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Structural brain characteristics and gene co-expression analysis: A study with outcome label from normal cognition to mild cognitive impairment

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ABSTRACT

Background: Longitudinal studies reported that some elderly people with normal cognition (NC) converted to mild cognitive impairment (MCI), and some remained normal state (NC_S). The underlying factor for this difference conversion of NC is worthy of exploration

Methods: Eighty-three NC participants were tracked for eight years. Thirty participants transitioned from NC to MCI (NC_MCI). The remaining 53 participants retained an NC_S. The structural brain features and genetic expression of the 83 NC participants were obtained. We applied weighted gene co-expression network analysis (WGCNA) to inquire into the co-expression network of those. Mediator effect analysis of regulatory roles was conducted to inquire into the associations between brain measures, expression values, and clinical scores.

Results: The main results are: 1) 20 brain features and 740 gene expression had significant differences between the two groups, 2) one module including 187 genes had the most correlation with cortical thickness of left superior temporal sulcus (L.STS), 3) NFKBIA and RARA genes were the top two genes that made the greatest contribution to L.STS thickness, and 4) mediating effect was found between the L.STS thickness, the NFKBIA and RARA expression levels, and clinical scores.

Conclusion: Our results provide a theoretical foundation based on gene expression and brain imaging for the factors of NC with different outcomes.

1. Introduction

Mild cognitive impairment (MCI) is a transition state between normal cognition and Alzheimer's disease (AD) (Morris, Storandt, Miller, Mckeel, Price, Rubin, and Berg, 2001; Stephan, Hunter, Harris, Llewellyn, Siervo, Matthews, and Brayne, 2012). MCI entails more cognitive and memory decline than normal ageing (Levey, Lah, Goldstein, Steenland, and Bliwise, 2006). Longitudinal studies found that some elderly people with normal cognition (NC) maintained an NC state, and some elderly people developed MCI during follow-up (Morris and Price, 2001). The reason for this difference in conversion from NC is worth exploring. According to the longitudinal follow-up labels of NC, we wanted to determine whether certain factors of this different conversion of NC, such as imaging genetics characteristics, would be evident in individuals when they were in a normal cognitive state. Based on such a problem, we designed this study.

Structural abnormalities of brain morphology, such as a decrease or increase in cortical thickness in particular brain regions, are an important cause of MCI or AD (Filippi et al., 2020; Lerch, Pruessner, Zijdenbos, Hampel, Teipel, and Evans, 2005; Querbes et al., 2009). Aberrant cortical thickness is a potential marker to track neuropathological prefiguration of AD progression (Reiter, Nielson, Smith, Weiss, Alfini, and Smith, 2015). A previous study determined that the thickness of the temporal cortex of MCI patients decreased significantly compared to that of healthy elderly individuals (Vivek, Howard, Lerch, Evans, Dorr, and Jehan, 2006). Beyond this, genetic factors might play a vital role in the degeneration of AD. Complex genetic risk factors are believed to contribute to 70% of AD risk. A largest *meta*-analysis has reported approximately twenty genes to have relationship with the progression and pathogenesis of MCI or AD (Lambert et al., 2013). In genetics, gene

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expression is the basic level at which genotype produce genotype. The genetic code stored in DNA is "translated" by gene expression, and the characteristics of gene expression produce the phenotype of an organism. However, it is unclear what aspects of genetic expression variation are associated with the abnormal cortical characteristics regarding MCI or AD.

Weighted gene co-expression network analysis (WGCNA) is an unsupervised method for clustering genes into co-expression modules (Langfelder and Horvath, 2008). This method can be applied to ascertain potential markers and has been employed in varieties of biological studies (Tao, Han, Yu, Wang, and Zhang, 2020). WGCNA was designed to divide thousands of genes into several modules and allow phenotypic and behavioral characteristics to be associated with tens of modules instead of thousands of individual variables. This method has been used widely in studies of various diseases, such as neurological and psychiatric disorders (Eugenia et al., 2018; Miller, Woltjer, Goodenbour, Horvath, and Geschwind, 2013; Wang et al., 2020). Recent studies have identified several hub genes which have relationship with AD or MCI using WGCNA (Hu, Yu, Zhou, Yin, Hu, Lu, and Hu, 2020; Zhu, Jia, Li, and Jia, 2020). Tang and Liu (2019) identified temporal characteristic networks in AD by WGCNA using expression data from peripheral blood. Otherwise, this method also offers an insight into a scientific co-expression network that may be closely related to the interesting phenotype. Thus, we employed WGCNA to research the associations among brain features, expression values, and clinical scores of NC with different conversion outcomes and preliminarily inquired into the factors affecting transformation of NC.

In this work, the hypothesis is that there is an association between structural brain characteristics and gene expression in patients with outcome label from normal cognition to mild cognitive impairment. 83 participants with NC who were enrolled in an 8-year longitudinally follow-up study were included. During the eight-year period, 30 participants transitioned from normal cognition to MCI (NC_MCI). The other 53 participants remained in a state of NC (NC_S). Differentially expressed genes and morphological characteristic of the cerebral cortex were identified. We employed WGCNA and identified the pivotal modules and genes which have relationship with NC_MCI. Then, we performed correlation and mediator effect analysis to inquire into deeper and subtler results.

