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D-glutamate, D-serine, and D-alanine differ in their roles in cognitive decline in patients with Alzheimer's disease or mild cognitive impairment

Chieh-Hsin Lin[a,b,c], Hui-Ting Yang[d], Hsien-Yuan Lane[b,e,f,*]

[a] Department of Psychiatry, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Kaohsiung, Taiwan
[b] Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan
[c] School of Medicine, Chang Gung University, Taoyuan, Taiwan
[d] School of Food Safety, Taipei Medical University, Taipei, Taiwan
[e] Department of Psychiatry & Brain Disease Research Center, China Medical University Hospital, Taichung, Taiwan
[f] Department of Psychology, College of Medical and Health Sciences, Asia University, Taichung, Taiwan

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ABSTRACTS

Background: D-amino acids have been recognized as bioactive substances in humans. D-Serine and D-alanine are co-agonists of *N*-methyl-D-aspartate receptors. Glutamate has been suggested to be involved in the pathophysiology of Alzheimer's disease (AD). This study aimed to explore the roles of amino acids, particularly D-amino acids, in cognitive decline among patients with AD or mild cognitive impairment (MCI).

Methods: We enrolled 144 patients: 20 amnestic MCI, 85 mild AD, 25 moderate AD, and 14 severe AD. Serum levels of amino acids were measured by high performance liquid chromatography and confirmed by D-amino acid oxidase assay. The cognitive function was mainly evaluated by Alzheimer's Disease Assessment Scale - Cognitive Subscale (ADAS-cog).

Results: ADAS-cog total scores were positively correlated with D-serine ($r = 0.186$, $p = 0.026$) and D-/Total-serine ratio ($r = 0.191$, $p = 0.022$). ADAS-cog behavior scores were negatively correlated with D-glutamate ($r = -0.177$, $p = 0.034$) and L-glutamate ($r = -0.250$, $p = 0.003$), but positively correlated with D-alanine ($r = 0.236$, $p = 0.005$) and D-/Total- alanine ratio ($r = 0.252$, $p = 0.002$). Among the 11 tasks of ADAS-cog, D-glutamate and D-serine were correlated with different items respectively, noteworthily in the opposite direction.

Conclusion: This is the first study suggesting that D-amino acids in blood may be correlated with ADAS-cog in different items and in the opposite direction. Lower D-glutamate and higher D-alanine levels may predict more behavioral symptoms. In summary, D-glutamate, D-serine and D-alanine play different and characteristic roles in AD. Further longitudinal studies are warranted to elucidate the function and interaction of D-amino acids in specific cognitive domains as well as various phases of dementia.

1. Introduction

The majority of amino acids in higher animals are thought to be L-enantiomers. However, D-amino acids have recently been recognized as bioactive substances in mammals, including humans (Ariyoshi et al., 2017).

Glutamate, an excitatory neurotransmitter, may also act as an endogenous neurotoxin. Excessive glutamatergic activation has been suggested to be involved in the pathophysiology of Alzheimer's disease (AD) (Danysz and Parsons, 2012; Wang and Reddy, 2017). Among glutamate receptors, NMDA receptors (NMDARs) are liable to be directly activated by numerous endogenous co-agonists such as glycine, D-serine, and D-alanine (Kleckner and Dingledine, 1988). D-serine may be responsible for controlling the extent of NMDAR-mediated neurotoxic insults observed in AD (Danysz and Parsons, 2012). Blood and cerebrospinal fluid (CSF) levels of D-serine were recently reported as distinctive for AD (Hashimoto et al., 2004; Lin et al., 2017; Madeira et al., 2015); however, the findings have been inconsistent across studies (Biemans et al., 2016). Further studies using a thorough cognitive assessment which is not influenced by demographic characteristics, such as education levels, are warranted.

