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Bridging Integrator 1 (*BIN1*) Genotypes Mediate Alzheimer's Disease Risk by Altering Neuronal Degeneration

Hui-Fu Wang[a], Yu Wan[b], Xiao-Ke Hao[c], Lei Cao[a], Xi-Chen Zhu[a], Teng Jiang[d], Meng-Shan Tan[b], Lin Tan[e], Dao-Qiang Zhang[c], Lan Tan[a,b,e,*], Jin-Tai Yu[a,b,*] and Alzheimer's Disease Neuroimaging Initiative[1]

[a]*Department of Neurology, Qingdao Municipal Hospital, Nanjing Medical University, China*
[b]*Department of Neurology, Qingdao Municipal Hospital, School of Medicine, Qingdao University, China*
[c]*Department of Computer Science and Engineering, Nanjing University of Aeronautics and Astronautics, Nanjing, China*
[d]*Department of Neurology, Nanjing First Hospital, Nanjing Medical University, China*
[e]*Department of Neurology, Qingdao Municipal Hospital, College of Medicine and Pharmaceutics, Ocean University of China, Qingdao, China*

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Abstract.
Background: Bridging integrator 1 (*BIN1*) has been identified as one of the most associated loci for Alzheimer's disease (AD), and recently was reported to modulate tau pathology to mediate AD *in vitro*. However, the effects of *BIN1* on the AD related biomarkers in AD continuum were not specifically assessed.
Objective: We explored the effects of *BIN1* loci on AD specific biomarkers (CSF proteins, brain structures, glucose and amyloid-β (Aβ) metabolisms) to investigate the role *BIN1* in AD pathogenesis.
Methods: We calculated the associations of *BIN1* loci with these markers at baseline and follow-up in multiple linear models in 812 ADNI subjects.
Results: *BIN1* loci were significantly associated with the levels of T-tau (rs744373: $p_c = 0.047$, rs13031703: $p_c = 0.042$) and P-tau (rs744373: $p_c = 0.044$, rs13031703: $p_c = 0.019$), but not with Aβ in CSF test. *BIN1* genotypes were strongly related to atrophy of hippocampus (rs7561528: $p_c = 0.011$), CA1 (rs1469980: $p_c = 0.029$) and parahippocampus (rs72838284, $p_c = 0.017$) on MRI, and to glucose metabolism on FDG-PET, but not to Aβ deposition on AV45-PET imaging. Furthermore, haplotype and subgroup analysis confirmed these significant findings. In addition, the loci associated with these markers were also identified to influence the risk for AD in the meta-analysis of 74 046 European individuals.
Conclusion: This study supported that *BIN1* contributes to the risk of AD by altering neural degeneration (abnormal tau, brain atrophy and glucose metabolism) but not Aβ pathology.

Keywords: Aβ deposition, Alzheimer's disease, *BIN1*, brain structure, cerebrospinal fluid, glucose metabolism

[1]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://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: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

*Correspondence to: Lan Tan, Department of Neurology, Qingdao Municipal Hospital, Nanjing Medical University, China. E-mail: dr.tanlan@163.com and Jin-Tai Yu, Department of Neurology, Qingdao Municipal Hospital, Nanjing Medical University, China. Tel.: +1 415 988 0162; Fax: +1 415 476 0679; E-mails: jintai.yu@ucsf.edu or yu-jintai@163.com.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in the elderly, accounting for 50% of all dementia [1]. It has been documented that genetic factors, along with environments, contribute to the pathogenesis of AD [2], and the bridging integrator 1 (*BIN1*), located in chromosome 2q14.3, has been identified as the most significantly associated risk gene with AD following *APOE* in Caucasian in large genome-wide association studies (GWAS) and meta-analysis [3–5]. Moreover, Liu et al. found *BIN1* rs744373 polymorphisms affected the risk for AD in East Asian population [6], and we also reported that genetic variants in *BIN1* were markedly linked to AD in Han Chinese [7, 8].

Regarding the mechanisms by which the *BIN1* genetic polymorphisms induce the onset of AD, Chapuis et al. discovered that *BIN1* genetic variations increased *BIN1* expression level, and the increase in *BIN1* expression modulated tau but not amyloid-β $(Aβ)_{42}$ induced neurotoxicity *in vitro* [9]. Otherwise, the insertion/deletion variant (rs59335482) was detected to associate with tau loads but not with Aβ loads in AD brains [9]. Likewise, *BIN1* protein expression was reported to be significantly linked to the amount of neurofibrillary tangles but not to either diffuse of neurotic plaques, or the amount of Aβ in the brain [10]. Furthermore, the low or over expression of *BIN1* did not influence AβPP processing in a neuroblastoma cell line [11]. From the evidence, it is possible that *BIN1* variations mediate the susceptibility of AD by altering the neuronal degeneration/injury markers (including total tau/phosphorylated tau in CSF, brain structures, and glucose hypometabolism on imaging) rather than the Aβ biomarkers (including $Aβ_{42\ \text{level}}$ in CSF and Aβ deposition on imaging) [12].