2. Materials and methods

2.1. Sample dataset

The sample data is from the ADNI database (Alzheimer's Disease Neuroimaging Initiative database; adni.loni.usc.edu). We followed up 92 participants with NC for approximately eight years. We found that 57 participants maintained a state of NC (NC_S), and 35 participants converted from NC to mild cognitive impairment (NC_MCI) after reviewing the latest visit records. After quality control, 9 subjects (4 in the NC_S group and 5 in the NC_MCI group) were removed.

Detailed information of ADNI, quality control and the timeline of the 30 conversions from NC to MCI are in Supplementary materials and Supplementary Table S1. Further information about inclusion and exclusion criteria used across the ADNI study can be found in the procedure manual (https://adni.loni.usc.edu/wp-content/uploads/2008/07/adni2-proceduresmanual.pdf).

2.2. Ethics approval statement

We confirm that all procedures performed in this study involving human participants were in accordance with the ethical standards of the ADNI consortium Ethics Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent Process was accomplished by ADNI database (adni.loni.usc.edu).

2.3. sMRI data processing

We employed FreeSurfer to conduct the full processing stream for structural MRI data (https://surfer.nmr.mgh.harvard.edu/) to process T1-weighted MRI images (Fischl et al., 2004; Fischl, Salat, Busa, Albert, and Dale, 2002). We firstly transfered the format of MRI image from DICOM to NIFTI. Using the BET, a brain extracting tool, we extracted brain from all T1 images (Smith, 2002). Then, we segmented grey matter, white matter and cerebrospinal fluid from brain images (Wilson, 2002) (Andersson et al., 2007). Thirdly, we registered the grey matter images to the standard template of ICBM152 and smoothed these images with an 8 mm Gaussian kernel using SPM8 (Statistical Parametric Mapping, https://www.fil.ion.ucl.ac.uk/spm). Finally, grey matter was divided into 148 regions (74 brain regions in each hemisphere) using aseg atlas information of FreeSurfer (Fischl, Sereno, And, and Dale, 1999). The detailed steps are in the Supplementary materials. We extracted 7 kinds of brain measures from each region. In the statistical analysis, we used a two-sample T test to obtain the differences of 7*148 features using the conversion time (the mean conversion time is 27.2 months) as a covariate.

2.4. Genetic expression data processing

15,481 gene expression (from peripheral venous blood) data corresponding to the 83 participants is also provided on the ADNI database. Differentially expressed genes (DEGs) were gained between groups applying a R (version 3.6.3) software package limma. DEGs and regions with difference measures were used to next analysis.

2.5. WGCNA analysis

We employed the WGCNA method in R software to explore the relationship between 1,036 (7*148) brain structural features and 15,481 gene expression levels. This is an approach that selects highly co-expression gene modules form DEGs and constructs associations between modules and phenotypes. DEGs were clustered into different modules. Then, the relationship of modules and cortical areas were established. Modules were visualized by using R package. Finally, we extracted genes from the most correlation module, which were used for the next analysis.

2.6. Enrichment analysis

WEB-based Gene Set Analysis Toolkit (https://www.webgestalt.org/) (Liao, Wang, Jaehnig, Shi, and Zhang, 2019) is a powerful tool for gene function annotation. Gene ontology (GO) terms analysis of 740 DEGs from the comparison between NC_S and NC_MCI and 187 genes from the most correlated module were carried out using this online tool. The parameters for the enrichment analysis are: enrichment method: ORA (over-representation enrichment analysis); organism: hsapiens; enrichment categories: geneontology_Biological_Process; ID type: gene symbol; reference list: all mapped entrez gene IDs from the selected platform affy_hc_g110; the reference list can be mapped to 1881 entrez gene IDs and 1335 IDs are annotated to the selected functional categories that are used as the reference for the enrichment analysis; FDR method: Benjamini-Hochberg correction (BH); significance Level: Top 10, $p < 0.01$.

2.7. Correlation analysis

Based on the enrichment analysis results, we extracted the expression values of genes from the top 10 GO biological processes or KEGG pathways that have relationships with the progression of MCI or AD. We also extracted the brain measures and clinical scores of the NC_MCI groups and performed correlation analysis on these values using IBM SPSS Statistics 22.

2.8. Mediator effect analysis

From the results of the correlation analysis, we performed mediating effect analysis using SPSSAU (version 20.0; https://spssau.com/). The mediator pathway that had an effect was defined as $X=>M=>Y$, where X is the independent variable (gene expression), Y is the dependent variable (clinical scores), and M is the mediating variable (brain measures). Z, a regulating variable, regulated the mediator effects on ways of $X=>M$ or/and $M=>Y$.

3. Results

3.1. Demographic results

Age and sex matching in NC_S and NC_MCI groups. Several clinical scores and cortical thickness of the left superior temporal sulcus (L.STS) have remarkable differences ($p < 0.01$, FDR correction, Table 1). The violin plots of between-group difference are shown in Fig. 4 (F-K).

