CHAPTER 7

Matrix metalloproteinases in Alzheimer’s disease and effects of concurrent cerebral amyloid angiopathy


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ABSTRACT

Matrix metalloproteinases (MMPs) are a family of enzymes able to degrade components of the extracellular matrix (ECM), which is important for normal blood-brain barrier (BBB) function. Their function is regulated by tissue inhibitors of matrix metalloproteinases (TIMPs). We investigated whether MMPs and TIMPs in CSF and plasma were altered in Alzheimer’s disease (AD) and vascular dementia (VaD), and whether this effect was modified by presence of microbleeds in AD patients. In addition, we assessed associations of MMPs and TIMPs with CSF amyloid-β_{1-42} (Aβ_{42}), tau and tau phosphorylated at threonine-181 (p-tau). We measured MMP2, MMP9, and MMP10, and TIMP1 and TIMP2 in CSF and plasma of 52 AD patients, 26 matched controls and 24 VaD patients. AD patients showed higher plasma MMP2 levels compared to VaD patients (p<0.05), and higher CSF MMP10 levels compared to controls (p<0.05). Microbleeds in AD were associated with lower CSF TIMP1, TIMP2 and MMP9 in a dose-response relation. In addition, CSF MMP2 was associated with p-tau (St.B 0.23, p<0.05), and CSF MMP10 with tau (St.B 0.38, p<0.001) and p-tau (St.B 0.40, p<0.001). Our findings suggest involvement of MMP2 and MMP10 in AD pathology. Lower levels of TIMPs in AD patients with microbleeds suggest less MMP inhibition in patients with concurrent cerebral amyloid angiopathy, which may lead to a more vulnerable blood-brain barrier in these patients.
1. Introduction

Alzheimer’s disease (AD) is characterized by accumulation of amyloid-β (Aβ) in senile plaques, but Aβ also accumulates in the cerebral vessel walls, referred to as cerebral amyloid angiopathy (CAA). CAA is a frequent cause of spontaneous intracerebral hemorrhage, and cerebral microbleeds on magnetic resonance imaging (MRI) have been associated with CAA. Microbleeds are visible as small black dots on T2*-weighted MRI. They are found in approximately 23% of AD patients, compared to only 5% in healthy elderly. In line with these findings, CAA is found more often in AD brains compared to normal aging brains at autopsy. We have shown previously that CSF amyloid-β₁-₄₂ (Aβ₄₂) was even lower in AD patients with microbleeds compared to those without. This suggests that the pathophysiological pathway of AD in patients with microbleeds, reflecting concurrent CAA pathology, may be partly different from the process in patients without microbleeds.

Matrix metalloproteinases (MMPs) are a family of enzymes able to degrade components of the extracellular matrix (ECM). The ECM is important for neural plasticity and normal blood-brain barrier (BBB) function, the latter being disturbed in CAA and VaD. MMPs are expressed in neurons, but also secreted by astrocytes and microglia. Their function is regulated by tissue inhibitors of matrix metalloproteinases (TIMPs). In addition to ECM degradation, it has been shown that Aβ induces production of several MMPs, and that MMPs are able to degrade Aβ in vitro as well as in APP-transgenic mice. Studies in CSF or plasma of AD, VaD and CAA patients are contradictory, as MMPs in CSF were either not measurable or not different between patient groups, while in other studies both higher and lower MMP concentrations have been reported. The frequently concurrent AD and CAA pathology in patients may account for the inconsistent results in these clinical studies. In this study we therefore analyzed concentrations of MMP2, -9, and -10 and TIMP-1 and -2 in plasma and CSF, to investigate whether MMPs play a role in AD or VaD, and whether this would be dependent on presence of microbleeds, as a marker of CAA, in AD.

