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Investigating the Link between Alpha-1 Antitrypsin Deficiency and Abdominal Aortic Aneurysms

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Background: Alpha-1-Antitrypsin (AAT) is one of the major plasmatic protease inhibitors. In the last decade, an association between Alpha-1-Antitrypsin Deficiency (AATD) and Abdominal Aortic Aneurysms (AAA) has been hypothesized. Multiple factors may be involved in AAA's etiopathogenesis, and an underlying structural defect of the extracellular matrix (ECM) is always present. AATD could be a reasonable risk factor for AAA because it is related to protease/antiprotease imbalance and enhanced ECM degradation of the vessel wall.
Methods: We performed genotyping of 138 patients hospitalized in the Vascular Surgery Division of the ASST-Spedali Civili di Brescia, Italy, for nontraumatic rupture of AAA. The second purpose was to observe the distribution of main nongenetic risk factors for AAA between patients with and without AATD.
Results: Out of 138 patients, 22 were found with AATD: 16 MS, 1 SS, 3 MZ, and 2 with a new rare AAT variant. When compared to the general Italian population, our cohort's frequency of deficient S allele was significantly higher (7.8 vs. 2.2% respectively, $P < 0.01$), whereas the deficient Z allele was similar (1.1 vs. 1.3% respectively, $P > 0.05$). Although we found no differences in age, gender, hypertension, diabetes, and smoke habits between AAA patients with and without AATD, hyperlipidemia was significantly less frequent in patients with AATD (46.4 vs. 12.5% respectively, $P < 0.05$).
Conclusions: In our AAA patients' cohort, the S allele frequency was higher than in the general Italian population. Our results support the hypothesis that AATD might be a risk factor for AAA.

The authors declare no conflicts of interest.

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Abbreviations: AAT, Alpha-1 Antitrypsin; AATD, Alpha-1 Antitrypsin Deficiency; AAA, Abdominal Aortic Aneurysm; ECM, Extracellular matrix; LDL, Low density lipoprotein; DBS, Dried Blood Spot; HS-CRP, High Sensitive C Reactive Protein; EDTA-Na_2, Ethylenediaminetetraacetic acid disodium salt dihydrate.

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INTRODUCTION

Alpha1-antitrypsin (AAT) is the main plasmatic protease inhibitor and plays a crucial role in protecting the connective tissue from the damage inflicted by proteinases, particularly neutrophil elastase. Inherited deficiency in AAT (AATD) is associated with various diseases such as lung emphysema and other respiratory diseases, liver diseases, panniculitis, kidney disease, heart disease, rheumatoid arthritis, and systemic vasculitis.[1–10]

In the last decade, associations between AATD and vascular disease, such as Abdominal Aortic Aneurysm (AAA), have been theorized.[11] However, further investigations are needed before this association can be confirmed.

Abdominal Aortic Aneurysm (AAA) is a vascular disease with a prevalence rate between 1% and 9% and 1–2.2% in men and women, respectively. In the last 2 decades, the incidence is progressively increased, and its rupture, the most life-threatening condition, is associated with an overall mortality rate between 65 and 85%. AAA causes about 8000 deaths per year in the UK and 15000 deaths per year in the USA, and it is responsible for an overall death percentage of 1–3% among men aged between 65 and 85 years living in developed countries.[12]

To reduce the AAA-related mortality is essential to make an early diagnosis through which it is possible to establish the appropriate follow-up regimen and timely plan surgery. For this purpose, it is important to identify all the possible risk factors for AAA development.[13] Although multiple factors may be involved in AAA's etiopathogenesis, an underlying structural defect of the extracellular matrix (ECM), resulting from a combination of genetic and environmental factors, is always present, and the loss of elastic fibres seems to be an early step in AAA formation.[14]

Among several described risk factors, those most relevant and acknowledged are tobacco smoking, hypertension, hyperlipidemia, diabetes, atherosclerosis, traumas, infections, inflammatory diseases, and connective disorders.[15–18] In this picture, AATD appears to be a good candidate as a risk factor for AAA's onset and development, as previously suggested.[19–23] The imbalance between elastase and antielastase caused by AATD provides an unopposed increase in Neutrophil Elastase amount, leading to progressive degradation of the ECM into the vascular wall endangering its mechanical stability and elasticity.[24,25] Moreover, the lack or decrease of the AAT antiinflammatory activity may induce a relative increase in proinflammatory cytokines' basal level, and the subsequent local and systemic inflammatory processes may contribute to the AAA development and progression.[26–29] Many evidences confirm that AAT may be implicated in blood vessel protection,[30–35] and in patients with AATD, an increased aortic wall stiffness has been shown.[36] For all these reasons, AAT was defined in 1994 as a "guardian of the vascular tissue."[37]

Despite the available data describing a possible role of AAT deficiency in the onset of vascular diseases, only a few studies have investigated the relationship between AATD and AAA. In 1994, Elzouki et al. reported 1 case of AAA among 30 patients with AATD.[38] Other studies showed conflicting results. Schardey et al. in a case-control and Cohen et al. in a case-finding study described a positive association between AAA and AATD,[19,39] while St. Jean, in a case-control study, and Hernandez and Elzouki, in the respective case-finding studies, did not find any significant correlation.[40–42] Hence, although biologically plausible, the link between AATD and AAA is still a matter of debate.

