ORIGINAL ARTICLE

Serum levels and genotype distribution of α_1-antitrypsin in the general population

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ABSTRACT

Rationale α1-Antitrypsin (AAT) deficiency is one of the commonest rare respiratory disorders worldwide. Diagnosis, assessment of risk for developing chronic obstructive pulmonary disease (COPD), and management of replacement therapy require the availability of precise and updated ranges for protein serum levels.

Objective This paper aims to provide ranges of serum AAT according to the main genotype classes in the general population.

Methods The authors correlated mean AAT serum levels with the main *SERPINA1* variants (M1Ala/M1Val (rs6647), M3 (rs1303), M2/M4 (rs709932), S (rs17580) and Z (rs28929474)) in 6057 individuals enrolled in the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA) cohort.

Results The following ranges (5th—95th percentile) of AAT were found in the serum (g/litre): 1.050—1.640 for PI*MM, 0.880—1.369 for PI*MS, 0.730—1.060 for PI*SS, 0.660—0.997 for PI*MZ and 0.490—0.660 for PI*SZ. There was very little overlap in AAT serum levels between genotype classes generally not believed to confer an enhanced health risk (MM and MS) and those associated with an intermediate AAT deficiency and a potentially mildly enhanced health risk (SS, MZ).

Conclusion This work resulted in three important findings: technically updated and narrower serum ranges for AAT according to PI genotype; a suggestion for a population-based 'protective threshold' of AAT serum level, used in decision-making for replacement therapy; and more precise ranges framing the intermediate AAT deficiency area, a potential target for future primary prevention.

Key messages

What is the key question?

► What are the ranges of serum α1-antitrypsin (AAT) level in the general population?

What is the bottom line?

► State-of-the-art methodologies allowed identification of AAT ranges according to the major genotypes, narrower than those previously available. Moreover, the authors defined the intermediate AAT deficiency area (0.92—0.49 g/litre) of particular interest being a possible target for future interventional options and clarified the longstanding controversy in the conversion from μM to g/litre of the 'protective threshold' of AAT serum level, used in decision-making for replacement therapy.

Why read on?

► It is important to clearly identify the protective threshold for AAT deficiency and, in turn, the serum level of AAT characterising patients with severe AAT deficiency. It is also important to correctly diagnose patients with intermediate AAT deficiency (mostly with the PI*MZ genotype).

INTRODUCTION

One of the few unambiguously ascertained individual risk factors for chronic obstructive pulmonary disease (COPD) is the serum level of α1-antitrypsin (AAT), which in turn is strongly determined by the AAT genotype variant system, classically named PI type. A large body of evidence suggests that the degree of risk for COPD is inversely related to the serum AAT level according to the hierarchy PI NullNull > PI ZZ > PI SZ > PI MZ.[1–4] Therefore, accuracy in AAT serum level determination is a relevant factor in COPD risk assessment. Above the area termed 'severe' AAT deficiency (AATD), bounded by the AAT protective threshold level of 11 μM[5] and at high risk for developing COPD, lies the area of 'intermediate' AATD, whose threshold has not been determined but is currently used as a proxy for the PI*MZ genotype. Notably, the currently used standard reference values for AAT in serum[6] show a broad and overlapping range of values for the PI MM, PI MZ, PI MS and PI SS classes and do not represent data from the general population. In the absence of such data, only AAT serum values below 11 μM are of use for the assessment of severe AATD and for COPD risk prediction, whereas meaningful reference values to classify intermediate AATD associated with different AATD genotypes are lacking. Careful evaluation of serum AAT concentration is the initial diagnostic test in patients with suspected AATD.[7] This measurement can be routinely performed in any clinical chemistry laboratory, and it is the determining factor that justifies further analysis such as genotyping and sequencing, which are performed in dedicated laboratories.[8] Thus, the need for updated reference intervals for AAT according to the different PI types is of clinical relevance. This is especially true for reference values related to PI MZ, which may also require clinical attention in the form of smoking counselling in light of evidence for an increased risk of developing airflow obstruction.[4 9]

The aim of this paper was to correlate serum AAT levels with the main PI variants, using current standards of measurement and diagnosis, including the molecular characterisation of the *SERPINA1* gene encoding AAT.[8] To the best of our knowledge, this information has not been published for a large general population sample.

We took advantage of the first follow-up examination of the (Swiss Cohort Study on Air Pollution and Lung Diseases in Adults) SAPALDIA cohort, which included 8047 people randomly selected from eight population registries representing the three major Swiss language regions, including both urban and rural areas.[10] The SAPALDIA biobank, which includes blood and DNA samples for more than 6000 people, was used to perform a previous study on the *SERPINA1* molecular characterisation of 1399 samples displaying reduced serum AAT levels.[11] In this study all 6057 samples from subjects who gave consent for genetic analyses, including the 1399 mentioned above, were investigated for normal M and deficient Z and S *SERPINA1* variants. The data presented were used to define the prevalence of those variants and the levels of serum AAT according to the main genotypes in the general population.

MATERIALS AND METHODS

Subjects

The SAPALDIA cohort has been previously described.[10] At the baseline in 1991 the subjects, who were 18–60 years old and predominantly Caucasian of Swiss nationality, were randomly selected from eight population registries. The current cross-sectional investigation of serum AAT is restricted to follow-up data collected in 2002–2003 when the biobank was established and includes 6057 subjects who donated blood and consented to genetic analysis. The study was approved by the Central Ethics Committee of the Swiss Academy of Medical Science and Cantonal Ethics Committees for each of the eight examination areas.

Serum analysis

AAT (g/litre) and C-reactive protein (CRP, mg/litre) concentrations were determined by latex-enhanced immunoturbidimetric assay (COBAS Integra analyzer, Roche Diagnostics, Indianapolis, Indiana, USA), a robust assay with principles that are perfectly comparable to those of nephelometry.[12] The interassay coefficient of variation (CV) was 3.6–4.6%; lower detection thresholds for the AAT and CRP assays were 0.21 g/litre and 1 mg/litre, respectively, and reference values were 0.9–2.0 g/litre and <8 mg/litre, respectively. Each new batch of antiserum was compared with previous batches for value recovery and proportionality in actual assays. A clarified, delipidated, commercially available serum calibrant (Calibrator f.a.s. Proteins, Roche Diagnostics) was used during the study; the same calibration batch, buffers and other reagents were used throughout the entire study.

