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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 α_1-antichymotrypsin and α_1-antitrypsin, and CSF levels of α_1-antichymotrypsin, α_1-antitrypsin, and neuroserpin along with three standard biomarkers (total tau, tau phosphorylated at threonine-181, and the A$\beta_{1\text{-}42}$) 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 α_1-antichymotrypsin and α_1-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 α_1-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 α_1-antichymotrypsin, neuroserpin, and A$\beta_{1\text{-}42}$ 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 α_1-antichymotrypsin were associated with the clinical diagnosis of Alzheimer disease (AD) and facilitated the diagnostic classification of AD vs controls. CSF serpin levels did not improve 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.[1] Inflammation is driven by cytokines (particularly interleukin [IL]-1) that are released from activated microglia and astrocytes,[2] and this in turn drives the expression of IL-6[3] and inducible nitric oxide synthase.[4] 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.[5] These include α_1-antichymotrypsin, α_1-antitrypsin, and neuroserpin. The important role of α_1-antichymotrypsin in the pathogenesis of AD was demon-

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strated by the finding of raised levels in brain homogenates from affected individuals[6]; the finding that it is tightly associated with virtually all β-amyloid plaques[7]; and the demonstration that it interacts with,[8,9] and affects the clearance of, $A\beta_{1\text{-}42}$.[10-14] Moreover elevated levels of plasma and CSF α_1-antichymotrypsin correlate with cognitive decline in elderly nondemented persons and those with AD.[15-17] Similarly, α_1-antitrypsin[18] is present in β-amyloid plaques of patients with AD, and isoforms of α_1-antitrypsin are significantly altered in CSF of affected individuals when compared with controls.[19,20] α_1-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 $A\beta_{1\text{-}42}$ peptide. This in turn prevents fibril formation and renders the $A\beta_{1\text{-}42}$ 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 α_1-antitrypsin, α_1-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 $A\beta_{1\text{-}42}$ 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.[30] 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[31] were used for probable AD. Probable dementia with Lewy bodies was diagnosed according to the DLB consensus criteria.[32] 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/μL. The CSF samples were centrifuged at 2,000*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*g* at 4 °C for 10 minutes. The aliquots were immediately frozen at −80 °C and stored until assayed.

Determination of the concentration of α_1-antichymotrypsin and α_1-antitrypsin. Plasma and CSF levels of α_1-antichymotrypsin and α_1-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 V on 1 mm 0.9% w/v agarose gels containing 11 mg/L anti-human α_1-antitrypsin antibody (DakoCytomation, Glostrup, Denmark), and 6.98 mg/L (for plasma analysis) and 2.79 mg/L (for CSF analysis) anti-human α_1-antichymotrypsin antibody (DakoCytomation, Glostrup, Denmark). Gels were pressed between filter paper and dried before staining with Coomassie blue. To quantify α_1-antitrypsin and α_1-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

Table 1 Demographic data, MMSE, albumin ratio, and ApoE4 frequency

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

* 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.

percentage (%CV) for the interbatch and intrabatch variability were 7.9% and 5.8%.

Determination of A$\beta_{1\text{-}42}$, T-tau, and P-tau. Total tau, P-tau, and A$\beta_{1\text{-}42}$ levels were determined using Luminex xMAP technology as described previously.[34] In brief, this technology is based on flow cytometric separation of antibody-coated microspheres that are labeled with a specific mixture of two fluorescent dyes. After binding of a biotinylated 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 A$\beta_{1\text{-}42}$ correlated well with the levels obtained by conventional ELISA measurements.[34] The intra-assay and inter-assay %CV for the multiparametric assay for A$\beta_{1\text{-}42}$, 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 antibody[35] 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 Na_2CO_3/$NaHCO_3$ 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 NaN_3). 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 concentration 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 NaN_3, 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 H_2SO_4, and the color reaction was quantified in a Thermo-max microplate reader (Molecular Devices) at 450 nm. The detection limit was 1 ng/mL, and the interplate and intraplate coefficients of variation were both less than 5%.

