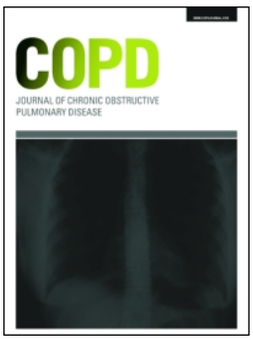

COPD: Journal of Chronic Obstructive Pulmonary Disease

ISSN: 1541-2555 (Print) 1541-2563 (Online) Journal homepage: http://www.tandfonline.com/loi/icop20

Alpha-1 Antitrypsin Deficiency Detection in a Portuguese Population

Leonor Meira, Rita Boaventura, Susana Seixas & Maria Sucena

To cite this article: Leonor Meira, Rita Boaventura, Susana Seixas & Maria Sucena (2018): Alpha-1 Antitrypsin Deficiency Detection in a Portuguese Population, COPD: Journal of Chronic Obstructive Pulmonary Disease, DOI: 10.1080/15412555.2017.1414779

To link to this article: https://doi.org/10.1080/15412555.2017.1414779

Published online: 02 Feb 2018.

COPD: JOURNAL OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE
2018, VOL. 0, NO. 0, 1–6
https://doi.org/10.1080/15412555.2017.1414779

Taylor & Francis
Taylor & Francis Group

Alpha-1 Antitrypsin Deficiency Detection in a Portuguese Population

Leonor Meira [a], Rita Boaventura[a], Susana Seixas[b,c], and Maria Sucena[a]

[a]Pulmonology Department, Centro Hospitalar de São João, Alameda Prof. Hernâni Monteiro Porto, Portugal; [b]Instituto de Investigação e Inovação em Saúde, Universidade do Porto (I3S), Porto, Portugal; [c]Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal

ABSTRACT

Alpha-1 antitrypsin deficiency (AATD) is an autosomal co-dominant disease characterised by low serum levels of this molecule. Its epidemiology remains unknown in many countries, mainly due to its underdiagnosed state and lack of patients' registries. We aim to evaluate and characterise a sample of Portuguese individuals tested for AATD, between 2006 and 2015, based on a retrospective analysis from the database of a laboratory offering AATD genetic diagnosis service. 1684 individuals were considered, covering almost every region in Portugal. Genetic diagnosis resulted from requests of clinicians from different areas of expertise, mainly pulmonology (35.5%). Most subjects could be distributed into more common genotypes: MZ (25.4%, $n = 427$), MS (15.5%, $n = 261$), SZ (11.2%, $n = 188$), ZZ (9.4%, $n = 158$) and SS (5.6%, $n = 95$). 9.5% of the subjects were found to carry at least one rare deleterious allele, including the recently described P_{Gaia}, $Q0_{Oliveira\ do\ Douro}$, $Q0_{Vila\ Real}$ and a novel S_{Gaia} variant. This study comprises 417 subjects (24.7%) with severe to very severe AATD and 761 carriers (45.2%), 22.7% of those identified by familial screening. The present study represents the most complete survey of AATD in Portugal so far and discloses a high rate of severe and very severe deficiency cases, attributed not only to ZZ and SZ genotypes but also to a large number of rare combinations with other null and deficiency alleles. It also uncovers a low awareness to AATD among the medical community, highlighting the need to create a Portuguese national registry and AATD guidelines and increase the awareness about this condition.

ARTICLE HISTORY
Received 7 September 2017
Accepted 4 December 2017

KEYWORDS
Alpha-1 antitrypsin deficiency (AATD); genetic screening; deficiency alleles; AATD registry

Abbreviations

AATD	Alpha-1 antitrypsin deficiency
AAT	Alpha-1 antitrypsin
COPD	Chronic obstructive pulmonary disease
IPATIMUP	Institute of Molecular Pathology and Immunology of the University of Porto
PI	Protease inhibitor
SERPIN	Superfamily of serine protease inhibitors

Introduction

Alpha-1 antitrypsin deficiency (AATD) is an autosomal co-dominant disease characterised by low circulating levels of this molecule in serum (1).

Alpha-1 antitrypsin (AAT) is a 52 kDa glycoprotein that belongs to the superfamily of serine protease inhibitors (SERPIN), which is mainly synthesised in the liver in addition to smaller contributions from leucocytes, lungs and intestine.

Amongst its various functions, such as an acute phase protein, and immunomodulatory and anti-inflammatory properties, AAT is an important neutrophil elastase inhibitor in the lower respiratory tract, protecting lung parenchyma from excessive destruction, which ultimately results in emphysema (2). Diminished levels of serum. protein and, in some cases, the accumulation of defective protein in the hepatocyte and other tissues, will increase the risk of developing premature onset of chronic obstructive pulmonary disease (COPD), liver cirrhosis in children and adults, and less frequently, relapsing panniculitis, systemic vasculitis and other inflammatory, autoimmune and neoplastic diseases (1,3).

