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Alpha-1 antitrypsin deficiency is significantly associated with atopy in asthmatic patients

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ABSTRACT

Background: Although the etiology and disease mechanisms of asthma and alpha-1 antitrypsin deficiency (AATD) are distinct, several reports indicate that asthma is common in AATD patients, however the relationships between asthma and AATD are poorly described in the literature.

Objectives: The aim of the study was to investigate in a cohort of outpatients affected by mild to moderate asthma the clinical features that may differentiate asthmatic patients with and without mutation on SERPINA1 gene.

Methods: Seven hundred thirty-five asthmatic outpatients underwent quantitative analysis of the serum level of alpha-1 antitrypsin. According to the literature only sixty-seven out of seven hundred thirty-five asthmatic patients were submitted to genetic analysis to identify AATD and non-AATD subjects. Fifty-eight patients were studied. Clinical and functional data, including lung function, atopy and bronchial hyperactivity, were recorded.

Results: The fifty-eight asthmatic patients were divided in AATD patients ($n = 22$) and non AATD patients ($n = 36$), according to genotype. The presence of atopy was significantly higher in patients with AATD than in those without AATD (91% vs. 64%; $p = 0.031$). AATD patients reported allergic manifestations more than non AATD patients (77% vs. 47%; $p = 0.030$).

Conclusion: Our study shows that the presence of atopy in asthmatic patients with AATD is significantly higher than in asthmatic patients without gene mutation. In addition, a higher percentage of AATD patients self-reported allergic manifestations. No significant differences in respiratory symptoms, physical examination, disease severity or inflammation markers were found between AATD patients and non AATD patients.

Abbreviations: AATD: Alpha-1 antitrypsin deficiency; AAT: Alpha-1 antitrypsin; ACT: Asthma control test; BPT: Bronchial provocation test; CRP: C-reactive protein; FEV₁: Forced expiratory volume in one second; FEV₁/FVC: Forced expiratory volume in one second to forced vital capacity ratio; PD₂₀: Provocation dose 20%; PI: Proteinase inhibitor; SD: Standard deviation; WAO: World Allergy Organization

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Introduction

Alpha-1 antitrypsin deficiency (AATD) is the most common hereditary disorder involving lungs in adults. It is characterized by a decreased serum concentration of the alpha-1 antitrypsin (AAT) protein, due to mutations in the highly polymorphic *SERPINA1* gene, located on the long arm of the chromosome 14 (1,2). This gene has many different alleles that produce different amounts of AAT. The three most common alleles are M (normal), S (AAT intermediate serum levels) and the severely deficient Z (1,2). Since AATD

is a co-dominant disease, subjects with a pathological allele in heterozygosity, has an intermediate AATD. The presence of numerous potentially variants is clinically relevant to determine the best treatment of the patient.

Several reports indicate that asthma is common in AATD patients (3,4). It is of note that AAT inhibits airway inflammation induced by neutrophil elastase, and its loss may predispose to an increased release of potent chemotactic agents acting on neutrophils and to the development of chronic airway hyperreactivity (5,6). On the other hand, underdiagnoses of AATD

