Mol Neurobiol (2017) 54:1111–1118
DOI 10.1007/s12035-016-9718-4

Impacts of CD33 Genetic Variations on the Atrophy Rates of Hippocampus and Parahippocampal Gyrus in Normal Aging and Mild Cognitive Impairment

Wen-Ying Wang[1] · Ying Liu[1] · Hui-Fu Wang[2] · Lin Tan[3] · Fu-Rong Sun[1] · Meng-Shan Tan[1] · Chen-Chen Tan[1] · Teng Jiang[4] · Lan Tan[1,2] · Jin-Tai Yu[1,5] · Alzheimer's Disease Neuroimaging Initiative

Received: 20 November 2015 / Accepted: 12 January 2016 / Published online: 23 January 2016

Abstract The cluster of differentiation 33 (*CD33*) has been proved as a susceptibility locus associated with late-onset Alzheimer's disease (LOAD) based on recent genetic studies. Numerous studies have shown that multiple neuroimaging measures are potent predictors of AD risk and progression, and these measures are also affected by genetic variations in AD. Figuring out the association between CD33 genetic variations and AD-related brain atrophy may shed light on the underlying mechanisms of CD33-related AD pathogenesis. Thus, we investigated the influence of *CD33* genotypes on AD-related brain atrophy to clarify the possible means by which *CD33* impacts AD. A total of 48 individuals with probable AD, 483 mild cognitive impairment, and 281 cognitively normal controls were recruited from the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset. We investigated the influence of *CD33* SNPs on hippocampal volume, parahippocampal gyrus volume, posterior cingulate volume, middle temporal volume, hippocampus CA1 subregion volume, and entorhinal cortex thickness. We found that brain regions significantly affected by *CD33* genetic variations were restricted to hippocampal and parahippocampal gyrus in hybrid population, which were further validated in subpopulation (MCI and NC) analysis. These findings reaffirm the importance of the hippocampal and parahippocampal gyrus in

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (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

Wen-Ying Wang and Ying Liu contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s12035-016-9718-4) contains supplementary material, which is available to authorized users.

✉ Lan Tan
dr.tanlan@163.com

✉ Jin-Tai Yu
yu-jintai@163.com

Alzheimer's Disease Neuroimaging Initiative

[1] Department of Neurology, Qingdao Municipal Hospital, School of Medicine, Qingdao University, No. 5 Donghai Middle Road, Qingdao Shandong Province 266071, China

[2] Department of Neurology, Qingdao Municipal Hospital, Nanjing Medical University, Qingdao, China

[3] College of Medicine and Pharmaceutics, Ocean University of China, Qingdao 266000, China

[4] Department of Neurology, Nanjing First Hospital, Nanjing Medical University, Nanjing, China

[5] Memory and Aging Center, Department of Neurology, University of California, San Francisco, CA, USA

AD pathogenesis, and present evidences for the *CD33* variations influence on the atrophy of specific AD-related brain structures. Our findings raise the possibility that *CD33* polymorphisms contribute to the AD risk by altering the neuronal degeneration of hippocampal and parahippocampal gyrus.

Keywords Alzheimer's disease · *CD33* · Genetics · Atrophy · Hippocampal · Parahippocampal gyrus

Introduction

Alzheimer's disease (AD), the prevalent cause of dementia, is the most common neurodegenerative disease among elderly, affecting almost 50 % of those over the age of 85 in America. AD exerts great global public health challenge for our generation: 2015 World Alzheimer Report estimated that 46.8 million people worldwide are living with dementia in 2015, and the number will almost double every 20 years, which will reach to 74.7 million in 2030 and 131.5 million in 2050 [1, 2]. Among them, AD makes up 50 to 70 % of cases. To date, there is no determined view on AD pathogenic mechanisms; epidemiologic studies failed to identify one single cause that could be sufficient to explain the pathogenesis of AD. Indeed, our understandings of AD have been greatly advanced by genetic studies, especially for the novel discoveries by the large genome-wide association studies. Genetic factors played a decisive role in the development of both early-onset AD (EOAD) and late-onset AD (LOAD) [3]. Thus, exploring for the mechanism underlying these genetic loci is critical for ascertaining the progressing steps in AD risk, which may lead to a new therapeutic target.

Recently, several large-scale genome-wide association studies (GWASs) have identified nine genes associated with AD risk. Herein, cluster of differentiation 33 (*CD33*), a member of sialic acid-binding immunoglobulin-like lectins (Siglecs) family, was regarded as a strong genetic locus associated with the risk of AD [4–6]. *CD33* is located on chromosome 19q13.33 in humans and has been successfully proved as a putative and novel AD protective loci in different ethnicities [3, 7, 8]. By contrast, our previous studies identified the minor allele (A) of the rs3865444 polymorphism within the *CD33* as a higher risk locus for LOAD in Han Chinese population [9]. Probably due to environmental and lifestyle differences in population, rs3865444 polymorphism provided a protective effect for AD in specific ethnic cohorts while conferred risk effect for AD in some other cohorts.

