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α_1 antitrypsin deficiency prevalence in patients with Chronic Obstructive Airways Disease: a screening program in Nord Sardinia.

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Introduction

All the rare diseases have in common two main problems: the delay in the diagnosis and the difficult in reaching adeguate samples in order to have significant studies on epidemiological, clinical and therapeutic aspects. This is true also for α_1 antitrypsin deficiency.

Although in the past years different scientific societies, headed by World Health Organization (WHO), have focused the importance of an early diagnosis in order to change the natural course of the disease, the main part of the *at risk* subjects have never been screened for the AAT hematic value in a lifetime, and we are referring to millions of individuals with respiratory diseases that necessitate a variety of sanitary tests and therapy.

If, obviously, the genetic component of the disease is not modifiable, the environmental factors, that have a crucial role in development of the disease, surely can be controlled and improved. Starting an healthy life style, for example smoking cessation and reduced exposure to pollution, preventing the respiratory infections in addition to pharmacological treatment can influence heavily the prognosis, quality of life and the socio economical cost of this disease. Reducing the gap between symptoms appearance and diagnosis become mandatory and the screening programs in general and in selected population are the right answer both to understand the right

frequency of this disease that is, more than rare, under diagnosed, and how modify its history.

Following these needs we have started in 2019 a screening program in Sardinia, an island in the Mediterranean Sea that has peculiar genetical characteristics with negative and positive features: for example a high frequency of rare diseases such as Multiple Sclerosis but also a numerous group of centenaries. This is a consequence of a geographical and anthropological isolation during the past centuries, especially in some rural area of our region and in addition to a low exposure to pollution, create a different condition in comparison to the peninsular Italian territory.

The comprehension of the clinical characteristics of the affected subjects is the first step in the path to improve the management of the α_1 antitrypsin deficiency patients.

Backgrounds

Hereditary Alfa one Antitrypsin deficiency

α_1 antitrypsin deficiency (AATD) is genetic disorder with a autosomal co-dominant transmission which predisposes mostly to pulmonary and hepatic disease, but also to different clinical manifestations and diseases that actually are not completely understand.

The protein

α_1 antitrypsin (AAT) is a glycoprotein belonging to the Serpin (SERine Protease Inhibitor) family and its responsible gene, called SERPINA 1, is located on the long arm of the chromosome 14.

More than 120 alleles are known but only 30 of them are associated with pathological presentations. Historically the phenotypes were classified by the PI* (for Protease Inhibitor) system and the following letters denoted the migration rate during an electrophoresis on a starch gel, M (*medium*), S (*slow*), F (*fast*), or Z (*very slow*)[1].

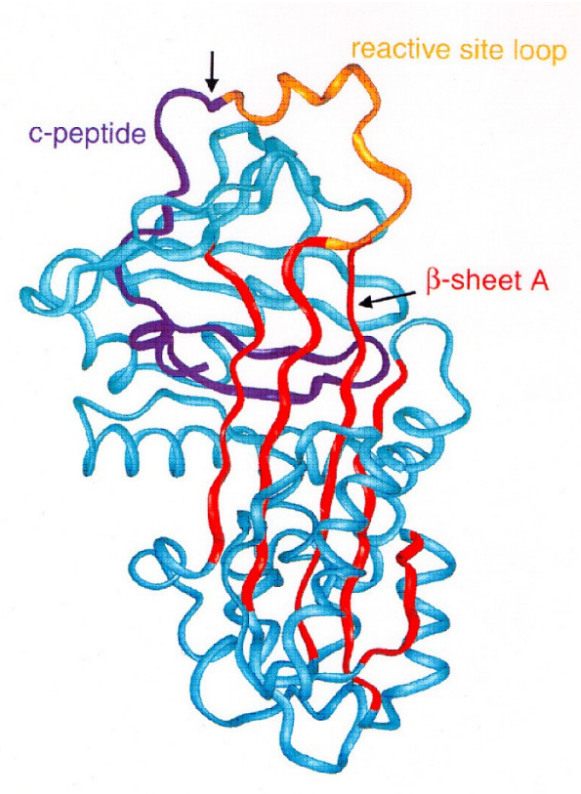

Fig. 1 AAT protein

Afterwards, when the migration procedure changed using a isoelectric point (isoelectric focusing, IEF, pH 4-5 on polyacrilamide gel) the nomenclature was maintained so the variant with an anodal migration were describe with the first letters of the alphabet and the one with a cathodal migration were associated with the last letters. Lately thanks to the genetic techniques it is possibile to describe the AAT allelic combination [2]. So, by convention, the phenotype describes the AAT protein expressed and are classified by the Pi* system based on the migration on isoelectric pH gradient, on the other hand the genotype demonstrated by allele-specific amplification, is classified by the PI* system.

The variants are usually categorized in 4 groups:

1. Normal, the AAT levels are within the normal range (20-53 μmol o 80-220 mg/dl) and genotype PI*MM
2. Deficient, the serum AAT is below 20 μmol and genotype PI*ZZ, PI*SZ and other rare variants as M-likes
3. Null, defined as Q0 and absent circulating protein
4. Dysfunctional, characterized by abnormal function of the protein, for example decrease binding to neutrophils elastase (PI*F) or thrombin inhibitory activity (Pittsburgh variant) [3].

AAT is mainly produced in the liver and lately secreted in the blood stream so the molecules can reach the lung by diffusion [4]. There is also a local production by alveolar macrophages, circulating monocytes and bronchial epithelial cells [5-8].

The main target is the neutrophil elastase (NE) and, through a suicide mechanism that implies its consume, AAT furnishes more than the 90% of the protection against the neutrophilic elastolytic burden in the deeper lung [9]. As the other serpins, AAT has a three β — sheets and a lateral chain where is located the reactive centre [10,11]. The bond with the NE causes the lateral chain rotation to the opposite side distorting and inactivating the elastase molecule and the AAT itself. In physiological condition, the abundance of AAT in the lung is able to guarantee an adeguate protection [12](Fig.2).

