Prevalence of alpha-1 antitrypsin deficiency and allele frequency in patients with COPD in Brazil

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ABSTRACT

Objective: To determine the prevalence of alpha 1-antitrypsin (AAT) deficiency (AATD), as well as allele frequency, in COPD patients in Brazil. Methods: This was a cross-sectional study involving 926 COPD patients 40 years of age or older, from five Brazilian states. All patients underwent determination of AAT levels in dried blood spot (DBS) samples by nephelometry. Those with DBS AAT levels ≤ 2.64 mg/dL underwent determination of serum AAT levels. Those with serum AAT levels of < 113 mg/dL underwent genotyping. In case of conflicting results, SERPINA1 gene sequencing was performed. Results: Of the 926 COPD patients studied, 85 had DBS AAT levels ≤ 2.64 mg/dL, and 24 (2.6% of the study sample) had serum AAT levels of < 113 mg/dL. Genotype distribution in this subset of 24 patients was as follows: PI*MS, in 3 (12.5%); PI*MZ, in 13 (54.2%); PI*SZ, in 1 (4.2%); PI*SS, in 1 (4.2%); and PI*ZZ, in 6 (25.0%). In the sample as a whole, the overall prevalence of AATD was 2.8% and the prevalence of the PI*ZZ genotype (severe AATD) was 0.8%. Conclusions: The prevalence of AATD in COPD patients in Brazil is similar to that found in most countries and reinforces the recommendation that AAT levels be measured in all COPD patients.

Keywords: alpha 1-antitrypsin deficiency/epidemiology; pulmonary disease, chronic obstructive/epidemiology; Alleles; alpha 1-antitrypsin.

INTRODUCTION

Alpha-1 antitrypsin (AAT) deficiency (AATD) is an autosomal codominant disorder primarily affecting the lungs and the liver.1,2 The incidence of AATD is 1 per 2,000-5,000 live births; analysis of a database of 4.4 billion people from 58 countries estimated that 116 million individuals have the MS or MZ phenotype and that 3.4 million have the SS, SZ, or ZZ phenotype.3,4 AAT is a glycoprotein consisting of a chain of 394 amino acids and three carbohydrate side chains, being considered the prototype of a superfamily of proteins known as serpins (serine protease inhibitors). Also known as protease inhibitor (PI), AAT is encoded by the SERPINA1 gene, located on the long arm of chromosome 14 (14q32.1), and inhibits neutrophil elastase, trypsin, and protease-3.5,6

Although smoking is a major cause of airflow obstruction, it is estimated that only 15-30% of smokers develop COPD7-9 Despite the clear association between smoking and COPD, the effects of smoking vary across individuals.10 Studies have shown that AATD can increase the impact of smoking on the lungs, resulting in an increased rate of decline in lung function and early emphysema in smokers. Mutant S and Z alleles are the most commonly involved in severe AATD.11,12

The fact that the Brazilian population is racially diverse and includes immigrants from European countries where the frequency of alleles involved in early lung changes is high suggests that AATD is underdiagnosed in the country. Despite the estimated 5–7 million COPD patients in Brazil,13 the prevalence of AATD in this population remains unknown, as does allele frequency. Therefore, the objective of the present study was to assess the prevalence of AATD, as well as allele frequency, in COPD patients from five Brazilian states.
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METHODS

Study design

The present study was approved by the Research Ethics Committee of the Federal University of São Paulo Hospital São Paulo (Protocol no. 0633/10), located in the city of São Paulo, Brazil, as well as by the research ethics committees of all participating centers. Between July of 2011 and August of 2012, 1,073 COPD patients followed at any of the six participating centers (two in northeastern Brazil, two in southeastern Brazil, one in southern Brazil, and one in central-western Brazil) were evaluated.

Patients

The inclusion criteria were as follows: being 40 years of age or older; having been diagnosed with COPD (on the basis of clinical history and spirometry results, including a post-bronchodilator percent predicted FEV1/FVC ratio—FEV1/FVC%—below the lower limit of normal); and having been stable for at least four weeks. The exclusion criteria were as follows: having been diagnosed with any other lung disease or systemic disease that can increase serum AAT levels (including infections and inflammatory processes); having previously been diagnosed with AATD; being a relative of an index case of AATD; and having asthma (Figure 1).

The goal was to include 200 COPD patients from each participating center. At the end of the study period, no more patients were added to the study, regardless of whether or not the desired number of patients had been attained for each center.

