



# The associations of serum valine with mild cognitive impairment and Alzheimer's disease

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## Abstract

**Background** The introduction of metabolomics makes it possible to study the characteristic changes of peripheral metabolism in Alzheimer's disease (AD). Recent studies have found that the levels of valine are related to mild cognitive impairment (MCI) and AD.

**Aims** This study aimed to further clarify the characteristics of valine levels in MCI and AD.

**Methods** A total of 786 participants from the Alzheimer's Disease Neuroimaging Initiative-1 (ADNI-1) cohort were selected to evaluate the relationships between serum valine and cerebrospinal fluid (CSF) biomarkers, brain structure (magnetic resonance imaging, MRI), cerebral glucose metabolism (<sup>18</sup>F-fluorodeoxyglucose-positron emission tomography, FDG-PET), and cognitive declines, through different cognitive subgroups.

**Results** Serum valine was decreased in patients with AD compared with cognitive normal (CN) and stable MCI (sMCI), and in progressive MCI (pMCI) compared with CN. Serum valine was negatively correlated with CSF total tau (t-tau) and phosphorylated tau (p-tau) in pMCI. Serum valine significantly predicted conversion from MCI to AD. In addition, serum valine was related to the rate of change of cerebral glucose metabolism during the follow-up period in pMCI.

**Conclusions** Serum valine may be a peripheral biomarker of pMCI and AD, and its level predicts the progression of MCI to AD. Our study may help to reveal the metabolic changes during AD disease trajectory and its relationship to clinical phenotype.

**Keywords** Alzheimer's disease · Cerebral glucose metabolism · Cognition · Mild cognitive impairment · Serum valine

## Background

Alzheimer's disease (AD) is the most prominent cause of dementia in the elderly, which the mechanism of disease development and progression is unclear. Growing evidence supports the concept that severe metabolic dysfunction is a marker and the cause of AD [1]. Researchers have applied metabolomics to examine alterations in blood metabolite profiles in AD patients. Such studies may not only identify peripheral biomarkers but also identify key metabolic

pathways to AD pathogenesis [2–5]. To enhance the systems-level data available, the Alzheimer's Disease Metabolomics Consortium (ADMC) in partnership with the Alzheimer's Disease Neuroimaging Initiative (ADNI) is creating a database to interrogate global metabolomics changes for patients in the ADNI-1 cohort [6].

Branched-chain amino acids (BCAAs) are amino acids possessing an aliphatic side chain with a branch, which can easily cross the blood–brain barrier (BBB) [7], and convert to glutamate by a transamination reaction catalyzed by branched-chain aminotransferases in the presence of  $\alpha$ -ketoglutarate. Glutamate is an excitatory neurotransmitter in the mammalian brain, which is related to the process of memory and learning [8]. It has been estimated that BCAAs provided at least one-third of the nitrogen for brain glutamate [9].  $\gamma$ -aminobutyric acid (GABA) is an inhibitory neurotransmitter synthesized by decarboxylation of glutamate. Therefore, the disorder of BCAAs levels significantly affect

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the overall function of the central nervous system, especially the balance between excitation and inhibition [9]. Interestingly, disrupted excitatory glutamate signaling is strongly implicated in AD as well as several other neurodegenerative disorders [10].

There are three known proteinogenic BCAAs, valine, leucine, and isoleucine. A study in mice indicated that brain uptake of valine was more rapid than that of other BCAAs [11]. The study on the concentration of amino acids in cerebrospinal fluid (CSF) showed that valine in AD was significantly lower than that in healthy controls [12]. It has been found that the level of serum valine has changed during the symptomatic stage of AD, and was related to the decline of cognitive function and the change of ventricular volume [13]. Given that decreased serum valine levels have also been detected in frontotemporal dementia [14], whether serum valine can be a serum-specific marker of AD needs to be further explored.

In the present study, we detected serum valine levels in different cognitive subgroups, and then explored whether serum valine could offer predictive value for future disease progression, also examined the relationship between serum valine and CSF core markers, cognition, brain structure and metabolism in AD, as measured by the Mini-Mental State Examination (MMSE), Alzheimer's Disease Assessment Scale cognitive subscale (ADAS-cog 13), magnetic resonance imaging (MRI), and <sup>18</sup>F-fluorodeoxyglucose positron emission tomography (FDG-PET).

## Materials and methods

### Database description

Data used in the preparation of this article were obtained from the ADNI database (<http://adni.loni.usc.edu>). ADNI was launched in 2003 as a public-private partnership, led by principal investigator Michael W. Weiner, MD (the most recent information on the ADNI is available at <http://www.adni-info.org>). The ADNI participants have been recruited from more than 50 sites across the United States and Canada. Regional ethical committees of all participating institutions approved the ADNI. All study participants provided written informed consent. In this study, we profiled baseline serum samples from the ADNI-1 cohort where vast data exist on each patient including cognitive decline and imaging changes over many years, information on CSF markers, genetics, and other omics data. Further information can be found at <http://www.adni-info.org>.

From the database, we selected all participants who were aged 55 to 90 years, had completed at least 6 years of education, were fluent in Spanish or English, and had no substantial neurological disease other than AD, who had baseline

serum valine samples provided by ADNI-1 and completed lumbar puncture, MMSE, ADAS-cog, Clinical Dementia Rating (CDR) scale, MRI, and FDG-PET. According to clinical and behavioral measures provided by the ADNI-1, selected individuals were classified as cognitively normal (CN,  $n = 225$ ), stable MCI (sMCI,  $n = 181$ ), progressive MCI (pMCI,  $n = 195$ ), and dementia due to AD ( $n = 185$ ).

### Classification criteria

The criteria for CN included an MMSE score of 24 or higher, where lower scores indicate more impairment and higher scores less impairment (0–30), and a CDR score of 0, where lower scores indicate less impairment and higher scores more impairment (0–3) [15, 16]. The criteria for MCI included the presence of a subjective memory complaint, with an MMSE score between 24 and 30, a CDR of 0.5, preserved activities of daily living, and an absence of dementia [17]. Patients with AD dementia fulfilled the National Institute of Neurological Communicative Disorders and Stroke-Alzheimer Disease and Related Disorders Association criteria for probable AD, had MMSE scores between 20 and 26, and a CDR of 0.5 or 1.0 [18]. We defined sMCI as MCI subjects not progressing to AD during at least 2 years of follow-up and pMCI as MCI subjects progressing to AD at any time during follow-up [19]. We excluded subjects who were diagnosed with MCI at baseline but reverted to CN during follow-up, as well as subjects who were diagnosed with AD at baseline but reverted to MCI during follow-up. (Further information about the inclusion/exclusion criteria may be found at [www.adni-info.org](http://www.adni-info.org), accessed May 2021.)