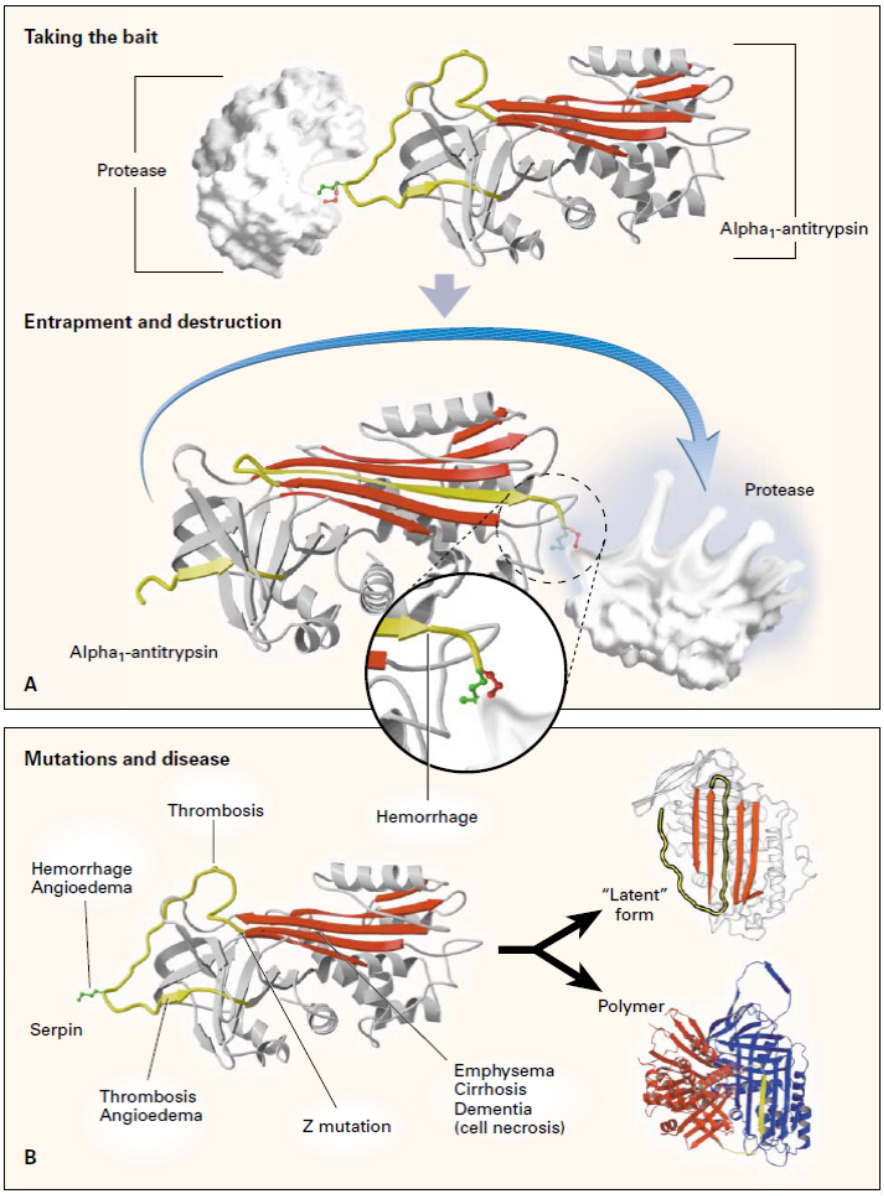

Figure 2. AAT Inactivating mechanism of neutrophil elastase (A), mutations and pathologies (B). *From Carrell, Lomas N Engl J Med 2002*

The protective threshold effective against the elastolytic activity in the lung is supposed to be 11 µmol (o 50 mg/dl) [13].

AAT also possesses anti-inflammatory capabilities that extend beyond its anti-protease role. In fact, AAT is involved in regulating the

CD14 expression [14], inhibition of TNFα gene up-regulation [15], and inhibition of lipopolysaccharide activation of human monocytes and neutrophil migration in vitro [16,17]. In addition, AAT has been shown to down-regulate apoptosis [18] and to inhibit antiprotenase 3 antibody activation of neutrophils [19].

In addition to the control on chemotactic neutrophilic expression due to interleukin 8 (CXCL8) and leukotriene B4 (LTB4) [20, 21], the AAT, thanks to its methionine residues, is a powerful antioxidant and it is able to suppress the superoxide production by activated neutrophils [22]. Among the pro-inflammatory molecules, TNFα is a cytokine with different functions including neutrophil activation, up regulation of adhesion molecules and stimulation of the production and release of other cytokines. AAT initially facilitates acute responses of the endothelium to TNFα, followed by selective inhibition of TNFα - Induced-self amplification, which may assist the vasculature in the resolution of chronic inflammation in both native and oxidized form [23,24].

All these mechanisms, even if it not completely clear, can explain the variegate clinical presentation of the AATD.

Pathophysiology

It is possible to recognize two major pathological mechanisms: the loss of a physiological function and the gain of a toxic action. In

the lung we mostly observe the effects of the protective function reduction, on the other hand, the hepatic damage is correlated to the cytotoxicity due to the intracellular accumulation of altered proteins.

The gain of a toxic function is related to specific genotypes, in particular in the Z alleles where the substitution of a lysine for a glutamic acid at position 342 widens the tertiary structure of the protein and allows polymerization by linking through the reactive loop different molecules in an irreversible process [25-27]. The polymers generate inclusions inside the endoplasmic reticulum of hepatocytes which, if not degraded, can cause clinical liver disease [28-29]. The retention of the polymers may be due to an alteration of the Z-type proteins and its chaperone, calnexin [30], to an irregular response to the unfolded protein [31], probably because the well ordered polymeric structures are not seen as misfolded [32] and to a damaged autophagy [33]. Intracellular inclusion were observed with other AATD phenotypes such as M_{malton} [34] and S_{iiyama} [35].

