Alpha-1 Antitrypsin MZ Heterozygosity is a Clinical and Biologic Endotype of Chronic Obstructive Pulmonary Disease

Auyon J. Ghosh[1,2], Brian D. Hobbs[1,2,3], Matthew Moll[1,2], Aabida Saferali[1], Adel El-Boueiz[1,2,3], Jeong H. Yun[1,2,3], Frank Sciurba[4], Lucas Barwick[5], Andrew H. Limper[6], Kevin Flaherty[7], Gerard Criner[8], Kevin K. Brown[9], Robert Wise[10], Fernando J. Martinez[11], David Lomas[12], Peter J. Castaldi[1,3], Vincent J. Carey[1,3], Dawn L. DeMeo[1,2,3], Michael H. Cho[1,2,3], Edwin K. Silverman[1,2,3], Craig P. Hersh[1,2,3]

[1]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA; [2]Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA; [3]Harvard Medical School, Boston, MA: [4]Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Pittsburgh, Pittsburgh, PA; [5]The Emmes Company, Rockville, MD; [6]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Mayo Clinic, Rochester, MN; [7]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Healthy System, Ann Arbor, MI; [8]Department of Thoracic Medicine and Surgery, Temple University, Philadelphia, PA; [9]Department of Medicine, National Jewish Health, Denver, CO; [10]Division of Pulmonary and Critical Care Medicine, Johns Hopkins School of Medicine, Baltimore, MD; [11]Division of Pulmonary and Critical Care Medicine, Department of Medicine, Weill Cornell Medicine, New York, NY; [12]UCL Respiratory Medicine, University College London, London, UK

Corresponding author:
Craig P. Hersh, MD, MPH
Channing Division of Network Medicine
Brigham and Women's Hospital
181 Longwood Avenue
Boston, MA 02115
617-525-0729
craig.hersh@channing.harvard.edu

Abstract

Rationale: Multiple studies have demonstrated an increased risk of chronic obstructive pulmonary disease (COPD) in heterozygous carriers of the alpha-1 antitrypsin Z allele. However, it is not known if MZ subjects with COPD are phenotypically different compared to non-carriers (MM genotype) with COPD. We hypothesized that MZ subjects with COPD have different clinical and gene expression features compared to MM subjects with COPD.

Methods: Genotypes of *SERPINA1*, the gene encoding alpha-1 antitrypsin, were ascertained from whole genome sequencing data in three independent cohorts. We compared clinical and chest CT scan imaging outcomes between MM and MZ subjects with COPD in each of the three cohorts and combined the results in a meta-analysis. We performed differential expression analysis between MM and MZ subjects using RNA sequencing on lung tissue and whole blood and examined the results with pathway enrichment.

Results: There were a total of 287 MZ subjects with COPD and 6,190 MM subjects with COPD between the three cohorts. MZ subjects with COPD had consistently lower FEV_1 % predicted and greater emphysema on chest CT scans compared to MM subjects with COPD. In a meta-analysis, adjusted FEV1 was 3.8% lower (95% CI -6.07, -1.53) and emphysema (at -950HU) was 3.7% greater (95% CI 1.52, 5.91) in MZ subjects. We found only one gene, *PGF*, differentially expressed in lung tissue between MZ subjects compared to MM subjects. Using the nominally significant differentially expressed genes, we found that the peroxisome and KRAS signaling downregulation pathways were enriched.

Conclusions: Alpha-1 antitrypsin MZ heterozygous carriers with COPD had lower lung function and more emphysema compared to 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.

Background

The debate surrounding the risk of chronic obstructive pulmonary disease (COPD) in heterozygous carriers of the protease inhibitor (PI) Z allele of *SERPINA1*, the gene encoding alpha-1 antitrypsin (AAT), has been debated in the literature over the past two decades. Current evidence has clearly demonstrated an increased risk of COPD, particularly in cigarette smokers(1–3). The mechanism of this increased risk remains unclear, as serum AAT levels in heterozygous carriers (PI MZ genotype) are usually above the established threshold for deficiency(4). Accumulation of the Z-AAT protein has been shown to promote inflammation, independent of the deficit in inhibition of neutrophil elastase(5). However, it is not known what biologic effects, including systemic and lung tissue-specific gene expression, the mutant allele and dysfunctional protein may have in affected individuals.

Many heterozygous carriers (PI MZ genotype) are identified by alpha-1 testing in patients presenting with established COPD or emphysema. However, this diagnosis 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 non-carriers (PI MM genotype)(6). COPD in MZ individuals is poorly understood(7). For example, while augmentation therapy is not currently indicated in MZ carriers with COPD, it is being increasingly prescribed in this population(8). Understanding the clinical and biologic differences in COPD between individuals with MZ and MM genotypes 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 (TOPMed) program, we were able to assemble a large population of MZ individuals with COPD across three independent cohorts(9). We sought to identify clinical characteristics, including spirometry and chest computed tomography (CT) measures, that differ between MZ and MM individuals with COPD. In addition, we explored the differences in lung tissue and whole blood gene expression between the two genotypes and the associated pathways. We hypothesized that MZ individuals with COPD with have distinct clinical and biologic features compared to MM individuals with COPD, defining a COPD endotype.

