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Shared effects of the clusterin gene on the default mode network among individuals at risk for Alzheimer's disease

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Summary

Aims: To explore the common effects of the clusterin (*CLU*) rs11136000 variant on the default mode network (DMN) in amnestic mild cognitive impairment (aMCI) subjects and remitted geriatric depression (RGD) subjects.

Methods: Fifty-one aMCI subjects, 38 RGD subjects, and 64 cognitively normal elderly subjects underwent resting-state fMRI scans and neuropsychological tests at both baseline and a 35-month follow-up. Posterior cingulate cortex seed-based functional connectivity (FC) analysis was used to obtain the DMN patterns.

Results: A *CLU* gene×disease×time interaction for aMCI subjects was mainly detected in the core cortical midline structures of the DMN, and the interaction for RGD subjects was mainly detected in the limbic system. However, they overlapped in two frontal regions, where consistent effects of the *CLU* gene on FC alterations were found between aMCI and RGD groups. Furthermore, the alterations of FC with frontal, parietal, and limbic regions compensated for episodic memory impairments in *CLU*-CT/TT carriers, while no such compensation was found in *CLU*-CC carriers.

Conclusion: The *CLU* gene could consistently affect the DMN FC with frontal regions among individuals at risk for Alzheimer's disease, and the *CLU*-T allele was associated with more compensatory neural processes in DMN changes.

KEYWORDS

Alzheimer's disease, clusterin, default mode network, geriatric depression, mild cognitive impairment

1 | INTRODUCTION

Clusterin (*CLU*), an extracellular chaperone protein, is richly expressed within the central nervous system and plays an important role in Alzheimer's disease (AD) pathogenesis.[1] *CLU* can inhibit amyloid beta (Aβ) deposition and facilitate Aβ transport across the blood-brain barrier.[2] The C allele of the rs11136000 single nucleotide polymorphism in the *CLU* gene is the third strongest genetic risk factor for sporadic AD.[3] The C allele confers greater cognitive impairments and a 1.16 greater odds of developing sporadic AD than the T allele.[4] In a sense, the minor T allele has been considered as a protective factor.

Using resting-state fMRI, a series of functionally relevant resting-state networks were identified according to the temporal correlations between intrinsic fluctuations of blood-oxygen-level-dependent signals in different brain areas, also known as functional connectivity (FC).[5] Altered FC patterns of the default mode network (DMN) have been demonstrated across the AD spectrum and can predict the severity and progression of AD.[6,7] Studies exploring the association between Aβ deposition and DMN patterns have suggested that aberrant DMN changes may be induced by Aβ deposition before the onset of any clinical symptoms.[8,9] As mentioned above, the *CLU* gene could affect Aβ pathology, and thus, it would not be surprising for there to exist a regulation of the *CLU* gene on DMN patterns. However,

our recent cross-sectional study did not find any impact of the *CLU* rs11136000 variant on the DMN itself in either healthy elderly subjects or subjects with amnestic mild cognitive impairment (aMCI, an intermediate state between normal aging and early AD that is characterized by episodic memory loss).[10] The reasons may include the limited sample size and the multiple regions-of-interest-based methods used in DMN reconstruction, both of which may reduce the sensitivity of DMN alterations.

Both aMCI and geriatric depression are very common in the elderly population and confer an increased risk for developing AD.[11] A high prevalence of depressive symptoms has been found among MCI patients,[12] and cognitive impairments are also common in depressed patients.[13] The coexistence of aMCI and depression significantly increase the risk for AD.[14] Furthermore, increased Aβ deposition has been shown not only in aMCI patients but also in geriatric depression patients.[15,16] As described above, increased Aβ deposition may induce aberrant DMN changes, and thus, DMN patterns have been investigated in MCI patients and geriatric depression patients. For example, both decreased FC from the posterior to anterior portions of DMN and increased FC with multiple brain regions have been identified in MCI patients.[17-19] Similarly, both decreased DMN FC with the frontal cortex and increased FC with the parietal cortex and the anterior cingulate cortex have been found in geriatric depression patients.[20,21] However, whether the DMN patterns in these subjects can be regulated by the *CLU* gene remains relatively unknown. This study hypothesized that the *CLU* gene could affect the DMN patterns in both aMCI subjects and geriatric depression subjects. In addition, in view of the distinct clinical link between MCI and geriatric depression, we also hypothesized that similar effects of the *CLU* gene on the DMN might exist between aMCI subjects and geriatric depression subjects.

