Reference and Interpretive Ranges for α₁-Antitrypsin Quantitation by Phenotype in Adult and Pediatric Populations

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Abstract

Laboratory evaluation of α₁-antitrypsin (A1AT) deficiency involves measurement of circulating A1AT protein (quantitation) and characterization of A1AT genetic polymorphisms (phenotyping or genotyping). This study compared adult and pediatric A1AT reference ranges in patients with nondeficiency alleles and examined A1AT concentrations in multiple other phenotypes. A1AT phenotype and quantitation were retrospectively collected on adult (n = 21,444) and pediatric (n = 2,469) samples that were submitted for laboratory evaluation of A1AT deficiency. The 95% reference ranges for normal adult and pediatric populations with the M/M phenotype were determined to be 100 to 273 mg/dL (18.4-50.2 μmol/L) and 93 to 251 mg/dL (17.1-46.2 μmol/L), respectively (P < .0001). Decreased concentrations of A1AT correlated with heterozygosity and homozygosity for the S and Z alleles in both the adult and pediatric groups. Other rare alleles, such as I, were also associated with decreased concentrations of A1AT, particularly in the context of a Z allele, and may warrant monitoring for symptoms of deficiency.

α₁-Antitrypsin (A1AT) is a 52-kDa serine protease inhibitor that is produced by the liver, circulates in the plasma, and diffuses into the lungs.¹,² It functions to inhibit neutrophil elastase in tissues with significant neutrophil burdens, such as the lungs.³ Deficiency of A1AT occurs with a relatively high prevalence and is caused by genetic variations in the A1AT gene.⁴ The gene encoding A1AT can exist as one of more than 100 different alleles and can be classified as normal, deficient, null, or dysfunctional.² Many alleles are associated with normal A1AT concentration and function. The M allele is the most common quantitatively and functionally normal variant, and accounts for approximately 95% of the allele frequency in most ethnic groups.⁵ The 2 most prevalent deficiency alleles, which result in decreased circulating concentrations of A1AT, are designated as Z and S. The Z allele produces a protein that polymerizes to form inclusion bodies within the hepatocytes, such that very little is secreted into the circulation.⁶-⁸ The S allele also results in quantitative deficiency because of intracellular degradation.⁹,¹⁰ Patients with the Z and/or S alleles are at risk of developing expiratory airflow obstruction because of panlobular emphysema and/or bronchiectasis, that is, chronic obstructive pulmonary disease (COPD) caused by unimpeded neutrophil elastase activity.¹¹-¹³ Patients with A1AT deficiency and a history of smoking or other inhalational exposures may have accelerated incidence of pulmonary manifestations.¹⁴,¹⁵ In addition, patients with the Z allele are also at increased risk for liver cirrhosis, caused by accumulation of polymerized A1AT within the hepatocyte.⁷ Null alleles are those that result in undetectable circulating protein caused by production of either unstable transcript or
protein. Dysfunctional alleles produce a sufficient quantity of protein, but with reduced elastase inhibitory activity.

Diagnosis of A1AT deficiency is a 2-step process. Serum concentrations of A1AT are measured to detect a quantitative deficiency, and A1AT is characterized by phenotype or genotype to identify the cause of the low A1AT concentration. Identification of the specific A1AT alleles confirms the genetic deficiency and provides information as to whether the patient is at risk for liver damage in addition to lung manifestations.

The clinical features that trigger testing are broad. In adults, a decreased α₁ fraction on serum protein electrophoresis, evidence of certain pulmonary obstructive defects such as emphysema or COPD, unexplained liver disease, or a family history of emphysema or liver disease are some of the clinical features that warrant suspicion of A1AT deficiency. Significant COPD is not generally evident until later in life, and rarely triggers evaluation in young children. We undertook this study to verify adult and pediatric reference ranges for A1AT quantitation in the nondeficient reference population. In addition, we established 95th percentile ranges for various A1AT phenotype groups to which we will refer throughout the article as interpretive ranges for phenotypes associated with A1AT deficiency.

