Determinants of Airflow Obstruction in Severe Alpha 1-Antitrypsin Deficiency

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ABSTRACT

Rationale: Severe alpha 1-antitrypsin deficiency is an autosomal recessive genetic condition associated with an increased, but variable, risk for chronic obstructive pulmonary disease (COPD).

Objective: To assess the impact of chronic bronchitis, pneumonia, asthma, and sex on the development of COPD in individuals with severe alpha 1-antitrypsin deficiency.

Methods: The Alpha 1-Antitrypsin Genetic Modifier Study is a multi-center family-based cohort study designed to study the genetic and epidemiological determinants of COPD in alpha 1-antitrypsin deficiency. 378 individuals (age range: 33-80), confirmed to be homozygous for the SERPINA1 Z mutation, were included in the analyses.

Measurements and Main Results: The primary outcomes of interest were a quantitative outcome, forced expiratory volume in one second (FEV_1) as a percent of predicted, and a qualitative outcome, severe airflow obstruction (FEV_1 < 50% predicted). In multivariate analysis of the overall cohort, cigarette smoking, sex, asthma, chronic bronchitis and pneumonia were risk factors for reduced FEV_1 percent predicted and severe airflow obstruction ($p<0.01$). Index cases had lower FEV_1 values, higher smoking histories, and more reports of asthma, pneumonia, and asthma before age 16 compared to non-index cases ($p<0.01$). Men had lower pre- and post-bronchodilator FEV_1 percent predicted measures than women ($p<0.0001$); the lowest FEV_1 values were observed in men reporting a history of childhood asthma (26.9%$\pm$9.1). This trend for more severe obstruction in men remained when index and non-index groups were examined separately, with males representing the majority of non-index individuals with airflow obstruction (71%). Chronic bronchitis (OR 3.8, CI: 1.8-12.0) and a physician's report of asthma (OR 4.2, CI: 1.4-13.1) were predictors of severe airflow obstruction in multivariate analysis of non-index men but not women.

Conclusion: In individuals with severe alpha 1-antitrypsin deficiency, sex, asthma, chronic bronchitis and pneumonia are risk factors for severe COPD, in addition to cigarette smoking. These results suggest that amongst severely AAT deficient subjects, men, individuals with symptoms of chronic bronchitis, and/or a past diagnosis of asthma or pneumonia may benefit from closer monitoring and potentially earlier treatment.

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a complex disease characterized by variable susceptibility; this variability is likely determined by genes interacting with other predisposing host factors and environmental exposures. Homozygosity for the Z mutation in the SERPINA1 gene is an autosomal recessive genetic risk factor for COPD due to alpha 1-antitrypsin deficiency. Although at high genetic risk, individuals with two severe deficiency alleles (e.g. PI ZZ) have marked variability in the extent of COPD, consistent with the hypothesis that other factors may be relevant to the development of obstructive lung disease.

Alpha 1-antitrypsin (AAT) is the main plasma inhibitor of neutrophil elastase, and the inherited deficiency of AAT has been clearly proven to be associated with the development of early-onset emphysema [1]. In the presence of AAT deficiency, cigarette smoking is an important contributor to accelerated lung function decline, and the extent of lung injury is usually disproportionate to the amount smoked. The Z allele of the AAT gene is associated with a marked decrement in serum AAT levels, but studies of PI Z individuals have demonstrated substantial variability of lung disease in smokers. In individuals who have never smoked cigarettes, there is also wide variability for the development of airflow obstruction [2,3]. Factors such as asthma, pneumonia, other childhood respiratory illnesses [4-6], chronic bronchitis, differential impact of gender/sex biology, and modifier genes all may have a role in the development of lung disease in AAT deficient individuals.

The vast majority of individuals with severe alpha 1-antitrypsin deficiency are identified after being diagnosed with lung or liver disease; based on gene frequency estimates, most PI ZZ subjects remain unidentified. The ascertainment scheme involved in identifying most PI ZZ subjects with existing liver or lung disease has biased insight into the natural history of PI ZZ individuals. As part of our AAT Genetic Modifier Study, we ascertained individuals based upon the presence of two Z alleles (irrespective of lung/liver disease) and investigated characteristics that may be associated with obstructive lung disease in PI ZZ siblings and other relatives. Focusing on predictive characteristics in non-index PI ZZ subjects partially avoids the issues of ascertainment bias and likely provides a more accurate view of natural history of obstructive lung disease in PI ZZ individuals. However, since non-index PI ZZ subjects are typically (but not always) relatives of individuals with lung and/or liver disease, the findings may not be completely generalizable to the entire PI ZZ population.

We have assembled a large cohort of PI ZZ index and non-index subjects in families who have undergone standardized phenotyping with a questionnaire and spirometry. The careful phenotyping and inclusion of index and non-index individuals provides a unique opportunity to test the hypothesis that ascertainment, gender, asthma, chronic bronchitis and pneumonia significantly and independently are associated with airflow obstruction in PI ZZ subjects.

METHODS

Study sites and participants

The AAT Genetic Modifier Study is a multi-center study in collaboration with the Alpha-1 Foundation, designed to study the genetic epidemiology of lung disease in PI ZZ individuals. The study protocol was reviewed by the individual Institutional Review Boards at each site.

378 Caucasian PI ZZ subjects in 167 families were enrolled in the AAT Genetic Modifier Study. At least one PI ZZ sibling pair was necessary for a family to be eligible, with both siblings at least 30 years of age. All participants provided written informed consent. The study protocol included a questionnaire, spirometry and a blood sample for DNA and alpha 1-antitrypsin studies.

Ascertainment scheme and proband designation

Ascertainment of eligible sibling pairs was based upon confirmed homozygosity for the Z allele at the SERPINA1 locus (PI ZZ). The index case was the first person in the family diagnosed with AAT deficiency (irrespective of liver or lung disease), based upon questionnaire responses. Each family only had one index case; all other ZZ family members were classified as non-index. Due to death or non-participation of some index cases, index cases were not available for 27 families.

Questionnaire

Each participant completed a computer-based modified version of the ATS-DLD Epidemiology Questionnaire [7]. The specific questions for defining the presence or absence of chronic bronchitis, wheezing, asthma and pneumonia are summarized in the online supplement. Pack-years of cigarette smoking were calculated by multiplying the number of years smoked times the average number of daily cigarettes smoked, divided by 20. 'Ever smokers' answered affirmative to the question 'Have you ever smoked cigarettes'; no was defined as less than 20 packs of cigarettes or 12 oz of tobacco in a lifetime or less than a cigarette a day for 1 year. 'Current smokers' answered negative to 'Have you stopped smoking (as of one month ago).'