In the lung it is possible to describe a proteases-antiproteases unbalance, an amplification of the chemotaxis induced inflammation and a mechanical damage. The lack of AAT causes an uncontrolled serine protease proteolytic activity, mostly of the NE that is involved in the lung destruction [36] and in the alteration of the immune response by cleaving complement receptors and immunoglobulins [37, 38], interfering with ciliary mobility [39] and inactivate anti proteases such

as secretory leucoprotease inhibitor (SPLI) and elafin [40,41]. The elastase burden is enhanced by lung infections and cigarette smoking with an acceleration of the pulmonary damage and may be also consequent to a functional deficiency as the AAT is oxidized and inactivated by cigarette smoke.

The inflammation can also be fueled by enhanced neutrophilic recruitment to the lung secondary to different stimuli. The binding of free NE and alveolar macrophage causes their leukotriene B4 (LTB4) liberation with an increased chemotactic activity for neutrophils that has been demonstrated in the sputum of COPD patients [42]. Increased release of LTB4 by macrophages has been demonstrated in AATD patients [43,44] with blood levels that correlate with exacerbation frequency [47]. On the other hand, LTB4 decreases with augmentation therapy [46]. In addition, human neutrophil peptide (HNP), usually neutralized by AAT, in case of deficiency, can work as a pro-inflammatory molecule by releasing macrophage LTB4 synergically with NE and by interleukin 8 (IL-8), a ligand for the CXCR1 receptor on the neutrophil surface [47]. In COPD patient sputum, LTB4 has been estimated to contribute about 47% of the neutrophilic chemotactic activity and IL-8 for the 31% [42]. AAT polymers, that can be produced also within the lung, appear to fuel the inflammation in AATD. In particular, Z-type polymers are chemotactic for human neutrophils *in vitro* studies [48-50] and stimulate myeloperoxidase

release and neutrophil adhesion [50]. Moreover, elastin fragments have been found to drive the chemotactic activity and the monocyte recruitment in response to cigarette smoke in a murine model of emphysema. The use of a monoclonal antibody against elastin fragments interrupts the chemotaxis [51].

Finally the mechanical damage: the alveolar wall is made of elastic fibers that, in physiological conditions, share equally the stretching force so the maximum of tension is never reached. But, if some of the fibers are damaged, such as after an exposition to NE, the load that every fiber should sustain increases gradually reaching the breaking point [52]. This dynamic stress causes an acceleration of the parenchymal loss.

Epidemiology

AATD shows a different prevalence and frequency worldwide depending on the migration drifts. In Europe prevalence in general population varies from 1 in 1368 in Denmark to 1 in 58319 in Poland and in Italy is rated in 1 in 2000 - 5000 [53].

These numbers are higher from the diagnosed patients and it supposed that more than 90% of the affected subjects are undiagnosed. In fact, it has been estimated that 185 million subjects

carried one deficient allele (MS or MZ) and 5.5 million subject carried two deficient alleles (SS, SZ and ZZ) [54-56].

In clinical practice the majority of the severe deficient patients are associated with the Z allele, followed by S and then the so called "rare variant". Different studies point out that in specific geographical aerea these variant are not so rare and they could be more frequent where the PI*Z frequency is lower, such as in Sardinia, some Mediterranean and North African countries and Japan [57-60].

The prevalence of rare variants established by Ferrarotti *et al.* in 2005 in Italy was 1.2% and among them the more frequent is PI*M_{malton} (Fig 3) [58].

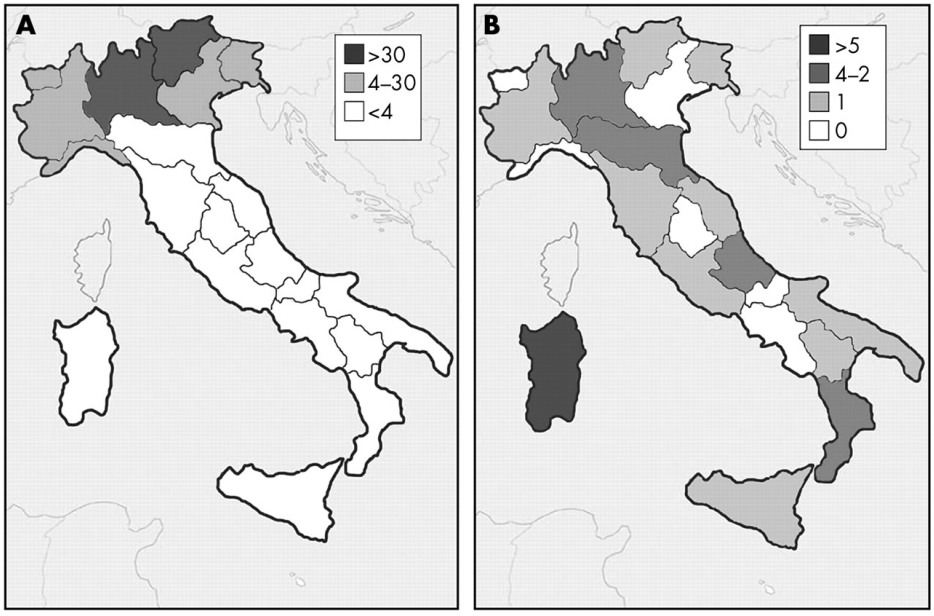

Figura 3: Geographical distribution of PI*ZZ genotype (A) and of rare variants carriers (B). ***From Ferrarotti et al. J Med Genet. 2005***

Beyond the general population the scientific interest was focus on the impact on the affected subjects. In fact 1% of subjects with chronic

obstructive pulmonary disease (COPD) has been estimated to suffer from AATD [61] most of them undiagnosed. Different screening programs were performed on COPD patients in different clinical setting trying to improve the diagnosis rate in this population where a more specific pharmaceutical and non approach could influence the clinical course of the disease.

Diagnosis

The first step in the diagnosis path is the quantitative evaluation of the serum AAT. Nowadays it is mostly performed by nephelometry and it is possibile to safely use dried blood spot (DBS) to centralize the samples.

In 2012 Ferrarotti and colleagues summarized the AAT level in general population following the genotype and identified the threshold of 1.1 g/l as the optimal value to recognize the genotypes caring at least an S or a Z [62]. AAT is a phase acute protein so its value con be influenced by the inflammation status of the subject so it is mandatory to evaluate biochemical markers such as C-reactive protein (CRP) when testing for AAT level.

