The landscape of m6A regulators in multiple brain regions of Alzheimer's Disease

Zijie Liu
Harbin Medical University
Qing Xia
Harbin Medical University
Xue Zhao
Harbin Medical University
Feifei Zheng
Harbin Medical University
Jiaying Xiao
Harbin Medical University
FangLiang Ge
Harbin Medical University
Dayong Wang (wangdayonghmu@126.com)
Harbin Medical University https://orcid.org/0000-0002-9672-3347
Xu Gao
Harbin Medical University

Research Article

Keywords: Alzheimer's disease, m6A, brain regions, FTO, YTHDF2

Posted Date: September 21st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1839644/v2

Abstract

Alzheimer's disease research has been conducted for many years, yet no effective cure methods have been found. N6-methyladenosine (m6A) RNA methylation, an essential posttranscriptional regulation mechanism, has been discovered to affect essential neurobiological processes, such as brain cell development and ageing, which are closely related to neurodegenerative diseases such as Alzheimer's disease. The relationship between Alzheimer's disease and the m6A mechanism still needs further investigation. Our work evaluated the alteration profile of m6A regulators and their influences on Alzheimer's disease in 4 brain regions: the postcentral gyrus, superior frontal gyrus, hippocampus, and entorhinal cortex. We found that the expression levels of the m6A regulators FTO, ELAVL1, and YTHDF2 were altered in Alzheimer's disease and were related to pathological development and cognitive levels. We also assessed the pathways and biological processes related to m6A regulators via the GSVA method, and pathways including N glycan metabolism, amino acid metabolism, and protein metabolism pathways were found to be affected by AD-related m6A regulators. We also found different m6A modification patterns in AD samples among different brain regions, mainly due to differences in m6A readers. Finally, we further evaluated the importance of AD-related regulators based on the WGCNA method, assessed their potential targets based on correlation relationships, and constructed diagnostic models in 3 of all 4 regions using hub regulators, including FTO, YTHDC1, YTHDC2, etc., and their potential targets. This work aims to provide a reference for the follow-up study of m6A and Alzheimer's disease.

Introduction

Alzheimer's disease was first reported by Dr. Alzheimer more than 100 years ago [1], yet researchers are still trying to find a cure and it remains a huge burden on society. Population ageing is making AD (Alzheimer's disease) one of the greatest challenges globally [2]. The diagnosis of Alzheimer's disease is complex. From the earliest NINCDS-ADRDA criteria [3] to subsequent revisions [4], the unique diagnosis pipeline reflects the specificity of the disease. Patients should first be diagnosed with dementia and then require a comprehensive evaluation, including assessment of the levels of biomarkers and the presence of pathological damage to be diagnosed with AD. There are various hypotheses about the pathological cause of Alzheimer's disease. The NINCDS-ADRDA guideline includes Aβ, neurofibrillary tangles, tau protein phosphorylation, and neuritic plaques as pathological diagnostic markers [5], which are also the most recognized hypotheses for the causes of neuropathology and targets of current drug development research [6]. Last year, the Aβ monoclonal antibody drug aducanumab became the first FDA-approved drug targeting the neuropathological cause of Alzheimer's disease [7]. Although the results of aducanumab to improve the cognitive status of patients are still controversial, it does represent an advance in the concept of AD treatment study from symptomatic treatment to targeting neuropathology causes. However, the failure of many other neuropathology-targeting drugs [8, 9] also suggests the need to research the pathological mechanism and drug targets of Alzheimer's disease from different perspectives.

N6-methyladenine (m6A) modification is the most abundant epigenetic modification of eukaryotic mRNA. The m6A mechanism has been reported to participate in the regulation of various mRNA behaviours, including translation [10], degradation [11], splicing [12], and nuclear export [13]. Regulators of m6A that have been discovered to date are divided into 3 types: writers, erasers, and readers. The m6A modification is reversible and dynamic and has been reported to play an indispensable role in the brain, which must execute complex processes that require a flexible regulation method. There have been several recent studies related to m6A and brain function: whole transcriptome sequencing of methylated RNA performed in the mouse medial prefrontal cortex (mPFC) revealed that m6A is involved in the consolidation of fear-related memory [14]. Me-RIP sequencing of mouse forebrain synapses also suggested the essential role of m6A modification in synaptic functioning [15]. FTO, a m6A eraser, has also been reported to be essential for memory formation in the hippocampus [16]. There are also studies reporting a relationship between m6A modification and cell senescence through several genes, including p21 [17], p53, p27 [18], and AGO2 [19]. Considering the relationship between m6A modification, brain development, and ageing, there may be important links between m6A and Alzheimer's disease.

There are already several studies on the relationship between m6A regulators and Alzheimer's disease, including METTL3 [20, 21] and FTO [21–23], without considering their function as m6A regulators. The reader hnRNPG has been reported to be associated with frontotemporal dementia, another neurodegenerative disease that represents tauopathy [24]. There are also recent studies about m6A modification participating in processes of brain development and ageing [25], which are closely related to Alzheimer's disease. A study also suggested that m6A modification profiles are altered in the brains of an Alzheimer's disease mouse model [20, 25]. Although studies have suggested a relationship between Alzheimer's disease and the m6A mechanism, a detailed mechanism between m6A regulators and AD in the human brain is still absent.

In this study, we systematically evaluated the relationship between m6A regulators and AD and found potential target genes of hub m6A regulators that might affect disease progression and pathology among 4 different brain regions. We also assessed the validity of the relations between regulators and targets by constructing a diagnostic model (Fig. 1). We hope this research can provide a preliminary understanding of AD aetiology research and drug development from an epitranscriptome perspective.

Materials And Methods

Data collection

AD transcriptome datasets were obtained from the Gene Expression Omnibus (GEO) database. GSE48350 was used for the primary analysis, containing 253 samples of 4 brain regions (postcentral gyrus, superior frontal gyrus, hippocampus, and entorhinal cortex). Samples from patients under the age of 60 were not used in the study to balance the age difference between the AD group and the control group. Part of the GSE28146 (30 samples from the hippocampus divided into four groups due to Braak stages) and GSE118553 (98 samples from the entorhinal cortex and 115 samples from the frontal cortex

divided into four groups due to Braak stages) datasets were used as validation datasets. The R package GEOquery [26] was used to obtain the array matrix and detailed sample information.

Collection of m6A regulators

Regulator gene sets were manually obtained from past research [27], and 23 regulators were analysed in this study, including 2 erasers (FTO and ALKBH5), 11 readers (YTHDF1, YTHDF2, YTHDF3, YTHDC1, YTHDC2, IGFBP1, IGFBP2, IGFBP3, HNRNPC, HNRNPA2B1, and ELAVL1), and 10 writers (METTL3, METTL14, WTAP, VIRMA, ZC3H13, METTL16, ZCCHC4, HAKAI, RBM15, and RBM15B).

Screening AD-related regulators

In this study, we considered regulators that were differentially expressed in AD groups and genes related to Braak stages and MMSE scores as AD-related regulators. The R package limma [28] was used to identify the DEGs between the AD and control groups. Limma is an R package based on the linear model to screen DEGs, commonly used in transcriptome profile studies. The R package psych was used to analyse the Spearman correlation between regulators and Braak stages [29] and MMSE scores [30]. Braak stages are established based on neurofibrillary tangles, which contain stages to , and a higher stage indicates more severe disease. MMSE (Mini-Mental State Examination) scores indicate the cognitive level, and higher scores indicate better cognition levels.

