


APOE genotype moderates the relationship between LRP1 polymorphism and cognition across the Alzheimer's disease spectrum via disturbing default mode network

Feifei Zang¹ | Yao Zhu¹ | Qianqian Zhang¹ | Chang Tan¹ | Qing Wang¹ | Chunming Xie^{1,2}  | on behalf of Alzheimer's Disease Neuroimaging Initiative*

¹Department of Neurology, Affiliated ZhongDa Hospital, School of Medicine, Southeast University, Nanjing, China

²Neuropsychiatric Institute, Affiliated ZhongDa Hospital, Southeast University, Nanjing, China

Correspondence

Chunming Xie, Department of Neurology, Affiliated Zhongda Hospital, School of Medicine, Southeast University, No.87 Dingjiaqiao Road, Nanjing, Jiangsu 210009, China.
Email: chmxie@163.com

Funding information

This study was granted funding by the National Key Projects for Research and Development Program of China (2016YFC1305800, 2016YFC1305802, CMX), the National Natural Science Foundation of China (81671256, 81871069, 82071204, CMX), the Key Project for Research and Development Program of Jiangsu Province (BE2018741, CMX), the Key Projects of Jiangsu Commission of Health (ZDB2020008, CMX), the Nanjing International Joint Research and Development Project (201715013), and the Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX19_0115).

Abstract

Aims: This study aims to investigate the mechanisms by which apolipoprotein E (APOE) genotype modulates the relationship between low-density lipoprotein receptor-related protein 1 (*LRP1*) rs1799986 variant on the default mode network (DMN) and cognition in Alzheimer's disease (AD) spectrum populations.

Methods: Cross-sectional 168 subjects of AD spectrum were obtained from Alzheimer's Disease Neuroimaging Initiative database with resting-state fMRI scans and neuropsychological scores data. Multivariable linear regression analysis was adopted to investigate the main effects and interaction of *LRP1* and disease on the DMN. Moderation and interactive analyses were performed to assess the relationships among *APOE*, *LRP1*, and cognition. A support vector machine model was used to classify AD spectrum with altered connectivity as an objective diagnostic biomarker.

Results: The main effects and interaction of *LRP1* and disease were mainly focused on the core hubs of frontal-parietal network. Several brain regions with altered connectivity were correlated with cognitive scores in *LRP1*-T carriers, but not in non-carriers. *APOE* regulated the effect of *LRP1* on cognitive performance. The functional connectivity of numerous brain regions within *LRP1*-T carriers yielded strong power for classifying AD spectrum.

Conclusion: These findings suggested *LRP1* could affect DMN and provided a stage-dependent neuroimaging biomarker for classifying AD spectrum populations.

KEYWORDS

Alzheimer's disease, apolipoprotein E, default mode network, low-density lipoprotein receptor-related protein 1

*The data used in preparation of this article were obtained from the Alzheimer's disease Neuroimaging Initiative (ADNI) database (<http://www.loni.ucla.edu/>). Investigators within the ADNI database contributed to the design and implementation of the ADNI and provided data but did not participate in the analysis or write this report. For a complete listing of ADNI investigators, please see http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *CNS Neuroscience & Therapeutics* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

Low-density lipoprotein receptor-related protein 1 (LRP1) is a large cell surface transmembrane receptor, highly expressed in the neurons, astrocytes, and vasculatures of the brain. It regulates the pathogenesis of Alzheimer's disease (AD).¹ Reportedly, up to 50 structurally diverse proteins including β -amyloid (A β) and apolipoprotein E (APOE) are ligands of LRP1.² Notably, LRP1 promotes A β clearance, maintains synaptic integrity, and regulates lipid metabolism in the brain.^{3,4} *LRP1* gene rs1799986 polymorphism in exon 3 has a silent mutation of C allele to T allele and generates three isoform genotypes including CC, TC, and TT. The T allele potentially confers a risk factor for developing sporadic AD. Nonetheless, the relationship of this variant with AD is elusive. Several previous studies reported that *LRP1* rs1799986 polymorphism was connected with late-onset AD.⁵⁻⁸ However, recent three genome-wide association studies failed to discover a significant impact of this variant on AD risk.⁹⁻¹¹

Despite the conflicting findings in polymorphism, one human postmortem study with brain tissues revealed that LRP1 levels from the middle frontal cortex were significantly reduced in AD patients compared with healthy controls. Also, LRP1 levels progressively decreased with the increasing age in controls, whereas a higher level of LRP1 correlated with later age of AD onset.¹² Elsewhere, another postmortem study found significantly decreased LRP1 levels in the hippocampus of mild cognitive impairment (MCI), an early stage of AD, compared with age-matched controls.¹³ These observations preliminarily suggest that the disrupted LRP1 levels might partly reflect brain function.

Accumulating evidence from animal studies indicates that LRP1 has been implicated in the process of A β and Tau pathology and related to cognitive function. Besides, LRP1 potentially acts predominantly over A β clearance in a mouse model of AD.¹⁴ In cerebral blood vessels, LRP1 importantly mediates the rapid removal of A β from the brain to transport across the blood-brain barrier; also, endothelial LRP1 may be treated as a potential target for the treatment of AD.¹⁵ Additionally, recent research identified that knockdown *LRP1* significantly reduced tau uptake in neurons and tau propagation between neurons. This implies a master regulatory role of LRP1 in tau pathology, thereby providing a novel therapeutic target for tau-related neurodegenerative diseases.¹⁶ Drug trials also have revealed that low-dose pioglitazone ameliorates learning and memory impairment by upregulating LRP1 expression in the hippocampus.¹⁷ Moreover, APOE- ϵ 4 mediates A β pathology based on its neuronal receptor LRP1¹⁸ and *LRP1* knockout prevents the increase of A β pathology caused by APOE- ϵ 4 expression.¹⁹ Therefore, LRP1 is a common factor modulating A β and tau metabolism to maintain brain homeostasis.

Default mode network (DMN) is an intrinsic brain networks that most consistently exists in healthy and disease populations; its deterioration acts as a tracking tool to monitor AD progression.^{20,21} A β accumulation preferentially commences in several core regions of DMN, including the precuneus, medial orbitofrontal, and posterior

cingulate cortex, and further affected brain connectivity within DMN.²² Nevertheless, information on how the *LRP1* gene polymorphism affects DMN in the AD spectrum is scant, despite being the regulatory effect of LRP1 on A β . Additionally, considering that APOE exacerbates A β pathology in an LRP1-dependent manner,¹⁸ investigating the role of APOE on the relationship between *LRP1* and cognition in AD spectrum is of importance.

Herein, we first assessed whether the *LRP1* genotype disturbed functional connectivity (FC) within DMN and affected cognitive performance across all subjects. Secondly, this work explored the relationships among APOE, *LRP1*, and cognition in the AD spectrum with moderation analysis. Thirdly, a support vector machine (SVM) model was employed to classify AD spectrum with the altered connectivity within DMN as an objective diagnostic biomarker, based on *LRP1* genotypes.