Single nucleotide polymorphism analysis

All subjects were typed for give SNPs: S (rs17580), Z (rs28929474), M1Ala/M1Val (rs6647), M3 (rs1303), M2/M4 (rs709932). Typing was performed by PCR with fluorescently labelled TaqMan probes (Vic or Fam labels) on a LigthCycler480 (Roche Diagnostics). All single nucleotide polymorphisms (SNPs) were in Hardy–Weinberg equilibrium. Further details on SNP analysis are available in the online data supplement.

Detection of rare deficient variants

The presence of rare deficient mutations was determined by sequencing the coding region of the *SERPINA1* gene, as previously described, on selected samples as reported by Zorzetto and coworkers.[11]

Statistical analysis

AAT concentrations were normally distributed and analysis of variance (ANOVA) was applied to compare means in different subgroups. Reference values covered the range from the 5th to the 95th percentile of AAT serum values. Linear and quantile regression was used to calculate adjusted means and percentiles. Covariates in the regression models were selected according to a former publication[13] and they were all significantly associated with AAT concentrations. The receiver operating characteristic (ROC) curve was used to estimate the predictive accuracy of serum AAT, and maximation of the Youden index (ie, the sum of sensitivity and specificity minus 1) defined the optimal threshold for discrimination of genotype classes. Bootstrapping procedures were used to estimate the 95% CIs of the optimal thresholds. Statistical analysis was performed with MedCalc 9.4.2.0 (MedCalc Software, Mariakerke, Belgium), Stata V.10.1 IC and SAS V.9.2.

RESULTS

As a first step we identified the number of subjects belonging to different *SERPINA1* genotype classes and determined their frequency in the general population (table 1). The PI*MM genotype accounted for 5398 individuals (89.12% of the overall population), whereas PI*MS was the second genotype in order of frequency (7.48%), followed by PI*MZ (2.36%). Only one subject carrying the PI*ZZ genotype (0.02%) was identified. The two classes defined in table 1 as rare variants and novel variants, accounting for 42 subjects (0.69%), were very heterogeneous groups of variants and were therefore excluded from further analyses on the relationship between AAT serum levels and *SERPINA1* genotypes. Nevertheless, means and ranges of AAT serum levels did not notably change if rare and novel variants were not excluded (data not shown). The frequencies of S and Z alleles in the three main Swiss language groups (German, French, Italian) are shown in online table E1 and are further described in the online data supplement.

Table 1 Frequency of the *SERPINA1* genotype classes detected in 6057 subjects from the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA) cohort

Genotype	PI*MM†	PI*MS	PI*SS	PI*MZ	PI*SZ	PI*ZZ	Rare variants‡	Novel variants§
Number of subjects	5398	453	10	143	10	1	34	8
Frequency (%)	89.12	7.48	0.16	2.36	0.17	0.02	0.56	0.13

†The PI*MM genotype class encompasses different combinations of normal M variants (M1Ala/Val, M3, M2/M4). Details are reported in the online data supplement.
‡This class includes subjects heterozygous for rare deficient variants, such as I, P_{lowell}, M_{malton}, $M_{Wurzburg}$ etc. These data were previously analysed in more detail.[11]
§This class includes novel putative deficient variants detected during *SERPINA1* gene sequencing. These data were previously analysed in more detail.[11]

Table 2 Unadjusted and adjusted means and intervals (5th and 95th percentiles) for α1-antitrypsin (AAT) serum concentration in the six main *SERPINA1* genotype classes

SERPINA1 genotype	Unadjusted AAT serum concentrations, N=5981*				Adjusted AAT serum concentrations,† N=5768‡			
	N	Mean, SD (g/litre)	5th percentile	95th percentile	N	Mean (g/litre)	5th percentile	95th percentile
MM	5366	1.298, 0.18	1.05	1.64	5175	1.298	1.079	1.572
MS	451	1.085, 0.16	0.88	1.37	438	1.082	0.902	1.312
SS	10	0.849, 0.10	0.73	1.06	10	0.823	0.735	1.009
MZ	143	0.805, 0.11	0.66	1.00	136	0.811	0.672	1.011
SZ	10	0.555, 0.06	0.49	0.66	9	0.554	0.480	0.638
ZZ	1	0.320, 0.00						

*Subjects with rare variants (42) and samples with missing AAT levels (34) were excluded.
†Adjusted for age, sex, area, alcohol consumption (yes/no), systolic blood pressure, body mass index, smoking habit (never, former, current) and C-reactive protein levels.
‡Additionally excluded were subjects with missing covariate data (212) and ZZ genotype due to insufficient frequency (1).

Unadjusted and adjusted means and reference intervals for AAT serum concentration in the six main *SERPINA1* genotype classes are presented in table 2. Adjusting for age, sex, study area, alcohol intake (yes vs no), systolic blood pressure, body mass index (BMI), smoking status (never, former, current) and CRP levels did not essentially alter the results. The 5th–95th percentiles were subsequently compared with previously reported American Thoracic Society (ATS)/European Respiratory Society (ERS) reference values[6] (table 3). The AAT serum level ranges determined in our investigation are markedly more narrow than those previously reported. Data are also graphically reported in figure E1, in which AAT serum concentrations are mathematically converted to μM.

Since AAT is a acute phase protein, we recalculated the reference intervals of AAT serum concentrations in subgroups of subjects according to systemic inflammatory status. A CRP value of 8 mg/litre, which is the upper normal limit for this protein as suggested by the equipment used in the present study, was used as the cutoff to stratify subjects as being without (<8 mg/litre) or with (≥8 mg/litre) systemic inflammation (table E2). Comparison of AAT means between the two CRP strata revealed higher values in the systemic inflammation stratum for all genotype classes. This difference was statistically significant in the PI*MM subgroup ($p<0.001$).

