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Matrix metalloproteinases are associated with brain atrophy in cognitively unimpaired individuals

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ABSTRACT

Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) have been linked to age-related neurodegeneration and Alzheimer's disease (AD), but their role in normal aging is poorly understood. We used linear mixed models to determine if baseline or rate of yearly change in cerebrospinal fluid (CSF) levels of MMP-2; MMP-3; MMP-10; TIMP-123 (composite of TIMP-1, TIMP-2, and TIMP-3); or TIMP-4 predicted changes in bilateral entorhinal cortex thickness, hippocampal volume, or lateral ventricle volume in cognitively unimpaired individuals. We also assessed effects on the CSF AD biomarkers amyloid-β_{42} and phosphorylated tau$_{181}$. Low baseline levels of MMP-3 predicted larger ventricle volumes and more entorhinal cortex thinning. Increased CSF MMP-2 levels over time predicted more entorhinal thinning, hippocampal atrophy, and ventricular expansion, while increased TIMP-123 over time predicted ventricular expansion. No MMP/TIMPs predicted changes in CSF AD biomarkers. Notably, we show for the first time that longitudinal increases in MMP-2 and TIMP-123 levels may predict age-associated brain atrophy. In conclusion, MMPs and TIMPs may play a role in brain atrophy in cognitively unimpaired aging.

1. Introduction

Matrix metalloproteinases (MMPs) are a family of proteases with important roles in tissue remodeling and neuroinflammation (Rivera et al., 2010). MMPs are expressed in the brain by astrocytes and microglia (Nuttall et al., 2007; Thorns et al., 2003) and tightly regulated by tissue inhibitors of MMPs (TIMPs). These proteins have been implicated in neurodegenerative diseases, such as Alzheimer's disease (AD; Rivera et al., 2019), and have been linked to cognitive decline, brain atrophy, and AD biomarkers, such as cerebrospinal

Abbreviations: Aβ_{42}, amyloid-β_{42}; AD, Alzheimer's disease; CSF, cerebrospinal fluid; FDR, false rate discovery; MCI, mild cognitive impairment; MMP, matrix metalloproteinase; MMSE, Mini Mental Status Examination; MRI, magnetic resonance imaging; REC, Regional Committee for Ethics in Medical Research in Norway; p-tau$_{181}$, phosphorylated tau$_{181}$; SD, standard deviation; SE, standard error; TIMP, tissue inhibitor of MMP; TMT, Trail Making Test

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fluid (CSF) amyloid-β_{42} ($A\beta_{42}$) and phosphorylated tau_{181} (p-tau_{181}). However, their role in normal brain aging is poorly understood, and the current study aims to elucidate their longitudinal effects on brain atrophy, CSF $A\beta_{42}$, and p-tau_{181} in a cognitively unimpaired cohort.

In cognitively unimpaired older adults, MMP-2 levels are reduced in those who progressed to mild cognitive impairment (MCI; Mattsson et al., 2013). In patients with MCI however, higher CSF levels of MMP-10 are associated with an increased risk of progression to dementia (Martino Adami et al., 2022). Elevated CSF MMP-10 levels have also been associated with disease progression in Parkinson's disease (Santaella et al., 2020). It has been suggested that these proteins, especially MMP-10, may be associated with general neurodegenerative pathways related to aging (Martino Adami et al., 2022). Indeed, elevated protein expression of MMP-10 has been associated with dermal aging and atherosclerosis (Lago and Puzzi, 2019; Montero et al., 2006). Changes in the balance between MMPs and TIMPs have also been linked to age-related vascular diseases and increased neuroinflammation in the aging brain (Brkic et al., 2015).

Expanding ventricles, reduced hippocampal volumes, and reduced cortical thickness in areas such as the entorhinal cortex are common findings in the aging human brain (Fjell et al., 2014; Fjell and Walhovd, 2010); these areas are also well known to be affected by age-related diseases such as AD. However, research on associations between MMPs/TIMPs and markers of brain atrophy in normal aging is sparse. In AD, MMP expression is increased in response to Aβ and tau pathology, and several MMPs play important roles in the degradation of Aβ and tau (Hernandez-Guillamon et al., 2015; Nübling et al., 2012; Yan et al., 2006).

CSF levels of MMPs and TIMPs have been studied as potential biomarkers for AD, with mixed results; for example TIMP-1 levels have been found to be higher, similar and lower in AD patients versus cognitively unimpaired controls (Lorenzl et al., 2003; Mroczko et al., 2014; Stomrud et al., 2010). Interestingly, CSF levels of selected MMPs and MMP/TIMP ratios are higher in cognitively unimpaired individuals with abnormal levels of CSF $A\beta_{42}$ and p-tau_{181} compared to those with normal biomarker levels. In these individuals, CSF levels of MMP-3 and MMP-9 also correlated with p-tau_{181} levels (Stomrud et al., 2010). Furthermore, in cognitively unimpaired individuals above 60 years of age, an exploratory study has suggested an Aβ-associated effect of CSF MMP-3 on brain atrophy (Mattsson et al., 2014). This indicates that MMPs might be involved in early pathogenesis of AD and that MMPs could be associated with Aβ- and tau-driven neurodegeneration prior to the development of cognitive decline. Moreover, high levels of circulating TIMP-1 have been associated with more hippocampal atrophy in patients with mild cognitive impairment and lower total brain volume cognitively unimpaired individuals (Abe et al., 2020; Romero et al., 2010). Circulating TIMP-1, MMP-2, and MMP-9 levels have been associated with higher prevalence of large white matter hyperintensities in patients with acute stroke and cognitively unimpaired controls (Corbin et al., 2014; Jiménez-Balado et al., 2021; Kim et al., 2014; Romero et al., 2010). This suggests a more general role for MMPs/TIMPs in aging and brain ischemia, beyond the link to AD pathology.

The main aim of the current study was to test whether baseline CSF levels of MMP-2, MMP-3, MMP-10, TIMP-4 or a composite measure of TIMP-1, TIMP-2, and TIMP-3 were associated with bilateral entorhinal cortex thinning, hippocampal atrophy, or ventricle volume expansion in normal aging. Further, we aimed to determine if the associations between these MMPs/TIMPs and brain atrophy were moderated by CSF $A\beta_{42}$ or p-tau_{181}. Finally, in a small subset of patients who had undergone two lumbar punctures, we explored whether changes in MMP/TIMP levels over time were associated with brain atrophy or changes in the CSF $A\beta_{42}$ or p-tau_{181} levels.

2. Materials and methods

2.1. Participants

We included 111 individuals from the COGNORM cohort (Idland et al., 2017), to which cognitively unimpaired individuals aged ≥65 years scheduled for elective gynecological, urological, or orthopedic surgery in spinal anesthesia were recruited during 2012–2013. Exclusion criteria for COGNORM were dementia, Parkinson's disease, previous stroke with sequela, or other neurological diseases likely to affect cognition at baseline. CSF samples were collected by the anesthesiologist before spinal anesthesia and the participants underwent brain magnetic resonance imaging (MRI) after surgery. We included all patients in COGNORM with measures of MMP/TIMPs in CSF and MRI of the brain from baseline. Patients were assessed with an extensive battery of cognitive tests before surgery, including the Mini Mental Status Examination (MMSE; Folstein et al., 1975); the Word List Memory Task (Morris et al., 1989); the Trail Making Tests A and B (TMT-A and TMT-B; Reitan, 1958); and phonetic and semantic verbal fluency tests (FAS test and animal naming test; Strauss et al., 2006). Patients were tested with a similar panel of cognitive tests annually and with MRIs biannually for up to 9.5 years. A subset of patients (n = 32) volunteered for a second lumbar puncture for CSF collection after an average of 4.75 years (range = 4.15–5.86 years). The study was conducted in accordance with the Declaration of Helsinki and approved by the Regional Committee for Ethics in Medical Research in Norway (REC South East 2011/2052). All participants provided written consent.

