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RESPIRATORY CARE

ORIGINAL RESEARCH

Alpha-1 Antitrypsin Deficiency Screening Using Serum Protein Electrophoresis

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Abstract

Background: Alpha-1 antitrypsin (AAT) is encoded by the polymorphic SERPINA1 gene, with pathogenic variants causing AAT deficiency. While being underrecognized, AAT deficiency can be screened through serum protein electrophoresis (SPE) to detect mutations. This study aimed to evaluate the effectiveness of an SPE-based screening protocol for identifying SERPINA1 mutations and diagnosing AAT deficiency.

Methods: This study involved analyzing all SPE tests over one year at the Hospital Clínico Universitario "Lozano Blesa" (Zaragoza, Spain). AAT concentration was measured in samples with <3% alpha-1 globulin band, selecting those with <100 mg/dL as potential study participants. Participants provided blood samples for the genetic analysis of the SERPINA1 gene.

Results: Out of 12,460 SPE tests analyzed, 175 had alpha-1 globulin bands <3%, and 70 cases had AAT concentrations <100 mg/dL. Of these cases, 39 subjects participated in the study. The mean AAT concentration was 78.8 mg/dL. Genetic analysis showed 87.2% had SERPINA1 mutations, with common genotypes being PI*MS, PI*MZ, and PI*SZ.

Conclusions: This study confirms the efficacy of SPE as a potential screening strategy for detecting mutations in the SERPINA1 gene. It can facilitate opportunistic diagnosis of AAT deficiency, promoting early detection and treatment.

Keywords: alpha-1 antitrypsin deficiency, electrophoresis, liver diseases, genotype, lung diseases, genetic diseases.

Introduction

Alpha-1 antitrypsin (AAT) is an acute phase protein encoded by the SERPINA1 gene (serine protease inhibitor, group A, member 1), located in chromosome 14 (14q31-32.3).[1,2] The SERPINA1 gene consists of 7 exons, of which 4 are coding (II, III, IV, and V) and 3 are noncoding (Ia, Ib, Ic), along with 6 introns.[3] This gene exhibits high polymorphism, with over 150 genetic alleles identified to date; however, the majority remain poorly characterized.[4,5] Although the liver predominantly synthesizes over 80% of AAT, smaller amounts are also produced by other cells such as those in the lungs or pancreas.[1,6] AAT is the main serum inhibitory protease, and its main function is to inhibit enzymes that can cause damage and inflammation in healthy tissues because of overactivation of proteases.[6]

Pathogenic variants within the SERPINA1 gene are responsible for AAT deficiency,[2] which is typified by a diminished serum concentration of AAT. This deficiency arises from protein variants that may either spontaneously

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polymerize, preventing their release into circulation, or exhibit reduced enzymatic activity.[3] In the general population, normal alleles, designated as M, are present in 85–90% of individuals and correspond to the Pi*MM genotype, which expresses 100% of AAT. The most common deficiency alleles are Pi*S and Pi*Z, with a prevalence of 5–10% and 1–3% in white populations, respectively. Thus, the resulting genotypes include MM, MS, SS, MZ, SZ, and ZZ. The prevalence of COPD varies among these genotypes, with higher frequencies of the deficient genotypes Pi*MZ, Pi*SZ, and Pi*ZZ observed, whereas Pi*MM shows a significant decrease. Based on current data, Pi*MS carriers are not considered at risk for disease. The Pi*SS genotype, being rare (<1%) in Europe, has limited and somewhat controversial evidence regarding its association with COPD or liver disease. If this genotype were to imply an increased risk, it would likely do so in combination with factors such as smoking.[1,7] The imbalance produced by the enzyme deficiency causes lung tissue degradation because of neutrophil elastase, contributing to the development of COPD and early emphysema.[8] In addition, this deficit is also a risk factor for other types of diseases, such as liver disease. In this case it is caused by the intrahepatocytic accumulation of polymerized and unsecreted AAT. This is a characteristic of the polymerogenic Z allele, but other polymerogenic alleles occur, such as Mmalton.[8,9]