3.2. Differential brain measures and genes

We extracted seven kinds of measures in each brain region. Therefore, we obtained 1036 features of each participant, of which 20 features have remarkable differences ($p < 0.01$, FDR correction), such as the cortical thickness of L.STS. Most of the measures, shown in Supplementary materials: Fig.S1 and Supplementary Table S2, are significant increase in NC_MCI group compared with NC_S group. 740 differential genes were obtained between the NC_S and NC_MCI groups ($\log_2$FC = 1.5, adjusted $p < 0.05$, Benjamini-Hochberg correction) (Supplementary Table S3).

3.3. WGCNA and enrichment analysis results

To ensure a scale-free network, a power $\beta = 4$ was selected as the soft-thresholding (Supplementary materials: Fig. S2). A total of 5 modules (Fig. 1(A)) were identified through hierarchical clustering. Fig. 1(B) shows the heatmap of all genes. Fig. 1(C) and (D) show the eigengene adjacency heatmap and dendrogram of five modules. Fig. 2 shows the module-trait relationships. We observed that the blue the 'blue' module (contained 187 genes) had the most correlation with L.STS thickness ($r = 0.37$, $p = 5*10^{-4}$).

As for the enrichment analysis, 740 differential genes were mainly enriched in protein kinase C signaling, anoikis, pigment metabolic process and neural precursor.

Table 1
Demographic information.

	NC		
	NC_S (n = 53)	NC_MCI (n = 30)	*p* values
Sex (M/F)	53 (21/32)	30 (16/14)	0.227
Age	75.45 ± 5.96	77.57 ± 6.24	0.131
Right/Left handed	53 (48/5)	30 (26/4)	0.669
L.STS thickness	0.48 ± 0.08	0.54 ± 0.07	0.0023**
PHS	0.4587 ± 0.1784	0.5995 ± 0.2241	0.149
ADNI-MEM	1.2 ± 0.34	0.78 ± 0.26	0.0036**
ADNI-EF	0.93 ± 0.14	0.82 ± 0.16	0.491
ADNI-LAN	0.86 ± 0.21	0.84 ± 0.19	0.844
ADNI-VS	0.23 ± 0.08	0.17 ± 0.09	0.246
ADAS-TOTAL13	8.25 ± 1.96	10.96 ± 2.51	0.009**
CDR	0.09 ± 0.019	0.167 ± 0.024	0.001**
MMSE	29.09 ± 1.06	28.63 ± 1.847	0.217

Data are presented as the mean ± sd; p values were obtained from two-sample *T*-tests and 5000 bootstrapping tests (**: p < 0.01, FDR correction). NC: normal cognition; NC_S: maintained a state of NC; NC_MCI: converted from NC to mild cognitive impairment in longitudinal tracking; L.STS: left superior temporal sulcus; PHS: polygenic hazard score; The remaining abbreviations are in Supplementary materials.

cell proliferation (Supplementary materials: Fig. S3). 187 input genes in 'blue' module are unambiguously mapped to 182 unique entrez gene IDs and 5 user IDs could not be mapped to any entrez gene ID. The GO enrichment analysis is based upon the 182 unique entrez gene IDs. These genes were mainly enriched in the NIK/NF-kappa B signaling, interleukin-13 and −4 production, membrane lipid metabolic process, and regulation of peptide secretion (Fig. 3). We extracted the top 10 genes of the enrichment analyses results for the next correlation analysis.

3.4. Correlation analysis results

Correlation analysis with the purpose of finding the correlation between brain characteristics and gene expression, exploring which gene contributing the most to the cortex thickness of L.STS. We obtained two genes those have significant correlation with L.STS thickness: 4792 (nuclear factor kappa-B inhibitor alpha [NFKBIA]: $r = 0.411$, $p < 0.00001$) and 5914 (retinoic acid receptor alpha [RARA]: $r = 0.36$, $p < 0.00001$). The two genetic expression values had no significant correlation with clinical scores. However, cortex thickness of L.STS had a remarkable correlation with ADNI_MEM and CDR scores. The details are shown in Fig. 4 (A-E).

3.5. Regulatory mediator effects

Based on the results of the correlation analysis, both the expression values of the NFKBIA and RARA genes and clinical scores were significantly correlated with L.STS thickness. This motivated us to explore whether there is a mediating effect of L.STS thickness between the expression of the two genes and clinical scores. In this study, we applied ADNI_MEM as the dependent variable in regulatory mediator effects analysis. We found that L.STS cortex thickness assuredly serves as a complete mediator between the ADNI_MEM scores and genetic expression levels. The mediator pathway that had an effect was defined as X=>M=>Y, where X is the independent variable (RARA gene expression), Y is the dependent variable (clinical ADNI_MEM scores), M is the mediating variable (cortical thickness of left superior temporal sulcus (L.STS)), and Z is a regulating variable (high, average or low expression levels of NFKBIA gene) which regulated the mediator effects on ways of X =>M and M => Y pathways (Fig. 5A). Correspondingly, when X is the independent variable (NFKBIA gene expression), Y is the dependent variable (clinical ADNI_MEM scores), M is the mediating variable (cortical thickness of L.STS), and Z is a regulating variable (high, average or low expression levels of RARA gene) which regulated the mediator effects on ways of M => Y pathways (Fig. 5B). Details are in Supplementary Table S4.