2. Materials and methods

2.1 Patients

All patients were selected from our memory clinic based Amsterdam dementia cohort. We included 52 patients with probable AD (26 patients with and 26 patients without microbleeds; matched for age and sex) and 24 VaD patients. 26 patients with subjective memory complaints without microbleeds were matched for age and sex to the AD patients, and served as control group. Inclusion criteria were presence of 3T MRI with T2*-weighted imaging and availability of both CSF and plasma samples. The current sample partly overlapped with an earlier publication.
All patients underwent extensive dementia screening at baseline, including physical and neurological examination, EEG, MRI and laboratory tests. Neuropsychological assessment was performed, and included Mini Mental State Examination (MMSE) for global cognition. Diagnoses were made by consensus in a multidisciplinary meeting. Probable AD was diagnosed according to the core clinical NIA-AA criteria. National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l’Enseignement en Neurosciences (NINDS-AIREN) criteriawere used for VaD, and all patients met the VCI criteria for probable (n=21) or possible (n=3) VaD. The label of subjective memory complaints was used when results of all clinical examinations were normal, and there was no psychiatric diagnosis. The study was approved by the local ethical review board and all subjects gave written informed consent for the use of their clinical data for research purposes.

2.2 CSF and blood biochemical analysis

CSF was obtained by lumbar puncture using a 25-gauge needle, and collected in 10 mL polypropylene tubes (Sarstedt, Nümbrecht, Germany). Part of the CSF was used for routine analysis including leukocyte count, erythrocyte count, glucose concentration, and total protein concentration. Within two hours, the remaining CSF was centrifuged at 1800g for 10 minutes at 4°C, and transferred to new polypropylene tubes. It was stored up to two months at -20°C until Aβ42, total tau and p-tau analysis, or immediately stored at -80°C until further analysis. Blood was collected in 6 mL EDTA tubes on the same day as the CSF was obtained. Samples were centrifuged at 1800g for 10 minutes at 4°C. Next, they were aliquoted into Sarstedt polypropylene tubes and stored at -80°C until further analysis. CSF was analyzed without knowledge of clinical diagnoses. CSF Aβ42, tau and p-tau were measured with commercially available ELISAs (Innotest β-amyloid (1-42)), Innotest hTAU-Ag and Innotest Phosphotau (181P); Innogenetics, Ghent, Belgium) on a routine basis as described in detail before. MMP2, MMP9, and MMP10, and TIMP1 and TIMP2 were analyzed in plasma and CSF using multiplex beads-based immunoassays (MILLIPLEX MAP kits, Merck) according to manufacturer’s instructions. Two different panels were used; one panel for the three MMPs, and one for the two TIMPs. Dilution factors were 1:10 for plasma MMPs, none for CSF MMPs, and 1:50 for TIMPs in plasma as well as CSF. All samples were run in duplicate. Inter-assay CVs were 19.5±2.7% for MMP2, 20.7±2.7% for MMP9, 21.9±3.8% for MMP10, 13.0±3.2% for TIMP1 and 13.1±2.2 for TIMP2, as analyzed in three quality control samples used in all 7 runs. Lower limits of detection (based on the first standard of the standard curve with a different value from the blank) were 206 pg/mL for MMP2, 5 pg/mL for MMP9, 9 pg/mL for MMP10, 78 pg/mL for TIMP1 and 49 pg/mL for TIMP2. Of 6 patients (3 AD and 3 VaD) there was no CSF available for MMP and TIMP analysis. In addition, based on high duplicate CVs (i.e. >30%), some samples were excluded: for MMP2 in plasma n=5 and CSF n=1 (3% of all 198 measurements), for MMP9 in plasma n=4 and CSF n=8 (6%), for MMP10 in plasma n=2 and CSF n=9 (6%), for TIMP1 in plasma n=2 and CSF n=3 (3%), and for TIMP2 in plasma n=4 and CSF n=3 (4%).
2.3 MRI acquisition and assessment of microbleeds

MRI was performed on a whole-body 3T magnetic resonance (MR) system (Signa HDxt, General Electric, Milwaukee, WI, USA) using an 8-channel phased-array head coil. The MRI protocol included axial 2-dimensional T2*-weighted gradient-echo echo-planar imaging (EPI; matrix 256x480, field of view [FOV] 25x19 cm2, slice thickness 3.0 mm, repetition time [TR] 5300 ms, echo time [TE] 25 ms, 2 excitations) for rating of microbleeds. In addition, the MRI protocol included 3-dimensional fluid-attenuated inversion-recovery (FLAIR; matrix 224x224, FOV 25x25 cm2, slice thickness 1.2 cm, TR 8000 m, TE 140 ms) for rating of white matter hyperintensities (WMH), and 3-dimensional T1-weighted fast spoiled gradient recalled echo-based sequence (FSPGR; matrix 256x256, FOV 25x25 cm2, slice thickness 1 mm, TR 708 ms) with oblique reconstructions for rating of atrophy.

