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Case report

Clinical characterization of a novel alpha1-antitrypsin null variant: $PiQ0_{Heidelberg}$

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ABSTRACT

The clinical characterization of a null variant of *SERPINA1* – $PiQ0_{Heidelberg}$ – resulting in alpha1-antitrypsin (AAT) deficiency is described. This rare mutation (c.-5+5 G > A) has been previously identified but not clinically described. The 77 year-old female patient had GOLD-3, Group B COPD, severe destructive panlobular emphysema and newly observed respiratory failure on exertion at the time the genetic analysis was performed. Serum AAT level was 0.1 g/L (reference 0.9–2.0 g/L). Isoelectric focusing showed only the Z-protein indicating that this was a null mutation. The patient has started AAT replacement. Early screening and identification of AAT deficiency would allow for earlier intervention.

Abbreviations

AAT	alpha1 antitrypsin
AATD	alpha1 antitrypsin deficiency
CAT	chronic obstructive pulmonary disease assessment test
COPD	chronic obstructive pulmonary disease
DLCO	diffusion capacity of carbon monoxide
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
GOLD	Global Initiative for Chronic Obstructive Lung Disease
IEF	isoelectric focusing
mMRC	Medical Research Council Breathlessness Scale
RV	residual volume
VA	alveolar volume

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VCmax maximum vital capacity

1. Introduction

Alpha1-antitrypsin (AAT) is a primary serum anti-protease that is predominantly synthesized in the liver and is transported to the lungs via the circulatory system. In the lungs, AAT provides most of the counterbalance to the activity of elastases, especially neutrophil elastase (NE). AAT deficiency (AATD) disrupts the balance and allows less-regulated elastase activity which can lead to destruction of lung parenchyma and deterioration of gas exchange over time [1].

AATD is a rare hereditary condition. AAT is encoded for by the *SERPINA1* gene and the majority of the population have the M-allele of this gene which is associated with normal serum levels of AAT. The most common pathogenic alleles associated with reduced serum AAT are the S- (40% reduction in serum AAT) and Z-alleles (90% reduction in serum AAT) [2]. Additionally, more than 120 mutations of the *SERPINA1* gene have been identified [3] of which approximately 40% are associated with some degree of AATD [4]. These mutations may account for up to 17% of clinical cases of AATD [5,6].

Despite the identification of this large number of variants and the potential clinical significance of these variants, AATD remains an underdiagnosed disease. In this case report, the clinical characterization of a rare mutation (c.-5+5 G > A) identified in Heidelberg ($PiQ0_{Heidelberg}$) is described. A previous screening study identified this mutation but did not describe its clinical character [7].

2. Clinical presentation

Written informed consent was obtained from the patient described below for their anonymized information to be published in this article. Ethics committee approval was not sought for this study because The University of Heidelberg does not require ethical approval for reporting individual cases. This study was completed in accordance with the Helsinki Declaration as revised in 2013.

The patient in this case study is a 77-year-old female with no family history of lung pathology and no history of occupational exposure to hazardous substances. She has a history of regular cigarette smoking for two years at the age of 23 and infrequent smoking since the age of 55 - one cigarette per month. In addition, the patient reported environmental exposure to an open fireplace for more than 15 years. The patient's primary diagnosis was chronic obstructive pulmonary disease (COPD). Secondary diagnoses included hypertension, hypercholesterolemia, coronary artery disease, first degree tricuspid regurgitation, 50% stenosis of the right carotid artery, and type 1 diabetes mellitus (since 1988).

On her first presentation (October 2020) at the Thoracic Clinic at the University of Heidelberg, the patient reported a history of exertional dyspnea for six years that had intensified in the last six months. The patient had undergone pulmonary rehabilitation in 2017 and had been treated with corticosteroids on an outpatient basis for an exacerbation of COPD in May of 2020. The patient reported regular participation in a pulmonary exercise group.

The patient had no dyspnea at rest but had exertional dyspnea after climbing two flights of stairs. The patient reported occasional coughing with minimal whitish sputum production and no hemoptysis. Current COPD medications are beclomethasone dipropionate 100 μg/formoterol fumarate dihydrate 6 μg twice a day (Foster 100/6 metered dose inhaler, Chiesi Farmaceutici SpA, Parma, Italy), tiotropium bromide 18 μg once daily (Spiriva Handihaler, Boehringer Ingelheim, Ingelheim am Rhein, Germany), and fenoterol hydrobromide 50 μg/ipratropium bromide 20 μg (Berodual N, Boehringer Ingelheim) as needed.

