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Evidence of altered phosphatidylcholine metabolism in Alzheimer's disease

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ABSTRACT

Abberant lipid metabolism is implicated in Alzheimer's disease (AD) pathophysiology, but the connections between AD and lipid metabolic pathways are not fully understood. To investigate plasma lipids in AD, a multiplatform screen (n = 35 by liquid chromatography–mass spectrometry and n = 35 by nuclear magnetic resonance) was developed, which enabled the comprehensive analysis of plasma from 3 groups (individuals with AD, individuals with mild cognitive impairment (MCI), and age-matched controls). This screen identified 3 phosphatidylcholine (PC) molecules that were significantly diminished in AD cases. In a subsequent validation study (n = 141), PC variation in a bigger sample set was investigated, and the same 3 PCs were found to be significantly lower in AD patients: PC 16:0/20:5 ($p < 0.001$), 16:0/22:6 ($p < 0.05$), and 18:0/22:6 ($p < 0.01$). A receiver operating characteristic (ROC) analysis of the PCs, combined with apolipoprotein E (ApoE) data, produced an area under the curve predictive value of 0.828. Confirmatory investigations into the background biochemistry indiciated no significant change in plasma levels of 3 additional PCs of similar structure, total choline containing compounds or total plasma omega fatty acids, adding to the evidence that specific PCs play a role in AD pathology.

1. Introduction

It is estimated that there are 24 million persons worldwide with Alzheimer's disease (AD), a figure that is expected to double every 20 years until at least 2040 (Mayeux and Stern, 2012). The size of the affected population and the nature of the disease poses a huge challenge to patient health and care organizations around the world. However, the full physiological mechanism of AD is yet to be fully elucidated, and there is thus a need to identify both disease-relevant pathways for targeted treatment as well as molecular markers to aid in clinical diagnosis and monitoring disease progression (Jack et al., 2011).

Previous research has indicated that lipid molecules play a role in AD, and these have frequently been reported at abnormal concentrations in AD tissue (Bradley et al., 2012; Mangialasche et al., 2012; Wang et al., 2012). Many of these prior studies were conducted using a targeted experimental design, in which known pathways of disease were investigated in a focused manner, based on previous hypotheses of disease pathogenesis. Such targeted approaches have provided evidence suggesting a link between AD and high-density lipoproteins (HDLs) and related proteins in plasma (Di Paolo and Kim, 2011; Han et al., 2011; Lovestone et al., 1996; Orešič et al., 2011; Thambisetty et al., 2010; Whiley and Legido-Quigley, 2011). In addition, the lipoproteins apolioprotein E (ApoE) and apolipoprotein J (ApoJ) have been linked to AD via both genetic and proteomic studies

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(Shi et al., 2012; Thambisetty et al., 2010). ApoJ has been found as a component of HDL and is thought to be a chaperone of amyloid protein, a protein known to be heavily involved in the pathology of AD (Hye et al., 2006; Thambisetty et al., 2010).

An alternative to such targeted discovery is the use of non-targeted small-molecule analysis, commonly termed metabolomics. In contrast to targeted studies, metabolomics attempts to analyze an expansive range of lipids and small metabolites. In metabolomics studies, small-molecule (size <1000–1500 Da) fingerprints are collected, and subsequent data mining can provide unexpected leads into the biochemistry of the disease. The range of molecules studied depends on the analytical platform used and on the applied methodology, with certain combinations of techniques achieving higher specificities (Martin et al., 2007; Whiley et al., 2012).

Non-targeted analysis of AD has previously been reported (Greenberg et al., 2009; Han et al., 2011; Orešič et al., 2011). The majority of these previous studies focus on plasma samples, analyzed either by liquid chromatography–mass spectrometry (LC-MS) (Greenberg et al., 2009; Orešič et al., 2011) or direct infusion mass spectrometry (DIMS) (Han et al., 2011). Interestingly, the significant molecular features identified in these previous works were lipid molecules. One of these studies reported an increase in ceramide (CM16) levels and a decrease in sphingolipid (SP16) levels in the plasma of AD patients (Han et al., 2011). Another investigation identified a phosphatidylcholine (PC), PC 16:0/16:0, as 1 of a cluster of 3 metabolites thought to be predictive markers of AD development in individuals with mild cognitive impairment (MCI) (Orešič et al., 2011). The third example reported a number of bile acids (GCA, GDCA, and GCDCA) that increase in MCI and AD plasma (Greenberg et al., 2009). The latter publication went on to recommend further investigation into the lipid fraction of the AD metabolome.

