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Proteomic correlates of cortical thickness in cognitively normal individuals with normal and abnormal cerebrospinal fluid beta-amyloid$_{1-42}$

Laura L. Ekblad[a,b,1,*], Pieter Jelle Visser[a,c,d], Betty M. Tijms[a], for the Alzheimerś Disease Neuroimaging Initiative[2]

[a] Department of Neurology, Alzheimer Center Amsterdam, Amsterdam Neuroscience, Amsterdam, the Netherlands
[b] Turku PET Centre, Turku University Hospital and University of Turku, Turku, Finland
[c] Alzheimer Center Limburg, School for Mental Health and Neuroscience, Maastricht University, Maastricht, the Netherlands
[d] Department of Neurobiology, Care Sciences and Society, Division of Neurogeriatrics, Karolinska Institutet, Stockholm, Sweden

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ABSTRACT

Cortical atrophy is an early feature of Alzheimerś disease (AD). The biological processes associated with variability in cortical thickness remain largely unknown. We studied 220 cerebrospinal fluid (CSF) proteins to evaluate biological pathways associated with cortical thickness in 34 brain regions in 79 cognitively normal older individuals with normal (>192 ng/L, $n = 47$), and abnormal (≤192 ng/L, $n = 32$) CSF beta-amyloid$_{1-42}$ ($A\beta_{42}$). Interactions for $A\beta_{42}$ status were tested. Panther GeneOntology and Cytoscape ClueGO analyses were used to evaluate biological processes associated with regional cortical thickness. 170 (77.3 %) proteins related with cortical thickness in at least 1 brain region across the total group, and 171 (77.7 %) proteins showed $A\beta_{42}$ specific associations. Higher levels of proteins related to axonal and synaptic integrity, amyloid accumulation, and inflammation were associated with thinner cortex in lateral temporal regions, the rostral anterior cingulum, the lateral occipital cortex and the pars opercularis only in the abnormal $A\beta_{42}$ group. Alterations in CSF proteomics are associated with a regional cortical atrophy in the earliest stages of AD.

1. Introduction

Alzheimerś disease (AD) is the cause for approximately 50%–70 % of all dementia cases worldwide (Qiu et al., 2009; Scheltens et al., 2016). One of the earliest changes in AD, when cognitive functioning is still intact, is the aggregation of beta-amyloid ($A\beta$) into plaques in the brain (Jack et al., 2018). As the disease progresses, neuronal loss, i.e. brain atrophy occurs, which is associated with cognitive decline (Bakkour et al., 2009; Jack et al., 2016; Liu et al., 2010). Thinning of the cortex also occurs in normal aging, albeit at slower rates (Dicks et al., 2019; Fjell et al., 2014). At this point the precise biological processes related to interindividual variations in cortical thickness remain unclear. Such knowledge could potentially help in developing therapies that slow or prevent cognitive decline.

One approach to study on-going biological processes in the brain is with cerebrospinal fluid (CSF) proteomics (Pedrero-Prieto et al., 2020; Wesenhagen et al., 2019). Previous studies have demonstrated changes in the CSF proteome already in the cognitively normal stage (Spellman et al., 2015; Tijms et al., 2020). One previous study tested associations between 70 targeted CSF proteins and cortical thickness rates in cognitively normal older adults, in 7 *a priori* defined AD-related regions of interest (Mattsson et al., 2014). They showed that in addition to the estab-

Abbreviations: AD, Alzheimerś disease; $A\beta$, beta-amyloid; CAA, cerebral amyloid angiopathy; CSF, cerebrospinal fluid; p-tau, phospho-tau; t-tau, total tau; WMH, white matter hyperintensity.

* Corresponding author at: Department of Neurology, Alzheimer Center Amsterdam, Amsterdam Neuroscience, De Boelelaan 1118, 1081 HZ Amsterdam, Vrije Universiteit Amsterdam, Amsterdam UMC, the Netherlands.
E-mail address: llekbl@utu.fi (L.L. Ekblad).
[1] Permanent address: Turku PET Centre, c/o Turku University Hospital, P.O. Box 52, 20521 Turku, Finland.

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lished CSF biomarkers of AD – elevated phospho-tau (p-tau) and low Aβ_{42} – also higher levels of 5 other proteins were associated with regional cerebral atrophy over a follow-up of up to 4 years. In addition, they found Aβ modulated effects between 18 different proteins and regional cortical atrophy rates, and that higher levels of the majority of these proteins were associated with atrophy rates mainly in individuals with abnormal CSF Aβ_{42}. Hence, CSF proteomics seems to convey information on cortical thickness, but it remains unclear how this association differs in normal aging and in preclinical AD. Furthermore, it remains unknown which proteins may be associated with other brain areas, which were not included by the previous study. In AD atrophy of more widespread regions across the cortex, including the frontal cortex, can predict conversion from MCI to AD, also in individuals without abnormal amyloid at baseline (Ten Kate et al., 2017; Whitwell et al., 2008). In addition, since the study by Mattsson et al. only examined how baseline proteins predicted longitudinal brain atrophy rates, the cross-sectional associations between cortical thickness and the CSF proteome have not been explored previously. We hypothesized that cognitively normal individuals who are biomarker positive for Aβ_{42} would show different biological pathways that associate with regional cerebral atrophy than those who are Aβ_{42} negative.

To test this hypothesis, we utilized the cross-sectional CSF proteomic and cortical thickness results from ADNI and investigated which biological processes were associated with regional cortical thickness in cognitively normal individuals, and whether such relationships depended on amyloid status.

2. Methods

2.1. Study design and participants

Data used in the preparation of this article were obtained from the ADNI database (http://adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD.

We included 79 cognitively normal participants of ADNI who had both CSF proteomic data and cortical thickness measurements from brain MRIs available. All the participants were from the baseline ADNI-1 study. The participants were considered the have abnormal amyloid (i.e. Aβ+), when their CSF Aβ_{42} levels were <192 ng/L (Shaw et al., 2009) (see details on CSF markers below). Normal cognition was defined in ADNI by the following criteria: (1) no subjective cognitive complaints; (2) MMSE $\geq$ 24; (3) Clinical Dementia Rating (CDR) = 0, and (4) normal performance (education-adjusted) in delayed recall on the Logical Memory II subscale of the Wechsler Memory Scale–Revised; (5) no significant impairment in cognitive functions or activities of daily living (Petersen et al., 2010).

2.2. MRI analysis

In ADNI-1, 3D T1-weighted scans were performed on 1.5 T scanners using standardized protocols at each site (Jack et al., 2008). The cortical thickness measures and white matter hyperintensity (WMH) volume were obtained from the ADNI website. MRI image processing and measuring cortical thickness and WMHs from the images have been described in detail at http://adni.loni.usc.edu. The ADNI cortical thickness measures that are presented as standardized z-scores include 34 different cortical regions averaged over the left and right hemispheres defined by the Desikan-Killiany atlas. The 34 brain regions included in the analyses were: Banks SuperiorTemporal Sulcus, Caudal Anterior Cingulate, Caudal Middle Frontal, Cuneus, Entorhinal Cortex, Frontal Pole, Fusiform Gyrus, Inferior Parietal, Inferior Temporal, Insula, Isthmus Cingulate, Lateral Occipital, Lateral Orbital Frontal, Lingual Gyrus, Medial Orbital Frontal, Middle Temporal Gyrus, Paracentral Gyrus, Parahippocampus, Pars Opercularis, Pars Orbitalis, Pars Triangularis, Pericalcarine, Postcentral Gyrus, Posterior Cingulate, Precentral Gyrus, Precuneus, Rostral Anterior Cingulate, Rostral Middle Frontal, Superior Frontal, Superior Parietal, Superior Temporal, Supramarginal Gyrus, Temporal Pole, Transverse Temporal.