The patient was admitted to the hospital in November 2020 for evaluation regarding endoscopic lung volume reduction. At admission, the patient was classified as Global Initiative for Chronic Obstructive Lung Disease grade 3 (GOLD-3), Group B indicating a

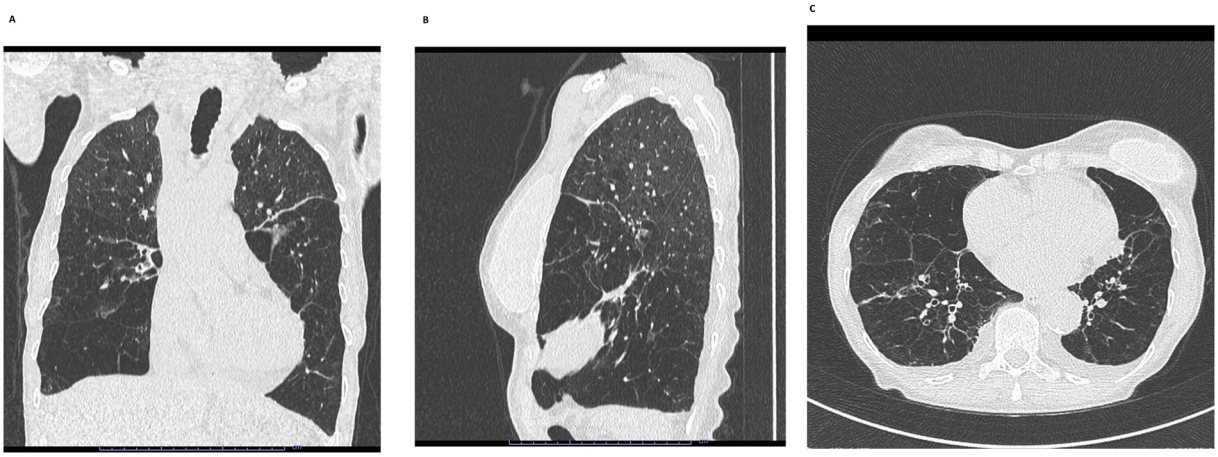

Fig. 1. Thin-sliced CT-scan of the lungs in coronal (A), sagittal (B) and axial (C) planes. The predominant finding was severe destructive panlobular emphysema of the inferior lobes.

forced expiratory volume (FEV_1)/forced vital capacity (FVC) ratio of less than 0.70 and FEV_1 of 30–49% of normal with symptoms but a low risk of exacerbation [8]. This patient had an FEV_1 of 46% of the predicted value. The patient was also rated a Grade 3 on the modified Medical Research Council Breathlessness Scale (mMRC: range 0–4) [9] and 29 on the COPD Assessment Test (CAT: range 0–40) [10].

At this visit, the patient had severe destructive panlobular emphysema (Fig. 1) and newly observed respiratory failure on exertion and de-oxygenation on exertion. Body plethysmography showed substantial deficits in maximum vital capacity (VC_{max}: 1.6 L; 63% of predicted) FEV_1 (0.9 L; 42% of predicted), and Tiffeneau index (FEV_1/VC_{max}: 55% of predicted). Residual volume (RV: 3.2 L; 144% of predicted) was elevated. Diffusion capacity of carbon monoxide (DLCO) was found to be 40% of predicted and DLCO/alveolar volume (VA) was 67% of predicted indicated compromised pulmonary gas exchange. Routine laboratory tests were unremarkable except for elevated hemoglobin: 16.0 g/dL (lab standard range 12–15 g/dL). AATD genetic testing was initiated at this visit.

The AATD genetic analysis was performed at the German AAT Laboratory at the University of Marburg. We have described the laboratory methods in detail elsewhere [11–13]. AAT levels were measured by nephelometry. The patient's dried blood spot samples were tested using the Progenika A1AT genotyping kit (Progenika Biopharma, S,A, Derio, Spain) on the Luminex 200 (Luminex, Austin, TX, USA). This multiplex PCR (polymerase chain reaction) and hybridization test allows the simultaneous identification of 14 alleles [14]. The results from this patient showed Pi*MZ genotype.

Genotyping was followed by isoelectric focusing (IEF). IEF is used for samples that indicate other mutations may be present. On the IEF gel, only the Z-protein could be identified, suggesting a null mutation. Routinely, samples with inconsistent results from the PCR and IEF tests or with indications of a rare mutation, are sent for whole gene sequencing. For sequencing, we shipped the DBS samples to a reference laboratory (Progenika Biopharma, Spain, (Next Generation Sequencing)).

For this patient, serum AAT level was 0.1 g/L (normal values 0.9–2.0 g/L). IEF indicated that only the Z-protein was visible (data not shown). The other allele produced no protein that could be detected in plasma. Genotyping showed that the patient was P*iZ/(c. −5+5 G > A). This mutation (c.-5+5 G > A) was previously described by Graham et al. [7].

