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Nuclear Magnetic Resonance Analysis Implicates Sex-Specific Dysregulation of the Blood Lipids in Alzheimer's Disease: A Retrospective Health-Controlled Study

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Objective The aging demographic landscape worldwide portends a heightened prevalence of neurodegenerative disorders. Foremost among these is Alzheimer's disease (AD), the foremost cause of dementia in older adults. The shortage of efficacious therapies and early diagnostic indicators underscores the imperative to identify non-invasive biomarkers for early detection and disease monitoring. Recently, blood metabolites have emerged as promising candidates for AD biomarkers.

Methods Leveraging nuclear magnetic resonance (NMR) spectroscopy on plasma specimens, we conducted a cross-sectional study encompassing 35 AD patients and 35 age-matched healthy controls. Cognitive function was evaluated using the mini-mental state examination in all participants, followed by peripheral blood sample collection. We utilized univariate and multivariate analyses to perform targeted lipidomic profiling via NMR spectroscopy.

Results Our study revealed significant differences in the expression profiles of low-density lipoprotein-associated subfractions in females and high-density lipoprotein-associated subfractions in males between AD patients and healthy controls (all $p<0.05$). However, there was no significant metabolite overlap between males and females. Furthermore, receiver operating characteristic curve analysis demonstrated that the combination of lipid metabolites had good diagnostic values (all area under the curve>0.70; $p<0.05$).

Conclusion Our findings suggest that the blood plasma samples using NMR hold promise in distinguishing between AD patients and healthy controls, with significant clinical implications for advancing AD diagnostic methodologies.

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Keywords Alzheimer; Lipoproteins; Nuclear magnetic resonance spectroscopy; Biomarkers.

INTRODUCTION

Alzheimer's disease (AD), the most prevalent form of dementia, is a neurodegenerative condition characterized by cognitive decline and progressive memory impairment.[1] Projections from the World Health Organization indicate that in the forthcoming decades, neurodegenerative disorders will surpass cardiovascular diseases as the leading cause of mor-

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tality in developed nations.[2] Notably, women are disproportionately affected by AD, constituting nearly two-thirds of AD cases in the United States.[3-5] The wealth of preclinical and clinical data underscores a heightened intrinsic risk of AD among women. Thus, further investigations into the underlying biological mechanisms driving this sex disparity are warranted to enhance our understanding of AD pathogenesis across sex.[6]

The major neuropathological hallmark of AD is the extracellular deposits of amyloid protein and neurofibrillary tangles. Current clinical diagnosis of AD focuses on the measurement of total tau or phosphorylated tau and amyloid-β in cerebrospinal fluid (CSF), positron emission tomography (PET) imaging of brain amyloid, or inflammation, structural imaging of the hippocampus with magnetic resonance imaging (MRI), and neuropsychological tests.[7,8] However, both PET imaging and CSF measurements have significant hurdles. They are expensive, time-consuming, invasive, and have limited availability. Therefore, there is an excellent need for easily accessible, non-invasive, and cost-effective biomarkers, preferably blood tests.

Blood is a complex tissue containing many organ proteins and is easily collected via non-invasive and safe procedures. Therefore, blood is a valid potential source for screening biomarkers.[9-11] Dysfunction of blood-brain barrier permeability has been reported in AD, implying that protein alterations may occur between the brain and peripheral bloodstream.[12] Cerebral lipids comprise at least 50% of dry brain weight and are the essential structural components of neuronal cell membranes.[13] Accumulating evidence has demonstrated that cerebral lipid peroxidation was an early event in AD. Abundant lipid particles are present in the brain glial cells of AD patients, indicating abnormal lipid metabolism.[14] Numerous blood metabolomic studies have highlighted the role of lipid compounds, such as phosphatidylcholines, in AD. Blood metabolites have been surveyed intensively as promising AD biomarkers and may help identify new therapeutic targets.[8,15-17]

The lipidomics and metabolomics approach in biomedical research can be based on diverse analytical platforms for lipid/metabolite profiling, the two leading analytical strategies being nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS).[18] NMR spectroscopy has accurately measured the subclass distribution, size, and number of circulating lipoprotein particles.[19,20] NMR spectroscopy has rarely been used in "omic" AD-related studies.[21-23]

To our knowledge, there is still a lack of studies on sex differences in metabolites associated with lipid subfractions in AD. Therefore, in this study, we aimed to present our NMR studies of human blood plasma samples obtained from AD patients and healthy controls, emphasizing the disease-specific sex differences in plasma lipid metabolism levels. In particular, we hypothesized that AD patients would have unique lipid metabolism changes than healthy controls.

