High-Throughput Metabolomics for Discovering Potential Biomarkers and Identifying Metabolic Mechanisms in Aging and Alzheimer's Disease

Kun Xie
Harbin Medical University School of Public Health
Qi Qin
Capital Medical University
Zhiping Long
Harbin Medical University School of Public Health
Yihui Yang
Harbin Medical University School of Public Health
Chenghai Peng
Harbin Medical University Fourth Hospital
Chunyang Xi
Second Affiliated Hospital of Harbin Medical University
Liangliang Li
Harbin Medical University School of Public Health
Zhen Wu
Harbin Medical University School of Public Health
Volontovich Daria
Harbin Medical University School of Public Health
Yashuang Zhao
Harbin Medical University School of Public Health
Fan Wang (yifan.701@163.com)
Harbin Medical University School of Public Health https://orcid.org/0000-0002-9869-9504
Maoqing Wang
Harbin Medical University School of Public Health

Research

Keywords: aging, Alzheimer's disease, mild cognitive impairment, metabolic biomarkers, metabolomics

Posted Date: August 26th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-60073/v1

Version of Record: A version of this preprint was published at Frontiers in Cell and Developmental Biology on February 25th, 2021. See the published version at https://doi.org/10.3389/fcell.2021.602887.

Abstract

Background and Aims: Alzheimer's disease (AD) is an aging-related neurodegenerative disease. The current diagnosis of AD may fail to identify a substantial number of asymptomatic individuals who will progress to AD. We aimed to investigate the metabolic mechanisms of aging and AD and to identify potential biomarkers for the early screening of AD in a natural aging population.

Methods: To analyse the plasma metabolites related to aging, we conducted an untargeted metabolomics analysis using ultra-high-performance liquid chromatography/quadrupole time-of-flight mass spectrometry in a two-stage cross-sectional study. Spearman's correlation analysis and random forest were applied to model the relationship between age and each metabolite. Moreover, systematic reviews of metabolomics studies of AD in the PubMed, Cochrane and Embase databases were searched to extract the differential metabolites and altered pathways from original studies. Pathway enrichment analysis was conducted using Mummichog.

Results: In total, 669 metabolites were significantly altered with aging, and thirteen pathways were enriched and correlated with aging. Five metabolites (palmitic acid, stearic acid, linoleic acid, glutamine, and oleic acid) were identified as potential biomarkers for AD based on a systematic review. Arginine and histidine were considered candidate monitoring markers of disease progression in the mild cognitive impairment (MCI) population. Moreover, three pathways (purine metabolism, arginine and proline metabolism, and the TCA cycle) were shared between aging and AD. Arginine and proline metabolism play a key role in the progression from CN to MCI and to AD in the natural aging population. Three metabolites, 16-a-hydroxypregnenolone, stearic acid and PC (16:0/22:5(4Z,7Z,10Z,13Z,16Z)), were finally proposed as potential markers of AD in the natural aging population.

Conclusion: The underlying mechanism shared between aging and AD and the potential biomarkers for AD diagnosis were proposed based on multistep comparative analysis.

Introduction

Alzheimer's disease (AD) is an aging-related neurodegenerative disease characterized by senile plaques caused by amyloid beta (Aß) and neurofibrillar tangles containing hyperphosphorylated tau-protein. Current estimates suggest that 44 million people live with dementia worldwide. This figure is predicted to more than triple by 2050 as the population ages; at this time, the annual cost of dementia in the USA alone may exceed US$600 billion (1, 2). Mild cognitive impairment (MCI, "amnestic MCI" is seen as a prodromal stage of Alzheimer's disease) represents the clinically diagnosed pre-dementia stage. Several studies have shown that the pathological changes of AD begin several years before the onset of evident memory impairment (3, 4). However, the current diagnosis is based on clinical symptoms of AD combined with pathological alterations, such as a decrease of cerebrospinal fluid Aß42 or an increase in p-tau or t-tau protein, which may not identify a substantial number of asymptomatic individuals who will develop AD later. Thus, it is urgent to investigate the physiology of AD and/or MCI and detect early biomarkers to improve the quality of life of those affected by this disease.

Although genetic factors involved in the development of AD have been identified, studies have also suggested that aging is the major risk factor. After the age of 60 years, the incidence of AD was found to double with every 5-year increase in age (5). Although the mechanisms triggering alterations associated with both aging and AD are not completely understood, they have been simultaneously divided into at least two aspects: oxidative stress and inflammation. First, increasing oxidative stress is observed in the contexts of aging, AD and/or MCI in terms of membrane lipids (6–8), proteins (9, 10) and mitochondrial DNA (11, 12). Second, inflammation is found to be a considerable driving force of aging and AD. In addition, recent studies have identified common histological changes (13, 14) and modulation of

neurotransmission (15), including hypofunction of the cholinergic system, in AD and aging (16, 17). Thus, the relationship between aging and AD can be revealed.

Metabolomics has been widely used to provide an overall description of metabolic profiles in pathological or physiological processes (18). Metabolomics offers quantitative measurement of final products downstream of interactions among genes, proteins and various influences. Compared with genomics and proteomics, metabolomics is regarded as an optimal platform to describe a dynamic physiological process and integral disease response. Evidence has shown that changes in metabolites are significant early indicators of diseases (19). A growing body of literature has already reported distinct perturbed sets of metabolites in the contexts of aging and AD (20–23). Thus, metabolomics is also a promising tool to systematically assess changes of small molecules in both natural aging and AD populations and provides clues essential for the early diagnosis of AD. However, little effort to date has been made to detect and compare the metabolites and metabolic pathways between AD and natural aging.

Therefore, we carried out a metabolomics analysis of aging-related metabolites and a systematic review of differential metabolites related to MCI and AD. Based on these findings, we aimed to explore similar metabolomic signatures among AD, MCI and aging, which could help explain the high incidence of AD in older populations and suggest novel markers to identify the earliest phase of AD in the natural aging population.

Materials And Methods

I) Global plasma metabolic profiling analysis of aging in a two-stage cross-sectional study

Study population and sample collection

Subjects were enrolled from orthopaedic and ophthalmic patients attending the Second Affiliated Hospital of Harbin Medical University and community-dwelling individuals from the Xiangfang community and surrounding villages in the city of Harbin. The training set consisted of 119 participants enrolled in the first half year of 2010, while the testing set of 64 individuals was enrolled in the next half year. All subjects completed a comprehensive questionnaire to obtain information about sociodemographic characteristics, lifestyle, and history of some diseases, such as diabetes, hypertension and heart disease. Fasting peripheral venous blood (5 ml) was collected using an EDTA tube and treated with centrifugation for 10 min at 3000 rpm and 4 °C. Then, samples were frozen at − 80 °C prior to measurement.

