

# Small airways dysfunction is associated to Alpha-1 Antitrypsin Deficiency in patients with asthma

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## Research article

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## Abstract

**Background** Alpha-1 Antitrypsin Deficiency (AATD) is a hereditary genetic disorder involving lungs in adults, characterized by low serum concentration of the protein alpha-1 antitrypsin (AAT). Several reports indicate that asthma is common in AATD patients, but there are only few data on respiratory function in asthmatic patients with intermediate AATD. The aim of the study is to evaluate lung function in asthmatic outpatients with AATD vs. asthmatic subjects without AATD.

**Methods** We enrolled 57 outpatients affected by mild to moderate asthma from the University Hospital of Parma, Italy. We submitted to genetic analysis the asthmatic outpatients with a serum AAT concentration <113 mg/dL and those with relatives affected by AATD. Subsequently, the study population was split into AATD and not AATD subjects. We assessed lung function through a flow-sensing spirometer and the small airways parameters through an impulse oscillometry system.

**Results** The values of FVC (% predicted) and of the RV/TLC (%) ratio were respectively lower and higher in patients with AATD vs. patients without AATD, showing a significantly greater air trapping ( $p = 0.014$  and  $p = 0.017$  respectively). Moreover, patients with AATD in comparison to patients without AATD showed lower FEV<sub>3</sub> (% predicted) and FEV<sub>6</sub> (L) values. A significant and positive correlation ( $p=0.041$ ;  $r=0.894$ ) occurred between the RV/TLC ratio and the years of smoking in the group of asthmatic patients with AATD.

**Conclusions** AATD predisposes asthmatic heterozygote patients with PI\**MZ*, PI\**MS* and intermediate levels of AAT to small airways dysfunction and to increased values of pulmonary air trapping.

## Background

Alpha-1 Antitrypsin Deficiency (AATD) is a genetic condition that predisposes subjects to pulmonary diseases. It is characterized by a reduced serum concentration of the Alpha-1 Antitrypsin (AAT) protein. The relationship between AATD and respiratory diseases has been a topic of research activity since this deficiency was discovered in the early 1960s.<sup>1</sup> Previous studies suggested an association between AATD and asthma;<sup>2,3,4</sup> on this basis, the literature recommends that all adult-onset asthmatic patients should be screened for AATD.<sup>5,6</sup>

Pulmonary function has been studied especially in patients affected by Chronic Obstructive Pulmonary Disease (COPD) and severe AATD,<sup>7,8</sup> but only a few data are available on respiratory function in asthmatic patients with intermediate AATD. The relative importance of intermediate AATD, mainly caused by heterozygosity of pathological alleles in the SERPINA1 gene, in the pathogenesis of COPD in adult subjects is still controversial.<sup>9</sup>

We hypothesized that asthmatic patients affected by AATD could have abnormal spirometry and oscillometry values compared to asthmatic patients without AATD. The objective of this study was therefore to evaluate lung function in asthmatic outpatients affected by AATD vs. asthmatic subjects without AATD.

## Methods

### Study Subjects

We enrolled 57 non-severe asthmatic outpatients, 18 years of age or older, with a diagnosis of bronchial asthma, in stable clinical conditions, from the University Hospital of Parma (Italy).

Asthma was diagnosed based on the combined presence of respiratory symptoms, reversible airflow obstruction and bronchial hyperactivity, as assessed by the methacholine (MCh) test. Patients with other concomitant lung diseases were excluded from the study.

### Measurements

We recorded the following data in all patients: anthropometric variables (sex, age, body mass index – BMI in kg/m<sup>2</sup>), smoking habits (current/former), number of packs per year. We used the Asthma Control Test (ACT)<sup>10</sup> to assess symptoms and asthma-related morbidity. All asthmatic outpatients underwent a quantitative analysis of the serum concentration of AAT and of C-reactive Protein (CRP). Outpatients with a AAT serum concentration < 113 mg/dL, and with a concomitant normal serum CRP concentration, and patients with relatives identified as AATD were submitted to genetic analysis, i.e. to the sequencing of the SERPINA1 gene, consistently with the recent guidelines.<sup>11</sup> On the basis of the genetic test results, the study population was split in two groups: patients with a pathological genotype and patients with a non-pathological genotype (PI\**MM*). Patients were recruited during scheduled office visits.