With these previous results in mind, we developed a non-targeted lipidomics to investigate plasma lipid species in AD. In the study described here, an initial metabolite screen involving LC-MS and nuclear magnetic resonance (NMR) profiling was performed, and the resultant data were analyzed using multivariate statistical modeling. The results of this "screen" phase indicated that 3 lipid phosphatidylcholine molecules (PC16:0/20:5, PC16:0/22:6, and 18:0/22:6) significantly decrease in AD plasma compared to controls. We then performed a multiplatform "validation", designed to both confirm the findings, as well as provide further biological reasoning regarding the changes observed. Fig. 1 illustrates the overall study design and the individual analytical stages incorporated into each phase (screen and validation).

2. Methods

2.1. Sample cohorts

Plasma for the 2 experimental phases (screen and validation) was collected from 2 clinical cohorts, the AddNeuroMed cohort and the King's College London Dementia Case Register (DCR). Ethical approval was awarded for all cohorts in the corresponding centers of collection. The cohorts are described in full in the Supplementary Information section and are summarized in Supplementary Table S1. Further details regarding sample collection and the AddNueroMed and DCR cohorts can be found elsewhere (Hye et al., 2006; Lovestone et al., 2009; Simmons et al., 2009; Thambisetty et al., 2010).

2.2. Procedures

The study was divided into 2 major sections, designated throughout as "screen" (the initial screening phase) and "validation" (the subsequent confirmatory phase).

2.2.1. Screen phase

The screen phase of the study used 2 analytical platforms (LC-MS and NMR) to perform non-targeted analysis. LC-MS analysis was completed in a cohort of 35 age- and sex-matched human plasma samples (10 AD, 10 MCI, 15 control) (Supplementary Table S1). A complete method description is provided in the Supplementary Information section (under the heading "Screen LC-MS").

A separate set of 35 samples (13 AD, 12 MCI, 10 control) (Supplementary Table S1) was analyzed by ^{1}H-NMR spectroscopy at 600.29 MHz using a Bruker Advance 600 spectrometer (Bruker Biospin, Coventry, UK). Standard 1D, Carr-Purcell-Meiboom-Gill spin-echo (CPMG), and J-resolved (JRES) spectra were acquired for metabolomics and metabolite measurements. Further details regarding sample preparation and analysis are described in the Supplementary Information.

2.2.2. Validation phase

Validation was completed in 3 parts. First, a comprehensive LC-MS lipidomics method was applied to a larger sample set to confirm the initial PC observations from the screen phase; then,

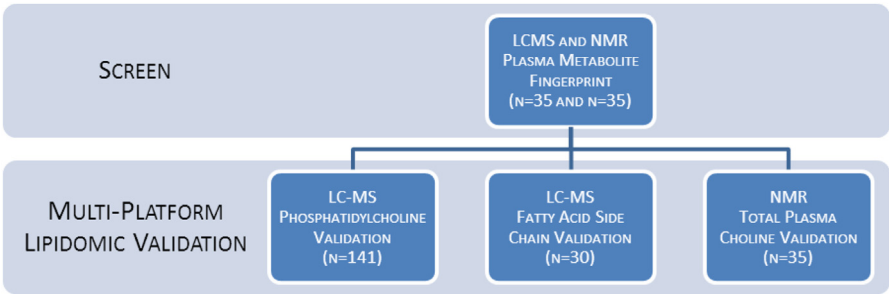


Fig. 1. Experimental pipeline overview. Initially a typical liquid chromatography–mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) metabolite screen led to the identification of 3 phosphatidylcholine (PC) molecules that significantly decrease in individuals with Alzheimer's disease (AD) compared to controls. This led to a comprehensive multiplatform lipidomic analysis consisting of 3 major components. During NMR analysis of patient plasma, particular attention was focused on total choline-containing molecules, a vital component of the phosphatidylcholine molecules identified in the screen experimental section. LC-MS analysis of plasma fatty acids (arachidonic acid, docosahexaenoic acid, and eicosapentaenoic acid) was also conducted. Again, as with NMR analysis of choline species, these fatty acid species are components of phosphatidylcholine structures indentified in the screen experimental section. Finally, comprehensive lipidomic validation was conducted with a large sample cohort. Here a specially developed lipidomic LC-MS method was applied to increased sample numbers. The method is able to detect >3000 lipid markers from a single plasma extraction.

total fatty acid analysis estimation by LC-MS was performed, and finally, total choline-containing compound analysis by NMR was completed to provide some background information as to the source of the PC reduction.

2.2.2.1. Phosphatidylcholine analysis by LC-MS lipidomics. LC-MS lipidomic phosphatidylcholine analysis was completed in 141 age and sex-matched samples (42 AD, 50 MCI, 49 control) (Supplementary Table S1). Sample preparation, reagents and analytical conditions used were as described in a previous article and are summarized in Supplementary Information (Whiley et al., 2012). The samples were analyzed using the LC-MS instrument conditions described in the LC-MS screen section of the Supplementary Information.

2.2.2.2. Total plasma fatty acid analysis. A total of 30 plasma samples were analyzed for major ω-fatty acids. Analysis of total AA, DHA, and EPA was completed using a validated extraction and LC-MS method (Salm et al., 2011). Adjustments to the method are elaborated upon in the Supplementary Information.

