Cerebrospinal Fluid Profile of Lipid Mediators In Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) develops into dementia over a period of several years, during which subjective cognitive impairment (SCI) and mild cognitive impairment (MCI) are used as intermediary diagnoses of increasing severity. Chronic neuroinflammation resulting from insufficient resolution is involved in the pathogenesis of AD and is associated with cognitive impairment. Specialized pro-resolving lipid mediators (LMs) that promote the resolution of inflammation may be valuable markers in AD diagnosis and as therapeutic targets. Liquid chromatography-tandem mass spectrometry was used to analyze pro-resolving and pro-inflammatory LMs in cerebrospinal fluid (CSF) from patients with cognitive impairment ranging from subjective impairment to a diagnosis of AD, and correlated to cognition, CSF tau and β-amyloid (Aβ). Resolvin (Rv) D4, neuroprotectin D1 (NPD1), maresin 1 (MaR1), and RvE4 were lower in AD and/or MCI compared to SCI. The pro-inflammatory LTB4 and 15-HETE were higher in AD and MCI, respectively, while PGD2 and PGE2 were decreased in AD, compared to SCI. RvD4 was also negatively correlated to AD tangle biomarkers. In this exploratory study of the lipidome in CSF of AD, MCI, and SCI, the results indicate a shift in the LM profile from pro-resolving to pro-inflammatory in progression to AD, suggesting that it may be of use as a biomarker when followed by confirmation by replication studies.

Introduction

Alzheimer's disease (AD) is the most common dementia in the aged population (Prince et al. 2013). The disease progression is insidious and takes over decades to develop dementia. The pathology in the AD brain includes neuronal and synapse loss, widespread deposits of senile plaques consisting of β-amyloid (Aβ) peptide and neurofibrillary tangles of phosphorylated (p)-tau protein, and activated microglia (Maccioni et al. 2001; Heneka et al. 2015; Scheltens et al. 2016). A commonly used nomenclature of increasing severity starts with subjective cognitive impairment (SCI) (Reisberg et al. 2008), then mild cognitive impairment (MCI) (Albert et al. 2011), and finally, dementia due to AD (Jack et al. 2018). The mini-mental state examination (MMSE) is a widely used test of cognitive function, and the severity of AD is commonly assessed by MMSE (Arevalo-Rodriguez et al. 2015), although other tests are increasingly used, such as Montreal Cognitive Assessment (MoCA) (Luis et al. 2009). Since the diagnosis of SCI is based on the individual experiences of memory problems, clinical assessments, including MMSE scores, are within the normal range (Reisberg et al. 2008), while MCI is diagnosed as decreased cognitive function with minimal or no functional decline (Albert et al. 2011). In severe dementia, not only cognitive and mental functions but also communication ability and mobility are impaired (Winblad et al. 2004).

Molecular biomarkers in cerebrospinal fluid (CSF) of Aβ and tau pathology are used to facilitate AD diagnosis (Olsson et al. 2016). However, AD is a heterogeneous and multifactorial disease and should be regarded from a broader perspective than from only Aβ and p-tau (Lue et al. 1996). It is important to expand the range of molecular factors used as biomarkers reflecting other mechanisms of the pathogenesis, such as inflammation and metabolic alterations. Inflammatory responses in AD are well-known (McGeer and McGeer 1995; Heneka et al. 2015), and there is accumulating evidence of its occurrence early in the disease process (Rodriguez-Vieitez et al. 2016). Aside from being regulated by

cytokines and chemokines, inflammation engages a prominent network of lipid mediators (LMs) with well-known bioactivities, such as the fever response (Coceani et al. 1986; Kozak and Fraifeld 2004) and pain (Juan 1978). LMs also play an essential role in the resolution of inflammation and the initiation of tissue restoration, an active process regulated by specialized pro-resolving LMs. These include the lipoxins (LX), protectins (PD), resolvins (Rv), and maresins (MaR), which are derived from the omega-3 and -6 polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA), arachidonic acid (AA), and eicosapentaenoic acid (EPA) (Buckley et al. 2014; Serhan et al. 2014). Although it is a relatively new field of research, studies on various pathologies revealed LM involvement (Gonzalez-Gay et al. 2008).