This study recruited cognitively normal elderly subjects, aMCI subjects, and remitted geriatric depression (RGD) subjects. Each group was divided into *CLU*-CC carriers and *CLU*-CT/TT carriers. All subjects underwent resting-state fMRI scans and neuropsychological tests at both baseline and a 35-month follow-up. A series of quantitative DMN FC analyses were performed. The aims of this study were (i) to explore the effects of the *CLU* rs11136000 variant on the longitudinal changing patterns of the DMN in aMCI, RGD, and cognitively normal elderly subjects; (ii) to determine whether aMCI and RGD share a common regulation of the DMN by the *CLU*; and (iii) to identify the behavioral significance of *CLU* gene-related modulation of the DMN.

2 | METHODS

2.1 | Participants

This study was approved by the Affiliated ZhongDa Hospital of Southeast University Research Ethics Committee. Chinese Han participants were recruited using community health screening and media advertisements. Written informed consent was provided by each participant. We initially recruited 87 aMCI subjects, 72 RGD subjects, and 135 cognitively normal elderly subjects. During the follow-up period, 36 aMCI subjects and 34 RGD subjects were lost due to the development of neurological or other psychiatric diseases, relocation to other cities, passing by, nonresponders, and subjective unwillingness. The follow-ups of cognitively normal elderly subjects were paused after comparative numbers of aMCI subjects, RGD subjects, and cognitively normal elderly subjects were obtained. A total of 51 aMCI subjects, 38 RGD subjects, and 64 cognitively normal elderly subjects underwent resting-state fMRI scans and neuropsychological tests at both baseline and follow-up. The mean follow-up period was 35 months. Eight aMCI subjects, five RGD subjects, and four cognitively normal elderly subjects were excluded after the evaluation of head motion artifacts. During the follow-up period, six aMCI subjects converted to cognitively normal elderly subjects and nine cognitively normal elderly subjects converted to aMCI subjects. These subjects were also excluded to decrease the heterogeneity in each group. Finally, there were 37 aMCI subjects, 33 RGD subjects, and 51 control subjects. In the remaining RGD subjects, the average age of onset of depression was 60±9.43 years, and 28 subjects developed depression after the age of 50 years, also known as late-onset depression.[22] These groups were matched for the duration of the follow-up period.

2.2 | Neuropsychological assessments

Each subject underwent a standardized diagnostic evaluation, including demographic information, medical history, and examination of neurological and mental status. See Supporting information.

2.3 | Inclusion and exclusion criteria

All aMCI subjects were included according to the diagnostic criteria proposed by Petersen et al.[23] and others.[24] The inclusion criteria for RGD subjects and control subjects were described previously.[25] See Supporting information.

2.4 | *CLU* genotyping

See Supporting information. In consideration of a dominant model of minor T allele effects,[26,27] each group was divided into *CLU*-CC carriers and *CLU*-CT/TT carriers.

2.5 | Magnetic resonance imaging procedures and image preprocessing

See Supporting information. Because the global signal has recently been thought to be related to major neuronal components, the global signal was not regressed in the present study.[28-30]

2.6 | FC analysis

A 5-mm-radius sphere centered at posterior cingulate cortex (PCC) hub [Montreal Neurological Institute (MNI) space: −2, −45, 34] served as a seed region. For each subject, a mean time series of the seed region was computed as the reference time course. Pearson cross-correlation analysis was carried out between the seed time course and

the time course of the whole-brain voxels. A Fisher's *z*-transformation was applied to improve the normality of the correlation coefficients $z = 0.5 \times ln\frac{1+r}{1-r}$. Finally, individual maps of the DMN were obtained.

2.7 | Statistical analysis

2.7.1 | Demographic and neuropsychological data

Analysis of variance (ANOVA) and chi-square tests (applied for the comparisons of gender and *ApoE* status) were used to compare the demographic data. Mixed analysis of covariance (ANCOVA), with disease, gene, and time as fixed factors, was used to analyze the neuropsychological performances among the six groups with statistically significant differences ($P<.05$), controlling for age, gender, years of education, and *ApoE* status. Two-sample *t* tests and chi-square tests (applied in the comparisons of gender and *ApoE* status) were used for the comparison of the demographic data between included subjects and excluded subjects with significance of $P<.05$. As described in our previous study,[29] the individual raw scores of each cognitive test (except for MMSE) were transformed to *Z* scores. See Supporting information. All statistical procedures utilized SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA).