Spirometry

A full description of the spirometry methods is provided in the online supplement. Pre- and post-bronchodilator spirometry testing was performed according to American Thoracic Society standards[8] with the Jaeger Masterscope PC Spirometer system (Jaeger, Hoechberg,Germany). For 16 subjects who had already undergone lung transplantation or lung volume reduction at the time of the study visit, spirometry that antedated the surgical procedure was obtained and the age used for analyses was the age at spirometry. Percent predicted values were calculated using predicted equations of Crapo and colleagues [9]. Unless indicated, data are presented for pre-bronchodilator FEV_1. Bronchodilator reversibility was defined as the difference between absolute post- and pre-bronchodilator FEV_1 divided by baseline pre-bronchodilator FEV_1.

Confirmation of AAT phenotype

Blood spot cards for alpha 1-antitrypsin studies were made from peripheral blood samples collected in EDTA. PI phenotyping was performed using the blood spot cards; specimens were assayed in polyacrylamide gels at pH 4.2-4.9 as previously described [10]. The phenotype results were reported only if two independent interpretations agreed. Genotyping was performed using the ARMS (Amplification and Refractory Mutation System) technology for the detection of point mutations [11]. A genotype of PI ZZ was

assigned if the Z mutation was detected at position 342 and normal sequence was not detected.

Statistics

All computations were performed using the SAS statistical package (SAS Statistical Institute, Cary, North Carolina) on a SUN Microsystem. Chi-square and Student's t tests were used to compare counts and means respectively. The Mann-Whitney test was performed for non-normally distributed covariates. Covariates considered for inclusion in regression models included binary variables (asthma (yes=1, no=0), sex (male=1, female=0), ever-smoking status (yes=1, no=0), chronic bronchitis (yes=1, no=0), physician's diagnosis of asthma (yes=1, no=0), physician's diagnosis of pneumonia (yes=1, no=0), index case in family (yes=1, no=0)) and continuous variables (age, pack-years of smoking). Multiple regression models were performed in SAS using the MIXED procedure in order to control for the correlation between family members.

RESULTS

The 378 confirmed PI ZZ individuals represented 372 siblings, 2 PI ZZ parents, 1 PI ZZ uncle and 3 adult children of PI ZZ individuals. Individuals were ascertained for the study on the basis of the PI ZZ genotype, and all genotypes were confirmed. There were also 50 parents (47 MZ, 1 SZ, 2 missing PI type) enrolled in these families who were not included in the epidemiological analysis. Characteristics of the PI ZZ individuals in this cohort are provided in Table 1.

Table 1: General demographics of the 378 PI ZZ individuals*

Demographics	All (N=378)	Index (N=140)	Non-Index (N=238)	p value Index vs. Non-index
Sex (female)	205 (54%)	73 (52%)	132 (55%)	0.53
Age (SD)	52.2 (±9.7)	52.9 (9.5)	51.7 (9.8)	0.25
FEV_1 % predicted(SD)**	65.9 (±33.5)	49.0 (28.5)	72.3 (32.7)	<0.0001
Ratiox100 (SD)**	55.1 (±20.7)	43.9 (17.3)	61.4(19.8)	<0.0001
Smoking status Ever smoker Current Smoker	 233 (62%) 13 (3%)	 105 (75%) 1 (<1%)	 128 (54%) 12 (5%)	 <0.0001 0.03
Pack-years for smokers Median (IQR)	16 (7-24)	17.3(9.5-25.0)	15 (6-22)	0.02
Age of onset smoking Median (IQR)	17 (15-19)	17 (15-19)	17 (15-19)	0.70
Lung trouble before age 16	93 (25%)	41 (29%)	52 (22%)	0.11
History of wheezing	244(65%)	102 (73%)	142 (60%)	0.01
Doctor confirmed asthma	139 (37%)	64 (46%)	75 (32%)	0.006
Asthma before age 16	20 (5%)	13 (9%)	7 (3%)	0.008
History of pneumonia	220 (58%)	95 (68%)	125 (53%)	0.004
Pneumonia before age 16	47 (12%)	17 (12%)	30 (13%)	0.90
Chronic bronchitis	83 (22%)	33 (24%)	50 (21%)	0.56

*Includes 372 siblings, 2 parents, 1 uncle, 3 children of siblings
**post-bronchodilator

Although 62% of this cohort reported a history of cigarette smoking, only 3% were current smokers. The mean pack-years of cigarette smoking were 18.2 for the smokers (median 17) and the majority started smoking during adolescence. Sixteen participants had undergone lung transplantation and/or lung volume reduction for the surgical management of emphysema. More than half of the participants reported a history of physician-diagnosed pneumonia, 37% reported physician-diagnosed asthma, and 22% met the criteria for chronic bronchitis.

For all PI ZZ participants, FEV_1 as a percent of predicted was graphed as a function of age and stratified by smoking history (Figure 1). As expected, the mean FEV_1 was higher

for non-smokers (non-smokers versus smokers p<0.0001). However, marked variability in FEV_1 values was noted, with some life-time non-smokers demonstrating airflow obstruction before age 50. 61% of the cohort met the criteria for GOLD Stage 2 or higher. We also examined the mean spirometric values and demographic features by index case designation (index or non-index). There was no significant difference between the percentage of men and women classified as an index or non-index subject (Table 1). Index subjects had significantly lower spirometric measures and slightly higher pack-years of cigarette smoking than non-index subjects. There were more non-index subjects who were current smokers. Although there was no difference in the report of general lung problems before age 16, more index subjects reported a history of asthma before age 16. Although the number of individuals were small, asthma diagnosis prior to 16 was a significant predictor of airflow obstruction, with an odds ratio of 4.5 (95% 1.6-12.6, p=0.004), with 15 of the 20 individuals with asthma before age 16 having severe COPD. The mean FEV_1 for subjects with asthma before age 16 was 39.8% predicted ($\pm$ 24.9 % predicted). More index subjects reported a history of physician-diagnosed asthma and physician-diagnosed pneumonia but not chronic bronchitis symptoms (Table 1).

