Simultaneous Phenotyping and Quantification of α-1-Antitrypsin by Liquid Chromatography/Tandem Mass Spectrometry

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BACKGROUND: α-1-Antitrypsin (A1AT)6 deficiency results from a genetic disorder at 2 common loci. Diagnosis requires quantification of A1AT and subsequent identification of the specific variant. The current algorithm of laboratory testing for the diagnosis of A1AT deficiency uses a combination of quantification (nephelometry), genotyping, and/or phenotyping. We developed a multiple reaction monitoring liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for simultaneous quantification of A1AT and identification of the 2 most common deficiency alleles present in 95% of the patients with A1AT deficiency.

METHOD: Serum samples (n = 40) were digested with trypsin, and appropriate 13C/15N-labeled standard peptides were added. We performed LC-MS/MS analysis with a 0.5- by 150-mm C18 column and H2O:acetonitrile:n-propanol:formic acid (A:98:1:1:0.2 and B:10:80:10:0.2; flow 12 μL/min) mobile phase in positive ion mode on a TSQ Quantum triple quadrupole MS system. We measured the A1AT concentration by comparison to a calibration curve and determined the phenotype by the presence or absence of variant peptides. We compared the results to the current phenotyping assay by isoelectric focusing (IEF) and the immunonephelometry quantitative assay.

RESULTS: For A1AT allele detection, in 39 of 40 samples the LC-MS/MS results were identical to those obtained by IEF gel electrophoresis. The single discrepant result was rerun by IEF at a lower dilution, and the results were in concordance. The A1AT quantification by LC-MS/MS also compared favorably with nephelometry.

CONCLUSIONS: The LC-MS/MS method correlates well with current phenotyping and nephelometric assays and has the potential to improve the laboratory diagnosis of genetic A1AT deficiency.

α-1-Antitrypsin (A1AT) is a member of the serine protease inhibitor (SERPIN) family. A1AT is produced in the liver and transported to the lungs, where it functions to inhibit neutrophil elastase (1). After binding, elastase cleaves a reactive loop of A1AT, resulting in a portion of A1AT inserting into the β-sheet structure of elastase. A1AT is then trapped against elastase, leading to lysosomal degradation of the A1AT/elastase complex (2–5).

A1AT is a relatively polymorphic gene, with >100 polymorphisms identified (6). These mutations result in decreased plasma concentrations of A1AT (7). A lack of functional A1AT leads to uncontrolled proteolytic tissue damage mediated by elastase (8), a disorder known as A1AT deficiency. The 2 most common disease-associated alleles found in patients with genetic A1AT deficiency are the S and Z alleles. The S allele contains a substitution of valine for glutamate at codon 288 (E288V), resulting in production of an unstable transcript and decreased protein expression (9, 10). Individuals who are homozygous for the S allele are at risk for developing chronic obstructive pulmonary disease (COPD) due to proteolytic damage of the lung tissue. The Z variation consists of a glutamate-to-lysine substitution at codon 366 (E366K) (11). This mutation causes a conformational change such that A1AT polymers form within the hepatocytes. The polymerized...
A1AT is not efficiently secreted, leading to decreased circulating concentration and an increased risk for COPD, as with the S allele (11). The polymerization of the Z variant, however, also damages the hepatocyte (12). Individuals who are Z/Z homozygotes may also develop liver cirrhosis, possibly requiring transplantation (13).

The diagnosis of A1AT deficiency requires quantification of circulating A1AT and confirmation of the presence of A1AT disease alleles. Quantification may be accomplished by a variety of methods, the most commonly used techniques being immunonephelometry and antitryptic activity (14). Identification of the deficient alleles can be performed by phenotyping and genotyping. Phenotyping uses isoelectric focusing (IEF) gel electrophoresis to separate the serum proteins based on charge. The specific A1AT alleles are identified based on migration patterns within the gel (15, 16). In contrast, genotyping uses molecular approaches to identify the mutations at the DNA level (17, 18). Some genotyping techniques, including allele-specific amplification and melting-curve analysis, detect only the S and Z alleles, whereas others, such as sequencing, have the ability to identify any mutation, although this is usually restricted to exons. The various phenotyping and genotyping assays have advantages and disadvantages. For this reason, most algorithms proposed for the diagnosis of A1AT deficiency recommend a combination of laboratory tests (19). Our laboratory has proposed an algorithm in which genotyping and A1AT quantification are performed as the first level of testing, with phenotyping used as a reflex test in cases where the A1AT concentration does not fall into the range expected for the given genotype (19). Given the various pieces of information required to make a diagnosis of A1AT deficiency, a multiplex assay capable of simultaneously quantifying A1AT and identifying the deficiency alleles would be a substantial improvement over the current laboratory testing paradigm.