4. Discussion

The main results of this study are: 1) 20 brain features and 740 gene expression had significant differences between the two groups, 2) one module including 187 genes had the most correlation with cortical thickness of left superior temporal sulcus (L.STS), 3) NFKBIA and RARA were the top two genes that made the greatest contribution to L.STS thickness, and 4) mediating effect were found between the L.STS thickness, the NFKBIA and RARA expression levels, and clinical scores. Taken together, the factors influencing the conversion of MCI in elderly people have relationship with the thickness of L.STS and expression of NFKBIA and RARA genes.

Previous studies have suggested that changes in cortical thickness and grey matter volume are powerful biomarkers for AD progression (Dickerson and Wolk, 2012; Holbrook et al., 2020). Accurate measurement of cortical thickness or grey matter volume across multiple regions may provide signatures of the disease specific enough to be helpful to the early diagnosis of AD (McEvoy et al., 2009). In this study, 20 significantly different characteristics (80%) mainly focused on cortex

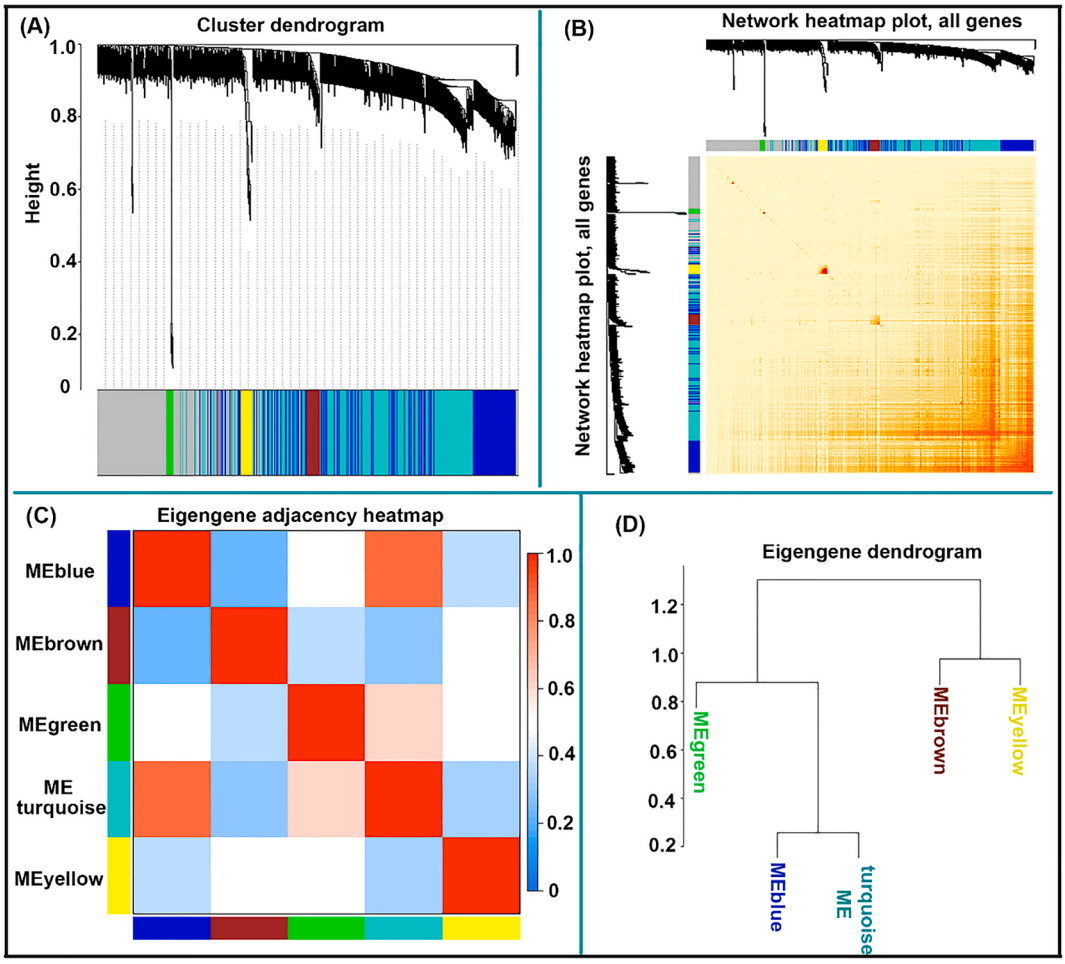

Fig. 1. WGCNA results. (A) cluster dendrogram of all genes; (B) network heatmap of all genes; (C) adjacency heatmap of five modules; (D) eigengene dendrogram of five modules.