2.3. Measurements of the CSF proteins

Data for the CSF proteomic data used in this article was obtained from the ADNI targeted mass spectrometry (multiple reaction monitoring, MRM) analyses (Spellman et al., 2015). The finalized 'Normalized Intensity' data was used for the analyses (for the detailed explanation of the normalization procedure, please see the "Biomarkers Consortium CSF Proteomics MRM data set" in the "Data Primer" document at adni.loni.ucla.edu). The ADNI MRM panel consists of altogether 567 peptides detected in the CSF that represent 222 unique proteins. The peptides included in the ADNI MRM panel were based on the work of the Biomarkers Consortium CSF Proteomics Project which aimed to evaluate the usage of a multiplexed mass-spectrometry based approach to qualify candidate CSF biomarkers for AD. The peptides representing CSF proteins were chosen for the panel based on their relevance to AD, their previous detection in CSF, and based on results from the Rules Based Medicine (RBM) multiplex immunoassay analyses of the ADNI CSF samples. In addition to the peptides and/or proteins identified by a thorough literature search, the panel was supplemented with peptides representing proteins that serve as inflammatory markers and that were identified as potentially interesting by the RBM study that preceded the MRM study. (Spellman et al., 2015). Information on protein assessment and quality control is described at http://adni.loni.usc.edu/data-samples/biospecimen-data/. We excluded APOD and APOB, since the MRM fragments did not correlate with the RBM analyses. CSF A$\beta_{1\text{-}42}$ and t-tau levels were measured at the ADNI Biomarker Core laboratory at the University of Pennsylvania Medical Center with the multiplex xMAP luminex platform (Luminex Corp, Austin, TX) with the INNOBIA AlzBio3 kit (Innogenetics, Ghent, Belgium) (Toledo et al., 2013). Neurogranin, BACE1 and alpha-synuclein (SNCA) were measured with ELISA. Altogether 220 CSF proteins were included in the analyses.

2.4. Statistical analysis

Box plots and histograms of the CSF protein levels and the cortical thickness measurements were inspected visually to detect possible outliers and skewed distributions. We removed outliers: 5 because they had levels below zero in CSF: CRP (n = 1), SLITKR1 (n = 2), PRDX3 (n = 1) and TGFB1 (n = 1), and 1 outlier was detected to have GNR levels >5 standard deviations (SD) from the mean. The right-skewed distributions of p-tau, t-tau, TGFB1, CAT, CFH, IGFBP2, PRDX2, PRDX6, SNCA, and HBA1 were corrected with a logarithmic transformation, and the left-skewed distributions of C8B, MELFT, ENO2, CCL5, MIF and BASP1 by using a square transformation to achieve approximately normal distributions. Group comparisons to detect differences between the Aβ- and Aβ+ groups in study characteristics, CSF protein levels and cortical thickness were performed with Studentś t-test or with Wilcoxon rank order sums test for continuous variables and with χ^2 tests for categorical variables. Next, we studied the relationships between 220 CSF

protein levels and thickness of 34 different brain regions, averaged over the left and right hemispheres, with Pearson's correlation in the total study population ($n = 79$). We then evaluated interaction effects of Aβ status on the association between each CSF protein and cortical thickness in the 34 brain regions with linear regression models. When the interaction term was significant ($p < 0.1$), we repeated analyses stratified for Aβ status.

Additional analyses were performed to evaluate if APOE genotype or WMHs would influence our results. The correlation between WMHs and cortical thickness in each region was analyzed with Spearman's correlation, because of the skewed distribution of WMH volumes. Differences in cortical thickness between APOEε4 carriers and non—carriers in the Aβ- and Aβ+ groups were assessed with ANOVA. Interaction testing for WMHs and APOE genotype on the association between cortical thickness and CSF proteins was even performed.

Statistical significance was set at $p < 0.05$ for all other analyses except interactions, where statistical significance was set at $p < 0.1$. All analyses were performed with R version 3.6.1 (2019-07-05) – "Action of the Toes."

2.5. Protein pathway analysis

To detect biological pathways associated with proteins that were related to cortical thickness we performed pathway enrichment analyses in the Panther GeneOntology database (http://geneontology.org) for each brain region, and stratified according to whether proteins showed an interaction effect (separate for Aβ+ and Aβ- only, according to which group showed significant associations in the stratified analyses) or not, in which case we considered the total group. More specifically, the proteins were considered for pathway analyses according to the following criteria (Supplemental Table 2):

1) No interaction: correlations which showed a similar direction in the Aβ+ and the Aβ- groups (no interaction and $p \leq 0.05$ in the total group);
2) Interaction: correlations which showed a significant interaction that was driven by both Aβ+ and Aβ- groups;
3) Aβ only: correlations which showed a significant interaction and were only driven by the Aβ+ group, defined as $p \leq 0.05$ in the Aβ+ group and $p \geq 0.05$ in the Aβ- group;
4) Normal CSF only: correlations which showed a significant interaction and were only driven by the Aβ- group, defined as $p \leq 0.05$ in the Aβ- group and $p \geq 0.05$ in the Aβ+ group.

We only considered pathways that shared 10 or more genes that coded a protein detected in the CSF. Finally, we used ClueGO (version v2.5.5, within Cytoscape version 3.7.2) (Bindea et al., 2009) to study whether the pathways identified were functionally related to each other.

3. Results

3.1. Demographics

In total, the study population consisted of 47 individuals with normal cognition and Aβ- and 32 with normal cognition and Aβ+. The characteristics of the study population according to Aβ status are shown in Table 1. The mean age of the study population was 75.7 years (SD 5.6). 46.8 % (37 of 79) were women and 26.6 % (21 of 79) carried at least 1 *APOE* ε4 allele. There were no differences between the Aβ groups in age ($p = 0.59$), sex ($p = 0.995$), MMSE score ($p = 0.29$) or WMHs ($p = .88$). The Aβ+ group included more *APOE* ε4 carriers than the Aβ- group ($p = 0.0004$) (Table 1).

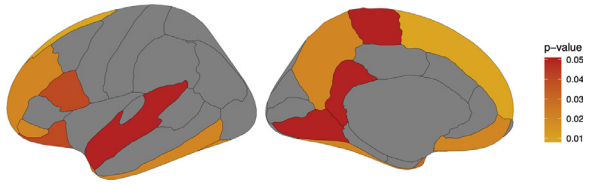

Fig. 1. Brain regions with statistically different cortical thickness z-scores between Aβ- and Aβ+ groups. Colored regions indicate brain regions where the Aβ+ group had lower cortical thickness than the Aβ- group, assessed with Student's t-test ($p < 0.05$). Brighter colors indicate smaller *p*-values.