### Spirometry

We used a flow-sensing spirometer connected to a computer for data analysis (Vmax22 and 6200; Sensor-Medics; Yorba Linda, CA, USA) to measure lung parameters. Forced expiratory volume in the 1st second (FEV<sub>1</sub>) and forced vital capacity (FVC) were recorded and expressed as absolute values (in liters, L) and as percentage of a predicted value (% predicted). The FEV<sub>1</sub>/FVC value was recorded as a ratio. Total lung capacity (TLC) was obtained as the sum of functional residual capacity and the linked inspiratory capacity (IC). Residual volume (RV) value was obtained by subtracting vital capacity from TLC. The ratio of residual volume to total lung capacity (RV/TLC) was also recorded as index of lung air trapping. Diffusing capacity for carbon monoxide (DLCO) and transfer coefficient of the lung for carbon monoxide (KCO) were measured as a percentage of predicted value (% predicted). At least 3 measurements were taken for each spirometry test and lung volume variable to ensure data reproducibility.<sup>12</sup>

In order to measure the smaller airway contributions, forced expiratory volume in 3 seconds (FEV<sub>3</sub>, in L and % predicted) and forced expiratory volume in 6 seconds (FEV<sub>6</sub>, in L) were recorded. The FEV<sub>3</sub>/FVC, FEV<sub>6</sub>/FVC and FEV<sub>3</sub>/FEV<sub>6</sub> values were recorded as ratio and were considered as measures able to detect small airway dysfunctions (SAD). Moreover, we recorded maximal expiratory flow-rates at 25, 50 and 75% of the vital capacity (MEF<sub>25</sub>, MEF<sub>50</sub>, MEF<sub>75</sub>, expressed as L/s and as % predicted).

#### Impulse Oscillometry

Impulse Oscillometry (IOS) was performed using the Jaeger MasterScreen-IOS instrument (Carefusion Technologies, San Diego, CA, USA) as per standard recommendations.<sup>13</sup> Patients were asked to wear a nose clip and were seated during tidal breathing with their neck slightly extended and their lips sealed tightly around the mouthpiece, while firmly supporting their cheeks with their hands. The procedure was repeated at least 3 times, each lasting 30 s, and mean values were chosen. Respiratory resistances at 5 and 20 Hz (R5 and R20, in kPa/[L/s]) were used as index of total and proximal airway resistance, respectively, and the fall in resistance from 5 to 20 Hz (R5-R20 in kPa/[L/s]) was considered an index for the resistance of peripheral airways. The reactance at 5 Hz (X5 in kPa/[L/s]) and the resonant frequency (F<sub>res</sub> in kPa/[L/s]) were considered representative markers of peripheral airway dysfunction. Moreover, impedance at 5 Hz (Z5 in kPa/[L/s]) and the area of reactance (AX in kPa/[L/s]) were measured.

## Statistical Analysis

A Kolmogorov-Smirnov test was used to assess the normality of distribution in all variables.

Group data with normal distribution are presented as mean ± SD, while data with non-normal distribution are presented as median values (1st quartile; 3rd quartile). Comparisons of means among groups were performed through the analysis of variance (t-tests) for continuous variables. The non parametric Kruskal-Wallis test was used for data with non-Gaussian distribution.

For correlation analysis, the Pearson or Spearman correlation coefficients were used for linear or normally distributed variables and for non linear or non normally distributed variables, respectively. Receiver operating characteristic (ROC) curves were generated to calculate the area under the curve (AUC) with 95% confidence interval (CI) and to select the best cut-off value with the related sensibility and specificity.

A p value < 0.05 was considered statistically significant. Statistical analysis was performed using the SPSS Statistics version 25.0 software package (IBM, Armonk, New York, USA).

## Results

In asthmatic patients, the mean age was 57 ± 15 years and 54% were female subjects; median serum AAT concentration was 109 mg/dL. Asthmatic subjects were classified as patients with AATD (n = 22) and without AATD (n = 35) according to their PI\* (Proteinase Inhibitor). The frequency of deficient genotypes was: 11 patients with the PI\*MS genotype, 9 with the PI\*MZ genotype, only one with the PI\*MM<sub>Malton</sub> and the PI\*SS genotype (Table 1). Median values of AAT concentration were lower in patients affected by AATD vs. PI\*MM patients (97.8 mg/dL vs. 111 mg/dL, p = 0.000), while values of BMI were higher in patients affected by AATD vs. PI\*MM patients (27.5 kg/m<sup>2</sup> vs. 25 kg/m<sup>2</sup>, p = 0.007). Demographic and clinical characteristics of the patients are summarized in Table 2. No significant differences were observed in pack/years data and mean age at smoking onset between groups with and without genetic AATD. Forty-three asthmatic patients (75% of cases) showed atopy, with skin-test positive for common aeroallergens, 20 were with AATD (91% of cases) and 23 without AATD (66% of cases) (p = 0.031).

