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Blood BMP6 Associated with Cognitive Performance and Alzheimer's Disease Diagnosis: A Longitudinal Study of Elders

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Abstract.
Background: Bone morphogenetic protein (BMP) plays important roles in the pathology of Alzheimer's disease (AD).
Objective: We sought blood BMP6 involved in the processes underlying cognitive decline and detected them in association with AD.
Methods: A total of 309 participants in Shanghai Mental Health Center (SMHC) and 547 participants in Alzheimer's disease Neuroimaging Initiative (ADNI) cohort were included. Blood BMP6 and cognitive functions were measured in all subjects of both cohorts at baseline, and in 482 subjects of ADNI cohort after one year. A total of 300 subjects in ADNI cohort were detected cerebrospinal fluid (CSF) tau biomarker, and 244 received 1-year follow-up.
Results: AD patients had lower levels of blood BMP6 compared to normal controls, and BMP6 was positively associated with cognitive functions. Longitudinal BMP6 combing with *APOE* genotype could distinguish probable AD from normal controls. The influence of blood BMP6 on cognition was modulated by tau pathology.
Conclusion: Blood BMP6 was associated with cognitive performance and identified as a potential predictor for probable AD.

Keywords: Alzheimer's disease, APOE, BMP6, mild cognitive impairment, prediction, tau pathology

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia worldwide and is characterized by extracellular deposits of amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles of tau protein in brain [1]. Easily accessible biomarkers of AD pathology are essential for detecting individuals with the greatest risk of developing mild cognitive impairment (MCI) or AD. The ratios of cerebrospinal fluid (CSF) total tau/$A\beta_{42}$ and phosphorylated tau/$A\beta_{42}$ can predict cognitive decline [2], but require invasive procedures and patient acceptance. Blood-based

[1]These authors contributed equally to this work.

[2]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (https://adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: https://adni.loni.usc.edu/wpcontent/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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cytokines associated AD pathology have significant advantages of time efficiency and reduced invasiveness. It has been demonstrated that neither plasma nor serum $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ consistently differ between AD patients and normal controls [3]. Elevated levels of phosphorylated tau is thought to be more specific to AD than total tau [4]. Besides above biomarkers, there are still lack of other valuable and meaningful markers.

Bone morphogenetic protein (BMP), a member of the β-transforming growth factor (TGF-β) subfamily, has important effects on neuronal differentiation and axonal growth [5]. The BMPs, more than 20 at the last count, are discovered in bone tissue, which activate the canonical small mother against decapentaplegic (Smad) pathway in the brain via their type I and type II Serine/Threonine kinase receptors. BMP6, is demonstrated increased in AD pathology, accompanied by impaired neurogenesis [6]. This information implicates that BMP6 has an important role in AD pathology, but there is still lack of research on characteristics of peripheral BMP6 in AD patients. Therefore, we aimed to identify blood BMP6 expression in AD patients and relationship with global cognition. We analyzed the data for blood BMP6 of 309 participants in Shanghai Mental Health Center (SMHC) cohort and 547 participants in Alzheimer's disease Neuroimaging Initiative (ADNI) cohort. To additionally test the prediction effect of blood BMP6 for AD and whether the influences of blood BMP6 on cognition were modulated by AD core pathology in ADNI cohort.

MATERIALS AND METHODS

309 participants were derived from SMHC cohort, which included 103 probable AD, 92 mild cognitive impairment (MCI), and 114 cognitively normal controls (NC) between March 2011 and April 2018. All participants were Mandarin-speaking Han Chinese and were older than 55 years old. Subjects underwent a screening process that included medical history, physical and neurological examination, and cognitive assessment by a face-to-face interview at baseline. Some were assessed at a 1-year follow-up. All participants had scores on the Hachinski ischemia scale of < 4, and no history of significant systemic or psychiatric conditions or traumatic brain injuries that could compromise brain function. The Beijing version of the Montreal Cognitive Assessment (MoCA) [7] and Petersen Mini-Mental States Examination (MMSE) [8] were used to assess cognitive function. Probable AD was diagnosed according to the criteria from the Diagnostic and Statistical Manual for Mental Disorders IV (DSM IV), and from the National Institute of Neurologic and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) [9]. MCI was diagnosed according to Petersen's criteria [10]. Based on cognitive test scores, subjects displaying memory deficit or additional deficit in another domain were included in the MCI group [11]. Cognitively normal controls had no history of cognitive decline, neurologic disorders, or uncontrolled systemic medical disorders.

Data collection in SMHC cohort was carried out in accordance with the recommendations of the Shanghai Mental Health Center ethical standards committee on human experimentation.

