Lipid signaling and inflammation: metabolomics for better diagnosis and treatment strategy

Yang, W.

Chapter III

Association of oxidative stress and inflammatory metabolites with Alzheimer's disease cerebrospinal fluid biomarkers in mild cognitive impairment

Based on:

Shahzad Ahmad*, Wei Yang*, Adelina Orellana, Lutz Frölich, Itziar de Rojas, Silvia Gil, Mercè Boada, Isabel Hernández, Lucrezia Hausner, Margot H.M. Bakker, Alfredo Cabrera-Socorro, Najaf Amin, Alfredo Ramírez, Agustín Ruiz, Cornelia M. Van Duijn, Thomas Hankemeier

Association of oxidative stress and inflammatory metabolites with Alzheimer's disease cerebrospinal fluid biomarkers in mild cognitive impairment

Manuscript in preparation

*Authors contributed equally

Abstract

Background: Isoprostanes and prostaglandins are biomarkers for oxidative stress and inflammation. Their role in AD pathophysiology is yet unknown. In the current study, we aim to identify the association of isoprostanes and prostaglandins with the Amyloid, Tau, Neurodegeneration (ATN) biomarkers (Aβ-42, p-tau, and t-tau) of AD pathophysiology in mild cognitive impairment subjects.

Methods: Targeted metabolomics profiling of 16 isoprostanes and 12 prostaglandins was performed using liquid chromatography-mass spectrometry (LCMS) in 142 paired plasma-CSF samples from the Barcelona-based memory clinic Fundació ACE cohort and 40 CSF samples from Heidelberg/Manheim clinical centers of mild cognitive impairment (MCI) patients. Linear regression was used to evaluate the association of metabolites with CSF levels of ATN biomarkers in the overall sample and stratified by the *APOE* genotype. False discovery rate (FDR) control was used to adjust for multiple testing. We further evaluated the role of metabolites in MCI to AD progression in the ACE cohort.

Results: CSF levels of two isoprostanes and 1 prostaglandin were associated with tau pathology (p-tau) and neurodegeneration marker (t-tau). CSF levels of a prostaglandin PGF2α and an isoprostane 5-iPF2α VI showed significant association (*FDR* <0.05) with p-tau after multiple testing, while increased levels of an isoprostane 8,12-iso-iPF2α VI was significantly associated with increased levels of both p-tau and total tau levels in CSF. In *APOE* stratified analysis, PGF2α was found positively associated with p-tau and t-tau in only *APOE* ε4 carriers while 5-iPF2α VI showed a positive significant association with both p-tau and t-tau in only *APOE* ε33 carriers. For 8,12- iso-iPF2α VI, it showed a positive association with both p-tau and t-tau in *APOE* ε33 carriers and showed a positive association with t-tau in *APOE* ε3 carriers. None of the metabolites showed evidence of association with MCI to AD progression.

Conclusions: Oxidative stress and inflammatory biomarkers are correlated with biomarkers of neurodegeneration during the prodromal stage of AD, which may be influenced by *APOE* genotype.

Introduction

Oxidative stress represents a series of adaptive responses as a result of the insufficiency of the antioxidant system counteracting the oxidant system [1]. Characterized by the excessive production of free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS), oxidative stress results in cellular injury, which has been involved in various disorders including neurodegenerative diseases [2,3] such as Alzheimer's disease (AD) [4-6]. Besides the tissue damage, oxidative stress may also influence blood-brain integrity which may also activate neuro-inflammation [7], an early-stage process in AD pathophysiology [8,9].

A large number of studies have shown elevated CSF and plasma levels of isoprostanes and prostaglandins in AD [5,10-16], but their relation with established ATN biomarkers [17] is not yet studied during the prodromal phase of AD or linked to the progression from MCI to AD. To study the oxidative stress and inflammatory pathways during the prodromal phase of AD, i.e. mild cognitive impairment (MCI), we profiled a set of isoprostanes and prostaglandins in both cerebrospinal fluid (CSF) and plasma. Isoprostanes are prostaglandin-like metabolites produced by a free radical-mediated phospholipid peroxidation [18] and are established biomarkers of oxidative stress [19]. Together with their isomeric prostaglandins, pro-inflammatory metabolite s [20], they reflect oxidative stress combined with inflammatory status [21]. Since apolipoprotein E (*APOE*) may enhance oxidative stress and inflammation [22,23], examining the role of *APOE* of the relationship between oxidative stress and inflammatory markers and AD pathology may be relevant.

Our study aims to determine whether oxidative stress and inflammation-related metabolites in the prostaglandin and isoprostane pathway in CSF and plasma, are associated with amyloid-beta 42 ($A\beta_{42}$ [A]), phosphorylated-tau (*p-tau [T]*), and total tau (t-tau [N]) levels in CSF during the prodromal phase of AD. We further studied the interaction of metabolites with the *APOE* genotype in association with ATN biomarkers and their role in MCI to AD progression.

Methods

Study populations

Study participants included in the analyses came from two cohorts of the Alzheimer's Disease Apolipoprotein Pathology for Treatment Elucidation and Development (ADAPTED) consortium, including Barcelona-based memory clinic Fundació ACE (142 CSF-plasma paired samples) and Heidelberg/Mannheim memory clinic (40 CSF samples). Both cohorts had obtained their approvals from their respective medical ethical committees, and informed consents are available from all participants which permit the use of phenotype and biomarker information for research purposes. MCI patients with complete information on age at blood collection, sex, body mass index (BMI), lipid-lowering medication use, as well as AD biomarkers in CSF (i.e., Aβ-42, p-tau, and total tau) were selected for both studies.

-Fundació ACE cohort

Patient recruitment and assessment from the Fundació ACE (ACE) cohort was carried out at the Memory Disorders Unit from Fundació ACE, Institut Català de Neurociènces Aplicades, Barcelona, Spain between 2016 to 2017 [24]. The diagnosis was assigned for each patient by consensus among neurologists, neuropsychologists, and social workers at a case conference. All the MCI patients fulfilled the MCI Petersen's diagnostic criteria [25,26] including subjective memory complaints, decline from normal general cognition, preserved performance in activities of daily living, absence of dementia, and a measurable impairment in one or more cognitive functions, with or without a deficit in other cognitive domains (amnestic MCI: single domain or amnestic MCI: multiple domains). In the subsequent follow-up of MCI patients, dementia diagnosis was performed based on the DSM-V criteria [27]. Classification of the cognitive deficits within the dementia group was classified according to the 2011 National Institute of Aging- Alzheimer´s Association (NIA-AA) [28] for Alzheimer´s disease; the National Institute of Neurological Disorder and Stroke and Association Internationale pour la Recherche et l'Enseignement in Neurosciences criteria(NINDS-AIREN) [29] for vascular dementia; Frontotemporal Dementia [30], and for Lewy body dementia [31]. Paired CSF and plasma samples were collected from fasted patients using clinically recommended approaches. Lumbar puncture (LP) was used for CSF

collection from the patient's intervertebral space of L3-L4 according to standard recommendations [32] and the procedure was performed by experienced neurologists under local anesthesia (1% mepivacaine) of the patient in a sitting position. Two tubes (10-ml polypropylene tube, Sarstedt ref 62610018) of CSF were obtained passively of which, one tube for basic biochemistry analysis including glucose, total proteins, proteinogram, and cell type and cell number. The second CSF tube was aliquoted into polypropylene tubes (Sarstedt ref 72694007) after being centrifuged (2000xg 10 min at 4 °C) and finally stored at -80°C. This was performed within 2 hours after CSF collection. For AD biomarker analysis on the sample collection day, an aliquot was thawed at room temperature and vortexed for 5-10 seconds followed by CSF Aβ1-42, total tau, and p-tau level determination using commercially available enzyme-linked immunosorbent assays, namely Innotest Aβ1-42, Innotest hTAU Ag and Innotest PHOSPHO-TAU (181P) (Innotest, Fujirebio Europe) [32,33].