To date, it has been documented that CSF Aβ and tau proteins were strongly associated with Aβ and tau pathology in brain, respectively. Recently, multiple neuroimaging measures were proposed as new crucial markers for AD in biological research and clinical trials for their strong associations with AD pathophysiological process [13, 14]. These measures also appeared to be shaped by genetic influences with heritability estimates as high as 80% [15]. The increasing evidence that candidate gene for AD also impacted CSF and neuroimaging markers further confirmed the role of these genetic factors in AD and suggested mechanisms through which they might modulate the onset of AD. In this study, we genotyped *BIN1* loci and explored their associations with AD specific CSF and neuroimaging markers to ascertain whether *BIN1* loci polymorphisms were associated with the neuronal degeneration/injury biomarkers, but not with the Aβ deposition in AD pathogenesis.

MATERIALS AND METHODS

ADNI dataset and subjects

The Alzheimer's Disease Neuroimaging Initiative (ADNI) is a large, multicenter, longitudinal neuroimaging study, launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations [16]. The initial goal of ADNI is to recruit 800 subjects, but ADNI has been followed by ADNI-GO and ADNI-2. To date the three protocols have covered more than 1,500 adults, ages 55 to 90 years, to participate in the research, including cognitively normal (CN) older individuals, mild cognitive impairment (MCI), and early dementia patients with due to AD [17]. However, only 812 participants were genotyped using the Illumina HumanOmni Express BeadChip. Finally, 281 CN, 483 MCI, and 48 AD patients were included in our study. The study was approved by the institutional review boards of all participating centers and written informed consent was obtained from all participants or authorized representatives.

SNPs selection

BIN1 genotypes were extracted from the ADNI PLINK format data [18]. Thus far, four *BIN1* loci (rs744373, rs7561528, rs59335482, and rs6733839) have been reported to be strongly associated with AD in GWAS [4, 5, 9, 19], and these loci neighbored with each other and located in the upstream of *BIN1* gene. Therefore, the region adjacent to top SNP (rs744373 $\pm$ 10 kp) within upstream of *BIN1*, covering the four loci, were treated as our region of interest in this study (Supplementary Figure 1). We then performed the quality control (QC) procedures using PLINK software, and the inclusion criteria were as follows: minimum call rates >90%, minimum minor allele frequencies (MAF) >0.01, Hardy-Weinberg equilibrium test $p > 0.001$. Finally, using tagger methods on Haploview 4.2 platform, we selected other 6 loci, along with the known 3 loci, as our targeted *BIN1* loci in this study (Supplementary Table 1).

CSF proteins

The cerebrospinal fluid (CSF) data used in this study were downloaded from ADNI dataset. The methods for CSF acquisition and measurement have been reported previously [20]. Briefly, CSF samples were collected into collection tubes, and then transferred into polypropylene transfer tubes followed by freezing on dry ice within 1 h after collection, and transported overnight to the ADNI Biomarker Core laboratory at the University of Pennsylvania Medical Center in dry ice. Preparation of aliquots (0.5 ml) from these samples was done after thawing (1 h) at room temperature and gentle mixing. The aliquots were stored in bar code–labeled polypropylene vials at –80°C. CSF proteins, such as $A\beta_{1\text{-}42}$, T-tau, and P-tau181p, were calculated in every CSF baseline aliquots on the multiplex xMAPLuminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use only reagents) immunoassay kit-based reagents. Full details of this combination of immunoassay reagents and analytical platform are described elsewhere [20].

Brain structures on MRI

The MRI volumes of brain structures used in our study were from the UCSF data in ADNI dataset (https://ida.loni.usc.edu/pages/access/studyData.jsp). The cerebral image segmentation and analysis were performed with the FreeSurfer version 5.1 (http://surfer.nmr.mgh.harvard.edu/) based on the 2010 Desikan-Killany atlas [21]. This process mainly included motion correction and averaging of multiple volumetric T1 weighted images (when more than one is available), removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles) [22], intensity normalization, tessellation of the gray matter white matter boundary, automated topology correction, and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class. The technical details of these procedures are described in prior publications [23]. Here, we selected the most associated brain regions with AD, such as hippocampus, parahippocampus, middle temporal and entorhinal cortex as our regions of interest (ROI) to analyze their associations with *BIN1* genotypes, and we also assessed CA1, the most associated substructure in hippocampus with the AD specific amnestic syndrome [24].

Glucose metabolism on FDG-PET

The information regarding glucose metabolism was from the UC Berkeley and Lawrence Berkeley National Laboratory (http://adni.loni.usc.edu/data-samples/access-data/) [25]. In this laboratory, five regions (left and right angular gyrus, bilateral posterior cingulate, left and right temporal gyrus) were treated as metaROIs to analysis. The brief procedures were as follows. Firstly, PET data was downloaded from LONI (http://loni.usc.edu/). These images were then spatially normalized in SPM to the MNI PET template. The mean counts from the metaROIs for each subject's FDG scans at each time point were extracted and the intensity values were computed with SPM subroutines. Finally, the mean of the top 50% of voxels within a hand-drawn pons/cerebellar vermis region that was hand-drawn on a T1 template in MNI space was extracted; and each metaROI mean was normalized by dividing it by pons/vermis reference region mean. The detailed process and quality control have been described elsewhere [25, 26].

