On line supplement: SZ alpha-1 antitrypsin deficiency and pulmonary disease: more like MZ, not like ZZ".

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Supplementary methodology.

Study design

AAT typing 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)[1] and/or confirmatory genotyping[2] performed through the National Targeted Detection Program for AATD, at Beaumont Hospital, Dublin, Ireland, with AAT levels measured by turbidimetry. Patient data from the registry were exported and pseudo-anonymised using study-specific identification numbers (ID). Inclusion in the final analysis was restricted to the MZ, SZ and ZZ genotypes and required availability of all key data variables: age, sex, ascertainment-mode, pack-year/smoking history, height, weight and AAT level at time of diagnosis, as well as pulmonary function test results (PFT) including both absolute and percentage predicted (pp); forced expiratory volume in 1 seconds (FEV_{1pp}), forced vital capacity (FVC), FEV_1/FVC ratio. Diffusion capacity for carbon monoxide ($DLCO_{pp}$) was assessed where available. Where key variables were missing, chart reviews were undertaken to identify required data at the time of the first registry entry. Individuals diagnosed due to pulmonary disease or pulmonary symptoms were designated “lung-index” cases with those diagnosed due to alternative causes (family screening, liver disease, panniculitis) designated “nonlung-index”. “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]. Patients were further categorised by AAT levels by “above” or “below” the PPT (11μM or 0.57g/L). Analyses of the effect of the PPT were restricted to the SZ genotype only (as the only cohort encompassing levels both above- and below- the PPT).

Pulmonary function

Spirometry results were exported from the National Irish AATD Registry. These data are imported from the database of the Department of Pulmonary Physiology, following each clinical review at the National Centre for Expertise for AATD,

Beaumont Hospital, Dublin, Ireland. Spirometry and diffusion capacity were measured according to the standardised ATS/ERS guidelines [3 4].

CT data

CT reports included in the National Irish AATD Registry were reviewed and presence of visually-defined emphysema was recorded as a binary outcome.

Statistical analysis

All analyses were performed in RStudio Version 1.1.463 (www.cran.r-project.com). One single measurement for each individual – that from the first registry entry - were compared. For all analyses a p value of <0.05 was considered statistically significant. Continuous data were validated for normality using the Shapiro-Wilks test. Normally and non-normally distributed data were analysed by Student t test and Mann-Whitney U test respectively, with analyses corrected for multiplicity using Bonferroni's method in the univariate analyses comparing baseline characteristics (e.g. table 1 and supplementary table 3). Correlations were measured using Pearson's method and Spearman's method for normal and non-normal data respectively. Linear mixed model analyses were used to perform multivariable analyses (*lmer* function in R) modelling predictors and confounders as fixed effects and subject ID as a random effect. Analyses comparing percentage predicted values were adjusted for age and pack-years with analyses comparing the FEV_1/FVC ratio also adjusted for sex, height and weight. For calculation of estimated effects of genotypes the SZ genotype was modelled as the reference factor with MZ and ZZ as comparators to estimate the magnitude of effect on outcomes relative to SZ. Other categorical variables were coded with the presumed lowest risk lowest category as the reference factor (e.g. never-smoker, non-lung-index) with higher risk factors as comparators. Adjusted odds ratios (OR) for binary outcomes were calculated using binomial logistic regression in generalised mixed models (*glm* function in R). The effect of genotype on lung function was assessed in stratified analyses first comparing genotypes by smoking status [(never-smoker SZ vs MS and ZZ), then (ever-smoker SZ vs MZ and ZZ)] and then in secondary analyses stratifying by smoking and age over 50 years of age to enrich for age-related decline [(age >50 and never-smoker SZ vs MZ and ZZ), then (age >50 and ever-smoker SZ vs MZ and

ZZ)].

Supplementary results

The relationship of age and FEV_{1pp} by genotype

The genotype-specific correlation of age and decline in predicted lung airflow (FEV_{1pp}) was examined in never-smokers to remove the confounding effect of smoking. No significant correlation between age and FEV_1pp was seen in MZ or SZ cohorts (rho 0.01, p = 0.9 and R -0.09, 95% CI; -0.35 to 0.17, p = 0.49 respectively), whilst a statistically significant correlation was seen in the ZZ cohort (rho -0.51, p < 0.0001, figure 1). Univariate regression analyses demonstrated no difference in slope for MZs compared to SZs (FEV_{1pp} +0.13%/year ± 0.24 vs SZ, p = 0.59) while a significant difference was seen for ZZs vs SZs (FEV_{1pp} -0.71%/year ± 0.23, p = 0.002).