After the AATD diagnosis, the patient was seen again in February 2021. At that visit the patient was observed to have no dyspnea at rest, but dyspnea on exertion had progressed from the previous visit. Exertional dyspnea was seen after climbing one flight of stairs and was now limiting the patient's physical activities. The patient reported no cough, no sputum production and no hemoptysis at this visit. Since November 2020, the patient has been on long-term oxygen therapy on exertion at level 3. Blood gas analysis showed $PaO_2 = 68$ mmHg (normal range 80–100 mmHg), oxygen saturation = 94% (normal range 95–100%), pH = 7.43 (normal rang 7.35–7.45) and $PaCO_2 = 41$ mmHg (normal range 35–45 mmHg).

Body plethysmography at this visit showed $VC_{max} = 1.5$ L (58% of predicted); FEV1 = 0.9 L (42% of predicted), Tiffeneau index 58% of predicted, RV = 3.6 L (161% of predicted) and TLC = 4.8 L (94% of predicted. Diffusion capacity was lower than the previous visit. DLCO was 33% of the predicted value and DLCO/VA was 63% of the predicted value.

Since AATD can affect the liver as well as the lungs, the patient had a liver ultrasound with FibroScan (Echosens, Waltham, MA, USA) to assess the presence of fibrosis (6 July 2021). Liver stiffness was measured at 7.5 kPa which translates into no or mild fibrosis. In addition, transaminase levels were within normal limits.

Augmentation therapy with AAT (Prolastin, Grifols Deutschland GmbH, Frankfurt, Germany) was requested and approved for this patient. She has received this therapy for four weeks starting in April 2021. AAT augmentation therapy has been well-tolerated by the patient and initiation of home infusion therapy is planned.

3. Discussion

In this article, we report the case of a COPD patient with AATD who was found by genetic analysis to have the Z allele in combination with the variant c. −5+5 G > A. This mutation has been previously described but it has not been clinically characterized [7]. In the original report, Graham et al. speculated that this mutation would be deleterious. They proposed that the five-base-pair shift in the splice region would lead to truncation of the AAT protein. Another variant at this splice site have been associated with a null phenotype [15].

In this case report, this variant was identified as a null variant and has been designated $Q0_{Heidelberg}$. In this patient, this variant was identified along with a Z-allele by IEF and genetic analysis. This patient was found to have very low serum AAT levels and significant COPD despite not having a history of heavy smoking. This patient was identified at an advanced age (77 years) and has been started on AAT augmentation therapy.

AATD remains an underdiagnosed disease due to the lack of widespread testing. Expanded testing especially in patients with onset of COPD at a young age or without a history of heavy smoking could potentially identify AATD patients early in the course of their disease. Early detection of AATD could help to avoid or delay some of the long-term sequalae. Early intervention and treatment, including smoking cessation assistance and, if appropriate, AAT augmentation therapy, may improve long-term outcomes. Many mutant variants of SERPINA1 have been identified but some have not been clinically characterized. When SERPINA1 variants are possible or suspected, assessment of the patient's medical history, lab values and genetic testing may help shed light on the underlying etiology and provide guidance for treatment.

4. Conclusions

- This patient was found to have a previously identified null mutation of the *SERPINA1* gene: $PiQ0_{Heidelberg.}$ This mutation has not been clinically characterized.

- The 77-year-old patient with the $PiQ0_{Heidelberg}$ variant showed severe COPD without a history of heavy smoking. Serum AAT level was 0.1 g/L (normal values 0.9–2.0 g/L). The patient is undergoing AAT augmentation therapy.
- AATD is underdiagnosed. Wider testing could help delay some clinical sequalae by allowing earlier therapeutic and lifestyle interventions.

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Declaration of competing interest

Maria A. Presotto, Martina Veith, Kai Schlamp, Markus Polke and Felix F.J. Herth have no conflicts of interest. Franziska Trudzinski reported personal fees from GlaxoSmithKline, Novartis and CSL Behring, outside the submitted work. Ralf Eberhardt received personal fees from Pfizer, Pulmonx, Olympus and Broncus outside the submitted work. Frederik Trinkmann received travel support from Actelion, Berlin Chemie, Boehringer Ingelheim, Chiesi, Novartis, Mundipharma, and TEVA, as well as speaker or consultation fees from AstraZeneca, Berlin Chemie, Boehringer Ingelheim, Bristol-Myers Squibb, Chiesi, GlaxoSmithKline, Novartis, Roche, and Sanofi-Aventis, all outside the submitted work.

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