As a next step we assessed the accuracy of predicting genotype classes which are not believed to represent a risk for developing emphysema (PI*MM and PI*MS) and those associated with intermediate AATD and arguably a slightly increased risk for developing emphysema (PI*SS and PI*MZ) from AAT concentrations using ROC statistics.[4 14] For this analysis we included the rare variant carriers of the respective groups to get a representative sample for the general population. The findings were highly accurate for the area under the curve (AUC = 0.9907) (figure E2). The optimal threshold according to the Youden index provided a cutoff at 1.00 g/litre AAT level (95% CI 0.97 to 1.06), which presents a sensitivity of 95.8% and a specificity of 94.8%. For discrimination between PI*MM and any other genotype carrying at least one S or Z allele an optimal cutoff at 1.10 g/litre was determined (73.4% sensibility, 88.5% specificity). The impact of sex, smoking status and CRP levels on these genotype discriminations are described in the online data supplement (table E3).

Finally we analysed the influence of the different PI*M subtypes on AAT serum level. This result is reported in the online data supplement (table E4 and figure E3).

Table 3 Comparison between two published ranges (5th–95th percentiles) of α1-antitrypsin serum levels according to different phenotypes (PI),[1] one in μM and one in g/litre,[6] and the range, according to different genotypes (PI*), as deduced by our analysis[2]

Phenotype–genotype	Units	Reference ranges[1]	Present paper[2]
PI MM–PI*MM	μM	20–48	20.2–31.5
	g/l	1.50–3.50	1.05–1.64
PI SS–PI*SS	μM	15–33	14.0–20.4
	g/l	1.00–2.00	0.73–1.06
PI MZ–PI*MZ	μM	17–33	12.7–19.2
	g/l	0.90–2.10	0.66–1.00
PI SZ–PI*SZ	μM	8–16	9.4–12.7
	g/l	0.75–1.20	0.49–0.66

The g/litre values in our analysis were mathematically converted to μM, based on a molecular weight of 52 kDa. Note that the PI*MS data are not present because this genotype was not included in the original American Thoracic Society/European Respiratory Society guidelines.

DISCUSSION

This study ideally represents the most valid setting to date to derive reference values for serum AAT by genotype group in the general population. We applied state-of-the-art technology for the assessment of serum AAT and *SERPINA1* genotypes in the Swiss population, which is a combination of three language groups that adequately represent the genetic structure of the European population.[15] To the best of our knowledge, only a few studies have been performed in the general population that measure circulating AAT protein[7] or *SERPINA1* gene variants[9] or both.[16 17] The most comparable study is the Copenhagen City Heart study, a longitudinal survey of 7963 subjects from Copenhagen who were genotyped for PI*Z and PI*S but in whom only a small sample of AAT concentrations in blood were measured (n=592).[9] In the study by Sveger,[16] blood from 200 000 infants was drawn for simultaneous AAT determination by semi-quantitative electroimmunoassay and Pi typing with isoelectric focusing. However, the analytical methods used in this study were out of date and therefore these data can no longer be used as a reference. The study by Silverman *et al*[17] applied an automated immunoassay to measure AAT in plasma samples from 20 000 blood donors from the St Louis area. Plasma samples that met criteria of <50% of plasma pool reactivity were examined by isoelectric focusing to determine PI type. The reported St Louis Z allele frequency was 0.0116, but no data on the reference values for the concentration of AAT in plasma were extrapolated. In summary, current AAT serum level–genotype relationships seem obsolete as updated diagnostic standards for AATD have never been applied to a general population sample.

Our work generated a number of outputs. First, a more precise allele and genotype frequency was identified for *SERPINA1* variants in Switzerland's general population. We calculated an updated Pi*S gene frequency of 0.0401 whereas that of PI*Z was 0.0130. Compared with previous estimates for this population,[18]

PI*S frequency was similar whereas PI*Z frequency was slightly higher. Further discussion of this topic is provided in the online data supplement.

A second major outcome of the study was the analysis of AAT serum concentration and genotypes of the *SERPINA1* gene in a large cohort representative of the general population. This enabled the extrapolation of updated protein ranges according to the main normal and deficient genotype classes and evaluation of whether reference values need to be specific for sex, smoking status and CRP level.

To date, no epidemiological data on AAT serum concentration according to the *SERPINA1* genotype have been reported for the general population. Some studies identified mean values or reference intervals of AAT serum concentration but they were limited to target groups, mostly COPD cases or populations in which the frequency of AATD is low, such as Japanese[19] and Korean populations,[20] or in patients with a peculiar clinical phenotype, such as Peyronie's disease,[21] or characteristic cohorts, such as paediatric subjects[7] and pregnant women.[22] The use of advanced technologies makes this study innovative. These technologies include SNP detection for genotyping and the exclusion of potentially confounding genetic factors, that is, deficient variants other than S and Z alleles, from the final analysis. The presence of other variables that could affect the AAT concentration in serum were considered and adjusted for in an additional analysis. This resulted in much narrower serum AAT ranges than those presented in the ATS/ERS consensus document,[6] with a drastic reduction in the overlap among genotypes (table 3). Moreover, the mean values of AAT concentration according to *SERPINA1* genotypical classes (table 2), were lower than those reported so far,[9] likely due to a smaller upper dispersion of measurements. Although 70–80% of the variation in total AAT serum concentration is explained by the Pi type (after age and sex adjustment),[23] other factors can influence variation.[13] Since AAT is an acute-phase reactant, inflammatory status may increase the serum level of AAT.[11 13 24] As shown in table E2, when SAPALDIA subjects were stratified according to the presence or absence of an inflammatory condition, most *SERPINA1* genotypes showed even narrower ranges, particularly those with CRP <8 mg/litre. However, ranges for individuals with elevated CRP were generally higher. In the real world of routine AAT serum measurement, our data do not justify systematic measurement of the inflammatory status and the stratified ranges can be used only in reference laboratories.[8]

Other factors that impact AAT concentration variability include active smoking[25] and age.[21] An in-depth analysis of the same SAPALDIA population has shown that an inter-relationship among circulating AAT, smoke exposure, gender and systemic inflammatory status exists.[13] However, in this study with narrower diagnostic purposes, we demonstrated that inclusion of sex and current smoking status to predict intermediate deficiency genotypes was not necessary. We also addressed a further putative factor for AAT serum concentration variability, that is, the intrinsic effect of the different PI*M subtypes (figure E3). The effect of this variable seems to be negligible because only two PI*MZ haplotypes displayed significant changes in AAT serum concentration.

