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Case report

Clinical presentations of four patients with rare Alpha 1 Antitrypsin variants identified in a single US center

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ABSTRACT

Alpha 1 Antitrypsin Deficiency (AATD) is a rare condition primarily associated with lung complications and liver disease. As disease symptoms are similar to those in other respiratory conditions, patients generally experience long delays before receiving an accurate diagnosis and treatment. AATD results from mutations in the *SERPINA1* gene that encodes Alpha 1 Antitrypsin (AAT). Over 500 single-nucleotide variants have been reported in mutation databases; however, there is increasing interest in the clinical significance of rare and novel *SERPINA1* variants. In this case series of four patients from a single US center, next-generation sequencing (NGS) was used to guide AATD diagnosis. Four distinct rare variants of *SERPINA1* (P289S; I50N; E204K; H262Y) were identified, three of which were found in patients with advanced chronic obstructive pulmonary disease (COPD)/emphysema. Computational modeling predicted these mutations to have potentially deleterious effects, a finding supported by AAT levels that were comparable with those seen in individuals heterozygous for the most common deficiency allele (PI*MZ). The remaining mutation (E204K) was found in a patient with a cerebral aneurysm; potential links between *SERPINA1* variants and neurological conditions, such as cerebral aneurysm and arterial dissections, have been previously reported in individuals with heterozygous AATD phenotypes (PI*MS and PI*MZ). Novel and rare variants, often not detected by basic AATD diagnostic tests, have the potential to contribute to the development of COPD and emphysema. Detection of these variants can be enhanced by NGS, and modeling techniques can help determine if variants are pathogenic, thereby enabling a quicker, more accurate AATD diagnosis.

1. Introduction

Alpha 1 Antitrypsin (AAT), a serine protease inhibitor encoded by the *SERPINA1 gene*, is predominantly produced by hepatocytes and usually present in human blood at levels of 150–350 mg/dL [1]. The primary function of AAT is to regulate serine proteases and protect the lungs from elastolytic damage by inhibiting the proteolytic enzyme neutrophil elastase [2]. Sequence variants of the *SERPINA1* gene can result in low serum levels of AAT or subtle structural changes to the protein that reduce its functionality [3]. This deficit in functional AAT results in Alpha 1 Antitrypsin Deficiency (AATD), a condition associated with lung tissue destruction, emphysema and liver disease [4].

AATD is inherited as an autosomal codominant condition, which can be characterized by several homozygous and heterozygous variant combinations [5]. The functional wild-type allele is known as PI*M and the most common deficiency alleles are PI*Z and PI*S. AAT is a highly diverse polymorphic protein and clinical manifestations of AATD are associated with various allele combinations, such as PI*ZZ, PI*SZ, PI*SS and PI*MS, which are often characterized by low levels of AAT [6]. Patients homozygous for the PI*Z allele typically have AAT levels below 45 mg/dL. These patients frequently develop severe emphysema, specifically when they smoke cigarettes [1]. The PI*Z allele, a Glu342Lys substitution, leads to aggregation of misfolded AAT protein that accumulates in hepatocytes, which predisposes individuals to liver as well as lung disease [7,8]. To date, over 500 single-nucleotide variants (SNVs) have been reported and over 70 sequence variants have been identified as clinically significant [9].

AATD diagnosis can be challenging; under-recognition and

Abbreviations: AAT, Alpha 1 Antitrypsin; AATD, Alpha 1 Antitrypsin Deficiency; CIDP, chronic inflammatory demyelinating polyneuropathy; COPD, chronic obstructive pulmonary disease; CT, computed tomography; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; IEF, isoelectric focusing; NGS, next-generation sequencing; PCR, polymerase chain reaction.

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misdiagnosis are very common and patients often experience long delays between the onset of symptoms and initial diagnosis. Average delays of 6 years have been reported, due in part to a lack of awareness of AATD and the similarity of symptoms to other respiratory diseases [10]. AAT augmentation therapy is the only available treatment that addresses disease etiology in patients with AATD and findings from the RAPID clinical trial program support its efficacy in slowing disease progression, providing evidence of a disease modifying effect [11,12]. Early intervention is important and raising awareness of AATD among physicians can lead to patients with signs/symptoms of AATD being identified earlier, and thus leading to earlier diagnosis and intervention. However, more extensive genetic testing, particularly next-generation sequencing (NGS), and raising awareness of AATD among physicians may be required to further identify rare/novel genotypes and determine the best course of treatment for patients [9,10,13]. The increasing improvements and availability of DNA testing techniques is helping to improve diagnosis of AATD, which is crucial to allow active intervention with measures such as smoking cessation and perhaps AAT therapy to prevent further destruction of the lung tissue.