2 | METHODS

2.1 | Participants

Cross-sectional data were downloaded from the Alzheimer's disease Neuroimaging Initiative (ADNI) database (<http://adni.loni.usc.edu>) before March 21, 2021. Moreover, resting-state functional magnetic resonance imaging (rs-fMRI) images were downloaded from ADNI-1, ADNI-GO, ADNI-2, and ADNI-3 projects. If two or more rs-fMRI scans were performed at baseline, the first available scan was included for analysis ($n = 184$). Corrupted image sequences ($n = 6$), image quality control failure ($n = 4$), and excessive head motion ($n = 6$) were excluded. In total, 168 participants including 55 CN, 45 subjective cognitive decline (SCD), 42 MCI, and 26 mild AD participants were enrolled for the final analysis. Mini-Mental State Examination (MMSE) was adopted as a measure of general cognition since it was available across all participants.²³ In addition, demographic and genetic information were obtained from the ADNI database. Genetic genotyping for APOE and *LRP1* was performed as previously described.²⁴ Participants with at least one ϵ 4 allele were categorized into APOE- ϵ 4 carriers (APOE ϵ 4⁺), while others without ϵ 4 alleles were categorized into APOE- ϵ 4 non-carriers (APOE ϵ 4⁻). Similarly, those with at least one T allele were categorized into *LRP1*-T carriers (*LRP1* T⁺), while others without T allele were categorized into *LRP1*-T non-carriers (*LRP1* T⁻). Hardy-Weinberg equilibrium (HWE) test for each gene was calculated. Flow chart was shown in Figure S1.

2.2 | Statistics analyses

2.2.1 | Demographic and neuropsychological data analysis

First, the Shapiro-Wilk test was adopted to assess the data normality of continuous variables. A non-parametric test was analyzed

when data distributions were not normal. Levene's test was examined to assess the homogeneity of variance. One-way analysis of variance (ANOVA) was separately used to compare the group differences of age, years of education, and MMSE scores. Non-parametric Kruskal-Wallis test was used if the Shapiro-Wilk test or Levene's test $p < 0.05$. Chi-square tests were applied to compare the group differences of gender, *APOE*- $\epsilon 4$ status and *LRP1*-T status. The significant level was set at $p < 0.05$. Post hoc analyses with Bonferroni correction ($p < 0.05/6 = 0.0084$) were essential in establishing the significance between any two groups. All statistical analyses were performed using SPSS 22.0 software (SPSS, Inc., Chicago, IL, USA).

2.2.2 | DMN functional connectivity analysis

A voxel-wise one-sample t-test was performed on the subject-specific maps to achieve a t-map (DMN pattern, FDR corrected, $p < 0.001$), which was shown in Figure S2. This pattern was converted to a binary map. Of note, voxels outside of gray matter would be excluded. Subsequently, an overlap mask was generated by combining the above binary map and gray matter mask to prevent any spurious effects from white matter and ventricles. After controlling nuisance variables of age, gender, education, and *APOE* genotype, multivariable linear regression analysis was employed to investigate the effects of *LRP1* genotype, disease status, and *LRP1* \times disease interaction on the DMN within the above overlap mask (3dRegAna, AFNI).

The cluster-level threshold corrected for multiple comparisons was derived using Monte Carlo simulation of the random noise distribution in the data using the latest 3dClustSim program with the -acf function in AFNI [overlap DMN mask correction (39,543 voxels), voxel-level $p < 0.01$, cluster-level $\alpha < 0.05$, $\kappa > 113$ voxels, cluster size > 3051 mm³; https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html]

2.2.3 | Behavioral correlations

After controlling covariate effects of age, gender, years of education and *APOE*- $\epsilon 4$ status, linear regression analyses across all the subjects were performed. This was to investigate the correlation between behavior performance and averaged FC extracted from each region of interest identified by the main and interactive effect of *LRP1* genotype and disease status on DMN. Then, we separately analyzed the correlations of regional FC to behavior performance, specifically for *LRP1*-T carriers and non-carriers.

2.2.4 | Moderation and interactive effect analysis

A simple linear moderation effect model under PROCESS macro for SPSS (model 1)²⁵ was performed to address whether *APOE* genotype

moderates the genotype effect of *LRP1* on MMSE; or whether *LRP1* genotype moderates the genotype of *APOE* on MMSE, controlling for age, gender, and education. This was geared toward exploring the potential relationship between *LRP1* and *APOE*. Herein, either of the dichotomous *LRP1* genotype (*LRP1* T⁺ and T) or *APOE* genotype (*APOE* $\epsilon 4^+$ and $\epsilon 4^-$) represents the independent variable, while another one represents moderator. MMSE represents the dependent variable. If the effect of independent variable size or sign on dependent variable varies with moderator, the effect of the independent variable on dependent variable is considered to be moderated by the moderator.

Secondly, analysis of covariance (ANCOVA), with *LRP1* genotype and *APOE* genotype, was used as dichotomous fixed factors, adjusting for potential confounding variables of age, gender and education, to illustrate the interactive effect of *LRP1* and *APOE* on MMSE across all subjects.

2.2.5 | Neuroimaging biomarker of FC for classifying AD spectrum disease

The extracted mean FC from each region of interest was considered a predictive variable to classify the AD spectrum among all subjects. Further, this regional FC was adopted to classify the AD spectrum in separate *LRP1*-T carriers and non-carriers. The receiver operating characteristic (ROC) curve was used to calculate the area under the curve (AUC) with the better predictive effect as AUC close to 1. To verify the accuracy of the classification, a leave-one-out cross-validation method was used in the linear support vector machine (SVM) model implemented under MATLAB LIBSVM library²⁶ and repeated 10,000 times permutation tests for the limited sample size. Unless specifically emphasized, the statistical significance was set at $p < 0.05$.

3 | RESULTS

3.1 | Demographic characteristics

No differences were observed in gender and education among all participants (all p values > 0.05). A significant decreasing trend was noted in age along the disease process, and the main difference existed between the MCI or AD groups and the CN group. While acknowledging no significance in *LRP1*-T status, a slight marginal differential trend was noted ($p = 0.052$). Regarding *APOE*- $\epsilon 4$ status, a remarkable discrepancy was noted with a tendency that the proportion of $\epsilon 4$ allele increased as the disease progressed. Furthermore, the MCI and AD groups showed a more significant decrease in MMSE scores; however, the SCD subjects had higher MMSE scores than the CN group. No genotype frequency deviated from HWE (*LRP1*, $\chi^2 = 2.455$, $p = 0.117$; *APOE*, $\chi^2 = 1.580$, $p = 0.209$). The characteristics of participants are summarized in Table 1.

3.2 | Main and interactive effects of *LRP1* genotype and disease status on the DMN

The main effect of the disease status on the DMN was observed in the left temporoparietal joint (LTPJ), left inferior parietal cortex (LIPC), left and right posterior cingulate cortex (LPCC and RPCC), left retrosplenial cortex (LRSC), left precuneus/cuneus (LPCUN/LCUN), and right precuneus/cuneus (RPCUN/RCUN) (Figure 1A). Interestingly, the FC changes demonstrated a dynamic robust pattern along the entire disease process. The distribution pattern of FC in the LTPJ, LIPC, LPCC, and LRSC showed an inverted-U shape, whereas FC changes in the LPCUN/LCUN, RPCC, and RPCUN/RCUN showed a U-shape pattern (Figure 1B). The main effect of the *LRP1* genotype on the DMN was located in the left middle frontal gyrus (LMFG), LPCC, and RPCC (Figure 1C). In contrast with *LRP1*-T non-carriers, *LRP1*-T carriers exhibited a stronger FC strength in these brain areas (Figure 1D).