All MRI characteristics were rated without knowledge of clinical diagnoses. Microbleeds were defined as round hypointense foci in brain parenchyma up to 10 mm, and were counted in lobar and non-lobar locations. Lesions in sulci possibly representing flow voids, as well as symmetrical lesions in the basal ganglia, suggesting iron or calcium deposits, were excluded. Hypointensities inside infarcts were not regarded as microbleeds, but as probable hemorrhagic transformations. We classified number of microbleeds in no microbleeds, 1 microbleed, or ≥2 microbleeds, as described before. WMH were rated using the Fazekas scale. Medial temporal lobe atrophy (MTA) was rated using a 5-point rating scale. Global cortical atrophy (GCA) was rated using a 4-point rating scale.

2.4 Statistical analysis

For statistical analysis, SPSS 21.0 (IBM for Windows) was used. All CSF biochemical markers were log-transformed for statistical analyses because of skewed distributions. CSF and plasma concentrations were evaluated separately, and in addition, we calculated the CSF/plasma ratio of each MMP and TIMP. We assessed demographic differences between groups using chi-squared test, Student’s T-test, or ANOVA when appropriate. We compared differences in biomarker concentrations between diagnostic groups (AD, VaD and controls) using age- and sex-adjusted univariate general linear models (GLM) with post-hoc Bonferroni adjustments. Subsequently, we assessed the relationship of microbleeds (independent variable) with MMPs and TIMPs (dependent variables, all entered in separate models), using age- and sex adjusted univariate GLM analysis. As the distribution of number of microbleeds was skewed (i.e. few patients had many microbleeds), microbleed categories were entered as continuous variable. In this analysis p for trend represents a dose-response effect of number of microbleeds on the dependent variable. Only AD patients (including three VaD patients with a clinical diagnosis of possible VaD /possible AD) were included in this analysis. Finally, we examined associations of MMPs and TIMPs with CSF AD biomarkers using linear regression analysis, adjusted for age and sex. MMP or TIMP was entered as dependent variable, while each AD biomarker was entered as independent variable (all in separate models). To estimate
standardized B’s for each diagnostic group we repeated these analyses including terms for diagnosis (dummy variables) and interaction terms of the biomarker of interest (i.e. A\(\beta\)42, total tau or p-tau) with diagnosis. In general, statistical significance was set at \(p<0.05\).

3. Results

Table 1 shows baseline characteristics of the study population according to diagnosis. Groups were reasonably well matched for age and gender. As expected, MMSE values were lower in the dementia groups compared to controls. CSF A\(\beta\)42 was lower, while total tau and p-tau levels were higher in the AD group compared with controls and VaD patients. VaD patients had slightly lower CSF A\(\beta\)42 levels than controls, but comparable tau and p-tau values. VaD patients had more microbleeds compared to the subgroup of AD patients with microbleeds (median [IQR] 7 [1-40] vs. 1 [1-6], \(p<0.05\)). AD patients had mostly lobar microbleeds, while VaD patients showed microbleeds in predominantly non-lobar locations (\(p<0.05\)). Fifteen VaD patients (63%) showed only small vessel disease (i.e. extensive white matter hyperintensities and/or multiple lacunes) on MRI. One patient (4%) had a large vessel infarct, and eight patients (33%) had small as well as large vessel disease.

