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The *CD33* genotype associated cognitive performance was bidirectionally modulated by intrinsic functional connectivity in the Alzheimer's disease spectrum

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ABSTRACT

CD33 is a susceptibility locus for late-onset Alzheimer's disease (AD). However, how the neural mechanism of *CD33* affects cognition in the AD spectrum population remains unclear. We aimed to investigate the primary and interactive effects of the *CD33* (rs3865444) genotype on brain function in patients with AD using global functional connectivity density (gFCD) mapping via resting-state functional magnetic resonance imaging. Furthermore, we used a conditional process analysis to identify the relationship among the *CD33* genotype, gFCD, and cognition performance across the AD spectrum population. Compared to cognitively normal (CN) and mild cognitively impaired (MCI) subjects, patients with AD showed higher gFCD in the default mode network, and the *CD33* genotype primarily influenced brain function in the fronto-striatal circuit. Importantly, an interaction between the *CD33* genotype and AD was observed in the parahippocampal gyrus. During disease progression, the gFCD trajectories of the *CD33* A + allele gradually decreased, whereas those of the *CD33* CC allele displayed an inverted U-shaped curve. Furthermore, gFCD in the dorsal anterior cingulate cortex positively mediated the relationship between the *CD33* genotype and cognition, while gFCD in the precuneus bidirectionally moderated the mediation in the AD spectrum. These findings provide new insights into the neural mechanisms underlying the influence of the *CD33* genotype on cognitive performance and highlight the importance of precise therapeutic strategies for high-risk AD populations.

1. Introduction

Alzheimer's disease (AD) is complex and is one of the most common neurodegenerative diseases in the elderly [1]. Emerging evidence suggests that neuroinflammation plays an important role in the pathogenesis of AD and may contribute to AD pathogenesis to the same extent as plaques and tangles do [2]. Large-scale genome-wide association studies (GWAS) have identified the *CD33* gene as a risk factor for AD [3]. The *CD33* gene encodes a microglial surface receptor. The active site of the CD33 protein contains a conserved arginine residue, which is positively charged at physiological pH. CD33 plays an important role in the mutation and differential expression of microglia [4]. Importantly, macrophages isolated from subjects carrying heterozygous and homozygous *CD33* mutations had reduced levels of β-amyloid (Aβ) phagocytosis, which may increase the risk of AD [5]. Additionally, *CD33* rs3865444 CC allele carriers had increased Aβ deposition in the AD brain (detected by Pittsburgh compound B (PiB) - positron emission tomography (PET)) [5]. Furthermore, higher CD33 expression levels have been associated with cognitive impairment in AD patients [6], and the *CD33* rs3865444 A allele seems to protect against postreproductive cognitive decline [7]. However, these observations have been recently challenged by a follow-up study that found associations of cognitive decline with the *CD33 gene* in only one female cohort [8], and these associations were not identified in a meta-analysis based on four large-

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[2] Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.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 the analysis or writing of this report. A complete list of members of the ADNI is available at http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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scale GWAS [9]. Hence, these inconsistent findings indicate that there is a more complex relationship between the *CD33* gene and cognitive performance.

Imaging phenotypes, such as intermediate phenotypes, might connect a mechanistic pathway of genetic variation to cognitive performance [10]. Recently, the influence of the *CD33* genotype on brain atrophy and metabolism has been identified using structural MRI and fluorodeoxyglucose PET imaging data [11,12]. The results demonstrated that the *CD33* rs3865444 CC allele was associated with smaller intracranial volume, reduced brain metabolism, and gray matter atrophy in the prefrontal cortex of the pooled groups, but this association was not present in single AD or mild cognitively impaired (MCI) groups [11]. In addition, the *CD33* rs3865444 variant did not affect the volumes of cortical thickness in the hippocampus, posterior cingulate, and entorhinal cortex [12]. However, to date, no study has investigated how the *CD33* genotype influences brain function and cognitive performance at the network level.

Currently, we applied global functional connectivity density (gFCD) mapping, a data-driven, voxel-wise method, to measure the amount of functional connections in the whole brain using resting-state functional MRI (R-fMRI) data [13]. This was done to identify the impact of *CD33* rs3865444 polymorphisms and their interactions, with disease status throughout the whole brain of MCI and AD patients as well as cognitively normal (CN) subjects. Importantly, we integrated gene-brain-cognition models to investigate how the brain functional imaging features served as potential intermediate phenotypes in the relationship between the *CD33* genotype and cognitive impairment in the AD spectrum population (including MCI and AD) [14]. We predict that the gFCD plays a significant role linking the *CD33* gene with cognition in the AD spectrum.

