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ORIGINAL ARTICLE

Alpha-1 Antitrypsin MZ Heterozygosity Is an Endotype of Chronic Obstructive Pulmonary Disease

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Abstract

Rationale: Multiple studies have demonstrated an increased risk of chronic obstructive pulmonary disease (COPD) in heterozygous carriers of the AAT (alpha-1 antitrypsin) Z allele. However, it is not known if MZ subjects with COPD are phenotypically different from noncarriers (MM genotype) with COPD.

Objectives: To assess if MZ subjects with COPD have different clinical features compared with MM subjects with COPD.

Methods: Genotypes of *SERPINA1* were ascertained by using whole-genome sequencing data in three independent studies. We compared outcomes between MM subjects with COPD and MZ subjects with COPD in each study and combined the results in a meta-analysis. We performed longitudinal and survival analyses to compare outcomes in MM and MZ subjects with COPD over time.

Measurements and Main Results: We included 290 MZ subjects with COPD and 6,184 MM subjects with COPD across the three studies. MZ subjects had a lower FEV_1% predicted and greater quantitative emphysema on chest computed tomography scans compared with MM subjects. In a meta-analysis, the FEV_1 was 3.9% lower (95% confidence interval [CI], −6.55% to −1.26%) and emphysema (the percentage of lung attenuation areas <-950 HU) was 4.14% greater (95% CI, 1.44% to 6.84%) in MZ subjects. We found one gene, *PGF* (placental growth factor), to be differentially expressed in lung tissue from one study between MZ subjects and MM subjects.

Conclusions: Carriers of the AAT Z allele (those who were MZ heterozygous) with COPD had lower lung function and more emphysema than MM subjects with COPD. Taken with the subtle differences in gene expression between the two groups, our findings suggest that MZ subjects represent an endotype of COPD.

Keywords: chronic obstructive pulmonary disease; alpha-1 antitrypsin; meta-analysis; RNA sequencing

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

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At a Glance Commentary

Scientific Knowledge on the Subject: It is not known whether MZ subjects with chronic obstructive pulmonary disease (COPD) are phenotypically different from noncarriers (MM genotype) with COPD.

What This Study Adds to the Field: Those who are MZ heterozygous and have COPD had lower lung function and more emphysema than MM subjects with COPD and also had subtle differences in gene expression. Our findings suggest that MZ subjects represent an endotype of COPD.

The debate surrounding the risk of chronic obstructive pulmonary disease (COPD) in heterozygous carriers of the protease inhibitor Z allele of *SERPINA1*, the gene encoding AAT (alpha-1 antitrypsin), has been considered repeatedly over the past 5 decades. Current evidence has clearly demonstrated an increased risk of COPD among MZ subjects who are cigarette smokers (1–3). The mechanism of this increased risk remains unclear, as serum AAT concentrations in heterozygous carriers (MZ genotype) are substantially greater than those in severe AAT deficiency (4). Accumulation of the Z-AAT protein has been shown to promote inflammation independently of the deficit in inhibition of neutrophil elastase (5). However, it is not known what effects, aside from an increased risk of COPD, the mutant allele and dysfunctional protein may have in MZ individuals with COPD.

Many heterozygous carriers are identified by AAT testing in patients presenting with established COPD or emphysema. However, this diagnosis currently has no effect on the evaluation or management of lung disease in MZ individuals, as their COPD has been assumed to be similar to COPD in noncarriers (MM genotype) (6). COPD in MZ individuals is poorly understood (7, 8). For example, whereas augmentation therapy is not currently indicated in MZ individuals with COPD, it has been prescribed in this population (9). Understanding the clinical and biological differences in COPD between individuals with the MZ genotype and individuals with the MM genotype could have implications for prognosis and management, as well as informing future clinical trials.

Leveraging whole-genome sequencing (WGS) data from the NHLBI Trans-Omics in Precision Medicine program, we were able to assemble a large population of MZ individuals with COPD across three independent studies (10). We sought to identify clinical and imaging characteristics, including spirometry and chest computed tomography (CT) measures, that differ between MZ individuals with COPD and MM individuals with COPD. We tested for differences in longitudinal outcomes and survival between the two genotypes. In addition, we explored the differences in lung tissue and whole-blood gene expression between the two genotypes, the associated pathways, and deconvoluted cell types. We hypothesized that MZ individuals with COPD would have clinical and biological features distinct from those of MM individuals with COPD, defining a COPD endotype.

Methods

Study Participants

Study participants from the COPDGene (Genetic Epidemiology of COPD), ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints), and LTRC (Lung Tissue Research Consortium) studies were included. Further details regarding recruitment have been previously published (11–13). Institutional review boards approved the studies at all participating institutions, and all participants

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Data sharing statement: COPDGene (Genetic Epidemiology of Chronic Obstructive Pulmonary Disease [COPD]), ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints), and LTRC (Lung Tissue Research Consortium) study data are available on the National Center for Biotechnology Information Database of Genotypes and Phenotypes under the accession numbers phs000179.v6.p2 and phs000765.v3.p2 (COPDGene), phs001252.v1.p1 (ECLIPSE), and phs001662.v1.p1 (LTRC).

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provided written, informed consent per study protocols. We defined COPD as an FEV_1/FVC ratio <0.70. Subjects with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage 1–4 COPD were included from the COPDGene and LTRC studies, whereas only subjects with GOLD stage 2–4 COPD were available from the ECLIPSE study. Subjects in the ECLIPSE study were followed for approximately 8 years, whereas subjects in the COPDGene study were asked to participate in 5-year (phase 2) and 10-year (phase 3) follow-up visits.