APOE genotyping was performed in the ACE cohort. The patient's whole blood was obtained for DNA extraction using DNA Chemagen technology (Perkin Elmer). Then TaqMan probes analysis (Real-Time PCR QuantStudio3, Thermofisher) was applied to characterize the *APOE* genotype of the patient.

-Heidelberg/Mannheim memory clinic sample

Heidelberg/Mannheim memory clinic cohort included 40 MCI patients between 2012 to 2016 at the Memory Clinic of the Central Institute of Mental Health (Mannheim, Germany), providing 40 CSF samples in the study. Patients were recruited by detailed medical history, physical and neuropsychiatric examination, and standard serum laboratory assessment excluding subjects with neuropsychiatric or general medical causes of impaired cognition. Therefore, all MCI patients met the MCI Petersen's diagnostic criteria [25,26] , including subjective memory complaints, normal general cognition, only minimally impaired performance in instrumental activities of daily living, absence of dementia, and a measurable impairment in one or more cognitive domains. Cognitive impairment was defined as performance below 1.2 standard deviation in one or more cognitive domains in standard neuropsychological test battery [34](test battery of the Consortium to Establish a Registry for Alzheimer Disease (CERAD) [35]plus the Wechsler memory scale – logical memory (WMS) immediate and delayed recall [36], and the trail making test A (TMT-A) and

B (TMT-B) [37]. CSF collected by lumbar puncture was used for biomarker assessment for amyloid determination, and the results of the individual patient were discussed at a case conference attended by geriatric psychiatrists and neuropsychologists. The diagnosis of MCI due to AD or prodromal AD [38] was assigned by consensus. CSF samples were collected and aliquoted for storage at -80ºC. Determination of Aβ1–42, p-tau, and t-tau were performed based on standardized protocols in the Neurochemistry Laboratory at the Department of Neurology, University Medical School, Göttingen. CSF levels of p-tau and CSF levels of Aβ1–42 were both quantitatively determined using a commercially available ELISA kit [INNOTEST® PHOSPHO-TAU(181P), Innogenetics] and a commercially available ELISA kit [INNOTEST®β- AMYLOID (1–42) Innogenetics] respectively.

Illumina GSA1.0 SharedCustom Content bead array was applied for *APOE* genotyping. *APOE* genotype determination was performed using GenomeStudio 2.0 software and data were exported in PLINK format.

Metabolomics profiling

All CSF and plasma samples of both cohorts were analyzed using a ultra-high-performance LC (UHPLC)–MS/MS based approach profiling oxidative stress and inflammatory metabolites including isoprostanes and prostaglandins [39,40].

Samples were stored at -80°C, thawed on ice, and randomized prior to analysis. The sample volume of CSF aliquot and plasma aliquot is 350 µL and 150 µL respectively. The remains were pooled and used for quality control (QC) samples. CSF samples were dried under the vacuum, spiked with deuterated internal standards (ISTDs) and antioxidant (BHT:EDTA 1:1, 0.2 mg/mL) and then extracted with a mixture of 1-butanol:ethyl acetate (1:1, v/v). After the supernatant was collected and dried, samples were reconstituted using a mixture of methanol:water (70:30, v/v). Plasma samples were prepared with the same ISTDs and antioxidant with extra acidifying buffer of 0.2M citric acid and 0.1M disodium hydrogen phosphate (pH 4.5). Then liquid-liquid extraction was performed with a mixture of 1-butanol:ethyl acetate (1:1, v/v) and samples were vortexed followed by centrifugation and collection of the upper organic phase for evaporation. Dried samples were reconstituted with a mixture of ice-cold methanol:water (70:30, v/v). All reconstituted samples were measured using a Shimadzu LCMS-8050 system (Shimadzu, Japan).

For both plasma and CSF samples, LC-MS analyses were performed with high pH run and low pH run using two aliquots from each reconstituted sample. The high pH run targets 24 lysophosphatidic acid species of which results were published elsewhere. The low pH run targets 16 isoprostanes and their isomeric prostanoids as well as some nitro-free fatty acids. For low pH run, samples were measured using an Acquity BEH C18 column (2.1 × 50 mm, 1.7 µm, Waters) with a tertiary mobile phase system of (A) water with 0.1 % acetic acid, (B) 75 % acetonitrile with 25 % methanol and 0.1 % acetic acid, and (C) 100% isopropanol. Dynamic multiple reaction monitoring (dMRM) mode with fast polarity switching was selected for MS acquisition.

QC samples and blank samples were injected together with study samples to ensure data quality. Metabolites showing a relative standard deviation (RSD) no more than 30% on corrected peak areas in QC samples were used as a criterion for metabolite export and further analysis. After QC correction, 9 and 2 metabolites in CSF and plasma, respectively, were used for further data analysis (**Supplementary Table 1**). We detected two isoprostanes in both CSF and plasma including 8-iso-PGF2α and 8,12-iso-iPF2α-VI. Metabolites exclusively detected in CSF samples included three prostaglandins and four isoprostanes. The inverse rank transformation was performed to normalize the distribution of metabolites in both cohorts.

Association of AD biomarkers with metabolites in CSF and plasma

We performed linear regression to assess the association of Aβ-42, p-tau, and t-tau with the isoprostanes and prostaglandins profiled in paired CSF and plasma samples from the ACE cohort and only CSF samples from the Heidelberg-Manheim memory clinic. Levels of Aβ-42, p-tau, and t-tau in CSF were used as an outcome variable in the regression model, and the analyses were adjusted for age, sex, body mass index (BMI), and lipid-lowering medications. The inverse rank transformation was applied to normalize the distribution of both CSF AD biomarkers (Aβ-42, p-tau, and t-tau) and metabolite levels in CSF and plasma.

Meta-analysis of regression analysis

A meta-analysis of regression analysis results of the two cohorts was performed using METAL software [41] using the inverse-variance fixed-effect model. Meta-analysis results of association were also corrected for multiple testing separately for each AD biomarker using

false discovery rate (*FDR*) by Benjamini and Hochberg method [42] and findings with *FDR* < 0.05 were considered significant in overall analysis. All analyses were performed in R (https://www.r-project.org/).

APOE stratified regression analysis

To identify *APOE* specific associations of metabolites with AD biomarkers, *APOE* stratified analysis was performed in both participating cohorts based on three *APOE* strata including *APOE 44/34/24*, *APOE 33,* and *APOE 22/23*. In the stratified analysis, subjects with *APOE 24* genotype were pooled with patients having *APOE 44/34* genotypes based on their similar risk profiles as reported in an earlier study [43]. *APOE* stratified analysis results were reported as a combined meta-analysis of both datasets (ACE cohort and Heidelberg/Manheim cohort) included in the current study. Due to the smaller number of *APOE 22/23* carriers in these two datasets, a combined regression analysis was performed, aggregating all *APOE 22/23* carriers from two cohorts. The combined analysis for *APOE* ε2 stratum was additionally adjusted for cohort information in the tested model. The multiple testing correction was performed using *FDR < 0.05* based on Benjamin and Hochberg method [42].

