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Plasma Phospholipid and Sphingolipid Alterations in *Presenilin1* Mutation Carriers: A Pilot Study

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Abstract

Background and Objective—Aberrant lipid metabolism has been implicated in sporadic Alzheimer's disease (AD). The current study investigated plasma phospholipid and sphingolipid profiles in individuals carrying *PSEN1* mutations responsible for autosomal dominant AD (ADAD).

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SUPPLEMENTARY MATERIAL

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Methods—Study participants evaluated were from the Perth and Melbourne sites of the Dominantly Inherited Alzheimer Network (DIAN) study. Plasma phospholipid and sphingolipid profiles were measured using liquid chromatography coupled with mass spectrometry in 20 *PSEN1* mutation carriers (MC; eight of whom were symptomatic and twelve asymptomatic, based on Clinical Dementia Rating scores) and compared with six non carriers (NC) using linear mixed models. Further, AD gold standard biomarker data obtained from the DIAN database were correlated with lipid species significantly altered between MC and NC, using Spearman's correlation coefficient.

Results—One-hundred and thirty-nine plasma phospholipid and sphingolipid species were measured. Significantly altered species in MC compared to NC primarily belonged to choline and ethanolamine containing phospholipid classes and ceramides. Further phosphatidylcholine species (34:6, 36:5, 40:6) correlated with cerebrospinal fluid tau ($p < 0.05$), and plasmalogen ethanolamine species (34:2, 36:4) correlated with both cerebrospinal fluid tau and brain amyloid load within the MC group ($p < 0.05$).

Conclusion—These findings indicate altered phospholipid and sphingolipid metabolism in ADAD and provide insight into the pathomolecular changes occurring with ADAD pathogenesis. Further, findings reported in this study allow comparison of lipid alterations in ADAD with those reported previously in sporadic AD. The findings observed in the current pilot study warrant validation in the larger DIAN cohort.

Keywords

Alzheimer's disease; biomarkers; familial Alzheimer's disease; phospholipids; sphingolipids

INTRODUCTION

Alzheimer's disease (AD), a progressive neurodegenerative disorder characterized by its neuropathological hallmarks (amyloid plaques and neurofibrillary tangles), is the most common form of dementia and yet definitive diagnosis can only be performed postmortem by confirming the presence of these pathologies. Investigating lipid alterations during AD pathogenesis may serve as an alternative to the proteomic approaches channeled toward the development of a protein-based 'blood test' for the early diagnosis of AD.

Given the basic functions of phospholipids and sphingolipids [1], numerous studies have reported lipid abnormalities in sporadic AD [2–9]. Interestingly, recent reports particularly highlight altered blood phospholipid and sphingolipid metabolism in sporadic AD pathology [8, 10–12]. However, blood phospholipid and sphingolipid alterations in the autosomal dominant form of AD (ADAD) have not been investigated. Mutations present in genes *APP*, *PSEN 1* or *2* are responsible for ADAD [13]. Based on previous reports [2, 14–19] (Fig. 1), we hypothesized that individuals carrying mutations responsible for ADAD have altered blood phospholipid and sphingolipid profiles compared to non carriers.

Using liquid chromatography coupled with mass-spectrometry, we report phospholipid alterations in ADAD mutation carriers. Additionally, associations between the gold standard biomarkers of AD and phospholipid concentrations were investigated in the MC group to

track lipid alterations with disease progression [20]. Findings from the current study provide insight into the biochemical changes that accompany ADAD pathogenesis.

