Cytokine Genotype and Phenotype Effects on Lung Function Decline in Firefighters

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Objective: *We conducted this study to evaluate the association of cytokine genotypes and sputum concentrations on longitudinal decline in lung function in firefighters.* ***Methods:*** *In 67 firefighters with at least four pulmonary function tests, DNA was analyzed for functional polymorphisms of interleukin (IL)-1β, IL-1 receptor antagonist (IL-1RA), IL-8, IL-10, tumor necrosis factor-alpha (TNF-α) genes, and sputum evaluated for cytokine concentration by ELISA.* ***Results:*** *The annual rate of FEV1 decline was greater in firefighters with TT genotypes at IL-10 (−819) (P = 0.009) and with CT or TT genotypes at IL-1RA (2018) (P = 0.050). These genotypes were not associated with concentrations of sputum cytokine, but increased IL-1RA was associated with a slower rate of FEV1 decline (P = 0.025), as was increased sputum macrophage count (P = 0.002).* ***Conclusions:*** *Cytokine genotypes were associated with the rate of FEV1 decline but did not alter concentrations of sputum cytokine. Increased sputum IL-1RA may be protective.* (J Occup Environ Med. *2007;49:282–288*)

Firefighters demonstrate an extensive variability in the rate of decline in lung function, measured by forced expiratory volume in 1 second (FEV_1). Accelerated rate of decline (≥60 mL/yr), not directly related to smoking or occupational smoke exposure, has been observed in certain individuals,[1,2] far exceeding the average annual rate of decline in FEV_1 of approximately 20 to 30 mL in the general population.[3] The accelerated rate of decline in lung function may have significant clinical consequences, including the potential to develop chronic obstructive pulmonary disease (COPD). COPD is present in more than 9% of people aged 30 to 80 years who had never smoked and in 16% of people in their 60s who had never smoked.[4] Prior to development of COPD, there is a subclinical phase during which individuals suffer accelerated loss of pulmonary function.[5] A disproportionately increased rate of decline in FEV_1 may be associated with increased baseline lung inflammation, which is regulated by the interplay of several factors including genetic susceptibility, environmental factors, and patterns of occupational exposure.[2,6,7]

Cytokines are secreted by epithelial and inflammatory cells including macrophages, neutrophils, and lymphocytes. Associations between lung disease and/or lung function decline, and single nucleotide polymorphisms (SNPs) in cytokine-related genes have been reported in several studies, including our previous study of interleukin-10 (IL-10) SNPs in firefighters.[6–10] Interleukin-1beta, interleukin-8, (IL-1β and IL-8), and

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tumor necrosis factor-alpha (TNF-α) are proinflammatory and are associated with increased inflammation in several disease states involving the lungs, such as COPD, asthma, acute lung injury, and fibrosis.[11–15] Interleukin-1 receptor antagonist (IL-1RA) competitively binds IL-1β, thus reducing its inflammatory effect. IL-10 is an anti-inflammatory cytokine that directly regulates the secretion of several proinflammatory cytokines.

Our primary study hypothesis was that chronic lung inflammation, as measured by altered inflammatory mediators in sputum and increased neutrophils and/or macrophages, is an intermediate step in the process of accelerated loss of lung function, and this relationship is modified by genetic factors. In the current study, we evaluated cytokine SNPs to determine whether the genotype effects on decline in lung function are mediated through phenotypic concentrations of cytokine mediators and differential concentrations of inflammatory cells in the sputum.

Materials and Methods

Study Population and Pulmonary Testing

The study was approved by the University of Arizona Human Subjects Institutional Review Board and was carried out at the Phoenix Fire Department (PFD) Health Center in Phoenix, AZ. A convenience sample of 67 firefighters was recruited. All subjects were current nonsmokers, were actively involved in responding to fires but had not been exposed to smoke for at least four days, and had at least four pulmonary function tests (PFTs) within 7 years prior to entry into the study. Informed study and HIPAA (Health Insurance Portability and Accountability Act) consents were obtained from all study volunteers during enrollment. Study enrollment and sample collection took place from June 2004 through December 2004. A self-administered questionnaire collected information on occupational exposures and medical history, including cigarette smoking and respiratory and cardiovascular symptoms during the most recent year only. Clinically relevant longitudinal data on PFTs, height, and weight at the time of pulmonary testing were collected from PFD medical records. Spirometry was performed as a part of annual medical screening examinations at the PFD using flow spirometry (Spirotech S401 [Graseby Andersen, Smyrna, GA] from 1998–2001, and Puritan Bennett Renaissance II [Puritan Bennett, Pleasanton, CA] from 2001–2005), corrected for temperature and barometric pressure.