2.2. CSF sampling and biochemical analyses

CSF was collected in polypropylene tubes, centrifuged, and aliquoted before storage at −80 °C (Idland et al., 2017). CSF $A\beta_{42}$ and p-tau_{181} concentrations were measured using INNOTEST enzyme-linked immunosorbent assays (Fujirebio) at Sahlgrenska University Hospital (Mölndal, Sweden). For the subset of patients with CSF from two time points, CSF $A\beta_{42}$ and p-tau_{181} concentrations were analyzed in baseline and follow-up samples in parallel using Lumipulse assays (Fujirebio), as previously described (Gobom et al., 2022). CSF MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, MMP-13, TIMP-1, TIMP-2, TIMP-3, and TIMP-4 were determined simultaneously using a multiplex Disocvery Assay on a Luminex xMAP instrument at Eve Technologies (Calgary, Canada). All samples were measured in duplicate. Samples were sent on dry ice to the respective laboratories without any information about the patients. Markers that were detectable in less than 70% of samples (MMP-1, MMP-7, MMP-9, and MMP-12) were dichotomized as detectable/not-detectable. MMP-8 and MMP-13 were not detectable in any markers and were excluded from further analysis.

2.3. MRI acquisition and processing

T1-weigthed MPRAGE 3D images were acquired by a 1.5T Siemens Avanto scanner using a 12-channel head coil (repetition time = 2400 ms, echo time = 3.79 ms, field of view = 240 mm, slice thickness = 1.20 mm, and pixel size = 1.25 × 1.25 mm). Images were transformed to the Brain Imaging Data Structure format (Gorgolewski et al., 2016) and processed in FreeSurfer (https://surfer.nmr.mgh.harvard.edu/fswiki; Dale et al., 1999; Fischl et al., 1999) with the longitudinal FreeSurfer v.7.1.0 stream (Reuter et al., 2012). In short, images were processed first using the cross-sectional stream, involving the removal of nonbrain tissues, Talairach transformation, intensity correction, tissue and volumetric segmentation, cortical surface reconstruction, and cortical parcellation. Then, for

each patient an unbiased within-subject template space based on all cross-sectional images was created using a robust, inverse consistent registration (Reuter et al., 2010). To increase the reliability and statistical power of the cortical thickness estimates, the processing of each time point was then reinitialized using common information from the within-subject template. Entorhinal thickness, hippocampal volume, and lateral ventricles volume, averaged across both hemispheres, were selected as regions of interest due to their role in aging and age-related diseases such as AD.

2.4. Statistical analysis

We investigated correlations between all measured CSF biomarkers, see Appendix A: Fig. A1 for the Pearson's correlation matrix. TIMP-1, TIMP-2, and TIMP-3 were highly intercorrelated: Pearson's r = 0.77 for TIMP-1 and TIMP-2; r = 0.66 for TIMP-1 and TIMP-3; and r = 0.73 for TIMP-2 and TIMP-3. We created a composite variable, TIMP-123, by standardizing the TIMP-1, TIMP-2, and TIMP-3 levels and averaging the standardized scores. Analyses were run with each MMP/TIMP (MMP-2, MMP-3, MMP-10, TIMP-123, or TIMP-4) as an independent variable. We performed sensitivity analyses by repeating all analyses excluding 18 patients who either (1) had poor baseline MMSE results, (2) had poor TMT-A or TMT-B results, or (3) were offered referral for further cognitive testing. All analyses were run in the R-environment (R Core Team, 2022) using the corrplot (Wei and Simko, 2021), ggplot2 (Wickham, 2016), lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), and sjPlot (Lüdecke, 2022) packages.

We used linear mixed models to investigate the effects of baseline levels and rate of change in MMP/TIMP levels on brain atrophy (measured by longitudinal change in bilateral entorhinal cortex thickness, bilateral hippocampal volume, and bilateral ventricles volumes). We calculated the rate of change in MMP/TIMP levels as: (MMP/TIMP level at follow up – MMP/TIMP level at baseline)/time in years between lumbar punctures. All linear mixed models included sex and age at baseline as covariates and patient identifiers as random intercepts; for patients with *APOE* status available (n = 94), we ran models including *APOE* ε4 status as a covariate. For models with volumetric measures as the outcome, estimated intracranial volume was also included as a covariate. For the MMPs that were dichotomized, we investigated the effects of high (i.e. detectable) versus low (i.e. non detectable) levels. For models with a significant interaction between baseline MMP/TIMP × time on brain atrophy, we ran additional models controlling for interactions with (INNOTEST-measured) CSF $A\beta_{42}$ or p-tau_{181} (MMP/TIMP × CSF $A\beta_{42}$ × time and MMP/TIMP × CSF p-tau_{181} × time). We applied false discovery rate (FDR)-correction across all brain regions for each MMP/TIMP considering q-values (p_{FDR}) < 0.05 significant.

For the subset of patients with two lumbar punctures, we performed paired-samples t-tests to investigate the changes in CSF biomarkers at follow-up compared to baseline. The effect of baseline levels and rate of change in MMP/TIMP levels on changes on (Lumipulse-measured) CSF $A\beta_{42}$ and p-tau_{181} levels were assessed with linear mixed models with each MMP/TIMP as an independent variable and either CSF $A\beta_{42}$ or p-tau_{181} as the dependent variable. We applied FDR-correction across each AD biomarker for each MMP/TIMP considering q-values (p_{FDR}) < 0.05 significant.

3. Results

3.1. Cohort characteristics

The baseline characteristics of the cohort are presented in Table 1.

Table 1
Cohort characteristics at baseline

	N	(%)
N	111	(100.0)
Women	52	(46.9)
Detectable MMP-1	35	(31.5)
Detectable MMP-7	18	(16.2)
Detectable MMP-9	29	(26.1)
Detectable MMP-12	72	(64.9)
	Median	(First quartile; third quartile)
Age	72	(68; 77)
MMSE	29	(28; 30)
CSF $A\beta_{42}$ (pg/mL)[a]	764	(514; 864)
CSF p-tau_{181} (pg/mL)[a]	58	(46; 70)
MMP-2 (ng/mL)	46.2	(41.0; 53.3)
MMP-3 (pg/mL)	202.0	(152.4; 278.1)
MMP-10 (pg/mL)	14.1	(7.8; 23.6)
TIMP-123	−0.15	(−0.7; 0.5)
TIMP-4 (ng/mL)	1.5	(1.3; 1.8)

Key: $A\beta_{42}$, amyloid-β_{42}; CSF, cerebrospinal fluid; p-tau_{181}, phosphorylated tau_{181}; MMP, matrix metalloproteinases; TIMP, tissue inhibitors of MMP.
[a] Measured by the INNOTEST assay. N = 107.

3.2. Relationship between MMPs, TIMPs, and AD biomarkers

The Pearson's correlation matrix for all measured CSF biomarkers ($A\beta_{42}$, p-tau_{181}, MMP-2, MMP-3, MMP-10, TIMP-123, and TIMP-4) is presented in Fig. 1. CSF $A\beta_{42}$ levels were weakly correlated with TIMP-4, but not with any other markers. CSF p-tau_{181} levels were weakly/moderately correlated with all measured MMPs and TIMPs.

