

# $\alpha_1$ -Antitrypsin Deficiency: Situation in Spain and Development of a Screening Program

Cristian de la Roza, Beatriz Lara, Sara Vilà, and Marc Miravittles

Servicio de Neumología, Institut Clínic del Tórax, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Red Respira RTIC 03/11 ISCIII, Hospital Clínic, Barcelona, Spain.

Studies undertaken in Spain indicate that 9% of the general population aged between 40 and 70 years is affected by chronic obstructive pulmonary disease (COPD). Although tobacco smoke is the causative factor in more than 90% of cases, it is estimated that only 10% to 20% of smokers develop COPD. This may be explained by the existence of genetic or environmental factors that modulate the toxic effects of tobacco. The best known genetic factor is  $\alpha_1$ -antitrypsin deficiency, which is associated with an increased risk of developing pulmonary emphysema in smokers. The most recent guidelines from both the World Health Organization and the American Thoracic Society/European Respiratory Society recommend the establishment of screening programs for the detection of  $\alpha_1$ -antitrypsin deficiency in patients with COPD. This strategy is crucial in Spain, where the disease is under diagnosed, mainly due to a low index of suspicion among doctors.

**Key words:**  $\alpha_1$ -Antitrypsin deficiency. Diagnosis. Prevalence. COPD.

Déficit de alfa-1-antitripsina. Situación en España y desarrollo de un programa de detección de casos

Estudios realizados en España señalan que un 9% de la población general de entre 40 y 70 años está afectada de enfermedad pulmonar obstructiva crónica (EPOC). El humo del tabaco es el factor causal en más del 90% de los casos; sin embargo, se ha estimado que sólo un 10-20% de los fumadores desarrollan EPOC. La causa se puede encontrar en la existencia de factores genéticos o ambientales que modulan el efecto tóxico del tabaco. El factor genético más conocido es el déficit de alfa-1-antitripsina, que comporta un riesgo aumentado de desarrollar enfisema pulmonar en fumadores. Tanto la Organización Mundial de la Salud como la American Thoracic Society/European Respiratory Society en sus recientes normativas recomiendan establecer programas de detección del déficit de alfa-1-antitripsina en pacientes con EPOC. Esta estrategia es crucial en España, donde la enfermedad está infradiagnosticada, sobre todo debido a un bajo índice de sospecha entre los médicos.

**Palabras clave:** Déficit de alfa-1-antitripsina. Diagnóstico. Prevalencia. EPOC.

## Introduction

Chronic obstructive pulmonary disease (COPD) is a major public health problem. Studies undertaken in Spain indicate that 9% of the general population aged between 40 and 70 years is affected by COPD.<sup>1</sup> This indicates that in Spain more than a million individuals in this age group have the disease, whose clinical manifestation is pulmonary emphysema in most cases.

COPD is characterized by a slow, progressive obstruction of airflow due to the loss of elasticity caused by destruction of the pulmonary parenchyma and obstruction of the peripheral airways. Tobacco smoke is the causative factor in more than 90% of cases in westernized countries.<sup>2</sup> Despite the clear association between smoking and airflow obstruction, there is a

notable variation between individuals in their response to tobacco smoke.<sup>3,4</sup> This indicates that additional genetic or environmental factors exist that can contribute to the development of COPD.

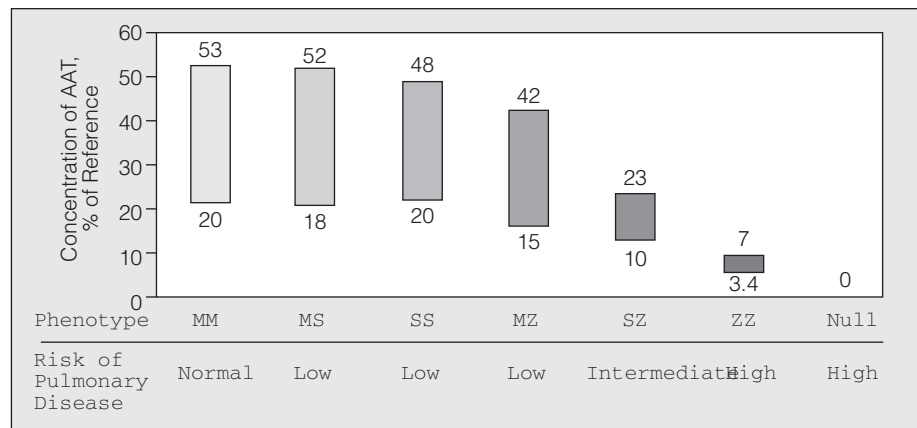
Extensive data from epidemiologic and clinical studies indicate a hereditary input in the development of COPD.<sup>5-15</sup> Genetic factors can alter the impact of tobacco smoke on the lung. Thus, such factors will be critical in determining the rate of deterioration of lung function and will affect which patients will develop COPD and the severity of the disease in those patients. This is suggested by case-control studies that have shown an increase in the prevalence of COPD in the families of cases compared with the families of controls, a finding that could not be explained on the basis of other known risk factors.<sup>5-7</sup> There is also a higher prevalence of lung function abnormalities in the children of patients with COPD than in the patients' spouses.<sup>5,8-15</sup> Cross-sectional studies have shown that increasing genetic distance is associated with a reduction in the prevalence of the disease and less similarity in terms of lung function.<sup>6,13,16</sup> Although the

