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The Effects of Inflammation on Alpha 1 Antitrypsin Levels in a National Screening Cohort

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ABSTRACT

Alpha 1 Antitrypsin (AAT) is a highly polymorphic serum protein. Several genetic variants are associated with varying degrees of decreased serum levels; however, these levels can rise in response to infection, inflammation, injury and estrogen levels. Although the effect of inflammation is well established, it has never been studied quantitatively with respect to specific genotypes in a large representative sample. Using data from a national AAT deficiency-targeted screening cohort, we evaluated AAT levels of patients with normal and deficiency genotypes in response to inflammation, indicated by elevated serum C-reactive protein (CRP). Additionally, we utilized a regression analysis to adjust for the effect of inflammation for each genotype. Across all stratified genotype groups, increased AAT levels were observed in patients with CRP ≥5 mg/L. Different AAT phenotypes reacted differently in the acute phase; M showed a strong response and Z a reduced reaction. Nevertheless, we discovered that inflammation significantly masked clinically relevant base AAT levels in some PI*MZ individuals; approximately a quarter of PI*MZ samples showed signs of inflammation. Median AAT levels (mg/dL) in the presence of inflammation are given for several genotypes; numbers in parentheses are levels from the cohort without inflammation/adjusted levels from the cohort with inflammation using the newly devised algorithm: PI*MM: 162 (142/140); PI*MS: 136 (117/115); PI*MZ: 104 (85/89); PI*MF: 161 (132/141); PI*SS: 115 (96/91); PI*SZ: 66 (54/50). We conclude that simultaneous determinations of CRP and AAT levels, and genotyping are clinically valuable in defining AAT variants and that the effect of inflammation can be adjusted for.

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Alpha 1 Antitrypsin deficiency; screening; C-reactive protein; inflammation; testing

Introduction

Alpha 1 Antitrypsin Deficiency (AATD) has been well established as a genetic condition that predisposes individuals to lung disease (1). Alpha 1 Antitrypsin (AAT) is a member of the serpin-superfamily of proteinase inhibitors (2). Its major biochemical activity is the regulation of neutrophil elastase; its site of production is the liver. AATD is caused by mutations in the *SERPINA1* gene, which encodes AAT. The most common 'normal' allele is designated as 'M'; the 'S' and 'Z' alleles are the most commonly identified loss-of-function or deficiency variants in Caucasians; different combinations of these alleles lead to varying levels of circulating AAT (1). Using nephelometry, serum AAT levels are usually in the range 83–220 mg/dL (1). Patients homozygous for the Z variant (PI*ZZ) have the most severe AATD, with levels typically below 43 mg/dL (1). Moreover, *SERPINA1* is a highly polymorphic gene, with a number of rare and novel alleles previously discovered that are associated with varying serum levels (3, 4).

In severe AATD, patients present with respiratory symptoms due to degradation of lung tissue from uninhibited neutrophil elastase activity, which eventually leads to emphysema. Individuals with the Z allele are also predisposed to liver disease (5). Individuals with AATD may present with childhood liver disease or the condition may become symptomatic in middle-age, with a wide range of symptoms indicative of asthma, COPD, emphysema, and chronic bronchitis, making the condition difficult to diagnose based on clinical symptoms alone (6, 7). It has been predicted that <10% of Americans with severe deficiency (serum AAT levels below the protective threshold value of 50 mg/dL if measured by nephelometry or 80 mg/dL if measured by radial immunodiffusion) are recognized clinically as having AATD (8). On average, patients visit three different physicians before receiving an accurate diagnosis of AATD (6).

In the general population, common heterozygous *SERPINA1* genotypes (i.e., PI*MS and PI*MZ) have been associated with a slightly increased risk of chronic obstructive pulmonary disease (COPD) (9). Moreover, there is evidence that PI*MZ smokers are particularly at risk of respiratory disease; a recent study indicated that PI*MZ patients who smoke are at a greater risk of airflow obstruction and COPD than equivalent PI*MM individuals (10). However, patients with intermediate deficiency are often not identified as such, as their AAT levels may be found to be in the "normal" range, precluding further genotypic/phenotypic assessment (11).