Serum AAT levels are primarily defined by mutations in *SERPINA1* gene located at chromosome 14q32.1. Chief normal alleles are usually named as M-types (e.g. M1, M2 and M3) and the most common deficient alleles as Z (p.Glu342Lys) and S (p.Glu264Lys) (1,3). Nevertheless, to date there are more than a hundred variants described, including normal, deficiency and null alleles.

Homozygosity for the Z allele can result in very low AAT serum concentrations (10–15%), and SZ and MZ genotypes result in low to intermediate serum AAT concentrations (35 and 70%, respectively). Most AATD-related patients are linked with the ZZ genotype (96%), and the remaining to SZ, null and about other 30 rare genetic combinations of deficiency and null alleles (1,4).

Characterised over 50 years ago, (5) AATD is considered one of the most common hereditary disorders, but its epidemiology remains unknown in many countries, mainly due to its underdiagnosed state and a lack of registries of patients already identified (6–8). In recent years, some countries are trying to fill this gap by creating diagnose and management guidelines and national patient registries (9).

Nonetheless, some authors have managed to extrapolate, based on available studies, Z and S worldwide distributions.

CONTACT Leonor Meira lo.meira@gmail.com Pulmonology Department, Centro Hospitalar de São João, Alameda Prof. Hernâni Monteiro 4200-319 Porto, Portugal.

Both alleles have uneven dispersals with estimated prevalence varying between different countries of a continent, and also within those countries' regions. In Europe, maximum Z allele frequencies (>2%) were found in the south of Scandinavia, Denmark and Baltic Republics, and a significantly high frequency has also been described in the Iberian Peninsula. The S allele was also found to be significantly increased in the Iberian Peninsula with its frequency reaching >15% (10).

In Portugal, it is estimated that 1:5249 individuals (2,000 individuals) have a ZZ genotype, and that 1:281 individuals (37,400 individuals) have a SZ genotype. Multiple rare alleles have been identified in Portugal but their frequencies in random population are still unknown (10–13). Nonetheless, the real burden of the disease is still unrecognised since there is no national registry.

The number of people at risk of developing this disease supersedes by far the number of people diagnosed, so it is mandatory to build guidelines and expand our knowledge on disease epidemiology.

In this study, we aim to evaluate and characterise a sample of Portuguese individuals tested for AATD, between the years 2006 and 2015 (10 years), based on a retrospective analysis of the database from a laboratory offering an AATD genetic diagnosis service (Institute of Molecular Pathology and Immunology of the University of Porto, IPATIMUP, Porto, Portugal).

Methods

We conducted a retrospective study of all samples sent to IPATIMUP between 2006 and 2015 for the purpose of AATD diagnosis. IPATIMUP is a private non-profit association of public utility devoted mainly to health sciences, also aiming at translating genetics and molecular pathology research findings into high-quality diagnosis services. Specifically, for the purpose of AATD genetic testing, there is no standard form or any mandatory requirement to include any clinical information or AAT serum levels in the AATD genotyping request. Therefore, the data accessible for this study comprised only AAT phenotyping or genotyping results, patient age at the time of diagnosis and health entities requesting the AATD genetic diagnosis. In most cases, whole blood samples were provided and, less frequently, plasma and/or genomic DNA were sent for phenotyping and/or genotyping, respectively. All data analysis was conducted anonymously using SPSS (Version 21): simple descriptive statistics of mean and standard deviation for continuous data and frequencies and percentages for categorical data.

Prior to 2008, AATD genetic diagnosis included mainly the protein phenotyping by isoelectric focusing (IEF) (14) and *SERPINA1* sequencing to identify rare protein variants or complex family cases suspect to carry null alleles (15,16). From 2008 to 2012, in addition to IEF, a screening of common mutations (p.Arg101His, p.Ala213Val, p.Glu264Val and p.Glu342Lys) by multiplex polymerase chain reaction (17) was carried out in homozygous phenotypes to discard the presence of rare deficiency and null alleles. After 2012, IEF and genotyping of common mutations was routinely applied to all cases. Since 2008, *SERPINA1* sequencing is performed to confirm cases carrying rare alleles.

The study has been conducted in accordance with the ethical standards of the involved institutions and the Helsinki Declaration. As a retrospective study, no patient was identified (treated as anonymised samples) and only a few variables were considered in the analysis (see description below). AATD diagnosis was undertaken with patient consent.

