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# Air Trapping Is Associated with Heterozygosity for Alpha-1 Antitrypsin Mutations in Patients with Asthma

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**Keywords**
Alpha-1 antitrypsin deficiency · Asthma · Lung function tests · Proteinase inhibitor · Genotype

**Abstract**
***Background:*** Alpha-1 antitrypsin deficiency (AATD) is a hereditary disorder involving lungs, characterized by low serum concentration of the protein alpha-1 antitrypsin (AAT) also called proteinase inhibitor (PI). Asthma is common in AATD patients, but there are only few data on respiratory function in asthmatic patients with AATD. ***Objectives:*** The aim of the study was to evaluate lung function in asthmatic outpatients with mutation in the *SERPINA1* gene coding for AAT versus asthmatic subjects without mutation. ***Methods:*** We performed the quantitative analysis of the serum concentration of AAT in 600 outpatients affected by mild to moderate asthma from the University Hospital of Parma, Italy. Fifty-seven of them underwent the genetic analysis subsequently; they were subdivided into mutated and non-mutated subjects. All the mutated patients had a heterozygous genotype, except 1 (PI*SS). We assessed the lung function through a flow-sensing spirometer and the small airway parameters through an impulse oscillometry system. ***Results:*** The values of forced vital capacity (% predicted) and those of the residual volume to total lung capacity ratio (%) were, respectively, lower and higher in patients mutated versus patients without mutation, showing a significantly greater air trapping ($p = 0.014$ and $p = 0.017$, respectively). Moreover, patients with mutation in comparison to patients without mutation showed lower forced expiratory volume in 3 s (% predicted) and forced expiratory volume in 6 s (L) spirometric values, reflecting a smaller airways contribution. ***Conclusions:*** In asthmatic patients, heterozygosity for AAT with PI*MZ and PI*MS genotypes was associated with small airway dysfunction and with lung air trapping.



## Introduction

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 [1]. Previous studies suggested an

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association between AATD and asthma [2–4]; on this basis, the literature data recommend that all adult-onset asthmatic patients should be screened for AATD [5, 6]. Pulmonary function has been studied especially in patients affected by chronic obstructive pulmonary disease and severe AATD [7, 8]; a few data are available on respiratory function in asthmatic patients with AATD.

We hypothesized that asthmatic patients affected by AATD could have abnormal spirometric and oscillometric values compared to asthmatic patients without AATD. Therefore, the first objective of this study was to evaluate lung function in asthmatic outpatients affected by mutation in the *SERPINA1* gene coding for AAT versus asthmatic subjects without mutation. Subsequently, we focused on the heterozygous patients with PI*MZ and PI*MS following our study population genotypes.

## Materials and Methods

*Study Subjects and Data Collection*

This study has been performed at the University of Parma (Italy) over a period of 12 months between September 2018 and 2019, during the scheduled office visits. We enrolled 57 mild-to-moderate asthmatic outpatients, 18 years of age or older, of both genders. Asthma diagnosis was based on the combined presence of respiratory symptoms, reversible airflow obstruction, and bronchial hyperactivity, as assessed by the methacholine test [9, 10]. Patients with other concomitant lung diseases (BPCO, interstitial lung disease or bronchiectasis) were excluded from the study. No patient was found to have emphysema or severe exacerbations. We recorded the following data in all asthmatic patients: anthropometric variables (sex, age, BMI in kg/m$^2$), smoking habits (former/nonsmoker), number of packs per year, AAT serum concentration, and score of Asthma Control Test (ACT) [11] to assess symptoms and asthma-related morbidity. None of the patients was an active smoker at the time of enrollment in the study. Table 1 summarizes the features of mutated asthmatic patients.The study was approved by the Hospital Ethics Committee of North Emilia Area (approval number: 33503, dated September 4, 2018) in agreement with the Declaration of Helsinki. Written informed consent was obtained from all participants before inclusion.

*Study Design*

This study is observational, with a data collection prospective and retrospective. Following our clinical procedure, 600 asthmatic outpatients underwent a concomitant quantitative analysis of the serum concentrations of AAT (mg/dL) and C-reactive protein. The C-reactive protein (mg/L) was used as internal control of analysis [12]. Consistently with the literature data [6, 13], 52 out of 600 asthmatic patients were submitted to sequencing of the *SERPINA1* gene due to a serum AAT concentration ≤113 mg/dL. We performed the genetic analysis also in 9 asthmatic patients with serum AAT concentration >113 mg/dL but with the presence of a clinical and/or family history that could be related to AAT deficiency [12]. We excluded 6 patients with respiratory comorbidities from the study.

Furthermore, the relatives of asthmatic patients with mutation in the *SERPINA1* gene underwent genetic analysis [6]. Only 2 of them were included in our study as asthmatic. They were heterozygous, with PI*MS and PI*MZ genotypes, and their serum AAT concentration was >113 mg/dL.