### Serum valine

Morning fasting blood samples from the baseline visit were included in the study. Serum valine was measured with a targeted metabolomics approach using the Absolute IDQ-p180 kit (BIOCRA TES Life Science AG, Innsbruck, Austria), with ultra-performance liquid chromatography (UPLC)/MS/MS system [Acquity UPLC (Waters), TQ-S triple quadrupole MS/MS (Waters)]. The Absolute IDQ-p180 kit has been fully validated according to European Medicine Agency Guidelines on bioanalytical method validation. In addition, plates include an automated technical validation to approve the validity of the run and provide verification of the actual performance of the applied quantitative procedure including instrumental analysis. The technical validation of each analyzed kit plate was performed using Met IDQ software based on results obtained and defined acceptance criteria for blank, zero samples, calibration standards, and curves, low/medium/high-level quality control samples, and measured signal intensity of internal standards over the plate [13, 20].

This is a highly useful platform that was used in hundreds of publications, including several studies in AD [2, 21, 22].

### CSF measurements

Lumbar puncture was performed in the mornings after an overnight fast. CSF amyloid- $\beta$  42 (A $\beta$ 42), total-tau (t-tau), and phosphorylated-tau at threonine 181 (p-tau) were measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX, USA) and Innogenetics INNO-BIA Alz-Bio3 (Innogenetics, Ghent, Belgium) immunoassay reagents as described previously [22]. All of the CSF data used in this study were obtained from the ADNI files “UPENN-BIOMK5–8.csv” and “FAGANLAB\_07\_15\_2015.csv” (accessed May 2021). Further details of ADNI methods for CSF acquisition and measurements and quality control procedures can be found at [www.adni-info.org](http://www.adni-info.org).

### Cognitive assessment

Global cognitive performance was assessed by MMSE and ADAS-cog 13 scores. We had selected MMSE and ADAS-cog 13 scores at five time points: baseline, 12 months, 24 months, 36 months, and 48 months. The data used in this study was obtained from the ADNI files “MMSE.csv” and “ADAS\_ADNI1.csv” (accessed May 2021).

### Neuroimaging methods

Structural brain images were acquired using 1.5-T MRI imaging systems with T1-weighted MRI scans using a sagittal volumetric magnetization-prepared rapid acquisition gradient-echo sequence. We used hippocampal and ventricular volume to represent neurodegeneration and had selected the imaging data at five time points: baseline, 12 months, 24 months, 36 months, and 48 months. These data came from the ADNI files “FOXLABBSI\_08\_04\_17.csv” and “UCSDVOL.csv,” (accessed May 2021). Further details for ADNI image acquisition and processing can be found at [www.adni-info.org/methods](http://www.adni-info.org/methods).

### FDG-PET

FDG-PET was used to investigate cerebral glucose metabolism. Acquisition and processing of PET imaging data in ADNI had described in detail elsewhere [21]. In short, the mean counts of the lateral and medial prefrontal, anterior, and posterior cingulate regions, as well as lateral parietal and lateral temporal regions were used to estimate FDG standardized uptake value ratio (SUVR) value for each participant. FDG-PET image data were acquired at baseline and 12 months, 24 months, 36 months, and 48 months.

### Statistical methods

Analysis of covariance (ANOVA) and chi-square analyses were performed to test for significant differences between groups on baseline demographics. We tested associations between serum valine and diagnostic groups using analysis of variance.

Spearman correlations were used to assess relationships between serum valine and other core AD biomarkers. We calculated diagnostic accuracies of each biomarker using area under the receiver operating characteristic curve (ROC) analysis. Bootstrapping method was used to assess the potential differences between two area under the curves (AUCs) derived from all pairs of two different biomarkers.

The associations of serum valine with the incidence of AD were evaluated by calculating hazard ratios (HRs) with 95% confidence intervals (CIs) using Cox proportional hazard regression analysis with adjustment for age and sex. Serum valine was categorized into two groups by the median of each biomarker when conducting Cox proportional hazard regression analysis.

Associations of serum valine level with longitudinal cognition, brain structure, and brain metabolism were tested with linear mixed-effects models. The intercepts (baseline values) and slopes (rates of change) were then used as outcomes in linear regression models with valine as a predictor (adjusted for age and gender, and for education for MMSE and ADAS-cog 13, and for intracranial volume for hippocampal and ventricular volumes) within diagnostic groups. All statistics were performed using SAS 9.4 and SPSS version 21. Statistical significance was defined as  $p < 0.05$  for all analyses.

## Results

### Demographic results

The demographics and biomarker characteristics of the study subjects are presented in Table 1. There was no difference in age among the groups. Compared with AD group, there were significantly fewer female subjects in sMCI group ( $p < 0.01$ ). The educational levels in AD group were lower than those in other diagnostic groups ( $p < 0.05$  for all). Compared with CN and sMCI, CSF A $\beta$ 42 levels were significantly lower in pMCI and AD, and CSF t-tau and p-tau were significantly higher in pMCI and AD, but there was no significant difference between pMCI and AD. The mean levels of MMSE, ADAS-cog 13, hippocampal volume, and FDG-PET (SUVR) were significantly different among the diagnostic groups. Ventricular volume was significantly higher in patients with AD compared with CN and sMCI, and lower in CN compared with other diagnostic groups (Table 1).

## Serum valine in different diagnostic groups

Serum valine levels were significantly lower in patients with AD ( $278.05 \pm 50.4 \mu\text{M}$ ) compared with CN ( $300.92 \pm 66.27 \mu\text{M}$ ) ( $p < 0.01$ ) and sMCI ( $297.89 \pm 61.79 \mu\text{M}$ ) ( $p < 0.05$ ). Lower serum valine levels were also found in pMCI ( $284.99 \pm 57.95 \mu\text{M}$ ) compared with CN ( $300.92 \pm 66.27 \mu\text{M}$ ) ( $p < 0.05$ ). However, there were no differences between CN and sMCI as well as between sMCI and pMCI, and similarly between pMCI and AD (Fig. 1).