3.2. Differences between Aβ- and Aβ+ groups in cortical thickness and CSF protein levels

Compared to the Aβ- group, the Aβ+ group had thinner cortical thickness in the fusiform gyrus ($p = 0.02$), the inferior temporal cortex ($p = 0.02$), the lateral orbital frontal cortex ($p = 0.04$), the medial orbital frontal cortex ($p = 0.03$), pars orbitalis ($p = 0.02$), the precuneus ($p = 0.02$), the rostral middle frontal cortex ($p = 0.02$), and in the superior frontal cortex ($p = 0.009$) (Fig. 1). Furthermore, compared to Aβ-, the Aβ+ group had higher CSF levels of p-tau ($p = 0.001$), total tau (t-tau) ($p < 0.0001$) (Table 1), and neurogranin (Aβ- mean [SD]: 276.7 [201.3]; Aβ+: 410.0 [234.2], $p = 0.008$), SPP1 (Aβ-: 26.1 [4.3]; Aβ+: 29.1 [5.5]; $p = 0.008$), FGF4 (Aβ-: 1.56 [0.16], Aβ+: 1.65 [0.15]; $p = 0.02$), FABP3 (Aβ-: 10.0 [0.3], Aβ+: 10.2 [0.4]; $p = 0.02$), CHI3L1 (Aβ-: 20.7 [0.5], Aβ+: 20.9 [0.5], $p = 0.04$), and ENO2 (Aβ-: 11.5 [0.3], Aβ+: 11.6 [0.4], $p = 0.03$). No proteins with significantly decreased levels in the Aβ+ group were observed.

3.3. Relationships between CSF proteins and regional cortical thickness in the total group

Of the core CSF biomarkers of AD (A$\beta_{42,}$ t-tau and p-tau) lower CSF Aβ_{42} levels were related to thinner cortex in the inferior temporal ($r = 0.24$, $p = 0.04$) and the fusiform gyri ($r = 0.22$, $p = 0.046$) across the total group. Higher levels of both CSF t-tau and p-tau were related with thinner cortex in 16 of the 34 brain regions. Although p-tau and t-tau strongly correlate (Spearman's correlation coefficient $r = 0.80$, $p < 0.0001$), we observed 6 regions that specifically correlated with p-tau (entorhinal cortex, lateral orbital frontal, lingual gyrus, pars opercularis, posterior cingulate, transverse temporal), and 2 regions that specifically correlated with t-tau (lateral occipital and pars orbitalis). (Supplemental fig. 1). In addition, higher levels neurogranin were associated with a thinner cortex 22 of 34 brain regions, and neurofilament light in 20 of 34 brain regions.

In the proteomics data, we observed relationships between 170 proteins and cortical thickness in 1 or more brain regions across the group, ranging from 4 proteins in the rostral anterior cingulate to 105 proteins in the superior parietal cortex (Supplemental Table 1). 166 (97.6%) of the 170 proteins correlated negatively with cortical thickness i.e. a higher CSF protein level was associated with a thinner cortex. Only 4 proteins: Aβ_{42}, AGRP (agouti related neuropeptide), FGF4 (fibroblast growth factor 4) and LEP (leptin) showed only positive correlations with cortical thickness, indicating that lower levels of these proteins were associated with a thinner cortex.

The parahippocampus was the only region where thinner cortex was related with *lower* levels of 42 proteins. The 5 proteins that most strongly correlated with cortical thickness in the parahippocampus were CHBG (chromogranin B, $r = 0.3$, $p = 0.007$), NRXN1 (neurexin-1, $r = 0.3$, $p = 0.008$), NCAM1 (neural cell adhesion molecule 1, $r = 0.29$, $p = 0.009$), NCAN (neurocan,

Table 1
Characteristics of the study population

	Aβ-	Aβ+	p-value
n	47	32	
age (y)	75.4 (5.7)	76.1(5.6)	0.59
female (%)	22/46.8	15/46.9	0.995
APOE4 (%)	5/10.6	16/50.0	0.0004
MMSE score	29.0 (1.1)	29.2 (1.0)	0.29
Phospho-tau (pg/mL)	20.4 (9.4)	31.0 (15.4)	0.001
Total tau (pg/mL)	56.7 (13.5)	84.6 (32.3)	<0.001
White matter hyperintensity volume (mL)	0.69 (2.06)	0.89 (1.99)	0.85

The results are shown as mean (SD) for continuous variables, and as n/% for categorical variables. *P*-values assessed with Student's t-test for age, with the Wilcoxon rank order sum test for MMSE and white matter hyperintensity volume, phospho-tau and total tau because of a skewed distribution, and with the χ^2 test for the categorical variables.

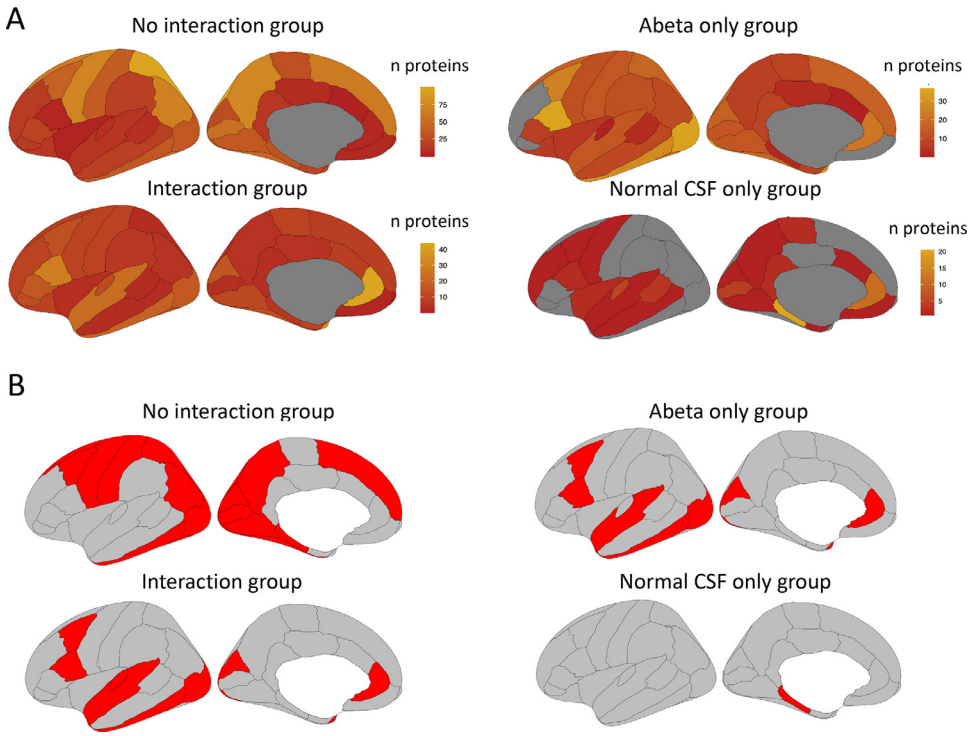

Fig. 2. Brain regions with significant protein-cortical thickness correlations and biological pathways. (A) Number of proteins that correlated with cortical thickness in each brain region according to Aβ status interaction analyses. (B) Brain regions where protein groups were related to biological pathways on Panther GeneOntology analyses.