Correlation analysis among regulators

TheR package Hmisc was used to analyse the Pearson correlation between m6A regulators in different brain regions. The R package corrplot was used for visualization.

Evaluation of the alteration of biological processes

The activation levels of biological pathways were analysed using the GSVA method [31]. GSVA is a GSE method that can evaluate the activity of gene sets in an unsupervised manner and can be used to estimate pathway activity. The KEGG pathway and REACTOME pathway gene sets were downloaded from the MSigDB database. The difference in the GSVA scores of the pathways was evaluated using limma.

Consensus clustering

To evaluate whether there were different m6A modification patterns in AD samples, the R package ConsensusClusterPlus [32] was used to perform consensus clustering in AD samples from the 4 brain regions. Consensus clustering is a method for evaluating the adequate number and robustness of clustering using iterative subsampling and clustering.

WGCNA

A weighted coexpression network was constructed using the WGCNA [33] package. Significant regulators in the network (GS > 0.2 and MM > 0.65 with a module) and significant modules ($p < 0.1$ for phenotype correlation) were analysed. WGCNA is a method to weigh the coexpression network so that the whole can be aggregated into different modules, and then the genes and phenotypes can be studied through the modules [34]. The hub genes (GS > 0.2, MM > 0.65) in the corresponding modules of key regulators were selected and considered important regulatory relationship pairs for the disease, and follow-up analysis was carried out.

Potential m6A-regulated genes and confidence evaluation

For the potential regulator-gene regulation relationship discovery, the Pearson correlation between regulators and these genes was measured, and correlations with $p<0.1$ were considered potential targets. For these relations, we assessed their confidence in the following aspects: whether the gene transcripts were m6A or m6Am modified; if there was evidence that the regulators could bind the target transcripts or if perturbation of the regulators could affect the target genes in transcript abundance, m6A modification level, and translation level. The m6A2Targets database [35] was used to determine the regulators' perturbation effects and binding potential was evaluated from high-throughput sequencing data. The human m6A modification profile was obtained from a recent study [36]. The supplementary tables that contain information about m6A- or m6Am-modified genes were downloaded for the research.

Lasso regression

Key regulators and their potential targets were used to fit a LASSO penalized logistic model to diagnose AD. LASSO [37] penalized logistic regression was fitted by the glmnet package in R, and the penalty coefficient of λ minimizing the binomial deviance was chosen. To retain more variables, λ-1 was used as the penalty coefficient to construct the final model. For the constructed model for each brain region, the same-brain region data of GSE28146 and GSE118553 were used as the validation dataset.

Results

AD-related m6A regulators vary among different brain regions

To evaluate whether m6A regulators are related to AD, we performed gene differential expression analysis between AD and healthy cases in different brain regions (Fig. 2A, p values shown in Supplementary Table 1). The differential expression of regulators varied among different brain regions. In the postcentral gyrus region, we found that two writers, VIRMA and RBM15, were downregulated in the AD group. In the superior frontal gyrus, ZCCHC4 and HNRNPNPA2B1 were significantly upregulated in AD. A large number of regulators of different types were found to be differentially expressed in the hippocampi of AD patients. Two writers (RBM15 and METTL3) were found to be downregulated in AD samples. Regarding readers, IGFBP1 and ELAVL1 were upregulated, and YTHDC1 and YTHDF2 were downregulated in the hippocampus of AD samples. FTO, an eraser, was also found to be downregulated in AD samples in the hippocampus. In the entorhinal cortex region, three readers (IGFBP3, YTHDC1, and

YTHDC2) were upregulated to various degrees. Only YTHDC1 was differentially expressed across multiple brain regions, and the propensity of changes varied. In addition to the hippocampus, the other three brain regions had an obvious tendency towards certain regulator types. In the PG region, m6A writers tended to be downregulated in the AD group. Writers and readers tended to be upregulated in the AD patients' SFG region. m6A readers were likely to be upregulated in the EC region.

In addition to DEG exploration, we also assessed which regulators might influence the pathological progression of AD. We investigated the relationship between the expression levels of m6A regulators and cognition level (MMSE score) and neuropathology progression stage (Braak stage). Multiple regulators were considered to affect the cognitive level or pathological progression among different brain regions (Fig. 2B, p values shown in Supplementary Table 2). For erasers, the level of the FTO transcript in the PG region was found to be associated with both cognitive and neuropathological progression and negatively correlated with disease progression. The level of FTO transcript in the HP region was also negatively correlated with neuropathology progression. For readers, ELAVL1 was positively correlated with neuropathology progression in both the SFG and HP regions. The expression levels of YTHDF2 in both HP and EC regions were correlated with pathological progression and tended to have opposite effects and were also positively correlated with cognitive levels in the PG region. Among writers, the levels of HAKAI in HP and EC regions were negatively correlated with Braak score and MMSE score, respectively. There are many genes with altered expression in AD patients compared to controls that are related to pathology and cognition, which may indicate critical regulatory roles in AD, such as downregulation of FTO and YTHDF2 and upregulation of ELAVL1, which also occurs with more severe neuropathological progression in AD patients' HP region. Many genes correlating with cognition level and neuropathology were not previously found in DEG screening. Despite no expression change in erasers between groups in the PG region, FTO and ALKBH5 were found to correlate with Braak stages and MMSE scores. Similar results were found in other brain regions, suggesting that there may be potential contributions of regulators to the pathological deterioration of AD that do not have significant differences in expression levels between the control and disease groups.

Considering that the interactions between regulators are very common, we also measured the correlation between regulators in different brain regions ($p \leq 0.005$, Fig. 3A-H, Supplementary Table 3). Correlations between regulators were common in all brain regions and were mostly positive. However, the correlation status between different types of regulators varied in different brain regions. In the PG region, the correlations between writers and readers were extensive, while erasers were only related to readers. In the SFG region, writers and readers were still commonly correlated. There was an increase in the number of correlations among writers compared to the PG region, while the correlation between readers decreased. Erasers were not only related to readers; there was a correlation between FTO and a writer, HAKAI. The interaction profile in the HP region was similar to that in the SFG region, presenting a strong correlation between writers and writers. Although the correlation between readers in the HP region was weaker, ALKBH5 (eraser) was strongly related to m6A writers. In the EC region, the correlations among regulators were the weakest, but the erasers represented a strong

correlation with writers and readers, which might indicate a special role of the m6A eraser. In conclusion, the correlation of regulators varies in different brain regions. Considering the different functions of each brain region, this may be due to different pathogenic mechanisms of different types of m6A regulators.

m6A regulators influence multiple essential biological processes

To explore which biological processes may be affected by AD-related m6A regulators, we used the GSVA method to measure the activation of KEGG and REACTOME biological pathways and assessed the changes in these pathways in AD and their correlation with regulators (Fig. 3, Supplementary Fig. 1A-D, Supplementary Table 4). Several pathways were found with altered activation levels in AD in different brain regions ($p \leq 0.1$, 164 in PG, 420 in SFG, 686 in HP, and 713 in EC). Among them, 23 pathways were found to have different activation states in all 4 brain regions, including several metabolic pathways, such as N-glycan biosynthesis, lipid metabolism, amino acid metabolism, and protein export pathways. Several signalling pathways were also involved, such as G protein activation and beta-gamma signalling, chemokine receptor binding, and NTRK2 and NTRK3 activation via RAS. We also evaluated which pathways were potentially regulated by m6A regulators related to AD pathology (DEGs and genes associated with Braak stage and MMSE score) in the corresponding brain regions. The Pearson correlations between regulators and the GSVA scores of biological pathways were calculated. Among the 23 pathways, 13 were found to be associated with at least one of the disease-related regulators in all brain regions ($p \leq 0.05$, Fig. 4, Supplementary Fig. 1H-I, Supplementary Table 5), which were mainly REACTOME pathways (11 out of 13). The pathways included were most metabolic pathways described previously (N glycan, amino acid, and protein metabolism) and were associated with most disease-related regulators in the hippocampus.

