Clarification of the Risk of Chronic Obstructive Pulmonary Disease in α_1 -Antitrypsin Deficiency PiMZ Heterozygotes

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Abstract

Rationale: Severe α_1 -antitrypsin deficiency (typically PiZZ homozygosity) is associated with a significantly increased risk of airflow obstruction and emphysema but the risk of chronic obstructive pulmonary disease (COPD) in PiMZ heterozygotes remains uncertain.

Objectives: This was a family-based study to determine the risk of COPD in PiMZ individuals.

Methods: We compared 99 PiMM and 89 PiMZ nonindex subjects recruited from 51 index probands who were confirmed PiMZ heterozygotes and also had a diagnosis of COPD Global Initiative for Chronic Obstructive Lung Disease stage II–IV. The primary outcome measures of interest were quantitative variables of pre- and post-bronchodilator FEV₁/FVC ratio, FEV₁ (liters), FEV₁ (% predicted), forced expiratory flow midexpiratory phase (FEF_{25–75}; liters per second), FEF_{25–75} (% predicted), and a categorical outcome of COPD.

Measurements and Main Results: PiMZ heterozygotes compared with PiMM individuals had a reduced median (interquartile range) post-bronchodilator FEV₁ (% predicted) (92.0 [75.6–105.4] vs. 98.6 [85.5–109.7]; $P = 0.04$), FEV₁/FVC ratio (0.75 [0.66–0.79] vs. 0.78 [0.73–0.83]; $P = 0.004$), and FEF_{25–75} (% predicted) (63.84 [38.45–84.35] vs. 72.8 [55.5–97.7]; $P = 0.0013$) compared with PiMM individuals. This effect was abrogated in never-smoking and accentuated in ever-smoking PiMZ individuals. PiMZ heterozygosity was associated with an adjusted odds ratio for COPD of 5.18 (95% confidence interval, 1.27–21.15; $P = 0.02$) and this was higher (odds

ratio, 10.65; 95% confidence interval, 2.17–52.29; $P = 0.004$) in ever-smoking individuals.

Conclusions: These results indicate that PiMZ heterozygotes have significantly more airflow obstruction and COPD than PiMM individuals and cigarette smoke exposure exerts a significant modifier effect.

Keywords: α_1 -antitrypsin; heterozygote; chronic obstructive pulmonary disease

At a Glance Summary

Scientific Knowledge on the Subject: The risk of chronic obstructive pulmonary disease in α_1 -antitrypsin deficiency PiMZ heterozygotes is uncertain. An accurate risk estimate has been difficult to ascertain because of various design and methodologic weaknesses, including selection bias and lack of rigorous control for environmental influences, such as cigarette smoking, in previous studies.

What This Study Adds to the Field: This family-based study gives protection from selection bias and shows that PiMZ individuals have a greater degree of airflow obstruction than PiMM individuals with a similar degree of cigarette smoke exposure. Ever-smoking PiMZs have an increased risk for chronic obstructive pulmonary disease and this risk is attenuated in never-smokers.

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Chronic obstructive pulmonary disease (COPD) represents a major health burden worldwide. It is estimated that as many as 12.6 million people are diagnosed with COPD in the United States and COPD is the third leading cause of death in the United States, with approximately 100,000 deaths annually (1). Cigarette smoke exposure is a significant environmental risk factor for COPD, and severe α_1 -antitrypsin (AAT) deficiency is a proven genetic risk factor for COPD (2).

AAT is a serine protease inhibitor encoded by the SERPINA1 locus on chromosome 14q32.1 (3). It functions as the major circulating antiprotease, which protects the tissues from indiscriminate proteolytic attack. The most common deficiency-associated alleles are PI*S and PI*Z. The Z mutation in the AAT (SERPINA1) gene encodes a misfolded variant of AAT that accumulates intracellularly in the endoplasmic reticulum of hepatocytes and other AAT-producing cells. PiZZ individuals are severely deficient in AAT. This loss of functional AAT leads to the unopposed action of proteases, primarily neutrophil elastase (NE), contributing to PiZZ-related emphysema (4). PiMZ individuals have intermediate levels of plasma AAT, approximately 60% of normal. If a dose-dependent relationship exists, it is biologically plausible that PiMZ individuals may be at an increased risk of COPD. Nonsmoking, asymptomatic PiMZ individuals with normal lung function have a higher IL-8-related neutrophilic burden (5) but whether there is a significantly increased risk of lung disease in PiMZ heterozygotes has yet to be definitively determined. The estimated prevalence of the Z deficiency allele is 10.5 per 1,000 in the United States and 21.8 per 1,000 in Ireland, with approximately 6 million and 170,000 individuals in these respective populations estimated to be PiMZ carriers (6, 7).