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Correspondence: Dr. C. de la Roza.  
Servicio de Neumología. Hospital Clínic.  
Villarroel, 170. 08036 Barcelona. España.  
E-mail: croza@clinic.ub.es

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**Figure 1.** Intervals of serum concentrations of  $\alpha_1$ -antitrypsin (AAT) according to phenotype. The x-axis shows the most frequent PI phenotypes and the y-axis shows the plasma concentrations expressed by each phenotype as a percentage of the reference value. The length of the bars corresponds to the prevalence of each phenotype; the cut-off point for protection is 35%. Below this value are all PIZZ individuals and a third of PISZ individuals. There is a large overlap between the values of the PIMS, PIMZ, and PISS phenotypes.



results of some of those studies reveal grouping of cases of COPD within families, there is no clear mendelian pattern of inheritance.

Among the genetic factors implicated in the development of COPD, the clearest example is  $\alpha_1$ -antitrypsin (AAT) deficiency, which is associated with a greatly increased risk of developing pulmonary emphysema at an early age, particularly in smokers.<sup>17</sup> In this review we attempt to highlight the most relevant clinical aspects of this deficiency. In particular, we draw attention to the importance of early diagnosis, since AAT deficiency is a disease in which measures can be taken to slow the progress of emphysema, including replacement therapy with AAT derived from the plasma of donors in those individuals in whom such treatment is indicated.

### $\alpha_1$ -Antitrypsin Variants

The discovery by Laurell and Erikson<sup>18</sup> that patients with low plasma concentrations of  $\alpha$ -globulins had an increased prevalence of emphysema represented the first evidence of a genetic risk factor for the development of COPD. AAT is a potent antiprotease and one of the few enzymes that can inhibit neutrophil elastase, an important enzyme in the pathogenesis and development of COPD. Although AAT is mainly synthesized in the liver, it is also produced by alveolar macrophages and peripheral blood monocytes.<sup>19</sup> It is a highly polymorphic protein, with more than 75 variants identified using electrophoresis and isoelectric focusing methods.<sup>20-22</sup> Although the majority of these variants lack clinical significance, some 30 of them may have pathologic consequences. The variants are classified according to their rate of electrophoretic migration in a magnetic field as M (medium), F (fast), and S (slow). When other variants were discovered, they were designated with the initial letters of the alphabet for the variants that migrated towards the anode and the last letters of the alphabet for those that migrated towards the cathode.

From a clinical perspective, it is useful to classify AAT variants in 3 categories (Figure 1):

1. Normal, characterized by plasma concentrations of AAT in the normal range and that are not associated

with an increased risk of developing lung or liver disease. This category essentially includes the M genotypic variant and its subvariants.

2. Deficient, characterized by low but not undetectable concentrations of AAT. This group mainly includes the S and Z variants. The Z variant expresses approximately 10% to 20% AAT and the S variant, which is more common in the Mediterranean region, expresses approximately 50% to 60% AAT.

3. Null, associated with undetectable concentrations of AAT in plasma and increased risk of developing emphysema.

Most cases of AAT deficiency are caused by homozygosis of the deficient allele PIZ or by combinations of the 2 most common deficient alleles, PiS and PiZ.