SERPINA1 Genotype Ascertainment

Genotypes were extracted for the *SERPINA1* Z allele (chr14:94378610; rs28929474), S allele (chr14:94380925; rs17580), F allele (chr14:94381049; rs28929470), and I allele (chr14:94383051; rs28931570) by using bcftools (version 1.10.2, samtools). Those who were heterozygous for the S, F, and I alleles and those who were homozygous for the Z, S, F, and I alleles were excluded. Those who were heterozygous for the Z allele were defined as having an MZ genotype. Individuals who were neither heterozygous nor homozygous for the Z, S, F, and I alleles were defined as having an MM genotype. We compared the WGS-ascertained genotype with the TaqMan genotype, the genome-wide association study (GWAS) genotype, and the isoelectric focusing genotype available in the COPDGene study; one subject with discordant genotypes was removed.

Statistical Analysis

We compared MZ individuals with COPD with MM individuals with COPD in terms of demographics, lung function, respiratory symptoms (available in the COPDGene and ECLIPSE studies), and chest CT scan measurements by using a Student's *t* test for continuous variables and a chi-square test for proportions. Statistical analyses were performed using R (version 4.0.0, R Foundation for Statistical Computing). Multivariable regression was performed, with models for FEV_1% predicted being adjusted for age, self-reported race, sex, current smoking status, smoking pack-years, and body mass index, and models for CT scan measures of emphysema were additionally adjusted for the scanner model. Sensitivity analyses included adjustment for the first five principal components for genetic ancestry in addition to the base models. Effect estimates were combined in a random effects meta-analysis. Mean differences (Hedges' g) and 95% confidence intervals (CIs) are reported. Meta-analysis and forest plotting were performed by using the meta package (14). Survival analyses that used parametric models with the Weibull distribution adjusted for covariates as above; the body mass index, airflow obstruction, dyspnea, and exercise (BODE) index; and Kaplan-Meier curves were obtained by using the survival and survminer packages (15, 16). Longitudinal models were adjusted for baseline values. Additional details on the methods for differential expression, pathway analysis, and cell deconvolution are provided in the online supplement. Briefly, we performed cell deconvolution on the bulk lung tissue gene expression data from the LTRC study to estimate the frequencies of immune-cell types, including neutrophils, B cells, T cells, and macrophages.

Results

Subject Characteristics

We identified 162 MZ individuals with COPD in the COPDGene study, 25 MZ individuals with COPD in the LTRC study, and 100 MZ individuals with COPD in the ECLIPSE study (Tables 1–3). In the COPDGene study, MZ individuals with COPD were older, more likely to be non-Hispanic White, and less likely to be current smokers. There was no difference in the proportion of female subjects or the lifetime smoking intensity in pack-years. In the LTRC study, MZ individuals with COPD were younger, but otherwise, there was no difference in sex, race, current smoking status, or smoking pack-years. In the ECLIPSE study, there was no difference in demographics between MZ individuals with COPD and MM individuals with COPD.

Lung Function and Respiratory Symptoms

In all three studies, MZ individuals with COPD had a lower FEV_1% predicted (COPDGene: MM, 57.73% vs. MZ, 52.59% [$P = 0.005$]; LTRC: MM, 54.05% vs. MZ, 38.09% [$P = 0.002$]; ECLIPSE: MM, 48.52% vs. MZ, 43.55% [$P = 0.002$]) and a lower FEV_1/FVC ratio. There was no difference in the $D_{L_{CO}}$% predicted in the COPDGene and LTRC studies (the $D_{L_{CO}}$ was not available in the ECLIPSE study). In the COPDGene study (Table 1), MZ subjects had a statistically significantly higher Modified Medical Research Council (MMRC) dyspnea score than MM subjects, but the difference did not meet the minimum clinically significant difference threshold (minimum clinically important difference for MMRC, ~1). There was no difference in the St. George's Respiratory Questionnaire (SGRQ) total score, 6-minute-walk distance (6MWD), BODE index, exacerbation frequency, or chronic bronchitis status (data not shown). The chi-square test for the trend showed a significant difference in GOLD stages between MZ individuals with COPD and MM individuals with COPD, with MZ individuals tending to be in more severe GOLD stages.

In the LTRC study (Table 2), PI MZ individuals had a higher SGRQ total score, but there was no difference in the 6MWD. The chi-square test for the trend likewise showed a difference in GOLD stages between the two groups.

In the ECLIPSE study (Table 3), MZ individuals had a higher BODE index and MMRC dyspnea score, but the difference in the MMRC dyspnea score similarly did not meet the threshold for a minimum clinically significant difference. There was no difference in the SGRQ total score, 6MWD, exacerbation frequency, or chronic bronchitis status. The chi-square test for the trend similarly showed a significant difference in GOLD stages between the two groups.

Chest CT Scan Characteristics

In all three studies, MZ individuals with COPD had a higher percentage of emphysema as defined by the percentage of voxels with CT attenuation ≤ -950 Hounsfield units at inspiratory CT (COPDGene: MM, 11.57% vs. MZ, 15.69% [$P < 0.001$]; LTRC: MM, 17.67% vs. MZ, 32.11% [$P < 0.001$]; ECLIPSE: MM, 17.03% vs. MZ, 22.67%, [$P < 0.001$]) and had lower CT attenuation at the 15th percentile of the lung CT histogram (Perc15) (Tables 1–3). There was no difference in CT airway measures, including the square root of the wall area of a theoretical airway with an internal perimeter 10 mm and the segmental airway wall thickness, in any of the three studies. Quantitative emphysema distribution metrics were available in the COPDGene study. We found that MZ individuals had a lower ratio of the upper lobe to lower lobe percentage of emphysema (difference in ratio, 0.46; 95% CI, 0.02–0.90;