MATERIALS AND METHODS

Study design

The longitudinal multicenter Dominantly Inherited Alzheimer Network (DIAN) study enrolls adult offspring of a biological parent carrying a mutation responsible for ADAD. Offspring who inherited the mutation (mutation carrier; MC) and those who did not (non carrier; NC) are both enrolled in the DIAN Study. Participants with a medical or psychiatric illness that would interfere in completing initial and follow-up assessments or requiring nursing home-level care were not included in the study. Enrolled asymptomatic participants who inherited the mutation have an expected time (observed in years) to onset of symptoms (EYO). EYO is an estimated parameter and is calculated as the difference between each participant's age and their parent's age at symptom onset (AAO) [21]. Asymptomatic participants who have an EYO between +3 to -3 years are followed up annually, while the remaining participants are followed up every three years [22]. The current study only evaluated participants from two sites of the DIAN cohort at baseline, namely, the McCusker Alzheimer Research Foundation (MARF) in Perth and the Mental Health Research Institute (MHRI) in Melbourne. All participants or their caregivers provided informed consent, and the Ethics Committee of the Hollywood Private Hospital (WA), Edith Cowan University and the University of Western Australia approved the study.

Sample collection

Fasted plasma samples were collected according to the protocol described by Bateman et al. [22] and stored at -80°C before analysis began. Plasma samples from 26 participants belonging to 12 families with *PSEN1* mutations (20 MC and 6 NC; mutations listed in Table 1) were analyzed. A subset of eight MC and two NC were recruited from the Perth DIAN center, while the remaining participants belonged to the Melbourne DIAN center. Further, among the 20 MC, based on the Clinical Dementia Rating (CDR) score [23], eight MC were symptomatic (CDR >0) while 12 MC were asymptomatic (CDR = 0).

Measurement of lipid species

Plasma lipid extractions were prepared using the modified Bligh and Dyer method [24, 25], from an initial volume of 150 µl. The lipid extracts were appropriately diluted, spiked with internal standards and analyzed using a QTRAP 4000 (AB Sciex) mass spectrometer (MS) with an electrospray ionization source, coupled to a Shimadzu high performance liquid chromatography (HPLC) system. Separation of individual polar lipid species by normal phase HPLC was carried out using a Phenomenex Luna 3µ-silica column (i.d. 150 × 2.0 mm) and have been previously described elsewhere [26]. Multiple reaction monitoring (MRM) transition were set up for each specie measured, and species were quantified by normalizing their observed intensity with intensities obtained from synthetic internal standards of known concentrations (PE 14:0/14:0, PC 14:0/14:0, PI diC8, C17-Cer d18:1/17:0, C12-SM d18:1/12:0 purchased from Avanti Polar Lipids, USA and Echelon

Biosciences, USA). Single assays were run for each sample however repeat extraction from pooled control plasma was used as quality control for reproducibility checks (cv <15%).

Neuroimaging and neuropsychological data collection

Neuroimaging and neuropsychological data were obtained from the DIAN database. Brain amyloid load was defined as the standard uptake value ratio (SUVR) of the ligand Pittsburgh compound B (PiB) using positron emission tomography (PET) in the precuneus with the brainstem as the reference region. Two NC and two Sym MC did not undergo neuroimaging. CDR scores were employed to evaluate asymptomatic and symptomatic *PSEN1* MC.

Protocols for the above mentioned assays have been described previously by Bateman et al. [22]. Mini-Mental State Examination (MMSE) scores were recorded as a measure of general cognitive function [27].

CSF A β_{42} and tau measurements

Cerebrospinal fluid (CSF) amyloid- β 42 (A β_{42}), total-tau (t-tau), and tau phosphorylated at threonine 181 (p-tau) concentrations were obtained from the DIAN database. CSF A β_{42} and tau species were measured using INNO-BIA AlzBio3™ (Fujirebio, formerly Innogenetics, Ghent, Belgium) as described previously [22]. Two NC did not undergo lumbar puncture and therefore their CSF A β_{42} and tau concentration data were not available.

Statistical analysis

Plasma phospholipids measured from MC were compared with NC using linear mixed models with measurements grouped according to family identification to accommodate within-family correlations. Analyses were based on the square roots of the lipid concentrations to normalise data and were adjusted for gender, *APOE* allele status, site (MARF and MHRI) and age or expected years to symptom onset (EYO). Models were compared using likelihood ratio tests. Correlations between lipid species and the current gold standard biomarkers (CSF A β and tau concentrations and brain amyloid load using PiB-PET) were based on Spearman's correlation coefficient. Analyses were carried out in TIBCO Spotfire S+ version 8.2 (TIBCO Software Inc., Palo Alto, California) and IBM® SPSS® version 20.