Regarding to the neuropathology mechanisms of CD33 in AD, there was no explicit answer. Numerous studies have reported that *CD33* might impair microglia-mediated Aβ clearance and participate in the AD pathology [10, 11]. To date, understanding the disease mechanism of AD had been tremendously advanced by the development of clinical and neuroimaging biomarkers (measured by tau/phosphorylated tau in CSF, cortical atrophy on MRI and FDG-PET, CSF Aβ42, and amyloid PET imaging) [12–14]. These multiple neuroimaging measures may provide a foothold for the neuropathological changes through brain structural and developmental alterations. It is of note that magnetic resonance imaging (MRI) markers of cortical volume and hippocampal volume are important predictors of neurodegeneration and may reflect some potential pathologic roles in AD [15]. Bradshaw and colleagues had demonstrated that the *CD33* functional polymorphism (rs3865444) could elevate *CD33* expression level and further influence neurotic amyloid plaques in the brains of older individuals at autopsy [11]. Even so, little is known about the influence of genetic variations in *CD33* on neurodegenerative features. Based on these findings, we thus supposed that investigating the relationship between *CD33* genetic locus and neuroimaging markers may help to ascertain the potential mechanism of *CD33* in modulating AD. To explore the potential functional and neuroimaging influence of the *CD33* locus on AD, we accessed genotyped and archived samples from Alzheimer's Disease Neuroimaging Initiative (ADNI).

Methods

ADNI

The data source of this article was ADNI database (https://ida.loni.usc.edu/), which was launched by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies, and nonprofit organizations in 2003. The initial goal of ADNI was to recruit 800 subjects to test if serial imaging techniques, fluid biomarkers, and clinical and neuropsychological assessment can be combined to detect MCI and AD at earlier stages. However, ADNI-1 has been followed by ADNI-GO and ADNI-2 (see http://www.adni-info.org for up-to-date information). To date, these three protocols have recruited over 1500 adults, age from 55 to 90, to the research, consisting of NC, MCI, and individuals with AD. All the Institutional Review Boards of all participating sites at their respective institutions approved the study. Before the start of the study, signed written informed consent was obtained from all ADNI participants.

Participants

Participants were considered for enrollment if they met criteria protocolled in the ADNI-1 and ADNI-2/GO dates (http://www.adni-info.org/scientists/adnistudyprocedures.aspx). The normal healthy subjects (NC) had average MMSE scores of

29.07 ± 1.15, ADAS-Cog scores of 9.06 ± 4.23, and CDR sum of boxes scores of 0.03 ± 0.13. MCI subjects had mild memory complaints and MMSE scores of 27.89 ± 1.69, ADAS-Cog scores of 15.30 ± 6.65, and CDR sum of boxes scores of 1.44 ± 0.87. Patients who met National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria were defined as probable AD. On average, participants with AD had MMSE scores of 22.96 ± 2.03, ADAS-Cog scores of 29.80 ± 8.44, and CDR sum of boxes scores of 4.44 ± 1.69. The subjects were excluded if they had any serious neurological disease other than possible AD, any history of brain lesions or trauma, or psychoactive medication use (including antidepressants, neuroleptics, chronic anxiolytics, or sedative hypnotics). The final dataset for the present analysis comprised 812 individuals, including 281 NC and 483 MCI patients and 48 AD. Table 1 lists the detailed demographics of all these subjects divided to three diagnostic groups (NC, MCI, and AD).

SNP Selection

We extracted the SNP genotypes of *CD33* from the ADNI GWAS PLINK format data [16]. The quality control (QC) procedures were performed using PLINK version 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/) software. The SNPs were excluded if they suffered from at least one of the following deficiencies: minimum call rates <95 %, minimum minor allele frequencies <0.001, Hardy-Weinberg equilibrium test $p < 1 \times 10^{-3}$. After quality control procedures, in the theoretical 15 possible haplotypes, only 10 (including rs3865444 and rs3826656) were found to be common alleles (minimum minor allele frequencies >0.001). Finally, nine loci (rs3865444, rs3826656, rs1803254, rs113464261, 8112072, 73932888, 34813869, 1354106, 1399839) were selected as our targeted *CD33* loci in further analysis, which captured the 90 % of common variations in *CD33* (Table 2, Supplementary Fig. S1) using Haploview 4.2 platform.