AAT deficiency is probably one of the most common, underrecognized, hereditary conditions worldwide, with only a small minority (≈2%) of individuals currently detected. This may be caused by the clinical variability of the presentation of AAT deficiency, with a large proportion of patients having no clinical symptoms. Moreover, many patients are misclassified as tobacco-related COPD or alcohol-related cirrhosis.[1,10] At present, intravenous AAT replacement therapy is the only effective pharmacologic treatment for AAT deficiency. However, this option only addresses the pulmonary manifestations and not the possible liver disease.[9] Other types of treatments, such as gene therapy, although promising, are still under investigation.[11,12]

There is no doubt of the importance of diagnosing patients with AAT deficiency to start treatment early and diagnose relatives if appropriate. The strategy for diagnosis consists of the combination of genotyping and quantification of AAT.[13] However, in a recent study by this group, the effectiveness of serum protein electrophoresis (SPE) as a screening strategy for the detection of mutations in the SERPINA1 gene and opportunistic diagnosis of AAT deficiency was highlighted.[14]

The objective of this investigation was to assess the efficacy of a screening protocol designed for the identification of SERPINA1 gene mutations and the subsequent diagnosis of AAT deficiency. This procedure relies on the incidental observation of diminished values within the alpha-1 globulin band of the SPE.

QUICK LOOK

Current knowledge

Alpha-1 antitrypsin (AAT) deficiency is caused by pathogenic variants in the SERPINA1 gene. This deficiency is associated with pulmonary diseases such as COPD and emphysema, as well as hepatic pathologies. Approximately 2% of AAT deficiency cases are diagnosed because of clinical variability.

What this paper contributes to our knowledge

AAT deficiency results from pathogenic variants in the SERPINA1 gene, leading to reduced protein levels. This condition is linked to COPD, emphysema, and liver disease because of protein accumulation. Less than 2% of cases are diagnosed owing to clinical variability. Serum protein electrophoresis is a potentially effective method for identifying SERPINA1 mutations and diagnosing AAT deficiency.

Methods

Subjects

This study was performed on all routine SPE requests from primary care and specialist consultations, analyzed during one year at the Biochemistry Laboratory of the Hospital Clínico Universitario "Lozano Blesa" in Zaragoza. AAT concentration was determined in those SPE whose alpha-1 globulin band was <3%, which is established as the lower limit of normality in our laboratory. Both AAT and SPE were performed on the same sample. When the AAT concentration was <100 mg/dL, they were classified as positive by screening and selected as potential study participants, as this cutoff is the established lower limit of normality in our laboratory, in accordance with guidelines from the Mayo Clinic. After identifying potential cases of AAT deficiency, they were contacted by telephone. Those who agreed to participate in the study had a blood sample taken, and an interview was conducted to collect family history.

The study was carried out following the principles of the Helsinki Declaration and was approved by the Research Ethics Committee of the Community of Aragón (C.I. PI20/219; Approval Date: v3.14/05/2020). All participants provided written informed consent, ensuring that they were fully aware of the study's objectives. This process involved detailed discussions with participants, allowing them to ask questions and seek clarification before agreeing to participate. Furthermore, participants were informed of their right to withdraw from the study at any time without any impact on their medical care.

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Methods

All blood samples of genetic tests were collected using tubes Vacutest® with K_3EDTA 5.4 mg (Vacutest Kima srl, Arzergrande, Italy). The tubes were labeled and were afterward processed and stored in appropriate conditions. Blood samples for SPE and AAT measurement were collected using Vacumed tubes with clot activator and separation gel (FL Medical, Torreglia, Italy).

SPE was carried out in a volume of 30 μL and consisted of applying an average voltage of 20,000 V to the ends of the capillaries for 10–30 s. This generated an electric field to separate the proteins from the serum of the patients. The separation was monitored using ultraviolet-visible light at a wavelength of 214 nm. Serum AAT concentration was determined by immunonephelometry method using BN*II and BN ProSpec® systems.