Table 1. AAT MM versus MZ Subjects with COPD in the COPDGene Study

Characteristic	MM	MZ	*P*
n	3,964	165	—
Age, yr, mean (SD)	62.83 (8.67)	65.91 (8.01)	<0.001
Sex, F, *n* (%)	1,734 (43.7)	76 (46.1)	0.612
Non-Hispanic White, *n* (%)	2,985 (75.3)	153 (92.7)	<0.001
Current smoker, *n* (%)	1,789 (45.1)	37 (22.4)	<0.001
Smoking pack-years, mean (SD)	51.41 (27.21)	51.34 (27.32)	0.972
Post-bronchodilator FEV_1% predicted, mean (SD)	57.73 (22.74)	52.69 (22.58)	0.005
FEV_1/FVC, mean (SD)	0.53 (0.13)	0.49 (0.14)	<0.001
D_{LCO}% predicted, mean (SD)	65.36 (22.82)	64.05 (21.83)	0.654
BODE index, mean (SD)	3.02 (2.59)	3.42 (2.75)	0.066
6-min-walk distance, feet, mean (SD)	1,233.61 (407.66)	1,202.50 (410.96)	0.352
SGRQ total score, mean (SD)	36.61 (22.98)	39.19 (23.22)	0.158
MMRC dyspnea score, mean (SD)	1.88 (1.47)	2.16 (1.47)	0.019
GOLD stage, *n* (%)			
1	704 (17.8)	26 (15.8)	0.028
2	1,725 (43.5)	60 (36.4)	
3	1,017 (25.7)	45 (27.3)	
4	518 (13.1)	34 (20.6)	
Percentage of emphysema, mean (SD)	11.57 (12.15)	15.69 (14.12)	<0.001
Perc15, mean (SD)	−933.77 (28.37)	−942.47 (27.95)	<0.001
Pi10, mean (SD)	2.64 (0.60)	2.58 (0.51)	0.240
Segmental airway wall thickness, mean (SD)	1.13 (0.24)	1.12 (0.23)	0.840

Definition of abbreviations: AAT = alpha-1 antitrypsin; BODE = body mass index, airflow obstruction, dyspnea, and exercise; COPD = chronic obstructive pulmonary disease; COPDGene = Genetic Epidemiology of COPD; CT = computed tomography; GOLD = Global Initiative for Chronic Obstructive Lung Disease; MM = PI MM genotype; MMRC = Modified Medical Research Council; MZ = PI MZ genotype; Perc15 = CT attenuation at the 15th percentile of the lung CT histogram; Pi10 = square root of the wall area of a theoretical airway with an internal perimeter of 10 mm; SGRQ = St. George's Respiratory Questionnaire.
The percentage of emphysema is the percentage of lung voxels with CT attenuation ⩽−950 Hounsfield units at inspiratory CT.

Table 2. AAT MM versus MZ Subjects with COPD in the LTRC Study

Characteristic	MM	MZ	*P*
n	536	25	—
Age, yr, mean (SD)	65.14 (9.54)	60.56 (7.41)	0.018
Non-Hispanic White, *n* (%)	486 (90.7)	24 (96.0)	0.582
Sex, F, *n* (%)	239 (44.6)	13 (52.0)	0.601
Current smoker, *n* (%)	41 (8.1)	1 (4.2)	0.754
Smoking pack-years, mean (SD)	47.25 (33.18)	36.70 (23.23)	0.124
Prebronchodilator FEV_1% predicted, mean (SD)	54.05 (25.01)	38.09 (22.98)	0.002
Prebronchodilator FEV_1/FVC, mean (SD)	0.51 (0.14)	0.40 (0.17)	<0.001
D_{LCO}% predicted, mean (SD)	47.75 (18.63)	42.07 (21.83)	0.165
6-min-walk distance, feet, mean (SD)	1,114.29 (344.70)	992.28 (382.86)	0.125
SGRQ total score, mean (SD)	35.64 (24.11)	51.29 (24.32)	0.002
GOLD stage, *n* (%)			
1	114 (21.3)	1 (4.0)	0.002
2	219 (40.9)	9 (36.0)	
3	89 (16.6)	2 (8.0)	
4	114 (21.3)	13 (52.0)	
Percentage of emphysema, mean (SD)	17.67 (13.67)	32.11 (16.29)	<0.001
Perc15, mean (SD)	−945.14 (36.71)	−974.21 (24.00)	0.004
Average of right and left mainstem wall area percentage, mean (SD)	44.02 (5.04)	43.23 (4.46)	0.611
Average of right and left mainstem wall thickness, mean (SD)	2.18 (0.32)	2.09 (0.30)	0.415

Definition of abbreviations: AAT = alpha-1 antitrypsin; COPD = chronic obstructive pulmonary disease; CT = computed tomography; GOLD = Global Initiative for Chronic Obstructive Lung Disease; LTRC = Lung Tissue Research Consortium; MM = PI MM genotype; MZ = PI MZ genotype; Perc15 = CT attenuation at the 15th percentile of the lung CT histogram; SGRQ = St. George's Respiratory Questionnaire.
The percentage of emphysema is the percentage of lung voxels with CT attenuation ⩽−950 Hounsfield units at inspiratory CT.

