The Associations of Serum Valine with Mild Cognitive Impairment and Alzheimer's Disease

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Research Article

Keywords: Alzheimer's disease, cerebral glucose metabolism, cognition, mild cognitive impairment, serum valine

Posted Date: September 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-887019/v1

Version of Record: A version of this preprint was published at Aging Clinical and Experimental Research on April 1st, 2022. See the published version at https://doi.org/10.1007/s40520-022-02120-0.

Abstract

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, but its characteristics in MCI and AD need to be further clarified. 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 (^{18}F-fluorodeoxyglucose-positron emission tomography, FDG-PET), and cognitive declines, through different cognitive subgroups. We found that (1) 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; (2) serum valine was negatively correlated with CSF total tau (t-tau) and phosphorylated tau (p-tau) in pMCI; (3) serum valine significantly predicted conversion from MCI to AD; (4) serum valine was related to the rate of change of cerebral glucose metabolism during the follow-up period in pMCI. We speculated 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.

Introduction

Alzheimer's disease (AD) is the most prominent cause of dementia in the elderly, but the mechanism of the occurrence and development of the disease is unclear. There is growing evidence that severe metabolic dysfunction is a marker and the cause of AD [1]. Recent studies 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 intrinsic to AD pathogenesis [2–5]. The Alzheimer's Disease Metabolomics Consortium (ADMC) is working with the Alzheimer's Disease Neuroimaging Initiative (ADNI) to add metabolomics data to the vast collection of data collected for this cohort. The data collected through the ADMC will provide a resource to interrogate global metabolomics changes within the ADNI-1 cohort, to enhance the systems-level data available [6].

Branched-chain amino acids (BCAAs) are amino acids possessing an aliphatic side chain with a branch, which are abundant in human body and comprise almost one-third of all amino acids present in humans. Initial experiments with radiolabeled amino acids in rats proved that BCAAs readily cross the blood-brain barrier (BBB) [7]. Glutamate is an excitatory neurotransmitter in mammalian brain that contributes to memory and learning processes [8]. It has been estimated that at least one-third of cerebral glutamate contains nitrogen derived from BCAAs [9]. γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter, which is synthesized by decarboxylation of glutamate. Thus, BCAAs are also indirectly involved in their synthesis. Therefore, the disorder of BCAAs levels significantly affect the overall function of the central nervous system, especially the balance between excitation and inhibition [9].

Valine belongs to BCAAs. A study in mice indicated that brain uptake of valine was more rapid than that of other amino acids [10]. 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 [11]. 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 [12]. However, it is still unknown whether serum valine has diagnostic significance for AD, and whether valine can predict the progression of the disease.

In the present study, we tested the hypotheses that serum valine decreased in mild cognitive impairment (MCI) and AD and could offer predictive value for future disease progression in MCI subjects, 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 ^{18}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 (range, 0-30), and a CDR score of 0, where lower scores indicate less impairment and higher scores more impairment (range, 0-3) [13,14]. 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 [15]. 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 [16]. 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 [17]. 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, 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 [12,18]. This is a highly useful platform that was used in hundreds of publications, including several studies in AD [19,20,2].

CSF Measurements

Lumbar puncture was performed in the mornings after an overnight fast. CSF amyloid-β 42 (Aβ42), 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 AlzBio3 (Innogenetics, Ghent, Belgium) immunoassay reagents as described previously [21]. All of the CSF data used in this study were obtained from the ADNI files “UPENNBIOMK5–8.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.

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.

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 [22]. 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 Aβ42 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±50.4 μM) compared with CN (300.92±66.27 μM) ($P<0.01$) and sMCI (297.89±61.79 μM) ($P<0.05$). Lower serum valine levels were also found in pMCI (284.99±57.95 μM) compared with CN (300.92±66.27 μM) ($P<0.05$). However, there were no differences between CN and sMCI as well as between sMCI and pMCI, and similarly between pMCI and AD (Figure 1).

Serum valine in relation to CSF Aβ and tau

There was no significant correlation between CSF Aβ42 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, $r=0.088$, $p=0.406$) (Figure 2A). Valine was negatively correlated with CSF t-tau ($r=-0.260$, $p=0.01$) (Figure 2B) and p-tau ($r=-0.231$, $p=0.023$) in pMCI (Figure 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) (Figure 2B and 2C).