The genetic analysis of coding exons and adjacent intronic regions of the SERPINA1 (ENST00000448921.5) gene was conducted utilizing the primers detailed in Table 1. First, DNA extraction was performed and the exon region to be analyzed was amplified. The polymerase chain reaction conditions were 5 min of preamplification at 95°C, 35 cycles of amplification (30 s at 95°C, 30 s at 60°C, and 30 s at 72°C), and, finally, a postamplification cycle (10 min at 72°C). After polymerase chain reaction, we verified the amplification by performing agarose gel electrophoresis to confirm the presence of the amplified exon before proceeding with sequencing.

T1

Subsequently, exon sequencing was performed by capillary electrophoresis (ABI 3500 XL from Applied Biosystems, Foster City, CA, USA). The DNA polymerase used was VWR Taq DNA Polymerase (Cat. No.: 733-1301).

Statistical analysis

The statistical model used in this work was descriptive. Mean, standard deviation, minimum, and maximum were calculated to the quantitative variables. Percentages were calculated to qualitative variables. All statistical analyses were performed using SPSS statistics v22.0 (IBM) and Jamovi v2.0 (The Jamovi project [2023]. jamovi [Version 2.3] [computer software]).

Results

In total, 12,460 SPE were analyzed and 175 had alpha-1 globulin band quantification <3%. The AAT concentration was <100 mg/dL in 70 cases, so they were classified as positive by screening and selected as potential study participants. Three participants died before the start of the study and 28 refused to be included; therefore, 39 finally participated (Fig. 1). The age of the studied subjects followed a normal distribution, with a minimum of 16, a maximum of 81, and a mean of 51.3 years (SD of 16.0); 18 were women (45%) and 21 were men (55%).

F1

Regarding the parameters determined, the mean AAT concentration was 78.8 mg/dL (SD of 14.8) with a maximum of 99.6 mg/dL and a minimum of 47.2 mg/dL. Alpha-1 globulin fraction values are very narrow, with a mean of 2.69% (SD of 0.20), a minimum of 2.3%, and a maximum of 3.0%. A significant relationship was found between both variables ($P = .02$). We conducted an analysis of the clinical traits exhibited by both participants and their family members. It is noteworthy that 7 participants had pulmonary pathology, 3 had hepatic pathology, 2 had infertility, and 1 had pericarditis. Regarding familial clinical features, 12 participants in our cohort reported notable health conditions among their first-degree relatives, including colon, liver, and pulmonary cancer; unspecified pulmonary disease; and bronchitis or asthma. Pulmonary fibrosis and low lung capacity are observed, although these are considered unusual presentations in individuals with AAT deficiency (Table 2).

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T2

Subsequently, a genetic investigation was conducted, revealing mutations in 87.2% (34 out of 39) of the

Table 1. Sequence of the primers used for sequencing the SERPINA1 gene (ENST00000448921.5)

Exon	*Forward primer*	*Reverse primer*
4	GTACTTGGCACAGGCTGGTT	ATGCATTGCCAAGGAGAGTT
5	GAGGGATGTGTGTCGTCAAG	TAGCAGTGACCCAGGGATGT
6	TAGTGTGGGTGGAGGACACA	CAGCCTGGGTCTTCATTTGT
7	GTGACAGGGAGGGAGAGGAT	CTGTTACCTGGAGCCCACAT