Aβ deposition on AV45-PET

The Aβ deposition imaging data with amyloid tracer, florbetapir (AV-45), were obtained from UC Berkeley – AV45 analysis dataset on website (http://adni.loni.usc.edu/data-samples/access-data/). The institute used a native-space MRI scan for each subject that is segmented with Freesurfer (version 4.5.0) to define cortical grey matter ROI (frontal, anterior/posterior cingulate, lateral parietal, lateral temporal) that make up a summary cortical ROI [27, 28]. At the same time, they also defined whole cerebellum as reference region. They then applied each florbetapir scan to the corresponding MRI and calculate the mean florbetapir uptake within the cortical and reference region. Finally, florbetapir standard uptake value ratios (SUVRs) were created by averaging across the four cortical regions and dividing this cortical summary ROI by the whole cerebellum.

Statistical analysis

All statistical analyses were performed by R 3.12 (http://www.r-project.org/) and PLINK 1.07 (http://pngu.mgh.harvard.edu/wpurcell/plink/). Differences in continuous variables (age, education years, cognitive scores, volume, etc.) were examined using one-way analysis of variance (ANOVA), and categorical data (gender, *APOE* ε4 status) were tested using chi-square test. We used a multiple linear regression model which considered age, gender, education years, and *APOE* ε4 status as covariates to estimate possible correlation between *BIN1* genotypes and these various endophenotypes at baseline in the entire cohort. Furthermore, we computed the effects of *BIN1* loci on the change percentage of these above phenotypes in the longitudinal study in a reduced sample due to the loss of data in the follow-up. Given that Bonferroni correction was inappropriate due to the non-independence of these tests [29], the false discovery rate (FDR) based on the method developed by Hochberg and Benjamini [30] was used to control for multiple test. Statistical significance was considered for FDR-corrected $p_c < 0.05$. We further detected the correlation between these *BIN1* loci and these suggestive phenotypes in the haplotype-based association analysis, and in subgroup analysis to identify that at which stage *BIN1* loci impacted these pathological markers in the AD pathogenesis. Finally, we investigated the association of the significant loci in our study with the risk for AD in a meta-analysis of GWAS from 74,046 individuals of European descent [4].

RESULTS

Characteristics of included subjects

The information about these included subjects is listed in Table 1. In total, 281 CN (145 women, 74.51 ± 5.56 years), 483 MCI (201 women, 72.28 ± 7.45 years), and 48 AD patients (18 women, 75.51 ± 9.23 years) were recruited in this study. As expected, the frequency of the *APOE* ε4 allele in AD subgroup (44.8%) was significantly higher than that in MCI (27.1%) and CN group (14.9%). Compared to CN and MCI subjects, AD dementia patients displayed the worst cognitive function ($p < 0.01$) on these neuropsychological scales (CDR-SB, MMSE, ADAS-cog, RAVLT). Likewise, AD patients showed more severe atrophy in hippocampus, middle temporal and entorhinal cortex than MCI and CN individuals on structural neuroimaging (MRI). In addition, AD patients had the lowest cerebral glucose metabolism rate for glucose (CMRgl) followed by MCI and CN individuals using FDG-PET methods, and the highest Aβ tracer retention on amyloid PET.

Table 1
The characteristics of the ADNI subjects at baseline

Characteristics	CN		MCI		AD		p^*
Age (years)	281	74.51 ± 5.56	483	72.28 ± 7.45	48	75.51 ± 9.23	<0.01
Gender (male/female)	281	136/145	483	282/201	48	30/18	0.02
Education (years)	281	16.41 ± 2.66	483	15.98 ± 2.82	48	15.73 ± 2.62	0.08
APOE ε4 (0/1/2)	281	204/70/7	483	262/180/41	48	14/25/9	<0.01
CDR-SB	207	0.03 ± 0.13	406	1.44 ± 0.87	47	4.44 ± 1.69	<0.01
MMSE	281	29.07 ± 1.15	483	27.89 ± 1.69	48	22.96 ± 2.03	<0.01
ADAS-cog	281	9.06 ± 4.23	480	15.30 ± 6.65	48	29.80 ± 8.44	<0.01
RAVLT	280	44.83 ± 9.60	483	36.16 ± 10.86	47	22.32 ± 7.84	<0.01
FAQ	281	0.17 ± 0.66	481	2.85 ± 3.99	48	12.6 ± 7.14	<0.01
Hippocampus (mm^3)	257	7344 ± 895	422	6996 ± 1126	39	5757 ± 948	<0.01
Middle Temporal (mm^3)	257	20298 ± 2600	422	20186 ± 2735	39	17776 ± 3230	<0.01
Entorhinal (mm^3)	257	3803 ± 650	422	3610 ± 723	39	2919 ± 705	<0.01
CMRgl	207	6.55 ± 0.55	406	6.32 ± 0.64	47	5.30 ± 0.72	<0.01
SUVR	152	1.12 ± 0.19	323	1.20 ± 0.22	46	1.39 ± 0.22	<0.01