Table 3. AAT MM versus MZ Subjects with COPD in the ECLIPSE Study

Characteristic	MM	MZ	*P*
n	1,684	100	—
Age, yr, mean (SD)	63.31 (7.15)	63.19 (6.40)	0.870
Sex, F, *n* (%)	587 (34.9)	32 (32.0)	0.635
Non-Hispanic White *n* (%)	1,647 (97.8)	96 (96.0)	0.409
Current smoker *n* (%)	610 (36.2)	27 (27.0)	0.078
Smoking pack-years, mean (SD)	48.35 (27.00)	47.21 (25.85)	0.682
Post-bronchodilator FEV_1% predicted, mean (SD)	48.52 (15.54)	43.55 (16.48)	0.002
FEV_1/FVC, mean (SD)	0.45 (0.11)	0.41 (0.11)	<0.001
BODE index, mean (SD)	3.13 (2.08)	3.81 (2.42)	0.002
6-min-walk distance, feet, mean (SD)	1,219.71 (398.89)	1,194.72 (404.09)	0.553
SGRQ total score, mean (SD)	48.34 (18.37)	49.82 (18.80)	0.437
MMRC dyspnea score, mean (SD)	1.67 (1.05)	1.89 (1.16)	0.043
GOLD stage, *n* (%)			
2	750 (44.5)	34 (34.0)	0.001
3	720 (42.8)	41 (41.0)	
4	214 (12.7)	25 (25.0)	
Percentage of emphysema, mean (SD)	17.03 (11.98)	22.67 (13.06)	<0.001
Perc15, mean (SD)	−949.93 (50.30)	−970.79 (45.73)	<0.001
Pi10, mean (SD)	4.40 (0.19)	4.38 (0.20)	0.325
Segmental wall area percentage, mean (SD)	65.77 (4.09)	64.91 (3.78)	0.048

Definition of abbreviations: AAT = alpha-1 antitrypsin; BODE = body mass index, airflow obstruction, dyspnea, and exercise; COPD = chronic obstructive pulmonary disease; CT = computed tomography; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; GOLD = Global Initiative for Chronic Obstructive Lung Disease; MM = PI MM genotype; MMRC = Modified Medical Research Council; MZ = PI MZ genotype; Perc15 = CT attenuation at the 15th percentile of the lung CT histogram; Pi10 = square root of the wall area of a theoretical airway with an internal perimeter of 10 mm; SGRQ = St. George's Respiratory Questionnaire.
The percentage of emphysema is the percentage of lung voxels with CT attenuation ⩽−950 Hounsfield units at inspiratory CT.

$P = 0.04$), implying a higher burden of lower lobe versus upper lobe disease in MZ individuals compared with MM individuals.

Multivariable Analysis and Meta-analysis

We performed multivariable analyses for the FEV_1% predicted and the two CT emphysema measures: the percentage of emphysema and Perc15 (Table 4). After adjustment for covariates, the MZ genotype was associated with a lower FEV_1% predicted in all three studies (COPDGene: −3.18%, $P = 0.069$; LTRC: −11.25%, $P = 0.022$; ECLIPSE: −3.46%, $P = 0.029$), but the effect size was not statistically significant in the COPDGene study. We performed meta-analysis on these results by using a random-effect model, which showed a mean difference of −3.9 (95% CI, −6.55 to −1.26) for MZ individuals compared with MM individuals, without significant heterogeneity between studies being shown (Figure 1A). In sensitivity analyses, the effect of having an MZ genotype on the FEV_1 persisted in the ECLIPSE and LTRC studies but was no longer significant in the COPDGene study

Table 4. Multivariable Analysis of Effect of MZ on Lung Function and Radiographic Emphysema

Measure by Study	β	SE	*P*
FEV_1% predicted			
LTRC	−11.25	4.89	0.0219
ECLIPSE	−3.46	1.58	0.0287
COPDGene	−3.18	1.75	0.0689
Percentage of emphysema			
LTRC	11.20	3.59	0.002
ECLIPSE	3.84	1.15	<0.001
COPDGene	2.66	0.832	0.0015
Perc15			
LTRC	−23.83	9.69	0.0145
ECLIPSE	−15.33	5.03	0.0024
COPDGene	−4.51	1.82	0.0133

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; COPDGene = Genetic Epidemiology of COPD; CT = computed tomography; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; LTRC = Lung Tissue Research Consortium; Perc15 = CT attenuation at the 15th percentile of the lung CT histogram.
Data are presented as linear regression effect estimates (β) and SEs. All regression models adjusted for age, race, sex, current smoking status, and smoking pack-years. Imaging outcome models additionally adjusted for body mass index and the scanner model. The percentage of emphysema is the percentage of lung voxels with CT attenuation ⩽−950 Hounsfield units at inspiratory CT.

