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Plasma and CSF serpins in Alzheimer disease and dementia with Lewy bodies

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ABSTRACT

Objective: Serine protease inhibitors (serpins), the acute phase reactants and regulators of the proteolytic processing of proteins, have been recognized as potential contributors to the pathogenesis of Alzheimer disease (AD). We measured plasma and CSF levels of serpins in controls and patients with dementia.

Methods: Using rocket immunoelectrophoresis, ELISA, and Luminex xMAP technology, we analyzed plasma levels of α1-antichymotrypsin and α1-antitrypsin, and CSF levels of α1-antichymotrypsin, α1-antitrypsin, and neuroserpin along with three standard biomarkers (total tau, tau phosphorylated at threonine-181, and the Aβ1-42) in patients with AD (n = 258), patients with dementia with Lewy bodies (DLB; n = 38), and age-matched controls (n = 37).

Results: The level of CSF neuroserpin was significantly higher in AD compared with controls and DLB, whereas CSF α1-antichymotrypsin and α1-antitrypsin were significantly higher in both AD and DLB groups than in controls. Results from logistic regression analyses demonstrate a relationship between higher CSF levels of α1-antichymotrypsin and neuroserpin and increased predicted probability and odds ratios (ORs) of AD (OR 5.3, 95% CI 1.3 to 20.8 and OR 3.3, CI 1.3 to 8.8). Furthermore, a logistic regression model based on CSF α1-antichymotrypsin, neuroserpin, and Aβ1-42 enabled us to discriminate between AD patients and controls with a sensitivity of 94.7% and a specificity of 77.8%.

Conclusions: Higher CSF levels of neuroserpin and α1-antichymotrypsin were associated with the clinical diagnosis of Alzheimer disease (AD) and facilitated the diagnostic classification of AD vs dementia with Lewy bodies. Neurology® 2007;69:1–1

GLOSSARY

AAT = α1-antitrypsin; ACT = α1-antichymotrypsin; AD = Alzheimer disease; ApoE = apolipoprotein E; AUC = area under the curve; BBB = blood–brain barrier; COPD = chronic obstructive pulmonary disease; %CV = coefficients of variation percentage; DLB = dementia with Lewy bodies; IL = interleukin; MMSE = Mini-Mental State Examination; NSAIDs = nonsteroidal anti-inflammatory drugs; OR = odds ratio; P-tau = tau phosphorylated at threonine-181; ROC = receiver operating characteristic; T-tau = total tau.

In addition to β-amyloid plaques and neurofibrillary tangles, the pathology of Alzheimer disease (AD) is characterized by excessive inflammation. Inflammation is driven by cytokines (particularly interleukin [IL]-1) that are released from activated microglia and astrocytes, and this in turn drives the expression of IL-6 and inducible nitric oxide synthase. Neuronal proteases that are released as part of the inflammatory response are controlled by a variety of inhibitors including members of the serine protease inhibitor (serpin) superfamily. These include α1-antichymotrypsin, α1-antitrypsin, and neuroserpin. The important role of α1-antichymotrypsin in the pathogenesis of AD was demon-
trated by the finding of raised levels in brain homogenates from affected individuals\(^2\); the finding that it is tightly associated with virtually all \(\beta\)-amyloid plaques\(^3\); and the demonstration that it interacts with, and affects the clearance of, \(\alpha_\beta_{1-42}\).\(^{10,14}\) Moreover elevated levels of plasma and CSF \(\alpha_\tau\)-antichymotrypsin correlate with cognitive decline in elderly nondemented persons and those with AD.\(^{15,17}\) Similarly, \(\alpha_\tau\)-antitrypsin\(^18\) is present in \(\beta\)-amyloid plaques of patients with AD, and isoforms of \(\alpha_\tau\)-antitrypsin are significantly altered in CSF of affected individuals when compared with controls.\(^{19,20}\) \(\alpha_\tau\)-Antitrypsin levels are increased in AD plasma and correlate with heme oxygenase-1 activity and cognitive decline.\(^{21}\)

It has been shown that mutants of the neuron-specific serpin, neuroserpin, underlie the inclusion body dementia familial encephalopathy with neuroserpin inclusion bodies.\(^{22,23}\) Neuroserpin is expressed throughout the nervous system\(^24\) and inhibits serine proteases such as the tissue plasminogen activator; urokinase-type plasminogen activator; and, to a lesser extent, plasmin.\(^{25-27}\) Recently, we have shown that neuroserpin is a plaque-associated protein in the brains of patients with AD and that it forms a 1:1 binary complex with the \(\alpha_\beta_{1-42}\) peptide. This in turn prevents fibril formation and renders the \(\alpha_\beta_{1-42}\) peptide less toxic to neuronal cells.\(^{28}\) Whether neuroserpin is present within the CSF or is involved in AD pathogenesis has hitherto been unknown.