Univariate and multivariate models were evaluated as predictors of FEV_1 (percent predicted) in all subjects, revealing that in addition to cigarette smoking and index case status, pneumonia, asthma, chronic bronchitis and male gender were associated with lower FEV_1 (Tables 2a, 2b). Because spirometric prediction equations may not capture all of the variation in FEV_1 in AAT deficiency, we repeated the analyses using the percent predicted equations proposed by Hankinson et al[12]. Using the Hankinson predicted equations, our results remained robust for the overall cohort and for the non-index subgroup; the effect estimates for sex were attenuated only for the index case subgroup analysis.

Table 2: Univariable and multivariable predictors for FEV_1 (percent predicted) in the overall cohort, index subject and non-index subjects

2a. Univariable Predictors*

	Overall			Index			Non-index		
	Beta	95% CI	p value	Beta	95% CI	p value	Beta	95% CI	p value
Sex	-19.69	-13.17 to -26.21	<0.0001	-11.30	-20.53 to -2.06	0.02	-23.49	-31.36 to-15.63	<0.001
Pack-years	-1.10	-0.13 to -0.89	<0.0001	-0.72	-0.98 to -0.46	<0.0001	-1.19	-1.49 to -0.89	<0.0001
Ever smoking	-29.26	-35.58 to -22.94	<0.0001	-25.31	-35.32 to -15.30	<0.0001	-24.64	-32.42 to -16.86	<0.0001
Asthma	-19.08	-25.87 to -12.30	<0.0001	-7.46	-16.83 to 1.92	0.12	-21.14	-29.77 to -12.52	<0.0001
Pneumonia	-21.25	-27.70 to -14.70	<0.0001	-17.33	-26.99 to -7.68	0.0005	-18.09	-26.15 to -10.02	<0.0001
Chronic Bronchitis	-17.264	-25.28 to -9.25	<0.0001	-4.67	-15.83 to 6.31	0.40	-23.83	-33.64 to -14.03	<0.0001
Index	-26.34	-32.74 to -19.73	<0.0001	---	---	---	---	---	---

*Univariate model includes sex (male=1, female=0), pack-years of cigarettes, ever-smoking (yes=1, no=0), asthma (yes=1, no=0), pneumonia (yes=1, no=0) , chronic bronchitis (yes=1, no=0), index case (yes=1, no=0)

** Odd ratio estimate for pack-years is for each pack-year smoked

2b. Multivariable predictors*

	Overall			Index			Non-index		
	Beta	95% CI	p value	Beta	95% CI	p value	Beta	95% CI	p value
Sex	-15.51	-20.58 to -10.43	<0.0001	-8.57	-16.63 to -0.52	0.04	-19.53	-26.10 to -12.96	<0.0001
Pack-years	-0.62	-0.84 to -0.40	<0.0001	-0.51	-0.80 to -0.22	0.0007	-0.67	-1.01 to -0.32	0.0002
Ever smoking	-10.36	-16.86 to -3.85	0.002	-13.33	-24.34 to -2.33	0.02	-8.62	-17.04 to -0.20	0.04
Asthma	-12.47	-17.79 to -7,15	<0.0001	-7.33	-15.49 to 0.83	0.08	-15.00	-22.09 to -7.90	<0.0001
Pneumonia	-14.38	-19.51 to -9.25	<0.0001	-14.63	-23.11 to -6.15	0.0009	-14.49	-20.98 to -8.00	<0.0001
Chronic bronchitis	-9.26	-15.35 to -3.16	0.003	-5.24	-14.70 to 4.22	0.28	-11.34	-19.43 to -3.26	0.006
Index	-15.19	-20.54 to -9.84	<0.0001			---			---

*Multivariate model includes sex (male=1, female=0), pack-years of cigarettes, ever-smoking (yes=1, no=0), asthma (yes=1, no=0), pneumonia (yes=1, no=0) , chronic bronchitis (yes=1, no=0), index case (yes=1, no=0).
** Odd ratio estimate for pack-years is for each pack-year smoked

We also evaluated bronchodilator reversibility in this cohort and observed that the mean bronchodilator responsiveness (measured as the percent change in the absolute lung function as a function of the baseline FEV_1) was 6.7%. As expected, bronchodilator responsiveness was associated with lower FEV_1 ($p<0.0001$). In univariate models, significant predictors of bronchodilator responsiveness included pneumonia ($p=0.01$), male sex ($p=0.03$), and pack-years of smoking ($p=0.0008$); there was a trend for physician-diagnosed asthma ($p=0.06$). In a multivariate model that included sex, pack-years of smoking, asthma and pneumonia, only sex, pack-years of smoking and pneumonia remained significant predictors ($p<0.05$)

Logistic regression analysis was performed to identify predictors of moderate to severe airflow obstruction in all subjects (Table 3a, 3b); these models for severe COPD demonstrated the same significant predictors as FEV_1 analyzed as a quantitative outcome. The odds for severe airflow obstruction in the overall cohort were highest for index cases, males, individuals with a history of cigarette smoking, and subjects with a history of asthma, chronic bronchitis, and/or pneumonia.

Table 3. Univariable and multivariable models for predicting severe airflow obstruction in the overall cohort, index subject and non-index subjects

3a. Univariable predictors

	All OR (95% CI)	Index OR (95% CI)	Non-Index OR (95% CI)
Number with severe airflow obstruction	158/377 (42%)	90/140 (64%)	68/237 (27%)
Predictor			
Age	1.01 (0.99-1.03)	1.07 (0.97-1.05)	1.01 (0.98-1.04)
Sex	3.14 (2.05-4.80)	2.43 (1.18-4.97)	4.59 (2.49-8.46)
Pack-years of cigarettes	1.09 (1.06-1.11)	1.09 (1.05-1.13)	1.08 (1.05-1.10)
Ever smoking status	6.89 (4.14-11.47)	5.54 (2.43-12.62)	6.44 (3.47-12.90)
History of asthma	3.14 (2.03-4.85)	2.43 (1.18-5.01)	3.33 (1.78-5.84)
History of pneumonia	3.57 (2.28-5.58)	3.50 (1.66-7.37)	3.16 (1.71-5.81)
Chronic bronchitis	2.98 (1.80-4.93)	2.02 (0.83-4.90)	4.18 (2.17-8.05)
Index case	4.47 (2.86-6.99)	---	---