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 Figure 3A), pMCI (Table 2 and Figure 3B), and AD (Table 2 and Figure 3C). While the diagnostic accuracy of valine for sMCI (Table 2 and Figure 3A), pMCI (Table 2 and Figure 3B), and AD (Table 2 and Figure 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 Figure 3A and C). The combination of valine, t-tau, and p-tau did not significantly improve diagnostic accuracy for pMCI (Table 2 and Figure 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 individuals progressed to MCI or AD and 195 MCI participants progressed to AD during follow-up. We investigated whether serum valine predicted conversion from CN to MCI or AD and from MCI to AD. Cox proportional hazard models were established using 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 cutoff (adjusted for age and sex). Serum valine did not predict conversion from CN to MCI or AD ($P=0.12$) (Figure 4A). However, MCI patients with lower valine (≤291pg/ml) progressed much more rapidly to AD than those with higher valine (>291pg/ml) ($P=0.04$) (Figure 4B).

Serum valine in relation to cognition

In each diagnostic group, serum valine did not correlate with baseline MMSE (CN, β=-0.00022, p=0.495; sMCI, β=0.00082, p=0.544; pMCI, β=-0.00032, p=0.727; AD, β=0.00295, p=0.053) (Figure 5A) and ADAS-cog13 (CN, β=0.00121, p=0.683; sMCIβ=-0.00140, p=0.811; pMCI, β=0.00137, p=0.793; AD, β=-0.01335, p=0.149) (Figure 5C). Similarly, it was not associated with the rates of change of MMSE (CN, β=-0.00009, p=0.256; sMCI, β=0.00051, p=0.536; pMCI, β=-0.00030, p=0.841; AD, β=0.00463, p=0.145) (Figure 5B) and ADAS-cog 13 (CN, β=0.00003, p=0.931; sMCI, β=-0.00021, p=0.918; pMCI, β=-0.00023, p=0.943; AD, β=-0.00778, p=0.131) (Figure 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 volumeas measured by MRI, and brain metabolism as measured by FDG-PET (SUVR). Serum valine did not correlate with baseline FDG-PET (CN, β=-9.3e-7, p=0.958; sMCI, β=0.00001, p=0.961; pMCI, β=-0.00008, p=0.371; AD, β=0.00012, p=0.382) (Figure 6A), ventricular volume (CN, β=-14.43002, p=0.455; sMCI, β=-11.01430, p=0.694; pMCI, β=3.14764, p=0.915; AD, β=10.54132, p=0.766) (Figure 6C), and hippocampal volume (CN, β=1.07287, p=0.119; sMCI, β=1.72319, p=0.073; pMCI, β=-0.92409, p=0.396; AD, β=-1.44042, p=0.208) (Figure 6E) in any diagnostic group. Serum valine was correlated with rates of change of FDG-PET (β=0.00004, p=0.016) (Figure 6B) in pMCI, but not in the other groups (CN, β=-4.2e-6, p=0.851; sMCI, β=0.00001, p=0.477; AD, β=0.00001, p=0.794) (Figure 6B). There was also no correlation between serum valine and the rate of change in ventricular volume (CN, β=-0.79444, p=0.505; sMCI, β=-1.93837, p=0.369; pMCI, β=1.19231, p=0.680; AD, β=-2.41099, p=0.549) (Figure 6D) and hippocampal volume (CN, β=0.02611, p=0.479; sMCI, β=-0.02792, p=0.743; pMCI, β=-0.09965, p=0.205; AD, β=0.00245, p=0.990) (Figure 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 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.

Blood metabolomics is an attractive tool for agnostic exploration of disease pathways because metabolites are small molecules that reflect the interaction of genetic and environmental factors, readily cross the BBB and their levels are modifiable through dietary or pharmacological interventions [23]. Some studies support the hypothesis 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, quickly offers amino groups for the synthesis of glutamate, thus maintaining brain nitrogen homeostasis and indirectly affecting excitatory neurotransmitters [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 [11].

Previous studies showed that serum valine levels were significantly lower in patients with AD than in healthy controls [11, 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. 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 9m (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. It is worth noting that lower valine levels have also been detected in serum samples from Huntington's disease, frontotemporal dementia, and other neurodegenerative diseases [29, 30]. Unfortunately, in this study, we failed to rule out the possible effects of nutritional status and drugs, it may have impacted the measured serum valine concentrations.