MRI Structure

The ADNI MRIs used in this research were acquired at multiple sites using a GE Health-care (Buckinghamshire, England), Siemens Medical Solutions USA (Atlanta, GA), or Philips Electronics 1.5 T system (Philips Electronics North America, Sunnyvale, CA). These regional volumes on MRI were all downloaded from the ADNI dataset, which is provided by the University of California, San Francisco (UCSF) medical center. The institute used FreeSurfer version 5.1(http://surfer.nmr.mgh.harvard.edu/) to segment and analyze the cerebral imaged based on the 2010 Desikan-Killany atlas. For the details, the imaging processing can be found in previous studies [17, 18]. In addition, cortical thickness measurement was derived from calculating the distance between the gray and white matter surfaces at each point (per hemisphere) [19]. In this study, we selected the regions of interest (ROIs) (including the hippocampus, parahippocampus gyrus, posterior cingulate, middle temporal, hippocampus CA1 subregion, and the entorhinal cortex) to analyze the relationship between *CD33* and AD. These ROIs had been found to be strongly associated with AD based on recently literature searches [14, 15, 20]. To make sure the accumulated ROIs volume loss exceeded the incurred measurement errors, we used both baseline and longitudinal data (2-year follow-up) from ADNI to investigate the association between each SNP and neuroimaging phenotypes. According to 2-year follow-up data, we used the volume ratio of follow-up to baseline as the calculated value.

Data Analysis

Additive model was used to investigate the association between *CD33* and AD. The means and standard deviations (SD) were used to assess the information of all datasets for continuous variables and proportions for categorical variables. One-way analysis of variance (ANOVA) was used to compare differences in continuous sociodemographic variables, and chi-squared test was used to check for the categorical data. The associations between the volume/thickness (baseline data and follow-up changes) and *CD33* genotypes were assessed using linear regression models, which consider age, gender, education years, and APOEe4 status as covariates in total sample. Ninety-five percent confidence interval (CI) was used to explore this association further. All these statistical analyses were performed by R 3.12 (http://www.r-project.org/) and PLINK 1.07 (http://pngu.mgh.harvard.edu/wpurcell/plink/).

Controlled by the false discovery rate (FDR), the multiple hypothesis testing was used to avoid the nonindependence of Bonferroni correction [21]. The criterion for significant difference was $Pc < 0.05$ according to FDR-correction. Further validation was obtained by replicated these positive *CD33* loci (findings in total group) in the subgroups population. For these positive *CD33* loci findings in total group, we replicate the significant brain regions in the subgroup population to investigate that at which stage these variations impacted the neurodegeneration markers. Finally, we investigated the correlations between these new positive loci in our study and AD in a large database from a meta-analysis of GWAS in 74,046 individuals of European descent. According to the collected data at baseline and 24 months, there is some conversion or diagnosis change during the 24-month follow-up, and these conversions or diagnosis change may effect on the results. Moreover, it would be meaningful to stratify the MCI group in MCI converting to dementia versus MCI nonconverting to dementia; hence, more studies are needed to perform to discover this relation.

Table 1 The characteristics of the ADNI subjects at baseline

Characteristics	CN		MCI		AD		*P* value*
Age (years)	281	74.51 ± 5.56	483	72.28 ± 7.45	48	75.51 ± 9.23	–
Gender (male/female)	281	136/145	483	282/201	48	30/18	–
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	20,298 ± 2600	422	20,186 ± 2735	39	17,776 ± 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

Data are given as mean ± standard deviation unless otherwise indicated

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-squared test

Results

Demographic and Clinical Characteristics

Table 1 lists the detailed demographics and multiple memory scores of all these subjects divided to three diagnostic groups (NC, MCI, and AD). Hippocampus, middle temporal, and entorhinal cortex volume exhibited significant differences across groups ($P < 0.01$). As expected, hippocampal volume exhibited a decreasing trend in the course of AD progression (NC < MCI < AD, $P < 0.01$). Also as expected, APOEe4+ was more prevalent in AD group than MIC and NC group ($P < 0.01$): 34 of 48 AD participants (72.9 %) showing one or more APOEe4 allele compared with 221 of 483 MCI participants (45.8 %) and 77 of 281 NC participants (27.4 %). Meanwhile, AD patients performed worse than MCI and NC in memory tests on the Clinical Dementia Rating scale sum of boxes (CDRSB), the Alzheimer's Disease Assessment Scale