As second step there are quantitative tests, namely phenotyping or genotyping. The first consist in the migration of the protein

following the isoeletrict point, the second utilizes the CPR technique to identify manly S and Z mutations but can also detect rare variants.

In 2017 the ERS proposes a diagnostic algorithm starting from the quantitative test to the genotyping and gene sequencing (Fig 4)[63].

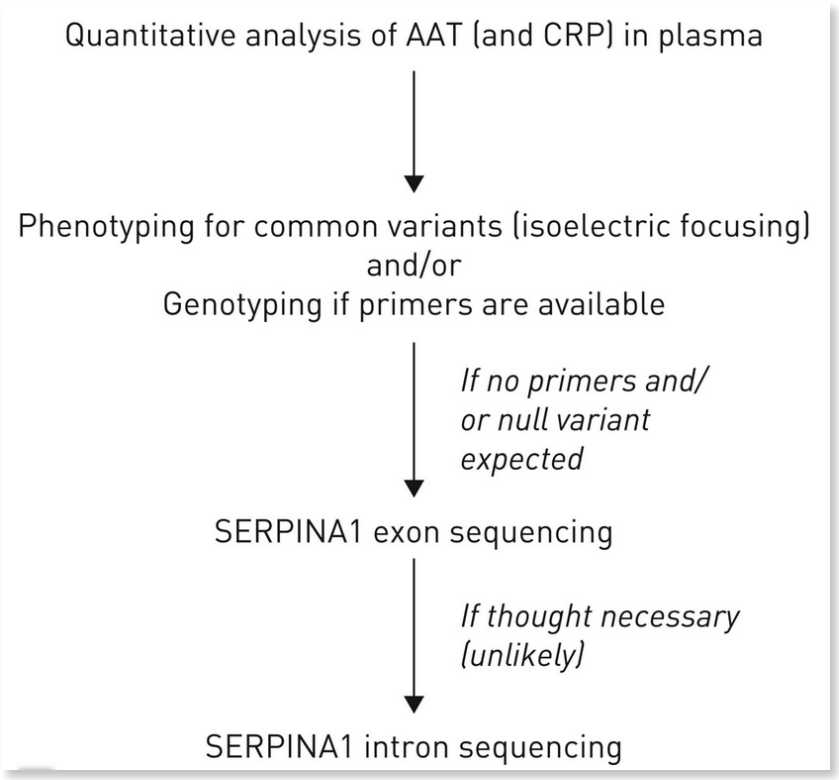

FIGURE 4. Algorithm for laboratory testing of α1-antitrypsin deficiency (AATD). This algorithm describes the current practice of how members of the task force treat patients with AATD and is not provided as a general recommendation. AAT: α1-antitrypsin; CRP: C-reactive protein. ***From Miravitlles et al. Eur Respir J 2017; 50: 1700610***

Once identified an index case should be proposed a family testing and an adeguate genetic counseling in order to explain all the characters of this hereditary disease.

Clinical aspects and therapy

AAT deficiency has been historically associated to pulmonary and hepatological conditions such as chronic hepatitis, cirrhosis and hepatoma [64,65], vasculitis (in particular anti cytoplasmatic antibody-positive such as Granulomatosis with polyangioitis [66-68) and skin diseases (especially panniculitis [69]). Nevertheless other diseases are thought to be correlated to AAT deficiency [70] such as glomerulonephritis [71], celiac disease [72], pancreatitis [73], lung, colon-rectal and bladder cancer [74] and intracranial and abdominal aneurysm [75] and fibromuscolar dysplasia [76].

Pulmonary involvement

The classical clinical phenotype is defined by a pan-lobular emphysema with a basal predominance and an early onset COPD, usually in the 4th or 5th decade depending on exposure factors as smoking habits, but the pulmonary involvement has fully demonstrated to be far more heterogeneous.

The different registries, both at national and international level [77,78], and the follow-up of the Swedish cohort [79] have allowed to identify different sort of lung damages and consequent clinical presentations. It wides from asymptomatic to more classic COPD

features, common also in not AATD subjects, such as cough, dyspnea, wheezing, bronchiectasis, chronic bronchitis, bacterial colonization, frequent exacerbations, impaired health status and a degree of reversibility of airflow obstruction [80-83]. Furthermore the radiological aspects are more heterogeneous than we could expect. In fact only 20% of chest radiographs in PI*ZZ showed basal emphysema and 15% were normal [84] and in computer tomography series the 64% of the 102 PI*ZZ subject demonstrates basal emphysema but 36% has apical predominance. [85]

The variety of clinical and radiological patterns persuades the different guidelines to recommend AAT testing in all the subject with COPD irrespective of age and severity and adult onset asthma [86-89]

Treatment

Currently only specific treatment for AATD is the augmentation therapy. It consist of the intravenous administration of AAT with the aim of raising the serum level above the pulmonary protective threshold and, consequently, positively influence the pathological mechanism of lung disease.

For many years the augmentation therapy was given without clear scientific evidences but recently different studies and metanalysis have demonstrated its value. The ERS statement group in 2017

examined the RCT trials and the observational studies [63] and affirmed that in severe AATD augmentation therapy reduces the emphysema progression.

The elimination of negative factor such as smoking habits or working exposure and a strictly control of the pulmonary disease are fundamental in the evolution of the disease mostly in the early phases when the lung tissue is less involved.

Purpose

Starting from a AAT hematic screening in subjects affected by chronic obstructive airways diseases in Nord Sardinia, the project has the aim to describe in a selected population the AATD incidence, the allelic prevalence and its clinical and genetic characteristics.

The knowledge of the current estimated prevalence is the first step in the characterization of any disease because it helps to define a patient "*typical*" profile and consequently its recognization. Its importance is magnified even further in AATD and similar rare conditions, in which only a prompt suspects can lead to a timely diagnosis that is, unfortunately, still an unmeet need.

The interaction among specialists, general practitioners and patients is the only practicable way to widen the comprehension of the disease and to start the changes needed to improve the quality of life and prognosis of this subjects and their relatives.

Methods

We made an observational screening study in patients with pulmonary diseases followed in outpatients clinic of the Respiratory Disease Department of the Sassari University in Sardina, Italy.