2. Materials and methods

2.1. Participants

All data sources used in this article were obtained from the public Alzheimer's Disease Neuroimaging Initiative (ADNI) database (https://ida.loni.usc.edu). The inclusion and exclusion criteria of all ADNI participants are available at http://www.adni-info.org. Subjects were selected according to the following criteria: Caucasian, R-fMRI scan availability, 3D T1-weighted MRI scan availability (for spatial normalization), SNP rs3865444 genotypes of *CD33*, Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog), and Mini-Mental State Examination (MMSE) score. The different imaging modalities were acquired no more than 6 months apart from each other. According to our criteria, 214 subjects were included in the first step. Thereafter, 25 subjects were excluded due to poor signals in the R-fMRI image scans (N = 17), excessive head motion (translations > 2 mm or rotation > 2°) (N = 6), and failed spatial normalization (N = 2). Finally, 189 participants, comprising 67 with CN, 93 with MCI, and 29 with AD, were finally analyzed.

2.2. ADNI

The ADNI was launched in 2003 as a joint effort by the National Institute on Aging, National Institute of Biomedical Imaging and Bioengineering, Food and Drug Administration, private pharmaceutical companies, and non-profit organizations. The goal of the ADNI was to investigate whether the combination of neuroimaging, biological markers, and clinical and neuropsychological assessments could accurately detect disease progression in MCI and AD patients [15]. Ethical approval was obtained by the ADNI investigators (http://www.adni-info.org/pdfs/adni_protocol_9_19_08.pdf). Institutional Review Boards of all participating sites at their respective institutions approved the study. All participants provided written informed consent before the start of the study.

2.3. MRI data acquisition

All R-fMRI scans were performed using a single-shot T2-weighted echo-planar imaging pulse sequence in a Philips 3 T MRI scanner, with an eight-channel head coil. The R-fMRI parameters were as follows: repetition time (TR) = 3000 ms, echo time (TE) = 30 ms, flip angle (FA) = 80°, acquisition matrix = 64 × 64, field of view (FOV) = 240 × 240 mm, thickness = 3.3 mm, gap = 0 mm, and number of slices = 48. In addition, 3D T1 MRI images were obtained using a magnetization-prepared gradient echo (MPRAGE) sequence with a spatial resolution of $1 \times 1 \times 1.2\,mm^3$. The detailed MRI scanner protocols can be found at http://adni.loni.usc.edu/methods/documents/mri-protocols/.

2.4. R-fMRI data preprocessing

The functional data were preprocessed using SPM12 toolkit (http://www.fil.ion.ucl.ac.uk/spm) and MATLAB version 7.10 (The MathWorks, Inc., Natick, MA, USA). The preprocessing steps were conducted using the BRAinNetome Toolkit (http://www.brainnetome.org). The structural images were segmented (VBM toolbox in SPM) and co-registered with resting functional images. The segmented gray matter image was applied as a covariate after smoothing (the same as functional maps 8 mm Gaussian kernel). The fMRI scans were preprocessed in the following manner. The first ten volumes of the scanning session were discarded for the fMRI signal to reach equilibration. The remaining 130 volumes were corrected for slice timing, realigned, and subsequently spatially normalized to diffeomorphic high-dimensional registration as implemented in the DARTEL toolbox using the default settings and resampling to $3 \times 3 \times 3\,mm^3$ cubic voxels. The BOLD signal was low-pass filtered (0.01-0.1 Hz) and detrended. We also calculated the frame-wise displacement (FD), which reflected the mismatch of volume to volume head position [16]. The mean FD was also applied as a covariate in the imaging analyses. Next, the six motion parameters, the white matter signal, and the cerebrospinal fluid (CSF) signal, were removed from the data through linear regression.

2.5. gFCD calculation

We calculated the gFCD of each voxel using the in-house script according to the method described by Tomasi and Volkow [13]. Briefly, the gFCD at a given voxel (x_0) was computed as the global number of functional connections using Pearson's linear correlation between x_0 and all other voxels; voxels pairs with a correlation coefficient > 0.6 were considered functionally connected. In addition, calculating the gFCD in the gray matter regions was restricted with a signal-to-noise ratio > 50% to minimize unwanted effects from susceptibility-related signal-loss artifacts [13]. To increase the normality of the distribution, grand mean scaling of gFCDs was performed by dividing by the mean value of qualified voxels of the whole brain. Finally, the normalized gFCDs maps were spatially smoothened with an $8 \times 8 \times 8\,mm^3$ Gaussian kernel. Because the threshold of the Pearson correlation coefficient ($r = 0.6$) was arbitrarily selected, we also validated the reliability of our results using r = 0.5 and r = 0.7 thresholds.

2.6. Statistical analysis

2.6.1. Demographic information and neuropsychological characteristics

The 3 × 2 (3 groups, 2 genotypes) analysis of variance and chi-square tests were used to compare the demographic data among the groups using the Statistical Package for the Social Sciences version 20.0 (SPSS, Inc., Chicago, IL, USA). The statistical threshold was set at a $p < 0.05$. Cognitive performance was assessed by the Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-cog) scores across all subjects.