Here we report a case series of four patients from Temple University Hospital in whom NGS was used to identify novel variants of *SERPINA1*.

2. Case series

This study, investigating rare/novel *SERPINA1* variants, was approved by the Institutional Review Board of Temple University (Philadelphia, PA) and all patients provided prior consent for use of laboratory data for research purposes [9]. Patients were referred from the Lewis Katz School of Medicine, Temple University, Philadelphia, Pennsylvania to the DNA_1 Advanced Alpha-1 Screening Program™ (Biocerna, Fulton, MD) for detailed AATD testing if patients had clinical conditions associated with AATD and/or low AAT levels. NGS was utilized to detect rare/novel variants if discordance existed between the patient's AAT level and the targeted genotyping results; detailed cases were compiled only for patients with novel/rare variants. Quantitative analysis of antigenic serum AAT levels was performed by radial immunodiffusion (normal range: 150–400 mg/dL) at Temple University and all genetic and isoelectric focusing (IEF) analyses were performed centrally at Biocerna [9]. Qualitative assessment of AATD genotype was carried out by real-time polymerase chain reaction (PCR) targeted genotyping (TaqMan®: Thermo Fisher Scientific, Waltham, MA) and phenotype was determined by IEF (Hydragel 18 A1AT IEF kit, Sebia USA, Norcross, GA). Comprehensive screening using NGS was performed to identify mutations in the *SERPINA1* gene. Detailed methodology has been previously published where novel variants described here were subject to a previous computational analysis [9]. In brief, predictive software was used to evaluate the pathogenicity of all missense SNVs; methods included a support vector machine (SVM) program, PolyPhen-2 (Harvard University, Cambridge, MA), and FoldX (Center for Genomic Regulation, Barcelona, Spain). In total, four cases referred from Temple Lung Center were found to have rare variants of *SERPINA1* and are the basis for this case series.

2.1. Case 1: P289S

A 60-year-old white man who presented with a history of increasing shortness of breath, cough and sputum production, which began 6–8 years ago. He had smoked since his teenage years but stopped 6 years ago at age 56 years, totaling a cigarette load of approximately 50 pack-years. A pulmonary function test revealed a moderately severe degree of airway obstruction and a forced expiratory volume in 1 s (FEV_1) of 1.37 L (31% of predicted normal) and a FEV_1/forced vital capacity (FVC) ratio of 0.31, demonstrating advanced airways obstruction consistent with chronic obstructive pulmonary disease (COPD). Following this, the patient started to receive augmentation therapy at age 62 with Zemaira® 60 mg/kg each week. Severe emphysematous changes throughout both lungs, with a predominance in the lung bases, were seen on a computed tomography (CT) scan of the patient's chest. AAT levels of 80 mg/dL were recorded, which is below the normal level [1]. IEF identified a normal M pattern, suggesting that this patient had a low-expressing normal phenotype or was a heterozygous carrier of a non-expressing allele. NGS showed a substitution of nucleotide 9007 (cDNA [14]) from C > T of the *SERPINA1* gene, resulting in substitution of proline [P] for serine [S] at amino acid position 289 in exon 4 (P289S, accession number in National Institute of Health Database of Single Nucleotide Polymorphisms [dbSNP]: rs779938258; Fig. 1A). The patient's genotype was therefore PI*M/P289S. Structural analysis showed that proline 289 is located in a hydrophobic region of the *SERPINA1* gene at a key hinge point between two beta strands of the AAT protein, and suggested that a change to serine may lead to structural instability. Since IEF only identified the M allele, the protein produced by the C987T (cDNA [14]) variant is either, unstable and quickly eliminated from the peripheral circulation, or the mutant protein has the same IEF pattern as the normal M protein.