Of note, whether D-glutamate (Ariyoshi et al., 2017) and D-alanine (D'Aniello et al., 1992) may also affect cognitive aging deserves studies too. Decreased total glutamate levels measured by proton magnetic resonance spectroscopy (MRS) were found in the brain of patients with amnestic mild cognitive impairment (MCI) (Zeydan et al., 2017).

* Corresponding author at: Department of Psychiatry, China Medical University Hospital, No. 2, Yuh-Der Road, Taichung 404, Taiwan.
E-mail address: hylane@gmail.com (H.-Y. Lane).

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Riluzole, a glutamate modulator, reversed the age-related gene expression changes in a rodent study (Pereira et al., 2017). However, the role of D-glutamate in cognitive aging remains unclear. L-glutamate is more widely occurring in nature, but D-glutamate occurs in some mammalian tissues such as liver and blood (Heresco-Levy et al., 2007; Raj et al., 2001; Wang et al., 2019), and in the cell walls of bacteria (such as *E. coli*) which can synthesize it from L-glutamate with glutamate racemase (Liu et al., 1998).

In addition to D-serine, D-alanine is also a main substrate of D-amino acid oxidase (DAO) and another full agonist of the NMDAR co-agonist site (Koga et al., 2017; Lin et al., 2014). However, studies investigating the role of D-alanine on cognitive aging are lacking too. This study aimed to explore the roles of amino acids, particularly D-amino acids, in the cognitive decline in patients with AD or MCI.

2. Materials and methods

2.1. Participants

All participants were recruited from the outpatient departments and evaluated thoroughly by research physicians from Kaohsiung Chang Gung Memorial Hospital and China Medical University Hospital in Taiwan. This study was carried out in accordance with the recommendations of Good Clinical Practice (GCP), Institutional review boards of Kaohsiung Chang Gung Memorial Hospital and China Medical University Hospital, Taiwan. The study was approved by the institutional review boards of the two hospitals. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

All participants were 50–100-year-old Han Chinese who were physically healthy with normal blood routine and biochemical tests. Participants were recruited if they [1] satisfied NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) (McKhann et al., 1984) criteria for probable AD and had a Clinical Dementia Rating (CDR) (Morris, 1993) score of 1 (mild AD), 2 (moderate AD), or 3 (severe AD), or criteria for amnestic MCI (Lu et al., 2009), [2] had adequate education for effective communication, [3] were able to complete the assessments of the study, and [4] agreed to join the study and provided written informed consent. Exclusion criteria included major medical, neurological, or psychiatric conditions other than AD; delirium symptoms; substance abuse or dependence (including alcohol); Hachinski Ischemic Score > 4; history of significant cerebrovascular disease; severe hearing or visual impairment; and being unable to follow protocol.

AD patients with and without anti-dementia drugs were both recruited. AD patients without anti-dementia drugs were free from those medications for three months or longer. For patients with anti-dementia drugs treatment, those medications had been maintained for three months or longer with unchanged dose. Medication history was determined by reviewing medical records, contacting other health care providers, and interviewing the participants and their family or caregivers.

2.2. Cognitive function assessments

The cognitive function of the participants was evaluated by CDR, Mini-Mental State Examination (MMSE) (Folstein et al., 1975) and Alzheimer's Disease Assessment Scale- Cognitive Subscale (ADAS-cog) (Rosen et al., 1984). CDR is widely used for the evaluation of the global severity and staging of dementia (Rockwood et al., 2000). CDR reflects both the cognitive and global impairment of the participants. MMSE is a commonly used tool for the measurement of cognitive function of dementia (Folstein et al., 1975). However, CDR is unable to reveal the precise deficits of cognitive function. MMSE is influenced by age and education (Crum et al., 1993), therefore limiting its use. In contrast, ADAS-cog is the most popular tool for the measurement of cognitive function in clinical studies and trials. The ADAS-cog scale has two parts: one for cognitive functions and the other for non-cognitive functions such as mood and behavior. The part for cognitive functions consists of 11 tasks, including Word Recall Task, Naming Objects and Fingers, Following Commands, Constructional Praxis, Ideational Praxis, Orientation, Word Recognition Task, Remembering Test Directions, Spoken Language, Comprehension and Word-Finding Difficulty. Higher score of ADAS-cog represents the greater cognitive dysfunction. ADAS-cog is less influenced by age, gender or education compared to MMSE (Liu et al., 2002).