To validate the findings from our cohort and to explore the causal relationships, data of 482 elderly including 54 NC, 544 MCI, and 84 definite AD was downloaded from ADNI database (https://adni.loni.usc.edu). ADNI is a multi-site dataset designed to test clinical, imaging, genetic, and biochemical biomarkers of AD, which was launched in 2003. Data collection and sharing in ADNI were approved by institutional review boards of all participating institutions, and written informed consent was obtained from all participants or their guardians according to the Declaration of Helsinki. The participants are older adults aged 55–90 years. Each participant underwent an in-person interview for health and neuropsychological assessments at baseline and at annual follow-up. MMSE and Alzheimer's Disease Assessment Scale-Cognitive section (ADAS-cog) were used to assess cognitive function. The inclusion criteria were as follows: NC: MMSE scores between 24–30, Clinical Dementia Rating (CDR) of 0; MCI subjects: MMSE scores between 24–30, with memory complaint or other cognitive impairments, a CDR of 0.5, and preserved activities of daily living; Mild AD subjects: MMSE scores between 20–26, CDR of 0.5–1.0, meeting NINCDS/ADRDA criteria for probable AD [9], and CSF $A\beta < 976.6$ pg/ml. In this study, we chose subjects from ADNI database who had blood BMP6 protein measurement at baseline and 1-year follow-up.

Measurement of blood BMP6 and CSF AD biomarkers

In SMHC cohort, peripheral blood samples were collected in the fasting state from all subjects by

venipuncture into a coagulation promoting tube. Samples were collected and centrifuged at 3000 *g* for 20 min at 4°C, and serum was stored at –80°C. Enzyme linked immunosorbent assay kits for BMP6 (HEB037) and noggin (HEN036) (Bogoo Biological, Shanghai, China) were used to determine the serum levels of BMP6. Concentrations were expressed as ng/ml for BMP6.

In ADNI cohort, procedure of plasma protein data collection and measurement was explained in detail elsewhere (https://adni.loni.ucla.edu/wp-content/uploads/2010/11/BC_Plasma_Proteomics_Data_Primer.pdf). In brief, plasma proteins including BMP6 protein were measured in a subset of ethylene diamine tetraacetic acid (EDTA) plasma samples (obtained in the morning following an overnight fast), using a 190-analyte multiplex immunoassay panel. The panel, referred to as the human discovery map, was developed on the Luminex xMAP platform by Rules-Based Medicine (RBM) to contain multiple proteins [12]. Furthermore, CSF AD biomarkers including $A\beta_{42}$, phosphorylated tau (p-tau), and total tau, were detected. The ADNI used the fully automated and highly standardized Roche Elecsys immunoassay to assess AD biomarkers. Aβ+ individuals had a CSF $A\beta_{1\text{-}42} < 976.6$ pg/ml [13].

APOE genotype

DNA was extracted with the QIAamp®DNA Blood Mini Kit and amplified by the polymerase chain reaction (PCR) with forward primers 14 5'-AC GGCTGTCCAAGGAGCTG-3' (rs429358) and 5'-CTCCGCGATGCCGATGAC-3' 15 (rs7412). *APOE* genotype was performed through Restriction Fragment Length Polymorphism (RFLP) technology.

Statistical analyses

The data was not normally distributed; therefore, statistical significance was assessed using non-parametric tests. Demographics, physical disease, cognitive scores, and blood cytokine were analyzed using Mann-Whitney U test for continuous variables and a χ^2 test for categorical variables. BMP6 change rate was defined as the ratio of (follow-up BMP6 - Baseline BMP6)/Baseline BMP6, which reflected the annual change in BMP6 in ADNI cohort. Longitudinal change of BMP6 was plotted against age to see the annual difference between NC and AD groups. The Spearman correlation coefficient was used to explore the associations of blood BMP6 with cognitive scores.

Modulation analysis was used to test whether the association between BMP6 and follow-up cognitive function was modulated by AD core pathology. This analysis was performed using the PROCESS macro for SPSS [14]. Age, education years, sex, and *APOE4* genotype were included as covariates. Mean center was used for construction of continuous variables. Significance was determined through 95% bias corrected confidence intervals from bootstrapping of 1000 iterations. Significant interactions were probed using simple slopes analyses, which provided information about the deferential associations between variables, depending on the levels (i.e., high or low) of predictor variables. 'High' levels of each variable were computed as one standard deviation above the mean, and 'low' levels were computed as zero [15].

The least absolute shrinkage and selection operator (LASSO) regression through R software was applied for selection of BMP6 and demographics data to distinguish NC and AD subjects in ADNI cohort. This machine-learning algorithm identified the variables which could predict a given dependent variable and allowed optimal variable weights for this prediction. Randomly assigned 65% of all subjects, were involved in the train set, and the remaining 35% were involved in the test set. A 5-fold cross-validation procedure served to optimize the penalization and weighted parameters of this LASSO model. We estimated the discriminatory efficacy of this model in the train set by quantifying the area under curve (AUC) of the receiver-operator characteristic (ROC), and verified the generalizability of this model in the test set [16].