2.2.2.3. NMR investigation of total choline. The analyses performed in screen NMR (n = 35) were later used to measure total choline-containing metabolites. The peaks at 3.224 ppm corresponded to choline and phosphocholine, whereas that at 3.238 ppm corresponded to glycerphosphocholine. Peaks were normalized via ratio to creatinine (Carrola et al., 2011; Duarte et al., 2009).

2.3. Statistical analysis

2.3.1. Screen analysis

Following screen analysis, LC-MS raw data were aligned and normalized using Waters MarkerLynx software (Waters Corporation, Milford, MA). NMR data underwent phasing and baseline correction using XwinNMR 3.5 (Bruker Analytik, Rheinstetten, Germany) and were imported into Matlab 7 (Mathworks, Natwick, MA) for normalization.

The final data were transferred to SIMCA-P+ 12.0 (UMetrics, Umeå, Sweden), where it was subjected to multivariate data modeling, including pareto-scaled PCA and orthogonal partial least-squares discriminate analysis (OPLS-DA) with corresponding S-Plot analysis. Mann–Whitney *U* tests were then performed in SPSS (SPSS Inc, Chicago, IL) to calculate significance.

2.3.2. Validation

LC-MS data were analysed using Waters QuanLynx software (Waters Corporation, Milford, MA). Peak ratios were calculated alongside the internal standard. FA validation data underwent Mann–Whitney *U* tests for significance. PC data underwent logarithmic transformation, and were tested for normal distribution using the Shapiro–Wilks test. After this, significance values were calculated using the Student *t* test. Total plasma choline NMR data also underwent a Mann–Whitney *U* test for significance.

Logistic regression ROC was used to investigate the association of each of the 3 PCs with disease status after adjusting for the number of ApoE e4 alleles, as well as correlating with age and sex. APOE allelic status was determined following a standard procedure. Normalized peak areas for the 3 PCs were log transformed before logistic regression analysis. Different models using different predictor combinations were tested, and the best predictor combination was selected by step-wise regression using the Akaike information criterion. ROC curves with area under the curve (AUC) statistic and 95% confidence intervals (CIs) for each model were recorded. Analyses were performed in STATA 10 (StataCorp 2007. Stata Statistical Software: Release 10; College Station, TX).

3. Results

3.1. Screen results

The initial phase of the study was divided into 2 experiments, incorporating both LC-MS and NMR technologies. LC-MS and NMR analysis was performed on 2 separate sample sets each consisting of 35 age- and sex-matched samples (Supplementary Table S1). After this, multivariate data modeling was completed, including principal component analysis (PCA) and OPLS-DA. Models created from the NMR data revealed no significant metabolite changes attributable to disease type.

LC-MS modeling (Fig. 2B) demonstrated AD patient group isolation from control and MCI. From the OPLS-DA, the corresponding correlation power S-Plot (Fig. 2C) was used to generate a list of features of interest that were of importance to the discriminatory model.

Statistical data analysis was then performed on the features selected from the S-Plot model (Fig. 2C), to investigate their behavior in more detail. Of these, 3 features with an MS mass to charge ratio of 780.5538 ($p < 0.001$), 806.5694 ($p < 0.05$), and 834.6007 ($p < 0.005$) displayed an overall reducing trend in the direction control > MCI > AD (Supplementary Table S2). The 3 molecules were identified using a combination of accurate mass database matching and MS/MS fragmentation patterns (Fig. S1). (Rainville et al., 2007; Zhao et al., 2011). Final identification revealed that these were PC16:0/20:5 ($C_{44}H_{78}NO_8P$, 780.5538Da) PC16:0/22:6 ($C_{46}H_{80}NO_8P$, 806.5694Da), and PC18:0/22:6 ($C_{48}H_{84}NO_8P$, 834.6007Da).

3.2. Validation results

The validation phase was split into 3 analytical sections (Fig. 1). The first of these used an LC-MS lipidomics platform to validate the PC alterations observed in the screen results. After this confirmation, analysis was completed to provide insight into the behavior of molecules metabolically relevant to PC alterations. This was achieved by investigating individual total ω-fatty acids (PC side chains) via LC-MS, along with an investigation into total choline-containing molecules (PC head group) using NMR.