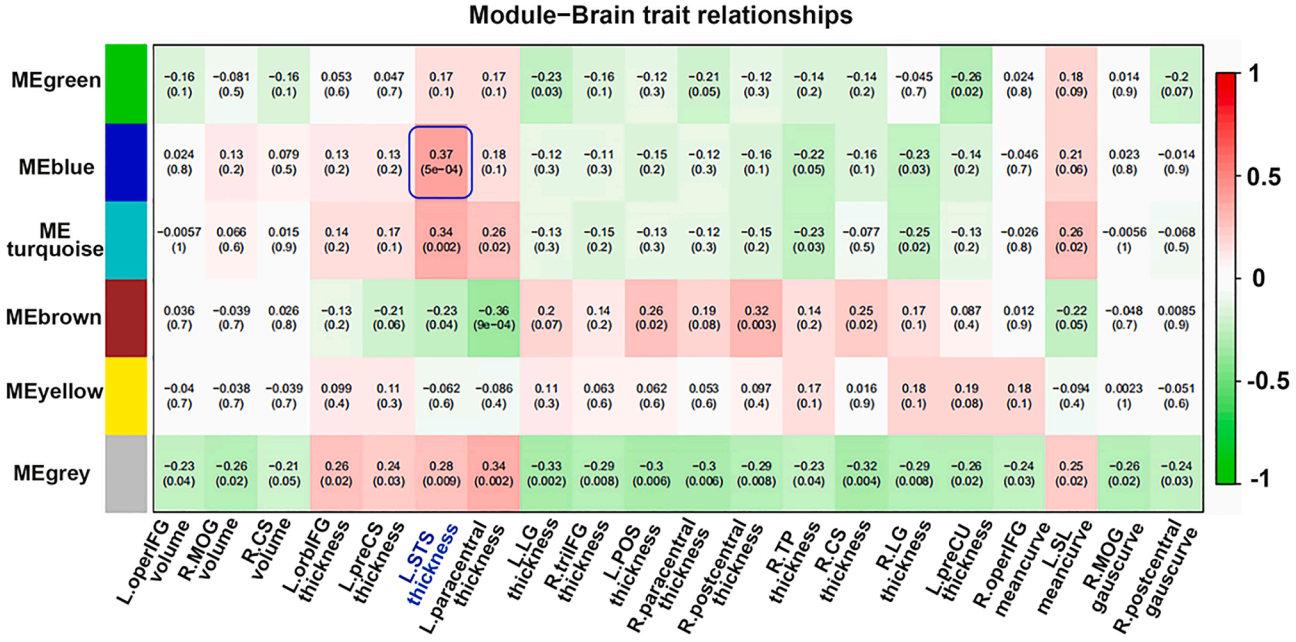

Fig. 2. Gene expression modules and brain traits relationship ($p < 0.01$, FDR correction). L.STS: left superior temporal sulcus. The remaining abbreviations are in Supplementary Table S2.

