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Identification of a novel alpha1-antitrypsin variant

Camille de Seynes [a, *], C. Ged [b], H. de Verneuil [b], N. Chollet [d], M. Balduyck [c], C. Raherison [a]

[a] Department of Respiratory Diseases, Bordeaux University, France
[b] Department of Biochemistry and Molecular Biology, Bordeaux University, France
[c] Department of Biochemistry and Molecular Biology, Lille University, France
[d] 130 rue de Pessac, 33000 Bordeaux, France

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ABSTRACT

Alpha-1-antitrypsin deficiency (A1ATD) is a genetic condition caused by SERPINA1 mutations, which results into decreased protease inhibitor activity in the serum and predisposes to emphysema and/or to liver disease due to accumulation of the abnormal protein in the hepatic cells. In most cases the clinical manifestations of A1ATD are associated with PIZZ (p.Glu366Lys; p.Glu366Lys (p.Glu342Lys; p.Glu342-Lys)) or PISZ (p.Glu288Val; p.Glu366Lys (p.Glu264Val; p.Glu342Lys)) genotype, less frequently, deficient or null alleles may be present in compound heterozygous or homozygous A1AT deficient patients.

We report the identification of a novel alpha1-antitrypsin variant in a 64-year old woman presenting with dyspnea on exertion. Imaging revealed bilateral bronchiectasis associated with moderate panacinar emphysema. The pulmonary function tests (PFTs) were subnormal but hypoxemia was noticed and A1AT quantitative analysis revealed a severe deficiency. DNA sequencing showed compound heterozygosity for the PIZ variant and a novel missense variant p.Phe232Leu (p.Phe208Leu). No specific treatment was proposed since PFTs were within the normal range at this stage of the disease. Close follow-up of pulmonary and hepatic parameters was recommended.

1. Introduction

Alpha1-antitrypsin (A1AT) is a 52-kDa single-chain glycoprotein composed of 394 amino acid residues and 3 asparagine-linked complex carbohydrate side chains. A1AT is produced predominantly by hepatocytes, and the synthesis also occurs in mononuclear phagocytes, neutrophils, as well as airway and intestinal epithelial cells [1]. The primary function of A1AT is the regulation of serine proteases, and the main site of action is the lung where it protects the alveolar tissues from proteolytic degradation by neutrophil elastase mainly during inflammatory responses [2]. When deficient, the A1AT may accumulate within the endoplasmic reticulum (ER) of hepatocytes leading to cirrhosis.

Since 1953, following the publication of the seminal paper by Laurell and Eriksson [3], there have been substantial advances in the understanding of genetic abnormalities related to A1AT deficiency.

The A1AT deficiency (A1ATD) is an underdiagnosed genetic condition that affects approximately 1 in 2500 to 1 in 5000 individuals and predisposes to early-onset emphysema and liver disease. The transmission is autosomal recessive. The disease is characterized by low serum levels of A1AT, and caused by mutations within the polymorphic SERPINA1 gene (SERine Proteinase INhibitor) which is organized in three noncoding (1a, 1b and 1c) exons and four (2, 3, 4 and 5) coding exons and located on the long arm of chromosome 14 (14q32.1).

To date, over 100 A1AT variants have been described and most of them can be identified using isoelectric focusing electrophoresis, commonly referred as the “phenotyping” method. The serum level of A1AT is strongly determined by the A1AT genotype variant system, classically named PI (Protease Inhibitor) assigned with an alphabetical designation based on A1AT mobility in an electrophoretic field at alkaline pH. The different A1AT variants that have been identified up to now are classified into four major categories for clinical purposes: normal, deficient, null and dysfunctional. The normal A1AT genotype named PIMM, (allelic frequency 94–96% of the Caucasian population), is characterized by normal protein serum levels (0.9–2.0g/L or 20–48 μM) [4]. The most common variants known to cause A1ATD are the dysfunctional PIZ (Nomenclature of the different variants mentioned in the paper are

* Corresponding author. Department of Respiratory Diseases, Bordeaux University, 33000 Bordeaux, France.
E-mail address: camdeseynes@gmail.com (C. de Seynes).