Buccal Cell Collection and SNP Analysis

Cheek cells were collected with buccal swabs, stored in cell lysis solution, and kept refrigerated until further processing. DNA extraction was performed with the Puregene DNA extraction kit (Gentra Systems, Inc., Minneapolis, MN). The resultant DNA pellet was washed twice in 75% ethanol and air-dried before being rehydrated in a low-TE (10 mmol/L Tris, 1.0 mmol/L EDTA, pH 8.0) buffer and stored until SNP analysis. SNP analysis was performed at the National Institute for Occupational Safety and Health (NIOSH) in Morgantown, WV. Genotyping was performed on genomic DNA using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique and a 5′ nuclease PCR assay (TaqMan, Applied Biosystems, Foster City, CA). IL-1RA (+2018) genotyping was performed using PCR-RFLP under the conditions described previously.[16] PCR products were electrophoresed using a 10% polyacrylamide-TBE gel (Bio-Rad Laboratories, Inc., Hercules, CA) at 150 V for 30 minutes and visualized by ultraviolet (UV) illumination after staining with ethidium bromide. Primers and probes for IL-8 (−251), IL-1β (+3953), IL-10 (−819), and TNF-α −308 were designed using 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 2 × TaqMan Universal Master Mix (PE Applied Biosystems, Foster City, CA), 200 nM of probe, and 900 nM of primer. Cycling conditions were 50°C for 2 minutes, 95°C for 10 minutes, followed by 50 cycles at 92°C for 30 seconds and 60°C for 1 minute. Amplification was performed using an iCycler IQ (Bio-Rad Laboratories, Inc., Hercules, CA) real-time thermal cycler. All subjects who could be successfully genotyped were included in the study. Negative controls were used within each run of PCR amplification. A random selection of 10% of all samples was repeated to ensure laboratory quality control.

Sputum Induction and Analysis

Subjects were asked to breathe 3% saline mist aerosolized through a DeVilbiss Ultra-Neb 99HD ultrasonic nebulizer (Somerset, PA) for 30 minutes. Every 2 minutes, subjects were encouraged to discard saliva to minimize salivary contamination and then cough up phlegm. The induced sputum sample was transported to the University of Arizona on ice for subsequent processing. Sputum samples were diluted by adding an equal volume of 10% Sputolysin (Calbiochem, San Diego, CA) and mixed gently until a uniform sputum solution was formed. A small amount of this solution was separated to perform cellular differential counts. Supernatant was then separated by centrifugation at 1900 rpm for 15 minutes and frozen at −80°C for later analysis of cytokines. Total cell count was performed with a hemocytometer and trypan blue stain (Sigma Chemical Co., St. Louis, MO). A portion of this sample was also cytocentrifuged (Shandon Cytospin, Thermo Shandon, Pittsburgh, PA) and stained with Diff-Quik (Dade Behring AG, Switzerland) for differential analysis. A total of 200

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nonsquamous cells were counted on each slide, and the number of each cell type is presented as a percentage of the total.

Supernatants were analyzed in duplicates for levels of IL-1β, IL-1RA, IL-8, IL-10, and TNF-α using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Inc., Minneapolis, MN). A uniform initial dilution was performed on all samples to maximize the number of concentrations measured within the standard range for each assay. Total protein concentration was estimated using a bicinchoninic acid (BCA) assay in a microplate format (Sigma-Aldrich Corp., St. Louis, MO). For statistical analysis, measured concentrations lower than the detectable limits were replaced with a concentration of half of the sensitivity for that assay.

