IL10 Polymorphisms Are Associated with Airflow Obstruction in Severe α_1-Antitrypsin Deficiency

Dawn L. DeMeo[1], Edward J. Campbell[2], Alan F. Barker[3], Mark L. Brantly[4], Edward Eden[5], N. Gerard McElvaney[6], Stephen I. Rennard[7], Robert A. Sandhaus[8], James M. Stocks[9], James K. Stoller[10], Charlie Strange[11], Gerard Turino[5], and Edwin K. Silverman[1]

[1]Channing Laboratory and Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, Massachusetts; [2]University of Utah, Salt Lake City, Utah; [3]Oregon Health and Science University, Portland, Oregon, [4]University of Florida, Gainesville, Florida, [5]St. Luke's/Roosevelt Hospital, New York, New York; [6]Beaumont Hospital, Dublin, Ireland; [7]University of Nebraska, Omaha, Nebraska; [8]National Jewish Medical and Research Center, Denver, Colorado; [9]University of Texas at Tyler, Tyler, Texas; [10]Cleveland Clinic, Cleveland, Ohio; and [11]Medical University of South Carolina, Charleston, South Carolina

Severe α_1-antitrypsin (AAT) deficiency is a proven genetic risk factor for chronic obstructive pulmonary disease (COPD), especially in individuals who smoke. There is marked variability in the development of lung disease in individuals homozygous (PI ZZ) for this autosomal recessive condition, suggesting that modifier genes could be important. We hypothesized that genetic determinants of obstructive lung disease may be modifiers of airflow obstruction in individuals with severe AAT deficiency. To identify modifier genes, we performed family-based association analyses for 10 genes previously associated with asthma and/or COPD, including *IL10*, *TNF*, *GSTP1*, *NOS1*, *NOS3*, *SERPINA3*, *SERPINE2*, *SFTPB*, *TGFB1*, and *EPHX1*. All analyses were performed in a cohort of 378 PI ZZ individuals from 167 families. Quantitative spirometric phenotypes included forced expiratory volume in one second (FEV_1) and the ratio of FEV_1/forced vital capacity (FVC). A qualitative phenotype of moderate-to-severe COPD was defined for individuals with $FEV_1 \leqslant 50$ percent predicted. Six of 11 single-nucleotide polymorphisms (SNPs) in *IL10* (P = 0.0005–0.05) and 3 of 5 SNPs in *TNF* (P = 0.01–0.05) were associated with FEV_1 and/or FEV_1/FVC. *IL10* SNPs also demonstrated association with the qualitative COPD phenotype. When phenotypes of individuals with a physician's diagnosis of asthma were excluded, *IL10* SNPs remained significantly associated, suggesting that the association with airflow obstruction was independent of an association with asthma. Haplotype analysis of *IL10* SNPs suggested the strongest association with *IL10* promoter SNPs. *IL10* is likely an important modifier gene for the development of COPD in individuals with severe AAT deficiency.

Keywords: chronic obstructive pulmonary disease; genetic modifiers; interleukin 10; family-based association analysis

CLINICAL RELEVANCE

The identification of *IL10* as a potential modifier gene for chronic obstructive pulmonary disease susceptibility provides insight into additional inflammatory pathways to consider in α_1-antitrypsin deficiency, and may result in improved risk assessment and clinical management of individuals who are homozygous for the Z deficiency allele.

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the United States. Severe α_1-antitrypsin (AAT) deficiency (MIM 107400) is a known monogenic cause of COPD that affects approximately 1% of individuals with COPD. Severe AAT deficiency usually results from a homozygous mutation of a single nucleotide base pair in the serum peptidase inhibitor clade A member 1 (*SERPINA1*) gene, also known as the Protease Inhibitor (PI) locus, resulting in a Glu-to-Lys substitution at amino acid 342 in the α_1-antitrypsin protein. Individuals who are homozygous for the Z deficiency allele (PI ZZ) are at increased risk for severe, early-onset COPD, especially if they smoke cigarettes. However, there is wide variability in the development of COPD among PI ZZ individuals, suggesting a contribution of modifier genes, in addition to environmental exposures.

The hypothesis that rare Mendelian diseases have a complex phenotypic expression influenced by modifier genes is not a novel concept in lung disease. Cystic fibrosis (CF), also an autosomal recessive disorder that occurs at a frequency similar to that of AAT deficiency, demonstrates similar variability in pulmonary function phenotypes. After evaluation of 10 genes previously suggested as modifier genes of CF, Drumm and colleagues identified polymorphisms in the *TGFB1* gene as modifiers of disease severity in individuals with CF (1). In individuals with severe AAT deficiency, there have been no systematic investigations of multiple modifier genes and lung function phenotypes in large, carefully phenotyped family-based cohorts.