2.6.2. Group-level analysis of the gFCD pattern

Voxel-wise comparisons of gFCD mapping were conducted using a 3 × 2 (Disease × *CD33* Genotype) analysis of covariance with age, sex, apolipoprotein E (*APOE*) genotype, years of education, and voxel-wise gray matter image as nuisance covariates. The *APOE* genotype was included as a covariate in all of our analyses, as this gene is the main genetic risk factor for sporadic AD and can have an interactive effect with *CD33* on cognitive decline [17]. Furthermore, *APOE* has been implicated in Aβ-induced neuroinflammation [18].The voxel level significant threshold was set at $p < 0.005$ and was corrected for multiple comparisons at cluster-level with the latest version of 3dClustSim program in AFNI_16.3.00 (gray matter mask correction (67541 voxels) at voxel level $p < 0.005$, cluster level $\alpha < 0.001$, $\kappa > 61$, and cluster size > 1647 mm^3; https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html).

2.6.3. Correlation of behavioral scores with the gFCD

To investigate the behavioral significance of gFCDs, the mean gFCD signals were extracted from the regions of interest (ROIs) that had significant effects on disease, *CD33* genotype, and their interaction with gFCD. Then, a partial correlation analysis was conducted to examine the relationships between the altered gFCDs and the ADAS-Cog scores in patients in the AD spectrum population (MCI and AD groups), after controlling for the effects of group, age, gender, *APOE* genotype, and years of education. Significance was set at $p < 0.05$ after correcting for multiple comparisons with a false discovery rate (FDR).

2.6.4. Mediation and moderation analyses

To identify whether and how the imaging phenotype affected the association between *CD33* genotype and cognitive performance in the AD spectrum, mediation and moderation analyses were employed. The regions of interest (ROIs) of gFCDs were selected in the significant regions that correlated with ADAS-cog scores. In all the models used, the independent variable (X) was the *CD33* genotype (the *CD33* rs3865444 A + allele was coded as 1, whereas the CC allele was coded as -1 for all the analyses), and the dependent variable (Y) was the ADAS-cog scores. We included group, gender, age, APOE status, and years of education as covariates. For the moderation analysis, we used a simple moderation model from PROCESS Marco in SPSS (Model 1 in version 2.16.3) [19]. In this model, the ROIs of gFCD were the moderators (W), which were entered separately. All models included 5000 bootstrap samples with a bias-corrected bootstrap confidence interval (CI). When the moderate effect was significant, a floodlight analysis [20] was conducted to detect significant interactions. At that point, the degree to which the gFCD value enhanced or weakened the association between the *CD33* genotype and cognitive performance was determined using the Johnson-Neyman's region of significance approach [21]. For the mediation analysis, PROCESS model 4 was used [22], and the mediator (M) was the gFCD. This model was based on 10,000 bootstrap samples with a bias-corrected bootstrap CI. The indirect effect was determined as significant at 95% CI and did not include zero (with a null hypothesis that there would be no indirect effect). The direct effect between X and Y was not a necessary prerequisite for mediation [23].

2.6.5. Conditional process analysis

Because both the mediator and moderator were involved in the association between *CD33* genotype and ADAS-Cog scores (see the results below), and more importantly, to further investigate whether the size of the mediated effect is dependent on the moderator, an integrated model named conditional process analysis was employed [24] (PROCESS model 7). The model is constructed using the following two equations:

$$\hat{M} = i_M + a_1\,X + a_2\,W + a_3XW + a_4U_1 + a_5U_2 + a_6U_3 + a_7U_4 + a_8U_5 \quad (1)$$

$$\hat{Y} = i_Y + c'\,X + b_1\,M + b_2U_1 + b_3U_2 + b_4U_3 + b_5U_4 + b_6U_5 \quad (2)$$

where X is the independent variable (the *CD33* genotype), Y is the dependent variable (the ADAS-cog scores), M is the mediator (the gFCD in the dorsal anterior cingulate cortex), W is the moderator (the gFCD in the precuneus), and U_1, U_2, U_3, U_4 and U_5 are the group, gender, education, age, and APOE genotype, respectively. This model yielded three major results. First, the *direct effect* of X on Y was independent of the effect of the mediator (*c*'). Second, the *index of moderated mediation* (a_3b_1) was identified, which was conducted with 10,000 bootstrap samples for a bias-corrected bootstrap CI [24], and the mediation was moderated if the 95% CI did not include zero. Third, the *conditional indirect effect* of X on Y was at values of the moderator; in PROCESS, the W values were the mean and below/above a standard deviation (SD) from the mean. If the 95% CI for the conditional indirect effect did not straddle zero, the M effect of X on Y at the value of W was significant.

3. Results

3.1. Demographic information and neuropsychological characteristics

No significant differences were observed in age, sex, and years of education between the disease and *CD33* genotype groups (all $p > 0.05$). No significant differences in ADAS-cog scores were observed between the two *CD33* genotype groups ($p = 0.198$), but the disease effect on MMSE and ADAS-cog scores was significant ($p < 0.001$). The MMSE score was significantly negative correlated with the ADAS-cog score in each group ($r = 0.43$, $p < 0.001$); hence, we used only the ADAS-cog score as a behavioral trait in the next analyses (see Table 1).