Statistical Analysis

STATA 9.0 (StataCorp, College Station, TX) and SPSS 14.0 (SPSS, Inc., Chicago, IL) were used for all statistical analyses. The longitudinal change in lung function (FEV_1 slope) was calculated based on the best FEV_1 measure from annual spirometry testing.[17] Simple linear regression with the least squared method of line fitting was used to calculate the slope of the relationship between FEV_1 and age at PFT for each individual. If a subject had a >15% annual change in FEV_1 between 2 years that was inconsistent with other measures and had a poor acceptability or reproducibility score, the PFT data from that time point was excluded from the calculation of the slope. The genotype frequencies of IL-1β 3953, IL-1RA 2018, IL-8 −251, IL-10 −819, and TNF-α −308 were tested to assess whether each genotype was in Hardy-Weinberg equilibrium. Sputum cytokines and cell counts were log(e)-transformed or dichotomized (above vs below detection limit) for analyses. Pearson and Spearman rank correlation tests were used to assess linear relations between variables. Associations between cytokine level and genotypes were tested using Kruskal-Wallis tests. Differences in mean FEV_1 slope and mean log values of normally distributed variables by gender, ethnic group, smoking status (ever vs never), and asthma status (ever vs never) were analyzed using *t* tests or analysis of variance (ANOVA). Multiple regression models were used to assess the relationship between sputum cytokine levels and FEV_1 slope after adjusting for covariates, including race/ethnic group, gender, baseline age, baseline FEV_1, weight change during years of follow-up, smoking status, asthma status, and sputum protein concentration. To correct for variability in the dilution of sputum samples during collection, ratios of IL-1β, IL-RA, and IL-8 to protein were computed and log(e)-transformed. Likelihood ratio tests were used to compare nested models, and regression diagnostics were performed.

Approximately 70% of all PFTs performed met the American Thoracic Society (ATS) acceptability and reproducibility criteria.[17] For an additional 18% of PFTs, there were no data on whether spirometry met any criteria. Analyses were run on all firefighters and on a subset of 36 who had at least 3 years of PFTs that met the criteria.

Results

Gender, age, ethnicity, years of service, and pulmonary function data for the 67 firefighters who met the inclusion criteria are summarized in Table 1. The population was primarily white, non-Hispanic, and male. FEV_1 slope averaged −0.033 (±0.059) L/yr. Approximately 18% of the firefighters were ex-smokers, and 18% reported ever having

TABLE 1
Characteristics of the Study Participants (*N*= 67)

	***N* (%)**	
Male	64 (96)	
Race/Ethnicity		
White, non-Hispanic	52 (78)	
Hispanic	7 (10)	
African American	4 (6)	
Other	4 (6)	
Ever-smokers	12 (18)	
Physician-confirmed asthma	12 (18)	
	Mean ± SD	**Range**
Age (yr) at baseline PFT	38.6 ± 7.8	20.3–59.4
Years of service at Phoenix Fire Department	16.6 ± 8.4	3–32
FEV_1 (L) at baseline	4.16 ± 0.70	2.82–5.93
FVC (L) at baseline	5.17 ± 0.87	3.38–7.76
FEV_1 slope (L/yr)	−0.033 ± 0.059	−0.146–0.153
IL-1β (pg/mL)	253.6 ± 546.8	11.1–4506
IL-1RA (ng/mL)	26.7 ± 29.8	5.1–172.6
IL- 8 (pg/mL)	5.3 ± 11.1	0.4–90.6
IL-10 (pg/mL)	4.76 ± 12.8	0.28–101
TNF-α (pg/mL)	2.28 ± 5.01	0.1–24.2
Sputum total cell count (million/mL)	2.87 ± 1.71	0.78–8.2
% Neutrophils	32.3 ± 20.0	0.45–88.2
% Monocytes	45.7 ± 18.4	7.8–91.8
% Lymphocytes	1.01 ± 1.27	0–5.3
% Eosinophils	0.60 ± 1.08	0–6.5
% Nonsquamous cells	20.4 ± 14.3	0–54.0

PFT = pulmonary function test; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; IL-1β = interleukin-1beta; IL-1RA = interleukin-1 receptor antagonist; TNF-α = tumor necrosis factor-alpha.

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asthma. Almost 50% reported recurrent lung, throat, or nose irritation after smoke exposure in the past year (not shown). FEV_1 slope was inversely related to baseline FEV_1 ($P = 0.002$) and change in weight ($P = 0.05$), and positively related to asthma status ($P = 0.023$). There was no significant relation between slope and gender, baseline age, height, baseline body mass index, or smoking status. The seven Hispanic firefighters had a steeper mean decline in FEV_1 (-0.071 ± 0.047 L/yr) than firefighters of other ethnic backgrounds, although this difference was not statistically significant ($P = 0.344$).