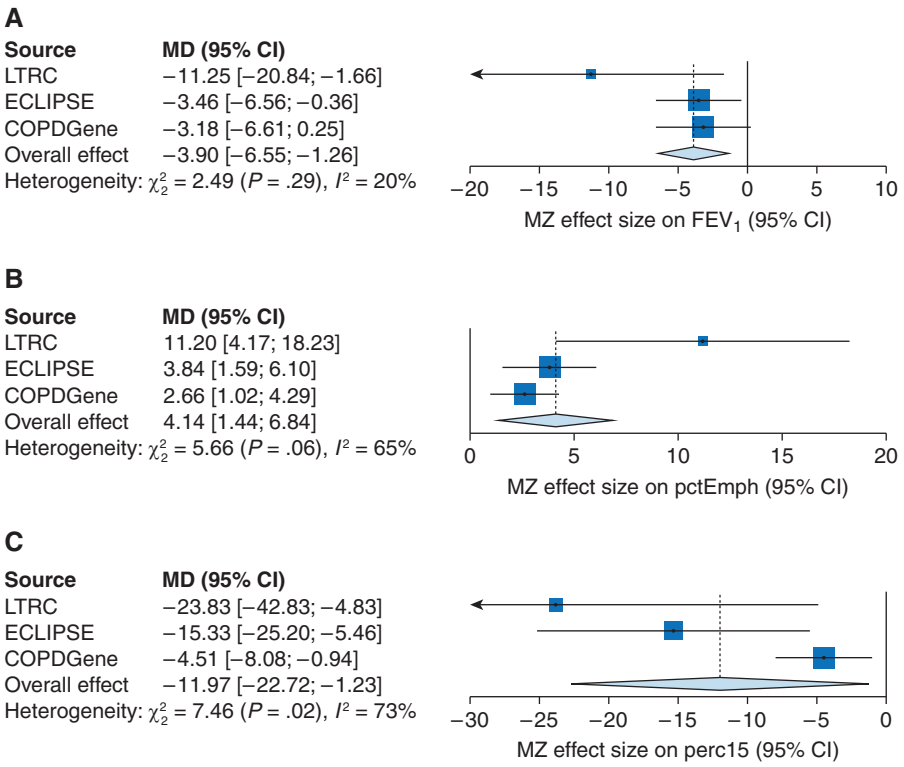

Figure 1. Meta-analysis of MZ effect sizes for lung function and computed tomographic emphysema. Meta-analyses of lung function and computed tomographic emphysema outcomes across the LTRC (Lung Tissue Research Consortium), ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints), and COPDGene (Genetic Epidemiology of COPD) studies were conducted. MDs and 95% CIs are shown. Heterogeneity was estimated by using the DerSimonian-Laird method. (*A*) Meta-analysis of effect sizes of MZ genotype on the FEV_1% predicted. (*B*) Meta-analysis of effect sizes of the MZ genotype on the pctEmph. (*C*) Meta-analysis of effect sizes of the MZ genotype on the perc15. CI = confidence interval; COPD = chronic obstructive pulmonary disease; MD = mean difference; pctEmph = percentage of emphysema; perc15 = computed tomographic attenuation at the 15th percentile of the lung computed tomographic histogram.

after adjusting for the first five principal components for genetic ancestry.

After adjustment for covariates, the MZ genotype was associated with a higher percentage of emphysema and a lower Perc15. We performed the meta-analysis as above and similarly found a consistently higher percentage of emphysema (4.14%; 95% CI, 1.44% to 6.84%) and a lower Perc15 (−11.97; 95% CI, −22.72 to −1.23) in subjects with the MZ genotype (Figures 1B and 1C). Notably, there was moderate heterogeneity between studies for the emphysema measures. In sensitivity analyses adjusting for genetic ancestry, the effect of the MZ genotype on the percentage of emphysema persisted across all three study cohorts (COPDGene: 2.39%, $P = 0.005$; LTRC: 9.84%, $P = 0.011$; ECLIPSE: 4.26%, $P = 0.001$).

Survival Analysis and Longitudinal Analysis

Survival data were available from the ECLIPSE and COPDGene studies, in which subjects were followed for approximately 8 years and 10 years, respectively. Kaplan-Meier curves are displayed in Figure 2. In the COPDGene study, there was lower survival rate in MZ individuals with COPD in the unadjusted model (Figure 2A). When using a parametric model fit to the Weibull distribution adjusted for covariates, the hazard ratio for MZ individuals with COPD compared with MM individuals with COPD was 0.88 (95% CI, 0.68–1.14; $P = 0.34$). Similarly, there was no difference in survival in the ECLIPSE study (hazard ratio, 1.43; 95% CI, 0.85–2.42; $P = 0.177$; Figure 2B).

For the COPDGene study, we tested the effect of the MZ genotype on the change in the FEV_1% predicted, percentage of emphysema, and Perc15. There were 82 MZ individuals with COPD with data available at phase 2 and 28 subjects with data available at phase 3, with 2,011 and 674 MM individuals with COPD at each phase, respectively. We found that MZ individuals had a greater decline in the FEV_1% predicted from baseline to phase 2 (*see* Table E1 in the online supplement) (−2.58%; 95% CI, −4.99 to −0.19; $P = 0.04$) and a greater change in the adjusted lung density from baseline to phase 2 (−3.14 g/L; 95% CI, −6.13 to −0.16 g/L; $P = 0.04$). However, after adjusting for the principal components of genetic ancestry, there was no difference in the change in the FEV_1% predicted or adjusted lung density from baseline to phase 2. Similarly, we found no difference in the change in the FEV_1% predicted or adjusted lung density from baseline to phase 3 between the two groups.

For the ECLIPSE study, we tested the effect of the MZ genotype on the annual rate of FEV_1 decline, which was previously estimated by using a random-effect model adjusted for age, sex, height, weight, and current smoking status (17). In univariate, multivariable, and sensitivity analyses adjusted for the principal components of genetic ancestry, there was no