2.3. Laboratory assessments

The blood sampling was done during 8–12 AM after fasting for more than eight hours. 10 mL of blood was collected by personnel trained in phlebotomy using sterile technique. The blood specimens were processed immediately by centrifugation at 1000 ×*g*. After centrifugation, serum was quickly dissected, immediately stored at −80 °C until further measurement.

2.4. Amino acids levels measurement

Serum was firstly extracted by methanol (1:3, by volume), then filtered after 15 min centrifugation (1500 ×*g*) with nylon membranes (0.45 mM, Minisart SRP4, Sartorius, Germany). The filtrate was diluted with proper amount of 20% methanol then derivatized with N-isobutyl-L-cysteine (IBC) and O-phthaldialdehyde (OPA) mixture for 5 min then injected into high performance liquid chromatography (HPLC, L-7100 Pump, L7250 Autosampler, L-7250, with L7480 fluorescence Detector, Hitachi, Japan) for analysis. Analytical column (Grom-Sil OPA-2, 5 µm, 250 mm * 4 mm, Part No: GSOP 20512S2504, SAP No: 5113679, Grace, US) with guard column (Grom-Sil OPA-2, 5 µm, 10 mm × 4 mm, Part No: GSOP20512v0104V, Grace, US) were used for the determination. Isocratic elution of mobile phase A (23 mM sodium acetate, pH 6.0) and B (50 mL acetonitrile in 600 mL methanol) were performed under fluorescence detection (excitation 260 nm, emission 455 nm), respectively. Retention time of each amino acid was L-glutamine, 25.5 min; D-glutamine, 27.3 min; L-serine, 33.6 min; D-serine, 35.8 min; glycine, 41.5 min; L-alanine, 47.2 min; D-alanine, 50.3 min, respectively. All amino acids levels were double-checked by performing HPLC analyses for two times in order to confirm that the peaks were not artifact.

We also used 10 µL D-amino acid oxidase (DAO) (2 mg/mL) mixed with 40 µL serum sample for the verification of D-amino acids levels (Kato et al., 2011). After serial gradient heating, 150 mL methanol was added and centrifuged under 15,000 ×*g* at 4 °C for 15 min. The filtrate was injected into the HPLC when reacting with OPA. The results are shown in Supplementary Table 1 and Supplementary Fig. 1. D-Serine and D-alanine levels were markedly decreased after DAO addition. The levels of L-form amino acids, D-glutamate and glycine were slightly decreased, representing the diluting effect in the test.

2.5. Statistical analysis

All participants' demographic and clinical characteristics and amino acids levels are shown as number (percentage) or mean ± SD. The relationships among variables were analyzed by Pearson Correlation. A p value < 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 22.0 (SPSS Inc.).

3. Results

A total of 144 patients were enrolled: 20 amnestic MCI patients (CDR = 0.5), 85 mild AD patients (CDR = 1), 25 moderate AD patients (CDR = 2), and 14 severe AD patients (CDR = 3). Their gender, age, education, CDR, MMSE, ADAS-cog total and ADAS-cog behavior scores,

Table 1
Characteristics of patients with cognitive decline (N = 144).