AD brains demonstrate lower levels of pro-resolving LMs than healthy controls (Lukiw et al. 2005; Wang et al. 2015; Zhu et al. 2016), while the expression of their receptors is increased (Wang et al. 2015; Emre et al. 2020). *In vitro* studies demonstrate that pro-resolving LMs improve cell survival, reduce Aβ production in neuronal models (Lukiw et al. 2005; Medeiros et al. 2013; Dunn et al. 2015; Zhu et al. 2016; Lee et al. 2020; Wang et al. 2021), and down-regulate inflammation and increase Aβ phagocytosis in glia (Lukiw et al. 2005; Medeiros et al. 2013; Dunn et al. 2015; Zhu et al. 2016; Lee et al. 2020; Wang et al. 2021). Reduction of AD pathologies and attenuation of cognitive impairment (Medeiros et al. 2013; Dunn et al. 2015; Kantarci et al. 2018; Yin et al. 2019; Lee et al. 2020) have been shown in *in vivo* models. To pave the way for future treatments and biomarkers based on the resolution of inflammation, we aimed at analyzing the pro-inflammatory and pro-resolving lipidome in CSF in cohorts of AD, MCI, or SCI patients and how the lipidome is associated with cognitive dysfunction and biomarkers of plaques and tangles. In view of sex differences in the prevalence of AD and lipid metabolism, we addressed sex-dependent alterations of lipidome in relation to cognitive impairment.

Results

CSF samples from cases with different degrees of memory dysfunction according to objective tests (AD or MCI) or subjective memory complaints (SCI) were analyzed by LC-MS/MS with regard to bioactive LMs, their fatty acid precursors, and intermediate derivatives. The median detected level and interquartile range for each LM within the diagnostic groups are presented in **Table S1**.

Differences in lipids between diagnostic groups

The analysis of LMs in CSF samples showed that levels of the pro-resolving LMs RvD4 and NPD1 were lower in the AD ($P < 0.00005$ and $P < 0.05$, respectively) and MCI ($P < 0.0005$ and $P < 0.05$, respectively) group compared to the SCI group (Fig. 1), whereas levels of the pro-inflammatory LM LTB_4 were higher in AD ($P < 0.001$) and MCI ($P < 0.05$) compared to SCI (Fig. 1). Comparing the diagnostic groups within the male and female group separately showed that for RvD4, there was a significant difference between AD and SCI for both women and men, but that the difference between MCI and SCI was seen only in women (**Fig. S3**). In the case of LTB4, higher levels in AD than in SCI were seen in women, whereas the increase in MCI compared with SCI reached statistical significance only in men (**Fig. S3**). The differences seen for NPD1 were small and not seen upon analyzing male and female groups separately. On the other hand,

the levels of RvE1 were reduced in men with AD compared to SCI (**Fig. S3**), but not in women or when analyzing both men and women together. Similarly, the levels of RvD3 were lower in MCI compared to SCI in women (**Fig. S3**), but not in men or in both groups together.

The pro-resolving LMs MaR1 ($P < 0.005$) (Fig. 1) and RvE4 ($P < 0.005$) (Fig. 1) were lower in CSF samples from MCI patients compared to SCI cases. The difference in MaR1 seems to be due mainly to a difference in men (**Fig. S3**), whereas this difference was not seen for either men or women. Interestingly, the levels of the pro-inflammatory LXA_4 were lower in men with MCI than in SCI (**Fig. S3**), a finding not seen when analyzing men and women together.

The levels of PGD_2 ($P < 0.0005$) and PGE_2 ($P < 0.0001$) were lower in MCI patients compared to SCI, and these differences were seen both in women and men (**Fig. S3**). In addition, the PGE_2 levels were lower in AD compared to SCI ($P < 0.005$) (Fig. 1), a difference also seen in women (**Fig. S3**).

The levels of the intermediate LM precursor 17-hydroxydocosahexaenoic acid (17-DHA) were higher in AD than in MCI ($P < 0.01$), and levels of 15-hydroxyeicosatetraenoic acid (15-HETE) were higher in AD ($P < 0.01$) and MCI ($P < 0.01$) than in SCI. The difference between AD and SCI reached statistical significance in women (**Fig. S3**). Regarding the n-3 and n-6 PUFA precursors, AA, EPA, or DHA, there were no significant differences between the three diagnostic groups, except in the case of DHA, where the levels in men were lower in AD compared to SCI (**Fig. S3**).

Correlations to cognitive function, CSF biomarkers of plaque and tangle pathology

Correlative relationships were investigated using the Spearman rank-order test. The complete results from the analysis of correlations, including all LMs and PUFAs, can be seen in **Table S2**.