Regulators closely related to most of these pathways (associated with more than half of the 13 pathways) in 4 regions had a significant difference. In the PG region, YTHDF2, YTHDF3, and FTO were found to be correlated with more than half of 13 pathways. The key regulators found to be correlated with most of the 13 pathways in other regions were different (SFG: HNRPA2B1; Hippocampus: YTHDF2 and FTO and RBM15; EC: HNRNPC). Most of these regulators (other than FTO and RBM15) were readers. Considering that YTHDF2 and FTO played important roles in 2 of the 4 brain regions, there might be an important relationship between these regulators and AD.

Identification of different m6A modification patterns in AD patients

To investigate whether there were different patterns in AD patients' m6A profiles,
we performed consensus clustering using the m6A regulator expression profile of AD patients in different brain regions to discover an adequate number of groups. The clustering results among the 4 brain regions were divided into 2 patterns. The PG and SFG regions were divided into 3 clusters, while the HP and EC regions were divided into 2 clusters (Fig. 5, Supplementary Fig. 3, Supplementary Table 6). To explore the reason for the above groupings, we assessed the differentially expressed regulators in different clusters of each brain region and evaluated similarities and the differences between regions (Supplementary Table 7). For the 3 clusters in each PG region and SFG region, 8 regulators (HNRNPA2B1,

HNRNPC, IGFBP2, IGFBP3, YTHDC1, YTHDC2, YTHDF1, and ZC3H13) had similar expression patterns (Fig. 5B and D). For the 2 clusters in each of the HP and EC regions, HNRNPA2B1 and IGFBP3 had similar expression patterns (Fig. 5F and H). The above regulators were all readers except ZC3H13.

To explore the biological differences in the different modification patterns, we conducted a difference analysis on the GSVA scores of biological pathways between different clusters in each brain region. We found that several KEGG (PG: 98; SFG: 136; hippocampus: 81; EC: 8) and REACTOME pathways (PG: 797; SFG: 1095; hippocampus: 703; EC: 72) had different activation states between different m6A groups, some with the same trend among multiple regions ($p < 0.1$). We assessed pathways that had common changes in the PG region and the SFG region, which were all divided into 3 m6A clusters. Of these two regions, 23 KEGG pathways and 189 REACTOMEs had similar trends among the three clusters, such as the Alzheimer pathway, the autophagy pathway, the oxidative phosphorylation pathway, and the TCA cycle pathway (Fig. 6A-B). There were differences in 81 KEGG pathways and 703 REACTOME pathways between the 2 different modification patterns in the HP region, including the Alzheimer pathway, autophagy pathway, and TCA cycle pathway (Fig. 6C). There were few differentially activated pathways in the EC region. Only 8 KEGG pathways and 72 REACTOME pathways had differences, presumably because of the lack of AD samples in this region. In summary, we found that different m6A modification patterns could be distinguished in AD patients among different brain regions, with several pathways that were commonly changed, including autophagy, oxidative phosphorylation, and the TCA cycle pathway.

Evaluation of the regulatory importance of m6A regulators based on WGCNA

To assess the importance of AD-related m6A regulators in the regulatory network, we used the WGCNA method to construct a weighted coexpression network for each brain region. The importance of the regulators was evaluated based on the gene significance (GS) of the AD-control group and module membership (MM) (Fig. 7), representing the regulators' relation with disease and gene modules, respectively. Regulators with $GS > 0.2$ and $MM > 0.65$ for a module were selected, and their GS values with all phenotypes and MM values with all modules are shown in Supplementary Fig. 3. Several important regulators related to the AD/control phenotype were found (PG: FTO; SFG: RBM15B and FTO; Hippocampus: RBM15, FTO, YTHDF2, YTHDC1, and IGFBP1; EC: YTHDC1 and YTHDC2). To assess the disease-related biological processes that might be modulated by the above regulators, we evaluated whether these hub regulators were related to modules related to the AD/control phenotype ($p < 0.1$). After the assessment, the PG region did not obtain disease-related modules related to essential regulators. In the SFG region, RBM15B was found to be related to the grey60 module, and FTO was related to the blue module. In the HP region, RBM15, YTHDC1, and IGFBP1 were related to the turquoise module, while FTO and YTHDF2 were related to the blue module. In the EC region, YTHDC1 and YTHDC2 were found to be related to the light cyan and dark red modules, respectively. The hub genes ($MM > 0.65$ and $GS > 0.2$) in each corresponding module were selected for the following analysis. We selected the hub genes correlated with the corresponding regulators above ($p < 0.05$) and obtained potential relationships between regulators and hub genes. We also evaluated the reliability of the possible

regulatory relation based on the Me-RIP seq of the human brain and the m6A2targets database. After evaluation, we obtained reliable regulation relations between regulators, disease-related gene modules, and target genes in the corresponding modules that correlated with AD-related genes in each brain region (Supplementary Fig. 4-6). Of these genes, several genes, including TMEM59L, RTN4RL2, SNCB, etc., have been reported to affect AD pathology in past studies [38-41] and are potentially regulated by regulators, including FTO. In conclusion, we obtained key AD-related regulators and regulatory relationships with their target genes that may have a decisive impact on AD.

Construction of the diagnosis model using hub regulators and related genes

For the hub regulators and their potential targets obtained in the previous step, we tried to use the above genes to fit diagnostic models to evaluate the importance and relationship with AD in each brain region. Specifically, the hub regulators and genes related to corresponding regulators ($p < 0.005$) were used to fit diagnostic models, which were disease-related and highly correlated with disease-related modules. We performed Lasso penalized logistic regression to construct the diagnostic model in each brain region. Then, the diagnostic model was validated using the validation dataset.