In this study, we also analysed the limits of the so-called 'protective threshold' and we tried to address the controversy and confusion about the expression of serum AAT concentration. The term 'protective threshold' derives from evidence that subjects with AATD and an AAT serum level above the threshold are at reduced risk of developing emphysema. This is not merely a theoretical cutoff because it is considered the decisional cutoff below which subjects with AATD are eligible for AAT replacement therapy.[6] Therefore, it is a very important concept in AATD patient management. An excellent discussion on this topic by Tonelli and Brantly has recently been published.[26] The concept was originally developed by Hutchinson *et al*[27] and Stockley,[28] based on evidence that subjects displaying the PI SZ phenotype had a reduced risk of developing emphysema compared with those displaying the PI ZZ phenotype[3] and are therefore seldom suitable for replacement therapy. The threshold was fixed at the serum AAT level corresponding to 0.8 g/litre, measured by radial immunodiffusion. A few years later, to resolve the lack of standardisation among laboratories that caused so much confusion in the definition of the AAT measurements, a highly purified AAT standard, expressed as μM, was introduced.[29] In the same report, the protective threshold using the highly purified AAT standard determined by nephelometry was fixed at 11 μM; that is, the 10th percentile of the AAT serum range for subjects with PI SZ, which is considered adequate to protect the lungs from proteolytic attack. Since then, in countries where the AAT concentration was expressed as g/litre, the 0.8 threshold was often considered equivalent to 11 μM. However, radial immunodiffusion cannot be considered equivalent to nephelometry because the former, obsolete method overestimates the real AAT concentration by about

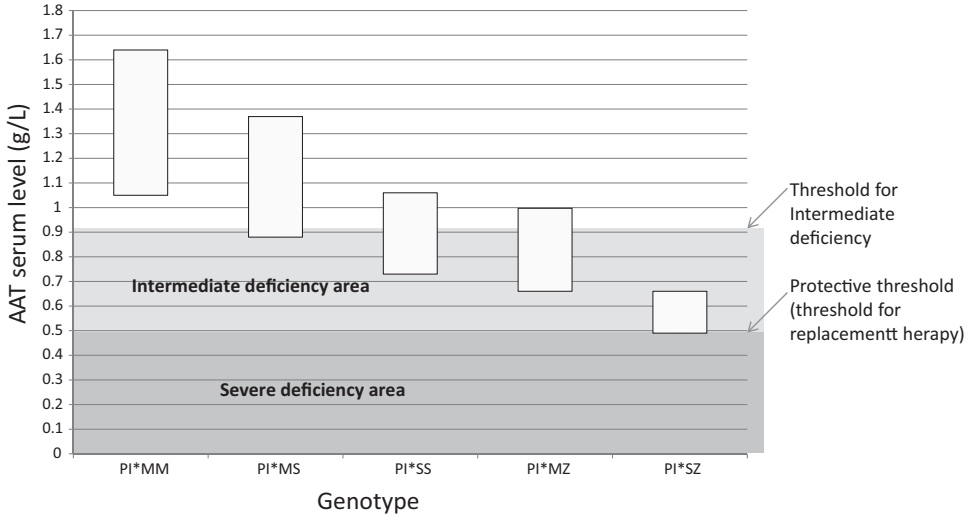

Figure 1 Suggested areas corresponding to severe α1-antitrypsin (AAT) deficiency (below the protective threshold) and intermediate AAT deficiency (above the protective threshold and below the 10th percentile of the AAT range for subjects carrying the PI*MS genotype). Bars represent 5th/95th percentiles of AAT serum levels.

50%.[30] The value corresponding to the 10th percentile of AAT serum concentration for the PI*SZ group in SAPALDIA, which is suitable to derive threshold values in the general population, is 0.49 g/litre.

Intermediate deficiency is a term usually referred to as synonymous with the PI*MZ genotype, which may represent a slightly increased risk of developing COPD.[4] We believe that correct diagnosis of subjects carrying the PI*MZ genotype is a critical issue for a number of reasons. First, having been identified as a group at risk of developing COPD, they are subjects particularly suitable for an effective prevention and smoking cessation campaign, as suggested by an increased rate of attempting to quit smoking following genetic testing.[31] Second, correct diagnosis is mandatory for genetic counselling. Third, subjects with COPD carrying the PI*MZ genotype could be suitable for future, specific therapeutic interventions. Expressed as a range of serum levels, we propose that corresponding values stretch from the protective threshold (0.49 g/litre) to the 10th percentile of the AAT concentration range for subjects carrying the PI*MS genotype, who are believed not to be at risk of developing emphysema,[14] which would correspond to 0.92 g/litre in SAPALDIA. This area includes 87% of subjects carrying the PI*MZ genotype in our cohort. The reported thresholds and related areas are depicted in figure 1.

One of the aims of this paper was to provide a clear cutoff, below which suspicion of AATD is reasonable, and to resolve the controversy around this issue. The choice of the AAT cutoff, below which samples should be selected for PI pheno/genotyping, has important financial and clinical implications. The cutoffs determined by individual laboratories currently range between 1.00 and 1.30 g/litre and they strongly depend on specific requirements. For example, the clinical importance of PI*MS detection is considered to be far less than PI*MZ detection due to the different risks for emphysema for the two genotypes.[4 14] Therefore, we reported two different cutoffs, one focused on avoiding the omission of deficient S or Z alleles (1.10 g/litre) and the second set to identify genotypes at a likely increased risk of emphysema (1.00 g/litre). We also considered the previously reported cutoff of 1.13 g/litre,[32] which is still useful since no Z alleles (and PI*SS) have been found in individuals with AAT blood levels above this level (100% sensitivity and 78.6% specificity for detecting AATD genotypes), while 31% of all assigned subjects with the PI*MS genotype show AAT blood levels higher than 1.13 g/litre (78.6% sensitivity and 82.6% specificity for detecting any deficient S or Z allele).