METHODS

Participants

We recruited older people with AD from Tianjin Anding Hospital (Tianjin Mental Health Center) and geriatric health from communities in Tianjin City. Participants were interviewed for diagnosis, demographic and clinical data, laboratory examination, and cognitive tests. The experimental group included 35 cases, and the health control included 35 cases. The inclusion and exclusion criteria for the participants were as follows: the experimental group was defined by 1) the initial diagnosis of AD is based on the clinical criteria of the National Institute on Aging and Alzheimer's Association.[24] The clinical standard is mainly based on the core clinical symptoms of patients, combined with a physical examination of the nervous system and mental and psychological examinations for diagnosis, 2) age ≥50 years, male and female, 3) mini-mental state examination (MMSE) total score <24, and 4) informed consent signed by the patient or guardian. Exclusion criteria were 1) severe or acute medical illness within the three months preceding the study and 2) neurological disorders such as delirium, Parkinson's disease, aphasia, or multiple sclerosis.

The health control criteria included 1) age ≥50 years, male and female, 2) MMSE total score ≥24, and 3) capacity to provide informed consent. Exclusion criteria were 1) severe or acute medical illness within the 3 months preceding the study, 2) neurological disorders such as dementia, delirium, Parkinson's disease, aphasia, or multiple sclerosis.

The study had received ethics approval from the Tianjin Anding Hospital (Approval Number: 2020-01), and all participants or guardians provided written informed consent.

Blood collection

Fasting morning peripheral blood samples were collected from all participants using ethylenediaminetetraacetic acid anticoagulant tube. Plasma was obtained by centrifugation at 1,000 rpm for 10 min. All samples were stored at -80°C until NMR analysis.

NMR spectroscopic measurements

NMR samples were allowed to thaw for approximately an hour on ice and transferred to 3 mm (600 MHz) NMR tubes (Bruker Biospin, Billerica, MA, USA) for NMR analysis. All blood samples collected were tested for 114 lipid indicators. The detection process was standard operating procedure.[25,26] NMR data were obtained at 4°C under specific spectral conditions (cycle time 5 s, 100 Carr-Purcell-Meiboom-Gill [CPMG] p pulses, total mixing time 55.56 ms, 16 K data points, total

acquisition time 0.85 s, spectral width 16 ppm) and rotation at 3,600 Hz rate and rotor synchronization collected by CPMG method.

Statistical analysis

Results were displayed as the mean±standard deviation and analyzed using SPSS Statistics 23 (IBM Corp., Armonk, NY, USA). The Student's t-test analyzed the two groups' demographic information and clinical features. The receiver operating characteristic (ROC) curves were utilized to investigate the area under the curve (AUC) values for lipid metabolites in plasma from AD patients and healthy controls. We performed a false discovery rate correction to adjust for multiple tests. No metabolites passed the false discovery rate correction. All p-values were two two-tailed at the significance level of <0.05.

RESULTS

Sample and baseline characteristics

Clinical data of the participants were provided in Table 1. There were no statistically significant differences in age, body mass index (BMI), and total MMSE scores between males and females in the healthy control group (all p>0.05). There were also no differences in age and BMI between males and females in the AD patient group, except for the MMSE total score (p<0.05). Table 2 provided an overview of the clinical characteristics between AD patients and healthy controls, separated by sex: 21 were male and 49 were female. There were no significant differences in age and BMI between healthy controls and AD patients (all p>0.05). In addition, the MMSE total scores of AD patients were significantly lower than those of healthy controls (both p<0.05).

Sex-associated differences in plasma lipid metabolism levels

First, to identify sex-related differences, statistical comparisons of lipid metabolite levels were examined between males and females in AD patients and healthy controls, respectively (Table 3). The levels of the three metabolites in the healthy controls differed significantly by sex. As shown in Table 3, the levels of 2 high-density lipoprotein (HDL) subfractions (HDL-2 cholesterol [H2CH] and HDL-3 apolipoprotein-A1 [H3A1]) were higher in females than in males, while low-density lipoprotein cholesterol/high-density lipoprotein cholesterol (LDHD) levels were just the opposite, with males higher than females. The levels of the four metabolites-HDL-3 phospholipids (H3PL), HDL-4 phospholipids (H4PL), H3A1, and HDL-3 apolipoprotein-A2 (H3A2) differed significantly by sex in the AD patients. All four above metabolites were present in higher levels in females than males, and all belonged to HDL subfractions.