Table 1  
AAT genotypes distribution among asthmatic patients

	AATD
Subjects, nr (%)	22/57 (38.6)
MS, nr (%)	11 (50.0)
MZ, nr (%)	9 (41.0)
MM <sub>Malton</sub> , nr (%)	1 (4.5)
SS, nr (%)	1 (4.5)
Data are shown as number of patients (%).	

Table 2  
Demographic and laboratory data, smoking habits in reference to presence/absence of genetic AATD

<b>Variables</b>	<b>Asthmatic patients</b>	<b>AATD</b>	<b>Non AATD</b>
Subjects, nr	57	22	35
BMI, kg/m <sup>2</sup>	26.0[24.0;29.0]	27.5[25.0;30.0] <sup>a</sup>	25.0[23.0;28.0]
Age, years	57 ± 15	57 ± 16	56 ± 15
Women, %	54	64	46
Former smokers, nr (%)	13(23)	5(23)	8(23)
Pack/years, nr	10[4;20]	10[8;28]	8[3;19]
Years of smoking, nr	19 ± 11	21 ± 10	19 ± 12
AAT concentration, mg/dL	109.0[97.9;112.5]	97.8[83.2;107.0] <sup>b</sup>	111.0[105.0;115.0]
Atopy, nr (%)	43(75)	20(91) <sup>c</sup>	23(66)
ACT, total score	25[23;25]	24[23;25]	25[23;25]
Data are shown as number of patients (%), means ± SD or medians [1st quartile; 3rd quartile].			
Boldface variables are statistically significant.			
Abbreviations: BMI, body mass index; AAT, serum α <sub>1</sub> -antitrypsin; ACT, asthma control test.			
<sup>a</sup> p value = 0.007 vs. non AATD.			
<sup>b</sup> p value < 0.001 vs. non AATD.			
<sup>c</sup> p value = 0.031 vs. non AATD.			

The results of the respiratory function tests are summarized in Table 3. FVC (%) and the RV/TLC ratio (%) were respectively lower and higher in patients with AATD than in those without AATD, showing a significantly greater air trapping (p = 0.014 and p = 0.017, respectively), as shown Fig. 1. We did not find any significant difference in reference to other variables.

Table 3  
Lung function tests in reference to presence/absence of genetic AATD

<b>Variables</b>	<b>Asthmatic patients</b>	<b>AATD</b>	<b>Non AATD</b>
Subjects, nr	57	22	35
FEV <sub>1</sub> , L	2.53 ± 0.90	2.30 ± 0.87	2.67 ± 0.90
FEV <sub>1</sub> , % predicted	92.7 ± 15.2	88.9 ± 13.9	95.0 ± 15.6
FVC, L	3.55 ± 1.13	3.16 ± 1.02	3.80 ± 1.14
FVC, % predicted	108.2 ± 16.2	101.6 ± 14.8 <sup>a</sup>	112.3 ± 15.9
FEV <sub>1</sub> /FVC, %	71.0 ± 9.9	72.6 ± 8.6	70.0 ± 10.6
TLC, L	5.86 ± 1.18	5.50 ± 1.13	6.08 ± 1.16
TLC, % predicted	106.7 ± 12.6	104.6 ± 11.3	107.9 ± 13.4
RV, L	2.25 ± 0.61	2.30 ± 0.55	2.22 ± 0.65
RV, % predicted	115.5 ± 26.9	121.4 ± 26.7	111.9 ± 26.7
RV/TLC, %	38.8 ± 10.1	42.8 ± 9.9 <sup>b</sup>	36.3 ± 9.5
DLCO, % predicted	92.2 ± 13.4	90.3 ± 13.4	93.4 ± 13.4
KCO, % predicted	97.0[87.0;109.4]	96.0[86.8;109.0]	97.0[87.0;111.0]
Data are shown as means ± SD or medians [1st quartile; 3rd quartile].			
Boldface variables are statistically significant.			
Abbreviations: FEV <sub>1</sub> , forced expiratory volume in one second; FVC, forced vital capacity; FEV <sub>1</sub> / FVC, forced expiratory volume in one second to forced vital capacity ratio; TLC, total lung capacity; RV, residual volume; RV / TLC, residual volume to total lung capacity ratio; DLCO, diffusing capacity for carbon monoxide; KCO, transfer coefficient of the lung for carbon monoxide.			
<sup>a</sup> p value = 0.014 vs. non AATD.			
<sup>b</sup> p value = 0.017 vs. non AATD.			

Table 4 summarizes the small airways values measured through oscillometry and spirometry in asthmatic patients. Patients with AATD showed lower values of FEV<sub>3</sub> (% predicted) and FEV<sub>6</sub> (L) in comparison to those without AATD (Figs. 2 and 3).