3.2.1. Lipidomics results

Plasma samples (n = 141) from a separate larger cohort underwent LC-MS lipidomic analysis. Samples were age and sex matched across the 3 subject groups (Supplementary Table S1). The method used has previously been published (Whiley et al., 2012), and has been shown to measure amounts of >4500 metabolite species, particularly lipids. Supplementary Table S3 lists the PC species analyzed and the observed results. Three PCs demonstrated significant reductions in AD compared with controls (16:0/20:5 ($p < 0.001$), 16:0/22:6 ($p < 0.05$), and 18:0/22:6 ($p < 0.005$)). These data are presented in Fig. 3A. To demonstrate the specificity of the findings for these particular PCs, 3 further PC species PC (16:0/20:4; $p = 0.226$), PC (18:1/22:6; $p = 0.188$), and PC (18:0/20:4; $p = 0.075$) were analyzed. These were chosen based on similarities in side chain arrangement, molecular size, and LC-MS retention time to the 3 PCs of significance. On statistical analysis, no significant changes were observed between groups for this chosen set, suggesting that the discriminatory result was specific to the 3 PCs of significance.

The 3 PC species that exhibited a significant decrease in AD compared with controls then underwent a receiver operating characteristic (ROC) analysis (Fig. 3B). Subject ApoE gene data was fitted to the analysis providing a standard comparison area under the curve (AUC) of 0.667. The individual mass peak area ratios

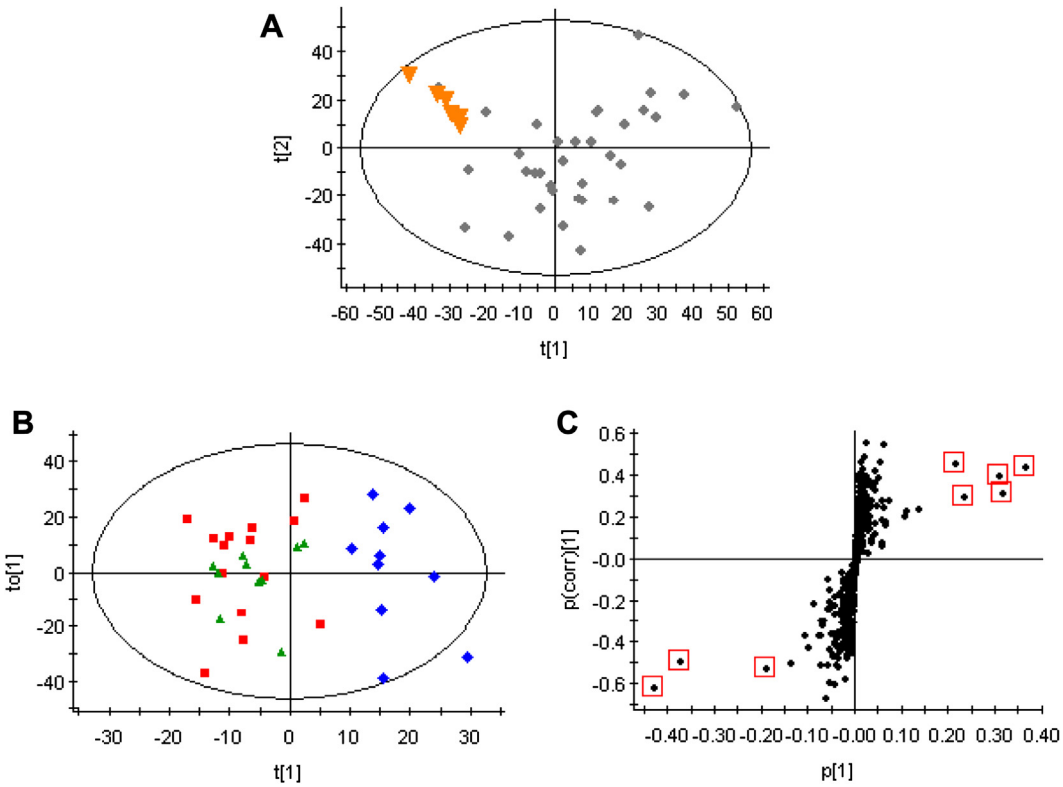


Fig. 2. Results of the multivariate data analysis completed with SIMCA-P+ 12.0 software, (Umetrics, Umeå, Sweden). (A) Unsupervised principal components analysis (PCA) with 41 samples (15 from controls, 10 from individuals with mild cognitive impairment [MCI], and 10 from individuals with Alzheimer's disease [AD]). Orange inverted triangle signifies a repeat extraction of a plasma pool used as a quality control (QC) (n = 6). The QCs cluster in same area of the PCA, suggesting across-run reproducibility. (B) Supervised orthogonal partial least-squares discriminate analysis (OPLS-DA) of all 3 groups (n = 35). Red square and green triangles relate to control (n = 15) and MCI (n = 10), individuals, respectively. Blue diamonds relate to individuals with AD (n = 10). A clear intergroup separation is achieved when observing AD samples. (C) S-Plot corresponding to the OPLS-DA in B. Highlighted in red boxes are masses with the $p[1]$ value of >0.15, which underwent further investigation. The 3 features in the bottom left of the S-Plot correspond to the 3 phosphatidylcholine species shown to significantly decrease in AD during the screen experimental phase.

underwent analysis alongside ApoE data, with an improvement observed for all masses. The most promising was ApoE + Mass780 with an AUC of 0.782. ApoE combined with Mass806 and ApoE combined with Mass834 provided an AUC of 0.746 and 0.756, respectively. Combining all masses alongside ApoE provided the best predictive properties in the ROC analysis, with an AUC of 0.828.