Association of APOE with metabolite levels

To evaluate the association of *APOE* genotype with metabolites measured in CSF, we also performed the association of metabolites with *APOE* using regression analysis. In this analysis, we compared levels of metabolites in CSF to *APOE ε4* (*APOE 44/34/24*) versus *APOE ε33* carriers and *APOE ε2* (22/23) versus *APOE ε33* carriers using regression adjusted for the age, sex, BMI, and lipid-lowering medications. Analysis results were reported for each cohort as well as their combined meta-analysis.

MCI to AD dementia progression analysis

Follow-up information was available for 138 out of 142 MCI patients of the ACE cohort, of which 43 MCI progressed into AD dementia (31%) while 95 MCI did not convert to AD dementia. The mean follow-up time in AD converters was 1.42 years (SD = 0.53) and 1.58 years (SD = 0.73) in non-AD converters. In the ACE cohort, 11 MCI patients also progressed to other types of dementia including vascular dementia (n = 6), semantic dementia (n = 1), Parkinson dementia (n = 1), Lewy Body dementia (n = 2), and frontal

temporal dementia (n = 1). We analyzed the association of metabolites with MCI to AD dementia progression using cox proportional hazard model adjusted for age at blood collection, sex, BMI, and lipid-lowering medication used.

Results

The general characteristic of the ACE (discovery) and Heidelberg/Mannheim (replication) cohorts are given in **Table 1**. The patients of the ACE cohort of Barcelona are on average 3 years older (P = 0.042). The proportion of women is similar between the two cohorts (P = 0.747). The proportion of patients treated with lipid-lowering medication in the ACE cohort (44%) is 1.6 times increased compared to that in the Heidelberg/Mannheim series of patients, which is borderline non-significant (P = 0.055). Aβ-42, p-tau, and t-tau in CSF levels were not significantly different between two cohorts.

Table 1: Population descriptive

	ACE cohort	Heidelberg/Mannheim cohort	P-value of difference
MCI patients (N)	142	40	
Metabolomics profiling tissue	CSF and Plasma	CSF	
Age (SD) blood collection, years	71.94(7.74)	68.85(8.50)	0.042
Female (%)	74(52%)	22(55%)	0.747
Body Mass index (SD)	26.46(3.74)	25.85(3.61)	0.353
Lipid lowering medication user (%)	63(44%)	11(27%)	0.055
Amyloid-beta 42 in pg/mL (SD)	791.59 (337.36)	690.84 (397.13)	0.151
P-Tau in pg/mL (SD)	71.37 (37.30)	63.17 (29.96)	0.153
Total tau in pg/mL (SD)	478.82 (253.45)	380.95 (326.97)	0.124
APOE genotype N (%)			
APOE 44/34/24	50 (35%)	18(45%)	
APOE 33	81(57%)	18(45%)	
APOE 22/23	11(8%)	4(10%)	

Abbreviations: MCI, mild cognitive impairment; SD, Standard deviation, CSF: Cerebrospinal fluid; *APOE*, apolipoprotein E gene

Association of metabolites with ATN biomarkers in overall sample

Results of association analysis of metabolites measured in CSF with ATN biomarker (Aβ-42,p-tau, and t-tau) levels in CSF are provided in **Table 2 (**Aβ-42), Table 3 (p-tau) and Table 4 (t-tau) . In the meta-analysis of association of metabolites with Aβ-42 (Table2), none of the metabolites studied were significantly associated when adjusting for multiple testing. Three metabolites showed marginal significant evidence of association with Aβ-42 including PGE2 (β = 0.200, $P = 1.33x10^{-2}$), PGF2α (β = 0.223, $P = 3.36x10^{-2}$) and 8-12-iso-iPF2α VI (β = 0.165, $P = 3.74x10^{-2}$) that was consistent across cohorts but all had *FDR* > 0.05. In meta-analysis of the two cohorts metabolites with p-tau (**Table 3**), two isoprostanes i.e., 8,12-iso-iPF2α VI (β = 0.275, $P = 2.19x10^{-4}$), 5-iPF2α VI (β = 0.216, $P = 1.26x10^{-2}$) and a prostaglandin i.e., PGF2α (β = 0.273, $P = 6.07x10^{-3}$) showed significant association (*FDR* < 0.05) with p-tau levels in CSF. Although for PGF2α and 5-iPF2α VI, the findings were not marginally significant in one of the two cohorts, the regression coefficient were very similar for 5-iPF2α VI across the cohorts, except for PGF2α. The latter metabolite was significant only in the Heidelberg/Mannheim sample (smaller samples) with an almost 3 time increased regression coefficient than in the larger cohort, suggesting the finding may be a false positive (I^2 = 53.4, heterogeneity *P* =0.143). **Table 4** shows that 8,12-iso-iPF2α VI (β = 0.228, $P = 2.84x10^{-3}$) is also significantly associated with t-tau levels at *FDR* < 0.05, PGF2α (β = 0.241, $P = 1.99x10^{-2}$) shows nominal association that is driven by the smaller sample Heidelberg/Mannheim but 5-iPF2α VI is not significantly associated. The heatmap of overall meta-analysis of regression analysis results of these metabolites with Aβ-42, p-tau and t-tau levels in CSF is shown in **Figure1**. Associations for PGF2α, 5-iPF2α VI, and 8,12-iso-iPF2α VI were similar across the ATN markers, but were strongest and most *FDR* significant for p-Tau. In plasma-based metabolic measurements, only two metabolites (8,12-iso-iPF2α VI, 8-iso-PGF2α) were detected in more than 60 percent of participants. None of these two metabolites showed association with Aβ-42, p-tau and total tau **(Supplementary table 2)**

Table 2: Association of cerebrospinal fluid (CSF) level of metabolites with Aβ-42 levels in CSF

	ACE cohort			Heidelberg/Mannheim samples			Meta-analysis				
	β	**SE**	***P-value***	**β**	**SE**	***P-value***	**β**	**SE**	**Direction**	***P-value***	***FDR***
PGE2	0.167	0.093	7.34E-02	0.302	0.164	7.47E-02	0.200	0.081	++	1.33E-02	1.12E-01
PGF2α	0.173	0.132	1.93E-01	0.309	0.173	8.32E-02	0.223	0.105	++	3.36E-02	1.12E-01
8,12-iso-iPF2α VI	0.099	0.087	2.58E-01	0.482	0.191	1.65E-02	0.165	0.079	++	3.74E-02	1.12E-01
5-iPF2α VI	0.116	0.099	2.44E-01	0.230	0.192	2.40E-01	0.140	0.088	++	1.12E-01	2.36E-01
8-iso-PGF2α (15-F2t-IsoP)	0.139	0.115	2.30E-01	0.123	0.196	5.33E-01	0.135	0.099	++	1.74E-01	2.36E-01
2,3-dinor-8-iso-PGF2α	0.131	0.123	2.87E-01	0.155	0.187	4.14E-01	0.138	0.103	++	1.78E-01	2.36E-01
PGA2	0.124	0.112	2.67E-01	0.132	0.181	4.71E-01	0.127	0.095	++	1.83E-01	2.36E-01
8-iso-PGE1	0.090	0.084	2.87E-01	-0.114	0.154	4.65E-01	0.043	0.074	-+	5.60E-01	6.21E-01
8-iso-PGA1	0.050	0.088	5.67E-01	-0.007	0.176	9.67E-01	0.039	0.078	-+	6.21E-01	6.21E-01