*Univariate model includes sex (male=1, female=0), pack-years of cigarettes, ever-smoking (yes=1, no=0), asthma (yes=1, no=0), pneumonia (yes=1, no=0), chronic bronchitis (yes=1, no=0), index case (yes=1, no=0)
** Estimate for pack-years is for each pack-year smoked

3b. Multivariable predictors

Predictor	All OR (95% CI)	Index OR (95% CI)	Non-Index OR (95% CI)
Sex	4.05 (2.63-7.45)	2.64 (1.05-6.53)	5.51 (2.57-11.81)
Pack-years of cigarettes	1.06 (1.03-1.09)	1.10 (1.04-1.16)	1.04 (1.00-1.08)
Ever smoking status	2.75 (1.27-5.97)	1.51 (0.42-5.43)	3.66 (1.36-9.87)
History of asthma	3.24 (1.81-5.81)	3.70 (1.45-9.44)	3.18 (1.47-6.89)
History of pneumonia	4.09 (2.25-7.45)	4.71 (1.80-12.33)	3.89 (1.78-8.50)
Chronic bronchitis	2.63 (1.37-5.07)	2.77 (0.90-8.47)	2.78 (1.22-6.35)
Index case	3.53 (1.996-6.239)	---	---

*Multivariate model includes sex (male=1, female=0), pack-years of cigarettes, ever-smoking (yes=1, no=0), asthma (yes=1, no=0), pneumonia (yes=1, no=0) , chronic bronchitis (yes=1, no=0), index case (yes=1, no=0)
** Estimate for pack-years is for each pack-year smoked

Because being the index case in a family was a strong predictor of reduced FEV_1 and severe airflow obstruction, we parsed the group by index case status (index versus non-index) and performed the same covariate modeling in the stratified cohort (Tables 2 and 3). In multivariate analysis, smoking, sex, a history of asthma, and a history of pneumonia were predictors of severe airflow obstruction in both index and non-index

individuals, but chronic bronchitis was a significant predictor of airflow obstruction only in non-index individuals (Table 3b). Similar results were obtained for FEV_1 as a quantitative outcome (Table 2b), although asthma was not significantly associated in index cases (p=0.08).

Subsequently, we investigated the characteristics of the 378 PI ZZ individuals stratified by sex, as sex was a highly significant predictor of both FEV_1 and severe airflow obstruction in univariate and multivariate models. The overall demographics for men and women are presented in Table 4 (and supplemental Tables 1 and 2).

Table 4: Sex-specific demographic and clinical characteristics in PI ZZ subjects

Demographic	Male (N=173)	Female (N=205)	p value
Age	52.0 (±9.1)	52.4(±10.2)	0.69
FEV_1 % predicted (post-bronchodilator)	55.4(±32.2)	74.9(±31.9)	<0.0001
Ratio x100 (post-bronchodilator)	48.2(±20.6)	61.1(±18.9)	<0.0001
Smoking status Ever smoker	114	119	0.12
Pack-years (smokers)	20.9(±14.7)	15.6(±13.9)	0.005
Age of onset smoking	17.0(±3.4)	18.6(±5.7)	0.008

Although there was no difference in the percentage of men versus women who reported a history of cigarette smoking, on average men smoked a mean of 5.3 pack-years more and started smoking at a younger age. Overall, men had significantly lower values for FEV_1 (percent predicted) and FEV_1/FVC (Table 4); this trend persisted even among the non-smokers , with men who were non-smokers having lower pre- and post bronchodilator spirometric measures than women (for pre-bronchodilator FEV_1 (72.2% predicted in men versus 86.3% predicted in women, p=0.003)and for pre-bronchodilator FEV_1/FVC (58.7 in men versus 69.2 in women, p=0.0006), with similar results for post-bronchodilator spirometry with $p \leq 0.002$). For all PI ZZ men and women, FEV_1 (percent predicted) was graphed separately for smokers and non-smokers as a function of age and stratified by sex (Figure 2a-smokers, Figure 2b-non-smokers), further demonstrating this marked variation in FEV_1 for women and men. We repeated the analyses using the percent predicted equations proposed by Hankinson et al[12]. Our results remained robust regardless of the predicted models used. There were no differences between the percentages of men and women who reported "lung problems before age 16", wheezing, physician diagnosis of asthma or pneumonia, although there was a significant trend for men to report chronic bronchitis more frequently (p=0.05) (data not shown). The most marked contrast for mean FEV_1 was for those individuals reporting a history of asthma before age 16 (men-26.9% predicted, women-55.0% predicted , p=0.03).

We examined the predictors of airflow obstruction in non-index men and women to determine if there were sex differences in the predictors of severe airflow obstruction. Despite a similar sex distribution of men and women in the index and non-index

categories, female index and non-index subjects had higher FEV_1 and FEV_1 /FVC values compared to men.

For the analysis of airflow obstruction, we focused on the non-index subjects. Only 15% of the female non-index subjects had severe airflow obstruction. The predictors of severe airflow obstruction were the same for male and female non-index subjects on univariate analysis; on multivariate analysis asthma and chronic bronchitis remained robust predictors of airflow obstruction only in men (Table 5a, 5b).

Table 5. Univariable and multivariable models for predicting severe airflow obstruction in the 237 PI ZZ non-index men and women

5a. Univariable predictors

	Men OR (95% CI)	Women OR (95% CI)
Number with severe airflow obstruction	48/106	20/131
Predictor		
Pack-years of cigarettes	1.07 (1.04-1.11)	1.07 (1.02-1.11)
Ever smoking status	6.14 (2.51-15.00)	7.72 (2.14-27.86)
History of asthma	6.70 (2.54-17.72)	2.89(1.10-7.62)
History of chronic bronchitis	5.20 (1.96-13.82)	3.20 (1.11-9.23)
History of pneumonia	4.61 (2.02-10.53)	2.95 (1.00-8.66)

*Univariate model includes sex (male=1), pack-years of cigarettes, ever-smoking (yes=1), asthma (yes=1), pneumonia (yes=1), chronic bronchitis (yes=1), index case (yes=1)

** Estimate for pack-years is for each pack-year smoked

5b. Multivariable predictors

Predictor	Men OR (95% CI)	Women OR (95% CI)
Pack-years of cigarettes	1.05 (1.00-1.10)	1.03 (0.98-1.08)
Ever smoking status	2.64 (0.65-10.68)	5.41 (1.20-24.41)
History of asthma	4.24 (1.38-13.07)	2.44 (0.81-7.33)
History of chronic bronchitis	3.76 (1.18-11.97)	1.95 (0.56-6.84)
History of pneumonia	4.14 (1.49-11.54)	3.51 (1.04-11.85)