2.7.2 | Group-level intrinsic connectivity analysis

To analyze the interaction between the *CLU* genotype, disease, and time on the DMN, a whole-brain voxel-wise mixed ANCOVA with *CLU* genotype (*CLU*-CC and *CLU*-CT/TT), disease status (control and aMCI or RGD), and time point (baseline and follow-up) as fixed factors was performed, controlling for age, gender, and years of education (using the full factorial option in SPM8). *ApoE* status was also treated as a covariate. The thresholds were set at a corrected $P<.05$ determined by Monte Carlo simulation for multiple comparisons in the whole brain (voxel-wise $P<.01$, FWHM=6 mm, cluster size>1080 mm^3). Then, the average FC strength of each significant region associated with the *CLU* genotype×disease×time point interaction was extracted in each subgroup at baseline and follow-up using the Resting-State fMRI Data Analysis Toolkit (REST) 1.7. Post hoc tests were performed to explore the difference in FC between groups and longitudinal changes (baseline vs follow-up) of FC within each group using SPSS 19.0 software. The significance level for post hoc tests was set at $P<.05$. It should be noted that ANCOVA was performed in aMCI subjects (aMCI vs control) and RGD subjects (RGD vs control) respectively. Then, the overlaps and nonoverlaps between the interactive regions in aMCI and RGD subjects were further analyzed.

2.7.3 | Correlative analysis between FC alterations and episodic memory impairments

Correlation analyses were performed between the longitudinal changes of FC in each interactive region and the longitudinal changes in episodic memory scores in each group. It should be noted that the correlative analysis was focused on episodic memory for the following reasons. First, in the present study, episodic memory was the most impaired cognitive domain in both aMCI and RGD subjects. Second, many studies have suggested that episodic memory is subserved by the DMN.[31-33] All statistical procedures for correlation analysis utilized SPSS 19.0 software. The significance level for correlation analysis was set at $P<.05$.

3 | RESULTS

3.1 | Demographic and neuropsychological data

As shown in Table 1, compared with control subjects, RGD subjects had less years of education. No other significant difference was observed in the demographic characteristics or the *ApoE* status between all groups. A significant main effect of disease was shown in the neuropsychological data. Compared with control subjects, both aMCI subjects and RGD subjects displayed impairments in all cognitive domains, except the information processing speed for RGD subjects. Compared with aMCI subjects, RGD subjects displayed poorer performance in all cognitive domains except for visuospatial function. A main effect of the *CLU* gene was also shown. *CLU*-CC carriers exhibited poorer performance in information processing speed than *CLU*-CT/TT carriers. Furthermore, a main effect of time revealed longitudinal decreases in MMSE and executive function scores in the whole sample during the follow-up period. There was no significant interaction of disease, *CLU* gene, and time on neuropsychological data (data on interactions of any two factors were not shown). Finally, it should be noted that although some subjects were excluded during analyses, there was no significant demographic difference between included subjects and excluded subjects (Table S1).

3.2 | DMN FC data

The main effects of *CLU* gene, disease, and time are shown in Figures S1 and S2. While comparing aMCI subjects and control subjects, the *CLU* gene×disease×time interaction was detected in the frontal cortex, parietal cortex, and cingulate cortex, most of which belong to the core cortical midline structures of the DMN (Figure 1A). While comparing RGD subjects and control subjects, the *CLU* gene×disease×time interaction was primarily observed in the limbic regions and small parts of cerebral cortex (Figure 1B). Specifically, the overlaps between the interactive regions in aMCI subjects and those in RGD subjects were found in the left middle frontal gyrus (LMFG) and the left medial orbital frontal gyrus (LMOFG; Figure 1C and Table 2).

Post hoc test: For each overlapping region, no significant difference in FC at baseline or FC changes during follow-up were shown between control subjects, aMCI subjects, and RGD subjects (Table S2). Then, these three groups were divided into six subgroups according to the *CLU* genotype. The six subgroups displayed no significant difference in FC at baseline but did exhibit a significant difference of FC changes during follow-up (Table 3). First, significant regulation by the *CLU* gene on the DMN was shown in each group. In the control group, *CLU*-CT/TT carriers displayed greater increases of FC with the LMFG than *CLU*-CC carriers ($P=.003$). In the aMCI group, *CLU*-CT/TT carriers displayed greater decreases in FC with the LMOFG than