Abbreviations: SE: standard error; FDR: false discovery rate

Direction: positive (+) or negative (-) in ACE cohort (left symbol) and in Heidelberg cohort (right symbol)

Table 3: Association of cerebrospinal fluid (CSF) level of metabolites with p-tau levels in CSF

	ACE cohort			Heidelberg/Mannheim samples			Meta-analysis				
	β	**SE**	***P-value***	**β**	**SE**	***P-value***	**β**	**SE**	**Direction**	***P-value***	***FDR***
8,12-iso-iPF2α VI	0.227	0.081	5.63E-03	0.555	0.194	7.23E-03	0.275	0.075	++	2.19E-04	1.97E-03
PGF2α	0.162	0.125	1.99E-01	0.464	0.164	7.76E-03	0.273	0.100	++	6.07E-03	2.73E-02
5-iPF2α VI	0.209	0.096	3.14E-02	0.248	0.201	2.26E-01	0.216	0.087	++	1.26E-02	3.79E-02
2,3-dinor-8-iso-PGF2α	0.046	0.121	7.07E-01	0.327	0.190	9.42E-02	0.127	0.102	++	2.14E-01	4.82E-01
8-iso-PGE1	-0.089	0.080	2.69E-01	0.014	0.157	9.30E-01	-0.068	0.071	+-	3.43E-01	5.43E-01
PGA2	-0.069	0.110	5.33E-01	-0.137	0.187	4.68E-01	-0.087	0.095	--	3.62E-01	5.43E-01
8-iso-PGA1	-0.068	0.083	4.11E-01	0.159	0.183	3.91E-01	-0.030	0.076	+-	6.95E-01	8.64E-01
8-iso-PGF2α (15-F2t-IsoP)	0.080	0.113	4.82E-01	-0.131	0.201	5.19E-01	0.029	0.099	-+	7.68E-01	8.64E-01
PGE2	-0.064	0.089	4.74E-01	0.302	0.173	9.00E-02	0.013	0.079	+-	8.73E-01	8.73E-01

Abbreviations: SE: standard error; FDR: false discovery rate

Direction: positive (+) or negative (-) in ACE cohort (left symbol) and in Heidelberg cohort (right symbol)

Table 4: Association of cerebrospinal fluid (CSF) level of metabolites with t-tau levels in CSF

	ACE cohort			Heidelberg/Mannheim samples			Meta-analysis				
	β	SE	*P-value*	β	SE	*P-value*	β	SE	Direction	*P-value*	*FDR*
8,12-iso-iPF2α VI	0.195	0.082	1.90E-02	0.457	0.213	3.93E-02	0.228	0.077	++	2.84E-03	2.55E-02
PGF2α	0.190	0.126	1.34E-01	0.349	0.182	6.40E-02	0.241	0.104	++	1.99E-02	8.96E-02
5-iPF2α VI	0.147	0.095	1.25E-01	0.127	0.215	5.59E-01	0.144	0.087	++	9.86E-02	2.96E-01
8-iso-PGE1	-0.071	0.081	3.84E-01	-0.228	0.169	1.88E-01	-0.100	0.073	--	1.71E-01	3.58E-01
PGA2	-0.049	0.108	6.50E-01	-0.339	0.189	8.19E-02	-0.121	0.094	--	1.99E-01	3.58E-01
2,3-dinor-8-iso-PGF2α	0.032	0.119	7.86E-01	0.282	0.203	1.73E-01	0.096	0.103	++	3.49E-01	5.23E-01
8-iso-PGF2α (15-F2t-IsoP)	0.054	0.111	6.30E-01	-0.278	0.208	1.90E-01	-0.020	0.098	-+	8.35E-01	8.44E-01
8-iso-PGA1	-0.082	0.084	3.29E-01	0.318	0.187	9.79E-02	-0.015	0.076	+-	8.42E-01	8.44E-01
PGE2	-0.086	0.089	3.39E-01	0.279	0.184	1.37E-01	-0.016	0.080	+-	8.44E-01	8.44E-01

Abbreviations: SE: standard error; FDR: false discovery rate

Direction: positive (+) or negative (-) in ACE cohort (left symbol) and in Heidelberg cohort (right symbol).

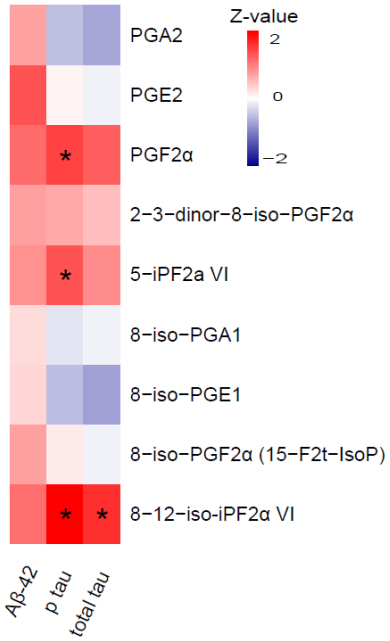


Figure 1: Heatmap of overall meta-analysis of regression analysis results of oxidative stress metabolites with Aβ-42, p-tau and t-tau levels in cerebrospinal fluid (CSF). Note: Star indicate significant association with false discovery rate <0.05.

Association of metabolites with ATN biomarkers in APOE stratified analysis

To study the role of *APOE* genotype on associations of metabolites in CSF with AD pathology biomarkers, *APOE* stratified analyses were performed (**Figure 2)**. Of the three metabolites (PGF2α, 5-iPF2α VI , 8-12-iso-iPF2α VI) which showed association with p-tau levels in CSF in the overall analysis, PGF2α showed a positive association with p-tau (β = 0.619, $P = 3.53x10^{-4}$) and total tau (β = 0.523, $P = 3.47x10^{-3}$) in only *APOE* ε4 carriers. Although the heterogeneity p-value is not significant (*APOE* ε4 strata: P-tau = 0.27, t-tau = 0.44), but beta values are high in the smaller cohort, indicating that association is maybe driven by one cohort. The association of 5-iPF2α VI with p-tau (β = 0.308, $P = 4.52x10^{-3}$) and total-tau (β = 0.288, $P = 1.06x10^{-2}$) was significant only *APOE ε33* carriers. The isoprostane 8,12-iso-iPF2α VI showed association to p-tau in both *APOE ε33* (β = 0.293, *P* = 1.60x10-3)and *APOE ε4* carriers *(*β = 0.395, *P* = 2.59x10-3*)*, while with total tau (*APOE* ε33: β = 0.298, *P* = 3.14x10-3) in only *APOE ε33* carriers. 8,12-iso-iPF2α VI show a negative regression coefficients with p-tau and total tau in *APOE ε*22/23 carriers. In the association analysis of the metabolite levels with *APOE* genotypes (**Supplementary table 3 and 4**), we did not observe association of oxidative stress and inflammatory related metabolites with *APOE ε4* and *APOE ε2.*