Spirometry

The reference values for calculating percent predicted FVC, percent predicted FEV1, and FEV1/FVC% were based on the National Health and Nutrition Examination Survey equations. Spirometry was performed with a portable spirometer (Easy One®; ndd Medical Technologies, Inc., Andover, MA, USA). At all participating centers, the American Thoracic Society acceptability and reproducibility criteria were used.

Quantification of AAT

The study was divided into three phases. In the first phase, all patients underwent determination of AAT levels in dried blood spot (DBS) samples in order to identify those with a possible diagnosis of AATD. In the second phase, patients with DBS AAT levels ≤ 2.64 mg/dL (suspected AATD) underwent determination of serum AAT levels. Finally, in the third phase, patients with serum AAT levels of < 113 mg/dL underwent genotyping. In case of conflicting results between serum AAT measurements and genotyping, genetic sequencing was performed (Figure 2). To determine the sensitivity and specificity of the eluate method, Zillmer et al. used the bootstrap resampling method, comparing the AAT values measured in serum with those measured in eluates from DBS samples in order to determine a cut-off point for AAT values in eluates; the value obtained was 2.02 mg/dL (97% CI: 1.45-2.64). All patients whose DBS AAT levels were below 2.64 mg/dL underwent measurement of serum AAT levels in order to prevent AATD from going undiagnosed.

Genotyping

Blood samples were collected with the use of filter paper cards (Whatman 903, lot W101; Whatman/GE Healthcare, Florham Park, NJ, USA). They were transported to the Federal University of São Paulo Hospital São Paulo Central Laboratory, located in the city of São Paulo, Brazil, under temperature-controlled conditions (i.e., at a constant temperature of −20°C, in accordance with applicable International Air Transport Association regulations. All cards were stored at −20°C for subsequent analysis (determination of DBS AAT levels, genotyping, and SERPINA1 gene sequencing).

Serum and eluate samples were analyzed on a Siemens BNII system (Siemens Healthcare, Indianapolis, IN, USA) in July of 2012.

Figure 1. Flowchart of the patients included in the study and their distribution, by participating center. UNIFESP: Universidade Federal de São Paulo; HSPE-SP: Hospital do Servidor Público Estadual de São Paulo; HGG: Hospital Geral de Goiânia; HSPE-SP: Hospital de Messejana; HOF: Hospital Otávio de Freitas; PUC: Pontifícia Universidade Católica; SP: Brazilian state of São Paulo; GO: Brazilian state of Goiás; CE: Brazilian state of Ceará; PE: Brazilian state of Pernambuco; and RS: Brazilian state of Rio Grande do Sul.
For DNA extraction, DBS samples were removed from the cards with a 6-mm paper punch, DNA being extracted with the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany), in accordance with the manufacturer instructions. For identification of S and Z alleles in exons 3 and 5, respectively, real-time PCR was used with TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific Inc., Waltham, MA, USA). All patients with AATD but without S and Z alleles underwent SERPINA1 gene sequencing (exons 2-5) in order to identify other polymorphisms described in the literature.

**Statistical analysis**

Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were expressed as absolute numbers and proportions. Data were entered into an Oracle database and analyzed with the Statistical Package for the Social Sciences, version 18.0 for Windows (SPSS Inc., Chicago, IL, USA).

**RESULTS**

Of the 1,073 patients who were being followed at any of the six participating centers during the study period, 926 met the eligibility criteria and were therefore included in the study (Figure 1). The demographic characteristics of the patients included in the present study are shown in Table 1. There was no statistically significant difference between males and females regarding the prevalence of AATD, and two thirds of the participants were White. Although former smokers predominated (having accounted for 83.9% of the study sample), 36 (3.7%) of the patients had never smoked; 410 patients (44.3% of the sample) had a body mass index within normal limits, and 56 (6%) were underweight. As expected for patients with COPD, FEV₁/FVC% and percent predicted FEV₁ were low, characterizing obstructive lung disease.

Of the 926 COPD patients included in the present study, 85 had DBS AAT levels ≤ 2.64 mg/dL, therefore being suspected of having AATD. Of those 85 patients, 2 died. Therefore, 83 patients underwent determination of serum AAT levels. Of those 83 patients, 24 had serum AAT levels < 113 mg/dL and therefore underwent genotyping. Genotype distribution was as follows: PI*MS, in 3 (12.5%); PI*MZ, in 13 (54.2%); PI*SS, in 1 (4.2%); PI*SZ, in 1 (4.2%); and PI*ZZ, in 6 (25%). Although determination of serum AAT levels was not performed in the 2 patients who had DBS AAT levels ≤ 2.64 mg/dL and died, genotyping and SERPINA1 gene sequencing were performed from the previously collected DBS samples, which had been stored at −20°C for subsequent analysis. Genotype distribution was as follows: PI*M₁I, in 1; and PI*ZZ,
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in 1 (Figure 2). Given the discrepant results and the deaths, genetic sequencing was performed, and the following genotypes were found: PI*M,Z; PI*M,I; and PI*ZZ. These genotypes were included in a second analysis of allele frequency, and the results were as follows: PI*M, 28.8%; PI*M,, 3.8%; PI*S, 11.5%; PI*Z, 53.8%; and PI*I, 1.9%.