The statistical significance of all tests was set at a two-sided p value < 0.05. All analyses were performed using SPSS 17.0 or R version 4.0.3.

RESULTS

Demographic and clinical variables

As for theSMHC cohort, demographics and cognitive scores for a total of 309 subjects including probable AD ($n = 103$), MCI ($n = 92$), and NC ($n = 114$) subjects at baseline were shown Table 1. The mean age of this study sample was 71.02 years, the mean educational years was 10.77, and males accounted for 40.78%. After 1 year, 163 subjects were assessed by MoCA test and 127 were assessed by MMSE test, and some subjects showed changes of cognitive functions including the conversion of NC

Table 1
Characteristics of participants in SMHC cohort and ADNI cohort

Characteristic	SMHC cohort				*p* value	ADNI cohort				*p*
	Total	NC	MCI	AD		Total	NC	MCI	AD	
Baseline										
N	309	114	92	103		482	54	344	84	
Age(y)	71.02 ± 7.49	70.29 ± 6.20	72.59 ± 7.32	70.43 ± 8.70	0.015	75.17 ± 7.25	75.52 ± 5.87	75.15 ± 7.34	75.04 ± 7.73	0.967
Male/Female	126/183	52/62	41/51	33/70	0.086	296/186	28/26	223/121	45/39	0.051
Education (y)	10.77 ± 4.13	11.09 ± 3.54	10.49 ± 4.73	10.67 ± 4.19	0.814	15.64 ± 2.94	15.59 ± 2.85	15.71 ± 2.96	15.37 ± 2.96	0.521
Hypertension(Y/N)	120/189	49/65	39/53	32/70	0.140	216/266	33/21	144/200	39/45	0.029
Diabetes(Y/N)	46/263	17/97	16/76	13/90	0.646	10/472	1/53	8/336	1/83	0.801
APOE (ε4/non-ε4)	/	/	/	/	/	253/229	5/49	187/157	61/23	< 0.001
BloodBMP6 (ng/ml)	0.52 ± 0.22	0.57 ± 0.21	0.54 ± 0.19	0.46 ± 0.25	0.008	0.87 ± 0.30	0.93 ± 0.23	0.85 ± 0.30	0.88 ± 0.33	0.042
MoCA	17.76 ± 9.68	26.39 ± 1.96	20.49 ± 3.11	5.76 ± 5.86	< 0.001	/	/	/	/	/
MMSE	18.79 ± 10.86 (*n* = 219)	28.13 ± 1.61 (*n* = 75)	25.56 ± 3.43 (*n* = 45)	8.64 ± 7.95 (*n* = 99)	< 0.001	26.64 ± 2.34	28.98 ± 1.17	27.05 ± 1.78	23.48 ± 1.87	< 0.001
ADAS-cog	/	/	/	/	/	12.00 ± 5.59	6.28 ± 2.85	11.41 ± 4.43	18.08 ± 5.86	< 0.001
1-year follow-up										
N	163	83	37	43		482	61	276	145	
Diagnosis change		stable NC (*n* = 64) MCI to NC (*n* = 19)	stable MCI (*n* = 21) NC to MCI (*n* = 16)	stable AD (*n* = 38) MCI to AD (*n* = 5)			stable N (*n* = 54) MCI to NC (*n* = 7)	stable MCI (*n* = 276)	stable AD (*n* = 84) MCI to AD (*n* = 61)	
1-year BMP6 (ng/ml)	/	/	/	/	/	0.90 ± 0.30	1.00 ± 0.039	0.93 ± 0.015	0.706 ± 0.031	< 0.001
BMP6 changerate	/	/	/	/	/	0.63 ± 0.46	0.09 ± 0.22	0.13 ± 0.49	–0.07 ± 0.47	< 0.001
1-year MoCA	18.69 ± 10.70	26.65 ± 2.06	20.08 ± 2.87	2.14 ± 4.36	< 0.001	/	/	/	/	/
1-year MMSE	19.76 ± 12.16 (*n* = 127)	28.44 ± 1.44 (*n* = 64)	25.11 ± 4.81 (*n* = 22)	1.58 ± 4.70 (*n* = 41)	< 0.001	25.76 ± 3.91	29.10 ± 1.18	26.96 ± 2.48	22.08 ± 4.19	< 0.001
1-year ADAS-cog	/	/	/	/	/	13.37 ± 7.86	5.73 ± 2.70	11.58 ± 5.30	20.01 ± 8.64	< 0.001
2-year follow-up										
N	/	/	/	/		425	62	174	189	
Diagnosis change	/	/	/	/			stable NC (*n* = 50) MCI to NC (*n* = 12)	stable MCI (*n* = 172) NC to MCI (*n* = 2)	stable AD (*n* = 76) MCI to AD (*n* = 113)	
1-year MMSE	/	/	/	/	/	24.73 ± 5.00 (*n* = 411)	29.27 ± 1.00 (*n* = 59)	27.11 ± 2.85 (164)	21.24 ± 4.88 (*n* = 188)	< 0.001
1-year ADAS-cog	/	/	/	/	/	15.41 ± 9.95 (*n* = 409)	5.64 ± 2.61 (*n* = 59)	11.47 ± 5.28 (*n* = 164)	21.98 ± 10.21 (*n* = 186)	< 0.001