Disease-specific sex differences in plasma lipid metabolism levels

Disease-specific sex differences in lipid metabolite levels were performed by comparing males and females. Of the metabolites detected, 9 (8%) showed sex-related differences in males (total apolipoprotein-A2 [TPA2], HDL apolipoprotein-A2 [HDA2], VLDL-5 triglycerides [V5TG], HDL-1 cholesterol [H1CH], HDL-4 cholesterol [H4CH], HDL-1 free cholesterol [H1FC], H4PL, HDL-4 apolipoprotein-A1 [H4A1], HDL-4 apolipoprotein-A2 [H4A2]). As shown in Table 4, the levels of 1 very low-density lipoprotein (VLDL) subfraction (VLDL-5

Table 1. Demographic and clinical characteristics of AD patients and healthy controls

Characteristics	Health controls (N=35)		p	AD patients (N=35)		p
	Male (N=12)	Female (N=23)		Male (N=9)	Female (N=26)	
Age (yr)	71.08±7.86	70.52±5.12	0.826	72.44±7.02	72.00±7.26	0.874
BMI (kg/m^2)	25.60±3.82	24.06±3.20	0.215	22.47±3.44	23.62±3.85	0.434
MMSE	27.83±2.98	27.04±3.05	0.469	5.33±6.69	12.46±5.75	0.004

Values are presented as mean±standard deviation unless otherwise indicated. AD, Alzheimer's disease; BMI, body mass index; MMSE, mini-mental state examination

Table 2. Demographic and clinical characteristics of AD patients and healthy controls stratified by sex

Characteristics	Male (N=21)		p	Female (N=49)		p
	Patients (N=9)	Health controls (N=12)		Patients (N=26)	Health controls (N=23)	
Age (yr)	72.44±7.02	71.08±7.86	0.686	72.00±7.26	70.52±5.12	0.420
BMI (kg/m^2)	22.47±3.44	25.60±3.82	0.068	23.62±3.85	24.06±3.20	0.667
MMSE	5.33±6.69	27.83±2.98	<0.001	12.46±5.75	27.04±3.05	<0.001

Values are mean±standard deviation or percentage unless otherwise indicated. Continuous variables were analyzed by Student's t-test. AD, Alzheimer's disease; BMI, body mass index; MMSE, mini-mental state examination

Table 3. Plasma levels of representative lipid metabolites with significant sex-associated differences

Metabolic indices	Male	Female	t	p
Health controls				
LDHD	2.33±0.61	1.91±0.56	2.047	0.049
H2CH	7.60±1.85	9.03±1.80	-2.218	0.034
H3A1	23.69±4.00	26.56±3.82	-2.077	0.046
Patients				
H3PL	13.84±2.48	16.13±2.75	-2.199	0.035
H4PL	22.32±2.91	26.13±5.46	-2.634	0.014
H3A1	23.53±3.39	26.80±3.78	-2.293	0.028
H3A2	4.86±0.64	5.63±0.94	-2.280	0.029

Values are presented as mean±standard deviation unless otherwise indicated. LDHD, LDL-cholesterol/HDL-cholesterol; H2CH, HDL-2 cholesterol; H3A1, HDL-3 apolipoprotein-A1; H3PL, HDL-3 phospholipids; H4PL, HDL-4 phospholipids; H3A2, HDL-3 apolipoprotein-A2; LDL, low-density lipoprotein; HDL, high-density lipoprotein

triglycerides [V5TG]) and 2 HDL subfractions (H1CH and H1FC) were higher in AD patients than in healthy controls. In contrast, the remaining 6 metabolites, including TPA2 and 5 HDL subfractions (HDA2, H4CH, H4PL, H4A1, and H4A2), were significantly lower in AD patients than in healthy controls. In females, as shown in Table 4, 23 (21%) metabolites showed sex-related differences (LDL-cholesterol [LDCH], LDHD, LDL particle number [LDPN], LDL-1 particle number [L1PN], LDL-2 particle number [L2PN], LDL-3 particle number [L3PN], LDL free cholesterol [LDFC], LDL phospholipids [LDPL], LDL apolipoprotein-B [LDAB], LDL-2 triglycerides [L2TG], LDL-3 triglycerides [L3TG], LDL-1 cholesterol [L1CH], LDL-2 cholesterol [L2CH], LDL-3 cholesterol [L3CH], LDL-1 free cholesterol [L1FC], LDL-2 free cholesterol [L2FC], LDL-3 free cholesterol [L3FC], LDL-1 phospholipids [L1PL], LDL-2 phospholipids [L2PL], LDL-3 phospholipids [L3PL], LDL-1 apolipoprotein-B [L1AB], LDL-2 apolipoprotein-B [L2AB], LDL-3 apolipoprotein-B [L3AB]). More notably, these metabolites were all associated with LDL and higher in AD patients than in healthy controls. On the other hand, it should be noted that no significant metabolites overlapped between the male and the female groups.