## Serum valine in relation to CSF A $\beta$ and tau

There was no significant correlation between CSF A $\beta$ 42 and serum valine in different diagnostic groups (CN,  $r = 0.071$ ,  $p = 0.513$ ; sMCI,  $r = 0.044$ ,  $p = 0.717$ ; pMCI,  $r = 0.130$ ,  $p = 0.207$ ; AD,  $r = 0.088$ ,  $p = 0.406$ ) (Fig. 2A). Valine was negatively correlated with CSF t-tau ( $r = -0.260$ ,  $p = 0.01$ ) (Fig. 2B) and p-tau ( $r = -0.231$ ,  $p = 0.023$ ) in pMCI (Fig. 2C), but not in CN ( $r = 0.075$ ,  $p = 0.491$  for t-tau;  $r = 0.052$ ,  $p = 0.637$  for p-tau), sMCI ( $r = 0.134$ ,  $p = 0.270$  for t-tau;  $r = 0.133$ ,  $p = 0.274$  for p-tau), and AD ( $r = 0.106$ ,  $p = 0.316$  for t-tau;  $r = 0.118$ ,  $p = 0.265$  for p-tau) (Fig. 2B, C).

## Diagnostic accuracy of serum valine, CSF t-tau, and p-tau

ROC analyses were performed to detect serum valine, CSF t-tau, and p-tau related to clinical diagnoses in sMCI, pMCI,

and AD. Compared to CN, CSF t-tau and p-tau showed significant diagnostic accuracy for sMCI (Table 2 and Fig. 3A), pMCI (Table 2 and Fig. 3B), and AD (Table 2 and Fig. 3C). While the diagnostic accuracy of valine for sMCI (Table 2 and Fig. 3A), pMCI (Table 2 and Fig. 3B), and AD (Table 2 and Fig. 3C) was not statistically significant. Compared to t-tau or p-tau alone, the combination of valine, t-tau, or p-tau provided a higher diagnostic accuracy for sMCI and AD, although not statistically significant (Table 2 and Fig. 3A and C). The combination of valine, t-tau, and p-tau did not significantly improve diagnostic accuracy for pMCI (Table 2 and Fig. 3B).

## Could serum valine predict conversion from CN to MCI or AD and from MCI to AD?

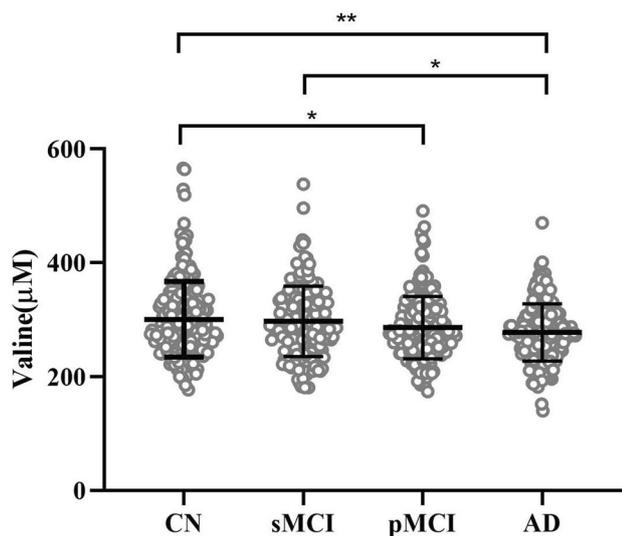
Among the subjects with longitudinal assessments, 44 CN participants progressed to MCI or AD and 195 MCI participants progressed to AD during follow-up. To investigate whether serum valine could predict the conversion from CN to MCI or AD and from MCI to AD, Cox proportional hazard models were performed for serum valine as a continuous variable. HRs were then calculated for serum valine as a dichotomized variable using median values of serum valine as a threshold (adjusted for age and sex). Serum valine did not significantly predict conversion from CN to MCI or AD ( $p = 0.12$ ) (Fig. 4A). While individuals with lower valine, corresponding to MCI participants whose valine values were  $\leq 291$  pg/ml, progressed much more rapidly to AD than those MCI participants with higher values ( $> 291$  pg/ml, corresponding to the higher values of valine) ( $p = 0.04$ ) (Fig. 4B).

**Table 1** Demographics of subjects at baseline

	CN	sMCI	pMCI	AD
Age	75.82(5.02)	75.07(7.66)	74.45(7.09)	75.24(7.46)
Gender, Female	107(47.56%)	62(34.25%) <sup>d</sup>	73(37.44%)	90(48.65%) <sup>b</sup>
Education	16.1(2.86) <sup>d</sup>	15.42(3.31) <sup>d</sup>	15.81(2.80) <sup>d</sup>	14.63(3.13) <sup>a,b,c</sup>
CSF A $\beta$ 42	1136.18(451.11) <sup>b,c,d</sup>	952.92(454.67) <sup>a,c,d</sup>	688.46(320.01) <sup>a,b</sup>	640.47(305.30) <sup>a,b</sup>
CSF t-tau	234.88(87.97) <sup>b,c,d</sup>	295.4(171.09) <sup>a,c,d</sup>	333.85(116.11) <sup>a,b</sup>	355.05(133.86) <sup>a,b</sup>
CSF p-tau	21.83(9.09) <sup>b,c,d</sup>	28.88(18.40) <sup>a,c,d</sup>	33.43(13.39) <sup>a,b</sup>	35.91(15.60) <sup>a,b</sup>
ADAS-Cog13	9.49(4.20) <sup>b,c,d</sup>	16.83(6.08) <sup>a,c,d</sup>	20.98(5.55) <sup>a,b,d</sup>	29.16(7.57) <sup>a,b,c</sup>
MMSE	29.11(1.00) <sup>b,c,d</sup>	27.24(1.80) <sup>a,c,d</sup>	26.73(1.71) <sup>a,b,d</sup>	23.28(2.04) <sup>a,b,c</sup>
Hippocampus	7235.17(907.05) <sup>b,c,d</sup>	6726.86(1012.62) <sup>a,c,d</sup>	6053.48(1015.49) <sup>a,b,d</sup>	5600.99(1012.16) <sup>a,b,c</sup>
Ventricles	35,375.83(19,829.12) <sup>b,c,d</sup>	42,938.77(24,103.23) <sup>a,d</sup>	46,592.12(23,148.57) <sup>a</sup>	50,077.37(25,265.24) <sup>a,b</sup>
FDG-PET	1.28(0.12) <sup>b,c,d</sup>	1.23(0.13) <sup>a,c,d</sup>	1.16(0.10) <sup>a,b,d</sup>	1.07(0.13) <sup>a,b,c</sup>