Table 2 shows the demographic characteristics of a subset of 24 patients with DBS AAT levels ≤ 2.64 mg/dL and serum AAT levels < 113 mg/dL. As expected, the patients with the PI*ZZ genotype were younger and had lower serum AAT levels than those with other genotypes (p < 0.001). However, there were no differences in gender, smoking history, spirometric values, Medical Research Council scale scores, or COPD Assessment Test scores across patient genotypes.

Among the 926 COPD patients included in the present study, the overall prevalence of AATD was 2.8% and the prevalence of the PI*ZZ genotype (severe AATD) was 0.8%. Analysis of allele frequency in the subgroup of patients with serum AAT levels < 113 mg/dL (including the alleles found in the 2 patients who died, whose DBS AAT levels were ≤ 2.64 mg/dL) revealed frequencies of 53.8%, 11.5%, and 1.9% for the Z, S, and I alleles, respectively (Table 3). Table 4 shows the genotype prevalence among the five regions of Brazil.

**DISCUSSION**

This is the first study to show the prevalence of AATD, as well as allele frequency, in a population of COPD patients in Brazil. The prevalence of AATD (i.e., 2.8%) and the Z allele (i.e., 0.8%) was found to be similar to that found in other countries. (18,19)

The decision to use the maximum confidence interval of the cut-off point for AAT values in eluates was due to the need to identify all individuals who were suspected of having AATD and should therefore undergo genotyping, thus minimizing the chances of not identifying patients with AATD. Although AATD is one of the most common genetic diseases, the prevalence of AATD is low. However, failure to identify any patient with AATD would have had an impact on the final results of the present study. Nevertheless, the use of the maximum confidence interval width resulted in more patients being re-evaluated. (17) The cut-off point of 113 mg/dL was used in an attempt to

Table 1. Demographic characteristics of the 926 COPD patients included in the present study.

| Characteristic          | COPD patients (N = 926) |
|------------------------|-------------------------|
| Gender                 |                         |
| Male                   | 522 (56.4)              |
| Female                 | 404 (43.6)              |
| Age, mean ± SD         |                         |
| Age, years             | 67.3 ± 10.5             |
| Race/ethnicity, n (%)  |                         |
| White                  | 612 (66.1)              |
| Non-White              | 314 (33.9)              |
| Smoking status, n (%)  |                         |
| Smokers                | 113 (12.2)              |
| Former smokers         | 777 (83.9)              |
| Never smokers          | 36 (3.9)                |
| Lung function, mean ± SD|                        |
| FEV1/FVC               | 0.45 ± 0.10             |
| FEV1/FVC, % of predicted| 61.0 ± 13.8            |
| FVC, L                 | 2.43 ± 0.76             |
| FVC, % of predicted    | 75.6 ± 20.2             |
| FEV1, L                | 1.12 ± 0.45             |
| FEV1, % of predicted   | 42.9 ± 17.0             |

Table 2. Demographic characteristics of a subset of 24 COPD patients with serum alpha 1-antitrypsin levels < 113 mg/dL, by genotype.

| Characteristic          | PI*M, S, Z | PI*M, Z | PI*S, Z | PI*Z, Z | p* |
|------------------------|------------|---------|---------|---------|----|
| Male gender, n (%)     | 2 (16.7)   | 7 (58.3)| 1 (8.3) | 1 (8.3) | 0.07|
| Age, years             | 69.3 ± 9.4 | 69.0 ± 10.1| 59.0 | 74.0 | 47.0 ± 2.3 | < 0.001|
| Smoking history, pack-years| 55.0 | 53.5 ± 41.1| 40.0 | 12.6 | 19.1 ± 16.7 | 0.07|
| Post-BD FEV1, % of predicted | 33.8 ± 8.3| 41.1 ± 14.0| 54.7 | 45.8 | 37.5 ± 19.9 | 0.63|
| FEV1/FVC               | 49.4 ± 5.8 | 57.4 ± 9.0| 59.0 | 56.5 | 55.7 ± 12.6 | 0.92|
| Serum AAT, mg/dL       | 100 ± 13.5 | 93.7 ± 14.0| 93.8 | 66.0 | 27.1 ± 4.8 | < 0.001|
| MRC scale score        | 2.6 ± 1.1 | 2.7 ± 1.0| 3.0 | 2.0 | 3.3 ± 1.6 | 0.27|
| CAT score, total       | 20.3 ± 6.4 | 16.6 ± 7.3| 30 | 18 | 17.8 ± 6.3 | 0.42|

Genotypes include those in 2 patients who died (PI*M,I and PI*ZZ) before undergoing determination of serum alpha 1-antitrypsin levels. PI: protease inhibitor.

