

RESEARCH ARTICLE

Differences in plasma metabolites related to Alzheimer's disease, *APOE* ϵ 4 status, and ethnicity

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Abstract

Introduction: We investigated metabolites in plasma to capture systemic biochemical changes associated with Alzheimer's disease (AD).

Methods: Metabolites in plasma were measured in 59 AD cases and 60 healthy participants of African American (AA), Caribbean Hispanic (CH), and non-Hispanic white (NHW) ancestry using untargeted liquid-chromatography-based ultra-high-resolution mass spectrometry. Metabolite differences between AD and healthy, ethnic groups and apolipoprotein E gene (*APOE*) ϵ 4 status were analyzed. Untargeted network analysis identified pathways enriched in AD-associated metabolites.

Results: A total of 5929 annotated metabolites were measured. Partial least squares discriminant analysis (PLS-DA) inferred that AD clustered separately from healthy controls (area under the curve [AUC] = 0.9816); discriminating pathways included glycerophospholipid, sphingolipid, and non-essential amino acid (alanine, aspartate, glutamate) metabolism. Metabolic features in AA clustered differently from CH and NHW (AUC = 0.9275), and differed between *APOE* ϵ 4 carriers and non-carriers (AUC = 0.9972).

Discussion: Metabolites, specifically lipids, were associated with AD, *APOE* ϵ 4, and ethnic group. Metabolite profiling can identify perturbed AD pathways, but genetic and ancestral background need to be considered.

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KEYWORDS

Alzheimer's disease, APOE ϵ 4, metabolite profile differences in Alzheimer's disease, metabolomics, multi-ethnic, plasma

1 | INTRODUCTION

The understanding of the pathogenesis of Alzheimer's disease (AD) is incomplete. Genetic and molecular mechanisms have been proposed, but no single gene variant or molecular mechanism can fully explain the complexity of this disorder. Thus, it is likely that genetic variants affect downstream metabolic pathways. Finding systemic molecular changes between disease and healthy states could identify biological mechanisms, potentially leading to early diagnosis and therapeutic development and possibly successful interventions.

Metabolomics is a method of deep molecular phenotyping that represents the underlying biochemical and physiological layer of the genome, transcriptome, and proteome. Metabolomics provides a precise and comprehensive analysis of phenotypic abnormalities in which the individual components are physiologically described. This snapshot of the metabolic state of individuals is possible due to advances in high-resolution mass spectrometric platforms with high sensitivity and specificity¹ that can strengthen the study at the human population level. For this study, we conducted untargeted metabolomics profile analyses in plasma to identify endogenous and exogenous metabolites associated with clinical AD, and to identify differences by racial/ethnic group and APOE genotype.

2 | METHODS

2.1 | Participants

The Washington Heights, Inwood Columbia Aging Project (WHICAP) has recruited a representative community-based group of individuals age 65 years or older through a collaborative effort with the Centers for Medicare and Medicaid and through the use of marketing rolls. The composition of the cohort reflected the community surrounding Columbia University Medical Center and comprises non-Hispanic whites (24%), African Americans (28%), and Caribbean Hispanics (48%), and 67% of the cohort are women. After obtaining informed consent, participants were interviewed in either English or Spanish. During each assessment, participants received a standardized neuropsychological test battery and medical interview. Blood was drawn, bar-coded, and brought to the laboratory within 2 hours of collection for DNA extraction and storage of plasma and serum. All of the medical, neurological, psychiatric, and neuropsychological data collected were reviewed at a consensus conference by clinicians who are experienced in the diagnosis of AD and related dementias. Diagnosis of clinical AD was based on accepted criteria.²

For this investigation, fresh plasma was obtained, processed, and frozen within 2 hours from individuals who had been evaluated at least twice to ensure stability of the clinical diagnosis AD and the lack of dementia in healthy controls. These individuals were equally divided between the three ethnic groups: African American, Caribbean Hispanic, and non-Hispanic white, and between cases and controls (Table 1).

2.2 | Metabolomics acquisition and analyses

We acquired metabolites by conducting untargeted metabolomics from the plasma in 119 cases and controls from the WHICAP cohort using a previously standardized method.³ Untargeted LC-based ultra-high-resolution mass spectrometry (LC-UHRMS) allowed deep phenotyping of the human metabolome, providing measures of metabolites in most Kyoto Encyclopedia of Genes and Genomes (KEGG) human metabolic pathways.⁴ After chromatographic separation using methods previously described,⁵ each sample was injected on each column (HILIC with positive electrospray ionization [ESI] and C18 with negative ESI) three times, to obtain three technical replicates per column. Data were processed through a computational pipeline that leverages open-source feature detection and peak alignment software, apLCMS⁶ and xMSanalyzer,⁷ to create a feature table with the mass-to-charge (m/z) ratio, retention time, and the abundance of each ion in each sample.⁶⁻⁸ The feature table was used for statistical analyses followed by untargeted pathway analysis using the software Mummichog.⁸ The LC-UHRMS platform detects >1500 chemical signals that arise from core nutrient metabolism, lipids, the microbiome, diet-derived chemicals, pharmaceuticals, and environmental contaminants, as well as >100,000 untargeted features.⁹ For simplicity, we refer to the mass spectral features as metabolites. Results described as annotations refer to level 4 and level 5 confidence of feature identification by criteria of Schymanski et al.¹⁰ Results described as identifications refer to level 1 confidence (accurate mass, retention time, and MS/MS fragmentation matching authentic standards).