Measurement data are expressed by mean and standard error.  $p$  values indicate the values assessed with analyses of variance for each variable, where a contingency chi-square was performed. Post hoc analysis provided significant differences between groups: <sup>a</sup>from CN; <sup>b</sup>from sMCI; <sup>c</sup>from pMCI; <sup>d</sup>from AD

MMSE mini-mental state examination; ADAS-cog Alzheimer's disease assessment scale-cog; FDG-PET <sup>18</sup>F-fluorodeoxyglucose positron emission tomography; CN healthy controls; sMCI stable mild cognitive impairment; pMCI progressive mild cognitive impairment; AD Alzheimer's disease



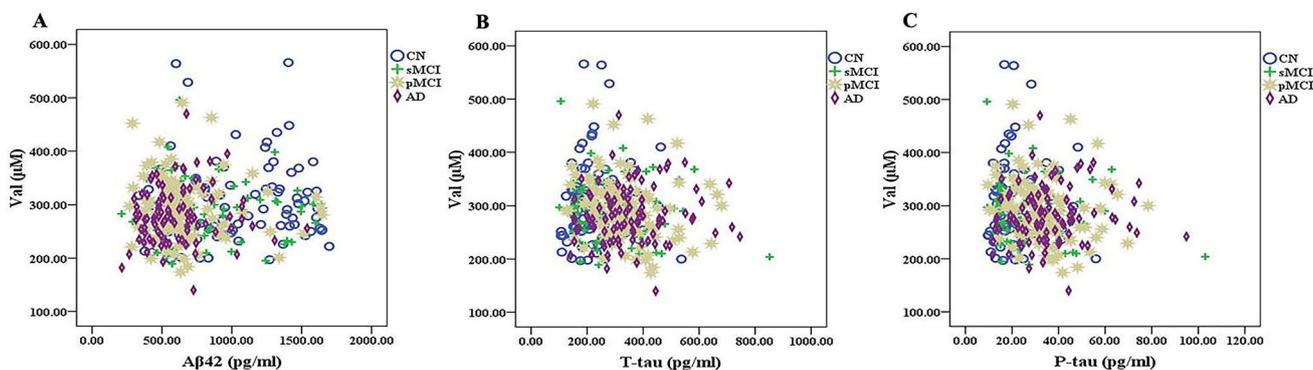
**Fig. 1** Serum valine levels in different diagnostic groups. Differences between groups were tested by analysis of variance. \* $p < 0.05$ ; \*\* $p < 0.01$ . Abbreviations: *CN* healthy controls; *sMCI* stable mild cognitive impairment; *pMCI* progressive mild cognitive impairment; *AD* Alzheimer’s disease

### Serum valine in relation to cognition

In each diagnostic group, serum valine did not correlate with baseline MMSE (*CN*,  $\beta = -0.00022$ ,  $p = 0.495$ ; *sMCI*,  $\beta = 0.00082$ ,  $p = 0.544$ ; *pMCI*,  $\beta = -0.00032$ ,  $p = 0.727$ ; *AD*,  $\beta = 0.00295$ ,  $p = 0.053$ ) (Fig. 5A) and ADAS-cog 13 (*CN*,  $\beta = 0.00121$ ,  $p = 0.683$ ; *sMCI*,  $\beta = -0.00140$ ,  $p = 0.811$ ; *pMCI*,  $\beta = 0.00137$ ,  $p = 0.793$ ; *AD*,  $\beta = -0.01335$ ,  $p = 0.149$ ) (Fig. 5C). Similarly, it was not associated with the rates of change of MMSE (*CN*,  $\beta = -0.00009$ ,  $p = 0.256$ ; *sMCI*,  $\beta = 0.00051$ ,  $p = 0.536$ ; *pMCI*,  $\beta = -0.00030$ ,  $p = 0.841$ ; *AD*,  $\beta = 0.00463$ ,  $p = 0.145$ ) (Fig. 5B) and ADAS-cog 13 (*CN*,  $\beta = 0.00003$ ,  $p = 0.931$ ; *sMCI*,  $\beta = -0.00021$ ,  $p = 0.918$ ; *pMCI*,  $\beta = -0.00023$ ,  $p = 0.943$ ; *AD*,  $\beta = -0.00778$ ,  $p = 0.131$ ) (Fig. 5D) during follow-up.

### Serum valine in relation to brain structure and metabolism

Finally, we examined whether serum valine was associated with hippocampal volume, ventricular volume as



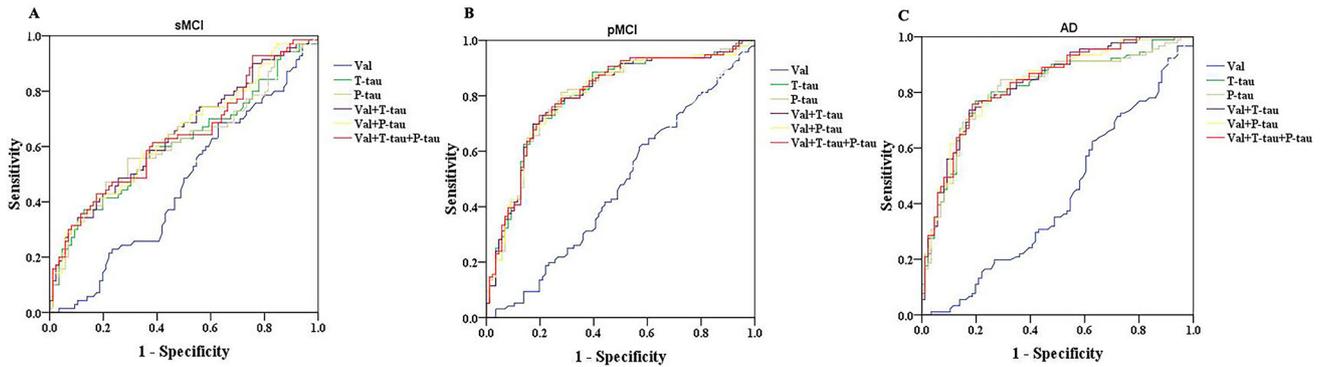
**Fig. 2** Serum valine in relation to CSF A $\beta$  and tau. Correlations between serum valine levels and CSF A $\beta$ 42 (A), CSF t-tau (B), and CSF p-tau (C) in different diagnostic groups. Abbreviations: *CN*

healthy controls; *sMCI* stable mild cognitive impairment; *pMCI* progressive mild cognitive impairment; *AD* Alzheimer’s disease