The interactive effects of *LRP1* genotype and disease status on the DMN were also discovered in the LMFG, LPCC, RPCC, LRSC, and right dorsolateral prefrontal cortex (RDLPFC) (Figure 1E). More importantly, unlike *LRP1*-T non-carriers, the FC of the *LRP1*-T carriers displayed opposite trajectory changes in these brain regions across

the entire disease process, specifically between the SCD and MCI stages. The *LRP1*-T carriers showed increased connectivity in the LMFG and decreased connectivity in the LPCC, LRSC, RDLPFC, and RPCC; nevertheless, the *LRP1*-T non-carriers demonstrated a relatively stable change with the progression of disease (Figure 1F). The main effects and interaction of *LRP1* genotype and disease status were majorly focused at the core hubs of the frontal-parietal network. Comprehensive descriptions of brain regions and their FC differences among different groups are illustrated in Tables S1 and S2.

3.3 | Relationship between altered FC and cognitive performance

As shown in Figure 2, the linear regression analyses indicated that disrupted FCs in brain regions of LMFG, RPCC, and LIPC were significantly correlated with the MMSE scores across all groups in the *LRP1*-T carriers but not in the non-carriers. The FC of LMFG negatively correlated with the MMSE score, indicating an increased FC corresponding to a declining cognitive score. Nonetheless, the FCs of RPCC and LIPC positively correlated with MMSE scores, that is, greater FCs corresponded to higher cognitive scores.

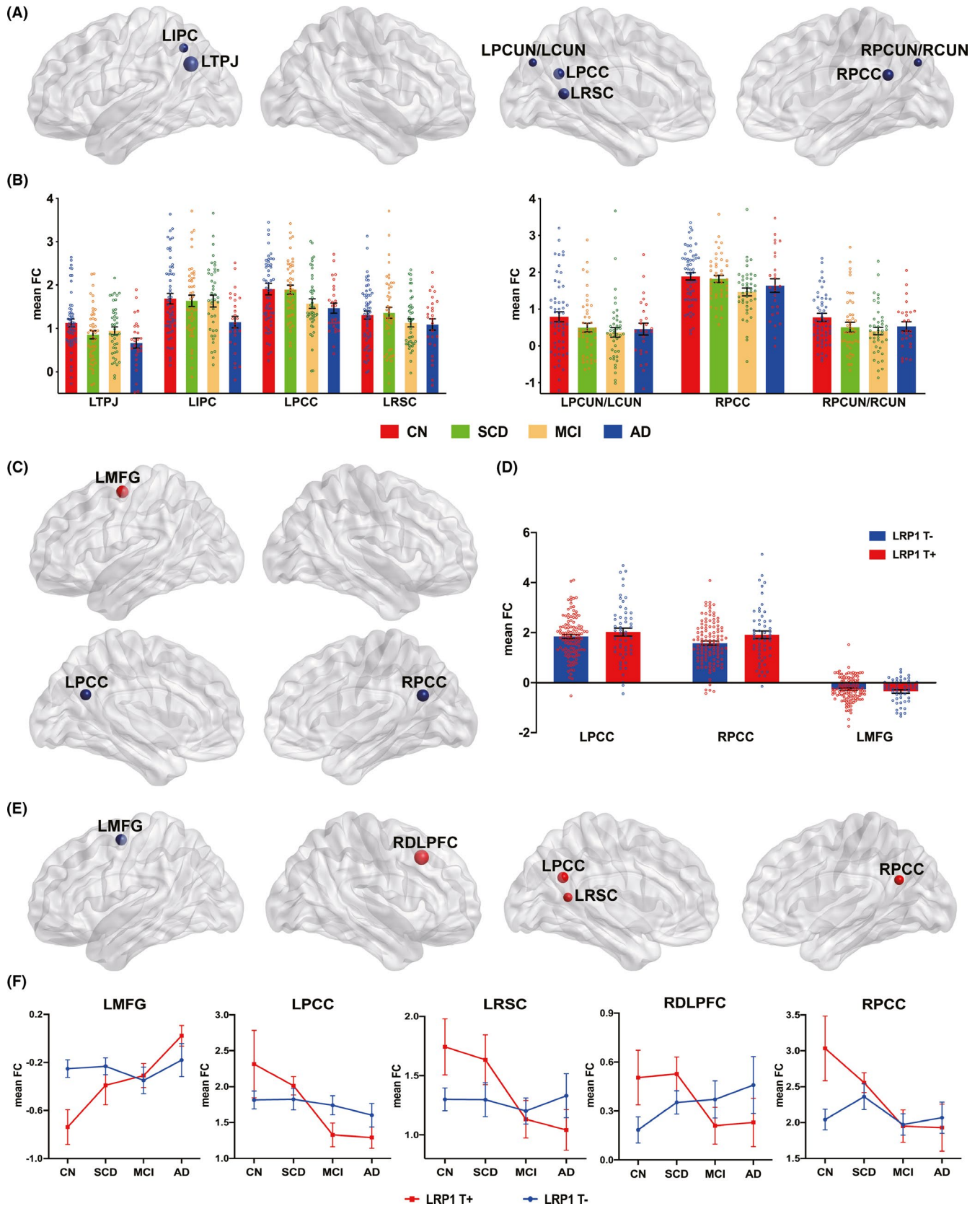
TABLE 1 Demographic, genetic and clinical data across all groups

Items	CN (n = 55)	SCD (n = 45)	MCI (n = 42)	AD (n = 26)	p value
Age (y)	78.58 ± 6.12	75.53 ± 5.43	73.69 ± 6.20 ^a	71.92 ± 7.74 ^b	<0.001
Gender (M/F)	27/28	15/30	25/17	13/13	0.103 [†]
Education (y)	15.96 ± 2.62	16.64 ± 2.67	15.86 ± 3.01	15.08 ± 2.64	0.082
APOE-ε4 status (+/-)	14/41	12/33 ^d	16/26 ^e	19/7 ^b	<0.001 [†]
<i>LRP1</i> -T status (+/-)	10/45	16/29	17/25	11/15	0.052 [†]
MMSE	28.75 ± 1.49	29.00 ± 1.26	27.17 ± 3.81 ^c	22.65 ± 3.25 ^{bde}	<0.001

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E; CN, cognitively normal; *LRP1*, low-density lipoprotein receptor-related protein 1; M/F, male/female; MCI, mild cognitive impairment; MMSE, mini-mental state examination; SCD, subjective cognitive decline.

[†]p values were obtained using χ^2 test; other p values were obtained by one-way ANOVA, but if the Shapiro-Wilk test or the Levene's homogeneity of variance test $p < 0.05$, values were acquired by Kruskal-Wallis test. Descriptive statistical values were presented as numbers for categorical variables and mean ± standard deviation for continuous variables. Post hoc analyses were performed with Bonferroni correction ($p < 0.05/6 = 0.0084$): ^a, statistical difference between MCI group and CN group; ^b, statistical difference between AD group and CN group; ^c, statistical difference between MCI group and SCD group; ^d, statistical difference between AD group and SCD group; ^e, statistical difference between AD group and MCI group. No difference was found between SCD group and CN group.