Correlation and clustering analysis

The correlation analysis using the BioCPR-A tool,[27] after sex stratification, was shown in Supplementary Figures 1 and 2. Moreover, the metabolic combinations of diagnostic potential were negatively associated with MMSE scores in both males and females (Supplementary Table 1). Further, the 36 differential metabolites were further analyzed for clustering analysis. The results suggested that these compounds were mainly

Table 4. Plasma levels of representative lipid metabolites with significant disease-specific sex differences

Metabolic indices	Patients	Health controls	t	p
Male				
TPA2	25.91±2.93	29.07±2.32	2.760	0.012
HDA2	26.55±2.83	29.64±2.24	2.804	0.011
V5TG	2.89±0.50	2.18±0.67	-2.656	0.016
H1CH	21.78±6.01	16.72±4.38	-2.237	0.037
H4CH	16.92±2.94	19.82±1.80	2.806	0.011
H1FC	4.88±1.30	3.79±1.08	-2.099	0.011
H4PL	22.32±2.91	25.91±2.11	3.286	0.004
H4A1	65.10±9.24	74.99±4.08	3.000	0.013
H4A2	14.88±2.98	18.65±1.66	3.704	0.002
Female				
LDCH	133.11±32.51	112.08±34.72	-2.189	0.034
LDHD	2.25±0.60	1.91±0.56	-2.085	0.043
LDPN	1,544.74±332.87	1,318.53±324.22	-2.403	0.020
L1PN	252.28±58.15	212.50±63.10	-2.314	0.025
L2PN	216.69±69.58	169.93±47.98	-2.703	0.010
L3PN	246.98±77.02	187.73±62.80	-2.927	0.005
LDFC	38.34±8.58	31.89±10.00	-2.432	0.019
LDPL	73.80±15.41	62.32±17.36	-2.453	0.018
LDAB	84.96±18.31	72.52±17.83	-2.403	0.020
L2TG	2.80±0.73	2.26±0.61	-2.795	0.007
L3TG	2.39±0.63	1.73±0.75	-3.322	0.002
L1CH	26.29±6.24	22.04±6.92	-2.260	0.028
L2CH	21.55±7.92	16.42±5.59	-2.584	0.013
L3CH	23.87±8.11	18.01±7.13	-2.672	0.010
L1FC	7.84±1.75	6.64±1.97	-2.257	0.029
L2FC	6.53±2.29	5.27±1.73	-2.141	0.037
L3FC	7.59±2.13	6.21±2.25	-2.202	0.033
L1PL	14.53±3.18	12.36±3.58	-2.244	0.030
L2PL	11.94±3.90	9.34±2.72	-2.673	0.010
L3PL	12.88±3.92	9.93±3.51	-2.761	0.008
L1AB	13.89±3.20	11.69±3.47	-2.313	0.025
L2AB	11.92±3.83	9.35±2.64	-2.703	0.010
L3AB	13.58±4.24	10.32±3.45	-2.927	0.005

Values are presented as mean±standard deviation unless otherwise indicated. TPA2, total apolipoprotein-A2; HDA2, HDL apolipoprotein-A2; V5TG, VLDL-5 triglycerides; H1CH, HDL-1 cholesterol; H4CH, HDL-4 cholesterol; H1FC, HDL-1 free cholesterol; H4PL, HDL-4 phospholipids; H4A1, HDL-4 apolipoprotein-A1; H4A2, HDL-4 apolipoprotein-A2; LDCH, LDL-cholesterol; LDHD, LDL-cholesterol/HDL-cholesterol; LDPN, LDL particle number; L1PN, LDL-1 particle number; L2PN, LDL-2 particle number; L3PN, LDL-3 particle number; LDFC, LDL free cholesterol; LDPL, LDL phospholipids; LDAB, LDL apolipoprotein-B; L2TG, LDL-2 triglycerides; L3TG, LDL-3 triglycerides; L1CH, LDL-1 cholesterol; L2CH, LDL-2 cholesterol; L3CH, LDL-3 cholesterol; L1FC, LDL-1 free cholesterol; L2FC, LDL-2 free cholesterol; L3FC, LDL-3 free cholesterol; L1PL, LDL-1 phospholipids; L2PL, LDL-2 phospholipids; L3PL, LDL-3 phospholipids; L1AB, LDL-1 apolipoprotein-B; L2AB, LDL-2 apolipoprotein-B; L3AB, LDL-3 apolipoprotein-B; VLDL, very low-density lipoprotein; HDL, high-density lipoprotein; LDL, low-density lipoprotein