Mass spectrometry (MS) is emerging as a powerful tool for the identification and quantification of human plasma proteins. Transferrin (carbohydrate-deficient glycoprotein syndrome) and transthyretin (familial amyloidosis) provide good examples of MS applied to intact protein analysis (20, 21). Other groups have shown that peptides from protease digests can be used to quantify proteins by use of liquid chromatography/tandem MS (LC-MS/MS) (22–25). We have applied this approach for the simultaneous determination of A1AT deficiency and detection of S and Z A1AT alleles, including the determination of zygosity.

Materials and Methods

REAGENTS
We purchased research-grade ammonium bicarbonate (NH₄HCO₃), trifluoroethanol (TFE), iodoacetamide (IAA), trifluoroacetic acid (TFA), and TPCK-treated trypsin (T-1426) from Sigma-Aldrich; dithiothreitol (DTT) and formic acid from Fluka; fetal calf serum (FCS) from Invitrogen (Gibco 10437-028); Zwittergent detergent 3-16 (Z 3-16) from Calbiochem; and highly purified A1AT reference material from Athens Research & Technology (16-16-011609). Water, acetonitrile, n-propanol, and dimethylformamide (DMF) were HPLC grade. Isotopically labeled peptide standards were synthesized in the Mayo Proteomics Core; their purity was found by analytical HPLC to be ≥90%. Designations and labeling strategy are shown in Table 1. We prepared stock peptide solutions in DMF at 1 mmol/L concentrations. We used a molecular weight of 52 kDa in all A1AT calculations (26) and an absorbivity for A1AT of 4.33 (A280, 1%, 1 cm) for quantification of the protein standard (27).

TRYPsin DIgESTion PROtocols
Lyophilized, purified human A1AT was dissolved in water or FCS at appropriate concentrations. A1AT solution (5 µL, or 5 µL serum) was denatured with 25 µL TFE and diluted with 25 µL 100 mmol/L NH₄HCO₃, and 5 µL of 200 mmol/L DTT was added and incubated for 30 min at 55 °C. The reduced samples were treated with 10 µL of 200 mmol/L IAA and incubated for 1 h at room temperature in the dark with shaking. The samples were diluted with 400 µL of 100 mmol/L NH₄HCO₃, treated with 25 µL of 1 g/L trypsin (approximately 1:20 wt:wt), and incubated for exactly 6 h at 37 °C. Digestion was terminated with 10 µL of 10% formic acid (Fluka) after 6 h. The prepared samples were stored at −20 °C before analysis.

IDENTIFICATION OF SIGNATURE TRYPTIC A1AT PEPTIDES
We analyzed the pure A1AT digest by LC-MS/MS and identified signature peptides that contained the S and Z allele mutations (for LC-MS/MS conditions, see Supplemental Data Materials, which accompany the online version of this article at http://www.clinchem.org/content/vol57/issue8). For the S (E287V, LQHLNELTHDIITK) and Z (E365K, AVLTIDK) alleles, the choice of peptides was dictated by the location of the mutations. In addition, we selected a proteotypic peptide—a peptide that is unique to a single protein—for the quantification of A1AT (SASLHLPK). SASLHLPK was selected because it does not contain any known polymorphisms, has an amino acid sequence specific to A1AT, does not contain any glycosylation sites, and fragments readily to provide a good product ion for LC-MS/MS analysis. Fragment ions
identified from the LC-MS/MS data of these 3 peptides determined the optimal transition states for A1AT quantification and the S and Z variant analysis. All selected peptides were synthesized as unlabeled and [13C6 15N]leucine labeled for use as internal standards.

STUDY POPULATION
We obtained serum samples (n = 40) from individuals referred to the Mayo Clinic Immunology Laboratory for evaluation of possible A1AT deficiency. All samples were submitted for A1AT quantification and phenotyping and were analyzed after approval by an institutional review board.