Many previous studies have sought to clarify the risk of COPD in PiMZ heterozygotes, but the published literature is conflicting (8). An increased risk of COPD was found in a meta-analysis with a compound odds ratio (OR) of 2.31 (95% confidence interval [CI], 1.6–3.4). However, although case-control studies showed an increased OR for COPD, this was not confirmed in cross-sectional studies. The major problems with these studies include small sample sizes; selection bias in

choosing cases and control subjects; inconsistent use of objective spirometric criteria in the diagnosis of COPD; and lack of rigorous control for age, sex, ethnicity, and cigarette smoking (9). Given the economic burden of COPD (10) and the potential numbers of PiMZ individuals at risk, an accurate elucidation of this risk is urgently needed. We therefore undertook a family-based study to ascertain whether there is an increased risk of COPD in PiMZ heterozygotes.

We hypothesized that PiMZ heterozygosity is associated with reduced lung function and an increased risk of COPD among relatives of PiMZ COPD probands. Some of the results of these studies have been previously reported in 2013 in the form of an abstract at the American Thoracic Society Conference in Philadelphia (11).

Methods

Study Design and Participants

We performed a family-based study to determine the risk of COPD in PiMZ heterozygotes. Index probands were identified from the Irish National Targeted Detection Program (7). The index proband was the first person in the family diagnosed with intermediate AAT deficiency (MZ heterozygosity) and Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage II–IV COPD (as defined by the GOLD stage [post-bronchodilator (BD) FEV₁/FVC, <0.7; post-BD FEV₁, <80 % predicted]) to enrich for families with a predisposition to COPD. All participants were at least 30 years of age and all available nonindex family members were recruited. Participants were excluded if they had a diagnosis of interstitial lung disease, previous thoracic surgery (including lobectomy, lung transplant, or lung volume reduction surgery), or current pregnancy. Study visits were scheduled at least 4 weeks after any respiratory tract infection.

The study protocol included a questionnaire, spirometry (before and after inhaled salbutamol/albuterol), and a blood sample for AAT studies. All participants provided written informed consent, and appropriate ethical approval was obtained from the hospital's institutional review board.

Questionnaire

Each participant completed a modified version of the American Thoracic Society Division of Lung Disease (DLD) Epidemiology Questionnaire (12). Pack-years of cigarette smoking were calculated by multiplying the number of years smoked by the average number of daily cigarettes smoked, divided by 20. "Never smoker" was defined as less than 20 packs of cigarettes or 12 oz of tobacco in a lifetime or less than one cigarette a day for 1 year. Passive cigarette smoke exposure was assessed by childhood, occupational, and household second-hand smoke exposure.

Spirometry

Pre- and post-BD spirometry testing was performed according to American Thoracic Society standards (13) with the EasyOne Spirometer: Model 2001–2S (NDD Medical Technologies, Zürich, Switzerland). Percentage predicted values were calculated using European Respiratory Society–ECCS (The European Community of Coal and Steel) (1993) reference equations (14).

Determination of AAT Phenotype

A 10-ml venous blood sample was obtained from each subject. Characterization of AAT phenotypes was performed using the Hydrasys electrophoresis platform (Sebia) and the Hydragel 18 A1AT Isofocusing kit (Sebia, Evry, France) (15). AAT levels were measured by nephelometry (Siemens Dade-Behring BN II, Tarrytown, NY).

Statistical Analysis

Although the text focuses mainly on post-BD spirometry, the results of comparable analyses based on pre-BD data are included in the online supplement.

To avoid selection bias, PiMZ probands with COPD were excluded from the analysis. We used the family-based association test (FBAT) implemented in the pedigree-based association testing program (version 3.6; <http://www.biostat.harvard.edu/clange/default.htm>) (16). Analyses for FEV₁/FVC ratio, FEV₁ (liters), and forced expiratory flow midexpiratory phase (FEF_{25–75}; liters per second) were adjusted for age, sex, height, smoking status (ever vs. never), and pack-years. Analyses for FEV₁ (% predicted) and FEF_{25–75} (% predicted) were adjusted for smoking status and pack-years. Analyses for the binary outcome of COPD were adjusted for age, sex, smoking status, and pack-years.