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summarized in Table 1) and PINull are characterized by a complete absence of A1AT in the plasma. Rare A1AT deficient variants account for 1,44% of A1ATD individuals [5]. A large body of evidence suggests that chronic obstructive pulmonary disease (COPD) risk is inversely related to blood A1AT concentration with the following hierarchy: PINullNull > PIZZ > PISZ > PIMZ [6]. A few A1AT variants, including the most frequent PIZ and rare variants are prone to misfolding and/or formation of toxic polymers which accumulate in the endoplasmic reticulum of hepatocytes. Such deleterious gain of function variants may be associated with reduced circulating A1AT levels and predisposition to neonatal jaundice, cirrhosis, and increased risk of hepatocellular carcinoma [7]. Polymers of A1AT form within the lung as a result of local inflammation and exposure to cigarette smoke. The pathogenesis of emphysema associated with A1AT deficiency is then a combination of loss of antiprotease function combined with pro-inflammatory properties of intrapulmonary polymers [8].

Significant advances in molecular diagnosis on genomic DNA, extracted mainly from circulating mononuclear blood cells ("genotyping"), allowed to elaborate a diagnosis strategy. Known mutations may be detected by amplification or direct sequencing. In a truly A1AT deficient patient, the lack of identification of at least one mutant allele presupposes the presence of a new variant. In this case, a gene scan is performed. The pertinence of this approach is that genotyping by primer-specific PCR is most often rapid, unequivocal and provides straightforward identification of the most common alleles associated with A1ATD. The knowledge of A1AT concentration helps in identifying samples with potentially rare deficient alleles not recognized by a genotyping method restricted to S and Z alleles (primer-specific PCR). In this situation, i.e. discordance between A1AT concentration and routine genotype determination, the diagnosis strategy may be implemented with the use of isoelectric focusing or direct antielastolytic activity measurement [9] as a reference test to document the presence of an atypical A1AT variant [10].

This strategy did allow the identification of a new SERPINA1 variant as described in this report.

2. Case report

A 64-year-old woman presented to her pneumologist with an initial complaint of dyspnea on exertion that had developed ten years prior and had progressed to shortness of breath while doing her daily chores, especially when she was bending over. She also reported a chronic cough with one or two exacerbations per winter for few years. She had never smoked. She experienced significant inorganic dust exposure while working in a chocolate factory. She owns a cat, a dog, 3 budgerigars and 3 doves. She had no familial history of pulmonary or liver disease.

Her medical history was notable only for hypothyroidism and a pericarditis in May 2015. Her physical examination revealed pulmonary auscultation that was remarkable for basal crackles. The remainder of her physical examination was otherwise unremarkable. She only reported swollen legs in the summer. Her body mass index was 27 (60kg, 147cm). She had no on-going treatment except levothyroxine.

The cardiac examination was within normal limits.

Pulmonary function testing at presentation demonstrated distal airflow obstruction: forced expiratory volume in 1 s (FEV_1) was 1.65 L (100% of the predicted value), forced vital capacity (FVC) was 2.25 L (112% of the predicted value), the FEV_1:FVC ratio was 70.9%, forced expiratory flow over 25%–75% of the expired volume (FEF_{25-75}) was 1.44 L per second (56% of the predicted value), total lung capacity (TLC) measured by plethysmography was 4.54 L (116% of the predicted value), and residual lung volume (RV) was 1.83 L (108% of the predicted value). There was no response to an inhaled bronchodilator. The diffusing capacity of the lung for carbon monoxide (D_LCO) (hemoglobin-adjusted) was 12.6 ml/min/mmHg (69% of the predicted value). Arterial blood gas analysis revealed isolated hypoxemia (PaO2 60 mmHg). The 6-Minute Walk Test was optimal (330m - 99% of the theory), with a saturation at 93% at the beginning remaining stable all along the exercise.

A computed tomography scan of the chest demonstrated bilateral bronchiectasis associated with moderate panacinar emphysema.