CN, cognitively normal; MCI, mild cognition impairment; AD, Alzheimer's disease; CDR-SB, Clinical Dementia Rating sum of boxes; ADAS-cog, Alzheimer's disease Assessment Scale Cognition; MMSE, Mini-Mental State Exam; RAVLT, Rey Auditory Verbal Learning Test; FAQ, Functional Activities Questionnaire; CMRgl, Cerebral Metabolism Rate for glucose measured with fluorodeoxyglucose-positron emission tomography (FDG-PET). SUVR, florbetapir standard uptake value ratios on amyloid imaging. $^*p$ values for continuous variables are from one-way analysis of variance (ANOVA). p values for categorical data are from chi-square test. Data are given as mean ± standard deviation unless otherwise indicated.

CSF markers and BIN1 genotypes

We firstly compared the levels of Aβ, T-tau, and P-tau of different *BIN1* genotypes in one-way ANOVA, and observed that Aβ did not show any evident difference between these genotypes, while tau showed significant difference among the three genotypes at rs13031703 (T-tau: $p = 0.003$; P-tau: $p = 0.001$) and rs744373 (T-tau, $p = 0.029$; P-tau: $p = 0.008$) in ANOVA test and in *post hoc* analysis (Supplementary Table 2A). Likewise, we did not discover any marked relation of Aβ levels to *BIN1* genotypes, whereas we discovered significant relations between tau (T-tau and P-tau) and *BIN1* loci (Fig. 1A; Supplementary Table 2B) in multiple linear models. Both T-tau and P-tau showed remarkable association with rs13031703 (T-tau $p = 0.005$, P-tau $p = 0.002$) and rs744373 (T-tau $p = 0.01$, P-tau $p = 0.01$), and these association achieved the significant level (rs13031703: T-tau $p_c = 0.042$, P-tau $p_c = 0.019$; rs744373: T-tau $p_c = 0.047$, P-tau $p_c = 0.042$) in the FDR test (Fig. 1B–E).

Moreover, we performed linkage disequilibrium (LD) analysis and discovered that rs13031703, rs7561528, and rs72838284 were in LD (Supplementary Figure 2). In the haplotype-based analysis, the haplotype (TGT) was observed to significantly relate to the levels of T-tau ($p = 0.004$) and P-tau ($p = 0.003$). In addition, we conducted subgroup analysis to ascertain whether *BIN1* loci modified the levels of CSF markers in AD, MCI, or CN subgroup, and observed rs72838284 ($p_c = 0.025$), rs744373 ($p_c = 0.025$), and rs7561528 ($p_c = 0.025$) greatly altered the levels of T-tau, and rs13031703 extremely altered the level of P-tau ($p = 5.72 \times 10^{-5}$, $p_c = 0.001$); however, none of these loci significantly altered the level of Aβ in the early AD subgroup. *BIN1* genetic polymorphisms did not alter the levels of Aβ and tau in MCI and CN subgroup (Supplementary Table 2C). Finally, among the four SNPs (rs13031703, rs72838284,