2.2. Case 2: I50N

A 62-year-old white man who was referred for evaluation and therapy at Temple University Hospital. He had a 6-year history of bronchitis and asthma-like symptoms, with increasing shortness of breath over the last 2 years. Before quitting smoking at the age of 47 years, the patient had been a mild smoker, smoking 3–4 cigars per week over 5 years. Pulmonary function results showed a FEV_1 of 1.58 L (54.9% of predicted normal) and a FEV_1/FVC ratio of 0.72, which was consistent with a mild restrictive/obstructive pattern. AAT levels of 78.0 and 83.2 mg/dL were recorded and a computed tomography (CT) scan of his lungs showed tubular bronchiectasis and diffuse bronchial wall thickening. Several chest x-rays showed bibasilar atelectasis. The IEF pattern did not provide a clear classification and was tentatively designated PI*MX. NGS identified the presence of the M3 allele; the second allele contains a nucleotide change at position 5585 (cDNA [14]) from T > A, leading to the substitution of isoleucine [I] to asparagine [N] at amino acid 50 in exon 2 (I50N, rs1275309068; Fig. 1B). The patient's genotype was thus PI*M3/I50N. Structural analysis showed that the change in the hydrophobic core from isoleucine to asparagine, which contains a polar uncharged side chain, is likely to result in instability of the protein and a possible decrease in half-life in the peripheral circulation [9]. The patient was diagnosed with chronic inflammatory demyelinating polyneuropathy (CIDP) at the age of 54. Initially, the patient was treated with prednisone (20 mg/day) but as his condition deteriorated, intravenous infusions of immunoglobulin 100 mg every other week were introduced at age 64. The patient also received mycophenolate mofetil (CellCept®) 750 mg/day and a 6-month regimen of cyclophosphamide (Cytoxan®), but Cytoxan® was discontinued as there was no observed benefit for this condition. Following the diagnosis of AATD, the patient started receiving intravenous infusions of AAT (Zemaira®) at age 62. Initially, this was started at 90 mg/kg per week and then switched to 180 mg/kg every other week.

2.3. Case 3: E204K

A 62-year-old white woman was referred to the Neurosurgical Department of Temple University Hospital after presenting at another hospital with acute mental status change; she had no previous medical history. A cerebral angiogram revealed a 3.5 mm aneurysm of the anterior communicating artery with a wide base and extensive subarachnoidal hemorrhage. The patient's aneurysm was successfully coiled, and the hemorrhage was stopped. Follow up appointments over 9 years showed that the coil was still in place and the cerebral vasculature was normal with no medical problems. Although a pulmonary function test was not performed, there were no reported respiratory problems. The patient had never smoked. Several chest x-rays taken during the