Specifically, the presence of systemic inflammation at the time of sample collection for AATD testing may prevent the

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identification of deficiency genotypes due to the nature of AAT as an acute phase reactant. This is due to the fact that labs may only reflex the sample to genetic or phenotype tests based on initial AAT levels. Blood levels of AAT can increase by 75–100% in response to inflammation, infection and injury (12). AAT's acute phase response is logical in the sense that more of the protein would be required in response to increased production of neutrophil-expressed elastase, in line with increased neutrophil activity in the acute phase, e.g., in response to infection.

In the clinical setting, acute phase reactants play a crucial role in the diagnosis of various disease states; a commonly used clinical indicator of inflammation is C-reactive protein (CRP). Similarly to AAT, CRP has an important role in the body's response to infection, and has several functions that include promoting agglutination, activation of complement cascades and binding to multiple microorganism-expressed ligands (13). Although elevation of CRP alone cannot identify a particular disease state, it is an important, widely used clinical tool in the diagnosis and monitoring of a wide range of inflammatory conditions (14).

Of interest is that AAT has been shown to be similarly elevated to CRP in the acute phase; this was previously found to be the case in systemic lupus erythematosus (15). In addition, a study in patients with normal or heterozygous *SERPINA1* genotypes, showed that AAT elevation in the acute phase parallels that of CRP, with this relationship unaffected by several single nucleotide polymorphisms in the CRP gene (16). Moreover, several studies have noted that inflammation, as indicated by elevated CRP, can particularly affect observed AAT levels in genotypes associated with intermediate deficiency (3, 11, 16, 17). It has previously been suggested that simultaneous determination of AAT and CRP may be useful in identifying patients with AAT variants, particularly in patients with genotypes associated with intermediate AATD (16). To build on previous research, we sought to investigate the individual relationships between AAT and CRP for different *SERPINA1* genotypes, in a large US-wide screening program. We also sought to assess whether the increase in AAT as a result of inflammation could be quantified and adjusted for.

Methods

Data collection

Data included in this report were generated from the DNA$_1$ Advanced Alpha-1 Screening Program™ (CSL Behring). Developed by Biocerna (Fulton, MD; on behalf of CSL Behring), the aim of this testing program is to improve diagnosis of AATD; US-based physicians can submit samples to Biocerna for testing of suspected AATD patients or for retesting of previously diagnosed patients. Blood samples are submitted on serum separator cards. In addition, consent for use of laboratory data for research purposes was obtained from all patients included in this analysis.

Quantitative analysis of AAT and CRP levels was performed using samples obtained from serum separator cards paired with Roche™ immunoassays, AAT2 and C-Reactive Protein gen 3 (Basel, Switzerland) (normal AAT & CRP ranges: 90–200 mg/dL and <5 mg/L, respectively as per the manufacturer's instructions). Qualitative assessment of AATD genotype was conducted using targeted genotyping (TaqMan®: Thermo Fisher Scientific; Waltham; Massachusetts, United States). Variant alleles screened for by targeted genotyping included S, F and Z; all samples with deficiency variants underwent secondary isoelectric focusing (IEF) analysis (Hydragel 18 A1AT IEF isofocusing kit, Sebia USA, Norcross, GA) as a final confirmatory step.

The DNA1 Advanced Alpha-1 Screening Program™ utilizes next-generation sequencing (NGS) to identify rare/novel genotypes when discordance is found between genotyping and AAT serum levels. For the purposes of the present investigation (i.e., impact of inflammation on AAT levels), only targeted genotyping results are presented.

Statistical analysis

All statistical analyses were performed using Stata version 13.1 (StataCorp LP, College Station, Texas, United States).

In order to evaluate the clinical impact of inflammation on AAT concentrations, our analysis used threshold values of 80 mg/dL to define "low"; this threshold is in line with the lower limit for "normal" AAT levels (measured by nephelometry) provided in the 2003 ATS/ERS statement on Standards for the Diagnosis and Management of Individuals with Alpha 1 Antitrypsin Deficiency (1). The data were grouped by genotype for all patients and then categorized into those with normal CRP (<5 mg/L) and those with high CRP (≥5 mg/L) (Table 1). For each genotype, the average increase in AAT from imputed normal to elevated CRP was calculated and presented as mean and confidence interval (Table 1). A univariate regression analysis was conducted to determine the correlation between AAT and CRP levels.

