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Investigating associations between blood metabolites, later life brain imaging measures, and genetic risk for Alzheimer's disease

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Abstract

Background Identifying blood-based signatures of brain health and preclinical pathology may offer insights into early disease mechanisms and highlight avenues for intervention. Here, we systematically profiled associations between blood metabolites and whole-brain volume, hippocampal volume, and amyloid-β status among participants of Insight 46—the neuroscience sub-study of the National Survey of Health and Development (NSHD). We additionally explored whether key metabolites were associated with polygenic risk for Alzheimer's disease (AD).

Methods Following quality control, levels of 1019 metabolites—detected with liquid chromatography-mass spectrometry—were available for 1740 participants at age 60–64. Metabolite data were subsequently clustered into modules of co-expressed metabolites using weighted coexpression network analysis. Accompanying MRI and amyloid-PET imaging data were present for 437 participants (age 69–71). Regression analyses tested relationships between metabolite measures—modules and hub metabolites—and imaging outcomes. Hub metabolites were defined as metabolites that were highly connected within significant ($p_{FDR} < 0.05$) modules or were identified as a hub in a previous analysis on cognitive function in the same cohort. Regression models included adjustments for age, sex, *APOE* genotype, lipid medication use, childhood cognitive ability, and social factors. Finally, associations were tested between AD polygenic risk scores (PRS), including and excluding the *APOE* region, and metabolites and modules that significantly associated ($p_{FDR} < 0.05$) with an imaging outcome ($N = 1638$).

Results In the fully adjusted model, three lipid modules were associated with a brain volume measure ($p_{FDR} < 0.05$): one enriched in sphingolipids (hippocampal volume: $ß = 0.14$, 95% CI = [0.055,0.23]), one in several fatty acid pathways (whole-brain volume: $ß = -0.072$, 95%CI = [−0.12, −0.026]), and another in diacylglycerols and

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phosphatidylethanolamines (whole-brain volume: $\beta = -0.066$, 95% CI = [−0.11, −0.020]). Twenty-two hub metabolites were associated ($p_{FDR} < 0.05$) with an imaging outcome (whole-brain volume: 22; hippocampal volume: 4). Some nominal associations were reported for amyloid-β, and with an AD PRS in our genetic analysis, but none survived multiple testing correction.

Conclusions Our findings highlight key metabolites, with functions in membrane integrity and cell signalling, that associated with structural brain measures in later life. Future research should focus on replicating this work and interrogating causality.

Keywords Metabolites, Dementia, Brain imaging, Ageing, Polygenic scores, Birth cohort, Weighted-gene coexpression network analysis, Alzheimer's disease

Background

Brain changes accompanying ageing are varied and can include pathologies that lead to cognitive impairment, the commonest of which is Alzheimer's disease (AD). Identifying non-invasive and scalable markers of brain health and pathology in later life, including but not limited to those associated with AD, would be valuable for research and therapeutic trials. This has led to large efforts in detecting blood-based markers, with candidates such as neurofilament light and phosphorylated-tau showing particular promise [1]. Blood metabolites—the products of chemical reactions occurring in the body—may also present as potential candidates for this goal. Due to their proximity to core biological processes, they are uniquely placed to capture physiological changes and may allow insights into the processes associated with emergence of disease [2]. Additionally, since they are potentially modifiable [3, 4], they could represent possible targets for intervention.

Existing research has identified associations between several metabolite classes and imaging markers related to neurodegeneration, including particular lipids and amino acids [5–9]. However, these studies have been directed towards clinical cohorts, where pathology is already advanced. Additionally, little is known about the involvement of groups of interrelated metabolites; using systems-level approaches could offer an improved understanding of these complex relationships and facilitate the identification of candidate markers. We previously employed a systems-level approach to explore the metabolic correlates of late midlife cognitive function in the Medical Research Council National Survey of Health and Development (NSHD; the British 1946 birth cohort) [10]. We identified groups of highly coexpressed metabolites that associated with cognitive outcomes and key metabolites within these to explore further, including acylcarnitines, modified nucleotides and amino acids, vitamins, and sphingolipids, although many associations with late midlife cognitive outcomes were explained by social factors and childhood cognitive ability [10].

Here, we aimed to investigate the early metabolic correlates of later life brain imaging measures relevant to AD and neurodegeneration—Aβ pathology, whole-brain volume, and hippocampal volume—using metabolite data collected at age 60–64 and imaging data measured 5–11 years later. To provide a deeper understanding on the nature of potential relationships and how they may contribute to AD risk, we explored whether any key metabolites were additionally associated with polygenic risk for AD.

Methods

Participants

The NSHD is a broadly representative birth cohort study, originally following 5362 individuals since their birth in mainland Britain during one week in March 1946 [11]. At age 69–71, 502 participants enrolled in Insight 46, the neuroscience sub-study. At a University College London clinic, they underwent comprehensive clinical and cognitive tests, MRI, and ^{18}F-florbetapir positron emission tomography (PET) imaging [12, 13]. Compared to the full NSHD cohort, participants of Insight 46 were of slightly higher cognitive ability, more socially advantaged, and of better overall health [13]. Further details on participant eligibility and recruitment can be found elsewhere [12].

Insight 46 participants with full metabolite data, and who completed the scanning procedure and were dementia-free, were included for module and hub metabolite analyses ($N = 437$; 47.6% female, 18.9% Aβ-positive). For PRS analyses, NSHD participants with metabolomics and genetic data were included ($N = 1638$; 50.4% female).

Ethical approval was obtained from the National Research Ethics Service Committee London (14/LO/1173). All participants provided written informed consent.

Materials

Metabolite quality control

At age 60–64, blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes by trained research nurses (96% fasted). Samples were stored at – 80 °C.