Results

Overall, 1684 subjects covering almost every region in Portugal (except Azores and Alentejo regions) were tested for AATD. The majority of AATD diagnosis requests came from the Northern region ($n = 1096$), followed by Central region ($n = 272$), Lisbon and Tejo's valley ($n = 160$), Algarve ($n = 111$) and Madeira Island ($n = 9$), as shown in Figure 1. One patient was excluded due to lack of data.

AATD genetic diagnosis requests came from clinicians from different areas of expertise. Pulmonology represented the major field of medical experience ($n = 598$, 35.5%), closely followed by Paediatrics ($n = 559$, 33.2%), and in to a lesser extent by Gastroenterology ($n = 113$, 6.7%), General Practice ($n = 93$, 5.5%) and Internal Medicine ($n = 91$, 5.4%). A large variety of health facilities requested AATD testing but these were mainly public (95.7%). Among all patients tested, 17.9% ($n = 301$) could be reported as identified during familial screening, in which 34.9% ($n = 105$) were requested by Paediatrics, 29.2% ($n = 88$) by Pulmonology and the remaining 35.9% were not specified or requested by other medical specialities.

The mean patient age at the time of diagnosis was 31.5 years (±23.8); 58.9% ($n = 992$) were male and 40.3% ($n = 678$) were female; in 13 cases the patient´s sex was not identified. Most patients, 59.4%, were at least 18 years old, with a mean age of 46.6 (±23.8; range: 18–84), of which more than half were male ($n = 573$; 57.2%). Regarding the underage patients (<18 years old, $n = 573$), 62.2% were male and the mean age was 5.2 years (±5.1). In 110 patients (6.5%), it was not possible to determine age due to lack of data.

The average number of requests per year was 187.1, with 2008 and 2015 being the years with more and less requests (227 and 123, respectively).

In most instances, cases sent for AATD diagnosis carried at least one deficiency allele ($n = 1289$; 76.6%). A large percentage of these subjects was found to have an MZ (25.4%, $n = 427$) or MS (15.5%, $n = 261$) genotype, whereas SZ (11.2%, $n = 188$), ZZ (9.4%, $n = 158$) and SS (5.6%, $n = 95$) were less prevalent genotypes (Figure 2). Noticeably, a considerable fraction of cases could be included in the MM (23.4%, $n = 394$) category and thus they were not associated to AATD. The majority were adults followed in pulmonology clinic ($n = 124$) or were children <18 years old ($n = 128$, 22 were new-borns). 86 cases were performed on familial screening basis and most were requested by Pulmonology or Paediatrics/Neonatology physicians ($n = 34$ and $n = 23$, respectively).

Remarkably, 9.5% of cases were linked to rare deficiency and null alleles in hetero- or homozygosity (Table 1). These included M_{Malton} or $M_{Palermo}$ ($n = 64$), $Q0_{Ourém}$ ($n = 28$), I ($n = 20$), P_{Lowell} ($n = 17$), $M_{Heerlen}$ ($n = 7$), $M_{Würzburg}$ ($n = 6$), T ($n = 4$), $Z_{Augsburg}$ ($n = 4$), $Q0_{Faro}$ ($n = 2$), and $Q0_{Lisboa}$ and $Q0_{Gaia}$, (one case each). Three novel mutations were identified during the