Results

Data from 10,429 patients were categorized by genotype, with corresponding median AAT and CRP levels calculated (Table 1). The majority of the total population (83%) had the normal (PI*MM) AAT genotype, with a median AAT level of 148.4 mg/dL (Table 1). The most common variants were PI*MS (8.1%) and PI*MZ (6.6%), with median AAT levels of 123.0 and 89.2 mg/dL, respectively (Table 1). Homozygosity for the severe deficiency allele (PI*ZZ) was observed in 0.7% of the total population, with an unknown median AAT level due to more than half of these patients having readings below the assay detection limit (20 mg/dL) (Tables 1 and 2). A number of other AATD genotypes were also identified—PI*MF, PI*FF, PI*SS, PI*SF, PI*SZ and PI*ZF—these were associated with varying median AAT levels (Table 1). Overall, median AAT levels and interquartile ranges were consistent with average levels reported in previous population studies (Table 1) (11, 18).

AAT and CRP by genotype

The patients were divided between normal (<5 mg/L) and elevated (≥5 mg/L) CRP levels. Approximately one-third of patients (32%) had CRP levels ≥5 mg/L, indicative of inflammation (Table 1). Distributions of AAT levels with normal and elevated CRP for the most common genotypes (PI*MM, PI*MZ

Table 1. Median AAT and CRP levels by AAT genotype, for all patients and for the subsets with inflammation (CRP ≥5 mg/L) or without inflammation (CRP <5 mg/L).

Genotype	All	PI*MM	PI*MS	PI*MZ	PI*MF	PI*SS	PI*SF	PI*SZ	PI*ZF	PI*ZZ
Patients, n	10429	8651	844	688	68	34	5	60	6	73
All patients (regardless of CRP level)										
AAT level, mg/dL	143.1 [123.7, 163.4]	148.4 [131.4, 167.4]	123.0 [108.9, 138.9]	89.2 [78.4, 103.4]	139.0 [124.1, 159.7]	98.2 [88.8, 114.1]	118.7 [117.5, 133.6]	55.7 [47.3, 65.1]	79.2 [66.7, 81.5]	0.0 [0.0, 29]
CRP level, mg/L	2.63 [1.0,6.5]	2.74 [1.0, 6.7]	2.2 [0.8, 5.8]	2.0 [0.8, 5.0]	2.1 [0.6, 7.2]	2.1 [0.9 6.0]	3.9 [2.7, 7.5]	1.6 [0.6, 3.9]	4.5 [2.5, 6.8]	2.3 [0.9, 6.0]
Normal CRP (<5 mg/L)										
Patients, n	7103	5804	600	519	48	24	3	52	3	50
AAT level, mg/dL	137.1 [118.8, 155.5]	142 [126.9, 159.2]	116.7 [105.0, 131.4]	85.4 [75.8, 96.6]	132 [119.4, 145.0]	96.3 [88.2, 107.5]	118.7 [117.5, 133.6]	53.7 [46.4, 61.0]	66.7 [64.5, 79.2]	0 [0.0, 28.2]
CRP level, mg/L	1.4 [0.6,2.7]	1.5 [0.7, 2.8]	1.3 [0.6, 2.6]	1.2 [0.6, 2.4]	1.1 [0.5, 2.2]	1.2 [0.3, 2.3]	2.7 [1.3, 3.9]	1.3 [0.6, 2.6]	2.5 [1.1, 3.0]	1.7 [0.6, 2.5]
Abnormal CRP (≥5 mg/L)										
Patients, n	3326	2847	244	169	20	10	2	8	3	23
AAT level, mg/dL	158.0 [138.5, 182.4]	162.0 [145.1, 186.3]	136.4 [121.5, 154.6]	104.4 [91.9, 116.0]	160.7 [147.4, 168.0]	115.2 [101.2, 130.7]	145.4 [113.4, 177.5]	65.7 [62.5, 70.3]	81.5 [79.2, 90.0]	0.0 [0, 41.3]
CRP level, mg/L	9.6 [6.8,16.2]	9.7 [6.8, 16.4]	9.5 [6.8, 17.6]	8.8 [6.7, 12.5]	10.7 [7.7, 12.6]	9.2 [6.3, 21.1]	11.6 [7.5, 15.8]	8.8 [6.8, 13.6]	6.8 [6.0, 8.2]	9 [6.4, 11.8]
AAT increase, mg/dL (95% CI), with ↑CRP	22.9 (21.5, 24.2)	21.1 (19.8, 22.4)	19.1 (15.6, 22.8)	18.4 (15.1, 21.9)	26.0 (15.7, 36.9)	17.7 (3.2, 34.9)	19.9 (−20.2, 60.0)	12.6 (4.3, 21.6)	14.8 (−0.1, 27.5)	0.0 (0.0, 0.0)