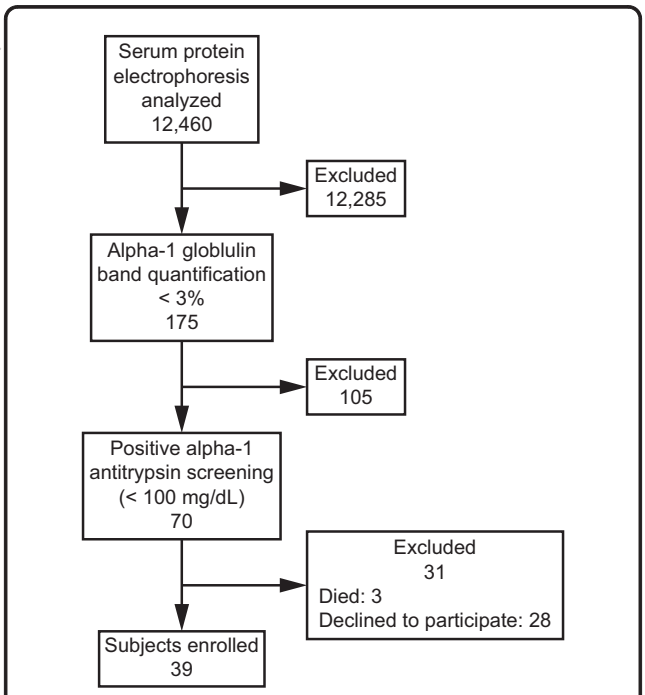

Fig. 1. Diagram of the algorithm followed to select participants for the genetic study. AAT, alpha-1 antitrypsin.

Table 2. General characteristics of patients and relatives

Patient	Age	Gender	Alpha-1 globulin (%)	Tobacco exposure	Patient's symptoms	First-degree relatives' symptoms
1	73	F	2.7	No	No	No
2	37	M	2.7	Yes	Hepatitis B	No
3	50	M	2.7	No	No	No
4	78	M	2.7	Yes	No	No
5	21	F	2.9	No	No	No
6	56	M	2.4	No	No	Colon and pulmonary cancer
7	74	F	2.9	No	COPD	No
8	74	M	3.0	Yes	COPD	No
9	43	F	2.7	No	No	No
10	20	M	3.0	No	No	No
11	63	M	2.9	Yes	Hemochromatosis, COPD	No
12	51	F	2.5	No	Low lung capacity	Asthma
13	54	F	2.4	No	S. Gilbert	Cholangiocarcinoma
14	45	M	2.5	No	No	Fatty liver
15	63	F	2.8	No	No	No
16	42	M	2.8	No	Pulmonary emphysema	No
17	59	M	2.6	No	No	Liver cancer
18	60	F	2.8	No	Pericarditis	No
19	63	M	2.7	Yes	No	No
20	45	F	2.4	Yes	No	Pulmonary disease
21	51	F	2.7	Yes	No	Pulmonary cancer
22	38	M	2.7	Yes	Infertility	Chronic bronchitis
23	47	F	2.9	Yes	No	No
24	31	M	2.9	No	No	No
25	31	F	2.5	No	Fatigue chronic syndrome	Digestive cancer
26	52	F	2.3	No	Infertility	Cardiac pathology
27	44	F	3	Yes	Pulmonary fibrosis	Pulmonary and liver cancer
28	62	M	2.8	No	No	No
29	62	M	2.3	No	No	No
30	52	F	2.6	Yes	No	No
31	68	M	2.8	No	Hepatitis C	No
32	81	M	2.5	Yes	Bronchitis	No
33	16	F	2.4	No	No	No
34	43	M	2.4	No	No	Fatty liver, cirrhosis
35	45	M	2.9	Yes	No	No
36	59	F	2.8	No	No	No
37	69	F	2.9	No	No	No
38	30	M	2.8	No	No	No
39	49	M	2.6	No	No	No

Descriptive table of the physical characteristics of each patient, as well as exposure to tobacco, clinical symptoms of the patient and family members. alpha-1 %, percentage of alpha-1 fraction of the proteinogram; F, female, M, male.

participants examined. Hence, within this cohort, the vast majority of deviations in AAT concentration were indicative of mutations occurring within the exons of the SERPINA1 gene. Ten participants were heterozygous for the c.863A>T (p.Glu288Val) mutation (PI*MS) and 17 for the c.1096G>A (p.Glu366Lys) mutation (PI*MZ). Six participants were double heterozygotes with c.863A>T/c.1096G>A mutations (Pi*SZ), and only one subject had the c.863A>T (p.Glu288Val) mutation in homozygosity (Pi*SS). In the other 5 participants, we found no clinically relevant mutations (Table 3). T3

Among our participants, we correlated the AAT concentrations with the genotype detected. The Pi*MS genotype has a mean AAT of 82.1 ± 14.9 mg/dL, range 98.2–56.2; the Pi*MZ has mean 81.2 ± 9.4 mg/dL, range 99.6–67.6, and the Pi*SZ has mean 57.7 ± 10.9 mg/dL, range 72.3–47.2. The Pi*SS genotype presents a single case with AAT of 94.4 mg/dL (Fig. 2). No statistical correlation was found between AAT values and the genotype detected. F2 AQ: 4

Discussion

In this study we attempted to evaluate the efficacy of a screening protocol based on the incidental observation of decreased values within the alpha-1 band of the SPE. Thus, it would allow the identification of SERPINA1 gene mutations and the subsequent diagnosis of AAT deficiency.