Materials and Methods

Serum Samples

All results of A1AT phenotype and quantitation were retrospectively collected on adult (≥18 y) (n = 21,444) and pediatric (<18 years) (n = 2,469) samples submitted for clinical testing from the Mayo Clinic and Mayo Medical Laboratories (Rochester, MN) between January 2008 and December 2009. Quantitative and phenotype results were always obtained from the same sample. If patients were tested more than once, only the first set of results was included in the analysis, thereby yielding 21,406 adult and 2,461 pediatric results. No medical records were accessed for this study.

A1AT

A1AT was quantitated by means of immunonephelometry on a Siemens Nephelometer II (Siemens Healthcare Diagnostics, Tarrytown, NJ) with commercially available standard and monospecific antisera (Siemens) according to the manufacturer’s instructions. Phenotype analysis was performed with isoelectric focusing and immunofixation (Sebia, Norcross, GA). Samples were isoelectrofocused using an agarose gel and immunofixed with peroxidase-conjugated anti-A1AT antiserum. Patient samples were assessed according to the manufacturer’s instructions; controls of known A1AT phenotypes were included for comparison purposes. Three laboratory technicians interpreted each result to reach consensus. If no consensus was met, or a rare phenotype was suspected, the sample was reanalyzed next to an electrophoresis lane that contained an appropriate control.

Reference and Interpretive Ranges and Statistics

Phenotypes that we considered comparable were grouped together to have sufficient data for the rarer phenotypes. The percentage of each phenotype (or group) for pediatric and adult patients was compared with the χ² test (or Fisher exact test) where appropriate. Distributions of the quantitative results were compared between adults and children using the Kolmogorov-Smirnov test for phenotypes with at least 30 samples. Reference ranges (2.5th and 97.5th percentiles) were calculated within each adult and pediatric phenotype of sufficient sample size. Quantile regression was used to assess whether the estimated percentiles were significantly associated with age or gender. Using the QUANTREG procedure in the SAS software (version 9, SAS Institute, Cary, NC), the percentiles were estimated using a bootstrap resampling procedure with replacement with 10,000 replicates. P values less than .05 were considered statistically significant.

Results

Phenotype Distributions

All A1AT phenotype and quantitative results were collected from routine clinical testing over a 2-year period. If duplicate testing was ordered on the same patient, only the first set of laboratory results was included in the analysis. The alleles that our clinical laboratory can identify, in part, are described in Table 1. For many of these alleles, the specific amino acid sequence variation is known and is indicated. In addition, it is indicated if the alleles are associated with reduced A1AT serum concentrations.

The frequencies of the phenotypes observed in the 21,406 adult and 2,461 pediatric samples are tabulated in Table 2. As expected, the most common phenotype identified was that of M/M, comprising slightly more than 80% of the adult and pediatric groups. The distribution of samples homozygous for the Z allele was different between the adult and pediatric cohorts. The percentage of Z/Z homozygotes in pediatric samples was nearly 3-fold higher. In the pediatric group, the Z/Z phenotype was the most prevalent deficiency phenotype, followed by the S/Z and S/S. The M/S and M/Z heterozygotes were detected in 8.0% and
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7.4% of the adult samples, respectively, and in 6.0% and 5.5% of the pediatric samples, respectively. The other most commonly identified deficiency allele was the I allele, but this was less than 1.0% of the total cohort. In addition to the common M alleles, individuals with rare alleles considered to be nondeficient were identified in combination with M, Z, and S (Table 2). A small number of adult samples were also identified to carry only the F allele. Lastly, in 38 adults (0.2%) and 8 children (0.3%), 3 alleles were identified (data not shown). These results were presumed to reflect blood transfusion or replacement therapy and were excluded from further analysis.