MMSE

Our analyses of correlations suggest that for several lipids, high levels are associated with are protection against the deterioration of cognition seen in AD as assessed by the MMSE test (**Table S2a**). Analysis of the entire cohort showed that the levels of RvD4 displayed the strongest correlation to cognition as evaluated by the MMSE test (r = 0.29, $P < 0.001$). Other lipids showing a positive correlation to MMSE when including all three diagnostic groups were DHA (r = 0.21), EPA (r = 0.18) and PGE_2 (r = 0.18), and RvD1 (r = 0.17), all with a significance level of $P < 0.05$. Separating the cases according to diagnosis provided stronger correlative relationships. In the group of AD cases, the strongest correlations to MMSE were by DHA and 14-HDHA (r = 0.53, $P < 0.0005$ for both; Fig. 2), EPA (r = 0.51, $P < 0.001$; Fig. 2), AA (r = 0.42, $P < 0.01$; Fig. 2), and 20-HDHA (r = 0.33, $P < 0.05$). Among the cases diagnosed with MCI, only one negative correlation was found with MMSE, *i.e.*, MaR1 (r = -0.32, $P < 0.05$). Cases diagnosed with SCI showed positive correlations between MMSE and RvD4 (r = 0.42, $P < 0.005$; Fig. 2), RvD1 (r = 0.36, $P < 0.01$; Fig. 2), RvE4 (r = 0.34, $P < 0.05$), and LTB_4 (r = 0.29, $P < 0.05$).

$A\beta_{42}$

Analysis of all diagnostic groups together showed that the CSF levels of $A\beta_{42}$ were positively correlated to the levels of RvD4 (r = 0.29, $P < 0.001$), RvE1 (r = 0.23, $P < 0.01$), RvD1 (r = 0.18, $P < 0.05$), and NPD1 (r = 0.18, $P < 0.05$) (**Table S2b**). The analysis of correlations according to diagnostic group showed a positive correlation between $A\beta_{42}$ and 12-HETE (r = 0.42, $P < 0.01$), LXA_4 (r = 0.35, $P < 0.05$), LTB_4 (r = 0.33, $P < 0.05$), and RvE4 (r = 0.32, $P < 0.05$) among the AD cases. In cases diagnosed with SCI, there was a positive correlation between $A\beta_{42}$ and RvE1 (r = -0.27, $P < 0.05$).

t-tau and p-tau

The CSF levels of the tangle biomarkers t-tau and p-tau showed weak correlative relationships to the lipids analyzed (**Tables S2c-S2d**). Analysis of the entire cohort showed a negative correlation between RvD4 and t-tau (r = -0.17, $P < 0.05$) while there was no correlation to p-tau. The analysis according to the diagnostic group showed that for AD cases, MaR1 was negatively correlated to t-tau (r = -0.35, $P < 0.05$), and PGD_2 was positively correlated to p-tau (r = 0.32, $P < 0.05$). In cases diagnosed with MCI, there was a negative correlation between the levels t-tau and those of LXA_4 and 12-HETE (r = -0.33, $P < 0.05$ and r = -0.32, $P < 0.05$, respectively) and between the levels t-tau and LXA_4 (r = -0.33, $P < 0.05$). There was no correlation to the CSF levels of t- or p-tau within the SCI group.

Discussion

The inflammatory lipidome consists of pro-inflammatory LMs and also of LMs that end and resolve inflammation while promoting restoration and regeneration of the tissue, *i.e.*, healing (Serhan et al. 2007). We have previously shown decreased levels of pro-resolving LMs in the human AD brain (Lukiw et al. 2005; Wang et al. 2015; Zhu et al. 2016). Two of these LMs, LXA_4 and RvD1, were present in lower levels in the CSF of AD patients compared to those without clinical evidence of memory deficits, *i.e.*, diagnosed with SCI, and were positively correlated to the scores from the MMSE test (Wang et al. 2015). A decrease in RvD1 in the CSF of AD patients was not seen in the present study, possibly due to the greater specificity of LC-MS/MS compared to immunochemical assays in which signals may consist of several compounds with molecular similarities. The LC-MS/MS analysis showed that the levels of RvD4 and NPD1 were lower in both AD and MCI patients compared to SCI patients, while RvE4 and MaR1 were lower in MCI patients only. The differences between the diagnostic groups in RvD4 were also seen in a gender-separated comparison, but this was not evident for NPD1. The difference in MaR1 levels was statistically significant only in men, thus contributing most to the difference seen for all cases. Further analysis within male and female groups showed some additional differences in pro-resolving LMs, such as for RvD3, which was lower in women with MCI than with SCI, for RvE1 that was lower in men with AD than with SCI, and for LXA_4 that was reduced in men with MCI compared to men with SCI. Moreover, DHA levels in CSF were reduced in men with AD. Both intermediate precursors, 17-HDHA and 15-HETE were higher in AD, either compared to MCI or to SCI.