Although the Z variant of AAT (Glu342Lys) also gives rise to a protein with deficient antiproteolytic function, the principal deficiency is in the concentration of the protein as a result of incorrect processing in the rough endoplasmic reticulum of the liver, leading to polymerization and formation of aggregates inside the hepatocytes, where it causes liver disease.<sup>23</sup>

The S variant (Glu264Val) occurs in around 25% of individuals in populations from southern Europe<sup>24,25</sup> and in homozygosis leads to concentrations of AAT corresponding to 60% of normal values. It has not been demonstrated that homozygosis for this variant is associated with an increased risk of developing COPD.

Individuals with the rare allelic combination SZ are less common than MS or MZ heterozygotes and have AAT concentrations that are approximately 40% of normal values. The risk of developing pulmonary disease in those patients has been investigated in numerous studies using a variety of methods. Case-control studies have not revealed an increase in the proportion of individuals with the SZ phenotype in patients with COPD compared with control groups.<sup>26,27</sup> In contrast, population-based studies have shown an increased risk of developing COPD in those individuals if they are smokers, with levels of obstruction similar to homozygous PIZ individuals, while this risk was not present in nonsmokers.<sup>28-30</sup> Finally, a recently published

metaanalysis of 6 studies demonstrated an increased risk of developing COPD in individuals with the combination SZ, with an odds ratio of 3.26 (95% confidence interval, 1.24-8.57).<sup>31</sup> However, due to the limited number of SZ individuals for whom information was available on smoking habit, the authors were unable to calculate the odds ratios separately for smokers and nonsmokers.

In summary, various studies have highlighted smoking as the main risk factor for the development of pulmonary emphysema in individuals with an SZ phenotype, while the risk in nonsmokers appears not to differ from the general population.

In addition to the most common deficient variants, DNA sequencing techniques have identified other deficient allelic variants. Eight variants of the P family of alleles have been described, some of which can only be differentiated using a technique for determination of the phenotype by isoelectric focusing and others also require analysis of the genotype since they display the same isoelectric point.<sup>32,33</sup> Among these, only PI Plovel and PI Pduarte have been associated with reduced concentrations of AAT and pulmonary emphysema.<sup>32-34</sup> In a study performed in Spain, the PI Plovel allele was detected in members of 5 families from different geographic areas, suggesting that the prevalence of this allele may be higher than initially thought.<sup>35</sup> The earlier failure to detect this allele could have been related to incorrect interpretation of isoelectric focusing patterns.

The allelic variant PI Mpalermo has been described in various families from the Mediterranean area.<sup>35,36</sup> This variant is characterized by a deletion of 3 base pairs in the position that codes for Phe51 or Phe52 and presents an AAT concentration similar to that associated with the PIZ allele.

Another rare deficient variant known as Mvall d'hebron, which was first characterized in Spain, arises from the Pro369Ser substitution.<sup>37</sup>

An additional new variant identified in Spain, known as PI Ybarcelona, is characterized by substitutions at positions Asp256 in exon 3 and Pro391 in exon 5.<sup>38</sup> Homozygosity for this variant gives rise to a severe AAT deficiency, with very low concentrations of AAT, and is clinically indistinguishable from the severe deficiency associated with PIZZ, since it leads to early pulmonary emphysema. Individuals who are heterozygous for this variant present intermediate concentrations of AAT and a risk of pulmonary disease that is similar to MZ individuals.<sup>39</sup>

### Deficient Z Variant

Individuals who are homozygous for the deficient Z variant have circulating concentrations of AAT that are less than 15% of normal values and an accelerated rate of deterioration of lung function, even in the absence of smoking.<sup>40,41</sup> However, pulmonary emphysema develops at an earlier age in those individuals who are also smokers.<sup>42,43</sup> The low plasma and tissue concentrations of AAT are insufficient to protect the connective tissue of the lung from the action of neutrophil proteases.

Although the PIZZ phenotype is undoubtedly a genetic risk factor for the development of COPD, there is considerable variation in the clinical expression of the deficiency.<sup>44,45</sup> This variability is not entirely attributable to the difference in exposure to tobacco smoke, since the rate of deterioration of lung function in ZZ individuals who are nonsmokers is also highly variable.<sup>41</sup>

It is possible that other mutations could act as modifiers of the clinical course of homozygous ZZ individuals. A study has linked a polymorphism (C774T) in the gene for endothelial nitric oxide synthase with the risk of developing COPD in ZZ individuals.<sup>46</sup> Nitric oxide may play a role in the pathogenesis of COPD because it regulates vascular tone and the tone of the airways in the lung and also facilitates adhesion of leukocytes to the endothelium. The 774T allele is significantly less common in ZZ individuals whose forced expiratory volume in the first second (FEV<sub>1</sub>) is less than 35% of predicted than in those ZZ subjects whose FEV<sub>1</sub> is greater than 35%.<sup>46</sup>