On the basis of the genetic test results, the study population was divided into 2 groups: 35 patients with a non-pathological genotype (PI*MM) and 22 patients with a pathological genotype. The subsequent comparisons were performed between the heterozygous genotypes PI*MS and PI*MZ, following the prevalence of heterozygosity for AAT of the study population. The participant selection process is shown in the Consort diagram (shown in Fig. 1).

*Spirometry*

We used a flow-sensing spirometer connected to a computer for data analysis ($V_{max}$22 and 6200, SensorMedics; Yorba Linda, CA, USA) to measure lung function parameters through plethysmographic technique. Forced expiratory volume in the 1st second ($FEV_1$) and forced vital capacity (FVC) were recorded and expressed as absolute values (in liters, L) and as percentage of a predicted value (% predicted). The $FEV_1$/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. 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 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 [14].

In order to measure the smaller airway contributions, forced expiratory volume in 3 seconds ($FEV_3$, in L and % predicted) and forced expiratory volume in 6 seconds ($FEV_6$, in L) were recorded. The $FEV_3$/FVC, $FEV_6$/FVC, and $FEV_3$/$FEV_6$ values were recorded as ratio and were considered as measures able to detect small airway dysfunction (SAD) [15]. Moreover, we recorded maximal expiratory flow-rates at 25, 50, and 75% of the vital capacity ($MEF_{25}$, $MEF_{50}$, and $MEF_{75}$, expressed as L/s and as % predicted).

*Impulse Oscillometry*

Impulse oscillometry was performed using the Jaeger MasterScreen-IOS instrument (Carefusion Technologies, San Diego, CA, USA) as per standard recommendations [16]. 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 as an index for the resistance of peripheral airways. The reactance at 5 Hz (X5 in kPa/[L/s]) and the resonant frequency ($F_{res}$ in kPa/[L/s]) were considered as representative markers of peripheral airway dysfunction. Moreover, impedance at 5 Hz (Z5 in kPa/[L/s]) and the area of reactance (in kPa/[L/s]) were measured.

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**Table 1.** Features of mutated asthmatic patients

| | Age, years | Sex | Genotype | ACT, *n* | FVC, % | $FEV_1$, % | $FEV_1$/FVC, % | Atopy Y/N | Eosinophils, $\times 10^3/\mu L$ | IgE tot., UI/mL | Smoking history | Cardiovascular diseases Y/N | Gastroesophageal reflux disease Y/N | Allergic manifestations | Exacerbation/year | Treatment schedule |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient 1 | 31 | M | MS | 23 | 105.00 | 107.00 | 84.98 | Y | 0.43 | 223.00 | No | N | N | Rhinitis | 0 | ICS + LABA |
| Patient 2 | 67 | F | MS | 24 | 117.00 | 89.00 | 63.60 | Y | 0.51 | 93.00 | No | N | Y | Rhinitis | 0 | ICS + LABA |
| Patient 3 | 22 | M | MS | 24 | 83.00 | 84.00 | 86.06 | Y | 0.07 | 73.00 | No | N | N | Rhinoconjunctivitis | 0 | ICS + LABA |
| Patient 4 | 65 | F | MS | 16 | 103.00 | 91.00 | 74.47 | Y | 0.36 | 27.90 | No | Y | Y | Oculorhinitis | 1 | ICS + LABA |
| Patient 5 | 61 | M | MS | 25 | 72.00 | 69.00 | 77.05 | Y | 0.59 | 48.00 | Ex | N | N | No | 1 | ICS + LABA |
| Patient 6 | 60 | F | MS | 24 | 124.20 | 109.00 | 73.93 | Y | 0.15 | 158.00 | No | Y | Y | Oculorhinitis | 0 | ICS + LABA |
| Patient 7 | 71 | F | MS | 22 | 98.00 | 90.00 | 73.85 | Y | 1.13 | 2,030.00 | Ex | N | N | Oculorhinitis | 0 | ICS + LABA |
| Patient 8 | 54 | F | MS | 23 | 128.00 | 105.00 | 70.39 | Y | 0.41 | 79.00 | No | N | N | Rhinoconjunctivitis | 0 | ICS + LABA |
| Patient 9 | 77 | M | MS | 24 | 81.00 | 68.00 | 65.06 | N | 0.06 | 121.00 | No | N | N | No | 0 | ICS + LABA |
| Patient 10 | 51 | F | MS | 20 | 103.00 | 98.00 | 80.90 | Y | 0.21 | 142.00 | No | N | N | Oculorhinitis, rhinoconjunctivitis | 1 | ICS + LABA |
| Patient 11 | 48 | F | MS | 25 | 103.00 | 87.00 | 72.37 | Y | 0.19 | 62.50 | No | Y | N | Rhinoconjunctivitis | 0 | ICS + LABA |
| Patient 12 | 61 | M | MZ | 25 | 114.60 | 95.70 | 66.00 | Y | 0.48 | 932.00 | No | Y | N | Oculorhinitis | 0 | ICS + LABA |
| Patient 13 | 48 | M | MZ | 25 | 91.00 | 90.00 | 80.56 | Y | 0.30 | 9.00 | No | Y | N | Oculorhinitis | 0 | ICS + LABA |
| Patient 14 | 56 | F | MZ | 23 | 103.00 | 87.00 | 72.00 | Y | 0.48 | 97.40 | No | Y | Y | Nasopharyngitis, oculorhinitis | 0 | ICS + LABA |
| Patient 15 | 26 | F | MZ | 25 | 97.20 | 97.30 | 87.53 | Y | 0.28 | 99.90 | No | N | N | No | 0 | ICS + LABA |
| Patient 16 | 53 | F | MZ | 25 | 114.00 | 102.00 | 75.56 | Y | 0.14 | 355.00 | No | N | N | Oculorhinitis | 0 | ICS + LABA |
| Patient 17 | 73 | F | MZ | 25 | 114.00 | 90.00 | 62.97 | N | 0.09 | 10.20 | No | N | N | No | 0 | ICS + LABA |
| Patient 18 | 74 | F | MZ | 23 | 97.00 | 75.00 | 63.20 | Y | 0.40 | 234.00 | No | N | N | Oculorhinitis | 0 | ICS + LABA |
| Patient 19 | 85 | F | MZ | 18 | 71.00 | 57.00 | 64.00 | Y | 1.26 | 242.00 | No | Y | N | Rhinosinusitis | 1 | ICS + LABA |
| Patient 20 | 69 | M | MZ | 24 | 92.00 | 84.00 | 70.82 | Y | 0.10 | 828.00 | Ex | Y | N | Rhinosinusitis | 1 | ICS + LABA |
| Patient 21 | 43 | M | MM-malton | 23 | 97.00 | 64.00 | 54.47 | Y | 0.11 | 1,082.00 | Ex | N | Y | Oculorhinitis | 0 | ICS + LABA, LAMA |
| Patient 22 | 62 | F | SS | 25 | 114.50 | 106.90 | 78.63 | Y | 0.01 | 14.20 | Ex | N | N | No | 1 | ICS + LABA |