All of the SNPs analyzed were in Hardy-Weinberg equilibrium. The associations between SNPs and decline in FEV_1 are listed in Table 2. In univariate analysis, the mean FEV_1 slope was significantly lower in firefighters possessing the TT genotype of the IL-10 (−819) polymorphism ($n = 3$, -0.125 ± 0.027 L/yr), compared with those with a CC ($n = 33$, -0.020 ± 0.061) or CT genotype ($n = 31$, -0.038 ± 0.051) ($P = 0.009$). Mean slope was also lower in subjects possessing a CT or TT genotype of the IL-1RA (+2018) SNP, compared with subjects with a CC genotype ($n = 31$, mean slope $= -0.048 \pm 0.050$ and $n = 36$, mean slope -0.020 ± 0.064 L/yr, respectively), although this difference was of marginal significance ($P = 0.050$). In the subset analysis on the 36 subjects who met acceptability and reproducibility criteria on a sufficient number of PFTs for calculating slope, only the relationship between slope and IL-10 (−819) genotype was significant ($P = 0.041$).

Sputum concentrations of IL-1β, IL-1RA, IL-8, IL-10, and TNF-α did not vary by their corresponding genotype (Table 3), with or without adjustment for protein concentration of the sample. More generally, none of the SNPs assayed was associated with sputum concentrations of any cytokines measured. In addition, there was no difference in the ratio of IL-1β to IL-1RA in the sputum by IL-1β 3953 or IL-1RA 2018 genotype. However, the mean log(e) of the absolute neutrophil count was significantly lower in individuals carrying the TT genotype at IL-8 (−251) (3.38 ± 1.41), compared with firefighters with either the AA (4.50 ± 0.89) or AT (4.31 ± 1.05) genotypes ($P = 0.009$). Also, the mean log(e) value of sputum protein concentration was significantly higher in individuals with the AA genotype at IL-8 (−251) compared to individuals with the AT genotype ($P = 0.009$). Sputum concentrations of IL-1β were highly correlated with sputum IL-1RA, IL-8, IL-10, TNF-α, total protein, and neutrophil count (Spearman rank correlation, all $P < 0.005$).

Controlling for ethnicity, gender, baseline age, baseline FEV_1, asthma status, smoking status, and weight change on multiple regression analysis, sputum concentrations of IL-1β, IL-1RA, IL-8, IL-10, and TNF-α, adjusted for sputum protein

TABLE 2
Mean FEV_1 Slope (L/yr) by Genotype at Specific SNPs

SNP	Genotype	*n*	Mean Slope ± SD	*P* Values ANOVA
IL-1β (3953)	CC	39	−0.036 ± 0.058	0.095
	CT	23	−0.040 ± 0.055	
	TT	5	0.022 ± 0.075	
IL-1RA (2018)	CC	36	−0.020 ± 0.064	0.050
	CT+TT	31	−0.048 ± 0.050	
IL-8 (−251)	TT	18	−0.027 ± 0.052	0.890
	AT	35	−0.035 ± 0.067	
	AA	14	−0.036 ± 0.051	
IL-10 (−819)	CC	33	−0.020 ± 0.061	0.009
	CT	31	−0.038 ± 0.051	
	TT	3	−0.125 ± 0.027	
TNF-α (−308)	GG	48	−0.030 ± 0.058	0.100
	AG	16	−0.052 ± 0.046	
	AA	3	0.024 ± 0.113	

SNPs = single nucleotide polymorphisms; IL-1β = interleukin-1beta; IL-1RA = interleukin-1 receptor antagonist; TNF-α = tumor necrosis factor-alpha.

TABLE 3
Relationship Between Sputum Cytokine Concentrations (pg/mL) and Genotype (Kruskal-Wallis Test)

SNP	Genotype	*n*	Median Concentration (25th, 75th Percentiles)	*P* Value
IL-1β 3953	CC	39	164 (82, 256)	0.814
	CT	23	104 (65, 268)	
	TT	5	124 (110, 158)	
IL-1RA 2018	CC	36	20001(12591, 30053)	0.371
	CT	29	16214 (12707, 29008)	
	TT	2	9673 (7723, 11623)	
IL-8 −251	TT	18	2895 (1523, 3293)	0.371
	AT	35	2987 (2313, 5434)	
	AA	14	3408 (1178, 7467)	
IL-10 −819	CC	33	0.62 (0.5, 6.28)	0.431
	CT	31	0.56 (0.5, 3.60)	
	TT	3	0.50 (0.48, 9.80)	
TNF-α −308	GG	48	0.13 (0.12, 1.06)	0.688
	GA	16	0.12 (0.12, 0.14)	
	AA	3	0.13 (0.12, 13.40)	

SNP = single nucleotide polymorphism; IL-1β = interleukin-1beta;. IL-1RA = interleukin-1 receptor antagonist; TNF-α = tumor necrosis factor alpha.