3.2.2. NMR of plasma and total amounts of choline-containing lipids

NMR data were applied to investigate the levels of total choline (for example, phosphocholine and glycerophoshphocholine-containing compounds) in plasma (refer to Fig. 5 for the participation of these compound classes in choline homeostasis). This was completed to see if an imbalance in PC species was linked to an overall imbalance of choline metabolism. As highlighted in Supplementary Fig. S2, these metabolite groups showed no significant inter–disease group variation.

3.2.3. Plasma ω–fatty acid profiling

To investigate whether the observations in individual PC species were linked to fatty acid side chains, total plasma ω-fatty acid profiles for arachidonic acid (20:4n6, AA), docosahexaenoic acid (22:6n3, DHA), and eicosapentaenoic acid (20:5n3, EPA) were obtained using a previously validated method (Salm et al., 2011). Analysis was performed on a group of 30 patients (10 per group; control, AD, and MCI subjects) that was age and gender matched across all 3 groups. Inter–disease group variation was non-significant for AA and DHA; however a moderate decrease was seen for EPA ($p = 0.023$) when comparing Control subjects versus subjects with AD.

4. Discussion

Here we report a significant reduction of 3 PC species in patients with AD compared with controls, which have not previously been linked to AD (PC16:0/20:5 ($p < 0.001$), PC16:0/22:6 ($p < 0.05$), and PC18:0/22:6 $p < 0.005$). The reduction was observed in 2 different sample sets, using 2 different LC-MS methods (in the screen and validation phases). In the validation result, the overall trend (i.e., control>MCI>AD) suggested a specific PC decline linked to cognition. Three PC species (PC16:0/20:4, PC18:1/22:6, and PC18:0/20:4) selected based on similar side chain composition, size, and LC retention time and chosen as negative controls were also quantified; however, these were found not to differ across the disease classes.

4.1. Predictive properties of phosphatidylcholine in AD

To provide an estimate of their clinical relevance, an ROC analysis was performed on the 3 PCs, to test their predictive properties. One of the accepted genetic risk factor markers of AD is that of the presence of 1 of the 2 ApoE ε_4 gene alleles (Ashford, 2004), and thus patient ApoE data was included in the ROC analysis as a comparison tool. In the case of the LC-MS validation sample set (n = 141), ApoE data alone gave an area under the curve of 0.667. In comparison, the 3 PCs combined without ApoE data returned an area under the curve of 0.786. When combined with the 3 lipid peak area ratio findings, the area rose to 0.828, suggesting a predictive potential of more than 80%. 80% is an important benchmark, as it is currently regarded as a minimum requirement for AD biomarker molecules (Biagioni and Galvin, 2011; The Ronald and Nancy Reagan Research Institute of the Alzheimer's