Table 2 Characteristics of nine SNPs finally selected for our analysis

N	SNP	Chr	Minor allele	Allele change	Position	*P* value	MAF[a]	Protective or risk factor
1	rs3865444	19	A	C → A	2 KB upstream variant, 5 prime UTR variant	0.8473	0.298	Protective (Caucasian population) Risk (Han Chinese)
2	rs3826656	19	G	A → G	Intron variant, upstream variant 2 KB	0.9664	0.264	Protective(North Han Chinese, African Americans) Risk (East Asian, eastern Chinese)
3	rs1803254	19	C	G → C	NC transcript variant, UTR variant 3 prime	0.2345	0.068	–
4	rs113464261	19	C	A → C	Intron variant	0.4856	0.036	–
5	rs8112072	19	G	A → G	Intron variant	1.0	0.02	–
6	rs73932888	19	C	T → C	Intron variant	1.0	0.018	–
7	rs34813869	19	G	A → G	Intron variant.	0.3491	0.326	–
8	rs1354106	19	G	T → G	Intron variant	0.629	0.329	–
9	rs1399839	19	C	T → G	Intron variant	0.3887	0.494	–

NF not found in ADNI database, *MAF* minor allele frequency, *SNP* single nucleotide polymorphism

[a] MAF data was calculated using Haploview software

(ADAS), Mini-Mental State Examination (MMSE), the Rey' Auditory Verbal Learning Test (RAVLT), and Functional Activities Questionnaire (FAQ) ($P < 0.01$).

Impacts of CD33 Genetic Variants on Subcortical Volume in Hybrid Group

Here, we selected several subcortical areas as ROI, including hippocampus, parahippocampus gyrus, posterior cingulate, middle temporal, hippocampus CA1 subregion, and the entorhinal cortex. At baseline, C allele of rs7393288 showed trend of association with smaller thickness of left entorhinal area and larger volume of left hippocampal CA1 region. Rs8112072 G allele carriers showed trend of smaller volume left parahippocampal region and hippocampal CA1 region than A allele homozygotes subjects (AA > GA > GG). In addition, rs1803254 showed a trend of being associated with baseline left middle temporal area volume, with minor C allele carriers showing smaller left middle temporal area volume (GG > CG > CC); the C allele carriers of rs113464261 were associated with larger volume of right hippocampus in the hybrid group. But, to our disappointment, all these associations failed to survive the FDR correction (Supplementary Table S1). Remarkably, we found more atrophy of left hippocampal for individuals carrying variations in rs8112072 (G allele, $P = 0.0005$) and rs73932888 (C allele, $P = 0.0036$). C allele of rs113464261 was significantly associated with smaller volume of left hippocampus ($P = 0.0086$) and left parahippocampus ($P = 0.0047$). The associations were still significant after FDR correction (Fig. 1).

In the follow-up analysis study, only rs1803254 (C allele, $P = 0.0005$) showed significant association with atrophy rate of right hippocampal CA1 region after FDR correction (Fig. 2e, f). It is worthy to note that G allele of rs3826656 showed trends to be associated with faster atrophy rate of left parahippocampus and rs1399839 showed trends of associations with slower atrophy rate of right hippocampal CA1 region. In addition, C allele of rs113464261 also showed trends to be related with slowing down the atrophy of left entorhinal. However, no associations were significant after FDR correction.

To validate our results and to facilitate comparisons, we also selected the hippocampal and parahippocampus as our ROI and independently tested its association with *CD33* variations in NC and MCI individuals, respectively (Fig. 2).

Impacts of CD33 Genetic Variants on Hippocampal and Parahippocampus in NC Individuals

In the NC group, the association between rs113464261 and left hippocampus volume gyrus at baseline was validated in the NC group ($P = 0.0365$, Fig. 2b). In addition, the association between variations in rs113464261 and larger volume of left parahippocampal gyrus at baseline was also further validated in the NC population ($P = 0.0459$, Fig. 2d). In addition, since the brain volume varied between NC individuals and MCI/AD individuals, NC may be the major contributor of the overall larger volume of left parahippocampal gyrus in the hybrid population (NC + MCI + AD).

Impacts of CD33 Genetic Variants on Hippocampal and Parahippocampus in MCI Individuals

The associations between two *CD33* loci (rs8112072 G allele and rs73932888 C allele) and larger baseline atrophy volume of left hippocampus gyrus were further validated in the MCI population (Fig. 2a, c). In MCI subgroup, C allele of rs1803254 was associated with faster atrophy rate of right hippocampus CA1 (Fig. 2f). As we expected, the overall atrophy rate of hippocampus in the MCI population was faster than that in hybrid population.