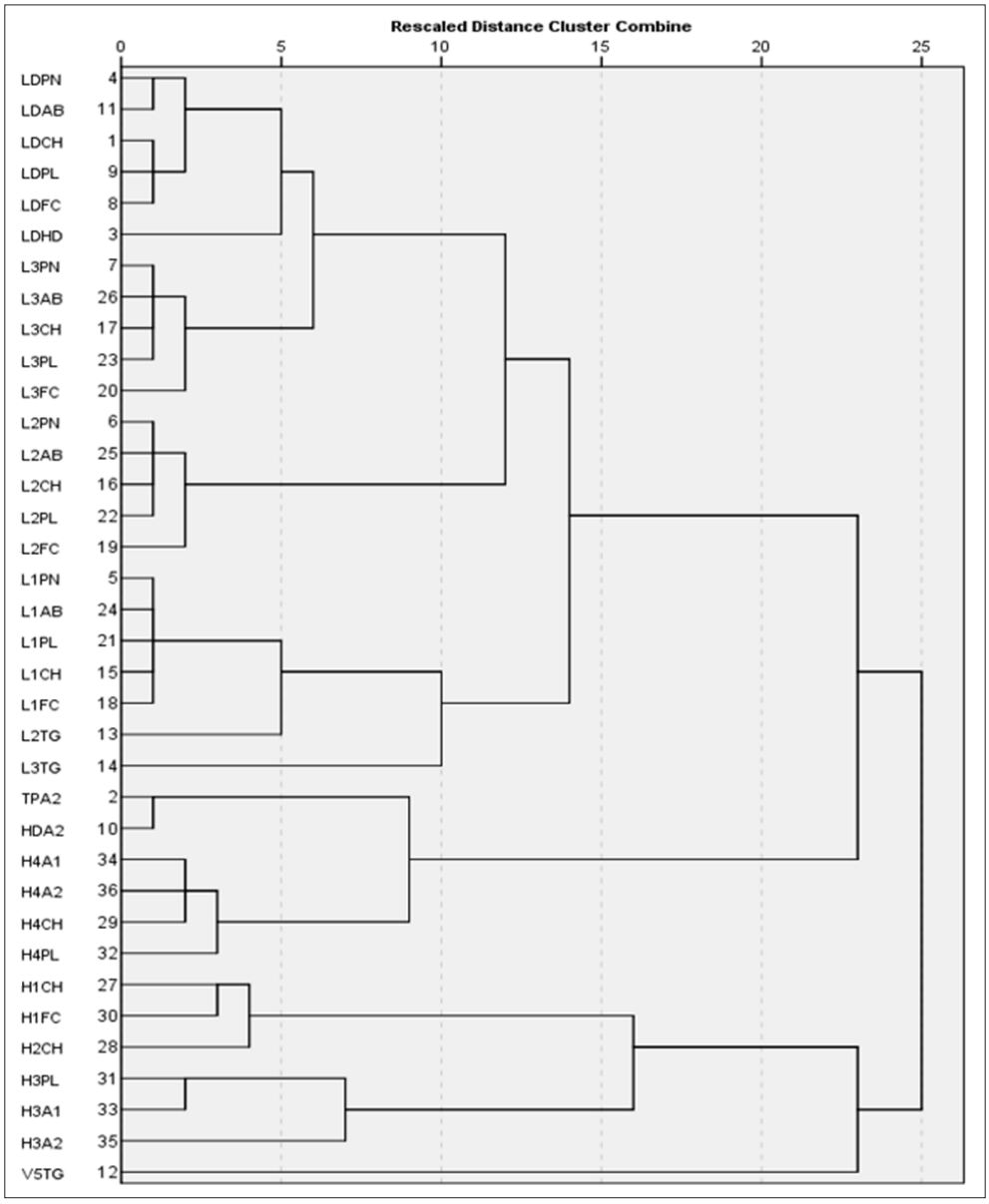

Figure 1. Clustering analysis of differential metabolites. LDPN, LDL particle number; LDAB, LDL apolipoprotein-B; LDCH, LDL-cholesterol; LDPL, LDL phospholipids; LDFC, LDL free cholesterol; LDHD, LDL-cholesterol/HDL-cholesterol; L3PN, LDL-3 particle number; L3AB, LDL-3 apolipoprotein-B; L3CH, LDL-3 cholesterol; L3PL, LDL-3 phospholipids; L3FC, LDL-3 free cholesterol; L2PN, LDL-2 particle number; L2AB, LDL-2 apolipoprotein-B; L2CH, LDL-2 cholesterol; L2PL, LDL-2 phospholipids; L2FC, LDL-2 free cholesterol; L1PN, LDL-1 particle number; L1AB, LDL-1 apolipoprotein-B; L1PL, LDL-1 phospholipids; L1CH, LDL-1 cholesterol; L1FC, LDL-1 free cholesterol; L2TG, LDL-2 triglycerides; L3TG, LDL-3 triglycerides; TPA2, total apolipoprotein-A2; HDA2, HDL apolipoprotein-A2; H4A1, HDL-4 apolipoprotein-A1; H4A2, HDL-4 apolipoprotein-A2; H4CH, HDL-4 cholesterol; H4PL, HDL-4 phospholipids; H1CH, HDL-1 cholesterol; H1FC, HDL-1 free cholesterol; H2CH, HDL-2 cholesterol; H3PL, HDL-3 phospholipids; H3A1, HDL-3 apolipoprotein-A1; H3A2, HDL-3 apolipoprotein-A2; V5TG, VLDL-5 triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein.