3.3. Effect of baseline MMP/TIMP levels on brain atrophy

The models assessing the effects of baseline MMP/TIMP levels on brain atrophy are summarized in Table 2. Higher baseline levels of MMP-3 were associated with less cortical thinning in the entorhinal cortex ($\beta = 3.6 \times 10^{-5}$, $p = 0.02$) and a smaller expansion of bilateral ventricle volume over time ($\beta = -5.5 \times 10^{-1}$, $p = 0.02$), see Fig. 2. Lower baseline levels of TIMP-123 were associated with more hippocampus atrophy, but this did not survive FDR-correction ($\beta = -5.4$, $p = 0.13$). Higher baseline levels of TIMP-4 were associated with a larger expansion of bilateral ventricle volume over time, but this did not survive FDR-correction ($\beta = 1.7 \times 10^{-1}$, $p = 0.06$). For patients with *APOE* status available, the models were run again including *APOE* ε4 status as a covariate. *APOE* ε4 status was not significantly associated with any MMP/TIMP, and inclusion of this covariate did not significantly affect the effects of baseline MMP/TIMP level on the brain atrophy measures. For each MMP/TIMP, we investigated the effects of baseline levels on whole brain atrophy, see Appendices B1; B5.

Detectable levels of MMP-1 were associated with smaller expansion of ventricle volume over time ($\beta = -2.2 \times 10^{2}$, corrected $p < 0.01$), but not with less entorhinal thinning or hippocampus atrophy. MMP-7, MMP-9, and MMP-12 were not associated with any measures of brain atrophy.

3.3.1. Effects of CSF $A\beta_{42}$ and p-tau_{181} on MMP/TIMP-related brain atrophy

To determine if the effect of MMP-3 on brain atrophy was independent of and/or moderated by the AD CSF biomarkers, linear mixed models controlling for the interactions with (INNOTEST-measured) $A\beta_{42}$ × time and p-tau_{181} × time were run. The effect of MMP-3 × time on bilateral entorhinal thickness remained significant when controlling for interactions with CSF $A\beta_{42}$ and p-tau_{181}. There were no significant interactions between MMP-3 and the AD biomarkers.

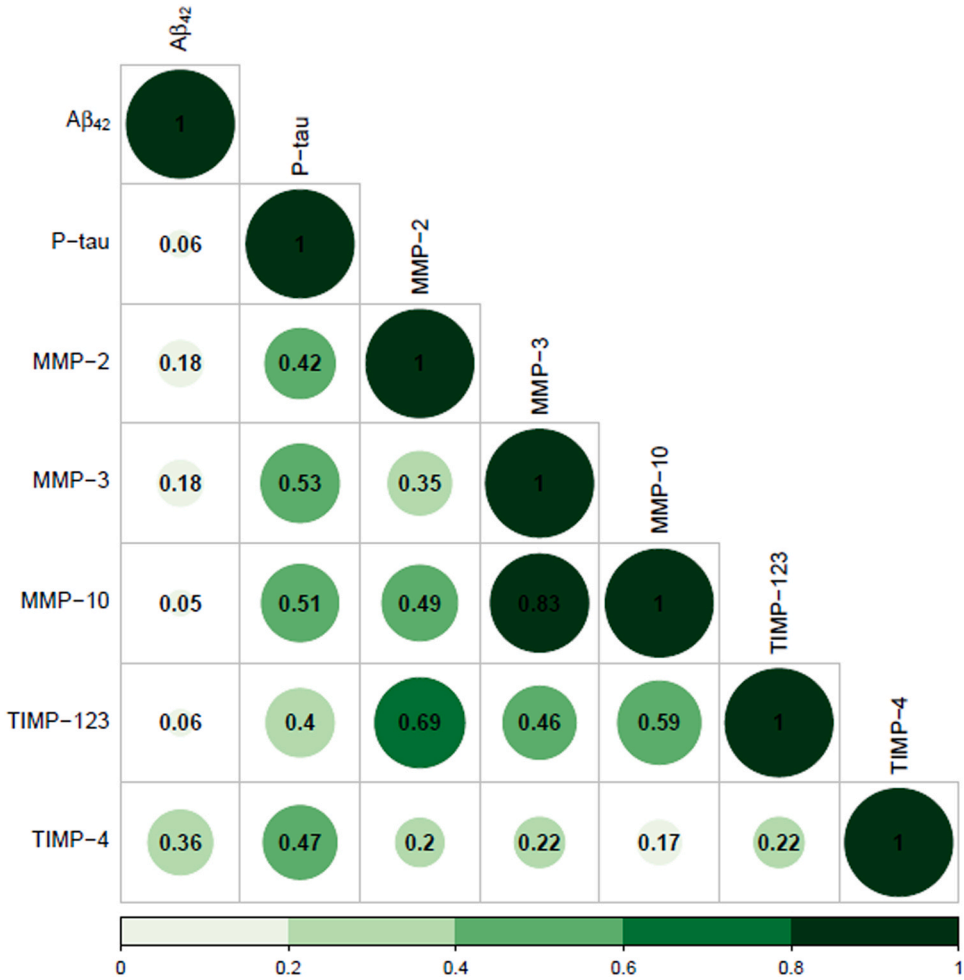

Fig. 1. Pearson correlation matrix for the CSF biomarkers. Darker colors and larger circles indicate stronger relationships. Abbreviations: CSF, cerebrospinal fluid.

When controlling for the effect of $A\beta_{42}$ × time, the effect of MMP-3 × time on bilateral ventricle volume was no longer significant ($\beta = -4.0 \times 10^{1}$, $p = 0.26$), while there was a significant effect of $A\beta_{42}$ × time on ventricle volume ($\beta = -1.2 \times 10^{2}$, $p < 0.01$), with higher baseline $A\beta_{42}$ predicting less ventricle volume expansion. There were no significant interactions between MMP-3 and the AD biomarkers.

3.4. Effect of yearly change in MMP/TIMP levels on brain atrophy

At follow-up, all MMP levels were significantly increased from baseline, whereas there was no difference in average TIMP-123 or TIMP-4 levels at follow-up compared to baseline, see Table 3. All follow-up levels were correlated with the baseline measurements, see Table 3.

Table 2
The effects of baseline MMP/TIMP levels on brain atrophy

CSF protein	Brain region	B	Standard error	*p*	p_{FDR}
MMP-2 × time	Entorhinal cortex	-3.3×10^{-7}	2.0×10^{-7}	0.10	
	Hippocampus	-3.5×10^{-4}	2.4×10^{-4}	0.15	
	Ventricles	0.5×10^{-3}	3.2×10^{-3}	0.15	
MMP-3 × time	Entorhinal cortex	3.6×10^{-5}	1.4×10^{-5}	**< 0.01**	**0.02**
	Hippocampus	8.4×10^{-3}	1.7×10^{-2}	0.62	0.62
	Ventricles	-5.5×10^{-1}	2.2×10^{-1}	**0.01**	**0.02**
MMP-10 × time	Entorhinal cortex	1.1×10^{-4}	1.5×10^{-4}	0.47	
	Hippocampus	1.5×10^{-2}	1.8×10^{-1}	0.93	
	Ventricles	−1.9	2.3	0.40	
TIMP-123 × time	Entorhinal cortex	-3.0×10^{-3}	2.2×10^{-3}	0.17	0.26
	Hippocampus	−5.4	2.6	**0.04**	0.13
	Ventricles	2.2×10^{1}	3.5×10^{1}	0.52	0.53
TIMP-4 × time	Entorhinal cortex	1.5×10^{-6}	4.4×10^{-6}	0.73	0.90
	Hippocampus	6.8×10^{-4}	5.4×10^{-4}	0.90	0.90
	Ventricles	1.7×10^{-1}	7.1×10^{-2}	**0.02**	0.06

Key: MMP, matrix metalloproteinases; TIMP, tissue inhibitors of MMP; *p*FDR, q-values false discovery rate.
Significant p-values are highlighted in bold.