A study has shown that there was no obvious correlation between CSF valine and CSF Aβ42, t-tau, and p-tau [28]. In this study, we found that there was no correlation between serum valine and CSF Aβ42. However, in pMCI group, serum valine was negatively correlated with CSF t-tau and p-tau, and the causal relationship between valine and tau pathology was still not clear. In the future, we need to further search for their potential biochemical pathways.

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. 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 [12]. Here, we demonstrated 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. Therefore, future studies need to verify the predictive value of valine on the disease progression of subjects with normal cognition.

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 [12]. 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*) ε4 were not corrected in this study, (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. Up to now, we have not found any study to explore the relationship between serum valine and brain glucose metabolism. 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. More and more studies have shown that insulin resistance, obesity, and diabetes are risk factors for AD. BCAAs (including valine) played a central role in metabolism and were related to insulin resistance, 2 Type diabetes, and obesity [31]. Therefore, we speculate that valine may affect brain metabolism through insulin resistance.

Limitations

Firstly, our research did not link alterations in valine levels in the blood to that in the brain, it is therefore difficult to assess whether a peripheral signal associated with disease status is also reflected in the brain. Secondly, the effect of confounds

like medications impacts metabolomics findings in significant ways and must be addressed carefully. Thirdly, The ADNI observational cohort was designed to be typical of participants who enroll in clinical trials but is not necessarily representative of the broader community as would be found in epidemiologically derived samples. Because ADNI is a largely white sample with high mean education, the present results should not be generalized to community-based populations without further investigation. It will be important to repeat these analyses in more socioeconomically, educationally, and racially diverse samples. The present study of cross-sectional associations is unable to support causal inferences regarding directionality [32, 33]. Finally, as many of the metabolites that are associated with AD are interconnected through metabolic pathways, cofactors, and common intermediates, changes to one metabolite can entail several others, as well as have downstream effects on other coregulated pathways.

Abbreviations

Aβ, amyloid-β; AD, Alzheimer's disease; ADAS-cog, Alzheimer's disease assessment scale-cognitive subscale; ADMC, Alzheimer's disease Metabolomics Consortium; ADNI, Alzheimer's disease Neuroimaging Initiative; ANOVA, analysis of covariance; APOE, apolipoprotein E; AUC, area under the receiver operator characteristics curve; BBB, blood-brain barrier; BCAAs, branched-chain amino acids; BMI, Body Mass Index; CDR, Clinical Dementia Rating scale; CIs, confidence intervals, CN, cognitive normal; CSF, cerebrospinal fluid; FDG-PET, ^{18}F-fluorodeoxyglucose-positron emission tomography; GABA, γ-aminobutyric acid; HRs, hazard ratios; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; NMR, nuclear magnetic resonance; pMCI, progressive mild cognitive impairment; ROC, receiver operating curve; sMCI, stable mild cognitive impairment; SUVR, standardized uptake value ratio; UPLC, ultra-performance liquid chromatography

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. Therefore, serum valine may be a peripheral blood biomarker for the progression of MCI. 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.

Declarations

Acknowledgments

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc; Cogstate; Eisai Inc; Elan Pharmaceuticals, Inc; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc; Fujirebio; GE Healthcare; IXICO Ltd; Janssen Alzheimer Immunotherapy Research & Development, LLC; Johnson & Johnson Pharmaceutical Research & Development LLC; Lumosity; Lundbeck; Merck & Co, Inc; Meso Scale Diagnostics, LLC; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

Funding

Not applicable.

Competing Interests

The authors declare no competing interests.

Data and Materials Availability

Data generated and analyzed during this study are included in the published article and its supplementary information files.

Code Availability

Not applicable.

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.

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.

Consent for publication

Not applicable.