The clinical expression of AAT deficiency is also modified by polymorphisms in glutathione S-transferase P1, a subfamily of glutathione S-transferase that is widely expressed in all types of epithelial cells, including those of the lung,<sup>47,48</sup> and that participates in the detoxification of electrophilic substances and products of oxidative stress caused by tobacco smoke.<sup>49</sup> The polymorphism that gives rise to an isoleucine (Ile) to valine (Val) substitution at position 105 can alter the activity of the enzyme since it is located very close to the binding site of the enzyme for the substrates on which it acts. A case-control study has shown that the presence of at least 1 Val allele at position 105 in smokers with an AAT deficiency carries with it an increased risk of deterioration of lung function.<sup>50</sup> However, polymorphisms in this gene do not appear to influence the severity of the deterioration of lung function in patients with COPD who have normal concentrations of AAT. This appears to suggest that the protective role of the Ile105 variant of the enzyme in homozygosity is lower than that due to AAT, meaning that the substitution would not have clinical repercussions in smokers with normal concentrations of AAT.

### Epidemiology of $\alpha_1$ -Antitrypsin Deficiency

The Z allele of AAT is predominant in populations from northern Europe and is less common in populations from southern Europe and Asia, and in black populations.<sup>51</sup> In Spain, a gene frequency for the Z allele of 1.5% has been observed in the general population,<sup>24,25</sup> meaning that for a population of approximately 40 million people, it could be expected that 8000 individuals have the severely deficient form associated with homozygosity for PIZZ. In a study performed in Spain, the number of individuals with the 5 most common deficient phenotypes (PIMS, PIMZ, PISS, PISZ, and PIZZ) was calculated based on the results of the main epidemiologic studies published on the frequency of AAT deficiency.<sup>52</sup> According to those calculations, there could be more than 9 million individuals who are carriers of those alleles: 80% with

the PIMS phenotype and the remainder PIMZ (13%), PISS (4.7%), PISZ (1.6%), and PIZZ (0.1%). In the same study, estimates of the penetrance of the PIZZ phenotype indicated that there could be 2526 adults with COPD and 4030 individuals (children and adults) with chronic liver disease associated with this phenotype.

The need to generate unified criteria throughout Spain for the instigation of replacement therapy and follow-up led to the creation in 1993 of the Spanish Registry of Patients with AAT Deficiency.<sup>53</sup> The initial aims were to identify the characteristics and frequency of AAT deficiency in Spain, establish guidelines for treatment and follow-up in patients with the deficiency, offer information to doctors who treat those patients, improve understanding and interest in the disease, and attempt to reduce underdiagnosis. From the outset, this registry formed a working group of the Respiratory Failure and Sleep Disorders assembly of the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR). The organization is made up of 2 coordinators, an advisory committee, and 87 participating hospitals distributed throughout Spain and Andorra.

The prevalence of AAT deficiency calculated from gene frequency data in Spain contrasts with the number in the Spanish patient registry, which, since its foundation in 1993, includes 410 patients from 16 out of the 17 autonomous communities.<sup>53-55</sup> Despite the detection of AAT deficiency in Spain being similar to, or possibly higher than, detection in other European countries,<sup>56</sup> it remains low when compared with the estimated number of patients.

The Spanish registry collects information on the functional evolution of the registered patients, the frequency of replacement therapy, and the possible appearance of adverse effects of this treatment. The advisory committee meets periodically to evaluate and analyze the development of the registry database and update guidelines on treatment and follow-up. The committee also organizes an annual open meeting as part of the SEPAR national conference. In 1999, the Spanish Registry of Patients with AAT Deficiency joined with the international registry for this disease (Alpha One International Registry), which includes patients from 20 countries on 4 continents,<sup>57</sup> and since 2002 the Spanish registry has used a new online system for data collection as part of the official web site of SEPAR, allowing doctors throughout Spain to introduce data on patients via the Internet ([www.separ.es/air](http://www.separ.es/air)).