Discussion

We performed a multimodal MRI neuroimaging biomarker analysis of ADNI subjects to uncover the potential mechanism of *CD33* gene in AD progression. In our study, we found that these selected *CD33* genetic SNPs were significantly associated with advanced atrophy of left hippocampal gyrus (rs73932888 and rs8112072) and CA1 region (rs1803254). Meanwhile, there was also an inconsistent result that one SNP (rs113464261) was significantly related to a relatively preserved of hippocampal and para hippocampal gyrus. While we failed to find an association between the rs3865444 (the locus that reported to be related with AD repeatedly) and the neuroimaging biomarkers, we predict, based on our results, that *CD33* genetic variants is likely to modulate AD risk by virtue of their influence on the hippocampus, which is reported to be highly associated with memory scores [22, 23]. Furthermore, our group had reported that lower CD33 protein levels were significantly associated with more advanced cognitive decline [24], which may indicate the CD33 role in the cerebral areas in charge of memory.

CD33 located at chromosome 19q13.33, approximately 6 Mb from *APOE* which is the strongest genetic risk factor in the LOAD. It is commonly accepted that CD33 is mainly expressed on the phagocytes including microglia cells, which are considered to have the potential to uptake and degrade of Aβ, and thus prevents the deposition of amyloid plaque in the brain of AD participants [25]. Using the large sample size, our findings are supportive of a relationship between hippocampal atrophy and *CD33* gene. Previous literatures concerning neuroinflammation in AD had shown the colocalization of microglial cells with the neuropathology of AD was conducted within hippocampus [26]. Findings from the recent studies