In conclusion, we provided values for serum AAT level according to the major genotype classes in the general population. In addition, these data have helped us to address controversies related to the different opinions in the definition of limits for the 'protective threshold' and to define a useful range for intermediate AATD. We believe that these findings will be helpful in the future for the investigation of AATD-related risk for COPD and for a more precise definition of when to implement AAT replacement therapy. Finally, the reported data show that the gene–environmental analysis is critical in the ongoing SAPALDIA longitudinal assessment of the impact of *SERPINA1* on pulmonary health.

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REFERENCES

1. **Fregonese L,** Stolk J, Frants RR, *et al*. Alpha1-antitrypsin null mutations and severity of emphysema. *Respir Med* 2008;**102**:876–84.
2. **Ferrarotti I,** Baccheschi J, Zorzetto M, *et al*. Prevalence and phenotype of subjects carrying rare variants in the Italian registry for alpha1-antitrypsin deficiency. *J Med Genet* 2005;**42**:282–7.
3. **Turino GM,** Barker AF, Brantly ML, *et al*. Clinical features in individuals with PI*SZ phenotype of alpha1-antitrypsin deficiency. Alpha1-antitrypsin Deficiency Registry Study Group. *Am J Respir Crit Care Med* 1996;**154**:1718–25.
4. **Hersh CP,** Dahl M, Ly NP, *et al*. Chronic obstructive pulmonary disease in alpha 1-antitrypsin PI MZ heterozygotes: a meta-analysis. *Thorax* 2004;**59**:843–9.
5. **Wewers MD,** Casolaro MA, Sellers SE, *et al*. Replacement therapy for alpha1-antitrypsin deficiency associated with emphysema. *N Engl J Med* 1987;**316**:1055–62.
6. **American Thoracic Society, European Respiratory Society.** American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha1-antitrypsin deficiency. *Am J Respir Crit Care Med* 2003;**168**:818–900.
7. **Steiner SJ,** Gupta SK, Croffie JM, *et al*. Serum levels of alpha1-antitrypsin predict phenotypic expression of the alpha1-antitrypsin gene. *Dig Dis Sci* 2003;**48**:1793–7.
8. **Miravitlles M,** Herr C, Ferrarotti F, *et al*. Laboratory testing of individuals with severe alpha1-antitrypsin in three European centres. *Eur Respir J* 2010;**35**:960–8.

9. **Dahl M,** Tybjaerg-Hansen A, Lange P, *et al*. Change in lung function and morbidity from chronic obstructive pulmonary disease in alpha1-antitrypsin MZ heterozygotes: a longitudinal study of the general population. *Ann Intern Med* 2002;**136**:270—9.
10. **Ackerman-Liebrich U,** Kuna-Dibbert B, Probst-Hensch NM, *et al*. Follow-up of the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA 2) 1991—2003: methods and characterization of participants. *Soz Praventivmed* 2005;**50**:1—19.
11. **Zorzetto M,** Russi EW, Senn O, *et al*. SERPINA1 gene variants in subjects from the general population with reduced alpha1-antitrypsin level. *Clin Chem* 2008;**54**:1331—8.
12. **Ledue TB,** Collins MF. Development and validation of 14 human serum protein assays on the Roche cobas(®) c 501. *J Clin Lab Anal* 2011;**25**:52—60.
13. **Senn O,** Russi EW, Schindler C, *et al*. Circulating alpha 1-antitrypsin in the general population: determinants and association with lung function. *Respir Res* 2008;**9**:35.
14. **Dahl M,** Hersh CP, Ly NP, *et al*. The protease inhibitor PI*S allele and COPD: a meta-analysis. *Eur Respir J* 2005;**26**:67—76.
15. **Lao O,** Lu TT, Nothnagel M, *et al*. Correlation between genetic and geographic structure in Europe. *Curr Biol* 2008;**18**:1241—8.
16. **Sveger T.** Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. *N Engl J Med* 1976;**294**:1316—21.
17. **Silverman EK,** Miletich JP, Pierce JA, *et al*. Alpha-1-Antitrypsin deficiency. High prevalence in the St. Louis area determined by direct population screening. *Am Rev Respir Dis* 1989;**140**:961—6.
18. **de Serres FJ.** Worldwide racial and ethnic distribution of alpha1-antitrypsin deficiency: summary of an analysis of published genetic epidemiologic surveys. *Chest* 2002;**122**:1818—29.
19. **Kwok JS,** Lawton JW, Yew WW, *et al*. Protease inhibitor phenotypes and serum alpha 1-antitrypsin levels in patients with COPD: a study from Hong Kong. *Respirology* 2004;**9**:265—70.
20. **Kim CH,** Yim JJ, Yoo CG, *et al*. Alpha-antitrypsin genotypes in Korean patients with chronic obstructive pulmonary disease. *Respirology* 2005;**10**:223—8.
21. **Hauck EW,** Hauptmann A, Haag SM, *et al*. Alpha 1-antitrypsin levels and genetic variation of the alpha 1-antitrypsin gene in Peyronie's disease. *Eur Urol* 2004;**43**:623—8.
22. **Lisowska-Myjak B,** Sygitowicz G, Wolf B, *et al*. Serum alpha-1-antitrypsin concentration during normal and diabetic pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2001;**99**:53—6.
23. **Silvermann EK,** Province MA, Campbell EJ, *et al*. Family study of a1-antitrypsin deficiency: effects of cigarette smoking, measured genotype, and their interaction on pulmonary function and biochemical traits. *Genet Epidemiol* 1992;**9**:317—31.
24. **Ottaviani S,** Gorrini M, Scabini R, *et al*. C reactive protein and alpha 1-antitrypsin: relationship between levels and gene variants. *Transl Res* 2011;**157**:332—8.
25. **Ashley MJ,** Corey P, Chan-Yeung M. Smoking, dust exposure, and serum alpha 1-antitrypsin. *Am Rev Respir Dis* 1980;**121**:783—8.
26. **Tonelli AR,** Brantly ML. Augmentation therapy in alpha1-antitrypsin deficiency: advances and controversies. *Ther Adv Respir Dis* 2010;**4**:289—312.
27. **Hutchinson DC,** Tobin MJ, Cook PJ. Alpha 1 antitrypsin deficiency: clinical and physiological features in heterozygotes of Pi type SZ. A survey by the British Thoracic Association. *Br J Dis Chest* 1983;**77**:28—34.
28. **Stockley RA.** Proteolytic enzymes, their inhibitors and lung diseases. *Clin Sci (Lond)* 1983;**64**:119—26.
29. **Brantly ML,** Wittes JT, Vogelmeier CF, *et al*. Use of a highly purified a1-antitrypsin standard to establish ranges for the common normal and deficient a1-antitrypsin phenotypes. *Chest* 1991;**100**:703—7.
30. **Liappis N.** Study on the determination of IgA, IgG, IgM, α_1-antitrypsin, haptoglobin and transferrin with the kinetic nephelometric method. Comparison with the radial immunodiffusion. *Klin Padiatr* 1980;**192**:370—8.
31. **Carpenter MJ,** Strange C, Jones Y, *et al*. Does genetic testing result in behavioral health change? Changes in smoking behavior following testing for alpha1-antitrypsin deficiency. *Ann Behav Med* 2007;**33**:22—8.
32. **Gorrini M,** Ferrarotti I, Lupi A, *et al*. Validation of a rapid, simple method to measure alpha1-antitrypsin in human dried blood spots. *Clin Chem* 2006;**52**:899—901.