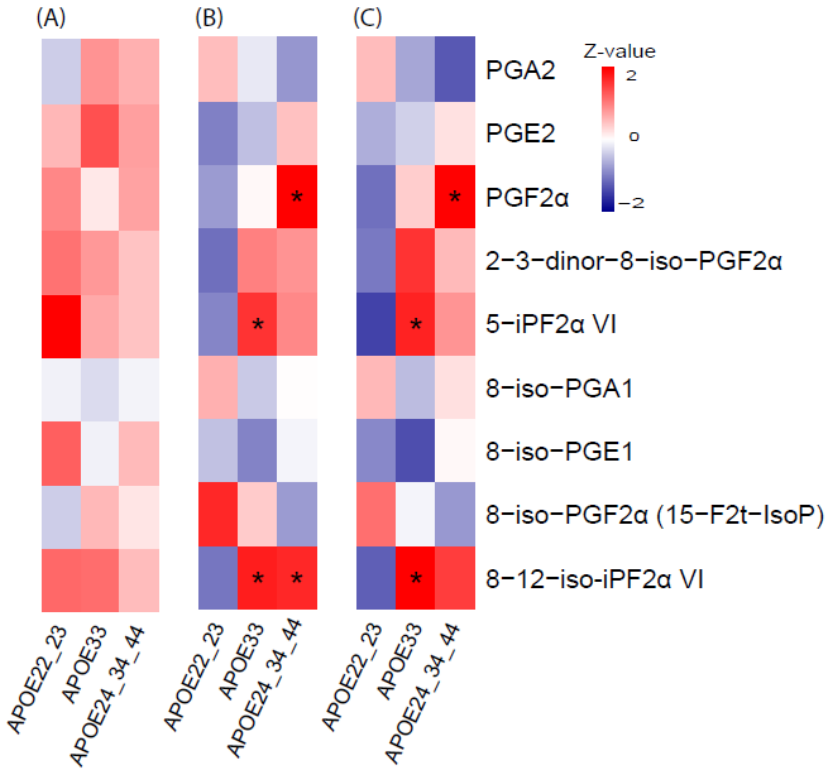


Figure 2: Heatmap of *APOE* stratified meta-analysis of regression analysis results of oxidative stress metabolites with Aβ-42 (A), p-tau (B) and t-tau (C) levels in in cerebrospinal fluid. Note: Star indicate significant association with false discovery rate < 0.05 in each stratum

Role of metabolites in MCI to AD progression

The metabolites were also tested for their association with MCI to AD progression in CSF (**Table 5**) and plasma (**Supplementary table 5**). We did not observe significant association of metabolite levels with MCI to AD progression.

Table 5: Association of cerebrospinal fluid (CSF) level of metabolites with MCI to AD progression

	β	SE	*P-value*	FDR
PGF2α	-0.420	0.263	1.10E-01	5.18E-01
8-iso-PGA1	-0.228	0.188	2.25E-01	5.18E-01
8,12-iso-iPF2α VI	-0.205	0.186	2.68E-01	5.18E-01
PGA2	0.237	0.214	2.69E-01	5.18E-01
5-iPF2α VI	-0.196	0.192	3.09E-01	5.18E-01
8-iso-PGF2α (15-F2t-IsoP)	-0.221	0.258	3.90E-01	5.18E-01
2,3-dinor-8-iso-PGF2α	-0.209	0.249	4.03E-01	5.18E-01
8-iso-PGE1	-0.103	0.182	5.70E-01	6.41E-01
PGE2	-0.003	0.184	9.88E-01	9.88E-01

Abbreviations: SE: standard error; FDR: false discovery rate

Discussion

We identified a significant positive association of an isoprostane 8,12-iso-iPF2α VI with p-tau and t-tau levels in CSF, while an isoprostane (5-iPF2α VI) and a prostaglandin (PGF2α) showed significant association with only p-tau levels in the overall analysis. In the APOE stratified analysis, PGF2α and 5-iPF2α VI showed significant association with p-tau and t-tau in only APOE ε4 and APOE 33 carriers, respectively. Whereas 8,12-iso-iPF2α VI showed association with p-tau and t-tau in APOE ε4 and APOE ε33 carriers.

Isoprostanes are the products of lipid peroxidation and established markers of oxidative stress [19]. Our findings are in line with earlier studies reporting the association of an isoprostane 8,12-iso-iPF2α-VI with MCI [5] and AD [11] and CSF levels of tau and amyloid in AD patients [10]. The *APOE* can modify the association of 8,12-iso-iPF2α-VI with p-tau and total tau is a unique observation of our study. Increasing evidence suggests that oxidative stress enhances the phosphorylation [44] and polymerization of tau [45], which is in line with the findings of our study. Moreover, studies have reported increased levels of oxidative stress markers with neurodegeneration [46,47]. Considering the CSF levels of p-tau and total tau as biomarkers of neurodegeneration and AD progression [48], our findings suggest that isoprostanes may increase during early phase of AD development. We did not observe any association of plasma level of isoprostane 8,12-iso-iPF2α VI with CSF levels of Aβ-42, p-

tau and total-tau which might be due to no correlation (P = 0.37) between CSF and plasma levels of this specific isoprostane in our study.

A prostaglandin PGF2α associates significantly with both p-tau and total tau in only *APOE* ε4 carriers. Due to the large regression coefficient estimate in a smaller Heidelberg/Mannheim sample, this finding should be considered carefully and need further evaluation in other studies. Although the p-value of heterogeneity was not significant for the meta-analysis in the association of this marker with p-tau and total-tau, PGF2α is one of the most important prostanoids with wide-ranging functions in inflammation, cardiovascular function, and smooth muscle contraction [21,49]. PGF2α is a product of arachidonic acid metabolism which can be generated through enzymatic mediation by cyclooxygenase-2 (COX-2) and Prostaglandin F Synthase (PGFS) or via autooxidation. In the central nervous system, prostamide/prostaglandin F synthase and CBR1 both involve the production of PGF2α of which CBR1 is possibly the predominant one [50-52]. Oxidative stress as a crucial pathogenesis in AD has been reported to be associated with increased levels of cytotoxic carbonyl products which consequently induce elevated level of CBR1 enzyme in the brain [53]. Carbonyls from lipid peroxidation modify tau proteins and result in consequent aggregation of phosphorylated tau [54,55]. Therefore, this may suggest the mechanistic relationship among tau proteins, carbonyl compounds, PGF2α production via CBR1 and oxidative stress in *APOE* ε4 stratified analysis. On the other hand, PGF2α together with F2-series isoprostanes (e.g. 2,3-dinor-8-iso-PGF2α; 5-iPF2α VI; 8,12-iso-iPF2α VI) showed positive association with p-tau and t-tau in the APOE ε4 group (Figure 2). This may indicate the potentially active contribution of autooxidation pathway mediated PGF2α production. Future mechanistic investigations on which pathway is more actively involved in PGF2α generation should be performed to shed light on the association of prostaglandin/isoprostane generation with APOE genotype as well as ATN biomarkers.

Limitations

One of the major limitations of our study is our limited sample size which challenged our *APOE* stratified analyses. For two study cohorts included in this study, meta-analysis of

association of metabolites with AD pathology biomarkers was only available for CSF samples due to the unavailability of plasma samples from Heidelberg/Mannheim memory clinic cohort. In addition, we had short follow-up of the MCI patients of which the sample size is also limited for patients with progression from MCI to AD.