divided into 4 clusters (Figure 1). The differential metabolites in cluster I was composed of LDPN, LDAB, LDCH, LDPL, LDFC, LDHD, L3PN, L3AB, L3CH, L3PL, L3FC, L2PN, L2AB, L2CH, L2PL, L2FC, L1PN, L1AB, L1PL, L1CH, L1FC, L2TG, and L3TG. The metabolites of cluster II include TPA2, HDA2, H4A1, H4A2, H4CH, and H4PL. The metabolites of cluster III include H1CH, H1FC, H2CH, H3PL, H3A1, and H3A2. Moreover, cluster IV only consisted of V5TG.

The ROC curve analysis of lipid metabolites

ROC curves were analyzed to evaluate the diagnostic value of lipid metabolites for AD. ROC curves of 8 lipid metabolites in males were shown in Figure 2A. We found an AUC of 0.82 (95% confidence interval [CI] is 0.63 to 1.00) for V5TG levels with a p-value of 0.016 (Figure 2A and Supplementary Table 2). Except for V5TG, no individual ROC curves provided an acceptable AUC. We thus decided to determine whether the

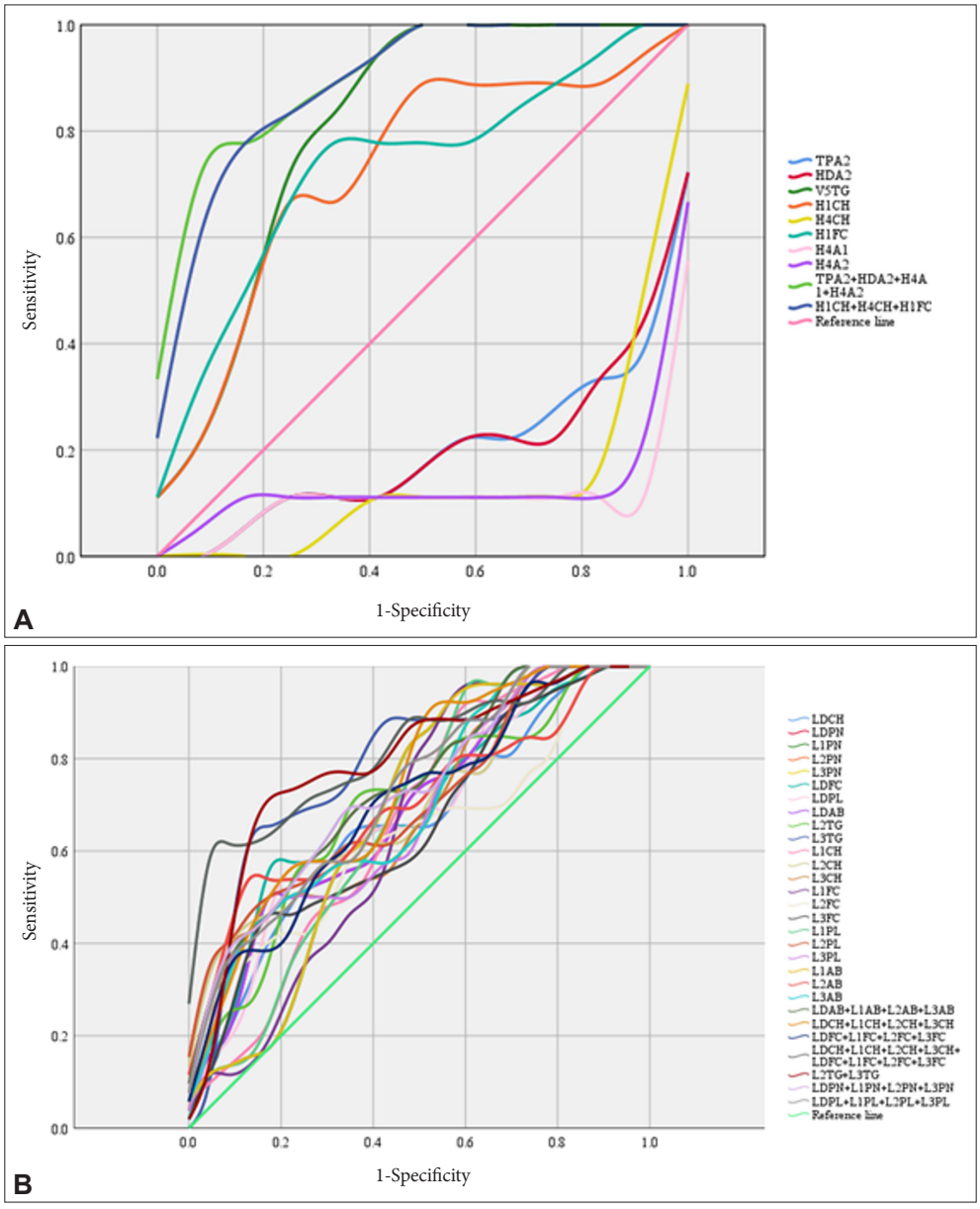

Figure 2. The ROC curve of lipid metabolites. A: The ROC curve of lipid metabolites in the male group. B: The ROC curve of lipid metabolites in the female group. TPA2, total apolipoprotein-A2; HDA2, HDL apolipoprotein-A2; V5TG, VLDL-5 triglycerides; H1CH, HDL-1 cholesterol; H4CH, HDL-4 cholesterol; H1FC, HDL-1 free cholesterol; H4A1, HDL-4 apolipoprotein-A1; H4A2, HDL-4 apolipoprotein-A2; LDCH, LDL-cholesterol; LDPN, LDL particle number; L1PN, LDL-1 particle number; L2PN, LDL-2 particle number; L3PN, LDL-3 particle number; LDFC, LDL free cholesterol; LDPL, LDL phospholipids; LDAB, LDL apolipoprotein-B; L2TG, LDL-2 triglycerides; L3TG, LDL-3 triglycerides; L1CH, LDL-1 cholesterol; L2CH, LDL-2 cholesterol; L3CH, LDL-3 cholesterol; L1FC, LDL-1 free cholesterol; L2FC, LDL-2 free cholesterol; L3FC, LDL-3 free cholesterol; L1PL, LDL-1 phospholipids; L2PL, LDL-2 phospholipids; L3PL, LDL-3 phospholipids; L1AB, LDL-1 apolipoprotein-B; L2AB, LDL-2 apolipoprotein-B; L3AB, LDL-3 apolipoprotein-B; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; ROC, receiver operating characteristic.