In addition to being genetically at risk for the development of early-onset COPD, individuals with AAT deficiency often report a history of asthma (2, 3). Although it is possible that early-onset COPD may be mistakenly diagnosed as asthma, this misdiagnosis is unlikely in children with AAT deficiency. In the AAT Genetic Modifier Study, we evaluated single-nucleotide polymorphisms (SNPs) from a panel of genes previously associated with COPD and/or asthma in individuals without AAT deficiency. These genes included interleukin-10 (*IL10*) (4–11), tumor necrosis factor-α (*TNF*) (12–19), transforming growth factor-β1 (*TGFB1*) (20, 21), microsomal epoxide hydrolase (*EPHX1*) (22–24), serum peptidase inhibitor clade E member 2 (*SERPINE2*) (25), glutathione s-transferase pi 1 (*GSTP1*) (26, 27), nitric oxide synthase 1 (*NOS1*) (28, 29), serum peptidase inhibitor clade A member 3 (commonly referred to as α_1-antichymotrypsin) (*SERPINA3*) (30, 31), and surfactant protein B (*SFTPB*) (32, 33). Nitric oxide synthase 3 (*NOS3*) was also included, as it has been previously investigated as a modifier of lung disease in AAT deficiency (34). We hypothesized that

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Correspondence and requests for reprints should be addressed to Dawn L. DeMeo, M.D., M.P.H., Brigham and Women's Hospital, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115. E-mail: dawn.demeo@channing.harvard.edu

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polymorphisms in genes previously associated with asthma and/or COPD may be modifiers of COPD in individuals genetically at risk for COPD due to severe AAT deficiency.

MATERIALS AND METHODS

The AAT Genetic Modifier Study

The AAT Genetic Modifier Study is a multi-center family-based cohort study performed in collaboration with the Alpha-1 Foundation to identify genes that modify COPD susceptibility in PI ZZ individuals (35). Ascertainment of eligible sibling pairs was based upon homozygosity for the Z allele at the *SERPINA1* locus (PI ZZ). Given that we were interested in genetic modifiers in ZZ individuals, the genetic association analysis included only the spirometric phenotypes of 378 PI ZZ individuals, although genotypes were included for all 428 subjects (i.e., genotypes from 50 parents were included) to reconstruct genetic transmissions within families. All participants provided written informed consent, and the study protocol was approved by individual Institutional Review Boards at each site.

Questionnaire and Spirometry

Each participant completed a computer-based modified version of the ATS-DLD Epidemiology Questionnaire (36), which included questions about the reason the participant was being tested for AAT deficiency, as well as questions about personal smoking history and asthma, as previously described (35). Asthma was defined by an affirmative answer to the questions "Have you ever had asthma?" and "Was it confirmed by a doctor?" Pack-years of cigarette smoking were calculated by multiplying the number of years smoking times the number of daily cigarettes smoked, divided by 20.

Spirometry testing was performed in all centers by trained research assistants. All centers received a Jaeger Masterscope PC Spirometer system (Jaeger, Hoechberg, Germany) for this study, and spirometric quality control was performed (35). For the 16 subjects who had already undergone surgical management of emphysema (lung transplantation and/or lung volume reduction surgery) at the time of the interview, spirometry that antedated the surgical procedure was obtained (although blood collection for DNA occurred at the time of the enrollment visit). In all models, age used in analyses was the age at spirometry.

Pre- and post-bronchodilator study spirometry was performed according to American Thoracic Society standards (37). Percent predicted values for FEV_1 were calculated using equations of Crapo and colleagues for white subjects (38).

We defined a qualitative phenotype for COPD using post-bronchodilator spirometry values. Moderate-to-severe COPD was defined as FEV_1 less than or equal to 50 percent predicted (FEV_1 criteria for GOLD stages 3 and 4). For the qualitative analysis of COPD, we excluded phenotypic data from individuals 60 years of age and older with a history of cigarette smoking, since severe airflow obstruction in older surviving smokers may not be indicative of a severe clinical course.

