HHS Public Access

Author manuscript

Neurobiol Aging. Author manuscript; available in PMC 2021 February 01.

Published in final edited form as:

Neurobiol Aging. 2020 February ; 86: 123–133. doi:10.1016/j.neurobiolaging.2019.10.014.

Brain and blood metabolome for Alzheimer's dementia: Findings from a targeted metabolomics analysis

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Abstract

The development of Alzheimer's dementia (AD) accompanies both central and peripheral metabolic disturbance, but the metabolic basis underlying AD and metabolic markers predictive of AD risk remain to be determined. It is also unclear whether the metabolic changes in peripheral blood and brain are overlapping in relation to AD. The current study addresses these questions by targeted metabolomics in both ante-mortem blood and post-mortem brain samples in two community-based longitudinal cohorts of aging and dementia. We found that higher serum levels of three acylcarnitines, including decanoylcarnitine [C10], pimelylcarnitine [C7-DC], and tetradecadienylcarnitine [C14:2], significantly predicts a lower risk of incident AD (composite HR = 0.368, 95% CI [0.207, 0.653]) after an average of 4.5-year follow-up, independent of age, sex, and education. In addition, baseline serum levels of ten glycerophospholipids, one amino acid, and five acylcarnitines predict the longitudinal change in cognitive functions. Moreover, 28 brain metabolites were associated with AD phenotypes. Of the putative metabolites identified in serum and brain, four metabolites (3 glycerophospholipids [PC aa C30:0, PC ae C34:0, PC ae C36:1] and 1 acylarnitine [C14:2]) were present in both post-mortem brain and ante-mortem blood, but only one metabolite (C14:2) was associated with AD in the same direction (i.e., protective). Partial correlation and network analyses suggest a potential tissue-specific regulation of metabolism, although other alternatives exist. Together, we identified significant associations of both central and peripheral metabolites with AD phenotypes, but there seems to be little overlap between the two tissues.

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Conflicts of interest: The authors have no conflicts of interest to declare.

1. All authors have disclosed that:
(a) All authors do not have potential conflicts of interest, including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence (bias) their work.
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3. The data presented in this manuscript have not been published or are being considered for publication elsewhere in whole or part, in any language, except as an abstract. We will not submit the manuscript to anywhere else while it is being considered by Neurobiology of Aging.
4. All authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data.

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Keywords

Alzheimer's Dementia; targeted metabolomics; cognitive decline; AD neuropathology; postmortem brain metabolomics

Introduction

Metabolic dysfunction, both peripherally and centrally, is a core feature of Alzheimer's dementia (AD) [1]. Metabolomics is an emerging omics technology that simultaneously identifies and quantifies hundreds to thousands of small metabolites in tissues or biofluids [2]. These small molecules represent the end products of gene-environment interactions [3], and hold promise for discovering novel biomarkers and disease mechanisms. Indeed, several epidemiological studies have reported associations of serum or plasma metabolites with AD [4–6], such as sphingolipids [7–9], phosphatidylcholine [7,10], glycerophospholipids [9], amine biomarkers [11], bile acids [12,13], and branched-chain amino acids [14]. A panel of 10-plasma lipids was also proposed to predict incident AD [10], although results were mixed [15–17]. In a recent targeted metabolomics analysis [9] including 207 serum samples and 44 postmortem brain samples, 26 brain metabolites were found to discriminate AD from cognitive normal with high accuracy. Associations of brain region-specific metabolites with AD and cognitive performance have also been reported [18,19]. While these findings support an important relationship between metabolic disturbance and AD, existing results are largely inconsistent, and no conclusive metabolites have been identified so far. Moreover, the incremental value of blood metabolites over conventional clinical factors in AD risk prediction has not been evaluated in previous studies. Further, little research has been conducted to examine the relationship between circulating metabolites and brain metabolites from same individuals. A systematic examination of the relationship between brain and blood metabolites with quantitative cognitive and neuropathological traits for AD is also lacking.

Leveraging the comprehensive clinical and neuropathological phenotypes as well as metabolomic data in both ante-mortem serum and post-mortem brain in two community-based longitudinal cohorts of aging and dementia (the Religious Orders Study and Rush Memory and Aging Project [ROSMAP]) [20,21], our goals here are to: (i) identify blood metabolites predictive of AD risk, independent of known clinical factors; (ii) identify brain metabolites associated with various measures for AD neuropathology; (iii) explore the blood-brain connections by examining the potential overlapping or co-regulation between blood and brain metabolites from same individuals. Findings of this research are likely to identify novel biomarkers for AD and may shed light on the role of metabolic disturbances in AD.

Materials and Methods

Study populations

Participants are community-based older persons who were enrolled in two ongoing longitudinal clinical- pathologic cohort studies of aging and dementia, the ROS (Religious

Orders Study) and MAP (Rush Memory and Aging Project). Detailed study design and methods for both studies (named ROSMAP) had been described previously [22]. Briefly, ROS (1994-ongoing) enrolls older Catholic priests, nuns, and brothers from more than 40 groups across the United States. MAP (1997-ongoing) recruits participants primarily from retirement communities throughout northeastern Illinois. These participants were free of known dementia at the time of enrollment and agreed to annual clinical assessments as well as brain donation after death. All participants signed an informed consent, a resource sharing consent, and an anatomical gift act for organ donation. The two studies were conducted using same protocols for participant recruitments, clinical assessments, and pathological examinations by a same team of investigators, and thus can be combined for data analysis. Both studies were approved by an Institutional Review Board of Rush University Medical Center. Data are available for sharing at www.radc.rush.edu.

Quantitative clinical and neuropathological phenotypes collected in ROSMAP

During the annual clinical visit, each participant receives a comprehensive evaluation of cognition functions via a battery of 21 cognitive performance tests. Seventeen of these tests across a range of cognitive abilities are used to construct a global composite measure of cognitive function, and summarized as 5 cognitive domains including episode memory, working memory, semantic member, perceptual speed, and visuospatial ability [23]. These summary measures have the advantage of minimizing floor and ceiling effects, and other sources of random variability. Information for demographics, lifestyle, sleep, medical condition, disease history, well-being, and medications is also recorded. Plasma and serum samples were collected, aliquoted, and stored at −80°C for future use.

Besides the comprehensive clinical phenotypes, the two studies also collected various quantitative measures for AD neuropathology across multiple brain regions, including quantitative measures of AD pathology by histochemistry across 5 brain regions (e.g., counts of neuritic plaque burden [24], neurofibrillary tangles [25], and global AD pathology burden [25]) and immunohistochemistry across 8 brain regions (e.g., β-amyloid load [26] and PHFtau tangle density [27]), semiquantitative measure of neurofibrillary tangle pathology (e.g., Braak stage [28]), and neuritic plaque (e.g., CERAD score [29]), and pathologic diagnosis of AD assessed based on the NIA-Reagan criteria [30]. Detailed methods for neuropathological examinations and variable constructions were described previously [31–35] and updated recently [22].

In our study, we comprehensively investigated the association between post-mortem brain metabolites with all these AD neuropathological measures, and examined ante-mortem serum metabolites that are predictive of cognitive status/function changes.