The objective of the study were:

- To assess AATD prevalence and to estimate the different allelic variants frequency
- To describe the clinical and demographic characteristics of the patients

Population

We enrolled subjects aged more than 18 years old affected by COPD, asthma, bronchiectasis and pneumothorax from 2017 to 2019.

The sample will be described at the enrollment by demographic (age, gender, BMI), anamnestic (smoking habits, familiarities), clinical (pulmonary and hepatic diseases, symptoms) radiological (High Resolution Computed tomography, HRTC), functional (respiratory functional tests) and laboratorial (C reactive protein, CRP) characteristics. All the patients underwent a hematic sample analysis in order to evaluate AAT serum level.

The limit to advance into the diagnostic path was 128 mg/dl. This value allows to recognize different heterozygosis alleles and, in a certain ways, correct the not normal CRP values in some subjects.

Based on the serum AAT levels, individuals were classified as no deficiency, intermediate deficiency and severe deficiency.

The samples of deficient patients were sent to the Anatomic Department of the University of Cagliari by dried blood spot (DBS) or hematic samples in order to define the genotype.

Statistical Analysis

Categorical variables were described by frequencies and percentages and compared using the Chi-squared test. Continuous variables were expressed as mean ± SD or median and interquartile rate and compared between groups using the T-test or Fischer test where appropriate.

Prevalence comparisons were made with Binomial Test [William J. Conover (1971) Practical nonparametric statistics. New York: John Wiley & Sons].

To evaluate allelic mutation influence on pulmonary diseases first *Generalized Linear Models (GLM*) were adopted for each one and then a Wald Test was performed (alpha=0.05).

Mutation effect on spirometric values was evaluated by Nonmetric Multidimensional Scaling (NMDS) and PERMANOVA where appropriate .

Data were processed using SPSS 28.0.1 (IBM SPSS, Inc., Chicago, IL, USA).

Results

Population

A total of 469 subjects was enrolled in our study, of these, 268 (57%) subjects were male and 201 were females.

The mean age of the sample was 67.96 ± 14.24 years old and the mean BMI was 26,46 ± 14,24.

290 (61%) subjects were active or former smokers, with a mean tobacco consumption of 29.56 ± 35.45 pack-years.

Asthma was reported as main familial disease (3%) and only 2 subject referred a AATD case between relatives (0.4%).

62.5% were diagnosed with COPD, followed by asthma (23.7%) and emphysema (19.2%).

The mean AAT value observed was 155.83 ± 46,68 with a mean CPR of 3,17 ± 15,05. (Table 1)

Gender	Male	268 (57%)
Mean age (yo)		67.96 ± 14.24
BMI (mean, Kg/m^2)		26,46 ± 14,24
Smoking habits	Never	119 (25%)
	Yes	91 (19%)
	Ex	199 (42%)
	Pack years	29.56 ± 35.45

Familial disease	Asthma	14 (3%)
	Emphysema	7 (1,5%)
	Bronchiectasis	1 (0,2%)
	COPD	6 (1.3%)
	AATD	2 (0,4%)
Diseases	Hepatological	7 (1,5%)
	Asthma	111 (23.7%)
	Emphysema	90 (19.2%)
	Bronchiectasis	74 (15.6%)
	COPD	293 (62.5%)
AAT (mg/dl)		155.83 ± 46,68
CRP (mg/dl)		3,17 ± 15,05

Table 1. General characteristics of the population.

Complete lung function tests population

Limiting to the subjects with complete pulmonary function data we have 265 patients with male prevalence (56%) and similar characteristics to the general population (Table 2).

Mean age, (years old)	68 (60-77)	
Gender (F/M), %	44/56	
BMI, Kg/m^2	25.7 (22.2-30.1)	
Smoking habits %	Never	27
	Ex	53
	Yes.	20
FEV_1, %	68 (52-86)	
FVC, %	79 (65-93)	
FEV_1/FVC, %	76 (62-98)	
VR, %	143 (111-186)	
TLC, %	106 (93-125)	
COPD, %	66	
ASTHMA, %	26	
ENPHYSEMA, %	26	
BRONCHIETASIS, %	15	
CRP, mg/L	0.69 (0.31-1.92)	
AAT, mg/dL	150 (125-175)	

Table 2. Characteristic of subjects with complete lung function test

Allelic frequency among the deficient group

71 patients (15% of the sample) had an AAT value lower than 128 mg/dl and underwent to genotyping process. 38 of them (53,5%) showed a mono or bi allelic mutations of the SERPINA 1 gene.

Nine combination was observed: PI* MM, PI*M/M Malton, PI*MS, PI*MZ, PI*M $_{Malton}$ M $_{Malton}$, PI*ZZ, PI*SS, PI*M $_{Malton}$S e PI*ZS. (Table 3)

Genetic allelic combination n= 71 (n, %)	
M M	33 (46.47%)
M M $_{Malton}$	15 (21.12%)
M S	14 (19.71%)
M Z	3 (1.4%)
M $_{Malton}$ homozigosis	2 (2.81%)
S M $_{Malton}$	1 (1,4%)
S S	1 (1,4%)
Z Z	1 (1,4%)
S Z	1 (1,4%)