Data are expressed as median [Interquartile range]; AAT = alpha-1 antitrypsin; CRP = C-reactive protein.

Table 2. Numbers of patients classified as having normal (≥80 mg/dL) or low (<80 mg/dL) AAT levels, by AAT genotype and CRP level. *PI*ZZ patients with normal levels were receiving AAT therapy; AAT = alpha-1 antitrypsin; CRP = C-reactive protein.

AAT (mg/dL)	All	PI*MM	PI*MS	PI*MZ	PI*MF	PI*SS	PI*SF	PI*SZ	PI*ZF	PI*ZZ
	All patients (regardless of CRP level), *n* (%)									
Normal	10,094 (96.7)	8,642 (99.9)	837 (99.2)	493 (71.7)	68 (100.0)	32 (94.1)	5 (100.0)	2 (3.5)	2 (33.3))	10* (13.7)
<80	335 (3.3)	9 (0.1)	7 (0.8)	195 (28.3)	0 (0.0)	2 (5.9)	0 (0.0)	55 (96.5)	4 (66.7)	63 (86.3)
	Normal CRP (<5 mg/L), *n* (%)									
Normal	6,810 (95.8)	5,796 (99.9)	593 (98.8)	338 (65.1)	48 (100.0)	22 (91.6)	3 (100.0)	4 (6.7)	0 (0.0)	6 (12.0)
<80	293 (4.2)	8 (0.1)	7 (1.2)	181 (34.9)	0 (0.0)	2 (8.4)	0 (0.0)	48 (92.3)	3 (100.0)	44 (88.0)
	Abnormal CRP (≥5 mg/L), *n* (%)									
Normal	3,284 (98.7)	2846 (99.96)	244 (100.0)	155 (91.7)	20 (100.0)	10 (100.0)	2 (100.0)	1 (12.5)	2 (66.7)	4 (17.4)
<80	42 (1.3)	1 (0.04)	0 (0.0)	14 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	7 (87.5)	1 (33.3)	19 (82.6)

and PI*MS) are shown in Figure 1; note that the distributions shift to the right, i.e., higher AAT levels with increasing CRP.

For all genotypes combined, the mean increase in the AAT level for those patients with CRP ≥5 mg/L vs. those with CRP <5 mg/L was 22.9 mg/L (95% confidence interval: 21.5, 24.2; Table 1).

A breakdown of AAT levels classified by genotype and between normal and elevated CRP is available in Table 2. There were 337 (3.2%) patients in the total population who demonstrated low (<80 mg/dL) serum AAT levels, with 96.5% (55/57) and 86.3% (63/73) of PI*SZ and PI*ZZ patients, respectively, falling under this threshold (Table 2). Interestingly, 195 of the patients falling below 80 mg/dL were PI*MZ (28.3% of all PI*MZ patients) and markedly fewer patients with elevated CRP than those with normal CRP showed low AAT levels (14/155 vs. 181/338, respectively; Table 2). Additionally, a minority (n = 9, 0.1%) of PI*MM patients were found to have AAT levels <80 mg/dL.

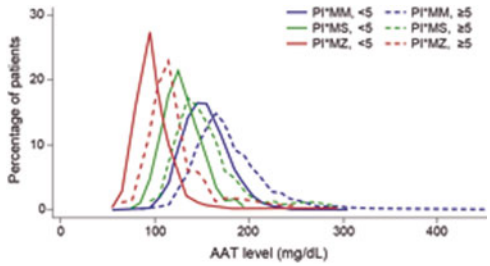

Figure 1. Example of observed AAT distribution according to genotype (PI*MM, PI*MS and PI*MZ.

