Risk of Lung Disease in PI MZ Heterozygotes

Current Status and Future Research Directions

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Abstract

The potential for increased chronic obstructive pulmonary disease (COPD) risk among PI MZ subjects was initially recognized decades ago. However, despite many studies of this topic, it has remained controversial whether such increased risk exists. Several recent studies in large populations strongly support increased risk for COPD among PI MZ subjects. This increased PI MZ risk will need to be understood in the context of other identified COPD genetic determinants and investigations of COPD phenotypic heterogeneity.

Keywords: alpha-1 antitrypsin deficiency; heterozygote risk; chronic obstructive pulmonary disease; genetics; association analysis

Supported by National Institutes of Health Grants R01 HL089856, P01 HL105339, R01 HL113264, P01 HL114501, and R01 HL111759 (E.K.S.).

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Ann Am Thorac Soc Vol 13, Supplement 4, pp S341–S345, Aug 2016

DOI: 10.1513/AnnalsATS.201507-437KV
Internet address: www.atsjournals.org

Severe alpha-1 antitrypsin (AAT) deficiency, caused by the inheritance of two deficiency alleles at the *SERPINA1* locus, is a proven genetic risk factor for chronic obstructive pulmonary disease (COPD) in a small percentage of patients with COPD. However, there has been ongoing controversy regarding the potential risk of lung disease in heterozygotes for a single deficiency allele, most commonly individuals with one normal (i.e., PI*M) and one abnormal (i.e., PI*Z) *SERPINA1* allele (i.e., PI MZ genotype). Because PI MZ heterozygotes are so much more common than PI ZZ individuals (approximately 1/30 vs. 1/2,500 in the United States), even a moderate increase in COPD risk among PI MZ subjects could have a major public health impact.

We review the history of the PI MZ controversy and recent research in this area. We also discuss progress in other aspects of COPD genetics research and in COPD subtyping, which could provide important insights into COPD risk for PI MZ individuals going forward.

History of the PI MZ Controversy

Soon after the discovery by Laurell and Eriksson that severe AAT deficiency (1), typically caused by homozygosity for the PI*Z allele, was associated with increased COPD risk, investigators tried to determine whether PI MZ heterozygotes were also more susceptible to COPD (2, 3). For example, Kueppers and colleagues reported an increased prevalence of PI MZ subjects among COPD cases compared with control subjects in 1969 (3). They compared the PI MZ frequency in 98 COPD cases to two control groups—one with 88 and the other with 100 subjects.

Many other case-control studies followed, most with small sample sizes. Hersh and colleagues performed a metaanalysis of these case-control studies, and they found an increased risk for COPD among PI MZ subjects (4). However, they also found that population-based studies of pulmonary function values have usually reported similar FEV_1 values in PI MZ and PI MM subjects. It was difficult to reconcile the results from these two different types of studies, and the risk for lung disease in PI MZ subjects remained unclear.

In retrospect, many of these early studies suffered from recurring challenges of the complex disease genetics "candidate gene era" of the 1970s to early 2000s. During this era, more than 70 candidate genes were studied in COPD case-control genetic association studies, most with inconsistent results (5, 6). The inconsistency of these results led to some compelling lessons from the candidate gene era. First, large sample sizes are required for adequately powered genetic association studies. Second, analytical quality control approaches are required, including assessment of Hardy-Weinberg equilibrium (appropriate genotype distributions based on allele frequencies) in control subjects and adjustment for potential differences in genetic ancestry (population stratification) between case and control subjects. Stringent levels of statistical significance are required to adjust for multiple statistical testing. For

GWAS, statistical significance levels of P less than 5×10^{-8} are generally accepted to demonstrate genome-wide significance. The appropriate significance level for candidate gene studies is debatable, but because many candidate gene studies can be performed in the same study population, using the genome-wide significance threshold is not unreasonable. Finally, replication of association results in multiple cohorts is essential. None of the PI MZ studies from the candidate gene era fulfilled all of these quality-control criteria.

Recent Studies of PI MZ Risk for Chronic Obstructive Pulmonary Disease

Since the Hersh metaanalysis was published in 2004, several other key studies of PI MZ risk for COPD have been published (Table 1). Sørheim and colleagues assessed PI MZ risk in two well-powered study populations (7). The GenKOLS study from Bergen, Norway included 834 COPD cases and 835 smoking control subjects with normal spirometry; a total of 78 PI MZ subjects were identified. The International COPD Genetics Network (ICGN) was a family-based study including 984 COPD probands and 1,723 relatives; 115 PI MZ subjects were found. Phenotypes analyzed for genetic association included post-bronchodilator spirometric measures of FEV_1 and FEV_1/FVC and densitometric measures of emphysema at −950 HU using chest computed tomography (CT). Multiple linear regression was performed to assess the impact of PI MZ genotype on these COPD-related phenotypes, adjusting for relevant covariates (including familial correlations in the ICGN).