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Tables

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%)[d]	73(37.44%)	90(48.65%)[b]
Education	16.1(2.86)[d]	15.42(3.31)[d]	15.81(2.80)[d]	14.63(3.13)[a,b,c]
CSF Aβ42	1136.18(451.11)[b,c,d]	952.92(454.67)[a,c,d]	688.46(320.01)[a,b]	640.47(305.30)[a,b]
CSF t-tau	234.88(87.97)[b,c,d]	295.4(171.09)[a,c,d]	333.85(116.11)[a,b]	355.05(133.86)[a,b]
CSF p-tau	21.83(9.09)[b,c,d]	28.88(18.40)[a,c,d]	33.43(13.39)[a,b]	35.91(15.60)[a,b]
ADAS-Cog13	9.49(4.20)[b,c,d]	16.83(6.08)[a,c,d]	20.98(5.55)[a,b,d]	29.16(7.57)[a,b,c]
MMSE	29.11(1.00)[b,c,d]	27.24(1.80)[a,c,d]	26.73(1.71)[a,b,d]	23.28(2.04)[a,b,c]
Hippocampus	7235.17(907.05)[b,c,d]	6726.86(1012.62)[a,c,d]	6053.48(1015.49)[a,b,d]	5600.99(1012.16)[a,b,c]
Ventricles	35375.83(19829.12)[b,c,d]	42938.77(24103.23)[a,d]	46592.12(23148.57)[a]	50077.37(25265.24)[a,b]
FDG-PET	1.28(0.12)[b,c,d]	1.23(0.13)[a,c,d]	1.16(0.10)[a,b,d]	1.07(0.13)[a,b,c]

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: [a]from CN; [b]from sMCI; [c]from pMCI; [d]from AD. Abbreviations: MMSE, Mini-mental State Examination; ADAS-cog, Alzheimer's disease assessment scale-cog; FDG-PET, ^{18}F-fluorodeoxyglucose positron emission tomography; 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
sMCI	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)
pMCI	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)
AD	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)

Abbreviations: 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.