Genotyping

Genes were selected on the basis of previous associations with asthma and/or COPD. SNPs were selected by linkage disequilibrium (LD) tagging using the algorithm implemented in Tagger (http://www.broad.mit.edu/mpg/tagger/; data run March 2006) (39) with an r^2 cutoff of 0.8 based on HapMap data for white subjects. Seventy-five SNPs in 10 candidate genes were genotyped using a multiplex approach with the SEQUENOM platform. All data were evaluated for familial inconsistencies using the PEDCHECK program (40).

Statistical Methods for Genetic Association Analysis

We used the family-based association test (FBAT) as implemented in the pedigree-based association testing (PBAT) program (version 3.3; http://biosun1.harvard.edu/~clange/pbat.html), as previously described (41). The FBAT statistic conditions on the sufficient statistic by Rabinowitz and Laird (42), which is constructed for each family separately by taking family size and the correlation of genotypes within families into account (43). We performed all single SNP and haplotype analyses assuming an additive model, under the null hypothesis of no linkage and no association. Analyses for FEV_1 percent predicted and FEV_1/FVC were adjusted for sex, "ever-smoking" status (yes/no), pack-years of cigarette smoking, and current cigarette smoking (yes/no). For SNPs demonstrating significant association, we also performed PBAT analyses incorporating a SNP-by-pack-years of smoking interaction term. P values less than or equal to 0.05 are reported. We also performed association analysis for FEV_1 as a percent of predicted and FEV_1/FVC after excluding phenotypic values of 139 individuals who reported a physician's diagnosis of asthma. Directionality of the effect on the phenotype of the genotype of the minor allele was obtained from the FBAT Z statistic.

IL10 Protein Levels

We measured IL10 protein levels in a subset of 312 PI ZZ individuals using the R&D Human IL10 High Sensitivity ELISA kit (R&D Systems, Minneapolis, MN). All samples were assayed in duplicate. Forty-one duplicate sample assays with a coefficient of variation (CV) greater than 10% were excluded from the analysis, as were five duplicate sample assay values greater than 50 pg/ml (the upper limit of our standard curve). Attempts to repeat 31 samples with high CV values failed, likely due to the repeat freeze thaw cycle of serum; we were unable to repeat the remaining 15 due to insufficient sample volume. Therefore, 266 subjects had IL10 protein measurements included for further analysis. Undetectable IL10 protein levels were valued as 0 in our analysis.

Additional detail for MATERIALS AND METHODS is provided in the online supplement.

RESULTS

All PBAT analyses were performed with phenotypic information from 378 PI ZZ individuals in 167 families (Table 1). Of note, only 22 individuals reported current oral steroid use, so systemic steroid use was not included in any of the models. We investigated genetic association for 75 SNPs in 10 candidate genes selected from the literature demonstrating prior association with COPD and/or asthma intermediate phenotypes (Table 2, and Table E1 in the online supplement). All SNPs were in Hardy-Weinberg equilibrium.

Nine SNPs in three genes (*IL10*, *TNF*, and *SERPINE2*) demonstrated significant association with pre- and/or post-bronchodilator FEV_1 percent of predicted ($P \leqslant 0.05$, Table 3). When an SNP-by-pack-years of smoking interaction term was included, the findings remained robust for SNPs in *IL10* and *TNF* ($P \leqslant 0.05$, data not shown). In additive models for pre- and post-bronchodilator FEV_1/FVC, 6 SNPs in three genes (*IL10*, *TNF*, *EPHX1*) demonstrated significant association ($P \leqslant 0.05$, Table 3). When an SNP-by-smoking interaction term was included, the findings remained significant only for *IL10* and *EPHX1* SNPs ($P \leqslant 0.05$, data not shown).

TABLE 1. DEMOGRAPHICS FOR 378 PI ZZ INDIVIDUALS

Demographics	
Sex (female)	205 (54%)
Age, yr (± SD)	52.2 (± 9.7)
$FEV_{1\%}$ predicted (± SD)*	65.9 (± 33.5)
FEV_1/FVC (± SD)*	0.551 (± 0.207)
Smoking status	
Ever smoker	233 (62%)
Current smoker	13 (3%)
Pack-years for ever smokers (± SD)	18.2 (± 14.5)
Physician-confirmed asthma	139 (37%)

Definition of abbreviations: FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; PI ZZ, individuals homozygous for the Z deficiency allele.

Data include 372 siblings, 2 parents, 1 uncle, and 3 children of siblings.

* Post-bronchodilator values, which were available for 360 subjects.