Table 4

Values of small airways measured by impulse oscillometry and spirometry in reference to presence/absence of genetic AATD

Variables	Asthmatic patients	AATD	Non AATD
Subjects, nr	57	22	35
Z5, kPa/(L/s)	0.47 ± 0.16	0.51 ± 0.15	0.43 ± 0.17
R5-R20, kPa/(L/s)	0.07 ± 0.08	0.08 ± 0.09	0.07 ± 0.07
R5, kPa/(L/s)	0.43 ± 0.14	0.47 ± 0.12	0.40 ± 0.15
R20, kPa/(L/s)	0.36 ± 0.10	0.39 ± 0.07	0.34 ± 0.12
AX, kPa/(L/s)	0.58[0.27;1.37]	0.64[0.35;1.56]	0.53[0.16;1.14]
X5, kPa/(L/s)	-0.15[-0.22;-0.11]	-0.15[-0.28;-0.13]	-0.14[-0.20;-0.08]
F <sub>Res</sub> , Hz	16.00 ± 5.65	17.16 ± 5.43	15.03 ± 5.75
FEV <sub>3</sub> , L	3.12 ± 1.12	2.73 ± 1.05	3.38 ± 1.10
FEV <sub>3</sub> , % predicted	91.8 ± 14.9	85.6 ± 16.9 <sup>a</sup>	95.9 ± 12.1
FEV <sub>6</sub> , L	3.24 ± 1.13	2.75 ± 0.91 <sup>b</sup>	3.55 ± 1.16
FEV <sub>3</sub> /FVC, %	89.5 ± 4.8	88.5 ± 5.7	90.2 ± 4.1
FEV <sub>6</sub> /FVC, %	95.4 ± 3.7	94.5 ± 4.3	96.0 ± 3.1
FEV <sub>3</sub> /FEV <sub>6</sub> , %	92.4[89.9;93.5]	91.1[88.6;93.1]	93.1[91.8;93.7]
MEF <sub>25</sub> , L/s	0.57[0.34;0.94]	0.50[0.20;0.65]	0.58[0.42;0.99]
MEF <sub>25</sub> , % predicted	37.4[31.2;55.3]	35.1[21.1;43.5]	40.5[32.7;58.3]
MEF <sub>50</sub> , L/s	2.15[1.51;3.22]	2.09[0.70;2.61]	2.15[1.76;3.38]
MEF <sub>50</sub> , % predicted	52.2[42.7;68.6]	50.6[22.0;69.0]	53.3[45.3;71.5]
MEF <sub>75</sub> , L/s	4.87 ± 2.20	4.43 ± 2.49	5.15 ± 1.97
MEF <sub>75</sub> , % predicted	78.1 ± 27.7	73.4 ± 32.5	81.2 ± 24.2
MEF <sub>75/25</sub> , L/s	1.62[1.07;2.59]	1.50[0.53;1.78]	1.70[1.21;2.73]
MEF <sub>75/25</sub> , % predicted	58.7 ± 24.3	54.3 ± 29.2	61.6 ± 20.5
Data are shown as means ± SD or medians [1st quartile; 3rd quartile].			
Boldface variables are statistically significant.			
Abbreviations: Z5, impedance at 5 Hz; R5, resistance at 5 Hz; R20, resistance at 20 Hz; AX, area of reactance; X5, reactance at 5 Hz; F <sub>Res</sub> , Resonant Frequency; FEV <sub>3</sub> , forced expiratory volume in three second; FEV <sub>6</sub> , forced expiratory volume in six second; MEF <sub>25</sub> , MEF <sub>50</sub> , MEF <sub>75</sub> , maximal expiratory flow-rates at 25, 50 and 75% of the inspiratory vital capacity.			
<sup>a</sup> p value = 0.018 vs. non AATD.			
<sup>b</sup> p value = 0.020 vs. non AATD.			

Significant results obtained by grouping the patients according to their PI\* genotype are summarized in Table 5. No difference in lung function test results and in general characteristics was observed between groups with PI\*MS and PI\*MZ genotypes, with the exception of the median values of AAT concentration (100 vs. 90.7 mg/dL, p = 0.016). However, we found an increased value of the RV/TLC ratio (%) in the subgroup of asthmatic patients with the PI\*MS genotype compared to the PI\*MM genotype (p = 0.043). The mean values of FEV<sub>6</sub> (L) were 2.73 L and 3.55 L in the PI\*MS and PI\*MM genotypes respectively, but the difference did not reach a statistical significance (p = 0.060).

Table 5  
General characteristics and lung function tests among the different genotypes.