3.1 Differences in MMP and TIMP levels between diagnostic groups

All concentrations were above the lower limit of detection in all patients, in plasma as well as in CSF. Table 2 shows all MMP and TIMP levels by diagnostic group. CSF MMP10 showed the most evident differences between groups (\(p<0.01\), as shown in figure 1. Post-hoc tests showed higher CSF MMP10 levels in AD patients compared to controls (median [IQR] 25 [16-35] vs. 13 [10-20] pg/mL, \(p=0.02\)). Moreover, the CSF/plasma ratio of MMP10 was higher in AD patients compared to the other two groups (0.06 [0.04-0.10] vs. 0.03 [0.02-0.07] for controls and 0.04 [0.02-0.05] for VaD patients; \(p<0.01\)). Plasma MMP10 showed no differences between the groups.

MMP2 in plasma showed differences between groups (\(p=0.01\); figure 1), while in CSF differences were not significant (\(p=0.09\)). Post-hoc tests showed higher plasma MMP2 levels in AD patients compared to VaD patients (60 [48-76] vs. 52 [36-67] ng/mL, \(p=0.02\)), but no significant difference with controls (\(p=0.12\)). Plasma and CSF MMP9, TIMP1 and TIMP2 and their CSF/plasma ratios were not different between diagnostic groups. Results remained the same after exclusion of the three VaD patients with mixed pathology.
Table 1. Baseline characteristics of diagnostic groups

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>AD</th>
<th>VaD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 26</td>
<td>n = 52</td>
<td>n = 24</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>65±6</td>
<td>67±7</td>
<td>68±6</td>
</tr>
<tr>
<td><strong>Sex, n (%) female</strong></td>
<td>11 (42%)</td>
<td>20 (39%)</td>
<td>8 (33%)</td>
</tr>
<tr>
<td><strong>MMSE</strong></td>
<td>28±2</td>
<td>20±5*</td>
<td>24±4*</td>
</tr>
<tr>
<td><strong>CSF Aβ42</strong></td>
<td>874±243</td>
<td>477±174*</td>
<td>666±237*</td>
</tr>
<tr>
<td><strong>CSF tau</strong></td>
<td>275±124</td>
<td>645±416*</td>
<td>338±189</td>
</tr>
<tr>
<td><strong>CSF p-tau</strong></td>
<td>50±15</td>
<td>96±54**</td>
<td>48±20</td>
</tr>
<tr>
<td><strong>MBs, presence, n (%)</strong></td>
<td>0 (0%)</td>
<td>26 (50%)#</td>
<td>20 (83%)</td>
</tr>
<tr>
<td><strong>MBs, median (IQR)</strong></td>
<td>n/a</td>
<td>1 (1-6)³ a</td>
<td>7 (1-40)</td>
</tr>
<tr>
<td><strong>MBs, localisation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strictly lobar, n (%)³</td>
<td>n/a</td>
<td>17 (65)%²</td>
<td>7 (35%)</td>
</tr>
<tr>
<td>Any non-lobar, n (%)³</td>
<td>n/a</td>
<td>9 (35)%²</td>
<td>13 (65%)</td>
</tr>
<tr>
<td><strong>GCA score</strong></td>
<td>0.5±0.6</td>
<td>1.2±0.6*</td>
<td>1.9±0.7*</td>
</tr>
<tr>
<td><strong>MTA score</strong></td>
<td>0.4±0.4</td>
<td>1.5±0.9*</td>
<td>1.4±0.8*</td>
</tr>
<tr>
<td><strong>Large vessel infarct, n (%)</strong></td>
<td>n/a</td>
<td>1 (2%)³</td>
<td>9 (38%)</td>
</tr>
<tr>
<td><strong>Multiple lacunes, n (%)</strong></td>
<td>n/a</td>
<td>2 (4%)³</td>
<td>23 (96%)</td>
</tr>
<tr>
<td><strong>WMH score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0, n (%)</td>
<td>9 (35%)</td>
<td>10 (19%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1, n (%)</td>
<td>15 (58%)</td>
<td>27 (52%)</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>2, n (%)</td>
<td>2 (8%)</td>
<td>12 (23%)</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>3, n (%)</td>
<td>0 (0%)</td>
<td>3 (6%)</td>
<td>18 (75%)</td>
</tr>
</tbody>
</table>

*median (IQR) of subgroup of AD patients with microbleeds (n=26)

² percentage of total number of patients with microbleeds within diagnostic group

³ Multiple lacunes were defined as presence of ≥2 lacunes. Eight VaD patients with multiple lacunes also showed a large vessel infarct on MRI.