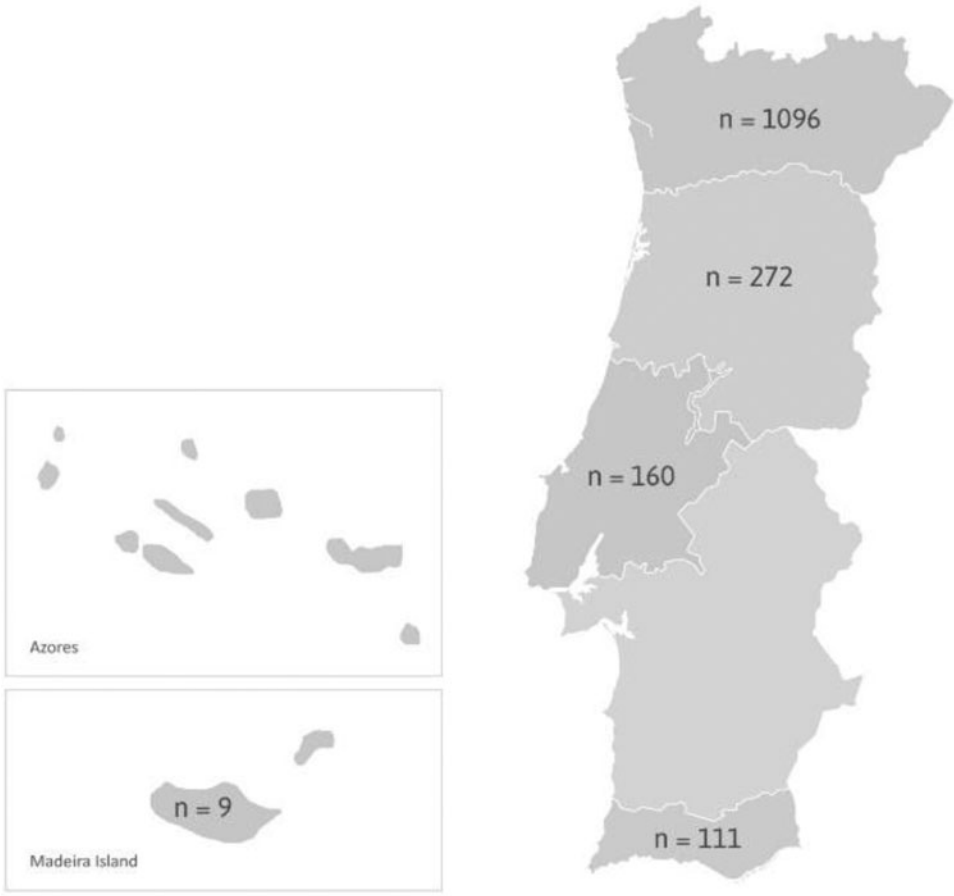

Figure 1. Number of patients tested for AATD per Portuguese major geographic regions.

time period of this study and were already described in literature: P_{Gaia} (identified in 2009), $Q0_{Oliveira\ do\ Douro}$ (identified in 2012) and $Q0_{Vila\ Real}$ (identified in 2013) (13). Another rare variant S_{Gaia} was identified more recently (2015) and characterised as the result of a p.Leu118Phe mutation in an S allele background. The p.Leu118 Phe was predicted by bioinformatics tools (18) as probably damaging (score 0.965) and accordingly, corresponding protein band in IEF seemed slightly reduced when compared to regular S alleles (not shown).

Three rare alleles not linked to AATD were also identified in a few subjects: an S-like allele (p.Ser47Arg) was detected twice, and $I_{euskadi}$ (p.Arg281del) and V (p.Gly148Arg) alleles were identified once each (13).

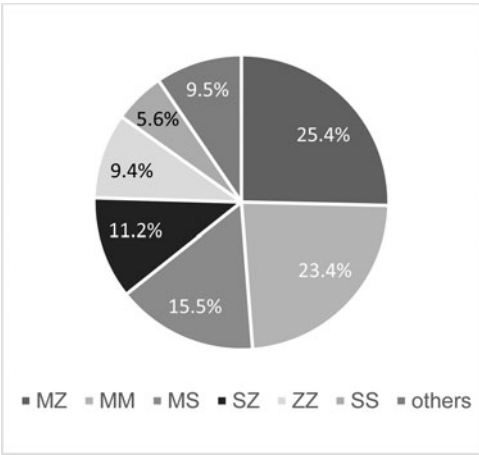

Figure 2. Genotypes of AATD found in the studied population.

Overall, this study comprises 417 subjects (24.7%) with severe to very severe AATD.

A large number of carriers (subjects with M allele sampled with one deficiency allele) were found ($n = 761$). In 22.7% of those, familial screening was the reason for testing.

Discussion

The present study describes the most recent and complete data for AATD in Portugal. In a 10 years' time frame, from 2006 to 2015, 417 individuals (almost 25% of tested cases) were confirmed by IPATIMUP to have severe or very severe AATD. These included 158 ZZ cases, 188 SZ and different combinations of rare and null alleles ($n = 71$).

Even though this study does not cover all AATD cases identified in Portugal and it is likely to be more representative of North and Central regions, the numbers are far from the projected ZZ and SZ prevalence in Portugal (approximately 2,000 and 37,000 individuals, respectively), highlighting that this genetic disease is, as in many other European countries, an underdiagnosed condition. However, our numbers of ZZ subjects identified in Portugal ($n = 158$; 9.4%) seem to exceed the percentages reported in many other countries worldwide (8).

Surprisingly, a considerable fraction of our cases (9.5%) could be associated to the presence of rare deficiency and null

Table 1. Genotypes associated to rare deficiency and null alleles identified in this study.