$p < 0.05$ The significance of difference among groups was examined by Mann-Whitney test (for continuous variable) and Pearson's Chi-squared test (for categorical variable). BMP6 change rate: (1-year BMP6-Baseline BMP6)/Baseline BMP6.

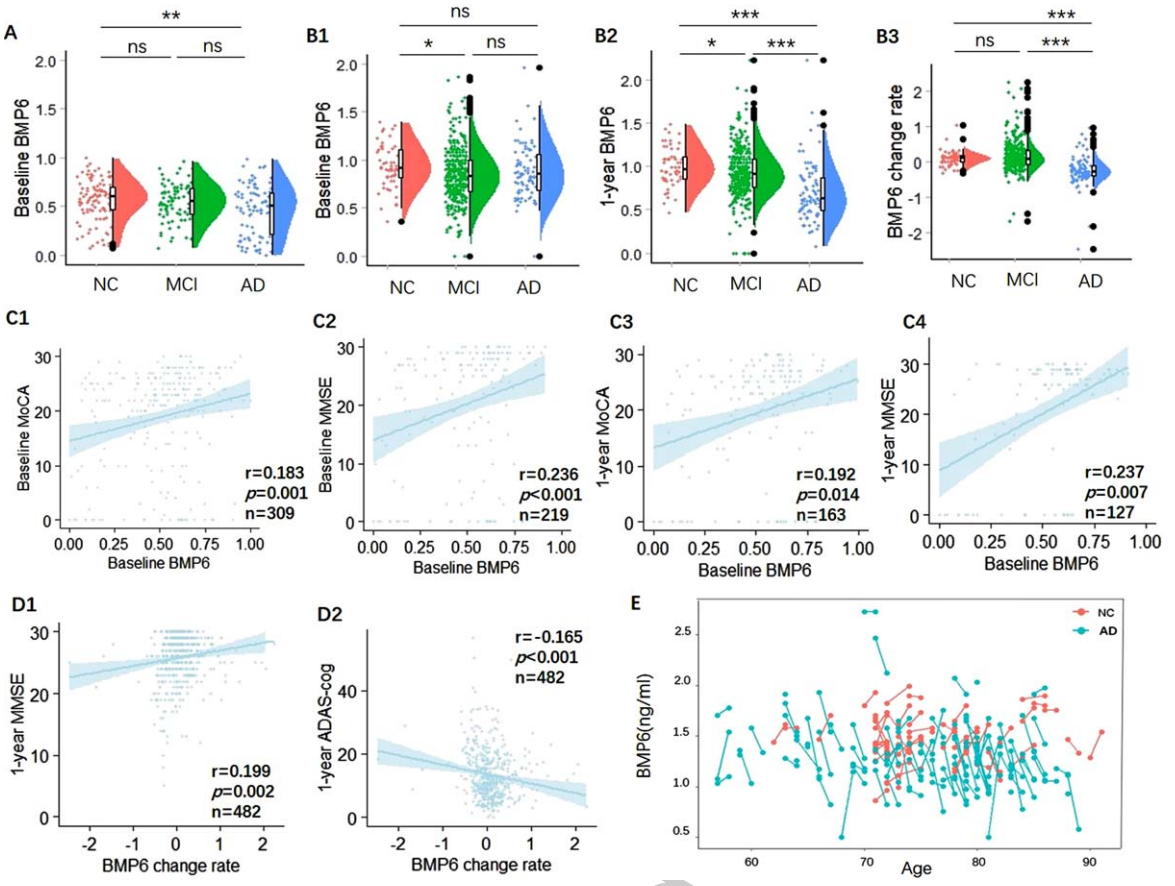

Fig. 1. There was significant difference of blood BMP6 between NC and probable AD groups in SMHC cohort (A). There was not significant difference of blood BMP6 between NC and definite AD groups at baseline (B1), but significant differences of 1-year BMP6 (B2) and BMP6 change rate (B3) between NC and definite AD groups in ADNI cohort. Correlation plots showed positive correlations between blood BMP6 and cognitive scores including baseline MoCA (C1), baseline MMSE (C2), 1-year MoCA (C3), and 1-year MMSE (C4) in SMHC cohort. Correlation plots showed positive correlation between blood BMP6 change rate and 1-year MMSE (D1), and negative correlation between blood BMP6 change rate and 1-year ADAS-cog (D2) in ADNI cohort. Longitudinal BMP6 plotted against age in definite AD and NC groups between baseline and 1-year follow-up (E) in ADNI cohort.

to MCI, the conversion of MCI to probable AD, and the reversion of MCI to NC (Table 1). As expected, differences in cognitive scores were observed.