$r = 0.29$, $p = 0.009$), and NRGN (neurogranin, $r = 0.29$, $p = 0.01$). In Panther GeneOntology analyses the 6 most significant biological pathways (sorted according to smallest FDR-corrected *p*-value) for the proteins that correlated with the parahippocampus in the total group were secretion (GO:0046903), nervous system development (GO:0007399), generation of neurons (GO:0048699), neuron projection development (GO:0031175), neuron development (GO:0048666) and neurogenesis (GO:0022008). (data not shown)

3.4. Aβ dependent relationships between protein levels and cortical thickness

We repeated analyses to test whether the observed associations were dependent on amyloid status. In total we observed that the CSF protein-cortical thickness relationships depended on Aβ status (*p*-value for the interaction term for Aβ status <0.1) for 171 of 220 (77.7%) proteins in at least 1 brain region, and for all 34 brain regions (Supplemental Table 1, Fig. 2A). Stratified analyses showed that in all of these brain areas, thinner cortex was related to higher CSF protein levels in the Aβ+ group for all proteins, apart from FGF4 that showed lower levels with thinner cortex in 18 brain regions in the Aβ+ group. While no interactions were found for t-tau, we observed an interaction for p-tau and Aβ status on cortical thickness in 4 brain regions: the cuneus, the parahippocampus, pars orbitalis, and the temporal pole. In these regions higher p-tau levels showed a trend toward a correlation with a thinner cortex only in the Aβ+ group, but the correlation was statistically significant only in the temporal pole ($r = -0.38$, $p = 0.03$).

Enrichment analyses with Panther GeneOntology and ClueGO showed distinct biological processes for the groups of proteins that were significantly correlated with cortical thickness in the Aβ+ group only in altogether 8 brain regions (Fig. 2B, Table 2). Panther GeneOntology enrichment analyses showed biological pathways related to synaptic and axonal integrity, to amyloid fibril formation and neurofibrillary tangle assembly, and to inflammatory processes (Supplemental figure 2, Supplemental Table 3). ClueGO functional analysis showed that the proteins that correlated with cortical thickness in the occipital regions (lateral occipital cortex and cuneus) were involved in biological pathways associated with apoptosis, blood coagulation and lipid

Table 2
Proteins that correlated with cortical thickness only in the Aβ+ group

Caudal middle frontal			Cuneus			Inferior temporal			Lateral occipital			Pars opercularis			Rostral anterior cingulate			Temporal pole		
Protein	*r*	*p*	Protein	*r*	*p*	Protein	*r*	*p*	Protein	*r*	*p*	Protein	*r*	*p*	Protein	*r*	*p*	Protein	*r*	*p*
VEGFA	-0.51	0.002	VCAM1	-0.53	0.001	FGF4	0.59	0.000	F3	-0.56	0.001	C2	-0.48	0.006	MCAM	-0.56	0.001	VCAM1	-0.61	0.000
F3	-0.49	0.003	SORT1	-0.51	0.002	IL3	-0.56	0.001	MMP3	-0.55	0.001	APOA1	-0.43	0.010	COCH	-0.55	0.001	FGF4	0.59	0.000
SPON1	-0.48	0.005	TNFRSF1B	-0.49	0.003	PGF	-0.55	0.001	S100B	-0.54	0.001	C8B	-0.45	0.011	CHI3L1	-0.5	0.004	BTD	-0.55	0.001
SHBG	-0.44	0.008	S100B	-0.48	0.004	BTD	-0.49	0.004	PGF	-0.54	0.001	APOC3	-0.43	0.011	BTD	-0.5	0.004	COCH	-0.52	0.002
CSF1	-0.43	0.011	F3	-0.48	0.004	VEGFA	-0.47	0.005	APOC3	-0.53	0.001	C6	-0.43	0.015	B2M	-0.49	0.004	VEGFA	-0.48	0.004
CCL4	-0.43	0.011	VEGFA	-0.46	0.007	IL6R	-0.47	0.005	BTD	-0.52	0.002	AHSG	-0.42	0.017	ANGPT2	-0.47	0.005	CLU	-0.48	0.005
SERPINF1	-0.44	0.012	CD40	-0.43	0.011	F3	-0.46	0.007	IL3	-0.5	0.003	FBLN1	-0.42	0.017	CD40	-0.47	0.005	GFAP	-0.48	0.006
GOT2	-0.43	0.014	TFF3	-0.41	0.015	SPON1	-0.46	0.007	SORT1	-0.5	0.003	CP	-0.42	0.017	VEGFA	-0.46	0.007	NEFL	-0.41	0.007
APOC3	-0.41	0.016	ICAM1	-0.4	0.018	COCH	-0.45	0.009	CTBS	-0.5	0.003	EXTL2	-0.41	0.018	APOE	-0.46	0.007	CTBS	-0.47	0.007
AXL	-0.41	0.016	FTL	-0.39	0.021	CTBS	-0.45	0.010	ICAM1	-0.48	0.004	GC	-0.41	0.021	IL18BP	-0.46	0.008	PRDX1	-0.46	0.009
TGFA	-0.41	0.017	PRDX6	-0.4	0.025	TGFA	-0.43	0.012	ADIPOQ	-0.48	0.004	EFEMP1	-0.41	0.021	CD14	-0.44	0.011	IL3	-0.44	0.009
CTSL	-0.41	0.019	APOC3	-0.38	0.025	MOG	-0.44	0.013	APOA1	-0.47	0.005	A1BG	-0.4	0.022	AGRP	0.43	0.011	IL6R	-0.44	0.009
BTD	-0.41	0.019	SERPINE1	-0.38	0.027	CLU	-0.43	0.014	SERPINF1	-0.46	0.008	HPX	-0.4	0.022	CLU	-0.43	0.013	ACE	-0.41	0.015
S100B	-0.4	0.020	MB	-0.37	0.030	VASN	-0.43	0.014	TNFSF10	-0.42	0.012	SERPINA3	-0.4	0.023	VCAM1	-0.41	0.015	TNFRSF1B	-0.41	0.016
EXTL2	-0.39	0.026	APOA1	-0.37	0.030	LAMB2	-0.43	0.015	PRDX1	-0.43	0.013	CTBS	-0.4	0.024	CNTN2	-0.43	0.015	VASN	-0.42	0.016
PRDX1	-0.39	0.028	TNFSF10	-0.35	0.041	HBEGF	-0.41	0.016	SERPINA3	-0.43	0.013	CCL8	-0.38	0.027	CNTN1	-0.39	0.025	CD40	-0.4	0.018
CLSTN1	-0.39	0.028				S100B	-0.4	0.019	C5	-0.43	0.015	C5	-0.39	0.028	TIMP1	-0.39	0.028	DAG1	-0.41	0.019
CLU	-0.38	0.030				NCSTN	-0.41	0.019	CNTN2	-0.42	0.016	ADIPOQ	-0.37	0.030	DAG1	-0.38	0.034	LTBP2	-0.41	0.020
ICAM1	-0.37	0.033				SERPINF1	-0.41	0.020	EXTL2	-0.42	0.016	BTD	-0.38	0.032	NCAM2	-0.37	0.040	S100B	-0.4	0.020
NCSTN	-0.37	0.036				PTGDS	-0.41	0.020	VASN	-0.42	0.017	CRP	-0.38	0.035	SPON1	-0.36	0.042	NCSTN	-0.4	0.024
ALDOA	-0.37	0.038				AGRP	0.38	0.028	COCH	-0.42	0.017	CLU	-0.37	0.035	CNDP1	-0.36	0.043	AXL	-0.39	0.024
FGF4	0.36	0.038				ACE	-0.37	0.032	PRDX6	-0.41	0.021	AFM	-0.37	0.037	PCSK1	-0.36	0.045	APOC3	-0.38	0.027
APOA1	-0.36	0.039				PRDX1	-0.38	0.034	HBEGF	-0.38	0.025	PRDX1	-0.36	0.042	F3	-0.35	0.045	F3	-0.38	0.027
VSNL1	-0.45	0.045				ITIH5	-0.37	0.036	C6	-0.39	0.026	CA1	-0.36	0.046				PRDX6	-0.39	0.029
IL3	-0.34	0.049				IL18BP	-0.37	0.039	FBLN1	-0.39	0.027	COCH	-0.35	0.046				EXTL2	-0.38	0.031
						CTSL	-0.35	0.048	PRDX2	-0.39	0.027	PLG	-0.41	0.020				B2M	-0.37	0.037
									KLK6	-0.38	0.030	PPY	-0.49	0.003				TGFA	-0.36	0.038
									HPX	-0.38	0.032	KNG1	-0.36	0.044				SERPINA3	-0.37	0.039
									SERPINA4	-0.37	0.037	ITIH1	-0.43	0.015				CNTN1	-0.36	0.041
									C8B	-0.37	0.038	PGLYRP2	-0.4	0.024				PRDX2	-0.36	0.042
									VWF	-0.35	0.041	PGF	-0.38	0.027				APOA1	-0.35	0.044
									SERPINA1_rbm	-0.34	0.047	ICAM1	-0.4	0.019				CNTN2	-0.36	0.044
									MB	-0.34	0.047	SERPINA4	-0.4	0.024				VCAM1	-0.61	0.000
									CTSL	-0.35	0.049	SERPINF1	-0.36	0.043				FGF4	0.59	0.000
												SHBG	-0.44	0.009				BTD	-0.55	0.001
												TNFSF10	-0.36	0.039						