Several factors may influence the levels of lipids and give rise to the different results seen in men and women, including diet, age, sex hormones, and the ability to synthesize lipids. Indeed, the ability to synthesize long-chain fatty acids was shown to be higher for women than men, as suggested by a higher conversion rate of α-linoleic acid (ALA) to DHA and EPA (Burdge and Wootton 2002). In a study on mice (Rodriguez-Navas et al. 2016), females had higher brain levels of PUFAs than males, both after a Western-style high fat diet and regular chow diet, while plasma levels were similar.

Although analyses of LTB_4 in CSF have been performed since the eighties (Westcott et al. 1987), the significance of its presence in CSF in the context of AD is not known. We show that LTB_4 in CSF of both AD and MCI patients was slightly higher but statistically significant than in SCI and positively correlated to the levels of $A\beta_{42}$ in AD patients. In studies on multiple sclerosis (MS) (Neu et al. 1992), higher levels of LTB_4 were found in the CSF of MS patients compared to controls, suggesting LTB_4 as an indicator of inflammation in the brain. LTB_4 increased the production of Aβ in neurons in culture (Joshi et al. 2014), providing a direct link to the molecular pathology in AD. In addition, we found that the CSF levels of 15-HETE, an intermediary in the synthesis of LTB_4, were slightly higher but statistically significant in women with AD compared to women with SCI diagnosis and negatively correlated to MMSE scores. Yao *et al*. previously detected increased levels of 15-HETE in CSF from AD patients (Yao et al. 2005).

The pro-inflammatory LMs PGD_2 and PGE_2 were lower in the CSF of MCI patients compared to SCI, and in the case of PGE_2, also reduced in AD patients compared to SCI. Analysis of human *post mortem* entorhinal cortex showed higher levels of PGD_2 in AD compared to non-demented controls (Zhu et al. 2016), and studies on CSF samples showed higher levels of PGE_2 in patients with probable AD (Montine et al. 1999) and in MCI patients but lower in AD (Combrinck et al. 2006). PGD_2 synthetase and the PGD_2 receptor DP1 were upregulated in plaque-associated glia in *post mortem* AD brains and an AD mouse model (Mohri et al. 2007). PGD_2 mediated neuronal cell death in *in vitro* cocultures of neurons and microglia exposed to $A\beta_{42}$ (Bate et al. 2006). PGE_2 is increased in the CSF of patients with severe MS (Prüss et al. 2013). In a mouse model of AD, PGE_2 was shown to mediate TNF-α- and presenilin (PS)1/2-dependent deposition of Aβ (Guan et al. 2019). The literature thus suggests that PGD_2 and PGE_2 play harmful roles in AD. The explanation for our findings of lower levels of these factors in the CSF of AD patients will need further studies. However, in addition to the role of PG's at the initiation of an inflammatory response (Serhan and Savill 2005), it is hypothesized that there is a post-resolution immunological activity during which PGE_2 may exert modulatory and anti-inflammatory effects (Feehan and Gilroy 2019). The reduced levels of PGE_2 in CSF from AD and MCI patients could thus be seen as a deficit for the post-resolution stage.

Our novel finding of the presence of several bioactive LMs in human CSF highlight their abundance, and the present data on decreased levels of MaR1, NPD1, RvD3, RvD4, RvE1, RvE4, and LXA_4 in patients with cognitive dysfunction are in line with our previous research showing impaired resolution in AD. Of these, NPD1 is the most well-studied, and beneficial effects in the brain have been shown (Lukiw et al. 2005;

Bazan 2009; Stark and Bazan 2011), as well as direct protection on human neuronal cells (Zhu et al. 2016). NPD1 here showed a positive correlation to the CSF levels of $A\beta_{42}$, known to be decreased in AD patients.