2.3 | Metabolomics data analysis

Raw metabolite intensities were corrected for batch effects using ComBat.¹¹ Metabolites missing in >30% of the samples were excluded from further analysis. Metabolite values missing or of low quality were imputed to half the value of the lowest level of detection for each

metabolite.¹² Intensities were quantile normalized, log-transformed, and auto-scaled to normalize distributions.¹³

Principal component analyses (PCA) of normalized values were used to estimate stratification by ethnic/racial group, disease status, and APOE ϵ 4. Partial least squares discriminant analysis (PLS-DA) implemented in the mixOmics¹⁴ package was used to conduct supervised dimension reduction analysis to obtain a linear combination of the features that correlated with variability by ethnic/racial group, disease status, and APOE ϵ 4 in the metabolomics data.

We tested the association of individual metabolites with AD adjusting for age, sex, APOE ϵ 4, and ethnic/racial group. In addition, we tested the association of these metabolites with AD independently in each ethnic/racial group (defined by PCAs and ancestry analysis of GWAS data) to identify both ancestry-specific and population-based metabolomics signatures in AD. Then, we tested the association of APOE ϵ 4 with metabolite intensities, adjusting for age and sex. For association testing of independent metabolites, we declared nominal significance at $P < .05$. Nominally significant metabolites were subsequently tested for pathway enrichment. Pathways enriched for metabolites associated with traits of interest were detected using module-enrichment analysis implemented in mummichog.⁸ Statistical significance of enriched pathways was determined using a permutation test adjusting for multiple testing.^{15,16}

3 | RESULTS

Untargeted metabolomics profiles were completed in 119 individuals, including 40 African American, 40 Caribbean Hispanic, and 39 non-Hispanic white from the WHICAP cohort. Using the HILIC column with positive electrospray ionization (ESI), we identified >9700 features, of which 5929 were putatively annotated using xMSannotator. The features did not show batch-related variation, and a total of 6375 features were present in at least 70% of the samples studied, of which 1704 metabolites were annotated with xMSannotator confidence level 2 or 3. Using the C18 column with negative ESI, we measured >6700 features, of which 3759 were present in at least 70% of the samples.

3.1 | Metabolomic profiles of AD

We compared metabolites between AD and controls adjusted for age, sex, and ethnic/racial group, and we found several metabolic features nominally associated with AD (uncorrected $P < .05$) measured on both columns (Figure 1A, Table S2) as well as unique metabolic profiles (Table 2).

Although none of the individual metabolites reached experiment-wide significance after correcting for multiple testing, we identified several metabolites annotated with a confidence level 2 by xmsannotator that were associated with AD at $P < 10e-03$ (Table 2). In particular, we identified benzyl chloride, various adducts of benzenes and toluenes, omega 3 fatty acids, ceramide, and carnitines that are associated with AD.

RESEARCH IN CONTEXT

1. Systematic review: A systematic review of the literature (PubMed and Web of Science) revealed few studies with limited sample size studying unbiased metabolomics assays in Alzheimer's disease (AD). Sphingolipid, amino acid, and lipid metabolism were enriched in AD, but validation and replication were limited.
2. Interpretation: We conducted an unbiased plasma-metabolomics assay in a multi-ethnic population from the Washington Heights, Inwood, Columbia Aging Project (WHICAP). In addition to disease-specific profiles, we identified differences in metabolomics profiles by ethnic group and by apolipoprotein E gene (APOE) ϵ 4. We found several novel pathways enriched in metabolites associated with AD and confirmed previous findings in sphingolipid and amino acid metabolism pathways.
3. Future directions: Our results suggest that studies need to consider genetic and ancestral background to fully understand the metabolic heterogeneity in AD pathogenesis. Differences in ethnic group and genetic background will be needed to be fully investigated in order to identify robust profiles associated with AD.

In addition, features from the HILIC-positive data indicated that altered polyunsaturated fatty acid biosynthesis, alanine, aspartate, glutamate, glycerophospholipid, and sphingolipid metabolism (Figure 1C) were also associated with AD metabolome-wide. Data from the C18-negative column implicated altered glycolysis and gluconeogenesis, pyruvate, alanine, and aspartate metabolism in cases versus controls of AD. Individual metabolites that contributed to enrichment of these pathways are described in the Supplementary Material.

