Clarifying the Risk of Lung Disease in SZ α1-antitrypsin Deficiency

Authors:

Alessandro N Franciosi PhD[1,3], Brian D Hobbs MD[2], Oliver J McElvaney PhD[1,3], Kevin Molloy MD[1,3], Craig Hersh MD[2], Louise Clarke[5], Cedric Gunaratnam MD[1,3], Edwin K Silverman MD PhD[2], Tomás P Carroll PhD[1,4], Noel G McElvaney MD DSc[1,3]

Institutions:

[1]Irish Centre for Genetic Lung Disease, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin.

[2]Channing Division of Network Medicine and Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Boston, MA, 02115, USA.

[3]Department of Medicine, Beaumont Hospital, Dublin, Ireland.

[4]Alpha-1 Foundation Ireland, Royal College of Surgeons in Ireland, Dublin, Ireland.

[5]Department of Pulmonary Physiology, Beaumont Hospital, Dublin, Ireland.

Corresponding author:

Dr Tomás Carroll,

Alpha-1 Foundation Ireland,

Royal College of Surgeons in Ireland,

Beaumont Hospital,

Dublin 9, Ireland.

Email: tcarroll@rcsi.ie

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Phone: +353(1)8093876

Author Contributions:

Alessandro N Franciosi is the lead author, field investigator, performed all spirometry and sample collection in the family study, designed and performed the retrospective registry analysis, performed main statistical analysis, authored and edited the manuscript.

Brian D Hobbs performed the GMMAT analysis, is the lead statistical supervisor, consulted on study design and co-authored and edited the manuscript.

Oliver J McElvaney performed the plasma anti-neutrophil-elastase measurements and edited the final manuscript.

Kevin Molloy consulted on study design and execution, co-authored and edited the manuscript.

Craig Hersh consulted on study design, performed internal statistical review, co-authored and edited the manuscript.

Louise Clarke performed all pulmonary function tests, data collection and output for the retrospective registry analysis, and edited the manuscript.

Cedric Gunaratnam consulted on study design, assisted in patient identification and enrollment, and performed internal manuscript review and editing.

Edwin K Silverman consulted on study design and statistical methodology, performed internal statistical review and edited the final manuscript.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Tomás P Carroll is the corresponding author and project co-supervisor, consulted on study design and patient identification, performed AAT phenotyping and AAT level validation, oversaw interrogation and data output from the National AATD Registry, co-authored and edited the final manuscript.

Noel G McElvaney is the senior author, designed the study, supervised all interim and final analyses, co-authored and edited the final manuscript.

Funding:

This study was funded by a research grant awarded by the US Alpha-1 Foundation.

Running Header:

Clarifying the risk of lung disease in SZ-AATD.

Descriptor:

9.3 – COPD: Clinical Phenotypes

Word count:

3302

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

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Abstract (word count 250)

Rationale: The ZZ-genotype of α1-antitrypsin deficiency (AATD) is associated with COPD, even amongst never-smokers. The SZ-genotype is also considered severe, yet its effect on lung health remains unclear.

Objectives: To determine the effect of SZ-AATD on spirometry compared to a normal-risk population and determine the effect of smoking cessation in this genotype.

Methods: We prospectively enrolled 166 related individuals, removing lung-index cases to reduce bias, and compared spirometry between 70 SZ and 46 MM/MS individuals (controls). The effect of AAT levels on outcomes was assessed in 82 SZs (including lung-indexes). Subsequently, we analyzed retrospective SZ registry data to determine the effect of smoking cessation on spirometry decline (n=60) and plasma anti-neutrophil-elastase (anti-NE) capacity (n=20).

Measurements and Main Results: No difference between SZ and control never-smokers was seen. Ever-smoking was associated with a lower FEV_1pp (-14.3%, p=0.0092), and FEV_1/FVC Ratio (-0.075, p=0.0041) in SZ-AATD. No association was found between AAT-level and outcomes for SZ-AATD. Longitudinal analysis of 60 SZs demonstrated that COPD at baseline, but not former-smoking or AAT levels, predicted greater spirometry decline. Finally, anti-NE capacity did not differ between former and never-smokers (p=0.67).

Conclusions: SZ never-smokers demonstrated no increased risk of COPD regardless of AAT level. Smoking interacts with SZ-AATD to significantly increase airflow obstruction. Former-smoking alone is not associated with greater spirometry decline in SZ-AATD, suggesting cessation

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

attenuates the obstructive process. We found no evidence that the putative-protective-threshold or AAT-levels predict risk within the SZ genotype, raising further doubts over the need for intravenous-AAT augmentation in this cohort.

Word count: 3302

Keywords: COPD, PiSZ, Phenotype, Genotype

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Introduction

α1-antitrypsin deficiency (AATD) remains the most common monogenic risk factor for Chronic Obstructive Pulmonary Disease (COPD). AAT is a 52kDa protease inhibitor, encoded for by the *SERPINA1* gene on chromosome 14. As the main inhibitor of neutrophil elastase (NE), AAT is crucial in maintaining protease/anti-protease homeostasis and structural integrity of the lung parenchyma.

The risk of COPD in AATD is believed to be increased in individuals with AAT levels below the "putative protective threshold" of 0.57g/L (11μM - hereinafter referred to as the "PPT") (1), though this theory remains unproven. Intravenous augmentation therapy with exogenous AAT aims to achieve nadir levels above the PPT and has been shown to slow lung density loss in severe AATD (2-4). Of the most common AATD genotypes, only the SZ genotype produces an AAT level which straddles the PPT (1, 5). Consequently, understanding SZ-AATD is crucial to clarifying the contribution of AAT levels to the risk of COPD and the role of augmentation therapy in this genotype(6).

ZZ-AATD is a major risk factor for COPD (7, 8). Recent studies also demonstrate an increased risk of COPD in MZ-AATD smokers (9, 10). The evidence in SZ-AATD remains conflicted, with meta-analysis results suggesting an increased risk for COPD (11) and cohort studies demonstrating no definitive increased risk of lung disease in SZ subjects (10, 12). Previous studies have compared SZ to ZZ-AATD and MM-COPD cohorts (13, 14), but no study has compared SZ individuals with normal-risk populations.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

We report the results of a prospective, family-based study assessing the risk of COPD in SZ-AATD. We explore the area further by analyzing longitudinal registry data to assess the effect of smoking cessation on lung function decline and the activity of AAT in plasma.

Methods

Study design and participants

In part 1 of the study, we performed a prospective, observational, family-based study to determine the impact of SZ-AATD on lung health. Ethical approval was obtained from the institutional medical ethics board at Beaumont Hospital (REC #16/69). Individuals diagnosed with SZ-AATD through the Irish National Targeted Detection Program were offered inclusion in the study, with those diagnosed due to lung disease classified as “lung-index”. Those diagnosed for other reasons were designated “non-lung-index”. All first-degree, full-biological relatives of index cases were invited to participate. Based on the statistically most likely parental genotypes of an SZ individual being MS and MZ (15), we predicted that enrolment of index cases parents and siblings would provide a “control” cohort of MM and MS genotypes and a study cohort of SZ individuals (see figure 1).

Inclusion required age over 30 years, capacity to give informed consent and first-degree full-biological relative status required for participating relatives. Individuals with known confounders to spirometry measurements were excluded (interstitial lung disease, pulmonary/lobar resection, neuromuscular disease). Visits were scheduled at least 4 weeks after any respiratory tract infections or exacerbation of COPD.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

The family study protocol included spirometry, phlebotomy for AAT typing, quantification of serum AAT level, blood eosinophil counts, serum Immunoglobulin-E levels (IgE), liver function tests and surrogate markers of synthetic liver function (INR/Albumin). Pedigrees and smoking histories were documented at the time of testing. Participants were invited to complete a modified version of the American Thoracic Society Division of Lung Disease (ATS-DLD) questionnaire(16), either during recruitment using a handheld tablet, or by email using the © 2019 QuestionPro Survey Software. All participants were recruited and tested by a single field investigator to reduce interobserver bias.

The MS genotype has previously been shown not to be associated with an increased risk of COPD (11) or to be associated with worse airflow and lung function decline amongst those with COPD (17) when compared to MM individuals. Therefore, to increase the power of our analysis, MM and MS genotype individuals were first compared, and then pooled and designated "control". Conversely, the MZ genotype precipitates airflow obstruction by way of an interaction with smoking (9, 10, 18). As such, MZ individuals were excluded from the final analysis.

In part 2 of the study we performed a retrospective longitudinal analysis of SZ lung function data extracted from the Irish National AATD Registry to examine the effect of smoking cessation on lung function in SZ-AATD. Inclusion required at least two spirometry measurements over a time period of at least 24 months and documented "never-smoker" or "former-smoker' status at start of follow up. Patients with a primary diagnosis of AATD-unrelated conditions associated with lung function decline (e.g interstital lug disease) were excluded.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Spirometry

In the family study (part 1) spirometry was performed before and after administration of salbutamol (albuterol) according to the American Thoracic Society standards (19) using the EasyOne™ Diagnostic spirometer (Model 2001–2S, NDD Medical Technologies, Zurich, Switzerland). Percentage predicted values were calculated using the European Respiratory Society "European Coal and Steel" reference equations (20). All spirometry outcomes assessed relate to post-bronchodilator values.

Longitudinal spirometry data (part 2) was "real world" data recorded by the pulmonary function laboratory of a single, tertiary referral hospital, at the Irish Center for Genetic Lung Disease, Beaumont Hospital, Ireland and captured in the Irish National AATD Registry. Diffusion capacity for carbon monoxide (DLCO) was performed in accordance with the 2017 ATS/ERS standards for single-breath carbon monoxide uptake in the lung (21).

Smoking history

"Never-smoker" was defined as a lifetime cigarette consumption of fewer than 20 packs of cigarettes (each pack equalling 20 cigarettes), or less than 12ounces of tobacco. Pack-years were calculated multiplying the average daily number of cigarettes consumed by the number of years smoked and dividing by 20 [(average cigarettes x day*years smoked)/20].