LC-MS/MS ANALYSIS OF PATIENT SERUM SAMPLES
Serum was digested according to the method described above for purified A1AT, substituting 5 μL serum for the 5 μL purified A1AT. After digestion, we added dilutions of labeled peptides (designated*) into the digest mixture for final concentrations of 2 μmol/L (proteotypic*), 0.5 μmol/L (Mu-Z*, WT-Z*), and 1 μmol/L (Mu-S*, WT-S*) and loaded 2 μL onto the column. We performed LC-MS/MS as described in the online Supplemental Data Materials.

We identified S and Z A1AT alleles by comparing the response to labeled peptides, which were used to authenticate retention times and instrument response.

We obtained stock solutions of A1AT by dissolving highly purified, lyophilized A1AT from Athens Research & Technology in FCS at 1.0 –77 μmol/L. After digestion and LC-MS/MS analysis, we calculated area ratios for the unlabeled and labeled proteotypic peptides. We constructed calibration curves by plotting the peak area ratio against the A1AT concentration, bracketed samples by calibration curves pre- and postsamples, and determined intraassay and interassay imprecision by analyzing 3 samples at low, medium, and high A1AT concentrations 10 times each over 4 days. We determined the limit of quantitation (LOQ) by serial dilutions of a standard solution: we measured 6 replicates at each dilution and used the lowest concentration giving a CV ≤10% for the LOQ.

IDENTIFICATION OF S AND Z A1AT ALLELES BY PHENOTYPING
Phenotyping of A1AT was performed by use of the Hydragel A1AT Isofocusing system (Sebia) according to manufacturer’s instructions. Briefly, serum proteins were separated by isoelectric focusing on agarose gels, and A1AT was visualized by immunofixation using peroxidase-labeled A1AT antisera.

A1AT QUANTIFICATION BY NEPHELOMETRY
A1AT was quantified by nephelometry on a Behring Nephelometer II (Dade Behring) by use of commercially available reagents and standards (Siemens; A1AT reagent PSAZ15 and calibrator/standard OQIM15). All assays were performed according to manufacturer’s instructions.

Table 1. Peptide sequences, labeling, and MS/MS transitions monitored in the LC-MS/MS experiment.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Designation</th>
<th>Sequence</th>
<th>Transition, m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteotypic</td>
<td>Unlabeled</td>
<td>SASLHLPK</td>
<td>426.8 → 694.4</td>
</tr>
<tr>
<td></td>
<td>Labeled</td>
<td>SASLHLPK</td>
<td>429.5 → 700.2</td>
</tr>
<tr>
<td>Wild Type Z</td>
<td>Unlabeled</td>
<td>AVLTIDEK</td>
<td>444.8 → 718.4</td>
</tr>
<tr>
<td></td>
<td>Labeled</td>
<td>AVLTIDEK</td>
<td>448.4 → 725.5</td>
</tr>
<tr>
<td>Mutant Z</td>
<td>Unlabeled</td>
<td>AVLTIDKK</td>
<td>444.3 → 717.5</td>
</tr>
<tr>
<td></td>
<td>Labeled</td>
<td>AVLTIDKK</td>
<td>447.5 → 723.4</td>
</tr>
<tr>
<td>Wild Type S</td>
<td>Unlabeled</td>
<td>LQHLENELTHDIITK</td>
<td>602.0 → 781.9</td>
</tr>
<tr>
<td></td>
<td>Labeled</td>
<td>LQHLENELTHDIITK</td>
<td>604.7 → 785.7</td>
</tr>
<tr>
<td>Mutant S</td>
<td>Unlabeled</td>
<td>LQHLVNELTHDIITK</td>
<td>592.0 → 766.9</td>
</tr>
<tr>
<td></td>
<td>Labeled</td>
<td>LQHLVNELTHDIITK</td>
<td>594.5 → 770.5</td>
</tr>
</tbody>
</table>

* All peptides were synthesized in unlabeled and labeled forms by incorporation of a single [13C6 15N]leucine at the positions noted by bold underlined type.
**Results**