Table 1: Demographic Data and Baseline Lung Function in Probands, PiMM, and PiMZ Relatives

	Probands	MM Relatives	MZ Relatives	P Value PiMM vs. PiMZ Relatives
Number	51	99	89	N/A
Relationships of probands				
Siblings		60	49	
Children	N/A	36	34	N/A
Parents		3	6	
Age, yr, mean $\pm$ SD	64.8 $\pm$ 11.1	51.9 $\pm$ 14.5	53.4 $\pm$ 13.6	ns
Sex, % females	25 (49.0)	66 (66.7)	52 (58.4)	ns
Body mass index, kg/m ² (mean $\pm$ SD)	25.2 $\pm$ 5.4	26.1 $\pm$ 4.6	26.7 $\pm$ 6.0	ns
α_1 -Antitrypsin, g/L (mean $\pm$ SD)	0.88 $\pm$ 0.16	1.40 $\pm$ 0.28	0.92 $\pm$ 0.39	<0.0001
% Ever-smokers	88.2 (n = 45)	52.5 (n = 52)	58.4 (n = 52)	ns
% Never-smokers	11.8 (n = 6)	47.5 (n = 47)	41.6 (n = 37)	ns
% Passive smoking among never-smokers	100 (n = 6)	91.5 (n = 43)	94.6 (n = 35)	ns
Pack-years of smoking	40.0 (17–67)	21.4 (14.8–36.5)	23.8 (14.3–45.8)	ns
Post-bronchodilator				
FEV ₁ /FVC ratio	0.40 (0.32–0.59)	0.78 (0.73–0.83)	0.75 (0.66–0.79)	0.004
FEV ₁ , L	0.96 (0.71–1.24)	2.69 (2.15–3.14)	2.54 (1.84–3.19)	ns
FEV ₁ , % predicted	38.8 (27.2–68.7)	98.6 (85.5–109.7)	92.0 (75.6–105.4)	0.04
FEF _{25–75} , L/s	0.12 (0.08–0.24)	2.42 (1.86–3.33)	2.14 (1.01–2.89)	0.009
FEF _{25–75} , % predicted	12.1 (8.4–23.9)	72.8 (55.5–97.7)	63.8 (38.5–84.4)	0.001
Diagnosis of chronic obstructive pulmonary disease, n	51	5	19	0.0009

Definition of abbreviations: FEF_{25–75} = forced expiratory flow midexpiratory phase; N/A = not applicable; ns = nonsignificant.

“Never smoker” was defined as less than 20 packs of cigarettes or 12 oz of tobacco in a lifetime or less than one cigarette a day for 1 year.

Passive cigarette smoke exposure was assessed by childhood, occupational, and household second-hand smoke exposure.

P values are based on chi-square test for the categorical variables. For continuous data, the Student *t* test was used for normally distributed (presented as mean $\pm$ SD) and the Mann-Whitney *U* test for nonnormally distributed data (presented as median [interquartile range]).

A significant gene-by-smoking interaction was tested in pedigree-based association testing using the cross product term (Pi $\times$ ever-smoker).

Continuous data were validated for normality of distribution using the Shapiro-Wilk test. Normally and nonnormally distributed data were analyzed by Student *t* test and Mann-Whitney *U* test, respectively, and categorical variables by chi-square test. Generalized estimating equations (GEE) were used to estimate the OR for COPD, adjusted for familial correlation, age, sex, smoking status, and pack-years. Normality

assessments and GEE analyses were performed using R software version 3.0.0.