Figures

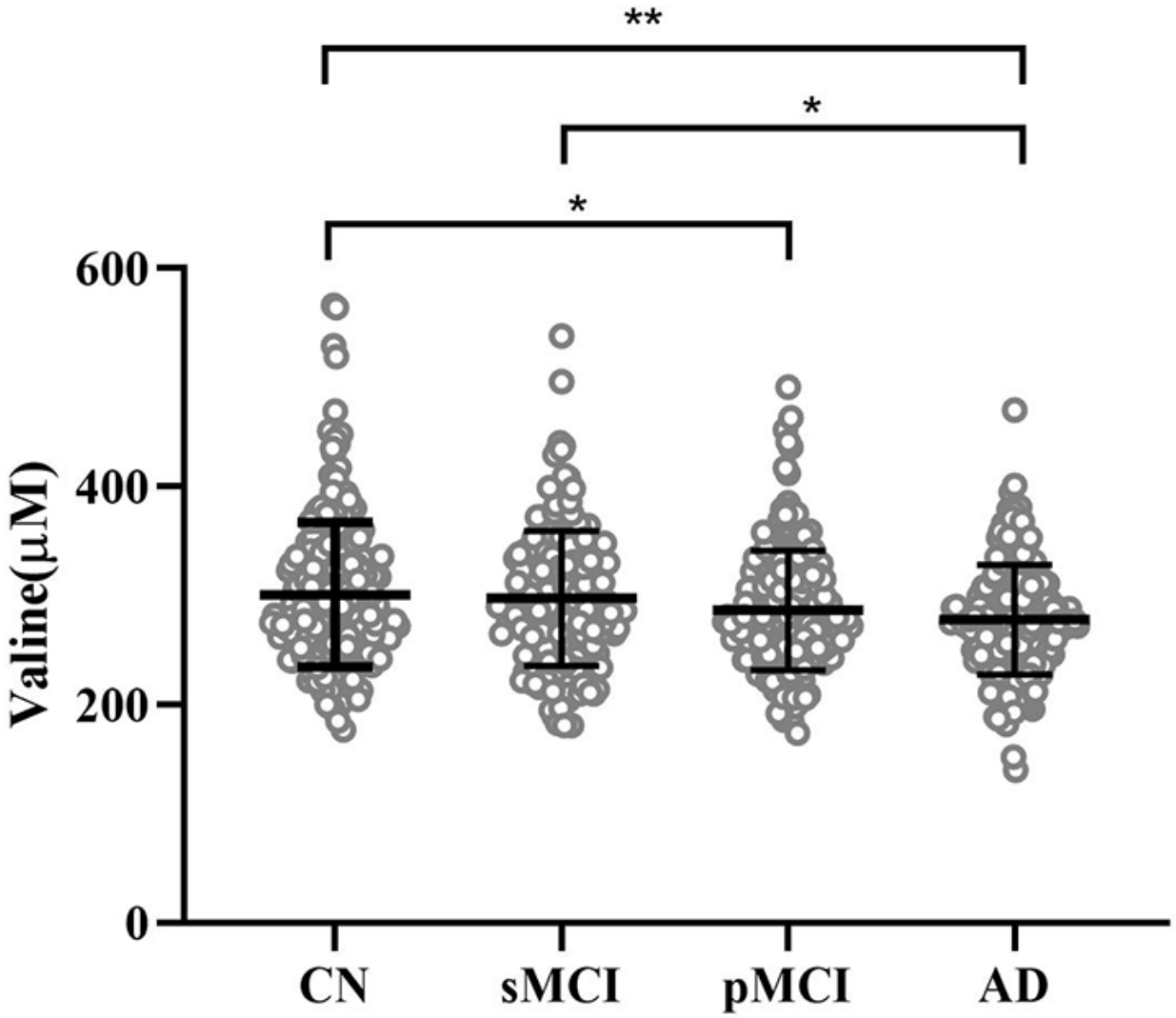

Figure 1

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.

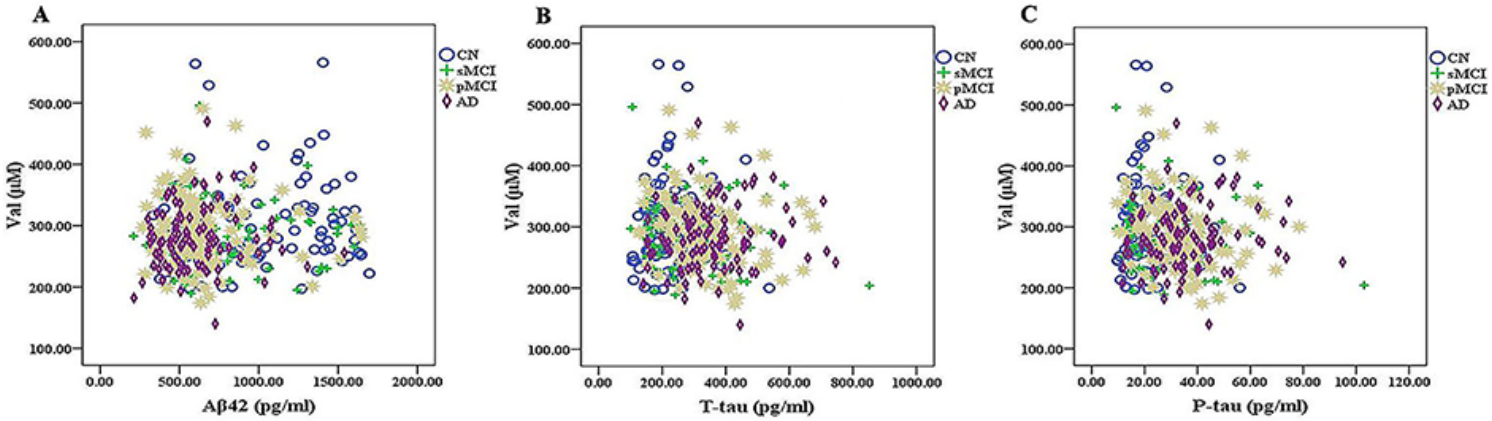

Figure 2

Serum valine in relation to CSF Aβ and tau Correlations between serum valine levels and CSF Aβ42 (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.