### **Risk of Emphysema in Heterozygous Carriers of $\alpha_1$ -Antitrypsin Deficiency (MZ, MS)**

The question of whether intermediate AAT deficiency is a risk factor for the development of COPD has generated a great deal of debate. The deficiency is mainly caused by the MS and MZ phenotypes, which are present in Caucasian populations at a rate of approximately 10% and 3%, respectively. In Spain, according to calculations of the number of individuals with the 5 most common deficiency phenotypes (PIMS, PIMZ, PISS, PISZ, and PIZZ), there could be 9 million carriers.<sup>52</sup>

Case-control studies have been performed in which the prevalence of phenotypes that give rise to intermediate concentrations in patients with COPD was compared with that found in patients without airflow obstruction. The results of many of those studies have demonstrated an increase in the prevalence of MZ heterozygotes in patients with COPD compared with the control group, with an odds ratio for the MZ form of between 1.5 and 5.<sup>58</sup>

Studies have also been performed in individuals from the general population on the risk associated with the MZ phenotype. In those studies, lung function was assessed in MZ individuals and compared with that of MM individuals. Such studies would be less susceptible to error, since all individuals are recruited in the same way and come from the same population. The results from the majority of these studies have not revealed significant differences in respiratory symptoms or lung function in MZ individuals compared with MM individuals. However, many of the studies were based on small samples and not all subjects had sufficient exposure to tobacco smoke.<sup>59-61</sup> Other studies performed in the general population have revealed differences between MZ individuals who were smokers and MM individuals in terms of lung function or loss of elastic recoil in the pulmonary parenchyma.<sup>62</sup>

In a recent study performed in the general population in which AAT concentrations and phenotype were assessed along with the annual rate of deterioration in FEV<sub>1</sub>, with a mean follow-up of 21 years, it was shown that deterioration of FEV<sub>1</sub> was 19% greater in MZ individuals than MM individuals (25 vs 21 mL;  $P=0.048$ ).<sup>63</sup> In addition, the rate of individuals with airflow obstruction was 19% in the group with an MZ phenotype, compared with 15% in the group with an MM phenotype ( $P=0.023$ ).

In conclusion, the results of case-control studies and some studies performed in the general population have indicated that the MZ genotype is a risk factor for the development of COPD, although the increase in risk is probably quite modest and only in smokers. The most relevant results regarding the risk of pulmonary disease in homozygotes are shown in Table 1.

Data available on the risk of developing diseases associated with the PIMS and PISS phenotypes are inconclusive, leading most authors to consider that these phenotypes do not represent an increased risk of developing pulmonary disease.<sup>70</sup>

Various studies have been performed to address the association between intermediate AAT deficiency and other pulmonary diseases, particularly bronchial asthma. A number of them have demonstrated that the presence of the Z allele in heterozygosis (PIMZ) gives rise to increased severity of asthma in children and adolescents.<sup>71,72</sup> A study performed in the adult population to assess the distribution of deficient AAT phenotypes in asthmatic patients did not reveal differences compared with the general population.<sup>73</sup> In addition, the deficient phenotypes in heterozygosis were not associated with greater severity or a different clinical expression of asthma in adults.

TABLE 1  
Studies of Intermediate  $\alpha_1$ -Antitrypsin Deficiency and Risk of Developing Chronic Obstructive Pulmonary Disease\*

Authors, y	Population	Study Type	Phenotypes, %	OR for MZ
Mittman et al, <sup>64</sup> 1974	350 with COPD 2830 controls	Case-control	13.7% MZ 2.9% MZ	3.8
Barnett et al, <sup>65</sup> 1975	107 with COPD 91 controls	Case-control	9.3% MZ 2.2% MZ	4.6
Cox et al, <sup>66</sup> 1976	114 with COPD 721 controls	Case-control	4.9% MZ 1.9% MZ	2.6 No difference in age
Shigeoka et al, <sup>67</sup> 1976	306 with COPD 196 controls	Case-control	3.9% MZ 1% MZ	4
Bartman et al, <sup>68</sup> 1985	526 with COPD 642 controls	Case-control	5.8% MZ 1.2% MZ	5 Functionally worse than MM
Janus et al, <sup>69</sup> 1988	190 with emphysema 1303 controls	Case-control	14.2% MZ 3.9% MZ	3.9
Lieberman et al, <sup>26</sup> 1986	965 with COPD 1380 controls	Case-control	8% MZ 2.8% MZ	3.3 No difference in age
Bruce et al, <sup>59</sup> 1984	143 MZ 143 controls	General population	No significant clinical or functional differences	
Cooper et al, <sup>62</sup> 1974	54 MZ 69 MM	General population	Additive effect of MZ and smoking on lung function	
Pride et al, <sup>60</sup> 1980	20 MZ 20 controls	General population	No significant clinical or functional differences	
Horton et al, <sup>61</sup> 1980	28 MZ 28 controls	General population (follow-up, 6 years)	Without significant clinical or functional differences, even after 6 years of follow-up	
Dahl et al, <sup>63</sup> 2002	451 MZ 8736 controls	General population (follow-up, 21 years)	Greater FEV <sub>1</sub> deterioration in MZ Can explain 2% of cases of COPD	