Statistical analysis

The metabolites detected in both the training and testing sets were considered the stably detected metabolites. Correlations between ion intensities of metabolites and age were calculated by Spearman's correlation analysis. The analytes with *P* values less than 0.05 were selected for the following statistical analyses. The set of aging-related metabolites was chosen according to the importance score given by random forest, in which age served as the dependent variable and all Spearman-correlated features served as independent variables. A total of 30 variables were assigned variable importance scores and identified with the HMDB database. All the Spearman-correlated metabolites were analysed for pathway enrichment analysis using Mummichog (http://mummichog.org/). All calculations were performed with the R statistical platform, version 3.4.4.

II) Systematic review of metabolomics studies of AD and MCI

Literature search strategy

We conducted an English language literature search for metabolomic studies of MCI and AD. The search was conducted in PubMed, Cochrane and Embase through August 27, 2018 with the search terms “Alzheimer disease”, “Alzheimer’s disease”, and “mild cognitive impairment ” combined with “metabolomics”, “metabonomics”, “GC-MS”, and “LC-MS”. Both automatic retrieval and manual retrieval were used for the literature search. In addition, we augmented the search by a snowball strategy, screening the references of original texts and reviews.

Quality assessment of individual studies

The QUADAS (24) (Quality Assessment of Diagnostic Accuracy Studies) is a well-established tool used for the appraisal of quality issues of omics-based studies investigating new diagnostic tests. QUADOMICS included sixteen questions, where the risk of bias could be appraised with respect to four aspects: “patient selection”, “index test”, “reference standard”, and “flow and timing” (25). Possible answers for each item were Y (criteria achieved), N (criteria not achieved), ? (unclear), and NA (not applicable).

Pathway enrichment analysis

We directly extracted the enriched pathways from original studies. Moreover, we conducted pathway enrichment analyses based on all the metabolites extracted from original studies using Mummichog. All these pathways were combined and categorized according to different biosamples (blood, urine, tissue, or CFS) or comparisons.

Results

I) Global plasma metabolic profiling analysis of aging in a two-stage cross-sectional study

Demographical characteristics of the study population

The training set involved 119 participants, aged 32 to 82 years, with an average age of 58.66 years and BMI of 24.10 kg m^{-2}. The testing set contained 64 people, aged from 25 to 85 years, with a mean age of 58.98 years and BMI of 23.93 kg m^{-2}. Other information on education, smoking, alcohol consumption, chronic diseases and exercises is shown in Table S1. No significant difference of any characteristics was observed.

Quality assessment of the metabolomics platform

Quality control (QC) samples were all clustered tightly in the two-stage cross-sectional detection. The relative standard deviations (RSDs; %) of the retention time and peak area ranged from 0 to 0.73 and 0.8 to 4, respectively, in the intra-batch assay and ranged from 0.1 to 2.7 and 1.5 to 6.2, respectively, in the inter-batch assay. The results showed that the stability of the UPC/Q-TOF MSMS platform was excellent throughout the run and was sufficient to ensure data quality for further global metabolic profiling analyses.

Multivariate analysis for plasma metabolic profiling analysis

The datasets with 12882 and 10705 variables in ESI^- and ESI^+, respectively, in the training population and 5394 and 7312 variables in ESI^- and ESI^+, respectively, in the testing set were detected based on UPLC/Q-TOF MS. Of these variables, 5545 variables in ESI^- and 5469 variables in ESI^+ were duplicated in the training set and testing set.

Identification of aging-related metabolites and pathway enrichment analysis

By including all duplicated metabolites in Spearman correlation analysis, we found that the ion intensities of 381 metabolites in ESI^- and 288 metabolites in ESI^+ were significantly associated with age (P values < 0.05, Excel 1&2). Then,

we employed an additional statistical method, random forest, to screen out the top 30 metabolites among all 669 metabolites (Table 1, Supplementary Fig. 1). Identification of these 30 metabolites was conducted by searching the HMDB metabolome database via a detection window of 50 ppm. Several classes of metabolites were observed twice or more often, such as dipeptides, long-chain fatty acids, triterpenoids, steroid glucuronide conjugates, fatty acid esters and phosphatidylcholines.

Table 1
Top 30 aging-related metabolites in plasma screened by random forest

Categories/Number	RT[a]	Mass	HMDB ID[b]	Metabolites' Name	Additive ion	ESI Mode
Dipeptides						
1	2.81	283.0861	HMDB0028853	Glycyl-Tyrosine	M + FA-H	ESI-
2	2.66	248.1024	HMDB0029060	Threoninyl-Glutamate	M + H	ESI+
3	2.67	265.1484	HMDB0029008	Phenylalanyl-Valine	M + H	ESI+
long-chain fatty acids						
4	3.71	307.1821	HMDB0000672	Hexadecanedioic acid	M + Na-2H	ESI-
5	3.81	257.1785	HMDB0000872	Tetradecanedioic acid	M-H	ESI-
6	**3.50**	**321.2046**	**HMDB0000827**	**Stearic acid**	**M + K-2H**	**ESI-**
Triterpenoids						
7	4.49	449.2562	HMDB0002385	Celastrol	M-H	ESI-
8	3.94	551.3198	HMDB0004309	Triterpenoid	M-H	ESI-
Steroid glucuronide conjugates						
9	3.61	597.3527	HMDB0002513	Lithocholate 3-O-glucuronide	M + FA-H	ESI-
10	3.4	565.3029	HMDB0002577	Cholic acid glucuronide	M-H20-H	ESI-
Fatty acid esters						
11	3.69	183.1395	HMDB0031272	Ethyl (E)-2-nonenoate	M-H	ESI-
12	3.57	465.2487	HMDB0029886	Sorbitan oleate	M + K-2H	ESI-
Phosphatidylcholine						
13	9.16	758.5624	HMDB0007880	PC(14:0/20:2(11Z,14Z))	M + H	ESI+
14	**9.42**	**846.5454**	**HMDB0007989**	**PC(16:0/22:5(4Z,7Z,10Z,13Z,16Z))**	**M + K**	**ESI+**
1,2-diacylglycerol-3-phosphates						
15	9.41	701.5504	HMDB0114824	1,2-diacylglycerol-3-phosphates		ESI+
1-acylglycerol-3-phosphates						
16	4.19	485.2756	HMDB0114752	LysoPA(22:4(7Z,10Z,13Z,16Z)/0:0)	M-H	ESI-
Gluco/mineralocorticoids, progestogins and derivatives						

[a]Rention time. [b]the HMDB identifier of the metabolite.