Taken together, these studies suggest that serpins may be associated with AD through initiating some of the neuropathologic changes and reflect the development of the disease. We therefore measured the CSF levels of \(\alpha_\tau\)-antitrypsin, \(\alpha_\tau\)-antichymotrypsin, and neuroserpin as well as the standard markers of AD, i.e., total tau (T-tau), tau phosphorylated at threonine-181 (P-tau), and \(\alpha_\beta_{1-42}\) in patients clinically diagnosed with AD, dementia with Lewy bodies (DLB), and in age-matched nondemented controls.

**METHODS**

**Patients.** Subjects with dementia who enrolled in this study (\(n = 296\)) are a sample of the patients that are included in the Malmö Alzheimer Study. Patients were seen in the Neuropsychiatric Clinic at Malmö University Hospital for evaluation of cognitive dysfunction between 1999 and 2003. Healthy elderly controls (\(n = 37\)) were recruited among relatives of health care personnel and through advertisements at senior citizen clubs. The cognitive status of the subjects was evaluated with the Mini-Mental State Examination (MMSE)\(^{29}\) and Alzheimer’s Disease Assessment Scale–Cognitive subscale.\(^{29}\) The criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, by the American Psychiatric Association (1994) were used for the clinical diagnosis of dementia, and the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association\(^{30}\) were used for probable AD. Probable dementia with Lewy bodies was diagnosed according to the DLB consensus criteria.\(^{31}\) All patients and controls underwent routine laboratory tests, including determination of the apolipoprotein E (ApoE) genotype and measurement of the CSF/serum albumin ratio as an indicator of the blood–brain barrier (BBB) function. In addition, the regular use (as prescribed by a physician) of nonsteroidal anti-inflammatory drugs (NSAIDs) and the presence of other chronic inflammatory diseases, such as atherosclerosis, chronic obstructive pulmonary disease (COPD), and rheumatoid diseases, were also recorded. This study was approved by the ethics committee of Lund University.

**Sample collection of blood and CSF.** Lumbar puncture was performed in the L3–L4 or L4–L5 interspace with the subject in the sitting position. The first milliliter of CSF was discarded, 1 mL was sent for cell analysis, and 10 mL was collected in plastic (polypropylene) tubes. All CSF samples were gently mixed to avoid possible gradient effects. No CSF sample contained more than 500 erythrocytes/\(\mu\)L. The CSF samples were centrifuged at 2,000 \(\times \)g at 4 °C for 10 minutes to eliminate cells and other insoluble material, and were then immediately frozen and stored at −80 °C pending biochemical analyses. Plasma and serum samples were collected at the same time as the lumbar puncture. Blood for plasma analysis was collected in tubes containing EDTA (B-D Vacutainer System, Franklin Lakes, NJ) and centrifuged at 2,000 \(\times \)g at 4 °C for 10 minutes. The aliquots were immediately frozen at −80 °C and stored until assayed.

**Determination of the concentration of \(\alpha_\tau\)-antichymotrypsin and \(\alpha_\tau\)-antitrypsin.** Plasma and CSF levels of \(\alpha_\tau\)-antichymotrypsin and \(\alpha_\tau\)-antitrypsin were determined using rocket immunoelectrophoresis as described by Laurell\(^33\) with in-house modifications. In brief, aliquots of plasma and CSF were run for 1.5 hours at 200 \(\times \)V on 1 mm 0.9% w/v agarose gels containing 11 mg/L anti-human \(\alpha_\tau\)-antitrypsin antibody (DakoCytomation, Glostrup, Denmark), and 6.98 mg/L (for plasma analysis) and 2.79 mg/L (for CSF analysis) anti-human \(\alpha_\tau\)-antichymotrypsin antibody (DakoCytomation, Glostrup, Denmark). Gels were pressed between filter paper and dried before staining with Coomassie Blue. To quantify \(\alpha_\tau\)-antitrypsin and \(\alpha_\tau\)-antichymotrypsin, the distance between the tip of the rocket-shaped immunoprecipitates and the application well was measured. Standard curves were generated by serial dilutions of a standard (Seronorm, Sero AS, Norway) that was run in parallel to samples on every gel. The coefficients of variation...
in 0.2 M Na2CO3/NaHCO3 pH 9.4 overnight at 4°C. After
3590) were coated with capture antibody diluted at 2
secondary antibody. The ELISA plates (Corning Inc. Costar
Prof. D. Lomas’ laboratory (1A10, 10B8, and 10G12) as the
monoclonal anti-human neuroserpin antibodies produced in

cients of variation were both less than 5%.
percentage (%CV) for the interbatch and intrabatch vari-
ing the xMAP technology as described previously.14 In brief, this
technology is based on flow cytomteric separation of
antibody-coated microspheres that are labeled with a speci-
c mixture of two fluorescent dyes. After binding of a bio-
tinylated reporter antibody, quantification is made by
binding of a third fluorochrome coupled to streptavidin. The

technique allows for simultaneous measurement of several
analytes in the same tube. The CSF levels of T-tau, P-tau,
and Ab1-42 correlated well with the levels obtained by con-
tventional ELISA measurements.15 The intra-assay and in-
assay %CV for the multiparametric assay for Ab1-42, T-tau,
and P-tau were less than 9%.