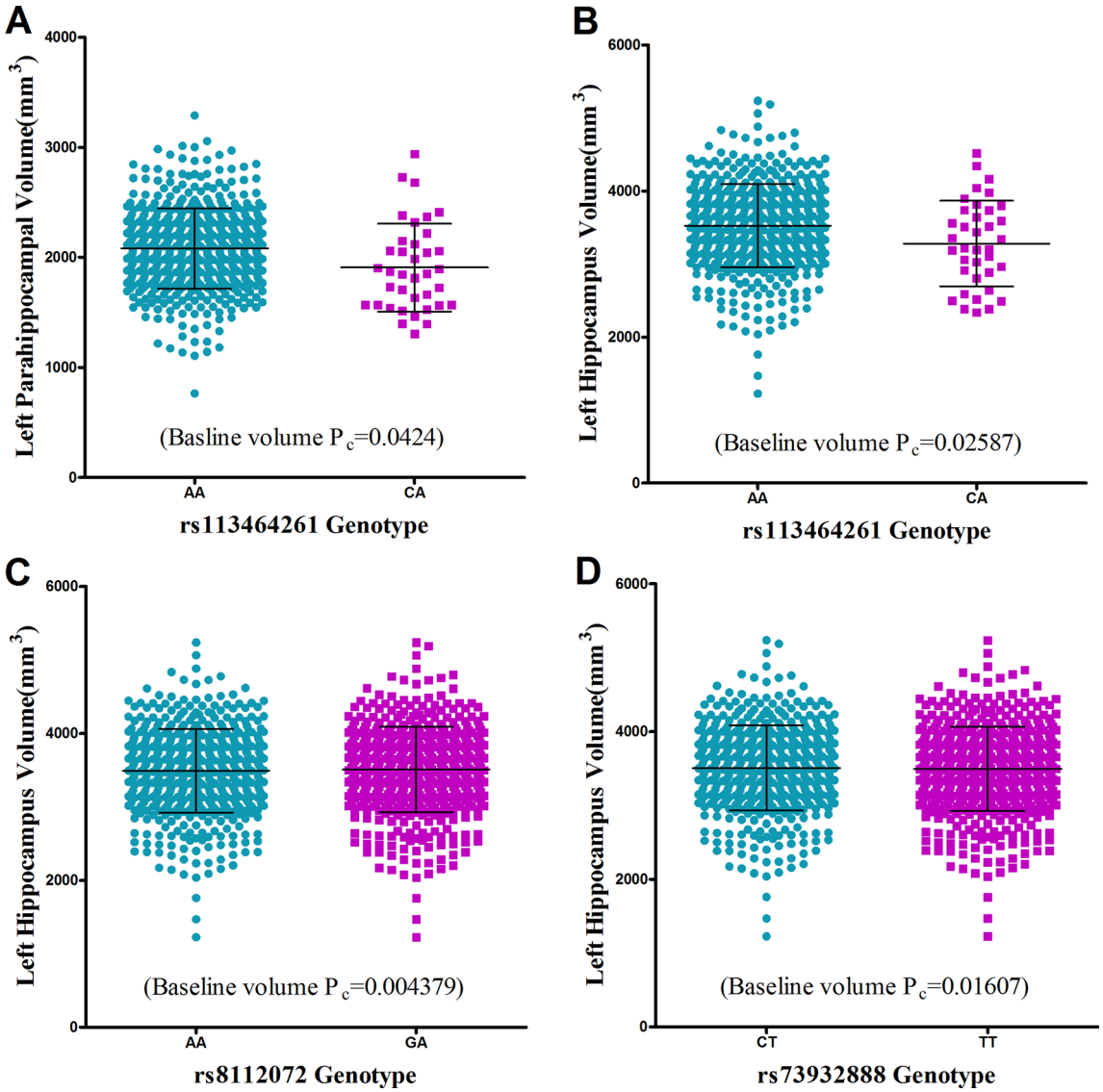

Fig. 1 The significant associations of *CD33* loci with atrophy rate of hippocampus and parahippocampal gyrus in the hybrid population. **a** rs113464261 was associated with left parahippocampal gyrus volume at baseline; **b** rs113464261 was associated with left hippocampus gyrus volume at baseline; **c** rs8112072 was associated with left hippocampus gyrus volume at baseline; **d** rs7393288 was associated with left hippocampus gyrus volume at baseline

also highlight the microglial-mediated neurodegeneration occurs in the hippocampal formation in the AD progression [27]. Researches in vivo [11] and vitro [10] all indicated that *CD33* mutation could directly inhibit the protein uptake by microglial and thus impair the Aβ clearance. Our observation might be explained by the hypothesis that CD33-related hippocampus and parahippocampal gyrus atrophy from structural MRI is the downstream consequences of the microglia cells expression [12]. In these regards, these findings offer clues to the mechanisms through which *CD33* genetic variants might influence cerebral atrophy on MRI through modulating the microglia cells expression which is seemed to interfere Aβ clearance. Besides, our previous research also found that APOEe4 was significantly associated with atrophic hippocampal volume [13], indicating the important role of hippocampus in cognitive decline and neurodegeneration in AD.

In the previous studies, the minor alleles of rs3865444 and rs3826656 were reported to be a protective factor for AD in Caucasian population. However, in our replication study in Han Chinese, we found that the minor allele of rs3865444 was a risk factor for AD in contrast [9]. The relationship between the other SNPs and AD were not reported yet.

Remarkably, a large-scale meta-analysis recently found that a novel risk allele for AD (rs3865444) on *CD33* were strongly associated with smaller intracranial volume but had no influence on the hippocampal volume [15]. They supposed that rs3865444 influenced AD probably due to the