**PHENOTYPING A1AT VARIANTS BY MASS SPECTROMETRY**

Online Supplemental Fig. 1 shows the sequence of A1AT and the tryptic peptides containing the E288V (S variant, designated Mu-S) and E366K substitutions (Z variant; Mu-Z). The corresponding wild-type peptides are designated WT-S and WT-Z, respectively, and indicate the M allele. The variant peptides, by virtue of the amino acid substitution, differ in mass and retention time from the wild-type peptides, thereby allowing MS detection. The transitions monitored for each peptide and internal standard peptides are shown in Table 1. Fig. 1 shows example chromatograms from control (M/M), M/Z, and S/S phenotypes. In a normal individual, only the wild-type alleles and no Mu-S or Mu-Z peptides are detected (Fig. 1A). In contrast, in a patient who is an M/Z heterozygote, the WT-Z, WT-S, and Mu-Z peptides are identified, but no Mu-S (Fig. 1B).

An individual heterozygous for M/S shows the Mu-S, WT-S, and WT-Z, but no Mu-Z (data not shown). For individuals who are S/S homozygotes, only 2 peptides will be identified: the WT-Z and the Mu-S peptides (Fig. 1C). The opposite would be true for a Z/Z homozygote (data not shown). By identifying each of the 4 peptides, we can determine if either of the common S and Z deficiency alleles are present.

As an initial validation of this method, we analyzed 40 serum samples by LC-MS/MS and IEF gel electrophoresis (phenotyping). Of these 40 samples, 39 showed exact correlation of the allele identification obtained by the 2 methods (online Supplemental Table 1). The single discrepant sample was identified as a Z/Z homozygote by IEF phenotyping, but as an M/Z heterozygote by LC-MS/MS. Further analysis of the LC-MS/MS data revealed that the wild-type M peptide peak was markedly smaller than generally observed in an M/Z heterozygote (data not shown). The sample
was reanalyzed by IEF gel electrophoresis at 1:5 instead of 1:10 dilution, and a band representing the M allele was observed.

**Absolute Quantification of A1AT by LC-MS/MS**

Online Supplemental Fig. 2 shows a calibration curve of the area ratio of unlabeled/labeled peptide vs μmol/L A1AT using high-purity A1AT from Athens Research & Technology (online Supplemental Fig. 3). Low, medium, and high A1AT patient samples were each analyzed 10 times on different days to assess the intraassay and interassay imprecision of the LC-MS/MS method. The imprecision results are summarized in Table 2. The intraassay imprecision (CV) was <5% for analysis of 10 replicates. The interassay imprecision was <5% over a 4-day period. These values compare well with the nephelometric assay, where intraassay imprecision was 5.4% and 5.0% for 0.22- and 23-μmol/L samples, respectively, and interassay imprecision was 5.3% and 3.2% for 19- and 41-μmol/L samples. The MS-based assay had an LOQ of 1.9 μmol/L. Triplicate analysis of a 9.6 μmol/L A1AT calibrator prepared from the Athens standard in FCS yielded mean (SD) 8.7 (0.3) μmol/L when measured by nephelometry, indicating that our standard was within 10% of the nephelometry calibrators with a slight negative bias.

We analyzed sera from 40 patients encompassing a range of A1AT concentrations by both LC-MS/MS and nephelometry. Fig. 2 shows the relationship between the 2 methods. Fig. 3 indicates that a bias exists in the LC-MS/MS method relative to the nephelometry method when the ratio of the 2 methods is plotted vs μmol/L A1AT. A 1.5 (0.2)–fold difference for controls is obtained with an even greater bias for samples containing variants. A Bland–Altman plot comparing the 2 methods is illustrated in online Supplemental Fig. 4.

**Discussion**

Repeated attempts to analyze intact A1AT proved unsuccessful, probably because A1AT is heavily glycosylated, in contrast to other proteins successfully analyzed in our laboratory (20, 21). In light of this, we developed a multiplexed LC-MS/MS method for detection of A1AT deficiency and associated allele identification. Our main goal in this work was to illustrate a method for accurately phenotyping A1AT. However, since multiple peptide targets can be analyzed in a multiple reaction monitoring (MRM) experiment, A1AT quantification is essentially free. The allele identification is by detection of the tryptic peptides that contain the S and Z variants and comparison to isotopically labeled internal standard peptides as a reference. For A1AT quantification, peak area ratios of the selected proteotypic peptide and internal standard peptide are compared to a calibration curve generated from au-