Phenotype-Segregated A1AT Reference and Interpretive Ranges

Reference ranges for adult and pediatric patients with the M/M phenotype were determined by calculating 95% reference intervals using quantile regression analysis. Overall, for the adult population, the reference range for nondeficiency M/M was calculated to be 100 to 273 mg/dL (18.4-50.2 μmol/L). A slight age dependence was noted, with the lower reference limit for 18 to 29 year olds at 97 mg/dL (17.8 μmol/L) increasing to 108 mg/dL (19.9 μmol/L) for those aged 75 years or older (data not shown). This trend was also observed when considering the entire age range; specifically, the pediatric 95% range was slightly lower at 93 to 251 mg/dL (17.1-46.2 μmol/L) compared with adults. In the pediatric group, a slight age-dependent narrowing was noted, with ranges calculated for those younger than 1 year of age vs those aged 10 to 17 years being 89 to 269 mg/dL (16.4-49.5 μmol/L) and 99 to 243 mg/dL (18.2-44.8 μmol/L), respectively. These trends were found to be statistically significant (P < .0001), but not likely clinically relevant because of the small differences in A1AT concentrations.

Table 3 also shows the associated interpretive 95% ranges for the M/S, M/Z, S/S, S/Z, and Z/Z phenotypes. In adults, the M/S phenotype had the mildest quantitative deficiency,
with the distribution slightly lower than the M/M phenotype. The S/S phenotype was also only mildly decreased, but to a greater extent than the M/S group. Of those individuals who carried the Z allele, M/Z heterozygotes had the mildest deficiency, but the median was lower than the M/M, M/S, and S/S groups. Following a similar trend, the concentration of A1AT was further decreased in the S/Z phenotype, followed by the Z/Z phenotype, which showed the most pronounced A1AT quantitative abnormality.

For the pediatric population, median and total ranges are shown for each phenotype, but the 95% ranges were only calculated for those groups with at least 30 individuals. In general, the association between A1AT quantitation and specific phenotype groups in pediatrics mimicked that of the adult cohort. Similar to the nondeficient reference ranges in the M/M populations, for those groups with a common deficiency allele, the quantitative distributions were statistically different between adults and pediatrics; however, these differences are also unlikely to be clinically significant (Table 3).

Figure 1 shows a graphic representation of the A1AT quantitative results for all phenotypes, including those with rare deficiency and nondeficiency alleles. Patients with A1AT concentrations less than 50 mg/dL (9.2 μmol/L) are at risk for A1AT deficiency disease. Because A1AT is an acute phase reactant, concentrations between 50 mg/dL (9.2 μmol/L) and 80 mg/dL (14.8 μmol/L) do not rule out a diagnosis of A1AT deficiency and may be a cause for concern about future clinical manifestations. Horizontal lines designating these concentrations, along with the percentage of results falling below 50 mg/dL (9.2 μmol/L) and 80 mg/dL (14.8 μmol/L) are shown in Figure 1.

![Box plots of α₁-antitrypsin (A1AT) quantitation by phenotype group. A, Adult, and B, pediatric distributions (next page). Boxes contain the central 50% of the data with the line and the circle inside the box representing the median and mean, respectively.](image-url)
In the adult population (Figure 1A), only a small number of samples in the M/M, M/S, and S/S phenotypes, 0.4%, 1.6%, and 16.7% respectively, had concentrations less than 80 mg/dL (14.8 μmol/L), consistent with the medians and 95% ranges shown in Table 3. In contrast, 28.3% of the M/Z group, 85.7% of the S/Z group, and 99.3% of the Z/Z group fell below the 80 mg/dL (14.8 μmol/L) cutoff. However, of these phenotypes, only the Z/Z homozygotes had a significant proportion of individuals with concentrations less than 50 mg/dL, consistent with a more quantitative abnormality. Within the groups of adults carrying the I allele, the range of A1AT concentrations in the I/M, I/P, and I/S groups is similar to the M/M and M/S groups, with no samples falling below 80 mg/dL (14.8 μmol/L). In heterozygous I/Z samples, however, 57.1% of samples fell below the 80 mg/dL (14.8 μmol/L) cutoff, but none had concentrations less than 50 mg/dL (9.2 μmol/L). However, the single adult sample that expressed protein only from the I allele was found to be deficient, with an A1AT concentration of 45 mg/dL (8.2 μmol/L).