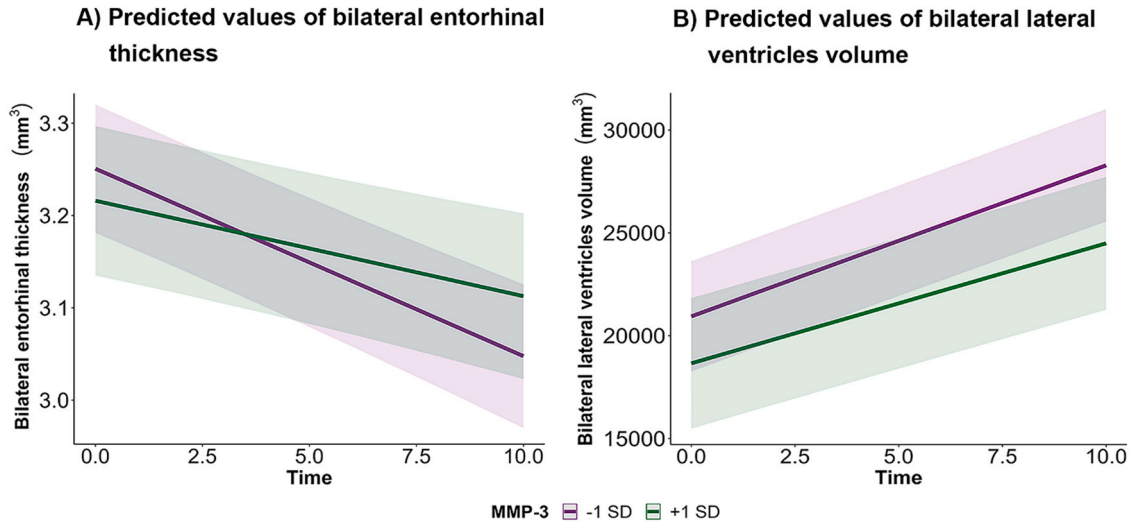

Fig. 2. The effect of baseline MMP-3 levels on (A) entorhinal thickness and (B) bilateral lateral ventricles volume over time. The purple line represents MMP-3 levels 1 SD below the mean, the green line represents MMP-3 levels 1 SD above the mean. Abbreviations: MMP, Matrix metalloproteinases; SD, standard deviation.

Table 3
Baseline and follow-up levels of CSF MMPs/TIMPs

CSF protein	Baseline level	Follow-up	Pearson's r	Mean difference	p^a
MMP-2 (ng/mL)	47.7 (9.0)	54.7 (11.9)	0.86	7.0 (6.2)	**< 0.01**
MMP-3 (pg/mL)	240.0 (21.8)	302.9 (128.5)	0.88	62.9 (62.0)	**< 0.01**
MMP-10 (pg/mL)	17.3 (11.1)	28.3 (16.9)	0.61	11.0 (13.4)	**< 0.01**
TIMP-123	− 0.01 (0.7)	0.08 (0.9)	0.55	0.09 (0.8)	0.52
TIMP-4 (ng/mL)	1.7 (0.3)	1.7 (0.3)	0.90	−0.04 (0.15)	0.11

Data is presented as mean (SD), N = 32.
Key: CSF, cerebrospinal fluid; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitors of MMP; SD, standard deviation.
Significant p-values are highlighted in bold.
[a] *p*-value is for a paired-samples t-test comparing baseline and follow-up levels.

The models assessing the effect of rate of change in MMP/TIMP levels on brain atrophy are summarized in Table 4. A larger increase in CSF MMP-2 levels over time was associated with more brain atrophy, both in terms of more entorhinal thinning ($\beta = -6.1 \times 10^{-6}$, $p < 0.01$), smaller hippocampal volume ($\beta = -6.4 \times 10^{-3}$, $p = 0.03$) and larger ventricle volume ($\beta = 7.2 \times 10^{-2}$, $p = 0.04$), see Fig. 3. A larger increase in CSF TIMP-123 levels from baseline was associated with more ventricle volume expansion over time ($\beta = 1.5 \times 10^{2}$, $p < 0.01$), see Fig. 3. A larger increase in MMP-10 was associated with more hippocampal atrophy over time, but this did not survive FDR-correction ($\beta = -5.1 \times 10^{-1}$, $p = 0.09$).

3.5. Effect of MMP/TIMP levels on change in AD biomarkers over time

Lumipulse CSF $A\beta_{42}$ and levels were significantly increased at follow-up (mean = 776 pg/mL, standard deviation [SD] = 280.5)

Table 4
The effects of yearly change in MMP/TIMP levels on brain atrophy

CSF protein	Brain region	B	Standard error	*p*	p_{FDR}
MMP-2 × time	Entorhinal cortex	-6.1×10^{-6}	2.0×10^{-6}	**< 0.01**	**< 0.01**
	Hippocampus	-5.4×10^{-3}	2.3×10^{-3}	**0.02**	**0.03**
	Ventricles	7.2×10^{-2}	3.4×10^{-2}	**0.04**	**0.04**
MMP-3 × time	Entorhinal cortex	3.5×10^{-5}	2.3×10^{-5}	0.14	
	Hippocampus	1.9×10^{-4}	2.7×10^{-2}	0.99	
	Ventricles	-4.5×10^{-1}	3.9×10^{-1}	0.26	
MMP-10 × time	Entorhinal cortex	-3.6×10^{-4}	2.1×10^{-4}	0.09	0.11
	Hippocampus	-5.1×10^{-1}	2.3×10^{-1}	**0.03**	0.09
	Ventricles	5.7	3.5	0.11	0.11
TIMP-123 × time	Entorhinal cortex	-5.3×10^{-3}	3.0×10^{-3}	0.08	0.08
	Hippocampus	−6.3	3.4	0.07	0.08
	Ventricles	1.5×10^{2}	4.8×10^{1}	**< 0.01**	**< 0.01**
TIMP-4 × time	Entorhinal cortex	-4.1×10^{-7}	9.8×10^{-6}	0.97	
	Hippocampus	-9.1×10^{-3}	1.1×10^{-2}	0.42	
	Ventricles	-2.3×10^{-1}	1.6×10^{-1}	0.17	

Key: CSF, cerebrospinal fluid; MMP, matrix metalloproteinases; *p*FDR, q-values false discovery rate; TIMP, tissue inhibitors of MMP.
Significant p-values are highlighted in bold.