	N	%
Gender, female	93	64.6
	Mean	SD
Age (year)	72.8	9.1
Education (year)	4.2	4.0
CDR	1.3	0.7
MMSE	16.3	6.1
ADAS-cog total score	25.8	14.0
ADAS-cog Behavior score	3.5	4.3
No. of patients using anti-dementia drugs	N	%
Total	29	20.1
No. and dose of anti-dementia drugs	N	Mean ± SD
Donepezil (dose)	21	9.5 ± 1.5
Rivastigmine (dose)	5	8.4 ± 1.3
Galantamine (dose)	2	16.0 ± 0.0
Memantine (dose)	1	20.0 ± NA
	Mean	SD
D-glutamate level (ng/mL)	849.080	690.409
L-glutamate level (ng/mL)	8804.263	6704.454
D/T-glutamate ratio	0.111	0.100
D-serine level (ng/mL)	49.971	29.342
L-serine level (ng/mL)	3437.136	1158.988
D/T-serine ratio	0.015	0.009
D-alanine level (ng/mL)	36.062	34.520
L-alanine level (ng/mL)	12,639.343	3650.388
D/T-alanine ratio	0.003	0.003
Glycine level (ng/mL)	4611.286	1950.635

Abbreviations: NA, not associated; CDR, Clinical Dementia Rating Scale; MMSE, Mini-Mental State Examination; ADAS-cog, Alzheimer's Disease Assessment Scale- Cognitive Subscale; D/T-glutamate ratio, D-glutamate/total glutamate ratio; D/T-serine ratio, D-serine/total serine ratio; D/T-alanine ratio, D-alanine/total alanine ratio.

and levels of amino acids are shown in Table 1. There were more women than men in this cohort (64.6%). Twenty-nine patients (20.1%) received anti-dementia agents (including AChEIs and memantine) (Table 1).

3.1. ADAS-cog total score and behavior score were correlated with different amino acids

The correlations among ADAS-cog total score, behavior score, and amino acid levels are shown in Table 2 and Fig. 1.

ADAS-cog total scores were positively correlated with D-serine levels ($r = 0.186$, $p = 0.026$) and D-/Total- serine ratio ($r = 0.191$, $p = 0.022$) (Table 2). The correlation between ADAS-cog total scores and D-glutamate levels appeared marginally significant ($r = -0.147$, $p = 0.079$), and so did the correlation between ADAS-cog total scores and D-alanine levels ($r = 0.140$, $p = 0.096$) (Table 2).

ADAS-cog behavior scores were negatively correlated with D-glutamate ($r = -0.177$, $p = 0.034$) and L-glutamate ($r = -0.250$, $p = 0.003$), but positively correlated with D-alanine ($r = 0.236$, $p = 0.005$) and D-/Total- alanine ratio ($r = 0.252$, $p = 0.002$) (Table 2).

Among all amino acids, D-glutamate was correlated with only L-glutamate ($r = 0.177$, $p = 0.034$), but not any of other amino acids. In contrast, L-glutamate was significantly correlated with L-serine and L-alanine. There was significant correlation between D-serine and D-alanine ($r = 0.287$, $p = 0.001$). L-Serine was also correlated with L-alanine and glycine. D-alanine was correlated with glycine (Table 2).

3.2. D-glutamate and D-serine referred to different tasks of ADAS-cog, in the opposite direction

Among the 11 items of ADAS-cog, D-glutamate had significantly negative correlations with item 2 ($r = -0.274$, $p = 0.001$), item 3 ($r = -0.217$, $p = 0.009$) and item 10 ($r = -0.165$, $p = 0.048$). D-serine had significantly positive correlations with item 1 ($r = 0.215$, $p = 0.010$), item 6 ($r = 0.242$, $p = 0.004$), item 10 ($r = 0.209$, $p = 0.012$) and item 11 ($r = 0.203$, $p = 0.015$). D-alanine showed no correlation with any of the items in ADAS-cog. Each item had significant correlations with other items (Table 3).

4. Discussion

To our knowledge, we are the first group to study the roles of D-glutamate and D-alanine in cognitive decline associated with dementia. The results suggest that D-glutamate and D-alanine play different roles from D-serine in cognitive decline in patients with AD or MCI. ADAS-cog total scores were positively correlated with D-serine levels. ADAS-cog total scores also showed a trend (albeit insignificant) to be related with D-alanine levels (positively) and D-glutamate (negatively) (Table 2).