Using Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS), levels of

1401 metabolites were detected and measured by Metabolon Inc. (Durham, NC, USA) among 1814 NSHD participants. All samples were received by Metabolon at the same time point. Metabolites were assigned to nine families (lipids, amino acids, xenobiotics, peptides, nucleotides, cofactors and vitamins, carbohydrates, energy and partially characterised molecules) and further organised into pathways based on their proposed biological function informed by the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database (Supplementary Table 1). Unknown metabolites were assigned to an "Unknown" family and pathway and denoted by a number prefixed by an "X"; these were included in all analyses.

Metabolite data underwent strict quality control (QC), as detailed in [10], resulting in 1019 metabolites (Supplementary Notes).

Genetic quality control

Initial QC and imputation were performed centrally by the NSHD study team (Supplementary Notes). For this analysis, we removed variants that were rare (MAF < 5%), with a low call rate (< 98%), or that deviated from Hardy–Weinberg equilibrium ($p < 1 \times 10^{-5}$). We additionally removed participants with a low call rate (< 98%), mismatching biological and reported sex, or that were related (PIHAT < 0.1). All QC was performed using PLINK v1.9 (https://www.cog-genomics.org/plink2) [14]. Following QC, genetic and metabolomic data were available for 1638 participants.

Scanning procedure

The scanning procedure and data processing were undertaken by the Insight 46 team. Aβ-PET and MRI were acquired contemporaneously using a single Biograph mMR 3 Tesla PET/MRI scanner (Siemens Healthcare) [12]. Aβ burden was quantified over 10 min, approximately 50 min after intravenous injection with ^{18}F-florbetapir (370 mBq. Standardised uptake value ratios (SUVRs) were derived using a grey matter cortical composite and an eroded subcortical white matter reference region. A cut-off of > 0.6104 was used to define Aβ positivity being the 99th percentile of the lower (Aβ-negative) Gaussian distribution [15]. Participants below this threshold were defined as Aβ-negative. Data were processed using an in-house pipeline, including attenuation correction using pseudo-CT [12]. For volumetric T1-weighted MRI images, visual QC was performed as detailed in [12] and processed using the following: MAPS [16] for whole-brain volume (with manual editing if needed), STEPS [17] for left and right hippocampal volumes (with manual editing if needed), and SPM12 (fil.ion.ucl.ac.uk/spm) [18] for total intracranial volume (TIV). Hippocampal volume was calculated as the mean volume of the left and right hippocampi.

Covariables

In line with our previous analysis in the full NSHD [10, 19], covariables were as follows: sex, blood collection information (clinic location, age, fasting status), age at scan, *APOE* genotype (ε4 carrier/non carrier; blood samples at age 53 or 69 years), BMI (60–64 years nurse visit), lipid medication (yes/no; self-reported use in 24 h preceding blood collection at 60–64 years), childhood cognitive ability (15 years), highest level of educational attainment (no qualifications/ 'O level'/ 'A level' or higher; 26 years), childhood socioeconomic position (SEP) (father's current or last known occupation categorised according to the UK Registrar General; 11 years), and midlife SEP (own occupation categorised as for childhood SEP; 53 years).

Statistical analysis

We previously imputed missing covariable data using multiple imputation chained Eqs. (100 iterations and 50 imputations) [20] in the full NSHD metabolomics dataset. Further details of missing data can be found in Table 1. Unless otherwise specified, we carried out all analyses in R version 3.6.0 (details of all software and packages used can be found in Supplementary Notes). A visual summary of our analytical pipeline can be found in Fig. 1.

Coexpression network analysis

To explore associations of clusters (termed "modules") of co-expressed metabolites, we applied weighted gene coexpression network analysis (WGCNA) [21–23] to metabolite data, as detailed previously [10]. First, metabolite data were adjusted for sex and blood clinic information, and the standardised residuals were used for WGCNA. Fourteen modules of highly connected metabolites were then identified, and the first principal component of each module (termed "module eigenvalue") was derived to allow for relationships between modules and outcomes to be examined. Overrepresentation analyses were conducted, using the hypergeometric test, to identify enriched pathways within the module and provide insight into potential biological function [10]. Modules were allocated an arbitrary colour name using the WGCNA package for ease of discussion.

Hub metabolites

Metabolites that are highly connected to their module (termed "hub metabolites") are likely to be functionally important and thus present as valuable marker