**Determination of Ab1-42, T-tau, and P-tau.** Total
tau, P-tau, and Ab1-42 levels were determined using Luminex
xMAP technology as described previously.14 In brief, this
technology is based on flow cytomteric separation of
antibody-coated microspheres that are labeled with a speci-
c mixture of two fluorescent dyes. After binding of a bio-
tinylated reporter antibody, quantification is made by
binding of a third fluorochrome coupled to streptavidin. The

technique allows for simultaneous measurement of several
analytes in the same tube. The CSF levels of T-tau, P-tau,
and Ab1-42 correlated well with the levels obtained by con-
tventional ELISA measurements.15 The intra-assay and in-
assay %CV for the multiparametric assay for Ab1-42, T-tau,
and P-tau were less than 9%.

**Determination of the concentration of neuroserpin.**
A sandwich ELISA was developed using the antigen-purified
fraction of a rabbit anti-human neuroserpin antibody16 as the
capture antibody and a pool of three high-affinity mouse
monoclonal anti-human neuroserpin antibodies produced in
Prof. D. Lomas’ laboratory (1A10, 10B8, and 10G12) as the
secondary antibody. The ELISA plates (Corning Inc. Costar
3590) were coated with capture antibody diluted at 2 µg/mL
in 0.2 M Na2CO3/NaHCO3 pH 9.4 overnight at 4°C. After
three washes (0.9% w/v NaCl, 0.05% v/v Tween20), the
wells were blocked for at least 1 hour at room temperature
with blocking buffer (phosphate-buffered saline, 0.25% w/v
bovine serum albumin, and 0.05% v/v Tween20, 0.025% w/v
NaNO3). Recombinant purified wild-type human neuroserpin
was used for the standard curve. It was sequentially diluted
1:2, 10 times in blocking buffer for a standard range of 500 to
1.0 ng/mL, and blocking buffer alone was used for the blank.
The CSF samples were diluted at an assay-dependent concen-
tration in the same blocking buffer. Standards and samples
were added to the plate and incubated at room temperature for
2 hours. After washing, the secondary antibody (monoclonal
pool, 333 ng/mL each antibody) diluted in blocking buffer was
added to the plate and further incubated at room temperature
for 2 hours. After washing, horseradish peroxidase-labeled
rabbit anti-mouse detection antibody (Sigma-Aldrich Co.,
Dorset, UK), diluted 1:20,000 in blocking buffer without NaN3,
was added to the plate and incubated at room temperature for 1
hour. The plate was washed again, and each well was treated
with developing solution (Sigma-Aldrich Co.) at room

temperature for 10 minutes. The reaction was stopped with 1 M
H2SO4, and the color reaction was quantified in a Thermo-max
microplate reader (Molecular Devices) at 450 nm. The detec-
tion limit was 1 ng/mL, and the interplate and intraplate coeffi-
cients of variation were both less than 5%.

**Statistical analysis.** Statistical analysis was performed us-
ing Statistica software (Series 1203b, version 6.1 for Win-
dows, Statsoft, Tulsa, OK), SPSS software (version 12.0.1 for
Windows, SPSS Inc., Chicago, IL), and GraphPad Prism soft-
ware (version 4 for Windows, GraphPad Software, Inc., San
Diego, CA). The Kruskal–Wallis test was used for compari-
sions between more than two groups, and if significance was
reached, groups were compared using the Mann–Whitney U
test with correction for multiple comparisons (Bonferroni).
Correlation coefficients were calculated using the test for
Spearman rank order correlations. Because of the lack of the
standardized reference values for the measured variables,
the median of each variable in the control group was used as a
cut-point for defining “high” levels of the variable. The associ-
ations between marker levels in controls and AD and dem-
entia with Lewy bodies were calculated as odds ratios (ORs)
with 95% CIs. The differences in Ab1-42 levels between the
controls and dementia patients were large. Therefore,
the inverse highest quintile of the control group was used as
the cut-point when comparing controls with the two
dementia groups. The two-sided χ2 test was used to test OR
significance and to test frequency differences among the
groups. With the attempt to discriminate between the study

groups using the analyzed markers, logistic regression analy-
ses were conducted with controls against AD and with AD
against DLB using a step-forward method. Variables were entered
based on a significant improvement in log likelihood
ratios in every model. To avoid problems with multi-
collinearity, highly correlated variables (significantly correlated
above r = 0.5) were excluded from the analysis. Receiver
operator characteristic (ROC) curves were created using the
averaged predicted probabilities for each model to show the
relationship between the logistic regression models’ specif-
icity and sensitivity. The results are expressed as mean ± SD
or median and range. P < 0.05 was considered significant.