Data are displayed as mean ± SD unless otherwise indicated. ANOVA with post-hoc Bonferroni corrections or Chi-squared tests were used when appropriate. For differences in number of microbleeds between AD with MBs and VaD the Mann-Whitney U test was performed. CSF Aβ42, total tau and p-tau were log-transformed for the analyses due to skewed values. For MTA score a 5-point rating scale was used,¹⁷ mean scores of left and right temporal lobe are displayed. For WMH score Fazekas scale was used.¹⁶

* p<0.05 compared with controls

¹² p<0.05 compared with VaD patients
Table 2 MMP and TIMP values by diagnostic groups

<table>
<thead>
<tr>
<th></th>
<th>Controls n=26</th>
<th>AD n=52</th>
<th>VaD n=24</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP2 (ng/mL)</td>
<td>49 (45-69)</td>
<td>60 (48-76) *</td>
<td>52 (36-67)</td>
</tr>
<tr>
<td>MMP9 (ng/mL)</td>
<td>16 (23-21)</td>
<td>14 (10-29)</td>
<td>12 (6-26)</td>
</tr>
<tr>
<td>MMP10 (pg/mL)</td>
<td>362 (297-548)</td>
<td>374 (288-522)</td>
<td>446 (314-575)</td>
</tr>
<tr>
<td>TIMP1 (ng/mL)</td>
<td>102 (94-139)</td>
<td>99 (86-117)</td>
<td>96 (87-124)</td>
</tr>
<tr>
<td>TIMP2 (ng/mL)</td>
<td>95 (84-111)</td>
<td>88 (82-100)</td>
<td>90 (82-114)</td>
</tr>
<tr>
<td><strong>CSF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP2 (ng/mL)</td>
<td>10 (8-11)</td>
<td>11 (9-12)</td>
<td>10 (8-11)</td>
</tr>
<tr>
<td>MMP9 (pg/mL)</td>
<td>17 (10-25)</td>
<td>15 (10-26)</td>
<td>15 (9-24)</td>
</tr>
<tr>
<td>MMP10 (pg/mL)</td>
<td>13 (10-20)</td>
<td>25 (16-35) *</td>
<td>20 (9-27)</td>
</tr>
<tr>
<td>TIMP1 (ng/mL)</td>
<td>62 (51-86)</td>
<td>79 (31-91)</td>
<td>76 (58-86)</td>
</tr>
<tr>
<td>TIMP2 (ng/mL)</td>
<td>89 (76-105)</td>
<td>101 (81-117)</td>
<td>86 (74-100)</td>
</tr>
</tbody>
</table>

Shown are analytes with significant differences between diagnostic groups: A) Plasma MMP2, and B) CSF MMP10. Analyses were performed using age- and sex-adjusted univariate GLM, with post-hoc Bonferroni adjustments, and were performed with log transformed values due to skewed distribution. Crude values are displayed in the graph (mean with 95% CI’s).

* AD > VaD, p = 0.02
* AD > control, p = 0.02

Figure 1. Bar graphs of plasma MMP2 and CSF MMP10 per diagnostic group.

Shown are analytes with significant differences between diagnostic groups: A) Plasma MMP2, and B) CSF MMP10. Analyses were performed using age- and sex-adjusted univariate GLM, with post-hoc Bonferroni adjustments, and were performed with log transformed values due to skewed distribution. Crude values are displayed in the graph (mean with 95% CI’s).