Variables	PI*MS	PI*MZ	PI*MM	PI*MS	PI*MM	PI*MZ	PI*MM
Subjects, nr	11	9	35	11	35	9	35
BMI, kg/m <sup>2</sup>	28.0 ± 2.6	29.3 ± 7.1	25.5 ± 4.2	28.0 ± 2.6	25.5 ± 4.2	29.3 ± 7.1	25.5 ± 4.2
AAT level, mg/dL	100.0[97.8;113.0]	90.7[77.2;99.9] <sup>a</sup>	111.0[105.0;115.0]	100.0[98.0;113.0]	111.0[105.0;115.0]	90.7[77.2;99.9] <sup>c</sup>	111.0[105.0;115.0]
FVC, L	3.12 ± 1.00	3.04 ± 1.05	3.80 ± 1.14	3.12 ± 1.00	3.80 ± 1.14	3.03 ± 1.05	3.80 ± 1.14
FVC, % predicted	102.7 ± 16.6	99.3 ± 14.2	112.3 ± 15.9	102.7 ± 16.6	109.0[103.6;127.0]	97.2[91.5;114.0] <sup>d</sup>	109.0[103.6;127.0]
TLC, L	5.39 ± 0.92	5.30 ± 0.89	6.08 ± 1.16	5.39 ± 0.92	6.08 ± 1.16	5.30 ± 0.86	5.91[5.12;6.95]
RV/TLC, %	43.2 ± 9.6	42.8 ± 11.5	36.3 ± 9.5	43.2 ± 9.6 <sup>b</sup>	36.3 ± 9.5	42.9 ± 11.5	36.3 ± 9.5
FEV <sub>3</sub> , % predicted	87.9 ± 21.3	82.9 ± 11.0	95.9 ± 12.1	87.9 ± 21.3	95.9 ± 12.1	82.9 ± 11.0 <sup>e</sup>	95.9 ± 12.1
FEV <sub>3</sub> , L	2.77 ± 1.15	2.70 ± 1.01	3.38 ± 1.09	2.77 ± 1.15	3.38 ± 1.09	2.70 ± 1.00	3.38 ± 1.09
FEV <sub>6</sub> , L	2.73 ± 0.87	2.78 ± 1.00	3.55 ± 1.16	2.73 ± 0.87	3.55 ± 1.16	2.77 ± 1.00	3.55 ± 1.16
R20, kPa/(L/s)	0.40 ± 0.08	0.38 ± 0.06	0.34 ± 0.12	0.39 ± 0.08	0.34 ± 0.12	0.38 ± 0.63	0.32[0.30;0.40]
R5-R20, kPa/(L/s)	0.08 ± 0.09	0.08 ± 0.09	0.07 ± 0.07	0.08 ± 0.09	0.07 ± 0.07	0.08 ± 0.09	0.07 ± 0.07

We found statistically significant differences when we compared lung function test results and general characteristics in asthmatic patients with the PI\*MZ genotype vs. those with the PI\*MM genotype. The median AAT concentration (90.7 mg/dL vs. 111 mg/dL,  $p = 0.000$ ) and FVC value (97.2% vs. 109% predicted,  $p = 0.040$ ) were lower, while the mean value of FEV<sub>3</sub> was 82.9 ± 11% vs. 95.9 ± 12% predicted ( $p = 0.007$ ) respectively, in both groups. The RV/TLC (%) ratio mean values were 42.9% and 36.3% in the PI\*MZ and PI\*MM genotypes respectively, without reaching a statistical significance ( $p = 0.079$ ).

The comparison between asthmatic patients with a PI\*MM genotype and grouped patients with PI\*MS and PI\*MZ genotypes revealed significant differences with reference to the FVC, RV/TLC, TLC, FEV<sub>3</sub>, FEV<sub>6</sub> and R20 values.

No difference in pulmonary function was found by splitting the AATD patients into smoker and non-smoker subgroups (data not shown).

We found a significant and positive correlation ( $p = 0.041$ ;  $r = 0.894$ ) between the RV/TLC ratio and the years of smoking in the group of asthmatic patients with AATD. We did not find the same correlation in the group of patients without AATD ( $p = 0.349$ ) (data not shown).

No significant correlation was found among AAT values, lung function test results and impulse oscillometry values in asthmatic patients with AATD.

The ROC curve has been calculated to set the value of the RV/TLC ratio able to suspect the presence of an AATD in asthmatic patients (Fig. 4), and revealed an AUC of 0.681 (standard error [SE] 0.070; 95% CI 0.543–0.819;  $p < 0.05$ ) with a RV/TLC % ratio cut-off value of 29.2 (sensitivity 0.864, specificity 0.429).