In conclusion, we obtained different diagnostic models in the regions above. No diagnostic model was constructed in the PG region because no key regulators or target modules were found in this region. For the validation dataset, we also constructed a diagnostic model using the genes from the penalized model obtained from the training dataset of the corresponding brain region. For the SFG region, RBM15B, FTO, and a total of 55 related genes were used to fit the model. With λ=-3.9, the model consisted of 13 genes, and the AUC was 0.958 (Fig. 8A-B). We used the frontal lobe data of GSE118553 as a validation dataset and it had an AUC of 0.98 (Fig. 8C-D). For the HP region, we used RBM15-, FTO-, YTHDF2-, YTHDC1-, IGFBP1-, and 4524-related genes for penalized regression. With λ=-6.1, the final model consists of 21 genes, and the AUC was 1. The AUC of the hippocampal validation datasets was also 1. For the EC region training dataset, we used YTHDC1, YTHDC2, and a total of 33 related genes for model fitting. With λ=-4.9, the model consisted of 8 genes, and the AUC was 1 (Fig. 8E-F). The entorhinal cortex data of GSE118553 were used for validation and had an AUC of 0.731 (Fig. 3F). In conclusion, highly reliable diagnostic models for the SFG, HP, and EC regions were obtained, indicating the consistency of the relationship between AD, regulators and their potential targets.

Discussion

N6-methyladenosine (m6A) RNA methylation is a very important epigenetic regulatory mechanism, and its role in neurodegenerative diseases is still under investigation. Although there have been some reports about the potential roles of m6A modification and regulators in AD, no studies have discussed the different mechanisms of regulators from a multi-region perspective. Here, we revealed key m6A regulators and their influence on AD in 4 brain regions through transcriptome mining and further identified important regulators and their potential targets based on weighted coexpression network analysis. We hope these

differences and similarities in m6A modification between the brain regions in AD can provide guidance for future studies.

Among the 4 brain regions investigated in this study, the superior frontal gyrus, hippocampus, and entorhinal cortex are regions reported to be pathologically invaded in AD progression, while the postcentral gyrus is relatively less pathologically affected [29]. However, a study also showed changes in ageing-related genes in the postcentral gyrus [42]. We first explored regulators that might affect AD through a variety of means. Differential expression analysis is one of the most common methods used in transcriptome research. We found that regulators obtained by DEG analysis varied among different brain regions. Although the changes varied among different kinds of regulators in the postcentral gyrus, superior frontal gyrus, and entorhinal cortex, their changes each focused on certain types of m6A regulators. Only the hippocampus showed different trends in regulator types, so there may be a more complex pathological mechanism of the m6A regulators in the hippocampus, which needs further investigation. Among them, the low expression of METTL3 in the hippocampus in AD has been reported in a previous article [21], which is consistent with our results. Other changes in the regulators found in the study still need further investigation. Braak staging and MMSE scores are commonly used as pathological progression and cognition indicators in AD and were also considered here to evaluate the relationship between m6A regulators and AD neuropathology. As a result, we found several regulators that were related to pathology, although they were not differentially expressed in the AD group in the postcentral gyrus, which may be due to less pathological effects of the postcentral gyrus and should be further studied to determine whether it is related to the early stage of AD or disease state transition.

We assessed the changes in the activation levels of biological pathways in each brain region using the GSVA method. The GSVA method has been commonly used in cancer research to study pathway changes in diseases, using a pathway-based dimensionality reduction method to improve the biological interpretability of transcriptome data in bioinformatic research. We obtained pathways that were commonly changed across brain regions and were potentially affected by AD-related m6A regulators and found that pathways such as protein export, N-glycan metabolism, and lipid metabolism may be affected by AD-related regulators in multiple brain regions. The alteration of the brain protein glycosylation profile of AD was recently reported, indicating that the N-glycan profile in the cortex and hippocampus of AD patients was altered [43], and our study showed that the m6A process might influence this process. APP and tau are the core proteins of current AD pathological hypotheses. Although there is no evidence that they are affected by glycosylation, their cellular and tissue localization plays an important role physically and pathologically [44, 45]. The effect of m6A dynamics on the protein export pathway may affect their transport at the cellular level. The allele of apolipoprotein E (APOE) has been reported as a risk factor for familial AD [46]. Here, the influence of m6A regulators on the lipid metabolism pathway was identified and may also be related to the pathological mechanism of APOE. The effect of G protein receptor-coupled kinases on AD pathology has also been reported [47]. In this study, we found that multiple G protein-related signalling pathways were changed in patients' brains in an m6A-related manner. For regulators found to be correlated with these biological processes, FTO, RBM15, YTHDF2, YTHDF3, YTHDC3, HNRNPC, and HNRNPA2B1 were found to play important roles in different brain regions. Among

them, HNRNPA2B1 was recently reported to affect the endocytosis of tau in microglial cells [48]. FTO has also been reported to be associated with AD [48]. Links between AD pathology and other regulators have not been reported and might need further study. However, several regulators have been reported to be associated with neurological functions. FTO was reported to be associated with memory[49] and neurogenesis [50]. YTHDF2's relationship with neurogenesis has also been reported [51], so their alteration might represent an underlying cause for neurodegeneration.

We also aimed to determine whether there were specific m6A modification patterns in different brain regions of AD patients. The consensus clustering method was used to provide a way to find a suitable clustering coefficient k by iterative clustering, which gives the adequate number of m6A clusters in each brain region. Similar work has also been conducted in several diseases, such as bladder cancer [52] and pancreatic cancer [53]. In this study, the number of modification patterns obtained in different brain regions differed, and the related biological processes also varied, but several pathways were found to be commonly affected by m6A regulators. Samples of both the postcentral gyrus and superior frontal gyrus were divided into three clusters, while samples of the hippocampus and entorhinal cortex were divided into two clusters. The expression patterns of several m6A readers were similar in regions that were divided into the same number of clusters, mainly readers. There were also several pathways with similar changes in activation state between clusters. In the postcentral gyrus and superior frontal gyrus, Alzheimer's pathways were both altered between m6A clusters, suggesting that m6A modification patterns were related to AD. There were changes in the autophagy pathway and TCA cycle pathway between m6A clusters in the postcentral gyrus, superior frontal gyrus, and hippocampus. The role of autophagy in AD has been studied from several perspectives, including PPARA-mediated autophagy [54] and mitophagy [55]. The differences in autophagy and the TCA cycle pathway between m6A modification patterns are worthy of further study in AD. Unfortunately, no differences in pathological data between different modification patterns were found (data not shown), but the differences among pathways suggest that different types of m6A modification in AD patients may require different intervention methods.

We also attempted to identify the hub regulators related to AD and assess their association with biological processes and target genes using the WGCNA method. WGCNA is a method that constructs weighted coexpression networks allowing genes to form meaningful modules for subsequent studies at the gene set level and has also been widely used in studies of a variety of diseases [53, 52]. The hub regulators related to AD were selected based on their correlations with the disease phenotypes and modules significantly related to AD. No regulators were screened out in the PG region, which may be associated with the milder influence of AD pathology in the PG region. Two regulators each in the SFG region (RBM15B and FTO) and EC regions (YTHDC1 and YTHDC2) were considered important, while multiple regulators were screened out in the HP region, including RBM15, FTO, YTHDF2, YTHDC1, and IGFBP1. Among these regulators, FTO, YTHDC1, and YTHDC2 are all thought to play important roles in multiple brain regions. We used the existing evidence to identify and assess essential target genes related to regulators, including evidence from the m6A2Targets database and the recently published m6A sequencing data from the m6A and m6Am modification landscape of human organs [35]. The

m6A2Targets database [36] provides evidence for the regulation of regulators to target genes based on a large amount of high-throughput sequencing data, covering the influence of the perturbation of regulators on changes in the expression, methylation, and translation levels of downstream genes and the interaction between regulators and target genes. Finally, to evaluate the importance of these genes, we attempted to fit diagnostic models with regulators and related hub genes and obtained stable models in the SFG, HP, and EC regions, indicating the confidence of the relationship between key genes and AD.