Table 1 Characteristics of participants included in this analysis

		Participants with genetic and metabolite data		Participants with imaging and metabolite data	
		Overall	**Missing (%)**	**Overall**	**Missing (%)**
n		1638		437	
Sex, *N* (%)	Male	812 (49.6)	0	229 (52.4)	0
	Female	826 (50.4)		208 (47.6)	
Age at scan (years), mean (SD)				70.7 (0.7)	0
APOE4 carrier, *N* (%)	Non-carrier	1044 (69.6)	8.5	306 (70.3)	0.5
	Carrier	455 (30.4)		129 (29.7)	
Amyloid status, *N* (%)	Negative			348 (81.1)	1.8
	Positive			81 (18.9)	
Hippocampal volume in ml, mean (SD)				3.1 (0.3)	0.5
Brain volume in ml, mean (SD)				1101.8 (99.3)	0.5
Total intracranial volume in ml, mean (SD)				1435.9 (132.2)	0.5
Age at blood collection (years), mean (SD)		63.2 (1.1)	0	63.3 (1.1)	0
Time between blood collection and imaging visit (years), mean (SD)				7.4 (1.3)	0
Lipid medication use (age 60–64), *N* (%)	No	1232 (75.2)	0	335 (76.7)	0
	Yes	406 (24.8)		102 (23.3)	
Body mass index (age 60–64) in kg/m^2, mean (SD)		27.8 (4.7)	0.1	27.4 (4.0)	0
Childhood cognitive ability (age 15)[a], *z*-score, mean (SD)		0.2 (0.8)	14.3	0.5 (0.7)	8.2
Childhood socioeconomic position (age 11), *N* (%)	Unskilled	80 (5.1)	5	18 (4.1)	0.5
	Partly skilled	274 (17.6)		64 (14.7)	
	Manual skilled	462 (29.7)		106 (24.4)	
	Nonmanual skilled	276 (17.7)		90 (20.7)	
	Intermediate	346 (22.2)		110 (25.3)	
	Professional	118 (7.6)		47 (10.8)	
Adult socioeconomic position (age 53), *N* (%)	Unskilled or Partly skilled[b]	205 (12.5)	0.4	26 (5.9)	0
	Manual skilled	238 (14.6)		40 (9.2)	
	Nonmanual skilled	380 (23.3)		91 (20.8)	
	Intermediate	680 (41.7)		225 (51.5)	
	Professional	129 (7.9)		55 (12.6)	
Highest educational attainment (age 26), *N* (%)	No qualification	446 (28.6)	4.9	65 (15.3)	3
	Up to GCSE	446 (28.6)		125 (29.5)	
	A-level or higher	665 (42.7)		234 (55.2)	

[a] Childhood cognitive ability *Z*-scores were calculated in the full National Survey of Health and Development cohort ($N = 5362$)

[b] Categories grouped due to low counts (for the purpose of this table only)

candidates [24]. We previously identified associations between 35 hubs, defined using correlations between metabolites and module eigenvalues exceeding $r = 0.65$ (termed "module membership"; kME), and cognitive outcomes in the NSHD [10]. As these metabolites were selected on the premise of showing associations with cognitive function, we additionally looked for hubs that may be important in brain imaging outcomes. To do this, we extracted any additional metabolites exceeding the 0.65 threshold [10] in modules showing significant ($p_{FDR} < 0.05$) associations in the present analysis.

Regression analysis

To allow for direct comparisons, we standardised all continuous predictors and outcomes to a mean of 0 and standard deviation of 1. We then tested relationships between (a) modules and (b) hub metabolites using linear regression (for whole-brain volume and hippocampal volume) and logistic regression (for Aβ status). Model 1 adjusted for basic covariables: sex, blood collection information, age at scan, *APOE* genotype, and total intracranial volume (for whole-brain volume and hippocampal volume only). As modules