TABLE 2. TEN CANDIDATE GENES INCLUDED AS POTENTIAL MODIFIERS OF COPD IN α_1-ANTITRYPSIN DEFICIENCY

Gene	Chromosome	Previous Association with Asthma	Previous Association with COPD	Number of SNPs Genotyped
IL10	1	Yes (6, 10, 11)	Yes (4)	11
EPHX1	1	No	Yes (22–24)	9
SFTPB	2	No	Yes (32, 33)	1
SERPINE2	2	No	Yes (25)	14
TNF	6	Yes (14, 16–18, 24)	Yes (12, 13, 55)	5
NOS3	7	No	Yes (34)*	5
GSTP1	11	Yes (56, 57)	Yes (26, 27)	2
NOS1	12	Yes (28, 29)	No	16
SERPINA3	14	No	Yes (30, 31)	6
TGFB1	19	Yes (58, 59)	Yes (20, 21)	6

Definition of abbreviations: COPD, chronic obstructive pulmonary disease; SNP, single-nucleotide polymorphism.

See Table E1 in the online supplement for extended description of the SNPs, including rs numbers and minor allele frequencies in our cohort.

* Suggested as a modifier gene for lung function in α_1-antitrypsin deficiency.

Approximately 37% of the cohort reported a physician's diagnosis of asthma. These 139 individuals had a mean pre-bronchodilator FEV_1 of 54.4 percent predicted (SD ± 28.8). The remaining 239 individuals without a physician's diagnosis of asthma had a mean FEV_1 of 72.4 percent predicted (SD ± 34.3); there was no difference in the mean age or pack-years by asthma status. We have previously observed that a physician's diagnosis of asthma is a strong predictor of lower FEV_1 in this cohort (35). Because of the consistency of the PBAT association findings for pre- and post-bronchodilator FEV_1 and FEV_1/FVC for multiple SNPs in *IL10*, we focused upon *IL10* SNPs and repeated the PBAT analysis for the *IL10* SNPs after excluding the phenotypic data for the 139 individuals who reported a physician's diagnosis of asthma. SNPs in *IL10* remained significantly associated with FEV_1 percent of predicted and FEV_1/FVC (Table E2).

The quantitative phenotype analyses performed likely represent association with COPD susceptibility, but they could also be influenced by FEV_1 variation within the normal range. Therefore, we subsequently performed a qualitative analysis to investigate whether any of the candidate genes demonstrated associations with severe COPD. For the qualitative COPD phenotype analysis, we excluded the phenotypic data for individuals who were over 60 years of age and who had a history of cigarette smoking (20 individuals excluded from the pre-bronchodilator analysis and 18 individuals excluded from post-bronchodilator analysis), since smokers that have survived to later ages with severe AAT deficiency might actually have protective genes, despite low lung function values at the time of study participation. The mean pre-bronchodilator percent predicted FEV_1 for the 138 individuals with moderate-to-severe COPD was 29.4 ± 10.6, versus a mean percent predicted FEV_1 of 81.7 ± 26.6 for those without moderate-to-severe COPD. Although there was no difference in the mean age for these two groups (53.3 yr for those with moderate-to-severe COPD versus 51.5 yr for those without, $P = 0.10$), subjects affected with moderate-to-severe COPD tended to have more extensive smoking histories (mean pack-year difference 18.8 [± 16.0] among subjects with moderate-to-severe COPD versus 6.9 [± 11.4] in the other subjects, $P < 0.0001$) and tended to be male ($P < 0.0001$), with men constituting 63% of those individuals affected with moderate-to-severe COPD. Of note, there was no age difference between men and women with moderate-to-severe COPD (52.9 yr for men versus 53.9 yr for women, $P = 0.60$.) PBAT analysis of the qualitative COPD phenotype revealed association with SNPs in *IL10* (rs1518110 $P = 0.02$ and rs1800871 $P \leqslant 0.03$, pre- and post-bronchodilator), *TNF* (rs769178 $P = 0.03$, pre-bronchodilator), *EPHX1* (rs1051740 $P = 0.04$, pre-bronchodilator), and *NOS1* (rs2682825 $P \leqslant 0.006$ and rs816361 $P \leqslant 0.01$, pre- and post-bronchodilator) (Table 4). Significant associations with any *NOS1* SNPs were not observed with FEV_1 or FEV_1/FVC as quantitative phenotypes.