<table>
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<tr>
<th>Sample</th>
<th>Concentration from nephelometry, μmol/L</th>
<th>Mean intraassay concentration, μmol/L</th>
<th>Intraassay imprecision, %</th>
<th>Mean intraassay concentration, μmol/L</th>
<th>Interassay imprecision, %</th>
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<td>n</td>
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<td>Patient 1</td>
<td>48.8</td>
<td>66.1</td>
<td>4.3</td>
<td>63.6</td>
<td>3.1</td>
</tr>
<tr>
<td>Patient 2</td>
<td>29.2</td>
<td>37.9</td>
<td>3.4</td>
<td>37.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Patient 3</td>
<td>4.6</td>
<td>6.4</td>
<td>4.1</td>
<td>6.3</td>
<td>2.6</td>
</tr>
</tbody>
</table>

**Fig. 2.** Comparison of A1AT quantitation between nephelometry and LC-MS/MS.

Ordinary least-squares regression analysis comparing the concentration of A1AT in serum samples (n = 40) measured by LC-MS/MS using the proteotypic peptide as described in “Materials and Methods.” The results from the LC-MS/MS method were compared to those obtained by nephelometry. Solid line indicates the regression and has a correlation coefficient of 0.91; dotted line indicates perfect correlation; dashed lines indicate 95% confidence limits.
authentic protein spiked into FCS at known concentrations. FCS was used as a background matrix to simulate human serum, as no human serum completely deficient in A1AT is available. Online Supplemental Fig. 5 indicates that no matrix effects result from the use of FCS.

The diagnosis of genetic A1AT deficiency requires more than quantification alone, because circulating concentrations of A1AT, an acute-phase protein, may increase during infections, even in individuals with a true deficiency (19, 28). In contrast, concentrations may decrease in the absence of a genetic mutation, such as with an unrelated liver disease or protein-losing enteropathy (19). For these reasons, identification of the deficiency alleles and quantification are both important to establish a diagnosis of genetic A1AT deficiency. Current testing methodologies center on nephelometry for quantification and phenotyping by IEF gel electrophoresis and DNA-based genotyping for detection of the deficiency alleles. Many laboratories also offer testing algorithms, generally recommending genotyping and quantification, with follow-up by phenotyping in cases of discrepant results (6). Although well accepted, these testing methodologies and algorithms can be problematic. Most genotyping assays are designed to specifically detect only the S and Z mutations. This is a substantial limitation, since other, rare deficiency alleles are not detected. Phenotyping has the advantage that many different alleles, not just Z and S, can be identified as long as the amino acid substitution results in an altered pI. Accurate identification of these variants requires well-characterized reference standards that are not commercially available for the rare deficiency alleles and must be developed and maintained by the individual clinical laboratory. In addition, phenotyping is a labor-intensive assay with relatively subjective interpretation. A comparison of genotyping and phenotyping revealed that approximately 70% of discrepancies between the 2 methodologies were due to phenotyping errors (19). The current diagnostic algorithms, which combine genotyping and phenotyping in a way to minimize the disadvantages of both methods (19) are, however, also problematic. A major disadvantage of most algorithms is the requirement for 2 separate blood samples—whole blood for genotyping and serum for quantification and phenotyping, if needed. This increases the volume of blood required for testing, which is a critical issue for the pediatric population, as Z/Z homozygotes may present with neonatal hepatitis syndrome shortly after birth. The requirement of 2 samples also increases the chances of a specimen mix-up. In addition, the genotyping assay has a relatively long turnaround time of 3–4 days. For those samples requiring confirmation by phenotyping, there is an additional time delay, as long as 48–72 h, before the result is available to the clinician.