Table 3. Allelic combination observed in the deficient subjects

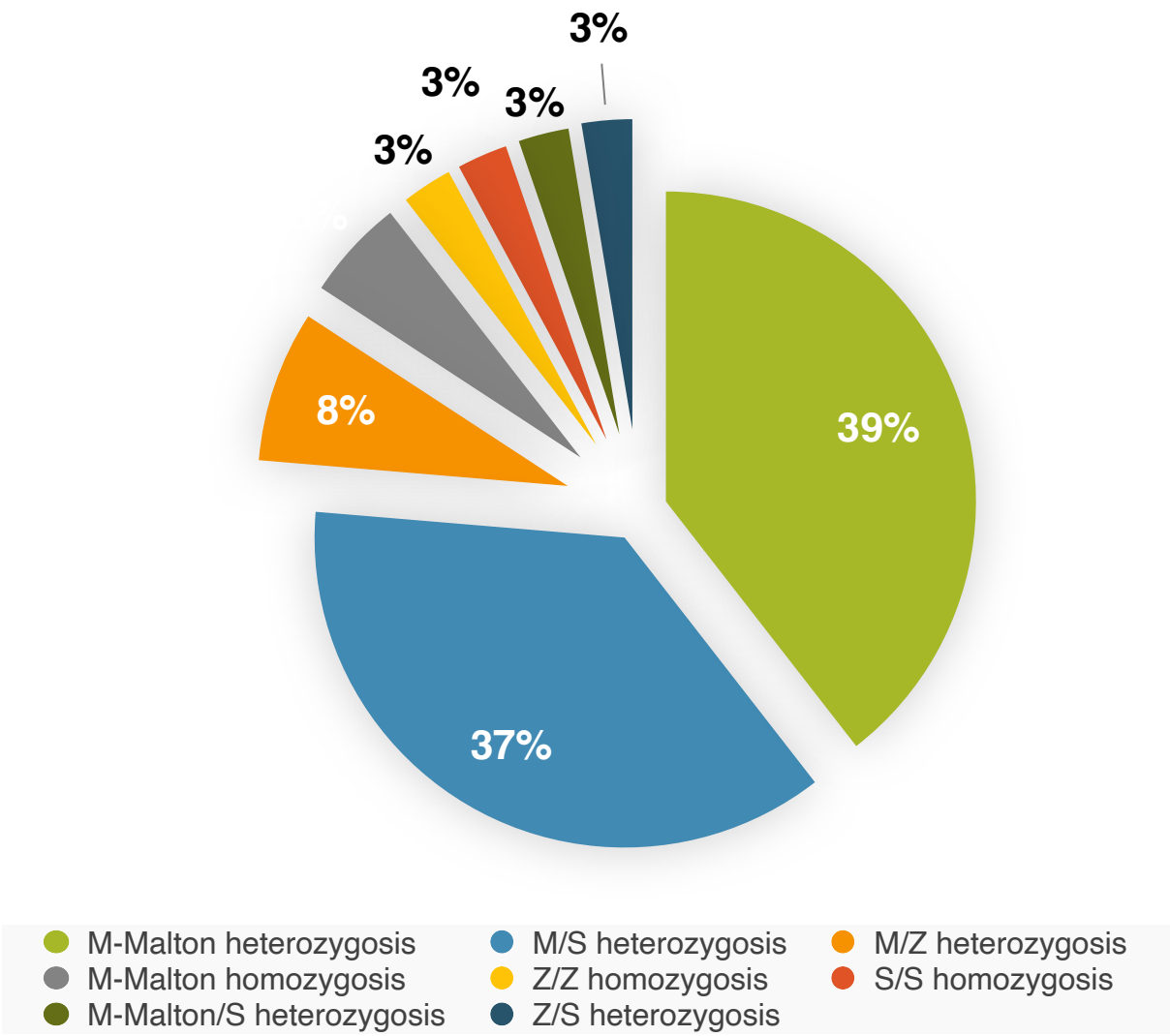

Fig 5. Frequency of the different allelic combination

Among the 38 subjects we observed 20 males and 18 females without significant difference in prevalence (p=0.8714).

The more frequent mutations observed were heterozygosis MM_{Malton} (39%) and the heterozygosis M S (37%). (Fig. 5)

M_{Malton} is the more carried mutation in heterozygosis and homozygosis (18 subjects). That is a frequent higher than expected and it possible to estimate in our Sardinian population (469 subjects) a prevalence of 3,84%.

AATD prevalence in COPD, asthma, bronchiectasis ed emphysema versus general population

In our sample of 469 subjects we observed 38 patients with AATD. It is possible to assess that the prevalence of AATD in the population affected by COPD, asthma, bronchiectasis and emphysema, in Sardinia, is 8.32%. That is significantly higher ($p<0,001$) than the general population (prevalence 0,00033%).

Influence on allelic mutation on clinical presentation

With the limitation of our small numbers of our sample we observed the following clinical presentation for every mutation. (Tab. 4)

	N	**%**
Hepatological disease	1	2.6
Asthma	13	34,2
Emphysema	7	18.4
Bronchiectasis	6	15.8
COPD	8	21.1
Other	4	7,9%

Tab 4. Clinical phenotypes in mutated group

	Asthma	COPD	Emphysema	Bronchiectasis	Other
S S		1			
Z Z			1	1	
M $_{Malton}$ homozigosis		1	1		1
M M $_{Malton}$	5	3	3		7
M S	6	2	2	5	2
M Z	1	1			2
S Z					1
S M $_{Malton}$	1				

Tab.5 Disease frequency for every allelic combination

Some of the patients presented overlap between different diseases, but the clinical presentation more observed was asthma and COPD (Fig.6).