Statistical analysis. Statistical analysis was performed using Statistica software (Series 1203b, version 6.1 for Windows, Statsoft, Tulsa, OK), SPSS software (version 12.0.1 for Windows, SPSS Inc., Chicago, IL), and GraphPad Prism software (version 4 for Windows, GraphPad Software, Inc., San Diego, CA). The Kruskal–Wallis test was used for comparisons 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 associations between marker levels in controls and AD and dementia with Lewy bodies were calculated as odds ratios (ORs) with 95% CIs. The differences in A$\beta_{1\text{-}42}$ 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 χ^2 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 analyses 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 multicollinearity, highly correlated variables (significantly correlated above $r = 0.5$) were excluded from the analysis. Receiver operating characteristic (ROC) curves were created using the averaged predicted probabilities for each model to show the relationship between the logistic regression models' specificity 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 significant difference in age at investigation, sex distribution, 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 2 Levels of AD markers and serpins

Marker	Controls, n = 37	AD, n = 258	DLB, n = 38
CSF T-tau, ng/L	307 (117–846)	539‡ (153–2,144)	330 (87–811)
CSF P-tau, ng/L	57 (38–112)	73§ (15–211)	68‖ (26–129)
CSF $A\beta_{1\text{-}42}$*, ng/L	754 (260–958)	397‡ (242–781)	463‡ (227–834)
P-tau/$A\beta_{1\text{-}42}$	7.6 (4.7–37.7)	18.8‡ (2.2–70.7)	15.2‡ (5.0–50.4)
Plasma ACT, mg/L	348 (232–600)	416‡ (196–1,256)	392 (256–1276)
CSF ACT, mg/L	2.5 (1.2–4.9)	3.6‡ (1.6–17.8)	3.4‡ (2.4–9.8)
Plasma AAT, g/L	1.36 (0.32–2.0)	1.52 (0.56–12.4)	1.59 (0.80–2.69)
CSF AAT, mg/L	7.6 (3.7–21.0)	10.8‡ (4.4–52.5)	10.2‖ (4.6–22.9)
CSF neuroserpin†, μg/L	7.41 (5.48–10.00)	9.30‡ (4.80–17.16)	8.06 (4.85–13.05)

Values are presented as median (range).
* CSF A $\beta_{1\text{-}42}$ 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 $p < 0.001$, $p < 0.01$, and $p < 0.05$ levels, compared with controls.
T-tau = total tau; P-tau = tau phosphorylated at threonine-181; ACT = α_1-antichymotrypsin; AAT = α_1-antitrypsin.

matoid disease and the regular use of NSAIDs was similar in all groups.

Levels of α_1-antichymotrypsin, α_1-antitrypsin, and neuroserpin. In both AD and DLB patient groups, we found higher levels of CSF α_1-antichymotrypsin (44%, $p < 0.001$ and 36%, $p < 0.001$) and α_1-antitrypsin (42.1%, $p < 0.001$ and 34.2%, $p < 0.05$) than in controls. Plasma levels of α_1-antichymotrypsin were elevated in the AD group (19.5%, $p < 0.05$) compared with controls, whereas plasma α_1-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 patients than in controls ($p < 0.001$) but did not differ in patients with DLB vs controls. Interestingly, CSF neuroserpin was the only marker that differed between the two groups of dementia patients. AD patients had 15.4% higher neuroserpin levels relative to the DLB patients ($p < 0.01$). There was considerable overlap, however, in CSF neuroserpin levels between AD and DLB patients.

When analyzed across all groups, women had higher plasma levels of α_1-antichymotrypsin than men (416 mg/L vs 392 mg/L, $p < 0.01$), whereas men had a higher albumin ratio (8.1 vs 6.3, $p < 0.001$) and higher CSF levels of α_1-antitrypsin (12.1 mg/L vs 9.9 mg/L, $p < 0.001$) and α_1-antichymotrypsin (3.6 mg/L vs 3.3 mg/L, $p < 0.05$) than women. We found no sex-associated differences in CSF levels of neuroserpin.