The table includes the collection of anamnestic data relating to the demographic data (age and sex), genotype, value of ACT, values of recent spirometric parameters (FVC%, $FEV_1$%, and $FEV_1$/FVC%), treatment, number of eosinophils in the blood, levels of IgE, smoking history, comorbidities, presence of atopy, and number of exacerbations/year. M, male; F, female; *n*, number; FVC, forced vital capacity; ACT, asthma control test; $FEV_1$, forced expiratory volume in 1 s; $FEV_1$/FVC, forced expiratory volume in 1 s to forced vital capacity ratio; Y, yes; N, no.

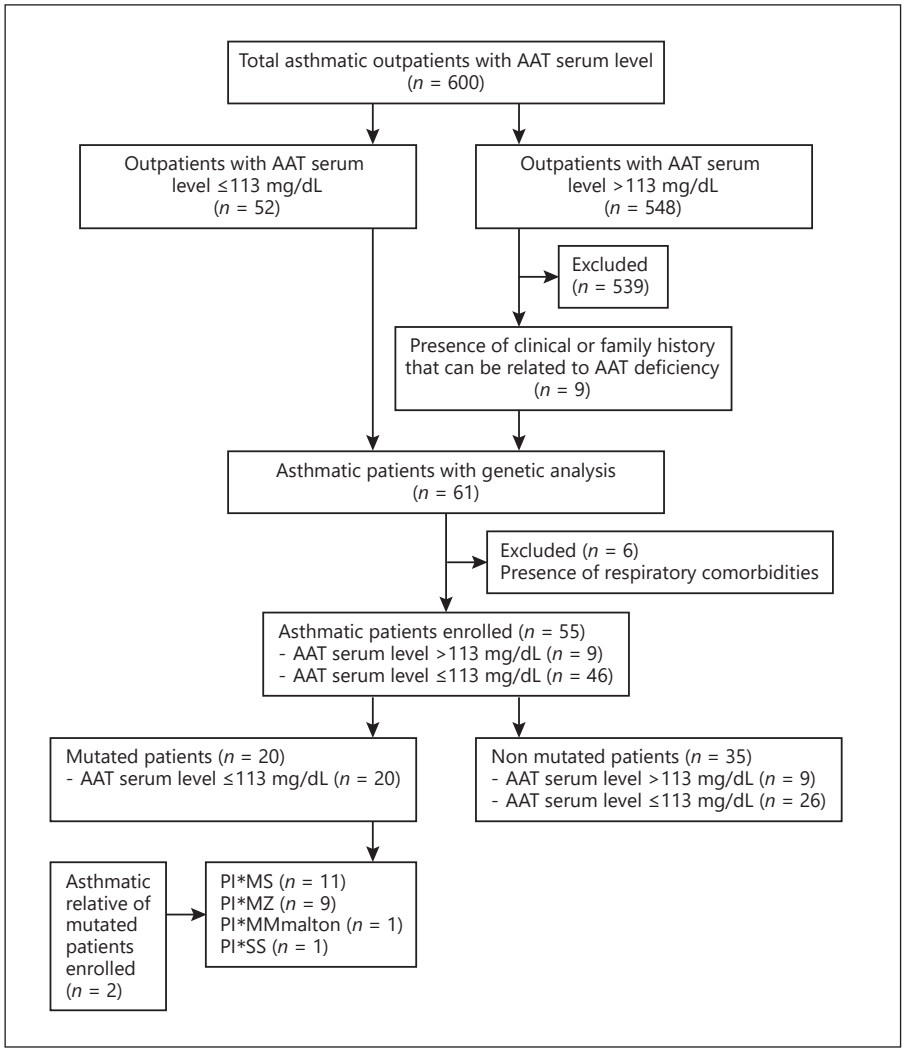


**Fig. 1.** Consort diagram of the study protocol. AAT, alpha-1 antitrypsin.

*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 ANOVA (*t* tests) for continuous variables. The non-parametric Kruskal-Wallis test was used for data with non-Gaussian distribution. $\chi^2$ tests and Fisher's exact tests were performed for qualitative variables.

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% CI and to select the best cutoff value with the related sensibility and specificity. Stepwise multiple regression analysis was used to determine the best predictor variables (age, sex, mutated y/n, atopy y/n, smoking habits, AAT serum level, asthma control test, R5–R20) for the RV/TLC ratio as dependent variables.

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

## Results

In all asthmatic patients, the mean age was 57 ± 15 years and 54% of patients were female subjects; the median serum AAT concentration was 108.0 [97.9; 111.5] mg/dL. Asthmatic subjects were classified as patients with mutation ($n$ = 22; 38.6%) and without mutation ($n$ = 35; 61.4%) according to their PI* (Proteinase Inhibitor) genotype. The frequency of deficient genotypes was 11 (19.3%) patients with the PI*MS genotype, 9 (15.8%) with the PI*MZ genotype, 1 (1.75%) patient with the PI*$MM_{Malton}$, and 1 (1.75%) patient with the PI*SS geno-