Table 3. Allele frequency in a subset of 24 COPD patients with serum alpha 1-antitrypsin levels < 113 mg/dL, including the alleles found in 2 patients with dried blood spot alpha 1-antitrypsin levels ≤ 2.64 mg/dL, both of whom died.

| Allele | n | % |
|--------|---|---|
| M      | 15 | 28.8|
| I      | 2  | 3.8 |
| S      | 6  | 11.5|
| Z      | 28 | 53.8|
| I      | 1  | 1.9 |
| Total  | 52 | 100|

Genotypes include those in 2 patients who died (PI*M,I and PI*ZZ) before undergoing determination of serum alpha 1-antitrypsin levels. PI: protease inhibitor.
identify not only patients with severe AATD but also those with moderate AATD. Despite methodological differences and the fact that not all participants underwent genotyping, our results regarding the PI*Z allele are similar to those found in the literature. The frequency of the mutant PI*Z allele and other AATD-associated alleles in the present study can be explained by the large number of immigrants from Europe, primarily from countries where the prevalence of AATD is high, such as Portugal and Italy.

Although DBS analysis is particularly useful as an initial screening test for AATD, it is not sufficient for a definitive diagnosis. Clinical history, physical examination findings, and family history should be taken into account when interpreting the results, which should be confirmed by measuring serum AAT levels in patients suspected of having AATD. If the results of DBS analysis are confirmed by serum ATT measurements, genotyping or phenotyping are necessary for a definitive diagnosis.

Health professionals providing care to patients with COPD should bear in mind that 2.8% of all COPD patients have some degree of AATD. Our study reinforces the knowledge that AATD is one of the most prevalent genetic diseases. Further studies are warranted, given that an AATD diagnosis can have a major impact on COPD prevention, especially in young smokers. In addition, our finding that the prevalence of the PI*ZZ genotype in the study population is 0.8% shows that severe AATD is present in patients with COPD in Brazil and reinforces the 1999 World Health Organization recommendation that "all COPD patients should be screened once for AATD using a quantitative test. Those with abnormal results on screening should undergo PI typing". Determination of AAT levels in COPD patients has also been recommended by the American Thoracic Society/European Respiratory Society and, more recently, the Canadian Thoracic Society. However, these patients typically present at a younger age (<45 years) with lower lobe emphysema. Family screening is useful for appropriate counseling. Few countries are as racially diverse as is Brazil, which is populated by a large number of immigrants, including Asians, Africans, Arabs, and, in particular, Europeans. The Portuguese brought centuries of genetic admixture among Europeans, including Celts, Romans, Germans, and Lusitanians. The differences in genotype prevalence among the five regions of Brazil might be due to the different immigrant origins.

One limitation of the present study is that not all participants underwent genotyping. However, serum AAT levels were measured in all patients with the use of the maximum confidence interval width, thus preventing AATD from going undiagnosed.

The prevalence of AATD in COPD patients in Brazil was found to be similar to that found in most countries, despite the racial diversity of the Brazilian population. The actual prevalence of AATD in this population can be best determined by investigating neonates. Genetic studies aimed at determining the ancestry of this population are critical in order to establish a correlation between mutated alleles and the actual ancestry of the individuals.

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Table 4. Genotypes involved in alpha 1-antitrypsin deficiency, distributed by mutation of the SERPINA1 gene (genotype) and by participating center.

| Brazilian region/state       | Genotype |
|------------------------------|----------|
|                              | PI*MZ    | PI*ZZ       | PI*MS    | PI*SS    | PI*SZ    | PI*M,1   |
| The northeast/Ceará          | 3        | 3           | 1        | -        | -        | -        |
| The northeast/Recife         | -        | -           | 1        | 1        | -        | -        |
| The central-west/Goiás       | -        | 5           | -        | -        | -        | -        |
| The southeast/São Paulo      | 3        | 3           | 1        | -        | -        | -        |
| The northeast/Rio Grande do Sul | 2      | 1           | 1        | -        | -        | -        |

PI: protease inhibitor.

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