**RESULTS Patient characteristics.** Table 1 gives the
demographic data, MMSE scores, albumin ratio, and
presence of the ApoE4 allele in patients with
dementia and in controls. There was no signifi-
cant difference in age at investigation, sex dis-
tribution, or CSF/serum albumin ratio (as a measure of the
BBB function). As expected, AD patients and
DLB patients had significantly lower MMSE
scores than controls. The distribution of the
ApoE4 allele between the groups was significantly
different. The occurrence of one or more of the
chronic inflammatory diseases arteriosclerosis,
chronic obstructive pulmonary disease, and rheu-

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>Sex, MF, n (%)</th>
<th>Age at investigation, mean ± SD</th>
<th>MMSE, mean ± SD</th>
<th>CSF/serum albumin ratio, mean ± SD</th>
<th>ApoE4 carriers, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>37</td>
<td>14/23 (38/62)</td>
<td>72.4 ± 7.5</td>
<td>29.1 ± 1.0</td>
<td>7.2 ± 2.7</td>
<td>27.0</td>
</tr>
<tr>
<td>AD</td>
<td>258</td>
<td>84/174 (33/67)</td>
<td>74.7 ± 6.3</td>
<td>21.4 ± 5.0*</td>
<td>7.5 ± 3.2</td>
<td>70.2*</td>
</tr>
<tr>
<td>DLB</td>
<td>38</td>
<td>19/19 (50/50)</td>
<td>75.8 ± 5.9</td>
<td>21.8 ± 4.7*</td>
<td>8.1 ± 4.3</td>
<td>55.3*</td>
</tr>
</tbody>
</table>

* Indicates a significant difference at the p<0.001 level, compared to controls. 
† ApoE4 carriers include both heterozygous and homozygous ApoE4 carriers.
MMSE = Mini-Mental State Examination; AD = Alzheimer disease; DLB = dementia with Lewy bodies.

Table 1 Demographic data, MMSE, albumin ratio, and ApoE4 frequency
matoid disease and the regular use of NSAIDs was similar in all groups.

**Levels of α₁-antichymotrypsin, α₁-antitrypsin, and neuroserpin.** In both AD and DLB patient groups, we found higher levels of CSF α₁-antichymotrypsin (44%, p < 0.001 and 36%, p < 0.001) and α₁-antitrypsin (42.1%, p < 0.001 and 34.2%, p < 0.05) than in controls. Plasma levels of α₁-antichymotrypsin were elevated in the AD group (19.5%, p < 0.05) compared with controls, whereas plasma α₁-antitrypsin levels did not differ between the groups (table 2). Plasma levels of neuroserpin were nondetectable. The CSF concentration of neuroserpin was 25.5% higher in the AD group than in controls (p < 0.001), p < 0.01). Plasma levels of neuroserpin were strongly associated with increased P-tau but lower Aβ₁₋₄₂ levels than controls; however, no difference was found between the two dementia groups. Previous studies have suggested that the P-tau/Aβ₁₋₄₂ ratio can improve the separation of AD and controls, so we also determined the P-tau/Aβ₁₋₄₂ ratio in all groups. As shown in table 2, both AD and DLB groups had significantly higher P-tau/Aβ₁₋₄₂ ratios than controls; however, no difference was found between the two groups with dementia.

**Intercorrelations between measured variables, age, and cognitive function.** Correlations between the measured markers, age, and cognitive function are given in table 3. We found a strong linkage between higher CSF levels of α₁-antichymotrypsin (p < 0.001) and α₁-antitrypsin (p < 0.001) and BBB dysfunction (increased CSF/serum albumin ratio) that was independent of diagnostic group (table 3). This linkage is supported by the correlations between the CSF/plasma ACT or CSF/plasma AAT ratio and the CSF/serum albumin ratio (r = 0.613 and r = 0.667, p < 0.001) (not shown in table 3). Lower MMSE scores were associated with increased CSF T-tau in AD (p < 0.001) and DLB (p < 0.05).

Among AD patients, higher CSF levels of neuroserpin were strongly associated with increased P-tau/Aβ₁₋₄₂ ratio (p < 0.001). BBB dysfunction (higher CSF/serum albumin ratio) and higher CSF α₁-antichymotrypsin were associated with lower T-tau (p < 0.05) in the DLB group. Lower cognitive performance was linked to higher levels of plasma α₁-antichymotrypsin in AD (p < 0.01).

### Table 2
Levels of AD markers and serpins

<table>
<thead>
<tr>
<th>Marker</th>
<th>Controls, n = 37</th>
<th>AD, n = 258</th>
<th>DLB, n = 38</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF T-tau, ng/L</td>
<td>307 (117–846)</td>
<td>539* (153–2,144)</td>
<td>330 (87–811)</td>
</tr>
<tr>
<td>CSF P-tau, ng/L</td>
<td>57 (38–112)</td>
<td>73* (15–211)</td>
<td>68 (26–129)</td>
</tr>
<tr>
<td>CSF Aβ₁₋₄₂, ng/L</td>
<td>754 (260–958)</td>
<td>397* (242–781)</td>
<td>463* (227–834)</td>
</tr>
<tr>
<td>P-tau/Aβ₁₋₄₂</td>
<td>7.6 (4.7–37.7)</td>
<td>18.8* (2.2–70.7)</td>
<td>15.2* (5.0–50.4)</td>
</tr>
<tr>
<td>Plasma ACT, mg/L</td>
<td>348 (232–600)</td>
<td>416* (196–1,256)</td>
<td>392 (256–1276)</td>
</tr>
<tr>
<td>CSF ACT, mg/L</td>
<td>2.5 (1.2–4.9)</td>
<td>3.6* (1.6–17.8)</td>
<td>3.4* (2.4–9.8)</td>
</tr>
<tr>
<td>Plasma AAT, g/L</td>
<td>1.36 (0.32–2.0)</td>
<td>1.52 (0.56–12.4)</td>
<td>1.59 (0.80–2.69)</td>
</tr>
<tr>
<td>Plasma ACT, mg/L</td>
<td>7.6 (3.7–21.0)</td>
<td>10.8* (4.4–59.2)</td>
<td>10.2* (4.6–22.9)</td>
</tr>
<tr>
<td>CSF neuroserpin*, µg/L</td>
<td>7.41 (5.48–10.00)</td>
<td>9.30* (4.80–17.16)</td>
<td>8.06 (4.85–13.05)</td>
</tr>
</tbody>
</table>