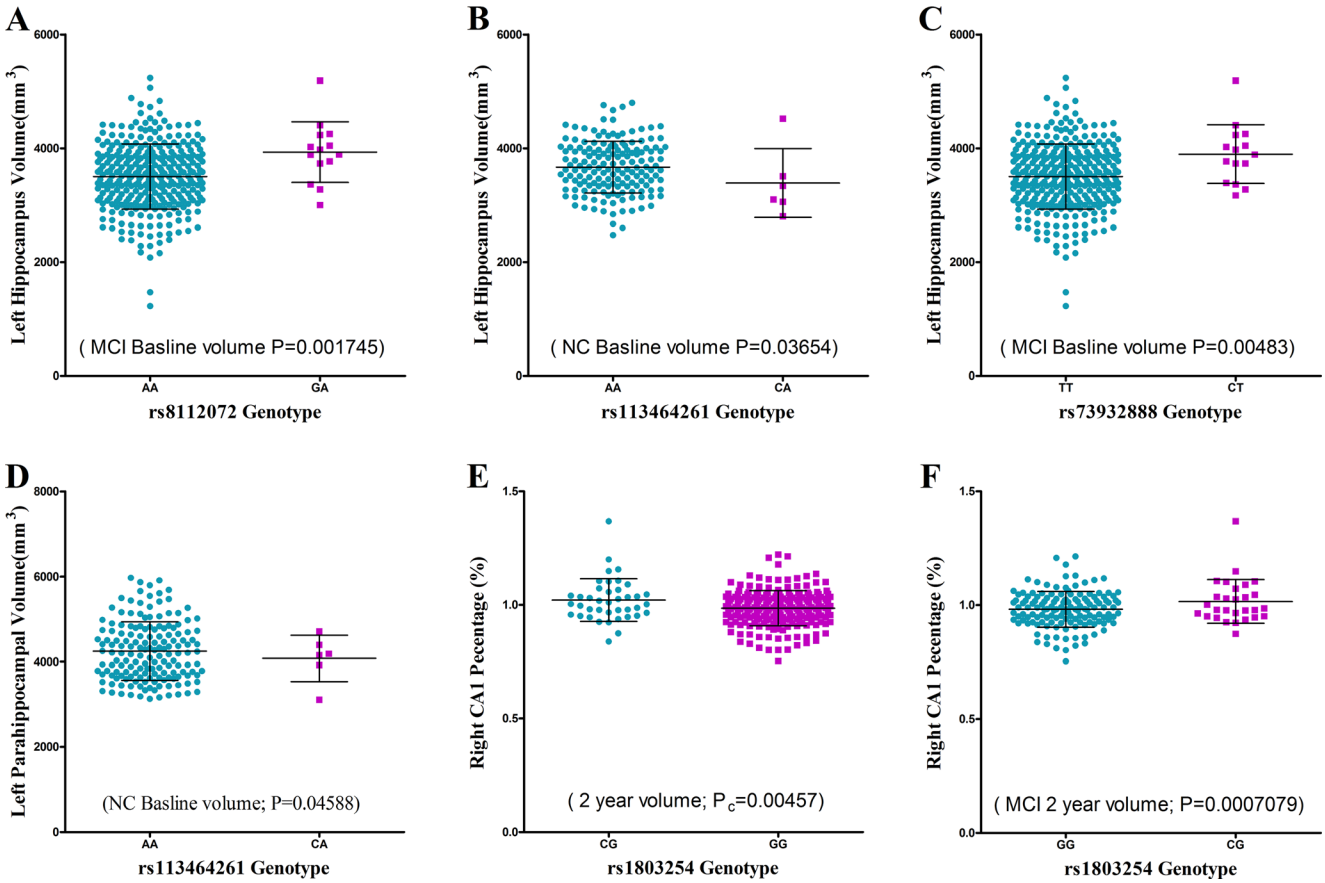

Fig. 2 The replicated results of the associations of *CD33* loci with hippocampus and parahippocampal gyrus volume in subgroup population. **a** rs8112072 was associated with left hippocampus gyrus volume at baseline in MCI population. **b** rs113464261 was associated with left hippocampus gyrus volume at baseline in NC population. **c** rs7393288 was associated with left hippocampus gyrus volume at baseline in MCI population. **d** rs113464261 was associated with left parahippocampal gyrus volume at baseline in NC population. **e** rs1803254 was associated with 2-year atrophy rate of right hippocampus CA1. **f** rs1803254 was associated with 2-year atrophy rate of right hippocampus CA1 in MCI population

involvement of this locus in brain maturation and reserve [15]. Owing to the different population included, we found a negative in rs3865444 but a positive result in other SNPs in *CD33*. Genetically, rs3865444 and rs3826656, located in the upstream of *CD33* gene, have been identified to be associated with the risk of AD in multicenter, large-scale GWAS studies [4–6]. The SNPs rs113464261, rs3865444, and rs3826656 are in almost complete linkage disequilibrium (rs3865444 and rs113464261 D' = 0.964; rs3826656 and rs113464261 D' = 1.0) (Supplementary Fig. 1). Despite the different results, our research lends strong support to their hypothesis that novel AD genetic risk variants (*CD33* SNPs and others) may associate with MRI markers of structural brain.

The crucial strength of our study arises from the imaging measures (quantitative traits (QTs) or continuous phenotypes), because QT association studies require less sample size and increased statistical power. Our study also has limitations. ADNI sample limited in AD sample size, and lager samples was still necessary. A follow-up of 2 years may be too short to detect the significant influence of *CD33* on AD. Besides, we selected only non-Hispanic (Caucasian) participants in order to avoid the population stratification effects. The polymorphisms of *CD33* differ in ethnically diverse populations; thus, the results replications in other ethnic populations are imperative. Future studies using genotype data are required to replicate our findings using dominant model and recessive model.

In conclusion, we investigated the association between *CD33* common variants and AD-related MRI neuroimaging phenotypes. Our study confirmed that *CD33* genetic variants were associated with the notable AD-related brain structures atrophy including parahippocampus and hippocampus on MRI imaging. The current findings provide underlying mechanisms by which *CD33* polymorphisms act on the risk for AD. In addition, these candidate loci can serve as targets in future large samples, replication studies.

Acknowledgments Data collection and sharing was funded by the ADNI (National Institutes of Health U01 AG024904). ADNI is funded by the National Institute on Aging; the National Institute of Biomedical

Imaging and Bioengineering; the Alzheimer's Association; the Alzheimer's Drug Discovery Foundation; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Co., F. Hoffmann-LaRoche Ltd and Genetech, Inc.; GE Healthcare; Innogenetics, NV; IXICO Ltd; Janssen Alzheimer Immunotherapy Research & Development LLC; Medpace, Inc; Merck & Co., Inc.; Meso Scale Diagnostics, LLC; NeuroRx Research; Novartis Pharmaceuticals, Co., Pfizer, Inc.; Piramal Imaging; Servier; Synarc Inc.; and Takeda Pharmaceutical Co. 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 was the Northern California Institute for Research and Education, and the study was coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of California, Los Angeles. This work was also supported by grants from the National Natural Science Foundation of China (81471309, 81171209, 81371406, 81501103, 81571245), the Shandong Provincial Outstanding Medical Academic Professional Program, Qingdao Key Health Discipline Development Fund, Qingdao Outstanding Health Professional Development Fund, and Shandong Provincial Collaborative Innovation Center for Neurodegenerative Disorders.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest.