**Table 2** AUC of biomarkers

	Val	T-tau	P-tau	Val + T-tau	Val + P-tau	Val + T-tau + P-tau
<i>sMCI</i>	0.462 (0.371–0.552) ( $p = 0.410$ )	0.610 (0.519–0.701) ( $p = 0.018$ )	0.614 (0.522–0.705) ( $p = 0.015$ )	0.644 (0.556–0.731) ( $p = 0.002$ )	0.642 (0.554–0.730) ( $p = 0.002$ )	0.637 (0.548–0.725) ( $p = 0.003$ )
<i>pMCI</i>	0.475 (0.390–0.560) ( $p = 0.558$ )	0.799 (0.733–0.865) ( $p < 0.001$ )	0.799 (0.733–0.866) ( $p < 0.001$ )	0.799 (0.732–0.865) ( $p < 0.001$ )	0.798 (0.732–0.865) ( $p < 0.001$ )	0.802 (0.736–0.868) ( $p < 0.001$ )
<i>AD</i>	0.425 (0.340–0.510) ( $p = 0.086$ )	0.814 (0.749–0.878) ( $p < 0.001$ )	0.812 (0.747–0.878) ( $p < 0.001$ )	0.831 (0.772–0.891) ( $p < 0.001$ )	0.830 (0.770–0.890) ( $p < 0.001$ )	0.832 (0.773–0.891) ( $p < 0.001$ )

AUC area under the receiver operator characteristics curve; Val Valine; T-tau total-tau; P-tau phosphorylated-tau; *sMCI* stable mild cognitive impairment; *pMCI* progressive mild cognitive impairment; *AD* Alzheimer’s disease



**Fig. 3** ROC analyses. ROC analyses were performed to test the levels of serum valine, CSF t-tau, and p-tau in relation to clinical diagnoses for sMCI (A), pMCI (B), and AD (C). Abbreviations: *Val* valine;

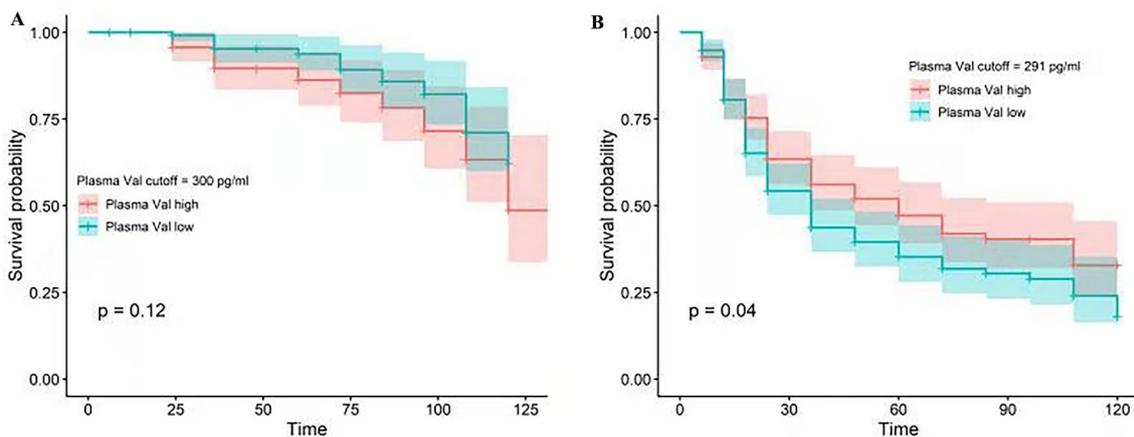
*sMCI* stable mild cognitive impairment; *pMCI* progressive mild cognitive impairment; *AD* Alzheimer's disease

measured by MRI, and brain metabolism as measured by FDG-PET (SUVr). Serum valine did not correlate with baseline FDG-PET (CN,  $\beta = -9.3e-7$ ,  $p = 0.958$ ; sMCI,  $\beta = 0.00001$ ,  $p = 0.961$ ; pMCI,  $\beta = -0.00008$ ,  $p = 0.371$ ; AD,  $\beta = 0.00012$ ,  $p = 0.382$ ) (Fig. 6A), ventricular volume (CN,  $\beta = -14.43002$ ,  $p = 0.455$ ; sMCI,  $\beta = -11.01430$ ,  $p = 0.694$ ; pMCI,  $\beta = 3.14764$ ,  $p = 0.915$ ; AD,  $\beta = 10.54132$ ,  $p = 0.766$ ) (Fig. 6C), and hippocampal volume (CN,  $\beta = 1.07287$ ,  $p = 0.119$ ; sMCI,  $\beta = 1.72319$ ,  $p = 0.073$ ; pMCI,  $\beta = -0.92409$ ,  $p = 0.396$ ; AD,  $\beta = -1.44042$ ,  $p = 0.208$ ) (Fig. 6E) in any diagnostic group. Serum valine was correlated with rates of change of FDG-PET ( $\beta = 0.00004$ ,  $p = 0.016$ ) (Fig. 6B) in pMCI, but not in the other groups (CN,  $\beta = -4.2e-6$ ,  $p = 0.851$ ; sMCI,  $\beta = 0.00001$ ,  $p = 0.477$ ; AD,  $\beta = 0.00001$ ,  $p = 0.794$ ) (Fig. 6B). There was also no correlation between serum valine and the rate of change in

ventricular volume (CN,  $\beta = -0.79444$ ,  $p = 0.505$ ; sMCI,  $\beta = -1.93837$ ,  $p = 0.369$ ; pMCI,  $\beta = 1.19231$ ,  $p = 0.680$ ; AD,  $\beta = -2.41099$ ,  $p = 0.549$ ) (Fig. 6D) and hippocampal volume (CN,  $\beta = 0.02611$ ,  $p = 0.479$ ; sMCI,  $\beta = -0.02792$ ,  $p = 0.743$ ; pMCI,  $\beta = -0.09965$ ,  $p = 0.205$ ; AD,  $\beta = 0.00245$ ,  $p = 0.990$ ) (Fig. 6F) during follow-up.