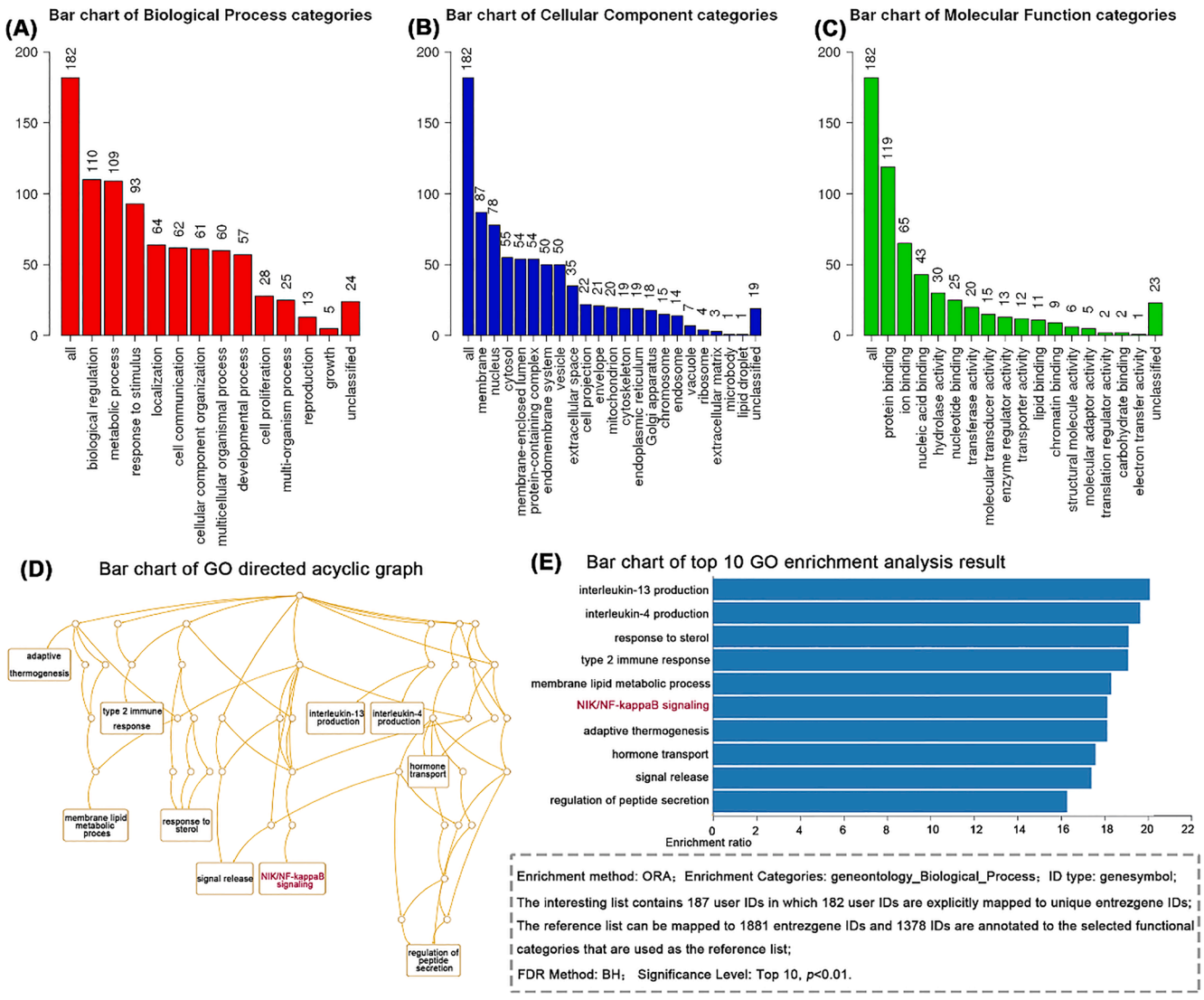

Fig. 3. Enrichment analysis esults of 187 differentially expressed genes. Bar chart of biological process categories (A), cellular component categories (B), molecular function categories (C), GO directed acyclic graph (D), and top 10 GO enrichment analysis result (E).

thickness and volume, such as the thickness and grey matter volume of several temporal and frontal lobes. This result is consistent with previous findings reporting remarkable changes in cortical thickness occurring in the temporal and frontal areas of MCI patients compared with normal controls (Im, Lee, Seo, Kim, Kim, and Na, 2008; Im et al., 2008; Sánchez-Benavides et al., 2010; Seo et al., 2007). These differences in cortical thickness of the temporal and frontal lobes may be one factor that potentially affect the degeneration from NC to MCI. In terms of gene expression, 740 genes were observed between the NC_S and NC_MCI groups. Berchtold et al., (2014) performed a microarray analysis and revealed extensive gene expression differences in a large proportion of brain regions in MCI patients relative to age-matched controls. Our result is consistent with this study. The 740 genes are mainly enriched in response to stimuli, multi-organism processes, developmental processes, and pigmentation. Majority of GO processes are associated with the pathogenesis of MCI. We chose a few genes involved in these GO biological processes to illustrate this relation. Calcium homeostasis modulator 1 (CALHM1) encodes a calcium channel that plays a role in formation of precursor protein of amyloid-β. Post-mortem studies have showed that amyloid-β abnormalities is a pivotal factor that lead to synaptic damage and cognitive decline in AD (Reddy, Manczak, Mao, Calkins, and Shirendeb, 2010). A polymorphism at CALHM1 gene locus has been reported to be related to susceptibility to late-onset AD (Nacmias et al., 2010). Neuronal calcium sensor 1 (NCS1) is a high-affinity calcium-binding protein that has been involved in the adjustment of calcium channels in dopaminergic signaling and axonal regeneration, synaptic plasticity mechanisms, learning and memory behaviors (Bandura and Feng, 2019). A previous study revealed that synapse loss potentially contributes the cognitive declines in ageing and AD, and the extensive declines in synaptic gene expression in normal ageing have suggested that the synaptic function might be hurt (Berchtold, Coleman, Cribbs, Rogers, Gillen, and Cotman, 2013).

WGCNA showed that 187 gene expression values had significant correlation with cortical thickness of L.STS. Enrichment analysis of 187 genes was mainly involved in the positive regulation of inflammatory responses, cellular responses to lipids, ageing, dopaminergic synapse activity, phagocytosis, regulation of cell activation, apoptotic cell clearance, cellular responses to lipopolysaccharides, biological oxidation, and the positive regulation of responses to external stimuli. Karch and Goate (2015) demonstrated that neuroinflammation and dysregulation of the immune response are central features of AD. Xu et al., (2020) explored the relationships between AD risk loci and lipid protein modules using WGCNA and found that lipid modules were correlated with AD risk loci implicated in the lipid metabolism and immune