The study did have several shortcomings. First, only 4 brain regions were analysed in the study. Considering the complexity of the human brain, more research is needed to determine the changes in m6A regulators in more brain regions. It is also important to compare the impact of m6A modification alterations in AD patients between different brain regions. Second, the m6A modification pattern clusters identified in the study may not reflect the actual state due to the lack of a sample size in each brain region, but we hope this study can provide insight into how to divide AD patients into different groups based on their m6A profile. Furthermore, the assessment of pathway activity using transcriptome data may not reflect the real status and requires further validation using other omics data and certain experiments. Finally, although there are studies between m6A modification and Alzheimer's disease based on mouse models, there are still no large-scale m6A sequencing data of AD patients' brains, so the evidence collected to assess the effectiveness of m6A regulation was only based on other platforms; therefore, they cannot reflect the real regulatory relationship, and further validation is needed.

Conclusion

In conclusion, this study provides insight into the potential regulatory mechanism of m6A regulators in AD, as well as their similarities and differences among brain regions. We hope this study can be used a reference for future research on the relationship between m6A modification and Alzheimer's disease.

Declarations

Funding

This work was supported in part by grants from National Natural Science Foundation of China (81701078), Natural Science Foundation of Heilongjiang Province of China (QC2017090), University Nursing Program for Young Scholars with Creative Talents in Heilongjiang Province (UNPYSCT-2016190), China postdoctoral science foundation (2016M600261, 2018T110317), The Innovative Science Research Project of Harbin Medical University (2016JCZX37), Heilongjiang Postdoctoral Financial Assistance (LBH-Z15163), Heilongjiang Touyan Innovation Team Program.

Competing interests

The authors declare that they have no competing interests.

Ethics approval

Not Applicable

Consent to participate

Not Applicable

Consent for publication

Not Applicable

Availability of data and materials

Not Applicable

Code availability

Not Applicable

Author' contributions

All authors contributed to the article. Dayong Wang and Xu Gao had the idea for the article. The first draft of the manuscript was written by Zijie Liu and Qing Xia, they contributed equally to this work. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

This work was supported in part by grants from National Natural Science Foundation of China (81701078), Natural Science Foundation of Heilongjiang Province of China (QC2017090), University Nursing Program for Young Scholars with Creative Talents in Heilongjiang Province (UN-PYSCT-2016190), China Postdoctoral Science Foundation (2016M600261, 2018T110317), The Innovative Science Research Project of Harbin Medical University (2016JCZX37), Heilongjiang Postdoctoral Financial Assistance (LBH-Z15163), Heilongjiang Touyan Innovation Team Program.

References

1. Alzheimer A (1907) Über eine eigenartige Erkrankung der Hirnrinde. Allgemeine Zeitschrift für Psychiatrie
2. Takizawa C, Thompson PL, van Walsem A, Faure C, Maier WC (2015) Epidemiological and economic burden of Alzheimer's disease: a systematic literature review of data across Europe and the United States of America. J Alzheimers Dis 43(4):1271–1284. doi:10.3233/JAD-141134
3. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34(7):939–944. doi:10.1212/wnl.34.7.939

4. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7(3):263–269. doi:10.1016/j.jalz.2011.03.005
5. Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, Duyckaerts C, Frosch MP, Masliah E, Mirra SS, Nelson PT, Schneider JA, Thal DR, Trojanowski JQ, Vinters HV, Hyman BT (2012) National Institute on A, Alzheimer's A National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol 123 (1):1–11. doi:10.1007/s00401-011-0910-3
6. Cummings J, Lee G, Zhong K, Fonseca J, Taghva K (2021) Alzheimer's disease drug development pipeline: 2021. Alzheimers Dement (N Y) 7(1):e12179. doi:10.1002/trc2.12179
7. Sevigny J, Chiao P, Bussiere T, Weinreb PH, Williams L, Maier M, Dunstan R, Salloway S, Chen T, Ling Y, O'Gorman J, Qian F, Arastu M, Li M, Chollate S, Brennan MS, Quintero-Monzon O, Scannevin RH, Arnold HM, Engber T, Rhodes K, Ferrero J, Hang Y, Mikulskis A, Grimm J, Hock C, Nitsch RM, Sandrock A (2016) The antibody aducanumab reduces Abeta plaques in Alzheimer's disease. Nature 537(7618):50–56. doi:10.1038/nature19323
8. Ostrowitzki S, Lasser RA, Dorflinger E, Scheltens P, Barkhof F, Nikolcheva T, Ashford E, Retout S, Hofmann C, Delmar P, Klein G, Andjelkovic M, Dubois B, Boada M, Blennow K, Santarelli L, Fontoura P, Investigators SCR (2017) A phase III randomized trial of gantenerumab in prodromal Alzheimer's disease. Alzheimers Res Ther 9(1):95. doi:10.1186/s13195-017-0318-y
9. Egan MF, Kost J, Voss T, Mukai Y, Aisen PS, Cummings JL, Tariot PN, Vellas B, van Dyck CH, Boada M, Zhang Y, Li W, Furtek C, Mahoney E, Harper Mozley L, Mo Y, Sur C, Michelson D (2019) Randomized Trial of Verubecestat for Prodromal Alzheimer's Disease. N Engl J Med 380(15):1408–1420. doi:10.1056/NEJMoa1812840
10. Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma H, Weng X, Chen K, Shi H, He C (2015) N(6)-methyladenosine Modulates Messenger RNA Translation Efficiency. Cell 161(6):1388–1399. doi:10.1016/j.cell.2015.05.014
11. Wang X, Lu Z, Gomez A, Hon GC, Yue Y, Han D, Fu Y, Parisien M, Dai Q, Jia G, Ren B, Pan T, He C (2014) N6-methyladenosine-dependent regulation of messenger RNA stability. Nature 505(7481):117–120. doi:10.1038/nature12730
12. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, Sorek R, Rechavi G (2012) Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature 485(7397):201–206. doi:10.1038/nature11112
13. Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, Vagbo CB, Shi Y, Wang WL, Song SH, Lu Z, Bosmans RP, Dai Q, Hao YJ, Yang X, Zhao WM, Tong WM, Wang XJ, Bogdan F, Furu K, Fu Y, Jia G, Zhao X, Liu J, Krokan HE, Klungland A, Yang YG, He C (2013) ALKBH5 is a mammalian RNA

demethylase that impacts RNA metabolism and mouse fertility. Mol Cell 49(1):18–29. doi:10.1016/j.molcel.2012.10.015

