a DNA isolation kit (Promega, Madison, WI). IL-1RN +2018 SNP was genotyped by PCR–restriction fragment length polymorphism technique. Other SNPs were genotyped using a 5'-nuclease real-time PCR assay. Primers and probes were designed, using the Assay-by-Design™ service (Applied Biosystems, Foster City, CA). PCR amplification was performed in a volume of 25 µl containing 10 ng genomic DNA, 12.5 µl 2X Taqman® Universal Master Mix, 200 nM of probe and 900 nM of primer using an iCycler ® IQ real-time thermal cycler (Biorad Laboratories, Hercules, CA). Cycling conditions were 50 C° for 2 min, 95 C° for 10 min, followed by 50 cycles at 92 C° for 30 sec and 60 C° for 1 min. Positive and negative controls were used within each run of PCR amplification and a random selection of 10% of all samples was genotyped to ensure laboratory quality control.

Statistical Methods

Differences between cases and controls with respect to demographic and other characteristics were evaluated using chi-square tests for discrete variables and two-sample t-tests for continuous variables. Potential associations between each SNP and disease were tested using chi-square tests for single SNP associations and Mantel-Haenszel chi-square tests for multiple SNP associations. The Breslow-Day test was performed to evaluate homogeneity of the odds ratios (OR). The Expectation-Maximization (EM) algorithm was used to determine haplotypes and their frequencies. A chi-square test was performed to determine haplotype-phenotype association. All statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC).

Results

A demographic summary of the study populations are presented in Table 1. The TNFα -238 variant showed a strong association with severe silicosis (OR=4.00). The frequency of the TNFα -308 and IL-1RA +2018 variants were increased in individuals with both moderate and severe disease. (Table 2). Gene-gene interactions, IL-1α by TNFα -238 and IL-1RA by TNFα -308, were associated with altered risk for silicosis (p=0.04 for both). While the presence of IL-1α and TNFα -238 variants were required to decrease the odds of moderate silicosis, TNFα -238 was associated with an increased odds of severe silicosis especially in those subjects without the IL-1α variant. For severe disease, the interaction between IL-1RA and TNFα -308 was driven by subjects with both variants. For moderate disease, the proportion of cases increased independently with the presence of either minor variant (Figure 1). Figure 2 shows the joint effect of genetic and environmental factors comparing with the only occupational exposure model.

There was no differences in individual genotype frequencies between PMF cases and controls (Table 3), as well as no statistically significant gene-gene interactions. However, individuals with the polygenotype of VEGF+405 /ICAM-1+241/ IL-6 -174 (C-A-G) were at a significantly higher risk of developing PMF (OR=3.4) than individuals with the other allelic combinations of these SNPs. There were no significant differences between the haplotypes and the reference haplotype in any of the comparisons.

Table 1: Characteristics of the participants in the silicosis and PMF groups

Silicosis	N	(Mean ± S.D.) Age	Years smoking	Years exposure
Controls	164	63.2 ± 8.0	20.4 ± 16.4	21.3 ± 13.3
Moderate	140	66.9 ± 9.2	20.5 ± 19.1	34.4 ± 10.1
Severe	185	68.7 ± 8.8	17.9 ± 18.4	34.2 ± 11.3
Overall	489	66.3 ± 9.0	19.5 ± 18.0	29.9 ± 13.2
PMF				
Controls	367	69.8 ± 8.9	17.7 ± 13.9	32.9 ± 11.6
PMF	375	70.2 ± 8.8	17.6 ± 14.1	33.3 ± 11.7

Table 2: Distribution of genotypes and allele frequencies in silicosis

Disease status	N(%)	N (%)	Frequency of A	OR (95% CI)
TNFα -308	**GG**	**AG+AA**		
Controls	75 (48.7)	79 (51.3)	0.266	1.00
Moderate	40 (29.2)	97 (70.8)	0.369	3.59 (2.0-6.4)
Severe	83 (52.9)	74 (47.1)	0.239	1.61 (0.9-2.8)
TNFα -238	**GG**	**AG+AA**	**Frequency of A**	
Controls	87 (54.4)	73 (45.6)		1.00
Moderate	91 (68.9)	41 (31.1)	0.159	0.52 (0.3-0.9)
Severe	42 (23.0)	141 (77.0)	0.399	4.00 (2.4-6.8)
IL-1RA +2018	**CC**	**CT+TT**	**Frequency of T**	
Controls	113 (72.0)	44 (28.0)	0.156	1.00
Moderate	54 (47.4)	60 (52.6)	0.346	2.54 (1.4-4.5)
Severe	95 (59.4)	65 (40.6)	0.219	2.01 (1.2-3.4)

Table3: Gene-gene interaction in PMF study

SNPs		Gene-gene interaction
TNFα -308	MMP2 -1306	
EGF +61	VEGF +405	VEGF+405 / ICAM-1 +241 / IL-6 -174
TGFβ1 -509	TNFα -238	C A G
IL-6 -174	IL-1α +4845	(OR=3.4, 95% CI: 1.3-8.8)
IL-1β -511	ICAM +241	

Figure 1: Gene-gene and gene-exposure interaction in silicosis

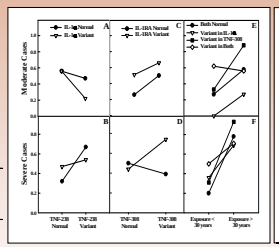
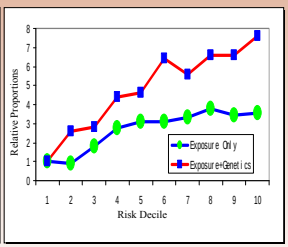


Figure 2: Influence of genetics and exposure on silicosis risk



Discussion

* A strong association between severe silicosis and the TNFα -238 variant indicate that this variant may act as a disease modifier and influence disease progression. The TNFα -308 and IL-1RA +2018 variants showed a relationship with both moderate and severe disease, suggesting that these variants affect susceptibility rather than extent of disease. Gene-gene interaction results showed both independent and interrelated effects of IL-1 and TNFα variants on susceptibility and extent of silicosis and also indicate that 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.

* No significant association was found between individual SNPs and PMF. However, three-way interaction analyses showed that the VEGF +405, ICAM-1 +241 and IL-6-174 SNPs interact synergistically to affect the occurrence of PMF. The combinatorial effect of these gene variants appears to mirror the interaction observed *in vivo* between VEGF, ICAM-1 and IL-6 proteins. It is possible that SNPs may influence the interaction and amplification process between these genes and play an important role in the pathogenesis of pulmonary fibrosis. Although the selection of candidate genes was based on their biological roles in disease process, it was limited to the genes/SNPs that have already been identified and characterized. There are potentially many as yet unidentified genetic variants that can contribute to disease risk. Moreover, inter-individual differences in the expression of selected genes may influence disease progression by acting as disease modifiers. However, our study design was not appropriate for such evaluation due to presence of only severe cases of PMF.