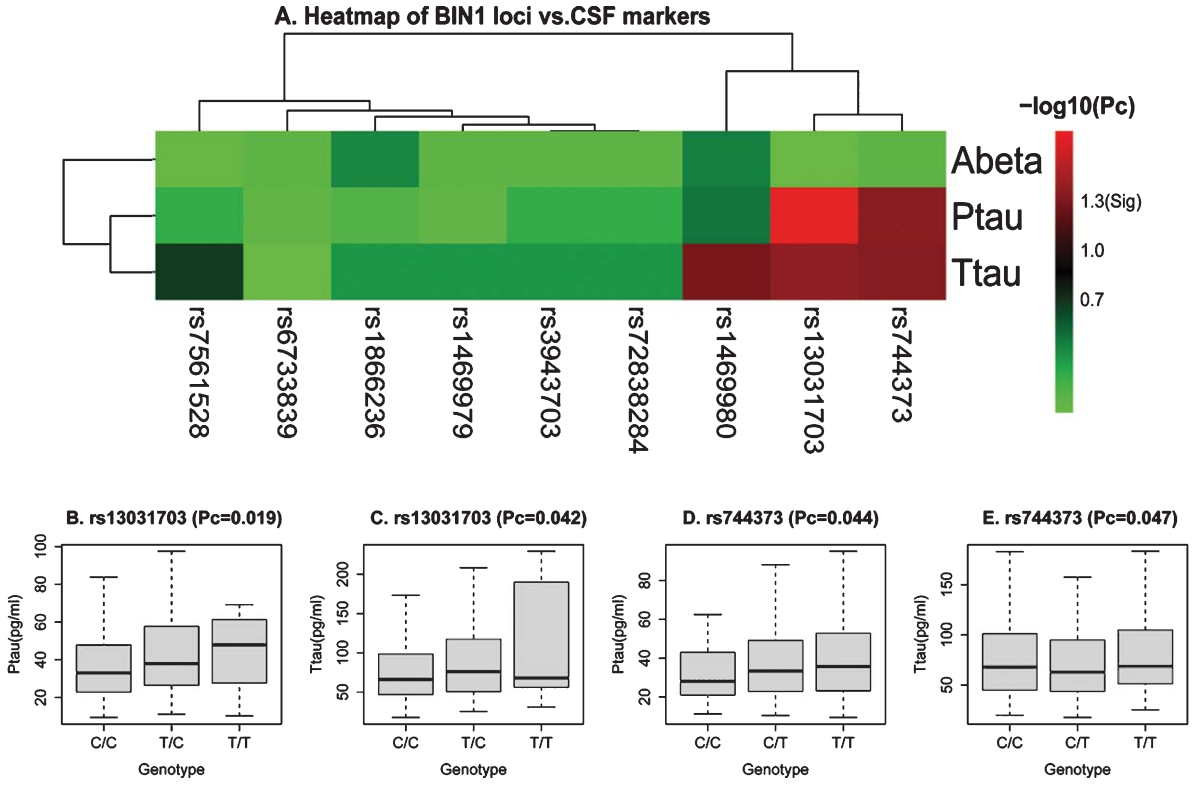

Fig. 1. The correlations between BIN1 loci and CSF markers. A) The statistical relations (FDR-corrected *p* values) between CSF proteins (rows) and BIN1 loci (columns); (B) Rs13031703 was associated with the level of T-tau at baseline; (C) Rs13031703 was associated with the level of P-tau at baseline; (D) Rs744373 was associated with the level of T-tau at baseline; (E) Rs744373 was associated with the level of P-tau at baseline.

rs744373, and rs7561528) related to the CSF proteins two loci (rs744373 and rs7561528) has been validated to be linked to AD in the previous GWAS, and rs13031703 ($p = 2.76 \times 10^{-6}$) and rs72838284 ($p = 3.169 \times 10^{-13}$) were also verified to associate with AD susceptibility in meta-analysis of 74,046 participants.

Brain structures and BIN1 genotypes

Secondly, we analyzed the association of these *BIN1* loci with AD related brain structures (hippocampus, parahippocampus, middle temporal, and entorhinal cortex) in a linear model which treated age, gender, education years, *APOE* ε4 status, and intracranial volume as covariates at baseline. Single nucleotide polymorphisms (SNPs) at rs72838284 were significantly associated with the volume of left ($p = 0.03$) and right parahippocampus ($p = 0.002$) respectively in cross-section analysis, but only the association with right parahippocampus ($p_c = 0.017$) still survived the FDR correction (Fig. 2A, B; Supplementary Table 3A); Besides, rs1409980 was related to the thickness of right entorhinal cortex at a marginal significance ($p = 0.009$, $p_c = 0.081$) at baseline. The variations at rs7561528 were markedly related to the right hippocampal atrophy rate ($p = 0.001$, $p_c = 0.011$) (Fig. 2C), and rs1469980 were remarkably correlated with the atrophy rate of right hippocampus substructure-CA1 ($p = 0.003$, $p_c = 0.029$) in the follow-up study in a decreased sample size (Fig. 2D, Supplementary Table 3A).

Furthermore, the haplotype (CAC) was significantly associated with the volume of right parahippocampus ($p = 0.002$) in haplotype-based analysis. Subgroup analysis discovered that rs7561528 and rs1469980 significantly linked to the atrophy rate of right hippocampus ($p = 0.009$, $p_c = 0.044$) and