Relationship between AAT and CRP

The relationship between AAT and CRP was found to be curved. A curved relationship was fitted using both a squared and a cubic term in the regression model. The overall relationship between the two variables was statistically significant (p < 0.001; Figure 2(A)). The initial regression model assumed a single relationship for all genotypes; however, it was predicted that genotype-specific regressions would be more accurate,

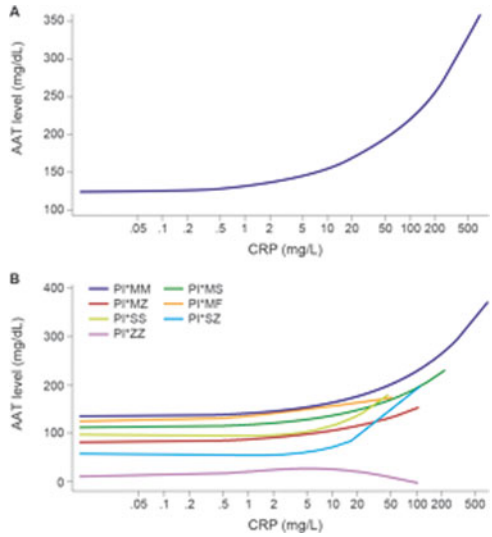

Figure 2. (A) Fitted relationship between AAT and CRP (all patients combined). (B)Fitted relationships between AAT level and CRP level for each AAT genotype, as derived from linear regression analysis.

as it was likely that the relationship between AAT and CRP varied between genotypes. The different relationships for each genotype were examined by including an interaction term in the regression model between genotype and CRP; the shape of the regression curve varied between genotypes, with the PI*ZZ genotype showing a markedly different relationship (Figure 2(B)).

Adjusting AAT for elevated CRP

The coefficients derived from the genotype-specific regression analyses (supplementary Table S1) were then used to create a "residual" value. This is a measure of how much the AAT values for a given person vary from those which might be expected for an average person with the same CRP value (on the log scale).

This was calculated as follows:

$$'\text{Residual AAT}' = \text{loge}(\text{measured AAT} + 100) - y$$

where:

$$y = b1 + b2.x + b3.x^2 + b4.x^3$$
$$x = \text{log10}\left(\text{crp} + 1\right)$$
$$\text{log10} = \text{log function (to base 10)}$$
$$\text{loge} = \text{natural log function (to base e)}$$

where:

$$b1, b2, b3, b4 = \text{regression coefficients (see supplementary Table S1)}$$

This enabled the calculation of "expected" AAT values for each genotype based on the median CRP value in the cohort of patients without inflammation (supplementary Table S2). From there, an AAT level with inflammation can be adjusted as follows:

$$\text{Adjusted AAT} = \exp\left(\text{expected value} + \text{residual}\right) - 100$$

where:

$$\exp = \text{exponential function}$$

Worked example:

An example of the conversion is shown below for a patient with a PI*MZ genotype. This example is from one selected actual patient from the database. This patient had a measured AAT of 91.47 mg/dL and a CRP of 22.65 mg/L. A calculation was performed to work out the patient's AAT level if their CRP level were equivalent to 1.44, the median value of all patients in the normal range (<5 mg/L), rather than 22.65.

The calculations were as follows:

$$x = \text{log10}\,(22.65 + 1) = 1.374$$
$$y = 5.19 + 0.114 \times 1.374 - 0.012 \times 1.374^2 + 0.018 \times 1.374^3 = 5.377$$
$$\text{Residual} = \text{loge}\,(91.47 + 100) - 5.377 = -0.123$$
$$\text{Adjusted AAT} = \exp\,(5.239 - 0.123) - 100 = 66.7$$

To summarize, a patient with a CRP of 23 mg/L, and an observed AAT of 91 mg/dL, would have a predicted AAT of 67 mg/dL if their CRP value were at the median CRP level of patients in the normal range (1.44 mg/L); this calculation is shown graphically in Figure 3(A).

Figure 3(B) shows the effect of applying genotype-specific adjustments to all patients with elevated CRP levels—the distribution shifts to the left (i.e., toward lower AAT levels). Table 3 shows a validation of the adjustment method—for each genotype the adjusted AAT levels we calculated agree strongly with the observed levels measured in the cohort of patients without inflammation.