RESULTS

Table 2 presents participant demographic data, APOE genotype, and MMSE scores at baseline. One-hundred and thirty-nine plasma phospholipid and sphingolipid species were investigated and broadly classified based on the head group present, namely choline (PC), ethanolamine (PE), and inositol (PI), while sphingolipids were classified as ceramide (Cer) and sphingomyelin (SM) (Supplementary Table 1). Based on the ester, ether, or vinyl ether linkage of the fatty acid to the SN¹ carbon of the phospholipid glycerol backbone, phospholipids were classified as phosphatidyl (Ptd), plasmalogen (e), and plasmenyl (or plasmalogen; p) [28].

Among the 139 plasma phospholipids and sphingolipids measured, species significantly different between MC and NC have been listed in Table 3 (graphs in Supplementary Figure

1). Within the aforementioned phospholipid groups, mainly PC and PE species were significantly altered in MC compared to NC. Plasmalogen species significantly different between MC and NC groups, increased in MC compared to NC ($p < 0.05$). While lower levels PtdC species were primarily observed in asymptomatic MC ($p < 0.05$), lyso PC species significantly altered between the two groups were elevated in MC compared to NC ($p < 0.05$). Further, an elevation in ceramide specie concentrations was observed in MC compared to NC ($p < 0.05$).

Within the MC group, correlations between significantly altered lipid species and gold standard biomarkers (brain amyloid load and CSF tau concentrations) were investigated to track lipid alterations with disease pathogenesis. A significant positive correlation was observed between CSF tau and lysoPC18e:0 (p-tau: $R = 0.450$, $p = 0.047$, $n = 20$), PC34:6 (p-tau: $R = 0.448$, $p = 0.048$, $n = 20$), PC36:5 (p-tau: $R = 0.453$, $p = 0.045$, $n = 20$; t-tau: $R = 0.517$, $p = 0.020$, $n = 20$), PC40:6 (p-tau: $R = 0.457$, $p = 0.043$, $n = 20$; t-tau: $R = 0.513$, $p = 0.021$, $n = 20$) and a negative correlation was observed between CSF tau and plasmalogen PE species 34:2 (p-tau: $R = -0.519$, $p = 0.019$, $n = 20$; t-tau: $R = -0.624$, $p = 0.003$, $n = 20$) and 36:4 (t-tau: $R = -0.472$, $p = 0.036$, $n = 20$), indicating the direction of alteration with increase in CSF tau (Supplementary Figure 2). Further a positive correlation trend was observed between plasmalogen PE species (34:2: $R = 0.441$, $p = 0.052$; 36:1: $R = 0.405$, $p = 0.077$; 36:4: $R = 0.383$, $p = 0.095$) and CSF A β . In addition, brain amyloid load measured via PiB-PET was observed to inversely correlate with plasmalogen PE species 34:2 ($R = -0.492$, $p = 0.038$, $n = 18$) and 36:4 ($R = -0.631$, $p = 0.005$, $n = 18$) (Supplementary Figure 3).

DISCUSSION

The current study reports altered plasma phospholipid and sphingolipid metabolism in individuals carrying ADAD related *PSEN1* mutations in a subset of the DIAN cohort. Primarily, alterations were observed in species belonging to the PC, PE, and Cer classes.

A significant decline in PtdC specie levels were observed in asymptomatic MC compared to NC. Further, within the MC group, PtdC species 34:6, 36:5, and 40:6 were found to positively correlate with CSF tau concentrations. These observations therefore indicate a decline in PtdC levels in the asymptomatic stage in MC compared to NC, followed by an increase in PtdC specie concentrations with disease progression (elevation in CSF tau concentrations) [20]. Interestingly, the above mentioned PtdC species have also been associated with sporadic AD pathology [8, 9].