Results

Demographic Data and Baseline Lung Function of Study Subjects

All study subjects were white. Fifty-one families were enrolled through index PiMZ probands with COPD. A total of 196 nonindex relatives were recruited, 8 of which were excluded because of a non-PiMZ or non-PiMM genotype (MS, *n* = 5; SZ, *n* = 3). The remaining relatives included 99 PiMM

and 89 PiMZ individuals. Table 1 shows demographic data and baseline characteristics for the study population and for probands and relatives separately. Index probands were excluded from further analysis. From this point forward, PiMM and PiMZ relatives are referred to as PiMM and PiMZ individuals. As expected, there was a difference in mean (SD) AAT (grams per deciliter) levels between PiMM (1.396 $\pm$ 0.275) and PiMZ (0.921 $\pm$ 0.389) individuals (*P* < 0.0001). There was no significant difference between PiMM and PiMZ individuals in terms of age, sex, body

Table 2: Post-bronchodilator Spirometry in Never-Smoking versus Ever-Smoking PiMM and PiMZ Individuals

Parameter	Never-Smokers			Ever-Smokers		
	PiMM	PiMZ	P Value	PiMM	PiMZ	P Value
FEV ₁ /FVC ratio	0.81 (0.75–0.85)	0.77 (0.73–0.83)	ns	0.77 (0.72–0.81)	0.71 (0.58–0.77)	0.001
FEV ₁ , L	2.69 (2.32–3.13)	2.98 (2.22–3.54)	ns	2.70 (2.11–3.13)	2.32 (1.64–3.02)	ns
FEV ₁ , % predicted	103.7 (90.3–112.5)	102.6 (93.2–114.8)	ns	96.4 (84.2–107.1)	84.6 (69.4–96.3)	0.0006
FEF _{25–75} , L/s	2.89 (2.15–3.49)	2.63 (1.84–3.52)	ns	2.18 (1.64–3.17)	1.68 (0.70–2.44)	0.003
FEF _{25–75} , % predicted	84.1 (70.7–100.8)	76.1 (63.6–99.4)	ns	64.8 (52.2–90.2)	47.7 (22.7–66.8)	0.0002

Definition of abbreviations: FEF_{25–75} = forced expiratory flow midexpiratory phase; ns = nonsignificant.

“Never smoker” was defined as less than 20 packs of cigarettes or 12 oz of tobacco in a lifetime or less than one cigarette a day for 1 year.

P values are based on the Mann-Whitney *U* test. Data presented as median (interquartile range).

Figure 1. Post-bronchodilator spirometry in ever- and never-smoking individuals. Post-bronchodilator FEV₁/FVC ratio (A), FEV₁ (% predicted) (B), and forced expiratory flow midexpiratory phase (FEF₂₅₋₇₅; % predicted) (C) in ever- versus never-smokers. “Never smoker” was defined as less than 20 packs of cigarettes or 12 oz of tobacco in a lifetime or less than one cigarette a day for 1 year.

mass index, ever-smoking status, and pack-year smoking history. Among never-smoking individuals, almost all recruits were exposed to passive cigarette smoke and only a small number of never-smoking PiMM ($n = 4$) and PiMZ ($n = 2$) individuals had no passive cigarette smoke exposure.

PiMZ individuals differed significantly from PiMM individuals in pre-BD FEV₁/FVC ratio ($P = 0.002$) and post-BD FEV₁/FVC ratio ($P = 0.004$). There was no significant difference in pre- and post-BD FEV₁ (liters) but there was a significant difference in post-BD FEV₁ (% predicted) between PiMZ and PiMM individuals ($P = 0.04$). There was a trend toward a reduction in pre-BD FEV₁ (% predicted) in PiMZ individuals ($P = 0.05$). FEF₂₅₋₇₅ (liters per second) was reduced pre-BD ($P = 0.03$) and post-BD ($P = 0.009$) in PiMZ subjects as was FEF₂₅₋₇₅ (% predicted) pre- ($P = 0.008$) and post-BD ($P = 0.001$) (Table 1).

Lung Function in PiMM and PiMZ Individuals Stratified by Cigarette Smoke Exposure

Ever-smokers versus never-smokers. To determine the effect of cigarette smoke exposure on the lung function of PiMZ individuals, PiMM and PiMZ individuals were subcategorized into ever-smokers and never-smokers. There was no difference in lung function in never-smoking PiMM versus PiMZ individuals (Table 2). Ever-smoking PiMZ individuals had a significantly lower pre-BD ($P = 0.0003$) and post-BD ($P = 0.001$) FEV₁/FVC ratio (Figure 1A). There was no significant difference in pre-BD ($P = 0.2$) and post-BD ($P = 0.09$) FEV₁ (liters) in ever-smoking PiMM versus PiMZ individuals. However, FEV₁ expressed as % of predicted volume was significantly lower both pre- ($P = 0.0009$) and post-BD ($P = 0.0006$) in ever-smoking PiMZ individuals (Figure 1B). This decrease in lung function was also reflected in the pre- and post-BD FEF₂₅₋₇₅ (liters per second) and FEF₂₅₋₇₅ (% predicted) in PiMZ individuals (Figure 1C).