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TABLE 4
Coefficients and *P* Values of Covariates in Multiple Regression Model of IL-1RA and FEV_1 Slope (Adjusted $r^2 = 0.383$).

	β Coefficient	*P* value
Hispanic vs Anglo	−0.060583	0.005
Black vs Anglo	−0.0210197	0.483
Other vs Anglo	−0.0236512	0.351
Male vs female	0.0703329	0.060
Baseline age (yrs)	−0.0016558	0.047
Baseline FEV_1 (L)	−0.0572772	0.000
Ever-asthma	0.0154093	0.395
Ever-smoker	0.0031879	0.860
Ln (IL-1RA/protein)	0.0191138	0.025
Weight change (lbs)	−0.0014736	0.014
Constant	0.1797462	0.004

FEV_1 = forced expiratory volume in 1 second; IL-1RA = interleukin-1 receptor antagonist.

TABLE 5
Coefficients and *P* Values of Covariates in a Multiple Regression Model of Absolute Macrophage Count and FEV_1 Slope (Adjusted $r^2 = 0.367$)

	β Coefficient	*P* value
Hispanic vs Anglo	−0.044334	0.036
Black vs Anglo	−0.0047429	0.877
Other vs Anglo	−0.0330834	0.201
Male vs Female	0.0534116	0.151
Baseline age (yrs)	−0.0012218	0.134
Baseline FEV_1 (L)	−0.0476359	0.000
Ever-asthma	0.0334439	0.055
Ever-smoker	0.0047825	0.793
Macrophage count (ln)	0.0296467	0.002
Weight change (lbs)	−0.0016618	0.006
Constant	0.0334846	0.651

FEV_1 = forced expiratory volume in 1 second.

concentration, were assessed individually as predictors of FEV_1 slope. Increased IL-1RA was significantly associated with a less rapid decline in FEV_1 ($P = 0.025$) (Table 4). None of the other cytokines was a significant predictor of FEV_1 slope.

In both univariate and multivariate models, log(e) total cell count was a positive predictor of FEV_1 slope ($P = 0.004$). The log(e) of the absolute macrophage count was also positively associated with FEV_1 slope in multivariate models ($P = 0.002$) (Table 5). There was no correlation between sputum cytokine concentrations and neutrophil, monocyte, or total cell counts.

Discussion

The cytokines evaluated in this study include both proinflammatory and anti-inflammatory cytokines, are known to be associated with COPD, and are influenced by both occupational and environmental factors.[15,18–21] We observed an association between genotypes of IL-10 (−819) and IL-1RA (2018) and longitudinal decline in FEV_1, demonstrating a genetic component to rate of loss of lung function in firefighters. In the case of IL-10, the −819 SNP is in the promoter region and therefore would be expected to influence concentration of the IL-10 protein. Although its role has not been clarified, the IL-1RA 2018 TT genotype appears to enhance in vitro and in vivo production of IL-1RA and production of plasma levels of IL-1β, independent of IL-1β polymorphisms.[7,22] However, neither SNP was associated with altered concentrations of cytokines in the sputum. Therefore, our results do not provide a clear mechanism by which the two SNPs influence rate of decline in lung function.

IL-10 is thought to play an important role in limiting the degree of inflammation through suppression of release of inflammatory cytokines.[23] Lower levels of IL-10 are seen in several inflammatory conditions including COPD, asthma, and environmental exposures such as smoking and smoke inhalation injury.[20] In the current study, an increased rate of decline in FEV_1 was associated with the presence of the TT genotype at IL-10 −819. Although there were only three firefighters with this genotype, all three had an extremely rapid rate of decline in lung function (from −0.094 to −0.137 L/yr). The existing literature is contradictory. The C allele has been associated with high levels of IL-10 production following in vitro stimulation of peripheral blood mononuclear cells,[24] suggesting a protective effect of the C allele on lung function decline, whereas in another study, the presence of a T allele was associated with a marked increase in plasma IL-10 concentrations.[25] In the current study, since sputum IL-10 concentrations did not vary by genotype, the mechanism for the relationship between the IL-10 −819 TT genotype and the decline in FEV_1 is unclear and should be tested in additional populations.