Values are presented as median (range).
* CSF Aβ₁₋₄₂ was obtained from n = 257 Alzheimer disease (AD) patients.
† CSF neuroserpin was obtained from n = 18 controls, n = 238 AD patients, and n = 37 dementia with Lewy body (DLB) patients.
‡, §, and † indicate a significant difference at the 0.05 levels, compared with controls.
T-tau = total tau; P-tau = tau phosphorylated at threonine-181; ACT = α₁-antichymotrypsin; AAT = α₁-antitrypsin.

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Table 3  Variable correlation matrix

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Age at investigation</th>
<th>MMSE</th>
<th>Albumin ratio</th>
<th>Plasma ACT</th>
<th>CSF ACT</th>
<th>Plasma AAT</th>
<th>CSF neuroserpin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin ratio</td>
<td>Controls</td>
<td>0.337*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td>AD</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.289*</td>
<td>0.131*</td>
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<tr>
<td></td>
<td>DLB</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>—</td>
</tr>
<tr>
<td>Plasma ACT</td>
<td>Controls</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.760*</td>
<td>—</td>
<td>—</td>
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<tr>
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<td>AD</td>
<td>—</td>
<td>—</td>
<td>−0.189*</td>
<td>−0.168*</td>
<td>—</td>
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<td>DLB</td>
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</tr>
<tr>
<td>CSF ACT</td>
<td>Controls</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.156‡</td>
<td>0.621*</td>
<td>0.183*</td>
<td>—</td>
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<tr>
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<td>AD</td>
<td>—</td>
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<td>—</td>
<td>—</td>
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<td>0.607*</td>
<td>0.122‡</td>
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<td>DLB</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>0.644*</td>
<td>—</td>
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<tr>
<td>Plasma AAT</td>
<td>Controls</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>AD</td>
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<td>0.289*</td>
<td>0.131*</td>
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<td>DLB</td>
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<tr>
<td>CSF AAT</td>
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<td>—</td>
<td>0.818*</td>
<td>—</td>
<td>0.747*</td>
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<td>AD</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.757*</td>
<td>—</td>
<td>0.607*</td>
<td>0.122‡</td>
</tr>
<tr>
<td></td>
<td>DLB</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>−0.325‡</td>
<td>0.740*</td>
<td>0.644*</td>
<td>—</td>
</tr>
<tr>
<td>CSF T-tau</td>
<td>Controls</td>
<td>—</td>
<td>—</td>
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<td>AD</td>
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<tr>
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<td>DLB</td>
<td>—</td>
<td>—</td>
<td>−0.344*</td>
<td>−0.430*</td>
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<td>−0.358*</td>
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<td>CSF P-tau</td>
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<td>−0.134‡</td>
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<td>DLB</td>
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<td>CSF Aβ1-42</td>
<td>Controls</td>
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<td>—</td>
<td>−0.129‡</td>
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<td>—</td>
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<tr>
<td>P-tau/Aβ1-42</td>
<td>Controls</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

* Correlation is significant at the 0.001 level.
† Correlation is significant at the 0.01 level.
‡ Correlation is significant at the 0.05 level.
— No significant correlation.

MMSE = Mini-Mental State Examination; ACT = α1-antichymotrypsin; AAT = α1-antitrypsin; AD = Alzheimer disease; DLB = dementia with Lewy bodies; T-tau = total tau; P-tau = tau phosphorylated at threonine-181.

and to higher levels of CSF α1-antitrypsin in DLB ($p < 0.05$) (table 3).

Associations between dementia type and levels of measured variables. The associations between the levels of analyzed plasma and CSF markers and clinically diagnosed AD and DLB are presented in table 4. The strongest association, i.e., highest OR, was found between low levels of Aβ1-42 and AD. Equally strong association was observed between AD and higher levels of CSF α1-antichymotrypsin, neuroserpin, and T-tau. Higher levels of plasma α1-antichymotrypsin, but not α1-antitrypsin, were also linked to AD. Lower CSF Aβ1-42 and higher CSF α1-antichymotrypsin levels showed the strongest association with DLB. In addition, higher plasma levels of α1-antitrypsin, but not α1-antichymotrypsin, were associated with DLB.