FIGURE 1 Main effect and interaction between disease status and *LRP1* genotype on the DMN across all subjects. (A) Brain regions with main effect of disease status on the DMN were identified and presented with node map. (B) Numerical representations of significant main effect of disease status on the DMN were illustrated in bar charts. (C) Brain regions significantly affected by *LRP1* genotypes on the DMN in *LRP1*-T carriers (*LRP1*^T) compared with *LRP1*-T non-carriers (*LRP1*^T) were plotted with node map. (D) Numerical representations of the significant main effect of *LRP1* genotype on the DMN were described in bar charts. (E) Brain regions with significant interactive effects between *LRP1* genotype and disease on the DMN. (F) Linear trend of functional connectivity (FC) drawn via line charts represents the significant interactive effects of *LRP1* genotype and disease status on the DMN. Notably, the trajectory changes of mean FC in *LRP1*-T carriers are opposite to *LRP1*-T non-carriers. Nodes' colors and sizes in Figure A, C, and E indicate the plus and minus sign and variance of F value. Bar represents mean and standard error of mean, and each dot represents averaged functional connectivity of each participant within significant brain regions in Figure B and D. *LRP1*, low-density lipoprotein receptor-related protein 1; DMN, default mode network; CN, cognitively normal; SCD, subjective cognitive decline; MCI, mild cognitive impairment; AD, Alzheimer's disease; FC, functional connectivity; LTPJ, left temporoparietal junction; LIPC, left inferior parietal cortex; LPCC, left posterior cingulate cortex; LRSC, left retrosplenial cortex; LPCUN/LCUN, left precuneus and left cuneus; RPCC, right posterior cingulate cortex; RPCUN/RCUN, right precuneus and right cuneus; LMFG, left middle frontal gyrus; RDLPFC, right dorsal lateral prefrontal cortex.



3.4 | Relationships among LRP1, APOE, and MMSE

The moderation effect analysis revealed that APOE genotype and LRP1 genotype regulated the effects of each other on cognitive

performance across all subjects. Figure 3A shows the moderation model of APOE genotype affecting the effect of LRP1 genotype on MMSE scores. As presented in Figure 3B, a relationship between LRP1 genotype and MMSE scores was moderated by

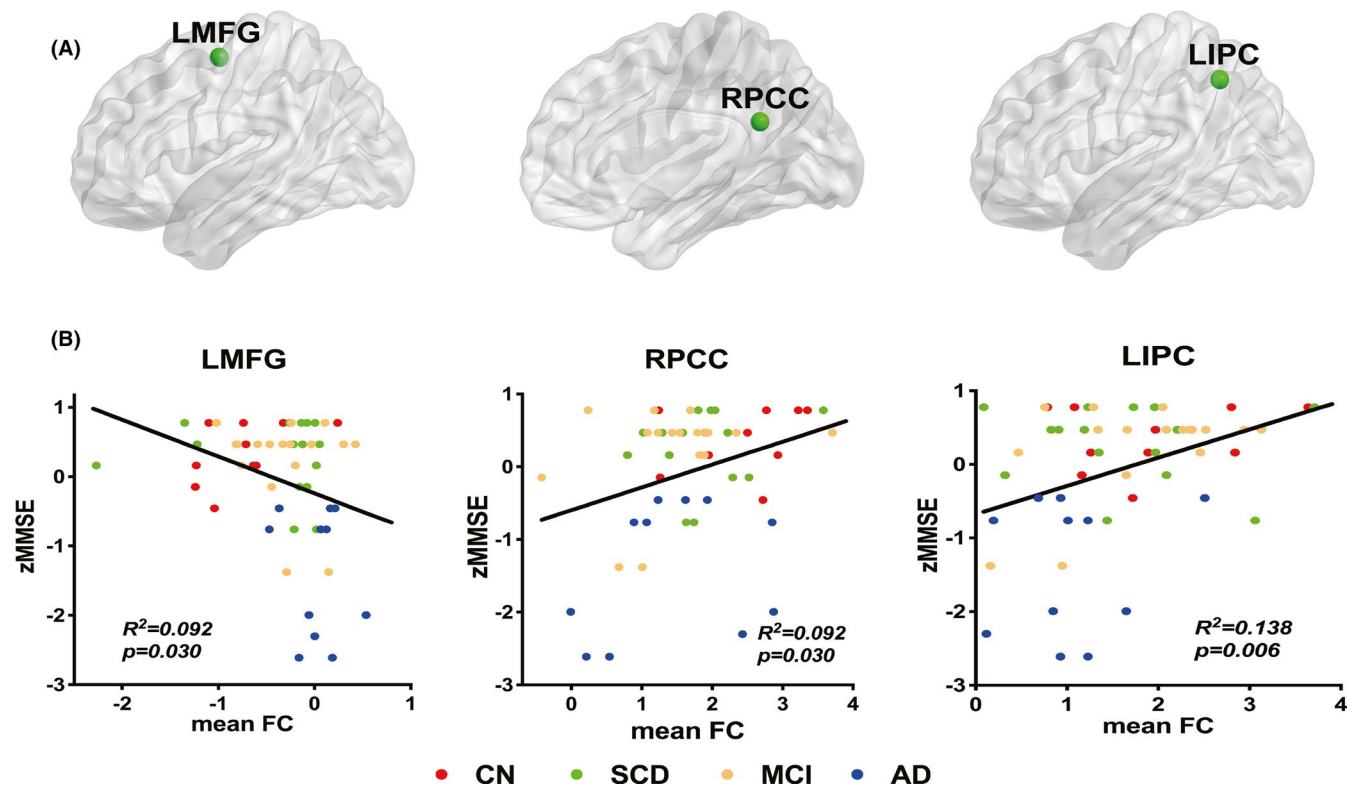


FIGURE 2 Linear regression analyses between functional connectivity and cognitive performance in the *LRP1*-T carriers. (A) Brain nodes diagrams represented altered functional connectivity (FC) values in the regions of LMFG, RPCC, and LIPC. (B) Brain regions of LMFG, RPCC, and LIPC with altered FC could significantly affect the MMSE scores in the *LRP1*-T carriers but not in *LRP1*-T non-carriers. The individual raw scores of MMSE were transformed to z scores to assure data normally distributed. Z transformation formula is $z = (x - \mu) / \sigma$, where x is a specific value, μ is the mean value, and σ is the standard deviation. The significance level was set at $p < 0.05$. LMFG, left middle frontal gyrus; RPCC, right posterior cingulate cortex; LIPC, left inferior parietal cortex; MMSE, mini-mental state examination.

APOE genotype; that is because the interaction of *LRP1* genotype and *APOE* genotype significantly affected cognitive performance ($\beta = 0.946$, $p = 0.004$). Similarly, Figure 3C displays the model of the *LRP1* genotype as a moderator affecting the casual correlation of *APOE* genotype to MMSE scores. The relationship between *APOE* genotype and MMSE scores depended on the moderation of the *LRP1* genotype (Figure 3D). Specifically, *APOE* genotype and *LRP1* genotype interaction significantly affected cognition ($\beta = 0.946$, $p = 0.004$). Therefore, the effects of *LRP1* and *APOE* on cognition are moderated dependent on each other.

Furthermore, the interactive effect analysis disclosed that the effect of *LRP1* genotype on MMSE scores was dependent on different levels of *APOE* genotype. Figure 3E presents the main effect of *LRP1* on MMSE was insignificant ($F = 0.510$, $p = 0.476$), yet that of *APOE* was significant ($F = 6.877$, $p = 0.010$). As evident in Figure 3F, the interactive effect of *LRP1* genotype and *APOE* genotype on MMSE scores was important ($F = 8.546$, $p = 0.004$). Since the *LRP1* genotype shows opposing effects at both levels of *APOE* genotype, the main effect of the *LRP1* genotype is balanced out. Nonetheless, the *LRP1* genotype affects MMSE (see moderation effect above). These details of moderation and interactive effect are shown in Tables S3 and S4.