IL-1β is a proinflammatory cytokine associated with pathways leading to cellular proliferation and a fibrotic end-response in chronic lung inflammation.[11] Its activity is counter-regulated by the naturally occurring antagonist IL-1RA.[7] Increased IL-1RA along with IL-1β in the lung has been reported in idiopathic pulmonary fibrosis,[12] asthma,[13] panbronchiolitis,[26] and acute respiratory distress syndrome.[27] In the current study, the presence of the IL-1RA 2018 T allele was marginally associated with a more rapid longitudinal decline in FEV_1. This is consistent with an elevated odds ratio of silicosis in exposed workers for carriers of the IL-1RA (2018) variant.[28] Although not significant in our study, the presence of a T allele at the 3953 site in the IL-1β gene has been associated with increased production of IL-1β following lipopolysaccharide (LPS) exposure,[29] whereas in another study, the CC genotype was associated with a nonsignificant increase in production of IL-1β.[22] Apparently, there is a significant interaction between the IL-1β and IL-1RA polymorphisms, and plasma levels of IL-1RA appear to be influenced by both IL-1β and IL-1RA genes.[30]

The association found in our study between increased sputum IL-1RA levels and a slower decline in FEV_1 is consistent with its role in the suppression of IL-1β-induced inflammation.[12,13] In a case-control

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study of smokers with rapid versus slow decline in lung function, there was a strong association with the IL-1β/IL-1RA haplotype.[7] Although the ratio of IL-1RA to IL-1β may provide a better measurement of inflammation than either one would alone,[31] only IL-1RA levels in the sputum were associated with a rate of decline in FEV_1 in our study.

To our knowledge, the current study is the first to report a decrease in sputum neutrophil count associated with the TT genotype at IL-8 (−251). A strong correlation between IL-8 and the percentage of neutrophils in the sputum was observed in this study and in the bronchoalveolar lavage (BAL) fluid from smokers and COPD patients in a study by Soler et al.[32] Although we found no association between this genotype and IL-8 levels in the sputum, other studies have reported decreased production of IL-8 associated with the TT genotype.[33] Elevated IL-8 has been associated with increased lung inflammation[14] and smoking,[15] and negatively associated with FEV_1/FVC in patients with COPD.[34]

Contrary to our expectations, we found an association between both increased sputum total cell count and macrophages and a slower decline in lung function after adjustment for other factors. This finding is in contrast to previous cross-sectional studies of smokers showing a correlation between the severity of airflow limitation and the severity of airway inflammation (specifically increased neutrophils, macrophages, and natural killer lymphocytes in bronchial biopsies) and increased macrophages and neutrophils associated with severe COPD.[35,36] In contrast to these populations, our firefighters are generally healthy, which may help to explain the observed differences.

There are a number of limitations to this study. We had a small number of subjects for a genetic study, making it difficult to validate the effects of the less common genotypes. In addition, by testing multiple hypotheses, it is possible that some of our significant findings may be due to chance. We found no relationship between cytokine genotypes and cytokine concentrations in the sputum and, hence, cannot provide a mechanism for the effect of genotypes on the rate of decline in FEV_1. There may be some confounding of sputum inflammation by acute exposures, although induction of sputum was not performed if the firefighter had been exposed to smoke within the previous 4 days. Other possible causes of longitudinal decline in lung function, such as decreased mucociliary clearance,[37] dilation of airspaces,[38] and loss of elastic recoil,[39,40] could not be directly assessed in this study. We were also unable to adequately characterize occupational exposures for the firefighters in this study. Certain products of combustion, especially oxides of sulfur, carbon and nitrogen, and organic compounds such as acrolein, are known to cause adverse respiratory effects.[8,41] Although longitudinal studies of firefighters from the 1970s and 1980s demonstrated an accelerated decline in lung function, most likely as a result of exposure to products of combustion,[42,43] more recent studies have reported mixed effects, with decrease in lung function attributed to a range of other factors including selection of and more pronounced respirator use.[44]

In conclusion, we found that the TT genotype at IL-10 (−819) was significantly associated with an increased rate of decline in FEV_1, and that the association between the T allele at IL-1RA (2018) and decline in lung function was of borderline significance. However, we failed to observe any significant functional effect of these SNPs on their respective concentrations of cytokine in the sputum. We also demonstrated that higher sputum IL-1RA levels, adjusted for protein, are associated with a less rapid annual FEV_1 decline in firefighters. Additional research is necessary in order to validate these findings in other populations to understand the mechanism by which these genotypes cause differential rates of decline in lung function. Future research should also involve better characterization of occupational exposures and evaluate the prospective ability of sputum IL-1RA concentration to predict subsequent decline in lung function in firefighters.

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