The relationship of smoking intensity and FEV_{1pp} by genotype

The effect of pack-years smoked on FEV_{1pp} was compared between genotypes in ever-smokers by linear mixed regression model to adjusted for age. The interaction between pack-years and genotype was examined. The interaction of the MZ genotype with pack-years on FEV_{1pp} did not differ from the SZ genotype (+0.08%/pack-year ±0.17, p = 0.63), however the ZZ genotype interaction with pack-years on FEV_{1pp} differed significantly to the SZ genotype (-0.39%/pack-year ± 0.19 vs SZ, p = 0.039) (supplementary figure 1).

Ascertainment mode (lung-index status).

The effect of lung-index status on FEV_1pp, examined in a mixed model adjusting for smoking (ever vs never), age and genotype was found to be -15.06% (95% CI; 19.59 to -9.88 vs non-lung-index, p < 0.0001) (supplementary table 1). The OR (adjusted for age, sex and pack-years smoked) of being lung-index case relative to SZs did not differ significantly for MZs (OR 1.28, 95% CI; 0.77 to 2.12, p = 0.32) but was significantly increased for ZZs (OR 2.11, 95% CI; 1.31 to 3,39, p < 0.001).

The putative protective threshold (PPT).

The effect of the PPT on outcomes in the SZ cohort was explored in two ways. The effect of AAT levels below the PPT on the OR of being lung-index was assessed in the SZ cohort, adjusting for age, sex and pack-years smoked. In this model, AAT levels below the PPT were not associated with an increased OR of lung-index status (OR 0.65, 95% CI; 0.29 to 1.48, p = 0.31). A linear mixed regression model was then fitted to assess the effect of AAT levels below the PPT on FEV_{1pp}, adjusting for packyears and lung-index status demonstrating no significant effect, (+4.98% ± 6.0 vs above-PPT cohort, p = 0.411).

CT Data

CT thorax reports were available for 448 individuals (MZ = 136, SZ = 102, ZZ = 210) (supplementary table 3). Visually-defined emphysema was reported significantly more frequently on CT scan of ZZ individuals (21.3% vs 15.7% vs 54.8%, p < 0.001). Among never-smokers 0/54 of MZ, 0/48 of SZ and 27/77 (35%) of ZZs had visually defined emphysema reported (p <0.001 for MZ vs ZZ and SZ vs ZZ). The OR of emphysema (adjusted for lung-index status, age and pack-years) being reported on CT relative to SZ individuals was not significant for MZs (OR 1.18, 95% CI; 0.49 to 2.80, p = 0.70) but was significant for ZZs (at 13.51, 95% CI; 6.19 to 29.47, p < 0.0001). When stratifying the analysis to only include ever-smokers, the OR of emphysema remained non-significant for MZs (OR 1.18, 95% CI; 0.49 to 2.85, p = 0.72) and reduced to 7.71 for ZZs (95% CI; 3.40 to 17.46, p < 0.0001) compared to SZs.

Supplementary tables

	Estimated FEV_{1pp} Effect	SEM	92.5%	97.5%	P value
MZ vs SZ	-1.79%	±3.16	-7.93	4.36	**0.57**
ZZ vs SZ	-16.84%	±3.02	-22.45	-10.63	**<0.0001**
Ever-smoker (vs never)	-17.64%	±2.42	-21.97	-12.52	**<0.0001**
Lung-index (vs not)	-15.06%	±2.42	-19.59	-9.88	**<0.0001**
Method Lmer Adjusted for age and random effect (ID)					

Supplementary table 1: mixed model assessment of the effect of genotype (relative to SZ), smoking history and ascertainment mode (index-status) on $FEV1_{pp}$ in the whole study population. No difference was observed between MZs and SZs. ZZ genotype, ever-smoking and lung-index ascertainment were all associated with worse $FEV1_{pp}$ ($p < 0.0001$ for all).