A1AT quantitative analysis revealed a severe deficiency (A1AT = 0.3g/L, normal range 0.9–2g/L). DNA sequencing showed heterozygosity for the Z variant and a novel missense variant p.Phe232Leu (p.Phe208Leu) (c.696 C > G, reference sequence NM_001002235) (Fig. 1). This variant had not been described in the literature and has not been reported in the international database HGMD (www.biobase-international.com/product/hgmd). There are no frequency data in the ESP (Exome Sequencing Project), EXAC (Exome Aggregation Consortium) and dbSNP databases (www.ncbi.nlm.nih.gov/SNP). The prediction softwares SIFT, Polyphen and MutationTaster, implemented through Alamut (Interactive Biosoftwares) predicted this variant as *deleterious*, *probably damaging* and *disease causing,* respectively. The patient was considered as a compound heterozygous, given the very low residual AAT concentration in plasma. No family studies were undertaken, since the parents had died while the proband was unique and had no child.

Hepatic and autoimmune biological tests were within normal limits.

In order to evidence pulmonary arterial hypertension given the elevated pressure on cardiac ultrasound (PASP 34+3 mmHg), the patient underwent right heart catheterization, which indicated an mPAP (mean pulmonary arterial pressure) of 15 mmHg with a PCWP (pulmonary capillary wedge pressure) of 5 mmHg; cardiac output was 4.39 L/min, cardiac index was 3 L/min/m^2 and PVR was 2.27 Wood units, which are considered within normal range.

3. Discussion

In single-gene disorders, like A1ATD, a gene mutation causes missing or dysfunctional protein synthesis and can lead to serious complications for the patient affected who may necessitate expensive lifelong care. Until now, therapeutic options have remained limited, cost-intensive (i.e. the supplementation therapy using plasma fractionation) or lacked effectiveness. Common therapies in COPD are prescribed, including pulmonary rehabilitation, vaccination and the use of inhaled pharmacotherapies (corticosteroids, β-agonists, anti-cholinergics). The treatment of liver disease associated with A1AT deficiency has been a model for the application of novel biomedical technologies, as reviewed by Lomas et al. [11].

Table 1
Nomenclature of SERPINA1 variants mentioned in the paper.

cDNA location NM_001002235	Protein location from the initiating met	Location on the secreted protein	Common name
c.1096 G > A 5th coding exon	p.Glu366Lys	Glu342Lys	PIZ
c.863 A > T 3rd coding exon	p.Glu288Val	Glu264Val	PIS
c.696 C > G 3rd coding exon	p.Phe232Leu	Phe208Leu	Novel variant

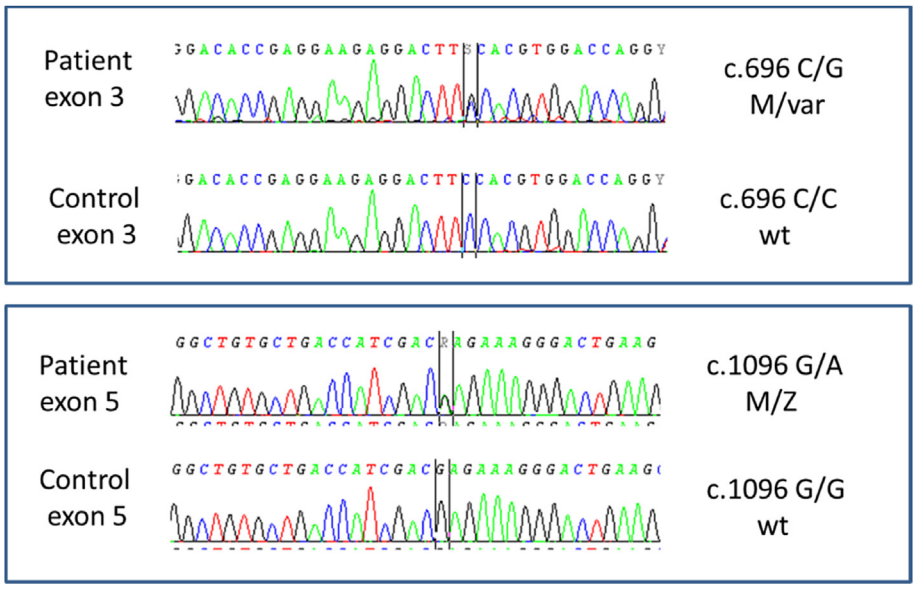

Fig. 1. Sanger sequencing profile of the known Z variant and the novel SERPINA1 variant.