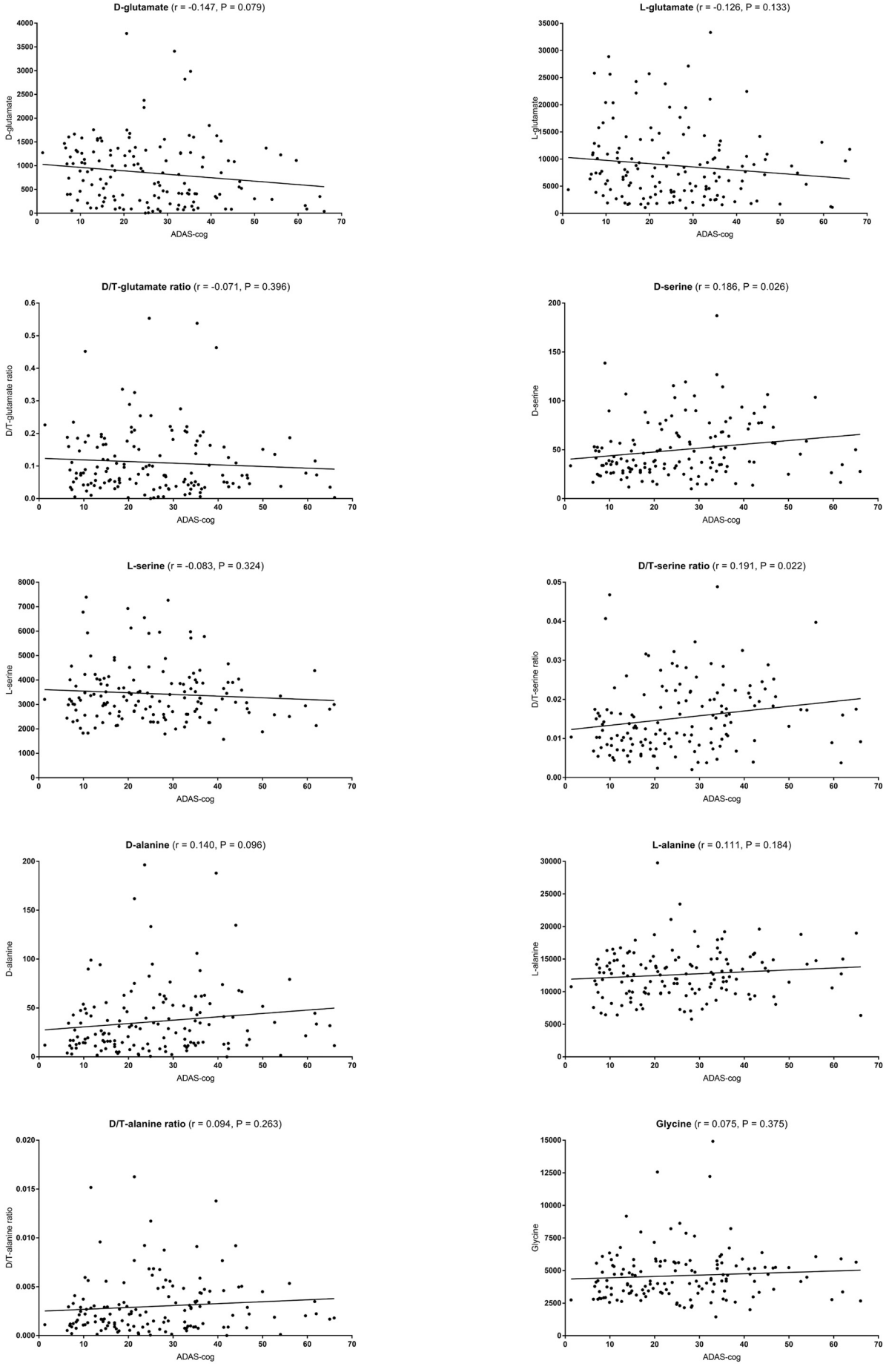
The ADAS-cog is the standard assessment tool used to measure cognitive dysfunction in clinical trials of AD; it consists of 11 items that are designed to measure diverse cognitive domains, including those associated with memory, language and praxis (Benge et al., 2009). Importantly, D-glutamate and D-serine levels were related to different items of ADAS-cog, in the opposite direction, while D-alanine showed no correlation with any of the ADAS-cog items. Among the 11 ADAS-

Table 2
Zero-order correlations among ADAS-cog total and behavioral scores and amino acids levels.