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**Table 2.** Demographic and laboratory data, smoking habits in reference to presence/absence of mutation in AAT

| Variables | Asthmatic patients | Mutated | Non-mutated |
|---|---|---|---|
| Subjects, *n* | 57 | 22 | 35 |
| BMI, kg/m$^2$ | 26.0 [24.0; 29.0] | **27.5 [25.0; 30.0]**[a] | 25.0 [23.0; 28.0] |
| Age, years | 57±15 | 57±16 | 56±15 |
| Women, % | 54 | 64 | 46 |
| Non-smokers, *n* (%) | 44 (77) | 17 (77) | 27 (77) |
| Former smokers, *n* (%) | 13 (23) | 5 (23) | 8 (23) |
| Pack/years, *n* | 10 [4; 20] | 10 [8; 28] | 8 [3; 19] |
| Years of smoking, *n* | 19±11 | 21±10 | 19±12 |
| AAT concentration, mg/dL | 108.0 [97.9; 111.5] | **97.8 [83.2; 106.5]**[b] | 111.0 [105.0; 115.0] |
| Atopy, *n* (%) | 43 (75) | **20 (91)**[c] | 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. *n*, number, AAT, alpha-1 antitrypsin; ACT, asthma control test. [a] $p$ value = 0.007 versus non-mutated. [b] $p$ value = 0.000 versus non-mutated. [c] $p$ value = 0.031 versus non-mutated.

type (shown in Fig. 2). The median values of AAT concentration were lower in patients with mutation versus PI*MM patients (97.8 vs. 111 mg/dL, $p$ = 0.000), while values of BMI were higher in patients with mutation versus PI*MM patients (27.5 vs. 25 kg/m$^2$, $p$ = 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 mutation. Forty-three asthmatic patients (75% of cases) showed atopy, with skin-test positive for common aeroallergens; 20 were with mutation (91% of cases) and 23 were without mutation (66% of cases) ($p$ = 0.031).

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 mutation than in those without mutation, showing a significantly greater air trapping ($p$ = 0.014 and $p$ = 0.017, respectively), as shown in Figure 3. We did not find any significant difference in other variables.

Table 4 summarizes the small airway values measured through oscillometry and spirometry in asthmatic patients. Patients with mutation showed lower values of $FEV_3$ (% predicted) and $FEV_6$ (L) in comparison to those without mutation (shown in Fig. 3, 4).