Adult samples heterozygous for the M allele and a rare nondeficiency allele had serum A1AT concentrations similar to homozygous M/M samples, with only 1.5% having concentrations less than 80 mg/dL (14.8 μmol/L). In addition, 7.1% of F/S and P/S heterozygotes had concentrations less than 80 mg/dL (14.8 μmol/L), which is not significantly different from the M/S group (P = .21). However, when a rare nondeficiency allele, specifically C, P, or F, is present in the context of a Z allele, 59.1% of samples fell below the 80 mg/dL (14.8 μmol/L) clinical cutoff, which is significantly greater than the percentage of M/Z heterozygotes (P = .003). In addition, close to 20% of this population also had concentrations less than 50 mg/dL (9.2 μmol/L). Lastly, in the small number of adults in

![Figure 1](cont) The percentages of the population falling below 80 mg/dL and 50 mg/dL are indicated along the x axis. # = presence of B, C, E, F, G, P, V, X as second allele. A1AT concentrations are given in conventional units; for conversion to Système International units (μmol/L), multiply by 0.184.
whom only the F allele was detected (n = 4), all individuals had concentrations higher than 80 mg/dL (14.8 μmol/L).

Similar trends can be seen in the pediatric population (Figure 1B). Of the common allele combinations (M/M, M/S, M/Z, S/S, S/Z, and Z/Z), only M/Z, S/Z, and Z/Z populations had a significant number of samples, 29.6%, 86.4%, and 97.9%, respectively, with A1AT concentrations below the 80 mg/dL (14.8 μmol/L) cutoff, with almost 95% of the Z/Z homozygotes also falling below 50 mg/dL (9.2 μmol/L). In addition, all pediatric samples with I/M, I/P, or I/S phenotypes had normal A1AT concentrations, whereas the 2 I/Z pediatric samples fell between the 50 mg/dL (9.2 μmol/L) and 80 mg/dL (14.8 μmol/L) cutoffs. Also similar to the adult cohort, all pediatric samples in which the samples were heterozygous for the M and another nondeficiency allele had A1AT concentrations higher than 80 mg/dL (14.8 μmol/L). However, in contrast to the adult population, the 2 F/S and P/S pediatric samples fell below the 80 mg/dL (14.8 μmol/L) cutoff, while both had A1AT concentrations greater than 50 mg/dL (9.2 μmol/L).

**Discussion**

We found differences between adult and pediatric reference and interpretive ranges that were statistically significant but unlikely to be clinically relevant. Therefore, our data suggest that the same reference range can be applied to both adult and pediatric populations. In addition, we found a slight increase in serum A1AT concentrations with increasing age in homozygous M allele adults but no such association existed in the other phenotype groups tested (M/S, M/Z, S/S, S/Z, and Z/Z). Therefore, age-independent reference ranges can be used.

Of note, the upper limit of the adult M/M reference range was found to be 273 mg/dL (50.2 μmol/L), a concentration that is higher than our clinical laboratory’s upper limit of 190 mg/dL (35 μmol/L) based on 120 healthy donors. The elevated upper limit in the current study likely reflects the fact that A1AT is an acute phase reactant and that this study population is not a healthy normal-donor group. Therefore, the data presented here should guide clinicians and laboratorians as to the possible ranges observed within each phenotype group. In investigations of deficiency the lower reference limit is generally not associated with clinical manifestations of A1AT deficiency. Our data suggest that perhaps a subpopulation in the M/Z group may be at risk, particularly in the context of another liver insult, such as alcoholic or viral hepatitis, or prolonged exposure to inhalants that may exacerbate damage to the pulmonary system.