## Discussion

The present study assessed the lung function in two groups of asthmatic patients with and without AATD and showed significantly lower FVC (%) values in AATD patients vs. subjects without AATD. Furthermore, we showed that the values of the RV/TLC ratio in AATD patients were significantly higher vs. subjects without AATD. Although these results should be interpreted with caution, they allow some speculation regarding the pathophysiology of pulmonary dysfunction in asthmatic patients affected by AATD. It is known that connective tissue destruction and pulmonary hyperinflation are the result of a protease-antiprotease imbalance and of proteolytic degradation of elastin and other extracellular matrix components of the respiratory tract by neutrophil elastase and other proteases, whose activity is unopposed and enhanced because of AATD in the pulmonary parenchyma and airways. Therefore, the significant differences observed in our spirometry-derived parameters FVC and RV/TLC ratio suggest primarily an insufflation component that probably represents a very early measurement of the airway obstruction in asthmatic patients with AATD.

The RV/TLC ratio has received little emphasis in studies focusing on pulmonary dysfunction in AATD subjects, while other lung function-related parameters have been taken into consideration: Vance et al.<sup>14</sup> demonstrated a reduction in FEV<sub>1</sub> and FEV<sub>1</sub>/FVC ratio, and an increased total pulmonary resistance in PI\*MZ subjects compared to PI\*MM control subjects. Other studies showed a decline in FEV<sub>1</sub> and KCO values in a group of AATD patients with PI\*MZ genotype not receiving any augmentation therapy.<sup>7</sup>

While FEV<sub>1</sub> values can indicate large airway obstructions, FEV<sub>3</sub> and FEV<sub>6</sub> values could better reflect smaller airway obstructions, and be a more sensitive measure to diagnose early airway obstruction.<sup>15</sup>

Our data suggest that the increased inflammation in asthmatic patients with AATD causes a more evident and early dysfunction and narrowing in the small airways, where FEV<sub>3</sub> and FEV<sub>6</sub> values were significantly lower compared to asthmatic non carrier patients. A study has recently suggested that the AAT protein plays relevant immune-modulating functions and might affect eosinophils, further explaining the occurrence of asthma in subjects with AATD.<sup>16</sup> On the other hand, no significant difference in R5-R20 values has been found. This may suggest that damage to elastic tissue in patients with AATD could be better revealed through spirometric evaluations of small airways performed with forced maneuvers compared to resting evaluations such as oscillometric measurements, because of the collapsibility of small airways.

Lutfi et al.<sup>17</sup> found that FEV<sub>3</sub> and FEV<sub>6</sub> are accurate and reliable alternatives to FVC in assessment of airway obstruction in asthmatic patients. Previous studies in young adults revealed through the oscillometry method an increased total pulmonary resistance in MZ subjects vs. the MM control group.<sup>18,19</sup>

The analysis of the results obtained by grouping the patients according to their PI\* genotype revealed that 50% and 41% of AATD asthmatic subjects had a PI\*MS and PI\*MZ genotype, respectively.

The prevalence in our data of deficient S and Z alleles and the prevalence of heterozygous forms are consistent with literature data. In more detail, the presence of S allele has been associated both with a high risk of non-specific bronchial hyperresponsiveness, and with a higher asthmatic disease vs. the general population.<sup>20,21</sup> Other studies showed a greater asthma severity in children and adolescents when associated to Z allele in the heterozygous form.<sup>22</sup> Eden et al. found a three-fold higher prevalence of asthma in the PI\*MZ group vs. the PI\*ZZ group.<sup>23</sup>

We found SAD and pulmonary insufflation not only among the groups of asthmatic patients classified as mutation carriers and non carriers, but also when we split the population in relation to the PI\*MS and PI\*MZ genotypes compared to the PI\*MM genotype. In addition, we did not find any significant difference in lung function test results between the PI\*MS and the PI\*MZ AATD genotypes; the only difference concerned the AAT protein concentration.

We did not find any significant difference in FEV<sub>1</sub>, FEV<sub>1</sub>/FVC, TLC and KCO values between PI\*MS asthmatic patients and PI\*MM patients, consistently with Miravittles et al.<sup>9</sup> However, our data showed a significant difference between the mean values of the RV/TLC ratio in the two groups of patients mentioned above, a parameter not measured by Miravittles et al.

None of our 57 patients was an active smoker at the time of enrollment in the study. Five subjects out of the 22 AATD patients were former smokers (23%) and eight subjects out of the 35 patients without AATD were former smokers (23%). There was no statistically significant difference in the AATD patients and the PI\*MM subjects with reference to their smoking habits. Finally, in the group of asthmatic patients with AATD, the RV/TLC ratio correlates significantly with the years of smoking; this correlation is not significant in the 35 asthmatic patients without AATD. These results highlight an increased risk for impaired lung function related to cigarette smoke exposure in asthmatic patients with AATD compared to asthmatic subjects without AATD.