Combination of different variables in logistic regression models. A logistic regression model was constructed in which we combined CSF α1-antichymotrypsin, P-tau, Aβ1-42, CSF neuroserpin, plasma α1-antichymotrypsin, and plasma α1-antitrypsin to predict the classifications of the studied subjects (table 5). For comparison, the analysis was conducted by adding to the model only the standard AD markers, i.e., P-tau, T-tau, and Aβ1-42. The combination of CSF Aβ1-42, α1-antichymotrypsin, and neuroserpin correctly discriminated clinically defined AD cases from controls with
We suspected that a reason for such low specificity is the differences in the size of the studied groups. Therefore, to minimize the effect of unequal group size, we randomly selected 50 AD cases in 10 independent selections and used the logistic regression method as described above. With a smaller difference in group size and using the three variables most frequently added to the model (CSF Aβ42, α1-antichymotrypsin, and neuroserpin), the averaged sensitivity of the classified AD cases was nearly the same as in the original analysis (96.5% vs 94.7%); however, the averaged specificity increased by 22% (55.6% vs 77.8%). For the model including only the standard markers, Aβ42 alone generated an averaged sensitivity of 93.2% and specificity of 83.5% for discrimination between AD and controls.

The same method was used to create a model for discrimination between the AD and DLB groups. Aβ42 and neuroserpin together correctly classified AD cases with 99.6% sensitivity, but with only 54% specificity. In view of the difference in size of the groups, we randomly selected 50 AD patients on 10 occasions to decrease the size of this group. Now Aβ42 and neuroserpin correctly classified AD patients with an average of 78.7% sensitivity and 57.4% specificity. The standard markers Aβ42, P-tau, and T-tau predicted the correct classification of AD with an averaged sensitivity of 75.5% and 88.8% specificity.

For the demonstration of the sensitivity and the specificity of the two logistic regression models, i.e., AD vs controls and AD vs DLB, we pooled and averaged the predicted probabilities, generated by the two models after running each 10 times, and used them to create ROC curves. As illustrated in figure, A, the areas under the curve (AUCs) generated from the ROC curves for AD vs controls using CSF Aβ42, α1-antichymotrypsin, and neuroserpin or standard AD markers alone were 0.973 (95% CI 0.926 to 1.019, p < 0.001) vs 0.933 (CI 0.867 to 0.999, p < 0.001). AUC, however, was somewhat smaller for the AD vs DLB classification, 0.777 (CI 0.664 to 0.890, p < 0.001) and 0.848 (CI 0.744 to 0.952, p < 0.001) (figure, B).

Table 4

<table>
<thead>
<tr>
<th>Marker</th>
<th>AD*</th>
<th>DLB*</th>
<th>95% CI</th>
<th>AD</th>
<th>DLB</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma ACT, mg/L</td>
<td>3.00</td>
<td>1.45</td>
<td>1.22-7.41</td>
<td>0.58-3.61</td>
<td>0.0153</td>
<td>0.4223</td>
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<tr>
<td>CSF ACT, mg/L</td>
<td>10.59</td>
<td>10.00</td>
<td>3.43-32.71</td>
<td>2.95-33.92</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma AAT, g/L</td>
<td>1.78</td>
<td>4.60</td>
<td>0.75-4.23</td>
<td>1.72-12.27</td>
<td>0.1901</td>
<td>0.0018</td>
</tr>
<tr>
<td>CSF AAT, mg/L</td>
<td>5.54</td>
<td>3.96</td>
<td>2.05-14.97</td>
<td>1.44-10.89</td>
<td>0.0004</td>
<td>0.0063</td>
</tr>
<tr>
<td>CSF neuroserpin, μg/L</td>
<td>10.75</td>
<td>2.36</td>
<td>2.71-42.73</td>
<td>0.74-7.56</td>
<td>0.0002</td>
<td>0.1426</td>
</tr>
<tr>
<td>T-tau, ng/L</td>
<td>10.89</td>
<td>1.17</td>
<td>3.25-36.48</td>
<td>0.47-2.90</td>
<td>&lt;0.0001</td>
<td>0.7342</td>
</tr>
<tr>
<td>P-tau, ng/L</td>
<td>2.41</td>
<td>3.96</td>
<td>0.96-6.05</td>
<td>1.44-10.89</td>
<td>0.0672</td>
<td>0.0063</td>
</tr>
<tr>
<td>Aβ1-42, ng/L</td>
<td>102.90</td>
<td>12.00</td>
<td>20.02-528.5</td>
<td>4.02-35.87</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Associations between the levels of measured variables and Alzheimer disease (AD) vs controls.
† Associations between the levels of measured variables and dementia with Lewy bodies (DLB) vs controls. Cutoffs based on median values among controls (table 2).
‡ Cutoff value based on the inverse highest quintile among controls (551.8 ng/L).