*Multivariate model includes sex (male=1), pack-years of cigarettes, ever-smoking (yes=1), asthma (yes=1), pneumonia (yes=1) , chronic bronchitis (yes=1), index case (yes=1)

** Estimate for pack-years is for each pack-year smoked

DISCUSSION

The development of COPD in the setting of severe alpha 1-antitrypsin (AAT) deficiency is highly variable. Host factors, such as modifier genes, likely interact with environmental factors to contribute to an individual's manifestations of lung disease. Many people are tested for AAT deficiency due to existing lung disease, and this ascertainment scheme biases observations of the natural history of lung function. Previous studies have raised concerns about the influence of ascertainment bias in studies designed to investigate determinants of lung disease in PI ZZ subjects. One important and unique feature of our cohort is that it represents the first large collection of sibling pairs and family members systematically enrolled in this study on the basis of homozygosity for the Z mutation of the SERPINA1 gene, irrespective of the presence of lung or liver disease. Evaluating the predictors of airflow obstruction in the non-index individuals in such families may provide insight into predictors of variable severity of COPD in alpha 1-antitrypsin deficient individuals.

Our study suggests that asthma, pneumonia, chronic bronchitis, and sex have the largest impact on the non-index subjects with alpha 1-antitrypsin deficiency, many of whom still have lung function in the normal range; asthma and chronic bronchitis are not significant predictors in the index individuals. When we focused on identifying risk factors for severe COPD, sex, smoking, pneumonia and chronic bronchitis all had odds ratios greater than 2, and these predictors could identify important pathways for genetic modifiers of COPD in AAT deficiency. A childhood history of asthma, although present in a small number of individuals in our study, was associated with the most severe COPD. Although the individual predictors that we investigated have been suggested as predictors of lung function in some prior studies of AAT deficient individuals, this observed difference in families for predictors of lung function between index and non-index individuals, as well as men and women, provides insight into the variable susceptibility to COPD in AAT deficiency as well as familial discordance for COPD, despite similar smoking histories.

Cigarette smoking is the most important risk factor for the development of emphysema in AAT deficient individuals, but the variability and dose-response to this exposure suggest the importance of genetic and other environmental factors. Similar to other investigators [13-18], we have observed that smoking was often but not always associated with lower FEV_1. Individuals with AAT deficiency who smoke often develop obstructive lung disease at an early age [16 14 13], but we have corroborated that some current and former smokers have preserved lung function [4]. We also observed that sex, smoking and a history of pneumonia were important predictors of lung function in index and non-index individuals. A physician diagnosis of asthma and chronic bronchitis contributed to severity of COPD in the non-index individuals. This observation in the non-index individuals, who tend to have higher FEV_1, provides insight into the types of symptoms (cough, sputum, and wheeze) that may portend susceptibility to lung function decline and COPD amongst ZZ individuals. Further research will be required to determine whether the increased risk for airflow obstruction among individuals with symptoms of chronic cough and phlegm and episodes of pneumonia could relate to bronchiectasis, which is also associated with AAT deficiency [19].

Airway hyperresponsiveness and wheezing may be susceptibility phenotypes for worse lung outcomes, especially in the setting of cigarette smoking. In this cohort, 25%

had at least a 10% increase in FEV_1 and this reversibility was associated with lower lung function. Although emphysema/COPD may be misdiagnosed as asthma in adults with alpha 1-antitrypsin deficiency[20], a diagnosis of asthma in childhood is unlikely to be due to emphysema/COPD. In individuals in Sweden identified with AATD as part of neonatal screening, 15% were given a diagnosis of asthma by age 22 and 29% self-reported recurrent wheezing episodes[21]. We observed that a physician diagnosis of asthma before age 16 was strongly associated with reduced FEV_1 and severe COPD in adulthood, suggesting asthma (or the presence of asthma-like symptoms) may define a particularly susceptible subset of PI ZZ individuals. In our study, the most profound reduction in lung function was among men with a history of asthma before age 16, and a physician diagnosis of asthma remained an important predictor of airflow obstruction in non-index case males (not females), suggesting that one potential difference between men and women with COPD may be related to lung inflammation. Inflammation associated with asthma may portend the development of airflow obstruction in later life through limiting the level of maximally achieved lung function or through accelerating lung function decline. Prior studies have demonstrated an association between asthmatic features and COPD in adults with AAT deficiency [5,6,22], and asthmatic symptoms in adults have been previously suggested as a risk factor for lower FEV_1 [18,3].

Pneumonia was associated with lower FEV_1 and severe airflow obstruction in individuals with AAT deficiency in our study cohort, although it is unclear if pneumonia precedes or follows COPD. Although pneumonia has been suggested as a risk factor for COPD in the past, [4] this has not been a consistent observation [3,15]. Interestingly, we observed that although a physician diagnosis of pneumonia was associated with lower lung function in both men and women, a report of pneumonia before age 16 was not, suggesting that pneumonia may be a consequence rather than a cause of COPD.

Sex has been reported as a risk factor for lower FEV_1 in some but not all prior studies of AAT deficient individuals. An increased risk for lung disease in males has been noted by various investigators (Tobin 1983, Larsson 1978, Piitulainen 1997). No significant effect was observed by Silverman and colleagues [4], but the small sample size in that study may have limited the ability to detect a gender effect. The higher number of males among identified PI Z individuals has been attributed to higher rates of smoking among males [18,23] but our current data and previous data in non-smokers [3] suggest that there are sex-based differences for susceptibility to lung disease in AAT deficiency additional to cigarette smoking. Males had lower spirometry measurements than females in our cohort, regardless of index case or smoking status, age, and/or a history of pneumonia, chronic bronchitis or asthma. Larsson and colleagues noted a low prevalence of COPD in non-smoking women in their cohort [14]. Tobin and colleagues observed wide variability in lung function amongst PI Z non-smokers but also noted that older female non-smokers had less limitation of lung function than older males. They also noted that FEV_1 percent predicted was lower in men than women and that the effect of gender and smoking were significant upon multiple regression analysis, although there was no significance of a sex-by-smoking interaction term [13]. Although we, like Tobin and colleagues, did not observe a significant interaction term for smoking and sex (results not shown), it may be more likely that the significant interaction will be modifier gene-by-sex factors. Other environmental exposures, such as occupational dust exposures, are also important to consider as potential modifiers of the risk for COPD in AAT deficiency. Piitulainen and

colleagues observed that male non-smokers were at increased risk for low lung function when compared to female non-smokers [3]. In a sex-stratified analysis they observed that age was an independent predictor of FEV_1 in both men and women but "wheeziness" was a predictor only in men. In our study, men have lower spirometric measures than women, and amongst the non-index cases, symptoms of chronic bronchitis and a physician diagnosis of asthma were significant predictors of severe airflow obstruction only for men. Although this may be because of the lower number of non-index women with airflow obstruction, as noted above men with alpha 1-antitrypsin deficiency may have increased inflammation associated with asthma and chronic bronchitis symptoms.