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,

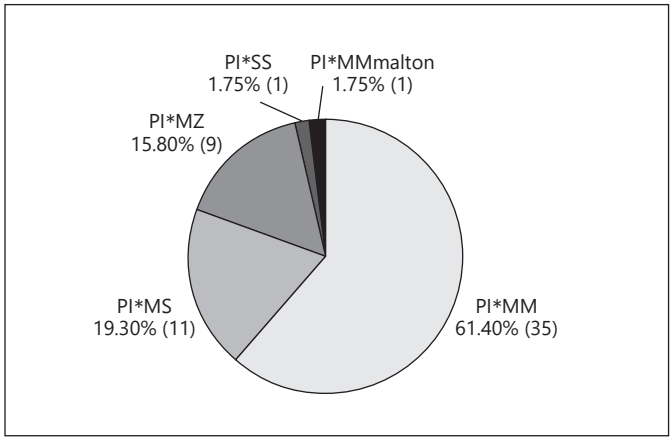


**Fig. 2.** AAT genotype distribution among asthmatic patients. AAT, alpha-1 antitrypsin.

$p$ = 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 $FEV_6$ (L) were 2.73 and 3.55 L in the PI*MS and PI*MM genotypes, respectively, but the difference was not statistically significant ($p$ = 0.060).

We found statistically significant differences when we compared lung function test results and general characteristics in asthmatic patients with the PI*MZ genotype versus those with the PI*MM genotype. The median AAT concentration (90.7 vs. 111 mg/dL, $p$ = 0.000) and FVC

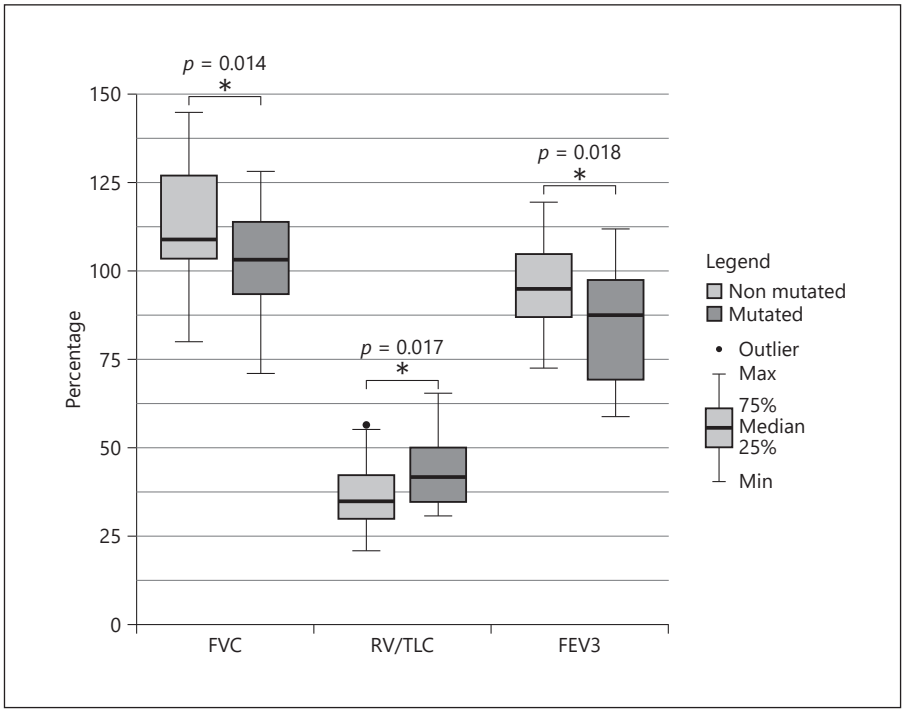


**Fig. 3.** FVC, RV/TLC ratio, and $FEV_3$ versus non-mutated. The outer edges of the box represent the interquartile range (1st and 3rd quartile), the solid line within the box is the median, and whiskers indicate the minimum and maximum values. Black dot represents an outlier of the data. * Indicates that the difference between the 2 groups is statistically significant. FVC, forced vital capacity; RV/TLC, ratio of residual volume to total lung capacity.

value (99.3 vs. 112.3% predicted, $p = 0.040$) were lower, while the mean value of $FEV_3$ was 82.9 ± 11 versus 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 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, $FEV_3$, $FEV_6$, and R20 values.

No difference in pulmonary function was found by splitting the mutated 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 mutation; we did not find the same correlation in the group of patients without mutation ($p = 0.349$) (data not shown). No significant correlation was found among AAT values, lung function test results and impulse oscillometry values in asthmatic patients with mutation. The ROC curve was calculated to set the value of the RV/TLC ratio able to be likely associated to the presence of mutation in *SERPINA1* gene in asthmatic patients (shown in Fig. 5), and revealed an area under the curve of 0.681 (standard error 0.070; 95% CI 0.543–0.819; $p < 0.05$) with an