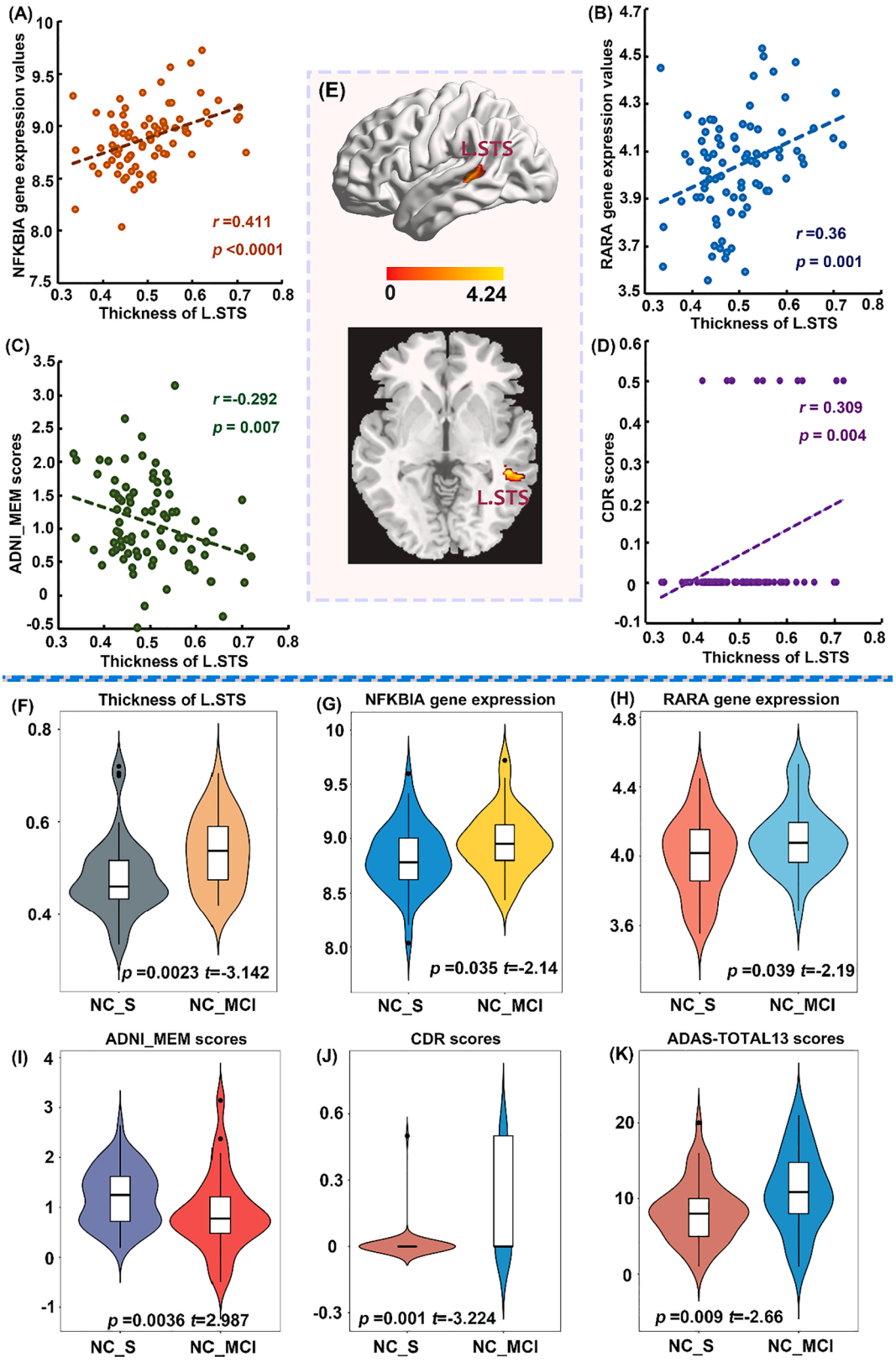

Fig. 4. The between-group differences and Pearson correlation of clinical scores, genetic expression values, and the thickness of L.STS. NFKBIA (A) and RARA (B) gene expression values and ADNI_MEM (C) and CDR (D) scores have remarkable correlations with the cortex thickness of L.STS;(E) is the difference region of L.STS. Between-group difference violin plots of thickness of L.STS (F), NFKBIA gene expression (G), RARA gene expression (H), ADNI_MEM score (I), CDR score (J), and ADAS-TOTAL13 score (K). L.STS: left superior temporal sulcus; NFKBIA: nuclear factor kappa-B inhibitor alpha; RARA: retinoic acid receptor alpha.

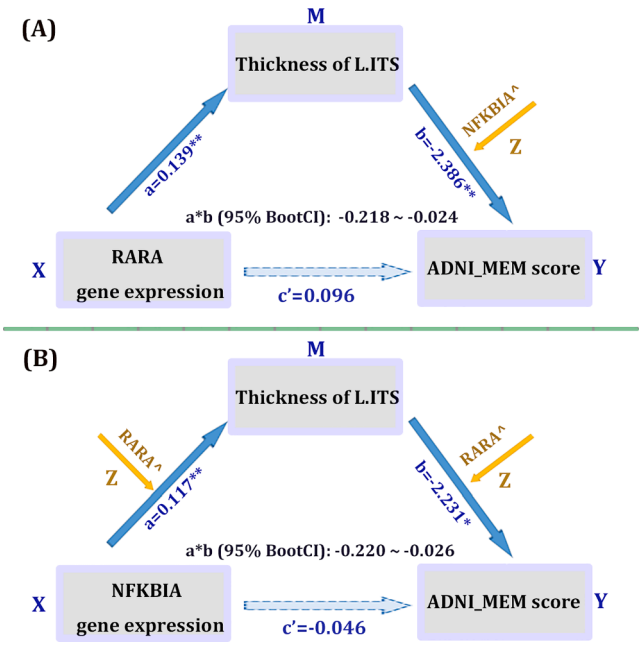

Fig. 5. Regulating mediator effects. Details are in Supplementary Table S4.

response. Hayes et al., (2002) explored the pathologic relationships among tau, amyloid β protein and microglial cell activity in AD and found that the microglial cell activation was prominently correlated with tau load. A previous study suggested that cortical sulcal morphology has a relationship with cognitive performance in elderly individuals (Liu et al., 2011). Our result suggested that the abnormal expression of 187 genes may influence the changes in L.STS cortical thickness and then promote the conversion from NC to MCI. There have been few previous studies on ITS cortical thickness. Therefore, we explored its adjacent structures. The STS links the middle temporal gyrus (MTG) and superior temporal gyrus (STG). STS deformation may reflect morphologic variations in the STG and MTG. Singh et al., (2006) found the most significant thickness difference within the MTG and STG in both the NC-MCI and MCI-AD comparisons. Scheff et al., (2011) observed synaptic loss in the ITG in MCI patients. These results indirectly supported the thickness changes in STS. To further explore the relationship between genetic expression and L.STS cortical thickness, we obtained the gene expression values from the top 10 GO biological processes or KEGG pathways that have relationships with the progression of MCI or AD. We found that the NFKBIA and RARA genes have the greatest contribution to L.STS cortical thickness based on correlation analysis.