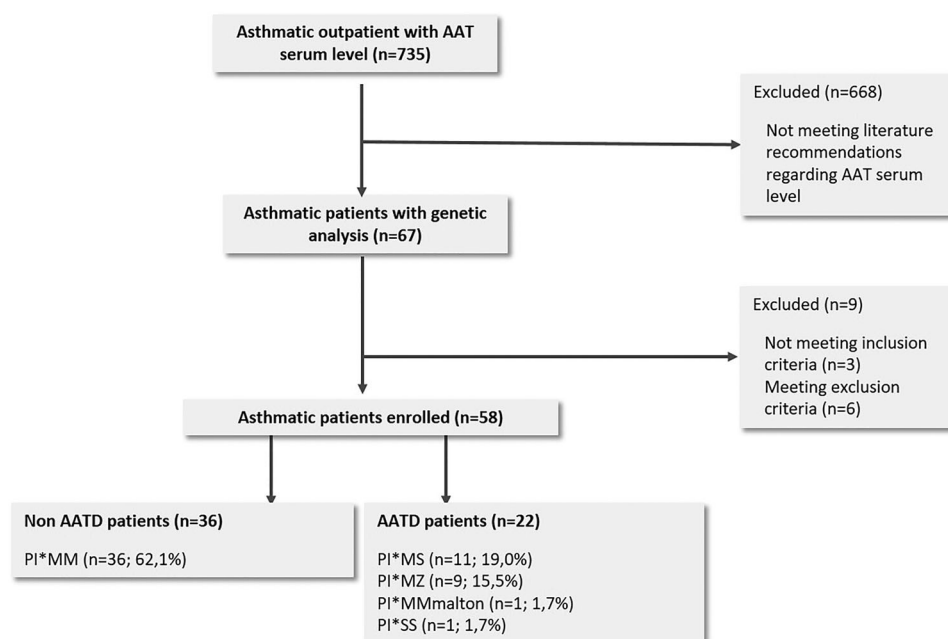


Figure 1. Consort diagram of the study protocol. Detailed representation of the enrollment of patients in the present study.

might stem from the misunderstanding that asthma and AATD are mutually exclusive disease entities. Actually, the etiology and disease mechanisms of asthma and AATD are distinct, but patients affected by AATD commonly first present with asthma-like symptoms, and initially receive asthma treatment (7). Accordingly, the relationship between asthma and AATD is complex and, up to now, poorly investigated.

We hypothesized that asthmatic patients with pathological gene mutations on *SERPINA1* gene may exhibit peculiar clinical features that differentiate them from asthmatic patients without gene mutations. The aim of this study was, therefore, to ascertain in a cohort of outpatients affected by mild to moderate asthma, whether or not patients with AATD may differ from those without AATD in terms of clinical and functional characteristics.

Materials and methods

Patients

This study was performed at the University Hospital of Parma (Italy). Over a period of 16 months between September 2018 and December 2019, seven hundred thirty-five outpatients affected by mild to moderate asthma underwent routinely a quantitative analysis of the serum level of AAT. The diagnosis of asthma was based on the history of characteristic symptom patterns and evidence of variable airflow limitation (8).

According to the literature, the requirement for submission to genetic analysis was the presence of AAT

serum level ≤ 113 mg/dL or serum level >113 mg/dL and the presence of a clinical symptoms and/or family history that can be related to AATD (9–11). Only sixty-seven (9%) out of seven hundred thirty-five asthmatic patients had the indication to be submitted to genetic analysis. Candidate participants to the study were patients meeting the following inclusion criteria: age >18 years, of both genders, with clinically stable bronchial asthma. The presence of concomitant lung diseases was an exclusion criterion. Accordingly, fifty-eight patients enter the study, 9 patients did not meet selection criteria and were excluded. On the basis of the genetic test results, the study population was later subdivided in two groups: 36 non AATD patients and 22 AATD patients. The participant selection process is shown in the flow chart (Figure 1).

The study was performed according to the Declaration of Helsinki. All the procedures were explained to the patients, who gave their written informed consent for the participation to the study. The Ethical Committee of the University Hospital of Parma, Italy, approved the protocol (approval number: 33503, dated September 04, 2018). All participants' data were anonymously analyzed and reported.

Measurements

In all patients, the Asthma Control Test (ACT) was used to assess symptoms and disease control (12). All the patients were interviewed about their smoking habits in order to record both the pack/years number

and years of smoking. Atopy, in accordance with the definition by World Allergy Organization (WAO) (13), was assessed through the prick tests for the most common inhalant allergens, allergic manifestations and serum total IgE concentration (expressed as UI/mL). Specific IgE were measured for patients who receive chronic antihistamine treatment. Patients with positive prick tests and/or high value for specific IgE (3.50 – 17.49 kU/L) for at least one allergen were considered atopic. The most common symptoms of allergy, such as oculorinitis and rhinoconjunctivitis were also recorded.

The AAT quantitative analysis was performed using an immune-nephelometric method (range of AAT normal serum level: 90–200 mg/dL) (10). A concomitant normal serum C-reactive protein (CRP) concentration was used as internal control of analysis (9). The genetic analysis for the sequencing of the *SERPINA1* gene was consistent with the recent guidelines (14). Patients' blood samples used for the genetic analysis were stored in EDTA-containing tubes or managed through the AlphaKitTM test, introduced in the Italian AATD screening program since 2006. The sample was analyzed by isoelectric focusing (to determine the phenotype), genotyping for the AATD variants S and Z through *SexAI/Hpy99I* RFLP, and sequencing of the AATD variants, which is required in case of discrepancies between AAT nephelometric level and genotyping (15). Blood eosinophils (expressed as cells/mm³ and as % on white blood cell) were measured as part of the complete blood count plus differential.