3.5 | Stage-dependent neuroimaging biomarker of FC for classifying AD spectrum population

As displayed in Figure 4, ROC analysis indicated that FC of numerous brain regions produced a strong power for classifying different disease stages specifically in the *LRP1*-T carriers but not in non-carriers. In the brain area of LMFG, FC was detected as the predictive variable with AUC of 0.744 to discriminate SCD from CN, AUC of 0.771 to discriminate MCI from CN, AUC of 0.891 to discriminate AD from CN, and AUC of 0.756 to discriminate AD from SCD. Also, FC of LIPC helped discriminate AD from MCI and FC of RPCC discriminate MCI from CN. Besides, all AUC values were more than 0.7, indicating the strong power of these predictive variables to discriminate disease stages.

4 | DISCUSSION

This study demonstrates the interactive effect of *LRP1* genotype and disease status on the DMN that primarily focused on the core hubs of the frontal-parietal network and shows the opposite trajectory changes of FC within DMN in the *LRP1*-T carriers compared

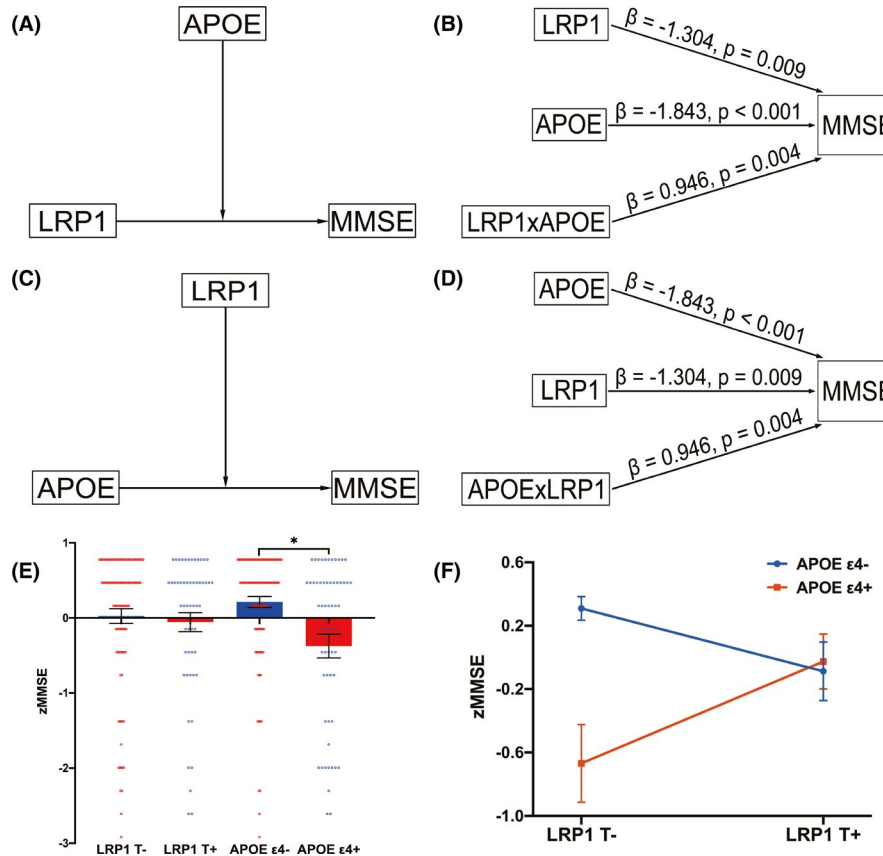


FIGURE 3 The relationships between *LRP1*, *APOE*, and MMSE across all subjects. (A) The moderation model of *APOE* genotype influencing the effect of *LRP1* genotype on MMSE scores. (B) The statistical diagram presents the effects of *LRP1* genotype, *APOE* genotype, and interaction of *LRP1* and *APOE* on MMSE scores. Moderation effect analysis revealed that *APOE* could modulate the relationship between *LRP1* and cognitive performance. (C) The moderation model of *LRP1* genotype influencing the effect of *APOE* genotype on MMSE scores. (D) The statistical diagram presents the effects of *APOE* genotype, *LRP1* genotype, and interaction of *LRP1* and *APOE* on MMSE scores. Moderation effect analysis revealed that *LRP1* could modulate the relationship between *APOE* and cognitive performance. (E) The interaction of *LRP1* genotype (T^-/T^+) and *APOE* genotype ($\epsilon 4^-/\epsilon 4^+$) on z-transformed MMSE values. Bar represents mean and standard error of mean, and each dot represents z-transformed MMSE scores of each participant within every genotype group. The main effect of *LRP1* on MMSE was insignificant ($F = 0.510$, $p = 0.476$), but that of *APOE* was significant ($F = 6.877$, $p = 0.010$). * $p < 0.05$. (F) The interactive effect of *LRP1* genotype (T^-/T^+) and *APOE* genotype ($\epsilon 4^-/\epsilon 4^+$) on MMSE scores was significant ($F = 8.546$, $p = 0.004$). Interactive effect analysis revealed that the effect of *LRP1* on MMSE was dependent on different levels of *APOE* genotype. *APOE*, apolipoprotein E; *LRP1*, low-density lipoprotein receptor-related protein 1; MMSE, mini-mental state examination.

with non-carriers. This dichotomous pattern suggests that these vulnerable brain regions across the AD spectrum undergo different temporal and spatial patterns of progression. More importantly, we found that FCs correlated with cognitive performance in the *LRP1*-T carriers but not in the *LRP1*-T non-carriers. *APOE* and *LRP1* could regulate the effect of each other on cognitive performance. Furthermore, we confirmed that the disrupted FCs potentially classify the AD spectrum population and act as a potential neuroimaging biomarker, specifically in the *LRP1*-T carriers. These findings imply that the *LRP1* gene rs1799986 variant polymorphism consistently affects the DMN FC changes across the AD spectrum population and provides a stage-dependent neuroimaging biomarker for early identification of the AD spectrum.

We detected the neural correlates of the *LRP1* genotype and disease status on the DMN along the AD spectrum. These brain regions belong to the frontal-parietal network, which governs the

cascade of attentional processes underlying the complex cognitive functions.²⁷⁻²⁹ Besides, they are vulnerable areas of AD progression, as reported formerly.³⁰⁻³³ Interestingly, the distribution pattern of FC in the LTPJ, LIPC, LPCC, and LRSC exhibited an inverted-U shape, suggesting that the altered FC strengths of these regions may compensate for cognitive decline in the MCI stage but decompensation occurred in the AD stage.³⁴ The FC changes in the LPCUN/LCUN, RPCC, and RPCUN/RCUN manifested a U-shape pattern, indicating a disruption in these regions-related networks at the early stage of AD; however, compensation occurred until the AD stage.^{35,36} Then, the FC strength of regions affected by the *LRP1* genotype presented an increased distributed tendency in the *LRP1*-T carriers compared with non-carriers. This T allele dose-dependent distribution suggested that compensation mechanism occurred in the LPCC, RPCC, and LMFG. Notably, the posterior cingulate cortex (PCC) is an identical brain area influenced by both the *LRP1* genotype and