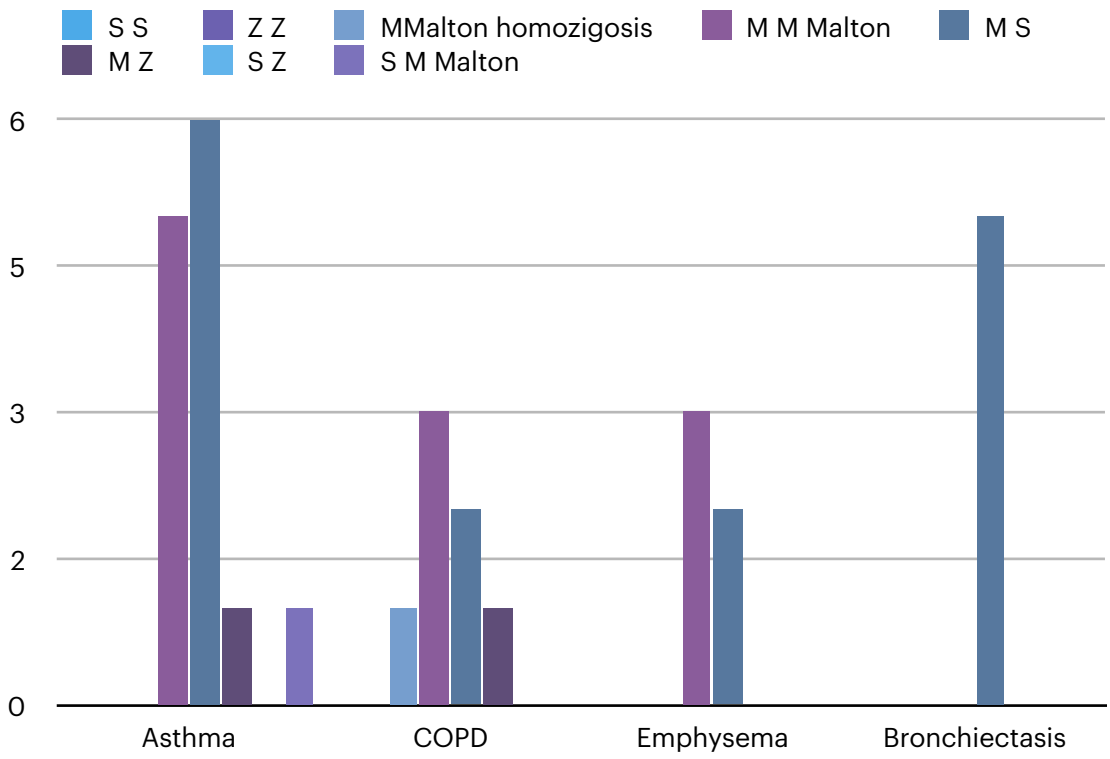

Fig.6 Clinical presentation in mutated group

The exact test of Fischer does not demonstrated any influence between allelic mutation and pulmonary disease (p=0.08874).

Allelic combination and FEV_1

In order to evaluate the correlation between mutation and functional tests we chose to confront the two numerous groups: PI*MS and P*M M_{Malton} (n=10 and 6 respectively).

The distribution inside the *Nonmetric Multidimensional Scaling* (NMDS) multivariate space was significantly representative with a

stress value of 0,0566. As showed in the figure 7, patients carried an S mutation (black triangle) and patients with an heterozygosis with M_{Malton} (white dot) are partially separated in the NMSD plot.

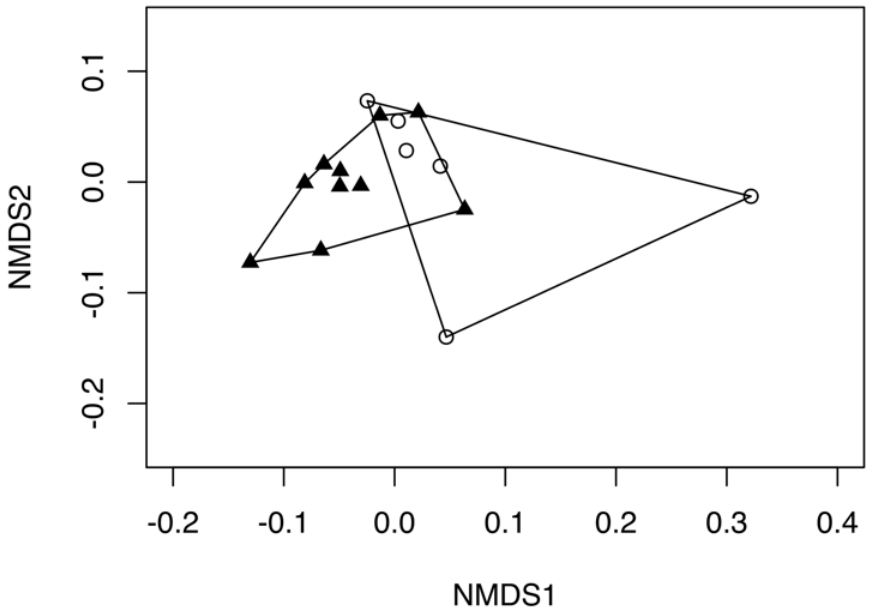

Fig.7 Multidimensional Scaling (NMDS) on spirometric pattern in patients with heterozygosis M/S (black triangles) e heterozygosis M. M_{Malton} (white dots).

This was also confirmed by the PERMANOVA analysis that showed a significantly difference between the two patterns. (PERMANOVA: F1,14=3.54, R2=0.20, p=0.02)

Discussion

The importance of an early diagnosis is out of doubt for any disease but it become critical in all the conditions which can be profoundly influenced by behavioral and pharmacological treatment even to prevent the disease development.

The screening programs have been at the center of the diagnosis both of symptomatic and asymptomatic subjects. Few years after the first identification from Eriksonn and Laurell in 1963 [90], screening programs were performed in addition of epidemiological strategies in order to assess the frequency of this new disease.

There are two approaches: population based screening, with unselected subjects, or among selected groups. Obviously random population samples can provide less biased data but they are difficult and expensive to perform. On the other hand testing selected groups can be even more useful if a “at risk” or “affected” sample is chosen because it is possibile to identify people in whom educational and pharmacological programs can improve the quality of life and prognosis.

In our study we decide to enroll patients with pulmonary diseases that the major international societies suggested to be tested for AATD: COPD, asthma, bronchiectasis and pneumothorax. 15% of the subjects were under the chosen cut off for serum AAT. This can be slightly an overestimation because we set a higher cut off than usual. In this way

we have tried to correct the influence of an underlying inflammatory state on the AATD value and, as described by Ferrarotti in 2012 [13], we have the chance to identify more subjects even with a single allele mutated.

Our data showed a AATD prevalence of 8.39% and considering the prevalence of COPD (approximatively 10-15% in Sardinia [91]) and of asthma (10%), there is a massive proportion of undiagnosed individuals in everyday clinica practice. These people need to be informed about the disease, its consequences and how modifying everyday life is possible to ameliorate quality of life and prognosis. Apart from the medical and ethic point of view, respiratory disease has a great impact on the health system both in direct and indirect ways. The health care expenses (drugs, inpatients and outpatients) rules the direct cost whereas the production lost form the indirect ones. European Respiratory Society estimated that in Europe the direct cost of COPD and asthma are respectively about €1000 and €2000 per patient per years with a total (direct and indirect) charge amounting to €82 billion [92]. The recognition of an AAT deficiency among the patient with obstructive respiratory disease allows a better treatment and counseling and could influence the huge costs of this diseases.