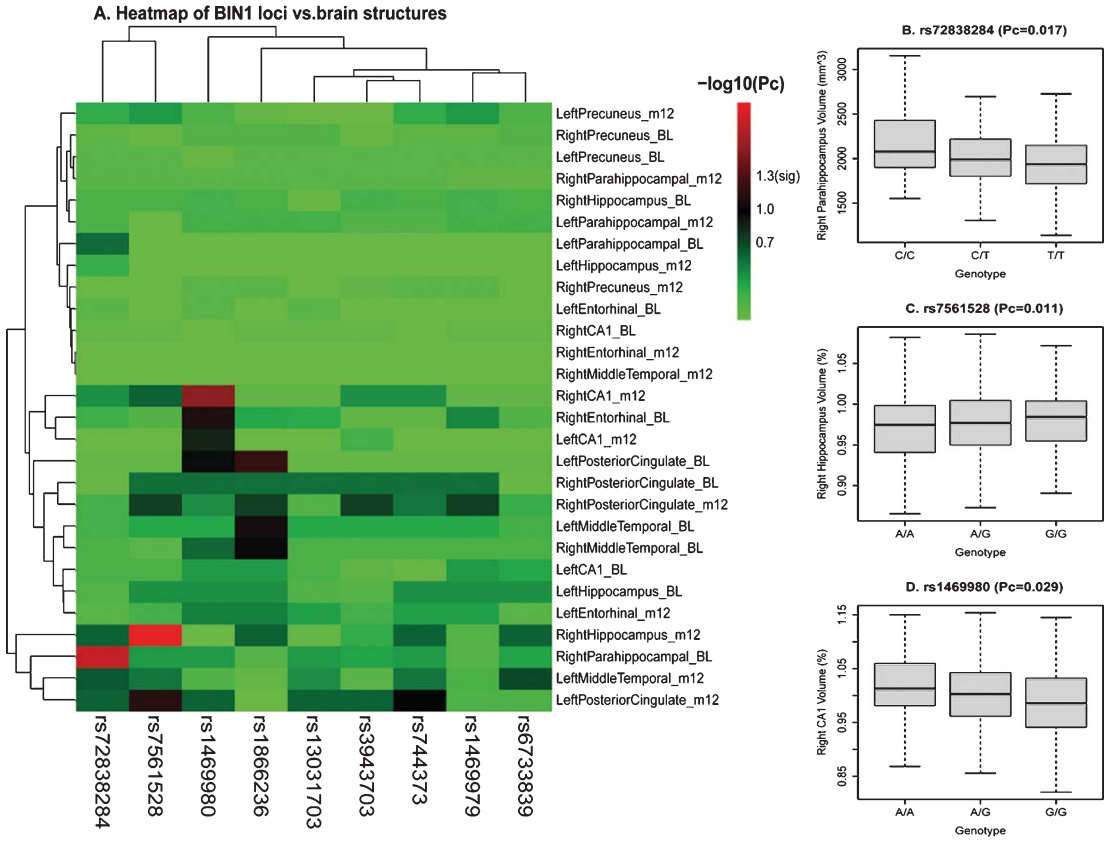

Fig. 2. The correlations between BIN1 loci and AD specific brain structure on MRI. (A) The statistical relations (FDR-corrected *p* values) between brain structures (rows) and BIN1 loci (columns); (B) Rs72838284 was associated with the volume of right parahippocampus at baseline; (C) Rs7561528 was associated with the atrophy rate of right hippocampus in the follow-up study; (D) Rs1469980 was associated with the atrophy rate of right CA1 in the follow-up study.

right CA1 ($p = 0.002$, $p_c = 0.015$) respectively in MCI subgroup in the follow-up study (Supplementary Table 3B). In this section, rs1469980, apart from rs7561528 and rs72838284, was the susceptibility locus for AD related brain structures, which was not associated with AD susceptibility ($p > 0.05$) in the large meta-analysis from 74,046 individuals.

Brain glucose metabolism and BIN1 genotypes

We then analyzed the influences of *BIN1* genotypes on cerebral metabolism rate of glucose (CMRgl) in amygdala, posterior cingulate and temporal cortex on FDG-PET imaging, and observed that the three genotypes at rs1469980 (GG, AG, and AA) had different metabolism rate in right angular ($p = 3.31 \times 10^{-4}$) at baseline, and the significant difference remained after FDR correction ($p_c = 0.003$) (Fig. 3A, B; Supplementary Table 4A). Likewise, subjects bearing the three genotypes at rs1469980 had different CMRgl in the bilateral temporal cortex (left: $p = 0.024$; right: $p = 0.001$) in cross-section analysis on FDG-PET, and the significant difference in the right temporal cortex ($p_c = 0.01$) remained after FDR correction (Fig. 3C; Supplementary Table 4A). In addition, rs3943703 was strongly related to the change of CMRgl in bilateral temporal cortex (left: $p = 0.03$; right: $p = 0.004$) in the follow-up study, but only the significant relationship to right temporal CMRgl ($p_c = 0.038$) still appeared in FDR test (Fig. 3D).

In addition, subgroup analysis detected that rs1469980 was significantly correlated with glucose metabolism of right angular ($p = 0.001$, $p_c = 0.008$) and temporal cortex ($p = 0.008$, $p_c = 0.074$) in MCI subgroup (Supplementary Table 4B). However, both of these positive loci were not revealed to link to AD risk in this large meta-analysis of 74,046 Caucasians.