Metabolomic data acquisition

Targeted metabolomics data were obtained using the Biocrates AbsoluteIDQ® p180 Kit (Biocrates Innsbruck, Austria). The kit includes 182 metabolites from five compound classes (20 amino acids, 21 biogenic amines, 40 acylcarnitines, 87 glycerophospholipids, and 14

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sphingolipids) as previously described [18]. Briefly, 10 μL serum sample, calibration standard, and QC sample were added to the appropriate wells of the 96-well extraction plate with 10 μL of the supplied internal standard solution. After drying, the sample was derivatized with phenyl isothiocyanate, then eluted with 5 mM ammonium acetate in methanol. Analysis of acylcarnitines, sphingolipids, and glycerophospholipids was performed by flow injection analysis (FIA), and amino acids and biogenic amines were analyzed by ultra-high-pressure liquid chromatography (UPLC) tandem MS method. For the purpose of peak integration, calibration, and concentration calculations, the FIA-MS/MS data and the UPLC-MS/MS data were analyzed via Biocrates MetIDQ™ software and TargetLynx™ software, respectively. A series of calibration concentration with methanol/water was adopted and the internal standard concentration was kept constant.

To quantify brain metabolites, each brain tissue sample (from dorsolateral prefrontal cortex) was diluted with 100 μL 1:1 methanol:water and homogenized. Further dilution was performed using 250 μL 3:1 methanolxhloroform. After centrifugation, the homogenized samples were refrigerated at −80°C. The concentrations of brain metabolites were measured using the same protocols as those used for serum metabolites measurement.

Metabolites data preprocessing and quality control

We obtained raw data for 182 metabolites in each of the 111 post-mortem brain tissue samples and 597 ante-mortem serum samples (from 560 ROSMAP participants, 31 of them were repeatedly measured). Of these 560 participants, we removed 11 subjects with dementia at the time of blood draw (baseline) and 19 subjects with missing follow-up data, leaving a total of 530 participants with complete clinical data. After further removing metabolites with more than 5% missing samples, 154 serum metabolites from 530 participants (433 no cognitive impairment [NCI] and 97 mild cognitive impairment [MCI] at baseline) and 153 brain metabolites from 111 brain donors (51 NCI, 32 MCI, and 28 AD) were included in the final analysis. Of these, 92 individuals had metabolomics data in both brain (153 metabolites) and blood (154 metabolites), of which 143 metabolites are present in both brain and blood samples. Figure 1A–1B summarize the blood and brain samples included in the current analysis. Missing values were replaced by LOD/2, where LOD denotes the lower limit of detection. Normalization was performed using probabilistic quotient normalization as previously described [36], followed by log (base 2) transformation before statistical analysis as described below.

Statistical analyses

We conducted statistical analyses to: (i) identify blood metabolites predictive of incident AD and cognitive changes over time, independent of traditional clinical factors; (ii) identify brain metabolites associated with various AD neuropathological measures; and (iii) explore the blood-brain connections by examining the potential overlapping or co-regulation between blood and brain metabolites from same individuals. Figure 1C depicts our analytical plan.

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Identifying blood metabolites predictive of incident AD and cognitive decline over time

Of the 530 non-dementia participants (433 NCI and 97 MCI) at baseline, 85 developed incident AD during an average of 4.5-year follow-up. To identify blood metabolites predictive of AD onset, we fitted Cox regression models, in which time to AD onset was the dependent variable, and metabolite concentration was the independent variable, adjusting for age at baseline, sex, education, and batch. To evaluate the combined effects of putative serum metabolites in predicting AD risk, we constructed a composite metabolite score using regression coefficients as weights. To examine whether the putative blood metabolites improve risk prediction beyond conventional risk factors (e.g., age, sex, and education), we calculated the integrated discrimination improvement (*IDI*) [37,38] using the R package *survIDINRI* [39]. IDI evaluates the improvement in prediction performance gained by adding a new biomarker to a set of traditional risk factors or predictors by calculating the difference in model-based probabilities for events and nonevents. *P*-value was obtained by 1,000 times permutations.

To identify blood metabolites predictive of cognitive decline over time, we fitted linear mixed models using the R package *lme4* [40]. In the model, the annual score in global cognition or one of the five cognitive domains (episode memory, working memory, semantic memory, perceptual speed, and visuospatial ability) was the dependent variable, and metabolites, visit time, as well as their interaction were the independent variables, adjusting for baseline age, sex, education, cognitive performance, and batch.

Identifying brain metabolites associated with AD phenotypes

To identify brain metabolites associated with AD phenotypes, we fitted linear regression, logistic regression or ordinal logistic regression models for a variety of AD-related traits, adjusting for age at death, sex, education, post mortem interval (PMI), and batch. These analyses included 153 brain metabolites from 111 subjects.

Potential brain-blood connections

Some circulating metabolites and brain metabolites could be connected either through the blood brain barrier or through sharing common mechanisms (e.g., metabolized by same enzymes). To explore the potential connections between brain and blood metabolites, we first plotted heat maps to visualize the potential overlap between putative metabolites identified in both blood and brain (FDR 10%) in relation to various AD-related clinical and neuropathological measures. To examine whether blood and brain metabolites are co-regulated, we calculated partial correlations (adjusting for age, sex, and education) for all 307 metabolites including 154 blood metabolites and 153 brain metabolites (of these, 143 metabolites were present in both blood and brain) assayed in 92 participants with both blood and brain samples. Further, we conducted the weighted gene co-expression network analysis (WGCNA) [41] to identify metabolites that are co-regulated. The co-regulated metabolite modules were visualized via *Cytoscape* [42]. To facilitate interpretation, we calculated the first principal component (PC) for each module and correlated them with cognitive and neuropathological phenotypes.

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Multiple testing correction

In the above-described analyses, multiple testing was corrected by false discovery rate (FDR) using the Benjamini-Hochberg procedure [43]. Given the high correlation between metabolites and the relatively small sample size, we used a less stringent cutoff (FDR 10%) to determine statistical significance, which indicates that among the metabolites we claimed to be significant, 10% of them could be false positives.

Results

Table 1 presents the clinical characteristics of study participants with ante-mortem blood metabolites data (N=530). All participants were free of known dementia at the time of blood draw (baseline). The mean (SD) age at baseline was 82 (7.4) years. Men accounted for 22% of the sample. The average follow-up period was 4.5 years (ranging from 1 to 9 years), during which 85 developed incident AD. Table 2 shows the AD neuropathological characteristics of post-mortem brain donors (N=111). The mean (SD) age of brain donors was 90 (5.8) years at the time of death. Men accounted for 28% of the sample. The mean (SD) post-mortem interval was 8.8 (6.8) hours. Table S1 shows the characteristics of the 92 participants with both brain and blood metabolomics data. The mean (SD) age was 87 (5.8) at baseline and 91 (5.9) at death. Men accounted for 27% of these participants.

Baseline serum metabolites predict risk of AD onset and cognitive decline over time

Of the 154 blood metabolites included in this analysis, higher baseline levels of three acylcarnitines, including decanoylcarnitine [C10] (HR = 0.625, 95% CI [0.468, 0.836]), pimelylcarnitine [C7-DC] (HR = 0.503, 95% CI [0.328, 0.769]), and tetradecadienylcarnitine [C14:2] (HR = 0.632, 95% CI [0.473, 0.844]), significantly predicted a lower risk of incident AD (average 4.5-year follow-up) after adjusting for age, sex, education, and batch (Figure 2A–2C). The combined effects of these three acylcarnitines were associated with over 60% reduction in risk for incident AD (HR = 0.368, 95% CI [0.207, 0.653]; Figure 2D). These three metabolites also significantly improved AD risk prediction beyond conventional clinical factors (IDI=0.03, p-value = 0.004, 95% CI [0.009, 0.084], Table S2). Moreover, higher levels of these three acylcarnitines were associated with a slower cognitive decline over time (Table 3, Table S3).