There have been many previous studies of risk factors for reduced lung function in PI ZZ individuals, but our study has several unique features. First, only subjects with a confirmed PI ZZ genotype were considered; second, we used standardized spirometry with the same spirometry system across centers to minimize technical variability from the spirometric measures; third, we collected a large number of non-index cases and adjusted for potential familial correlations within the multivariate models. Limitations include a potential effect of recall bias for childhood asthma as well as physician's diagnosis of pneumonia and asthma. There is also the potential for misclassification bias associated with COPD diagnosed as asthma. We do not have corroborating data such as chest radiological studies, sputum assessments for pneumonia or methacholine testing for asthma. Ascertainment bias is also important to consider as a limitation, although focusing on the observations in the non-index subjects partially broaches this bias. Although a complete understanding of the natural history of lung disease in PI ZZ individuals will require enrolling subjects without ascertainment bias from a large general population sample, our current study confirms that (in addition to cigarette smoking) sex, asthma, chronic bronchitis, and possibly pneumonia are risk factors for reduced FEV_1 and severe airflow obstruction in PI ZZ individuals. These results suggest that amongst those with severe AAT deficiency, men, individuals with symptoms of chronic bronchitis, and/or a past diagnosis of asthma or pneumonia may benefit from closer monitoring and potentially earlier treatment.

Genetic modifiers are likely important in the variable development of COPD in individuals with severe alpha 1-antitrypsin deficiency. We have identified several determinants of COPD in a large collection of siblings homozygous at the PI ZZ risk locus. Using family-based genetic methods these epidemiological factors may identify important genetic modifiers (such as asthma genetic modifiers of COPD in PI ZZ individuals) and key interactions (such as gene-by-sex) that will provide new insights into the pathophysiology and natural history of lung disease in PI ZZ alpha 1-antitrypsin deficiency.

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Figure Legends:

Figure 1: **FEV_1 Percent Predicted by age for 378 ZZ individuals stratified by smoking status.** This figure presents FEV_1 (percent predicted) as a function of age for 378 ZZ individuals stratified by smoking status. There is a wide variability of FEV_1 for both smokers and non-smokers, with some non-smokers demonstrating low lung function and some smokers demonstrating preserved lung function.

Figure 2a. **FEV_1 Percent Predicted by age for ZZ smokers stratified by sex.** This figure presents FEV_1 (percent predicted) as a function of age for smokers stratified by sex. Male smokers tend to have lower FEV_1 when compared to female smokers ($p<0.0001$).

Figure 2b. **FEV_1 Percent Predicted by age for ZZ non-smokers stratified by sex.** This figure presents FEV_1 (percent predicted) as a function of age for non-smokers stratified by sex. Male non-smokers tend to have lower FEV_1 when compared to female non-smokers ($p=0.003$).

REFERENCES

1. Laurell CB, Eriksson, S. The electrophoretic alpha 1-globulin pattern of serum in alpha 1-antitrypsin deficiency. *Scand J Clin Lab Invest* 1963;**15:**132-40.
2. Black LF, Kueppers F. Alpha1-antitrypsin deficiency in nonsmokers. *Am Rev Respir Dis* 1978;**117**(3)**:**421-8.
3. Piitulainen E, Tornling G, Eriksson S. Effect of age and occupational exposure to airway irritants on lung function in non-smoking individuals with alpha 1-antitrypsin deficiency (PiZZ). *Thorax* 1997;**52**(3)**:**244-8.
4. Silverman EK, Pierce JA, Province MA, Rao DC, Campbell EJ. Variability of pulmonary function in alpha-1-antitrypsin deficiency: clinical correlates. *Ann Intern Med* 1989;**111**(12)**:**982-91.
5. Eden E, Hammel J, Rouhani FN, et al. Asthma features in severe alpha1-antitrypsin deficiency: experience of the National Heart, Lung, and Blood Institute Registry. *Chest* 2003;**123**(3)**:**765-71.
6. Eden E, Mitchell D, Mehlman B, et al. Atopy, asthma, and emphysema in patients with severe alpha-1-antitrypysin deficiency. *Am J Respir Crit Care Med* 1997;**156**(1)**:**68-74.
7. Ferris BG. Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis* 1978;**118**(6 Pt 2)**:**1-120.
8. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;**152**(3)**:**1107-36.
9. Crapo RO, Morris AH, Gardner RM. Reference spirometric values using techniques and equipment that meet ATS recommendations. *Am Rev Respir Dis* 1981;**123**(6)**:**659-64.
10. Pierce JA, Eradio BG. Improved identification of antitrypsin phenotypes through isoelectric focusing with dithioerythritol. *J Lab Clin Med* 1979;**94**(6)**:**826-31.
11. Stockley RA, Campbell EJ. Alpha-1-antitrypsin genotyping with mouthwash specimens. *Eur Respir J* 2001;**17**(3)**:**356-9.
12. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999;**159**(1)**:**179-87.
13. Tobin MJ, Cook PJ, Hutchison DC. Alpha 1 antitrypsin deficiency: the clinical and physiological features of pulmonary emphysema in subjects homozygous for Pi type Z. A survey by the British Thoracic Association. *Br J Dis Chest* 1983;**77**(1)**:**14-27.
14. Larsson C. Natural history and life expectancy in severe alpha1-antitrypsin deficiency, Pi Z. *Acta Med Scand* 1978;**204**(5)**:**345-51.
15. Survival and FEV1 decline in individuals with severe deficiency of alpha1-antitrypsin. The Alpha-1-Antitrypsin Deficiency Registry Study Group. *Am J Respir Crit Care Med* 1998;**158**(1)**:**49-59.
16. Janus ED, Phillips NT, Carrell RW. Smoking, lung function, and alpha 1-antitrypsin deficiency. *Lancet* 1985;**1**(8421)**:**152-4.
17. Seersholm N, Kok-Jensen A, Dirksen A. Decline in FEV1 among patients with severe hereditary alpha 1-antitrypsin deficiency type PiZ. *Am J Respir Crit Care Med* 1995;**152**(6 Pt 1)**:**1922-5.