Bold, 3 metabolites related to both aging and AD, including 16-a-Hydroxypregnenolone, stearic acid, and PC(16:0/22:5(4Z,7Z,10Z,13Z,16Z)).

Categories/Number	RT[a]	Mass	HMDB ID[b]	Metabolites' Name	Additive ion	ESI Mode
17	**3.71**	**369.1899**	**HMDB0000315**	**16-a-Hydroxypregnenolone**	**M + K-2H**	**ESI-**
Phenylpropanoic acids						
18	2.59	145.0582	HMDB0001955	3-Phenylbutyric acid	M-H20-H	ESI-
Hydroxyindoles						
19	2.59	263.1037	HMDB0001238	N-Acetylserotonin	M + FA-H	ESI-
Hypoxanthines						
20	2.67	130.0577	HMDB0000897	7-Methylguanine	M-2H2O + H	ESI+
Acylcarnitines						
21	3.12	304.2231	HMDB0061634	3-hydroxyoctanoyl carnitine	M + H	ESI+
Phosphatidylglycerophosphates						
22	4.00	735.3353	HMDB0033168	(15a,20R)-Dihydroxypregn-4-en-3-one 20-[glucosyl-(1->4)-6-acetyl-glucoside]	M + K-2H	ESI-
Medium-chain fatty acids						
23	3.55	167.1402	HMDB0000947	Undecanoic acid	M-H20-H	ESI-
Oligopeptides						
24	3.83	444.273	HMDB0012936	Dynorphin B (10–13)	M-H	ESI-
Purine 2'-deoxyribonucleosides						
25	2.66	275.065	HMDB0000071	Deoxyinosine	M + Na	ESI+
Prostaglandins and related compounds						
26	3.73	315.189	HMDB0060046	15d PGD2	M-H20-H	ESI-
[a]Rention time. [b]the HMDB identifier of the metabolite.						
Bold, 3 metabolites related to both aging and AD, including 16-a-Hydroxypregnenolone, stearic acid, and PC(16:0/22:5(4Z,7Z,10Z,13Z,16Z)).						

Table 2 summarizes the pathways enriched by all metabolites correlated with aging using Mummichog. A total of 13 pathways were significantly perturbed with a P value < 0.05.

Table 2
Aging-related pathways enriched by spearman-correlated metabolites

Pathways' Name	Total No.	Hits No.	*P*-value
Carnitine shuttle[a]	15	4	0.021
Omega-3 fatty acid metabolism[a]	5	2	0.035
Ascorbate (Vitamin C) and Aldarate Metabolism[b]	2	2	0.007
Glutamate metabolism[b]	6	3	0.010
Biopterin metabolism[b]	10	4	0.012
TCA cycle[b]	3	2	0.019
Vitamin B3 (nicotinate and nicotinamide) metabolism[b]	15	5	0.021
Purine metabolism[b]	23	7	0.023
Tyrosine metabolism[b]	31	9	0.026
Aspartate and asparagine metabolism[b]	31	9	0.026
Carnitine shuttle[b]	12	4	0.031
Leukotriene metabolism[b]	21	6	0.044
Arginine and Proline Metabolism[b]	17	5	0.045
[a]Pathway enrichment conducted using negative-mode features.			
[b]Pathway enrichment conducted using positive-mode features.			

II) Systematic review of metabolomics studies of AD and MCI

Literature retrieval

In total, 494 articles from PubMed, 23 articles from Cochrane, and 64 articles from Embase were retrieved from automatic electronic searches. A total of 581 publications were examined through title and abstract screening. There were 486 records that were excluded because they were duplicates, reviews, or not related to the research topic (e.g., mechanisms or drug use exploration and technology assessment). Additionally, 9 articles were manually retrieved after searching references from original articles. After careful full-text screening, 67 studies were finally included in this systematic review (Supplementary Fig. 2).

Description of included studies

Table S2 summarizes the characteristics of the 67 included studies regarding the number of subjects, sample source, sample type, platforms and outcomes. Of the 67 studies, the number of AD cases ranged from 7 to 1356, the number of MCI cases ranged from 10 to 356, and the number of healthy controls ranged from 7 to 23882. The detection platform includes LC-MS in 28 studies, GC-MS in 13 studies, multiple platforms in 11 studies, mass spectrometry (MS) in 5 studies, nuclear magnetic resonance (NMR) in 2 studies, and other platforms in 8 studies. Various biosamples used included serum (18 studies), plasma (16 studies), brain tissue (11 studies), CSF (8 studies), CSF and plasma (5 studies), brain tissue and plasma (1 study), and others (4 studies). Additionally, 83.58% of the studies were case-control studies, and 41.79% of the subjects in these studies were European (Supplementary Fig. 3).

Quality assessment of eligible studies

According to the QUADOMICS tool, 30 out of 67 articles were unable to avoid overfitting due to the lack of a statistical approach such as cross-validation or an independent test set. All of the articles explored differences in biomarkers between overt cases and healthy individuals and could be categorized into preliminary phase 1 studies. Items with respect to the representative feature of the included subjects and the availability of the clinical data were not applicable for all articles. Table S3 presents a detailed assessment for all 67 studies.

Metabolites related to the occurrence and progression of AD and MCI

Table S4-8 presents the metabolites that were extracted as biological markers in original metabolomics studies. There were 830 altered analytes in the comparison of AD VS. CN, with 137 analytes reported twice or more often (Table S4). Tryptophan was the most commonly detected metabolite, followed by palmitic acid, arginine, and L-phenylalanine. The abundance of 290 metabolites was significantly altered in the comparison of MCI VS. CN (Table S5); tryptophan was detected 3 times, and 7 other metabolites, such as 5-hydroxytryptophan, L-phenylalanine and L-arginine, were identified twice. For AD VS. MCI, a total of 120 metabolites were affected, including tryptophan (reported 2 times) and histidine (2) (Table S6). In the prospective studies, 9 and 26 metabolites were found to be related to the progression from CN to AD (CN_AD) and MCI to AD (MCI_AD), respectively (Table S7-8).