Besides these three protective acylcarnitines described above, baseline serum levels of another 13 metabolites also predicted longitudinal change in global cognition or sub-domains of cognitive function (episode memory, perceptual speed, or semantic memory, Table 3). Of these, a higher baseline level of serum asparagine was associated with a faster decline in global cognition (β = −0.068, 95% CI [−0.112, −0.024]). In addition, a higher baseline level of serum PC ae C34:0 predicted a faster decline in global cognition and three cognitive domains (Figure 3). At the FDR level of 10%, no serum metabolite was associated with working memory or visuospatial ability, though. The complete information of these serum putative metabolites is in Table S3. Figure S1 summarizes all metabolites predictive of risk for AD onset or cognitive decline.

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Brain metabolites associated with AD phenotypes

Of the 153 brain metabolites analyzed, 28 metabolites from 4 compound classes (acylcamitines, amino acids, biogenic amines, and glycerophospholipids) were either positively or negatively associated with various measures for and AD neuropathology (Figure 4A). Figure 4B shows the number of these putative brain metabolites in each of these four compound classes. The complete information of these putative metabolites is shown in Table S4. Interestingly, two brain metabolites (symmetric dimethylarginine [SDMA] and threonine) were positively associated with almost all AD-related phenotypes, including autopsy-confirmed AD diagnosis, CERAD score, global AD pathology burden, amyloid beta, neuritic plaque. Figure 5 depicts the association of SDMA with various measures of AD neuropathological phenotypes. At FDR 10%, no metabolite was associated with neurofibrillary tangles, PHFtau tangle density, Braak stage. Figure S2 summarizes the associations of all brain metabolites with AD phenotypes.

Brain-blood connections

Figure 6 summarizes the relationship between all putative metabolites identified in blood (16 metabolites) and brain (28 metabolites) in relation to various measures for cognition and AD neuropathology. Of these, 4 metabolites, including one acylcarnitine (tetradecadienylcarnitine [C14:2]) and three glycerophospholipids (PC aa C30:0, PC ae C34:0, PC ae C36:1), showed significant associations with both AD neuropathology and cognitive changes. While tetradecadienylcarnitine was protective in both brain and blood, the three glycerophospholipids showed opposite directions in the two tissues.

Figure S3 shows that the within-tissue correlation was stronger than cross-tissue correlation (i.e., between brain and serum). With respect to the magnitude of correlations, Figure S4 shows a stronger within-brain correlation (median 0.208), followed by within-serum correlation (median 0.161), and cross brain-serum correlation (median 0.073). Cross-tissue co-regulated metabolic network analysis identified 7 distinct metabolic modules (3 in brain and 4 in blood, Figure S5). There was no module containing metabolites from both blood and brain, indicating that metabolites regulation could be tissue-specific. The co-regulated modules are shown in Figure S6. The correlations between these modules (represented by the first PC in each module) with cognitive or neuropathological phenotypes are shown in Figure S7. At raw p 0.05, one module (yellow) containing 30 metabolites from the brain was positively correlated with multiple AD neuropathological measures.

Discussion

In this targeted metabolomics analysis of metabolites in both ante-mortem blood and post-mortem brain, we identified 3 serum acylcarnitines that negatively predict the risk for incident AD and cognitive decline over time, independent of age, sex, and education. Another 13 serum metabolites predict longitudinal changes in cognition but not AD onset. Of the 153 metabolites in post-mortem brain tissue, 28 metabolites were associated with various cognitive and neuropathological measures. Among the 40 putative metabolites identified in blood and brain, only one metabolite (tetradecadienylcarnitine [C 14:2]) was associated with AD-related phenotypes in both tissues with a same direction. Network

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analysis implies tissue-specific metabolic regulation. Collectively, our results support an important role of metabolic disturbance in AD neuropathology and highlight the potential prognostic value of blood metabolites in AD risk prediction. Moreover, there seems to be little overlap between blood and brain metabolites, suggesting potential differences in peripheral and central metabolism in relation to AD pathology.

Of the putative metabolites identified in serum, we found that higher baseline levels of three acylcarnities predict a lower risk for AD onset and cognitive decline. Their combined effects were associated with over 60% decreased risk of AD onset after accounting for age, sex, and education. Acylcarnitines are a large class of metabolites that are the members of non-protein amino acid family. They play essential roles in long-chain fatty acids metabolism by serving as carriers to transport activated long-chain fatty acids into mitochondria for β-oxidation to provide energy for cellular functions [44]. Acylcarnitines are also involved in many other important physiological processes such as the metabolism of branched-chain amino acids [45], insulin resistance [46], cellular stress responses [47], and cholinergic neurotransmission [44]. In line with our findings, previous studies found that AD patients had lower serum levels of several acylcamitines (decanoylcarnitine, tetradecadienylcarnitine, hexenoylcarnitine, and malonylcarnitine) compared to cognitively normal controls [48,49]. Lower plasma concentrations of several acylcamitines, including decanoylcarnitine and tetradecadienylcarnitine, were also found in individuals with schizophrenia compared with healthy controls [50]. Besides acylcamitines, we found that a higher level of serum asparagine predicts a faster cognitive decline during follow-up. Asparagine is a non-essential amino acid that is involved in the metabolic control of neuronal functions [51]. For example, previous studies in mouse found that during aging, asparagine endopeptidase (AEP) is activated to induce tau aggregation and trigger neurodegeneration [52]. Moreover, inhibition of AEP appeared to have therapeutic effects against neurodegenerative diseases [53]. In addition, 10 glycerophospholipids, including 7 diacyl phosphatidylcholines, and 3 acly-alkyl phosphatidylcholines, were also predictive of longitudinal change in cognitive function. While higher levels of all these 7 diacyl phosphatidylcholines were predictive of accelerated cognitive decline, the directions of the 3 acly-alkyl phosphatidylcholines were mixed. Glycerophospholipids are the most common type of phospholipids, which are the major constituents of all cells, and also present in fat storage. The role of glycerophospholipids have been implicated in sensory axon guidance in the spinal cord [54] and neuronal morphology [55]. Consistent with our results, previous studies also reported that higher levels of serum glycerophospholipids predict incident AD and longitudinal cognitive decline [9].