CT results

Where available, chest CT reports by hospital radiologists were reviewed for documented evidence of emphysema and confounding diagnoses such as interstitial lung disease.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

AAT typing and serum quantification

Concomitant C-reactive protein (CRP) levels were measured to identify spuriously elevated AAT levels attributable to an acute phase response (5, 22). AAT phenotype was determined by immunofixation of serum glycoforms via isoelectric focusing, performed using the Hydrasys electrophoresis platform (Sebia) and the Hydragel 18 A1AT Isofocusing kit (Sebia, Evry, France)(23). DNA was collected for genotyping in the event of inconclusive phenotyping. AAT levels were measured by turbidimetry and internal quality control of assay accuracy was performed by comparing serial dilutions of the World Health Organisation standard ("WHO International Standard, 1st International Standard For Alpha-1-Antitrypsin, Plasma-Derived", NIBSC code: 05/162)(24) to measured values on the Olympus AU5800 platform (Olympus Corporation, Japan).

Anti-NE capacity

Exogenous NE (Elastin Products) was incubated with a range of concentrations of SZ plasma using NE alone as a control. Samples were mixed with FRET substrate and fluorescence was recorded by spectrophotometry. NE activity was quantified by comparison with a NE standard curve of known NE activity. Anti-NE capacity was calculated via the percentage inhibition of NE from the plot of the percentage of remaining activity versus the plasma concentrations (see full method in supplementary appendix).

Statistical analysis

Statistical analysis was performed in RStudio Version 1.1.463 running R v3.5.2 (www.cran.r-project.com). Continuous data were validated for normality of distribution using the Shapiro-

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Wilk test. Normally and non-normally distributed data were analyzed by Student t test and Mann-Whitney U test respectively. Categorical variables were analyzed by Chi-square test. Models with percentage predicted (pp) spirometry results were adjusted for smoking history (ever-smoking and pack-years). Absolute spirometry results (in liters), FEV_1/FVC Ratio, forced expiratory times (FET) and categorical COPD (defined as FEV_1/FVC Ratio <0.7 in all analyses) were also adjusted for age, height, weight, sex (and for FET, the measured FVC). Analyses were adjusted for multiplicity using the Bonferroni correction.

In the family study, lung-index cases were excluded from the final case-control analyses to remove referral bias. In SZ-only analyses lung-index cases were included and index status was modelled as a fixed effect. The effect of the SZ genotype on spirometry was defined in never-smokers and ever-smokers using mixed model analyses examining the covariates of age, genotype, smoking and the PPT to assess for differences between categories. We fit a linear mixed-effects model with predictors and confounders as fixed effects and a kinship coefficient matrix to model relatedness as a random effect (25). The association of the SZ genotype with COPD in all persons and in ever-smokers was performed with the GMMAT R package (26) using a logistic mixed model adjusted for age, sex, pack-years of smoking, and familial relatedness (27).

In the longitudinal study of persons with SZ-AATD, a linear mixed-model regression analysis modelling subject ID and time as random effects (each subject with independent slope and intercept) was used to calculate age, sex, height and weight adjusted slopes for each spirometric parameter against time (using lmer in R). Individual slopes were extracted using the random.effects output of lmer (figure 3) and assessed for association with categorical fixed

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

effects (never/former-smoker, COPD at baseline, AAT levels above/below the PPT and baseline presence of emphysema) in multi-variable linear mixed models (glm in R).

Results

Between November 1st 2016 and January 30th 2019, we enrolled 166 participants from 44 SZ genotype-containing families comprising of 82 SZ, 27 MS, 32 MZ, 19 MM, 4 ZZ and 2 MI individuals. Lung-index cases (12 SZ, 4 MZ, 1 ZZ) were excluded from the final case-control analyses to remove referral bias. No significant difference in AAT levels was seen between lung-index and non-lung-index SZs (median 0.60g/L vs 0.61g/L, $P = 0.476$) (table E1).

The characteristics of MM, MS and MZ participants are included in the supplemental data (table E2 & E3). Other than AAT levels ($P < 0.001$) no significant differences in baseline demographic, physiologic or biochemical data were found. Furthermore, only the SZ cohort demonstrated an AAT range inclusive of levels both above and below the PPT (figure E1) with 40.6% of non-lung-index SZ subjects having AAT levels below the PPT.

The case-control population characteristics are summarized in table 1. No significant age-related difference in spirometry between control and SZ cohorts was noted in univariate analysis (figure E2). Among ever-smokers a significant difference in lung function outcomes was demonstrated between control and SZ cohorts in univariate regression analysis, with FEV_1pp and FEV_1/FVC Ratio both more negatively correlated with pack-years (figure 2).

In the final linear mixed model analyses, no significant difference was noted in spirometry between never-smoker control and SZ cohorts (table 2). Conversely, smoking exerted a significantly greater effect on FEV_1pp (-14.23%, 95% CI: -24.94 to -3.52 $P = 0.009$), FEV_1/FVC

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Ratio (-0.075, 95% CI: -0.13 to -0.02, *P* = 0.0041) and FET (+2.83s, 95%CI: +0.75 to +4.91, *P* = 0.008) in ever-smoker SZ versus controls. These findings were validated in a sub-analysis which included SZ lung-index smokers (modelling lung-index status as a covariate: SZ FEV_1pp -13.59%, 95% CI: -24.33 to -2.86, *P* = 0.013) and accentuated in a sub-analysis of >20 pack-year smokers (SZ FEV_1pp -23.14%, 95% CI: -39.61 to -6.68, *P* = 0.006).

When further exploring the relationship of smoking to lung function, a significant interaction between pack-years smoking and SZ-AATD (compared to control) was found in ever-smokers. The effect of pack-years smoking on lung function in SZ versus controls was: FEV_1pp (-1.24 vs -0.0615, *P* < 0.001), FVCpp (-0.657 vs -0.126, *P* = 0.032) and FEV_1/FVC Ratio (-0.007 vs -0.003, *P* = 0.002) (table E4). No significant increased risk of categorical COPD was seen for SZ individuals compared to family-based controls (OR 3.1, 95% CI: 0.7 - 13.66, *P* = 0.14). The effect of the SZ genotype tended to be greater, though not statistically significant (OR 4.65, 95% CI: 0.51 - 42.3, *P* = 0.17), when restricting the analysis to ever-smokers.

Predictors of outcome for the SZ genotype

In the SZ-only analyses, smoking (pack-years) was associated with a decrease in all spirometry outcomes, greatest in FEV_1pp (-1.261 per pack-year, 95% CI; -1.61 to -0.915, *P* < 0.001) whilst lung-index status also predicted lower FEV1pp (-22.18%, 95% CI: -33.75 to -10.63, *P* < 0.001). AAT levels were not significantly associated with any spirometry outcome when modeled either as a dichotomous outcome using the putative protective threshold (PPT) or as numerical values in g/L (table 3 & E5).

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Respiratory Symptoms

Respiratory symptom data revealed no significant differences between non-lung-index SZ individuals and controls (table 4). Overall response rate for respiratory symptom questions was 80% (133/166) including 12/12 SZ lung-index (100%), 51/70 SZ non-lung-index (72%) and 29/47 (62%) control cases.

Chest CT results

By study completion, CT results from usual clinical follow up were available for 50/82 SZ (60.09%) participants (table E6). Emphysema was not reported in any never-smokers (0/17, mean age 51.35 ±12.69) compared with 33% (11/33) of ever-smokers (mean age 53.03 ±10.63) with this prevalence rising to 60% (9/15) among >20 pack-year smokers. Amongst those with CT evident emphysema, 32/33 (97%) demonstrated an upper zone predominant distribution, in contrast to the lower zone predominant distribution typical of ZZ-AATD.

Longitudinal lung function outcomes and anti-NE capacity in the SZ genotype

Data from SZ individuals enrolled in the Irish National AATD Registry was filtered according to the inclusion criteria, yielding 60 individuals (table 5). No difference in AAT levels was noted between never- and former-smokers (0.61g/L for both, P = 0.89).

Smoking status (ever vs never), baseline COPD, and AAT levels (above versus below the PPT) were assessed as predictors of slope of lung function (e.g., FEV_1 ml/year). In this model only COPD at baseline was associated with greater decline in FEV1 (-12.87 ml/year, 95% CI: -24.92 to -0.83, P = 0.041) (figure 3). Former-smoking (n=33) was not associated with greater decline in

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

FEV_1 (-3.81 ml/year vs never-smokers, *P* = 0.51) (table 6). Furthermore, below-PPT levels (table E7), quantitative AAT level or >20 pack-year smoking status were not associated with significant decline.

Anti-NE capacity compared by smoking status

Plasma from ten SZ never-smokers and ten SZ former-smokers – with former-smokers required to have quit for more than six months – was collected to compare anti-NE capacity following smoking cessation. The demographics of the anti-NE comparison cohort are included in the supplementary materials (table E8). No significant difference was demonstrated between the anti-NE capacity of never- and former-smokers (figure 4).

Discussion

Our findings suggest that SZ never-smokers are not at increased risk of lung disease and that neither the PPT nor AAT levels are useful for predicting risk in this genotype. Conversely, current-smoking SZ individuals are at a significantly increased risk of airflow obstruction compared to control smokers. Former-smoking alone was not associated with accelerated decline in our registry cohort, suggesting smoking cessation attenuates the interaction of smoking with SZ-AATD. A greater decline in FEV_1pp was seen in SZ former-smokers with COPD, a finding also described in non-AATD populations (28). To adequately assess whether former-smoker lung function decline differs between individuals with SZ-COPD and those with MM-COPD a study directly comparing the two would be required.

In our study we have accounted for the impact of referral bias to more precisely assess the risk for airflow obstruction for the SZ genotype compared to normal-risk genotypes. We present the

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

largest number of non-lung-index SZ individuals to date, and for the first time compare them to a well-matched control population. The adjusted OR for COPD in SZ smokers did not achieve statistical significance in our data (OR 4.65, *P* = 0.17). Nonetheless, a significant difference in both the FEV_1/FVC Ratio and the interaction effect of pack-years with SZ-AATD on FEV_1/FVC Ratio was demonstrated. Furthermore, SZ smokers who were found to have emphysema on CT imaging of the chest were noted to have an upper lobe predominant distribution of disease, as opposed to the lower zone predominant emphysema classically described in ZZ-AATD. These findings support those by previously published by other investigators in larger CT specific studies (29), suggesting that the pathophysiology of COPD in ZZ and SZ-AATD may differ significantly.