Table 3 illustrates the duplicate metabolites between AD VS. CN and CN_AD VS. CN. Six metabolites (palmitic acid, stearic acid, linoleic acid, glutamine, oleic acid, and myristic acid) were detected in both retrospective case-control and prospective nested case-control studies, and all of these metabolites were detected in serum. In particular, 5 (all except myristic acid) of these 6 metabolites have been found in brain tissue and can be potential biomarkers for AD diagnosis. Additionally, three metabolites (arginine, creatine, and histidine) were detected in both AD VS. MCI and MCI_AD VS. MCI (Table 3), and two (arginine and histidine) of them were found in CSF and can be considered monitoring markers of disease progression in the MCI population.

Table 3
Metabolic biomarkers of Alzheimer's disease and mild cognitive impairment replicated in both case-control and nested case-control studies

Metabolites' Name	HMDB ID	Biosample' type	
		AD VS. CN (reported frequency)	CN_AD VS. CN (reported frequency)
Palmitic acid	HMDB0000220	Brain, serum (7)	Serum (1)
Stearic acid	HMDB0000827	Brain, serum (3)	Serum (1)
Linoleic acid	HMDB0000673	Brain, serum (3)	Serum (1)
Glutamine	HMDB0000641	Brain, serum, urine (4)	Plasma (1)
Oleic acid	HMDB0000207	Brain, serum (5)	Serum (1)
Myristic acid	HMDB0000806	Serum (3)	Serum (1)
		AD VS. MCI (reported frequency)	MCI_AD VS. MCI (reported frequency)
Arginine	HMDB0000517	CSF (1)	Plasma (1)
Creatine	HMDB0000064	Serum (2)	Plasma (1)
Histidine	HMDB0000177	Serum (3)	CSF (1)
AD VS. CN, AD VS. MCI, the comparison in case-control studies;			
CN_AD VS. CN, MCI_AD VS. MCI, the comparison in nested case-control studies.			

Pathway intersection analysis among different types of biosamples

Based on the metabolites extracted from original studies, we performed pathway enrichment analyses by categorizing different types of samples (Table S9). Table S10 presents the 16, 48, 88, and 25 pathways of AD and MCI enriched in brain tissue, CSF, plasma, and serum, respectively. As shown in Fig. 1, there were 15 common pathways perturbed in all four types of samples, such as sphingolipid metabolism, butanoate metabolism, propanoate metabolism, pantothenate and CoA biosynthesis, aminoacyl-tRNA biosynthesis and some amino acid metabolism pathways. All 16 pathways in brain tissue and most pathways (28/48) in CSF could be detected in plasma.

Metabolic pathways associated with AD and MCI

As shown in Table S9, there are 53 pathways that exhibited significant alterations in the comparison of AD VS. CN. These pathways were mainly related to amino acid metabolism (including alanine, aspartate and glutamate metabolism and arginine and proline metabolism), tryptophan metabolism, the TCA cycle, and purine metabolism. Additional analysis for the comparison of MCI VS. CN showed 39 metabolic pathways associated with MCI, including lysine metabolism, tryptophan metabolism, polyamine metabolism, and the urea cycle. Comparison of AD VS. MCI presented 38 altered pathways.

As shown in Fig. 2, a total of 15 pathways were shared in the comparison between AD VS. CN and MCI VS. CN, which indicated that the metabolic mechanisms of AD and MCI share similar pathological alterations. Moreover, four pathways were shared between AD VS. MCI and MCI_AD VS. MCI, including lysine metabolism, polyamine metabolism,

catecholamine metabolism, and prostaglandin 2 biosynthesis and metabolism. These pathway alterations indicated pathological progression from MCI to AD.

Intersection analysis of metabolic pathways among AD, MCI, and aging

We compared the aging-related pathways in the two-stage metabolomics analysis with the AD pathways generated in this systematic review. As shown in Fig. 3, three pathways (including purine metabolism, arginine and proline metabolism, and the TCA cycle) revealed the common metabolic changes in AD and aging and provided explanations for why natural aging is closely related to the high incidence of AD. All of these pathways have been detected in brain tissue, CSF, serum, and plasma in the AD population. Additionally, two pathways (arginine and proline metabolism and the TCA cycle) were found to be duplicated between MCI VS. CN and aging-related pathways. In particular, arginine and proline metabolism were duplicated between MCI VS. MCI_AD and aging-related pathways, which indicated that this mechanism plays an important role in the progression from CN to MCI and to AD in the natural aging population.

Metabolic biomarkers of AD in the natural aging population

Furthermore, we compared the top 30 metabolites of aging with the metabolites of AD and MCI reported in previous studies. After comparing Table 1 with Table S4 and Table S5, 3 metabolites were identified that were related to both AD and aging, including 16-a-hydroxypregnenolone, stearic acid, and PC (16:0/22:5(4Z,7Z,10Z,13Z,16Z)), which can be considered metabolic biomarkers of AD in the natural aging population.

Discussion

AD is not a sign of normal aging, but with age, the probability of suffering from AD increases annually. Considering the dramatic aging of populations worldwide, it is of great importance to explore common mechanisms between AD and natural aging. The key finding of this study is the systematic comparison of the metabolic mechanisms of AD, MCI, and aging. We first revealed the metabolic mechanism and potential biomarkers of AD in a natural aging population.

Based on this independent metabolomics analysis in a two-stage cross-sectional study, we found metabolites and pathways associated with aging. Some metabolites were involved in elderly-related diseases, such as fatty acids associated with atherosclerosis, prostaglandins and related compounds associated with cardiovascular disease, and long-chain fatty acids associated with MCI and AD. Strikingly, pathways enriched by aging-related metabolites were consistent with the hypotheses of aging mechanisms in previous studies, such as oxidative stress and inflammation. The carnitine shuttle indicates a process in which long-chain fatty acids are converted to corresponding acylcarnitines (ACs) and transferred into the mitochondria for energy production. Yu et al. (22) investigated higher concentrations of ACs during aging, indicating that our bodies may protect us from oxidative stress through the carnitine shuttle, and lower levels of histidine reflected the body's response to oxidative stress. The ratio of lipoxin A4 to cysteinyl leukotrienes was found to be negatively associated with age and suggests reduced anti-inflammatory ability. Additionally, in our work, pathways altered in the elderly were also related to various amino acid pathways. Glutamate is the middle step from glutamine to α-ketoglutarate (αKG), and these two deamination steps are called glutaminolysis (26). Enhanced glutaminolysis controls both cell growth and autophagy, which is known to decline with age by simulating lysosomal translocation and activation of mTORC1. Previous detection of amino acids in the serum of normal healthy Japanese people revealed that the concentrations of aspartate, asparagine, and arginine increased with age in males, whereas the levels of tyrosine asparagine, arginine and proline increased with age in females, which together suggests that aspartate and asparagine metabolism and arginine and proline metabolism are related to aging (27). Furthermore, delayed

degradation of plasma tyrosine (precursor of dopamine) in the elderly may influence cognition disruption during aging (28, 29).