TABLE 1 Demographic and neuropsychological data

	Control		aMCI		RGD		*P*-value			
Items	*CLU*-CC (n=26)	*CLU*-CT/TT (n=25)	*CLU*-CC (n=24)	*CLU*-CT/TT (n=13)	*CLU*-CC (n=21)	*CLU*-CT/TT (n=12)	Disease	Gene	Time	Disease×Gene×Time interaction or chi-square
Age (y)	69.3±5.29	68.4±6.27	67.7±7.37	68.7±5.92	69.1±6.32	66.3±6.17	.518	.293	—	—
Education (y)	12.0±2.75	13.2±3.00	12.0±3.18	12.2±3.90	12.1±3.37	10.3±2.14	.019[b]	.715	—	—
Gender (male:female)	10:16	12:13	14:10	8:5	8:13	2:10	—	—	—	.156
ApoE status (ε4+:−)	6:20	3:22	9:15	3:10	2:19	1:11	—	—	—	.139
MMSE										
Baseline	28.4±1.06	28.2±1.55	27.2±2.74	26.8±1.80	27.6±1.12	27.7±1.44	<.001[a,b,c]	.299	.024	.757
Follow-up	28.0±1.54	28.4±1.58	26.1±3.53	25.4±4.54	27.2±1.78	27.1±1.62				
Episodic memory										
Baseline	0.58±0.50	0.52±0.57	−0.64±0.56	−0.73±0.65	−0.06±0.64	−0.26±0.71	<.001[a,b,c]	.301	.901	.698
Follow-up	0.54±0.57	0.62±0.61	−0.72±0.70	−0.89±0.80	−0.15±0.78	−0.06±0.65				
Executive function										
Baseline	0.25±0.43	0.51±0.71	−0.23±0.48	−0.46±0.58	0.03±0.58	0.04±0.42	<.001[a,b,c]	.806	.021	.800
Follow-up	0.14±0.52	0.30±0.74	−0.53±0.46	−0.67±0.94	−0.12±0.75	−0.01±0.59				
Visuospatial function										
Baseline	0.28±0.51	0.32±0.80	0.04±0.68	−0.33±0.84	−0.13±0.75	−0.26±0.73	.001[a,b]	.373	.960	.980
Follow-up	0.19±0.88	0.29±0.75	0.04±0.51	−0.21±1.19	−0.14±0.85	−0.28±1.30				
Information processing speed										
Baseline	0.22±0.70	0.44±0.84	−0.38±0.59	−0.37±0.49	0.20±0.75	0.26±0.82	<.001[a,c]	.046	.105	.606
Follow-up	−0.01±0.77	0.23±0.86	−0.65±0.66	−0.59±0.50	0.04±0.76	0.50±0.96				

ANOVA, analysis of variance; ANCOVA, analysis of covariance; MMSE, Mini-Mental State Examination.

[a]*P*<.05, aMCI subjects differs from control subjects.

[b]*P*<.05, RGD subjects differs from control subjects.

[c]*P*<.05, RGD subjects differs from aMCI subjects.

Values are presented as the mean±standard deviation (SD). Chi-square test was applied in the comparisons of gender and *ApoE* status. ANOVA was applied in the comparisons of age and years of education. ANCOVA (with disease, gene, and time as fixed factors) was applied in the comparisons of neuropsychological data, controlling for age, gender, years of education, and *ApoE* status.

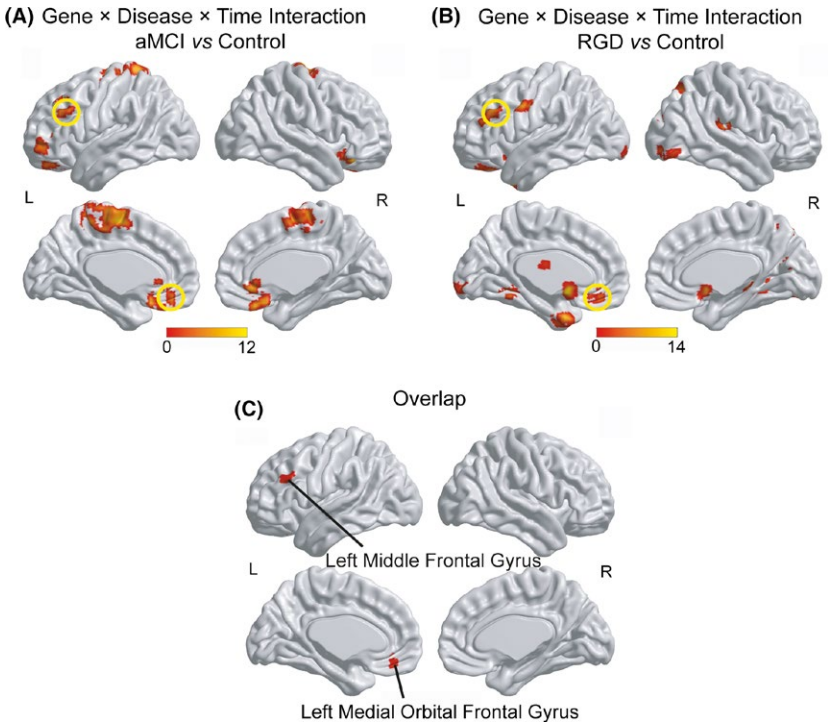

FIGURE 1 *CLU* gene×disease×time interaction on the DMN. (A) The *CLU* gene×disease×time interaction in aMCI subjects and control subjects. (B) The *CLU* gene×disease×time interaction in RGD subjects and control subjects. (C) The overlaps between the interactive regions in aMCI subjects and in RGD subjects. The thresholds were set at a corrected $P<.05$ determined by Monte Carlo simulation for multiple comparisons. L, left; R, right