	*FVC_{pp}			*FEV_1/FVC ratio			†$DLCO_{pp}$		
	Effect	**Std.Error**	**P value**	**Effect**	**Std.Error**	**P value**	**Effect**	**Std.Error**	**P value**
Never smokers	**(n = 199)**			**(n = 199)**			**(n = 135)**		
MZ vs SZ	-0.25%	±4.83	0.95	+0.04	±0.03	0.29	-3.45	±3.85	0.37
ZZ vs SZ	-4.97%	±3.66	0.16	-0.03	±0.03	0.38	-6.20	±3.81	0.11
Never smokers >50	**(n = 94)**			**(n = 94)**			**(n = 68)**		
MZ vs SZ	+4.25%	±6.06	0.49	+0.08	±0.04	**0.10**	-4.43	±5.34	0.41
ZZ vs SZ	-5.96%	±6.00	0.32	-0.06	±0.04	0.09	-13.53	±5.47	**0.013**
Smokers	**(n = 287)**			**(n = 287)**			**(n = 158)**		
MZ vs SZ	+1.39%	±3.68	0.71	-0.01	±0.02	0.80	-3.22	±3.81	0.39
ZZ vs SZ	-6.22%	±3.46	0.07	-0.15	±0.02	**<0.0001**	-16.78	±3.43	**<0.0001**
Smokers >50	**(n = 149)**			**(n = 149)**			**(n = 91)**		
MZ vs SZ	-4.80%	±5.19	0.36	+0.01	±0.03	0.78	-6.78	±5.32	0.22
ZZ vs SZ	-7.00%	±5.05	0.17	-0.12	±0.03	**<0.0001**	-21.10	±54.77	**<0.001**

Method: LMER
Adjusted for age, and lung-index status (and pack-years in smokers analyses), as well as sex, height and weight in FEV1/FVC ratio analyses
*Never-smokers, n=64 MZ, 56 SZ, 79 ZZ
*Never-smokers > Age 50, n=31 MZ, 28 SZ, 40 ZZ
*Ever-smokers, n= 92 MZ, 61 SZ, 134 ZZ
*Ever-Smoker > Age 50, n=54 MZ, 34 SZ, 63 ZZ
†Never-smokers, n=41 MZ, 47 SZ, 47 ZZ
†Never-smokers > Age 50, n=21 MZ, 25 SZ, 22 ZZ
†Ever-smokers, n= 43 MZ, 50 SZ, 65 ZZ
†Ever-Smoker > Age 50, n=25 MZ, 28 SZ, 38 ZZ

Supplementary table 2: Stratified mixed model analyses of estimated effect on lung function for the MZ and ZZ genotypes relative to the SZ genotype. No significant difference were seen between MZ and SZ cohorts for FVC_{pp}, FEV1/FVC ratio or $DLCO_{pp}$, Significant differences in both FEV1/FVC ratio or $DLCO_{pp}$ between SZs and ZZs were seen, most pronounced when stratifying by age over 50 or by ever smoking.

	MZ	SZ	ZZ	P value		
n	**136**	**102**	**210**	**MZ vs SZ**	**MZ vs ZZ**	**SZ vs ZZ**
Age (Y)	51.0 [42.0, 63.0]	50.5 [36.3, 59]	49.0 [41.0, 57.0]	0.226	0.049	0.644
Sex = Male (%)	54 (39.7)	50 (49.0)	116 (55.2)	0.152	**0.004**	0.301
Lung-index (%)	70 (51.5)	43 (42.2)	128 (61.0)	0.154	0.816	**0.001**
Ever-smoker (%)	82 (60.3)	54 (52.9)	133 (63.3)	0.256	0.569	0.788
Emphysema (%)	29 (21.3)	16 (15.7)	115 (54.8)	0.271	**<0.001**	**<0.001**
BMI (kg/m^2)	26 [23, 30]	27 [22.6, 30.9]	26. [22.7, 29.0]	0.824	0.383	0.216
$FEV1_{pp}$	90.5 [71.8, 108.3]	95 [82.5, 107]	70 [42.3, 99.0]	0.305	**<0.001**	**<0.001**
FVC_{pp}	106 [92.8, 116.5]	104.5 [95, 116.5]	102.0 [83.0, 115.0]	0.974	0.037	0.045
FEV_1/FVC ratio	0.72 [0.61, 0.81]	0.75 [0.68, 0.81]	0.56 [0.41, 0.77]	0.137	**<0.001**	**<0.001**
$DLCO_{pp}$	84 [73.3, 91.8]	87 [71.0, 96.3]	71 [52.0, 91.0]	0.343	**0.002**	**0.001**

Supplementary table 3: Demographics of CT data cohort. Data are presented as mean (±SD) for parametric, median + [IQR] for non-parametric and number (%) for categorical. Bonferroni-adjusted significance threshold $p < 0.005$.

Supplementary figure 1: Relationship of pack-years smoked to FEV_{1pp} at presentation, classified by genotype. In linear regressions adjusted for age, no significant difference in the slope of FEV_{1pp}:pack-years is seen between MZ and SZ individuals (FEV_{1pp} +0.08%/pack-year ±0.17, p = 0.63), with a significant difference in slope between SZs and ZZs seen (-0.39%/pack-year ±0.19 vs SZ, p = 0.039).

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