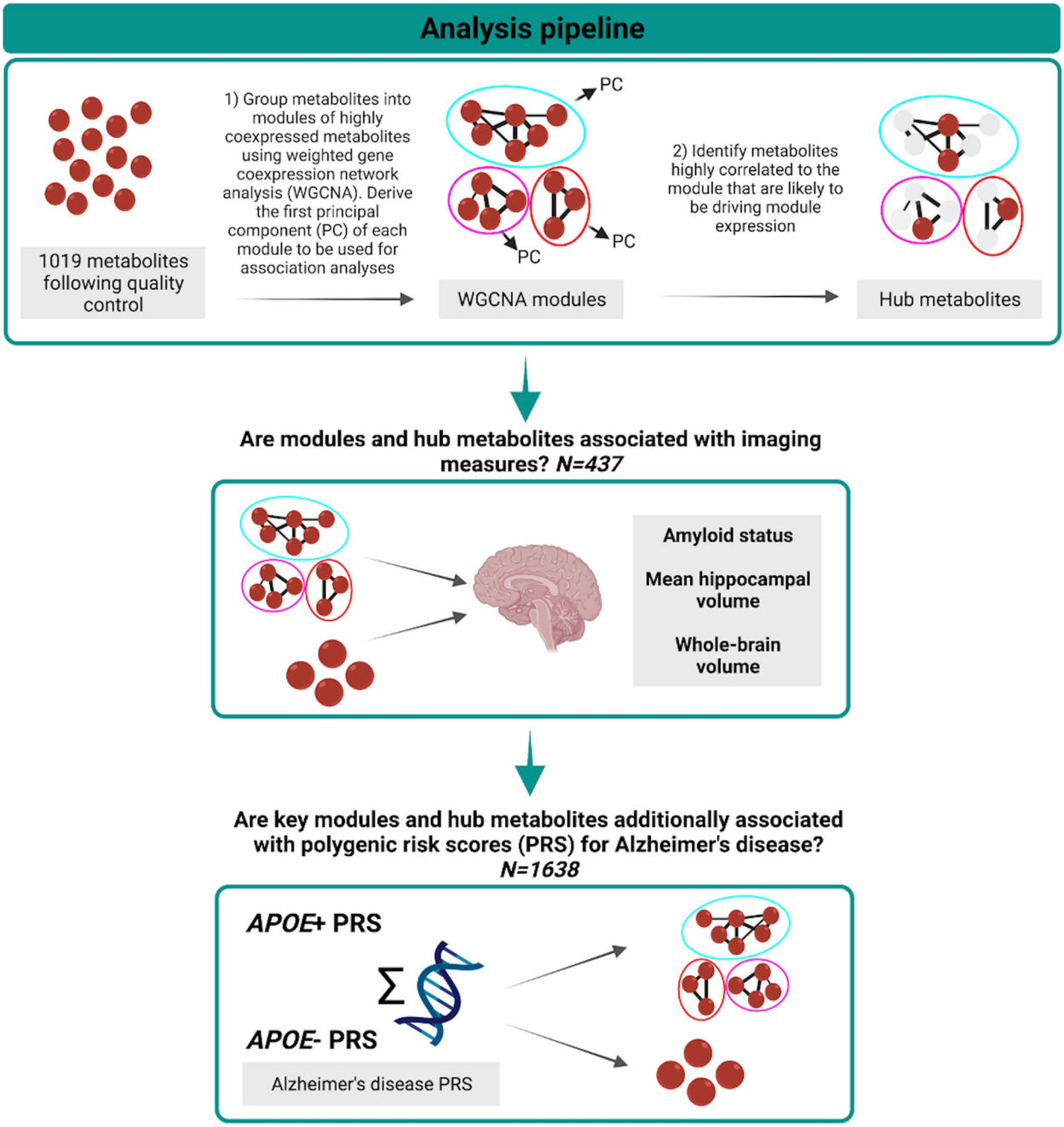


Fig. 1 Analysis pipeline

were already adjusted for sex and blood clinic information, these covariables were not additionally included for module analyses. Model 2 additionally adjusted for lipid-related factors: BMI and lipid medication use. Finally, model 3 further adjusted for childhood cognitive ability, educational attainment, and SEP (parental and midlife).

Analyses were conducted on each imputed dataset and pooled using Rubin's rules [25]. Regression assumptions were checked by examination of the residuals. We applied false discovery rate (FDR) correction using the Benjamini–Hochberg procedure [26] with an alpha = 0.05. FDR correction was applied separately to each analysis (module and hub) and each outcome.

Polygenic risk scores

To explore whether levels of key metabolites were influenced by genetic risk for AD, we investigated associations between AD polygenic risk scores (PRS)—a weighted sum of genetic variants associated with a trait or disease—and hub metabolites and modules that significantly associated with an imaging outcome ($p_{FDR} < 0.05$). We obtained genome-wide association study summary statistics from Kunkle et al. [27] ($N = 63{,}926$; 21,982 AD clinically ascertained cases, 41,944 controls), which were used as the base data for PRS analyses. Using PRSice-2 [28], we computed PRS in the NSHD, both including and excluding SNPs in the *APOE* region (chr 19, GRCh37 coordinates 44,912,079 to 45,912,079) [29]. Two *p*-value thresholds (P_T)—previously identified to be optimal for PRS including and excluding the *APOE* region—were used for SNP selection: 5×10^{-8} (suggested for *APOE* region included) and 0.1 (suggested for *APOE* region excluded) [30], resulting in four PRS. SNPs in linkage disequilibrium ($r^2 > 0.001$ within a 250-kb window) were clumped, and the SNP with the lowest *p*-value was retained.

We first standardised predictors and outcomes to a mean of 0 and standard deviation of 1. Then, we regressed PRS on key metabolites and modules, adjusting for sex, age, blood collection details, and seven genetic principal components (to control for population stratification). We applied FDR correction to each analysis—module and hub metabolite—using the Benjamini–Hochberg procedure [26] with an alpha = 0.05.

Additional analysis

We conducted several additional analyses to test the robustness of our findings (see Supplementary Notes for full details). In brief, we first investigated whether WGCNA modules, which were curated in the full NSHD, were preserved in the Insight 46 subset. Then, for our main analyses, we applied a more conservative Bonferroni correction to our findings (module: $p < 3.57 \times 10^{-3}$; hub metabolite: $p < 1.06 \times 10^{-3}$). Finally, we explored whether lifestyle and related factors (lifetime smoking, diet, exercise, blood pressure, and alcohol intake) explained any of our results.

Results

Participant characteristics

Participant characteristics can be found in Table 1 (see Supplementary Notes for characteristics split by Aβ status).

Metabolite coexpression network modules

Overall, we identified three modules that showed associations with brain volume outcomes ($p_{FDR} < 0.05$) and none ($p > 0.05$) with Aβ status. Full results can be found in Supplementary Table 2 and are visualised in Fig. 2. Results of the fully adjusted model are discussed hereafter.

We report associations between higher expression of two lipid modules and smaller whole-brain volumes: the brown module, enriched in diacylglycerol (DAG) and phosphatidylethanolamine (PE) pathways ($\beta = -0.066$, 95%CI = [−0.11, −0.019], $p = 0.006$, $p_{FDR} = 0.044$) and the blue module, enriched in various fatty acids pathways ($\beta = -0.072$, 95% CI = [−0.12, −0.026], $p = 0.0021$, $p_{FDR} = 0.035$). Higher expression of the yellow module, enriched in sphingolipid metabolism and related pathways, was associated with a larger hippocampal volume ($\beta = 0.14$, 95% CI = [0.055, 0.23], $p = 0.0017$, $p_{FDR} = 0.035$).