Low versus high cigarette smoke exposure. To examine the dose-response of lifetime smoking on lung function in ever-smoking PiMZ individuals, we stratified our study population by level of smoking exposure: a low-exposure (<20 pack-years of smoking) and a high-exposure (≥ 20 pack-years of smoking) group. There was

Table 3: Post-bronchodilator Spirometry in PiMM versus PiMZ Individuals Stratified by Levels of Smoking Exposure

Parameter	Low Exposure			High Exposure		
	PiMM	PiMZ	P Value	PiMM	PiMZ	P Value
FEV ₁ /FVC ratio	0.80 (0.76–0.83)	0.75 (0.67–0.77)	0.007	0.74 (0.69–0.78)	0.68 (0.56–0.78)	0.01
FEV ₁ , L	2.85 (2.55–3.49)	2.37 (1.89–3.03)	ns	2.59 (2.04–2.94)	2.31 (1.55–3.01)	ns
FEV ₁ , % predicted	97.2 (90.0–108.2)	89.1 (70.6–97.6)	0.02	94.3 (80.1–102.3)	81.1 (69.0–91.1)	0.01
FEF _{25–75} , L/s	3.08 (2.17–3.68)	1.95 (0.87–2.77)	0.01	1.97 (1.35–2.45)	1.40 (0.64–2.40)	ns
FEF _{25–75} , % predicted	86.4 (60.8–98.6)	55.4 (30.8–68.1)	0.002	56.6 (44.5–70.6)	41.4 (22.3–64.0)	0.02

Definition of abbreviations: FEF_{25–75} = forced expiratory flow midexpiratory phase; ns = non-significant.

A low exposure was defined as less than 20 pack-years of smoking and a high exposure as ≥ 20 pack-years of smoking.

P values are based on the Mann-Whitney U test. Data presented as median (interquartile range).

no difference in lifetime smoking intensity between the low-exposure PiMM (median [interquartile range] 10.25, [6–16.5]) versus PiMZ (10.75 [9.8–16.75] pack-years) category ($P = 0.5$) or between the high-exposure PiMM (31 [24–47.25]) versus PiMZ (40.38 [26.5–58.25]) category ($P = 0.1$). Cigarette smoke exposure was inversely proportional to quantitative measures of lung function including FEV₁/FVC ratio, FEV₁ (% predicted), and FEF_{25–75} (% predicted) (Table 3). PiMZ individuals in the low- and high-exposure group had a greater degree of airways obstruction (lower pre- and post-BD FEV₁/FVC ratio, FEV₁ [% predicted], FEF_{25–75} [liters per second], FEF_{25–75} [% predicted]), compared with PiMM individuals (Figures 2A–2C).

FBAT and GEE

The results of family-based association analysis between the Pi locus, lung function, and COPD are summarized in Table 4. In models adjusted for relevant covariates, PiMZ heterozygosity was associated with reduced pre- and post-BD FEV₁/FVC ratio, FEV₁ (liters), FEV₁ (% predicted), FEF_{25–75} (liters per second), and FEF_{25–75} (% predicted), indicating an increased risk of airflow obstruction in PiMZ individuals, and an increased risk of COPD, defined as GOLD stage 2 or greater (post-BD FEV₁/FVC, <0.7 ; post-BD FEV₁, <80 % predicted). Subjects with GOLD stage 1 were excluded from the analysis of COPD status. In stratified analyses, ever-smoking PiMZ heterozygotes had significantly reduced lung function and increased risk of COPD ($P = 0.0004$), whereas there was no increased risk in PiMZ never-smokers.