TABLE 1 Demographic characteristics of cases and controls analyzed in the study

	Cases	Controls
Caucasian(N)	19	20
Age	89.21301	93.00000
Sex (% female)	79%	60%
APOE allele frequency (%)	13.2	2.5
African American (N)	20	20
Age	86.38012	91.05000
Sex (% female)	80%	90%
APOE allele frequency (%)	30	17.5
Hispanic (N)	20	20
Age	83.80178	91.55000
Sex (% female)	85%	90%
APOE allele frequency (%)	15	10

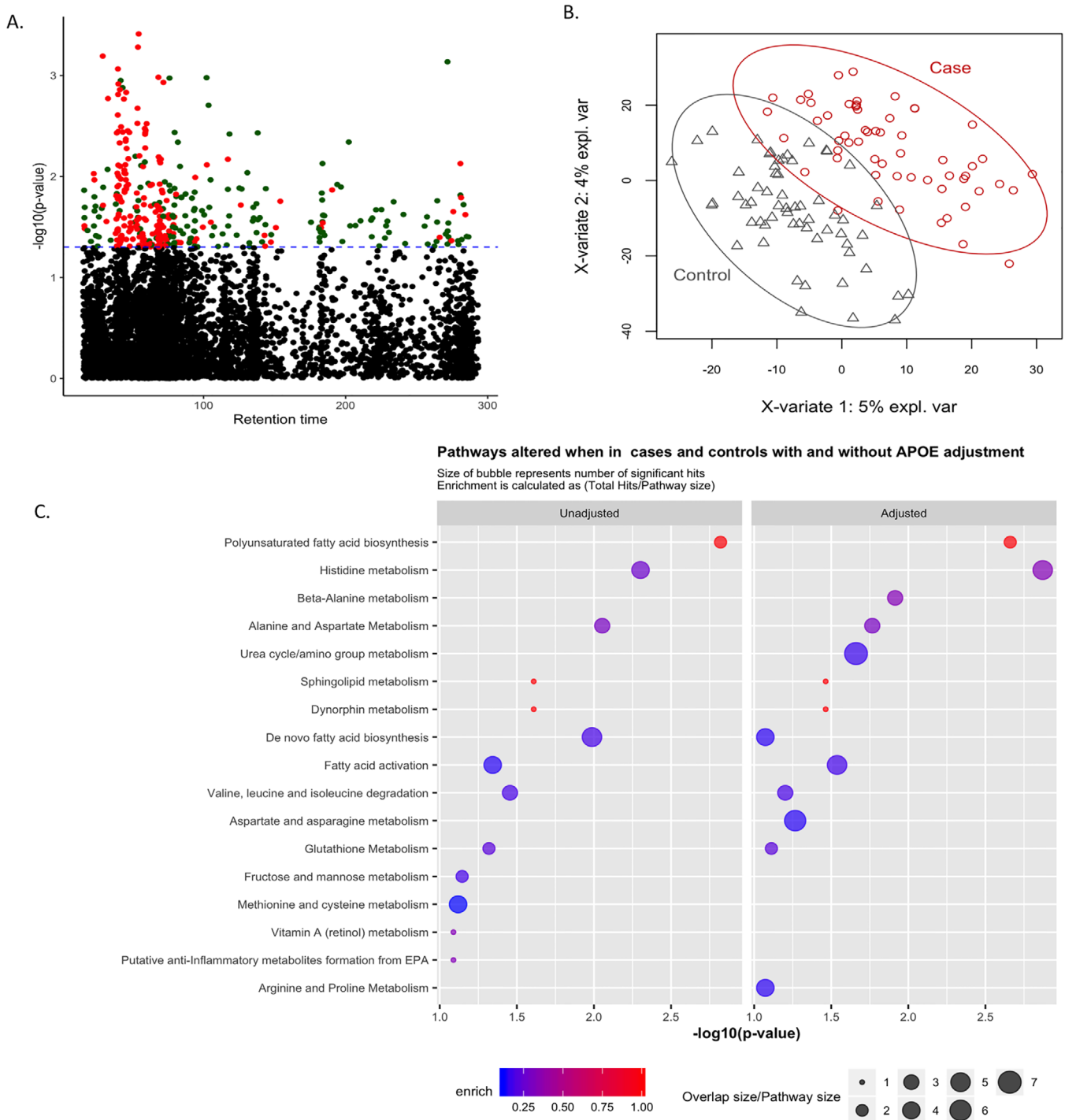


FIGURE 1 Metabolome wide association study in cases and controls of AD. (A) A metabolome wide association study (MWAS) found 382 features altered in cases and controls at nominal significance of $P < .05$. In red: features lower in cases, in green features higher in cases: (B). A PLS-DA shows metabolomic patterns different between cases and controls (unadjusted for age and sex variables). (C) Pathway analysis using mummichog shows most probable metabolic pathways altered between cases and controls. We tested enrichment of pathways among metabolites that were nominally significant in association with AD ($P < .05$) in models unadjusted and adjusted for APOE, respectively. Displayed pathways are enriched at $P < .05$, corrected for multiple testing