The proteins under each brain region represent protein-cortical thickness correlations which were modulated by Aβ status (the interaction term Aβ*protein was significant), and for which analyses stratified for Aβ status showed significant correlations ($p < 0.05$) only in the Aβ+ group. r = Pearsonś correlation coefficient for the correlation between CSF protein level and cortical thickness in each brain region.

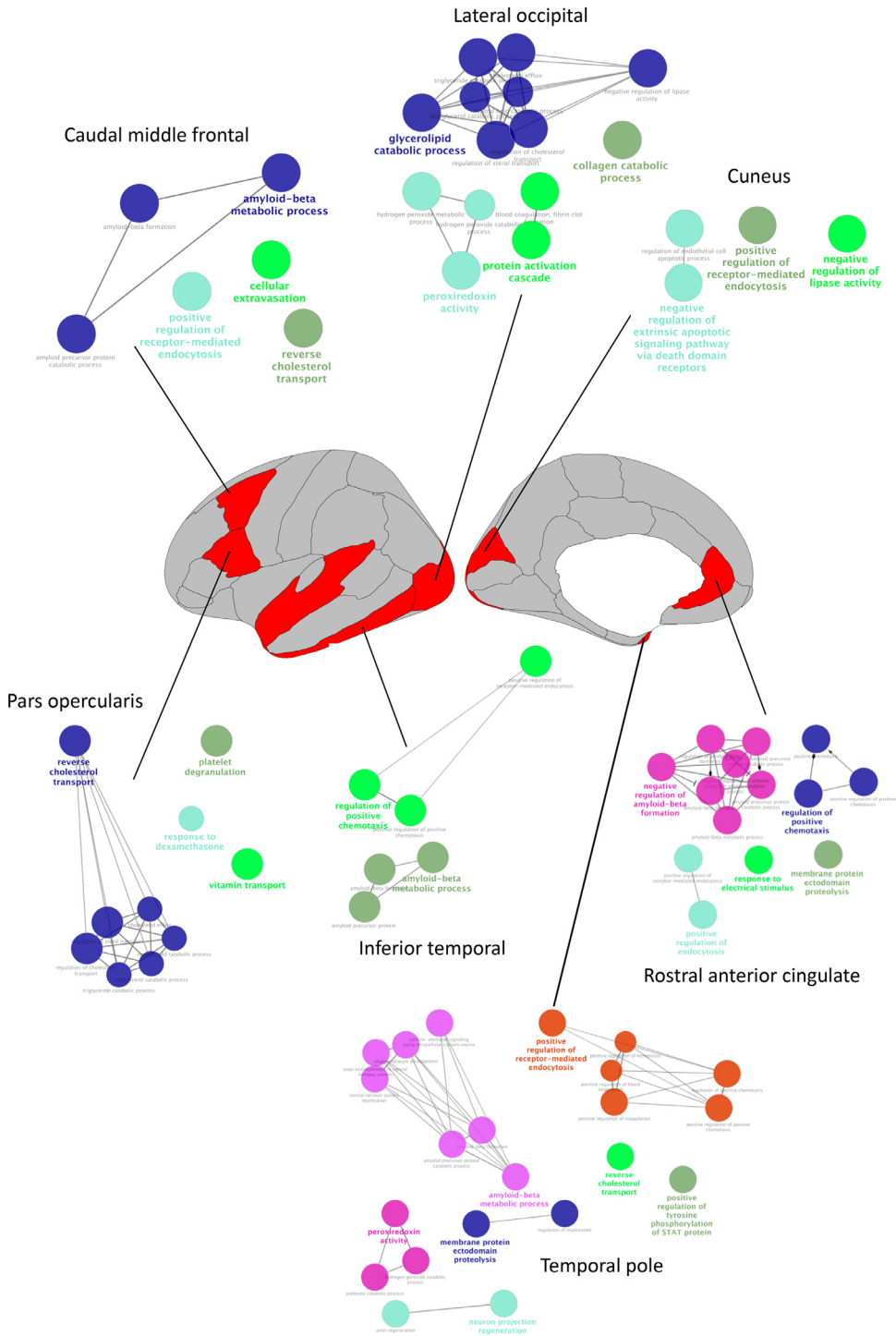

Fig. 3. Biological pathways on ClueGO functional analyses according to brain region in the Aβ+ group.

transport, whereas the temporal (inferior temporal and temporal pole) and frontal regions (caudal middle frontal and rostral anterior cingulate) were associated with pathways associated with amyloid-beta metabolic process and chemotaxis. Cortical thickness in the pars opercularis was associated with reverse cholesterol transport, platelet degranulation and vitamin transport (Fig. 3).

We also found groups of proteins that showed significant interactions for Aβ status, but that were not significantly correlated with either Aβ group in the stratified analyses, and these protein groups were found in the same brain regions as the proteins that correlated significantly only in the Aβ+ group (Fig. 2A and B, Supplemental Table 2). Panther GeneOntology enrichment results for these proteins are presented in the supplemental material (Supplemental Table 3, "interaction group.") The processes that were most significantly associated with these proteins were found to correlate with cortical thinning in the temporal pole, and they included interleukin-8 secretion and platelet degranulation.

Altogether 39 of 220 (17.7 %) proteins were significantly associated with cortical thickness in the normal Aβ group only (Supple-

mental Table 2). Higher levels of most of these proteins were associated with a thinner cortex only in 1 brain region. CTSL (cathepsin L1) showed associations in 7 brain regions, CALCA (calcitonin) in 4 brain regions, and NGF (beta-nerve growth factor) in 3 regions. Higher levels of 20 proteins associated with a thicker cortex in the parahippocampus. Enrichment analyses showed relations to biological processes only in the parahippocampus (Fig. 2B, Supplemental Table 3).