Conclusions

In our study, we showed the association of isoprostanes and prostaglandins with biomarkers of AD pathology (Aβ42,p-tau,t-tau). In *APOE* stratified analysis, we observed *APOE* 4 specific association of PGF2α with both p-tau and total tau biomarkers. Even though no significant differences of metabolite levels in CSF were found between different *APOE* carriers, the correlations between PGF2α, 5-iPF2α VI, 8,12-iso-iPF2α VI and tau biomarkers implicate the complexity of metabolite changes and tau pathology with the influence of *APOE* genotype. Our study provides insight into the role of oxidative stress and inflammatory metabolites during the prodromal phase of AD and role of *APOE* in modifying the association of these metabolites with biomarkers of AD pathophysiology.

Declarations

Ethics approval and consent to participate

Fundació ACE cohort has been approved by the ethic committee of the Hospital Clinic i Provincial de Barcelona in Barcelona, Spain in accordance with Spanish biomedical laws (Law 14/2007, July 3rd, about biomedical research; Royal Decree 1716/2011, November 18th) and followed the recommendations of the Declaration of Helsinki.

Consent for publication

All the samples from the Fundació ACE cohort and the Heidelberg/Mannheim memory clinic have the informed consent of the subjects that have donated them. In the Fundació ACE cohort, these protocols of consent have been approved previously by Ethic Committee

of the Hospital Clínic (HCB/2014/0494, HCB/2016/0571, HCB/2016/0835, HCB/2017/0125 and HCB/2018/0333). The protocols have been designed in agreement with the indications of the Sociedad Española de Neurologiaaccording to the current normative for the use of clinical data and biological material and surplus of the assisted process for the biomedicine research of neurodegenerative diseases.

Availability of data and materials

Data can only be available upon request for only research purposes. The availability of data would be possible in compliance with EU-GDPR rules. Data access requests can be made directly to the corresponding authors.

Acknowledgments

This study was funded by the ADAPTED: Alzheimer's Disease Apolipoprotein Pathology for Treatment Elucidation and Development consortium which has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under Grant Agreement No 115975. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and the European Federation of Pharmaceutical Industries and Associations. Part of this work was supported by the JPND EADB grant (German Federal Ministry of Education and Research, BMBF: 01ED1619A).

We acknowledge all patients participating in this study from Fundació ACE and Heidelberg-Mannheim memory clinic. We also want to thank the investigators from the Fundació ACE Barcelona Alzheimer Treatment and Research Center, for their close collaboration and continuous intellectual input. We are grateful to Laura Montrreal for her exceptional technical support. A.R. and M.B. receive support from the European Union/EFPIA Innovative Medicines Initiative Joint Undertaking ADAPTED and MOPEAD projects (Grants No. 115975 and 115985, respectively). M.B. S.G. and A.R. are also supported by national grants PI13/02434, PI16/01861, and PI17/01474. Acción Estratégica en Salud is integrated into the Spanish National R + D + I Plan and funded by ISCIII (Instituto de Salud Carlos III)-Subdirección General de Evaluación and the Fondo

Europeo de Desarrollo Regional (FEDER- "Una manera de Hacer Europa"). Wei Yang would like to acknowledge the Chinese Scholarship Council (CSC No. 201607060017).

References

1. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem. 2015;97:55-74.

2. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol. 2009;7(1):65-74.

3. Radi E, Formichi P, Battisti C, Federico A. Apoptosis and oxidative stress in neurodegenerative diseases. J Alzheimers Dis. 2014;42 Suppl 3:S125-152.

4. Pratico D. Oxidative stress hypothesis in Alzheimer's disease: a reappraisal. Trends Pharmacol Sci. 2008;29(12):609-615.

5. Pratico D, Clark CM, Liun F, Rokach J, Lee VY, Trojanowski JQ. Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. Arch Neurol. 2002;59(6):972-976.

6. de Leeuw FA, Peeters CFW, Kester MI, et al. Blood-based metabolic signatures in Alzheimer's disease. Alzheimers Dement (Amst). 2017;8:196-207.

7. Abdul-Muneer PM, Chandra N, Haorah J. Interactions of oxidative stress and neurovascular inflammation in the pathogenesis of traumatic brain injury. Mol Neurobiol. 2015;51(3):966-979.

8. Heneka MT, Carson MJ, El Khoury J, et al. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 2015;14(4):388-405.

9. Calsolaro V, Edison P. Neuroinflammation in Alzheimer's disease: Current evidence and future directions. Alzheimers Dement. 2016;12(6):719-732.

10. Pratico D, Clark CM, Lee VM, Trojanowski JQ, Rokach J, FitzGerald GA. Increased 8,12-iso-iPF2alpha-VI in Alzheimer's disease: correlation of a noninvasive index of lipid peroxidation with disease severity. Ann Neurol. 2000;48(5):809-812.

11. Pratico D, V MYL, Trojanowski JQ, Rokach J, Fitzgerald GA. Increased F2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo. FASEB J. 1998;12(15):1777-1783.

12. Montine TJ, Beal MF, Cudkowicz ME, et al. Increased CSF F2-isoprostane concentration in probable AD. Neurology. 1999;52(3):562-565.

13. Montine TJ, Markesbery WR, Morrow JD, Roberts LJ, 2nd. Cerebrospinal fluid F2-isoprostane levels are increased in Alzheimer's disease. Ann Neurol. 1998;44(3):410-413.

14. Montine TJ, Sidell KR, Crews BC, et al. Elevated CSF prostaglandin E2 levels in patients with probable AD. Neurology. 1999;53(7):1495-1498.

15. Combrinck M, Williams J, De Berardinis MA, et al. Levels of CSF prostaglandin E2, cognitive decline, and survival in Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2006;77(1):85-88.

16. Li G, Millard SP, Peskind ER, et al. Cross-sectional and longitudinal relationships between cerebrospinal fluid biomarkers and cognitive function in people without cognitive impairment from across the adult life span. JAMA Neurol. 2014;71(6):742-751.

17. Cummings J. The National Institute on Aging-Alzheimer's Association Framework on Alzheimer's disease: Application to clinical trials. Alzheimers Dement. 2019;15(1):172-178.

18. Rokach J, Kim S, Bellone S, et al. Total synthesis of isoprostanes: discovery and quantitation in biological systems. Chem Phys Lipids. 2004;128(1-2):35-56.

19. Pratico D, Lawson JA, Rokach J, FitzGerald GA. The isoprostanes in biology and medicine. Trends Endocrinol Metab. 2001;12(6):243-247.

20. Hein AM, O'Banion MK. Neuroinflammation and memory: the role of prostaglandins. Mol Neurobiol. 2009;40(1):15-32.

21. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol. 2011;31(5):986-1000.

22. Lauderback CM, Kanski J, Hackett JM, Maeda N, Kindy MS, Butterfield DA. Apolipoprotein E modulates Alzheimer's Abeta(1-42)-induced oxidative damage to synaptosomes in an allele-specific manner. Brain Res. 2002;924(1):90-97.

23. Parhizkar S, Holtzman DM. APOE mediated neuroinflammation and neurodegeneration in Alzheimer's disease. Semin Immunol. 2022:101594.

24. Boada M, Tarraga L, Hernandez I, et al. Design of a comprehensive Alzheimer's disease clinic and research center in Spain to meet critical patient and family needs. Alzheimers Dement. 2014;10(3):409-415.

25. Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med. 2004;256(3):183-194.

26. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol. 1999;56(3):303-308.