As for ADNI, a total of 482 subjects including definite AD ($n=84$), MCI ($n=344$), and NC ($n=54$) were involved at baseline (Table 1). Compared with SMHC cohort, the study sample was older (mean = 75.17 years), had more educational years (mean = 15.64 years), and had more males (61.41%). Individuals in definite AD group tended to be *APOE4* positive. All subjects received 1-year follow-up, and 425 subjects received 2-year follow-up which included 411 subjects were assessed by MMSE and 409 were assessed by ADAS-cog (Table 1). Some subjects showed changes of cognitive functions including the conversion of the conversion of MCI to AD and the reversion of MCI to NC (Table 1). Similarly, differences in cognitive scores were observed.

Blood levels of BMP6

As for SMHC cohort, blood levels of BMP6 protein were decreased significantly in probable AD than NC subjects (Table 1, Fig. 1). However, in ADNI database, there were no difference of baseline BMP6 levels between NC and definite AD groups. After a year, significant lower BMP6 levels were found in definite AD and MCI groups than NC group (Table 1, Fig. 1). Slopes representing the change of blood BMP6 between 2 years plotted against age. Individual longitudinal changes showed decreased rate of

BMP6 in definite AD subjects when compared to NC subjects (Fig. 1E).

In ANDI, a feature set including baseline BMP6, 1-year BMP6, *APOE4*, demographics, diabetes, and hypertension, was used to distinguish definite AD and NC subjects at 1-year and 2-year follow-up. The total sample was randomly divided into the train set (65%) and test set (35%), and feature selection was conducted by LASSO logistic regression. First, BMP6, 1-year BMP6, and the change rate of BMP6 were involved, and 1-year BMP6 was selected by LASSO model, which showed the prediction efficacy with AUC of 56–67% at 1-year follow-up (Fig. 2A1, A2) and 2-year follow-up (Fig. 2F1, F2). Second, *APOE*4 and demographics as features were involved, and *APOE*4 was selected by LASSO model which showed better prediction efficacy with AUC of 76–82% at 1-year follow-up (Fig. 2B1, B2) and 2-year follow-up (Fig. 2F1, F2). Last, all features were involved, and an overview of the weighted coefficients of the feature set (Fig. 2C2, G2) and the features selection process (Fig. 2C1, G1) were showed. 1-year BMP6 levels combining with *APOE4* displayed favorable prediction efficacy. ROC analysis revealed an AUC of 0.854 (95% confidence interval (CI), 0.789–0.919) in the train set and 0.892 (95%CI, 0.818–0.965) in the test set (Fig. 2D1, D2) after 1 year, and an AUC was 0.832 (95% CI, 0.764–0.899) in the train set and 0.803 (95%CI, 0.711–0.895) in the test set (Fig. 2H1, H2) after two years.

Association of blood BMP6 with cognitive functions

As SMHC cohort, we observed a significant correlation between baseline BMP6 and cognitive functions including MoCA and MMSE scores at baseline and 1 year follow-up (Fig. 1C1-C4). Similarly, as for ADNI cohort, a significant correlation between BMP6 change rate and cognitive functions including MMSE and ADAS-cog scores at 1-year follow-up was observed (Fig. 1D1, D2).

Moderator analyses

Blood BMP6 was a significant risk factor for cognitive impairment, and we next investigated whether BMP6 contributed to cognitive impairments via modulating tau pathology. As for ADNI cohort, a total of 300 subjects with both blood BMP6 and CSF tau tests were selected, which included 53 NC, 167 MCI, and 80 definite AD. The characteristics were showed in Table 2. We found the relationship between BMP6 change rate and 1-year global cognition was modulated by tau pathology including baseline tau (Fig. 3A, C), baseline p-tau (Fig. 3B, D), 1-year tau (Fig. 3E, G), and 1-year p-tau (Fig. 3F, H). First, the subjects with lower BMP6 change rate tended to have lower follow-up MMSE and ADAS-cog scores. Second, subjects with lower BMP6 change rate, higher baseline, or follow-up tau burden, tended to report lower follow-up MMSE and ADAS-cog scores.

DISCUSSION

The major findings of this study were that 1) AD patients had lower levels of blood BMP6 compared to normal controls; 2) BMP6 was positively associated with baseline or follow-up cognitive functions; 3) Longitudinal BMP6 combining with *APOE4* could distinguish AD from NC elderly; 4) the influence of BMP6 change rate on cognition was modulated by tau pathology. These findings consolidated the close relationships of BMP6 with cognitive function and tau pathology, supporting the hypothesis that BMP6 was identified as a potential predictor of AD.