In addition, there were 170 proteins that showed significant correlations in the total group and for which the interaction with Aβ status was not significant, suggesting that these proteins correlated with cortical thickness in a similar way for cognitively normal individuals with and without abnormal Aβ. (Fig. 2A, Supplemental Table 2, "no interaction group.") Panther GeneOntology analyses revealed meaningful biological pathways for these protein groups in 19 of the 34 studied brain regions, consisting of wide regions of the frontal, parietal, occipital and temporal cortices. (Fig. 2B, Supplemental Table 3). Biological pathways that associated with a thinner cortex in the total group included for example developmental pathways (for example developmental process (GO:0032502), central nervous system development (GO:0007417), and cellular developmental process (GO:0048869)), inflammatory pathways (for example cytokine-mediated signaling pathway (GO:0019221) and defense response (GO:0006952)), and regulatory pathways (for example negative regulation of cellular process (GO:0048523) and positive regulation of cell communication (GO:0010647)). Strongest associations for biological pathways (according to FDR-corrected *p*-value in Panther GeneOntology analyses) were found for the proteins that correlated with a thinner cortex in the superior parietal cortex. (Supplemental Table 3)

3.4.1. Correlations between CSF proteins and cortical thickness in the parahippocampus according to Aβ interactions

When interaction effects were taken into account, 1 brain area, the parahippocampal gyrus, showed Aβ independent associations between thinner cortex and lower levels of 32 proteins in the total group ("no interaction group,") and these included neurexin-1, neural cell adhesion molecule 1, neurocan, neurogranin and SPARCL1 (SPARC-like Protein 1) (Table 3). The Panther GeneOntology analyses showed that these proteins were related to biological processes including clustering of voltage-gated potassium channels, regulation of axon diameter, central nervous system myelination, and interleukin-8 secretion (Fig. 4, Supplemental Table 3). ClueGO analyses showed biological processes of neuron recognition, neuron maturation, neuron projection fasciculation, axon fasciculation, synapse maturation, and response to copper ion (Fig. 4). In addition, analyses stratified according to Aβ interaction effects showed significant associations between lower protein levels and thinner cortex (or, conversely, higher protein levels and thicker cortex) for altogether 20 proteins in the Aβ- group only, including CHGB (chromogranin B), CNTN1 (contactin-1), B2M (beta-2-microglobulin), MCAM (melanoma cell adhesion molecule) and COCH (cochlin) (Table 3). Panther GeneOntology analyses showed that these proteins were related to response to stimulus, response to stress and signaling (Supplemental Table 3).

3.5. Associations of white matter hyperintensity volume and APOE genotype with cortical thickness

WMH volume correlated with cortical thickness in the total group in only 5 of the 34 brain regions studied, i.e. the parahippocampus (r = -0.37, p = 0.008), lateral orbital frontal (r = -0.31, p = 0.006), fusiform gyrus (r = -0.30, p = 0.007), temporal pole (r = -0.28, p = 0.01) and medial orbital frontal regions (r = -0.24, p = 0.03). No interaction effects for WMHs on the association between regional cortical thickness and CSF protein levels were found for these 5 brain regions (all p-values ≥0.05, data not shown). There was no difference in cortical thickness between carriers and non–carriers of the APOE ε4 allele in any of the brain regions in neither the Aβ- nor the Aβ+ group. The interaction for APOE genotype on the association between cortical thickness was only significant ($p \leq 0.05$) for 5 proteins (CRP, IL3, HGF, NPPB and IL25) in the transverse temporal region. No other interaction effects for APOE genotype were found.

Table 3
Proteins that correlated with cortical thickness in the parahippocampus in the total group and in the Aβ- group

Parahippocampus					
Total group (no interaction), n = 79			Aβ- group, n = 47		
Protein	r	p	Protein	r	p
NRXN1	0,3	0.008	CHGB	0.47	0.001
NCAM1	0.29	0.009	CNTN1	0.46	0.001
NCAN	0.29	0.009	B2M	0.45	0.002
NRGN	0.29	0.010	MCAM	0.43	0.002
SPARCL1	0.28	0.011	COCH	0.42	0.004
CNTN2	0.28	0.011	UBB	0.39	0.006
MELTF	0.28	0.014	CD59	0.39	0.007
LEP	0.27	0.015	GOT1	0.38	0.009
TNFRSF21	0.27	0.015	APLP2	0.37	0.011
SOD1	0.27	0.017	APOE	0.37	0.011
FAM3C	0.27	0.018	CADM3	0.36	0.012
VGF	0.26	0.019	NEO1	0.36	0.014
CLSTN1	0.26	0.019	HBA1	0.35	0.015
ALDOA	0.26	0.020	IL6R	0.33	0.017
NPTXR	0.25	0.024	HBB	0.33	0.022
CST3	0.25	0.025	VEGFA	0.31	0.028
PDYN	0.25	0.026	CA1	0.32	0.029
PAM	0.25	0.026	PTAU	0.23	0.041
EXTL2	0.25	0.026	LAMB2	0.3	0.042
SEZ6L	0.25	0.028	PCSK1	0.3	0.043
ACE	0.25	0.028			
NRCAM	0.24	0.030			
NBL1	0.24	0.030			
FBLN1	0.24	0.032			
IL18BP	0.24	0.035			
SCG2	0.23	0.038			
PKM	0.23	0.039			
PLXDC1	0.23	0.040			
APP	0.23	0.042			
CACNA2D1	0.22	0.048			
CXCL10	0.22	0.049			
NPTX1	0.22	0.050			

The proteins represent protein-cortical thickness correlations which were not modulated by Aβ status (no interaction group), or which only showed significant correlations in the participants with normal CSF Aβ_{42} levels in analyses stratified according to interaction testing. r = Pearsonś correlation coefficient for the correlation between CSF protein level and cortical thickness in the parahippocampus. P-values uncorrected for multiple comparisons.

4. Discussion

Here, we studied with CSF proteomics which biological processes were associated with inter-individual differences in cortical thickness, and the influence of Aβ status on those relationships in cognitively normal older individuals. Across the group, we observed widespread associations with many proteins, including the established biomarkers of AD, p-tau and t-tau, and proteins that have been associated with AD and cerebral atrophy in previous studies i.e. neurogranin and neurofilament light (Moore et al., 2020; Pereira et al., 2017). Higher levels of the proteins that correlated with a thinner cortex in the total group independently of Aβ status were related to a number of biological processes, such as developmental pathways, inflammatory pathways and regula-