Study limits are represented by the low number of subjects and consequently by the low percentage of former smokers in the group of mutation carriers (23%). This percentage reflects the rate of former smokers in the general asthmatic population, ranging from 22–43%, as reported in literature.<sup>24</sup> It is well known that smoking not only potentiates lung injury, but also reduces the antiprotease activity of the AAT protein by approximately 2,000 times,<sup>25</sup> making it an important and avoidable player in the development of emphysema. Molloy et al. obtained similar results for COPD disease.<sup>26</sup>

## Conclusions

In conclusion, our data showed the presence of SAD, assessed by spirometry, and a significant pulmonary air trapping in asthmatic heterozygote patients with PI\*MZ, PI\*MS and intermediate levels of AAT protein, compared to non carrier asthmatic patients. The risk is associated to cigarette smoke exposure.

Further studies will be necessary to confirm if pulmonary insufflation and SAD could possibly be associated with a more rapid decline, in order to identify the patients most likely to benefit from an effective intervention.

## Abbreviations

AATD: Alpha-1 antitrypsin deficiency; AAT: Alpha-1 antitrypsin; ACT: Asthma control test; AUC: Area under the curve; AX: Area of reactance; BMI: Body mass index; COPD: Obstructive pulmonary disease; CRP: C-reactive protein; DLCO: Diffusing capacity for carbon monoxide; FEV<sub>1</sub>: Forced expiratory volume in one second; FEV<sub>3</sub>: Forced expiratory volume in three second; FEV<sub>6</sub>: Forced expiratory volume in six second; FEV<sub>1</sub> / FVC: Forced expiratory volume in one second to forced vital capacity ratio; FRes: Resonant Frequency; FVC: Forced vital capacity; IC: Inspiratory capacity; IOS: Impulse Oscillometry; KCO: Transfer coefficient of the lung for carbon monoxide; MEF25, MEF50, MEF75, maximal expiratory flow-rates at 25, 50 and 75% of the inspiratory vital capacity; MCh: Methacholine; PI: Proteinase inhibitor; ROC: Receiver operating characteristics; RV: Residual volume; RV / TLC: Residual volume to total lung capacity ratio; R5, resistance at 5 Hz; R20, resistance at 20 Hz; SAD: small airways dysfunction TLC: Total lung capacity; X5: Reactance at 5 Hz; Z5: Impedance at 5 Hz.

## Declarations

**Ethics approval and consent to participate**

This study was approved by Hospital Ethics Committee of North Emilia Wide Area (approval number: 33503, dated September 04<sup>th</sup>, 2018) in compliance with the Declaration of Helsinki along with established written informed consent. This research was carried out in accordance with the approved guidelines. Written informed consent was obtained from all participants before inclusion.

#### Consent for publication

Not applicable.

#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Competing interests

The authors declare that they have no competing interests

#### Funding

There was no funding source for this study.

#### Authors' contributions

CA and AM conceived and designed the study, had full access to all of the data, and took responsibility for the integrity of the data and the accuracy of the data analysis. AM, GM, FI, BG analyzed and contributed to the statistic of the data. GM and PR contributed to the collection clinical data. AM prepared and reviewed the manuscript. All other authors revised the manuscript and approved the final draft.

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## Figures

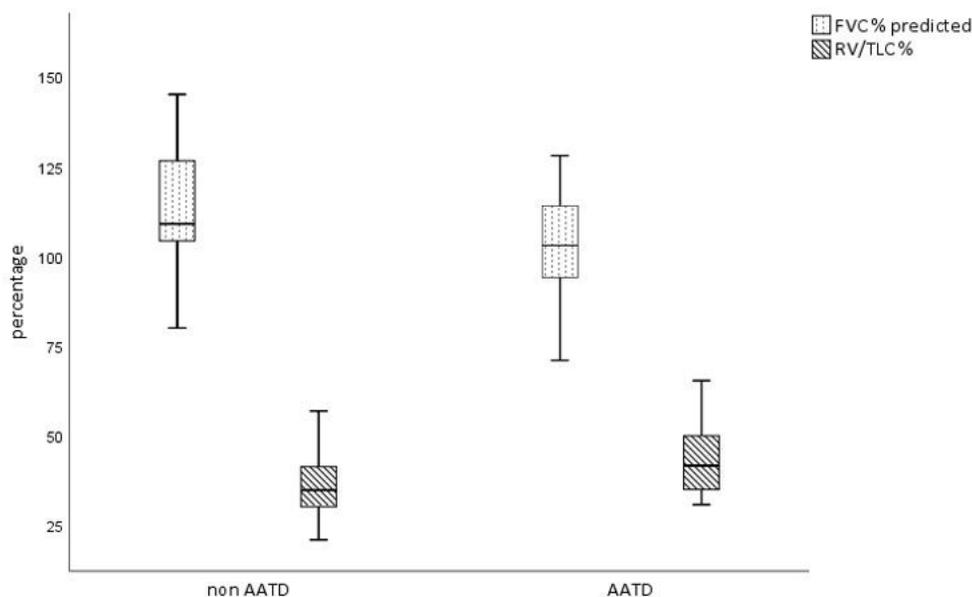


Figure 1. FVC and RV/TLC ratio vs. non AATD.