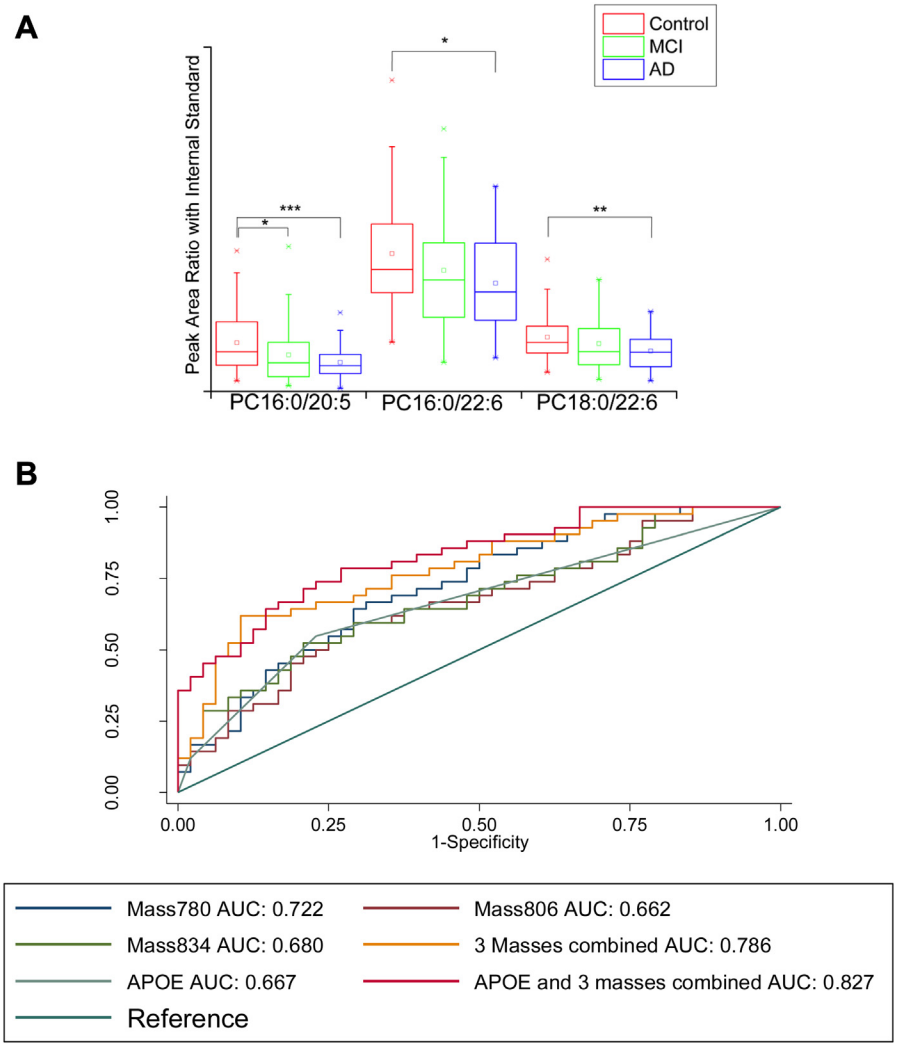


Fig. 3. An overview of the plasma phosphatidylcholine (PC) analysis and resultant predictive properties. (A) Box plots for the 3 significant PC species identified in the comprehensive lipidomic analysis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (B) Receiver operating characteristic (ROC) analysis results of the 3 peak area ratios for the 3 PC masses of interest (n = 141). Mass 780 (PC16:0/20:5) area under the curve (AUC) of 0.722, mass 806 (PC16:0/22:6) AUC of 0.662, and mass 834 (PC18:0/22:6) AUC of 0.680. Combining the 3 PC features into an ROC analysis returned an AUC of 0.788. Inclusion of the ApoE genotyping data within disease classes provided an AUC of 0.828. As a comparison, apolipoprotein E (ApoE) data used on its own yielded an AUC of 0.667.

Association, National Institute of Aging, 1998), thus suggesting an early potential for the identified PCs as predictive AD markers.

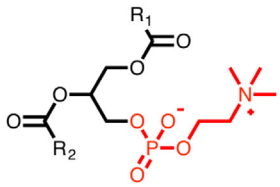
4.2. Phosphatidylcholine species decrease in AD plasma

PCs are a class of 1,2-diacylglycerophospholipids that are an essential component of cell membranes and that make up approximately 95% of the total choline compound pool in most tissues (Frisardi et al., 2011; Schaeffer et al., 2010). They have a common zwitterionic structure, with hydrophobic chains that vary in length and degree of saturation (Fig. 4), and have structural roles defined primarily by chain length (as chain length differences can affect cell membrane fluidity (Perttu et al., 2012)). Along with their structural roles, they are also found as components of lipoproteins, in particular, HDL. PCs interact with ApoE as part of the HDL group, and are implicated in cholesterol transport (Cramer et al., 2012). This is of interest, as ApoE is unambiguously associated with AD (Luft, 1997; Pericak-Vance et al., 1991), with the ApoEε_4 allele being the most significant susceptibility genetic factor (Ashford, 2004).

The enzymes that catalyze the breakdown of PC (Fig. 5) to phosphatidate (the phospholipase D or PLD enzymes) or to glycerophosphocholine and free fatty acids (phospholipase A2 or PLA_2 enzymes) have been directly associated with AD (Dennis et al., 2011; Li and Vance, 2008; Selvy et al., 2011). In addition, alterations in the reaction cascades of PLD enzymes, leading to aberrant phosphatidic acid (PA) signaling, have been linked to neurodegenerative processes, with activation of PLA_2-family enzymes by amyloid beta peptide in neurons, in turn releasing secondary lipid messengers such as AA (Sanchez-Mejia and Mucke, 2010).

PLA_2s also have a role in modification of the physical properties (such as fluidity) of cellular membranes; because amyloid precursor protein (APP) is a transmembrane protein, membrane fluidity may be of great importance to platelet formation in AD (Lee et al., 2011). Moreover, several previous works (Colangelo et al., 2002; Gattaz et al., 1995, 1996; Ross et al., 1998; Talbot et al., 2000) have shown that PLA_2 activity and expression in the AD brain and central nervous system seem to be correlated with the progression of AD. As such, there is strong consensus on the importance of lipid and glycerophospholipid pathways in AD pathology (Di Paolo and Kim, 2011).