3.2. Main effect of disease and the CD33 genotype on gFCD

As illustrated in Fig. 1A, the main effect of disease was located in the default mode network (DMN), including the bilateral medial prefrontal cortex (MPFC) and precuneus. Further analysis revealed that the gFCDs in the MPFC and the precuneus increased in the AD group compared to

Table 1
Demographic and neuropsychological data.

	CD33 rs3865444 A +			*CD33* rs3865444 CC			*F* or X^2	*p* values
	CN (n = 38)	MCI (n = 60)	AD (n = 17)	CN (n = 29)	MCI (n = 33)	AD (n = 12)		
Age	73.90 ± 6.48	71.24 ± 7.78	73.21 ± 7.76	72.75 ± 4.98	71.53 ± 6.54	72.89 ± 5.84	0.89	0.486
Gender (F/M)	14/24	32/28	8/9	15/14	13/20	7/5	4.18	0.523[†]
Years of education	16.23 ± 2.62	15.81 ± 2.85	15.76 ± 2.56	16.41 ± 2.38	16.57 ± 2.58	15.66 ± 3.08	0.56	0.734
ADAS-Cog	9.34 ± 4.06[c,d,e,f]	14.82 ± 6.01[a,b,e,f]	32.41 ± 9.75[a,b,c,d,f]	9.03 ± 3.97[c,d,e,f]	14.00 ± 7.22[a,b,e,f]	37.58 ± 8.48[a,b,c,d,e]	68.73	< 0.001
MMSE	28.63 ± 1.45[e,f]	27.86 ± 1.83 [e,f]	22.47 ± 2.48 [a,b,c,d]	28.93 ± 1.05 [e,f]	28.15 ± 1.50 [e,f]	22.25 ± 2.52 [a,b,c,d]	60.28	< 0.001

Notes: [†], *p* value was obtained using the chi-square test; other *p* values were obtained using one way ANOVA, measurement data are presented as the means ± standard deviation. [a] Compared with CN *CD33* A +, $p < 0.05$; [b] Compared with CN *CD33* CC, $p < 0.05$; [c] Compared with MCI *CD33* A +, $p < 0.05$; [d] Compared with MCI *CD33* CC, $p < 0.05$; [e] Compared with AD *CD3*3 A +, $p < 0.05$; f Compared with AD CD33 CC, $p < 0.05$. Abbreviations: CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's disease; ADAS-Cog, Alzheimer's disease assessment scale-cognitive subscale; MMSE, Mini-Mental State Examination.

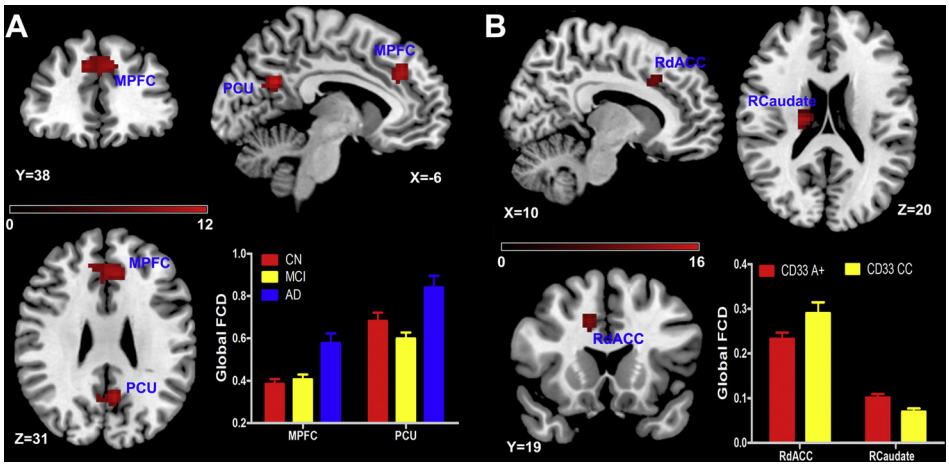

Fig. 1. The group differences (A) and CD33 genotype effect (B) on gFCD ($p <$ 0.005, $\alpha < 0.001$, 3dClustSim corrected).
The color bar represents *F* values. The quantitative histogram illustrates the group differences among the three groups in the MPFC and PCU (**A**) and the CD33 genotype effect on gFCD in the right caudate and dACC (**B**). Abbreviations: MPFC, medial prefrontal cortex; PCU, precuneus; RdACC, right dorsal anterior cingulate cortex; RCaudate, right caudate; CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's Disease; gFCD, global functional connectivity density.

the CN and MCI groups, but these gFCDs were not significantly different between the CN and MCI groups.

The main effect of the *CD33* genotype on gFCD was seen in the right dorsal anterior cingulate cortex (dACC) and right caudate. Further analysis showed that the gFCD in the right caudate was greater in the *CD33* rs3865444 A + group than in the *CD33* rs3865444 CC group, whereas the gFCD in the right dACC was weaker in the *CD33* rs3865444 A + group than in the *CD33* rs3865444 CC group (Fig. 1B).