14. Widagdo J, Zhao QY, Kempen MJ, Tan MC, Ratnu VS, Wei W, Leighton L, Spadaro PA, Edson J, Anggono V, Bredy TW (2016) Experience-Dependent Accumulation of N6-Methyladenosine in the Prefrontal Cortex Is Associated with Memory Processes in Mice. J Neurosci 36(25):6771–6777. doi:10.1523/JNEUROSCI.4053-15.2016
15. Merkurjev D, Hong WT, Iida K, Oomoto I, Goldie BJ, Yamaguti H, Ohara T, Kawaguchi SY, Hirano T, Martin KC, Pellegrini M, Wang DO (2018) Synaptic N(6)-methyladenosine (m(6)A) epitranscriptome reveals functional partitioning of localized transcripts. Nat Neurosci 21(7):1004–1014. doi:10.1038/s41593-018-0173-6
16. Walters BJ, Mercaldo V, Gillon CJ, Yip M, Neve RL, Boyce FM, Frankland PW, Josselyn SA (2017) The Role of The RNA Demethylase FTO (Fat Mass and Obesity-Associated) and mRNA Methylation in Hippocampal Memory Formation. Neuropsychopharmacology 42(7):1502–1510. doi:10.1038/npp.2017.31
17. Li Q, Li X, Tang H, Jiang B, Dou Y, Gorospe M, Wang W (2017) NSUN2-Mediated m5C Methylation and METTL3/METTL14-Mediated m6A Methylation Cooperatively Enhance p21 Translation. J Cell Biochem 118(9):2587–2598. doi:10.1002/jcb.25957
18. Lewinska A, Adamczyk-Grochala J, Deregowska A, Wnuk M (2017) Sulforaphane-Induced Cell Cycle Arrest and Senescence are accompanied by DNA Hypomethylation and Changes in microRNA Profile in Breast Cancer Cells. Theranostics 7(14):3461–3477. doi:10.7150/thno.20657
19. Min KW, Zealy RW, Davila S, Fomin M, Cummings JC, Makowsky D, McDowell CH, Thigpen H, Hafner M, Kwon SH, Georgescu C, Wren JD, Yoon JH (2018) Profiling of m6A RNA modifications identified an age-associated regulation of AGO2 mRNA stability. Aging Cell 17(3):e12753. doi:10.1111/acel.12753
20. Han M, Liu Z, Xu Y, Liu X, Wang D, Li F, Wang Y, Bi J (2020) Abnormality of m6A mRNA Methylation Is Involved in Alzheimer's Disease. Front Neurosci 14:98. doi:10.3389/fnins.2020.00098
21. Huang H, Camats-Perna J, Medeiros R, Anggono V, Widagdo J (2020) Altered Expression of the m6A Methyltransferase METTL3 in Alzheimer's Disease. eNeuro 7(5). doi:10.1523/ENEURO.0125-20.2020
22. Li H, Ren Y, Mao K, Hua F, Yang Y, Wei N, Yue C, Li D, Zhang H (2018) FTO is involved in Alzheimer's disease by targeting TSC1-mTOR-Tau signaling. Biochem Biophys Res Commun 498(1):234–239. doi:10.1016/j.bbrc.2018.02.201
23. Reitz C, Tosto G, Mayeux R, Luchsinger JA, Group N-LNFS, Alzheimer's Disease Neuroimaging I (2012) Genetic variants in the Fat and Obesity Associated (FTO) gene and risk of Alzheimer's disease. PLoS ONE 7(12):e50354. doi:10.1371/journal.pone.0050354
24. Wang Y, Wang J, Gao L, Stamm S, Andreadis A (2011) An SRp75/hnRNPG complex interacting with hnRNPE2 regulates the 5' splice site of tau exon 10, whose misregulation causes frontotemporal dementia. Gene 485(2):130–138. doi:10.1016/j.gene.2011.06.020

25. Shafik AM, Zhang F, Guo Z, Dai Q, Pajdzik K, Li Y, Kang Y, Yao B, Wu H, He C, Allen EG, Duan R, Jin P (2021) N6-methyladenosine dynamics in neurodevelopment and aging, and its potential role in Alzheimer's disease. Genome Biol 22(1):17. doi:10.1186/s13059-020-02249-z
26. Davis S, Meltzer PS (2007) GEOquery: a bridge between the Gene Expression Omnibus (GEO) and BioConductor. Bioinformatics 23(14):1846–1847. doi:10.1093/bioinformatics/btm254
27. Jiang X, Liu B, Nie Z, Duan L, Xiong Q, Jin Z, Yang C, Chen Y (2021) The role of m6A modification in the biological functions and diseases. Signal Transduct Target Ther 6(1):74. doi:10.1038/s41392-020-00450-x
28. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK (2015) limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43(7):e47. doi:10.1093/nar/gkv007
29. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82(4):239–259. doi:10.1007/BF00308809
30. Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12(3):189–198. doi:10.1016/0022-3956(75)90026-6
31. Hanzelmann S, Castelo R, Guinney J (2013) GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinformatics 14:7. doi:10.1186/1471-2105-14-7
32. Wilkerson MD, Hayes DN (2010) ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking. Bioinformatics 26(12):1572–1573. doi:10.1093/bioinformatics/btq170
33. Langfelder P, Horvath S (2008) WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9:559. doi:10.1186/1471-2105-9-559
34. Zhang B, Horvath S (2005) A general framework for weighted gene co-expression network analysis. Stat Appl Genet Mol Biol 4:Article17. doi:10.2202/1544-6115.1128
35. Liu J, Li K, Cai J, Zhang M, Zhang X, Xiong X, Meng H, Xu X, Huang Z, Peng J, Fan J, Yi C (2020) Landscape and Regulation of m(6)A and m(6)Am Methylome across Human and Mouse Tissues. Mol Cell 77(2):426–440e426. doi:10.1016/j.molcel.2019.09.032
36. Deng S, Zhang H, Zhu K, Li X, Ye Y, Li R, Liu X, Lin D, Zuo Z, Zheng J (2021) M6A2Target: a comprehensive database for targets of m6A writers, erasers and readers. Brief Bioinform 22(3). doi:10.1093/bib/bbaa055
37. Tibshirani R (1996) Regression Shrinkage and Selection Via the Lasso. 10.1111/j.2517-6161.1996.tb02080.x
38. Ullrich S, Munch A, Neumann S, Kremmer E, Tatzelt J, Lichtenthaler SF (2010) The novel membrane protein TMEM59 modulates complex glycosylation, cell surface expression, and secretion of the amyloid precursor protein. J Biol Chem 285(27):20664–20674. doi:10.1074/jbc.M109.055608
39. Kern F, Sarg B, Stasyk T, Hess D, Lindner H (2012) The Nogo receptor 2 is a novel substrate of Fbs1. Biochem Biophys Res Commun 417(3):977–981. doi:10.1016/j.bbrc.2011.12.050