CLU-CC carriers ($P=.030$). In the RGD group, *CLU*-CT/TT carriers also displayed greater decreases of FC with both the LMFG ($P=.033$) and the LMOFG ($P=.041$) than *CLU*-CC carriers. Second, the trends of longitudinal changes of FC were consistently affected by the *CLU* gene in the aMCI group and the RGD group. In the LMFG, while the control *CLU*-CC subgroup showed longitudinally decreased FC ($P=.024$), both the aMCI *CLU*-CC subgroup ($P=.047$) and the RGD *CLU*-CC subgroup ($P=.012$) displayed longitudinally increased FC; However, while longitudinally increased FC was found in the control *CLU*-CT/TT subgroup ($P=.038$), no significant change was found in the aMCI *CLU*-CT/TT subgroup or the RGD *CLU*-CT/TT subgroup (Figure 2A). In the LMOFG, longitudinal decrease trends of FC were observed in both the aMCI *CLU*-CT/TT subgroup ($P=.118$) and the RGD *CLU*-CT/TT subgroup ($P=.025$; Figure 2B). Third, the extents of longitudinal changes of FC were also consistently affected by the *CLU* gene in the aMCI group and the RGD group (Table 3). In the LMFG, compared with the control *CLU*-CC subgroup, both the aMCI *CLU*-CC subgroup ($P=.002$) and the RGD *CLU*-CC subgroup ($P=.001$) showed greater increases of FC. In the LMOFG, compared with the control *CLU*-CT/TT subgroup, both the aMCI *CLU*-CT/TT subgroup ($P=.024$) and the RGD *CLU*-CT/TT subgroup ($P=.008$) showed greater decreases of FC. On the other hand, in most of the nonoverlapping regions associated with the interaction, *CLU*-CT/TT carriers in both the aMCI group and the RGD group displayed significant longitudinally decreased FC (Figures S3 and S4).

3.3 | Longitudinal changes of DMN FC and behavioral significance

As shown in Table 4, for *CLU*-CT/TT carriers, five longitudinal changes of FC compensated for the declines in episodic memory. For example, in the control *CLU*-CT/TT subgroup, the longitudinal increase of FC with the LMFG was positively correlated with the changes in episodic memory scores ($r=.420$, $P=.046$, two-tailed; Figure 2C); greater increases of FC corresponded to lower episodic memory scores. In the aMCI *CLU*-CT/TT subgroup, greater decreases of FC in the LMOFG were associated with less decline of episodic memory scores ($r=-.650$, $P=.030$, two-tailed; Figure 2D). Only one significant correlation in *CLU*-CT/TT carriers showed harmful effects; decreased FC in the right parahippocampal gyrus was associated with greater decline of episodic memory scores in the RGD *CLU*-CT/TT subgroup (Figure S5). The only significant correlation in *CLU*-CC carriers was shown in the right cuneus in the control group, where the decreased FC was harmful for the changes of episodic memory.

4 | DISCUSSION

The present study is the first to show the effects of the *CLU* gene on the DMN in subjects at high risk of AD. Some adjustments were made relative to our previous study. First, PCC seed-based FC analysis was used to obtain DMN patterns. The PCC is a key region of the DMN, and the PCC seed-based FC analysis has been widely used in DMN construction.[31] Second, the present study employed longitudinal data from a larger sample size. One of the key strengths of the present study was that this study employed two different groups of subjects at high risk of AD. Despite the different core symptoms of aMCI subjects and RGD subjects, both displayed impaired performance in multiple cognitive domains. Differences and similarities were also found in the DMN patterns. The *CLU* gene×disease×time interaction for aMCI subjects was mainly detected in the core cortical midline structures of