Additionally, increased lyso PC levels were observed in the current study. This increase may indicate elevated phospholipase A2 (PLA2) activity (or increased exposure of the precursor 'PC' to PLA2) in AD pathology. PLA2 is involved in the cleavage of fatty acids from the SN²-carbon of the phospholipid glycerol backbone, giving rise to lyso PC and free fatty acids such as arachidonic acid and docosahexaenoic acid. At normal physiological concentrations, these phospholipid products serve as substrates for important signaling messengers (that influence cell proliferation, gene expression and signal transduction) such as eicosanoids and platelet activating factor [18], however, at higher concentrations cause

neurotoxicity and appear to have detergent-like properties resulting in dispersion of neural membrane [18, 29, 30]. In support of elevated lyso PC levels in the current study, elevated cerebrospinal lyso PC levels and PLA2 activity in the sporadic form of AD, has been reported previously [18, 31].

Elevated levels of plasmalogen species were mostly observed in asymptomatic MC when compared to NC. However, within the MC group, a decrease in plasmalogen species levels (PE 34p:2, 36p:4) was observed with an increase in brain amyloid load and CSF tau concentrations, therefore indicating a decline in plasmalogen concentrations with progression in disease pathogenesis. Plasmalogens have been reported to be protective against reactive oxygen species [32]. The general trend of elevated plasmalogen species in asymptomatic MC compared to NC observed in the current study may reflect a compensatory mechanism to cope with oxidative stress in the early stages of pathogenesis or the asymptomatic stage. However, with disease progression (increase in CSF tau concentrations and brain amyloid load) a decline in plasmalogen levels (PE 34p:2, 36p:4) was observed. Interestingly, decreased plasmalogen ethanolamine concentrations have also been reported in the serum of subjects with dementia of Alzheimer's type [19].

Additionally, ceramide is well known to induce apoptosis and its production has been reported to be induced by elevated levels of A β ₄₂ [14]. Concentrations of ceramide species (Cer.d18:1/24:0, Cer.d18:1/22:0, Cer.d18:1/20:0) were elevated in the MC group. Elevated ceramide levels observed in the current study show similar ceramide metabolism to that reported in sporadic AD [12, 33, 34].

It is acknowledged that the current study has its limitations. Being a pilot study, only a modest sample size of the DIAN was accessible and therefore multiple comparisons were not adjusted for. Additionally, although the gold standard biomarkers of AD and phospholipid levels are both known to be influenced by age [19, 35], correlations between lipid species and gold standard biomarkers of AD could not be adjusted for age due to sample size constraints. The DIAN cohort is a very unique and powerful cohort, comprising individuals carrying mutations responsible for ADAD, thus making them predestined to develop AD pathology; therefore presenting the opportunity to track biochemical changes that culminate in disease pathogenesis. However, due to the rare prevalence of ADAD, accessibility to an adequate sample size for pilot studies remains a challenge. The encouraging findings from the current pilot study therefore warrant the evaluation and validation of the aforementioned preliminary findings to the greater DIAN cohort with a larger range of EYO and mutations.

Exploring biomolecular alterations in ADAD individuals will not only provide insight into the mechanism of ADAD pathogenesis, but also allows the comparison of previous findings reported in sporadic AD with ADAD, thus supporting that ADAD serves as a suitable model for sporadic AD which can aid to identify potential biomarkers for the early diagnosis of sporadic AD pathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Rationale for hypothesis of phospholipid alterations in ADAD. The flowchart represents various interconnected mechanisms that could potentially contribute to aberrant lipid metabolism in ADAD [2, 14–19].

Table 1List of *PSEN1* mutations present in study participants

<i>PSEN1</i> mutations
PS1His163Arg
PS1Ser290Cys
PS1Leu271Val
PS1Ala079Val
PS1Leu219Pro
PS1Ser170Phe
PS1Met146Ile
PS1 exon9 deletion
PS1Met233Thr
PS1Gln222His
PS1Ser290Cys-exon9deletion
PS1Ile439Val/Ala713Thr

Table 2

Comparison of participant demographic characteristics, APOE genotype and mini mental state examination scores