Traditionally, one of the main challenges in diagnosing AAT deficiency has been the attitude of physicians toward diagnosing a disease for which effective treatment options are limited.[5,15] At present, it is important to note that replacement therapy with AAT is currently available for treating patients with evidence of progressive lung disease. Studies using sensitive measures of emphysema progression have demonstrated the effectiveness of this therapy in preserving lung tissue, which may also contribute to the preservation of lung function and overall structure.[16] Despite clinical guidelines, increased disease awareness, and the potential efficacy of new treatments, we continue to diagnose individuals

Table 3. AAT concentration, detected mutations in SERPINA1 gene, and associated genotypes

Patient	*AAT (mg/dL)*	*Mutated exon*	*Mutation*	*Genotype*
1	81.4	5	c.863A>T (p.Glu288Val) heterozygous	Pi*MS
2	81.2	–	No mutation	Pi*MM
3	98.2	5	c.863A>T (p.Glu288Val) heterozygous	Pi*MS
4	96.1	5	c.863A>T (p.Glu288Val) heterozygous	Pi*MS
5	81.1	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
6	70.3	5 and 7	c.863A>T (p.Glu288Val)/c.1096G>A (p.Glu366Lys)	Pi*SZ
7	97.1	–	No mutation	Pi*MM
8	99.6	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
9	94.4	–	No mutation	Pi*MM
10	95.9	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
11	78.1	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
12	55.7	5 and 7	c.863A>T (p.Glu288Val)/c.1096G>A (p.Glu366Lys)	Pi*SZ
13	49.9	5 and 7	c.863A>T (p.Glu288Val)/c.1096G>A (p.Glu366Lys)	Pi*SZ
14	72.9	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
15	75.7	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
16	77.6	5	c.863A>T (p.Glu288Val) heterozygous	Pi*MS
17	84.7	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
18	61.5	–	No mutation	Pi*MM
19	97.2	5	c.863A>T (p.Glu288Val) heterozygous	Pi*MS
20	70.4	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
21	88.3	5	c.863A>T (p.Glu288Val) heterozygous	Pi*MS
22	94.4	5	c.863A>T (p.Glu288Val) homozygous	Pi*SS
23	67.6	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
24	68.8	5	c.863A>T (p.Glu288Val) heterozygous	Pi*MS
25	72.3	5 and 7	c.863A>T (p.Glu288Val)/c.1096G>A (p.Glu366Lys)	Pi*SZ
26	47.2	5 and 7	c.863A>T (p.Glu288Val)/c.1096G>A (p.Glu366Lys)	Pi*SZ
27	83.4	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
28	56.2	5	c.863A>T (p.Glu288Val) heterozygous	Pi*MS
29	50.7	5 and 7	c.863A>T (p.Glu288Val)/c.1096G>A (p.Glu366Lys)	Pi*SZ
30	92.5	5	c.863A>T (p.Glu288Val) heterozygous	Pi*MS
31	97.2	–	No mutation	Pi*MM
32	76.5	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
33	73.5	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
34	64.6	5	c.863A>T (p.Glu288Val) heterozygous	Pi*MS
35	88.0	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
36	94.6	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
37	71.3	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
38	84.8	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ
39	81.5	7	c.1096G>A (p.Glu366Lys) heterozygous	Pi*MZ

The table is showing the specific mutations found in each patient and the exons in which they are located, as well as the associated phenotype.
AAT, alpha-1 antitrypsin.