Table 2
Brief clinical data, A1AT blood concentrations, and SERPINA1 genotype in families harbouring the novel SERPINA1 variant.

Patient identification	Family member	Clinical data	A1AT concentration	Genotype
This case 65 y (F)	unique	emphysema	0.3 g/L	Z/novel variant
SM.Th. 48y (M)	Father	unknown	0.46 g/L	S/novel variant
SM.V. 36 y (F)	Mother	asymptomatic	1.2 g/L	M/M
SM.E 8 y (M)	Twin boy	Asthma Gastroesophageal reflux	0.73 g/L	M/novel variant
SM. M 8 y (M)	Twin boy	asymptomatic	1.19 g/L	M/S

Different groups have assessed a range of chemical chaperones that stabilise intermediates on the PIZ folding pathway, with little success in clinical trial. Among the novel pharmacological options tested so far, the use of drugs targeting the regulation of autophagy (carbamazepine) [12] or proteasome function (bortezomib) [13] have been proposed. An attractive strategy, in this well characterized monogenic disease, is represented by gene therapy, particularly for patients with pulmonary dysfunction, where augmentation of functional A1AT levels in plasma might slow down respiratory disease development. *In vitro* and *in vivo* experimental A1AT gene transfer with viral vectors has resulted in enhanced A1AT serum levels and a promising safety profile [14]. Human clinical trials using intramuscular viral transfer with AAV1 and AAV2 vectors (Adeno-Associated Viral vectors) carrying the therapeutic A1AT gene, demonstrated its safety but did not achieve a protective level of A1AT in serum (A1AT>11 μM). The development of new approaches using patient-specific induced pluripotent stem cells reveals a potentially promising tool towards the setting of cell-based therapy against A1ATD [15].

The novel SERPINA1 variant, described in the patient, associated with a deficient PIZ variant (although heteroallelism could not be tested) is possibly responsible for the pulmonary disease described in this report, since A1AT circulating levels were lower than expected from an heterozygous status for the PIZ variant. Of note, we had previously identified this variant in an unrelated family, where it was associated with the defective S allele. The compound heterozygous patient was asymptomatic at the time of the genetic analysis (48-year-old); the genotype was a fortuitous finding in the father of twin-boys presenting with asthma. The respective A1AT blood concentration and genotype determinations including the novel A1AT variant are presented in Table 2. The direct measurement of plasma anti-elastase activity as well as the molecular characterisation of the corresponding mutant recombinant A1AT protein would be a nice documentation of the function of this novel variant.

Further support to the hypothesis of a novel dysfunctional variant relies on structural studies performed in cellular models expressing recombinant A1AT mutant proteins [16]. Structural modelling highlighted the critical role of 'gate' region with a network of hydrophobic interactions involved in the mobilisation of the reactive site loop and alternative conformations leading to polymerisation. The novel substitution p.Phe232Leu may loosen 'latch' interactions which constraint the A1AT reactive loop and facilitate core packing thus resulting in deleterious polymerisation.

4. Conclusion

This report which describes a novel missense variant, p.Phe232Leu, combined with the common Z defective variant of SERPINA1 gene, discovered in a symptomatic patient with emphysema, outlines the need for accurate gene analysis in such a common pathology. Further investigations like functional assays of the purified native mutant A1AT could be done to assess the probable dysfunctional capacity of this variant.

Extensive respiratory examinations were performed to evaluate the condition of the patient and therapeutic options. No augmentation therapy was proposed considering the subnormal results of the pulmonary functional tests but a close follow-up of pulmonary and hepatic parameters and morphology was recommended given the Z/Phe232Leu association which is at risk of liver disease.

The actual therapeutic perspectives in A1ATD are briefly mentioned with a special mention for innovative strategies which

may become applicable in a short future.

Written informed consent was obtained from the patient for publication of this case report.

Conflicts of interest

Participation in the European Respiratory Society (ERS) congress: registration, fees and stay costs supported by LFB Biomédicaments.

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