	NC	MC	Asym MC	Sym MC	p-value
Number F/M	5/1	13/7	8/4	5/3	0.681
Age (y) (Mean $\pm$ SD)	34.67 $\pm$ 14.36	37.85 $\pm$ 9.70	33.75 $\pm$ 6.53 [#]	44 $\pm$ 10.78	0.085
EYO (Mean $\pm$ SD)	14 $\pm$ 12.31	8.95 $\pm$ 8.81	13.17 $\pm$ 7.61 [#]	2.63 $\pm$ 6.61 [*]	0.024
APOE $\epsilon 4$ carrier %	0	25	16	37	0.202
MMSE (Mean $\pm$ SD)	28.80 $\pm$ 1.64	24.90 $\pm$ 5.34	27.75 $\pm$ 1.71 ^{###}	20.62 $\pm$ 6.18 ^{**}	0.001

Mutation carriers (MC) were classified as asymptomatic (Asym) and symptomatic (Sym) based on Clinical Dementia Rating scores. Statistical analysis of age, EYO, and MMSE between NC, Asym MC, and Sym MC were carried out using general linear models using Tukey's *post hoc* test while gender (F, female; M, male) and APOE $\epsilon 4$ % were carried out using χ^2 tests.

^{*,#} $p < 0.05$,

^{**,##} $p < 0.01$, and

^{***,###} $p < 0.001$.

^{*} represents comparison between MC and NC groups,

while [#] represents comparison within MC groups. APOE, apolipoprotein E; EYO, expected years to symptom onset; MMSE, Mini-Mental State Exam (range 0–30, with 30 a perfect score).

Table 3

Significantly different plasma lipid species between mutation carriers and non carriers