Allele	Mutation	Mean Serum level(a)	Associated genotype	Deficiency(b)
M_{Malton} or $M_{Palermo}$ (M_M)	p.Phe52del	12%	31 MM_M	Moderate (56%)
			16 SM_M	Severe (31%)
			14 ZM_M	Very severe (16%)
			1 $M_M M_M$	Very severe (12%)
			1 IM_M	Severe (31%)
			1 S-likeM_M	Moderate (56%)
$Q0_{Ourém}$ ($Q0_O$)	p.Leu353Phefs*24	0%	15 $MQ0_O$	Moderate (50%)
			5 $Q0_O Q0_O$	Very Severe (0%)
			4 $SQ0_O$	Severe (25%)
			4 $ZQ0_O$	Very Severe (8%)
I	p.Arg39Cys	50%	8 IZ	Severe (35%)
			6 SI	Moderate (50%)
			5 MI	Mild (75%)
			1 IM_M	Severe (31%)
P_{Lowell} (P_L)	p.Asp256Val	30%	10 MP_L	Mild (65%)
			5 SP_L	Moderate (40%)
			2 $P_L Z$	Severe (23%)
$M_{Heerlen}$ (M_H)	p.Pro369Leu	1–2%	4 ZM_H	Very severe (9%)
			3 MM_H	Moderate (52%)
$M_{Würzburg}$ (M_W)	p.Pro369Ser	15%	4 SM_W	Severe (33%)
			2 MM_W	Moderate (56%)
T	p.Glu264Val	50%	1 MT	Mild (75%)
			1 ST	Moderate (50%)
			1 TZ	Severe (35%)
			1 TZ_A	Severe (32.5%)
$Z_{Augsburg}$ (Z_A)	p.Glu342Lys	15%	2 ZZ_A	Very severe (17.5%)
			1 TZ_A	Severe (32.5%)
			1 MZ_A	Moderate (57.5%)
$Q0_{Faro}$ ($Q0_F$)	c.−5+ 2dupT	0%	2 $MQ0_F$	Moderate (50%)
$Q0_{Oliveira\ Douro}$ ($Q0_{OD}$)	p.Arg281Lysfs[a]17	0%	1 $MQ0_{OD}$	Moderate (50%)
			1 $SQQ0_{OD}$	Severe (25%)
$Q0_{Gaia}$ ($Q0_G$)	p.Leu263Pro	0%	2 $ZQ0_G$	Very severe (10%)
$Q0_{Lisboa}$ ($Q0_L$)	p.Thr68Ile	0%	1 $ZQ0_L$	Very severe (10%)
P_{Gaia} (P_G)	p.Glu162Gly	50%	1 SP_G	Moderate (50%)
$Q0_{Vila\ Real}$ ($Q0_{VR}$)	p.Met374Leufsa19	0%	1 $MQ0_{VR}$	Moderate (50%)
S_{Gaia} (S_G)	p.Leu118Phe	20%	1 MS_G	Mild (75%)

[a] Mean of estimated percentage of circulating AAT.
[b] The severity of AATD was extrapolated by averaging the estimated circulating AAT levels for each allele. Mild AATD: >60%; Moderate AATD: 40–60%, Severe AATD: 20–40%, Very severe AATD: <20%.

variants reported to segregate in Portuguese and other European populations at very low frequencies (below 0.1%) (13). A plausible explanation for this finding is related to IPATIMUP being considered an AATD reference laboratory in Portugal, where cases with incongruent data (clinical versus genetic) are sent. Another reasonable argument is that combining phenotyping and genotyping techniques for AATD diagnosis elicits the detection of most variants playing a role in this disease. Furthermore, in Portugal it is common practice by most clinicians in central hospitals to assess AAT serum levels prior to genotyping, which makes the studied sample naturally enriched with cases displaying reduced AAT concentrations.

Oddly, this study shows very similar Paediatric and Pulmonology referrals for AATD testing. However, it is important to note that AATD testing by pulmonologists is likely to be underestimated because, since 2009, the second largest tertiary hospital in Portugal started to concentrate the management of AATD requests in its genetic department. This comprises 10.2% of cases for which it was not possible to allocate a medical speciality requesting AATD genotyping. This limitation is also observed regarding familial screening data, with paediatric referrals having the higher number of requests.

Conversely, our study also suggests a lack of awareness of AATD by general practitioners and internal medicine physicians given that these only referenced a few subjects in spite of treating a large bulk of COPD patients. It is possible that besides their insufficient knowledge about the disease, this might be as well attributed to a low familiarity with AATD diagnostic tools (AAT serum quantification and genetic screening), contributing to the reduced numbers of AATD subjects identified, as also described in other studies (6,19–24).

Despite these limitations in recognising the importance of AATD screening in Portugal, our study shows that the mean age at time of diagnosis, after excluding a large number of subjects under 18, does not differ from other countries such as Poland, Italy, Germany, Spain and USA (45–49 years old) (7,22,25,26).