Effect of the ApoE4 allele, NSAID treatment, and inflammatory diseases on the levels of measured variables. Subjects were grouped according to the presence of the ApoE4 allele, and levels of the different variables were compared between ApoE4 carriers (n = 212) and noncarriers (n = 121). ApoE4 carriers had lower MMSE scores (22 vs 24, p < 0.001) and Aβ42 levels (392 ng/L vs 489 ng/L, p < 0.001) but higher levels of T-tau (531 ng/L vs 413 ng/L, p < 0.001), P-tau (75 ng/L vs 61 ng/L, p < 0.001), P-tau/Aβ42 ratio (19.9 vs 12.1, p < 0.001), and plasma α1-antitrypsin (1.54 g/L vs 1.36 g/L, p < 0.01). No difference was found in the levels of neuroserpin and α1-antichymotrypsin among ApoE4 carriers and noncarriers. We also found no differences in levels of measured variables when comparing subjects who had arteriosclerosis, COPD, or rheumatoid disease when compared with subjects without these disorders. NSAID treatment had also no effect on the levels of the measured markers.

DISCUSSION In the present study, we promote the hypothesis that the complex pathologies of AD and DLB are reflected in concentrations of plasma and CSF inflammatory markers. To
test this hypothesis, we investigated plasma and CSF levels of three members of the serpin family (α1-antitrypsin, α1-antichymotrypsin, and neuroserpin) and standard AD markers, in a large cohort of well-characterized AD and DLB patients and age-matched, nondemented controls.

Elevated ACT in brains, CSF, and serum from AD patients have been reported earlier, and plasma ACT was found to be increased in AD patients, even without alteration of levels of other acute phase proteins such as C-reactive protein and α1-antitrypsin. We found strong correlations between CSF levels of both ACT and AAT with the albumin CSF/serum ratio. This suggests that the levels of ACT and AAT found in CSF might be derived from the periphery due to BBB dysfunction and so may be an epiphenomenon rather than central to the pathogenesis of disease. Others have also reported increasing serum ACT levels with progression of AD and suggested ACT as a useful marker of disease severity. On the standard AD markers, our findings are also concordant with those of others who report decreased CSF Aβ1-42 but increased CSF T-tau and P-tau in individuals with AD. However,

### Table 5 Logistic regression models to discriminate between AD patients and controls, and AD patients and DLB patients

<table>
<thead>
<tr>
<th>Serpins in combination with standard AD markers</th>
<th>-2 log likelihood (p value in χ² test)</th>
<th>Coefficient</th>
<th>p Value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD patients vs controls</td>
<td>50.488 (&lt;0.001)</td>
<td>Aβ1-42</td>
<td>-0.010</td>
<td>&lt;0.001</td>
<td>0.990</td>
</tr>
<tr>
<td>Model 2</td>
<td>36.652 (&lt;0.001)</td>
<td>Aβ1-42</td>
<td>-0.011</td>
<td>0.001</td>
<td>0.989</td>
</tr>
<tr>
<td>Neuroserpin</td>
<td>1.112</td>
<td>0.004</td>
<td>3.042</td>
<td>1.415-6.537</td>
<td></td>
</tr>
<tr>
<td>Model 3</td>
<td>26.263 (0.001)</td>
<td>CSF ACT</td>
<td>1.664</td>
<td>0.017</td>
<td>5.283</td>
</tr>
<tr>
<td>Aβ1-42</td>
<td>-0.011</td>
<td>0.002</td>
<td>0.989</td>
<td>0.982-0.996</td>
<td></td>
</tr>
<tr>
<td>CSF neuroserpin</td>
<td>1.207</td>
<td>0.015</td>
<td>3.345</td>
<td>1.269-8.814</td>
<td></td>
</tr>
<tr>
<td>AD patients vs DLB patients</td>
<td>104.953 (0.001)</td>
<td>CSF neuroserpin</td>
<td>-0.415</td>
<td>0.004</td>
<td>0.660</td>
</tr>
<tr>
<td>Model 2</td>
<td>96.867 (0.004)</td>
<td>Aβ1-42</td>
<td>0.005</td>
<td>0.009</td>
<td>1.005</td>
</tr>
<tr>
<td>Neuroserpin</td>
<td>-0.413</td>
<td>0.006</td>
<td>0.661</td>
<td>0.491-0.891</td>
<td></td>
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<tr>
<td>Standard AD markers</td>
<td>65.193 (&lt;0.001)</td>
<td>Aβ1-42</td>
<td>-0.11</td>
<td>&lt;0.001</td>
<td>0.989</td>
</tr>
<tr>
<td>AD patients vs controls</td>
<td>103.381 (&lt;0.001)</td>
<td>T-tau</td>
<td>-0.004</td>
<td>0.001</td>
<td>0.996</td>
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<tr>
<td>Model 2</td>
<td>86.567 (&lt;0.001)</td>
<td>T-tau</td>
<td>-0.009</td>
<td>&lt;0.001</td>
<td>0.991</td>
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<td>P-tau</td>
<td>0.061</td>
<td>&lt;0.001</td>
<td>1.062</td>
<td>1.028-1.098</td>
<td></td>
</tr>
<tr>
<td>Model 3</td>
<td>81.011 (0.018)</td>
<td>T-tau</td>
<td>-0.009</td>
<td>&lt;0.001</td>
<td>0.991</td>
</tr>
<tr>
<td>P-tau</td>
<td>0.005</td>
<td>0.029</td>
<td>1.006</td>
<td>1.001-1.010</td>
<td></td>
</tr>
<tr>
<td>Aβ1-42</td>
<td>0.070</td>
<td>&lt;0.001</td>
<td>1.073</td>
<td>1.035-1.112</td>
<td></td>
</tr>
</tbody>
</table>