3.3. Interactive effect of disease and the CD33 genotype on gFCD

The effect of disease × *CD33* genotype interaction on gFCD was observed in the right parahippocampal gyrus (PHG). Further analysis revealed that during disease progression, the gFCD trajectories of the *CD33* A + allele decreased slowly (Fig. 2). By contrast, the trajectories of *CD33* CC allele represented an inverted U-shape, and the gFCD was significantly higher at the MCI stage but was lower in the CN and AD stages.

3.4. Behavioral significance of gFCD among all the AD spectrum populations

Partial correlation analyses revealed that the gFCDs in the MPFC, precuneus, and right dACC were positively correlated with ADAS-Cog scores among all the AD spectrum population (Fig. 3, MPFC, $r = 0.455$, $p <$ 0.001; precuneus, $r = 0.294$, $p =$ 0.001; right dACC, $r = 0.243$, $p =$ 0.008). The associations between the ADAS-cog scores and gFCD remained significant after FDR correction.

3.5. Mediation and moderation analyses

The mediation and moderation analyses results are presented in Fig. S1 and Tables S1 and S2. Specifically, the gFCD in dACC mediated the effect of *CD33* genotype on ADAS-cog score in all patients (indirect effect, β = -0.208, 95% CI = [-0.563, -0.018]). That is, a *CD33* rs3865444 A + carrier status and a higher gFCD in dACC might predict a lower cognitive impairment in the AD spectrum patients. No significant mediation occurred in the subgroup analyses. The moderation analyses revealed that the gFCD in the precuneus significantly moderated the relationship between *CD33* genotype and the ADAS-cog score among all AD spectrum patients ($R^2 = 0.64$, $F_{(8,113)} = 25.69$, $p < 0.001$). In addition, the moderation of the gFCD in the precuneus was significant in the MCI group ($R^2 = 0.26$, $F_{(7,85)} = 3.66$, $p <$ 0.001). The Johnson-Neyman's approach was then used to assess the regions of significant interaction. As illustrated in Fig. S1B and D, when the gFCD in the precuneus was below 0.04 (0.16 in MCI patients), the relationship between the *CD33* and ADAS-cog score was positive ($\beta(SE) = 2.50(1.28)$, $t = 1.97$, $p =$ 0.05), meaning that *CD33* rs3865444 A + carriers had a worse cognitive function than the *CD33* rs3865444 CC carriers. When the gFCD in the precuneus was increased to 0.58 (0.72 in MCI patients), the relationship between the *CD33* genotype and the ADAS-cog score was negative ($\beta(SE)$ = -1.43 (0.72), t = -1.98, $p =$ 0.05), implying that the *CD33* rs3865444 A + variant could have a protective effect whereas the *CD33* rs3865444 CC variant could have an aggravated effect on cognitive function. The moderate effects of the other ROIs were not significant ($p >$ 0.05).

3.6. Conditional process analysis

As illustrated in Fig. 4, the conditional process analysis revealed a significant bidirectional modulation pattern of gFCD on the relationship between the *CD33* genotype and ADAS-Cog scores among the AD spectrum. Specifically, the indirect effect of *CD33* genotype on cognitive performance through dACC was moderated by the values of gFCD in the precuneus; when the value of the gFCD in the precuneus was equal or greater than the mean, the indirect effect was significantly negative (W = 0.46, β = -0.19, CI_{95} = [-0.53, -0.02]; W = 0.71, β = -0.66, CI_{95} = [-1.32, -0.13]). By contrast, when the value of the gFCD in the precuneus was a SD below the mean (W = 0.20), the indirect effect was significantly positive (β = 0.27, CI_{95} = [0.01, 0.75]). The negative index of moderated mediation indicated that the indirect effect of the *CD33* genotype on cognition impairment was generally negative in the AD spectrum, but when the gFCD in the precuneus was sufficiently low, the negative indirect effect reversed. This finding indicated that the *CD33* rs3865444 CC allele is usually a risk factor for cognitive impairment, but it could also be a protective factor for cognitive impairment when the gFCD in the precuneus is low enough. Last, the direct effect of this model was not significant, indicating that, independent of the moderated mediation, the *CD33* genotype did not have a direct effect on cognitive performance in the AD spectrum patients. As the mediation is not significant in MCI and AD groups, no further conditional process analysis was performed.

3.7. Verification analyses

Because the threshold of the Pearson correlation coefficient (r = 0.6) was arbitrarily selected in the gFCD calculation, we also validated the reliability of our results using r = 0.5 and r = 0.7 as thresholds. As illustrated in Fig. S2-4, the main effects of the disease and *CD33* genotype were almost similar. For the interactive effect of the disease and *CD33* genotype, PHG was found in both correlation coefficient thresholds; additionally, an interactive effect was found in the right superior frontal gyrus (SFG) at $r = 0.5$.