The decreased levels of MaR1 in CSF of MCI cases can mainly be attributed to reduced levels in men with MCI. We previously found decreased levels of MaR1 in the hippocampus (Wang et al. 2015) and entorhinal cortex (Zhu et al. 2016) of AD patients and beneficial effects of MaR1 in several cellular models (Zhu et al. 2016; Wang et al. 2021). Surprisingly, there was a negative correlation of MaR1 to the MMSE scores in MCI patients, indicating a more complex nature of immune regulation in this heterogeneous group of patients than previously thought. However, in general, the correlative relationships between the lipids and cognition and AD CSF biomarkers indicated a positive role, where the levels of AA, DHA, and EPA all showed a comparatively strong positive correlation to cognition in AD cases, while within the group of SCI cases the LMs derived from DHA and EPA showed such a relationship. Of note, we, along with other researchers, consistently detect the presence of pro-resolving LMs in pathological as well as healthy tissues, which adds credibility to an evolving concept of the resolution pathway as an ever-present “care-taker-guardian” of the tissue rather than a response that is elicited only on demand. Studies in animal models of cancer (Sulciner et al. 2018; Panigrahy et al. 2019; Fishbein et al. 2021) provide a fascinating perspective on resolution as a defender of the tissue, adding further support to this concept in which future therapies for disorders that today are hard to treat may be found.

Our results uncovered alterations in the pro-resolving CSF lipidome during the dysfunctions of inflammatory resolution in AD. Our data demonstrate that it is possible to detect bioactive lipids in CSF samples and to show that pro-resolving LMs such as RvD4, NPD1, MaR1, and RvE1 are reduced in CSF samples from patients with cognitive dysfunction, supporting the disturbance of the resolution of inflammation in the brain. Some of these LMs and the PUFA precursors show positive correlations with MMSE test scores, indicating their relevance for cognitive function.

Limitations

This is an explorative study, original in that it uses LC-mass-spectrometry to analyze the pro-inflammatory and pro-resolving lipidome in samples from cases of AD and MCI as well as SCI in a reasonably large cohort considering the analysis method. However, further studies on a larger cohort are necessary. Although we suggest the use of the CSF lipidomic profile as a biomarker of cognitive decline, its usefulness as a novel biomarker must be determined in replication studies, including longitudinal observations of cognitive decline. We are in the process of performing such studies and hope that the results from the present study motivate other researchers to explore and hopefully confirm the association of alteration in CSF LMs that we believe can be seen in our data. Age and gender were included in our analyses, and the influence of gender on the abundance of LMs is also of importance and requires further investigation.

The majority of the differences observed reach the threshold of *P*-values 0.005 or 0.001, and in some cases even 0.0001. Considering the explorative nature and novel findings in our study, the analyses resulting in a *P*-value of > 0.005 should be interpreted with caution and as an impetus for further investigation rather than hard evidence. Although we did not perform a sensitivity power analysis prior to our investigation, we believe that the sample size in our cohort is large enough for an explorative study. Therefore, the fact that MaR1 and RvE4 were decreased only in MCI compared to SCI, whereas NPD1 and RvD4 were reduced in both AD and MCI, may be a reflection of a limited sample size.

Materials And Methods

Recruitment of study subjects

The study population consisted of 136 participants with SCI (n = 53; 33 female and 20 male), MCI (n = 43; 23 female and 20 male), or AD (n = 40; 24 female and 16 male) from the Memory Clinic at Karolinska University Hospital, Huddinge, Sweden. All participants gave informed consent and agreed to donate their CSF to the Gedoc biobank for scientific research. The study was approved by the Regional Human Ethics Committee of Stockholm (2011/680-31, 2014/1921-32, and 2020-02023). Table 1 lists the demographics of the study population. The recruitment procedure details are outlined in **Fig. S1**. Data on age, gender, cognition, CSF AD biomarkers ($A\beta_{42}$, total (t)-tau and phosphorylated (p)-tau), the mini-mental state examination (MMSE) test (Folstein et al. 1975), and clinical diagnosis were retrieved from the biobank database at the clinic. The CSF biomarker levels were determined by ELISAs (INNOTEST®, Innogenetics, Ghent, Belgium) with the following cut-off values indicating pathology: $A\beta_{42}$ < 550 pg/mL, t-tau > 400 pg/mL, and p-tau > 80 pg/mL. The ICD-10 criteria were used for AD diagnosis (Naik and Nygaard 2008), and the Winblad criteria were used for the diagnosis of MCI (Winblad et al. 2004). A diagnosis of SCI was established when clinical tests did not indicate pathology (Reisberg et al. 2008).

Table 1
Cohort characteristics.