That moved our intent to start a research aiming to confirm the possible association between AATD and AAA occurrence, leveraging a consistent population of patients with AAA afferent to the Vascular Surgery Division of the ASST-Spedali Civili of Brescia, 1 with the highest volume of the AAA repair surgeries in Italy.

METHODS

Study Design

To primarily investigate the association between AATD and AAA, we collected and analyzed data from consecutive patients hospitalized for planned AAA surgery in 1 year.

The second goal was to observe the distribution of the main risk factors in these patients with and without AATD.

A total of 138 patients with abdominal aortic aneurysms were recruited for this study. Participants were selected among patients who underwent surgery for AAA at the Vascular Surgery Unit of the Spedali Civili of Brescia. Patients with AAA of traumatic origin and subjects suffering from collagen diseases (e.g., Marfan syndrome) were excluded from the study.

A thorough anamnestic investigation was conducted in all patients to reconstruct their clinical history. Patients with a cigarette pack/year value equal to or greater than 10 were considered smokers. Patients with systolic systemic blood

pressure higher than 140 mm Hg and/or a diastolic higher than 90 mm Hg, or treated with antihypertensive drugs, were considered suffering from systemic arterial hypertension. Patients with fasting plasma glycaemia equal or greater than 126 mg/dl, found at least in 3 independent measurements or in therapy with oral hypoglycemic drugs or insulin, were considered to have diabetes. Patients with LDL cholesterol values greater than 160 mg/dl or in therapy for hypercholesterolemia were considered affected by hypercholesterolemia. Finally, patients with ischemic heart disease with a positive history of episodes of angina pectoris or previous myocardial infarction were considered to have ischemic heart disease.

Patients underwent blood sampling to perform Alpha1-antitrypsin and C-reactive protein plasma dosage and genotypization for the AAT gene.

Alpha1-antitrypsin Quantification

The Dried Blood Spot technique (DBS) has been adopted to collect blood samples for the AAT dosage.[43] Patients' fresh blood was taken through a venous puncture or a fingertip puncture and collected on filtered cards. The AAT assay was performed with the nephelometric technique.[44] A polyclonal antihuman AAT antibody of caprine origin has been used. A calibrator compares the amounts of the alpha1-antitrypsin present in the sample against a conventionally established value used as standard. To ensure that each sample would be equal to the others, 6 mm diameter discs from each card have been cut. The disc is left at room temperature overnight immersed in 266 μL of water to achieve a theoretical elution of 1:21. This elution is applied to samples from adult subjects with an average hematocrit value of 45%. Values obtained are regressed using different curves according to the nephelometric reading of the eluate.

C Reactive Protein Quantification

The high-sensitivity immunoturbidimetric C-Reactive protein (HS-CRP) method was used, in which the intensity of the scattered light depends crucially on the diameter and refractive index of the particles scattering the light.

The immunological reactions amplified by particles are based on this phenomenon. Antibodies are bound to polystyrene particles (latex) by adsorption or by covalent binding. The resulting particles have numerous binding points for the antigen. Their size is several times larger than the size of an isolated antigen-antibody complex. The binding of these structures by a few antigen molecules already leads to a significant increase in light scattering. With turbidimetry, the attenuation of the intensity of the incident light beam passing through the solution containing antigen/antibody aggregates is measured.

This method provides a predilution of standards and samples with a tris(hydroxymethyl)aminomethane buffer 16 mmol/L at pH 7.4. The second reagent of 150 μL volume is added, consisting of latex particles coated with monoclonal anti-C protein murine reactive monoclonal antibodies 0.1% in 50 mmol/L glycine buffer at pH 8.0. Blood samples were taken in tubes with added anticoagulant. Samples were then centrifuged at room temperature for 15 min at 3000 rpm. After separation, the plasma was divided into aliquots, which were analyzed.