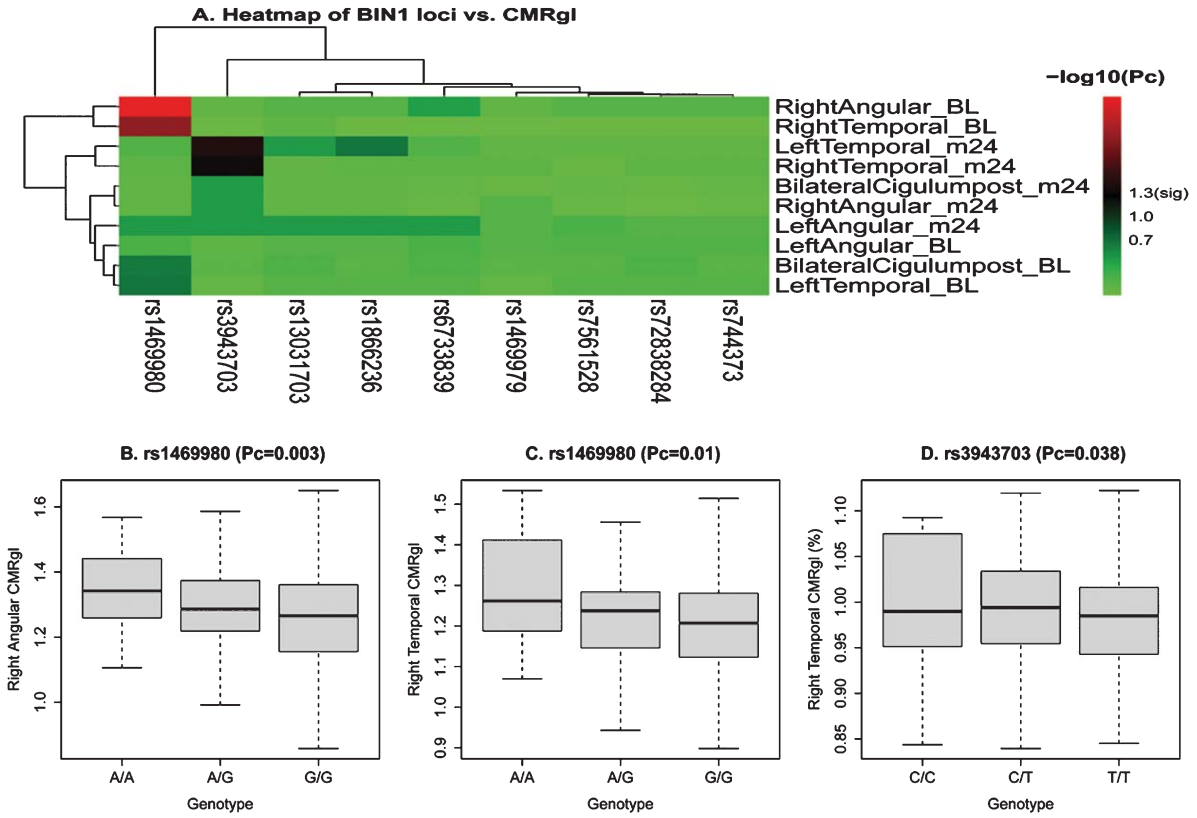

Fig. 3. The correlations between BIN1 loci and CMRgl on FDG-PET. (A) The statistical relations (FDR-corrected *p* values) between cerebral metabolisms for glucose (rows) and BIN1 loci (columns); (B) Rs1469980 was associated with the CMRgl of right angular at baseline; (C) Rs1469980 was associated with the CMRgl of right temporal cortex at baseline; (D) Rs3943703 was associated with the change percentage of CMRgl of left temporal cortex in the follow-up study.

Brain Aβ retention and BIN1 genotypes

Finally, we analyzed the associations of *BIN1* loci with Aβ retention in frontal, parietal, and temporal cortex and cingulate, as well as summary SUVR using the AV45-PET methods. None of these loci showed significant associations with Aβ retention in the above areas at baseline (Supplementary Table 5A). In the follow-up study, we observed remarkable relationships between rs1469980 and Aβ retention in frontal cortex ($p = 0.029$), cingulate ($p = 0.015$), parietal cortex ($p = 0.037$), and the summary SUVR ($p = 0.020$) on amyloid PET imaging; however, all these significant relations did not reach the significant level in the FDR test. Furthermore, subgroup analysis did not detect any significant relations between *BIN1* loci and Aβ retention in AD subgroup, nor in MCI or CN subgroup (Supplementary Table 5B, C).

DISCUSSION

Our study demonstrated that *BIN1* genotypes were significantly associated with the levels of tau protein, but not with Aβ in CSF test. The imaging-genetics analysis suggested that *BIN1* genetic variations were implicated in the volume loss of hippocampus, hippocampus subfield (CA1), and parahippocampus on MRI, and *BIN1* loci polymorphisms were linked to the CMRgl in angular and temporal cortex on FDG-PET. Furthermore, haplotype-based analysis and subgroup results confirmed these significant results. However, none of *BIN1* loci was identified to impact the Aβ deposition on amyloid PET imaging although there is a positive trend. Moreover, two new loci related to these biomarkers, which were not reported in previous literature, were identified to be associated with the risk of AD in the large meta-analysis including 74 046 individuals. These findings further confirmed that *BIN1* participated in the neuronal degeneration or injury, not in the Aβ deposition in the AD pathogenesis, leading to modulate the susceptibility of AD.