Using Fisher exact test, the unadjusted OR for COPD was 5.10 (95% CI, 1.81–14.33; $P = 0.0009$). GEE were used to adjust for

familial correlation with COPD as an outcome, adjusting for covariates including sex, ever-smoking status, age, and pack-years of smoking (Table 5). In the GEE models, only Pi genotype and age were significantly associated with COPD. Heterozygous PiMZ individuals had multivariate adjusted OR for COPD of 5.18 (95% CI, 1.27–21.15; $P = 0.02$). In stratified analyses, the unadjusted OR for COPD in ever-smoking PiMZ heterozygotes was 9.21 (95% CI, 2.43–52.47; $P = 0.0001$) and in a GEE model adjusting for age, sex, and pack-years of smoking was 10.65 (95% CI, 2.17–52.29; $P = 0.003$). In never-smoking individuals the OR (OR, 0.24; 95% CI, 0.01–5.21; $P = 0.2$) was not significantly increased.

The effect of genotype-by-environment interaction between Pi type and ever-smoking status on COPD risk was tested by adding a cross-product term (Pi $\times$ ever-smoking status) to the FBAT model. The genotype-by-ever-smoking interaction was statistically significant ($P = 0.005$).

Discussion

We have demonstrated that PiMZ heterozygotes are at an increased risk for impaired lung function and COPD and that this risk is strongly influenced by cigarette smoke exposure. The fact that never-smoking PiMZ heterozygotes did not develop lung disease in our study population demonstrates the importance of gene-by-smoking interactions in the genesis of COPD in PiMZ individuals. Severe AAT deficiency is the most convincingly proven genetic risk factor for COPD and significant gene-by-smoking interaction contributes to the pathogenesis of the disease (17). An accurate determination of the risk of COPD

in PiMZ heterozygotes and the contribution of environmental influences (primarily cigarette smoke) remains difficult to ascertain from the current published literature. This study addresses the various methodologic concerns of previous studies including the elimination of selection bias by excluding PiMZ probands, the use of objective spirometric criteria for the diagnosis of COPD, and careful control for age, sex, ethnicity, and cigarette smoking, and shows that MZs, a significant percentage of the general population, have an increased risk for development of COPD (9).

In our study, PiMZ heterozygotes had a reduced FEV₁/FVC ratio, and this decrease was accentuated in ever-smoking individuals. The FEV₁ % predicted was significantly lower in PiMZ individuals, particularly if they smoked, and the significant reduction in absolute (liters per second) and % predicted FEF_{25–75} authenticated the presence of airway obstruction in those with the PiMZ genotype. A population-based cohort study demonstrated that PiMZ heterozygotes had a rate of FEV₁ decline that was 19% greater than those with the PiMM genotype (18) but this finding was refuted by another study (19). A recent study examining the risk of airflow obstruction in PiMZ versus PiMM individuals in two large cohorts found that PiMZ heterozygosity was associated with 3.5% lower FEV₁/FVC ratio and 3.7% more emphysema on quantitative chest computed tomography (CT) scans in the case control study and 3.9% lower FEV₁/FVC ratio in the family study. There was more emphysema on chest CT scans in PiMZ individuals who had low cigarette smoke exposure (<20 pack-years) than in PiMM individuals. These findings are concordant with our demonstration that

Figure 2. Post-bronchodilator spirometry in low and high cigarette smoke exposure groups. Post-bronchodilator FEV₁/FVC ratio (A), FEV₁ (% predicted) (B), and forced expiratory flow midexpiratory phase (FEF_{25–75}, % predicted) (C) in low versus high smoke exposure groups. A low exposure was defined as less than 20 pack-years of smoking and a high exposure as greater than or equal to 20 pack-years of smoking.

PiMZ heterozygotes in the low-exposure group have a greater degree of airflow obstruction than PiMM individuals (20). However, both populations in this study consisted of current and ex-smokers and therefore only permitted conclusions to be drawn about ever-smoking PiMZ individuals.

Using the FBAT we were able to confirm the association between the PiMZ genotype and quantitative outcomes of lung function including the categorical outcome of COPD. The FBAT is robust against the effects of population stratification and given that it can be applied to any family structure regardless of missing parental data this makes it particularly useful for genetic association analysis of late-onset diseases, such as COPD (21). Seersholm and coworkers (22) found that PiMZ individuals have an increased risk of hospitalization with obstructive lung disease if they are first-degree relatives of PiZ index cases. This indicates that genetic and environmental factors likely interact to substantiate this increased risk. Using the FBAT statistic and stratifying subjects by ever- versus never-smoking status we were able to demonstrate that ever-smoking PiMZ heterozygotes had reduced lung function and increased risk of COPD. Using the cross-product term, $Pi \times$ ever-smoking, we demonstrated that a gene-by-smoking interaction exists in potentiating the risk of COPD in PiMZ heterozygotes.