3.2 | Metabolomic differences by ethnic/racial group

Within each ethnic/racial group, we found that African Americans with AD had altered glycolysis and amino acid metabolism as well as

polyunsaturated fatty acid metabolism. Non-Hispanic whites with AD had altered amino acid, fatty acid, and glycosphingolipid metabolism, and Caribbean Hispanic cases had altered amino acid metabolism (Figure 2). When restricting the analysis to controls, we that

TABLE 2 Individual metabolites associated with AD status and annotated with a confidence score > 2

<i>m/z</i>	Time	BETA	P	chemical_ID	Match Category	Theoretical <i>m/z</i>	delta_ppm	Name	Formula	Monoisotopic mass
127.03	53.80	-0.82	5.23E-04	HMDB59882	Unique	127.03	7.08	Benzyl chloride	C7H7Cl	126.02
121.10	271.70	0.79	7.31E-04	HMDB59877	Multiple	121.10	0.17	Propylbenzene	C9H12	120.09
				HMDB59819				o-Ethyltoluene	C9H12	120.09
				HMDB13733				124-Trimethylbenzene		
				HMDB40458				124-Tris (methylene) cyclohexane		
				HMDB59901				Hemimellitene		
				HMDB41924				Mesitylene		
594.58	68.20	-0.72	1.04E-03	HMDB59848	Unique	594.58	0.62	m-Ethyltoluene		
				HMDB34029				Isopropylbenzene		
				HMDB04951				Ceramide (d18:120:0)	C38H75NO3	593.57
291.20	41.60	0.72	1.12E-03	HMDB31098	Multiple	291.20	1.27	(9Z11E13E15Z)-4-Oxo-9111315-octadecatetraenoic acid	C18H26O3	290.19
302.23	41.10	-0.79	1.38E-03	HMDB13288	Multiple	302.23	0.93	Nonanoylcarnitine	C16H31NO4	301.23
				HMDB06320				26-Dimethylheptanoyl carnitine		

determined underlying metabolic differences in participants based on their ethnic/racial group were not driven by the presence of disease (Figure 3A, Table S1).

Compared with Caribbean Hispanics and non-Hispanic whites, PLS-DA suggested that features in African Americans clustered differently (Figure 3), including altered amino acid metabolic pathways such as arginine and proline metabolism, aspartate and asparagine metabolism, and glycine, serine, alanine, and threonine metabolism. Saturated fatty acid beta oxidation was also identified based on variable importance in the projection (VIP) scores from the PLS-DA (Figure 3).

3.3 | Metabolomic profiles associated with the APOE genotype

We also investigated metabolic differences driven by APOE ϵ 4. PLS-DA identified near-complete separation between APOE ϵ 4 carriers and non-carriers (Table S3). In contrast to pathways identified by AD diagnosis, the presence of APOE ϵ 4 was associated with changes in arachidonic acid metabolism, driven by features with putative matches to octadecatrienoic acid and eicosatrienoic acid (Figure 4A and B), changes in the pentose phosphate pathway, and in chondroitin sulfate. After adjusting for APOE ϵ 4, the pathways associated with AD were largely the same (Figure 1C).

4 | DISCUSSION

We compared untargeted metabolomics in patients with AD and healthy controls, equally divided between African Americans, Caribbean Hispanics, and non-Hispanic whites. The goal of this investigation was to identify metabolites associated with disease while adjusting for the strongest genetic risk factor, APOE status, and ethnic/racial group. Therefore, we used a simple case-control design equally stratified by ethnic/racial group.

We found significant differences comparing exogenous and endogenous metabolites in patients with AD and healthy controls. Similar differences were observed in a comparison of metabolic profiling in brain tissue from the middle frontal and inferior temporal gyri in healthy controls, patients with AD, and those with mild cognitive impairment (MCI) in the Baltimore Longitudinal Study.¹⁷ An untargeted approach has also been used to correctly classify patients with MCI or AD when compared with controls with 97.7% accuracy.¹⁸ However, metabolite analyses identified 22 putative biochemical pathways, including interlinked areas of metabolism (polyamine metabolism and L-arginine metabolism).

In this report, we identified several metabolites associated with AD that were altered in the fatty acid biosynthesis pathway. Of interest, dysregulation of unsaturated fatty acid metabolism was previously reported in brain tissue from patients with AD compared to healthy controls.¹⁷ The disruption in polyunsaturated fatty acid biosynthesis may have several downstream effects: inflammation, oxidative stress,