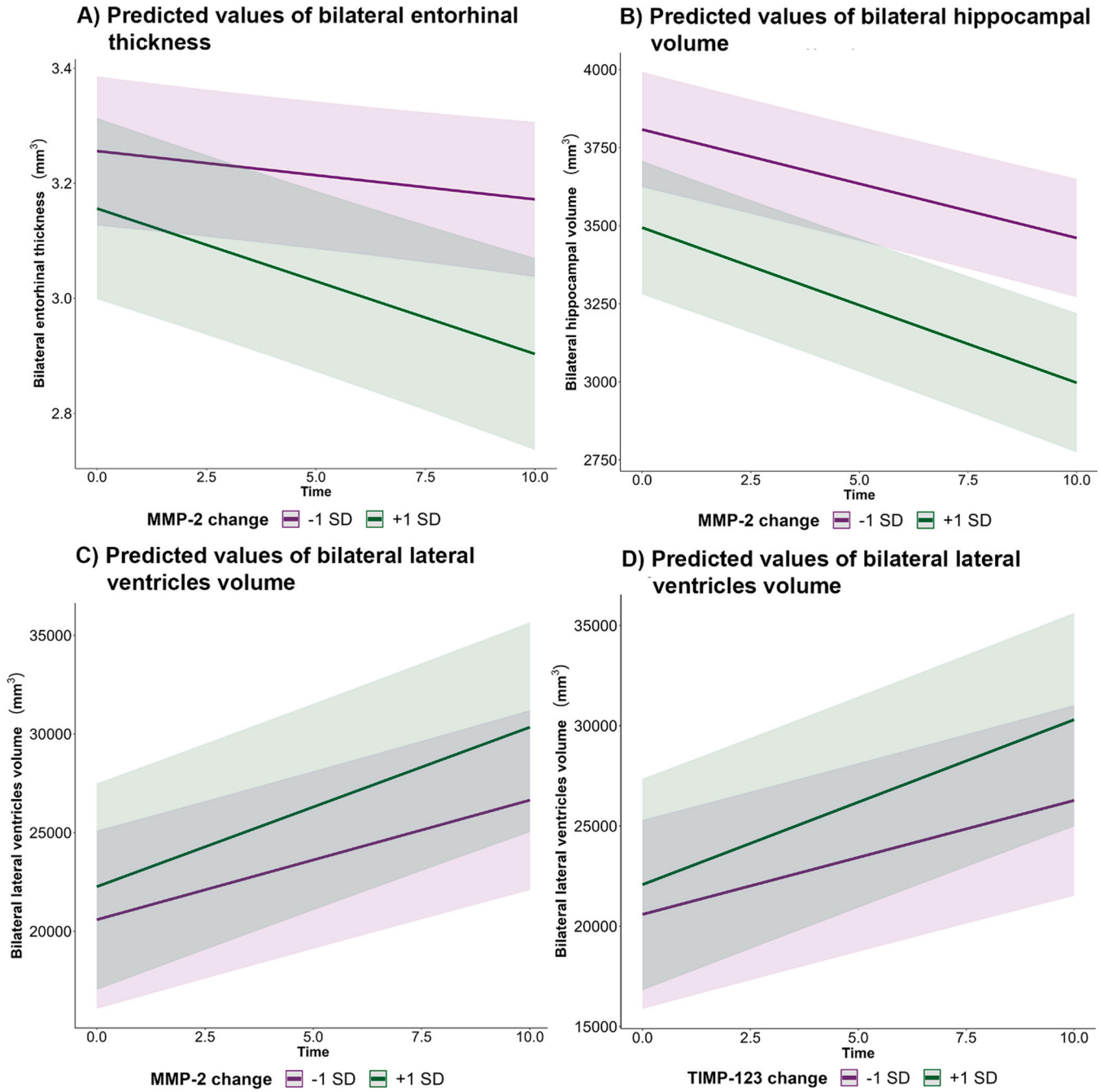

Fig. 3. The effect of yearly change in MMP-2 and TIMP-123 levels on brain atrophy. (A) The effect of MMP-2 change on entorhinal thickness, (B) the effect of MMP-2 change on hippocampal volume, (c) the effect of MMP-2 change on bilateral lateral ventricle volume, (D) the effect of TIMP-123 change on bilateral lateral ventricles volume over time. The purple line represents change from baseline 1 SD below the mean, the green line represents change from baseline 1 SD above the mean. Abbreviations: MMP, matrix metalloproteinase; SD, standard deviation; TIMP, tissue inhibitors of MMP.

compared to baseline (mean = 673.8 pg/mL, SD = 247.5, $p = 0.005$). Similarly, Lumipulse CSF p-tau$_{181}$ levels were significantly increased at follow-up (mean = 58.4 pg/mL, SD = 22.8) compared to baseline (mean = 51.3 pg/mL, SD = 18.5, $p < 0.001$). Baseline and follow-up levels were strongly correlated, Pearson's r = 0.75 for Aβ_{42} and r = 0.96 for p-tau$_{181}$.

The models assessing the effect of baseline MMP/TIMP levels on change in AD biomarkers over time are summarized in Table 5. A larger increase in MMP-2 levels over time was associated with a larger increase in p-tau$_{181}$ levels over time, but this did not survive FDR-correction ($\beta = 5.9 \times 10^{-5}$, $p = 0.08$).

3.6. Sensitivity analysis

We repeated all analyses excluding the patients (n = 18) with poor baseline MMSE, TMT-A, or TMT-B scores and the patients who were offered referral for further cognitive testing after baseline

Table 5
The effects of baseline MMP/TIMP levels on change in CSF $A\beta_{42}$ and p-tau_{181} over time

CSF protein	AD biomarker	B	Standard error	*p*	p_{FDR}
MMP-2 × time	$A\beta_{42}$	-2.6×10^{-4}	7.8×10^{-4}	0.74	0.74
	P-tau_{181}	5.9×10^{-5}	2.7×10^{-5}	**0.04**	0.08
MMP-3 × time	$A\beta_{42}$	-4.5×10^{-2}	5.8×10^{-2}	0.44	
	P-tau_{181}	-1.9×10^{-3}	2.2×10^{-3}	0.40	
MMP-10 × time	$A\beta_{42}$	3.2×10^{-1}	6.5×10^{-1}	0.62	
	P-tau_{181}	1.2×10^{-2}	2.4×10^{-2}	1.00	
TIMP-123 × time	$A\beta_{42}$	-1.1×10^{1}	9.8	0.27	
	P-tau_{181}	3.6×10^{-1}	3.7×10^{-1}	0.34	
TIMP-4 × time	$A\beta_{42}$	-2.5×10^{-3}	2.2×10^{-2}	0.91	
	P-tau_{181}	7.8×10^{-4}	8.0×10^{-4}	0.34	

Rate of yearly change in MMP/TIMP levels was not a significant predictor of changes in either CSF $A\beta_{42}$ or p-tau_{181}.
Key: $A\beta_{42}$, amyloid-β_{42}; AD, Alzheimer's disease; CSF, cerebrospinal fluid; MMP, matrix metalloproteinase; pFDR, q-values false discovery rate; SD, standard deviation; TIMP, tissue inhibitors of MMP.
Significant p-values are highlighted in bold.

testing. This did not alter the outcome of any analysis, see Appendix A: Sensitivity analysis.

4. Discussion

We found that baseline levels and yearly rate of change of selected MMP/TIMPs were associated with increased brain atrophy in a cognitively unimpaired older cohort. Specifically, we found that low baseline MMP-3 levels predicted more entorhinal cortical thinning and more ventricle volume expansion over time; the former was independent of the CSF AD biomarkers $A\beta_{42}$ and p-tau_{181}. In our longitudinal samples, we found that a larger increase in CSF MMP-2 from baseline predicted more entorhinal thinning, smaller hippocampal volumes, and more ventricle volume expansion over time. Finally, a larger increase in the composite variable CSF TIMP-123 from baseline was associated with larger bilateral ventricle volumes over time.