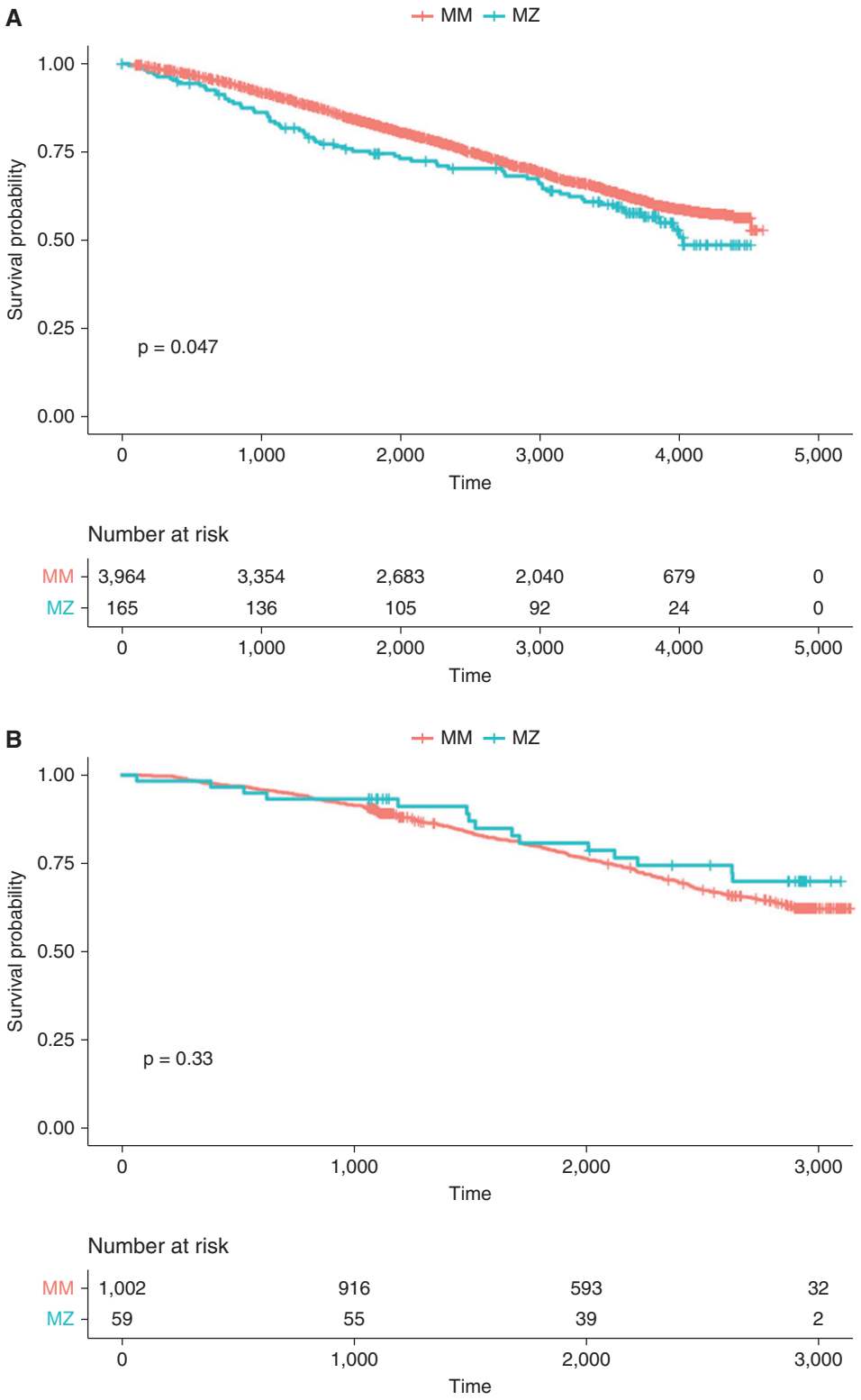

Figure 2. Survival in MM versus MZ subjects with chronic obstructive pulmonary disease (COPD) in the COPDGene (Genetic Epidemiology of COPD) and ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) studies. Kaplan-Meier curves demonstrating the survival probability in PI MM genotype subjects (orange lines) compared with PI MZ genotype subjects (teal lines) are shown. (*A*) Survival probability in the COPDGene study. (*B*) Survival probability in the ECLIPSE study.

difference in the rate of decline between the two groups.

Differential Expression, Pathway Analysis, and Cell Deconvolution

There were 54 MZ individuals with COPD with whole-blood RNA sequencing data available from phase 2 of the COPDGene study, and there were 22 MZ individuals with COPD with lung tissue RNA sequencing data available from the LTRC study, compared with 1,303 and 451 MM individuals with COPD, respectively. After filtering for low expression, there were 15,158 genes tested for differential expression in the COPDGene study and 15,548 genes tested for differential expression in the LTRC study. At a 5% false discovery rate (FDR), there were no genes differentially expressed in whole blood between MZ and MM individuals in the COPDGene study. There was one gene, *PGF* (placental growth factor), that was differentially expressed in lung tissue between MZ and MM individuals in the LTRC study (FDR and Bonferroni P value <0.05).

We selected the differentially expressed genes that reached nominal significance at $P < 0.001$ for pathway analysis. There were 17 genes that met this threshold in the COPDGene study and 44 genes that met the threshold in the LTRC study (Tables E2 and E3). There was no overlap between the two gene sets. By using the Molecular Signatures Database hallmark pathways as the reference pathway set, we found 11 pathways enriched in the differential expression gene set from the COPDGene study, including the unfolded protein response pathway, although none met the prespecified FDR of <5% (Figure 3A). We similarly found 11 pathways enriched in the differential expression gene set from the LTRC study. Two pathways, the peroxisome and inflammatory response pathways, overlapped between the two gene sets. The peroxisome pathway, in addition to the KRAS signaling–downregulated pathway, was significantly enriched (Figure 3B).

We performed cell-type deconvolution in the LTRC study by using gene expression profile references generated from publicly available lung single-cell RNA sequencing data (18). There were 50 different cell types available, but we focused on five immune-cell types previously associated with emphysema phenotypes (19). We found that there was a higher proportion of neutrophils (1.4% vs. 1.1%, $P = 0.036$) among MZ individuals with COPD than among MM individuals with COPD (Figure 3C). There was no difference