In studies of brain metabolites, we found that higher levels of symmetric dimethylarginine (SDMA) and threonine in brain were associated various measures for AD neuropathology, including autopsy-confirmed cognitive status, CERAD score, amyloid beta, neuritic plaque, and global AD pathology burden. SDMA is an endogenously generated inhibitor of nitric oxide synthase that is involved in inflammation [56] and oxidative stress [57], both of which have been implicated in AD pathogenesis [58]. An elevated serum level of SDMA has previously been associated with cardiovascular disease [59] and kidney disease [60]. Moreover, plasma asymmetric dimethylarginine (ADMA) was found to be increased in patients with schizophrenia and correlated with cognitive impairments [61]. Threonine was

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also positively associated with these AD-related traits. This is in line with the previous finding that an elevated level of threonine was found to affect neurotransmitter balance in the brain of rats [62]. In addition, we detected positive associations of AD neuropathological burden with many other amino acids, including aspartic acid, citrulline, threonine, glutamic acid, threonine, tryptophan, glutamine, tyrosine, phenylalanine, methionine, histidine, and alanine. These findings appear to be consistent with previous studies. For example, histidine is a precursor of brain histamine that is involved in AD and neuropsychiatric disorders in animal studies [63]. A higher level of aspartic acid in the brain was previously associated with dementia severity in human studies [64]. In a small case-control study, the concentrations of glycine, methionine, threonine, phenylalanine, and citrulline in CSF of dementia patients were higher than that in healthy controls [65]. We also found that higher levels of 10 brain glycerophospholipids were negatively associated with various AD neuropathological measures. This is consistent with a previous study showing that AD patients had decreased concentrations of brain glycerophospholipids than healthy controls [9]. For biogenic amines, we found significant associations of higher levels of alpha-amino adipic acid, creatinine or a lower level of putrescine with higher AD neuropathological burdens. Alpha-amino adipic acid is a degradation product of lysine in mammals [66]. It is a potential endogenous neuromodulator that can affect various elements of neurotransmission in human and rats [67]. Human studies have demonstrated that a higher level of serum alpha-amino adipic acid is associated with decreased cognitive function [68]. Putrescine is the metabolic product of amino acids which is toxic in large dosage. In line with our results, a previous study reported that AD patients had a lower level of putrescine in post-mortem brains compared to healthy controls [69].

Exploring the potential overlaps between blood and brain metabolites is important both scientifically and clinically. First, the current literature has little information regarding the relationship between circulating metabolites and brain metabolites from same individuals. Understanding the potential overlap or interaction between peripheral and central metabolism will enhance our understanding of the complex mechanisms underpinning AD pathology. Second, circulating metabolites assayed in ante-mortem samples could be used as biomarkers for AD risk prediction, whereas metabolites in postmortem brain tissue could help us understand disease mechanisms. Identifying metabolites present in both blood and brain (i.e., overlapping metabolites) is likely to lead to novel mechanistic markers for AD pathology. In our study, of the 40 putative metabolites (28 in brain and 16 in serum) associated with AD phenotypes, 4 metabolites (tetradecadienylcarnitine and 3 other glycerophospholipids) are present in both brain and serum. Of these, a higher level of tetradecadienylcarnitine in blood was associated with a slower cognitive decline, and a higher level of tetradecadienylcarnitine in brain was associated with a lower burden of AD neuropathology. However, the directions of the 3 glycerophospholipids (PC aa C30:0, PC ae C34:0, PC ae C36:1) were mixed, with brain glycerophospholipids showing protective effects on AD neuropathology, whereas serum glycerophospholipids showing risk for cognitive function. The opposite directions could be attributed to tissue-specific effects of metabolites or due to the stage difference in blood (ante-mortem) and brain (post-mortem) metabolites or other unknown reasons.

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In a previous study [9] including 44 brain tissue samples from three groups of individuals (15 AD, 14 NCI, and 15 asymptomatic AD) in the Baltimore Longitudinal Study of Aging (BLSA), a panel of 26 brain metabolites (largely sphingolipids and glycerophospholipids) was found to discriminate AD from NCI subjects with a high accuracy. The authors then assayed the serum levels of these 26 metabolites in participants from two longitudinal cohorts representing prodromal (the Alzheimer's Disease Neuroimaging Initiative, N = 767) and preclinical (BLSA, N=207) stages, and found that 25 of these metabolites in brain or blood are associated with either AD neuropathology or incident AD/cognitive decline. In our study, all these 26 metabolites are present in the serum sample, and 25 metabolites are present in the brain sample. However, only three brain metabolites, including PC ae C36:0, PC ae C34:0, and propionylcarnitine, were able to be replicated in our study at a same direction. In addition, the authors found that distinct sphingolipid species predict the incident AD and cognitive decline, but none of the 14 sphingolipids examined in our study was associated with AD-related traits at FDR 10%. This lack of replication could be due to multiple reasons, such as relatively small sample size, different study populations, heterogeneous AD phenotypes used in different studies, and inappropriate exclusion of metabolites by multiple testing adjustments, etc. Indeed, at a less stringent significant level (raw $p<0.05$), we did find that some sphingolipids (e.g., SM C16:1) were associated with autopsy-confirmed AD. Importantly, we found that only one metabolite (tetradecadienylcarnitine) showed a significant association with AD phenotypes in both blood and brain with the same direction. In support of this, our network analyses revealed that brain and blood metabolites are largely non-overlapping, indicating potential tissue-specific metabolic regulations. Thus, limiting brain metabolites in identifying metabolic predictors of AD progression may miss important prognostic markers.

Several limitations of our study should be noted. First, our targeted metabolomic analysis focused on a list of known metabolites, thus we might have missed some important unknown disease-related metabolites, and our conclusion that peripheral and central metabolism in relation to AD pathologies are tissue-specific may not necessarily be generalized to other/ unknown metabolites. Second, despite the relatively larger sample size in either brain or blood, we only had 92 subjects with both brain and blood samples. In addition, the average follow-up period of the participants in our analysis was about 4.5 years, which limits the number of cases, and thus statistical power of our analyses. Third, the present study only examined metabolite levels in one brain region (dorsolateral prefrontal cortex), whereas the metabolic signatures of AD could vary across different brain regions. Fourth, study participants included in the current analysis are highly educated European Caucasians, and our results may not be generalized to other racial/ethnic groups or population settings. Fifth, although we used a prospective design in the blood metabolites analysis, the identified associations between metabolites and AD risk do not necessarily imply causality. It is possible that the relationship between metabolic disturbance and risk for AD is bidirectional. Functional studies or advanced statistical models (e.g., Mendelian randomization) are required to establish the potential causal role of metabolic dysfunction in AD pathology. Sixth, our network analyses appeared to suggest potential tissue-specific metabolism, but as our blood samples were collected prior to death and the brain samples were collected after death, the lack of blood-brain overlapping or connection could also be due to the different

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stages of disease. Repeated measurements of ante-mortem blood metabolites including the one proximal to the death may help address the tissue- specific effects of metabolites. Finally, we did not include a replication cohort in the current study, since to our knowledge we are the first to perform metabolomics analyses in both brain and blood of same individuals. Our results await replications from future studies with similar clinical settings.

Nonetheless, our study has several strengths. Compared to previous studies [9] that included post-mortem brain tissue and/or blood samples, our study has a relatively larger sample size (111 brain tissue samples and 530 serum samples). Moreover, to our knowledge, the current study is the first to investigate AD-related metabolomic profiles in both brain and blood from a relatively larger number of individuals (N=92). Further, we examined the associations of metabolites with comprehensive cognitive and neuropathological phenotypes, including β-amyloid, neuritic plaques, global AD pathology burden, CERAD score, clinical diagnosis of Alzheimer's dementia, and cognitive functions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors would like to thank the participants of the Religious Orders Study and Memory and Aging Project studies and the staff of the Rush Alzheimer's Disease Center.