Methods

Study Participants

Study participants were obtained from the Genetic Epidemiology of COPD (COPDGene), Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE), and Lung Tissue Research Consortium (LTRC) studies. Further details regarding recruitment have been previously published(10–12). Institutional review boards approved the studies at all participating institutions and all patients provided written, informed consent per study protocols. We defined COPD as forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC) ratio < 0.70. Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage 1-4 subjects were included from COPDGene and LTRC, while only GOLD stage 2-4 subjects were available from ECLIPSE. Subjects in ECLIPSE were followed for approximately 3 years,

while subjects in COPDGene asked to participate in 5-year (Phase 2) and 10-year (Phase 3) follow up visits.

SERPINA1 Genotype Ascertainment

Whole genome sequencing (WGS) was performed to a median depth of 30X using DNA from blood, PCR-free library construction and Illumina HiSeq X technology. Genotypes were extracted for the Z allele (chr14:94378610; rs28929474), S allele (chr14:94380925; rs17580), F allele (chr14:94381049; rs28929470), and I allele (chr14:94383051; rs28931570) using bcftools (version 1.10.2). Heterozygotes for the S, F, and I alleles and homozygotes for the Z, S, F, and I alleles were excluded. Heterozygotes for the Z allele were defined as PI MZ genotype. Individuals who were neither heterozygous nor homozygous for the Z, S, F, and I alleles were defined as PI MM genotype. We compared WGS-ascertained genotype to Taqman genotyping available in COPDGene; 6 MZ subjects with discordant genotypes were removed.

Statistical Analysis

We compared PI MZ individuals with COPD to PI MM individuals by demographics, lung function, respiratory symptoms (available in COPDGene and ECLIPSE), and chest CT scan measurements, using Student's t test for continuous variables and Chi square test for proportions. Statistical analyses were performed using R (version 4.0.0). Multivariable regression was performed, with models for FEV_1 % predicted adjusted for age, race, sex, current smoking status, smoking pack-years, and BMI, and models for CT scan measures of emphysema were additionally adjusted for scanner model. Effect estimates were combined in a random effects meta-analysis, and heterogeneity was estimated using the DerSimonian-Laird method. Mean differences (Hedges' g) and 95% confidence intervals are reported. Meta-analysis and forest plots were performed using the meta package(13). Survival analysis, using Cox proportional hazard models adjusted for covariates as above and BODE index, and Kaplan-Meier curves were performed using the survival and survminer packages(14). Longitudinal models were adjusted for baseline outcome variable.

Differential Expression and Pathway Analysis

The methods of RNA data processing have been published previously(cite IPF/COPD overlap paper). Models were adjusted for age, race, sex, current smoking status, smoking pack-years, and library preparation batch. Differential expression using RNA sequencing data from whole blood in COPDGene was also adjusted for white blood cell count proportions. Surrogate variables were used to estimate other latent effects and included in the models as covariates. We used the Hallmark gene sets and pathways curated by the Molecular Signatures Database (MSigDB) as the reference for annotated gene sets. Multiple testing was controlled for with a false discovery rate (FDR) < 5%. We used the fgsea package to test for enrichment using differentially expressed genes with $p < 0.001$.

Results

Subject Characteristics

We identified 162 PI MZ individuals with COPD in COPDGene, 25 in LTRC, and 100 in ECLIPSE (Table 1-3). In COPDGene, PI 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 proportion of female subjects or lifetime smoking intensity in pack-years. In LTRC, PI MZ individuals with COPD were younger but otherwise there was no difference in sex, race, current smoking status, or smoking pack-years. In ECLIPSE, there was no difference in demographics between PI MZ and PI MM individuals with COPD.

Spirometry and Respiratory Symptoms

In all three study cohorts, PI MZ individuals with COPD had lower FEV_1 % predicted and FEV_1/FVC ratio. In COPDGene, PI MZ subjects had statistically significantly higher Modified Medical Research Council (MMRC) Dyspnea score compared to PI MM subjects, but the difference did not meet the minimum clinical difference threshold. There was no difference in St. George's Respiratory Questionnaire (SGRQ) total score, 6-minute walk distance (6MWD), or BODE index. The Chi square test for trend showed a significant difference in GOLD stage between PI MZ individuals with COPD and PI MM individuals with COPD.