As PC is a precursor for sphingomyelin (*via* the sphingomyelin synthases), our results are in agreement with the observed increase



PHOSPHATIDYLCHOLINE (PC)

PC	R_1	Source fatty acids	R_2	Source fatty acids
16:0/20:5	$-(CH_2)_{14}CH_3$	$CH_3(CH_2)_{14}COOH$ (palmitic)	$-(CH_2)_8(CH{=}CH)_5CH_3$	$CH_3(CH_2)_8(CH{=}CH)_5COOH$ (eicosapentanenoic)[a]
16:0/22:6	$-(CH_2)_{14}CH_3$	$CH_3(CH_2)_{14}COOH$ (palmitic)	$-(CH_2)_8(CH{=}CH)_6CH_3$	$CH_3(CH_2)_8(CH{=}CH)_6COOH$ (docosahexenoic)[b]
18:0/22:6	$-(CH_2)_{16}CH_3$	$CH_3(CH_2)_{16}COOH$ (stearic)	$-(CH_2)_8(CH{=}CH)_6CH_3$	$CH_3(CH_2)_8(CH{=}CH)_6COOH$ (docosahexaenoic)[b]

[a]IUPAC nomenclature: (5Z,8Z,11Z,14Z,17Z)-5,8,11,14,17-icosapentaenoic acid
[b](4Z, 7Z, 10Z, 13Z, 16Z, 19Z)-docosa-4,7,10,13,16,19-hexaenoic acid

Fig. 4. Phosphatidylcholine (PC) molecules reduced in plasma from individuals with Alzheimer's disease (AD) plasma versus control. This overview presents background information on the identified PC species, including their source side chains.