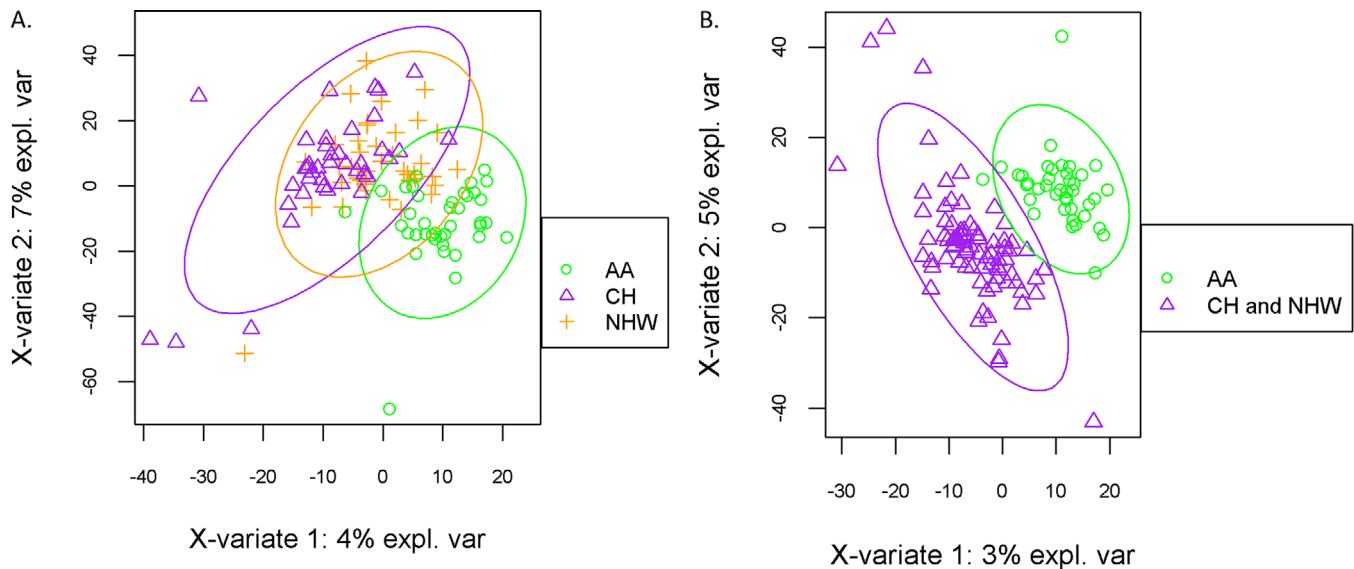


FIGURE 2 Ethnic differences. A bubble plot that shows pathways altered in controls of the three ethnic groups. Size of bubbles is proportional to overlap size of pathway and *P*-values are corrected for multiple testing

cell maintenance, or cell death. Several amino acid metabolism-related pathways were also enriched among metabolites that were associated with risk of AD. Lower levels of glutamate and aspartate, and higher levels of glutamine have been observed in the temporal cortex in postmortem samples from patients with AD.¹⁹ A different cohort also showed elevations in plasma levels of glutamine in AD patients.²⁰ We identified metabolites involved in dynorphin metabolism associated with AD, consistent with previous studies in postmortem samples from patients.²¹ Expression of dynorphin, an endogenous opioid peptide, increases with age and has been associated with cognitive impairment in rodent models.²²

We found ceramide to be significantly associated with AD, and it has been suggested that ceramide promotes neuronal apoptosis, $A\beta$ accumulation,²³ and has been seen at increased levels in patients with AD and co-morbid conditions.²⁴ In addition, lower levels of plasma acylcarnitine were found in AD patients compared to controls. Plasma acylcarnitines predict altered fatty acid beta oxidation and ketogenesis and have been reported to be depressed in patients with AD.²⁵ Metabolic features also differed significantly in the three ethnic/racial groups, especially among African Americans, despite the individuals being of similar in age and residing in the same geographic location. Very few studies have investigated the overall metabolic differences by ethnicity/race. Investigators from the Women's Health Initiative cohort identified significant differences in metabolite profiles between African Americans and individuals of European ancestry (https://www.ahajournals.org/doi/abs/10.1161/circ.139.suppl_1.MP55). Similar differences in amino acid and lipid metabolism were observed in a metabolic profiling study of serum metabolites in African American and European American patients with bladder cancer.²⁶ Thus, genetic and metabolic factors could help to explain ethnic/racial and racial disparities in the risk factors associated with AD. Similar pathways emerge when we assess difference by AD or ethnic/racial group, suggesting that

metabolome profiling from plasma could be used to identify racial differences in disease.

Differences between *APOE* $\epsilon 4$ carriers and non-carriers were apparent and remained when analyses were restricted to healthy individuals from all three ethnic/racial groups. Of interest, metabolites associated with AD were different from those associated with *APOE* $\epsilon 4$. Hexose phosphorylation and arachidonic acid metabolism pathways were most enriched among metabolites that were associated with *APOE* $\epsilon 4$. *APOE* $\epsilon 4$ carriers converting to MCI/AD had higher arachidonic acid /docosahexaenoic acid ratios in phospholipids compared to cognitively normal $\epsilon 4$ and non- $\epsilon 4$ carriers.²⁷ Alterations in plasma arachidonic acid were observed in the brains of $\epsilon 4$ carrier mice compared to non-carriers.²⁷

Taken together these results suggest that understanding of metabolic heterogeneity in AD pathogenesis may enable identification of biological mechanisms for specific subgroups with the disease. Namely, those carrying an *APOE* $\epsilon 4$ allele and among those of African or Hispanic ancestry. This study demonstrates the ability of untargeted metabolomics to reveal biochemical differences in plasma based on ethnicity/race, the presence of *APOE* $\epsilon 4$, and AD, thus informing study design for optimal power of discovery in larger studies.