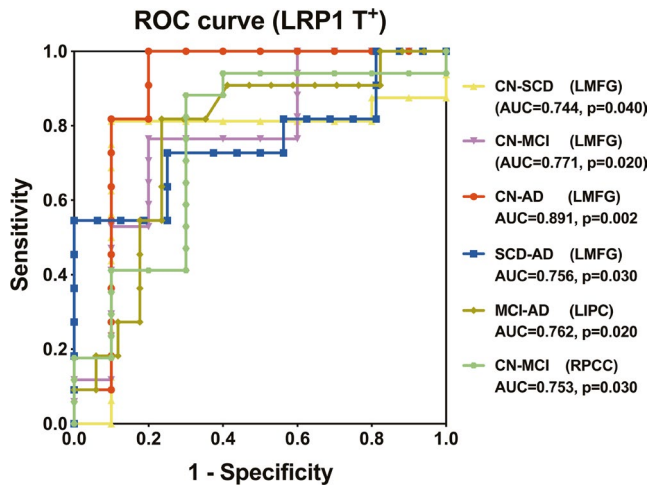


FIGURE 4 Stage-dependent neuroimaging biomarker for classifying AD spectrum population in the *LRP1-T* carriers. Only in the *LRP1-T* carriers, functional connectivity (FC) values of numerous brain regions were found to be predictive variables in classifying SCD from CN (AUC = 0.744, $p = 0.040$ for LMFG), MCI from CN (AUC = 0.771, $p = 0.020$ for LMFG), AD from CN (AUC = 0.891, $p = 0.002$ for LMFG), AD from SCD (AUC = 0.756, $p = 0.030$ for LMFG), AD from MCI (AUC = 0.762, $p = 0.020$ for LIPC), MCI from CN (AUC = 0.753, $p = 0.030$ for RPCC). ROC, receiver operating characteristic; AUC, area under curve; LMFG, left middle frontal gyrus; LIPC, left inferior parietal cortex; RPCC, right posterior cingulate cortex; CN, cognitively normal; SCD, subjective cognitive decline; MCI, mild cognitive impairment; AD, Alzheimer's disease.

disease status. As a crucial neural node within DMN, the PCC has been extensively studied in AD spectrum.³⁷⁻⁴⁰ It is considered one of the earliest regions to be affected by AD based on the proposed hypothesis of cascading network failure^{20,21} with early connectivity decreases³⁷ and directional receiving or transmitting information.³⁸

Specifically, the *LRP1-T* carriers and non-carriers yield opposite FC changes within DMN across the entire disease process, which might be interpreted by different neural mechanisms underlying AD progression. Notably, qualitative changes with different trajectories occurred in FC during the transition from SCD to MCI. This indicates that gene effects were mainly reflected in this selectively vulnerable transition process. Nevertheless, the genetic profile of pre-dementia is significantly underexplored. Genetic studies in longitudinal SCD and MCI follow-up may thus provide novel therapeutic targets and improve the existing knowledge of AD.⁴¹ The *LRP1-T* carriers revealed an increased FC in the LMFG and decreased FCs in the LPCC, LRSC, RDLPCF, and RPCC, whereas, the *LRP1-T* non-carriers presented a relatively stable change with the progression of disease. This *LRP1* genotype-related distribution suggested that the increased FC in LMFG likely occurs in *LRP1-T* carriers at a higher risk of AD, which is considered compensatory reallocation of cognitive resources since it is associated with better cognitive performance.⁴² However, the decreased FCs in LPCC, LRSC, RDLPCF, and RPCC seemingly occur in *LRP1-T* carriers at a higher risk of AD. This declining trajectory is broadly consistent with disease progression. On

the other hand, the FC of *LRP1-T* non-carriers has a relatively stable change across four groups, indicating that the *LRP1-C* allele might be a protective factor that prevents functional deterioration.

Moreover, the FCs of LMFG, RPCC, and LIPC correlated with MMSE scores in the *LRP1-T* carriers but not in the non-carriers. This indicates that the presence of risk T allele might partly influence the connection of the brain network with behavior performance. The FC of LMFG negatively correlated with MMSE, which might be explained as FC compensation for cognitive decline as above.⁴² Besides, we detected positive correlations of the RPCC and LIPC FCs with MMSE. This increased FC is broadly consistent with better cognitive performance at earlier disease stages. These findings highlight the role of the *LRP1* genotype in the intrinsic brain function of the AD spectrum population.

As mentioned above, *LRP1* is a key receptor of APOE, and APOE mediates A β pathology depending on *LRP1*.¹⁸ This means that their corresponding genes may be inextricably linked to each other. Previous research also verified that APOE- $\epsilon 4$ is linked to cognitive phenotypes across the AD spectrum.⁴³ In the present study, two simple moderation analyses identified that the *LRP1* genotype and APOE genotype could regulate the effect of each other on cognitive performance. This implies the importance of removing the APOE effect when investigating the relationships of *LRP1* with network and cognition. Further, an interactive effect analysis was performed; this confirmed that the impact of *LRP1* on cognitive scores depends on different levels (opposing direction) of the APOE genotype; hence, it is balanced out ($p > 0.05$) in the interaction model. APOE- $\epsilon 4^+$ could promote cognitive decline even with the absence of the *LRP1-T* allele, whereas, APOE- $\epsilon 4^-$ synergy with *LRP1-T* might act a protective role for a higher cognitive function. These findings indicate that APOE- $\epsilon 4$ is a consistent and strong genetic risk factor for AD. Besides, a strong APOE effect may bridge the connection between the *LRP1* genotype and the complex brain cognition as well as provide a novel outlook on the complex mechanism underlying gene-behavior interaction.

Several studies have confirmed that brain network variables could be used as predictors in the classification of diseases.⁴⁴⁻⁴⁶ The FC values have been applied in distinguishing AD, MCI from CN subjects.⁴⁷ Our group discovered that FCs of numerous brain regions including LMFG, LIPC, and RPCC could classify different disease stages of AD spectrum among all *LRP1-T* carriers but not in non-carriers. Specifically, the FC of LMFG could even classify SCD from the CN group with an AUC value of 0.744. Besides, all AUC values were more than 0.7, suggesting that FCs could yield high accuracy in classifying the AD spectrum. Also, the FCs of LMFG could discriminate SCD, MCI, AD from CN, and AD from SCD. Further, the SVM model cross-validated this discriminating capacity. This suggests that the LMFG may be a vulnerable region susceptible to genetic influence. It is noteworthy that no intrinsic connectivity could distinguish four groups at one time. As such, the disrupted FC can serve as a stage-dependent neuroimaging biomarker in classifying the AD spectrum.

This study has worth-mentioning limitations that should be addressed in future research. First, the study design is cross-sectional;

thus, incorporating follow-up data is essential to validate the predictive power of FCs for AD spectrum progression. Secondly, a recent study revealed temporal dynamic feature in AD⁴⁸; thus, a prominent future direction might be necessary to evaluate the effect of biological factors on FC dynamics across the progression of AD. Thirdly, LRP1 mediated vascular clearance of A β from the brain⁴⁹; hence, abnormal vascular function might induce cerebral hemodynamic dysregulation and further aggregate A β deposition. Previous studies have reported that microvascular lesions altered A β plaques⁵⁰; local cerebral blood flow responses to neuronal activity were impaired in AD⁵¹; and neurovascular dysregulation even occurred in middle-aged APOE- ϵ 4 carriers.⁵² Nevertheless, aerobic exercise significantly improved hippocampal blood flow in hypertensive APOE- ϵ 4 carriers, which might be beneficial for individuals at high risk of AD.⁵³ Therefore, future work will focus on cerebral vascular issues and design effective intervention strategies to delay the disease progression in preclinical stage of AD. Fourthly, hypotheses such as autophagy⁵⁴ and gut dysbiosis⁵⁵ represented distinct signaling pathways in AD, and more genes were implicated in A β clearance, including APOE,⁵⁶ clusterin,⁵⁷ α 2-Macroglobulin,⁵⁸ and triggering receptor expressed on myeloid cells 2.⁵⁹ Thus, future research will focus on pathway-based polygenic effects on brain networks that can better translate the underlying mechanism of AD.