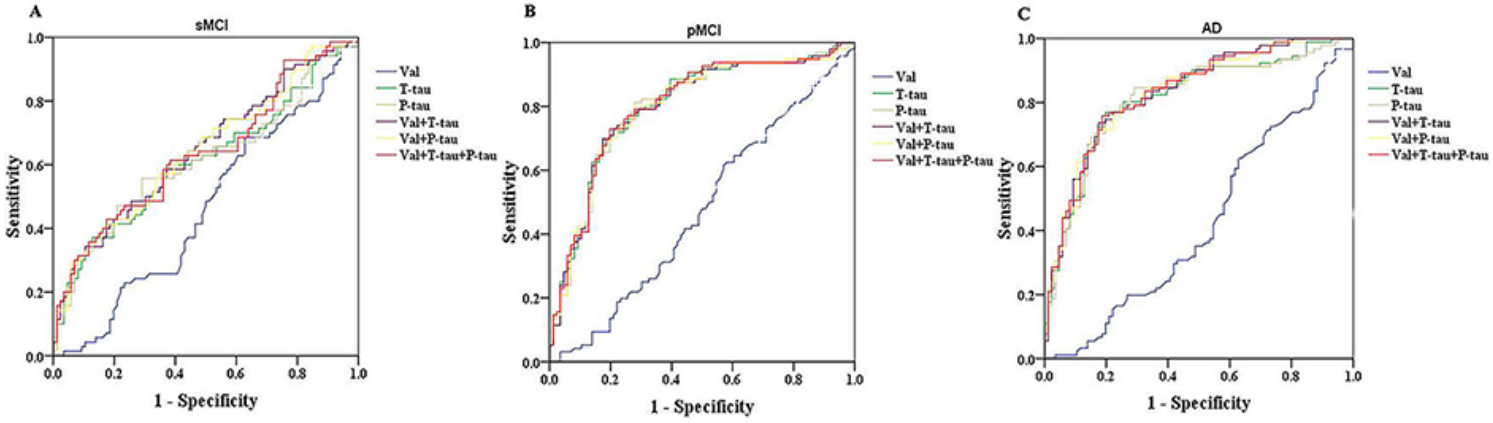

Figure 3

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.

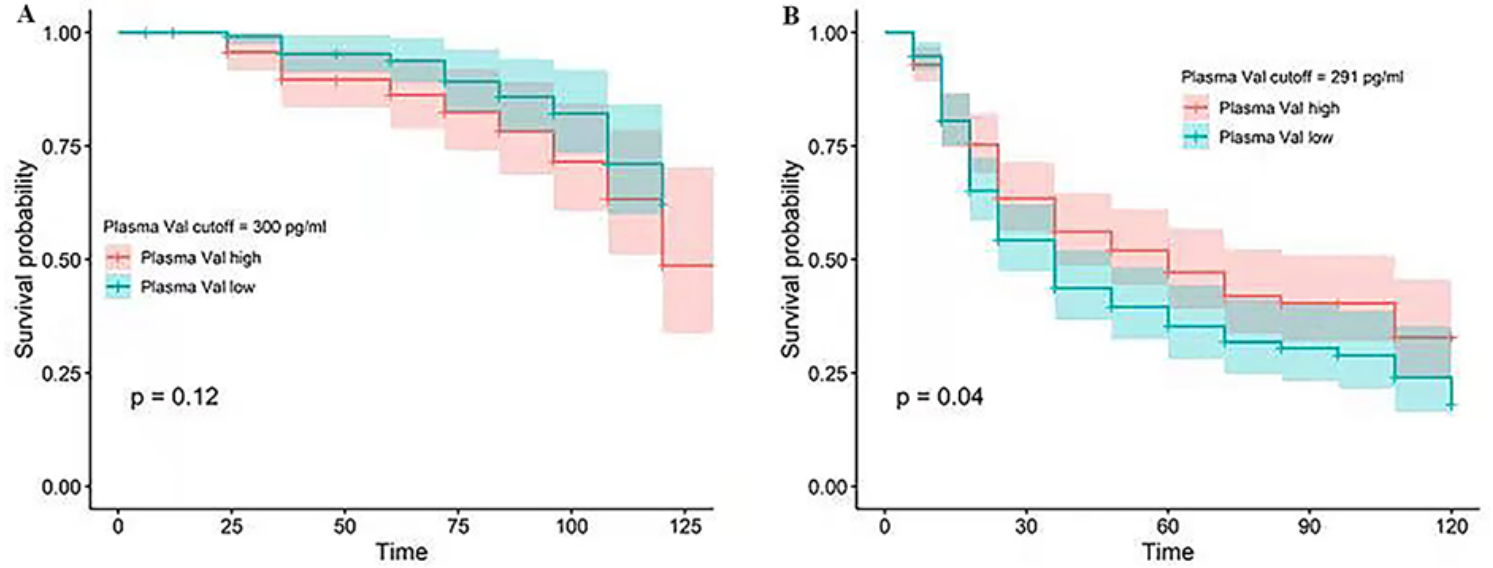

Figure 4

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 300pg/ml (CN) and 291pg/ml (MCI) for Val. Abbreviations: Val, valine.