Previous studies have proven that oxidative stress plays a vital role in the progression of AD (30, 31), as well as in MCI. The shared pathways between AD and MCI in our results reflected that MCI and AD have the same mechanisms. Carnosine, synthesized from ß-alanine, is elevated in AD patients as a result of oxidative stress (32). Glutathione, made up of cysteine, glycine and glutamate, was shown to have neuroprotective effects by reducing Aß-related oxidative stress via 4-hydroxynonenal (33) and attenuating amyloid fibrillation (34). Additional perturbed mechanisms associated with energy metabolism are pyruvate metabolism, the TCA cycle and glutathione metabolism (35–37). Furthermore, lipid metabolism is one of the most extensively implicated dysfunctions in the context of AD. Consistent with that, cholesterol and sphingolipid transport, saturated fatty acid metabolism and sphingolipid metabolism were disrupted in both AD and MCI. Moreover, disrupted amino acid pathways may be related to alterations of neurotransmitters. Arginine (38) and butanoate metabolism were shown to be related to the metabolism of an inhibitory neurotransmitter, gamma-amino butyric acid (GABA). Tryptophan can be converted to serotonin or participate in the kynurenine pathway (KP). In AD brains, upregulation of the KP may result in the depletion of serotonin, which is vital for cognition and learning (39). In our results, four pathways overlapped between AD VS. MCI and MCI_AD VS. MCI, including lysine metabolism, polyamine metabolism, catecholamine metabolism, and prostaglandin 2 biosynthesis and metabolism. Specific lysine residues within the microtubule-binding motif are the major sites of tau acetylation, which can inhibit tau function as a result of impaired tau–microtubule interactions and promote pathological tau aggregation (40). Changes of polyamine metabolism in the brain influence the progression of AD through several mechanisms, such as the regulation of cholinergic neurotransmission (41). These pathway alterations indicated progressive changes in the patients from MCI to AD.

As shown in Fig. 1, plasma presents the most complete metabolic changes compared with those of CSF and serum; in particular, plasma reflects all metabolic changes in the brain tissue of AD patients. Thus, plasma metabolites will most likely be the source of noninvasive diagnostic markers. Moreover, we still cannot ignore the fact that more than 50% of metabolic changes in plasma cannot be evidenced in brain tissue and may be caused by other dietary or environmental exposures. Thus, the uniqueness of plasma AD markers should be given more attention in clinical studies.

As illustrated in Table 3, some fatty acids were found to be altered in both retrospective and prospective studies. In astroglia, palmitic acid may stimulate ceramide synthesis by secreting signalling molecules such as cytokines and nitric oxide, resulting in Aβ accumulation and tau hyperphosphorylation (42). Similarly, stearic, linoleic, and oleic acids were proven to be related to the accumulation of both Aβ and tau in vitro (43, 44). Five metabolites (palmitic acid, stearic acid, linoleic acid, glutamine, and oleic acid) in serum/plasma have also been confirmed in brain tissue, which suggests their powerful potential for the noninvasive diagnosis of AD. Moreover, arginine, creatine and histidine were observed in both retrospective and prospective studies. Considering that arginine and histidine were altered in both CSF and plasma/serum, these two metabolites may act as noninvasive biomarkers for the MCI population to monitor the progression from MCI to AD.

The novel findings in our study are the metabolic pathways and biomarkers related to both aging and AD. Regarding the three shared pathways between aging and AD, the TCA cycle did not attract our attention because it is such an extensive metabolic pathway altered in diverse physiological and pathological processes, which include the preclinical stage of AD (36). Purine nucleoside phosphorylase (PNP) converts guanosine to guanine and inosine to hypoxanthine and is an important enzyme involved in purine metabolism. A study on astroglia reported a marked increase in PNP with aging (45), while another study observed increased PNP activity in patients with AD (46). Regarding arginine and proline metabolism, arginine is the central substance and serves as the only precursor of nitric oxide (NO). NO could react with superoxide (O2−) to produce peroxynitrite (ONOO−), and the latter is so active that it would experience ecleavage and generate reactive oxygen/nitrogen species (ROS/RNS) (47), which could occur in the process of natural aging. In addition, the brain

is much more vulnerable to nitroxidative stress than other tissues due to its high oxygen demand, weakened antioxidative ability and low proliferative trait of neurons, indicating that oxidative stress is involved in the initiation of AD in healthy individuals (15). Arginine could be metabolized to agmatine, which is involved in memory decline processes and can be found in both elderly and AD brain tissue (48). Arginine and proline metabolism contains several metabolic pathways we mentioned in the AD-related pathways, such glutathione, glycine, and polyamine metabolism. Evidence has shown that these pathways are related to aging (49–51). We can see that arginine and proline metabolism has been shown to play a role in prospective studies of the progression from no disease to MCI and eventually AD; likewise, arginine and proline metabolism appears in the pathways related to aging.

From the results of direct comparison of metabolite lists of aging and AD, there are three metabolites that were found to be duplicated: 16-a-hydroxypregnenolone, stearic acid and PC (16:0/22:5(4Z,7Z,10Z,13Z,16Z)). 16-alpha-hydroxypregnenolone is classified as a gluco/mineralocorticoid, a progestogin or aprogestogin derivative. Although no cytological mechanism studies have confirmed the role of 16-a-hydroxypregnenolone in aging and dementia, we observed that it was significantly associated with aging in our metabolomics analysis and altered in AD and MCI patients in population studies. Thus, experimental confirmation based on in vitro studies is urgently needed.