Patients underwent spirometry (Jager Vyntus APS - Vyntus, Carefusion, Germany) and both forced expiratory volume in the 1st second (FEV₁) and forced vital capacity (FVC) were recorded and expressed as absolute values (in liters, L) and as percentage of a predicted value (% predicted). The FEV₁/FVC value was recorded as a ratio. At least 3 measurements were taken for each spirometry test and lung volume variable to ensure data reproducibility (16). Bronchial provocation test with methacholine challenge was performed to measure the bronchial hyperreactivity degree, expressed as the dose inducing the 20% fall in FEV₁ (PD₂₀) (17). The fraction of exhaled nitric oxide (FeNO) was also measured in all the patients (NObreath; Bedfont, Rochester, UK) (18).

Statistical analysis

Unless otherwise stated, data are presented as mean ± SD. A Kolmogorov–Smirnov test was used to

assess the normality of distribution in all variables. Data with no normal distribution were reported as median values (1st quartile; 3rd quartile). Nonparametric Mann–Whitney test was used for data with non-Gaussian distribution.

Comparisons of means between groups were performed through *t* tests for quantitative variables, chi-square tests and Fisher exact tests for qualitative variables. The values of total IgE and PD₂₀ FEV₁ were log-transformed (to assure a normal distribution) and expressed as geometric means before comparison.

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

Results

The mean age ± SD value of the fifty-eight patients (53% female subjects) was 56.7 ± 15 years. Thirteen out of 58 patients (22%) were former smokers, no current smokers were enrolled in the study. In all patients, the median serum AAT concentration was 108.5 mg/dL ranging from 61 mg/dL to 159 mg/dL. Moreover, patients had a moderate airflow obstruction to normal lung function, FEV₁ value ranging from 57% to 134%. Demographic and clinical characteristics of the patients are summarized in Table 1.

Twenty-two out of 58 patients (38%) had AATD according to the PI (Proteinase Inhibitor) genotype. The frequency of deficient phenotypes was distributed as follows: eleven patients with PI*MS genotype (mean age ± SD = 55 ± 17 years, 54% female subjects), nine with PI*MZ genotype (mean age ± SD = 61 ± 17 years, 46% female subjects), one patient with PI*MM_{Malton} and one with the PI*SS genotype (Figure 1). By analyzing the results by grouping the patients according to the PI* genotype, no difference was observed in any anthropometric, clinical and functional characteristic, between PI*MS and PI*MZ patients, with the exception of the AAT median values (100 vs. 91 mg/dL; *p* = 0.016, Tables 2 and 3).

Patients with AATD did not differ from patients without AATD in any anthropometric, clinical and functional characteristic, except for atopy. When all patients were considered, forty-three asthmatic patients (74%) showed atopy, with skin-test positive for common aeroallergens. The presence of atopy was significantly higher in patients with AATD than in those without AATD (90.9% vs. 63.9%; chi-square test, *p* = 0.031; Table 4; Figure 2). Moreover, AATD patients reported a higher presence of the allergic

Table 1. Clinical characteristics, smoking habits, demographic and laboratory data according to presence/absence of genetic AATD.

	Asthmatic patients	Non AATD	AATD
Subjects, <i>n</i>	58	36	22
Age (years)	56.7 ± 15	56.4 ± 15	57.4 ± 16
Women (%)	53	36	64
Former smokers, <i>n</i> (%)	13 (22)	8 (22)	5 (23)
Pack/years, <i>n</i>	10 [4.0; 19.5]	8 [3.0; 18.8]	10 [8.00; 27.50]
Years of smoking, <i>n</i>	19.3 ± 11	18.5 ± 12	20.6 ± 9
AAT level (mg/dl)	108.5 [97.9; 112.3]	110.5 [105; 114.5]	98 [83.2; 107.0]*
PD ₂₀ FEV ₁ (mcg)	182 [108–323]	114[55–272]	346[200–631]
FEV ₁ (L)	2.48 ± 0.93	2.61 ± 0.89	2.28 ± 0.98
FEV ₁ (% predicted)	85.5 ± 16.1	88.2 ± 13.9	81.2 ± 18.8
FEV ₁ /FVC (%)	71.3 ± 9.4	70.5 ± 9.9	72.6 ± 8.6
FEV ₁ /FVC _{former smokers} (%)	68.8 ± 9.4	67.4 ± 9.5	71.0 ± 9.7
Eosinophils. cells/mm ³	0.23 [0.14; 0.39]	0.22 [0.15; 0.32]	0.29 [0.11; 0.48]
Eosinophils (%)	4 [2; 6]	3.8 [2.6; 5.1]	4.45 [1.65; 8.23]
IgE total (U/ml)	84.52[57.57–128.77]	80.04[48.42–141.18]	98.40[51.94–204.38]
ACT, total score	25 [23; 25]	25 [23; 25]	24 [23; 25]
FeNO (ppb)	20 [13.4; 35.9]	18.5 [12.5; 38.0]	22 [13.8; 36.5]