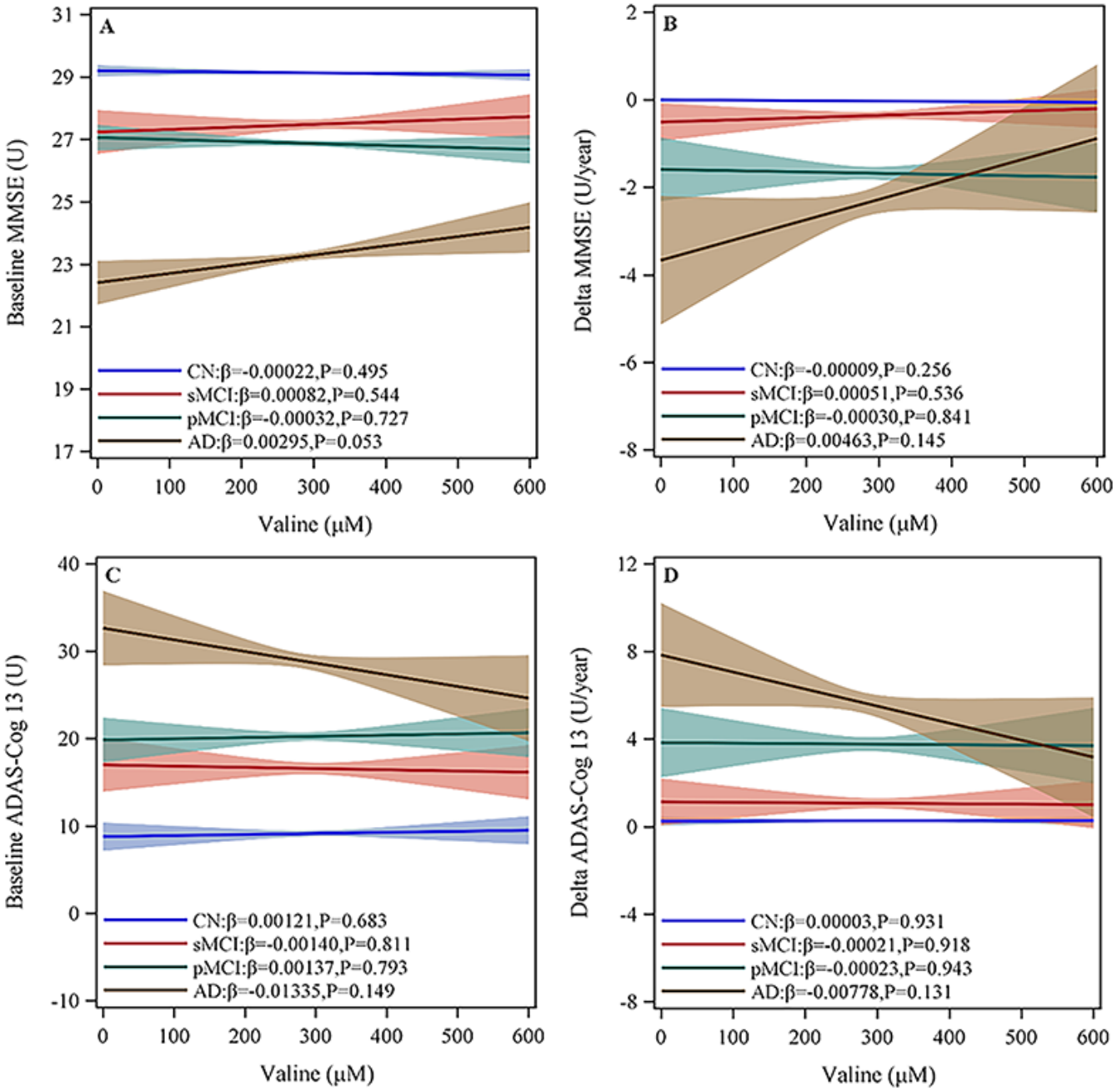

Figure 5

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.