18. Silverman EK, Miletich JP, Pierce JA, et al. Alpha-1-antitrypsin deficiency. High prevalence in the St. Louis area determined by direct population screening. *Am Rev Respir Dis* 1989;**140**(4)**:**961-6.
19. Dowson LJ, Guest PJ, Stockley RA. The relationship of chronic sputum expectoration to physiologic, radiologic, and health status characteristics in alpha(1)-antitrypsin deficiency (PiZ). *Chest* 2002;**122**(4)**:**1247-55.
20. Stoller JK, Smith P, Yang P, Spray J. Physical and social impact of alpha 1-antitrypsin deficiency: results of a survey. *Cleve Clin J Med* 1994;**61**(6)**:**461-7.
21. Piitulainen E, Sveger T. Respiratory symptoms and lung function in young adults with severe alpha(1)-antitrypsin deficiency (PiZZ). *Thorax* 2002;**57**(8)**:**705-8.
22. Eden E, Strange C, Holladay B, Xie L. Asthma and allergy in alpha-1 antitrypsin deficiency. *Respir Med* 2006;**100**(8)**:**1384-91.
23. Brantly ML, Paul LD, Miller BH, Falk RT, Wu M, Crystal RG. Clinical features and history of the destructive lung disease associated with alpha-1-antitrypsin deficiency of adults with pulmonary symptoms. *Am Rev Respir Dis* 1988;**138**(2)**:**327-36.

Figure 1:

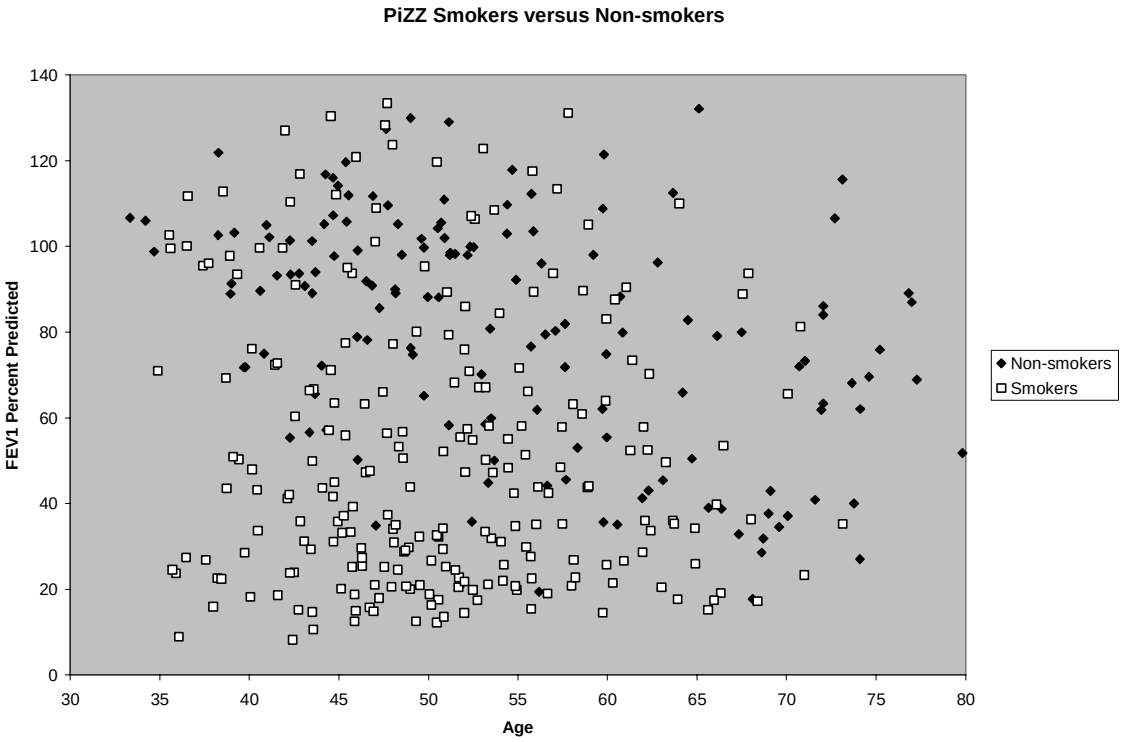

Figure 2a.