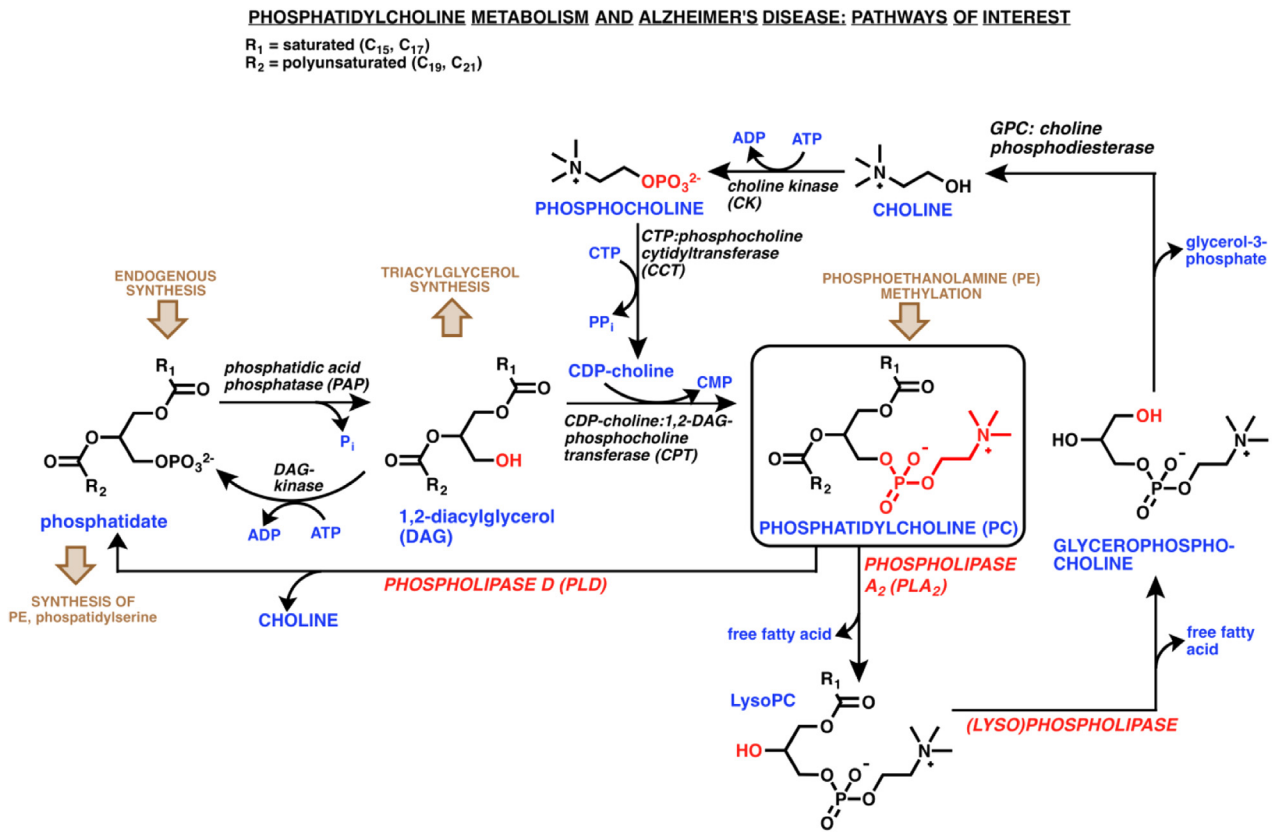


Fig. 5. Phosphatidylcholine (PC) metabolism and Alzheimer's disease (AD). This overview illustrates the major routes to PC biosynthesis, with an emphasis on steps involving choline-containing compounds and those that have been previously associated with the pathology of AD. Phosphatidate (phosphatidic acid [PA]) is synthesized endogenously from glycerol-3-phosphate in most tissues, and is a key signaling molecule and a precursor for other 1,2-diacylglycerophospholipids. PA is converted to 1,2-diacylglycerol (DAG), which is then fed into the CDP-choline Kennedy pathway to form PC (PC can also be formed in the liver from phosphatidylethanolamine [PE] in a multi-step pathway catalyzed by PE-methyl transferase). PC is subject to the action of a variety of phospholipases, 2 main families of which are the phospholipase D (PLD) and phospholipase A2 (PLA2): PLD enzymes hydrolyze PC to yield free choline (Ch) and PA, whereas PLA2 enzymes cleave acyl groups from the sn-2 position of PC to yield lysoPC.

in these molecules. Furthermore, Gaudin et al. found that 2 PCs (32:0 and 34:1) were decreased in senile plaques micro-extracted from the post-mortem AD brain. The group concluded that PC regulation was affected in AD, and that it could be linked to the roles of PLA_2 and PLD_1 in Aβ activation (Gaudin et al., 2012). Given the possibility that changes in PLA_2 activity may be causing the observed decrease in PCs, and the previous work relating DHA concentrations to AD, we also measured the total (combined both circulating free + bound to larger lipids) concentrations of AA (non-significant, $p > 0.05$), DHA (non-significant, $p > 0.05$), and EPA ($p < 0.05$) in AD plasma.

Although our results are in keeping with the general trend observed in previous studies (i.e., disruptions in glycerophospholipid pathways appear to be correlated with the prevalence of AD), as illustrated in Fig. 5, choline and phospholipid biochemistry are clearly complex. In addition, it can be seen in Fig. 4 that choline is a core component of PC species; therefore, data were collected to observe whether overall choline levels are influenced by disease state. Amounts of choline-containing compounds have been found to be correlated with AD pathology and membrane turnover during neuronal degeneration in specific regions of the brain (Kantarci et al., 2007). An alternative model hypothesises that that amyloid binding to membrane lipids is responsible for the disruption of membrane structures and frees up choline in the brain (Small et al., 2007). NMR analysis was used to ascertain whether the levels of total choline-containing species (such as PCs and GPCs) were significantly altered between disease classes. Our results indicated that overall plasma choline levels were randomly distributed, and showed no significant trend between groups.

4.3. Phosphatidylcholine 16:0/20:5

Of the 3 molecules reported to decrease in AD, PC16:0/20:5 was of the greatest significance. A PC with similar side chains (PC16:0/16:0) has been reported as part of a cluster analysis that found that 3 major molecules were contributors to progression from MCI to AD. These included the aforementioned PC16:0/16:0, alongside 2,4-dihydroxybutanoic acid and an unidentified molecule (Gonzalez-Dominguez et al., 2012; Orešič et al., 2011). This result takes on further relevance when considered in conjunction with the finding that a phospholipid species with chain C16:0 (the lysoPC form generated by the PLA_2 enzyme) is a precursor of the platelet-activating factor (PAF), which in turn is an important mediator in inflammation-related processes. Perhaps more interestingly, the C16:0 PAF has been shown to be toxic to neurons (Ryan et al., 2009). Han et al., in their metabolomics study, found sphingomyelins and ceramides with chains C22:0, C23:0, and C16:0 to be changed and highly correlated with AD (Han et al., 2011). In particular, chain 16:0 cer/sm ratio showed the highest correlation with AD. Interestingly, the negative control analysis of the similar PC16:0/20:4 resulted in a non-significant change, suggesting that specific PCs with a 16:0 side chain may be implicated in AD pathology.

As discussed earlier, phospholipase dysfunction has been implicated in the pathology of AD, and as illustrated in Fig. 5, altered phospholipase activity for selected PCs could lead to an over-metabolism of these molecules, and thereby a subsequent diminishment in plasma levels. The link among membrane breakdown, amyloid deposits, and neuronal dysfunction has been supported by publications from Dyrke et al. (Dyrks et al., 1988) and Pettigrew et al. (Pettegrew et al., 1988). These previously published results support the finding of abnormal PC levels in individuals with AD disease in our study, and suggest a possible relationship to amyloid deposits associated with the pathology of the disease. Our results would suggest that specific PCs, rather than PCs as a whole, may have a crucial role in AD and future disease research, and that these molecules could form the basis of a simple and targeted biochemical assay for use in clinical settings.

Disclosure statement

The authors declare no conflicts of interest is assotiated with this manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.08.001.

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