Funding sources: This work was supported by the National Institutes of Health grants RF1AG52476, P30AG10161, R01AG15819, R01AG17917, R01AG16042, U01AG46152, RF1AG36042, 1U01AG061359, R01AG36836, and R01AG42210. ROSMAP data can be requested at www.radc.rush.edu.

2. This work was supported by the National Institutes of Health grants RF1AG52476, P30AG10161, R01AG15819, R01AG17917, R01AG16042, U01AG46152, RF1AG36042, 1U01AG061359, R01AG36836, and R01AG42210.

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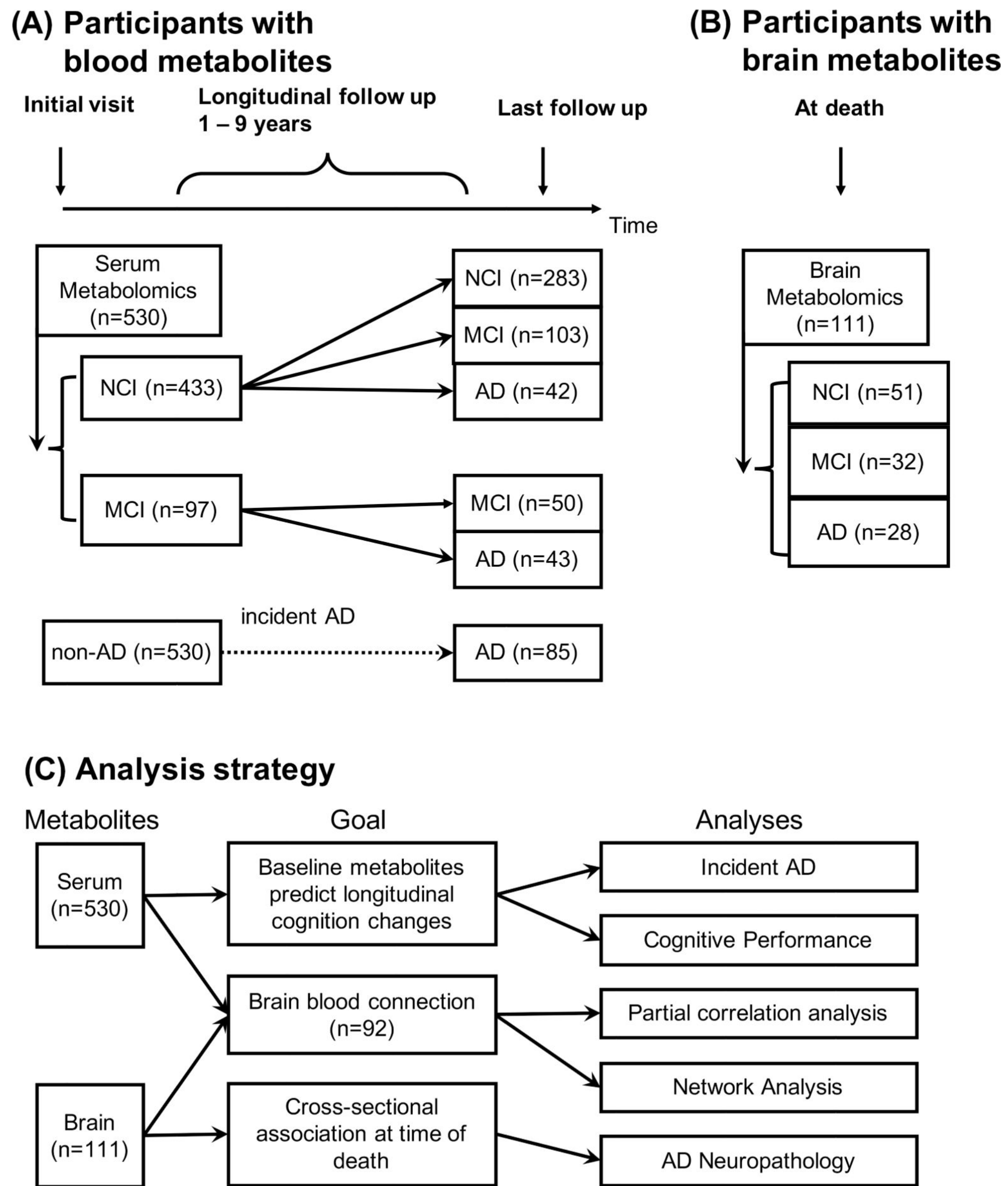

Figure 1,

Experimental design, sample size description, and statistical analysis strategy. **Figure IA** shows the experimental design, sample size description, and number of incident AD for the participants with blood samples; **Figure IB** shows the experimental design and sample size description for the participants with brain samples; and **Figure 1C** shows the overall statistical analysis strategy. For the analyses with serum or brain metabolites, outcome names were used in lieu of the association analysis with these outcomes.

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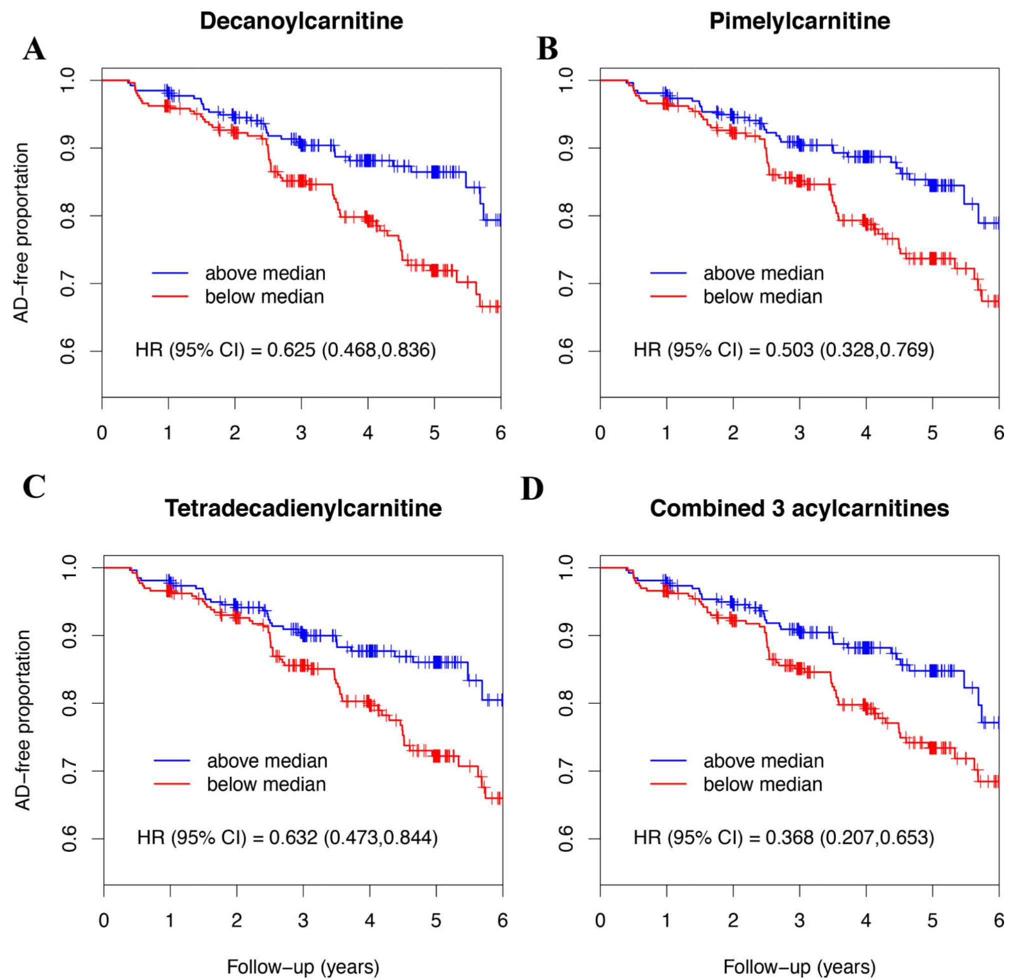

Figure 2.