CSF MMP-3 levels have previously been linked to amyloid pathology, as reduced levels of MMP-3 are found in CSF with low levels of $A\beta_{42}$ (Mlekusch and Humpel, 2009; Stomrud et al., 2010). However, in our cohort, the effect of baseline MMP-3 levels on entorhinal cortex atrophy was independent CSF $A\beta_{42}$ levels, suggesting that the effect of MMP-3 on brain atrophy is not driven by its connection with amyloid pathology. In the same vein, while MMP-3 previously has been linked to CSF t-tau and p-tau_{181} (Stomrud et al., 2010), we found that the effect of MMP-3 on brain atrophy was tau-independent. Beyond its connections to Aβ and tau pathology, MMP-3 has important roles in blood brain barrier permeability, inflammation, and apoptotic signaling. MMP-3 expression is increased in response to cellular stress signals, and MMP-3 triggers microglia to produce pro-inflammatory molecules in the extracellular space (Kim and Hwang, 2011). Moreover, MMP-3 might be responsible for organizing effective clearance of irreparably damaged neurons by triggering several apoptotic pathways (Kim and Hwang, 2011; Rosenberg, 2009). It is hypothesized that high MMP-3 might contribute to neurodegeneration in diseases such as AD through unchecked inflammation or apoptosis. However, elevated MMP-3 levels also have important roles in physiological processes such as remodeling of the extracellular matrix, synaptic plasticity, remyelination and learning (Kim et al., 2005; Meighan et al., 2006; Skuljec et al., 2011). We speculate that in our cognitively unimpaired cohort, low levels of MMP-3 might indicate impairment of these physiological roles, thus resulting in more atrophy over time (Kim et al., 2005; Meighan et al., 2006; Skuljec et al., 2011). However, the effect of baseline MMP-3 levels on ventricle volume expansion disappeared when controlling for the effect of $A\beta_{42}$ on brain atrophy over time. This is in line with an exploratory study on cognitively unimpaired older individuals suggesting an Aβ-associated effect of CSF MMP-3 on brain atrophy in the inferior temporal and inferior parietal cortices (Mattsson et al., 2014).

Previous research has linked CSF levels of MMP-2 in cognitively unimpaired older persons to increased risk of progression to mild cognitive impairment or dementia (Mattsson et al., 2013, 2014). Lower levels of CSF MMP-2 have been seen in cognitively normal patients who develop mild cognitive impairment and in patients with AD (Fagan and Perrin, 2012; Mattsson et al., 2013). As such, one could hypothesize that higher levels of CSF MMP-2 are protective and associated with less brain atrophy. However, in the current study, baseline MMP-2 levels were not associated with brain atrophy, which has also been found previously (Mattsson et al., 2014). Moreover, a larger increase in MMP-2 from baseline was associated with more brain atrophy over time on all measures. While this finding may appear in contradiction with previous studies, increased expression of MMP-2 has previously been documented in response to ischemic stroke and in associations with gliomas (Nie et al., 2014; Ramachandran et al., 2017; Zhang et al., 2019). Together with MMP-9, MMP-2 appears to promote and fine-tune neuroinflammatory processes such as the expression of chemokines and pro-inflammatory cytokines (Hannocks et al., 2019); thus it is possible that larger increases in MMP-2 over time associate with more brain atrophy because the MMP-2 levels increase in response to some underlying pro-inflammatory and neurodegenerative process.

Intriguingly, we found that a larger increase in CSF TIMP-123 levels from baseline was associated with larger ventricle volumes at follow-up. This is interesting as higher TIMP levels often are considered neuroprotective. For example, TIMP-1 has been found to protect against Aβ pathology and ameliorate cognition in model systems (Saha et al., 2020). However, increased expression of TIMP-1 is seen in many neuroinflammatory diseases and higher levels of CSF TIMP-2 are found across several neurodegenerative disorders such as AD, frontotemporal dementia, vascular dementia, and Parkinson's disease (Bjerke et al., 2011; Boström et al., 2021; Lorenzl et al., 2003). Such paradoxical effects are also reported for TIMP-3, where reduced levels of TIMP-3 have been linked to impaired cognition in mice (Baba et al., 2009), while higher levels of TIMP-3 have been linked to higher expression of Aβ and neurofibrillary tangles in the human brain (Dunckley et al., 2006; Hoe et al., 2007). Our results suggest that in normal aging, increased expression of TIMP-1, TIMP-2, and TIMP-3 is associated with accelerated brain atrophy. The underlying mechanisms of this effect should be further explored considering the TIMPs high number of binding sites and multiple physiological roles.

It is noteworthy that in this cohort high levels of MMP-3 associate with less brain atrophy, while increases in MMP-2 and TIMP-123 over time are associated with more brain atrophy. Both MMP-2 and MMP-3 activity are regulated by TIMP-1, TIMP-2, and TIMP-3 (Brew and Nagase, 2010). Moreover, MMP-2 and MMP-3 have previously been shown to be up- or downregulated together in response to neurodegenerative diseases or pro-inflammatory stimuli (Brkic et al., 2015). Our contrasting findings could be a selection effect driven by the participants with the longest follow-up times. However, it is also possible that upregulation of these MMPs and TIMPs have differential effects in the aging brain. The mechanisms driving these contrasting effects on brain atrophy should be further explored.

In line with previous studies, the measured MMP/TIMPs were more closely associated with p-tau_{181} than $A\beta_{42}$. None of the measured MMP/TIMPs could predict a change toward more pathological levels of AD biomarkers over time. Of note, this is a population with little evidence of AD pathology, as evident by the fact that most patients showed a small increase in CSF $A\beta_{42}$ levels at follow-up. In

addition, the sample consists of cognitively unimpaired participants, and the relationship with pathology biomarkers may be different in patients with AD. As such, MMP/TIMPs appear to have roles in aging-related brain atrophy that are independent of underlying neurodegenerative diseases such as AD.

A limitation of the current study is the relatively small cohort size. However, several follow-ups and considerable longitudinal data enhance our ability to detect change. The population is also very well characterized. Another limitation is the lack of paired plasma samples for the included patients. As there are several publications on circulating levels of MMP/TIMPs, the inclusion of paired plasma samples would have permitted us to better interpret our results considering these findings. Moreover, as several MMPs have important roles in blood–brain barrier permeability, markers on blood–brain barrier integrity would have contributed to our interpretations of the results. Finally, longitudinal information on change in the CSF biomarkers was available for only a small subsample, and these results should therefore be interpreted with caution. To our knowledge, this is the first study exploring the effects of changes in MMP/TIMP levels over time on neuroimaging and CSF biomarkers of neurodegeneration.

It is well established that MMP/TIMPs are involved in a plethora of both physiological (e.g. angiogenesis and neurogenesis) and pathophysiological processes (i.e. neuroinflammation and demyelination) in the brain, for a review see (Rempe et al., 2016). Establishing the mechanistic pathways linking alterations in MMP/TIMP levels to brain atrophy in cognitively unimpaired aging is beyond the scope of this paper. However, it is highly possible that imbalances in MMP or TIMP levels contribute to age-related neurodegeneration through neuroinflammation, as aging is associated with increased inflammation and MMPs are known to fine tune inflammatory processes (Rempe et al., 2016). Further research should explore whether low MMP-3 or increases in MMP-2 or TIMP-123 over time are associated with markers of neuroinflammation in cognitively unimpaired older individuals.