(N = 144)	1	2	3	4	5	6	7	8	9	10	11	12
1 ADAS-cog T	–											
2 ADAS-cog B	0.521#	–										
3 D-glutamate	−0.147	−0.177*	–									
4 L-glutamate	−0.126	−0.250**	0.177*	–								
5 D/T-glutamate ratio	−0.071	0.016	0.671#	−0.402#	–							
6 D-Serine	0.186*	0.082	0.101	0.027	0.135	–						
7 L-Serine	−0.083	−0.144	0.102	0.565#	−0.143	0.080	–					
8 D/T-serine ratio	0.191*	0.129	0.052	−0.208*	0.163	0.860#	−0.349#	–				
9 D-alanine	0.140	0.236**	0.003	−0.137	0.212*	0.287**	0.033	0.286**	–			
10 L-alanine	0.111	0.044	0.099	0.170*	−0.021	0.042	0.425#	−0.089	0.121	–		
11 D/T-alanine ratio	0.094	0.252**	−0.046	−0.197*	0.191*	0.247**	−0.103	0.292#	0.909#	−0.193*	–	
12 Glycine	0.075	0.014	0.089	0.137	0.030	0.162	0.438#	−0.021	0.179*	0.453#	0.022	–

Abbreviations: ADAS-cog T, Alzheimer's disease assessment scale-cognitive subscale total score; ADAS-cog B, Alzheimer's disease assessment scale-cognitive subscale behavioral score; D/T-glutamate ratio, D-glutamate/total glutamate ratio; D/T-serine ratio, D-serine/total serine ratio; D/T-alanine ratio, D-alanine/total alanine ratio.

* $p < 0.05$.
** $p < 0.01$.
\# $p < 0.001$.



(caption on next page)

Fig. 1. Correlations among ADAS-cog total scores and amino acids levels.

cog items, D-serine levels were positively correlated with item 1 (word recall task), item 6 (orientation), item 10 (comprehension) and item 11 (word-finding difficulty); in contrast, D-glutamate levels were negatively correlated with item 2 (naming objects and fingers), item 3 (following commands) and item 10 (comprehension) (Table 3). That is, patients with lower D-glutamate levels had higher scores in these three cognitive domains (more impaired). Of note, impairments in these three domains usually emerge at later phases of AD. In accordance, our recent study (Lin et al., 2017) also found that D-glutamate levels were highest in the healthy elderly (mean: 1620.4 ± 558.2 [SD] ng/mL), followed by MCI patients (1097.8 ± 284.0 ng/mL), patients with mild AD (1031.9 ± 775.8 ng/mL), and lowest in the patients with moderate-severe AD (598.3 ± 551.9 ng/mL).

Moreover, ADAS-cog behavior scores were also negatively correlated with D-glutamate, and positively correlated with D-alanine levels (Table 2). ADAS-cog behavior scores reflect the severity of behavioral and psychological symptoms of dementia (BPSD, including hallucinations, delusions, agitation, sundown syndrome…etc.). BPSD, occurring in most patients with dementia, are associated with worsening cognition and later stages of dementia (Huang et al., 2012). They cause great suffering in patients and caregivers, sometimes more so than the cognitive and functional decline (Tible et al., 2017). Psychosocial interventions may improve BPSD; however, medication is often required for more severe manifestations. To date, no pharmacological treatment has been demonstrated to be beneficial for the disease course (Tible et al., 2017), or approved by the U.S. Food and Drug Administration (FDA) for treating BPSD. Further, suitable biomarkers for assisting the diagnosis or predicting treatment response to neuroleptics or novel therapies, have been limited, particularly in peripheral blood (Bloniecki et al., 2014; Huang et al., 2012). Whether D-glutamate and D-alanine can serve as useful peripheral biomarkers (for early diagnosis and for prediction for treatment response), their relationships with CNS levels, as well as the underlying mechanisms deserve further studies. Moreover, whether modulating D-glutamate and D-alanine can help the treatment of AD, including BPSD, also need preclinical and clinical trials in the future.