combination of the lipid metabolites could reveal more accurate diagnostic and better predictive estimates than each metabolite marker itself. We tested apolipoprotein (apo)-associated metabolites (TPA2, HDA2, H4A1, and H4A2) and HDL cholesterol-associated metabolites (H1CH, H4CH, and H1FC), as shown in Supplementary Figure 3A and Supplementary Table 2, the AUC were 0.92 (95% CI, 0.80−1.00) and 0.90 (95% CI, 0.77−1.00), respectively.

ROC curves for each lipid metabolite from the females were performed, and all the individual ROC curves provided an acceptable AUC (Figure 2B and Supplementary Table 3). L3TG showed the best performance, with an AUC of 0.79 (95% CI 0.65−0.92). We also tested the combinations of the lipid metabolites, including LDL apo-B-associated metabolites (LDAB,

L1AB, L2AB, and L3AB), LDL cholesterol-associated metabolites (LDCH, L1CH, L2CH, and L3CH), LDL free cholesterol-associated metabolites (LDFC, L1FC, L2FC, and L3FC), LDL cholesterol and free cholesterol-associated metabolites (LDCH, L1CH, L2CH, L3CH, LDFC, L1FC, L2FC, and L3FC), LDL triglycerides -associated metabolites (L2TG and L3TG), LDL particle number-associated indicators (LDPN, L1PN, L2PN, and L3PN) and LDL phospholipids-associated metabolites (LDPL, L1PL, L2PL, and L3PL) to determine whether those achieved higher AUCs (Supplementary Figure 3B). As shown in Supplementary Table 3, the combination of multiple indicators improved the AUC, and LDL cholesterol plus free cholesterol-associated metabolites showed the best performance. For this composite biomarker, the AUC was 0.82 (95% CI, 0.71−0.94, p<0.001). All these data from the ROC curve showed that HDL subfractions in males and LDL subfractions in females might be prospective indicative parameters for AD.

DISCUSSION

To our best knowledge, this study is the first to conduct lipidomic analysis using NMR spectroscopy to reveal the disease-specific sex differences in AD and elderly control plasma samples. The main findings of our study were as follows: 1) the HDL-related subfractions levels were significantly higher in females than in males, 2) 7 HDL-related subfractions, 1 VLDL-related subfraction and apo-A2 were significantly different between AD patients and elderly controls in males, 3) 23 LDL-related subfractions were significantly higher in AD patients than in elderly controls in females, and 4) the combination of lipid metabolites had good diagnostic values.