27. Edition F. Diagnostic and statistical manual of mental disorders. Am Psychiatric Assoc. 2013;21(21):591-643.

28. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):263-269.

29. Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology. 1993;43(2):250-260.

30. Mesulam MM, Grossman M, Hillis A, Kertesz A, Weintraub S. The core and halo of primary progressive aphasia and semantic dementia. Ann Neurol. 2003;54 Suppl 5:S11-14.

31. McKeith IG, Boeve BF, Dickson DW, et al. Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. Neurology. 2017;89(1):88-100.

32. Vanderstichele H, Bibl M, Engelborghs S, et al. Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: a consensus paper from the Alzheimer's Biomarkers Standardization Initiative. Alzheimers Dement. 2012;8(1):65-73.

33. Blennow K, Zetterberg H. The application of cerebrospinal fluid biomarkers in early diagnosis of Alzheimer disease. Med Clin North Am. 2013;97(3):369-376.

34. Winblad B, Palmer K, Kivipelto M, et al. Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med. 2004;256(3):240-246.

35. Morris JC, Heyman A, Mohs RC, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. Neurology. 1989;39(9):1159-1165.

36. Walton D. The diagnostic and predictive accuracy of the wechsler memory scale in psychiatric patients over 65. J Ment Sci. 1958;104(437):1111-1118.

37. Reitan RM, Wolfson D. The Trail Making Test as an initial screening procedure for neuropsychological impairment in older children. Arch Clin Neuropsychol. 2004;19(2):281-288.

38. Kaerst L, Kuhlmann A, Wedekind D, Stoeck K, Lange P, Zerr I. Cerebrospinal fluid biomarkers in Alzheimer's disease, vascular dementia and ischemic stroke patients: a critical analysis. J Neurol. 2013;260(11):2722-2727.

39. Ahmad S, Orellana A, Kohler I, et al. Association of lysophosphatidic acids with cerebrospinal fluid biomarkers and progression to Alzheimer's disease. Alzheimers Res Ther. 2020;12(1):124.

40. Schoeman JC, Harms AC, van Weeghel M, Berger R, Vreeken RJ, Hankemeier T. Development and application of a UHPLC-MS/MS metabolomics based comprehensive systemic and tissue-specific screening method for inflammatory, oxidative and nitrosative stress. Anal Bioanal Chem. 2018;410(10):2551-2568.

41. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics. 2010;26(17):2190-2191.

42. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B (Methodological). 1995;57(1):289-300.

43. van der Lee SJ, Wolters FJ, Ikram MK, et al. The effect of APOE and other common genetic variants on the onset of Alzheimer's disease and dementia: a community-based cohort study. Lancet Neurol. 2018;17(5):434-444.

44. Zhu X, Rottkamp CA, Boux H, Takeda A, Perry G, Smith MA. Activation of p38 kinase links tau phosphorylation, oxidative stress, and cell cycle-related events in Alzheimer disease. J Neuropathol Exp Neurol. 2000;59(10):880-888.

45. Gamblin TC, King ME, Kuret J, Berry RW, Binder LI. Oxidative regulation of fatty acid-induced tau polymerization. Biochemistry. 2000;39(46):14203-14210.

46. Andersen JK. Oxidative stress in neurodegeneration: cause or consequence? Nat Med. 2004;10 Suppl:S18-25.

47. Rekatsina M, Paladini A, Piroli A, Zis P, Pergolizzi JV, Varrassi G. Pathophysiology and Therapeutic Perspectives of Oxidative Stress and Neurodegenerative Diseases: A Narrative Review. Adv Ther. 2020;37(1):113-139.

48. Schraen-Maschke S, Sergeant N, Dhaenens CM, et al. Tau as a biomarker of neurodegenerative diseases. Biomark Med. 2008;2(4):363-384.

49. Yu Y, Lucitt MB, Stubbe J, et al. Prostaglandin F2alpha elevates blood pressure and promotes atherosclerosis. Proc Natl Acad Sci U S A. 2009;106(19):7985-7990.

50. Yoshikawa K, Takei S, Hasegawa-Ishii S, et al. Preferential localization of prostamide/prostaglandin F synthase in myelin sheaths of the central nervous system. Brain Res. 2011;1367:22-32.

51. Hayashi H, Fujii Y, Watanabe K, Hayaishi O. Enzymatic formation of prostaglandin F2 alpha in human brain. Neurochem Res. 1990;15(4):385-392.

52. Schieber A, Frank RW, Ghisla S. Purification and properties of prostaglandin 9-ketoreductase from pig and human kidney. Identity with human carbonyl reductase. Eur J Biochem. 1992;206(2):491-502.

53. Balcz B, Kirchner L, Cairns N, Fountoulakis M, Lubec G. Increased brain protein levels of carbonyl reductase and alcohol dehydrogenase in Down syndrome and Alzheimer's disease. J Neural Transm Suppl. 2001(61):193-201.

54. Liu Q, Raina AK, Smith MA, Sayre LM, Perry G. Hydroxynonenal, toxic carbonyls, and Alzheimer disease. Mol Aspects Med. 2003;24(4-5):305-313.

55. Liu Q, Smith MA, Avila J, et al. Alzheimer-specific epitopes of tau represent lipid peroxidation-induced conformations. Free Radic Biol Med. 2005;38(6):746-754.

Supplementary files

Supplementary Table 1: List of oxidative stress metabolites in cerebrospinal fluid and plasma

Abbreviations	Name	Class	CSF	Plasma	Lipid maps ID
PGA2	Prostaglandin A2	Prostaglandin	Yes	No	LMFA03010035
PGE2	Prostaglandin E2	Prostaglandin	Yes	No	LMFA03010003
PGF2α	Prostaglandin F2α	Prostaglandin	Yes	No	LMFA03010002
2-3-dinor-8-iso-PGF2α	2,3-Dinor-8-iso-PGF2α	Isoprostane	Yes	No	LMFA03110010
5-iPF2α VI	(+/-) 5-iPF2α-VI	Isoprostane	Yes	No	LMFA03110011
8-iso-PGA1	8-iso-Prostaglandin A1	Isoprostane	Yes	No	LMFA03110008
8-iso-PGE1	8-iso-Prostaglandin E1	Isoprostane	Yes	No	LMFA03110002
8-iso-PGF2α (15-F2t-IsoP)	15-F2t-IsoP	Isoprostane	Yes	Yes	LMFA03110001
8-12-iso-iPF2α VI	8,12-iso--iPF2α-VI	Isoprostane	Yes	Yes	NA

Abbreviations: CSF, cerebrospinal fluid

Supplementary Table 2: Association of metabolites in plasma with amyloid-beta 42, P-Tau and total tau in ACE cohort