In 2005 de la Roza and colleagues started a severe disease (namely PI*ZZ) detection program among COPD patients in Spain and observed a 4.5% of the subjects with low AAT hematic value and 0.37% had a severe AATD [93]. These data were confirmed in a very

interestingly pilot program where the COPD patient were enrolled by family practice physicians [94]. In Germany in 2002 a similar program performed by general physician but enrolling individuals with different lung disease did not detect any severe case among the 1060 subjects [95] but in the USA within a similar population included by specialist and general practitioners, a higher diagnosed rate was observed (PI*ZZ 1 every 31 samples and 1 every nine was PI*MZ) [96]. In our population we identify one patient with PI*ZZ and one PI*SZ (each one 0.21% of total). The difference observed among these studies is easily explicated by the geographical distribution of the alleles worldwide as demonstrated in different epidemiological studies [57, 98].

What is really interesting, even if not surprisingly, is the frequency of a rare variants, M_{malton}. In the past years the role of the so called *rare* variants was reassessed: they are not so rare, with a prevalence described between 0.6 and 4.2% [58-60, 98 99]. The prevalence in our population, with the limitation of the sample size, is 3.84% supporting what reported for the Sardinian population in 2005 by Ferrarotti et al. [58] where it was described as the highest rate of rare variants in Italy.

M_{malton} is not only prevalent but it is the only rare variant we observed in our population. It is quite a common rare allele, in fact it was detected with high frequency in several studies in different countries (20% in Spain, 60% in Tunisia, 8% in Switzerland, and 35% in Italy) [58-60, 98, 99]. Furthermore in Sardinia M_{malton} is so recurrent

that has also a local designation, called $M_{cagliari}$ from the city where it was firstly described [100].

The abundance of AAT deficient variants leads to the hypothesis that they can carry a sort of protective mechanism through an inflammatory response amplification against infectious agents in the pre-antibiotic era [101]. Following this idea, rare variants have been selected where the more common deficient alleles were unfrequent and in this way preserving this theoretical biological advantage.

As long as our knowledge about AATD has grown, we have learnt that a pan lobular juvenile emphysema was only one of the many presentations that a medical doctor could meet. We selected our subjects among different lung diseases considered at risk and we meet a multifaceted pathology with different clinical aspect that easily mixed up. Asthma and COPD, also overlapping one into the other, were the more recurrent pulmonary diagnosis in the mutated group. Confronting subjects from the Italian and Spanish Registry Piras et al. found that the asthma prevalence was 9% [83], a value much lower from our 34.2% that is close to the 31% observed in the NHLBI registry [80] and the 36.6% in the Alpha One Foundation Research Network Registry [102] even if the subjects enrolled in these series are not similar.

The genetic mutation involved does not seem influence the clinical phenotype in our population. As told before, the environment and the personal habits affects deeply the AATD natural course but

some clinical aspects, such as a discordance among siblings [103] and the differences in lung function among similar patients [104-109], point up that there is surely more to be discovered. In these years we are enlightening new role for AAT in several inflammatory mechanism that could in the future explain the clinical complexity of this genetical condition.

So a specified allelic mutation does not lead to a specific clinical phenotype, but what happens regarding lung ventilatory function? The pulmonary function tests are routinely use to assess the gravity of the lung loss in several obstructive conditions. Although it was demonstrated that they are not so sensible and specific in evaluating the AATD pathological progression, they are simple, economical and repeatable with almost an easy access everywhere and limited contraindications comparing to more specific exams, for example CT scans.

The decline functional rata is variable among subject with severe AATD carrying the same mutation [110-112] depending on different external factor that can at least partially be modified.

In this study we have tried something different: compare the functional loss pattern between the two more frequent allelic combinations. With the limitation of a restricted sample, we observe a significative difference so we can speculate that a specific mutation could predispose to a determined pulmonary deterioration pattern.

Limitations

As author of this study, I am well aware of its limitation.

It depends mostly of the sample size and the missing data regarding lung ventilatory function and imaging.

The original study was drawn in late 2018 and in 2019 and it should comprehend different outpatients clinics all over the regional area and the screening should involved approximately 2000 subjects. At the enrollment in addition to the anamnestic data, informations from respiratory functional test and high resolution thoracic tomographic scans should have been collected. Furthermore we have scheduled to follow up the AATD subject with a frequency depending on their severity lung involvement.

From February 2020 Italy has been hitting by a series of waves from the COVID 2019 pandemia that still continue at the present time. The consequences on the Italian health system were disastrous at any levels and every strengths was focus on this fight. As consequence of the isolation protocol imposed in order to reduce the viral spreading, the outpatient clinics were closed and all their procedures were postpones. In addition international scientific societies recommended to not perform any lung ventilatory test for the high risk for the staff to viral exposition.

This affected obviously our study: the other outpatients clinics in Sardinia were not able to perform any enrollment and we have to

reduce our study to a single centre, the Respiratory Disease Outpatient Clinic of Sassari University. Moreover the data were often partial because of the impossibile task to perform CT Scan or a lung ventilatory test.

We have tried to correct as much as possibile the bias in our analysis, but more data are need to confirm and improve our observations.

Without any doubt reaching the population chosen in the original project could substantiate the results, the conclusions and overall the significancy of the study. Our ambition is to achieve in the next future what we have had programmed and to compare the new results with what we have observed until now.

Conclusion

Starting from a simple genetic mutation, α_1-antitrypsin deficiency is proved once again to be a variegate condition influenced by the environment, personal habits but also something more that is still unexplained.

This creates a real challenging task in the clinical practice both to general and specialistic physicians. Diagnose this disease promptly is essential because it is possibile to modify its evolution controlling some of the external factor and creating an information and supporting net around the patients and their family.

Speaking of respiratory affected patients, such as those involved in our study, this need became mandatory. Reducing or decrease the loss of pulmonary tissue and function is essential not only from the ethical and individual side, but also thinking about the social economical impact that the more frequent clinical phenotypes, COPD and asthma, have on our society.

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