The Kaplan Meier survival curves of three serum acylcarnitine metabolites that are predictive of risk for incident AD (FDR 10%). Subjects (N = 530) were dichotomized based on the median level of metabolite concentrations. HR denotes the hazard ratio per fold metabolite level increase.

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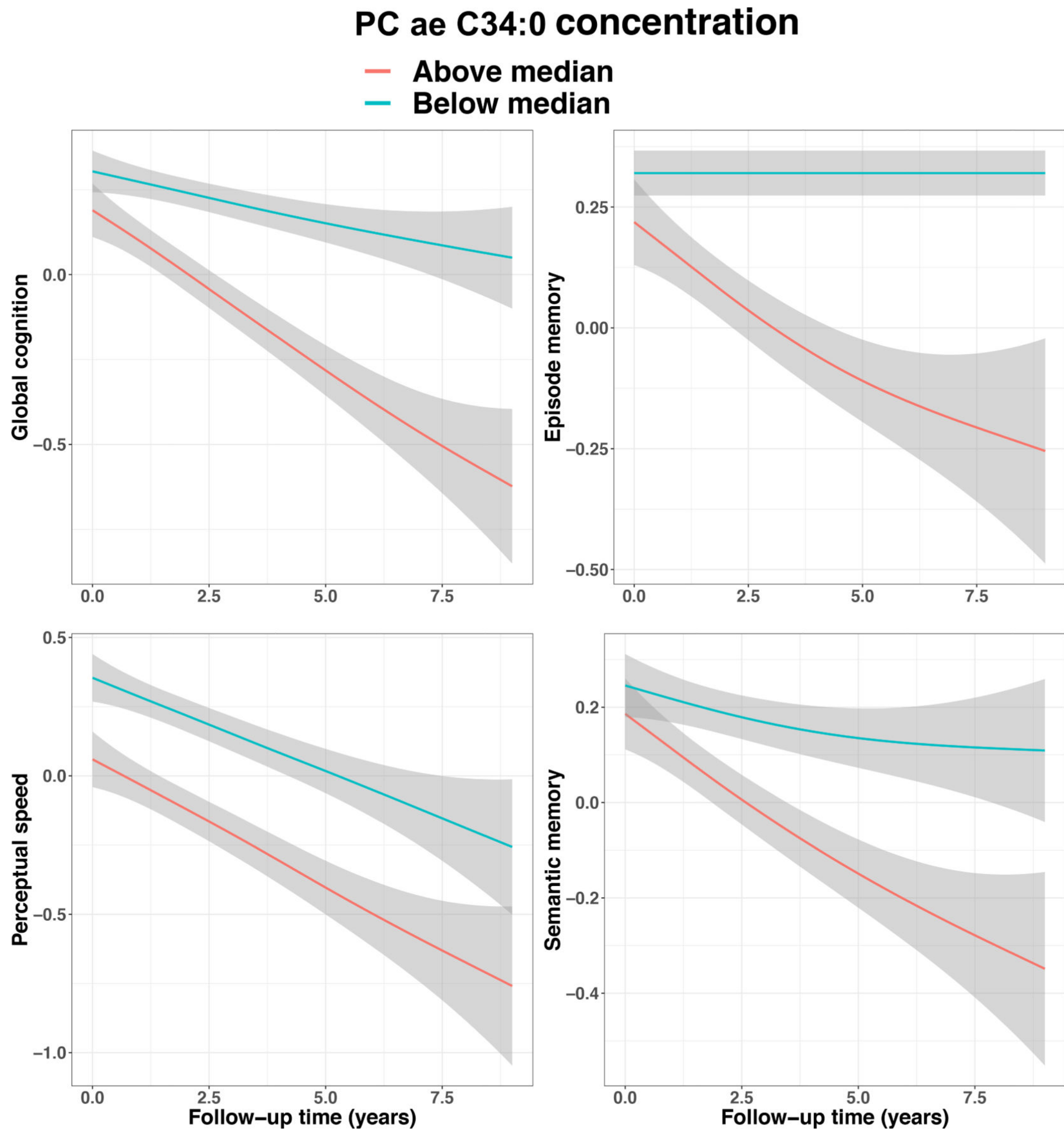

Figure 3.

Baseline serum level of PC ae C34:0 predicts cognitive decline over time (average 4.5-year follow-up). Shaded areas indicate standard errors for the trajectories. This analysis included 530 subjects who were NCI at baseline.

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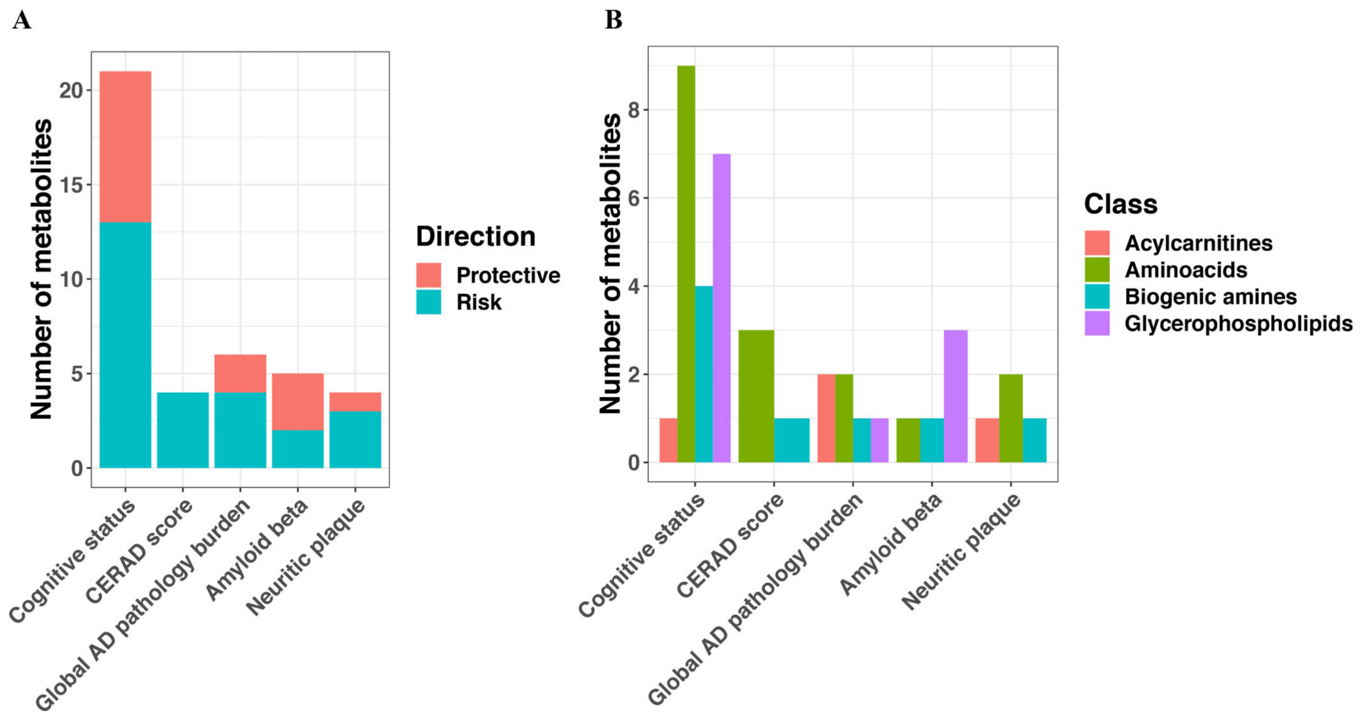

Figure 4.