TABLE 3. PEDIGREE-BASED ASSOCIATION TEST FOR FEV_1 (% PREDICTED) AND FEV_1/FVC

Gene	SNP	Minor Allele Frequency	FEV_1 Pre-bd	FEV_1 Post-bd	FEV_1/FVC Pre-bd	FEV_1/FVC Post-bd	Direction of Effect of Minor Allele on Lung Function Phenotypes
IL10	rs1800871 (−819)	0.25	**0.0009**	**0.0005**	**0.003**	**0.0005**	Decrease
	rs1518110	0.23	**0.004**	**0.004**	**0.004**	**0.001**	Decrease
	rs1800896 (−1082)	0.48	**0.02**	**0.02**	> 0.05	**0.05**	Increase
	rs1878672	0.48	**0.02**	**0.02**	> 0.05	> 0.05	Increase
	rs3024496	0.48	**0.02**	> 0.05	> 0.05	> 0.05	Increase
	rs3024498	0.29	**0.05**	> 0.05	> 0.05	> 0.05	Increase
TNF	rs1800610	0.07	**0.02**	> 0.05	> 0.05	> 0.05	Decrease
	rs769178	0.07	**0.01**	**0.04**	**0.05**	> 0.05	Decrease
	rs3091257	0.11	> 0.05	> 0.05	**0.05**	> 0.05	Decrease
SERPINE2	rs12476888	0.41	> 0.05	**0.05**	> 0.05	> 0.05	Decrease
EPHX1	rs2260863	0.29	> 0.05	> 0.05	**0.009**	**0.02**	Decrease

Definition of abbreviations: bd, bronchodilator; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; SNP, single-nucleotide polymorphism.

Exact *P* values by gene not presented if all SNPs for a gene have $P > 0.05$, and significant SNPs are presented in bold; each model was analyzed under the assumption of an additive mode of inheritance and adjusted for sex, ever-smoking, pack-years, pack-years^2, and current smoking.

TABLE 4. PEDIGREE-BASED ASSOCIATION TEST *P* VALUES FOR THOSE INDIVIDUALS AFFECTED WITH MODERATE-TO-SEVERE COPD

Gene	SNP	Moderate-to-Severe COPD Pre-bronchodilator	Moderate-to-Severe COPD Post-bronchodilator
IL10	rs1518110	0.02	0.02
	rs1800871	0.03	0.02
EPHX1	rs1051740	0.04	> 0.05
NOS1	rs2682825	0.006	0.005
	rs816361	0.003	0.01
TNF	rs769178	0.03	> 0.05

Definition of abbreviations: COPD, chronic obstructive pulmonary disease; SNP, single-nucleotide polymosrphism.

Each model was analyzed under the assumption of an additive mode of inheritance and adjusted for ever-smoking, pack-years, pack-years^2, and current smoking.

Given the consistent findings for *IL10* SNPs for quantitative and qualitative airflow obstruction phenotypes, we performed a sliding window haplotype analysis using the 11 SNPs genotyped in *IL10*, using 8, 4, 3, and 2 adjacent SNP sliding windows (Figure E1). This analysis revealed the most robust association with the *IL10* promoter SNP rs1800871 and the rs1518110 intronic SNP, which are in tight but not complete LD. A two-SNP haplotype analysis was also performed for rs1800896 (nucleotide −1082) and rs1800871 (nucleotide −819), the two nonadjacent *IL10* promoter SNPs that demonstrated significant association. The most highly associated haplotype was A at rs1800896 and T at rs1800871 with a haplotype frequency of 0.20; this haplotype was associated with the quantitative phenotypes FEV_1 percent predicted and FEV_1/FVC (AT haplotype individual P value < 0.001). Twenty-six individuals were homozygous at both the rs1800896 (AA, homozygous wild-type allele) and rs1800871 (TT, homozygous minor allele) loci (therefore AT haplotypes); the mean pre-bronchodilator FEV_1 of these individuals was 57.1 percent predicted versus 65.0 percent predicted for all other individuals, consistent with the direction of the effect of each individual allele (decrease in lung function for the rs1800896 wild-type allele and for the rs1800871 minor allele, Table 3) in the haplotype. Figure 1 demonstrates the high but not complete LD between all SNPs included in the haplotype analyses.