	AD			MCI			SCI		
	n = 40			n = 43			n = 53		
	(F = 24, M = 16)			(F = 23, M = 20)			(F = 33, M = 20)		
	Median	±	SEM	Median	±	SEM	Median	±	SEM
Age (y)	78.5	±	1.3	66	±	1.3	64	±	1.0
MMSE	24	±	0.5	28	±	0.2	29	±	0.2
$A\beta_{42}$	434.5	±	18.1	851	±	34.2	909	±	24.0
t-tau	59	±	4.4	40	±	3.0	37	±	1.6
p-tau	523	±	48.8	269	±	28.9	260	±	12.8

Data are described as median with interquartile (Q) range in pg/mL for amyloid β42 (Aβ42), total tau (t-tau), and phosphorylated tau (p-tau). AD = Alzheimer's disease; F = female, M = male, CSF = cerebrospinal fluid; MCI = mild cognitive impairment; MMSE = mini-mental state examination; SCI = subjective cognitive impairment; y = years

The clinical data were collected on the same or adjacent day of the lumbar puncture. The CSF samples were deoxygenated by induction of nitrogen gas for 10 sec to prevent lipid oxidation and aliquoted into low protein binding tubes, and the aliquots were kept at -80^{o}C until used. Prior to analysis, aliquots were thawed on ice.

Analysis of lipid mediators

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used to assess a total of 22 lipids in the CSF samples, including pro-resolving LMs (LXA_4, MaR1, MaR2, neuroprotectin D1 (NPD1), RvD1, RvD3, RvD4, RvE1, and RvE4), pro-inflammatory LMs (leukotriene B_4, LTB_4), prostaglandins (PGD2, PGE2, and PGF2α), their precursors (EPA, AA, and DHA) and the intermediate products in the metabolic pathways (14-hydroxy-docosahexaenoic acid (14-HDHA), 17-HDHA, 20-HDHA, 12-hydroxyeicosatetraenoic (12-HETE), 14-HETE and 15-HETE) (**Fig. S2**). Fatty acids were extracted from CSF samples using a liquid-liquid lipid extraction method based on chloroform-methanol extraction (Folch et al. 1957). Briefly, since the volume of CSF samples was small (< 700 µL), extraction was done by adding 9 ml of $CHCl_3$/MeOH = 2:1. Internal standard mix (PGD2-d4, LTB4-d4, 15-HETE-d8, EPA-d5, and AA-d8) was added. Then 2 ml of pH3.5 H_2O was added, the resulting upper aqueous phase discarded, and the bottom organic phase was dried down under a gentle N_2 gas stream. The lipid extract was re-constituted in 50 µL of MeOH/H_2O = 1:1 solvent and samples loaded onto a Xevo TQ-S equipped with Acquity I Class UPLC (Waters, Milford, MA, USA). Chromatographic separation was performed using CORTECS C18 2.7 µm column (4.6 x 100 mm; Waters, Milford, MA, USA). Initially, 56.2% of the mobile phase A (MeOH/H2O = 2:8, 0.01% AcA) gradually decreased to 25% for the first 8 min, then 3 min of

isocratic run, followed by 100% B (MeOH, 0.01% AcA) at 18.1 min. The isocratic run of 100% B till 25 min is followed. Finally, it comes back to the initial condition for 5 min. The capillary voltage was -2.5kV, desolvation temperature at 600^{o}C, desolvation gas flow at 1100 L/Hr, cone gas at 150 L/Hr, and nebulizer pressure at 7.0 Bar with the source temperature at 150^{o}C.

Statistical analysis

To investigate differences between diagnostic groups, Kruskal-Wallis was performed in Statistica v13 (Tibco, Palo Alto, USA), followed by Dunn's *post-hoc* test corrected for multiple comparisons. The association of LMs to cognition and AD biomarkers was tested with Spearman Rank Order Correlation in Statistica v13. A *P*-value of < 0.05 was considered statistically significant.

Statements And Declarations

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Competing Interest Statement: The authors declare that they have no competing interests.

Author Contributions: The study was designed by EH, MS, and NB. YW and MO took part in identifying the lipids to be analyzed, organized and handled the samples, and wrote a first draft of the manuscript. KVD, BJ, and MAIK extracted the lipids and analyzed the samples by LC-MS. YW, EH, and MS performed the statistical analysis. EH, MS, and NB edited and finalized the manuscript.

Data Availability: Data will be made available on reasonable request.