Lipid species	NC (mean $\pm$ SD)	Asym MC (mean $\pm$ SD)	Sym MC (mean $\pm$ SD)	Com. p
PCLyso18:0	4.23e-3 $\pm$ 1.95e-3	5.39e-3 $\pm$ 9.31e-4	5.95e-3 $\pm$ 1.47e-3 *	0.0808
PCLyso18:1	3.37e-3 $\pm$ 1.68e-3	4.52e-3 $\pm$ 7.41e-4 *	4.49e-3 $\pm$ 8.23e-4	0.0829
PCLyso18e:0	2.34e-4 $\pm$ 9.83e-5	2.90e-4 $\pm$ 6.46e-5	3.76e-4 $\pm$ 5.83e-5 *	0.0596
PC34:6	2.30e-4 $\pm$ 2.98e-5	1.84e-4 $\pm$ 4.72e-5 **	2.03e-4 $\pm$ 3.75e-5 **	0.0075
PC36:5	1.73e-2 $\pm$ 10.2e-3	1.22e-2 $\pm$ 4.02e-3 **	1.59e-2 $\pm$ 3.17e-3 **	0.0111
PC38:1	7.84e-4 $\pm$ 1.49e-4	7.72e-4 $\pm$ 1.18e-4 *	8.20e-4 $\pm$ 9.18e-5 **##	0.0047
PC38:5	2.90e-2 $\pm$ 7.53e-3	2.55e-2 $\pm$ 3.61e-3 *	2.90e-2 $\pm$ 4.38e-3 *	0.0426
PC40:5	7.46e-3 $\pm$ 1.66e-3	5.61e-3 $\pm$ 1.14e-3 *	7.20e-3 $\pm$ 1.87e-3	0.0391
PC40:6	1.59e-2 $\pm$ 7.39e-3	1.12e-2 $\pm$ 2.84e-3 *	1.59e-2 $\pm$ 4.39e-3	0.0754
PE34p:2	7.30e-5 $\pm$ 3.18e-5	1.02e-4 $\pm$ 3.06e-5 *	7.15e-5 $\pm$ 1.68e-5	0.0655
PE36p:1	7.83e-5 $\pm$ 3.59e-5	1.05e-4 $\pm$ 2.87e-5 *	8.19e-5 $\pm$ 1.69e-5	0.0845
PE36p:4	3.82e-4 $\pm$ 2.46e-4	5.24e-4 $\pm$ 2.13e-4 **	3.94e-4 $\pm$ 1.56e-4 *	0.0143
PE38:7	3.05e-5 $\pm$ 7.32e-6	4.64e-5 $\pm$ 1.18e-5 ***	4.05e-5 $\pm$ 1.61e-5 **	0.0005
PE40:3	1.00e-5 $\pm$ 4.89e-6	1.44e-5 $\pm$ 4.61e-6 *	1.47e-5 $\pm$ 6.37e-6 *	0.0339
PE40p:4	8.25e-5 $\pm$ 3.22e-5	9.34e-5 $\pm$ 2.11e-5	8.95e-5 $\pm$ 2.58e-5 *	0.0875
PE40p:5	1.09e-4 $\pm$ 4.21e-5	1.41e-4 $\pm$ 2.99e-5 *	1.25e-4 $\pm$ 3.10e-5 *	0.0439
PE40p:6	2.39e-4 $\pm$ 7.04e-5	3.14e-4 $\pm$ 8.35e-5	3.00e-4 $\pm$ 8.13e-5 *	0.0810
PE40p:7	1.34e-4 $\pm$ 3.99e-5	1.76e-4 $\pm$ 4.89e-5	1.96e-4 $\pm$ 8.43e-5 *	0.0360
PE42:2	1.09e-5 $\pm$ 4.37e-6	1.18e-5 $\pm$ 3.62e-6	1.51e-5 $\pm$ 6.13e-6 *	0.0690
PE42:4	7.94e-6 $\pm$ 2.31e-6	1.06e-5 $\pm$ 3.88e-6 *	1.19e-5 $\pm$ 4.81e-6	0.0564
PE42p:6	3.60e-5 $\pm$ 1.11e-5	4.63e-5 $\pm$ 1.32e-5 *	4.71e-5 $\pm$ 1.02e-5 *	0.0393
PE44:11	6.84e-6 $\pm$ 3.55e-6	9.54e-6 $\pm$ 3.49e-6 **	9.24e-6 $\pm$ 4.09e-6 ###	0.0007
PI38:6	5.14e-6 $\pm$ 9.39e-7	3.66e-6 $\pm$ 1.39e-6 **	6.84e-6 $\pm$ 4.39e-6	0.0110
Cer.d18:1/20:0	1.38e-5 $\pm$ 1.73e-6	1.95e-5 $\pm$ 6.35e-6 *	1.99e-5 $\pm$ 8.97e-6 *	0.0220
Cer.d18:1/22:0	1.19e-4 $\pm$ 3.94e-5	1.64e-4 $\pm$ 4.66e-5 *	1.48e-4 $\pm$ 5.45e-5	0.0959
Cer.d18:1/24:0	4.01e-4 $\pm$ 1.39e-4	5.52e-4 $\pm$ 1.85e-4	4.92e-4 $\pm$ 1.90e-4 *	0.0828
SM18/20:1	2.13e-3 $\pm$ 5.80e-4	2.56e-3 $\pm$ 6.20e-4	2.77e-3 $\pm$ 7.71e-4 *	0.0926

Plasma lipid species were measured using liquid chromatography coupled with mass spectrometry. The mutation carriers (MC) comprise both symptomatic (Sym, $n = 8$) and asymptomatic (Asym, $n = 12$) participants. Statistical analysis was carried out using linear mixed models with measurements grouped according to family identification to accommodate within-family correlations. The combined p -value (Com. p) for the three groups, namely non carriers (NC, $n = 6$), Sym MC, and Asym MC have been adjusted for age (or EYO, expected years to symptom onset), gender, *APOE* allele status, and DIAN site. All lipid species with a Com. $p < 0.1$ have been listed in the Table. The lipid concentrations are in $\mu\text{g/ml}$. Phospholipid species with 'p' represent plasmalogens, with 'e' represent plasmenyl and without 'p' or 'e' represent phosphatidyl linkages within this table. Boxplots are shown in Supplementary Figure 1, for the lipid species listed in this table.

*, #
 $p < 0.05$,

^{**}, ^{##} $p < 0.01$, and

^{***}, ^{###} $p < 0.001$.

* represents comparison between MC and NC groups,
while # represents comparison within MC groups.