Strikingly, studies have demonstrated the role of stearic acid and PC (16:0/22:5(4Z,7Z,10Z,13Z,16Z)) in the pathological process of AD. Together with a recent study demonstrating a close relationship between tau protein and inflammatory signalling in astrocytes (52), we assume a possible pathological process in astrocytes combining aging and AD via inflammatory and oxidative responses. Patil and Chan et al. (43) found that astroglia-mediated oxidative stress may be related to stearic and palmitic fatty acid-induced hyperphosphorylation of tau. Investigations have shown that in astrocytes, stearic acid promotes the release of inflammatory factors such as IL-6 and TNFα (53). PC(16:0/22:5(4Z,7Z,10Z,13Z,16Z)) can be classified as PtdCho, which can be synthesized from cytidine diphosphate choline (CDP-choline) and diacylglycerol and contains long-chain polyunsaturated fatty acids, which are important components of neuron membranes. Wurtman et al. (54) proposed that choline was used to synthesize both acetylcholine (Ach) and PtdCho. Therefore, PtdCho could be taken to maintain the level of Ach when the body experiences a shortage of choline. Choline deficiency could occur in the contexts of both aging and AD, resulting in depletion of PtdCho and death of cholinergic neurons. This “autocannibalism” hypothesis partially explained the selective vulnerability of the cholinergic system and provided clues regarding PtdCho as a shared metabolite of natural aging and AD.

The limitations of our study are that we did not conduct in vitro experiments to verify the overlapping mechanisms between aging and AD. Although we used training and testing sets to screen out the aging-related metabolites that can be stably detected, our metabolomics research was a non-targeted test. It is necessary to further verify and analyse the sensitivity and specificity of metabolic markers based on targeted quantitative detection of a larger sample in a cohort population.

In conclusion, this study is the first to comprehensively compare metabolites and pathways between aging and AD by utilizing metabolomic measurement and systematic review. We proposed potential noninvasive biomarkers for AD diagnosis and MCI monitoring based on retrospective and prospective population studies. More importantly, we revealed the key role of arginine and proline metabolism in the progression from a healthy status to MCI to AD in a natural aging population. In particular, we provided potential metabolic markers (16-a-hydroxypregnenolone, stearic acid, and PC(16:0/22:5(4Z,7Z,10Z,13Z,16Z)) of AD diagnosis for future validation in a natural aging population.

Abbreviations

Ach, acetylcholine; AD, Alzheimer’s disease; Aß, amyloid beta; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; CDP-choline, cytidine diphosphate choline; DHA, Docosahexaenoic acid; ESI, electrospray ionization; CN,

healthy controls; MS, mass spectrometry; MCI, mild cognitive impairment; NMR, nuclear magnetic resonance; NO, nitric oxide; QUADAS, Quality Assessment of Diagnostic Accuracy Studies; QC, quality-control; RSD, relative standard deviations.

Declarations

Competing interests

The authors have declared that no conflict of interest exists.

Ethics approval and consent to participate

We carried out this study after obtaining written informed consent from all the study subjects and approval from the Human Research and Ethics Committee of Harbin Medical University. All experiments, including relevant details, were performed in accordance with relevant guidelines and regulations.

Funding

This work was supported by grants from National Nature Science Foundation of China (81773503, 81973036), Scientific Research Foundation for the Returned Overseas Scholars of Heilongjiang Province (LC2018033), Capital Medical University (PYZ19137) and Capital's Funds for Health Improvement and Research (CFH 2020-4-1033).

Authors' contributions

F.W. and M.W. contributed to the study design, data interpretation, study supervision, and the acquisition of funding. Y.Z. contributed to critical revision of the manuscript for important intellectual content. Z.L. and Y.Y contributed to data processing, statistical analysis, and identification of differential metabolites. C.P., K.X. and H.Y. contributed to questionnaire and sample collection. Z.W., D.V. and C.X. contributed to sample preparation and metabolomics detection. Z.L., Y.Y and L.L. contributed to literature search, quality assessment of individual studies and data extraction. K.X. and Q.Q. contributed to manuscript preparation. All authors contributed to review and revision of the manuscript.

Consent for publication

Not applicable

Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

References

1. Lane CA, Hardy J, Schott JM. Alzheimer's disease. 2017;25.
2. Nichols E, Szoeke CEI, Vollset SE, Abbasi N, Abd-Allah F, Abdela J, et al. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet Neurology. 2019;18(1):88-106.
3. Fleisher AS, Chen K, Quiroz YT, Jakimovich LJ, Gomez MG, Langois CM, et al. Florbetapir PET analysis of amyloid-β deposition in the presenilin 1 E280A autosomal dominant Alzheimer's disease kindred: a cross-sectional study. Lancet neurology. 2012;11(12).
4. Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet neurology. 2010;9(1):119.

5. Mayeux R, Stern Y. Epidemiology of Alzheimer Disease. Cold Spring Harbor Perspectives in Medicine. 2012;2(8).
6. Markesbery WR, Lovell MA. Damage to lipids, proteins, DNA, and RNA in mild cognitive impairment. Archives of neurology. 2007;64(7):954-6.
7. Mielke MM, Lyketsos CG. Lipids and the pathogenesis of Alzheimer's disease: is there a link? International review of psychiatry (Abingdon, England). 2006;18(2):173-86.
8. Zhu Y, Carvey PM, Ling Z. Age-related changes in glutathione and glutathione-related enzymes in rat brain. Brain research. 2006;1090(1):35-44.
9. Greilberger J, Koidl C, Greilberger M, Lamprecht M, Schroecksnadel K, Leblhuber F, et al. Malondialdehyde, carbonyl proteins and albumin-disulphide as useful oxidative markers in mild cognitive impairment and Alzheimer's disease. Free radical research. 2008;42(7):633-8.
10. Poon HF, Shepherd HM, Reed TT, Calabrese V, Stella AM, Pennisi G, et al. Proteomics analysis provides insight into caloric restriction mediated oxidation and expression of brain proteins associated with age-related impaired cellular processes: Mitochondrial dysfunction, glutamate dysregulation and impaired protein synthesis. Neurobiology of aging. 2006;27(7):1020-34.
11. Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, et al. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nature genetics. 2006;38(5):515-7.
12. Wang J, Markesbery WR, Lovell MA. Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. Journal of neurochemistry. 2006;96(3):825-32.
13. Naslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, et al. Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. Jama. 2000;283(12):1571-7.
14. Ohm TG, Muller H, Braak H, Bohl J. Close-meshed prevalence rates of different stages as a tool to uncover the rate of Alzheimer's disease-related neurofibrillary changes. Neuroscience. 1995;64(1):209-17.
15. Kern A, Behl C. The unsolved relationship of brain aging and late-onset Alzheimer disease. Biochimica et biophysica acta. 2009;1790(10):1124-32.
16. Bartus RT. On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. Experimental neurology. 2000;163(2):495-529.
17. Gron G, Brandenburg I, Wunderlich AP, Riepe MW. Inhibition of hippocampal function in mild cognitive impairment: targeting the cholinergic hypothesis. Neurobiology of aging. 2006;27(1):78-87.
18. Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, et al. The human serum metabolome. PloS one. 2011;6(2):e16957.
19. Nicholson JK, Holmes E, Elliott P. The metabolome-wide association study: a new look at human disease risk factors. Journal of proteome research. 2008;7(9):3637-8.
20. Hertel J, Friedrich N, Wittfeld K, Pietzner M, Budde K, Van der Auwera S, et al. Measuring Biological Age via Metabonomics: The Metabolic Age Score. Journal of proteome research. 2016;15(2):400-10.
21. Rist MJ, Roth A, Frommherz L, Weinert CH, Kruger R, Merz B, et al. Metabolite patterns predicting sex and age in participants of the Karlsruhe Metabolomics and Nutrition (KarMeN) study. PloS one. 2017;12(8):e0183228.
22. Yu Z, Zhai G, Singmann P, He Y, Xu T, Prehn C, et al. Human serum metabolic profiles are age dependent. Aging cell. 2012;11(6):960-7.
23. Wilkins JM, Trushina E. Application of Metabolomics in Alzheimer's Disease. Frontiers in neurology. 2017;8:719.
24. Lumbreras B, Porta M, Marquez S, Pollan M, Parker LA, Hernandez-Aguado I. QUADOMICS: an adaptation of the Quality Assessment of Diagnostic Accuracy Assessment (QUADAS) for the evaluation of the methodological quality of studies on the diagnostic accuracy of '-omics'-based technologies. Clinical biochemistry. 2008;41(16-17):1316-25.

25. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Annals of internal medicine. 2011;155(8):529-36.
26. Duran RV, Oppliger W, Robitaille AM, Heiserich L, Skendaj R, Gottlieb E, et al. Glutaminolysis activates Rag-mTORC1 signaling. Molecular cell. 2012;47(3):349-58.
27. Kouchiwa T, Wada K, Uchiyama M, Kasezawa N, Niisato M, Murakami H, et al. Age-related changes in serum amino acids concentrations in healthy individuals. Clinical chemistry and laboratory medicine. 2012;50(5):861-70.
28. Berry AS, Shah VD, Baker SL, Vogel JW, O'Neil JP, Janabi M, et al. Aging Affects Dopaminergic Neural Mechanisms of Cognitive Flexibility. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2016;36(50):12559-69.
29. Rudman D, Abbasi AAL, 2013 #8}, Chaudry F, Mattson DE. Delayed plasma clearance of phenylalanine and tyrosine in elderly men. Journal of the American Geriatrics Society. 1991;39(1):33-8.
30. Huang WJ, Zhang X, Chen WW. Role of oxidative stress in Alzheimer's disease. Biomedical reports. 2016;4(5):519-22.
31. Perry G, Cash AD, Smith MA. Alzheimer Disease and Oxidative Stress. Journal of biomedicine & biotechnology. 2002;2(3):120-3.
32. Solis MY, Cooper S, Hobson RM, Artioli GG, Otaduy MC, Roschel H, et al. Effects of beta-alanine supplementation on brain homocarnosine/carnosine signal and cognitive function: an exploratory study. PloS one. 2015;10(4):e0123857.
33. Mark RJ, Lovell MA, Markesbery WR, Uchida K, Mattson MP. A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid beta-peptide. Journal of neurochemistry. 1997;68(1):255-64.
34. Wang SS, Chou SW, Liu KN, Wu CH. Effects of glutathione on amyloid fibrillation of hen egg-white lysozyme. International journal of biological macromolecules. 2009;45(4):321-9.
35. Isopi E, Granzotto A, Corona C, Bomba M, Ciavardelli D, Curcio M, et al. Pyruvate prevents the development of age-dependent cognitive deficits in a mouse model of Alzheimer's disease without reducing amyloid and tau pathology. Neurobiology of disease. 2015;81:214-24.
36. Atamna H, Frey WH, 2nd. Mechanisms of mitochondrial dysfunction and energy deficiency in Alzheimer's disease. Mitochondrion. 2007;7(5):297-310.
37. Liu H, Wang H, Shenvi S, Hagen TM, Liu RM. Glutathione metabolism during aging and in Alzheimer disease. Annals of the New York Academy of Sciences. 2004;1019:346-9.
38. Jo S, Yarishkin O, Hwang YJ, Chun YE, Park M, Woo DH, et al. GABA from reactive astrocytes impairs memory in mouse models of Alzheimer's disease. Nature medicine. 2014;20(8):886-96.
39. Griffin JW, Bradshaw PC. Amino Acid Catabolism in Alzheimer's Disease Brain: Friend or Foe? Oxidative medicine and cellular longevity. 2017;2017:5472792.
40. Cohen TJ, Guo JL, Hurtado DE, Kwong LK, Mills IP, Trojanowski JQ, et al. The acetylation of tau inhibits its function and promotes pathological tau aggregation. Nat Commun. 2011;2:252.
41. Mahajan UV, Varma VR, Griswold ME, Blackshear CT, An Y, Oommen AM, et al. Dysregulation of multiple metabolic networks related to brain transmethylation and polyamine pathways in Alzheimer disease: A targeted metabolomic and transcriptomic study. PLoS Med. 2020;17(1):e1003012.
42. Patil S, Melrose J, Chan C. Involvement of astroglial ceramide in palmitic acid-induced Alzheimer-like changes in primary neurons. The European journal of neuroscience. 2007;26(8):2131-41.
43. Patil S, Chan C. Palmitic and stearic fatty acids induce Alzheimer-like hyperphosphorylation of tau in primary rat cortical neurons. Neuroscience letters. 2005;384(3):288-93.
44. Amtul Z, Uhrig M, Wang L, Rozmahel RF, Beyreuther K. Detrimental effects of arachidonic acid and its metabolites in cellular and mouse models of Alzheimer's disease: structural insight. Neurobiology of aging. 2012;33(4):831.e21-31.