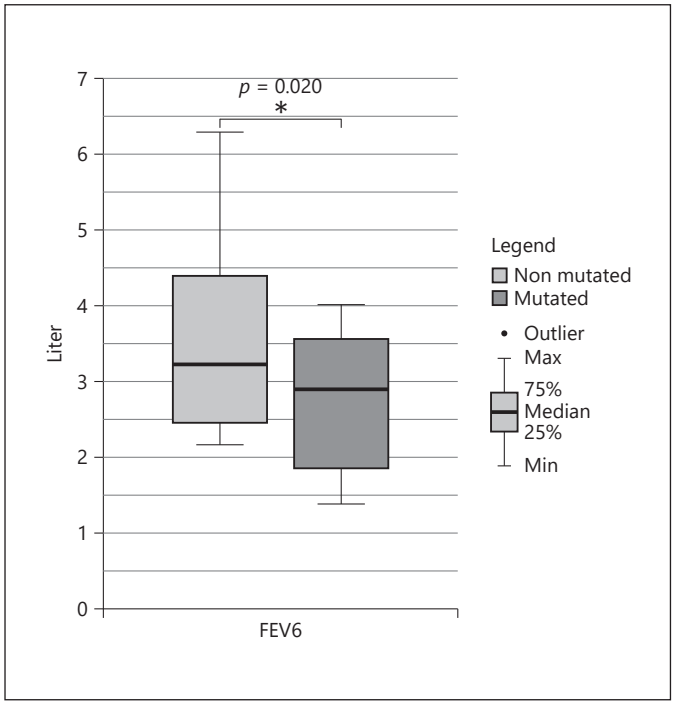


**Fig. 4.** $FEV_6$ versus non-mutated. The outer edges of the box represent the interquartile range (1st and 3rd quartile), the solid line within the box is the median, and whiskers indicate the minimum and maximum values. * Indicates that the difference between the 2 groups is statistically significant.

**Table 3.** Lung function tests in reference to presence/absence of mutation in AAT

| Variables | Asthmatic patients | Mutated | Non-mutated |
|---|---|---|---|
| Subjects, *n* | 57 | 22 | 35 |
| $FEV_1$, L | 2.53±0.90 | 2.30±0.87 | 2.67±0.90 |
| $FEV_1$, % 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**[a] | 112.3±15.9 |
| $FEV_1$/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**[b] | 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. *n*, number; $FEV_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; $FEV_1$/FVC, forced expiratory volume in 1 s 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; AAT, alpha-1 antitrypsin. [a] $p$ value = 0.014 versus non-mutated. [b] $p$ value = 0.017 versus non-mutated.

**Table 4.** Values of small airways measured by impulse oscillometry and spirometry in reference to presence/absence of mutation in AAT

| Variables | Asthmatic patients | Mutated | Non-mutated |
|---|---|---|---|
| Subjects, *n* | 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] |
| $F_{Res}$, Hz | 16.00±5.65 | 17.16±5.43 | 15.03±5.75 |
| $FEV_3$, L | 3.12±1.12 | 2.73±1.05 | 3.38±1.10 |
| $FEV_3$, % predicted | 91.8±14.9 | **85.6±16.9**[a] | 95.9±12.1 |
| $FEV_6$, L | 3.24±1.13 | **2.75±0.91**[b] | 3.55±1.16 |
| $FEV_3$/FVC, % | 89.5±4.8 | 88.5±5.7 | 90.2±4.1 |
| $FEV_6$/FVC, % | 95.4±3.7 | 94.5±4.3 | 96.0±3.1 |
| $FEV_3/FEV_6$, % | 92.4 [89.9; 93.5] | 91.1 [88.6; 93.1] | 93.1 [91.8; 93.7] |
| $MEF_{25}$, L/s | 0.57 [0.34; 0.94] | 0.50 [0.20; 0.65] | 0.58 [0.42; 0.99] |
| $MEF_{25}$, % predicted | 37.4 [31.2; 55.3] | 35.1 [21.1; 43.5] | 40.5 [32.7; 58.3] |
| $MEF_{50}$, L/s | 2.15 [1.51; 3.22] | 2.09 [0.70; 2.61] | 2.15 [1.76; 3.38] |
| $MEF_{50}$, % predicted | 52.2 [42.7; 68.6] | 50.6 [22.0; 69.0] | 53.3 [45.3; 71.5] |
| $MEF_{75}$, L/s | 4.87±2.20 | 4.43±2.49 | 5.15±1.97 |
| $MEF_{75}$, % predicted | 78.1±27.7 | 73.4±32.5 | 81.2±24.2 |
| $MEF_{75/25}$, L/s | 1.62 [1.07; 2.59] | 1.50 [0.53; 1.78] | 1.70 [1.21; 2.73] |
| $MEF_{75/25}$, % 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. *n*, number; Z5, impedance at 5 Hz; R5, resistance at 5 Hz; R20, resistance at 20 Hz; AX, area of reactance; X5, reactance at 5 Hz; $F_{Res}$, resonant frequency; $FEV_3$, forced expiratory volume in 3 s; $FEV_6$, forced expiratory volume in 6 s; $MEF_{25}$, $MEF_{50}$, and $MEF_{75}$,, maximal expiratory flow-rates at 25, 50, and 75% of the inspiratory vital capacity, respectively; AAT, alpha-1 antitrypsin. [a] $p$ value = 0.018 versus non-mutated. [b] $p$ value = 0.020 versus non-mutated.