In ECLIPSE, PI MZ individuals had higher BODE index and MMRC dyspnea score, but the difference in MMRC dyspnea score similarly did not meet the threshold for minimum clinical difference. There was no difference in SGRQ total score or 6MWD. The Chi square test for trend similarly showed a significant difference in GOLD stage between the two groups.

In LTRC, PI MZ individuals had higher SGRQ total score, but there was no difference in 6MWD. The Chi square test for trend likewise showed a difference in GOLD stage between the two groups.

Chest CT scan characteristics

In all three study cohorts, PI MZ individuals with COPD had higher percent emphysema, defined by percent of voxels with CT attenuation less than or equal to -950 Hounsfield units on inspiratory CT, and lower Perc 15, defined as CT attenuation at the 15^{th} percentile of the lung CT histogram (Tables 1-3). There was no difference in CT airway measures, including Pi10 and segmental airway wall thickness, in any of the three study cohorts.

Multivariable analysis and Meta-analysis

We performed multivariable analyses for FEV_1 % predicted and two CT emphysema measures, percent emphysema and Perc 15 (Table 4). After adjustment for covariates, PI MZ genotype was associated with lower FEV_1 % predicted in all three cohorts. The effect size was largest in LTRC, and similar between ECLIPSE and COPD, but the latter

was not statistically significant. We performed meta-analysis on these results using a random effects model, which showed a mean difference of -3.8 (95% CI -6.07, -1.53) for PI MZ individuals compared to PI MM individuals, without significant heterogeneity between studies (Figure 1A).

After adjustment, PI MZ genotype was associated with higher percent emphysema and lower Perc 15. We performed meta-analysis as above and similarly found consistently higher percent emphysema (3.71%, 95% CI 1.52, 5.91) and lower Perc 15 (-11.30, 95% CI -21.43, -1.17) in subjects with the PI MZ genotype (Figure 1B and 1C). Notably, there was moderate heterogeneity between studies for the emphysema measures.

Survival Analysis and Longitudinal Analysis

Survival data were available from ECLIPSE and COPDGene, where subjects were followed for approximately 3 years and 10 years, respectively. Kaplan-Meier curves are displayed in Figure 2. In COPDGene, while there was a trend toward lower survival in MZ individuals with COPD, the difference was not statistically significant (Figure 2A). Using a Cox proportional hazards model adjusted for covariates, the hazard ratio (HR) for MZ individuals with COPD compared to MM individuals with COPD was 1.14 (95% CI 0.87, 1.49; p = 0.31). Similarly, there was no difference in survival in ECLIPSE (HR 0.41, 95% CI 0.17, 1.01; p = 0.054; Figure 2B).

In COPDGene, we tested the effect of PI MZ genotype on change in FEV_1 % predicted, percent emphysema, and Perc 15. There were 81 MZ individuals with COPD with data available at Phase 2 and 28 subjects with data available at Phase 3, with 2011 and 674 MM individuals with COPD at each phase, respectively. We found that MZ individuals had greater decline in FEV_1 % predicted from baseline to Phase 2 (-2.53%, 95% CI -4.95, -0.11; p = 0.04) but there was no difference in change in percent emphysema from baseline to Phase 2. We found no difference in change in FEV_1 % predicted or percent emphysema from baseline to Phase 3 between the two groups.

In ECLIPSE, we tested the effect of PI MZ genotype on yearly rate of FEV_1 decline, estimated using a random effects model adjusted for age, sex, height, weight, and current smoking status(15). In both univariate and multivariable analyses, there was no difference in rate of decline between the two groups.

Differential Expression and Pathway Analysis

There were 54 MZ individuals with COPD with whole blood RNA sequencing data available from Phase 2 of COPDGene and there were 22 MZ individuals with COPD with lung tissue RNA sequencing data available from LTRC, compared to 1303 and 451 MM individuals with COPD, respectively. After filtering for low expression, there were 15,158 genes tested for differential expression in COPDGene and 15,548 genes tested in LTRC. At 5% FDR, there were no genes differentially expressed in whole blood between MZ and MM individuals in COPDGene. There was one gene, placental growth factor (*PGF*), that was differentially expressed in lung tissue between MZ and MM individuals in LTRC (FDR 5%).

We selected the differentially expressed genes that reached nominal significance of $p < 0.001$ for pathway analysis. There were 17 genes that met this threshold in COPDGene and 44 genes that met the threshold in LTRC (Supplemental Tables 1 and 2). There was no overlap between the two gene sets. Using the MSigDB Hallmark Pathways as the reference pathway set, we found 11 pathways enriched in the differential expression gene set from COPDGene, including the unfolded protein response pathway, though none were significantly enriched. We similarly found 11 pathways enriched in the differential expression gene set from LTRC. 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.