TABLE 2 Gene×disease×time interaction on DMN

Brain region	BA	Peak MNI coordinates *x*, *y*, *z* (mm)	Peak *F*-value	Cluster size (mm^3)
(1) aMCI vs control				
Bilateral medial frontal gyri	6	−6, −9, 60	11.1	15 255
Left medial orbital frontal gyrus	11	3, 30, −24	9.98	2349
Left middle frontal gyrus	45	−33, 45, −6	11.3	2727
Left postcentral gyrus	1	−19, −43, 71	8.58	3213
Right anterior cingulate gyrus	32	6, 33, −6	7.61	1161
Right inferior frontal gyrus	47	36, 30, −18	9.56	1755
(2) RGD vs control				
Left calcarine gyrus	18	−9, −99, −9	7.00	1107
Left caudate	25	−3, 6, −9	13.5	1350
Left lingual gyrus	19	−21, −48, −9	10.5	1269
Left medial orbital frontal gyrus	11	−6, 39, −21	8.52	2106
Left middle frontal gyrus	45	−48, 33, 36	11.8	1512
Left precentral gyrus	6	−60, 3, 36	11.3	1080
Left temporal pole	38	−18, 3, −33	13.1	2295
Left thalamus	-	−6, −15, 18	13.2	1620
Right cuneus	18	21, −81, 15	9.50	1080
Right inferior occipital gyrus	18	30, −81, −6	7.33	1215
Right insula	13	42, −27, 18	13.0	1296
Right parahippocampal gyrus	19	24, −33, −9	10.0	1377
Right superior parietal lobule	7	21, −66, 54	6.03	1107
(3) Overlap				
Left middle frontal gyrus	45			1323
Left medial orbital frontal gyrus	11			1863

DMN, default mode network; BA, Brodmann's area; MNI, Montreal Neurological Institute.
The thresholds were set at a corrected *P*<.05, determined by Monte Carlo simulation for multiple comparisons. The overlaps between the interactive regions in aMCI subjects and that in RGD subjects appear in bold.

TABLE 3 Comparisons of FC according to both diagnosis and gene

	Control		aMCI		RGD			
Brain region	*CLU*-CC	*CLU*-CT/TT	*CLU*-CC	*CLU*-CT/TT	*CLU*-CC	*CLU*-CT/TT	*F*-value	*P*-value
FC at baseline								
Left middle frontal gyrus	0.18±0.25	0.07±0.19	0.12±0.23	0.09±0.20	0.01±0.24	0.09±0.19	1.47	.204
Left medial orbital frontal gyrus	0.51±0.21	0.40±0.24	0.44±0.24	0.52±0.28	0.42±0.22	0.48±0.26	0.89	.487
Changes of FC (follow-up−baseline)								
Left middle frontal gyrus	−0.09±0.19	0.07±0.17[a]	0.09±0.21[a]	−0.01±0.21	0.10±0.16[a]	−0.05±0.22[c,d]	3.77	.003
Left medial orbital frontal gyrus	−0.06±0.22	0.03±0.23	0.03±0.27	−0.16±0.34[b,c]	−0.01±0.20	−0.20±0.27[b,c,d]	2.52	.033

FC, functional connectivity.
[a]*P*<.05 compared to the control *CLU*-CC subgroup.
[b]*P*<.05 compared to the control *CLU*-CT/TT subgroup.
[c]*P*<.05 compared to the aMCI *CLU*-CC subgroup.
[d]*P*<.05 compared to the RGD *CLU*-CC subgroup.
Values are presented as the mean±standard deviation (SD). ANOVA was applied in each comparison.