ONLINE DATA SUPPLEMENT

Serum levels and genotype distribution of alpha$_1$-antitrypsin in the general population

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SUPPLEMENTARY APPENDIX

Methods

SNP analysis

Results

Epidemiology of SERPINA1 Genotypes in Swiss language groups

ROC analysis for accuracy of predicting genotype classes

Analysis of normal M variant subtypes

Discussion

Frequency of SERPINA1 Gene variants in Switzerland

Effect of SERPINA1 M variants on AAT serum concentration

Methods

SNP analysis

The PCR conditions were identical for all applications: 0.125 μl of 20X working stock of SNPGenotypingAssay, 2.5 μl LightCycler 480 Probes Master (Roche Diagnostics), and 20 ng DNA sample, in a total volume of 5 μl. PCR cycling conditions were also identical for all assays: initial denaturation step of 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, and annealing at 60°C for 1 minute. After the amplification, melting curves were generated by slowly (ramp rate 2.2°C/second) cooling the sample to 40°C. The sequences of primers and probes are available on request. Subjects with low AAT serum levels who had been previously genotyped for S (rs17580) and Z (rs28929474) deficient variants by PCR-RFLP[E1], were re-analzyed by Taq-Man probes and had a good reproducibility score with an agreement of 99.7% and 99.6% for S and Z SNPs, respectively.

Results

Frequency of Z and S alleles in the Swiss language groups

We analyzed the frequency of S and Z alleles in the three language groups: German (n=3,288), French (n=1,938) and Italian (n=831) (Table E1). Interestingly, while the PI*Z allele was

homogeneously distributed among the three language groups, the frequency of the PI*S allele was significantly higher in the French subgroup (0.053) than in the German (0.036) and in the Italian (0.025) ($p<0.001$ for both comparisons).

ROC analysis for accuracy of predicting genotype classes

As we had previously reported differences in circulating AAT concentrations by sex, smoking status and CRP levels[E2], we tried to assess whether prediction of PI*SS or MZ genotypes could be improved by considering the influence of these factors on AAT blood levels. We found a marginal enhancement of prediction quality when adjusting for sex and current smoking status, but not for CRP (AUC=0.9927, p=0.05). We subsequently recalculated values for normal (PI*MM and PI*MS) vs. intermediate deficient genotype classes (PI*MZ and PI*SS) by sex and current smoking (Table E3). Despite slight differences in the stratified specific means, the impact of the genotype played a much bigger role than that of sex and current smoking.

Analysis of normal M variant subtypes

The analysis of the three SNPs for normal variants (M1Ala/M1Val - rs6647; M3 - rs1303; M2/M4 - rs709932) by haplotype reconstruction revealed 14 normal genotypic classes in the PI*MM group, 5 classes in the PI*MS group and 5 classes in the PI*MZ group (Table E4). Among these, the most common were PI*M1(Val)M1(Val), PI*M1(Ala)M1(Val), and M1(Val)M2 (frequencies of 0.28, 0.21, and 0.15, respectively). The reference intervals (5^{th}-95^{th} percentiles) for AAT serum concentration have been calculated in each group (Figure E3). Comparison of means within each group revealed only a significant difference between PI*M1(Val)Z and PI*M1(Ala)Z (0.819 vs. 0.754 g/L, p=0.003).

Discussion

Frequency of SERPINA1 Gene variants in Switzerland.

The estimated mean gene frequencies for PiS and PiZ in Switzerland are 0.0384 and 0.0073, respectively[E3] according to data based on the analysis of three Swiss cohorts[E4,E5] previously phenotyped for PI. The estimate is similar to ours for the S allele, but we obtained a slightly higher frequency for the Z allele. When we divided the population into the three language groups, we found evidence of a significantly higher frequency for the S allele in the French subgroup ($p<0.001$ for both comparisons, Table E1). This is in agreement with the hypothesis that the S mutation, which arose in the Portuguese population[E6], moved eastbound to the rest of Europe as a consequence of the late-glacial resettlement of Europe[E7], resulting in decreasing frequencies from southwest to the north and east.

Effect of SERPINA1 M variants on AAT serum concentration.

The normal variants of AAT, usually called M, are characterized by point mutations that neither change the phenotype nor the serum concentration of the AAT protein. The most common are M1Ala/M1Val (213Ala/213Val)[E8], M2 (213Val, 376Asp, and 101His)[E9], M3 (213Val and 376Asp)[E10], M4 (213Val and 101His)[E11]. It is well known that individuals bearing these mutations have normal AAT serum levels and that the protein functions normally as an inhibitor of neutrophil elastase.