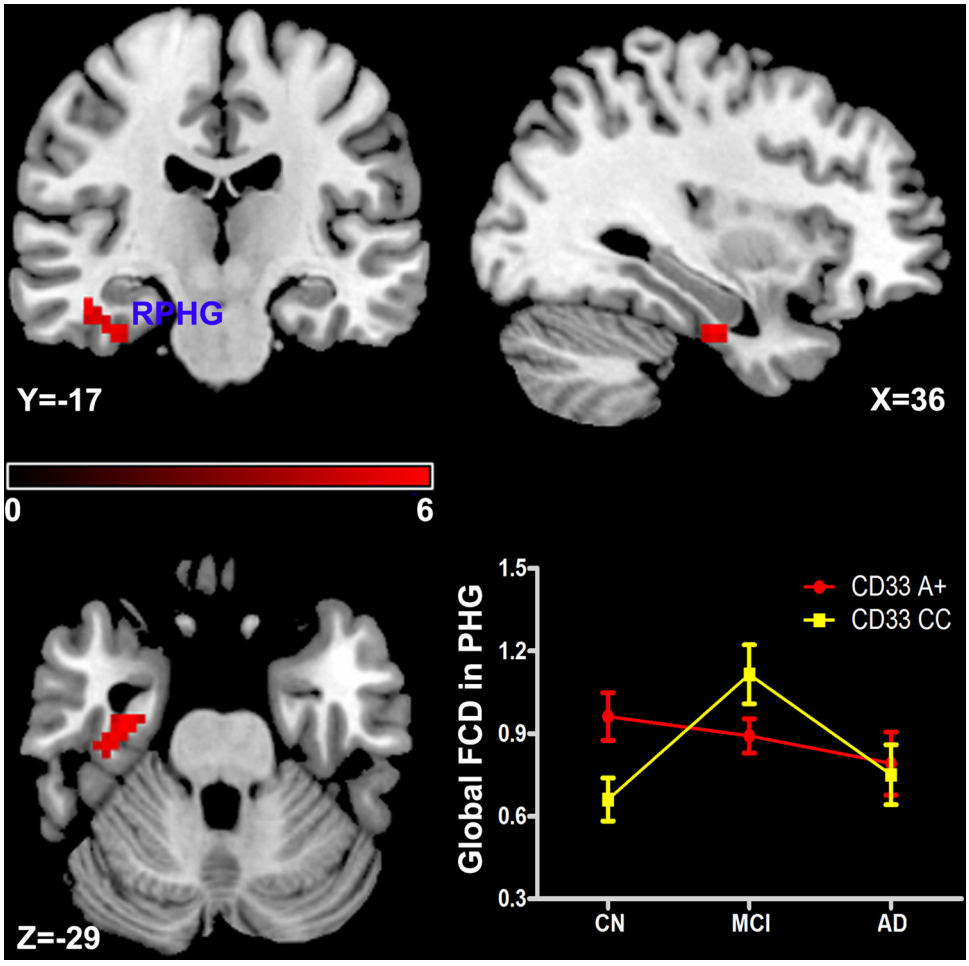

Fig. 2. The effect of disease × CD33 genotype on gFCD ($p < 0.005$, $\alpha < 0.001$, 3dClustSim corrected).
The color bar represents *F* values. The histogram quantitative illustrates the interactive effect between disease and *CD33* genotype in PHG. Abbreviations: PHG, parahippocampal gyrus; CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's disease; FCD, functional connectivity density.

4. Discussion

This study provides the first evidence that the neural effect of the *CD33* genotype on cognitive function is modulated by intrinsic brain function across the AD spectrum. The major findings of this study are as follows. First, the gFCDs in the DMN increased in AD patients compared to CN and MCI patients, whereas the regions where *CD33* influenced brain function were primarily located in the fronto-striatal circuits. Specifically, CD33 A + carriers showed enhanced gFCDs in the caudate and weakened gFCDs in the dACC. Second, the interactive effect of the *CD33* genotype and disease on the gFCD was found in the PHG region, which represented different patterns in CN and MCI patients but similar in AD patients. Third, a bidirectional neural moderated mediation of *CD33* on cognition was identified in the AD spectrum population. These results suggest that brain functional features may confer vulnerability to cognitive impairment among the AD spectrum in patients with different *CD33* genotypes.