Putative brain metabolites associated with measures for cognition and AD neuropathology (FDR 10%). (A) Dichotomized by direction of associations; (B) Associations based on compound classes.

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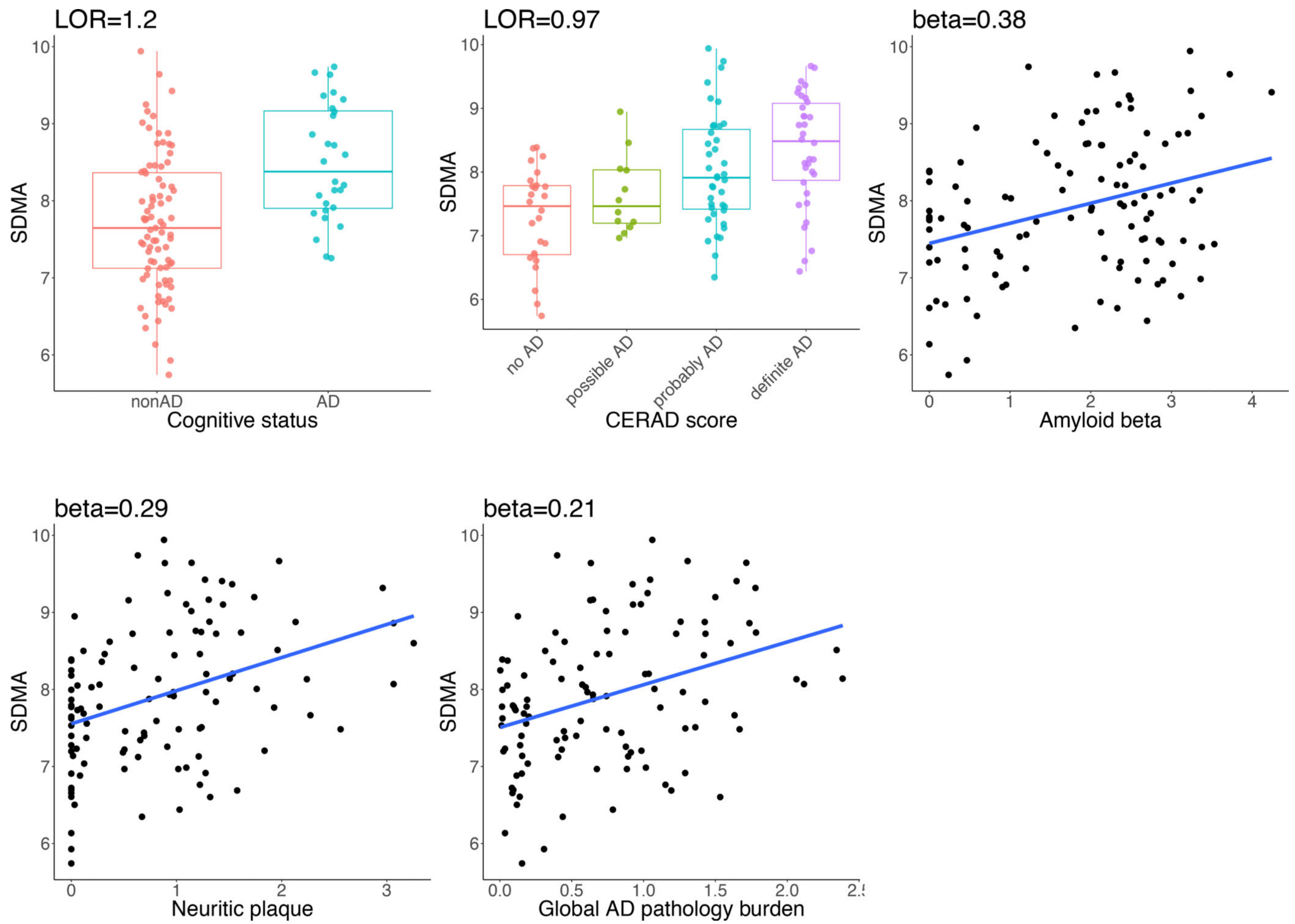

Figure 5.

Association of the symmetric dimethylarginine (SDMA) with various neuropathologic phenotypes for AD. LOR represents the log odds ratio of being in higher group per fold change of metabolite level, and beta represents the regression coefficient. This analysis included 111 subjects with brain samples.

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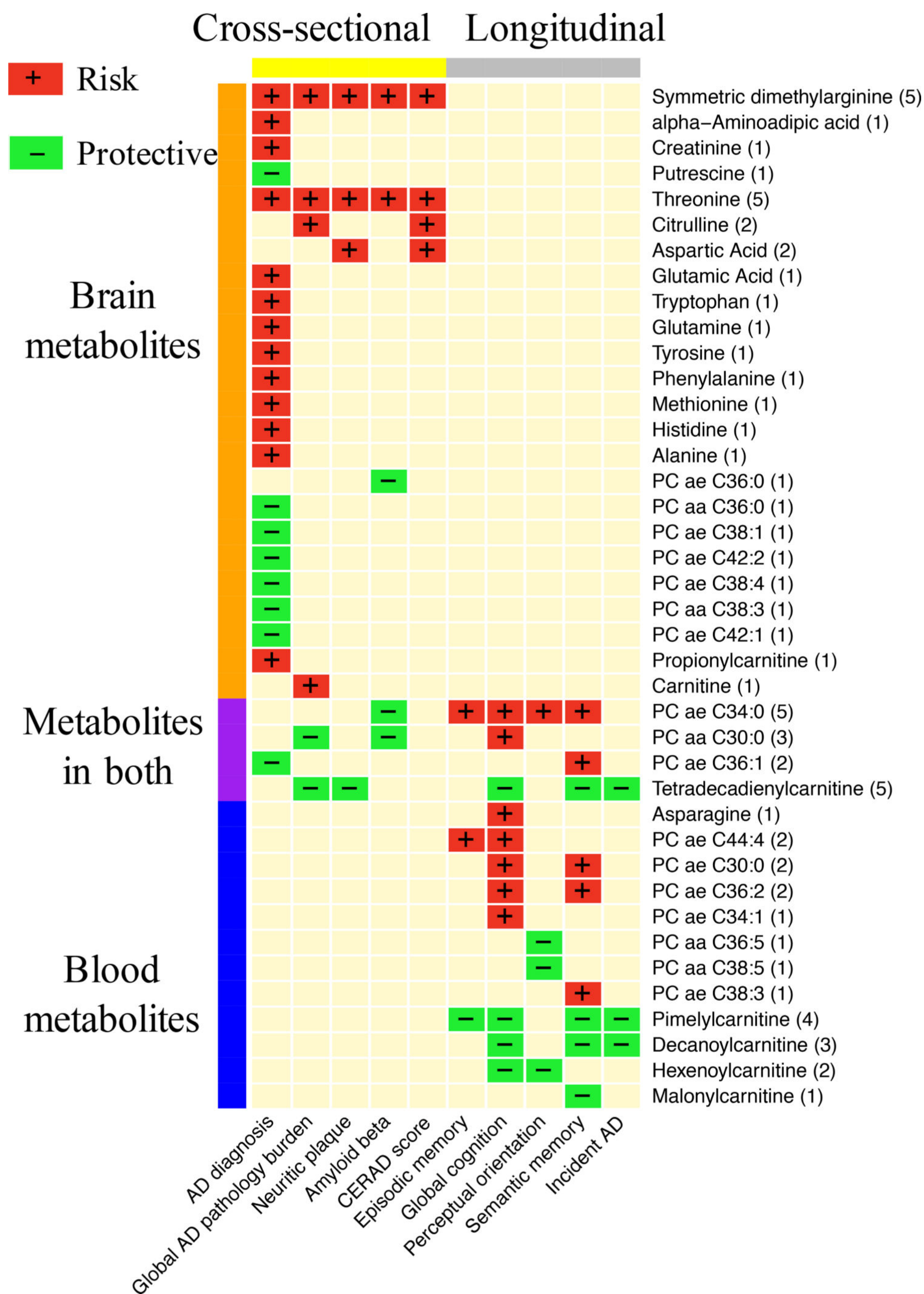