In our cohort the direction of effect of the −1082 A/G promoter polymorphism was for lower lung function with the wild-type allele and higher lung function with the mutant G (minor) allele (Table 3). The −1082 rs1800896 SNP has been associated with a functional effect on IL10 protein levels, with the A wild-type allele being associated with lower IL10 levels, and the G mutant allele being associated with higher IL10 levels. We measured serum IL10 levels in this cohort. In serum samples from 266 PI ZZ subjects, the overall mean CV of all of our samples was 3.8% (range of 0–9.6) after exclusion of 41 samples with CVs greater than 10% and 5 samples with values greater than our highest standard. The CV values for individuals on and not on augmentation therapy were not statistically different, at 4.1% (0–9.6%) and 3.5% (0–9.3%), respectively. After data quality assurance, we had measured IL10 levels in 266 individuals, 257 of whom had genotypes at the −1082 locus. The mean IL10 concentration in the overall cohort of 257 individuals was 2.40 (± 0.26) pg/ml (range 0–39.31 pg/ml). When parsed by use of AAT augmentation status, individuals on augmentation therapy had a mean IL10 concentration of 3.03 (± 0.50) pg/ml versus 1.80 (± 0.22) pg/ml for those not on augmentation therapy ($P = 0.02$). Individuals with the AA −1082 genotype (wild type) had low IL10 levels regardless of augmentation therapy use. However, individuals with one or two copies of the minor G allele had higher IL10 levels when compared with individuals not on augmentation therapy (Figure 2, Table E3).

DISCUSSION

Rare monogenic disorders such as AAT deficiency are often characterized by marked phenotypic variability, and genetic polymorphisms in additional genes may contribute to this

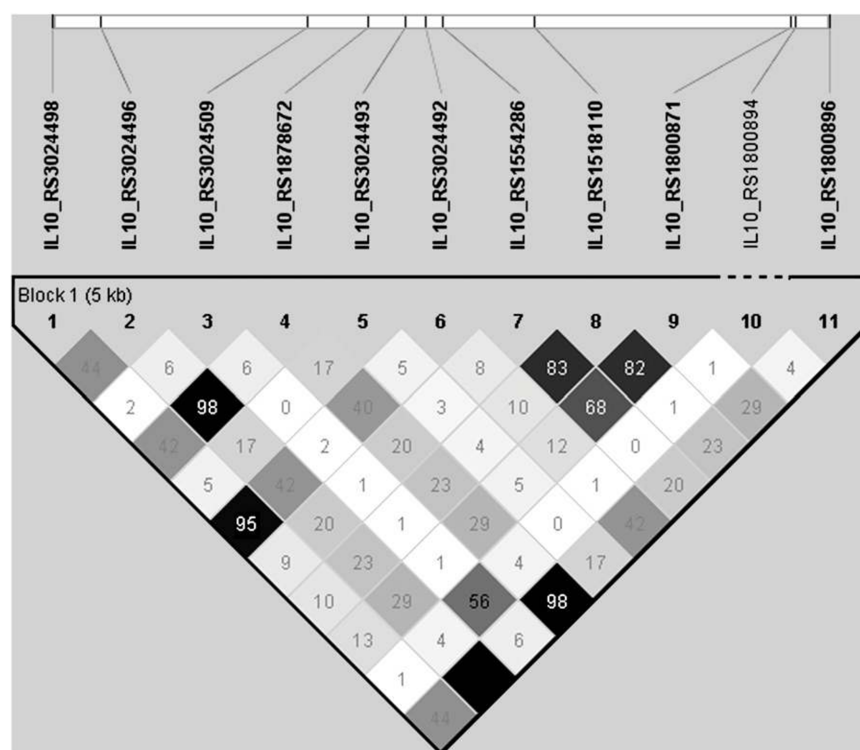

Figure 1. Linkage disequilibrium (LD) between the 11 SNPs analyzed for *IL10*. LD values are presented as r^2. r^2 between the rs1800871 promoter SNP and the rs1518110 intronic SNP was 0.82, suggesting tight but not complete LD.

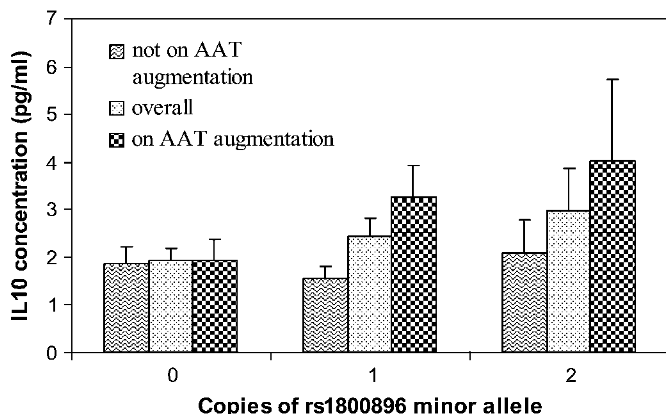

Figure 2. Serum IL10 levels in 257 individuals who are homozygous for the Z deficiency allele parsed by the *IL10* -1082 promoter SNP genotypes. These data demonstrated a trend for higher IL10 levels in individuals with one or two copies of the minor G allele, especially for individuals on α_1-antitrypsin augmentation therapy (*P* trend = 0.02 for one copy of minor allele for subjects on versus not on augmentation therapy; *P* trend = 0.28 for two copies of minor allele for subjects on versus no on augmentation therapy).