Data from 1 representative analysis (out of 10). AD = Alzheimer disease; DLB = dementia with Lewy bodies; ACT = α1-antichymotrypsin; T-tau = total tau; P-tau = tau phosphorylated at threonine-181.
DLB patients had also lower \( \text{A} \beta_{1-42} \) and higher P-tau concentrations when compared with controls. Recent studies have suggested that the P-tau/\( \text{A} \beta_{1-42} \) ratio is a sensitive and specific marker to differentiate AD patients from controls.\(^{36,37} \) We confirmed this finding in our cohort, but again showed that the P-tau/\( \text{A} \beta_{1-42} \) ratio did not differ significantly between the two dementia groups and therefore did not permit discrimination between AD and DLB. Therefore, measuring other proteins in CSF gains greater importance for the differential diagnosis of dementia. The present study is, however, cross-sectional, which limits us from drawing clear conclusions on the link between the levels of serpins and the severity of dementia. This is even made more difficult by the lack of adequate tools to stage DLB, because the MMSE score is not a direct measure of disease severity in DLB. Nevertheless, in agreement with previously published data, we found higher levels of CSF \( \alpha_1 \)-antichymotrypsin in AD\(^{15,44} \) and an association between higher plasma levels of \( \alpha_1 \)-antichymotrypsin and decline in cognitive function in individuals with AD. Importantly, this inverse relationship was restricted to \( \alpha_1 \)-antichymotrypsin because neither plasma and CSF levels of \( \alpha_1 \)-antitrypsin nor CSF levels of neuroserpin were linked to cognitive function in AD. Conversely, we were able to correlate higher levels of CSF \( \alpha_1 \)-antitrypsin to lower MMSE score in the DLB group, suggesting that different members of the serpin family might have different associations with cognitive function depending on the type of dementia. We therefore asked the question whether \( \alpha_1 \)-antichymotrypsin and \( \alpha_1 \)-antitrypsin can be used to distinguish patients with AD and DLB. Higher plasma \( \alpha_1 \)-antitrypsin levels were found to be associated with increased ORs of DLB when compared with AD, and higher plasma levels of \( \alpha_1 \)-antichymotrypsin were associated with an increased ORs of AD when compared with controls. However, neither \( \alpha_1 \)-antichymotrypsin nor \( \alpha_1 \)-antitrypsin alone was able to discriminate between patients with AD and DLB.

Neuroserpin, an axonally secreted regulator of the local extracellular proteolysis is involved in the reorganization of the synaptic connectivity during development and synapse plasticity in adults,\(^{26} \) and its levels in biologic fluids have so far not been established. In this study, we report that neuroserpin can be measured in the CSF of patients with dementia and elderly controls, and that its levels directly correlate to the CSF levels of T-tau. Expression of neuroserpin in regions of the brain that exhibit synaptic plasticity supports the hypothesis that this protein is a member of the group of extracellular protease inhibitors that orchestrate brain development, function, and anatomic integrity. The facts that neuroserpin was not detected in plasma and its CSF concentrations did not correlate with the albumin CSF/serum ratio suggest that neuroserpin is derived from brain tissue and therefore more specifically reflects processes within the brain than do ACT and AAT. It has been suggested that neuroserpin may stimulate neurite outgrowth in neuroendocrine cells by modulating cell migration and cell adhesion independent of its protease inhibitor function.\(^{58} \) The increase in T-tau CSF concentration is considered to reflect neuronal and axonal degeneration.\(^ {54} \) In fact, the level of CSF neuroserpin was found to be significantly higher in AD than in controls and DLB patients. Therefore, the observed correlation between CSF levels of T-tau and neuroserpin argues in favor of a potential relevance of neuroserpin as a marker of either neuronal degeneration or of a subsequent regenerative process of damaged neurons.
In view of the difference in serpin levels between individuals with AD, individuals with DLB, and nondemented controls, we combined the serpins with the standard AD markers in an attempt to discriminate between the three groups. Results from logistic regression analyses demonstrate a relationship between higher CSF levels of α₄-antichymotrypsin and neuroserpin and increased predicted probability and ORs of AD. Furthermore, a logistic regression model based on CSF α₄-antichymotrypsin, neuroserpin, and Aβ₁₋₄₂ enabled us to discriminate between AD patients and controls with a sensitivity and specificity comparable to standard markers. The levels of sensitivity and specificity that were derived in our analyses should, however, be viewed with caution until they are replicated.

ACKNOWLEDGMENT
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REFERENCES