The hypothesis that individuals with SZ-AATD, and particularly those with levels below the PPT, are at increased risk of COPD is based on previously reported levels of AAT in this genotype (1) coupled with evidence of physiological anomalies in asymptomatic SZ-AATD individuals (30), though the outcomes examined were not typical markers of COPD.

Whether genotype or AAT level is the greater predictor of lung disease in AATD is an ongoing point of discussion; however, the point may be moot. An overlap of AAT levels between SZ and ZZ cohorts is not seen outside of the acute phase, with ZZ levels not exceeding the PPT (1, 5, 22). Moreover, the S and Z isomers differ in their biochemical properties and interaction with NE (31, 32). Comparing different genotypes on the basis of AAT levels alone is therefore fundamentally flawed. Consequently, the true protective capacity of a given genotype is likely to be a composite effect of both the anti-NE/anti-inflammatory capacity and the inherent ability of that genotype to mount an increase in AAT levels during the acute phase which is

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

sufficient to meet the challenge of inflammatory insults. The evidence to date suggests that the ZZ genotype is insufficient on both these fronts whilst also promoting exaggerated protease activity (33-35). Other phenotypes such as SZ or MZ may have sufficient anti-NE/anti-inflammatory capacity in general but less ability than MMs to meet the chronic inflammatory challenges of cigarette smoking.

If a protective threshold exists within the SZ range, it is essential that it be assessed within a population of SZ individuals alone to remove confounding by other genotypes. In previous studies, when the PPT has been examined as a categorical variable of the SZ population, no association with worse clinical status has been demonstrated, with CT findings unrelated to AAT level, and indeed both physiology and symptoms often worse amongst those above the PPT (14, 29). Furthermore, previous studies have reported a relative minority of SZ participants (~10-20%) to have AAT levels below the PPT (1, 13, 14, 36). In these studies, acute phase response was not quantified and consequently the mean AAT levels may have been transiently elevated. Our data suggest that a significantly higher proportion of SZ individuals (40.6%) than previously described have resting AAT levels below the PPT, with a range of 0.4g/L to 0.74g/L when measured in the absence of acute phase response and using a highly purified AAT standard (24). In our analyses we have found no predictive value for the PPT or AAT levels in the SZ cohort.

To date, the only randomized placebo-controlled trial demonstrating the clinical efficacy of intravenous AAT (2) required participants to have AAT levels below the PPT. Of 180 participants, only two were SZ-AATD. As such, no clinical study has adequately assessed the benefits of intravenous AAT in SZ-AATD. Recommendations for the use of intravenous AAT

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

previously specified levels below the PPT as an indication for treatment (37, 38) whilst more recently recommending against its use in those actively smoking or those with the MZ genotype (39).

Despite these recommendations, intravenous AAT is prescribed to at least 1000 MZ and SZ individuals in the USA alone (40, 41) at an estimated annual cost of over US$80,000 per patient (42), or approximately US$80 million each year. Moreover, these cohorts report active-smoking rates of 7-11%, despite evidence that cigarette smoking may directly reduce the effectiveness of the therapy itself (43). Even more concerning is the fact that that some physicians prescribe intravenous augmentation for even the mild MS form of AATD (44).

Our results suggest that there is no increased risk of reduced lung function in never-smoking SZ-AATD and no evidence of CT-reported emphysema among never-smoking SZ individuals.

Identification of SZ individuals who smoke and subsequent smoking cessation should be a focus of care with the aim of preventing the onset of COPD. Furthermore, we found no evidence that AAT levels or the PPT are useful predictors of risk in this genotype. Therefore, this study raises significant questions as to the validity of the current PPT of 0.57g/L as an indication for therapy, or target for efficacy of such therapy. Consequently, there remains an absence of clinical evidence to support the need for augmentation therapy in SZ-AATD.

Acknowledgments

The authors would like to thank and acknowledge the many patients who gave up their time to make this research possible.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

References

1. Brantly ML, Wittes JT, Vogelmeier CF, Hubbard RC, Fells GA, Crystal RG. Use of a highly purified alpha 1-antitrypsin standard to establish ranges for the common normal and deficient alpha 1-antitrypsin phenotypes. *Chest* 1991; 100: 703-708.

2. Chapman KR, Burdon JG, Piitulainen E, Sandhaus RA, Seersholm N, Stocks JM, Stoel BC, Huang L, Yao Z, Edelman JM, McElvaney NG, Group RTS. Intravenous augmentation treatment and lung density in severe alpha1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. *Lancet* 2015; 386: 360-368.

3. McElvaney NG, Burdon J, Holmes M, Glanville A, Wark PA, Thompson PJ, Hernandez P, Chlumsky J, Teschler H, Ficker JH, Seersholm N, Altraja A, Makitaro R, Chorostowska-Wynimko J, Sanak M, Stoicescu PI, Piitulainen E, Vit O, Wencker M, Tortorici MA, Fries M, Edelman JM, Chapman KR, Group RET. Long-term efficacy and safety of alpha1 proteinase inhibitor treatment for emphysema caused by severe alpha1 antitrypsin deficiency: an open-label extension trial (RAPID-OLE). *Lancet Respir Med* 2017; 5: 51-60.

4. Wewers MD, Casolaro MA, Sellers SE, Swayze SC, McPhaul KM, Wittes JT, Crystal RG. Replacement therapy for alpha 1-antitrypsin deficiency associated with emphysema. *N Engl J Med* 1987; 316: 1055-1062.

5. Ferrarotti I, Thun GA, Zorzetto M, Ottaviani S, Imboden M, Schindler C, von Eckardstein A, Rohrer L, Rochat T, Russi EW, Probst-Hensch NM, Luisetti M. Serum levels and genotype distribution of alpha1-antitrypsin in the general population. *Thorax* 2012; 67: 669-674.

6. McElvaney GN, Sandhaus RA, Miravitlles M, Turino GM, Seersholm N, Wencker M, Stockley RA. Clinical considerations in individuals with Alpha-1 Antitrypsin PI*SZ genotype. *Eur Respir J* 2020.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

7. Piitulainen E, Tornling G, Eriksson S. Effect of age and occupational exposure to airway irritants on lung function in non-smoking individuals with alpha 1-antitrypsin deficiency (PiZZ). *Thorax* 1997; 52: 244-248.

8. McElvaney NG, Stoller JK, Buist AS, Prakash UB, Brantly ML, Schluchter MD, Crystal RD. Baseline characteristics of enrollees in the National Heart, Lung and Blood Institute Registry of alpha 1-antitrypsin deficiency. Alpha 1-Antitrypsin Deficiency Registry Study Group. *Chest* 1997; 111: 394-403.

9. Molloy K, Hersh CP, Morris VB, Carroll TP, O'Connor CA, Lasky-Su JA, Greene CM, O'Neill SJ, Silverman EK, McElvaney NG. Clarification of the risk of chronic obstructive pulmonary disease in alpha1-antitrypsin deficiency PiMZ heterozygotes. *Am J Respir Crit Care Med* 2014; 189: 419-427.

10. Foreman MG, Wilson C, DeMeo DL, Hersh CP, Beaty TH, Cho MH, Ziniti J, Curran-Everett D, Criner G, Hokanson JE, Brantly M, Rouhani FN, Sandhaus RA, Crapo JD, Silverman EK, Genetic Epidemiology of CI. Alpha-1 Antitrypsin PiMZ Genotype Is Associated with Chronic Obstructive Pulmonary Disease in Two Racial Groups. *Ann Am Thorac Soc* 2017; 14: 1280-1287.

11. Dahl M, Hersh CP, Ly NP, Berkey CS, Silverman EK, Nordestgaard BG. The protease inhibitor PI*S allele and COPD: a meta-analysis. *Eur Respir J* 2005; 26: 67-76.

12. Alvarez-Granda L, Cabero-Perez MJ, Bustamante-Ruiz A, Gonzalez-Lamuno D, Delgado-Rodriguez M, Garcia-Fuentes M. PI SZ phenotype in chronic obstructive pulmonary disease. *Thorax* 1997; 52: 659-661.

13. Turino GM, Barker AF, Brantly ML, Cohen AB, Connelly RP, Crystal RG, Eden E, Schluchter MD, Stoller JK. Clinical features of individuals with PI*SZ phenotype of alpha 1-antitrypsin deficiency. alpha 1-Antitrypsin Deficiency Registry Study Group. *Am J Respir Crit Care Med* 1996; 154: 1718-1725.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

14. Green CE, Vayalapra S, Hampson JA, Mukherjee D, Stockley RA, Turner AM. PiSZ alpha-1 antitrypsin deficiency (AATD): pulmonary phenotype and prognosis relative to PiZZ AATD and PiMM COPD. *Thorax* 2015; 70: 939-945.

15. Carroll TP, O'Connor CA, Floyd O, McPartlin J, Kelleher DP, O'Brien G, Dimitrov BD, Morris VB, Taggart CC, McElvaney NG. The prevalence of alpha-1 antitrypsin deficiency in Ireland. *Respir Res* 2011; 12: 91.

16. Ferris BG. Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis* 1978; 118: 1-120.

17. Ortega VE, Li X, O'Neal WK, Lackey L, Ampleford E, Hawkins GA, Grayeski PJ, Laederach A, Barjaktarevic I, Barr RG, Cooper C, Couper D, Han MK, Kanner RE, Kleerup EC, Martinez FJ, Paine Iii R, Peters SP, Pirozzi C, Rennard SI, Woodruff PG, Hoffman EA, Meyers DA, Bleecker ER, Subpopulations N, Intermediate Outcomes Measures in CS. The Effects of Rare SERPINA1 Variants on Lung Function and Emphysema in SPIROMICS. *Am J Respir Crit Care Med* 2019.

18. Silverman EK. Risk of Lung Disease in PI MZ Heterozygotes. Current Status and Future Research Directions. *Ann Am Thorac Soc* 2016; 13 Suppl 4: S341-345.

19. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995; 152: 1107-1136.

20. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993; 16: 5-40.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

21. Graham BL, Brusasco V, Burgos F, Cooper BG, Jensen R, Kendrick A, MacIntyre NR, Thompson BR, Wanger J. DLCO: adjust for lung volume, standardised reporting and interpretation. *Eur Respir J* 2017; 50.

22. Ottaviani S, Gorrini M, Scabini R, Kadija Z, Paracchini E, Mariani F, Ferrarotti I, Luisetti M. C reactive protein and alpha1-antitrypsin: relationship between levels and gene variants. *Transl Res* 2011; 157: 332-338.