"Glutamate excitotoxicity theory" is one of the possible pathogeneses of AD, particularly in the later phase (Danysz and Parsons, 2012; Huang et al., 2012; Wang and Reddy, 2017). Accordingly, in our recent study (Lin et al., 2017), L-glutamate levels appeared significantly higher in individuals with MCI (12,317.1 ± 7622.4 ng/mL), mild AD (9377.5 ± 6733.1 ng/mL), or moderate-severe AD (8549.7 ± 5551.8 ng/mL) than in healthy elderly (7057.1 ± 3915.1 ng/mL). On the other hand, as aforementioned, D-glutamate levels fell step by step from healthy elderly, MCI, mild AD, to later-phase AD. Further studies are warranted for verification, and future studies should measure D-glutamate and L-glutamate separately to distinguish their different (or opposite or compensatory) roles in the aging brain.

Blood and CSF levels of D-serine were recently reported distinctive for AD in some studies (Hashimoto et al., 2004; Lin et al., 2017; Madeira et al., 2015), but not in all (Biemans et al., 2016). Our recent study (Lin et al., 2017) showed that D-serine levels were lowest in healthy elderly with CDR = 0 (mean: 29.3 ± 9.9 ng/mL [SD]), higher in MCI individuals with CDR = 0.5 (38.4 ± 13.1 ng/mL) and patients with mild AD with CDR = 1 (47.0 ± 30.9 ng/mL) and highest in patients with moderate-severe AD with CDR = 2–3 (48.0 ± 26.3 ng/mL), albeit with statistical insignificance ($p = 0.062$, ANOVA test). In comparison, the present study demonstrated that the role of D-serine in cognitive aging became clearer, with statistical significance, while we applied a more comprehensive cognitive assessment tool, ADAS-cog. It has been indicated that several assessment tools differ in their precision in measuring the severity of dementia (Balsis et al., 2015). In the future, thorough assessments for both cognitive and non-cognitive fields are recommended. Possible confounding factors such as dementia severity and ethnicity also need to be taken into account.

Among all amino acids, there was no significant correlation between D-glutamate vs. each other amino acid except for L-glutamate. In contrast, L-glutamate was significantly correlated with L-serine and L-alanine. There was also significant correlation between D-serine and D-alanine. L-Serine was also correlated with L-alanine and glycine. D-alanine was correlated with glycine (Table 2). The findings also lend support to the unique roles of various amino acids.

While the function and potential interaction of the three D-amino acids (D-glutamate, D-serine, and D-alanine) in brain and its disorders such as AD is still an enigma, D-aspartate, which is involved in the regulation of hormone secretion and steroidogenesis in central nervous, neuroendocrine, and endocrine systems (Errico et al., 2009; Errico et al., 2014), also deserves studies on its possible role in cognitive aging (Punzo et al., 2016).

Table 3
Zero-order correlations among ADAS-cog sub-item scores and D-glutamate, D-serine, and D-alanine levels.