The focus on blood-based AD biomarkers had grown exponentially during the past decade. A series of studies had applied plasma profiling to detect AD from control subjects, which were related to the inflammatory response, lipid metabolism, and the microcirculation. These measurements showed substantial promise, but it remained uncertain whether these protein profiles had a definitive correlation with brain function [17]. In the present study, BMP6, as a member of TGF-β subfamily, influenced brain development and neurogenesis, and had a great impact on brain degenerative diseases, particularly AD [18]. Previous studies found BMP6 significantly increased in the hippocampus of AD patients and transgenic mouse model of familial AD [6]. The increase in BMP6 had been reported during healthy aging in wild-type mice, and the process might be exacerbated in pathological conditions such as AD that led to Aβ accumulation, microglia activation, and unfavorable inflammatory microenvironment [19, 20]. At present, there is lack of research on the expression of peripheral BMP6 in AD patients. We found a decrease of blood BMP6 both in probable AD of SMHC cohort and definite AD of ADNI cohort, compared to normal controls. As for ADNI cohort, BMP6 levels in definite AD did not differ from NC at baseline, but reduced significantly at 1-year follow-up,

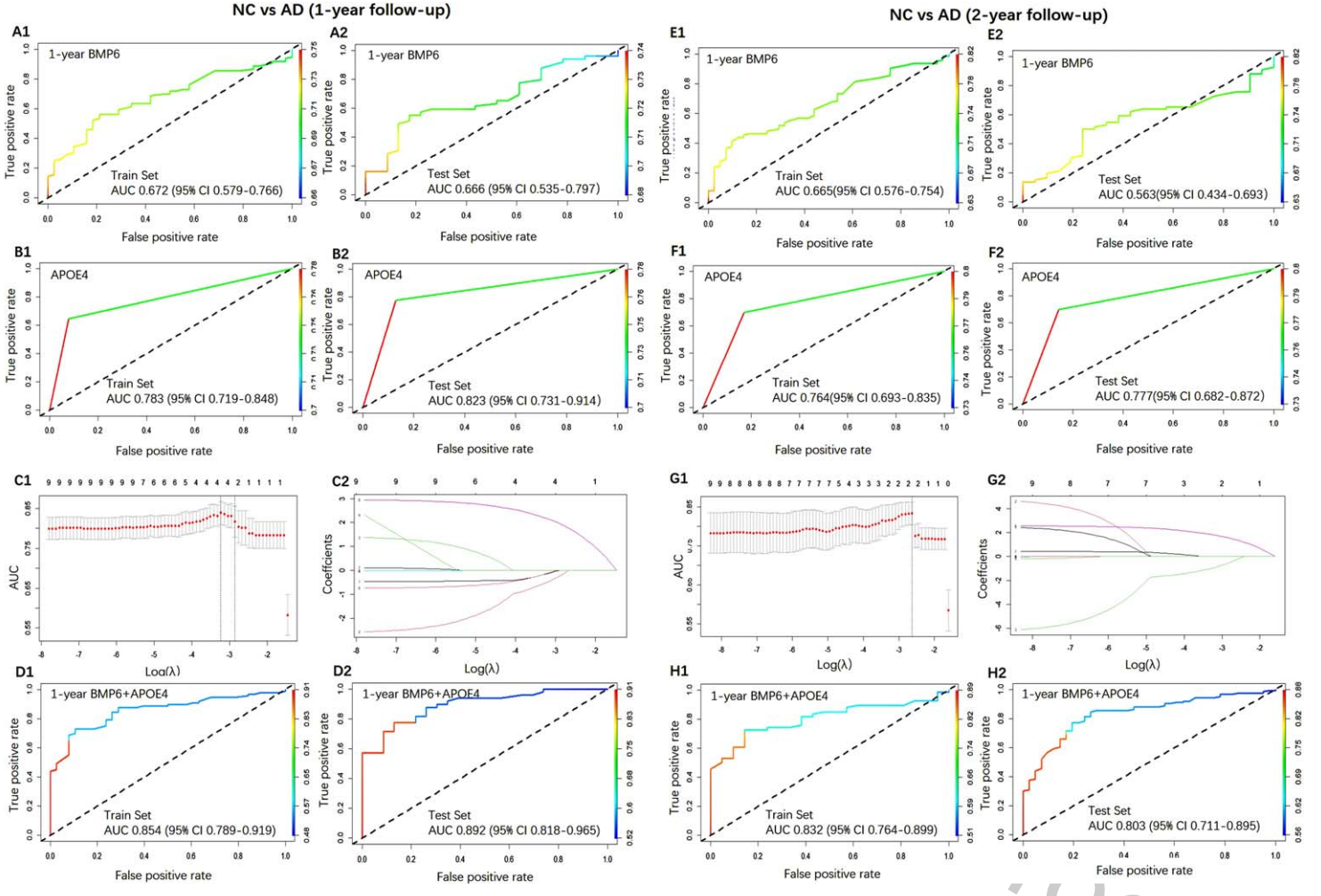

Fig. 2. In ADNI cohort, ROC curve analysis revealed the AUC for the diagnostic accuracy of 1-year blood BMP6 (A1, A2) or *APOE4* (B1, B2) to categorize definite AD patients from NC controls in train and test sets respectively at 1-year follow-up. ROC curve analysis revealed the AUC for the diagnostic accuracy of 1-year blood BMP6 (E1, E2) or *APOE4* (F1, F2) to categorize AD patients from NC controls in train and test sets respectively at 2-year follow-up. The