45. Zoref-Shani E, Bromberg Y, Lilling G, Gozes I, Brosh S, Sidi Y, et al. Developmental changes in purine nucleotide metabolism in cultured rat astroglia. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience. 1995;13(8):887-96.
46. Alonso-Andres P, Albasanz JL, Ferrer I, Martin M. Purine-related metabolites and their converting enzymes are altered in frontal, parietal and temporal cortex at early stages of Alzheimer's disease pathology. Brain pathology (Zurich, Switzerland). 2018;28(6):933-46.
47. Yi J, Horky LL, Friedlich AL, Shi Y, Rogers JT, Huang X. L-arginine and Alzheimer's disease. International journal of clinical and experimental pathology. 2009;2(3):211-38.
48. Liu P, Fleete MS, Jing Y, Collie ND, Curtis MA, Waldvogel HJ, et al. Altered arginine metabolism in Alzheimer's disease brains. Neurobiology of aging. 2014;35(9):1992-2003.
49. Weschawalit S, Thongthip S, Phutrakool P, Asawanonda P. Glutathione and its antiaging and antimelanogenic effects. Clinical, cosmetic and investigational dermatology. 2017;10:147-53.
50. Hashizume O, Ohnishi S, Mito T, Shimizu A, Ishikawa K, Nakada K, et al. Epigenetic regulation of the nuclear-coded GCAT and SHMT2 genes confers human age-associated mitochondrial respiration defects. Scientific reports. 2015;5:10434.
51. Minois N, Carmona-Gutierrez D, Madeo F. Polyamines in aging and disease. Aging-Us. 2011;3(8):716-32.
52. Lemoine L, Saint-Aubert L, Nennesmo I, Gillberg PG, Nordberg A. Cortical laminar tau deposits and activated astrocytes in Alzheimer's disease visualised by (3)H-THK5117 and (3)H-deprenyl autoradiography. Scientific reports. 2017;7:45496.
53. Gupta S, Knight AG, Gupta S, Keller JN, Bruce-Keller AJ. Saturated long-chain fatty acids activate inflammatory signaling in astrocytes. Journal of neurochemistry. 2012;120(6):1060-71.
54. Wurtman RJ. Choline metabolism as a basis for the selective vulnerability of cholinergic neurons. Trends in neurosciences. 1992;15(4):117-22.

Figures

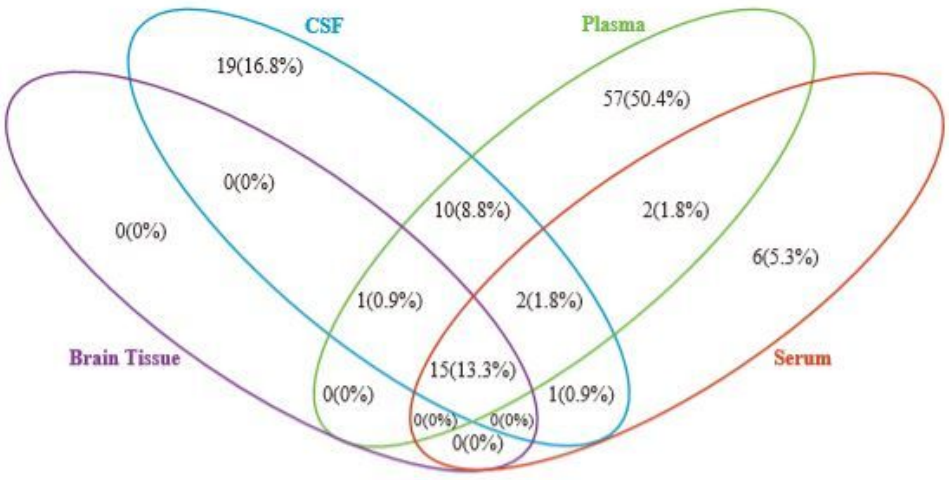

Figure 1

Venn diagram depicting common and unique pathways altered in Alzheimer's disease in different biosample types. Blue oval represents CSF, green oval represents plasma, purple oval represents brain tissue and red oval represents serum.

Figure 2

Venn diagram illustrating shared and unique pathways in retrospective and prospective studies. (A) Common pathways between AD VS. CN and MCI VS. CN. (B) Common pathways between AD VS. MCI and MCI VS. MCI_AD (conversion from MCI to AD).

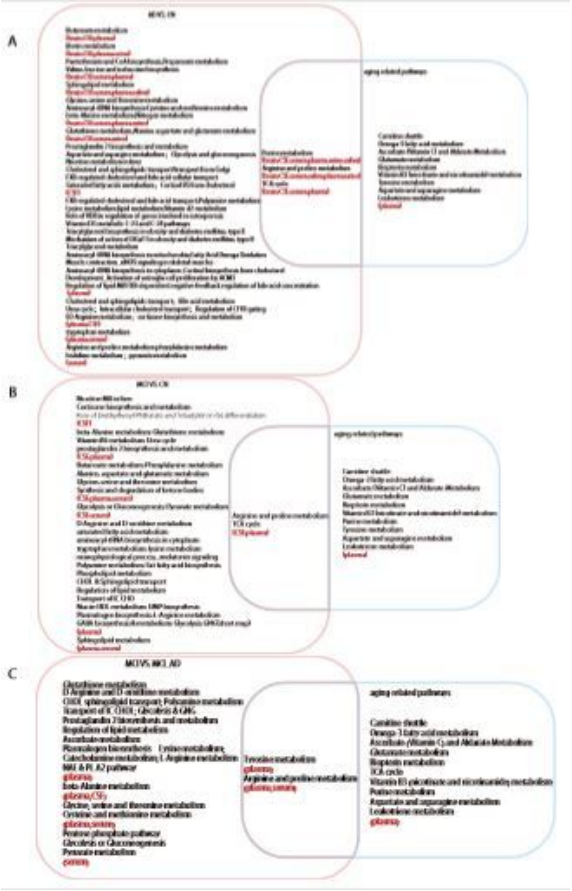

Figure 3

Intersection analysis among pathways of different comparisons and aging by Venn diagram. (A) Common pathways between AD VS. CN and aging. (B) Common pathways between MCI VS. CN and aging. (C) Common pathways between MCI VS. MCI_AD (conversion from MCI to AD) and aging.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Excel2.xlsx
- Excel1.xlsx
- SupplementaryFig.3.pdf
- SupplementaryFig.2.pdf
- SupplementaryFig.1.pdf
- SupplementaryTable10.docx
- SupplementaryTable9.docx
- SupplementaryTable8.docx
- SupplementaryTable7.docx
- SupplementaryTable6.docx
- SupplementaryTable5.docx
- SupplementaryTable4.docx

- SupplementaryTable3.docx
- SupplementaryTable2.docx
- SupplementaryTable1.docx