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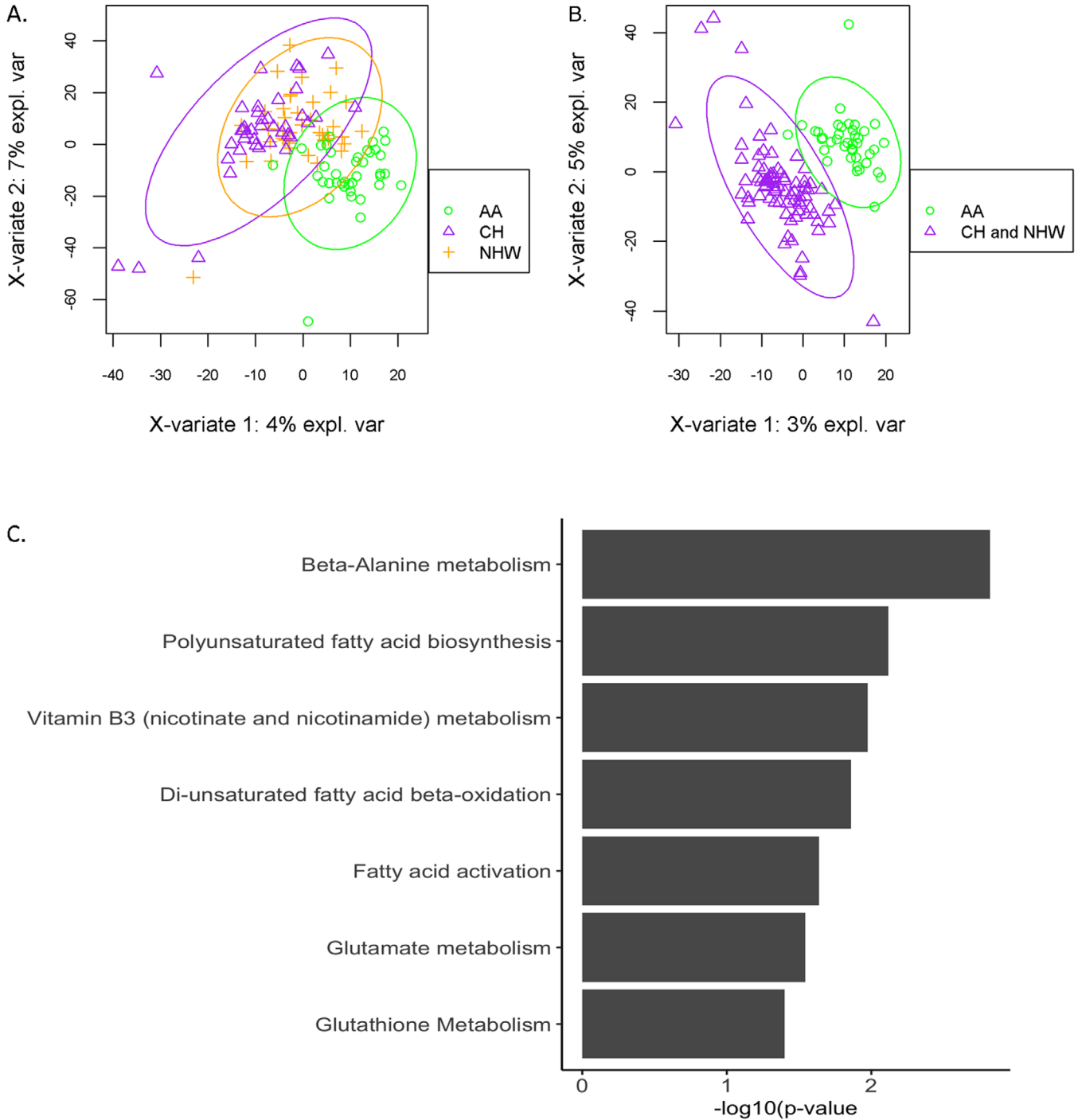


FIGURE 3 ***African Americans versus others. (A) A PLS-DA with all ethnic groups shows a African Americans differ from the Caucasian and Hispanic individuals. (B) A PLS-DA the shows differences when comparing AAs with all others. (C) Pathways analysis of features that are significantly different in cases and controls of African Americans