Figure 6.

Summarization of brain/blood metabolites associated with AD-related traits (FDR 10%). Each column represents a phenotype, with yellow color bar indicating cross-sectional AD-related traits at time of death and grey color bar indicating longitudinal cognitive phenotypes. Each row represents a metabolite, with orange color bar indicating brain metabolites; blue color bar indicating serum metabolites; and purple color indicating

metabolites that exist in both brain and blood. The total number of significant associations are denoted in ().

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Table 1.

Baseline characteristics of the study participants with blood samples.

Characteristics	**All (N=530)**	**Non-Converter (N=436)**	**Converter[*] (N=85)**	***p*-value[†]**
Age (years)	82 ± 7.4	81 ± 7.3	86 ± 5.9	6.0×10^{-12}
Male	114 (22%)	99 (22%)	15 (18%)	3.9×10^{-1}
Education (years)	16 ± 3.1	16 ± 3.1	16 ± 3.2	9.2×10^{-1}
APOE4 carrier	99 (19%)	73 (16%)	26 (32%)	2.0×10^{-3}
Duration (years)	4.5 ± 1.9	4.4 ± 1.9	5.1 ± 2.1	4.3×10^{-3}
Episode memory	0.28 ± 0.74	0.42 ± 0.63	−0.45 ± 0.83	4.9×10^{-15}
Perceptual speed	0.31 ± 0.72	0.37 ± 0.71	0.023 ± 0.7	7.5×10^{-5}
Visuospatial ability	0.22 ± 0.83	0.32 ± 0.8	−0.31 ± 0.79	7.7×10^{-10}
Semantic memory	0.24 ± 0.62	0.32 ± 0.58	−0.21 ± 0.67	4.7×10^{-10}
Working memory	0.12 ± 0.69	0.16 ± 0.7	−0.099 ± 0.63	8.3×10^{-4}
Global cognition	0.24 ± 0.54	0.34 ± 0.48	−0.27 ± 0.53	8.8×10^{-17}

[*]Among 530 participants free of AD at baseline, 85 developed incident AD by the last follow up (converters), and 436 remained free of AD (non-converter). 9 subjects were excluded because of dementia because of other causes.

[†]The *p*-value indicates the significance level comparing converters and non-converters.

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Table 2.

Clinical characteristics of brain donors at death.

Characteristics	All (N=111)	non-AD (N=83)	AD (N=28)	*p*-value*
Age (years)	90 ± 5.8	90 ± 5.7	91 ± 6.2	7.0×10^{-1}
Male	31 (28%)	27 (33%)	4 (14%)	8.8×10^{-2}
Education (years)	15 ± 3.2	15 ± 3.2	15 ± 3.2	8.5×10^{-1}
APOE4 carrier	23 (21%)	13 (16%)	10 (36%)	5.8×10^{-2}
PMI (hours)	8.8 ± 6.8	8.3 ± 6.7	10 ± 6.8	1.6×10^{-1}
CERAD score †	2.3 ± 1.1	2.5 ± 1.2	1.6 ± 0.83	9.0×10^{-5}
Amyloid-β plaques ‡	1.8 ± 1.1	1.6 ± 1.2	2.2 ± 0.77	2.5×10^{-3}
Neuritic plaques §	0.83 ± 0.79	0.64 ± 0.65	1.4 ± 0.89	1.2×10^{-4}
Braak stage **	3.7 ± 1.1	3.5 ± 1.1	4.2 ± 1.1	3.3×10^{-3}
PHF tau tangles ††	6.6 ± 7.8	4.3 ± 4.1	13 ± 12	3.0×10^{-4}
Neurofibrillary tangles ‡‡	0.63 ± 0.74	0.47 ± 0.5	1.1 ± 1.1	5.5×10^{-3}
Global AD pathology burden	0.73 ± 0.59	0.58 ± 0.49	1.2 ± 0.65	8.7×10^{-5}

[*] The *p*-value indicates the significance level comparing AD and non-AD.

[†] Semiquantitative measure of neuritic plaque.

[‡] Mean of amyloid-β score (square root transformed) in 8 brain regions by immunohistochemistry.

[§] Mean of neuritic plaque burden in 5 brain regions by histochemistry.

[**] Semiquantitative measure of neurofibrillary tangle.

[††] Mean of PHF tau tangles score in 8 brain regions by immunohistochemistry.

[‡‡] Mean of neurofibrillary tangle burden in 5 brain regions by histochemistry.

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Table 3.

Association of baseline serum metabolites with cognitive declines (FDR 10%).

Metabolites	Global cognition	Episode memory	Perceptual speed	Semantic memory
PC ae C34:0	–0.11	–0.10	–0.09	–0.10
PC ae C30:0	–0.08			–0.09
PC ae C36:2	–0.08			–0.11
Pimelylcamitine	0.04	0.05		0.05
Decanoylcarnitine	0.03			0.03
PC aa C30:0	–0.06			
Asparagine	–0.07			
PC ae C44:4	–0.08	–0.10		
Hexenoylcarnitine	0.06		0.08	
PC ae C36:1				–0.11
Tetradecadienylcarnitine	0.03			0.03
PC ae C34:1	–0.09			
PC aa C36:5			0.04	
PC aa C38:5			0.08	
PC ae C38:3				–0.09
Malonylcarnitine				0.04

[*] β coefficient in the table indicates per year change in cognitive function per fold change of metabolite level. A positive β indicates protective metabolites – higher metabolite level will improve longitudinal cognitive performance; and vice versa for negative β.