Overall, in these cohorts of patients, 761 were found to be carriers of a deficient allele with low or no recognised risk for developing AATD-related disorders (MZ or MS, respectively) and 394 did not carry any deficiency allele (MM). Although we are missing the information that motivated physicians to test these patients, this high number of subjects without disease risk may represent a fulfilment of the recommendations from several organisations like WHO and ERS for AATD testing in COPD patients and individuals with unexplained hepatic disease (1,12,27,28). Since no diagnostic algorithm exists in Portugal, some physicians could request phenotyping and/or genotyping without previous AAT serum level determination, explaining the high number of carriers and MM subjects.

Among individuals with severe to very severe AATD (AAT levels below 40%), we detected a relatively high amount of rare genotypes (71/417, 17%) which is superior to the 11% prevalence reported by Ferratori et al (29). In our sample, the vast majority of patients with rare alleles presented the p.Phe52del mutation (M_{Malton} and $M_{Palermo}$), found only once in homozygosity. Most of these cases were referenced by Pulmonology ($n = 21$) and Paediatrics ($n = 13$) and 12 were derived from familial screenings. Interestingly, this mutation was found to segregate at very low frequencies in Europeans (>0.01%) (30) and accordingly was identified in different regions in Portugal (North, Central and Tejo's valley regions).

Conversely, the $Q0_{Ourem}$ allele characterised by a p.Leu353Phefs*24 mutation in an M3 background was the second most prevalent rare variant ($n = 28$), appearing 5 times in homozygosity and being identified 13 instances during familial screenings. In contrast to the p.Phe52del mutation, the $Q0_{Ourem}$ allele was not detected in any large sequencing project of Europeans, confirming a probable origin in Portugal associated to a founder effect. This phenomena could explain the clustering in Central Portugal of several unrelated families with very severe AATD, as previously described (16).

Other prevalent rare alleles detected in our cohort, namely, the I, P_{Lowell}, $M_{Würzburg}$ and $M_{Heerlen}$ were all found to segregate within Europeans at very low frequencies (>0.01%) (30). Therefore, their discovery in our sample is most probably explained by an association of these alleles to clinical manifestations of AATD. The same may apply to the novel mutations identified exclusively in Portugal, in single families, which are likely to be extremely uncommon in healthy populations.

Although, IPATIMUP has been offering an AATD diagnosis service for more than decade, it remains as an informal reference laboratory, not having any special requirement for its execution. Given that there is no standard form to request AATD genotyping and each health centre in Portugal has its own requesting rules, in most instances, only basic sociodemographic data were made available to IPATIMUP such as age and gender. Indeed, this represents the major limitation of the current study that prevents an in-depth analysis of the existing correlations between AATD genotypes and the clinical manifestations of the disease.

Furthermore, in Portugal, there is no AATD screening program in course and available for the general population, which together with the considerably large variability of AATD manifestations, leads to a late diagnosis usually when patients already have symptoms (21). According to international guidelines, all patients with COPD, emphysema or asthma without fully reversible airflow obstruction are recommended for AATD testing (1,7,21,31). However, this evaluation is not being performed in early disease stages and, thus, worldwide detection rates are considerably lower than the expected *a priori* based in known frequencies of deficiency alleles (1,6,7,12,23,31–34). In order to promote preventive measures and lifestyle changes, familial screenings, genetic counselling and specific treatments, healthcare professionals must be aware first of this condition and diagnose it promptly. Only then a difference in the patient prognosis can be achievable, despite that no cure is currently available.

Hence, national registries of patients with rare diseases, such as AATD, are essential to know their epidemiology, genetic distribution and natural history (27), as well as associated clinical phenotypes (12,29,35). In addition, the identification of national reference laboratories specialised in AATD screening is also of extreme importance to improve the detection rates of this genetic condition.

In 2015, a study group of AAT deficiency was created in Portugal that is working towards the creation of a national registry as well as guidelines.

Conclusion

This study represents the most complete survey of AATD in Portugal so far and discloses a high rate of severe and very severe deficiency cases, attributed not only to ZZ and SZ genotypes but also a large number of rare combinations with other null and deficiency alleles. It also uncovers a low awareness to AATD among the medical community, particularly by general practitioners and internal medicine physicians, highlighting the needs to create a Portuguese national registry, our own AATD guidelines and to increase the knowledge about this condition for future reference to a speciality consultation and treatment, when suitable.

Acknowledgments

The authors would like to thank Renata Tamegão Rothwell for reviewing the manuscript.

Declaration of interest statement

The authors report no conflicts of interest in this work.