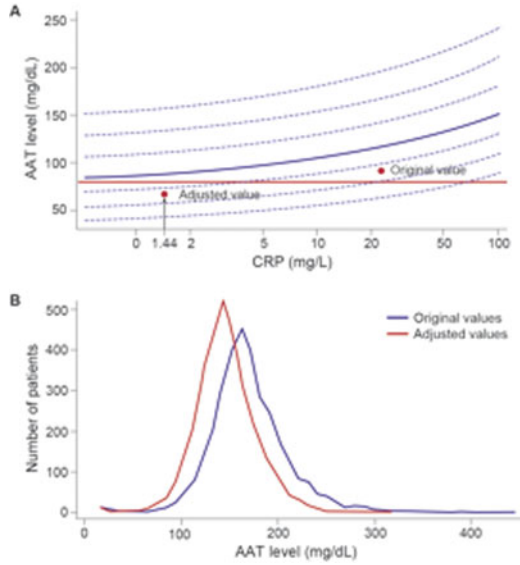

Figure 3. (A)Nomogram for adjusting AAT level in individuals with the PI*MZ genotype and inflammation (CRP ≥5 mg/L), with illustrated example: red lines = AAT 80–110 mg/dL range; solid blue line = overall regression curve for PI*MZ; dashed blue lines = regression curves with starting AAT values at increments of 25 mg/dL; 1.44 = median CRP level of all patients with normal CRP (i.e., <5 mg/L). (B) Distribution of AAT levels in all patients with inflammation (CRP ≥5 mg/L), before and after adjustment to cancel out the effect of inflammation.

Table 3. Validation of adjustment method—comparison of observed AAT levels from cohorts without inflammation with adjusted levels calculated using genotype-specific algorithms.

	Inflammation		No inflammation		Adjusted (inflammation cohort)	
Genotype	*N*	AAT levels (mg/dL)	*N*	AAT levels (mg/dL)	*N*	AAT levels (mg/dL)
PI*MM	2847	162 [145, 186]	5804	142 [127, 159]	2847	140 [125, 159]
PI*MS	244	136 [122, 155]	600	117 [105, 131]	244	115 [104, 132]
PI*MZ	169	104 [92, 116]	519	85 [76, 97]	169	89 [78, 98]
PI*MF	20	161 [(147, 168]	48	132 [119, 145]	20	141 [127, 149]
PI*SS	10	115 [101, 131]	24	96 [88, 107]	10	91 [82, 104]
PI*SZ	8	66 [63, 70]	52	54 [46, 61]	8	50 [47, 57]

Data are expressed as median [Interquartile range]; there were no PI*ZF patients with CRP ≥5 mg/L; AAT = alpha-1 antitrypsin.

Patients re-classified following adjustment

After applying the adjustment equations to all patients with elevated CRP (≥5 mg/L), some patients with AAT levels recorded as normal were re-categorized to low (<80 mg/dL; Table 2). Overall, forty patients with apparently normal AAT (0.4% of the total population) were adjusted to AAT <80 mg/dL (Table 4). PI*MZ patients comprised the majority of these patients ($n = 31$; 77.5%; Table 4).

Discussion

The present investigation explores the variability of AAT levels in the presence of an acute phase reaction, i.e., as indicated by the associated serum CRP levels. We found that AAT levels increase in all genotype categories to differing degrees in the acute phase. We also discovered that a quarter of PI*MZ individuals showed signs of inflammation. The PI*MZ cohort on average harbors AAT levels at near clinically-relevant low levels, as defined in the 2003 ATS/ERS statement on Standards for the Diagnosis and Management of Individuals with Alpha 1 Antitrypsin Deficiency.(1) Some PI*MZ individuals may therefore fall within the range for low, clinically relevant levels. Consistent with previous findings (3, 11, 16, 17), we have shown that, in patients with evidence of inflammation, AAT level testing shows higher result than would be seen in the absence of inflammation. This could lead to misdiagnosing of AATD in the absence of a concomitant test for inflammation and negatively impact a physician's ability to determine the "true" AAT level at baseline, which could put the patient at increased risk of lung disease.