Hub metabolites

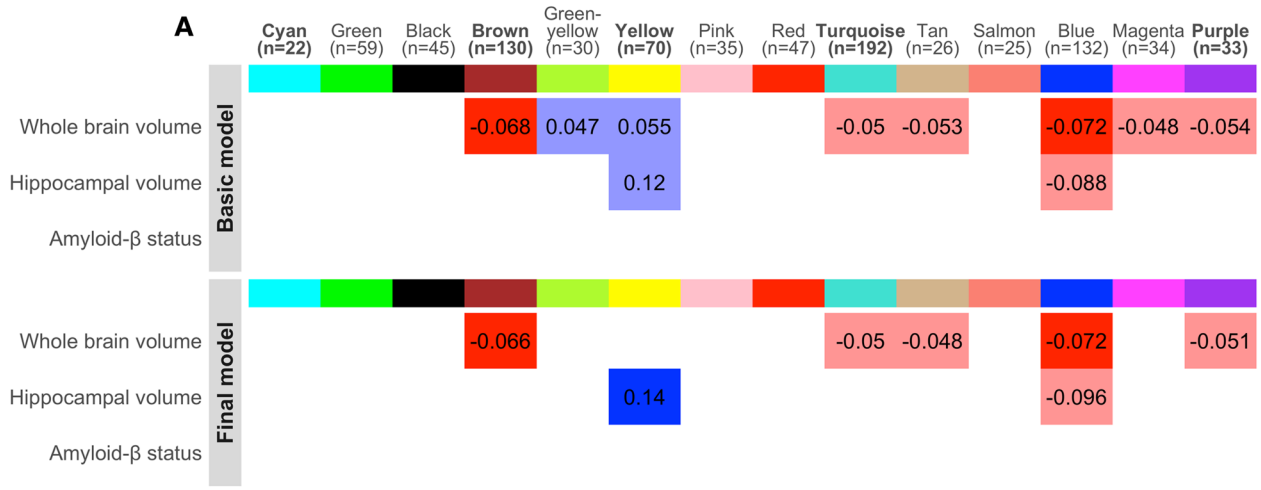
We explored associations between 81 metabolites that were highly connected (kME > 0.65) in significant modules identified in 3.2 and 35 that were identified to be hubs in our previous study on cognitive function. Across all models, we report 30 key metabolites after FDR correction, of which 13 were previously associated with cognitive outcomes (Fig. 3). No associations were detected for any metabolite and Aβ status after FDR correction, although some nominal associations were observed (3 cyan and 1 yellow metabolite; Supplementary Table 1 and Fig. 3).

In the fully adjusted model, 22 metabolites were associated ($p_{FDR} < 0.05$) with an imaging outcome (whole-brain volume: 22; hippocampal volume: 4). Of the 22, 10 metabolites belonged to the yellow module (pathways: sphingolipid metabolism) and were positively associated with larger brain volumes. Twelve metabolites were negatively associated with brain volumes: six belonging to the blue module (pathways: fatty acid (monohydroxy; dicarboxylate), long chain PUFA (n3 and n6), glycerolipid metabolism), four to the brown module (pathways: PE, phosphatidylcholine (PC), DAG), and two to additional modules identified in our previous analysis of cognitive function (pathways: fatty acid metabolism (acyl carnitine); methionine, cysteine, SAM and taurine metabolism).

Polygenic risk scores

We investigated whether modules and hub metabolites that associated with an imaging measure ($p_{FDR} < 0.05$) were also associated with polygenic risk for AD (*APOE* region included and excluded). We observed no significant relationships following FDR correction, and no relationships were observed for metabolite modules at either the nominal or adjusted level of significance.

A



B

Module	Pathway
cyan	Ascorbate and Aldarate Metabolism; Vitamin A Metabolism
green	Xanthine Metabolism; Benzoate Metabolism
black	Lysophospholipid; Fatty Acid Metabolism (Acyl Choline); Monoacylglycerol
brown	Diacylglycerol; Phosphatidylethanolamine (PE)
greenyellow	Plasmalogen; Ceramides
yellow	Sphingolipid Metabolism; Ceramides; Phosphatidylcholine (PC); Phosphatidylinositol (PI)
pink	Tryptophan Metabolism
red	Androgenic Steroids; Pregnenolone Steroids
turquoise	Histidine Metabolism; Urea cycle; Arginine and Proline Metabolism; Methionine, Cysteine, SAM and Taurine Metabolism; Tyrosine Metabolism; Polyamine Metabolism; Gamma-glutamyl Amino Acid; Leucine, Isoleucine and Valine Metabolism; Lysine Metabolism
salmon	Hemoglobin and Porphyrin Metabolism
blue	Long Chain Polyunsaturated Fatty Acid (n3 and n6); Long Chain Fatty Acids; Medium Chain Fatty Acid; Fatty Acid, Monohydroxy; Fatty Acid, Dicarboxylate; Endocannabinoid; Fatty Acid, Branched
magenta	Fatty Acid Metabolism (Acyl Carnitine)
purple	Fatty Acid Metabolism (Acyl Carnitine)

Fig. 2 Module results. **A** Heatmap showing relationships between modules and brain imaging outcomes in the basic model (model 1) and final model (model 3). The basic model was adjusted for age, sex, blood clinic location, and APOE genotype, and the final model additionally for BMI, lipid medication use, childhood cognitive ability, educational attainment, and SEP (parental and midlife). Tiles are coloured by effect direction (blue = associated with better outcomes, red = associated with worse outcomes). Effect sizes are presented inside the tiles. Associations significant after multiple testing correction are represented by a solid fill and nominal ($p < 0.05$) by a fainter fill. Module names in bold were additionally associated with a cognitive outcome in our previous analysis. Data for all models (1-3) are present in Supplementary Table 2. **B** Table presenting enriched pathways in each module, with the most highly enriched pathways presented first. No pathways were enriched for the tan module. Source data are available in [7]

At the nominal threshold, we report relationships between higher AD PRS and decreased levels of five hub metabolites (Fig. 3 and Supplementary Table 3). Three DAG and phosphoethanolamine hub metabolites were associated with the *APOE* AD PRS only ($P_T = 5 \times 10^{-8}$; β range = −0.061 to −0.050, p range = 0.012 to 0.04, $p_{FDR} > 0.05$). Two PUFA were associated with the non-*APOE* AD PRS ($P_T = 0.1$; DPA: $\beta = -0.077$, 95% CI = [0.13, −0.029], $p = 0.0018$; linolenate: $\beta = -0.054$, 95%CI = [−0.10, −0.0056], $p = 0.029$, $p_{FDR} > 0.05$); these associations weakened with the *APOE* region additionally included ($P_T = 0.1$; DPA: $\beta = -0.058$, 95% CI = [−0.11, −0.0097], $p = 0.019$, linolenate: $\beta = -0.040$, 95% CI = [−0.088, 0.0088], $p = 0.11$).