5. Conclusions

In conclusion, our results suggest that low CSF MMP-3 levels may predict age-associated brain atrophy, both dependent on and independent of Aβ pathology. Moreover, we show for the first time that increases in MMP-2 and the composite measure of TIMP-1, TIMP-2, and TIMP-3 over time may be good predictors of age-associated brain atrophy. The contrasting direction of these markers on brain atrophy should be explored further and selection effects should be ruled out. In our study, changes in these markers did not predict changes in the AD biomarkers CSF $A\beta_{42}$ or p-tau_{181} over time, implying that they can also reflect Aβ- and tau-independent age-associated neurodegenerative pathways.

Disclosure statement

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. The remaining authors declare no competing interests.

Declaration of Competing Interest

We declare that this work is original, has not been published before, and is not currently being considered for publication elsewhere. The manuscript is not currently under consideration for publication by any other journal, nor has it been previously published. The study was conducted in accordance with the Declaration of Helsinki and approved by the Regional Committee for Ethics in Medical Research in Norway. All co-authors agree with the content of this work.

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CRediT authorship contribution statement

Mari Aksnes: Conceptualization, Formal analysis, Data curation, Writing – original draft, Visualization. **Elettra Capogna**: Formal analysis, Data curation, Visualization. **Didac Vidal Piñero**: Formal

analysis, Supervision. **Farrukh Abbas Chaudhry**: Conceptualization, Funding acquisition. **Marius Myrstad**: Conceptualization, Resources. **Ane-Victoria Idland**: Investigation, Resources, Data curation. **Nathalie Bodd Halaas**: Investigation, Resources, Data curation. **Shams Dakhil**: Investigation, Resources, Data curation. **Kaj Blennow**: Investigation, Resources. **Henrik Zetterberg**: Investigation, Resources. **Kristine Beate Walhovd**: Funding acquisition, Resources. **Leiv Otto Watne**: Funding acquisition, Conceptualization, Investigation, Data curation, Resources. **Anders Martin Fjell**: Conceptualization, Funding acquisition, Resources. **All authors:** Writing – review & editing.

Appendix A

See Fig. A1.

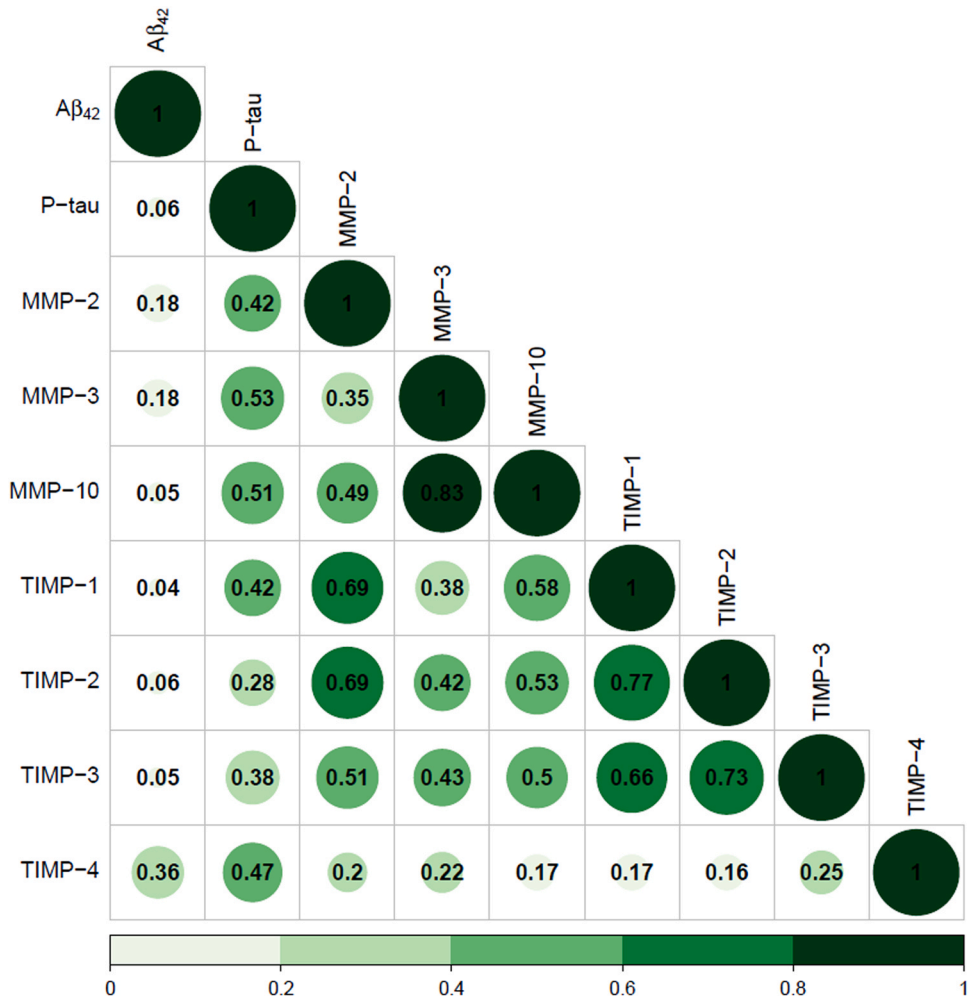

Fig. A1. Pearson correlation matrix for all measured CSF biomarkers. Darker colors and larger circles indicate a stronger relationship. Abbreviations: CSF, cerebrospinal fluid.

Sensitivity analysis

Cohort characteristics

The cohort characteristics of the entire cohort and the selected sample used in the sensitivity analysis are presented in Table A1. In the sensitivity analyses, the patients (n = 18) with poor baseline MMSE, TMT-A or TMT-B scores, and the patients who were offered referral for further cognitive testing after baseline testing are excluded.

Effect of baseline MMP/TIMP levels on brain atrophy

The models assessing the effects of baseline MMP/TIMP levels on brain atrophy in the selected sample are summarized in Table A2.

Effects of CSF Aβ42 and p-tau on MMP/TIMP-related brain atrophy

The effect of MMP-3 × time on bilateral entorhinal thickness remained significant when controlling for interactions with CSF $A\beta_{42}$ and p-tau. There were no significant interactions between MMP-3 and the AD biomarkers.

Table A1
Cohort characteristics at baseline

	All		Selected sample[a]	
N	111		93	
	N	(%)	N	(%)
Longitudinal CSF	32	(28.8)	31	(33.3)
Women	52	(46.9)	42	(45.2)
Detectable MMP-1	35	(31.5)	27	(29.0)
Detectable MMP-7	18	(16.2)	17	(18.3)
Detectable MMP-9	29	(26.1)	26	(28.0)
Detectable MMP-12	72	(64.9)	58	(62.4)
	Median	(First Q; third Q)	Median	(First Q; third Q)
Age	72	(68; 77)	71	(68; 75)
MMSE	29	(28; 30)	29	(29; 30)
CSF $A\beta_{42}$ (pg/mL)[b]	764	(514; 864)	731	(523; 859)
CSF p-tau (pg/mL)[b]	58	(46; 70)	57	(45; 69)
MMP-2 (ng/mL)	46.2	(41.0; 53.3)	45.7	(39.5; 52.7)
MMP-3 (pg/mL)	202.0	(152.4; 278.1)	197.4	(154.6; 265.5)
MMP-10 (pg/mL)	14.1	(7.8; 23.6)	14.1	(7.8; 21.7)
TIMP-123	−0.15	(−0.7; 0.5)	-0.2	(−0.7; 0.4)
TIMP-4 (ng/mL)	1.5	(1.3; 1.8)	1.5	(1.3; 1.8)

Key: $A\beta_{42}$, amyloid-β_{42}; CSF, cerebrospinal fluid; MMP, matrix metalloproteinases; MMSE, Mini Mental Status Examination; p-tau_{181}, phosphorylated tau_{181}; TIMP, tissue inhibitors of MMP; TMT, Trail Making Tests.
[a] Excluding the patients (n = 18) with poor baseline MMSE, TMT-A or TMT-B scores and the patients who were offered referral for further cognitive testing after baseline testing.
[b] Measured by the INNOTEST assay. N = 107 for the full sample, N = 93 for the selected sample. Q: quartile.