vertical dotted line points to the optimal λ value and the number of optimal predictors, and the pathway of coefficients among all parameters at 1-year follow-up (C1, C2) and at 2-year follow-up (G1, G2) respectively. The parameters including demographics, *APOE4*, baseline BMP6, 1-year BMP6, and BMP6 change rate were selected by LASSO model. In the train and test sets, ROC curve analysis revealed the AUC for the diagnostic accuracy of 1-year blood BMP6 combing *APOE4* to categorize AD patients from NC controls at 1-year follow-up (D1, D2) and 2-year follow-up (H1, H2) respectively.

Table 2
Characteristics of participants with blood BMP6 and CSF AD biomarkers in ADNIcohort

Characteristics	Total	NC	MCI	AD	p^*
N	300	53	167	80	
Age(y)	74.71 ± 7.37	75.59 ± 1.01	74.41 ± 0.57	74.75 ± 0.83	0.776
Male/Female	182.118	27/26	113/54	42/38	0.021
Education (y)	15.65 ± 2.92	15.51 ± 0.40	15.59 ± 0.23	15.21 ± 0.33	0.139
Hypertension(Y/N)	129/171	33/20	59/108	37/43	0.002
Diabetes(Y/N)	8/292	1/52	6/161	1/79	0.523
APOE (ε4/non-ε4)	153/147	5/48	89/78	59/21	<0.001
Blood BMP6 changerate	0.03 ± 0.52	0.10 ± 0.07	0.15 ± 0.04	-0.26 ± 0.06	<0.001
Baseline MMSE	26.39 ± 2.56	28.96 ± 0.24	26.97 ± 0.13	23.48 ± 0.19	<0.001
Baseline ADAS-cog	12.35 ± 6.15	6.32 ± 2.86	11.51 ± 4.65	18.08 ± 5.79	<0.001
Baseline CSF tau	306.45 ± 120.59	226.55 ± 72.49	307.21 ± 115.65	357.82 ± 128.52	<0.001
Baseline CSF p-tau	30.10 ± 13.90	19.94 ± 6.39	30.39 ± 13.38	36.24 ± 14.86	<0.001
1-year MMSE	25.31 ± 4.31	29.04 ± 0.46	26.11 ± 0.26	21.16 ± 0.38	<0.001
1-year ADAS-cog	13.87 ± 8.68	5.78 ± 0.92	12.53 ± 0.52	22.04 ± 0.75	<0.001
1-year CSF tau	315.89 ± 127.09 (*n* = 244)	229.40 ± 66.18 (*n* = 43)	320.83 ± 125.22 (*n* = 135)	362.14 ± 134.38 (*n* = 66)	<0.001
1-year CSF p-tau	30.69 ± 14.72 (*n* = 244)	19.98 ± 6.17(*n* = 43)	31.54 ± 14.52(*n* = 135)	35.94 ± 15.68(*n* = 66)	<0.001