CSF levels of T-tau, P-tau, and $A\beta_{1\text{-}42}$. Significantly higher CSF concentrations of T-tau and P-tau, but lower $A\beta_{1\text{-}42}$ concentrations, were found in the AD group compared with the control group (table 2). DLB patients also exhibited significantly higher concentrations of P-tau but lower $A\beta_{1\text{-}42}$ levels than controls; however, no difference was found between the two dementia groups. Previous studies have suggested that the P-tau/$A\beta_{1\text{-}42}$ ratio can improve the separation of AD and controls,[36,37] so we also determined the P-tau/$A\beta_{1\text{-}42}$ ratio in all groups. As shown in table 2, both AD and DLB groups had significantly higher P-tau/$A\beta_{1\text{-}42}$ 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 α_1-antichymotrypsin ($p < 0.001$) and α_1-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/$A\beta_{1\text{-}42}$ ratio ($p < 0.001$). BBB dysfunction (higher CSF/serum albumin ratio) and higher CSF α_1-antichymotrypsin were associated with lower T-tau ($p < 0.05$) in the DLB group. Lower cognitive performance was linked to higher levels of plasma α_1-antichymotrypsin in AD ($p < 0.01$)

Table 3 Variable correlation matrix

Variable	Group	Age at investigation	MMSE	Albumin ratio	Plasma ACT	CSF ACT	Plasma AAT	CSF neuroserpin
Albumin ratio	Controls	0.337‡	—					
	AD	—	—					
	DLB	—	—					
Plasma ACT	Controls	—	—	—				
	AD	—	−0.189†	−0.168†				
	DLB	—	—	—				
CSF ACT	Controls	—	—	0.760*	—			
	AD	0.156‡	—	0.621*	0.183†			
	DLB	0.164‡	—	0.694*	—			
Plasma AAT	Controls	—	—	—	—	—		
	AD	—	—	—	0.289*	0.131‡		
	DLB	—	—	—	—	—		
CSF AAT	Controls	—	—	0.818*	—	0.747*	—	
	AD	—	—	0.757*	—	0.607*	0.122‡	
	DLB	—	−0.325‡	0.740*	—	0.644*	—	
CSF T-tau	Controls	—	—	—	—	—	—	0.536‡
	AD	—	−0.211*	—	—	—	—	0.486*
	DLB	—	−0.344*	−0.430†	—	−0.358‡	—	0.517*
CSF P-tau	Controls	—	—	—	—	—	—	0.481‡
	AD	−0.148‡	−0.134‡	—	—	—	—	0.406*
	DLB	—	—	—	—	—	—	—
CSF A$\beta_{1\text{-}42}$	Controls	—	—	—	—	—	—	—
	AD	—	—	—	−0.129‡	—	—	—
	DLB	—	—	—	—	—	—	—
P-tau/A$\beta_{1\text{-}42}$	Controls	—	—	—	—	—	—	—
	AD	—	—	—	—	—	—	0.373*
	DLB	—	—	—	—	—	—	—

* 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 = α_1-antichymotrypsin; AAT = α_1-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 α_1-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 A$\beta_{1\text{-}42}$ and AD. Equally strong association was observed between AD and higher levels of CSF α_1-antichymotrypsin, neuroserpin, and T-tau. Higher levels of plasma α_1-antichymotrypsin, but not α_1-antitrypsin, were also linked to AD. Lower CSF A$\beta_{1\text{-}42}$ and higher CSF α_1-antichymotrypsin levels showed the strongest association with DLB. In addition, higher plasma levels of α_1-antitrypsin, but not α_1-antichymotrypsin, were associated with DLB.