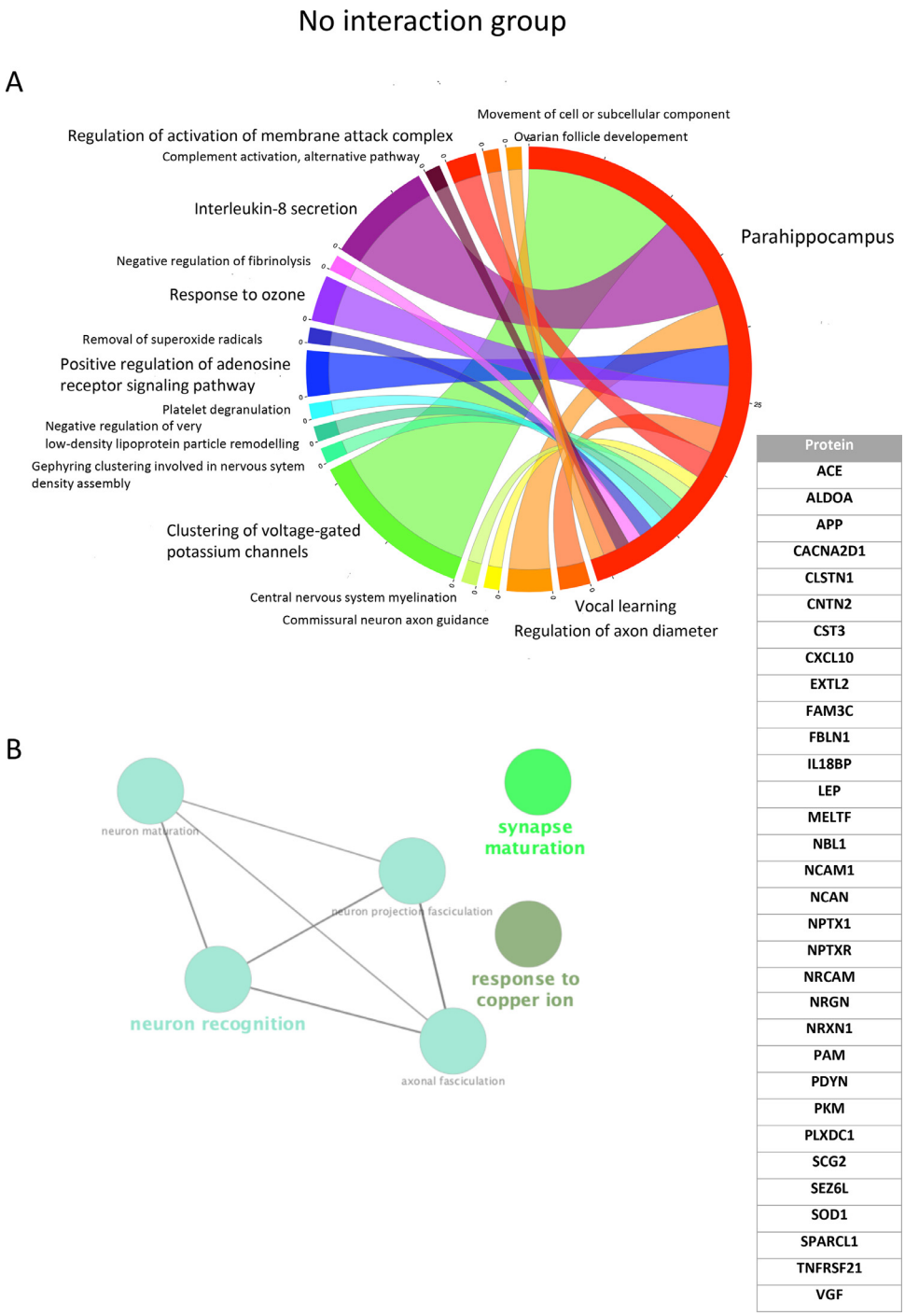

Fig. 4. Biological pathways represented by proteins that correlated with greater cortical thickness in the parahippocampus in the total study population which did not show an interaction for Aβ status. (A) Biological pathways according to Panther GeneOntology analyses; (B) Biological pathways according to ClueGO analyses.

tory pathways. A subset of brain areas, in particular in the lateral temporal regions, the rostral anterior cingulum, the caudal middle frontal cortex, the pars opercularis and the lateral occipital cortex, showed relationships with CSF proteins that were specific to the Aβ+ group. The biological processes that were specific to the Aβ+ group varied by brain region: thinner cortex in the occipital regions were related to apoptosis, blood coagulation and lipid transport, whereas the temporal and frontal regions were associated with amyloid-beta metabolic process and chemotaxis, consistent with early amyloid accumulating beginning in these regions. One brain area, the parahippocampal gyrus showed relationships between lower protein levels and thinner cortex (or, conversely, higher protein levels and thicker cortex), that were independent of Aβ status. Together these results imply that already in cognitively intact individuals changes in regional cortical thickness are reflected by changes in CSF proteins that point toward specific on-going biological processes in the brain, and that are modulated by the earliest sign of AD, the accumulation of Aβ in the brain.

Consistent with previous reports (Moore et al., 2020; Pereira et al., 2017), higher levels of CSF t-tau, p-tau, neurogranin and neurofilament light associated with a thinner cortex in the total group in regions including the medial temporal cortex, the precuneus and the superior temporal cortex. These are regions where atrophy is typically seen in the early stages of AD (Bakkour et al., 2009; Dickerson et al., 2009), and where cortical thinning has been shown to predict conversion to AD in cognitively normal individuals (Dickerson et al., 2011). Unexpectedly, considering that t-tau and p-tau show strong correlations, we observed some regional differences between t-tau and p-tau in the present study: higher t-tau was associated with a thinner cortex mainly in lateral temporal, occipital, parietal, prefrontal and medial frontal regions, whereas p-tau also showed associations with regions more specific to early AD (Braak & Braak, 1997), such as the entorhinal cortex and the posterior cingulum (Supplemental fig. 1). A previous study addressed the associations between CSF t-tau and p-tau and tau accumulation in the brain with [^{18}F]AV1451-PET (La Joie et al., 2018) and they also showed differences in anatomic areas. P-tau associated with [^{18}F]AV1451 uptake in the temporoparietal regions, and t-tau associated with [^{18}F]AV1451 in the prefrontal cortex (La Joie et al., 2018). Possibly, the similar pattern of association with cortical thickness we found suggests that p-tau and t-tau reflect different aspects of tangle pathology. This is in accordance with the A/T/N classification, in which CSF p-tau represents the presence of neurofibrillary tangles in the brain (the disruption of neurons containing neurofibrillary tangles which are first found in the transentorhinal regions in the early stages of AD), and t-tau may also represent neuronal injury (Jack et al., 2016). However, as there was substantial overlap of the regions where p-tau and t-tau correlated with cortical thickness, our results considering regional differences in p-tau and t-tau correlations warrant further investigation.

Furthermore, we found widespread associations of many other CSF proteins with cortical thickness across the total group, with 170 of the 220 proteins correlating with cortical thinning in at least 1 brain region independently of Aβ status, possibly reflecting processes associated with aging and not specifically with AD. The previous study that evaluated CSF proteomics with longitudinal rates of atrophy in AD signature regions over a follow-up of 4 years (Mattsson et al., 2014) discovered modest associations between CSF levels of p-tau, Aβ_{42}, ApoCIII, ApoD, A1M, ApoH and IL-16 and atrophy in at least 2 of the 7 regions they studied: the entorhinal cortex, the inferior parietal cortex, the middle temporal cortex, the precuneus, the posterior cingulate and the hippocampus. In the present study higher levels of ApoCIII associated with thinner cortex in the entorhinal cortex, precuneus, superior frontal and superior parietal cortices, the pericalcarine cortex and in the cuneus, and IL-16 and ApoH associated with a thinner cortex in the pericalcarine cortex, but no other brain regions. Because we included all brain regions, we further show that Aβ status modulated effects on protein-cortical thickness associations also in brain regions beyond the AD signature regions. In the Aβ stratified analyses we found 8 brain areas where groups of proteins were associated with cortical thinning only in the abnormal Aβ group, and where these protein groups represented meaningful biological pathways on enrichment analyses. These brain regions represented wider areas of the cortex than the AD signature regions that were examined by Mattsson et al., and included the caudal middle frontal, cuneus, inferior temporal, lateral occipital, pars opercularis and rostral anterior cingulate cortex. Mattsson and colleagues also found Aβ interactions and stratified analyses in their study showed higher baseline levels of ferritin, S100b, ApoCIII, ApoH and HGF to be associated with a faster atrophy rate in Aβ+ individuals only in the inferior parietal, inferior temporal, middle temporal and the posterior cingulate cortex. The differences between the previous study and ours are likely explained by the differences in study design, i.e. that we examined cross-sectional associations whereas Mattsson et al. focused on associations between baseline protein levels and longitudinal atrophy rates. Cross-sectional associations between CSF protein and cortical thickness may reflect on-going biological processes in the brain, whereas associations between baseline CSF proteins and longitudinal atrophy rates probably reflect proteins that are associated with accelerated neurodegeneration.