Funding

IPATIMUP integrates the i3S Research Unit, which is partially supported by the Portuguese Foundation for Science and Technology (FCT) and financed by the European Social Funds (COMPETE-FEDER) and National Funds (projects PEst-C/SAU/LA0003/2013 and POCI-01-0145-FEDER-007274). This work in IPATIMUP was also partially supported by Programa Operacional Regional do Norte (Norte 2020: NORTE-01-0145-FEDER-000029). This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

ORCID

Leonor Meira http://orcid.org/0000-0002-1841-4979

References

1. American Thoracic Society, European Respiratory Society. American thoracic society/European respiratory society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. Am J Respir Crit Care Med 2003;168(7):818–900. doi:10.1164/rccm.168.7.818.
2. Carrell RW, Lomas DA. Alpha1-antitrypsin deficiency–a model for conformational diseases. N Engl J Med 2002;346(1):45–53. doi:10.1056/NEJMra010772.
3. Vidal R, Blanco I, Casas F, Jardi R, Miravitlles M, Committee on the National Registry of Individuals with Alpha-1 Antitrypsin Deficiency. Guidelines for the diagnosis and management of alpha-1 antitrypsin deficiency. Arch Bronconeumol 2006;42(12):645–59. doi:10.1157/13095974.

4. Stoller JK, Aboussouan LS. A review of alpha1-antitrypsin deficiency. Am J Respir Crit Care Med 2012;185(3):246–59. doi:10.1164/rccm.201108-1428CI.
5. Laurell CB, Eriksson S. The electrophoretic alpha1-globulin pattern of serum in alpha1-antitrypsin deficiency. 1963. COPD 2013;10 Suppl 1:3–8. doi:10.3109/15412555.2013.771956.
6. Campos MA, Wanner A, Zhang G, Sandhaus RA. Trends in the diagnosis of symptomatic patients with alpha1-antitrypsin deficiency between 1968 and 2003. Chest 2005;128(3):1179–86. doi:10.1378/chest.128.3.1179.
7. Chorostowska-Wynimko J. Targeted screening programmes in COPD: how to identify individuals with alpha1-antitrypsin deficiency. Eur Respir Rev 2015;24(135):40–5. doi:10.1183/09059180.00010614.
8. Stoller JK, Brantly M. The challenge of detecting alpha-1 antitrypsin deficiency. COPD 2013;10 Suppl 1:26–34. doi:10.3109/15412555.2013.763782.
9. Stockley RA, Luisetti M, Miravitlles M, Piitulainen E, Fernandez P. Ongoing research in Europe: Alpha one international registry (air) objectives and development. Eur Respir J 2007;29(3):582–6. doi:10.1183/09031936.00053606.
10. Blanco I, de Serres FJ, Carcaba V, Lara B, Fernandez-Bustillo E. Alpha-1 antitrypsin deficiency pi*z and pi*s gene frequency distribution using on maps of the world by an inverse distance weighting (IDW) multivariate interpolation method. Hepat Mon 2012;12(10 hcc):e7434. doi:10.5812/hepatmon.7434.
11. Blanco I, de Serres FJ, Fernandez-Bustillo E, Lara B, Miravitlles M. Estimated numbers and prevalence of PI*S and PI*Z alleles of alpha1-antitrypsin deficiency in European countries. Eur Respir J 2006;27(1):77–84. doi:10.1183/09031936.06.00062305.
12. de Serres F, Blanco I. Role of alpha-1 antitrypsin in human health and disease. J Intern Med 2014;276(4):311–35. doi:10.1111/joim.12239.
13. Silva D, Oliveira MJ, Guimaraes M, Lima R, Gomes S, Seixas S. Alpha-1-antitrypsin (SERPINA1) mutation spectrum: three novel variants and haplotype characterization of rare deficiency alleles identified in Portugal. Respir Med 2016;116:8–18. doi:10.1016/j.rmed.2016.05.002.
14. Rocha J, Pinto D, Santos MT, Amorim A, Amil-Dias J, Cardoso-Rodrigues F, et al. Analysis of the allelic diversity of a (CA)n repeat polymorphism among alpha 1-antitrypsin gene products from northern Portugal. Hum Genet 1997;99(2):194–8.
15. Seixas S, Mendonca C, Costa F, Rocha J. Alpha1-antitrypsin null alleles: evidence for the recurrence of the L353fsX376 mutation and a novel G–>A transition in position +1 of intron IC affecting normal mRNA splicing. Clin Genet 2002;62(2):175–80. doi:10.1034/j.1399-0004.2002.620212.x.