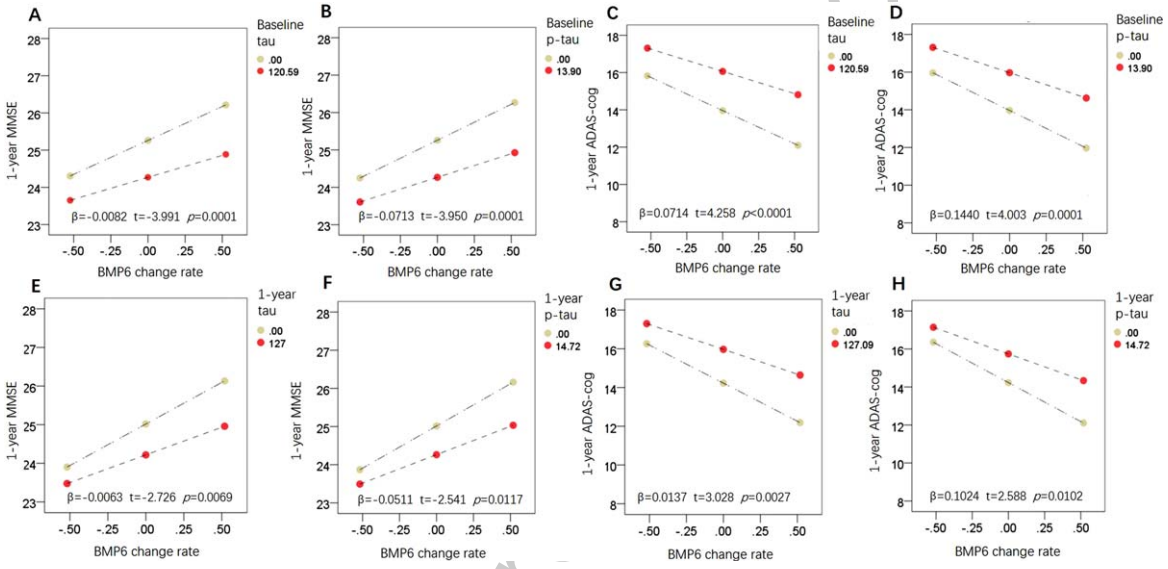

Fig. 3. In ADNI cohort, the relationship between blood BMP6 change rate and cognitive measures including 1-year global cognition measured by MMSE (A, B, E, F) as well as ADAS-cog (C, D, G, H), was modulated by tau pathology including baseline tau (A, C), baseline p-tau (B, D), 1-year tau (E, F), and 1-year p-tau (G, H). Subjects who experienced lower BMP6 change rate, higher baseline, or follow-up tau burden, reported lower follow-up cognitive function.

while in SMHC cohort, BMP6 levels in probable AD decreased significantly at baseline. The possible reason was that AD patients involved in ADNI cohort were mild, but severe in SMHC cohort. The changing pattern of BMP6 in peripheral blood and brain tissue was opposite, which was similar to Aβ in AD pathology. The possible reason was that BMP6 existed as hydrophobic homodimer/heterodimer in brain tissue and as hydrophilic monomer in body fluids [21]. Furthermore, the baseline levels of BMP6 in SMHC cohort and the changing rates of BMP6 in ADNI cohort, were both associated with global cognition. Above findings suggested that blood BMP6 had lower expression in AD patients and had a definitive correlation with cognitive function.

Previous research found *APOE*4 participants had 3.34 times the odds of developing AD within 17 years than *APOE* ε4- participants [22]. In ADNI cohort, we found *APOE4* alone could distinguish definite AD from normal elderly with the AUC > 75%, and blood

1-year BMP6 alone with the AUC of 56–67%. However, blood 1-year BMP6 combining with *APOE4* had diagnostic and prediction for definite AD with the AUC > 85% at 1-year follow-up and AUC > 80% at 2-year follow-up, which suggested longitudinal BMP6 combining *APOE4* as a potential diagnostic and predictive biomarker of cognitive decline in AD. These findings were thus less driven by the complex status of dementia. Although the dementia patients involved at baseline were diagnosed as AD through CSF AD biomarkers, dementia patients transforming from MCI were not assessed by CSF.

In ADNI cohort, we revealed that increased blood BMP6 contributed to cognitive impairment under a modulation of tau pathology. The elderly who experienced decreased BMP6 rate and high tau burden showed the lowest levels of cognitive function. As one of AD characteristics, tau protein was the most abundant microtubule-associated protein in the brain, and BMP/TGF-β pathway was not commonly discussed in relation to tau pathology [23]. Previous publications found the link between BMP and tau protein. Wu et al. revealed that BMP2 might affect injured facial nerve regeneration and levels of tau protein [24]. Lauzon et al. developed a small peptide derived from BMP9, which could inactivate GSK3β, a tau kinase [25]. The above research supported our finding that tau pathology could modulate the influence of blood BMP6 on cognitive impairment.

There were limitations in this study. First, the study sample of definite AD and NC was limited in ADNI cohort, which might introduce population heterogeneity bias. Second, CSF AD biomarkers and *APOE* genotype were adapted in ADNI cohort, but not in SMHC cohort, which made the loss of the validation about the modulation and ROC analysis. Third, dementia patients transforming from MCI were not assessed by CSF, which made the complex status of dementia at follow-up.

Conclusions

The present study indicates that AD patients have lower levels of blood BMP6 compared to normal controls, and BMP6 was positively associated with global cognition. Longitudinal BMP6 combing with *APOE4* could distinguish AD from NC elderly. The influence of BMP6 change rate on cognition was modulated by tau pathology. These findings demonstrate the relationship of blood BMP6 with cognitive performance and AD diagnosis.

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DATA AVAILABILITY

A complete listing of ADNI investigators can be found at: https://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

A complete listing of SMHC investigators can be obtained by email to the corresponding authors.

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