23. Zerimech F, Hennache G, Bellon F, Barouh G, Jacques Lafitte J, Porchet N, Balduyck M. Evaluation of a new Sebia isoelectrofocusing kit for alpha 1-antitrypsin phenotyping with the Hydrasys System. *Clin Chem Lab Med* 2008; 46: 260-263.

24. Thelwell C, Marszal E, Rigsby P, Longstaff C. An international collaborative study to establish the WHO 1st international standard for alpha-1-antitrypsin. *Vox Sang* 2011; 101: 83-89.

25. Therneau T. The lmekin function. 2018. Available from: https://cran.r-project.org/web/packages/coxme/vignettes/lmekin.pdf.

26. Chen H, Conomos MP. GMMAT: Generalized Linear Mixed Model Association Tests. ver 1.1.0. R version 3.6. 2019.)

27. Chen H, Wang C, Conomos MP, Stilp AM, Li Z, Sofer T, Szpiro AA, Chen W, Brehm JM, Celedon JC, Redline S, Papanicolaou GJ, Thornton TA, Laurie CC, Rice K, Lin X. Control for Population Structure and Relatedness for Binary Traits in Genetic Association Studies via Logistic Mixed Models. *Am J Hum Genet* 2016; 98: 653-666.

28. Bridevaux PO, Gerbase MW, Probst-Hensch NM, Schindler C, Gaspoz JM, Rochat T. Long-term decline in lung function, utilisation of care and quality of life in modified GOLD stage 1 COPD. *Thorax* 2008; 63: 768-774.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

29. Holme J, Stockley RA. CT scan appearance, densitometry, and health status in protease inhibitor SZ alpha1-antitrypsin deficiency. *Chest* 2009; 136: 1284-1290.

30. Larsson C, Dirksen H, Sundstrom G, Eriksson S. Lung function studies in asymptomatic individuals with moderately (Pi SZ) and severely (Pi Z) reduced levels of alpha1-antitrypsin. *Scand J Respir Dis* 1976; 57: 267-280.

31. Sinden NJ, Baker MJ, Smith DJ, Kreft JU, Dafforn TR, Stockley RA. alpha-1-antitrypsin variants and the proteinase/antiproteinase imbalance in chronic obstructive pulmonary disease. *Am J Physiol Lung Cell Mol Physiol* 2015; 308: L179-190.

32. Ogushi F, Hubbard RC, Fells GA, Casolaro MA, Curiel DT, Brantly ML, Crystal RG. Evaluation of the S-type of alpha-1-antitrypsin as an in vivo and in vitro inhibitor of neutrophil elastase. *Am Rev Respir Dis* 1988; 137: 364-370.

33. Mulgrew AT, Taggart CC, Lawless MW, Greene CM, Brantly ML, O'Neill SJ, McElvaney NG. Z alpha1-antitrypsin polymerizes in the lung and acts as a neutrophil chemoattractant. *Chest* 2004; 125: 1952-1957.

34. Bergin DA, Reeves EP, Meleady P, Henry M, McElvaney OJ, Carroll TP, Condron C, Chotirmall SH, Clynes M, O'Neill SJ, McElvaney NG. alpha-1 Antitrypsin regulates human neutrophil chemotaxis induced by soluble immune complexes and IL-8. *J Clin Invest* 2010; 120: 4236-4250.

35. Campbell EJ, Campbell MA, Boukedes SS, Owen CA. Quantum proteolysis by neutrophils: implications for pulmonary emphysema in alpha 1-antitrypsin deficiency. *J Clin Invest* 1999; 104: 337-344.

36. Seersholm N, Kok-Jensen A. Intermediate alpha 1-antitrypsin deficiency PiSZ: a risk factor for pulmonary emphysema? *Respir Med* 1998; 92: 241-245.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

37. American Thoracic S, European Respiratory S. American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. *Am J Respir Crit Care Med* 2003; 168: 818-900.

38. Marciniuk DD, Hernandez P, Balter M, Bourbeau J, Chapman KR, Ford GT, Lauzon JL, Maltais F, O'Donnell DE, Goodridge D, Strange C, Cave AJ, Curren K, Muthuri S, Canadian Thoracic Society CCAA-ADEWG. Alpha-1 antitrypsin deficiency targeted testing and augmentation therapy: a Canadian Thoracic Society clinical practice guideline. *Can Respir J* 2012; 19: 109-116.

39. Sandhaus RA, Turino G, Brantly ML, Campos M, Cross CE, Goodman K, Hogarth DK, Knight SL, Stocks JM, Stoller JK, Strange C, Teckman J. The Diagnosis and Management of Alpha-1 Antitrypsin Deficiency in the Adult. *Chronic Obstr Pulm Dis* 2016; 3: 668-682.

40. Holm KE, Mannino DM, Choate R, Sandhaus RA. Genotype is associated with smoking and other key health behaviors among individuals with alpha-1 antitrypsin deficiency-associated lung disease. *Respir Med* 2018; 143: 48-55.

41. Choate R, Mannino DM, Holm KE, Sandhaus RA. Comparing Patients with ZZ Versus SZ Alpha-1 Antitrypsin Deficiency: Findings from AlphaNet's Disease Management Program. *Chronic Obstr Pulm Dis* 2018; 6: 29-39.

42. Sieluk J, Levy J, Sandhaus RA, Silverman H, Holm KE, Mullins CD. Costs of Medical Care Among Augmentation Therapy Users and Non-Users with Alpha-1 Antitrypsin Deficiency in the United States. *Chronic Obstr Pulm Dis* 2018; 6: 6-16.

43. Taggart C, Cervantes-Laurean D, Kim G, McElvaney NG, Wehr N, Moss J, Levine RL. Oxidation of either methionine 351 or methionine 358 in alpha 1-antitrypsin causes loss of anti-neutrophil elastase activity. *J Biol Chem* 2000; 275: 27258-27265.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

44. Zaidi NS, Slocum P, Kearns S, Kristofek L. Demographics and Clinical Profile of Patients with Alpha-1 Antitrypsin Deficiency PiMZ and PiMS Genotypes Started on Augmentation Therapy. D103 ALPHA 1-ANTITRYPSIN DEFICIENCY: 50 YEARS OF PROGRESS: American Thoracic Society; 2017. p. A7388-A7388.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Figure legends:

Figure 1. Study population recruitment for the family study. SZ index cases (1) are invited to take part. Parents (2) and siblings (3) of index SZs are enrolled and predicted to provide SZ (orange) and MS/MM (control) participants for the comparison cohorts. MZs are excluded from analyses.

Figure 2. Univariate linear regression analyses of effect of pack years smoked on spirometry. A significant difference in effect on FEV_1pp (*P* =0.025) and FEV_1/FVC Ratio (*P* =0.026) is seen.

Figure 3. Rate of change of FEV1 (ml/year) per individual categorized by a) smoking status and b) baseline presence of COPD. Significant variability is demonstrated in both never- and former-smokers. Greater association with decline is seen in individuals with COPD at baseline (-12.87 ml/year, 95% CI -24.92 to -0.83, *P* = 0.041, by mixed model analysis).

Figure 4: Comparison of plasma anti-NE activity of never-smoking (n=10) and former-smoking (n=10) plasma **(**full methods in online supplement).

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Tables:

Table 1. Family study cohort characteristics

	Control	SZ	*P* Value
Subjects, *n*	46	70	
Age (years)	53.39 (13.59)	52.93 (10.93)	0.84
AAT level (g/L)	1.24 [1.13, 1.40]	0.60 [0.50, 0.68]	<0.001**
CRP (mg/L)	1.40 [1.05, 1.75]	1.75 [0.8, 3.0]	0.30
Male sex (%)	21 (45.7)	31 (44.3)	1
Ever-smoker (%)	24 (52.2)	33 (47.1)	0.73
Pack-years smoked (ever-smokers)	18 [8.25, 28.95]	14 [6, 22]	0.41
BMI (kg/m^2)	27.30 (3.94)	28.21 (4.93)	0.29
Height (cm)	167.91 (10.16)	169.39 (10.88)	0.47
Weight (kg)	77.04 (16.20)	81.36 (16.50)	0.17
FEV_1pp	99 [91, 112]	103.5 [92.25, 114]	0.51
FVCpp	113.27 (15.18)	112.20 (19.7)	0.76
FEV_1/FVC Ratio	0.74 [0.71, 0.79]	0.77 [0.71, 0.81]	0.20
$MMEF_{25-75}$pp	65.87 (27.09)	71.60 (31.66)	0.32
Positive post-BD response (%)	10 (21.7)	16 (23.5)	1
Hb (g/dl)	14.30 (1.18)	14.20 (1.15)	0.67
Serum eosinophil (per 10^9/L)	0.19 [0.11, 0.31]	0.16 [0.11, 0.26]	0.61
Bilirubin (µmol/L)	9 [7, 11]	9 [7, 10]	0.67
ALT (U/L)	24 [18, 34.75]	30 [23, 39]	0.025*
ALP (U/L)	80 [69, 100.25]	89.00 [74, 107]	0.07
GGT (U/L)	28 [24, 44.75]	29.5 [21, 48.5]	0.96
INR	1.08 [1.02, 1.14]	1.04 [1.00, 1.06]	0.005*
Albumin (g/L)	45 [42.75, 46]	44.50 [43, 47]	0.8
Immunoglobulin E (I.U/L)	35 [7.25, 114.25]	29 [15, 78]	0.84

Data are mean (+/-Std.Error) for parametric, median and [IQR] for non-parametric and percent (%) for categorical

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table 2. Family study main results: mean and (standard error)

	Control (n=46)	SZ (n=70)	*P* Value
Never-smokers			
FEV_1pp	Ref	+4.85 (3.57)	0.17
FEV_1 (L)	Ref	+0.14 (0.11)	0.19
FVCpp	Ref	-0.82 (5.15)	0.87
FVC (L)	Ref	+0.14 (0.14)	0.3
FEV_1/FVC	Ref	-0.02 (0.02)	0.29
$MMEF_{25-75}$pp	Ref	+9.86 (7.14)	0.17
$MMEF_{25-75}$(L)	Ref	+0.37 (0.25)	0.13
FET (s)	Ref	-1.29 (0.93)	0.17
Ever-smokers			
FEV_1pp	Ref	-14.23 (5.47)	0.009**
FEV_1 (L)	Ref	-0.367 (0.18)	0.045*
FVCpp	Ref	-8.830 (5.29)	0.09
FVC (L)	Ref	-0.231 (0.20)	0.26
FEV_1/FVC	Ref	-0.075 (0.03)	0.004**
$MMEF_{25-75}$pp	Ref	-8.502 (6.95)	0.22
$MMEF_{25-75}$(L)	Ref	-0.399 (0.27)	0.13
FET (s)	Ref	+2.830 (1.06)	0.008**

Ref = reference population

Data are mean and (standard error)

Method: *lmekin* mixed model

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table 3. Multivariate analysis of estimated effect of predictors on spirometry outcomes in the SZ cohort

(n=82)				
Outcome	**Covariate effect**			
	a. Ever-smoker	**b. Pack-years†**	**Lung-index**	**Below-PPT**
FEV_1pp	-15.63**	-1.261***	-22.182***	0.830
FVCpp	-5.12	-0.775***	-4.248	-1.496
$FEV_1/FVC^{\ddot{Y}}$	-0.07**	-0.006***	-0.125***	0.003
$MMEF_{25-75}$pp	-18.53**	-0.920***	-31.268***	-4.884
FET (s) ‡§	+2.54*	0.164***	1.269	1.129

When modelled without adjustment for pack-years

†When modelled with adjustment for pack-years + ever-smoker status

‡ Adjusted for age, sex, height, weight, pack-years.