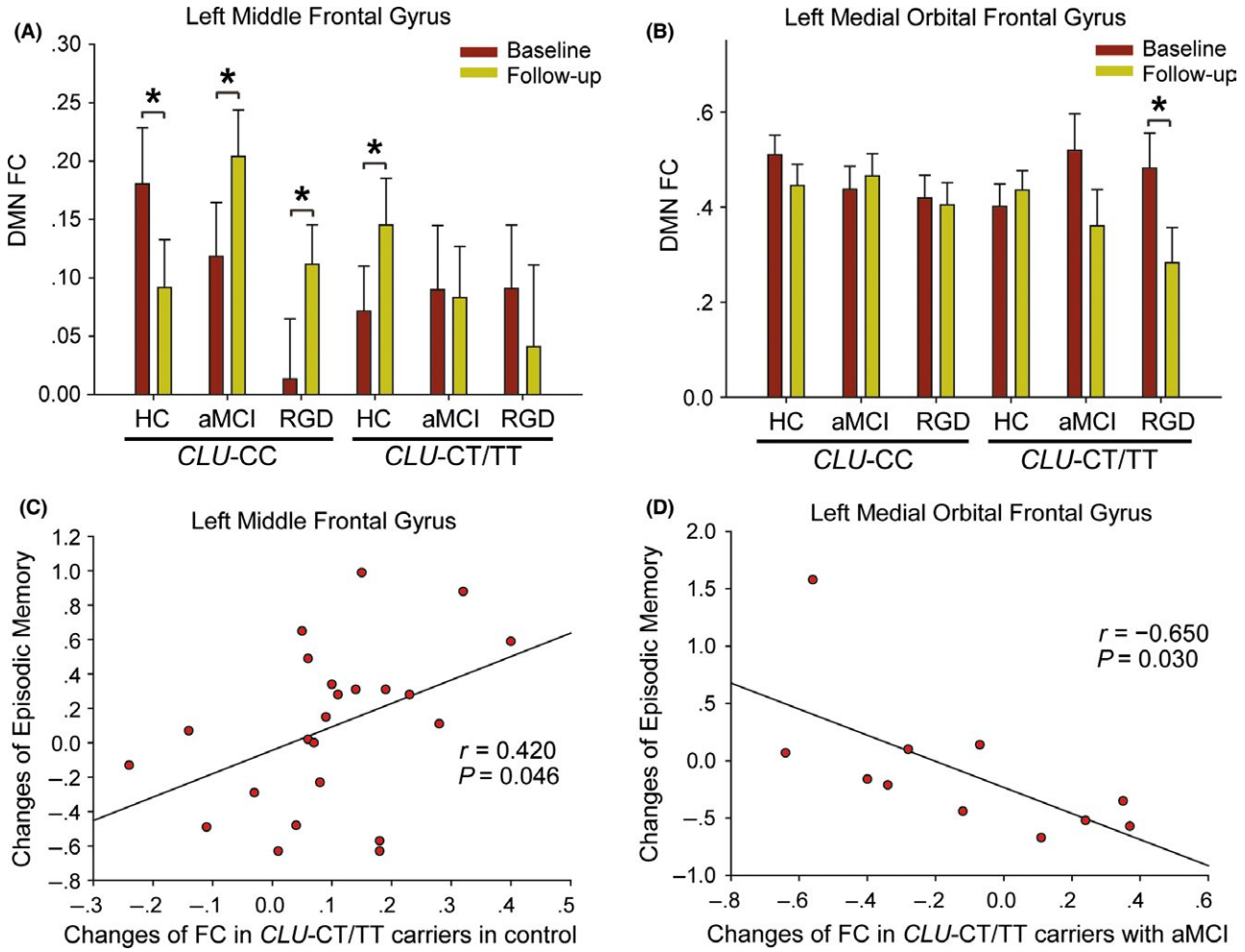

FIGURE 2 Post hoc tests and correlative analyses performed in overlapping regions. (A) In the left middle frontal gyrus, *CLU*-CC carriers in both the aMCI group and the RGD group displayed longitudinally increased FC. (B) In the left medial orbital frontal gyrus, *CLU*-CT/TT carriers in both the aMCI group and the RGD group displayed longitudinal decrease trends of FC. (C) For control *CLU*-CT/TT subgroup, greater increase of FC with the left middle frontal gyrus was associated with less decline in episodic memory scores (r=.420, P=.046, two-tailed). (D) For the aMCI *CLU*-CT/TT subgroup, greater decrease of FC with the left medial orbital frontal gyrus was associated with less decline in episodic memory scores (r=−.650, P=.030, two-tailed). * P<.05. DMN, default mode network; FC, functional connectivity

TABLE 4 Correlative analysis between DMN FC changes and impairments of episodic memory

Group	Brain regions	Longitudinal changes of FC	*r* value	*P*-value	Effects of FC changes on episodic memory
CLU-CC					
Control	Right cuneus	Decreased	.457	.022	Harmful
CLU-CT/TT					
Control	**Left middle frontal gyrus**	Increased	.420	.046	Compensatory
aMCI	**Left medial orbital frontal gyrus**	Decreased	−.650	.030	Compensatory
RGD	Right cuneus	Decreased	−.657	.039	Compensatory
RGD	Right parahippocampal gyrus	Decreased	.666	.018	Harmful
RGD	Right superior parietal lobule	Decreased	−.654	.040	Compensatory
aMCI	Right anterior cingulate	Decreased	−.592	.045	Compensatory

FC, functional connectivity; DMN, default mode network.
The overlaps between the interactive regions in aMCI subjects and in RGD subjects appear in bold.

the DMN, and the interaction in RGD subjects was mainly detected in the limbic system. However, the interactive regions of the two groups overlapped in frontal regions, suggesting that the *CLU* gene could consistently affect the DMN changing patterns in the frontal cortex among subjects at high risk of AD. Interestingly, using DTI, common deficits of structural connectivity patterns between aMCI and RGD patients were also shown in frontal regions in our previous study.[25] These data indicate that the frontal lobe plays an important role in the neural underpinnings of aMCI and RGD.