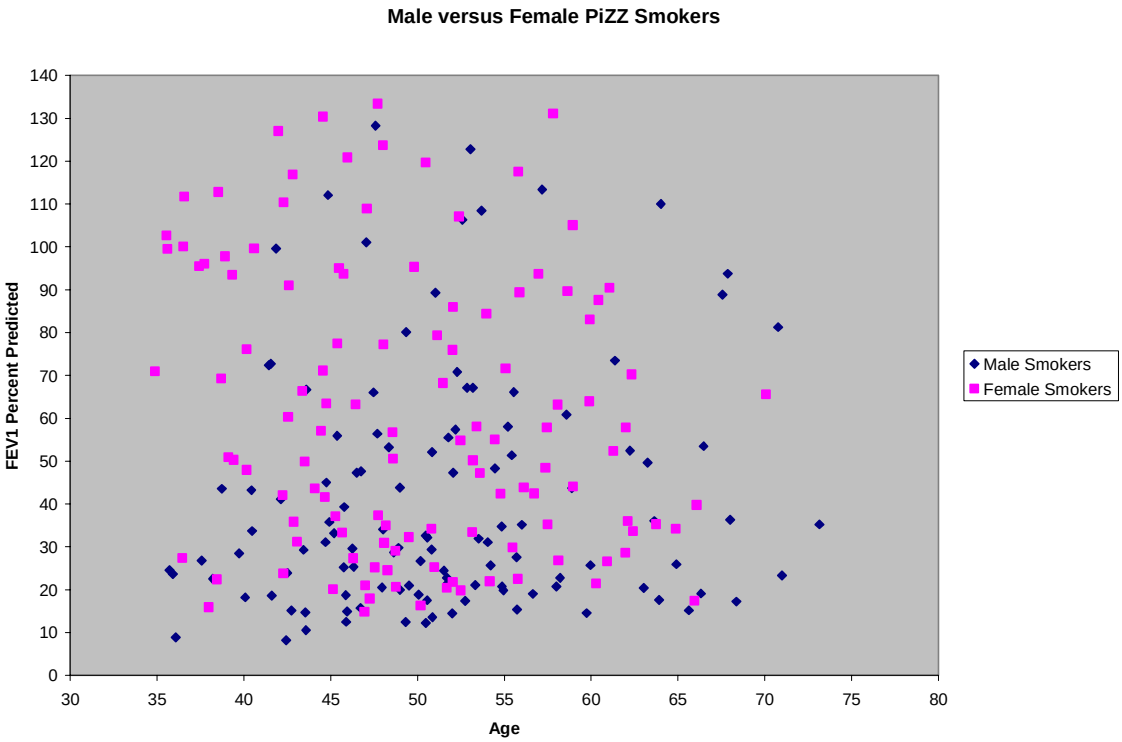

Figure 2b.

Determinants of Airflow Obstruction in Severe Alpha 1-Antitrypsin Deficiency

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Online Data Supplement

Detailed Methods

Study sites and participants

The AAT Genetic Modifier Study is a multi-center study in collaboration with the Alpha 1 Foundation, designed to identify genes that modify the risk of lung disease in PI ZZ individuals. The Sample Management and Data Analysis Center is at Brigham & Women's Hospital, Boston, MA; the Epidemiology Center is at the University of Utah. These two centers and ten other clinical centers identified PI ZZ individuals and enrolled eligible families for the present analysis. The study protocol was reviewed by the individual Institutional Review Boards at each site.

428 Caucasion family members in 167 families were enrolled in the AAT Genetic Modifier Study. At least one PI ZZ sibling pair was necessary for a family to be eligible, with both siblings at least 30 years of age. Parents of PI ZZ individuals and other PI ZZ family members were invited to participate. All participants provided written informed consent. The study protocol included a questionnaire, spirometry and a blood sample for DNA and alpha 1-antitrypsin studies.

Questionnaire

Each participant completed a computer-based modified version of the ATS-DLD Epidemiology Questionnaire(1), which was administered by a trained interviewer. This questionnaire included questions about the reason tested for AATD, personal smoking history, childhood and adult respiratory infections, asthma, and allergy. The specific questions for defining the presence or absence of chronic bronchitis, wheezing, asthma and pneumonia are summarized. Pack-years of cigarette smoking were calculated by

multiplying the number of years smoked times the average number of daily cigarettes smoked, divided by 20. Chronic bronchitis was defined as present when participants answered yes to both questions: “Do you usually cough like this on most days for 3 consecutive months or more during the year” and “Do you bring up phlegm like this on most days for 3 consecutive months or more during the year” combined with a response that these symptoms of cough and phlegm had been present for 2 or more years. Wheezing was defined as an affirmative answer to the question “Does your chest ever sound wheezing or whistling?” Asthma was defined by an affirmative answer to the questions “Have you ever had asthma?” and “Was it confirmed by a doctor?” Pneumonia was defined by an affirmative answer to the questions “Have you ever had pneumonia?” and “Was it confirmed by a doctor?”

Spirometry

Spirometry testing was performed with the Jaeger Masterscope PC Spirometer system (Jaeger, Hoechberg,Germany). Standardized training of all research assistants was performed. For subjects who had already undergone lung transplantation or lung volume reduction at the time of the study visit, spirometry that antedated the surgical procedure was obtained. In these participants, the age used for analyses was the age at spirometry.

Pre- and post-bronchodilator study spirometry was performed according to American Thoracic Society standards. After pre-bronchodilator spirometry, two puffs of albuterol (180 mg) were provided through a spacer, followed by repeat spirometry to obtain post-bronchodilator measures. We selected the highest FEV_1 value and the highest FVC from any effort to define pre and post-bronchodilator spirometric phenotypes. Percent predicted values were calculated using predicted equations of Crapo and colleagues(2).

Supplemental table 1: Sex-specific demographic and clinical characteristics in PI ZZ subjects

Demographic	Male (N=173)	Female (N=205)	P value
Age	52.0 (±9.1)	52.4(±10.2)	0.69
FEV_1 % predicted (post-bronchodilator)	55.4(±32.2)	74.9(±31.9)	<0.0001
Ratio (post-bronchodilator)	0.482(±0.206)	0.611(±0.189)	<0.0001
Smoking status Ever smoker	114	119	0.12
Pack-years (smokers)	20.9(±14.7)	15.6(±13.9)	0.005
Age of onset smoking	17.0(±3.4)	18.6(±5.7)	0.008
Lung trouble before age 16 N(%)	38 (22%)	55(27%)	0.27
History of wheezing N(%)	118 (68%)	126(61%)	0.17
History of Asthma N(%)	56 (32%)	83(40%)	0.10
Asthma before age 16 N(%)	8 (5%)	12 (6%)	0.60
Doctor confirmed hay fever N(%)	34(20%)	57(28%)	0.07
History of pneumonia N(%)	102(59%)	118 (58%)	0.78
Pneumonia before age 16 N(%)	22 (13%)	25 (12%)	0.88
Chronic bronchitis N(%)	46 (27%)	37 (18%)	0.046

**within gender distribution

Supplemental table 2:
Sex-specific FEV_1 and FEV_1/FVC by index case status

Spirometry Measure*	All Mean (SD)	Men Mean (SD)	Women Mean (SD)	P value for mean FEV_1 for Men versus Women
FEV_1				
Index	49.0 (±28.5)	42.9 (±26.8)	54.9 (±29.1)	0.02
Non-index	75.4 (±32.3)	63.2 (±33.0)	85.3 (±28.3)	<0.0001
FEV_1/FVC				
Index	0.439 (±0.173)	0.386 (±0.158)	0.489 (±0.173)	0.0006
Non-index	0.614 (±0.198)	0.541 (±0.211)	0.674 (±0.165)	<0.0001

*Post-bronchodilator FEV_1 percent of predicted

References

E1. Ferris, B. G. 1978. Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis* 118(6 Pt 2):1-120.

E2. Crapo, R. O., A. H. Morris, and R. M. Gardner. 1981. Reference spirometric values using techniques and equipment that meet ATS recommendations. *Am Rev Respir Dis* 123(6):659-64.