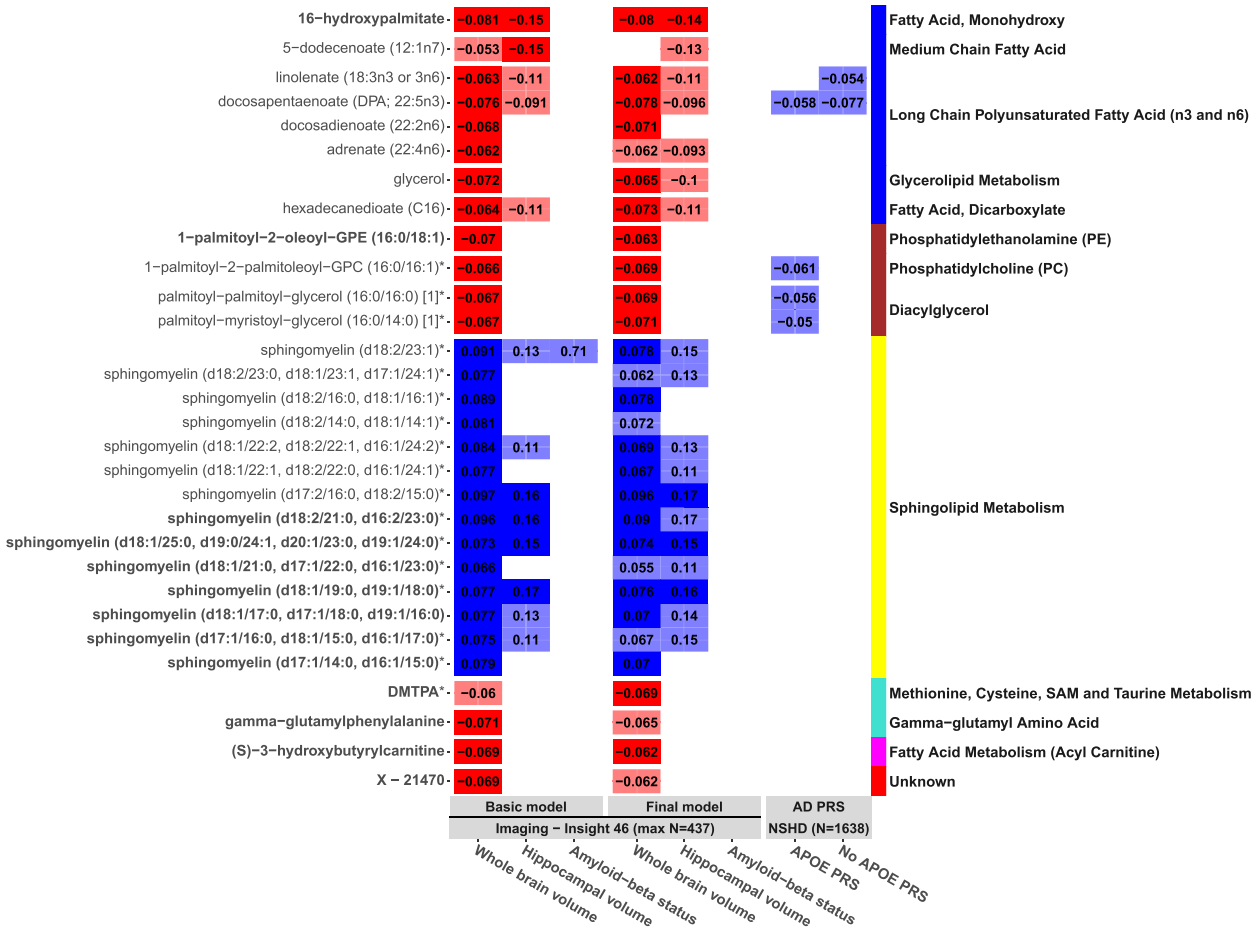


Fig. 3 Hub metabolite results. Heatmap showing relationships between key hub metabolites ($p_{FDR} < 0.05$) and brain imaging outcomes in the basic model (model 1) and final model (model 3), as well as relationships between these metabolites and Alzheimer's disease polygenic risk scores (best threshold shown). The basic model was adjusted for age, sex, blood clinic location, and APOE genotype, and the final model additionally for BMI, lipid medication use, childhood cognitive ability, educational attainment, and SEP (parental and midlife). Tiles are coloured by effect direction (blue = associated with better outcomes, red = associated with worse outcomes). Effect sizes are presented inside the tiles. Associations significant after multiple testing correction are represented by a solid fill and nominal ($p < 0.05$) by a fainter fill. Metabolites are organised by both module (indicated via the colour panel on the right) and pathway (specified next to the module colour panel). Metabolite names in bold were additionally associated with a cognitive outcome in our previous analysis. Data for all models (1-3) are present in Supplementary Table 1. AD, Alzheimer's disease; DMTPA, 2,3 dihydroxy-5-methylthio-4-pentenoic acid; NSHD, National Survey of Health and Development; PRS, polygenic risk score

Additional analysis

Full results from our additional analyses are discussed in Supplementary Notes. Briefly, modules showed moderate to large preservation in the Insight 46 subset of the NSHD (Supplementary Fig. 1). Following Bonferroni correction (module: $p < 3.57 \times 10^{-3}$; hub metabolite: $p < 1.06 \times 10^{-3}$), all modules and nine metabolites remained associated with an outcome, with 21 no longer reaching the adjusted level of significance. Further adjustment for life course factors resulted in minimal changes (Supplementary Tables 4 and 5).