Data are shown as number of patients (%), means ± SD, medians [1st quartile; 3rd quartile] or geometric mean {confidence intervals}.

Boldface variables are statistically significant.

Women (%) are calculated on the total of women in the whole asthmatic patients population.

Abbreviations: AATD, alpha-1 antitrypsin deficiency; *n*, number; AAT, serum α_1 -antitrypsin; FEV₁, forced expiratory volume in one second; FEV₁/FVC, forced expiratory volume in one second to forced vital capacity ratio; ACT, asthma control test; FeNO, fraction of exhaled nitric oxide.

**p* = 0.001 AATD vs. non AATD patients.

Table 2. Clinical characteristics, smoking habits, demographic and laboratory data vs. patients with PI*MS and PI*MZ.

Variables	PI*MS	PI*MZ
Subjects, <i>n</i>	11	9
Age (years)	55 ± 17	61 ± 17
Women (%)	54	46
Former smokers, <i>n</i> (%)	2(18)	1(11)
Pack/years, <i>n</i>	23 ± 18	20 ± 0
Years of smoking, <i>n</i>	28 ± 11	20 ± 0
AAT level (mg/dL)	100 [97.8; 113]	91[77.2; 99.9]*
PD ₂₀ FEV ₁ (mcg)	237 [147–401]	307[181–549]
FEV ₁ (L)	2.29 ± 1.07	2.3 ± 0.9
FEV ₁ (% predicted)	83.9 ± 22.8	77.7 ± 12.9
FEV ₁ /FVC (%)	74.8 ± 7.2	71.4 ± 8.6
Eosinophils (cells/mm ³)	0.37 ± 0.31	0.39 ± 0.36
Eosinophils (%)	5.5 ± 3.7	5.1 ± 3.5
IgE total (U/ml)	105.50[56.83–214.86]	96.52[27.11–555.57]
ACT, total score	24.0 ± 0.8	24.3 ± 0.9
FeNO (ppb)	30.5 ± 17.8	34.4 ± 36.1

Abbreviations: PI, protease inhibitor; *n*, number; AAT, serum α_1 -antitrypsin; FEV₁, forced expiratory volume in one second; FEV₁/FVC, forced expiratory volume in one second to forced vital capacity ratio; ACT, asthma control test; FeNO, fraction of exhaled nitric oxide.

Data are shown as number of patients (%), means ± SD, medians [1st quartile; 3rd quartile] or geometric mean {confidence intervals}.

Boldface variables are statistically significant.

**p* values = 0.016 PI*MS vs. PI*MZ patients.

Table 3. Clinical variables vs. patients with PI*MS and PI*MZ.

Variables	PI*MS	PI*MZ	<i>p</i> value	χ^2
Atopy, <i>n</i> (%)	10 (90.9)	8 (88.9)	1.000	0.022
No atopy, <i>n</i> (%)	1 (9.1)	2 (11.1)		
Seasonal allergies, <i>n</i> (%)	4 (80.0)	2 (50.0)	0.524	0.900
Perennial allergies, <i>n</i> (%)	1 (20.0)	2 (50.0)		
With allergic manifestation of oculorhinitis, <i>n</i> (%)	9 (81.8)	7 (77.8)	1.000	0.051
Without allergic manifestation of oculorhinitis, <i>n</i> (%)	2 (18.2)	2 (22.2)		

Abbreviations: PI, protease inhibitor; *n*, number.

Data are shown as number of patients (%).

manifestations of oculorhinitis than in those without AATD (77,3% vs. 47,2%; chi-square test, *p* = 0.030; Table 4; Figure 2). The majority of asthmatic patients

with AATD showed a cutaneous hypersensitivity to common seasonal aeroallergens respect to perennial allergies (70% vs. 30%).

Table 4. Clinical variables according to presence/absence of genetic AATD.