Combination of different variables in logistic regression models. A logistic regression model was constructed in which we combined CSF α_1-antichymotrypsin, P-tau, A$\beta_{1\text{-}42}$, CSF neuroserpin, plasma α_1-antichymotrypsin, and plasma α_1-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 A$\beta_{1\text{-}42}$. The combination of CSF A$\beta_{1\text{-}42}$, α_1-antichymotrypsin, and neuroserpin correctly discriminated clinically defined AD cases from controls with

Table 4 Associations between markers and dementia type

	Odds ratio		95% CI		*p* Value	
Marker	AD*	DLB†	AD	DLB	AD	DLB
Plasma ACT, mg/L	3.00	1.45	1.22–7.41	0.58–3.61	0.0153	0.4223
CSF ACT, mg/L	10.59	10.00	3.43–32.71	2.95–33.92	<0.0001	<0.0001
Plasma AAT, g/L	1.78	4.60	0.75–4.23	1.72–12.27	0.1901	0.0018
CSF AAT, mg/L	5.54	3.96	2.05–14.97	1.44–10.89	0.0004	0.0063
CSF neuroserpin, μg/L	10.75	2.36	2.71–42.73	0.74–7.56	0.0002	0.1426
T-tau, ng/L	10.89	1.17	3.25–36.48	0.47–2.90	<0.0001	0.7342
P-tau, ng/L	2.41	3.96	0.96–6.05	1.44–10.89	0.0672	0.0063
$A\beta_{1\text{-}42}$, ng/L‡	102.90	12.00	20.02–528.5	4.02–35.87	<0.0001	<0.0001

* 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).
ACT = α_1-antichymotrypsin; AAT = α_1-antitrypsin; T-tau = total tau; P-tau = tau phosphorylated at threonine-181.

96.5% sensitivity but only 55.6% specificity. 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 $A\beta_{1\text{-}42}$, α_1-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, $A\beta_{1\text{-}42}$ 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. $A\beta_{1\text{-}42}$ 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 $A\beta_{1\text{-}42}$ and neuroserpin correctly classified AD patients with an average of 78.7% sensitivity and 57.4% specificity. The standard markers $A\beta_{1\text{-}42}$, 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 $A\beta_{1\text{-}42}$, α_1-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).

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 $A\beta_{1\text{-}42}$ 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/$A\beta_{1\text{-}42}$ ratio (19.9 vs 12.1, $p < 0.001$), and plasma α_1-antitrypsin (1.54 g/L vs 1.36 g/L, $p < 0.01$). No difference was found in the levels of neuroserpin and α_1-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.[38-42] To

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

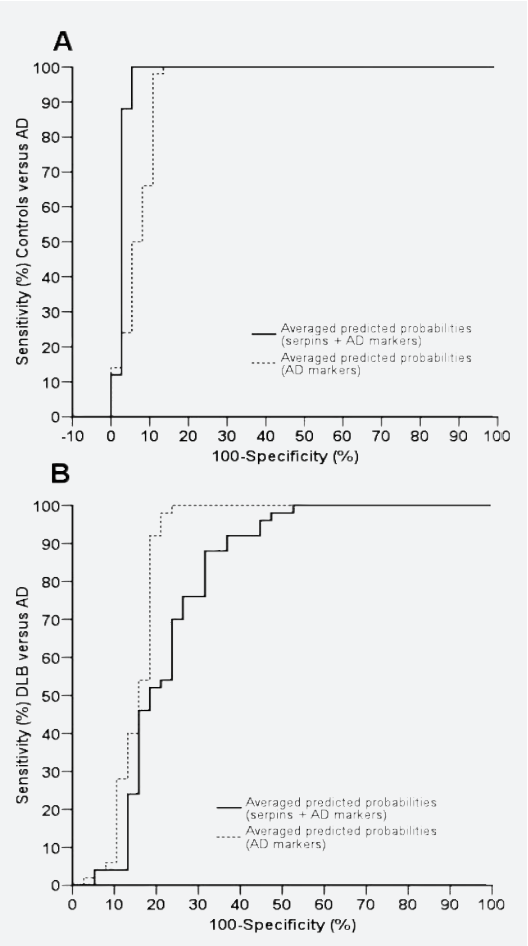
	−2 log likelihood (p value in χ^2 test)	Coefficient	p Value	Odds ratio	95% CI
Serpins in combination with standard AD markers					
AD patients vs controls					
Model 1	50.488 (<0.001)				
$A\beta_{1-42}$		−0.010	<0.001	0.990	0.985–0.995
Model 2	36.652 (<0.001)				
$A\beta_{1-42}$		−0.011	0.001	0.989	0.983–0.996
Neuroserpin		1.112	0.004	3.042	1.415–6.537
Model 3	26.263 (0.001)				
CSF ACT		1.664	0.017	5.283	1.343–20.781
$A\beta_{1-42}$		−0.011	0.002	0.989	0.982–0.996
CSF neuroserpin		1.207	0.015	3.345	1.269–8.814
AD patients vs DLB patients					
Model 1	104.953 (0.001)				
CSF neuroserpin		−0.415	0.004	0.660	0.498–0.876
Model 2	96.867 (0.004)				
$A\beta_{1-42}$		0.005	0.009	1.005	1.001–1.009
CSF neuroserpin		−0.413	0.006	0.661	0.491–0.891
Standard AD markers					
AD patients vs controls					
Model 1	65.193 (<0.001)				
$A\beta_{1-42}$		−0.11	<0.001	0.989	0.985–0.993
AD patients vs DLB patients					
Model 1	103.381 (<0.001)				
T-tau		−0.004	0.001	0.996	0.993–0.998
Model 2	86.567 (<0.001)				
T-tau		−0.009	<0.001	0.991	0.987–0.995
P-tau		0.061	<0.001	1.062	1.028–1.098
Model 3	81.011 (0.018)				
T-tau		−0.009	<0.001	0.991	0.987–0.995
P-tau		0.005	0.029	1.006	1.001–1.010
$A\beta_{1-42}$		0.070	<0.001	1.073	1.035–1.112