* p < 0.05
3.2 Effects of microbleeds

Figure 2 shows the results of the age- and sex adjusted GLM analysis to assess the relationship between amount of microbleeds (microbleed categories entered as continuous independent variable) and MMPs and TIMPs (dependent variables) in AD patients. There was a main effect of microbleeds for CSF TIMP1 and TIMP2, which were lowered in a dose response fashion (p for trend <0.05 for TIMP1 and <0.01 for TIMP2; figure 2). Patients with multiple microbleeds had similar levels of both TIMPs when compared to controls (median [IQR] in AD patients with multiple microbleeds was 62 [56-80] ng/mL for TIMP1 and 83 [63-97] ng/mL for TIMP2), thus levels were relatively lower in these patients. CSF MMP9 was similarly decreased with increasing number of microbleeds (p for trend < 0.05), but here patients without microbleeds had similar levels as controls, suggesting an absolute decrease with increasing number of microbleeds. Results remained essentially unchanged when we restricted the analysis to patients with lobar microbleeds (data not shown).

**Figure 2.** Relation of CSF TIMP2 and MMP9 with number of microbleeds in AD patients.

Displayed are A) CSF TIMP2 and B) MMP9 according to number of microbleeds in AD patients. Analyses were performed using age- and sex-adjusted univariate GLM, with number of microbleeds as independent variable (microbleed categories entered as continuous variable), and MMPs and TIMPs as dependent variables (entered in separate models). In this analysis p for trend < 0.05 represents a significant dose-response effect of number of microbleeds on the dependent variable. All analyses were performed with log transformed values due to skewed distribution, crude values are displayed in the graph (mean with 95% CI’s). Numbers per group are displayed below the graphs, some values were missing because there was no CSF available (n=3) or CV was >30% (n=3 for MMP9 and n=1 for TIMP2). Values of TIMP1 are not shown, as they were very similar to TIMP2.

* p for trend < 0.05  
** p for trend < 0.01
3.3 Association with AD biomarkers

We performed age- and sex adjusted linear regression analyses to assess associations between CSF AD biomarkers and MMPs and TIMPs. Figure 3 shows the main results. Aβ42 was not related to any of the MMPs or TIMPs. Across diagnostic groups, there was an association between p-tau and CSF MMP2 (standardized B [st.B] 0.27 [p<0.01]) and between both tau and p-tau and CSF MMP10 (st.B 0.41 [p<0.001] for tau, 0.42 [p<0.001] for p-tau). When we included diagnosis (as dummy variable) and interaction of tau or p-tau with diagnosis in the model to allow estimation of B’s per diagnostic group, significance for the association of p-tau with CSF MMP2 was lost, although the effect size was largest in the VaD group (st. B 0.35 [p=0.13]). The associations of tau and p-tau with MMP10 remained significant for AD patients (st.B 0.37 [p<0.05] for tau, 0.34 [p<0.05] for p-tau), while B’s for controls and VaD patients were smaller and non-significant.
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Figure 3. Scatter plots of associations of CSF MMP2 with p-tau and CSF MMP10 with tau.

Age- and sex-adjusted linear regression analysis was performed to assess the associations of MMPs and TIMPs with CSF AD biomarkers. Log transformed values were used due to skewed distribution; crude values are displayed in the graph. The striped lines and circles represent control subjects, the solid line and triangles AD patients, and the dotted line and diamonds the VaD patients. St.B’s for the total population were 0.27 (p<0.01) for the association of CSF p-tau with MMP2, and 0.41 (p<0.001) for CSF tau with MMP10. St.B’s (p-value) per diagnostic group were for p-tau~MMP2: controls 0.07 (0.83), AD 0.22 (0.13), VaD 0.35 (0.13); and for tau~MMP10: controls 0.32 (0.22), AD 0.37 (0.02), VaD 0.24 (0.29).

* p < 0.05
4. Discussion

In this study we found higher plasma MMP2 and CSF MMP10 levels in AD patients compared to VaD patients and controls. The observed associations of CSF MMP2 and MMP10 with CSF tau and p-tau suggest that they may be involved in tangle pathology or neuronal damage. Secondly, we found lower concentrations of CSF MMP9, TIMP1 and TIMP2 in the subgroup of AD patients with multiple microbleeds, suggesting their particular involvement in CAA.