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

| Variables | PI*MM | PI*MS | PI*MZ | PI*MS and PI*MZ |
|---|---|---|---|---|
| Subjects, *n* | 35 | 11 | 9 | 20 |
| BMI, kg/m$^2$ | 25.5±4.2 | 28.0±2.6 | 29.3±7.1 | **28.6±4.9**[f] |
| AAT level, mg/dL | 111.0 [105.0; 115.0] | 100.0 [97.8; 110.0] | **90.7 [77.2; 99.9]**[b, c] | **97.9 [90.8; 107.5]**[g] |
| FVC, L | 3.80±1.14 | 3.12±1.00 | 3.04±1.05 | **3.08±0.99**[h] |
| FVC, % predicted | 112.3±15.9 | 102.7±16.6 | **99.3±14.2**[d] | **101.2±15.2**[i] |
| TLC, L | 6.08±1.16 | 5.39±0.92 | 5.30±0.89 | **5.35±0.88**[j] |
| RV/TLC, % | 36.3±9.5 | **43.2±9.6**[a] | 42.9±11.5 | **43.1±10.2**[k] |
| $FEV_3$, % predicted | 95.9±12.1 | 87.9±21.3 | **82.9±11.0**[e] | **85.6±16.9**[l] |
| $FEV_3$, L | 3.38±1.09 | 2.77±1.15 | 2.70±1.00 | **2.73±1.10**[m] |
| $FEV_6$, L | 3.55±1.16 | 2.73±0.87 | 2.78±1.00 | **2.75±0.91**[n] |
| R20, kPa/(L/s) | 0.34±0.12 | 0.40±0.08 | 0.38±0.06 | **0.39±0.07**[o] |
| R5-R20, kPa/(L/s) | 0.07±0.07 | 0.08±0.09 | 0.08±0.09 | 0.08±0.08 |

Data are shown as mean ± SD or medians [1st quartile; 3rd quartile]. Boldface variables are statistically significant. *n*, number; AAT, alpha-1 antitrypsin; PI, proteinase inhibitor; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity. [a] *p* value = 0.043 PI*MM versus PI*MS patients. [b] *p* value = 0.016 PI*MS patients versus PI*MZ patients. [c] *p* value = 0.000. [d] *p* value = 0.040. [e] *p* value = 0.007 PI*MM versus PI*MZ patients. [f] *p* value = 0.019. [g] *p* value = 0.000. [h] *p* value = 0.023. [i] *p* value = 0.013. [j] *p* value = 0.040. [k] *p* value = 0.016. [l] *p* value = 0.018. [m] *p* value = 0.049. [n] *p* value = 0.020. [o] *p* value = 0.029 PI*MM versus PI*MS and MZ patients.

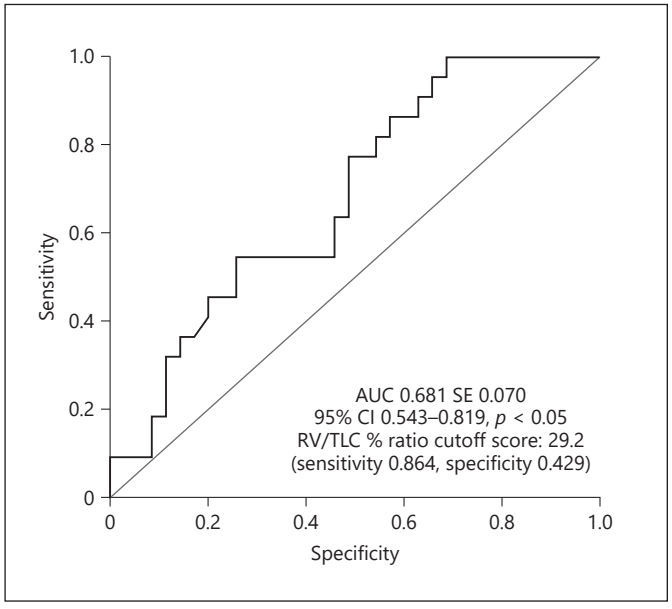


**Fig. 5.** ROC curve for RV/TLC ratio (%) calculated with presence of AAT mutation as test variable in asthmatic patients. The dashed line indicates the reference line. AUC, area under the curve; SE, standard error; AAT, alpha-1 antitrypsin; RV/TLC, ratio of residual volume to total lung capacity; ROC, receiver operating characteristics.

RV/TLC% ratio cutoff value of 29.2 (sensitivity 0.864, specificity 0.429).

The regression equation generated by stepwise multiple regression analysis for the RV/TLC ratio as dependent variable included only presence of mutation and age as independent variables. This model accounted for 43.7% of the total variance for the RV/TLC ratio. The equation generated is the following: RV/TLC ratio = 17.399 + 0.319 (age) + 6.151 (mutated y/n).