Data from 1 representative analysis (out of 10).
AD = Alzheimer disease; DLB = dementia with Lewy bodies; ACT = α_1-antichymotrypsin; T-tau = total tau; P-tau = tau phosphorylated at threonine-181.

test this hypothesis, we investigated plasma and CSF levels of three members of the serpin family (α_1-antitrypsin, α_1-antichymotrypsin, and neuroserpin) and standard AD markers, in a large cohort of well-characterized AD and DLB patients[31,32] and age-matched, nondemented controls.

Elevated ACT in brains,[6] CSF, and serum[43,44] 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 α_1-antitrypsin.[44,45] 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.[15] On the standard AD markers, our findings are also concordant with those of others who report decreased CSF $A\beta_{1-42}$[46-53] but increased CSF T-tau and P-tau in individuals with AD.[46,54-57] However,

Figure ROC curve analysis



Receiver operating characteristic (ROC) curve analysis of the discriminative ability of the predicted probabilities generated from the logistic regression model using serpins in combination with Alzheimer disease (AD) markers or AD markers alone, for the differentiation between AD and controls (A) and between AD and dementia with Lewy bodies (DLB) (B).

DLB patients had also lower $A\beta_{1\text{-}42}$ and higher P-tau concentrations when compared with controls. Recent studies have suggested that the P-tau/$A\beta_{1\text{-}42}$ 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/$A\beta_{1\text{-}42}$ 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 α_1-antichymotrypsin in AD[15,44] and an association between higher plasma levels of α_1-antichymotrypsin and decline in cognitive function in individuals with AD. Importantly, this inverse relationship was restricted to α_1-antichymotrypsin because neither plasma and CSF levels of α_1-antitrypsin nor CSF levels of neuroserpin were linked to cognitive function in AD. Conversely, we were able to correlate higher levels of CSF α_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 α_1-antichymotrypsin and α_1-antitrypsin can be used to distinguish patients with AD and DLB. Higher plasma α_1-antitrypsin levels were found to be associated with increased ORs of DLB when compared with AD, and higher plasma levels of α_1-antichymotrypsin were associated with an increased ORs of AD when compared with controls. However, neither α_1-antichymotrypsin nor α_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[59-61] in an attempt to discriminate between the three groups. Results from logistic regression analyses demonstrate a relationship between higher CSF levels of α_1-antichymotrypsin and neuroserpin and increased predicted probability and ORs of AD. Furthermore, a logistic regression model based on CSF α_1-antichymotrypsin, neuroserpin, and $A\beta_{1\text{-}42}$ 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.

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