40. Kurlawala Z, Shah PP, Shah C, Beverly LJ (2017) The STI and UBA Domains of UBQLN1 Are Critical Determinants of Substrate Interaction and Proteostasis. J Cell Biochem 118(8):2261–2270. doi:10.1002/jcb.25880
41. Younas N, Zafar S, Shafiq M, Noor A, Siegert A, Arora AS, Galkin A, Zafar A, Schmitz M, Stadelmann C, Andreoletti O, Ferrer I, Zerr I (2020) SFPQ and Tau: critical factors contributing to rapid progression of Alzheimer's disease. Acta Neuropathol 140(3):317–339. doi:10.1007/s00401-020-02178-y
42. Berchtold NC, Coleman PD, Cribbs DH, Rogers J, Gillen DL, Cotman CW (2013) Synaptic genes are extensively downregulated across multiple brain regions in normal human aging and Alzheimer's disease. Neurobiol Aging 34(6):1653–1661. doi:10.1016/j.neurobiolaging.2012.11.024
43. Gaunitz S, Tjernberg LO, Schedin-Weiss S (2021) The N-glycan profile in cortex and hippocampus is altered in Alzheimer disease. J Neurochem 159(2):292–304. doi:10.1111/jnc.15202
44. Haass C, Kaether C, Thinakaran G, Sisodia S (2012) Trafficking and proteolytic processing of APP. Cold Spring Harb Perspect Med 2(5):a006270. doi:10.1101/cshperspect.a006270
45. Eftekharzadeh B, Daigle JG, Kapinos LE, Coyne A, Schiantarelli J, Carlomagno Y, Cook C, Miller SJ, Dujardin S, Amaral AS, Grima JC, Bennett RE, Tepper K, DeTure M, Vanderburg CR, Corjuc BT, DeVos SL, Gonzalez JA, Chew J, Vidensky S, Gage FH, Mertens J, Troncoso J, Mandelkow E, Salvatella X, Lim RYH, Petrucelli L, Wegmann S, Rothstein JD, Hyman BT (2019) Tau Protein Disrupts Nucleocytoplasmic Transport in Alzheimer's Disease. Neuron 101(2):349. doi:10.1016/j.neuron.2018.12.031
46. Serrano-Pozo A, Das S, Hyman BT (2021) APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches. Lancet Neurol 20(1):68–80. doi:10.1016/S1474-4422(20)30412-9
47. Guimaraes TR, Swanson E, Kofler J, Thathiah A (2021) G protein-coupled receptor kinases are associated with Alzheimer's disease pathology. Neuropathol Appl Neurobiol 47(7):942–957. doi:10.1111/nan.12742
48. Jiang L, Lin W, Zhang C, Ash PEA, Verma M, Kwan J, van Vliet E, Yang Z, Cruz AL, Boudeau S, Maziuk BF, Lei S, Song J, Alvarez VE, Hovde S, Abisambra JF, Kuo MH, Kanaan N, Murray ME, Crary JF, Zhao J, Cheng JX, Petrucelli L, Li H, Emili A, Wolozin B (2021) Interaction of tau with HNRNPA2B1 and N(6)-methyladenosine RNA mediates the progression of tauopathy. Mol Cell 81(20):4209–4227e4212. doi:10.1016/j.molcel.2021.07.038
49. Shi H, Zhang X, Weng YL, Lu Z, Liu Y, Lu Z, Li J, Hao P, Zhang Y, Zhang F, Wu Y, Delgado JY, Su Y, Patel MJ, Cao X, Shen B, Huang X, Ming GL, Zhuang X, Song H, He C, Zhou T (2018) m(6)A facilitates hippocampus-dependent learning and memory through YTHDF1. Nature 563(7730):249–253. doi:10.1038/s41586-018-0666-1
50. Li L, Zang L, Zhang F, Chen J, Shen H, Shu L, Liang F, Feng C, Chen D, Tao H, Xu T, Li Z, Kang Y, Wu H, Tang L, Zhang P, Jin P, Shu Q, Li X (2017) Fat mass and obesity-associated (FTO) protein regulates adult neurogenesis. Hum Mol Genet 26(13):2398–2411. doi:10.1093/hmg/ddx128

51. Li M, Zhao X, Wang W, Shi H, Pan Q, Lu Z, Perez SP, Suganthan R, He C, Bjoras M, Klungland A (2018) Ythdf2-mediated m(6)A mRNA clearance modulates neural development in mice. Genome Biol 19(1):69. doi:10.1186/s13059-018-1436-y
52. Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD, Hinoue T, Laird PW, Hoadley KA, Akbani R, Castro MAA, Gibb EA, Kanchi RS, Gordenin DA, Shukla SA, Sanchez-Vega F, Hansel DE, Czerniak BA, Reuter VE, Su X, de Sa Carvalho B, Chagas VS, Mungall KL, Sadeghi S, Pedamallu CS, Lu Y, Klimczak LJ, Zhang J, Choo C, Ojesina AI, Bullman S, Leraas KM, Lichtenberg TM, Wu CJ, Schultz N, Getz G, Meyerson M, Mills GB, McConkey DJ, Network TR, Weinstein JN, Kwiatkowski DJ, Lerner SP (2018) Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. Cell 174(4):1033. doi:10.1016/j.cell.2018.07.036
53. Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, Miller DK, Christ AN, Bruxner TJ, Quinn MC, Nourse C, Murtaugh LC, Harliwong I, Idrisoglu S, Manning S, Nourbakhsh E, Wani S, Fink L, Holmes O, Chin V, Anderson MJ, Kazakoff S, Leonard C, Newell F, Waddell N, Wood S, Xu Q, Wilson PJ, Cloonan N, Kassahn KS, Taylor D, Quek K, Robertson A, Pantano L, Mincarelli L, Sanchez LN, Evers L, Wu J, Pinese M, Cowley MJ, Jones MD, Colvin EK, Nagrial AM, Humphrey ES, Chantrill LA, Mawson A, Humphris J, Chou A, Pajic M, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Lovell JA, Merrett ND, Toon CW, Epari K, Nguyen NQ, Barbour A, Zeps N, Moran-Jones K, Jamieson NB, Graham JS, Duthie F, Oien K, Hair J, Grutzmann R, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Rusev B, Capelli P, Salvia R, Tortora G, Mukhopadhyay D, Petersen GM, Munzy DM, Fisher WE, Karim SA, Eshleman JR, Hruban RH, Pilarsky C, Morton JP, Sansom OJ, Scarpa A, Musgrove EA, Bailey UM, Hofmann O, Sutherland RL, Wheeler DA, Gill AJ, Gibbs RA, Pearson JV, Waddell N, Biankin AV, Grimmond SM (2016) Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 531(7592):47–52. doi:10.1038/nature16965
54. Luo R, Su LY, Li G, Yang J, Liu Q, Yang LX, Zhang DF, Zhou H, Xu M, Fan Y, Li J, Yao YG (2020) Activation of PPARA-mediated autophagy reduces Alzheimer disease-like pathology and cognitive decline in a murine model. Autophagy 16(1):52–69. doi:10.1080/15548627.2019.1596488
55. Fang EF, Hou Y, Palikaras K, Adriaanse BA, Kerr JS, Yang B, Lautrup S, Hasan-Olive MM, Caponio D, Dan X, Rocktaschel P, Croteau DL, Akbari M, Greig NH, Fladby T, Nilsen H, Cader MZ, Mattson MP, Tavernarakis N, Bohr VA (2019) Mitophagy inhibits amyloid-beta and tau pathology and reverses cognitive deficits in models of Alzheimer's disease. Nat Neurosci 22(3):401–412. doi:10.1038/s41593-018-0332-9