Alpha1-antitrypsin Genotyping

To genotype the AAT gene, DNA has been extracted from the DBS collected samples using a commercial kit (DNA IQ System, Promega, Madison, Wisconsin) and maintained at 4°C. DNA analysis has been performed using the SeAI/Hpy991 RFLP technique. Amplification by PCR has been performed with the following primers:

- P3M (5'CTTCCAAACCTTCACCTGGT3') and P3P (5'GTCCTCATGAGCATGACGGCG3') for amplification of exon III S-variant; -5M (5'GAGCCCCTTGCTCGCCTGGATC3') and 5P (5'CAGGAAACATGAGGAGGATGATTTAC3') to amplify and search the exon V variant Z.[45]

The sequencing technique was necessary when a discrepancy between the results obtained with the AAT assay and the genotyping occurred. The CEQ 8800 Genetic Analysis System (Beckman Coulter) has been used.

Statistics

Continuous variables have been described as mean and standard deviation, and the differences between the 2 groups were calculated using the Student *t*-test. Differences between categorical variables were analyzed using Fisher's exact test applied to 2 × 2 contingency tables or binomial probability distribution analysis. The statistical analysis was performed using the "open source" statistical package R.[46] Statistical significance was declared with $P < 0.05$.

Table I. Anthropometric characteristics of the 138 patients

Anthropometric parameters	*Mean ± SD*
Age (years)	73 ± 10.3
Male Sex (%)	93.6
Female Sex (%)	6.4
Weight (kg)	73.2 ± 14.2
Height (m)	1.68 ± 0.06
BMI (kg/m^2)	25.9 ± 4.3

Table II. AAT genotyping results

AAT genotype of 138 patients	
No-deficit (n)	*With deficit (n)*
116 Pi-MM (84.06%)	**16** Pi-MS (11.59%) **3** Pi-MZ (2.17%) **1** Pi-SS (0.73%) **2** New rare variants (1.45%)

Table III. Comparison of our results with data for the Italian population

Pi	Studied population	Italian population	*P* value
S	6.5%	2.5%	<0.01
Z	1.1%	1.3%	NR

RESULTS

In 1 year, 138 patients have been enrolled. The average patients' age is 73 ± 10.3 years. Patients showed an average weight of 73.2 ± 14.2 kg and an average height of 1.68 ± 0.06 m. The average BMI value was 25.9 ± 4.3 kg/m2. The male gender was prevalent (93.6%) (Table I).

Twenty-two patients were found with AATD: 16 MS, 1 SS, 3 MZ, and 2 with a new rare AAT variant (Table II). In our cohort, when compared to the general Italian population,[47] deficient S allele frequency was significantly higher (7.8 vs. 2.2% respectively, $P < 0.01$), whereas the frequency of deficient Z allele was similar (1.1 vs. 1.3% respectively, $P > 0.05$) (Table III).

Both AAT and C reactive protein were tested to ensure that alpha1-antitrypsin values were not affected by systemic inflammation in patients. The AAT was significantly lower in the AATD group than in the non-AATD group (171.3 ± 63.29 mg/dl vs. 209.5 ± 81.59 mg/dl; $P < 0.05$). The HS-CRP values found in the 2 groups of patients do not show significant differences (2.4 ± 3.6 vs. 1.8 ± 2.3 mg/L). Even if the HS-CRP values are below the lower limit related to a systemic inflammatory condition, the calculated mean value higher than 2 mg/L in the AATD group of patients is suggestive of an increased cardiovascular risk condition.

Table IV. . Distribution of risk factors for AAA in the 138 patients divided into the 2 groups

AAA risk factor	*No-AATD N = 116*	*AATD N = 22*	*P value*
Smoking (%)	85	93.7	NR
Hypertension(%)	78	62	NR
Hypercholesterolemia (%)	46.4	12.5	<0.05
Diabetes (%)	17.5	12.5	NR
Ischemic cardiopathy (%)	32	20	NR

Subsequently, we observed the distribution of the known risk factors among the 138 patients divided into 2 groups: patients with AAA and no AATD-related genotype and patients with AAA and AATD. The 2 groups were homogeneous in terms of age (74.7 ± 11.6 for nondeficit patients and 69.5 ± 12.7 for patients with AAT deficiency), gender (89% male for patients without AAT deficiency and 70.2% for patients with AAT deficiency), weight (73.6 ± 14.4 kg for the nondeficit group and 71. 3 ± 14.6 kg for the group of patients with AAT deficiency), height (1.67 ± 0.06 m for patients without AAT deficiency and 1.71 ± 0.02 m for patients with AAT deficiency) and BMI (26.2 ± 4.2 kg/m2 for the group of patients without AAT deficiency and 24.3 ± 4.2 kg/m2 for the group of patients with AAT deficiency). Although we found no differences in terms of hypertension, diabetes, and smoke habits between AAA patients with and without AATD, hyperlipidemia was significantly less frequent in patients with AAA and AATD (12.5% vs. 46.4%, respectively, $P < 0.05$) (Table IV). Among them, 116 had normal genotype (PiMM), while 22 subjects showed at least 1 deficient allele for AAT: 16 Pi-MS, 1 Pi-SS, 3 Pi-MZ and 2 were M + rare variants. No Pi-ZZ subjects have been identified.