\*FEV<sub>1</sub> indicates forced expiratory volume in the first second; COPD, chronic obstructive pulmonary disease; OR, odds ratio.

### Screening for $\alpha_1$ -Antitrypsin Deficiency: Why, When, and How?

Population-based studies in Spain indicate that AAT deficiency is a disease that is underdiagnosed and that diagnosis is often delayed.<sup>54,55,74</sup>

Among all the causes of underdiagnosis, the most noteworthy is the attribution of emphysema to smoking alone without addressing the possibility of AAT deficiency. Some current guidelines still consider that deficiency should only be suspected in patients with severe emphysema who are young and/or nonsmokers<sup>75</sup>; however, those individuals only represent a small group of the total number of cases and many patients do not fit the criteria for severity.<sup>45</sup> In a study performed in Spain, the average delay in diagnosis, measured as the time between diagnosis of COPD and diagnosis of AAT deficiency, was found to be 10 years.<sup>76</sup>

The importance of early diagnosis resides in the possibility of undergoing a vigorous smoking cessation program and treatment of pulmonary disease, a family study to identify individuals at high risk who are at earlier stages in development of the disease, and the possibility of initiating AAT replacement therapy in individuals who meet the established criteria.

To date, the most commonly used techniques for the laboratory diagnosis of AAT deficiency include analysis of the serum concentration of AAT and determination of

the AAT phenotype by isoelectric focusing at a pH of 4.2 to 4.9.<sup>77,78</sup> Previous studies have assessed the efficacy of methods to screen for AAT deficiency. In a study to assess the effectiveness of quantification of the  $\alpha_1$  protein band, patients with severe AAT deficiency (PIZZ phenotype) consistently presented concentrations of  $\alpha_1$  globulins well below established normal limits.<sup>78</sup> However, due to the low prevalence of AAT deficiency and the various factors that can lead to reduced concentration of the serum proteins, low concentrations of the  $\alpha_1$  band are not always indicative of AAT deficiency. Under these circumstances, it is necessary to confirm the diagnosis through quantification of serum AAT by nephelometry and, if reduced values are obtained, analysis of the PI phenotype.

In 1997, the World Health Organization recommended determination of serum concentrations of AAT in all patients with COPD.<sup>79</sup> In patients in whom anomalous results are obtained in this screen, the phenotype should be determined. The American Thoracic Society and European Respiratory Society have prepared a joint consensus document on the diagnosis and treatment of AAT deficiency and recommend that diagnostic tests are performed in all adults with pulmonary emphysema or COPD (type A recommendation).<sup>80</sup>

Based on these recommendations, various countries have instigated screening programs (Table 2).<sup>81-84</sup> In Italy, screening has been performed through phenotyping with isoelectric focusing techniques in individuals for

whom there is an increased level of clinical suspicion or in family studies of AAT deficiency and the diagnostic yield has been high: 151 cases were detected from 1841 samples analyzed (8.2%), of which 118 were ZZ (78% of detected cases).<sup>81</sup>

In contrast, much lower detection rates have been observed in a program performed in Germany, with no homozygous individuals for the Z allele found from among 1060 screened individuals.<sup>82</sup> This is very likely to be due to the study population being made up of patients with all types of chronic respiratory diseases (asthma, bronchiectasia, and COPD), irrespective of the degree of suspicion of AAT deficiency.