References

1. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E (2011) Alzheimer's disease. Lancet 377:1019–1031
2. Chan KY, Wang W, Wu JJ et al (2013) Epidemiology of Alzheimer's disease and other forms of dementia in China, 1990-2010: a systematic review and analysis. Lancet 381:2016–2023
3. Jiang T, Yu JT, Hu N, Tan MS, Zhu XC, Tan L (2014) CD33 in Alzheimer's disease. Mol Neurobiol 49:529–535
4. Hollingworth P, Harold D, Sims R et al (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 43:429–435
5. Bertram L, Lange C, Mullin K et al (2008) Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. Am J Hum Genet 83:623–632
6. Naj AC, Jun G, Beecham GW et al (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet 43:436–441
7. Carrasquillo MM, Belbin O, Hunter TA et al (2011) Replication of EPHA1 and CD33 associations with late-onset Alzheimer's disease: a multi-centre case-control study. Mol Neurodegener 6:54
8. Chung SJ, Lee JH, Kim SY et al (2013) Association of GWAS top hits with late-onset Alzheimer disease in Korean population. Alzheimer Dis Assoc Disord 27:250–257
9. Tan L, Yu JT, Zhang W et al (2013) Association of GWAS-linked loci with late-onset Alzheimer's disease in a northern Han Chinese population. Alzheimers Dement 9:546–553
10. Griciuc A, Serrano-Pozo A, Parrado AR et al (2013) Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. Neuron 78:631–643
11. Bradshaw EM, Chibnik LB, Keenan BT et al (2013) CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. Nat Neurosci 16:848–850
12. Jack CR Jr, Knopman DS, Jagust WJ et al (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 9:119–128
13. Liu Y, Yu JT, Wang HF et al (2015) APOE genotype and neuroimaging markers of Alzheimer's disease: systematic review and meta-analysis. J Neurol Neurosurg Psychiatry 86:127–134
14. Liu Y, Tan L, Wang HF, et al. (2015) Multiple Effect of APOE Genotype on Clinical and Neuroimaging Biomarkers Across Alzheimer's Disease Spectrum. Mol Neurobiol.
15. Chauhan G, Adams HH, Bis JC et al (2015) Association of Alzheimer's disease GWAS loci with MRI markers of brain aging. Neurobiol Aging 36:1765, e1767-1716
16. Saykin AJ, Shen L, Foroud TM et al (2010) Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: Genetics core aims, progress, and plans. Alzheimers Dement 6:265–273
17. Fischl B, Salat DH, van der Kouwe AJ et al (2004) Sequence-independent segmentation of magnetic resonance images. Neuroimage 23(Suppl 1):S69–84
18. Fischl B, Salat DH, Busa E et al (2002) Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33:341–355
19. Fischl B, Dale AM (2000) Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proc Natl Acad Sci U S A 97:11050–11055
20. Liu Y, Yu JT, Wang HF et al (2014) Association between NME8 locus polymorphism and cognitive decline, cerebrospinal fluid and neuroimaging biomarkers in Alzheimer's disease. PLoS One 9:e114777
21. Biffi A, Anderson CD, Desikan RS et al (2010) Genetic variation and neuroimaging measures in Alzheimer disease. Arch Neurol 67:677–685
22. Wang WY, Yu JT, Liu Y et al (2015) Voxel-based meta-analysis of grey matter changes in Alzheimer's disease. Transl Neurodegener 4:6
23. Chang YT, Huang CW, Chang YH et al (2015) Amyloid burden in the hippocampus and default mode network: relationships with gray matter volume and cognitive performance in mild stage Alzheimer disease. Medicine (Baltimore) 94:e763
24. Hu N, Tan MS, Sun L et al (2014) Decreased expression of CD33 in peripheral mononuclear cells of Alzheimer's disease patients. Neurosci Lett 563:51–54
25. Tahara K, Kim HD, Jin JJ, Maxwell JA, Li L, Fukuchi K (2006) Role of toll-like receptor signalling in Abeta uptake and clearance. Brain 129:3006–3019
26. Sheng JG, Mrak RE, Griffin WS (1995) Microglial interleukin-1 alpha expression in brain regions in Alzheimer's disease: correlation with neuritic plaque distribution. Neuropathol Appl Neurobiol 21:290–301
27. Zeineh MM, Chen Y, Kitzler HH, Hammond R, Vogel H, Rutt BK (2015) Activated iron-containing microglia in the human hippocampus identified by magnetic resonance imaging in Alzheimer disease. Neurobiol Aging 36:2483–2500