Table A2
The effects of baseline MMP/TIMP levels on brain atrophy

CSF protein	Brain region	β	Standard error	*p*	p_{FDR}
MMP-2 × time	Entorhinal cortex	-4.0×10^{-7}	2.0×10^{-7}	0.05	0.15
	Hippocampus	2.2×10^{-1}	-3.1×10^{-4}	0.22	0.22
	Ventricles	4.7×10^{-3}	3.4×10^{-3}	0.17	0.22
MMP-3 × time	Entorhinal cortex	**4.0×10^{-5}**	**1.43×10^{-5}**	**< 0.01**	**0.01**
	Hippocampus	1.4×10^{-2}	1.8×10^{-2}	0.45	0.45
	Ventricles	**-6.1×10^{-1}**	**2.4×10^{-1}**	**0.01**	**0.04**
MMP-10 × time	Entorhinal cortex	1.1×10^{-4}	1.6×10^{-4}	0.49	
	Hippocampus	4.1×10^{-4}	2.0×10^{-1}	0.99	
	Ventricles	−2.3	2.5	0.35	
TIMP-123 × time	Entorhinal cortex	2.7×10^{-3}	2.2×10^{-3}	0.22	
	Hippocampus	−4.6	2.8	0.11	
	Ventricles	2.0×10^{1}	3.8×10^{1}	0.59	
TIMP-4 × time	Entorhinal cortex	1.9×10^{-6}	4.5×10^{-6}	0.68	0.78
	Hippocampus	1.6×10^{-3}	5.7×10^{-3}	0.78	0.78
	Ventricles	-1.7×10^{-1}	7.4×10^{-2}	**0.02**	0.07

For the dichotomized markers, detectable levels of MMP-1 were associated with more entorhinal cortex thinning ($\beta = 9.1 \times 10^{-2}$, corrected $p = 0.03$) and less ventricle volume expansion ($\beta = -2.2 \times 10^{2}$, corrected $p = 0.003$). MMP-7, MMP-9 and MMP-12 were not associated with any measures of brain atrophy.
Key: CSF, cerebrospinal fluid; MMP, matrix metalloproteinases; TIMP, tissue inhibitors of MMP; *pFDR, q-values false discovery rate.*
Significant p-values are highlighted in bold.

When controlling for the effect of $A\beta_{42}$ × time, the effect of MMP-3 × time on bilateral ventricle volume was no longer significant ($\beta = -4.2 \times 10^{1}$, $p = 0.25$), while there was a significant effect of $A\beta_{42}$ × time on ventricle volume ($\beta = -1.2 \times 10^{2}$, $p < 0.01$), with higher baseline $A\beta_{42}$ predicting less ventricle volume expansion. There were no significant interactions between MMP-3 and the AD biomarkers.

Effect of yearly change in MMP/TIMP levels on brain atrophy

The models assessing the effect of rate of change in MMP/TIMP levels on brain atrophy are summarized in Table A3.

Table A3
The effects of yearly change in MMP/TIMP levels on brain atrophy

CSF protein	Brain region	β	Standard error	*p*	p_{FDR}
MMP-2 × time	Entorhinal cortex	-6.2×10^{-6}	2.0×10^{-6}	**< 0.01**	**< 0.01**
	Hippocampus	-5.5×10^{-3}	2.3×10^{-3}	**0.02**	**0.03**
	Ventricles	7.5×10^{-2}	3.5×10^{-2}	**0.03**	**0.03**
MMP-3 × time	Entorhinal cortex	3.4×10^{-5}	2.3×10^{-5}	0.15	
	Hippocampus	-2.7×10^{-4}	2.7×10^{-2}	0.99	
	Ventricles	-4.4×10^{-1}	4.0×10^{-1}	0.27	
MMP-10 × time	Entorhinal cortex	-3.6×10^{-4}	2.1×10^{-4}	0.09	0.11
	Hippocampus	-5.1×10^{-1}	2.4×10^{-1}	0.03	0.10
	Ventricles	5.7	3.5	0.11	0.11
TIMP-123 × time	Entorhinal cortex	-5.3×10^{-3}	3.0×10^{-3}	0.08	0.08
	Hippocampus	−6.3	3.4	0.07	0.08
	Ventricles	1.5×10^{2}	4.9×10^{1}	**< 0.01**	**< 0.01**
TIMP-4 × time	Entorhinal cortex	-3.6×10^{-7}	9.9×10^{-6}	0.97	
	Hippocampus	-9.0×10^{-3}	1.1×10^{-2}	0.43	
	Ventricles	-2.3×10^{-1}	1.6×10^{-1}	0.17	

Key: CSF, cerebrospinal fluid; MMP, matrix metalloproteinases; TIMP, tissue inhibitors of MMP; *pFDR, q-values false discovery rate.*
Significant p-values are highlighted in bold.

Effect of MMP/TIMP levels on change in AD biomarkers over time

The models assessing the effect of baseline MMP/TIMP levels on change in AD biomarkers over time are summarized in Table A4.

Table A4
The effects of baseline MMP/TIMP levels on change in CSF $A\beta_{42}$ and p-tau over time

CSF protein	AD biomarker	β	Standard error	*p*
MMP-2 × time	$A\beta_{42}$	-2.9×10^{-4}	8.2×10^{-4}	0.73
	p-tau	5.6×10^{-5}	2.9×10^{-5}	0.06
MMP-3 × time	$A\beta_{42}$	-4.7×10^{-2}	6.1×10^{-2}	0.45
	p-tau	-2.2×10^{-3}	2.3×10^{-3}	0.34
MMP-10 × time	$A\beta_{42}$	-3.4×10^{-1}	6.8×10^{-1}	0.62
	p-tau	-4.3×10^{-3}	2.5×10^{-2}	0.87
TIMP-123 × time	$A\beta_{42}$	-1.2×10^{1}	1.0×10^{1}	0.25
	p-tau	2.9×10^{-1}	4.0×10^{-1}	0.48
TIMP-4 × time	$A\beta_{42}$	-3.0×10^{-3}	2.2×10^{-2}	0.90
	p-tau	7.1×10^{-4}	8.2×10^{-4}	0.39

Rate of yearly change in MMP/TIMP levels was not a significant predictor of changes in AD biomarkers over time.
Key: $A\beta_{42}$, amyloid-β_{42}; AD, Alzheimer's disease; CSF, cerebrospinal fluid; MMP, matrix metalloproteinases; p-tau, phosphorylated tau; TIMP, tissue inhibitors of MMP.

Appendix B. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neurobiolaging.2023.05.012.

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