variability. Although genetic modifiers of lung disease have been identified for cystic fibrosis (which occurs at about the same population frequency as AAT deficiency), there have been no published family-based genetic modifier studies of lung disease in AAT deficiency. AAT deficiency fits the criteria of a rare Mendelian disease with complex features: it is an autosomal recessive disorder with incomplete genotype/phenotype correlation. A strong gene-by-smoking interaction contributes to the genesis of lung disease in AAT deficiency, but even after controlling for cigarette smoking there is a broad range of phenotypic severity. A unique feature of our study is that this is a large collection of PI ZZ siblings investigated for modifier genes of AAT deficiency using family-based methods and uniform phenotyping. To identify modifier genes in the AAT Genetic Modifier Study, we have selected candidate genes from previous asthma and COPD genetic association studies.

The *IL10* SNP associations were the most consistent and significant associations that we observed with COPD phenotypes. IL10 is a potent anti-inflammatory cytokine that functions in part through limiting neutrophil release of pro-inflammatory cytokines such as TNF, IL8, and IP10. IL10 also inhibits the production of cytokines from monocytes including IL6, IL12, IL18, and TNF. Neutrophils are the key producers of neutrophil elastase in the lung, and the key inhibitor of this enzyme is AAT. In AAT-deficient individuals, especially those who smoke, unmitigated activity of neutrophil elastase likely contributes to the development of early-onset COPD. There have been data to suggest that IL10 may mitigate pulmonary inflammation by enhancing apoptosis of neutrophils (44). Some associations we observed with SNPs in other genes, especially *TNF*, could also prove to be valid genetic modifiers in future studies. Interestingly, recent work by Janciauskiene and colleagues demonstrated that AAT enhanced release of IL10 from human monocytes and inhibited endotoxin-mediated TNF-α release, potentially providing a pathophysiologic link between AAT, IL10, and TNF (45). In our study, although individuals homozygous for the wild-type IL10 −1082 polymorphism had low IL10 protein levels regardless of augmentation therapy, individuals with one or two copies of the minor G allele receiving augmentation therapy had higher IL10 levels when compared with individuals not on augmentation therapy. This provides an element of *in vivo* support for Janciauskiene and colleagues' (45) *in vitro* observation and also may suggest a potential pharmacogenetic effect associated with *IL10* polymorphisms and AAT augmentation therapy; this is a direction for future investigation.

The balance of proteases and antiproteases is crucial to maintaining the extracellular matrix of the lung. Matrix metalloproteinases (MMPs) such as MMP-9 may contribute to lung injury through degradation of type IV collagen; an imbalance between MMP-9 and its inhibitors such as tissue inhibitor of metalloproteinase 1 (TIMP-1) may contribute to the development of COPD (46, 47). IL10 has been observed to decrease MMP-9 and increase TIMP-1 in cigarette smokers (48). Thus, altered IL10 levels due to polymorphic variations in the gene may contribute to COPD in AAT deficiency due to combined imbalances in both protease and antiprotease pathways.

IL10 promoter polymorphisms and haplotypes have been associated with altered IL10 levels (49–51), and induced sputum IL10 levels in individuals with COPD have been noted to be significantly lower when compared with those of nonsmokers (52). There have been few studies of *IL10* polymorphisms in COPD. The *IL10* promoter polymorphism −1082 (but not −819) has been associated in a small study of COPD cases versus population controls (4), but this association was with the "high" producing G allele. Our data suggest the high producing G allele is associated with higher lung function in PI ZZ subjects. Given the importance of IL10 in many inflammatory processes relevant to asthma, functional *IL10* promoter polymorphisms have been more extensively evaluated in asthma. These data have relevance to our findings in AAT deficiency, as the individuals with the most severe COPD in our cohort often reported a physician's diagnosis of asthma, with the most severe cases of COPD among individuals reporting a diagnosis of childhood asthma (35). Previous studies have noted decreased serum IL10 levels in individuals with asthma versus control subjects (11), and IL10 levels in sputum are reduced in individuals with a history of asthma (52). *IL10* promoter haplotypes that have been associated with lower production of IL10 (−1082, −819, −592) have been associated with severe asthma (10). The most robust associations in our cohort were with the *IL10* promoter SNPs rs1800871 (at −819) and rs1800896 (at −1082), and the haplotypic combination of the −1082 SNP wild-type allele with the −819 SNP minor allele was strongly associated with lower lung function. Our genetic data and serum IL10 protein measures support that *IL10* polymorphisms may alter IL10 levels in AAT deficiency and contribute to the variable manifestations of COPD in AAT deficiency.