The other strength of the present work was that the participants were followed for an average of nearly 3 years. As the most important risk factor for AD,[34] aging could affect the DMN patterns at different stages of the disease. For example, aging-related decreases of the DMN FC have been widely reported in cognitively normal elderly subjects.[35,36] At the stage of MCI, both aging-related decreases of DMN FC in the frontal cortex and increases in the prefrontal cortex and the anterior cingulate cortex have been shown.[37] Moreover, the aging-related changes of DMN FC, including increases in the frontal cortex and decreases in major parts of the network, were accelerated in subjects with AD.[36] The present study also found both longitudinally increased DMN FC primarily in frontal regions and decreased FC in extensive regions. Notably, within the two disease groups, all of the significantly increased FC was shown in *CLU*-CC carriers, and all of the decreased FC with significances was shown in *CLU*-CT/TT carriers. The *CLU* gene-related distribution suggested that the increased FC in frontal regions tends to occur in subjects at a much higher risk of AD, that is, with both *CLU*-CC genotype and aMCI or RGD. On the other hand, disease groups with a protective factor, that is, the *CLU*-T allele, tended to display extensively decreased DMN FC.

Increased fMRI activity and FC in the frontal cortex have been generally considered as compensatory recruitment or reallocation of cognitive resources because they have been associated with relatively better performance in cognitive tasks.[38] However, several studies found that increased activity and FC were associated with relatively worse performance.[39,40] One study demonstrated that the reduction of brain activities could even improve cognitive function in aMCI patients.[39] Another study found that reduced resting-state FC was associated with increased task-related activity, which could compensate to maintain cognitive performance.[41] In the present study, for *CLU*-CT/TT carriers, both the increased FC with frontal regions in the control group and the decreased FC with parietal and limbic regions in the aMCI and RGD groups compensated for episodic memory declines. We concluded that the effects of FC changes in episodic memory depended not only on the FC changing patterns, that is, increases or decreases, but also on the risk factors associated with each subject, including the disease status and the *CLU* rs11136000 variant; *CLU*-CT/TT carriers tended to display increased DMN FC, especially in the frontal cortex, as compensatory neural processes during normal aging, but decreased FC in parietal and limbic regions as compensation after the onset of cognitive impairments. Increased FC compensation for cognitive decline may occur in normal aging. However, persistent hypersynchrony or hyperactivity could be harmful for cognitive development after the onset of cognitive impairments.[39,41] Thus, *CLU*-CT/TT carriers displayed decreased FC to compensate for cognitive impairments. This might explain the compensatory effects of both increased and decreased FC in these subjects. However, the significance of the correlative analyses should be validated in a larger sample, and we treat this interpretation with caution. Nevertheless, under similar conditions, that is, similar sample sizes and the same analytic procedure, *CLU*-CT/TT carriers displayed more compensatory FC alterations in the DMN relative to *CLU*-CC carriers.

Thus far, no study has explored the interaction of geriatric depression and the *CLU* gene on the incidence of dementia. However, the present study found that geriatric depression interacted with the *CLU* gene to affect the changing patterns in the DMN. Importantly, the interaction between geriatric depression and the *CLU* gene was highly similar to that between aMCI and the *CLU* gene in the frontal cortex. Even in the nonoverlapping regions, *CLU* gene-related changing trends of DMN FC were also similar between aMCI and RGD subjects. In summary, geriatric depression could interact with the *CLU* gene to result in aMCI-like alterations of the DMN. The interactive effects might be associated with Aβ pathology. Increased brain Aβ deposition has been shown in geriatric depression patients compared with healthy elderly.[16] Healthy elderly with depressive episodes also display high Aβ deposition.[42] Subjects with both geriatric depression and MCI even had Aβ deposition comparable to that in AD.[43] On the other hand, *CLU* gene could also modulate the brain Aβ loads.[44] Therefore, the coexistence of geriatric depression and *CLU* risk variants might considerably influence Aβ pathology, which could be reflected by the DMN patterns. Future studies investigating the interaction of geriatric depression and the *CLU* gene on the incidence of AD will help to confirm these synergistic effects.

The present study has several limitations. First, the sample size was small, especially for testing three-way interactions. During the follow-up period, nine subjects in the control group converted to aMCI subjects and nine subjects in the aMCI group converted to dementia subjects. However, the small number of converters limited further analyses comparing alterations of the DMN between converters and nonconverters. Second, although the interaction of geriatric depression and the *CLU* gene was interpreted based on Aβ pathology, the present study did not utilize any techniques to measure Aβ pathology. Finally, in the post hoc tests, comparisons between subgroups or time points were not corrected for multiple comparisons. Thus, these interpretations should be treated with caution.

5 | CONCLUSION

The *CLU* rs11136000 variant could consistently affect the changing of DMN patterns of aMCI and RGD subjects in the frontal cortex. The *CLU*-T allele was associated with more compensatory neural processes in DMN changes. The findings may help to focus attention to the frontal cortex when investigating the effects of the *CLU* gene on the brain. The interaction of the *CLU* gene and the diagnosis should be considered in future studies. Furthermore, the present findings also indicate that the *CLU* gene might interact with geriatric depression

and be related to risk of developing AD, which should be investigated in the future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

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