## Discussion

The present study assessed the lung function in 2 groups of mild-to-moderate asthmatic patients with and without mutation in *SERPINA1* gene and showed significantly lower FVC (%) values in mutated patients versus subjects without mutation. Furthermore, we showed that the values of the RV/TLC ratio in mutated patients versus subjects without mutation were significantly higher, with a cutoff value of 29.2%, calculated by the ROC curve, which helps us distinguish patients with mutation in *SERPINA1* gene with a sensitivity of 0.864 and a specificity of 0.429. Additionally, and most importantly, mutation together with age was the independent predictor for RV/TLC ratio values. Although the results we obtained in the spirometry-derived parameters FVC and RV/TLC ra-

tio should be interpreted with caution, they suggest the presence of air trapping, which is the marker of the airway obstruction in asthmatic patients with AATD. Hall et al. [17] found no significant difference in spirometry and static lung volumes in a population of asymptomatic nonsmoking adults with intermediate levels of AAT. Differences in study populations might explain the different results we obtained versus the study by Hall et al. [17].

Furthermore, in our study, there was no statistically significant difference both in mutated patients and in the PI*MM subjects when their smoking habits were considered (Table 2). In the group of asthmatic patients with mutation, the RV/TLC ratio correlates significantly with the years of smoking; this correlation was not significant in the 35 asthmatic patients without mutation. These results highlight an increased risk for impaired lung function related to cigarette smoke exposure in asthmatic patients with mutation compared to asthmatic subjects without mutation.

A study limitation is represented by the low number of subjects and consequently by the low percentage of former smokers in the group of mutated patients (5 and 23%, respectively). However, this percentage reflects the rate of former smokers in the general asthmatic population, ranging from 22 to 43%, as reported in literature [18]. The RV/TLC ratio has received little emphasis in studies focusing on pulmonary dysfunction in AATD subjects, while other lung function-related parameters ($FEV_1$, $FEV_1$/FVC ratio, and KCO) have been taken into consideration [7, 19].

While $FEV_1$ values can indicate large airway obstructions, $FEV_3$ and $FEV_6$ values, representing the latter fraction of forced exhalation, could better reflect smaller airway obstructions and be a more sensitive measure to diagnose early airway obstruction [20]. Furthermore, $FEV_3$ and $FEV_6$ are accurate and reliable alternatives to FVC in the assessment of airflow limitation in asthmatic patients [21].

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 $FEV_3$ and $FEV_6$ values were significantly lower compared to asthmatic patients without mutation. 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.

Following the prevalence of heterozygous forms in the study population, we focused on patients with PI*MS and the PI*MZ genotypes. The prevalence in our data of deficient S and Z alleles and the prevalence of heterozygous forms are expected results according to the trend observed in literature data [12, 22]. In more detail, the presence of S allele has been associated both with a high risk of nonspecific bronchial hyperresponsiveness and with a higher asthmatic disease versus the general population [23, 24]. Other studies showed a greater asthma severity in children and adolescents when associated to Z allele in the heterozygous form [25]. Eden et al. [26] found a 3-fold higher prevalence of asthma in the PI*MZ group versus the PI*ZZ group [26].

We found SAD and lung air trapping when PI*MS and PI*MZ genotypes were 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 genotypes; the only difference concerned the AAT protein concentration.

We did not find any significant difference in $FEV_1$, $FEV_1$/FVC, TLC, and KCO values between PI*MS asthmatic patients and PI*MM patients. This finding is consistent with Miravitlles et al. [27] results. In addition, our data showed a significant difference between the 2 groups of patients in the RV/TLC ratio values, a lung function parameter not evaluated by Miravitlles et al. [27].

Although we believe that the study population can reflect the features of asthmatic population, the limitation of our study, that it can be considered a pilot study, is the small sample population due to the involvement of a single center analysis. Therefore, will be needed further multicenter studies to confirm our results.

## Conclusions

Our data showed the presence of a significant pulmonary air trapping and of a SAD in asthmatic heterozygote patients with PI*MZ and PI*MS compared to PI*MM asthmatic patients. Further studies will be required to confirm if lung air trapping 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.

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## Statement of Ethics

This study was approved by the Hospital Ethics Committee of North Emilia Area (approval number: 33503, dated September 4, 2018) in agreement with the Declaration of Helsinki. This research was carried out in accordance with the approved guidelines. Written informed consent was obtained from all participants before inclusion.

## Conflict of Interest Statement

The authors declare that they have no competing interests.


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## Author Contributions

C.A. and A.M. 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. G.M., P.R., F.A., and M.L. contributed to the collection of clinical data. A.M., G.M., F.I., and B.G. analyzed and contributed to the statistic of the data. A.M. prepared and reviewed the manuscript, in consultation with F.A., G.M., and M.L. All other authors provided critical feedback and approved the final draft.

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