Variables	Non AATD	AATD	<i>p</i> value	χ^2
Atopy, <i>n</i> (%)	23 (63.9)	20 (90.9)	0.031^a	5.200
No atopy, <i>n</i> (%)	13 (36.1)	2 (9.1)		
Seasonal allergies, <i>n</i> (%)	2 (25.0)	7 (70.0)	0.153	3.600
Perennial allergies, <i>n</i> (%)	6 (75.0)	3 (30.0)		
With allergic manifestation of oculorhinitis, <i>n</i> (%)	17 (47.2)	17 (77.3)	0.030^b	5.084
Without allergic manifestation of oculorhinitis, <i>n</i> (%)	19 (52.8)	5 (22.7)		

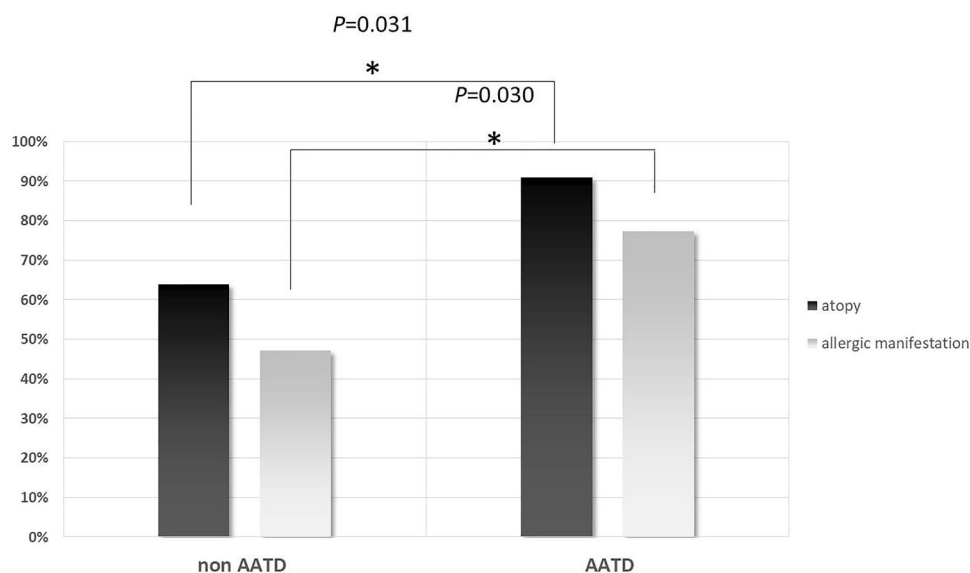
Abbreviations: AATD, alpha-1 antitrypsin deficiency; *n*, number.

Data are shown as number of patients (%).

Boldface variables are statistically significant.

^a*p* values < 0.05 AATD vs. non AATD patients.

^b*p* values <0.05 AATD vs. non AATD patients.

**Figure 2.** Presence of atopy and allergic manifestation in AATD vs. non AATD patients. AATD = Alpha-1 antitrypsin deficiency.

Discussion

The present study shows that the prevalence of atopy in patients with asthma and AATD differs significantly from that in asthmatic patients without AATD. This result is consistent with the findings obtained in the study of Eden et al. (6), showing that 48–51% of asthmatic patients with AATD were atopic. AAT plays relevant anti-infective, anti-inflammatory and anti-oxidant activities. An increased inflammatory mediator release as a result of the lack of AAT inhibition may lead to the development of chronic airway hyperreactivity and asthmatic state (6,19–21).

Subjects affected by AATD lack a major antiprotease defense against airway inflammation; they are also more susceptible to allergen-mediated asthma and to consequent progressive airway obstruction. Our results are in line with the study conducted in a population with a high incidence of bronchial asthma, such as the native population of Puerto Rico, where a higher prevalence of symptoms consistent with atopy was observed in PI*MS and PI*MZ subjects compared with PI*MM subjects (22).

A higher percentage of our asthmatic AATD subjects self-reported allergic manifestations, consistently with current literature (6). Notably, our results showed that the majority of asthmatic patients with AATD had a higher cutaneous hypersensitivity to common seasonal aeroallergens vs. perennial aeroallergens but without reaching a statistical significance, probably due to the small number of subjects included in subgroups. These results are consistent with other studies showing a prevalence of 1–40% of seasonal allergic rhinitis vs. 1–18% of perennial allergic rhinitis in the general population (23,24). The percentage of atopy and non-atopy in our asthmatic patients was 74% vs. 26% respectively. Burrows et al. found that the prevalence of atopy was about 40% in a general population study vs. 72% in asthmatic patients (25). The study by Postma et al. found a genetic susceptibility that contributes to bronchial hyperresponsiveness and atopy and that they are closely associated with bronchial inflammation, critical in the pathogenesis of asthma (26).