(N = 144)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 D-glutamate	–													
2 D-Serine	0.101	–												
3 D-alanine	0.003	0.287**	–											
4 Item 1	−0.062	0.215*	0.146	–										
5 Item 2	−0.274**	0.095	0.094	0.400#	–									
6 Item 3	−0.217**	−0.003	0.089	0.426#	0.371#	–								
7 Item 4	−0.037	0.014	0.074	0.481#	0.295#	0.303#	–							
8 Item 5	−0.093	−0.008	0.090	0.507#	0.406#	0.437#	0.457#	–						
9 Item 6	−0.132	0.242**	0.085	0.609#	0.468#	0.438#	0.346#	0.439#	–					
10 Item 7	−0.086	0.129	0.074	0.761#	0.439#	0.510#	0.366#	0.471#	0.603#	–				
11 Item 8	−0.133	0.134	0.035	0.394#	0.313#	0.535#	0.303#	0.389#	0.582#	0.439#	–			
12 Item 9	−0.098	0.099	0.097	0.437#	0.302#	0.495#	0.304#	0.420#	0.565#	0.431#	0.819#	–		
13 Item 10	−0.165*	0.209*	0.092	0.453#	0.329#	0.503#	0.261**	0.367#	0.572#	0.447#	0.818#	0.824#	–	
14 Item 11	−0.134	0.203*	0.101	0.417#	0.463#	0.509#	0.232**	0.437#	0.579#	0.494#	0.703#	0.760#	0.730#	–

Abbreviations: Item 1, Word Recall Task; Item 2, Naming Objects and Fingers; Item 3, Following Commands; Item 4, Constructional Praxis; Item 5, Ideational Praxis; Item 6, Orientation; Item 7, Word Recognition Task; Item 8, Remembering Test Directions; Item 9, Spoken Language; Item 10, Comprehension; Item 11, Word-Finding Difficulty.

* $p < 0.05$.

** $p < 0.01$.

\# $p < 0.001$.

This study has several limitations. Firstly, the finding from this study is limited by its cross-sectional design. Secondly, the peripheral blood-brain relationship of amino acids remains unknown. Thirdly, only Han Chinese populations were recruited in this study. The findings need to be tested in other populations. Fourthly, the sample size was only modest, perhaps hindering us from drawing definite conclusion. Lastly, daily diet may affect D-glutamate blood levels. D-glutamate is present in certain foods e.g., soybeans and also arises from the turnover of the intestinal microflora. All meats, poultry, fish, eggs, dairy products, seaweed and some protein-rich plant foods provide glutamate. Foods containing monosodium glutamate (MSG) have a low percentage of the D-glutamate. In contrast, fermented foods have relatively higher levels of D-glutamate (Rundlett and Armstrong, 1994). Daily diet should be controlled in future studies.

5. Conclusion

In conclusion, this study suggests that higher D-serine levels predict worse cognitive function, particularly in the domains of word recall task, orientation, comprehension, and word-finding difficulty. On the other hand, lower D-glutamate levels were associated with more impairments in naming objects and fingers, following commands, and comprehension (Table 3) and with more behavioral symptoms (Table 2). Differing from D-glutamate, higher D-alanine levels were associated with more behavioral symptoms (Table 2). In the future, the roles of these amino acids in modulating cognitive and behavior manifestations of AD deserve further elucidation by prospective longitudinal, larger-scale studies. It is worthy to test whether they could serve as biomarkers reflecting the function of NMDAR and progression of AD as well as predicting treatment response in cognitive and behavioral symptoms. Finally, it is also interesting to explore whether modulators of such amino acids can be developed as novel treatments for AD.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbb.2019.172760.

Ethical approval and consent to participate

This study was carried out in accordance with the recommendations of Good Clinical Practice (GCP), Institutional review boards of Kaohsiung Chang Gung Memorial Hospital and China Medical University Hospital, Taiwan. The study was approved by the institutional review boards of the two hospitals. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Consent for publication

All authors approved the submission and gave consent for publication.

Declaration of competing interest

All authors declare that they have no competing interest.

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Authors' contributions

CH Lin and HY Lane involved in conception and design, literature review, participants enrollment, data interpretation, and manuscript writing; HT Yang performed the laboratory procedures; All authors reviewed and approved the manuscript.

Availability of supporting data

The supporting data is available on request with adequate ethical approval document.

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