Both MMP2 and MMP10 have been implicated in AD before, although MMP2 has been more widely studied than MMP10. MMP2 is a gelatinase expressed by neurons and astrocytes, and is able to disrupt the BBB. The BBB in turn has been proposed to be important in both CAA and AD. In addition, MMP2 has been shown to increase after treatment with Aβ in cell cultures and to promote Aβ catabolism in mice, suggesting involvement in Aβ degradation as well. Clinical studies however have shown contradictory results. We found increased MMP2 in plasma of AD patients but no significant differences in CSF. Most previous studies measured MMP2 with gelatine zymography, which measures the activity instead of total protein concentration, as we did using immunoassays. The only previous study using an immunoassay for measurement of plasma MMP2 concentration similarly showed slightly elevated levels in a small set of AD patients compared to controls, although the difference did not reach significance.

CSF MMP10 (also known as stromelysin-2) has been implicated in inflammation, although the exact function in relation to AD is not yet known. In line with our results, MMP10 has previously been found to be increased in AD patients, and to correlate with CSF tau. Moreover, we also found a higher CSF/plasma ratio of MMP10 in AD patients compared to the other groups, which may be more informative on intrathecal origin and brain-specificity of this protein. As MMP10 is known to be highly expressed by activated microglia, it may be involved in the inflammatory response to neuronal damage. This would also be in line with a previous report, showing that MMP10 was produced in ischemic brain regions.

Interestingly, microbleeds in AD were associated with lower concentrations of CSF TIMP1, TIMP2 and MMP9 in a dose response relation. We are not aware of studies on TIMPs in CAA, and studies on TIMPs in relation to AD are sparse. One study showed a slight increase of CSF TIMP2 in AD patients compared to control subjects. In view of this previous study, TIMPs may play a role in amyloid pathology, or the inflammatory response on amyloid deposition. The relation between TIMPs and microbleeds we found suggests involvement in amyloid angiopathy in addition to AD type amyloid plaques. While in AD patients without evidence of co-existing amyloid angiopathy TIMPs may be slightly increased, the lower TIMP values in AD patients with microbleeds may imply relatively less MMP inhibition, leading to more pronounced BBB degradation, which has been found to be present in CAA. Presence of
MMP9 in microvessels has previously been associated with CAA and BBB leakage in studies with cell cultures and rodents. In clinical samples a slight increase has been found in CSF of VaD patients, while a trend towards lower MMP9 has been found in CSF with an AD profile (i.e. low Aβ42 and high tau and p-tau; based on cluster analysis). Why MMP9 is lowered in AD patients with more microbleeds needs to be clarified in future studies. In our previous study, with a partly overlapping patient sample, we found no difference in CSF/plasma albumin ratio between AD patients with and without microbleeds, providing no evidence for extensive BBB damage. This index is however a relatively crude measure of BBB integrity; more advanced imaging methods may be more sensitive to assess subtle changes.

Among the strengths of our study is that we included AD patients with and without microbleeds. As such, our results support the notion of additional disease processes in AD patients with microbleeds compared to those without. Our findings may partly explain the inconsistency of previous findings on MMPs in as well AD patients as VaD patients as both patient groups could have consisted partly of patients with concurrent CAA pathology, possibly confounding the results. However, especially the results of MMP9 have to be interpreted with care, as concentrations of MMP9 in CSF were low, and CVs were quite high. Although all analytes were above the lower limit of detection in our study, there have been problems with sensitivity in previous studies. Improvement of the sensitivity of the assays is therefore needed. Another limitation of our study is the fact that we measured total concentration of MMPs and TIMPs, instead of the active fraction of the concentration. One could argue that levels of active protein are more relevant to protein function than total concentration. The reliability of these assays using stored, frozen samples is however questionable, which made ELISA a more appropriate method for our samples.

In conclusion, our results suggest that MMP2 and MMP10 play a role in AD pathophysiology, while MMP inhibitors may play an important role in AD patients with concurrent CAA pathology. The relatively lower concentration of TIMPs in this subset of AD patients suggests a pathological mechanism leading to a more vulnerable blood-brain barrier in these patients. Whether TIMPs could be more directly linked to BBB damage in AD patients with concurrent CAA, or may be a therapeutic target for these patients is subject for further study.

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Reference List