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

REFERENCES

1. YoshidaM, HatanoN, NishiumiS, et al. Diagnosis of gastroenterological diseases by metabolome analysis using gas chromatography-mass spectrometry. *J Gastroenterol.* 2012;47(1):9-20.
2. McKhannGM, KnopmanDS, ChertkowH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic

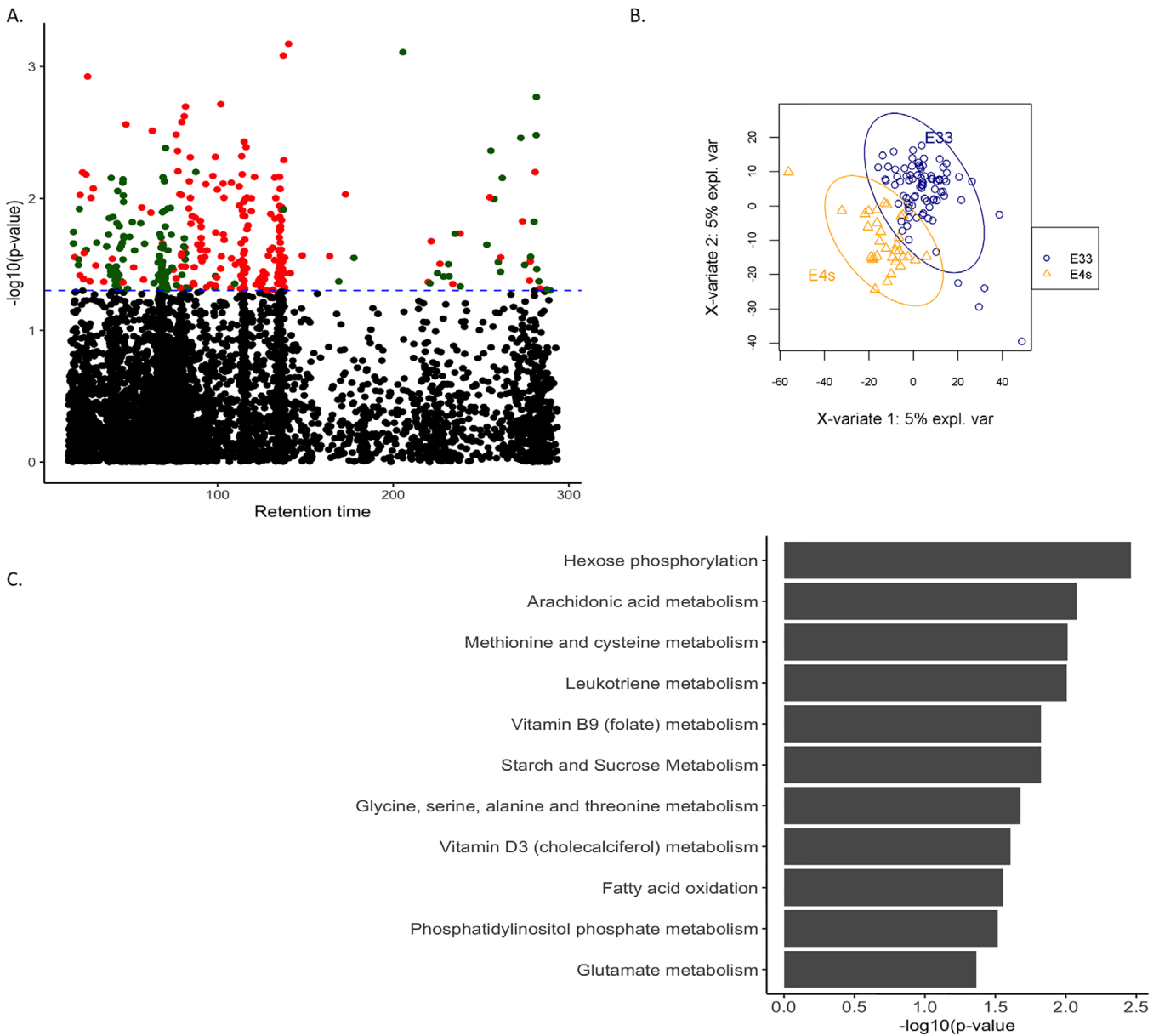


FIGURE 4 APOE status and metabolic differences. (A) MWAS shows features associated with APOE status. (B) PLS-DA showing separation between APOE $\epsilon 4$ carriers and non-carriers. (C) Metabolic pathways enriched in metabolites that are associated with APOE $\epsilon 4$ status at a nominal significance of $P < .05$

guidelines for Alzheimer's disease. *Alzheimer's Dement.* 2011;7(3):263-269.