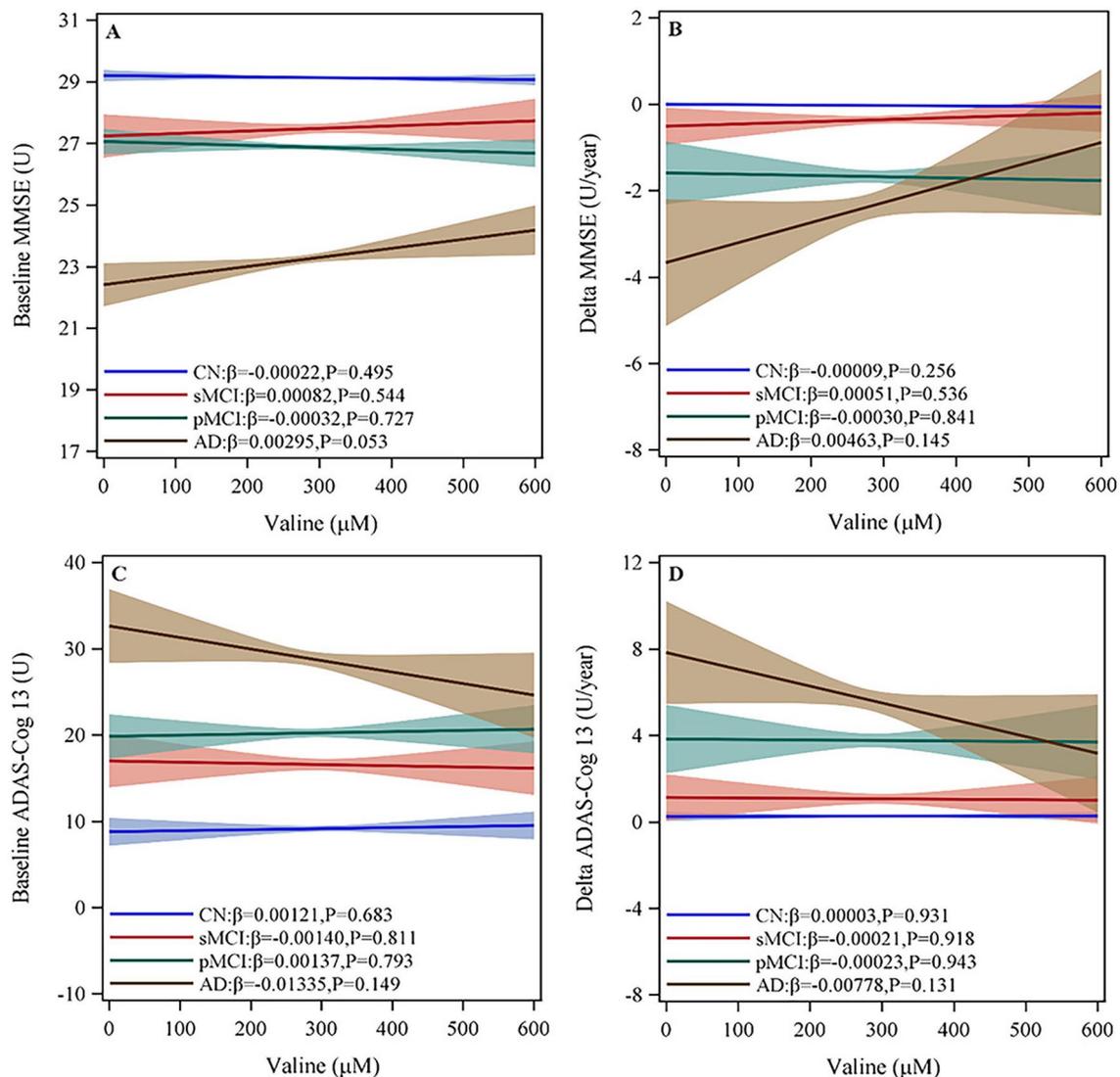
## Discussion

The present study investigated the characteristics of serum valine in participants with MCI and AD from the ADNI-1 cohort. We found that: (1) the levels of serum valine were significantly lower in AD compared with CN and sMCI, and lower serum valine levels were also found in pMCI compared with CN; (2) serum valine was negatively correlated



**Fig. 4** Baseline serum measures of valine as a predictor of conversion from CN to MCI or AD and MCI to AD. Cox proportional hazard regression analyses were used to evaluate the relationships between valine and the incidence of AD. Conversion from CN to MCI or

AD (A) and MCI to AD (B) as a function of serum valine measures (dichotomized at the median values) are shown. Analyses were adjusted for age, education, and gender. Cutoff values were 300 pg/ml (CN) and 291 pg/ml (MCI) for Val. Abbreviations: *Val* valine



**Fig. 5** Serum valine in relation to cognition and future cognitive change. MMSE and ADAS-cog 13 scores were used to assess overall cognitive abilities. MMSE and ADAS-cog 13 at baseline (**A**, **C**) and over time (**B**, **D**) as a function of baseline serum valine in different