§ Also adjusted for FVC (L).

All analyses include random variable (ID) and adjustment for kinship matrix.

Statistical model *lmekin*

* *P* Value <0.05, ** *P* <0.01, *** *P* <0.001

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table 4. Self-reported symptom and intervention requirements among family-study ATS-DLD respondents

	Control	SZ	*P* Value	Method
Subjects, *n*	29	51		
Symptoms				
Cough (%)	5 (17.2)	8 (15.7)	1	*Chi-square*
Phlegm (%)	10 (34.5)	6 (11.8)	0.031*	*Chi-square*
Wheeze (%)	14 (50.0)	25 (49.0)	1	*Chi-square*
mMRC dyspnoea scale (%)			0.54	*Chi-square*
N/A	0 (0.0)	2 (3.9)		
1	16 (55.2)	33 (64.7)		
2	11 (37.9)	12 (23.5)		
3	1 (3.4)	1 (2.0)		
4	1 (3.4)	3 (5.9)	0.67	
Flare of chest symptoms in past year (%)	9 (31.0)	17 (34.0)	0.98	*Chi-square*
Intervention requirement				
Antibiotics for chest in year (%)	8 (27.6)	8 (16.0)	0.35	*Chi-square*
Steroids for chest in year (%)	2 (6.9)	2 (4.0)	0.97	*Chi-square*

Ref = reference population

Data are mean and (standard deviation) or percent for categorical

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table 5. Longitudinal assessment cohort baseline characteristics

Characteristic	**Never-smoker SZ**	**Former-smoker SZ**	***P* Value**
Subjects, *n*	27	33	
Age at baseline (years)	46.78 (16.43)	48.15 (15.22)	0.74
AATD diagnosis age (years)	45.89 (16.33)	48.73 (15.65)	0.49
Sex (Male) (%)	9 (33.3)	17 (51.5)	0.25
Lung-Index (%)	2 (7.4)	17 (51.5)	0.001**
Follow up time (months)	63 [47, 79.5]	58 [36, 75]	0.29
Number of measurements	5.3 [2.81, 7.8]	5.62 [4.10, 9.01]	0.21
Pack Years Smoked	0	20 [7.5, 40]	-
AAT level - no CRP validation (g/L)	0.56 [0.50, 0.71]	0.58 [0.50, 0.69]	0.96
AAT level below PPT (%)	15 (55.6)	15 (45.5)	0.60
BMI (kg/m^2)	25.96 [22.11, 27.34]	27.57 [23.23, 30.48]	0.32
Baseline FEV_1/FVC <0.7 (%)	5 (18.5)	16 (48.5)	0.032*
Baseline FEV_1pp	100.00 [92.50, 108.00]	90.00 [67.80, 100.00]	0.007**
Baseline FVCpp	108.00 [101.00, 115.50]	104.00 [93.00, 123.00]	0.32
Baseline FEV_1/FVC Ratio	77.00 [71.50, 82.00]	70.00 [57.00, 78.00]	0.004**
Baseline DLCOpp	90.00 [78.50, 101.50]	83.00 [65.30, 93.25]	0.047*
CT emphysema (%)	0 (0.0)	11 (33.3)	0.002**

Data are mean (+/-Std.Error) for parametric, median and [IQR] for non-parametric and percent (%) for categorical

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table 6. Fixed effect of former-smoker status on lung function trends in the longitudinal registry cohort

	Never-smoker SZ	Former-smoker SZ	*P* Value	*Method*
Subjects, *n*	27	33		
ΔFEV_1 (ml/year)	Ref	-3.81 (5.71)	0.51	*glm*
ΔFVC (ml/year)	Ref	-4.47(5.53)	0.42	*glm*
$\Delta FEV_1/FVC$ (/year)	Ref	-0.01 (0.02)	0.69	*glm*
ΔDLCO (mL/min/mmHg /year)	Ref	+0.012 (0.07)	0.86	*glm*

Ref = reference population

Data are mean and (standard error)

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Figures:

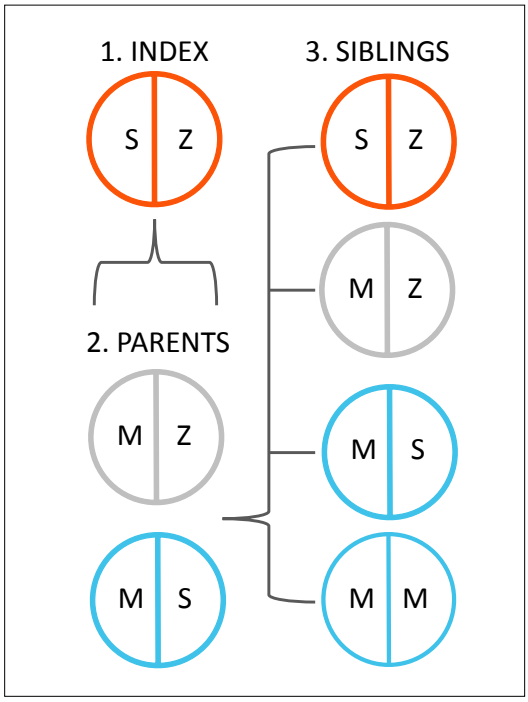

Figure 1.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

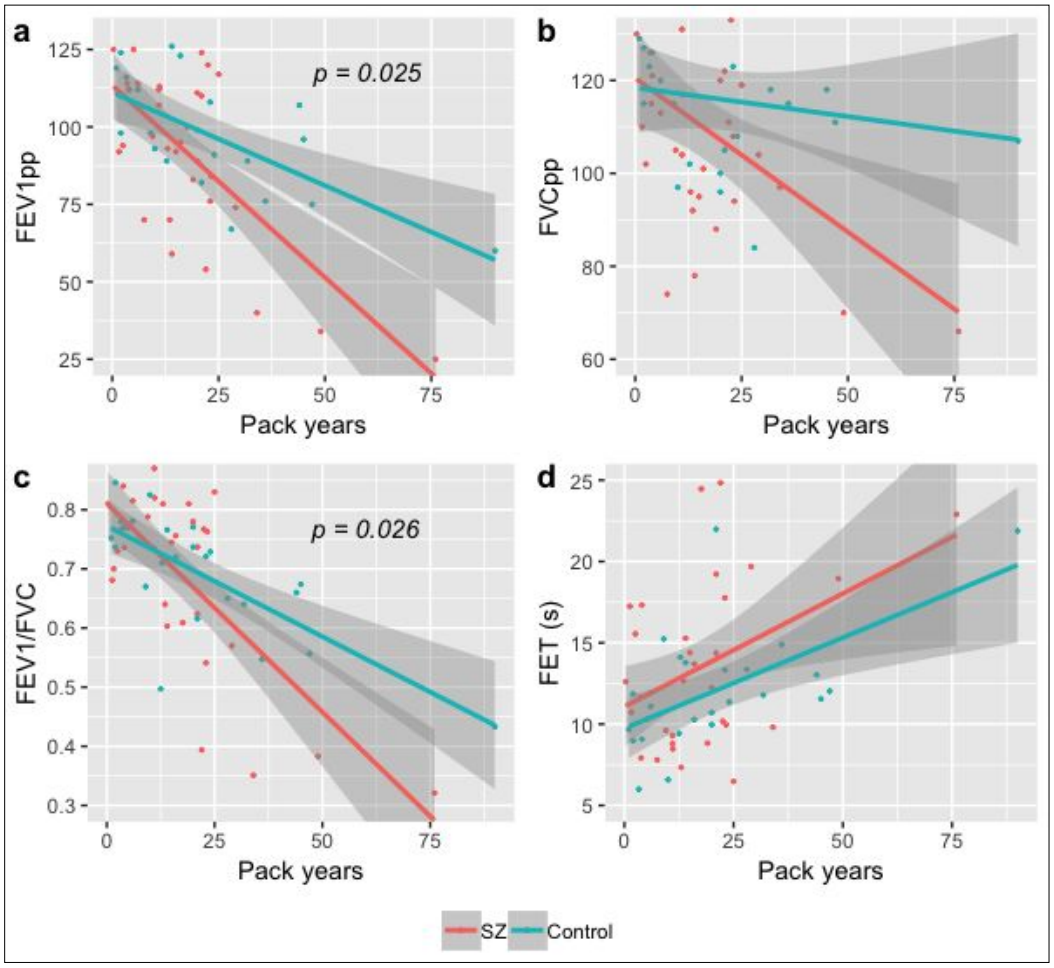

Figure 2.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

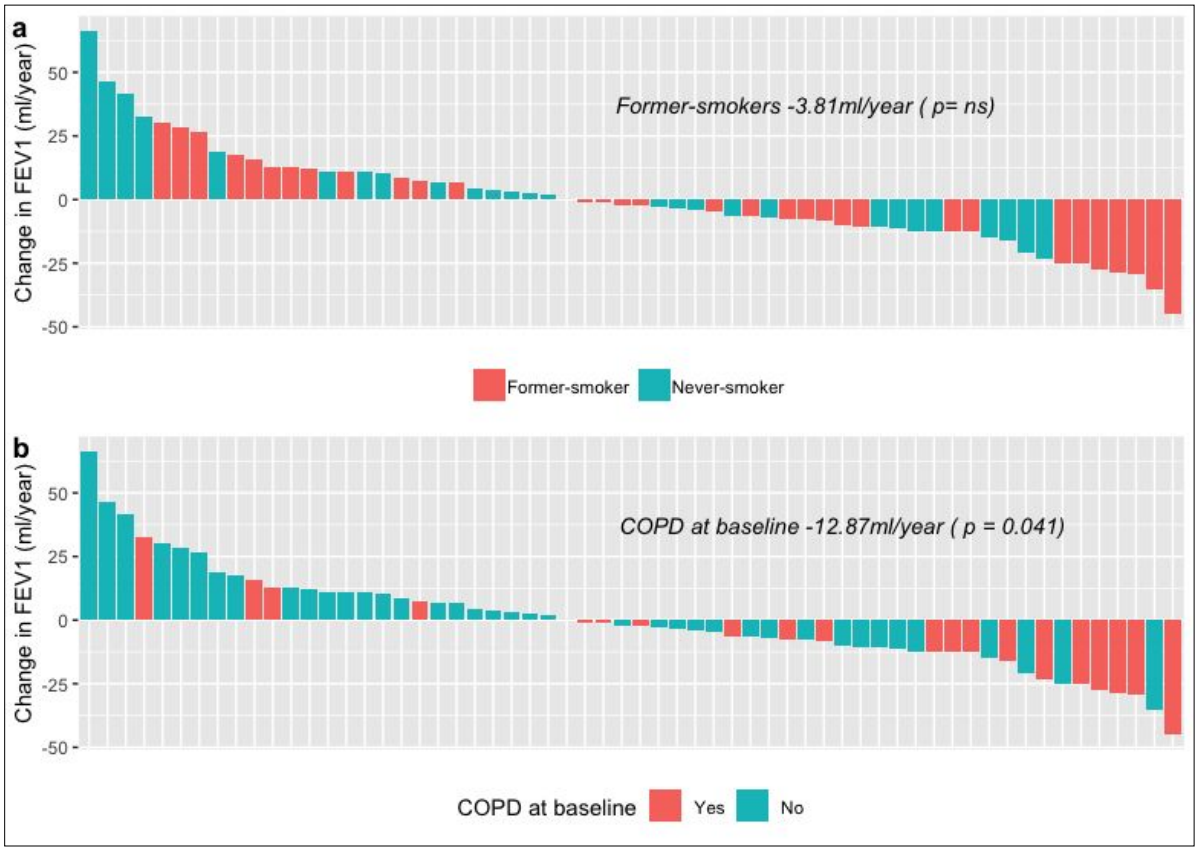

Figure 3.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

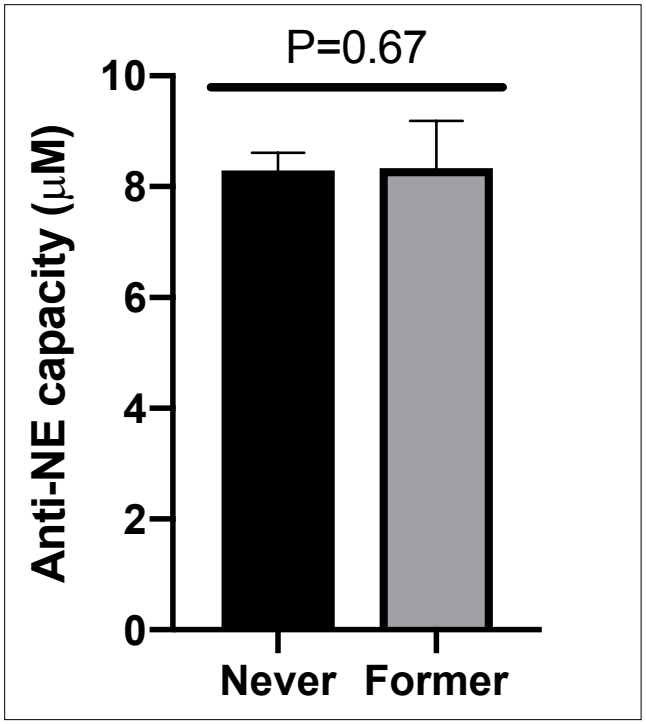

Figure 4.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Clarifying the Risk of Lung Disease in SZ α-1 antitrypsin Deficiency

Online Data Supplement.

Alessandro N Franciosi PhD

Brian D Hobbs MD

Oliver J McElvaney PhD

Kevin Molloy MD

Craig Hersh MD

Louise Clarke

Cedric Gunaratnam MD

Edwin K Silverman MD PhD

Tomás P Carroll PhD

Noel G McElvaney MD DSc

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table of Contents

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Supplementary Statistical Methodology

Demographic data, spirometry measurements and AAT levels in both the family study and registry study were available for all participants. In the longitudinal registry analysis, differences in the frequency and number of spirometry measurements between subjects were accounted for by modelling "time" (the timepoint for each measurement of lung function since the first measurement of lung function) as a random variable in the linear mixed models used to calculate individual slopes in lung function.

Statistical analysis was performed in RStudio Version 1.1.463 (www.cran.r-project.com). Continuous data were validated for normality of distribution using the Shapiro-Wilk test. Normally and non-normally distributed data were analyzed by Student t test and Mann-Whitney U test, respectively. For comparisons between more than two groups normally and non-normally distributed data were analyzed by ANOVA and Kruskal-Wallis test respectively. Categorical variables were analyzed by chi-square test. Models with percentage predicted (pp) spirometry results were adjusted for smoking history (ever-smoking and pack-years). Absolute spirometry results (in liters), FEV_1/FVC Ratio, forced expiratory times (FET) and categorical COPD (defined as post-bronchodilator FEV_1/FVC Ratio <0.7 in all analyses) were adjusted for smoking history, age, height, weight, sex (and for FET, the measured FVC). All participants in the two arms of the study were non-Hispanic white Irish, due to the family-based nature of the study and the European-Caucasian prevalence of S and Z alleles of AAT. Consequently, adjustment for ethnicity was not performed. A positive bronchodilator response was defined as a 12% and 200ml absolute increase in either FEV_1 or FVC(42).

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

In all comparative analyses categorical variables were coded with the supposed lower risk category (e.g. control/never-smoker/above-PPT AAT levels) as the reference factor (0) and proposed higher risk categories (e.g. SZ/smoker/Lung-Index/COPD) as the comparator (1).

In the family study we excluded lung-index cases from the final case-control analyses to remove referral bias. In SZ-only analyses we included lung-index cases and modelled index-status as a fixed effect. The effect of the SZ genotype on spirometry was defined in never-smokers and ever-smokers using mixed model analyses examining the covariates of age, genotype, smoking and the PPT to assess for differences between categories. We used the *lmekin* function in the *coxme* package in R, fitting a linear mixed-effects model with predictors and confounders as fixed effects and a kinship coefficient matrix to model relatedness as a random effect. The association of the SZ genotype with COPD in all persons and in ever-smokers was performed with the *GMMAT* R package (23) using a logistic mixed model adjusted for age, sex, pack-years of smoking, and familial relatedness.

In the longitudinal study of persons with SZ AATD, a linear mixed-model regression analysis modelling subject ID and time as random effects (subjects each with independent slope and intercept) was used to calculate age, sex, height and weight adjusted slopes for each PFT parameter against time (*lmer* in R). Individual slopes were extracted using the *random.effects* output of *lmer* package in R. Multi-variable linear mixed models (*glm* in R) were then performed to examine the association of categorical fixed effects (never- vs former-smoker, COPD at baseline vs Ratio $\geq$0.7 at baseline, AAT levels above/below the PPT and baseline presence of emphysema) with slope of lung function. Additional analyses examining association of pack-years smoked (as a continuous variable fixed effect) and smoking categorized as high/low

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

exposure (high ≥20 pack-years, low >0 <20 pack-years) were performed but did not yield significant results.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Anti-NE Capacity Measurement Methodology

7.5mls of peripheral venous blood was collected from each patient using a lithium heparin tube (Sarstedt Inc. Monovette®). Samples were centrifuged within 10 minutes of collection at 3000 RPM for 5 minutes. Plasma was then aliquoted in 500µL Eppendorf tubes and stored at -80℃. Each sample was only thawed once at the time of analysis.

To assess the anti-NE capacity of SZ AATD plasma, fixed concentrations of exogenous NE were incubated with a range of concentrations of plasma from never-smoking (n=10) and ever-smoking (N=10) SZ AATD patients at 37°C for 30min. NE with no plasma treatment acted as a control for each assay. Post-incubation, 1 µL of each plasma/NE reaction was mixed with 99µL of 1mM FRET substrate (Abz-APEEIMRRQ-EDDnp, Peptide Institute, Osaka, Japan) in assay buffer ([0.5 M NaCl]+[0.1% (v/v) Brij-35]+[0.1 M HEPES]; pH 7.8).

Fluorescence was recorded at excitation 320nm and emission 420nm at 21sec intervals for 17min at 28°C using a SpectraMax plate reader. NE activity was quantified by comparison with a NE standard curve (Elastin Products) of known NE activity. Anti-NE capacity was calculated via the percentage inhibition of NE from the plot of the percentage of remaining activity versus the plasma concentrations using GraphPad software (Prism 7.0).

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

The Putative Protective Threshold

The “Putative Protective Threshold” (abbreviated to PPT in our manuscript) alludes to the plasma level of AAT estimated to be required to confer sufficient anti-protease activity to protect an individual from accelerated lung degradation. It is accepted as both an indicator of need for intravenous AAT augmentation therapy and consequently as the target nadir level of AAT in circulation between dose administration of said therapy. This level, originally described as 11µM of AAT, equates to 0.57g/L (molecular weight of AAT = 52kDa) when measured by turbidimetry or nephelometry.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Online Data Supplement Figures and Tables

Figure E1: Family study participant serum AAT levels stratified by genotype and Lung/Non-Lung-index status demonstrating that only the SZ genotype is associated with levels straddling the "Putative Protective Threshold".