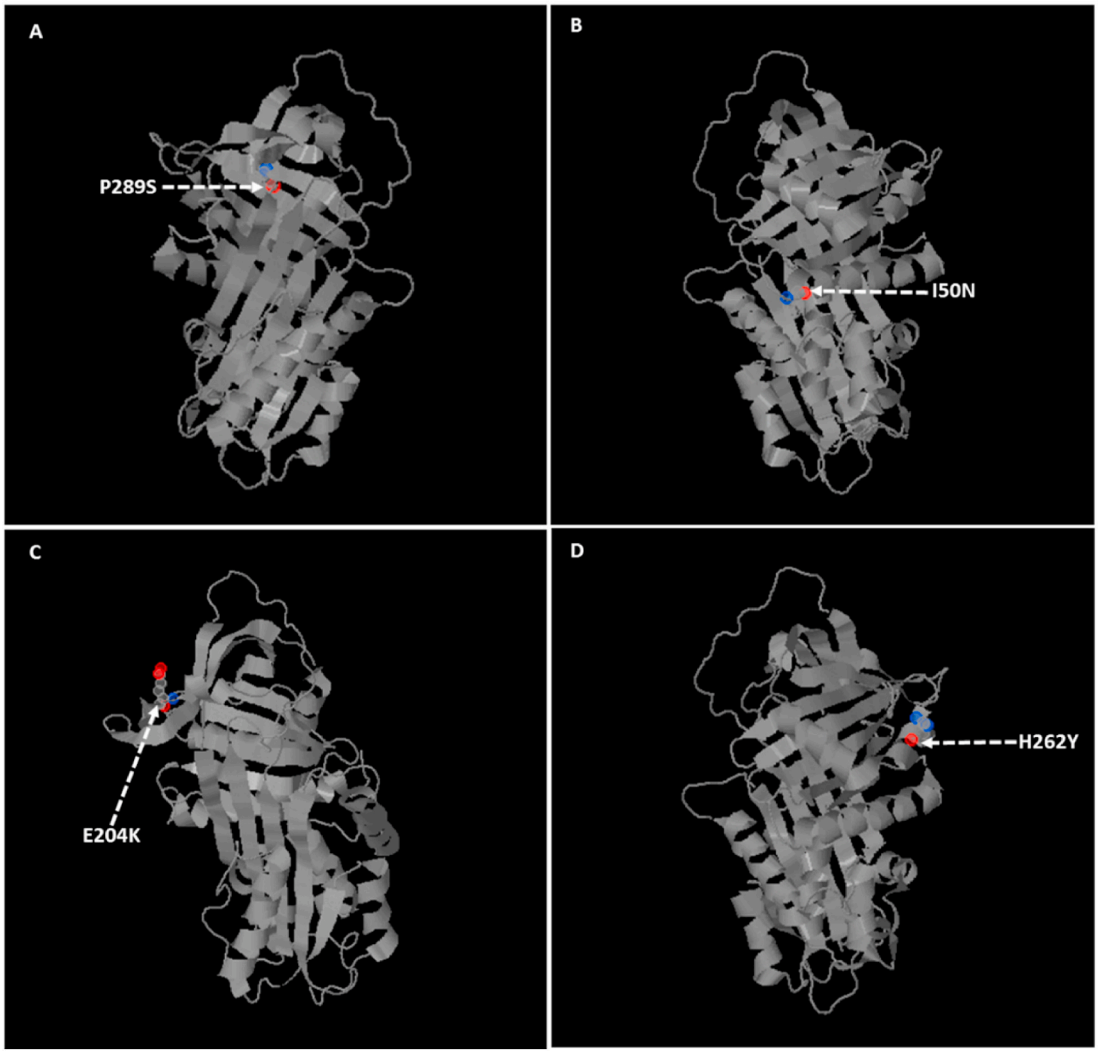

Fig. 1. PolyPhen-2 visualizations of mutation locations in the AAT protein: P289S (A); I50N (B); E204K (C); H262Y (D).
AAT, Alpha-1 antitrypsin.

patient's follow up appointments were normal and showed no emphysematous changes. In one x-ray, retro cardiac linear densities were recorded, which were thought to be plate-like atelectasis resulting from intubation during the coil procedure. AAT levels were 112 mg/dL and IEF showed an M2/M4 and M3 allele pattern (Fig. 2, lane 5). NGS revealed a base change of nucleotide 7496 (cDNA [14]) from G > A in the *SERPINA1* gene, leading to an amino acid change of glutamic acid [E] to lysine [K] at position 204 in exon 3 (E204K, rs199422208; Fig. 1C). The patient's genotype was therefore determined to be PI*M3/E204K/R101H (M2/M4). As position 204 is an exposed residue, protein disruption is not expected; however, it was considered possible that the substitution would impact the ability of the AAT protein to interact with serine proteases (i.e., elastase).

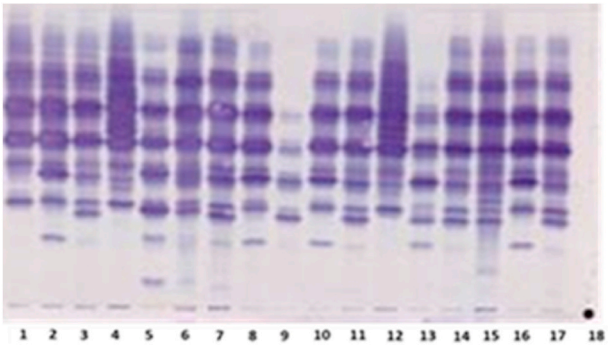

Fig. 2. IEF (Hydragel 18 A1AT IEF kit, Sebia USA, Norcross, GA) gel image; lane 5 – E204K
IEF, isoelectric focusing.

2.4. Case 4: H262Y

A 59-year-old African-American man was admitted to Temple Hospital with several serious cardiac, pulmonary and renal medical problems, including congestive heart failure, pulmonary hypertension, immunoglobulin A kappa multiple myeloma, chronic renal failure, a history of pulmonary embolus (previously treated), gastric ulcer and B12 deficiency. Due to his multiple organ failure a pulmonary function test could not be performed; however, a CT scan of the chest showed extensive emphysematous and fibrotic changes, in addition to, cystic bronchiectasis. The patient had a history of smoking cigarettes since his teenage years, accumulating a total of 40 pack-years. AAT levels were 74.8 mg/dL. NGS revealed the nucleotide change at position 7670 (cDNA [14]) from C > T. This results in the substitution of a highly preserved histidine [H] to tyrosine [Y] at position 262 in exon 3 of AAT (H262Y, rs149537225; Fig. 1D). The patient's genotype was therefore PI*M/H262Y. Structural analysis showed that the positively charged

side chain of histidine is connected to three hydrogen bonds with three neighboring amino acids and due to the large size of tyrosine, a disruption to the protein structure is predicted.