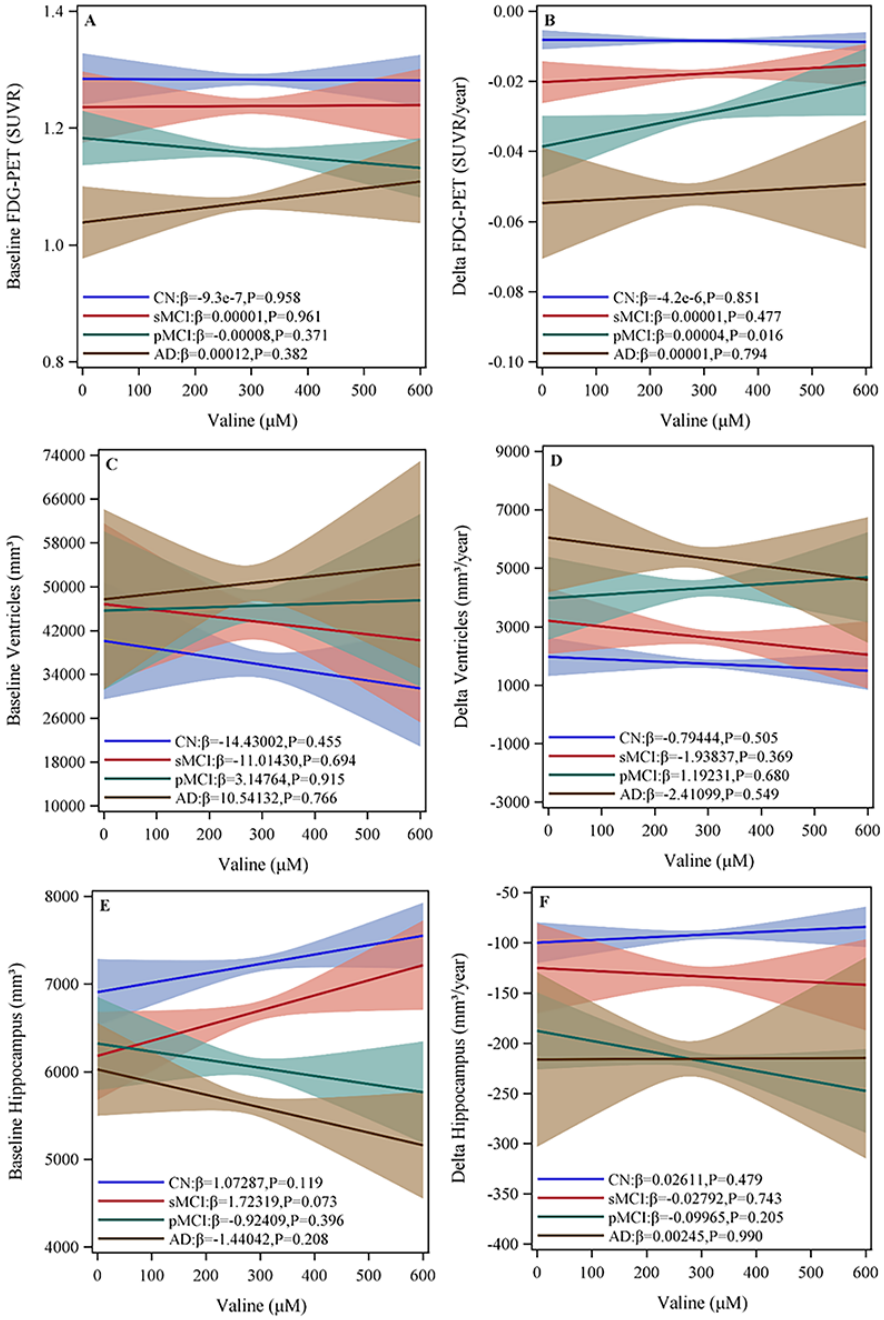

Figure 6

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 different diagnostic groups.
Abbreviations: FDG-PET, 18F-fluorodeoxyglucose positron emission tomography; CN, cognitively normal; sMCI, stable mild cognitive impairment; pMCI, progressive mild cognitive impairment; AD, Alzheimer's disease.