5 | CONCLUSION

In conclusion, this paper first found the LRP1 gene rs1799986 variant polymorphism could consistently affect DMN patterns across the AD spectrum population. APOE regulated the effect of LRP1 on cognitive performance. The disrupted FCs might be used as a stage-dependent neuroimaging biomarker in classifying the AD spectrum. These findings provide novel insights into the potential mechanism underlying cognitive impairment in AD spectrum progression, and the identification of intervention targets based on genetic risk variants may offer a sneak peek at future research direction.

ACKNOWLEDGEMENT

Data collection and sharing for this project were funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie, Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc; Biogen Idec Inc; Bristol-Myers Squibb Company; Eisai Inc; Elan Pharmaceuticals, Inc; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc; Fujirebio; GE Healthcare; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace,

Inc; Merck & Co., Inc; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc; Piramal Imaging; Servier; Synarc Inc; and Takeda Pharmaceutical Company. 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 (<http://www.fnih.org>). The grantee organization is the Northern California Institute for Research and Education, and the study is 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 Southern California.

CONFLICT OF INTEREST

No authors have any possible conflict of interest.

ORCID

Chunming Xie  <https://orcid.org/0000-0002-5978-9689>

REFERENCES

- Harris-White ME, Frautschy SA. Low density lipoprotein receptor-related proteins (LRPs), Alzheimer's and cognition. *Curr Drug Target CNS Neurol Disord*. 2005;4(5):469-480.
- Sagare AP, Deane R, Zlokovic BV. Low-density lipoprotein receptor-related protein 1: a physiological A β homeostatic mechanism with multiple therapeutic opportunities. *Pharmacol Ther*. 2012;136(1):94-105.
- Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science*. 1992;256(5054):184-185.
- Liu Q, Trotter J, Zhang J, et al. Neuronal LRP1 knockout in adult mice leads to impaired brain lipid metabolism and progressive, age-dependent synapse loss and neurodegeneration. *J Neurosci*. 2010;30(50):17068-17078.
- Kang DE, Saitoh T, Chen X, et al. Genetic association of the low-density lipoprotein receptor-related protein gene (LRP), an apolipoprotein E receptor, with late-onset Alzheimer's disease. *Neurology*. 1997;49(1):56-61.
- Lambert JC, Wavrant-De Vrièze F, Amouyel P, Chartier-Harlin MC. Association at LRP gene locus with sporadic late-onset Alzheimer's disease. *Lancet*. 1998;351(9118):1787-1788.
- Kolsch H, Ptok U, Mohamed I, et al. Association of the C766T polymorphism of the low-density lipoprotein receptor-related protein gene with Alzheimer's disease. *Am J Med Genet B Neuropsychiatr Genet*. 2003;121B(1):128-130.
- Bian L, Yang JD, Guo TW, et al. Association study of the A2M and LRP1 Genes with Alzheimer disease in the Han Chinese. *Biol Psychiatr*. 2005;58(9):731-737.
- Jansen IE, Savage JE, Watanabe K, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat Genet*. 2019;51(3):404-413.
- Kunkle BW, Grenier-Boley B, Sims R, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates A β , tau, immunity and lipid processing. *Nat Genet*. 2019;51(3):414-430.
- Marioni RE, Harris SE, Zhang Q et al. GWAS on family history of Alzheimer's disease. *Translational Psychiatry*. 2018;8(1):99.
- Kang DE, Pietrzik CU, Baum L, et al. Modulation of amyloid beta-protein clearance and Alzheimer's disease susceptibility by the LDL receptor-related protein pathway. *J Clin Investig*. 2000;106(9):1159-1166.