Stratum	Aβ-42			p-tau			t-tau		
Overall sample	**β**	**SE**	**P-value**	**β**	**SE**	**P-value**	**β**	**SE**	**P-value**
8,12-iso-iPF2α VI	0.180	0.111	1.07E-01	-0.115	0.110	2.96E-01	-0.144	0.107	1.83E-01
8-iso-PGF2α (15-F2t-IsoP)	0.040	0.089	6.52E-01	0.020	0.087	8.18E-01	0.026	0.086	7.61E-01
***APOE 4* stratum**									
8,12-iso-iPF2α VI	0.239	0.177	1.84E-01	-0.004	0.194	9.83E-01	-0.124	0.189	5.14E-01
8-iso-PGF2α (15-F2t-IsoP)	-0.124	0.158	4.37E-01	-0.031	0.170	8.58E-01	-0.037	0.167	8.27E-01
***APOE 33* stratum**									
8,12-iso-iPF2α VI	0.286	0.150	6.15E-02	-0.158	0.146	2.83E-01	-0.175	0.142	2.23E-01
8-iso-PGF2α (15-F2t-IsoP)	0.124	0.117	2.93E-01	0.010	0.112	9.27E-01	0.023	0.110	8.35E-01
***APOE* 2 stratum**									
8,12-iso-iPF2α VI	-0.143	0.432	7.58E-01	0.091	0.379	8.22E-01	0.195	0.329	5.85E-01
8-iso-PGF2α (15-F2t-IsoP)	-0.513	0.407	2.76E-01	0.526	0.327	1.83E-01	0.539	0.263	1.10E-01

Abbreviations: β, regression coefficient; SE, standard error

Supplementary Table 3: Association of metabolites in cerebrospinal fluid with *APOE* 22/23 (*APOE* 33 as reference)

	ACE cohort			Heidelberg/Mannheim samples			Meta-analysis				
Metabolite	β	SE	*P-value*	β	SE	*P-value*	β	SE	Direction	*P-value*	FDR
PGA2	-0.295	0.235	2.13E-01	0.309	0.516	5.57E-01	-0.191	0.214	-+	3.71E-01	5.57E-01
PGE2	-0.32	0.254	2.12E-01	-0.691	0.595	2.63E-01	-0.377	0.234	--	1.07E-01	2.53E-01
PGF2α	-0.029	0.218	8.95E-01	0.445	0.44	3.27E-01	0.064	0.195	-+	7.42E-01	8.08E-01
2,3-dinor-8-iso-PGF2α	0.502	0.23	3.17E-02	0.271	0.532	6.17E-01	0.466	0.211	++	2.73E-02	1.23E-01
5-iPF2α VI	0.369	0.262	1.62E-01	0.349	0.479	4.77E-01	0.364	0.23	++	1.12E-01	2.53E-01
8-iso-PGA1	0.302	0.334	3.68E-01	-0.05	0.538	9.27E-01	0.204	0.284	+-	4.72E-01	6.06E-01
8-iso-PGE1	0.01	0.302	9.74E-01	-0.273	0.513	6.02E-01	-0.063	0.26	+-	8.08E-01	8.08E-01
8-iso-PGF2α (15-F2t-IsoP)	0.241	0.227	2.92E-01	0.272	0.437	5.42E-01	0.247	0.201	++	2.19E-01	3.94E-01
8,12-iso-iPF2α VI	0.639	0.311	4.35E-02	0.5	0.462	2.96E-01	0.595	0.258	++	2.12E-02	1.23E-01

Abbreviations: APOE, Apolipoprotein E; β, regression coefficient; SE, standard error; FDR, false discovery rate

Note: Direction column indicates the direction of regression co-efficient of association in the ACE and Heidelberg/Mannheim cohort

Supplementary Table 4: Association of metabolites in cerebrospinal fluid with *APOE* 44/34/24 (*APOE* 33 as reference)

	ACE cohort			Heidelberg/Mannheim samples			Meta-analysis				
Metabolite	β	SE	*P-value*	β	SE	*P-value*	β	SE	Direction	*P-value*	FDR
PGA2	0.153	0.138	2.70E-01	-0.269	0.34	4.35E-01	0.093	0.128	+-	4.66E-01	9.81E-01
PGE2	0.108	0.167	5.16E-01	-0.351	0.346	3.18E-01	0.022	0.15	+-	8.84E-01	9.81E-01
PGF2α	0.008	0.119	9.47E-01	-0.093	0.346	7.90E-01	-0.003	0.112	+-	9.81E-01	9.81E-01
2,3-dinor-8-iso-PGF2α	-0.004	0.12	9.72E-01	0.207	0.33	5.35E-01	0.021	0.113	-+	8.56E-01	9.81E-01
5-iPF2α VI	0.113	0.154	4.64E-01	-0.195	0.314	5.39E-01	0.053	0.138	+-	7.00E-01	9.81E-01
8-iso-PGA1	-0.249	0.175	1.57E-01	-0.085	0.341	8.05E-01	-0.215	0.156	--	1.68E-01	9.81E-01
8-iso-PGE1	-0.175	0.187	3.52E-01	0.085	0.414	8.38E-01	-0.131	0.17	-+	4.43E-01	9.81E-01
8-iso-PGF2α (15-F2t-IsoP)	0.054	0.135	6.91E-01	-0.23	0.301	4.51E-01	0.006	0.123	+-	9.59E-01	9.81E-01
8-12-iPF2α VI	0.158	0.172	3.59E-01	-0.002	0.299	9.94E-01	0.118	0.149	+-	4.27E-01	9.81E-01

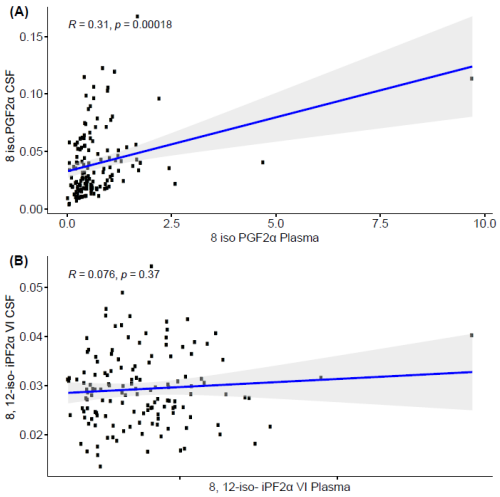
Abbreviations: APOE, Apolipoprotein E; β, regression coefficient; SE, standard error; FDR, false discovery rate

Note: Direction column indicates the direction of regression co-efficient of association in the ACE and Heidelberg/Mannheim cohort

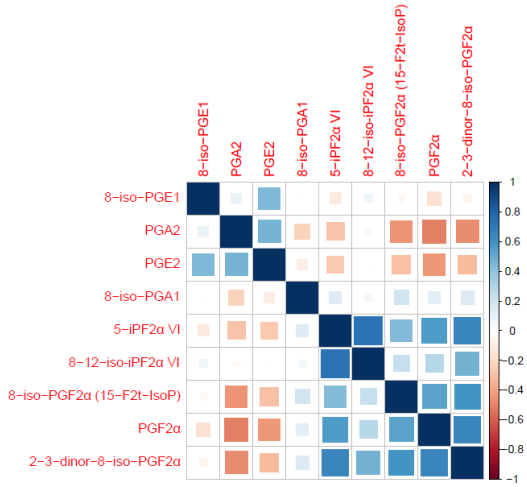
Supplementary Table 5: Association of plasma level of metabolites with MCI to AD progression in ACE cohort

	β	SE	P-value	FDR
8,12-iso-iPF2α VI	-0.138	0.223	5.37E-01	5.37E-01
8-iso-PGF2α (15-F2t-IsoP)	-0.213	0.175	2.23E-01	4.46E-01

Abbreviations: APOE, Apolipoprotein E; β, regression coefficient; SE, standard error; FDR, false discovery rate



Supplementary Figure 1: Correlation of metabolite levels between plasma and CSF.



Supplementary Figure 2: Correlation matrix of metabolites measured in CSF.