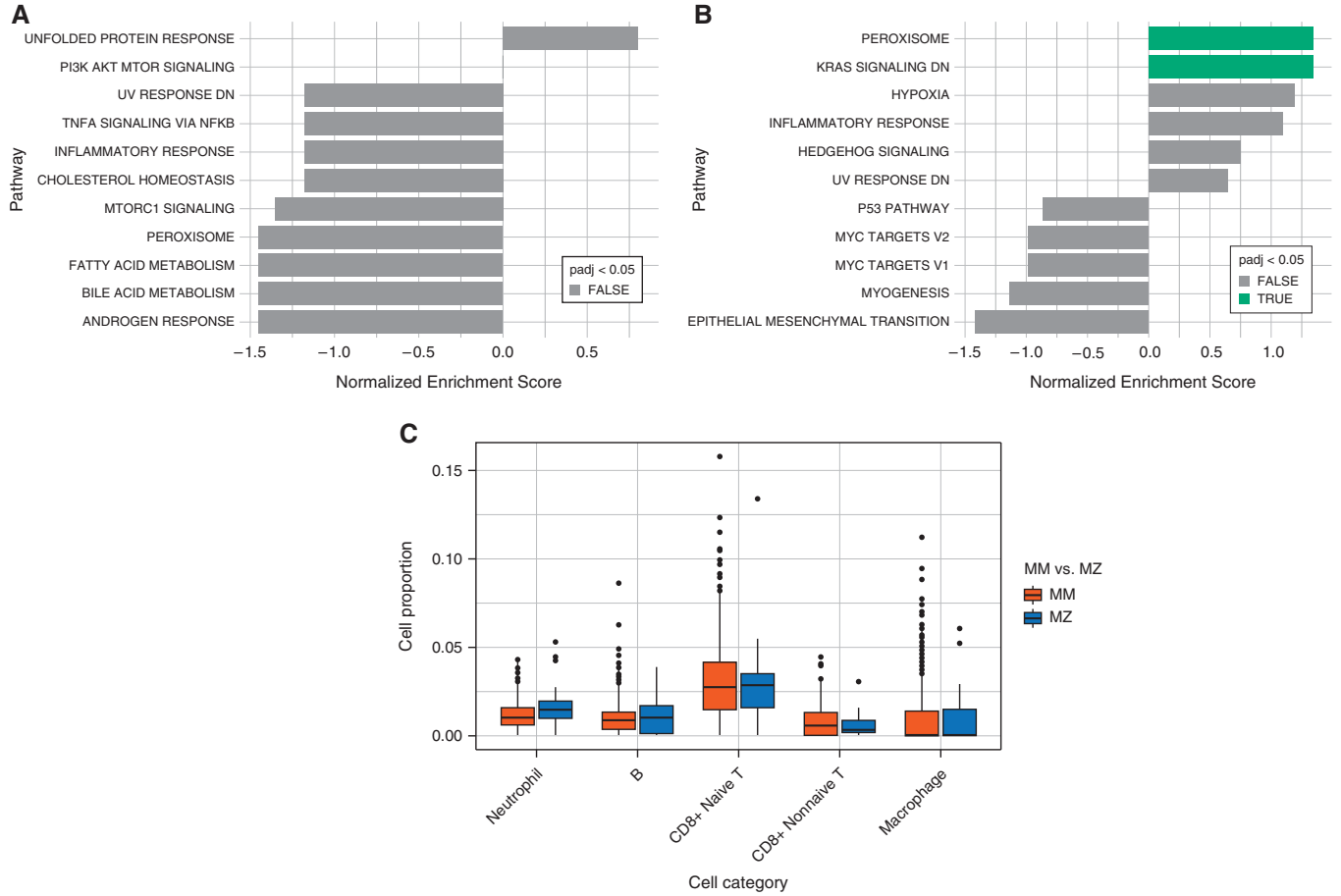

Figure 3. Pathway analysis of nominally significant differentially expressed genes and deconvoluted cell types between MM and MZ subjects with chronic obstructive pulmonary disease. Enrichment of Molecular Signatures Database hallmark pathways as determined by using nominally significant ($P < 0.001$) differentially expressed genes and selected cell-type deconvolution proportions are shown. (*A*) Pathways enriched in differentially expressed genes as determined by using RNA sequencing data from whole blood in the COPDGene (Genetic Epidemiology of Chronic Obstructive Pulmonary Disease) study. (*B*) Pathways enriched in differentially expressed genes as determined by using RNA sequencing data from lung tissue in the LTRC (Lung Tissue Research Consortium) study. (*C*) Selected deconvoluted cell-type proportions in the LTRC study, including neutrophils, B cells, $CD8^+$ naive T cells, $CD8^+$ nonnaive T cells, and macrophages. DN = down; padj = adjusted P value; UV = ultraviolet; V1 = version 1; V2 = version 2.

in the blood neutrophil counts in the COPDGene study.

Discussion

In this study, we report the results from the largest group of MZ individuals with COPD assembled to date. Compared with MM individuals with COPD, we found that MZ individuals with COPD had worse lung function and more emphysema on chest CT scans. Although we did not detect a statistically significant difference in mortality, we did observe that MZ individuals had a faster rate of decline in lung function in the COPDGene study. Finally, we compared gene expression in lung tissue and whole blood between the two groups. Although there was only one gene differentially expressed, we identified several pathways enriched in lung tissue gene expression. Taken together, these results establish Z allele heterozygosity as a clinically and biologically relevant COPD endotype. Our findings further add to the body of literature that clinicians may use to inform MZ individuals on the importance of smoking cessation and surveillance of AAT deficiency–associated comorbidities.

Several previous studies, including a meta-analysis from our group, have established the increased risk of COPD for MZ individuals who smoke cigarettes (1, 8, 20). However, to our knowledge, no prior study has investigated the differences between MZ and MM individuals with already established COPD. We have previously shown that, independent of the diagnosis of COPD, MZ individuals had lower lung function and more radiographic emphysema than MM individuals (2). A GWAS also from our group and another GWAS from SPIROMICS (Subpopulations and Intermediate Outcomes Measures in COPD Study) similarly showed the association of the Z allele with emphysema, which may have been driven by MZ individuals (21, 22). In the present study, we build on the results of these prior studies by focusing on these associations in MZ subjects with COPD. In addition, by leveraging WGS data from the NHLBI Trans-Omics in Precision Medicine program, we were able to combine results from three independent studies in a meta-analysis. Furthermore, by using WGS, we were able to identify and exclude individuals who were homozygous or heterozygous for other clinically important *SERPINA1* alleles, including the S, F, and I alleles.