Figure E2: Univariate linear regression analyses of effect of age on spirometry parameters. No significant difference in effect of increasing age on spirometry is demonstrated between SZ and control cohorts (*P* Value not significant for all).

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table E1. Comparison of lung-index and non-lung-index SZ participant characteristics

Characteristics	**SZ Non-lung-index**	**SZ lung-index**	***P* Value**
Subjects, *n*	70	12	
Age (years)	52.93 ±10.93	57.33 ±8.06	0.187
AAT level (g/L)	0.60 [0.50, 0.68]	0.61 [0.55, 0.66]	0.476
AAT level below PPT (%)	26 (40.6)	3 (25.0)	0.485
CRP (mg/L)	1.75 [0.8, 3.0]	2.95 [1.6, 10.48]	0.279
Male sex (%)	31 (44.3)	5 (41.7)	1.000
Ever-smoker (%)	33 (47.1)	10 (83.3)	0.045
Pack-years smoked[†] (smokers)	14 [6, 22]	34.5 [21, 41]	0.011**
BMI (kg/m^2)	28.21 ±4.93	26.29 ±4.80	0.214
Serum eosinophil (per 10^9/L)	0.16 [0.11, 0.26]	0.20 [0.10, 0.38]	0.650
Immunoglobulin E (I.U/L)	29 [15, 78]	27 [16.5, 60]	0.909
FEV_1pp	103.50 [92.25, 114.00]	53.50 [29.00, 82.75]	<0.001**
FVCpp	112.20 ±19.70	93.42 ±24.85	0.004**
FEV_1/FVC Ratio	0.77 [0.71, 0.81]	0.47 [0.35, 0.66]	<0.001**
$MMEF_{25-75}$pp	71 [54.5, 94.75]	13.5 [8, 25.75]	<0.001***
FET (s)	10.96 [8.55, 14.20]	13.55 [11.70, 18.11]	0.018
Positive Bronchodilator response (%)	16 (23.5)	7 (58.3)	0.035*

Data presented as mean ±Standard Error for parametric tests .

Non-parametric test results presented as median + [IQR].

Categorical data presented as number and (%).

Defined as 12% and 200ml increase in FEV_1 or FVC.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table E2. Comparison of MM and MS cohort characteristics

	MM	MS	*P* Value
Subjects, *n*	19	27	
Demographic			
Age (years)	54 [40.5, 62]	56 [41.5, 65.5]	0.554
AAT level (g/L)	1.40 ±0.16	1.19 ±0.14	<0.001**
CRP (mg/L)	1.20 [0.75, 1.50]	1.40 [1.20, 2.30]	0.081
Male sex (%)	9 (47.4)	12 (44.4)	1.000
Ever-smoker (%)	10 (52.6)	14 (51.9)	1.000
Pack-years smoked (smokers)	15.50 [3.97, 34]	18 [12.57, 23.75]	0.815
BMI (kg/m^2)	27.24 ±3.10	27.34 ±4.49	0.931
Height (cm)	168.11 ±10.59	167.78 ±10.05	0.916
Weight (kg)	76.21 ±14.46	77.62 ±17.56	0.775
Biochemical			
Hb (g/dl)	14.34 ±0.66	14.27 ±1.51	0.876
Serum eosinophil (per 10^9/L)	0.28 ±0.22	0.28 ±0.39	0.930
Bilirubin (µmol/L)	10 [7.75, 11.25]	8 [7, 9]	0.137
ALT (U/L)	26.5 [22.5, 36.75]	20 [16.75, 31.75]	0.188
ALP (U/L)	87.61 ±30.89	79.65 ±23.40	0.374
GGT (U/L)	35 [24, 47]	26 [24, 42.5]	0.466
INR	1.14 [1.07, 1.21]	1.05 [1.02, 1.09]	0.019*
Albumin (g/L)	45.31 ±2.09	43.70 ±2.43	0.043*
Immunoglobulin E (I.U/L)	44 [11, 87]	29 [6.50, 118]	0.755
Physiological			
FEV_1pp	97.68 ±16.98	102.12 ±14.53	0.352
FVCpp	112.58 ±12.62	113.77 ±17.03	0.798
FEV_1/FVC Ratio	0.74 [0.68, 0.79]	0.75 [0.71, 0.78]	0.721
$MMEFpp_{25-75}$pp	61.32 ±28.00	69.19 ±26.46	0.341

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

FET (s)	12.59 ±4.69	10.69 ±2.84	0.096
Positive Bronchodilator response (%)	5 (26)	5 (18.5)	0.788

Mean (±standard deviation) (% for categorical) are reported for parametric tests. Median and [IQR] are reported for non-parametric tests.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table E3. Baseline demographic, biometric and biochemical features of non-lung index participants (all genotypes)

	MM	MS	MZ	SZ	*P* Value
Subjects, *n*	19	27	28	70	
Demographic					
Age (years)	54 [40.5, 62]	56 [41.5, 65.5]	59 [43.75, 64.25]	54 [45.25, 61]	0.782
AAT level (g/L)	1.40 ±0.16	1.19 ±0.14	0.90 ±0.19	0.59 ±0.10	<0.001**
CRP (mg/L)	1.20 [0.75, 1.50]	1.40 [1.20, 2.30]	2.75 [0.83, 5.93]	1.75 [0.80, 3.0]	0.280
Male sex (%)	9 (47.4)	12 (44.4)	11 (39.3)	31 (44.3)	0.952
Ever-smoker (%)	10 (52.6)	14 (51.9)	16 (57.1)	33 (47.1)	0.835
Pack-years smoked (Smokers)	15.50 [3.97, 34]	18 [12.57, 23.75]	17.05 [7.75, 40.30]	14 [6, 22]	0.717
BMI (kg/m^2)	27.24 ±3.10	27.34 ±4.49	29.17 ±5.71	28.21 ±4.93	0.443
Height (cm)	168.11 ±10.59	167.78 ±10.05	167.25 ±9.04	169.39 ±10.88	0.782
Weight (kg)	76.21 ±14.46	77.62 ±17.56	81.86 ±18.32	81.36 ±16.50	0.515
Biochemical					
Hb (g/dl)	14.30 [14.1, 14.9]	14.70 [13.15, 15]	13.40 [13, 14.28]	14.20 [13.55, 14.95]	0.293
Eosinophils (per 10^{-9}/L)	0.20 [0.15, 0.37]	0.14 [0.10, 0.24]	0.18 [0.09, 0.27]	0.16 [0.11, 0.26]	0.602
Bilirubin (µmol/L)	10 [7.75, 11.25]	8 [7, 9]	7 [6, 13]	9 [7, 10]	0.395
ALT (U/L)	26.5 [22.5, 36.75]	20 [16.75, 31.75]	25.00 [19, 31]	30 [23, 39]	0.040*
ALP (U/L)	80.5 [69, 104.75]	80 [70.75, 97.25]	92 [77.25, 107]	89 [74, 107]	0.250
GGT (U/L)	35 [24, 47]	26 [24, 42.5]	25 [20.50, 34.50]	29.5 [21, 48.5]	0.807
INR	1.14 [1.07, 1.21]	1.05 [1.02, 1.09]	1.02 [0.97, 1.07]	1.04 [1.0, 1.06]	0.006*
Albumin (g/L)	45.31 ±2.09	43.70 ±2.43	45.29 ±3.07	44.62 ±3.47	0.358
IgE (I.U/L)	44 [11, 87]	29 [6.50, 118]	26 [6, 44]	29 [15, 78]	0.701

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Mean (±standard deviation) (% for categorical) are reported for parametric tests, with median and [IQR] for non-parametric tests (MI and ZZ participants not shown).

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table E4. Effect of Pack-years smoked on outcome by linear mixed model regression (first in stratified analyses and as then as an analysis of the interaction with the SZ genotype)

Outcome	Analysis	Effect	Std. Err	*P* Value
FEV_1pp	per Pack-year (Control)	-0.615	0.144	<0.001
	per Pack-year (SZ)	-1.240	0.226	<0.001
	Interaction Pack-years*SZ vs Control	**-0.640**	**0.268**	**0.017***
FEV_1 (L)†	per Pack-year (Control)	-0.010	0.004	0.033
	per Pack-year (SZ)	-0.036	0.008	<0.001
	Interaction Pack-years*SZ vs Control	**-0.025**	**0.008**	**0.0036****
FVCpp	per Pack-year (Control)	-0.126	0.152	0.410
	per Pack-year (SZ)	-0.657	0.219	0.003
	Interaction Pack-years*SZ vs Control	**-0.537**	**0.251**	**0.032***
FVC (L)†	per Pack-year (Control)	0.002	0.005	0.710
	per Pack-year (SZ)	-0.020	0.009	0.019
	Interaction Pack-years*SZ vs Control	**-0.023**	**0.009**	**0.013***
FEV_1FVC†	per Pack-year (Control)	-0.003	0.000	<0.001
	per Pack-year (SZ)	-0.007	0.001	<0.001
	Interaction Pack-years*SZ vs Control	**-0.004**	**0.001**	**0.0017****
MEF_{25-75}pp	per Pack-year (Control)	-0.878	0.204	<0.001
	per Pack-year (SZ)	-1.237	0.291	0.000
	Interaction Pack-years*SZ vs Control	-0.359	0.383	0.350
MEF_{25-75} (L) †	per Pack-year (Control)	-0.021	0.006	<0.001
	per Pack-year (SZ)	-0.042	0.012	<0.001
	Interaction Pack-years*SZ vs Control	-0.024	0.014	0.082
FET (s) †‡	per Pack-year (Control)	0.106	0.031	<0.001
	per Pack-year (SZ)	0.183	0.047	<0.001
	Interaction Pack-years*SZ vs Control	0.097	0.054	0.072

Adjusted for pack-years, random variable (ID) and relatedness.

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

†Adjusted for age, sex, height, weight, pack-years, random variable (ID) and relatedness.

‡ Also adjusted for FVC (L). Statistical method *lmekin* in *coxme* package in R.