13. Sultana R, Banks WA, Butterfield DA. Decreased levels of PSD95 and two associated proteins and increased levels of BCL2 and caspase 3 in hippocampus from subjects with amnesic mild cognitive impairment: Insights into their potential roles for loss of synapses and memory, accumulation of Abeta, and neurodegeneration in a prodromal stage of Alzheimer's disease. *J Neurosci Res*. 2010;88(3):469-477.
14. Van Gool B, Storck SE, Reekmans SM et al. LRP1 Has a predominant role in production over clearance of Abeta in a mouse model of Alzheimer's disease. *Mol Neurobiol*. 2019;56(10):7234-7245.
15. Storck SE, Pietrzik CU. Endothelial LRP1 - A potential target for the treatment of Alzheimer's disease : Theme: drug discovery, development and delivery in Alzheimer's disease guest Editor: Davide Brambilla. *Pharm Res*. 2017;34(12):2637-2651.
16. Rauch JN, Luna G, Guzman E, et al. LRP1 is a master regulator of tau uptake and spread. *Nature*. 2020;580(7803):381-385.
17. Seok H, Lee M, Shin E et al. Low-dose pioglitazone can ameliorate learning and memory impairment in a mouse model of dementia by increasing LRP1 expression in the hippocampus. *Sci Rep*. 2019;9(1):4414.
18. Tachibana M, Holm ML, Liu CC, et al. APOE4-mediated amyloid-beta pathology depends on its neuronal receptor LRP1. *J Clin Invest*. 2019;129(3):1272-1277.
19. Strickland MR, Holtzman DM. Dr. Jekyll and Mr. Hyde: ApoE explains opposing effects of neuronal LRP1. *J Clin Invest*. 2019;129(3):969-971.
20. Jones DT, Knopman DS, Gunter JL, et al. Cascading network failure across the Alzheimer's disease spectrum. *Brain*. 2016;139(Pt 2):547-562.
21. Damoiseaux JS, Prater KE, Miller BL, Greicius MD. Functional connectivity tracks clinical deterioration in Alzheimer's disease. *Neurobiol Aging*. 2012;33(4):828 e19-30.
22. Palmqvist S, Scholl M, Strandberg O et al. Earliest accumulation of beta-amyloid occurs within the default-mode network and concurrently affects brain connectivity. *Nat Commun*. 2017;8(1):1214.
23. Aisen PS, Petersen RC, Donohue M, Weiner MW. Alzheimer's disease neuroimaging initiative 2 clinical core: progress and plans. *Alzheimers Dement*. 2015;11(7):734-739.
24. Saykin AJ, Shen L, Foroud TM, et al. Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: Genetics core aims, progress, and plans. *Alzheimers Dement*. 2010;6(3):265-273.
25. Hayes AF, Rockwood NJ. Regression-based statistical mediation and moderation analysis in clinical research: Observations, recommendations, and implementation. *Behav Res Ther*. 2017;98:39-57.
26. Chang C, Lin C. LIBSVM: A library for support vector machines. *ACM Trans Intell Syst Technol*. 2011;2(3):1-27.
27. Johnson EL, King-Stephens D, Weber PB, Laxer KD, Lin JJ, Knight RT. Spectral imprints of working memory for everyday associations in the frontoparietal network. *Front Syst Neurosci*. 2018;12:65.
28. Duncan J. The structure of cognition: attentional episodes in mind and brain. *Neuron*. 2013;80(1):35-50.
29. Stoewer S, Ku SP, Goense J, et al. Frontoparietal activity with minimal decision and control in the awake macaque at 7 T. *Magn Reson Imaging*. 2010;28(8):1120-1128.
30. Qi H, Liu H, Hu H, He H, Zhao X. Primary disruption of the memory-related subsystems of the default mode network in Alzheimer's disease: resting-state functional connectivity MRI study. *Front Aging Neurosci*. 2018;10:344.
31. Dillen KNH, Jacobs HIL, Kukulja J, et al. Functional disintegration of the default mode network in prodromal Alzheimer's disease. *J Alzheimer's Dis*. 2017;59(1):169-187.
32. Dillen KNH, Jacobs HIL, Kukulja J, et al. Aberrant functional connectivity differentiates retrosplenial cortex from posterior cingulate cortex in prodromal Alzheimer's disease. *Neurobiol Aging*. 2016;44:114-126.
33. Celebi O, Uzdogan A, Oguz KK, et al. Default mode network connectivity is linked to cognitive functioning and CSF Abeta1-42 levels in Alzheimer's disease. *Archiv Gerontol Geriatr*. 2016;62:125-132.
34. Ye Q, Chen H, Su F, et al. An inverse U-shaped curve of resting-state networks in individuals at high risk of Alzheimer's disease. *J Clin Psychiatry*. 2018;79(2):17m11583.
35. Stern Y, Moeller JR, Anderson KE, et al. Different brain networks mediate task performance in normal aging and AD: defining compensation. *Neurology*. 2000;55(9):1291-1297.
36. Qi Z, Wu X, Wang Z, et al. Impairment and compensation co-exist in amnesic MCI default mode network. *NeuroImage*. 2010;50(1):48-55.
37. Yokoi T, Watanabe H, Yamaguchi H, et al. Involvement of the precuneus/posterior cingulate cortex is significant for the development of Alzheimer's disease: a PET (THK5351, PiB) and resting fMRI study. *Front Aging Neurosci*. 2018;10:304.
38. Yu E, Liao Z, Mao D, et al. Directed functional connectivity of posterior cingulate cortex and whole brain in Alzheimer's disease and mild cognitive impairment. *Curr Alzheimer Res*. 2017;14(6):628-635.
39. Yang H, Wang C, Zhang Y, et al. Disrupted causal connectivity anchored in the posterior cingulate cortex in amnesic mild cognitive impairment. *Front Neurol*. 2017;8:10.
40. Bai F, Watson DR, Yu H, Shi Y, Yuan Y, Zhang Z. Abnormal resting-state functional connectivity of posterior cingulate cortex in amnesic type mild cognitive impairment. *Brain Res*. 2009;1302:167-174.
41. Moreno-Grau S, Ruiz A. Genome research in pre-dementia stages of Alzheimer's disease. *Exp Rev Mol Med*. 2016;18:e11.
42. Obler LK, Rykhlevskaia E, Schnyer D, et al. Bilateral brain regions associated with naming in older adults. *Brain Lang*. 2010;113(3):113-123.
43. Saeed U, Mirza SS, MacIntosh BJ, et al. APOE-ε4 associates with hippocampal volume, learning, and memory across the spectrum of Alzheimer's disease and dementia with Lewy bodies. *Alzheimers Dement*. 2018;14(9):1137-1147.
44. Savini G, Pardini M, Castellazzi G, et al. Default mode network structural integrity and cerebellar connectivity predict information processing speed deficit in multiple sclerosis. *Front Cell Neurosci*. 2019;13:21.
45. Ruffle JK, Patel A, Giampietro V, et al. Functional brain networks and neuroanatomy underpinning nausea severity can predict nausea susceptibility using machine learning. *J Physiol*. 2019;597(6):1517-1529.
46. Espinoza FA, Liu J, Ciarochi J, et al. Dynamic functional network connectivity in Huntington's disease and its associations with motor and cognitive measures. *Hum Brain Mapp*. 2019;40(6):1955-1968.
47. Chen G, Ward BD, Xie C, et al. Classification of Alzheimer disease, mild cognitive impairment, and normal cognitive status with large-scale network analysis based on resting-state functional MR imaging. *Radiology*. 2011;259(1):213-221.
48. Gu Y, Lin Y, Huang L, et al. Abnormal dynamic functional connectivity in Alzheimer's disease. *CNS Neurosci Ther*. 2020;26(9):962-971.
49. Ramanathan A, Nelson AR, Sagare AP, Zlokovic BV. Impaired vascular-mediated clearance of brain amyloid beta in Alzheimer's disease: the role, regulation and restoration of LRP1. *Front Aging Neurosci*. 2015;7:136.
50. Zhang Y, Bander ED, Lee Y, Muoser C, Schaffer CB, Nishimura N. Microvessel occlusions alter amyloid-beta plaque morphology in a mouse model of Alzheimer's disease. *J Cerebral Blood Flow Metab*. 2020;40(10):2115-2131.
51. Liu D, Ahmet I, Griess B, Tweedie D, Greig NH, Mattson MP. Age-related impairment of cerebral blood flow response to K(ATP) channel opener in Alzheimer's disease mice with presenilin-1 mutation. *J Cerebral Blood Flow Metab*. 2021;41(7):1579-1591.
52. Dounavi ME, Low A, McKiernan EF. Evidence of cerebral hemodynamic dysregulation in middle-aged APOE ε4 carriers: The

- PREVENT-Dementia study. *J Cerebral Blood Flow Metab.* 2021. <https://doi.org/10.1177/0271678x211020863>. Online ahead of print.
53. Kaufman CS, Honea RA, Pleen J et al. Aerobic exercise improves hippocampal blood flow for hypertensive Apolipoprotein E4 carriers. *J Cerebral Blood Flow Metab.* 2021;41(8):2026–2037.
54. Kuang H, Tan CY, Tian HZ, et al. Exploring the bi-directional relationship between autophagy and Alzheimer's disease. *CNS Neurosci Ther.* 2020;26(2):155-166.
55. Cho J, Park YJ, Gonzales-Portillo B, et al. Gut dysbiosis in stroke and its implications on Alzheimer's disease-like cognitive dysfunction. *CNS Neurosci Ther.* 2021;27(5):505-514.
56. Cramer PE, Cirrito JR, Wesson DW, et al. ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models. *Science.* 2012;335(6075):1503-1506.
57. Mulder SD, Nielsen HM, Blankenstein MA, Eikelenboom P, Veerhuis R. Apolipoproteins E and J interfere with amyloid-beta uptake by primary human astrocytes and microglia in vitro. *Glia.* 2014;62(4):493-503.
58. Cascella R, Conti S, Tatini F, et al. Extracellular chaperones prevent Abeta42-induced toxicity in rat brains. *Biochem Biophys Acta.* 2013;1832(8):1217-1226.
59. Wang Y, Cella M, Mallinson K, et al. TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. *Cell.* 2015;160(6):1061-1071.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Zang F, Zhu Y, Zhang Q, Tan C, Wang Q, Xie C; Alzheimer's Disease Neuroimaging Initiative. APOE genotype moderates the relationship between LRP1 polymorphism and cognition across the Alzheimer's disease spectrum via disturbing default mode network. *CNS Neurosci Ther.* 2021;00:1–11. <https://doi.org/10.1111/cns.13716>