Our work builds on the previous literature on this subject (3, 11, 16, 17) but in a larger sample size. In particular, Ferrarotti and colleagues (2012) and Senn and colleagues (2008) utilized multivariate analyses that included CRP-adjustment to establish more accurate AAT reference ranges for different genotypes, and to explore the relationship between circulating AAT levels and forced expiratory volume in one second (FEV1) in the general population, respectively (11, 17). In addition, Ottaviani and colleagues (2011), in a similar manner to the present analysis, characterized the increase in CRP, as a marker of systemic inflammation, between three groups of *SERPINA1* genotypes: normal, intermediate deficiency and severe deficiency. Consistent with our findings, the impact of inflammation was found to be most clinically relevant in intermediate AAT deficiency variants (e.g., PI*MZ) (16). As Ottaviani and colleagues also found CRP to be closely correlated with increases in AAT, we sought to leverage a larger sample size to define the individual relationships between AAT and CRP for different *SERPINA1* genotypes, and establish whether the acute phase response of AAT could be adjusted for based on elevated CRP.

Table 4. Patients with inflammation (CRP ≥5 mg/L) re-categorized as having low (<80 mg/dL) or low-normal (≤110 mg/dL) AAT levels after adjustment to account for the effect of inflammation.

Genotype (%)	Adjusted AAT <80 mg/dL [n/N (%)]
PI*MM	1/2846 (0.4)
PI*MS	5/244 (2.1)
PI*MZ	31/155 (20.0)
PI*MF	0/20 (0.0)
PI*SS	2/10 (20.0)
PI*SZ	1/1 (100.0)
Additional patients out of total with inflammation	40 (1.2%)
Percentage out of all (10,429) patients	0.4%

There were no PI*ZF patients with CRP ≥5 mg/L; AAT = alpha-1 antitrypsin; CRP = C-reactive protein; *n* = number adjusted; *N* = total number of patients with inflammation in each genotype, omitting patients with original AAT values <80 mg/dL.

While the acute phase response of AAT and CRP is well established (3, 11, 12, 15–17), the correlation to the specific AAT genotypes has not previously been investigated in detail. Our results are based on a very large number (>10,000) of samples that were submitted by physicians for analysis of AAT. This algorithm, applied to a large number of samples and with all abnormal samples verified by at least two qualitative methods, provides high confidence in the reliability of our results. The stratification of patients into high and low CRP levels enabled us to clearly quantitate the effect of the acute phase reaction. Elevation of AAT by an average of 23 mg/L for all genotypes combined, confirmed a marked impact of inflammation on AAT levels. For the most common deficiency genotypes, this value is 18 mg/dL for PI*MZ and 19 mg/dL for PI*MS (Figure 1 shows a graphic representation). Additionally, the large sample size utilized in this study allowed us to create accurate adjustment algorithms specific to each genotype's response to inflammation. The accuracy of the adjustment method we present is demonstrated by Table 3, as the adjusted values are extremely close to the observed data from the cohort of patients without inflammation. We excluded PI*ZZ from this analysis because it shows only a small, often insignificant, elevation and most AAT levels associated with this genotype are below 20 mg/dL, which is the limit of the quantitative analysis.

It is of interest that the correlation of the blood levels of AAT and CRP was non-linear, suggesting that correlations differ at several quantitative levels, although the overall association remained highly significant ($p < 0.001$). There is no obvious explanation for this finding. One possibility is that the

quantitative responses of AAT and CRP are out of phase so that at a given time point, the peak concentration of both levels would not coincide. Only a longitudinal study where both proteins are followed over time could verify this possibility.

Of most practical importance is the finding that of all 688 PI*MZ samples, 493 (71.7%) had AAT levels above 80 mg/dL—this would fall within the common clinically-accepted normal range and therefore would be excluded from further, more specific, analysis. Moreover, there is evidence that the PI*MZ genotype may be clinically relevant in combination with other factors. For example, PI*MZ individuals are thought to be at increased risk of COPD with concurrent cigarette smoking (10, 19). PI*MZ individuals have also been shown to be at a greater risk of lung function decline related to occupational exposure to vapors, dusts, gases and fumes (VGDF), in combination with smoking (20). In addition, evidence suggests that the presence of the PI*MZ genotype in patients with higher levels of background inflammation (e.g., due to smoking or obesity) may accelerate lung function decline (21).