### Figure 1

FVC and RV/TLC ratio vs. non AATD.

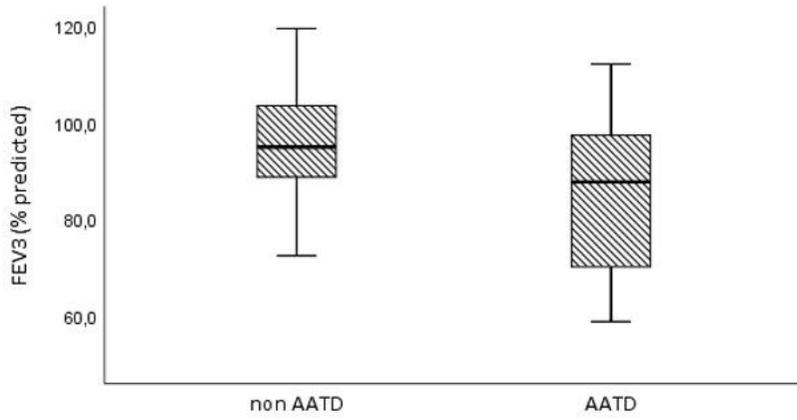


Figure 2. FEV<sub>3</sub> vs. non AATD.

Figure 2

FEV3 vs. non AATD.

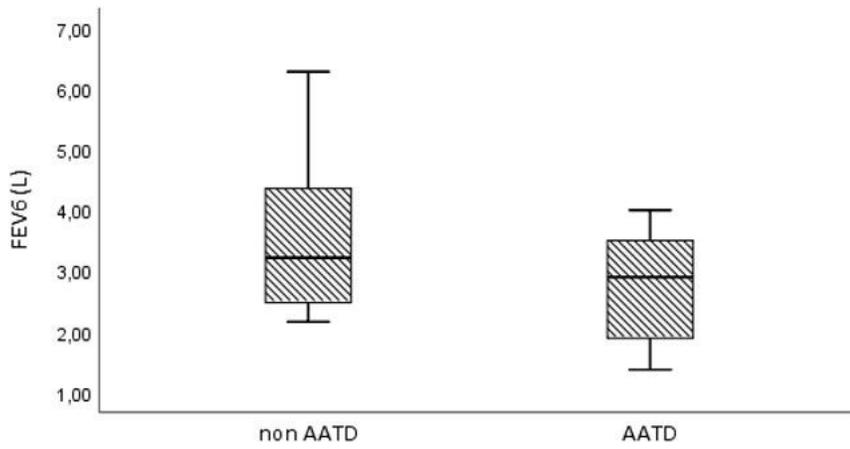


Fig. 3. FEV<sub>6</sub> vs. non AATD.

Figure 3

FEV6 vs. non AATD.

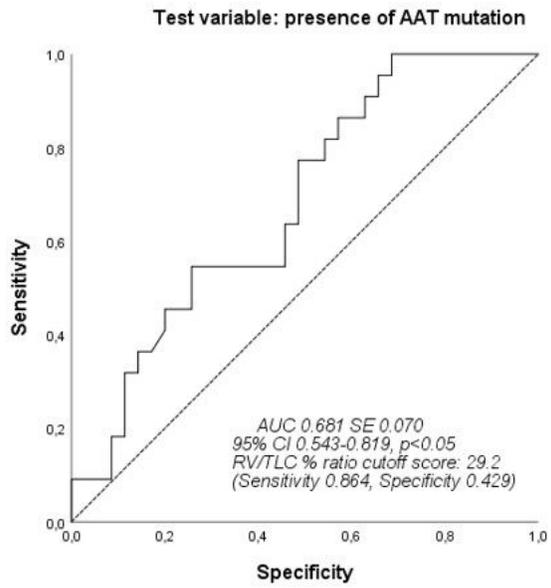


Figure 4

Receiver operating characteristic curve for RV/TLC (%) ratio calculated with presence of AAT mutation as test variable in asthmatic patients. The dashed line indicates the reference line. AUC, area under the curve; SE, standard error.