Evaluation of the crystallographic structure of AAT[E12] revealed that the substitution at position 101 occurs in helix D and the 376 substitution occurs in sheet 4B of the molecule, which are areas where changes are likely to cause minor conformational changes in the molecule.

Nevertheless, epidemiological studies of these normal variants are limited to their geographic distribution or prevalence data, and comparison among different genotypes, in terms of serum concentration of AAT, has never been performed. A side aim of this paper, was to confirm the absence of quantitative differences among PI*MM subtypes; therefore, an analysis of all possible combinations of M1, M2, M3, and M4 alleles in the cluster PI*MM, was performed. The effect of the M1(Ala) allele, in combination with the Z allele, in reducing the mean concentration of AAT in serum (Figure E3), should be considered with caution, because these data are not supported by biochemical explanations[E13].

Supplement References

E1. **Zorzetto M**, Russi EW, Senn O, *et al*. SERPINA1 gene variants in subjects from the general population with reduced alpha1-antitrypsin level. *Clin Chem* 2008; **54**:1331-8.

E2. **Senn O**, Russi EW, Schindler C, *et al*. Circulating alpha1-antitrypsin in the general population: determinants and association with lung function.Circulating alpha1-antitrypsin in the general population : determinants and association with lung function. *Respir Res* 2008;**9**:35

E3. **de Serres FJ**. Worldwide racial and ethnic distribution of alpha1-antitrypsin deficiency: summary of an analysis of published genetic epidemiologic surveys. *Chest* 2002;**122:**1818-29.

E4. **Scheffrahn W**, Ziggiotti E. Electrophoretic alpha 1-antitrypsin variation in the Swiss population. *Anthropol Anz* 1982;**40**:137-43.

E5. **Bär W**, Kratzer A. Polymorphism of alpha-1-antitrypsin (Pi) in the Swiss population determined by isoelectric focusing with an immobilized pH gradient. *Hum Hered* 1988;**38**:106-10.

E6. **Seixas S**, Garcia O, Trovoada MJ, *et al*. Patterns of haplotype diversity within the serpin gene cluster at 14q32.1: insights into the natural history of the alpha1-antitrypsin polymorphism. *Hum Genet* 2001;**108**:20-30.

E7. **Pereira L**, Richards M, Goios A, *et al*. High-resolution mtDNA evidence for the late-glacial resettlement of Europe from an Iberian refugium. *Genome Res* 2005;**15**:19-24.

E8. **NukiwaT**, Satoh K, Brantly ML, *et al*. Identification of a second mutation in the protein-coding sequence of the Z type alpha 1-antitrypsin gene. *J Biol Chem* 1986; **261**:15989-94.

E9. **Nukiwa T**, Brantly ML, Ogushi F, *et al*. Characterization of the gene and protein of the common alpha 1-antitrypsin normal M2 allele. *Am J Hum Genet* 1988;**43**:322-30.

E10. **Graham A**, Hayes K, Weidinger S, *et al*. Characterisation of the alpha-1-antitrypsin M3 gene, a normal variant. *Hum Genet* 1990;**85**:381-2.

E11. **Okayama H**, Holmes MD, Brantly ML, *et al*. Characterization of the coding sequence of the normal M4 alpha 1-antitrypsin gene. *Biochem Biophys Res Commun* 1989;**162**:1560-70.

E12. **Loebermann H**, Tokuoka R, Deisenhofer J, *et al*. Human alha1-proteinase inhibitor. Crystal structure analysis oft wo crystal modifications, molecular model and preliminary analysis oft he implications for function. *J Mol Biol*. 1984;**177**:531-57.

E13. **Nukiwa T**, Brantly M, Ogushi F, *et al.* Characterization of the M1(Ala213) type of alpha 1-antitrypsin, a newly recognized, common "normal" alpha 1-antitrypsin haplotype. *Biochemistry* 1987;**26**:5259-67.

Legends to Supplemental Figures

Figure E1 – Intervals (5^{th}-95^{th} percentiles) for unadjusted serum AAT levels in the main SERPINA1 genotypic classes;, 1^{st} -99^{th} percentiles are represented with lines, where possible. In this figure, the g/L values of our analysis were mathematically converted to μM, based on a molecular weight of 52kDa.

Figure E2: ROC curve for predicting MM/MS vs SS/MZ genotype classes from unadjusted AAT blood level (rare variants included).

Figure E3 – Intervals (5^{th}- 95^{th} percentiles) for AAT serum concentration in individuals stratified according to the haplotype reconstruction resulting in 24 genotypic classes.

Table E1 – Frequencies of Z and S alleles in the cohort and the three language groups

	Z allele (%)	S allele (%)
General population (n=6,057)	1.30	4.01
German subgroup (n=3,288)	1.22	3.65
French subgroup (n=1,938)	1.44	5.26
Italian subgroup (n=831)	1.26	2.53
p-value (chi-square-test)	0.61	<0.001

Table E2 – Intervals (5^{th}- 95^{th} percentiles) of AAT serum concentration (g/L) in individuals stratified according to genotype and CRP serum concentration. In the case of low number of samples, intervals were not calculated, and the lowest and highest values were reported.

	PI*MM		PI*MS		PI*SS		PI*MZ		PI*SZ	
	CRP<8	CRP≥8	CRP<8	CRP≥8	CRP<8	CRP≥8	CRP<8	CRP≥8	CRP<8	CRP≥8
N	5,092	274	426	25	9	1	136	7	10	0
5^{th} perc.	0.960	1.230	0.870	1.107			0.660		0.490	
95^{th} perc.	1.590	2.086	1.320	1.545			0.990		0.660	
Lowest value					0.730	1.060		0.820		
Highest value					0.910	1.060		1.060		

Table E3 - Influence of sex and current smoking status on unadjusted AAT (g/L) reference values

	n	median	5^{th}/95^{th} perc.
MM/MS all	5,848	1.26	1.01/1.63
MM/MS, male-smoker	791	1.29	1.01/1.59
MM/MS, female-smoker	663	1.36	1.07/1.74
MM/MS, male-nonsmoker	2,127	1.19	0.98/1.48
MM/MS, female-	2,267	1.28	1.03/1.69

nonsmoker			
SS/MZ, all	155	0.79	0.66/1.01
SS/MZ, male-smoker	16	0.79	0.69/1.02
SS/MZ, female-smoker	14	0.88	0.76/1.07
SS/MZ, male-nonsmoker	55	0.75	0.62/0.91
SS/MZ, female-nonsmoker	70	0.81	0.66/1.04