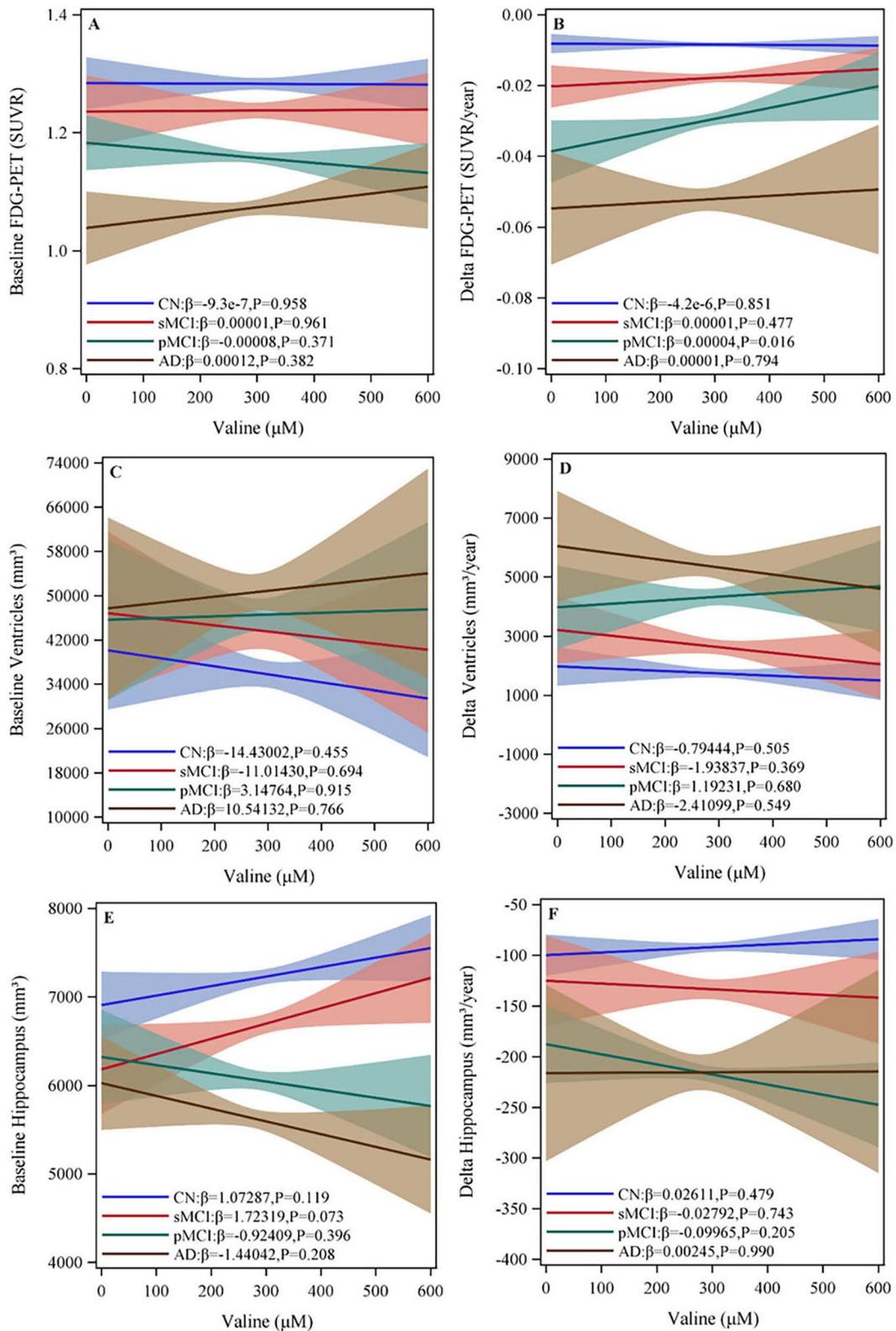
diagnostic groups. Abbreviations: *CN* healthy controls; *sMCI* stable mild cognitive impairment; *pMCI* progressive mild cognitive impairment; *AD* Alzheimer's disease

with CSF t-tau and p-tau in pMCI group; (3) the diagnostic accuracy of valine for MCI and AD was not statistically significant; (4) serum valine could predict conversion from MCI to AD; (5) serum valine was associated with low brain metabolism at follow-up in pMCI group.

Metabolites in the blood reflect the interaction of genetic and environmental factors, and their levels are modifiable through dietary or pharmacological interventions [23]. Researchers have proposed that AD is a systemic disease characterized by impaired glucose metabolism, mitochondrial dysfunction, and abnormal BCAAs metabolism [24, 25]. Valine, a branched-chain amino acid that cannot be synthesized endogenously and that must be supplied by the diet, plays an important role in maintaining brain nitrogen

homeostasis and neurotransmitter synthesis [26]. As early as 1990, a study of the amino acid composition of CSF showed that valine concentrations were significantly reduced in patients with AD compared to healthy controls [12].

Previous studies showed that serum valine levels were significantly lower in patients with AD than in healthy controls [12, 27]. To explore the changes of serum valine levels in the progression of AD disease, we divided MCI group into sMCI group and pMCI group according to whether they progressed to AD during follow-up. We found that serum valine levels of AD were lower than that of CN and sMCI, and serum valine levels of pMCI were lower than that of CN, but there was no significant difference between CN and sMCI as well as between sMCI and pMCI, suggesting that



**Fig. 6** Serum valine in relation to brain structure and metabolism. FDG-PET was used to evaluate metabolism. Hippocampal and ventricular volumes were used to assess neurodegeneration. FDG-PET, ventricular volumes, and hippocampal volumes at baseline (A, C, E) and over time (B, D, F) as a function of baseline serum valine in dif-

ferent diagnostic groups. Abbreviations: *FDG-PET*  $^{18}\text{F}$ -fluorodeoxyglucose positron emission tomography; *CN* cognitively normal; *sMCI* stable mild cognitive impairment; *pMCI* progressive mild cognitive impairment; *AD* Alzheimer's disease

serum valine changes slowly in the process of AD disease progression. A recent retrospective study using untargeted 1H nuclear magnetic resonance (NMR) spectroscopy-based metabolomics found that CSF valine levels decreased in patients with AD dementia, while no statistical difference was found between pre-dementia (mild cognitive impairment due to AD, MCI-AD) and non-AD patients [28]. However, another study using the untargeted metabolomics approaches to characterize the dynamic changes of serum metabolic profile of APP/PS1 double-transgenic mice (an AD mouse model) found that the levels of serum valine were upregulated in APP 9 m (APP/PS1 mice at the ages of 9 month) group mice [24]. We speculate that the possible reasons are the differences between human and animal model systems and the relatively small sample size of the study. Notably, valine is one of the nitrogen sources for the synthesis of the excitatory neurotransmitter glutamate, and disruption of glutamate signaling is closely associated with neurodegenerative diseases [10]. Lower valine levels have also been detected in serum samples from Huntington's disease, frontotemporal dementia, and other neurodegenerative diseases, making valine not a serum-specific marker of Alzheimer's disease [14, 29].