DISCUSSION

The documented results support the hypothesis of an increased frequency of AATD in patients with AAA. In addition, the presence of AATD seems to favor the occurrence of the vascular disease

even without a well-established risk factor for AAA development, such as hyperlipidemia.

The actual pathogenetic mechanism of abdominal aortic aneurysms remains partially unknown. Several studies have tried to find a link between the AAA and the AATD because the latter could be considered a risk factor due to the lack of inhibition of various proteolytic enzymes, particularly neutrophil elastase, leading to an imbalance between circulating levels of elastase and antielastase. AATD is associated with increased degradation of ECM proteins, and the loss of elastic fibers seems to be one of the initial stages of the abdominal aortic aneurysms development. AAT is also a protein with antiinflammatory properties, and its deficiency can favor an increase in plasma levels of inflammatory molecules, such as Il-6, Il-8, and TNFα, worsening the systemic inflammation state and promoting the AAA formation.

There is also evidence in the literature suggesting that AAT may preserve the vascular wall's integrity. Various studies show that patients with cerebral aneurysms and cervical artery dissection have an imbalance between elastase and antielastase due to AAT deficiency.[48–51] Other authors have highlighted how fibromuscular dysplasia of arterial walls is more common in patients with severe AATD.[52,53] Several reports suggest an association between AAT deficiency and stroke, myocardial infarction, coronary artery dissection, and atherosclerosis.[54–60] The AAT deficiency could also be related to an alteration in the aortic wall's mechanical properties and the formation of aneurysms in the mesenteric artery.[36,61,62] Despite the abundant data documenting the possible role of AAT in many vascular diseases, only a few studies have investigated the association between AAA and AATD. The high incidence of AAA and the remarkable mortality resulting from AAA rupture justify the need to better understand AATD and AAA's association. Due to the absence of distinctive symptomatology characterizing the abdominal aortic aneurysms up to their imminent rupture, their screening or early diagnosis is of great importance. One study has shown that in the next 4 years after screening, the mortality rate is halved in older men and regions with a prevalence of abdominal aortic aneurysm of 4% or higher, mainly by reducing the risk of rupture.[63] To ensure an early diagnosis in subjects most at risk of developing abdominal aortic aneurysm, it is necessary to identify all risk factors for this condition.

Our study's findings support the hypothesis of an association between alpha1-antitrypsin deficit and aneurysmal aortic pathology in accordance with the works of Schardey et al. and Cohen et al.[19,39] In contrast, Hernandez-Richter et al. and Elzouki et al. did not find an association between these 2 conditions.[38,41] However, the number of patients with AAA in these studies was much lower than ours. Finally, St Jean and co-workers' study also found no differences in the phenotypic distribution of AATD between the AAA patient group and the Pittsburgh population.[40] However, the phenotyping technique alone may have introduced a bias since, in many deficient variants, they can only be detected by genotyping.

CONCLUSION

Our results suggest that AATD should be considered a risk factor for developing abdominal aortic aneurysms, as we found a higher frequency of AATD in patients with AAA compared to the general population. Therefore, it would be advisable to genotype for AATD patients suffering from an abdominal aortic aneurysm with a nontraumatic aetiology and in the absence of collagen diseases and hyperlipidemia. This could be very helpful from an epidemiological perspective to identify potential AATD population clusters and for preventive and therapeutic purposes. We cannot rule out that patients undergoing surgery for AAA and (meanwhile or subsequently) identified as affected by AATD may not develop new aneurysmal pathologies in other aortic districts or its main branches. In this perspective, and if AAT concentrations are low enough to require it, supplementary therapy may be advisable for both preventive and therapeutic purposes. On the other hand, it can be advisable to perform an ultrasound examination of the abdomen in patients with AATD for the presence of AAA.

AUTHORS' CONTRIBUTIONS

Study design: LP.
Data collection: MP, CZ, and EB.
Data analysis: LT.
Interpretation of results: All authors.
Initial draft: LP.
Manuscript revision: All authors.

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AVAILABILITY OF DATA AND MATERIAL

All the available data and material have been provided in the Manuscript.

ETHICS APPROVAL

The study was performed in accordance with the Helsinki declaration and was approved by the local Institutional Ethic Committee.

CONSENT TO PARTICIPATE

All participants of the study signed written informed consent upon enrolling.

CONSENT FOR PUBLICATION

All participants of the study and the listed authors gave their consent for the publication of the Manuscript.

GUARANTOR STATEMENT

Prof. Laura Pini is the guarantor of the content of the Manuscript, including the data and analysis.

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