Our study found that the HDL-related subfractions were significantly higher in females than in males in AD patients and elderly controls. There are several differences in brain anatomy between males and females, with males having greater CSF, lateral ventricles, sulcal volumes, a higher proportion of white matter, and approximately 10% higher head size and brain volume than females.[28-31] In contrast, females have more gray matter and cerebral blood flow.[32,33] Sex differences in brain structure and function may lead to differences in biofluid results. For example, studies of CSF neurofilament light chain, a marker of large-caliber subcortical axonal degeneration, have consistently shown higher levels in males than in females,[34,35] even among cognitively unimpaired individuals without a neurodegenerative disease. The sex dimorphism of AD can be explained by many different biological risk factors, including genetic background (e.g., apolipoprotein E), the deviation of brain structure, inflammation, gliosis, and immune module (e.g., Triggering Receptor Expressed on Myeloid Cells 2 [TREM2]), and sex differences at the single cell level.[5,6,36,37] HDL particles include a variety of lipoprotein complexes that differ in size, structure, and function.[38,39] The brain makes HDL-like particles, but these particles have not been fully characterized.

Interestingly, we found 7 HDL-related subfractions, 1 VLDL-related subfraction, and apo-A2 significantly differed between AD patients and elderly controls in males. VLDL is a class of triglyceride-rich lipoproteins, and elevated VLDL may lead to hypertriglyceridemia or excess triglycerides in the blood, which puts one at risk for AD.[40] Different lipids in VLDL correlated differently with AD progression between males and females.[41] However, no relevant studies on sex differences in HDL-related subfraction and apo-A2 have been reported. Our analysis indicated that 23 LDL-related subfractions were significantly higher in AD patients than in elderly controls in females. As mentioned before, LDL, specifically LDL cholesterol content, poses a risk of developing cognitive impairment.[42]

Moreover, previous studies have found that small and medium LDL was associated with AD and mild cognitive impairment progression in females.[41] Interestingly, there was no significant metabolite overlap between the male and female groups, suggesting that lipoprotein metabolism in AD differs in females and males. Our findings highlighted the complexity of the role of HDL, VLDL, and LDL lipid content in AD and the sex-specific role it may play.

In addition, we demonstrated that the AUC areas of the combination of the lipid metabolites were all above 0.70, all of which had good diagnostic values. One such study from 2018 with 22,623 participants used NMR or MS metabolomics analysis to identify metabolites significantly correlated with dementia. This cohort study found that two VLDL-specific lipoprotein lipid subclasses, one VLDL (total cholesterol to total lipids ratio in very large VLDL) lipoprotein lipid subclass, and one HDL (the concentration of cholesterol esters relative to total lipids in large HDL), were associated with dementia risk.[43] Another study published in 2019 quantified 58 metabolites in each of the 311 samples. The results suggested that individuals with dementia and pre-diagnostic dementia were discriminated from controls using threonine and its relevant metabolic pathways.[44] Proitsi et al.[45] showed that a combination of blood lipid metabolites could help predict AD progression. Lipoproteins and their subfraction profiles have been associated with diverse diseases. Thus, there has been a great demand for measuring and quantifying lipoprotein profiles validly and accurately. NMR spectroscopy was sensitive to lipid chemistry and lipid micelle physics, enabling it to nondestructively measure the lipoprotein profiles of blood samples.[46] This present NMR-based metabolomics study found several metabolites significantly correlated with AD compared to controls, which may contribute to developing blood-based AD diagnostic and treatment tools.

Limitations of this study included the small sample size, which indicated an important direction for future studies. A larger trial may find more subtle differences in the concentration and particle size of lipoprotein subfractions caused by sex in AD. These differences could be due to chance, differences in dietary patterns, or genetic risk, suggesting that this area deserves further scrutiny, including possible gene-environment interaction.[47] The extent to which dysregulation of lipid metabolites in AD patients contributes to the occurrence and development of AD remains to be tested. Measurements of blood metabolomics in AD need to be cross-evaluated with measurements from different sources, such as those quantified from CSF,[48-51] saliva,[52] and brain tissues.[53] Metabolomics determined using NMR methods should also be compared with those assessed by other techniques, including in vivo MRI/magnetic resonance spectroscopy[54] and MS,[55] to construct complete AD-characterized metabolomics. In addition, this study with the cross-sectional design cannot lead to causality. Although our study found that the blood plasma samples using NMR hold promise in distinguishing between AD patients and healthy controls, these results cannot show their causal relationship and further longitudinal studies are needed.

In conclusion, our current study was among the first to conduct lipidomic analysis using NMR spectroscopy to reveal the disease-specific sex differences in AD and elderly control plasma samples. In addition, there was no significant metabolite overlap between the male and female groups. Last, ROC curve analysis was used to assess the diagnostic value of each differential lipoprotein metabolite and lipid metabolite combination, and the results showed that the AUC areas of the combinations of lipid metabolites were above 0.70, all of which had good diagnostic value. This study has important clinical implications because it demonstrates the potential to diagnose AD from a simple, non-invasive blood sample.

Supplementary Materials

The Supplement is available with this article at https://doi.org/10.30773/pi.2024.0164.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Author Contributions

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