Another study performed in the United States of America involved a screening program in 969 patients with diagnosis of emphysema, asthma, or chronic bronchitis, and the detection rates were 1 ZZ case per 31 samples, and 1 in 9 samples was heterozygous MZ.<sup>83</sup>

Such programs are possible thanks to the development of accurate techniques with which to quantify the concentrations of AAT and determine the genotype from samples of capillary blood on filter paper (Figure 2). Previous studies in Spain have

validated a specific immunonephelometry method for quantification of AAT in blood-spot samples on filter paper.<sup>85</sup> This has also been used for the detection of the most common genotypes associated with AAT deficiency, where it has been shown to be cost effective and applicable in large-scale screening programs.<sup>86</sup> Samples of this type only require a small amount of blood, which is obtained by extracting capillary blood from the fleshy part of the finger and placing it on filter paper before sending the sample for processing by conventional mail.

In Spain, this procedure is being used in an initiative from the Spanish Registry of Patients with AAT deficiency (Figure 3). In an initial phase it has been confirmed that the system for obtaining samples and sending them to the central laboratory is functioning properly. In addition, it has been demonstrated that the method is applicable, convenient, and well accepted by participating doctors and that it allows quantification of the serum concentration of AAT and detection of the Z allele.<sup>87</sup>

To date, samples have been analyzed from 2138 patients with COPD and 8 cases of homozygous PIZZ individuals have been detected.<sup>84</sup> This represents an

TABLE 2  
Screening Programs for  $\alpha_1$ -Antitrypsin Deficiency\*

Authors, y	Inclusion Criteria	Processing Protocol	Number	Deficient Phenotypes/ Genotypes	Heterozygotes
Luisetti et al, <sup>81</sup> 1999	Absence or reduction of values for the $\alpha_1$ band Plasma concentrations of AAT <80 mg/dL Early onset emphysema Family grouping of COPD cases Family studies of deficiency	Quantification by ELISA and phenotyping by isoelectric focusing in all samples	1841	Severe AAT deficiency 151 (8.2%) 118 (78%) PIZZ 17 (11%) PISZ 8 (5%) Null Null 4 (3%) PIZ Null 4 (3%) rare variants	
Wencker et al, <sup>82</sup> 2002	Patients with any type of chronic respiratory disease COPD (chronic bronchitis and pulmonary emphysema) Asthma Bronchiectasia	Quantification by ELISA and phenotyping by isoelectric focusing in all samples	1060	0 (0%) PIZZ 3 (0.28%) PISZ	36 (3.4%) PIMS 39 (3.7%) PIMZ
Brantly et al, <sup>83</sup> 2003	Patients with COPD Emphysema Asthma Chronic bronchitis	Quantification and subsequent genotyping in all samples	969	31 (3.2%) PIZZ 4 (0.4%) PISZ	107 (11%) PIMZ
De la Roza et al, <sup>84</sup> 2005	Patients with COPD, independently of the degree of suspicion of AAT deficiency, in whom the deficiency status was not known	First phase: quantification by ELISA and genotyping (Z allele) in all samples	971	5 (0.5%) PIZZ	23 (2.3%) PIMZ
		Second phase: quantification by ELISA Genotyping (S and Z alleles) only in samples with deficient concentrations	1167	3 (0.3%) PIZZ 3 (0.3%) PISZ 3 (0.3%) PISS	14 (1.2%) PIMZ 11 (0.9%) PIMS

\*Data are shown as number of patients (%). COPD indicates chronic obstructive pulmonary disease; ELISA, enzyme-linked immunosorbent assay; AAT,  $\alpha_1$ -antitrypsin.