In reporting results of our analyses as continuous outcomes, we have shown that PiMZ individuals have more airflow obstruction than PiMM individuals, particularly if they smoked, not necessarily that PiMZ individuals have more COPD. By using strict spirometric criteria for the diagnosis of COPD (post-BD FEV₁/FVC, <0.7; post-BD FEV₁, <80 % predicted) in our categorical analysis we substantiated an increased risk for COPD in PiMZ individuals. In our study, PiMZ heterozygosity was associated with an increased OR for COPD (OR 5.18; 95% CI, 1.27–21.15). In a meta-analysis by Hersh and coworkers (9), the compound OR for COPD in PiMZ heterozygotes was estimated at 2.31 (95% CI, 1.6–3.4). The magnitude of the effect estimate was lower in studies that adjusted for cigarette smoking (OR, 1.61; 95% CI, 0.92–2.81) than in those studies that did not (OR, 2.73; 95% CI, 1.86–4.01) (9). When we stratified our patients according to ever-smoking

Table 4: Family-based Analysis of Association between PiMZ Heterozygosity and Post-bronchodilator FEV₁/FVC Ratio, FEV₁ (Liters), FEV₁ (% Predicted), and COPD in Nonprobands

Characteristic	Covariates	All Relatives	Ever-Smoking Relatives*	Never-Smoking Relatives*	Direction of Effect [†]
Post-bronchodilator FEV ₁ /FVC ratio	Age, sex, height, ever-smoking status, and pack-years	0.0003	0.006	0.025	Decrease
FEV ₁ , L	Age, sex, height, ever-smoking status, and pack-years	0.043	0.003	ns	Decrease
FEV ₁ , % predicted	Ever-smoking status and pack-years	0.028	0.003	ns	Decrease
FEF _{25–75} , L/s	Age, sex, height, ever-smoking status, and pack-years	0.002	0.006	ns	Decrease
FEF _{25–75} , % predicted	Ever-smoking status and pack-years	0.006	0.020	ns	Decrease
COPD	Age, sex, ever-smoking status, and pack-years	0.0005	0.0004	ns	Increase

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; FEF_{25–75} = forced expiratory flow midexpiratory phase; ns = nonsignificant. *P* values are shown.

*Never smoker" was defined as less than 20 packs of cigarettes or 12 oz of tobacco in a lifetime or less than one cigarette a day for 1 year.

*Ever smoking status excluded from model.

[†]Direction of effect of Z allele on lung function phenotypes.

status, we found a higher OR for ever-smokers. The lack of a significant association with COPD in never-smoking relatives is unsurprising given that we show that PiMZ heterozygosity does not significantly affect spirometry in never-smoking relatives.

If cigarette smoke exposure interacts significantly with the PiMZ genotype, one would expect a different dose–response relationship between the pack-year smoking history and quantitative measures of lung function in PiMM and PiMZ individuals. PiMZ heterozygotes in the low cigarette smoke exposure (<20 pack-years) group had lower FEV₁/FVC ratio, FEV₁ (% predicted), and FEF_{25–75} than PiMM individuals. There was no significant difference between low-exposure PiMZ and high-exposure PiMM individuals, but there was a small but statistically significant difference between PiMM and PiMZ individuals in the high-exposure group and indicates that PiMZ individuals may be more susceptible to the effect of cigarette smoke. This emphasizes the importance of the gene–environmental interaction in the pathogenesis of PiMZ-related lung disease.

Cigarette smoke exposure has both loss of function and gain of function effects on M-AAT and Z-AAT, respectively (23). Oxidants in cigarette smoke and those spontaneously released by alveolar macrophages of cigarette smokers can inactivate the active site of AAT (notably methionine residue: 358), rendering it ineffective as an inhibitor of NE (24, 25). In

patients with normal levels of AAT, the AAT:NE association rate constant is significantly lower in smokers compared with nonsmokers and results in a twofold increase in the inhibition time of AAT for NE (26). Cigarette smoke increases Z-AAT polymerization resulting in further inactivation of its antiproteinase function and also acts to increase neutrophil influx into the lungs (27, 28). Polymerized Z-AAT increases the unfolded protein response and IL-8 production by monocytes, further potentiating the inflammatory burden (29). The inactivation of M-AAT on the one hand, and the enhanced polymerization of Z-AAT on the other hand, most likely act synergistically to lead to a greater risk of COPD in smoking PiMZ individuals.