Our LC-MS/MS method for diagnosis of A1AT deficiency offers several advantages over current A1AT laboratory testing. The LC-MS/MS method is similar to the phenotyping assay in the respect that both methods identify the deficiency alleles based on analysis of the circulating A1AT protein. The LC-MS/MS method has the advantage of being much less labor-intensive and has the capability to both quantify and phenotype A1AT in a single 20-min assay. This method also eliminates the subjective interpretation that surrounds phenotyping by electrophoresis. Despite these advantages, the LC-MS/MS method, in its current form, detects only the common S and Z mutations (M/M, M/S, M/Z, S/S, Z/Z, and S/Z genotypes). Although 95% of all A1AT deficient individuals are homozygous for the Z allele and would be detectable by this methodology (6), other mutations have been described that result in the clinical disease A1AT deficiency (26). Target peptides corresponding to the other rare deficiency alleles that phenotyping by IEF is capable of identifying will need to be added if the LC-MS/MS method is to completely replace phenotyping. For example, the next most common deficiency allele seen in our practice, the I allele, has an Arg63Cys substitution contained in the tryptic peptide ITPNLAEFAFSLYR (29). This conversion removes a tryptic cleavage site that would result in decreased amounts of this peptide after A1AT trypsin digestion. Additionally, 7 known variants reside in the next tryptic peptide QLAHQSNSTIFSVPSIATAFAMLSSLGTK, including L65P, S69F, F75 deletion, S77F, A84T, G91E, and T92I. However, this partic-
ular peptide and the adjacent tryptic peptide (ADT HDEILEGLNFINLTEIPAQIHEGFQELLR) contain the
known glycosylation sites (Asn70 and Asn170; in bold and underlined). Glycosylated peptides are not easily
detected by mass spectrometry without prior removal of the glycosylation. We did not observe any of the
tryptic peptides containing the glycosylation sites in our initial proteomics analysis (online Supplemental
Data Fig. 1). Optimizing the methodology to expand the number of deficiency alleles detected by the LC-
MS/MS method is a warranted next step.

We initially thought some type of quantification would be necessary to ascertain the S and Z variants.
However, we found that the presence of signal corresponding to the variant peptides was sufficient diag-
nostically. So although not needed for quantitative purposes, the presence of internal standards serves to
indicate only that the LC-MS/MS system is operating correctly. Utilizing Cohen’s $\kappa$ coefficient across the 6
genotypes of online Supplemental Table 1 yields a $\kappa$ value of 0.96, indicating excellent agreement between
IEF phenotyping and the LC-MS/MS phenotyping method (30).

Although in this work only a single transition was monitored for each peptide, future work should in-
clude additional transitions for each peptide to ensure specificity. We did not find interferences in any of our
studies utilizing a single transition. This assay has some advantage in this regard since A1AT is 1 of the 12 most
abundant plasma proteins, and only a tryptic peptide from another abundant plasma protein is likely to
interfere.

The mutant Z peptide we eventually monitored was AVLTIDKK, which is a missed cleavage. We moni-
tored both AVLTIDK and AVLTIDKK initially; however, the response for the fully tryptic AVLTIDK was
much less abundant than the semitryptic AVLTIDKK and thus we settled on only looking at the missed cleav-
age peptide AVLTIDKK. Trypsin missed cleavages ad-
jaent to basic residues are common and have already
been noted in the literature (31–34). Our studies
yielded similar results.

Although Fig. 2 shows a good correlation between nephelometry and the MRM approach, a 1.5 (0.2)–fold
positive bias does exist for control samples with a greater bias for variants (Fig. 3). Use of a surrogate
tryptic peptide for quantification of intact proteins measures all forms of a protein, both intact and de-
graded. Thus, it is not surprising that the MRM-based approach would routinely measure a higher concen-
tration than an antibody-based method, which recognizes a single epitope that is absent in degraded forms of
A1AT. The higher bias in the variants can be attributed
to the presence of even more degraded A1AT relative to
intact A1AT. A calibrator measured by both nephe-
ломetry and LC-MS/MS showed a slight negative bias
error of 10% on our highly purified A1AT (online Sup-
plemental Fig. 3), indicating that the positive bias of the
LC-MS/MS method is not due to any systematic error.

Although we cannot confirm it, it is likely that the
small amount of the M allele that was detected in the
single discrepant sample by LC-MS/MS was due to A1AT replacement therapy, a common treatment
given to individuals who are homozygous for the Z
allele. In this sample, the M protein was not detectable
in the IEF analysis at the usual 1:10 sample dilution, but
reanalysis at 1:5 dilution showed M was present, con-
firming the LC-MS/MS results.

In conclusion, the LC-MS/MS method described here provides unambiguous phenotyping of A1AT and
simultaneous quantification. As described, it is cur-
rently limited to the detection of the S and Z alleles that
comprise 95% of all cases. However, we envision phe-
notyping of other alleles with appropriate modifica-
tions. The positive quantification bias we found for
A1AT will likely exist for other proteins when this ap-
proach is compared to antibody-based assays.

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References