We were also able to support the contention that MZ individuals with COPD have a worse trajectory in lung function over time. Several population-based studies have assessed longitudinal lung function decline in MZ individuals compared with MM individuals and have shown mixed results (23). In a study by Dahl and colleagues (24), the authors examined over 9,000 Danish adults, including 451 MZ individuals, and found that the MZ individuals had a slightly greater annual decrease in the FEV_1, but interestingly, the effect was only statistically significant in nonsmokers. Two other studies, one by Silva and colleagues (25) and another by Thun and colleagues (26), did not find a difference in the rate of decline between MZ and MM individuals. Therefore, we were able to show an important prognostic difference in MZ individuals with COPD. The $D_{L_{CO}}$ has been considered as an additional prognostic indicator in clinical trials for therapies in AAT deficiency, particularly as a surrogate for radiographic emphysema (27). However, although we did see a difference in radiographic emphysema between MZ individuals with COPD and MM individuals with COPD, we did not observe a difference in the $D_{L_{CO}}$.

The mechanism for COPD risk and increased severity in MZ individuals is not known. Although AAT concentrations are reduced in MZ individuals, they remain above the reportedly protective threshold, which was established on the basis of the difference in COPD risk between SS and SZ individuals (6). Emphysema in severe AAT deficiency has traditionally been ascribed to the lack of neutrophil elastase inhibition, but there is increasing evidence that the Z-AAT protein may directly lead to stress in the endoplasmic reticulum, promoting inflammation, the unfolded protein response, and ultimately, apoptosis (5). In addition, polymerization of AAT protein due to the Z allele has been shown to inactivate free AAT and promote inflammation (28). We performed differential expression analysis by using RNA sequencing from whole blood and lung tissue to identify potential mechanisms for the clinical and imaging differences between MZ COPD and MM COPD. We found no genes that were differentially expressed between MZ individuals with and MM individuals with COPD in whole blood and only one gene, *PGF*, that was differentially expressed between the two groups in lung tissue, with higher expression being shown in MZ subjects than in MM subjects. *PGF* expression has been previously associated with COPD. In a study by Cheng and colleagues (29), the authors compared serum and BAL-fluid *PGF* expression and found that *PGF* expression was higher in both fluid compartments in subjects with COPD than in both smoking and nonsmoking control subjects. In addition, the authors observed increased expression of *PGF* in cultured bronchial epithelial cells in response to proinflammatory cytokines. Although *PGF* expression has been well studied in gestation, the biological function of *PGF* after gestation and in adulthood is not known (30). Given the association with *VEGF* and other factors associated with angiogenesis, *PGF* may be associated with aberrant vascular remodeling involved in the pathogenesis of emphysema (31–33).

The peroxisome pathway was one of the pathways that was significantly enriched on the basis of results obtained from differential expression in lung tissue from MZ and MM individuals with COPD. Peroxisomes contribute to many crucial metabolic processes, some of which have been implicated in systemic COPD pathology (34). Specifically, PPARs (peroxisome proliferator–activated receptors) have been shown to be reduced in the skeletal muscle of subjects with COPD compared with healthy control subjects. In addition, PPAR-associated gene expression has been shown to be lower in cachectic subjects (35). Muscle loss and cachexia are important clinical measures that have both been associated with worse COPD outcomes (36, 37). Although we did not observe peroxisome pathway enrichment in whole-blood gene expression, peroxisome pathway enrichment in lung tissue gene expression could represent an unrecognized role of dysregulated oxidation leading to inflammation and lung tissue destruction in MZ individuals with COPD. Given the association of the peroxisome pathway with immune regulation, the higher proportion of neutrophils in lung tissue in MZ individuals with COPD could account for the observed enrichment of the peroxisome pathway (38). The association of increased neutrophils in MZ individuals has been reported previously in the sputum of healthy, nonsmoking MZ individuals (39).

Although we were able to demonstrate several strengths, including three independent study populations, longitudinal data, and gene expression data from multiple tissues, we recognize that there are several limitations to our current study. First, although the Z allele frequency is highest in populations of European ancestry, two of the three studies likely overrepresent European ancestries and underrepresent participants from other ancestries (40). Specifically, there are ancestry-specific variants that impact AAT independently of the Z allele, which we did not account for. In addition, there is significant diversity in pathogenic variation, in the form of rare and singleton variants, in *SERPINA1* that we did not capture through the exclusions of the common pathogenic alleles (8). Second, the difference in gene expression between the two groups was subtle, which was likely due to the reduced sample size of subjects with RNA sequencing data compared with the overall MZ and MM subject numbers. Third, the longitudinal analyses were limited by a reduction in the sample size, and between-cohort comparisons are difficult because of the differences in follow-up methods and subsequent analysis. Future studies that are more adequately powered to detect differences over time will be helpful for clarifying the MZ disease progression profile. Finally, as with any genetic disease, there is a risk of genetic discrimination, further consideration of which is beyond the scope of the present study.

In summary, our study reveals important clinical and biologic differences between MZ individuals with COPD and MM individuals with COPD. We show that, in addition to lower lung function and increased emphysema, MZ individuals have greater lung function decline over time. We also show subtle but important differences in gene expression involving *PGF*, the peroxisome pathway, and a higher proportion of neutrophils that may account for the observed clinical variability. Mechanistic studies will be required to clearly delineate the biological processes underlying the MZ COPD endotype and to identify whether those with MZ COPD could be a potential patient population for future clinical trials of therapies for AAT deficiency. ■

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