Furthermore, in-line with previous reports (16), we have shown that active inflammation may further complicate the identification of PI*MZ individuals; however, we have also demonstrated that downward adjustment of AAT levels according to their specific CRP levels brings AAT back to expected levels. Therefore, in cases where AAT levels are found to be marginally above biologically relevant thresholds and CRP is abnormally high, future retesting of the patient's AAT levels may be clinically useful and advisable in order to identify heterozygotes. In addition, findings of a link between higher levels of background inflammation in PI*MZ individuals and accelerated lung function decline highlight the importance of identifying PI*MZ individuals with persistent inflammation, as these patients may require closer monitoring. In terms of clinical management, although AAT therapy is not currently recommended for PI*MZ patients (22, 23), correct identification may help to facilitate early intervention such as smoking cessation, avoidance of passive smoke and VGDF, and prompt treatment of respiratory infections. It is also essential for genetic counselling (22).

For patients with low (<80 mg/dL) and normal (≥ 80 mg/dL) AAT levels, the correction is also informative. There is a distinct possibility that previously unrecognized mutations of the PI*M allele may influence the AAT level. In our study, there were nine PI*MM patients with low AAT levels. This apparent discordance between targeted genotyping results and AAT levels may be explained by non-genetic causes. The DNA_1 testing program utilizes next-generation sequencing in all samples with discordant AAT levels and genotyping results before a definitive diagnosis of genotype is made to the treating physician. Frequencies of rare/novel genotypes within the targeted screening population will be reported elsewhere.

Limitations

Our study has some limitations. It is impossible to know the true baseline AAT levels of all patients unless additional patient samples were measured when inflammation in the patient had subsided; unfortunately, these data were unavailable due to the nature of the data collected through the screening program. That said, we had large sample sizes for the three main genotype categories of interest (PI*MM, PI*MS and PI*MZ) and we observed a universal bias towards higher median AAT levels when CRP testing suggested the patient was experiencing inflammation. Furthermore, the correlation of CRP and AAT does not apply for all genotypes. In particular, for PI*ZZ there is no correlation because the level of the Z protein rises only minimally in response to inflammation. This is likely due to accumulation of unsecreted, polymerized Z protein within hepatocytes, forming characteristic periodic acid–Schiff-positive inclusions (24). However, this does not affect our overall conclusions.

Additionally, there are a number of factors that may affect AAT levels, such as body mass index, estrogen and tobacco smoking (17). Due to the nature of the data collected in the screening program, we were unable to incorporate these as covariates into our analyses. However, this issue is lessened by the fact that *SERPINA1* genotype appears to be the major driver of serum AAT levels (25, 26). Finally, although CRP is used routinely as a marker of inflammation, there are rare genetic variations associated with varying effects on the levels of this protein (27); we cannot rule out the possible presence of such mutations in some patients included in the large population we studied.

Conclusions

Given the emerging evidence of the increased risk of respiratory disease in PI*MZ individuals who smoke or have other risk factors, identifying these specific patients with signs of respiratory disease is becoming more important in order to intervene early and implement smoking cessation and other lifestyle changes. Clinicians should be aware of the potential for AAT levels to be transiently elevated when inflammation is present (particularly in PI*MZ patients), due to its nature as an acute phase reactant. If a normal AAT level is found in a patient with suspected AATD and there is evidence of inflammation, such as an abnormal CRP result, it may be informative to retest the patient when the underlying causes of inflammation have abated. Also, an understanding of the underlying reflex strategy for an AATD test would help the physician to better understand the limitations of testing strategies that only conduct genetic testing or phenotyping on patient samples with abnormal AAT levels. In the presence of inflammation, these reflex strategies may not be effective and could lead to inaccurate diagnosis.

Declaration of interest

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C.L.S. is the guarantor of the article and takes responsibility for the integrity of the data and the accuracy of the data analysis. C.L.S. conceived the study, was involved in the collection and interpretation of the data, and reviewed the manuscript. A.P. assisted with data collection and reviewed the manuscript. F.K. participated in both the writing of the manuscript and with the interpretation of the data. All authors approved the manuscript for submission.

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Supplementary material

Supplemental data for this article can be accessed on the publisher's website.

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