4.1. Main effects of disease and the CD33 genotype on gFCD

It is well established that DMN is the most disrupted brain network in AD patients [25]. The DMN brain network is constrained by the fact that it depends on the seed-based approach instead of the characteristics of the network to identify and locate the topological organization of brain regions [13]. However, Sui et al recently reported that the long-range FCDs in MPFC, SFG, and supplementary motor area were

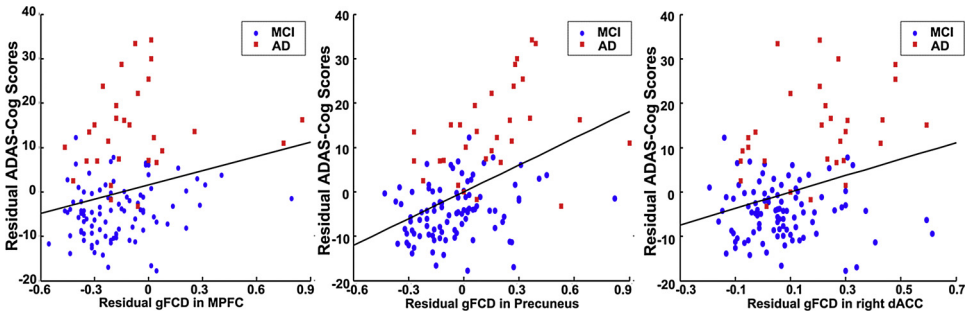

Fig. 3. The partial correlation analyses results. The correlation analyses examine the relationships between the altered gFCD and the ADAS-Cog scores in patients in the AD spectrum population (MCI and AD groups), after controlling for the effects of group, age, gender, *APOE* genotype, and years of education (blue plot for MCI and red square for AD). Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; MPFC, medial prefrontal cortex; dACC, dorsal anterior cingulate cortex; gFCD, global functional connectivity density; ADAS-Cog, Alzheimer's disease assessment scale - cognitive subscale.

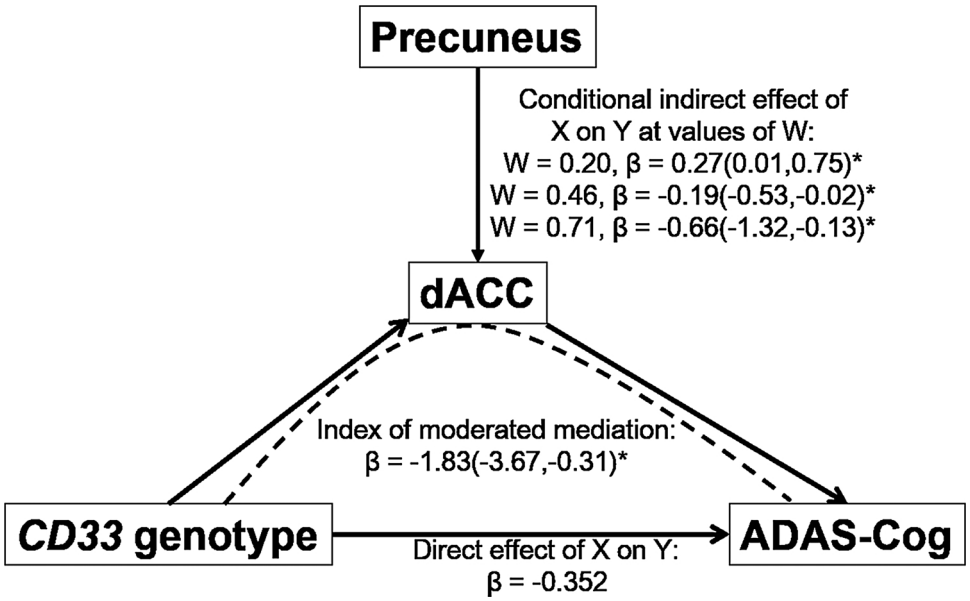

Fig. 4. The conditional process analysis revealed a bidirectionally moderated mediation of gFCD on the relationship between *CD33* genotype and ADAS-Cog score among AD spectrum patients.
X is *CD33* genotype, Y is ADAS-cog score, M is gFCD in dACC, and W is gFCD in precuneus. * The 95% bootstrap confidence interval does not straddle zero. Abbreviations: dACC, dorsal anterior cingulate cortex; ADAS-Cog, Alzheimer's Disease Assessment Scale – Cognitive subscale; gFCD, global functional connectivity density

increased in AD patients compared to MCI and CN patients [26]. Our findings also supported this notion and further identified that the abnormal gFCDs in the DMN were positively correlated with cognitive impairment in the AD spectrum. These results support the maladaptive mechanism in AD pathology, wherein the increased gFCD in the DMN reflects an unsuccessful attempt to recruit more preserved neural areas for AD pathological compensation [27]. The association between gFCD and cognitive performance would also suggest that the altered gFCD within the DMN may be a potential monitoring biomarker for AD progression.

The *CD33* rs3865444 A + allele is associated with reduced *CD33* expression in microglial cells in the healthy human brain, whereas higher levels of *CD33* expression were associated with higher amyloid burden in AD brains [5]. In addition, higher levels of *CD33* mRNA expression were associated with increasing AD pathology, and the *CD33* A + allele resulted in lower CD33 protein in temporal cortex samples [28]. However, the effects of the *CD33* genotype on brain function have rarely been studied at the network level. Currently, the *CD33* genotype influence on brain function was observed primarily in the fronto-striatal circuit, which is a critical pathway involved in complex cognition and emotion processing, including reward-based learning [29], decision making [30], emotional regulation [31], and executive control [32]. In addition, previous studies focused on the neural inflammation in the AD spectrum using PET and found an increased inflammation response in both the anterior cingulate cortex and striatum regions [33]. Interestingly, the *CD33* A + allele showed higher gFCD in the caudate but lower gFCD in the dACC compared to *CD33* CC allele, indicating that the *CD33* A + allele might resort to a balancing strategy to protect against cognitive impairment within the fronto-striatal circuits in the AD spectrum.