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Figures

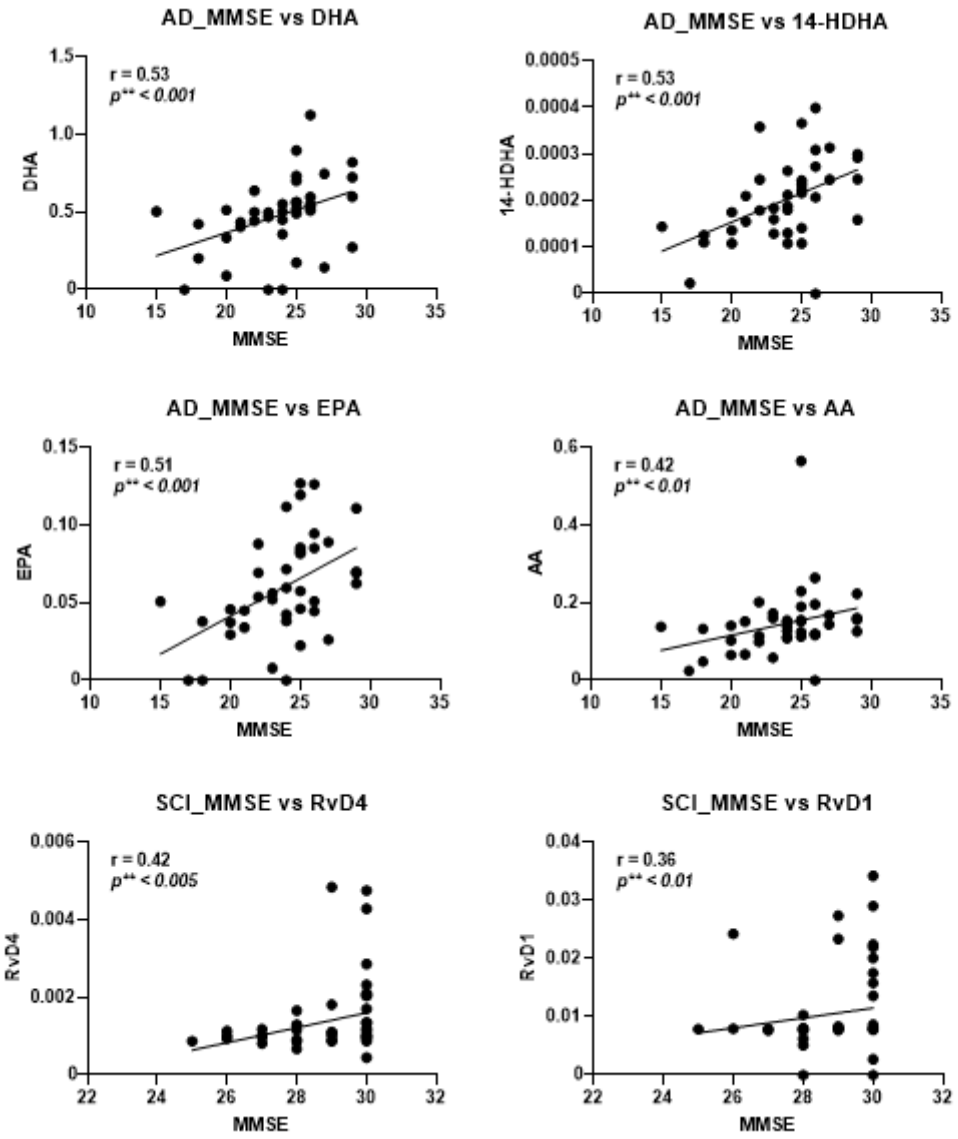

Figure 1

Pro-resolving LMs are reduced in CSF from MCI and AD patients while pro-inflammatory LMs show a mixed pattern. Lipid mediators (LMs) were assessed in the cerebrospinal fluid (CSF) samples from patients with Alzheimer's disease (AD) (n = 40), mild cognitive impairment (MCI) (n = 43) or subjective cognitive impairment (SCI) (n = 53), using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The levels of resolvin (Rv) D4 (D4) and neuroprotectin D1 (NPD1) were reduced in CSF from AD and MCI compared to SCI, while the levels of the pro-inflammatory LM leukotriene B4 (LTB4) levels were higher in AD. The levels of maresin 1 (MaR1) and RvE4 were significantly lower in MCI patients compared to SCI. The levels of the intermediate precursor for RvD4, NPD1, and MaR1, 17-hydroxy docosahexaenoic acid (17-HDHA), were higher in AD than in MCI, and the levels of the intermediate precursor 15-hydroxyeicosatetraenoic acid (15-HETE) were lower in SCI compared to MCI and AD. The levels of prostaglandin (PG) D_2 were lower in CSF from MCI patients compared with SCI, and the PGE_2 levels were lower in AD and MCI patients compared to SCI. Comparisons between groups were performed by Kruskal-

Wallis ANOVA with Dunn's multiple comparisons *post hoc* test (*$P < 0.05$, **$P < 0.005$, ***$P < 0.001$, ****$P < 0.0001$).