Discussion

In a population-based cohort, we identified three modules of coexpressed lipids (phospholipids and DAGs, fatty acids, and sphingolipids) that were associated with whole-brain or hippocampal volume and 22 highly connected metabolites within these that present as potential markers for additional study. We report no significant metabolic associations for Aβ status following multiple testing correction, nor for AD polygenic risk in our genetic analyses, although some relationships were seen at the nominal level. Taken together, these findings highlight associations between lipids and later life brain structure, with no strong evidence to suggest relationships are specific to AD-related pathology (as measured through Aβ-PET and PRS).

Fatty acids in whole-brain volume

First, we found that higher expression of the blue module—enriched in several fatty acid pathways—was associated with smaller whole-brain volumes. We identified

eight hub metabolites in this module, belonging to fatty acid pathways (long chain PUFA (n3 and n6); monohydroxy; dicarboxylate; medium chain), as well as glycerol from the glycerolipid metabolism pathway. These pathways are tightly linked: fatty acids and glycerol constitute phospholipids and triglycerides, and dicarboxylate and monohydroxy fatty acids are oxidative products of PUFA and other fatty acids.

Upregulation of this module and hub metabolites may thus represent changes in lipid metabolism, including enhanced lipid breakdown, accumulation of free fatty acids and glycerol in the blood, and alterations in fatty acid oxidation—all of which have been linked to neurodegeneration and AD [31–33]. Worth noting, however, is that while n6 PUFA have been typically linked to risk of neurodegenerative disease, n3 have been linked to decreased risk [34], although both are an area of contention [35]. Here, we identified one hub (docosapentaenoate; DPA, 22:5n3) to be an n3 fatty acid, contrasting with the general consensus among the literature. We additionally observed some suggestive evidence of an association between AD genetic risk and PUFA, particularly with the *APOE* region removed; a higher AD PRS was associated with decreased levels of two long chain PUFA hubs (linolenate (18:3n3 or 3n6) and DPA), albeit only at a nominal significance level. These effect directions align with those reported in the literature but were in the unexpected direction based on findings in our imaging analysis. While it is not possible to draw definitive conclusions, we believe these results highlight the need for further work in larger, independent samples.

We further identified two module hubs (16-hydroxypalmitate and hexadecanedioate) which are products of microsomal omega-oxidation—a minor oxidation pathway for fatty acids. Accumulation thus points to defects in mitochondrial β-oxidation pathways, perhaps induced by an overload of free fatty acids or vice versa [36, 37]. Notably, defective β-oxidation and compensatory omega-oxidation pathways are thought to induce oxidative stress [38, 39]—a key mechanism linked to neurodegeneration, which is marked among other things by reduction in brain volume [40]. Nevertheless, to our knowledge, these metabolites have not been linked to brain health and neurodegeneration previously, although they have been found to play a role in other phenotypes, such as mortality and blood pressure [36].

Phospholipids and DAGs in whole-brain volume

Similar to the module of fatty acids, higher expression of the brown module—enriched in PEs and DAGs—associated with smaller whole-brain volumes. These pathways have key roles in membrane structure and cell signalling: PEs are a class of phospholipid which form important components of cellular membranes [41], and are a precursor to DAGs, which are components of cellular membranes and secondary messengers [42]. We reported associations between higher expression of this module and worse short-term memory in our previous analysis of cognitive function in the NSHD, although these were mostly explained by BMI and lipid medication [10]. Here, our results were independent of these factors with minimal attenuations overall.

Within the module, we identified four hub metabolites—two phospholipids and two DAGs. Both subclasses have been previously linked to AD and neurodegeneration [43, 44], and higher blood levels have been hypothesised to represent alterations in membrane integrity and subsequent degradation [45, 46], although some associations in the opposite direction have also been reported [32, 47]. Three module hubs were additionally associated with an AD PRS at the nominal level; this relationship appeared to be driven predominantly by *APOE* and may therefore reflect pleiotropic pathways. Again, these were in the opposite direction to our findings for whole-brain volume and did not survive multiple testing correction, and so should be interpreted with caution.

Sphingolipids in hippocampal volume and whole-brain volume

We highlighted associations between higher levels of sphingolipids, a lipid class that contain important constituents of cellular membranes [48], and larger hippocampal and whole-brain volumes. Our findings were observed for sphingomyelins in particular, a subclass that are especially abundant in the CNS, where they form pivotal components of neuronal membranes and play key roles in signal transduction [49]. Given their biological role, it is unsurprising that sphingolipids have been previously linked to brain health and pathology [50, 51]. Here, we report associations between a module enriched in sphingolipids and hippocampal volume, as well as several sphingomyelin hub metabolites and whole-brain volume.

We previously reported similar findings for sphingolipids and several cognitive outcomes; however, after we adjusted for childhood cognitive ability and education in particular, these were entirely attenuated [10]. Interestingly, we did not observe the same pattern here, with relationships showing negligible attenuations overall, although it is worth noting that childhood cognitive ability and education show much weaker associations with structural brain measures and thus less likely to confound associations. We hypothesised that attenuations could represent earlier relationships we are unable to capture without longitudinal metabolite data, shared genetic or environmental underpinnings, or confounding by reverse causation, i.e. increased

sphingolipid levels may be consequential to higher cognitive function in early life, and education may be capturing shared components. With no earlier measure of brain volume, the latter could extend to our present findings, although previous research has linked sphingomyelins to longitudinal markers of pathology [50, 52, 53]. Another possibility is that sphingolipids may have different involvements in cognitive function and later life brain volume, or may be particularly important during sensitive age periods, for example in cognitive development as well as during vulnerable periods in later life with regard to structural integrity [48]. Expanding this to longitudinal data, alongside interrogating relationships using MR, will allow for a greater insight into our findings.