However, up until now it was believed that as long as the level of AAT in blood was above a putative protective threshold of around 11 μ M, the level associated with SZ individuals, there was no excess risk of COPD (30) for those with the “milder” forms of AAT deficiency compared with MM individuals. The findings in this study strongly suggest otherwise, namely that a combination of Z protein, less efficient at inhibiting NE and prone to polymerization, and M protein inactivated by cigarette smoking is a significant combination of risk factors for COPD.

Early detection of the PiMZ genotype in smoking individuals is of absolute clinical importance. Current guidelines issued by both the World Health Organization and the American Thoracic Society/European

Respiratory Society advocate targeted detection of patients with AAT deficiency (30, 31). However, there are still long delays in the diagnosis of AAT deficiency (32, 33). The timely detection of at-risk PiMZ individuals underscores the important public health implications, not only for the health of the affected individual but also for reducing the significant economic burden of COPD on the population as a whole. Smoking initiation rates are significantly lower in adolescents diagnosed with AAT deficiency (34) and smokers who test AAT deficient are significantly more likely to report a 24-hour quit attempt (35). Results from this study affirm that the best intervention in preventing obstructive lung disease in PiMZ heterozygotes is abstinence from cigarette smoking.

The strength of our family-based study includes protection from population stratification; an ethnically homogenous population in Ireland; and adjustment for covariates including age, sex, and cigarette smoking. In contrast to many previous studies of PiMZ risk, we included ever- and never-smokers. By using never-smokers as a reference group and stratifying ever-smoking patients by levels of smoking exposure, we were able to demonstrate the effects of PiMZ heterozygosity differ by level of smoking exposure, showing a gene-by-environment interaction. One of the limitations of this study is the absence of quantitative analysis of chest CT scans, which is the most sensitive and specific

Table 5: Generalized Estimating Equations for the Risk of Chronic Obstructive Pulmonary Disease in All Relatives and in Ever-Smoking Relatives*

Covariate	All Relatives		Ever-Smokers	
	OR (95% CI)	P Value	OR (95% CI)	P Value
PiMZ genotype	5.18 (1.27–21.15)	0.02	10.65 (2.17–52.29)	0.003
Age	1.04 (1.01–1.08)	0.02	1.05 (1.00–1.10)	0.03
Sex	1.35 (0.39–4.67)	0.63	1.84 (0.47–7.17)	0.38
Ever-smoker	6.33 (0.74–53.89)	0.09	N/A	N/A
Pack-years of smoking	1.02 (0.99–1.05)	0.14	1.02 (0.99–1.06)	0.17

Definition of abbreviations: CI = confidence interval; N/A = not applicable; OR = odds ratio.

*Generalized estimating equation models were adjusted for familial correlation.

measure of emphysema *in vivo* (36). Given our small sample size we were unable to assess the effect of passive cigarette smoke exposure and occupational exposures on lung function in PiMZ individuals. Of note, we focused on PiMZ and PiMM individuals who were ascertained through a PiMZ sibling with COPD. Thus, additional genetic risk factors for COPD may be present within the families selected in our study. A much larger study is

required to detect more subtle changes in lung function in never-smoking PiMZ individuals who have significant environmental exposures including passive inhalation of cigarette smoke and occupational irritants (37) and also to determine whether PiMZ is a moderate risk factor in all PiMZ smokers or a large risk factor in a subset of PiMZ smokers because of other genetic or environmental determinants.

Conclusions

In summary, we have specifically shown a gene-by-environment interaction in PiMZ heterozygotes leading to an increased risk of COPD in ever-smokers. There was no increased risk because of PiMZ genotype in never-smokers. This challenges some of the underlying tenets of the protease-antiprotease theory of the pathogenesis of COPD and makes it imperative to diagnose PiMZ heterozygotes early to avoid smoking initiation or provide another reason for smoking cessation to reduce the risk of COPD. ■

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