4.2. Interactive effect of the disease and CD33 genotype on gFCD

For the CN and MCI groups, the inverse pattern of the *CD33* genotype effects on brain function was observed in the right PHG, but this pattern was similar at the AD stage. During disease progression, the gFCD trajectories of the *CD33* A + allele decreased slowly, but the trajectories of the *CD33* CC allele represented an inverted U-shape, which was significantly higher at the MCI stage but lower in CN and AD stages. The PHG, an important region for environmental memory processing [34], exhibits significant structural atrophy and abnormal function in AD patients [35], even in the early stages of AD [36]. A previous study found that *CD33* genetic variants were associated with PHG volume in CN and MCI groups [12]. More recently, the increased functional synchrony in a resting-state brain might be attributed to early Aβ pathology in the early phase of AD transgenic mice [37]. However, there was no significant difference in the gFCDs between the *CD33* A + carriers and the *CD33* CC carriers in AD patients, indicating that the timing and stage-constrained genetic influences on AD pathology might be one of the reasons for the missing heritability in the AD stage.

4.3. Bidirectional modulation patterns of gFCD on the relationship between the CD33 genotype and cognition

Numerous imaging genetic studies have identified that neuroimaging features act as an intermediate phenotype from gene to behavior phenotype in psychiatric disorders [38]. Previous studies usually used mediation analysis and moderation analysis to investigate the imaging intermediate phenotypes in neuropsychiatric diseases [39]. Although the moderated mediation analysis has been employed widely in psychology research for years [40], no study has integrated mediation and moderation analyses in an imaging genetics study, this would be useful to further investigate the neural mechanisms underlying the gene and behavior [41]. Currently, we identified positive correlations between gFCDs in the MPFC, precuneus, and dACC and cognitive impairment in AD spectrum patients. We demonstrated both mediator and moderator effects in the relationship between the *CD33* genotype and cognitive performance; thus, the conditional process analysis was necessary to explore the intermediate effects of intrinsic brain function on the relationship between the *CD33* gene and behavior. Interesting, the conditional process analysis revealed indirect effects of the gFCD on the association between gene and cognition in the AD spectrum. In addition, the dACC is a core region involved in regulating cognitive control, reward-based learning, and conflict monitoring [42], while the precuneus plays an important role in episodic memory and consciousness processing [43,44]. Both regions are involved in AD pathology [45]. Previous studies have also reported that the *CD33* gene was mostly expressed in the microglia and was related to inflammatory responses in the brain [46]. Recent studies using PET imaging have found that inflammation in the ACC increased in AD and MCI patients [2,33]. These findings suggest that the *CD33* gene might influence cognition through the inflammatory response in ACC in the AD spectrum. The present findings support this notion and indicate that the brain

functional features of the gFCD in different regions play a different role in the relationship between gene and behavior. Specifically, the moderated mediator of *CD33* on cognition was bidirectional. This finding indicates that for the *CD33* A + carriers, the higher gFCD in the precuneus, combined with higher gFCD in the dACC, predicted less cognition impairment, whereas a much lower gFCD in the precuneus, combined with higher gFCD in the dACC, might also protect against cognitive decline in the AD spectrum with *CD33* CC alleles. These results suggested that the detection of the modulation pattern on brain function is needed for AD treatment in patients carrying different *CD33* genotypes. These findings expand our understanding of how neuroinflammation-related genes can affect brain function and further influence behavioral performance, and they also provide direct support for investigating the relationship in gene-brain-behavior in the AD spectrum.

4.4. Limitations

This study has some limitations. First, the results of this cross-sectional study did not enable an interpretation of a causal relationship between genetics and cognitive performance, thus highlighting the importance of conducting longitudinal studies in the future. Second, accumulating evidence has indicated that environmental factors may interact with genetic susceptibility to produce alterations in brain function and the final behavioral phenotype [39]. Future studies should examine gene-environment interactions on brain function and behavioral phenotype. Third, all participants enrolled in the present study were Caucasian. However, previous research has shown that the CD33 rs3865444 A allele is a risk factor rather than a protective allele in AD in the Han Chinese population [47]. Thus, the findings should be interpreted carefully in other populations.

4.5. Conclusion

In conclusion, we investigated the association of the *CD33* gene–brain-cognitive function in the AD spectrum. The gFCD acted as a bidirectional modulator in the association between the *CD33* genotype and cognitive performance in the AD spectrum. These findings provide new insights into the neural mechanisms underlying how the *CD33* genotype influences cognitive performance and highlight the importance of precise therapeutic strategies for AD high-risk populations.

Competing financial interests

All authors report no financial conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2019.108903.

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