Table E5. Linear mixed model assessment results of smoking and PPT interaction as predictors of outcome in the SZ cohort (n=82)

Variate-covariate interaction	Outcome Variable	Interaction Effect	St.Err	*P* Value
a. Ever-smoker*Below PPT	FEV_1pp	0.136	8.26	0.99
	FVCpp	-1.922	8.56	0.82
	FEV_1/FVC	0.034	0.04	0.44
	$MMEF_{25-75}$pp	22.454	12.24	0.07
	FET†	0.581	1.82	0.75
b. Pack-years*Below PPT	FEV_1pp	0.158	0.385	0.68
	FVCpp	0.091	0.392	0.82
	FEV_1/FVC	0.005	0.002	0.023
	$MMEF_{25-75}$pp	-0.042	0.578	0.94
	FET†	0.019	0.094	0.84

Co-variates: above/below-PPT, Lung-Index status, and in a) smoker status or b) pack year history.

Also adjusted for age, sex, height, weight.

† Also adjusted for age, sex, height, weight and FVC (L).

All analyses include random variable (ID) and adjustment for kinship matrix.

Statistical model *lmekin.*

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table E6. Characteristics of CT data cohort stratified by a) smoking status and b) lung-Index status

a. Characteristics of SZ CT-thorax cohort, stratified by smoker-status

Characteristics	**Never-smoker**	**Ever-smoker**	***P* Value**
Subjects, *n*	17	33	
Age (years)	51.35 (12.69)	53.03 (10.36)	0.62
Sex = Male (%)	7 (41.2)	17 (51.5)	0.69
Pack-years	0	19 [11.00, 31.00]	-
FEV_1pp	104.00 [98.00, 118.00]	84.00 [54.00, 107.00]	0.011*
Emphysema on CT (%)	0 (0.0)	11 (33.3)	0.020*

b. Characteristics of CT cohort stratified by lung-index status

Characteristics	**Non-Lung-Index**	**Lung-Index**	***P* Value**
Subjects, *n*	38	12	
Age (years)	50.92 (11.57)	57.33 (8.06)	0.08
Sex = male (%)	19 (50.0)	5 (41.7)	0.86
Ever-smoker (%)	23 (60.5)	10 (83.3)	0.27
Pack-years	5.75 [0.00, 17.20]	27.50 [10.75, 41.00]	0.011*
FEV_1pp	100.00 [84.00, 115.50]	53.50 [29.00, 82.75]	<0.001**
Emphysema on CT (%)	5 (13.2)	6 (50.0)	0.022**

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table E7. Multivariate analysis of predictors of lung function decline in a longitudinal follow up cohort of persons with SZ AATD (n=60)

Outcome	Coefficients:	Estimate	95% CI	Std.Err	*P* Value
ΔFEV_1 (ml/year)	Former-smoker (vs Never)	-3.81	-15.00 to 7.38	5.71	0.51
	Baseline COPD (vs normal)	-12.87	-24.92 to -0.83	6.15	0.041*
	AAT level below-PPT (vs above)	-2.09	-12.56 to 8.38	5.34	0.70
	Baseline Emphysema (vs none)	-0.73	-16.72 to 15.26	8.16	0.93
ΔFVC (ml/year)	Former-smoker (vs Never)	-4.47	-15.30 to 6.36	5.53	0.42
	Baseline COPD (vs normal)	-9.51	-21.18 to 2.16	5.95	0.12
	AAT level below-PPT (vs above)	1.87	-8.26 to 12.01	5.17	0.72
	Baseline Emphysema (vs none)	3.42	-12.07 to 18.90	7.90	0.67
ΔFEV_1/FVC Ratio (/year)	Former-smoker (vs Never)	-0.01	-0.04 to 0.03	0.02	0.69
	Baseline COPD (vs normal)	0.02	-0.02 to 0.06	0.02	0.32
	AAT level below-PPT (vs above)	-0.03	-0.06 to 0.01	0.02	0.13
	Baseline Emphysema (vs none)	-0.05	-0.10 to 0.00	0.02	0.06
ΔDLCO (mmol/mm Hg/min /year)	Former-smoker (vs Never)	0.012	-0.124 to 0.148	0.069	0.86
	Baseline COPD (vs normal)	0.070	-0.077 to 0.216	0.075	0.36
	AAT level below-PPT (vs above)	-0.084	-0.211 to 0.044	0.065	0.20
	Baseline Emphysema (vs none)	-0.188	-0.382 to 0.007	0.099	0.06

Outcome assessed was slope of lung function parameter against time. Slopes were determined for each individual using linear mixed models (*lmer* in R) adjusting for age, sex, weight and height (all individuals were Irish Caucasian therefore no adjustment for ethnicity was performed) as well as random effects of time and individual. The effect of categorical covariates (smoker status, baseline COPD, PPT, Emphysema) on slope was then assessed in a multivariate linear model (*glm* in R).

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Table E8. Patient characteristics for Anti-NE capacity comparison cohort. All individuals are SZ genotype. Mean and (Standard Deviation) shown (with % for categorical traits)

	Never-smoker	**Former-smoker**	***P* Value**
Subjects, *n*	10	10	
Age (years)	52.70 (13.59)	53.00 (11.97)	0.96
Male sex (%)	2 (20.0)	7 (58.3)	0.17
Pack years smoked	0.00	17.41 (10.70)	-
FEV1pp	107.10 (10.00)	90.91 (27.93)	0.100
Emphysema on CT (%)	0 (0.0)	3 (30.0)	0.28
AAT level (g/L)	0.61 (0.17)	0.58 (0.07)	0.57

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

Online supplement references

E1. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. Eur Respir J 2005;26:948-68.

E2. Chen H, Conomos MP. GMMAT: Generalized Linear Mixed Model Association Tests. ver 1.1.0. R version 3.6. 2019.)

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

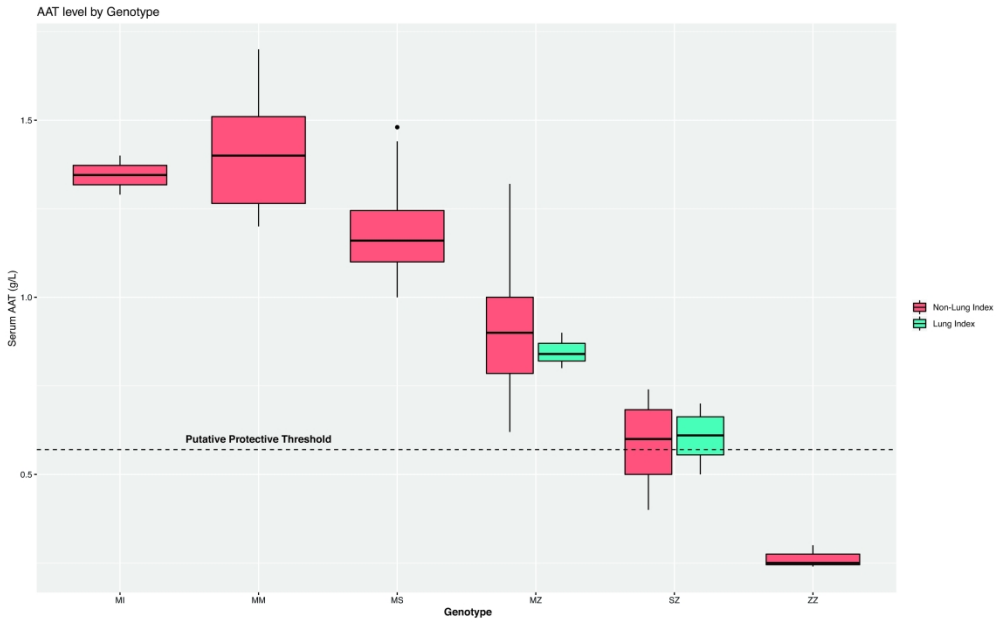

Figure E1: Family study participant serum AAT levels stratified by genotype and Lung/Non-Lung-index status demonstrating that only the SZ genotype is associated with levels straddling the "Putative Protective Threshold".

374x232mm (300 x 300 DPI)

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC

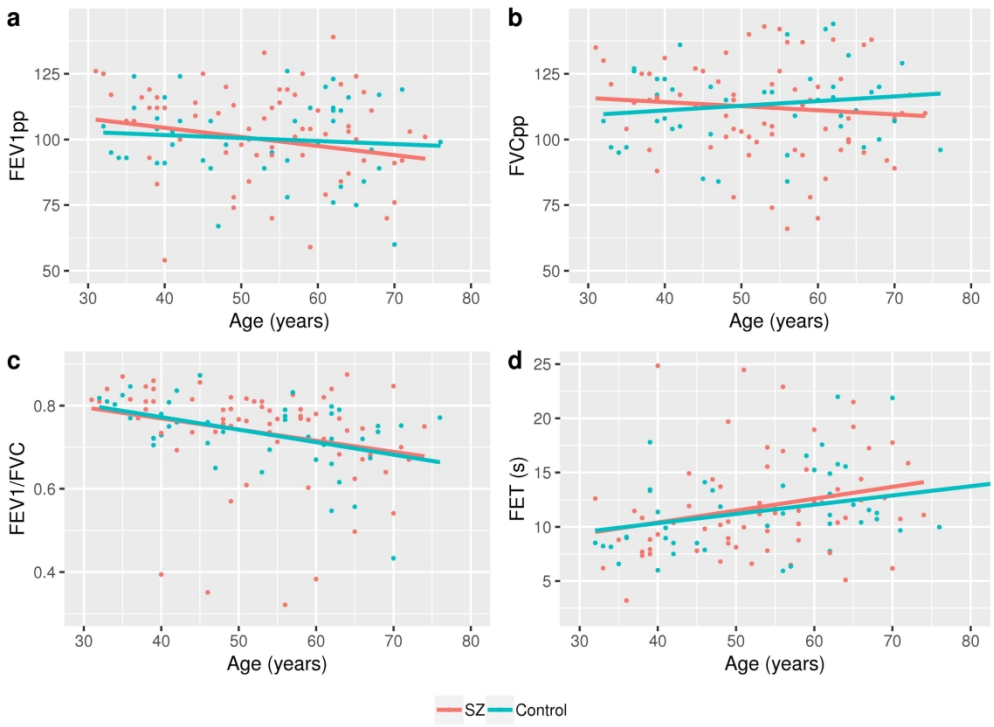

Figure E2: Univariate linear regression analyses of effect of age on spirometry parameters. No significant difference in effect of increasing age on spirometry is demonstrated between SZ and control cohorts (P Value not significant for all).

197x145mm (300 x 300 DPI)

AJRCCM Articles in Press. Published March 20, 2020 as 10.1164/rccm.202002-0262OC