These findings were partly consistent with the results of Kauwe et al. that *BIN1* loci were not associated with the level of Aβ and tau in CSF [31]; however, this study demonstrated that *BIN1* loci (rs13031703 and rs744373) was significantly associated with the level of tau, but not with the Aβ level. Apart from rs744373, different loci were assessed in these two studies, which may be the source of the different results. Moreover, this study detected *BIN1* genotypes were associated with the atrophy of hippocampus and hippocampus substructure (CA1) on MRI, which consisted with the findings of Zhang et al. [32]. We also observed that *BIN1* genetic variations were linked to the atrophy of the entorhinal thickness at a marginal level, and it further confirmed the relationship between *BIN1* and the entorhinal thickness that was reported by Biffi and colleagues [29].

Thus far, it has been identified that both Aβ and tau pathology could lead to neural degeneration (brain atrophy and glucose metabolism) [33–37]. Furthermore, the biomarker of CSF Aβ level and Aβ deposition on AV45-PET imaging are the strong evidence of AD diagnosis in clinical practice, and the Aβ deposition is more specific than abnormal tau in AD related cognitive impairment diagnosis [38]. This study identified that *BIN1* may modify the tau and neural degeneration markers, but not Aβ pathology to mediate the risk for AD, which was consistent with the findings about the involvement of *BIN1* in the pathogenesis of AD in previous reports. Chapuis et al. found that altered Amph expression, the *BIN1* ortholog, could modulate tau induced neurotoxicity, but cannot alter the Aβ induced neurotoxicity in *Drosophila*, in addition, the in/del variant (rs59335482) upstream the *BIN1* gene was associated with tau loads but not with $A\beta_{42}$ loads in the brains of AD patients [9]. Moreover, Holler and his colleagues reported that *BIN1* expression was remarkably correlated to the quantity of neurofibrillary tangles, but not to the quantity of Aβ amyloid in five different brain regions (hippocampus, inferior parietal, inferior temporal, and frontal cortex) in a sample containing 72 participants [10]. Furthermore, knockdown of *BIN1* gene or increased expression of *BIN1* did not influence the AβPP processing, although *BIN1* was established to be involved in the endocytosis, which is important for the processing of AβPP to amyloid peptides [11]. Here, CSF tau proteins, but not Aβ showed significant difference among these the subtypes of *BIN1* genotypes. All the above evidence, along with our findings, support that *BIN1* polymorphisms alter tau expression (neuronal degeneration/injury markers), but not Aβ accumulation, to mediate the risk of AD.

Genetically, several loci located in the upstream *BIN1* gene (rs744373, rs7561728, rs59335482, and rs6733839) have been identified to associate with the risk of AD in multi-center, large scale GWAS, replication and meta-analysis [4, 5, 39–42]. Although in/del variation (rs59335482) was not found in our study, the top GWAS SNP rs744373 could represent

rs59335482 for the two loci are in almost complete LD (D' = 0.98, $r^2 = 0.94$) [9]. Here, we observed the remarkable relations between rs744373 and tau, and rs7561528 and atrophy of hippocampus. Moreover, rs72838284, which showed significant association with the atrophy of parahippocampus, was also correlated with the risk for AD ($p = 3.169 \times 10^{-13}$) in the dataset of 74,046 individuals, and rs13031703, which significantly altered the levels of tau, was also validated to relate to the risk for AD in this large database.

Imaging genetics is an emergent transdisciplinary research field, in which genetic risk is assessed with imaging measures as quantitative traits (QTs) or continuous phenotypes; and CSF proteins also were treated as QTs in the study. QT association studies have increased statistical power and decreased sample size requirements, thus our study has advantages over traditional case-control designs [43, 44]. However, the CSF and neuroimaging data were available only in a subset of participants in some QT analyses, e.g., 85% of participants with MRI information, 55% with FDG-PET, and 70% with AV45-PET. Therefore, the meaningful findings at baseline were not verified in the follow-up study due to the decline in the sample size. On the other hand, brain volume and glucose metabolism rate start to decline before the onset of AD on the basis of the dynamic model of AD biomarkers, and the decline is more severe over time. Thus, the differences of brain structures and CMRgl markers may be more evident in follow-up stage, and it was more likely to be detected in the follow-up study. Besides, the ADNI data was restricted to Caucasians to avoid genetics stratification across ethnicities. The 9 loci in *BIN1*, however, have different frequencies in different races; therefore, our results cannot represent the other ethnicities, warranting the replications in other races.

CONCLUSION

In conclusion, our study confirmed that *BIN1* genotypes were significantly associated with the level of tau protein, but not with Aβ protein in CSF test; and *BIN1* loci were related to the atrophy of AD related brain structures on MRI, and to the CMRgl on FDG-PET, but not to the Aβ loads on amyloid imaging. These findings further supported the hypothesis that *BIN1* genetic variations modulate the alteration of the neuronal degeneration/injury biomarkers rather than the Aβ markers to influence the risk of AD.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-150972.

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