Discussion

In this study, we report the results from the largest group of PI MZ individuals with COPD assembled to date. Compared to PI MM individuals with COPD, we found that PI MZ individuals had worse lung function and more emphysema on chest CT scans. While we did not detect a statistically significant difference in mortality, we did observe that PI MZ individuals had faster decline in lung function. Finally, we compared gene expression in lung tissue and whole blood between the two groups. While 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.

Several previous studies, including a meta-analysis from our group, have established the increased risk of COPD for PI MZ individuals(16, 17). However, to our knowledge, no prior study has investigated the differences in PI MZ and PI MM individuals with already established COPD. We have previously shown that, independent of diagnosis of COPD, PI MZ individuals had lower lung function and more radiographic emphysema compared to PI MM individuals(2). A genome-wide association study also from our group similarly showed the association of PI MZ individuals and emphysema(18). In the present study, we build on the results of these prior studies by validating these associations in PI MZ subjects with COPD. In addition, by leveraging WGS from the NHLBI TOPMed Program, we were able combine results from three independent cohorts 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 S, F, and I alleles.

We were also able to identify that PI MZ individuals with COPD have a worse trajectory in lung function over time. Several population-based studies have assessed longitudinal lung function decline in PI MZ individuals compared to MM individuals with mixed results(19). In a study by Dahl et al, the authors examined over 9000 Danish adults, including 451 PI MZ individuals, and found the PI MZ individuals had slightly greater annual decrease in FEV_1, but interestingly the effect was only statistically significant in nonsmokers(20). Two other studies, one by Silva et al and another by Thun et al, did not find a difference in the rate of decline between PI MZ and PI MM individuals(21, 22). Therefore, we were able to show an important prognostic difference in PI MZ individuals with COPD.

The mechanism for COPD risk and increased severity in PI MZ individuals is not known. While AAT levels are reduced in PI MZ individuals, they remain above the protective threshold, established on the basis of the difference in COPD risk between PI SS and PI 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). We performed differential expression analysis using RNA sequencing from whole blood and lung tissue to identify potential mechanisms for the clinical and imaging differences between PI MZ and PI MM COPD. We found no genes differentially expressed between PI MZ and PI MM individuals with COPD in whole blood and only one gene, *PGF*, differentially expressed between the two groups in lung tissue, with higher expression in MZ subjects compared to MM. *PGF* expression has previously associated with COPD. In a study by Cheng et al, the authors compared serum and bronchoalveolar lavage fluid *PGF* expression and found that *PGF* expression was higher in both fluid compartments in subjects with COPD compared to both smoking and nonsmoking controls(23). In addition, the authors observed increased expression of *PGF* in cultured bronchial epithelial cells in response to pro-inflammatory cytokines. While *PGF* expression has been well studied in gestation, the biological function of *PGF* after gestation and in adulthood is not known(24). 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(25–27).

The peroxisome pathway was one of the pathways that was significantly enriched from the results obtained from differential expression in lung tissue from PI MZ and PI MM individuals with COPD. Peroxisomes contribute to many crucial metabolic processes, including fatty acid oxidation and free radical detoxification, some of which have been implicated in systemic COPD pathology(28). Specifically, peroxisome proliferator-activated receptors (PPARs) have been shown to be reduced in skeletal muscle of COPD subjects compared to healthy controls. In addition, PPAR-associated gene expression has been shown to be lower in cachectic subjects(29). Muscle loss and cachexia are important clinical measures that have both been associated with worse COPD outcomes(30, 31). While 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 PI MZ individuals with COPD.

While we were able to demonstrate several strengths, including multiple independent cohorts, longitudinal data, and gene expression data from multiple tissues, we recognize that there are several limitations to our current study. First, despite confidence in the WGS used to determine *SERPINA1* genotype, we were able to confirm genotype using traditional methods only in COPDGene, where the concordance rate was over 99%. Second, while the Z allele frequency is highest in populations of European ancestry, two of the three cohorts likely overrepresent European ancestries and underrepresent patients from other ancestries, thus limiting the generalizability of our results to non-European ancestral populations(32). Third, the difference in gene expression in between the two groups was subtle, likely due to the reduced sample size of subjects with RNA sequencing data compared to the overall PI MZ and PI MM

cohorts. Similarly, the longitudinal analyses were limited by reduction in sample size. Future studies that are more adequately powered to detect differences over time will be helpful to clarify the PI MZ disease progression profile.

In summary, our study reveals important clinical and biologic differences between PI MZ individuals with COPD compared to PI MM individuals with COPD. We show that, in addition to lower lung function and increased emphysema, PI MZ individuals have higher lung function decline over time. We also show subtle but important differences in gene expression involving *PGF* and the peroxisome pathway that may account for the observed clinical variability. Mechanistic studies will be required to clearly delineate the biologic processes underlying the MZ-COPD endotype and to identify potential targets for future clinical trials.

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