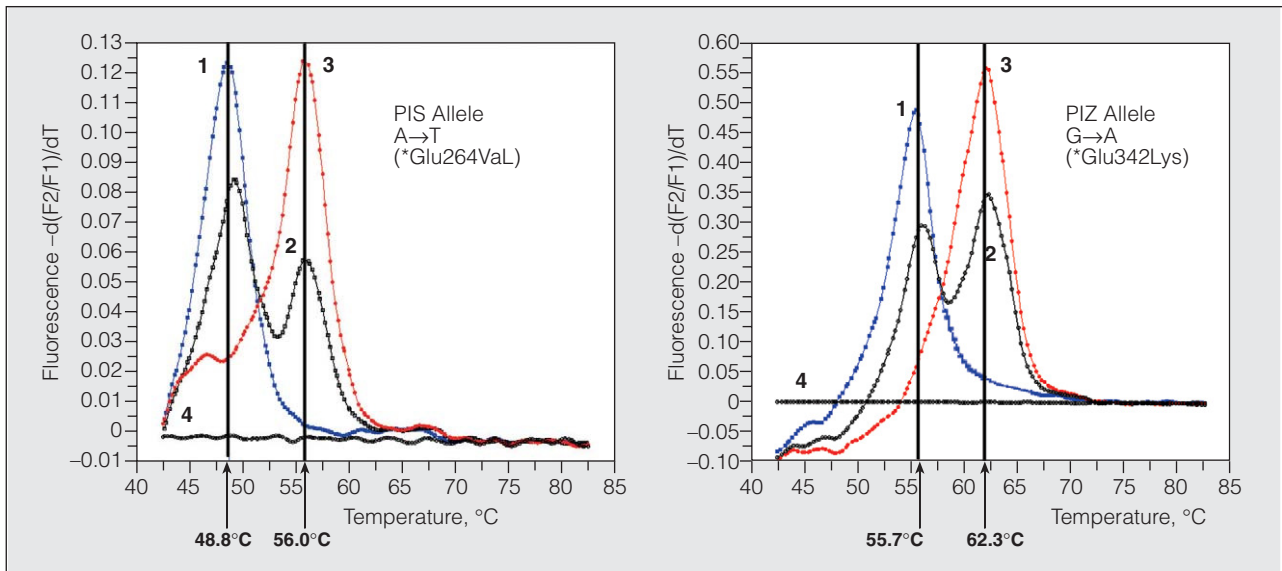


Figure 2. Results of genotyping for  $\alpha_1$ -antitrypsin (AAT) deficiency in dried blood spots using a LightCycler analyzer. Curves for the PIS genotype in the LightCycler: case 1, homozygote PISS; case 2, heterozygote PIMS; case 3, homozygote PIMM; and case 4, negative. Curves for the PIZ genotype in the LightCycler: case 1, homozygote PIZZ; case 2, heterozygote PIMZ; case 3, homozygote PIMM; and case 4, negative. A indicates adenine; T, thymine; Glu, glutamate; Val, valine; G, guanine; Lys, lysine. Adapted from Rodríguez.<sup>82</sup>

Patient's Initials <input type="text"/> <input type="text"/> <input type="text"/> <small>First Surname Second Surname First Name</small> Year of Birth (Year Only) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Sex Male <input type="checkbox"/> Female <input type="checkbox"/>	Name and Address of Doctor Name: ..... Hospital: ..... Address: ..... Town/City: ..... Fax: ..... E-mail: .....	S&S 903 <sup>tm</sup> LOT # W-941 <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Smoking Habit Smoker <input type="checkbox"/> Nonsmoker <input type="checkbox"/> Exsmoker <input type="checkbox"/> Symptoms Yes No Effort Dyspnea <input type="checkbox"/> <input type="checkbox"/> Intermittent Dyspnea <input type="checkbox"/> <input type="checkbox"/> Cough (>3 Months/Year for More Than 2 Years) <input type="checkbox"/> <input type="checkbox"/> Expectoration <input type="checkbox"/> <input type="checkbox"/>	Diagnosis <input type="checkbox"/> Asthma <input type="checkbox"/> Bronchiectasis <input type="checkbox"/> Chronic Bronchitis <input type="checkbox"/> COPD <input type="checkbox"/> Emphysema Family Members With $\alpha_1$ -Antitrypsin Deficiency Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown <input type="checkbox"/> Date of Blood Sample --/--/-- dd/mm/yy	

Figure 3. Questionnaire (translated into English) for collection of data from each patient along with filter paper disks used in the screening program for  $\alpha_1$ -antitrypsin deficiency.

intermediate rate of diagnosis between that obtained in Italy and Germany. When designing a screening program for detection of AAT deficiency, both the protocol for sample processing and the inclusion criteria for candidates must be taken into account, since both factors will have a decisive influence on the yield and cost of the program.

At the beginning of 2005, the Spanish registry began the Information and Detection of  $\alpha_1$ -Antitrypsin

Deficiency project, aimed at making diagnosis of the deficiency available to the greatest number of doctors who treat patients with COPD. This program provides information on the importance of early diagnosis, according to the guidelines of the World Health Organization and the American Thoracic Society/European Respiratory Society, in addition to providing the means with which to detect AAT deficiency in patients. Once patients are diagnosed and

included in the Spanish registry, it can be confirmed whether they meet the criteria for initiation of replacement therapy and whether or not to prescribe it.<sup>88</sup>

Detection of AAT deficiency is crucial to detect new cases in a country like Spain, where the disease has a low prevalence and, consequently, is associated with a low degree of suspicion in patients with COPD.

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