The FEV_1/FVC values in PI MZ subjects were lower than PI MM subjects in both the GenKOLS and ICGN populations at nominal levels of statistical significance ($P < 0.05$), but the FEV_1 values were not significantly different. CT emphysema values were lower in PI MZ subjects in GenKOLS but not in ICGN. Of interest, among subjects with less intensive smoking histories (<20 pack-years), PI MZ subjects had greater densitometric emphysema than PI MM subjects in both the GenKOLS and ICGN populations. These findings are suggestive of an increased risk of emphysema in a susceptible subset of PI MZ subjects after moderate smoke exposure; however, the small numbers of PI MZ subjects in these low pack-years groups limits the certainty of these results. Moreover, although the sample sizes in the GenKOLS and ICGN studies were reasonable, the rarity of the Z allele led to the inclusion of a modest number of PI MZ subjects, likely reducing power to detect significant genetic associations.

Molloy and colleagues recently leveraged a robust family-based study design to assess PI MZ risk for COPD (8). They identified 51 PI MZ subjects with moderate to severe COPD (Global Initiative for Chronic Obstructive Lung Disease spirometry grades 2–4) in Ireland, and they enrolled all available first-degree relatives of these COPD probands. They excluded the PI MZ probands from the analysis, focusing on the comparisons between 89 PI MZ relatives and 99 PI MM relatives. The PI MZ relatives had significantly lower FEV_1% predicted and FEV_1/FVC. However, these differences were driven by the current or ex-smokers; nonsmoking PI MZ subjects were not at increased risk for reduced spirometric values. A significant genotype-by-smoking interaction was confirmed using family-based association testing. Because these families were ascertained through PI MZ probands with COPD, they were potentially enriched for other genetic and environmental determinants of COPD.

The Sørheim and Molloy studies have strengthened the case for PI MZ risk for COPD—at least in current or ex-smokers. Recent results from the Genetic Epidemiology of COPD (COPDGene) (9) and Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS) (10) studies, published thus far only as abstracts from American Thoracic Society meetings, lend further credence to this increased risk. If the final published analyses from these additional large studies confirm their initial findings, the evidence for increased risk for COPD in PI MZ subjects will arguably be conclusive.

Progress in Chronic Obstructive Pulmonary Disease Genetics and Implications for PI MZ Risk

After the trials and tribulations of the candidate gene era in COPD genetics, the arrival of GWAS provided a welcome source of more consistent genetic association results for COPD (11). As noted above, to overcome the multiple statistical testing adjustments in GWAS, stringent levels of statistical significance (typically

Table 1. Key recent studies of PI MZ lung disease risk

Study Population	Study Design	Main Findings	Reference
GenKOLS (834 COPD cases and 835 smoking control subjects)	Case-control	Reduced FEV_1/FVC in PI MZ subjects Increased CT emphysema in PI MZ subjects	Sørheim et al. (2010) (7)
International COPD Genetics Network (984 probands with COPD and 1,723 relatives)	Nuclear families	Reduced FEV_1/FVC in PI MZ subjects Increased CT emphysema in PI MZ subjects with <20 pack-years	Sørheim et al. (2010) (7)
Irish families ascertained through a PI MZ proband with COPD (99 PI MM siblings and 89 PI MZ siblings)	Nuclear families	Reduced FEV_1 and FEV_1/FVC in PI MZ siblings No increased risk in nonsmoking PI MZ siblings Genotype-by-environment interaction between PI type and smoking	Molloy (2014) (8)

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; CT = computed tomography.

$P < 5 \times 10^{-8}$) are required. Large study populations, with metaanalysis of multiple studies, are usually used.

The COPD GWAS era began with the identification of the chromosome 15q25 region (containing genes encoding several components of the nicotinic acetylcholine receptor as well as the *IREB2* gene) (12) and the *HHIP* (hedgehog interacting protein) (13) region in 2009. Subsequently, the *FAM13A* and *RIN3* regions were associated with moderate to severe COPD (14, 15). Despite the smaller number of subjects with severe or very severe COPD, these more profoundly affected individuals may be enriched for COPD genetic determinants; Cho and colleagues found genome-wide significant associations of the *TGFB2* and *MMP12* regions with severe COPD (15).

In addition to these GWAS studies of COPD affection status, very large GWAS of spirometric phenotypes have identified more than 20 genetic determinants of lung function levels in the general population (16–18). Of interest, a substantial number of these lung function GWAS associations, including *HHIP*, *FAM13A*, and *TGFB2*, have also been associated with COPD.

GWAS of chest CT imaging phenotypes have also been performed. In a metaanalysis of the COPDGene, Bergen, Norway (GenKOLS), ECLIPSE, and NETT studies, Cho and colleagues identified multiple genome-wide significant associations for emphysema based on low attenuation areas at −950 HU (19). These significant associations included some previously reported COPD GWAS regions (e.g., *HHIP*, chrom 15q25, and *AGER*) and some novel regions of association as well (e.g., *DLC1*). Of interest, this emphysema GWAS also found a genome-wide significant association near the *SERPINA1* locus. Although the strongest evidence for association in the initial GWAS was located in the nearby *SERPINA10* gene, adjusting for the PI*Z allele attenuated this association signal. Because individuals with severe AAT deficiency (PI ZZ) were excluded from this GWAS analysis, this emphysema association is presumably being driven, at least in part, by PI MZ subjects in COPDGene.