Limited relationships were seen for Aβ status and AD polygenic risk

Interestingly, we saw limited metabolic relationships for Aβ status; no module showed associations ($p > 0.05$), and a handful of metabolites were associated at the nominal threshold only. Possibly, there are no robust associations between these metabolites and Aβ, or independent of *APOE*, although other work has reported differently [5, 7]. Alternatively, this may reflect power, particularly given the relatively young age of Insight 46 participants and the smaller sample that were Aβ PET-positive at this stage. Our measure of amyloid load (PET) reflects the deposition of fibrillar amyloid plaque; it is possible that measuring upstream, soluble forms of Aβ may both increase the numbers who are amyloid positive and allow for an exploration of whether different metabolic pathways are associated with different stages of β-amyloid formation. Further follow-up studies, including CSF measures of soluble Aβ burden, are planned. In addition, five metabolites were associated with an AD PRS at the nominal level, but none survived multiple testing correction, and no other metabolic associations were seen at either threshold. As modules and metabolites were selected for PRS analyses based on showing significant associations in our imaging analyses, which were observed for whole-brain or hippocampal volume, and not Aβ, this suggests that these metabolites may not be specific to AD. Alternatively, it may also reflect power; expanding our work in larger samples is warranted.

Strengths and limitations

Strengths of the study include age-matched cohort with information on a large range of confounders across the life course, including rarely available measures of cognitive development, as well as data on both imaging and subdomains of cognitive function. Moreover, the metabolomics data in this study represent a far more comprehensive proportion of the metabolome than in past clinical metabolomic studies of neuroimaging parameters [7, 53]. Nevertheless, the results of this study should be interpreted in the context of the following limitations. First, our findings may not extend to the general population. Study participants are all white and, compared to the full NSHD cohort, participants enrolled in Insight 46 were on average of slightly better self-rated health, cognitive ability, and SEP. Next, our findings may be subject to residual confounding; further study is needed to disentangle causal relationships. It is additionally possible that some metabolite degradation may have occurred despite storage at − 80 °C [54, 55]. However, strict quality control and best practices were implemented, and factors related to storage time and potential technical differences were adjusted. Finally, as there are currently few cohorts with genetic, serum LC–MS, and brain imaging data, we are not yet able to replicate our analyses elsewhere.

Conclusions

Our findings highlight relationships between groups of lipids and structural brain measures, as well as key metabolites within these that are likely to be driving associations. Future work should be directed towards understanding if these metabolites associate with longitudinal changes in brain volumes and whether relationships are causal; this could advance our understanding of brain health and neurodegeneration and reveal possible targets of intervention.

Abbreviations

Aβ	Amyloid-beta
AD	Alzheimer's disease
BMI	Body mass index
DAG	Diacylglycerol
EDTA	Ethylenediaminetetraacetic acid
FDR	False discovery rate
FLAIR	Weighted fluid-attenuated inversion recovery
KEGG	Kyoto Encyclopaedia of Genes and Genomes
kME	Module membership
LCMS	Liquid chromatography-mass spectrometry
MR	Mendelian randomisation
n3 and n6	Omega 3 and 6
NSHD	National Survey of Health and Development
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PRS	Polygenic risk score
PUFA	Polyunsaturated fatty acid
QC	Quality control
SEP	Socioeconomic position
SUVR	Standardised uptake volume ratio
WGCNA	Weighted gene coexpression network analysis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-023-01184-y.

Additional file 1: Supplementary file 1: Table 1. Full hub metabolite results (models 1-3).

Additional file 2: Supplementary file 2: Table 2. Full module analysis results (models 1-3).

Additional file 3: Supplementary file 3: Table 3. PRS results.

Additional file 4: Supplementary file 4: Table 4. Associations between metabolites, modules, outcomes and lifestyle factors.

Additional file 5: Supplementary file 5: Table 5. Associations between metabolites and modules and whole-brain volume following adjustment for smoking and alcohol intake.

Additional file 6: Supplementary file 6: Figure 1. A. Plot showing the Zsummary preservation statistics for each module against the module size. Thresholds for moderate and strong evidence of module preservation are indicated. B. Plot showing the median rank for module preservation. Modules are ranked from most preserved (lowest number) to least preserved (highest number).

Additional file 7: Supplementary file 7: Supplementary Notes. Supplementary Notes (metabolomics acquisition, metabolomic and genomic quality control, polygenic risk scores, software and packages used, additional analyses, participant characteristics split by amyloid status).

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Authors' contributions

RG, JMS, MR, and PP designed and set up the research and were responsible for the manuscript. RG performed the primary data analysis and wrote the first draft of the manuscript. MAS, AW, SNJ, DMS, TDP, CAL, IBM, DMC, CHS, WC, DLT, SK, NCF, JMS, MR, and PP were involved in data acquisition and generation. RG, JL, JX, AH, LG, MR, and PP provided input on data analysis and interpretation. All authors read and approved the final manuscript.

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Availability of data and materials

Anonymised data are available upon request to bona fide researchers (https://skylark.ucl.ac.uk/NSHD/doku.php). Analytical code can be accessed at https://github.com/beckigreen/metabolites-brain-ad-prs.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the National Research Ethics Service Committee London (14/LO/1173). The study was conducted in accordance with the Declaration of Helsinki, and all participants provided written informed consent.

Consent for publication

Not applicable.

Competing interests

MAS is currently a full-time employee of F. Hoffman-La Roche AG. However, her contributions to this research precede in time and are unrelated to her present employment. J.M.S. has received research funding from Avid Radiopharmaceuticals (a wholly owned subsidiary of Eli Lilly), has consulted for Roche Pharmaceuticals, Biogen, Merck, and Eli Lilly, given educational lectures sponsored by GE Healthcare, Eli Lilly, and Biogen. He is Clinical Advisor to UK DRI and Chief Medical Officer for ARUK.

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