3. Discussion

Identification of four distinct rare variants in patients presenting at a single center emphasizes the heterogeneity of the *SERPINA1* gene, and suggests that novel mutations may be more clinically significant than previously thought. Of the four mutations recorded, three were found in patients with advanced COPD/emphysema/bronchiectasis (P289S, I50N and H262Y), two of which (P289S, I50N) were shown by computational modeling to have potentially deleterious effects [9]. This is also supported by AAT levels, which were analogous to those observed in PI*MZ individuals [6,15]; individuals with this genotype and a history of smoking have a higher risk of developing COPD than equivalent individuals with a normal genotype (PI*MM) [16].

The remaining variant (E204K) was found in a patient with a ruptured cerebral aneurysm. Evidence of a link between AATD variants and cerebral aneurysm/arterial dissections has previously been reported. Heterozygous and homozygous AATD variants (PI*MS, PI*MZ and PI*ZZ) were more commonly found in patients with intracranial aneurysms than in the general population [17–19]. This mutation was previously predicted to be benign [9]; however, there may be a complex interaction between several minor mutations, including normal variants i.e., M2/M4. Furthermore, AAT is a protease inhibitor, in particular of elastase, but also collagenase and trypsin. Therefore, an imbalance in protease/antiprotease activity could result in degradation of the arterial wall, the main components of which are elastin and collagen, predisposing the vessel to aneurysm formation [17]. The I50N sequence variant was considered to be unusual due to a concomitant diagnosis of CIDP in this patient. AAT inhibits proteases that affect the function of the immune system, thus leading to complications in the process of inflammatory demyelination [20]. The protease inhibitor type M3 recorded in this patient has previously been linked to inflammatory demyelinating diseases of the peripheral and central nervous system [21], providing a link between AATD and CIDP.

We have previously argued [9] as have others [22] that molecular modeling can help to decide whether a mutation/sequence variation is pathogenic. We have used three common predictors of protein stability: SVM, Gibbs free energy changes/FoldX and Polyphen-2. If all three predictors were positive we considered the variation probably damaging; if 2 of 3 predictors were positive we considered it possibly deleterious, if one predictor was positive it was called possibly neutral and if all predictors were negative we considered it probably neutral [9]. Using this classification, the substitutions P289S and I50N would be considered probably deleterious and H262Y and E204K would be considered possibly neutral and probably neutral, retrospectively. This classification is an attempt to link the degree of protein instability to the possible clinical pathogenicity, but it is important to realize that the detection of a molecular variant is not a clinical diagnosis [23]. Considerations to connect a genetic molecular variant to a clinical diagnosis have been extensively discussed and recommendations by professional societies have been published [22,24]. To ascertain the genetic contribution to COPD/emphysema is particularly difficult because of the major role of environmental factors in this complex disorder.

Patients with AATD may need expensive long-term care. To date, treatment options have been limited or lacked effectiveness and diagnosis is challenging. Although AAT therapy is the only treatment for AATD, it is currently indicated only in patients with AATD and clinical evidence of emphysema, as observed in cases P289S and I50N. This limitation may require reconsideration to allow use in indications outside of AATD-associated emphysema. Little is known about disease manifestations associated with rare and novel variants in AATD, in particular whether development and progression of COPD/emphysema is analogous to that of 'common' deficiency variants i.e., PI*Z/PI*S [9]. Although detecting rare/novel mutations can be challenging, recent sequencing techniques (NGS) should be employed when standard PCR and IEF tests suggest a normal genotype in patients with low/normal AAT levels. Gene sequencing techniques are able to detect rare/novel genotypes, and in some cases, can help to determine the most suitable course of treatment for patients with AATD [3]. Nevertheless, it should be noted that sequencing techniques are generally expensive to run and less widely available in general laboratory/clinic settings than more basic tests. Although NGS represents a step forward in AATD testing in terms of it being less costly and higher throughput vs. Sanger sequencing, the aforementioned aspects represent obstacles to the applicability of sequencing in routine clinical practice and must be overcome in the future to provide better care for patients. Computational methodology has been highlighted to provide meaningful predictions of the pathogenic significance of novel mutations and identify areas for further investigation [22].

4. Conclusion

Novel and rare variants, often not detectable by basic AATD diagnostic tests, can contribute to the development of COPD/emphysema. Accurate determination of a patient's *SERPINA1* genotype can encourage the implementation of lifestyle changes (e.g., smoking cessation) and support access to AAT therapy, which can prevent further destruction of lung tissue and decrease the risk of emphysema development. Testing should also be offered to other family members to avoid any further risk of potential diagnosis delays. Pulmonologists should be aware of extrapulmonary conditions potentially linked to *SERPINA1* variants and AATD, such as CIDP and cerebral aneurysm.

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Declaration of competing interest

Declarations of interest: none.

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