ClueGO functional analyses *(Fig. 3)* showed biological processes for Aβ+ group specific proteins that were associated with cortical thickness in specific brain areas. For example, the temporal (inferior temporal and temporal pole) and frontal regions (caudal middle frontal and rostral anterior cingulate) were associated with pathways of Aβ metabolic process, chemotaxis, membrane protein ectodomain proteolysis and regulation of endocytosis. These brain regions seem to be amongst the brain areas that show early *in vivo* amyloid accumulation according to an amyloid-PET study (Grothe et al., 2017). In that study, brain amyloid accumulation was shown to begin in the inferior temporal cortex and in the rostral anterior cingulum, and to subsequently spread into the superior temporal gyrus, the prefrontal cortex, the inferior parietal and the lateral occipital cortex. The notion that Aβ metabolic process and inflammatory processes correlated with thickness in these specific brain areas suggests that aggregated Aβ already leads to decreases in cortical thickness, although combined amyloid, MRI and CSF studies would be necessary to further study this question. It is plausible to assume that in distinct brain areas different biological processes may be involved in determining cortical thickness. This might lead to differences between individual people in the vulnerability of specific brain areas. In addition, these findings may also reflect processes related to Aβ accumulation in the brain and very early atrophy related to preclinical AD.

We found that a thinner cortex in the lateral occipital region was associated with apoptosis, blood coagulation and lipid transport (*Fig. 3*). This seems to be line with neuropathological *(Masuda et al., 1988; Vinters & Gilbert, 1983)* and amyloid PET (*Johnson et al., 2007*) studies showing that the age-related deposition of Aβ in cerebral blood vessels, cerebral amyloid angiopathy (CAA), is mainly found in the occipital regions of the brain. The cognitively normal population included in the present study did not have a diagnosis of CAA. However, considering that CAA is an independent risk factor for cerebrovascular disease (*Smith & Greenberg, 2009*), our findings on CSF proteins that point toward biological pathways of blood coagulation suggest that there might already be subtle vascular changes occurring, which might be associated with cortical thinning in particular in the occipital cortex and protein leakage into the CSF. An interesting line of research in the future would be to evaluate associations between cerebrovascular pathology and the CSF proteome, but this research question is beyond the scope of our manuscript.

In line with our results in this cognitively normal population considering the parahippocampus and regional differences in protein-cortical thickness associations, a recent post mortem study reported regional differences in protein expression in brain slices of 4 brain regions (the entorhinal cortex, the parahippocampus, the lateral temporal cortex and the frontal cortex) in controls and across the AD spectrum (Mendonça et al., 2019), and upregulation of proteins in the parahippocampus in normal controls. They found that in medial temporal regions (entorhinal cortex and parahippocampus), the proteins that were upregulated in normal controls were down-regulated in AD cases (Mendonça et al., 2019). The proteins that were upregulated in the entorhinal cortex and the parahippocampus in normal controls were linked to energy production, protein translation and long-term potentiation. Interest-

ingly, we found that the parahippocampus stood out from all the other examined brain regions in the present study. Higher levels of 20 proteins were associated with a thicker cortex in the individuals with normal CSF Aβ levels, whereas the Aβ+ group showed correlations in the opposite direction. The parahippocampus was the only region that showed positive correlations between CSF proteins and cortical thickness, independently of Aβ status in the total study population, consisting of cognitively normal individuals. In the present study, the proteins that correlated with a thicker cortex in the parahippocampus represented biological pathways of neuron recognition and synapse maturation and development. The parahippocampus is adjacent to the dentate gyrus where adult neurogenesis takes place (Boldrini et al., 2018; Spalding et al., 2013). Of note, the dentate gyrus is a very small structure, and it is plausible to assume that changes in cortical thickness in the dentate gyrus might by captured by parahippocampal cortical thickness. Although only an assumption, it would be tempting to propose that the association between greater cortical thickness in the parahippocampus and a higher level of proteins associated with synapse maturation and neuron recognition could indicate a repair mechanism on neuronal functioning by these proteins in the CSF. Longitudinal studies should in the future further investigate whether these CSF proteins that correlate with a thicker cortex in the parahippocampus and smaller structures adjacent to it could be neuroprotective or if, conversely, the greater cortical thickness and the possible neurogenesis present in the dentate gyrus would result in higher levels of synaptic proteins in the CSF.

Our study had some limitations. Firstly, although the total sample size was almost a hundred, the group with abnormal amyloid was relatively small, which may have reduced statistical power for subgroup analyses. Still, we found large patterns of similar protein-cortical thickness correlations in specific brain regions that were significant for the Aβ+ group only, and that represented distinct biological pathways in enrichment analyses, suggesting that these findings are not driven by chance. Studies with larger sample sizes will be of use in repeating these results in future studies. Furthermore, at this point it is unclear what the concentrations of proteins in CSF exactly represent, although the pathway enrichment showed that higher levels of large groups of CSF proteins were associated with cortical thinning in both Aβ+ and Aβ- cognitively normal individuals, and that these groups of proteins pointed toward specific biological processes. This study is based on a panel of 220 CSF proteins which had been previously been selected according to specific criteria presented in the Methods section. Analyzing associations between a larger number of CSF proteins and cortical thickness in future studies may provide a wider picture of the biological pathways associated with cortical atrophy. In addition, since vascular pathology has been associated with cortical thickness *(Kim et al., 2020)* it is possible that vascular pathology such as WMHs, microbleeds or lacunar infarcts could have modulated the associations between regional cortical thickness and CSF proteins even though there was no difference in WMH volume between the Aβ groups. WMH volume was associated with cortical thickness in only 5 of the 34 brain regions studied. No interaction effects were found for the brain regions where WMHs correlated with cortical thickness. We therefore conclude that it is unlikely that WMHs affected our findings. A strength of this study is that we were able to study a large number of CSF proteins, that we used an unbiased approach including all cortical regions in our analyses, and that we used enrichment analyses to evaluate common biological pathways for groups of proteins instead of examining individual proteins.

In summary, we found that increased levels of proteins that were associated with a large entity of biological processes, such as regulatory, developmental and inflammatory pathways, are related with thinner cortex across the brain independently of Aβ, suggesting that these processes play an important role in age related cortical thinning. Furthermore, we identified groups of proteins that showed Aβ specific associations in typical AD regions, which may help in finding new therapeutic targets for treatment of AD. These findings emphasize the multifaceted pathologic process that underlie the development of AD that differs from normal aging and suggest that therapies may need to target multiple biological processes simultaneously to slow cognitive decline.

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Verification

We confirm that the submitted manuscript has not been published previously, that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Disclosure statement

The authors have no actual or potential conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2021.07.003.

CRediT authorship contribution statement

Laura L. Ekblad: Conceptualization, Formal analysis, Writing – original draft, Visualization. **Pieter Jelle Visser:** Conceptualization, Writing – review & editing. **Betty M. Tijms:** Conceptualization, Formal analysis, Writing – review & editing.

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