16. Vaz Rodrigues L, Costa F, Marques P, Mendonca C, Rocha J, Seixas S. Severe alpha-1 antitrypsin deficiency caused by Q0(Ourem) allele: clinical features, haplotype characterization and history. Clin Genet 2012;81(5):462–9. doi:10.1111/j.1399-0004.2011.01670.x.
17. Rieger S, Riemer H, Mannhalter C. Multiplex PCR assay for the detection of genetic variants of alpha1-antitrypsin. Clin Chem 1999;45(5):688–90.
18. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7(4):248–249 doi:10.1038/nmeth0410-248
19. Esquinas C, Barrecheguren M, Rodriguez E, Sucena M, Fernandez S, Miravitlles M. Degree of knowledge among physicians about the diagnosis and treatment of alpha-1 antitrypsin deficiency. Eur Respir J 2015;46(suppl 59):PA682. doi:10.1183/13993003.congress-2015.PA682
20. Esquinas C, Barrecheguren M, Sucena M, Rodriguez E, Fernandez S, Miravitlles M. Practice and knowledge about diagnosis and treatment of alpha-1 antitrypsin deficiency in Spain and Portugal. BMC Pulmon Med 2016;16:64. doi:10.1186/s12890-016-0222-4.
21. Greulich T, Ottaviani S, Bals R, Lepper PM, Vogelmeier C, Luisetti M, et al. Alpha1-antitrypsin deficiency - diagnostic testing and disease awareness in Germany and Italy. Respir Med 2013;107(9):1400–8. doi:10.1016/j.rmed.2013.04.023.
22. Kohnlein T, Janciauskiene S, Welte T. Diagnostic delay and clinical modifiers in alpha-1 antitrypsin deficiency. Ther Adv Respir Dis 2010;4(5):279–87. doi:10.1177/1753465810376407.
23. Stoller JK, Sandhaus RA, Turino G, Dickson R, Rodgers K, Strange C. Delay in diagnosis of alpha1-antitrypsin deficiency: a continuing problem. Chest 2005;128(4):1989–94. doi:10.1378/chest.128.4.1989.
24. Taliercio RM, Chatburn RL, Stoller JK. Knowledge of alpha-1 antitrypsin deficiency among internal medicine house officers and respiratory therapists: results of a survey. Respir Care 2010;55(3):322–7.
25. Piras B, Ferrarotti I, Lara B, Martinez MT, Bustamante A, Ottaviani S, et al. Clinical phenotypes of Italian and Spanish patients with alpha1-antitrypsin deficiency. Eur Respir J 2013;42(1):54–64. doi:10.1183/09031936.00104712.
26. Bornhorst JA, Greene DN, Ashwood ER, Grenache DG. Alpha1-antitrypsin phenotypes and associated serum protein concentrations in a large clinical population. Chest 2013;143(4):1000–8. doi:10.1378/chest.12-0564.
27. Boulyjenkov, V. Alpha 1-antitrypsin deficiency: memorandum from a WHO meeting. Bull World Health Organ 1997;75(5):397–415.
28. Marciniuk DD, Hernandez P, Balter M, Bourbeau J, Chapman KR, Ford GT, et al. Alpha-1 antitrypsin deficiency targeted testing and augmentation therapy: a Canadian Thoracic Society clinical practice guideline. Can Respir J 2012;19(2):109–16.
29. Ferrarotti I, Baccheschi J, Zorzetto M, Tinelli C, Corda L, Balbi B, et al. Prevalence and phenotype of subjects carrying rare variants in the Italian registry for alpha1-antitrypsin deficiency. J Med Genet 2005;42(3):282–7. doi:10.1136/jmg.2004.023903.
30. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536(7616):285–91. doi:10.1038/nature19057.
31. Lara B, de la Roza C, Vila S, Vidal R, Miravitlles M. Development and results of the Spanish registry of patients with alpha-1-antitrypsin deficiency. Int J Chron Obstruct Pulmon Dis 2007;2(3):393–8.
32. Spinola C, Bruges-Armas J, Pereira C, Brehm A, Spinola H. Alpha-1-antitrypsin deficiency in Madeira (Portugal): the highest prevalence in the world. Respir Med 2009;103(10):1498–502. doi:10.1016/j.rmed.2009.04.012.
33. Miravitlles M, Herr C, Ferrarotti I, Jardi R, Rodriguez-Frias F, Luisetti M, et al. Laboratory testing of individuals with severe alpha1-antitrypsin deficiency in three European centres. Eur Respir J 2010;35(5):960–8. doi:10.1183/09031936.00069709.
34. Kaplan A, Cosentino L. Alpha1-antitrypsin deficiency: forgotten etiology. Can Fam Physician 2010;56(1):19–24.
35. Seersholm N, Kok-Jensen A. Clinical features and prognosis of life time non-smokers with severe alpha 1-antitrypsin deficiency. Thorax 1998;53(4):265–8. doi:10.1136/thx.53.4.265.