None of the asthmatic patients included in the study had severe AATD, usually associated with

homozygosity deficit (e.g. PI*ZZ, PI*Q0Q0) or double heterozygous (e.g. PI*SZ, PI*ZQ0, PI*ZMmalton) with AAT serum level < 50 mg/dL (9); the majority of the 22 AATD patients (16 patients, 73%) had AAT levels ranging from 50 mg/dL to 100 mg/dL. This result is probably due to the fact that our patients with AATD showed a clinically stable asthma with moderate airflow obstruction to normal lung function and is in line to the finding that very low levels of AAT are usually associated to severe respiratory disease (27).

We did not find any significant difference in respiratory symptoms, physical examination, disease severity or inflammation markers in AATD patients and non AATD patients. This could justify the underdiagnosis of AATD in patients affected by bronchial asthma. Kelbel et al. studied a population of 226 patients with AATD included in the Alpha-1 Foundation Registry and found 36% asthma and 24% atopy among the diagnoses. Allergy specialists have been involved in the visit of 35% of participants but only 5% of them was diagnosed with AATD. These findings support the view that the allergy specialists should pay more attention to asthmatic patients in relation to the screening for AATD (28). The only clinical differences between asthmatic patients with and without AATD observed in our study consisted in the presence of atopy. In fact, AATD promotes atopy and therefore asthmatic patients with AATD are more atopic and exhibit more seasonal allergy symptoms vs. patients without AATD. Furthermore, we found a significant prevalence of seasonal atopy in patients with asthma and AATD with higher incidence of self-reported allergic manifestations compared to asthmatic patients without AATD. These results could have relevant clinical implication; however, future research is needed in a larger population to confirm these data.

The analysis of the results obtained by grouping patients according to their PI* genotype, showed that 91% of AATD asthmatic patients had PI*MS and PI*MZ genotypes (55% and 45%, respectively). No significant difference was found in clinical characteristics, or in asthma features between PI*MS and PI*MZ patients, with the exception of the AAT median value, as previously reported by Miravittles et al. (29). Consistently with Malerba et al. (30), we did not find any significant difference in PD₂₀ FEV₁ values between patients affected by AATD and patients without AATD.

The prevalence of heterozygous PI*MS and PI*MZ AAT deficiency forms both in the population of

asthmatic patients included in our study and in others (29,31) led us to hypothesize that it is likely to find asthmatic patients with mild AATD among the subjects that commonly refer to pneumologists. It is not clear whether mild AATD can impact asthma severity. No significant difference was observed in blood eosinophil value between PI*MS and PI*MZ patients. Our finding is in contrast with the results by Suárez Lorenzo et al. who have found peripheral eosinophils significantly lower in the PI*MS subjects in comparison to other genotypes (32). These conflicting results could be justified by our small study population.

The fact that our data did not show any significant clinical difference between PI*MS and PI*MZ asthmatic patients, suggests that S or Z alleles equally influence the occurrence of atopy, without any difference in symptom worsening. Additional studies would require the enrollment of subjects affected by severe asthma to assess a prevalence of severe AATD forms and to investigate any clinical difference in disease severity.

We acknowledge that our study has some limitations. First, this study doesn't include a control group of patients with atopy without asthma necessary to evaluate the effect of AATD on atopy and asthma. Furthermore, this is a small study from a single center study and therefore not generalizable. Most likely our results reflect characteristics of asthma populations and further multicenter studies are required to confirm our results.

Conclusion

In conclusion, our findings further support the view that AATD and asthma are pathological conditions strictly linked and that AATD can differently characterize the asthmatic population through a higher prevalence of allergic manifestations and atopy in these patients, compared to those without AATD. Furthermore, no clinical difference was detected in PI*MS compared to PI*MZ patients, suggesting that S and Z alleles affect in the same way, the occurrence of atopy and allergic manifestations.

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Ethics approval and consent to participate

This study was approved by the Hospital Ethics Committee of North Emilia Area (approval number: 33503, dated

September 04th, 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.

Consent for publication

Not applicable.

Disclosure statement

The authors declare that they have no competing interests.

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Availability of data and materials

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

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