with severe AAT deficiency in their mid-40s, often when they already have established COPD.[15,17] Furthermore, various studies have demonstrated that a COPD diagnosis in severe AAT deficiency is associated with higher mortality rates.[17,18] Currently, it is important to emphasize the diagnosis of AAT deficiency before COPD is established, allowing for early intervention and treatment before pulmonary function deteriorates.[17] However, this will not be feasible with current case identification programs, which focus on patients with emphysema, COPD, poorly responsive asthma, unexplained liver disease and panniculitis, as well as first-degree relatives of those diagnosed with AAT deficiency.[9,19,20]

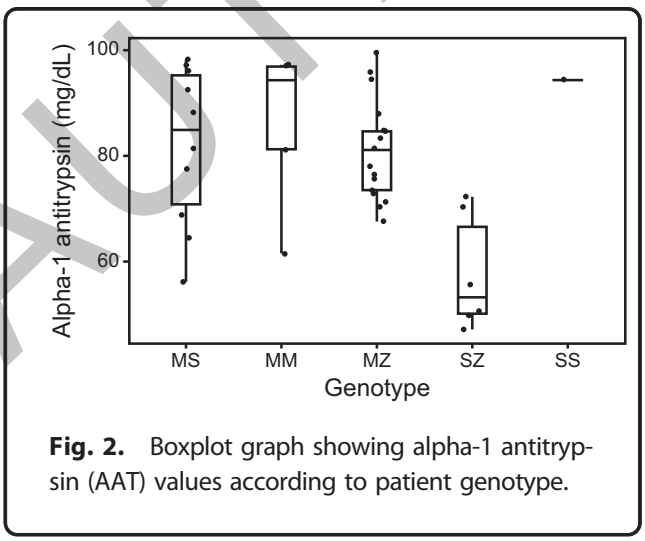

Fig. 2. Boxplot graph showing alpha-1 antitrypsin (AAT) values according to patient genotype.

Previous studies by our group, conducted with a small cohort, demonstrated that SPE is a useful tool for the opportunistic diagnosis of AAT deficiency.[14] This screening method would enable the diagnosis without relying on patients' clinical characteristics. Next, if this test is positive, more costly test would be conducted. The present study aims to validate these findings over an extended study period and with a larger participant pool, by implementing this screening method in our hospital center.

Out of a total of 12,460 patients screened, genetic testing was performed on 39 individuals, revealing that 87.2% of these tested subjects carried some form of mutation. Among these cases, 21% were identified as homozygotes or compound heterozygotes—individuals with 2 different mutations in the same gene, one inherited from each parent—indicating that these individuals could potentially be affected. Consequently, the efficacy of SPE as a potential screening method for the detection of mutations in the SERPINA1 gene is confirmed.

The utility of SPE as part of the AAT diagnostic algorithm has been investigated for several years, but they focus exclusively on the alpha-1 fraction, aiming to correlate it with AAT levels or directly with the patient's phenotype.[21–23] For instance, Slev et al used a cutoff of <2.1% to exclude patients without mutations (MM), which is more stringent than our cutoff of <3%, but achieved a similar sensitivity of 84.2%.[23] Lowering the cutoff for the alpha-1 fraction reduces the number of patients analyzed and genotypes detected. However, sequential analysis of AAT concentration in selected patients after SPE could mitigate this issue.

According to the European Respiratory Society, the optimal AAT threshold for distinguishing PI*MM from genotypes carrying at least one deficient S or Z allele is 110 mg/dL, with 73.4% sensitivity and 88.5% specificity.[20] However, serum levels observed in severe AAT deficiency are usually <57 mg/dL.[24] Despite this, numerous studies have been performed with different cutoff points to identify populations at higher clinical risk.[25–29] Since ZZ patients account for 95% of cases of severe AAT deficiency,[25] most studies focus on identifying Z alleles. On the one hand, Bornhorst et al and Donato et al studied cutoff points of 80 mg/dL and 85 mg/dL, respectively, to differentiate MM from ZZ patients, both showing high sensitivity and specifity.[26,27] On the other hand, Greulich et al assessed a 104 mg/dL cutoff point to differentiate ZZ patients from the rest[28] and Gorrini et al applied a 113 mg/dL cutoff point to detect SS patients and any Z allele,[29] both with a sensitivity close to 100%. Additionally, Ferraroti et al determined that the optimal cutoff point for the alpha-1 fraction was 3.4%, using a cutoff of 113 mg/dL for AAT.[22] These cutoffs, similar to those used in our study, achieved a sensitivity of 92.5% and a specificity of 63.8% (area under the receiver operating characteristic curve of 0.85). These sensitivity results would be close to ours, and the differences could be a result of the fact that we have studied fewer patients. In contrast to these studies, the effectiveness of alpha-1 globulin band and AAT levels in excluding heterozygous carriers is very limited.[25]