Similar observations can be made focusing on the phenotype groups containing an I allele, commonly thought to be a mild deficiency allele. Comparable to the M/M and M/S phenotypes, no samples were less than 80 mg/dL (14.8 μmol/L) in the I/M, I/P, and I/S groups. This would seem to indicate that the I allele causes a mild deficiency, perhaps even less severe than the S allele. However, the majority of the adult samples and both pediatric samples with the I/Z phenotype had A1AT concentrations between 50 mg/dL (9.2 μmol/L) and 80 mg/dL (14.8 μmol/L) similar to the S/Z phenotype. The single sample in which only the I allele was detected had a concentration of 45 mg/dL (8.3 μmol/L), suggesting a risk for clinical manifestations associated with a deficiency. Because null alleles cannot be detected with the phenotyping gels, it is not possible to determine whether the patient was I/I or I/null. The data, however, suggest that the I allele may be clinically relevant, particularly as an I/Z homozygote or in the context of a Z or null allele.

In the phenotype groups heterozygous for a rare nondeficient allele (C, F, or P) and an M allele, very few samples fell below the 80 mg/dL (14.8 μmol/L) cutoff. However when a rare nondeficient allele was combined with a Z allele, significantly more samples had A1AT concentrations below the 80 mg/dL (14.8 μmol/L) and 50 mg/dL (9.2 μmol/L) cutoffs compared with the M/Z phenotype. One could hypothesize that some of these rare alleles could preferentially form heteropolymers with the Z allele, leading...
to an overall decrease in A1AT secretion from the liver.\textsuperscript{28} Alternatively, it is possible that these alleles produce slightly less protein than the M allele, which only becomes apparent if paired with the severe Z deficiency allele. Regardless, patients who are nondeficient/Z phenotype may be more susceptible to clinical manifestations of a deficiency compared with those of M/Z phenotype.

Lastly, reports in the literature describe the F allele as deficient, dysfunctional, or nondeficient.\textsuperscript{4,29,30} In samples in which only an F allele was identified (n = 4), none fell below the deficiency cutoff, which would indicate that these patients are not at risk of manifesting clinical complications from deficiency. However, if the protein produced by the F allele is indeed dysfunctional, as reported in previous publications, this circulating concentration of A1AT may be insufficient to adequately inhibit neutrophil elastase.

It is estimated that 94% to 96% of white patients possess the M/M phenotype, with 2% to 4% being M/Z heterozygotes and 3% to 8% being M/S heterozygotes.\textsuperscript{22} As expected, our patient cohort included a higher proportion of non-M/M phenotypes because of referral bias. In addition, a higher percentage of Z/Z samples was observed in the pediatric compared with adult populations. We hypothesize that, because the Z/Z phenotype causes the most severe clinical symptoms, sometimes manifesting earlier in life, it is likely that a higher proportion of Z/Z patients are tested at a younger age. Conversely, we found a larger percentage of heterozygous M/S and M/Z samples in our adult population than in the pediatric population, possibly because of carrier status testing, especially if their spouse is known to have a deficiency or to be a carrier. Finally, a higher percentage of phenotypes heterozygous for M and a rare nondeficiency allele were present in the pediatric vs the adult samples. The reason for this difference is not readily apparent. In samples analyzed from infants, our laboratory noted the presence of extra anodal bands on electrophoresis that can be difficult to distinguish from the C allele (unpublished observation). This may, at least in part, explain the increased frequency of these specific rare nondeficient alleles in children.

There were 38 adults and 8 children whose phenotyping results consisted of 3 alleles, which we interpret as the likely result of a blood transfusion or A1AT replacement therapy.\textsuperscript{31,32} All triple-allele samples contained an M allele and the quantitation range of A1AT in these samples indicated no deficiency. Interestingly, not all samples with 3 identifiable alleles contained a Z allele, indicating that the third allele was unlikely to be present because of the treatment of deficiency. Regardless of the cause, all samples with triple alleles were excluded from our analysis.

In summary, this work outlines reference and interpretive quantitative ranges for multiple phenotypes of A1AT for both adult and pediatric populations. We suggest that laboratories can apply the same reference range for both adult and pediatric populations because of small concentration differences that are not likely to be clinically relevant. Patients who harbor at least 1 copy of the Z allele are most likely to have circulating concentrations of A1AT in the clinically deficient range. Therefore, these patients may be more susceptible to clinical manifestations of deficiency, especially when combined with environmental exposure or comorbidities that could exacerbate the deficiency manifestations.

References


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