AAT deficiency likely had a single evolutionary origin (53). As such, strengths of our study include limited genetic heterogeneity within the cohort, as all subjects were white and verified to be homozygous at the AAT locus (PI ZZ). Second, to avoid potential problems related to population stratification associated with a genetic study of unrelated PI ZZ individuals, we enrolled a large number of related individuals for a family-based association analysis. Limitations include that although we used an LD tagging approach to select polymorphisms for genotyping, the HapMap coverage of all of these genes may not be optimal; the number of SNPs tested in the 10 genes varied from 1 to 16. In addition, we have not formally adjusted for multiple statistical comparisons, although the association of the −819 SNP with post-bronchodilator FEV_1 and FEV_1/FVC would remain significant even after a highly conservative Bonferroni adjustment for testing 10 candidate genes or 75 SNPs. Lastly, we did not analyze occupational data on individuals in this cohort, which may represent an unmeasured

environmental exposure influencing spirometric outcomes. Future studies should focus on replication of our genetic association findings.

This is the first investigation demonstrating association of *IL10* with airflow obstruction in a family-based study of AAT deficiency; there have been few prior studies of genetic modifiers of severe AAT deficiency. Rodriguez and colleagues observed an increased frequency of the *GSTP1* 105Val polymorphism in a case-control study of 69 patients with AAT deficiency, 99 patients with COPD but without AAT deficiency, and 198 healthy control subjects (54). We did not demonstrate association with this polymorphism in our family-based analysis. Novoradovsky and colleagues investigated six polymorphisms in the *NOS3* gene in 339 individuals with AAT deficiency and 94 control subjects, observing increased frequency of the 774T and 894T alleles in AAT-deficient individuals with severe lung disease. Our genotyping of *NOS3* included the 894G/T polymorphism but we did not observe association for this or any other *NOS3* polymorphisms (34).

We conclude that *IL10* is a potential modifier gene of COPD in individuals with severe AAT deficiency. Only *IL10* SNPs demonstrated association with both quantitative and qualitative COPD-related phenotypes and remained associated when individuals with a prior asthma diagnosis were excluded, suggesting that the associations with *IL10* SNPs are not due to an independent association with asthma. Multiple modifier genes are likely relevant to the development of lung disease in AAT-deficient individuals. Identifying modifier genes for COPD in AAT deficiency may result in improved risk assessment and clinical management of patients with severe AAT deficiency, and may provide insight into the variable risk for COPD in non–AAT-deficient individuals.

Conflict of Interest Statement: E.K.S. received grant support, consulting fees, and honoraria from GlaxoSmithKline (GSK) for studies of COPD genetics, received a speaker fee from Wyeth for a talk on COPD genetics, and received honoraria from Bayer. J.K.S. served on an advisory board for Talecris regarding strategies to educate respiratory therapists about α_1-antitrypsin deficiency; grand rounds presentations were sponsored by ZLB Behring, Talecris, and Baxter. S.I.R. has participated as a speaker in scientific meetings and courses under the sponsorship of AstraZeneca (AZ) and GSK; serves on advisory boards for Altana, AZ, Dey, GSK, and Inspire; has conducted clinical trials for AZ, Centocor, GSK, Pfizer, Roche, and Sanofi; has served as a consultant for AZ, Centocor, GSK, Pfizer, and Roche; and a patent is pending on the use of PDE4 inhibitors in repair. S.I.R. is a coinventor of the patent owned by the University of Nebraska Medical Center. E.J.C. is an owner of Heredilab, Inc., which is a clinical diagnostic laboratory that specializes in testing for AAT deficiency; has consulted for Talecris in 2006–2007 ($24,000) and for Actopharma in 2006 ($10,000); and has served on an advisory board for CDC Behring in 2006–2007 ($6,500). None of the other authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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