Although densitometric emphysema measures provide valuable phenotypes for COPD assessment, they do not capture emphysema patterns effectively. Castaldi and colleagues performed automated texture-based assessment of emphysema patterns, such as centrilobular and panlobular emphysema, in the COPDGene population (20). Subsequent GWAS analysis identified both previously reported COPD GWAS associations (e.g., *MMP12*, *TGFB2*, and chrom 15q25 for moderate centrilobular emphysema) and novel associations (e.g., *VWA8* for panlobular emphysema). These local histogram emphysema measures have not yet been reported in severe AAT deficiency or PI MZ heterozygotes.

In parallel with these gene-finding efforts, the heterogeneity of COPD has been explored using multiple approaches. Because COPD is likely a heterogeneous syndrome rather than a single disease, the identification of subtypes—groups of subjects with different biological mechanisms of disease—could lead to improvements in diagnosis, prognosis, and treatment. Approaches to identify COPD subtypes have included imaging assessments (21), clinical assessments (22), and machine learning approaches (23). For example, Castaldi and colleagues applied K-means clustering to four COPD-related phenotypes in COPDGene: FEV_1% predicted, emphysema at −950 HU, emphysema distribution (upper vs. lower lung zones), and airway wall area. They found evidence for four clusters: resistant smokers, mild upper-lobe–predominant emphysema, airway-predominant COPD, and severe destructive emphysema (23). Of note, differences in genetic association with several known COPD GWAS genes were found with these clusters, including some stronger genetic effects than were observed in the entire study population. These results suggest that the identification of phenotypically more homogeneous subsets of subjects with COPD could lead to greater genetic influences. Such approaches have not yet been applied to severe AATD or PI MZ heterozygotes, but they could provide important insights into the variable development of lung disease for those individuals.

Unresolved Questions and Future Research Directions

Although an increased risk of PI MZ subjects for COPD is becoming more certain, multiple key questions remain. For example, it is unclear whether all PI MZ subjects are at similar COPD risk. An increased risk for COPD has been shown most clearly in current or ex-smokers; it remains unclear whether nonsmoking PI MZ subjects are at any increased risk. Other environmental factors, including occupational exposures (24), could also modify PI MZ COPD risk. The impact of other genetic determinants needs to be clarified. Genetic determinants of COPD in PI MM individuals could also influence COPD risk in PI MZ subjects; unique genetic determinants might modify COPD risk in PI MZ subjects as well. Male sex, childhood asthma, and pneumonia have been suggested as potential modifiers of lung disease risk in PI ZZ individuals (25); it will be important to determine whether these factors also influence COPD risk in PI MZ subjects. A recent report by Donato and colleagues suggested that there may be substantial variability in the relative amounts of Z and M protein in PI MZ subjects (26). They used a liquid chromatography/tandem mass spectrometry method to quantify the amounts of M protein and total AAT protein in serum samples; the Z protein level was calculated as the difference between total and M AAT protein levels. If persistent differences in the amounts of these isoforms of the AAT protein exist in different PI MZ subjects, it could influence their risk of COPD; further study will be required.

Studies of COPD subtypes in PI MZ subjects should be performed. It will be important to determine whether PI MZ subjects are at increased risk for emphysema, airway disease, or both component processes of COPD. Imaging, clinical, and machine learning approaches to subtyping COPD in PI MZ subjects will be essential.

The biological mechanisms for increased COPD risk in PI MZ risk will also need to be clarified. Is this increased risk related solely to the moderate reduction in circulating AAT levels, or are proinflammatory polymers of the Z protein, described by Mahadeva, Lomas and colleagues (27), key determinants of COPD pathogenesis in PI MZ subjects?

The clinical impact of demonstrating increased COPD risk in PI MZ subjects will need to be explored. Although screening with serum AAT protein levels identifies subjects with severe AAT deficiency (28), it is not an optimal approach to identify all PI MZ subjects. Thus, greater use of genotyping for *SERPINA1* deficiency alleles or protein phenotyping by isoelectric focusing may need to be considered. Although PI MZ smokers are at increased risk for COPD, it is unclear whether more intensive clinical

monitoring of such subjects will lead to improved outcomes. AAT augmentation therapy remains inappropriate for PI MZ subjects (29), but intensive counseling about the importance of avoiding smoking should be performed.

Conclusions

The evidence is mounting that PI MZ smokers are at increased risk for COPD. Key research questions concerning the impact of other genetic and environmental risk factors will need to be addressed to estimate this increased risk accurately in individual subjects. AAT augmentation therapy should not be used in PI MZ subjects (29); the current evidence demonstrating an increased COPD risk in PI MZ subjects does not impact this recommendation. However, intensive counseling to avoid starting smoking in nonsmokers, or to assist in smoking cessation for current smokers, should be performed. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The author thanks Dawn DeMeo, Craig Hersh, Peter Castaldi, and Michael Cho for helpful comments on this manuscript.

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