NFKBIA encodes an alpha member of the NF-kappa-B (NF-κB) inhibitor family, Neuronal degeneration in the AD brains has relationship with NF-κB activation (Granic, Dolga, Nijholt, van Dijk, and Eisel, 2009). Activated NF-κB can be detected in glial cells that neighbor Aβ plaque areas, and excessive activation of it could leads to an increase in proinflammatory cytokines (Chen, Zhang, Shi, Ai, Qi, and Hang, 2009; Zhang, Yu, Hui, Wu, Yin, Yang, and Xu, 2014). The RARA gene, representing nuclear retinoic acid receptor alpha, has been implicated in the regulation of development, apoptosis, differentiation, granulopoiesis, and transcription of clock genes. Wang et al. (2015) reported that RARA is a key gene of miRNA-138 that can modulate itself to facilitate tau hyperphosphorylation in AD models. The significant positive correlation between expression values of the NFKBIA and RARA and L.STS cortical thickness means that the higher the expression of the two genes, the greater the L.STS cortical thickness. The correlation analysis between L.STS cortical thickness and clinical scores showed that the greater the cortical thickness was, the worse the clinical manifestation was, indicated by measures such as higher CDR scores and lower memory scores. Reiter et al., (2015) reported that cortical thickness is a helpful biomarker to mark neuropathological symptoms of AD and that cortical change is a potential biomarker of AD degeneration. In the early stages of the disease, even in normal cognitive stages, gene expression and cortical thickness are quietly changing, further promoting the conversion from NC to MCI. Our results also implied that neither gene overexpression nor cortical areas that were too thick were good phenomena. However, the effect of gene expression on cortical thickness in the AD brains has rarely been studied. In the future, this effect may be a good topic.

In the correlation analysis between L.STS cortical thickness, clinical scores and expression values of the NFKBIA and RARA genes, we found that the thickness of L.STS was not only significantly correlated with the expression values of the NFKBIA and RARA genes but was also significantly correlated with the clinical measurements of ADNI_MEM scores. It suggested that L.STS thickness is closely related to expression of the NFKBIA and RARA genes and ADNI_MEM scores. Then, is L.STS thickness a link between genetic expression and ADNI_MEM scores and is this mediating effect regulated by the other gene? Our results showed that L.STS thickness has a complete mediating effect between ADNI_MEM scores and genetic expression; this finding implies that the effect of genetic expression on ADNI_MEM scores is mediated by L.STS thickness, although there is no direct effect of genetic expression on ADNI_MEM scores. Combining the results of the analysis of the mediating effect of regulatory roles, the different expression levels of RARA or NFKBIA prominently regulate the mediating effect pathway in which each gene was involved. As for the pathway of NFKBIA => L.STS thickness =>ADNI_MEM. When RARA gene expression was at an average or high level, the L.STS cortical thickness had effect on ADNI_MEM scores, suggesting that RARA gene expression regulated the mediating effect. In the pathway of RARA => L.STS thickness => ADNI_MEM, NFKBIA gene expression level had no regulatory mediating effect on the RARA => L.STS thickness sub-path. Also, when NFKBIA gene expression was at a low or average level, the mediation effect was non-existent; moreover, when NFKBIA gene expression was at a high level, the L.STS thickness had effect on ADNI_MEM scores, suggesting that NFKBIA gene expression regulates the sub-path of L.STS thickness =>ADNI_MEM.

A few concerns should be noticed. Firstly, we observed that cortical thickness of the L.STS is an intermedium between NFKBIA and RARA genetic expression and clinical scores in the conversion from NC to MCI in this study; however, further investigate need be held on specific regulatory mechanism. Secondly, which is the best method for co-expression module detection in WGCNA: still need for further verification. Thirdly, there are many factors that influence MCI or AD except genes and brain structure, such as physical or mental illness, and nutrition; We will examine the effects of these combined factors in the future. Finally, the sample size is also a concern.

5. Conclusion

We used WGCNA to study the association between gene expression and brain imaging features in NC participants with different conversion outcomes and observed that L.STS cortical thickness and NFKBIA and RARA genetic expression are associated with conversion from NC to MCI.

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CRediT authorship contribution statement

Suping Cai: Data curation, Writing – original draft. **Fan Yang:** Visualization. **Xuwen Wang:** Methodology, Software. **Sijia Wu:** Software, Validation. **Liyu Huang:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data used in preparation of this article were obtained from the ADNI database. A complete listing of ADNI investigators can be found at: https://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nlm.2022.107620.

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