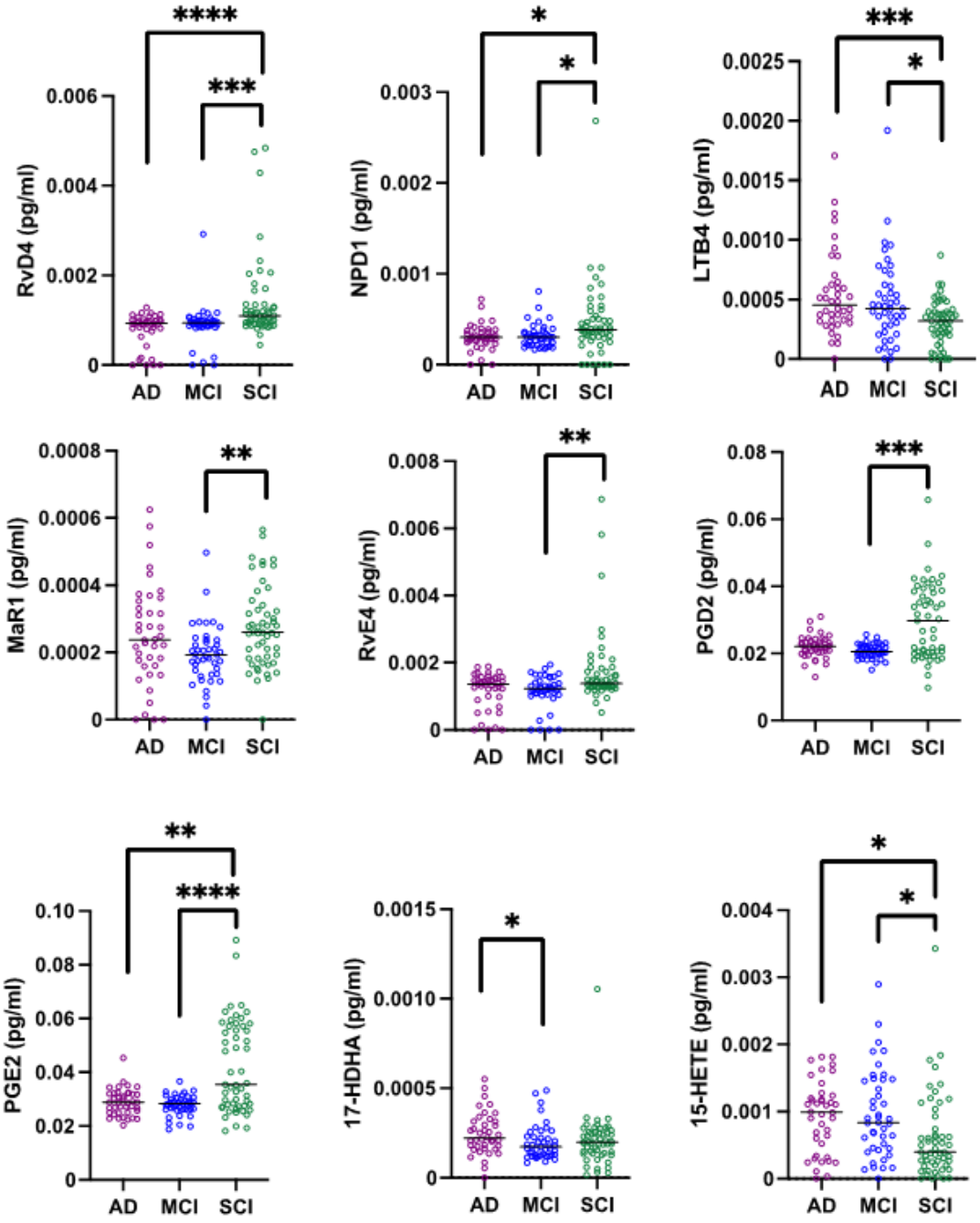

Figure 2

Correlations of bioactive LMs with cognitive function. The levels of lipid mediators (LMs) were correlated to the mini-mental state examination (MMSE) test scores and the r-value according to Spearman rank-

order test is given together with the *P*-value. Resolvin (Rv) D1 and RvD4 show positive correlation to the MMSE scores in the subjective cognitive impairment (SCI) group and when analyzing all three diagnostic groups together (**Table S2a**). The omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and the intermediate precursor 14-hydroxydocosahexaenoic acid (14-HDHA) are positively correlated to the MMSE scores in the Alzheimer's disease (AD) group. Also, levels of the omega-6 fatty acid arachidonic acid (AA) are positively correlated to the MMSE scores in the AD group

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