A study has shown that there was no obvious correlation between CSF valine and CSF A $\beta$ 42, t-tau, and p-tau [28]. In this study, we also found that there was no correlation between serum valine and CSF A $\beta$ 42. However, in pMCI group, serum valine was negatively correlated with CSF t-tau and p-tau. In transgenic mice expressing the pathogenic mutation P301L in the human tau gene (pR5 mice), astrocytes as well as glutamatergic and GABAergic neurons in the cortex were in a hypermetabolic state, whereas in the hippocampus, where expression levels of mutant human tau are the highest, glutamate levels were reduced and homeostasis was impaired [30]. We speculate that valine and tau pathology may be linked through glutamate metabolism. The specific causal relationship needs further research.

We next sought to test whether serum valine could improve the differential diagnosis of MCI and AD dementia in comparison to the traditional AD biomarkers, such as CSF t-tau and p-tau. Here, we confirmed that CSF t-tau and p-tau but not serum valine had significant diagnostic accuracy for MCI and AD. However, when compared with t-tau or p-tau alone, the diagnostic accuracy of serum valine combined with t-tau or p-tau for sMCI and AD tends to increase, suggesting that serum valine may be used in combination with other core biomarkers to improve diagnostic accuracy or promote early detection. The limited accessibility to brain histology hinders the availability of the gold standard for AD biomarkers. The field of AD diagnostic biomarkers is progressively approaching that of oncological biomarkers. Strategic Biomarker Roadmap has admitted a larger variety of reference standards based on construct validity. With the

A/T/N framework, biomarkers are examined and assessed for their individual contribution to an AD or non-AD profiles. In this study, we confirmed the diagnostic accuracy of tau biomarkers for AD, and the correlation of valine with t-tau and p-tau further validated the tau biomarkers [31]. A prospective study by Tynkkynen et al. has reported a significant association between lower serum valine levels and increased risk of AD, but this disappears after adjusting for Body Mass Index (BMI) and cholesterol-lowering medications [23]. In the Rotterdam and ERF studies, after adjusting for age at baseline, gender, education, and lipid-lowering medication, increased valine concentration was associated with decreased risk of AD [13]. Here, we found that serum valine could predict conversion from MCI to AD, but not from CN to MCI or AD. It is worth noting that during the follow-up period, the number of individuals who progressed from CN to MCI or AD is relatively small, the predictive value of valine for disease progression in cognitively normal subjects needs to be validated in a larger sample.

A previous study on CSF metabolomics demonstrated a significant correlation between valine CSF levels and cognitive decline, in particular considering MMSE at follow-up and the percentage change between baseline and follow-up MMSE [28]. A recent study of metabolite analysis of baseline fasting serum samples from the ADNI cohort also showed that the levels of serum valine were negatively associated with the ADAS-cog 13 at baseline, and in patients with up to 5 years of follow-up, it was also negatively associated with a faster cognitive decline and ventricular volume changes. In line with these data, the same study showed a positive association between serum valine levels and a higher general cognitive ability (g-factor, which is a general cognitive function phenotype created by principal component analysis of multiple cognitive tests), and a decreased risk of AD in the prospective ongoing population-based elderly Rotterdam cohort [13]. However, in the present study, serum valine was not correlated with cognitive function, hippocampal volume, and ventricle volume at baseline and during follow-up. The possible reasons are: (1) confounding factors such as medications, dietary supplements, and apolipoprotein E (*APOE*)  $\epsilon$ 4 were not corrected in this study, which may cause confounding bias; (2) the difference in follow-up time between studies. Therefore, the possible prognostic value of valine in the prediction of patient cognitive decline needs to be further clarified. More and more studies have shown that insulin resistance, obesity, and diabetes are risk factors for AD. BCAAs played a central role in metabolism and were related to insulin resistance, 2 Type diabetes, and obesity [32]. The carbon skeleton of the branched-chain keto acid derived from BCAAs can enter the TCA cycle as either acetyl CoA or succinyl CoA, hereby supporting the failing energy metabolism in AD [33, 34]. In the present study, we proved that serum valine was related

to changes in cortical glucose metabolism assessed by FDG-PET during follow-up in pMCI group, but not in the other cognitive subgroups. We speculate that the correlation of valine with cortical glucose metabolism may only occur at the end of AD disease progression.

## Limitations

Firstly, it is not certain whether the peripheral signals related to the disease status are also reflected in the brain because our research did not obtain the level of valine in the brain. Secondly, future research needs to exclude the influence of confounding factors such as drugs and participants' nutritional status. Finally, the participants in the ADNI-1 observational cohort are whites with high mean education, therefore, the present results need to be confirmed in more socioeconomically, educationally, and racially diverse samples [35, 36].

## Conclusions

In summary, we confirmed that the levels of serum valine were decreased in patients with pMCI and AD. Serum valine could predict conversion from MCI to AD, as well as correlated with cortical glucose metabolism assessed by FDG-PET during follow-up in pMCI. Therefore, serum valine may be a peripheral blood biomarker for the progression of MCI. However, due to the lack of significant diagnostic accuracy for MCI and AD and correlation with clinical and neuroimaging data, serum valine cannot be used as a specific biomarker of AD. In the future, valine needs to be located as a key biological pathway involved in the pathogenesis of AD to understand the potential role of valine and its interaction with other metabolites in triggering the occurrence and development of AD symptoms.

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**Author contributions** YX: analysis and interpretation of data, compose figures, and manuscript draft. JT: critical review of manuscript for intellectual content. SR: analysis and interpretation of data. XJ: analysis and interpretation of data. HZ: study concept, design, study supervision, and critical review of manuscript for intellectual content.

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**Availability of data and materials** The data supporting the conclusions of this article are available from the corresponding author upon request.

## Declarations

**Conflict of interest** The authors declare that they have no competing interests.

**Ethics approval and consent to participate** The ADNI study was approved by the Institutional Review Boards of all the participating institutions. Informed written consent was obtained from all subjects at each center.

**Human rights** The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki.

**Consent for publication** All authors approved the final manuscript for submission and gave consent for publication.

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