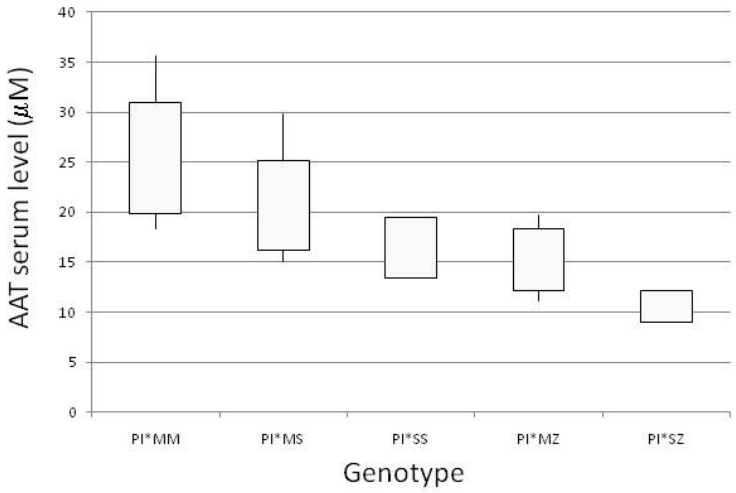
Table E4 – Frequencies of the genotypic classes

PI Group	**PI Genotype**	**Frequencies**
PI*MM		
	M1(Val) M1(Val)	0.2512
	M1(Ala) M1(Val)	0.1850
	M1(Val) M2 or M3 M4	0.1620
	M1(Val) M3	0.0908
	M1(Ala) M2	0.0559

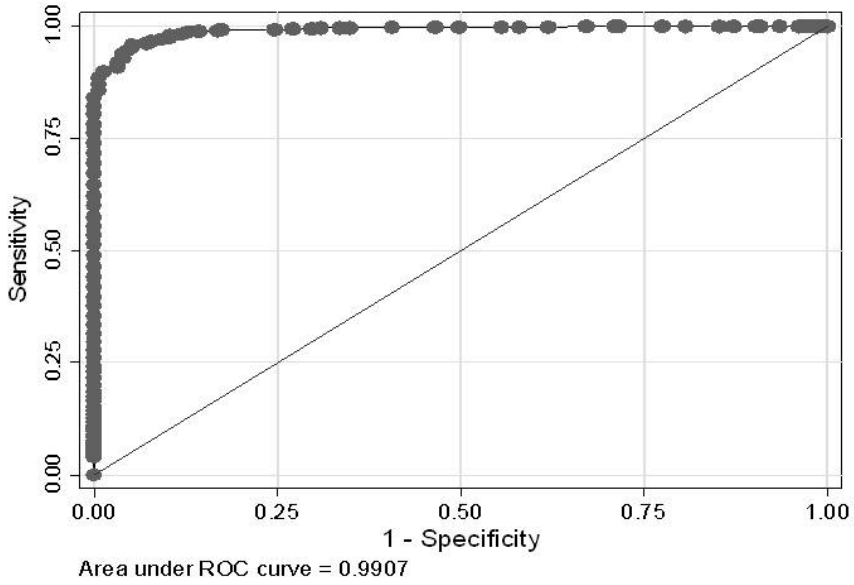
	M1(Ala) M1(Ala)	0.0351
	M1(Ala) M3	0.0328
	M2 M3	0.0266
	M2 M2	0.0253
	M1(Val) M4	0.0113
	M3 M3	0.0105
	M1(Ala) M4	0.0060
	M3 M4	0.0042
	M4 M4	0.0007
PI*MS		
	M1(Val) S	0.0379
	M1(Ala) S	0.0173
	M2 S	0.0110
	M3 S	0.0078
	M4 S	0.0013
PI*MZ		

	M1(Val) Z	0.0133
	M1(Ala) Z	0.0047
	M3 Z	0.0028
	M2 Z	0.0027
	M4 Z	0.0003

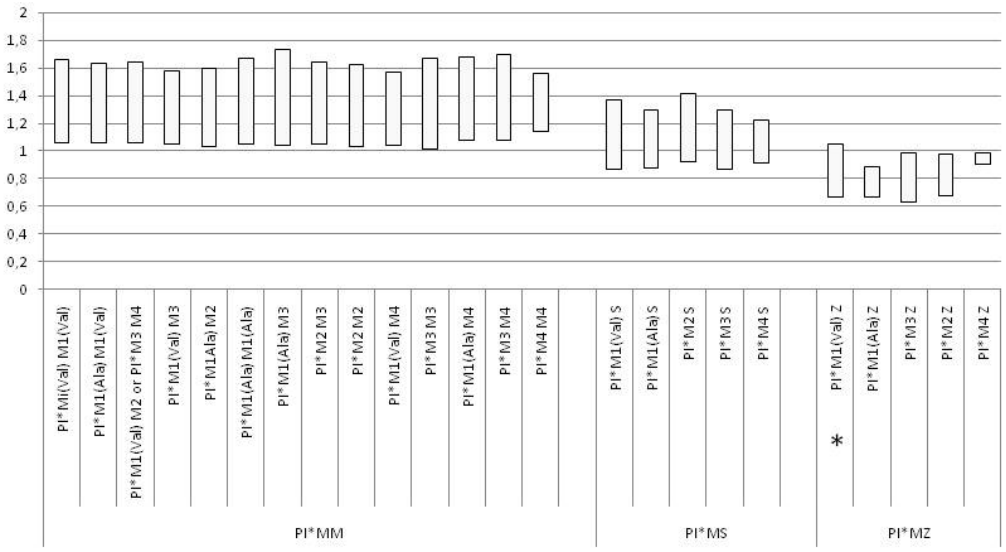
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



* p=0.003; PI*M1(Val) Z vs PI*M1(Ala) Z (0.819 and 0.754 g/L, respectively)