- SoltowQA, StrobelFH, MansfieldKG, WachtmanL, ParkY, JonesDP. High-performance metabolic profiling with dual chromatography-Fourier-transform mass spectrometry (DC-FTMS) for study of the exposome. *Metabolomics.* 2013;9(1 suppl):S132-S143.
- JonesDP, ParkY, ZieglerTR. Nutritional metabolomics: progress in addressing complexity in diet and health. *Ann Rev Nutr.* 2012;32:183-202.
- LiuKH, WalkerDI, UppalK, et al. High-resolution metabolomics assessment of military personnel: evaluating analytical strategies for chemical detection. *J Occup Environ Med.* 2016;58(8 suppl 1):S53-S61.
- YuT, ParkY, JohnsonJM, JonesDP. apLCMS-adaptive processing of high-resolution LC/MS data. *Bioinformatics.* 2009;25(15):1930-1936.

- UppalK, SoltowQA, StrobelFH, et al. xMSanalyzer: automated pipeline for improved feature detection and downstream analysis of large-scale, non-targeted metabolomics data. *BMC Bioinformatics.* 2013;14:15.
- LiS, ParkY, DuraisinghamS, et al. Predicting network activity from high throughput metabolomics. *PLoS Comput Biol.* 2013;9(7):e1003123.
- JohnsonJM, YuT, StrobelFH, JonesDP. A practical approach to detect unique metabolic patterns for personalized medicine. *Analyst.* 2010;135(11):2864-2870.
- SchymanskiEL, JeonJ, GuldeR, et al. Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ Sci Technol.* 2014;48(4):2097-2098.
- JohnsonWE, LiC, RabinovicA. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics.* 2007;8(1):118-127.

12. MenniC, FaumanE, Ertel, et al. Biomarkers for type 2 diabetes and impaired fasting glucose using a nontargeted metabolomics approach. *Diabetes*. 2013;62(12):4270-4276.
13. van denBergRA, HoefslootHC, WesterhuisJA, SmildeAK, van derWerfMJ. Centering, scaling, and transformations: improving the biological information content of metabolomics data. *BMC Genomics*. 2006;7:142.
14. RohartF, GautierB, SinghA, Le CaoKA. mixOmics: an R package for 'omics feature selection and multiple data integration. *PLoS Comput Biol*. 2017;13(11):e1005752.
15. BerrizGF, KingOD, BryantB, SanderC, RothFP. Characterizing gene sets with FuncAssociate. *Bioinformatics*. 2003;19(18):2502-2504.
16. HosackDA, DennisG, Jr, ShermanBT, LaneHC, LempickiRA. Identifying biological themes within lists of genes with EASE. *Genome Biol*. 2003;4(10):R70.
17. SnowdenSG, EbshianaAA, HyeA, et al. Association between fatty acid metabolism in the brain and Alzheimer disease neuropathology and cognitive performance: a nontargeted metabolomic study. *PLoS Med*. 2017;14(3):e1002266.
18. GrahamSF, ChevallierOP, ElliottCT, et al. Untargeted metabolomic analysis of human plasma indicates differentially affected polyamine and L-arginine metabolism in mild cognitive impairment subjects converting to Alzheimer's disease. *PLoS One*. 2015;10(3):e0119452.
19. GueliMC, TaibiG. Alzheimer's disease: amino acid levels and brain metabolic status. *Neurol Sci*. 2013;34(9):1575-1579.
20. NiedzwieckiM, WalkerD, HowellCJ, et al. High-resolution metabolomic profiling of Alzheimer's disease in plasma. *Ann Clin Transl Neurol*. 2019. In Press.
21. YakovlevaT, MarinovaZ, KuzminA, et al. Dysregulation of dynorphins in Alzheimer disease. *Neurobiol Aging*. 2007;28(11):1700-1708.
22. MenardC, HerzogH, SchwarzerC, QuirionR. Possible role of dynorphins in Alzheimer's disease and age-related cognitive deficits. *Neurodegener Dis*. 2014;13(2-3):82-85.
23. Jazvinscak JembrekM, HofPR, SimicG. Ceramides in Alzheimer's disease: key mediators of neuronal apoptosis induced by oxidative stress and abeta accumulation. *Oxid Med Cell Longev*. 2015;2015:346783.
24. FilippovV, SongMA, ZhangK, et al. Increased ceramide in brains with Alzheimer's and other neurodegenerative diseases. *J Alzheimers Dis*. 2012;29(3):537-547.
25. CiavardelliD, PirasF, ConsalvoA, et al. Medium-chain plasma acylcarnitines, ketone levels, cognition, and gray matter volumes in healthy elderly, mildly cognitively impaired, or Alzheimer's disease subjects. *Neurobiol Aging*. 2016;43:1-12.
26. VantakuV, DonepudiSR, PiyarathnaDWB, et al. Large-scale profiling of serum metabolites in African American and European American patients with bladder cancer reveals metabolic pathways associated with patient survival. *Cancer*. 2019;125(6):921-932.
27. AbdullahL, EvansJE, EmmerichT, et al. APOE epsilon4 specific imbalance of arachidonic acid and docosahexaenoic acid in serum phospholipids identifies individuals with preclinical Mild Cognitive Impairment/Alzheimer's Disease. *Aging (Albany NY)*. 2017;9(3):964-985.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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