Our findings indicate variability in AAT concentrations according to mutation type, closely aligning with expectations from the literature for Pi*MZ, Pi*SZ, and Pi*SS cases.[24] However, the Pi*MS group exhibits a mean AAT concentration of 82.1 mg/dL, notably lower than the expected 125 mg/dL. Analyzing the participants listed in Table 3, we observe that 3 individuals show particularly low AAT values, below the group mean. Although one subject has cirrhosis, which may account for their lower AAT level, the other 2 do not present any known medical conditions that would explain this reduction. Complete sequencing of the exon was performed, and no additional mutations were identified; thus, the cause of these unexpectedly low AAT concentrations in the Pi*MS group remains unknown. However, we hypothesize that this could be because of currently unrecognized intronic variants, environmental factors, or recent inflammation, supporting the need for further exploration of potential contributing factors.

Our study aligns with recent research on AAT deficiency detection, including the study by Cronin et al,[30] which also underscores the challenge of identifying this condition. Both studies employ similar detection schemes and cutoff points, recommending that an abnormally low peak in alpha globulins on SPE should prompt AAT measurement. Although our study confirms diagnosis through genetic testing, ensuring a precise and unequivocal result, their protocol involves phenotyping individuals with low AAT levels.

The underdiagnosis of AAT deficiency necessitates enhanced detection strategies, even when identification occurs incidentally. Integrating opportunistic screening methods, such as SPE, with systematic approaches may improve early detection and support tailored health recommendations, including smoking cessation.[30] Given the significant impact of AAT deficiency on individual health, early diagnosis can help alleviate symptoms or even prevent them from developing. In the era of emerging therapies for this disease, it is imperative that diagnosis advances alongside new therapy development. Therefore, in view of the results provided in studies mentioned above, it could be interesting to implement the SPE as the first step of the diagnostic algorithm for AAT deficiency. Furthermore, in the not-too-distant future, it may be pertinent to review neonatal screening programs as a means of early detection of this disease.[5]

Finally, several limitations of this study must be considered. First, AAT deficiency is classified as a rare disease, which led to a limited number of participants. Additionally, the Z allele, which is required for the ZZ genotype, has a low prevalence in white populations, estimated between 1% and 3%.[1] This combination of low allele prevalence and limited sample size may explain the absence of the less abundant but highly relevant ZZ genotype in the study. A larger sample size would enhance the statistical power and the robustness of the conclusions. Second, AAT is an acute-phase

reactant protein, which can be elevated in response to various etiologies including age, tissue necrosis, trauma, rheumatoid arthritis, bacterial infections, vasculitis, malignancy, or in pregnant women.[25] We did not account for participants' health status or measure C-reactive protein to assess the presence of an inflammatory process, which may have introduced bias in the AAT concentration measurements. However, this limitation is inherent in screening conducted within a hospital setting.

Conclusions

In conclusion, this study demonstrates the efficacy of SPE as a potential screening strategy for detecting mutations in the SERPINA1 gene. Implementing this method could facilitate subsequent testing and enable opportunistic diagnosis of AAT deficiency.

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Acknowledgments

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Authors' Contributions

M.Á.T.-C. and S.M. made substantial contributions to the conception of the work. J.J.P.A. made significant contributions to the data analysis and interpretation. J.P.-A. and A.G.-G. made significant contributions to the design of the work and the interpretation of data. J.P.-A. drafted the original manuscript. E.d.C.-D. and L.G.Z. substantially contributed to the revision of the manuscript drafts. All authors have approved the submitted version of the manuscript and agreed to be accountable for any part of the work.

Author Disclosure Statement

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