Figures

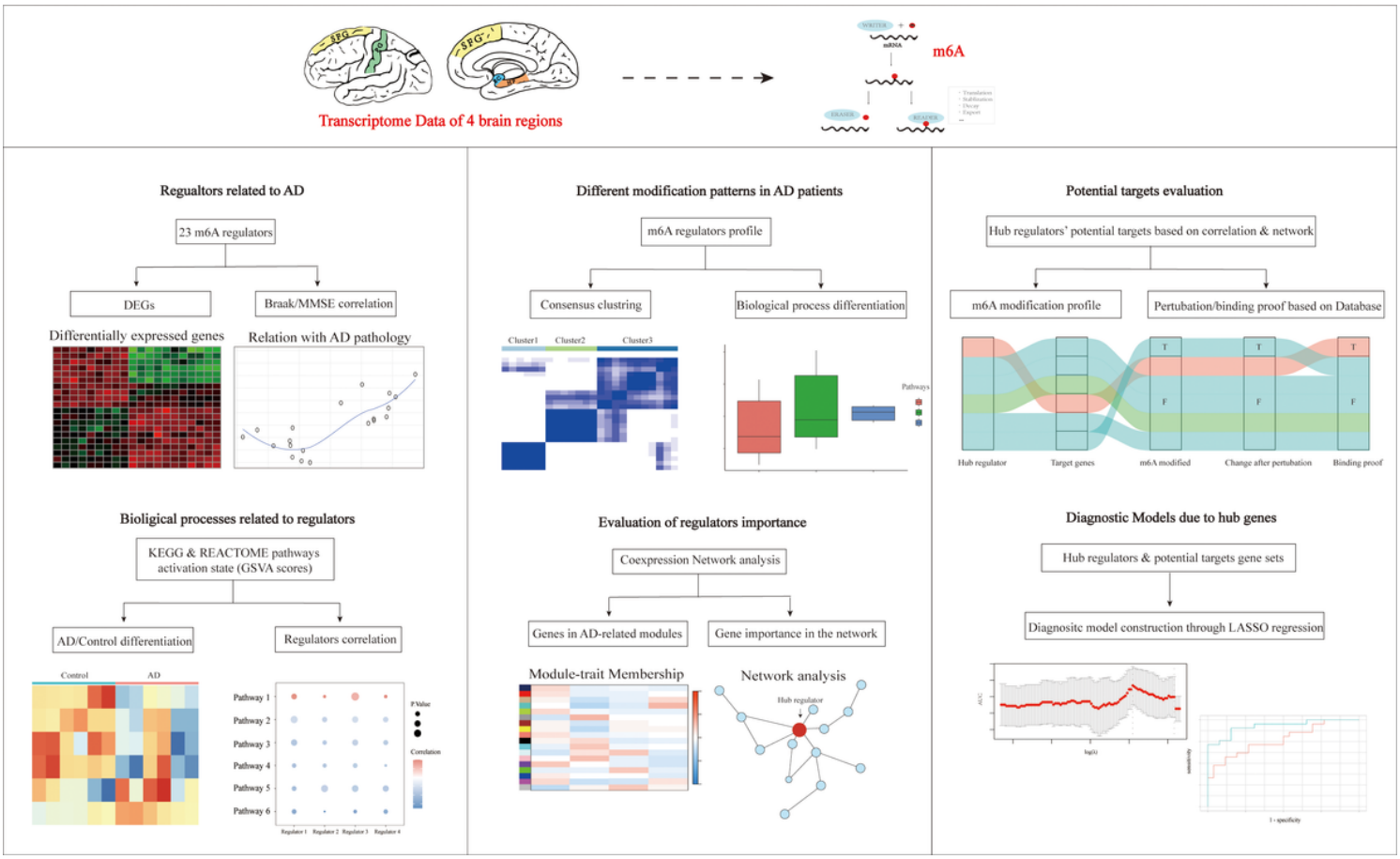

Figure 1

Graphical abstract of this study.

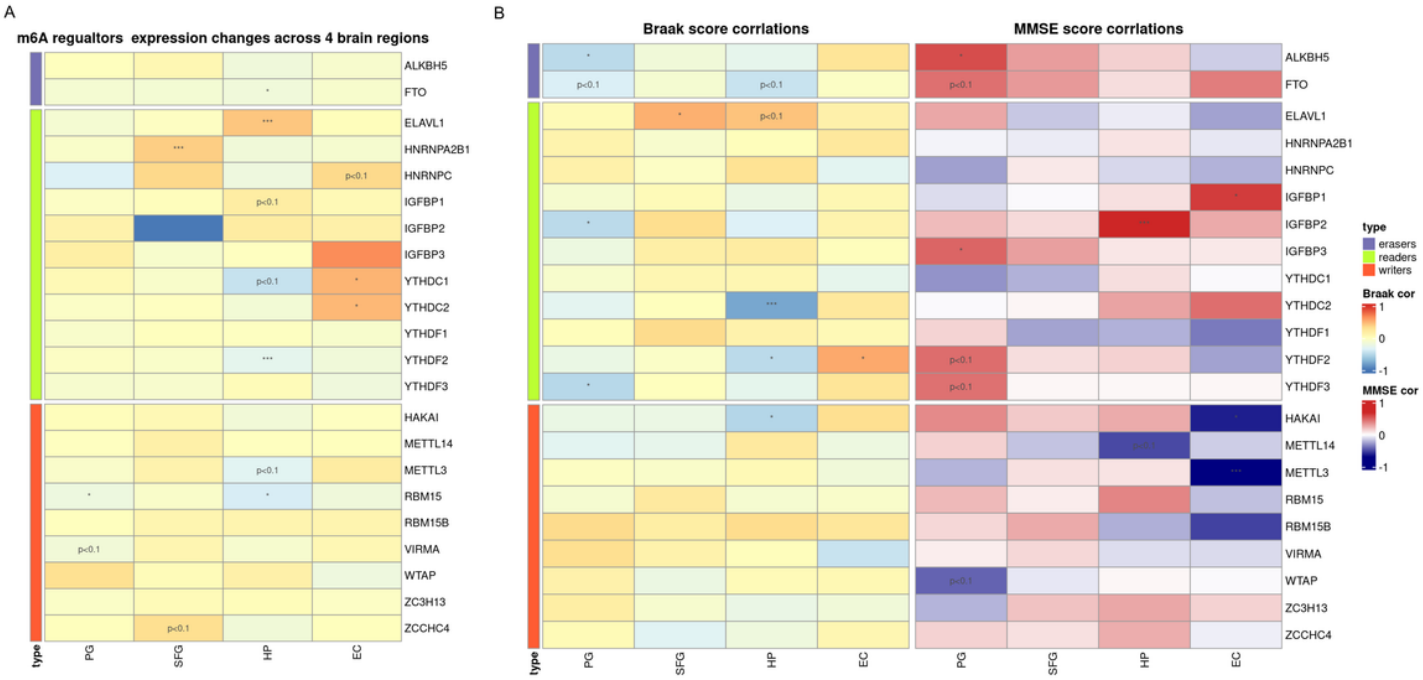

Figure 2

Identification of AD-related regulators. (A) Heatmap of m6A regulator expression and changes among 4 brain regions. (B) Heatmap of correlations between m6A regulators, Braak stages, and MMSE scores.

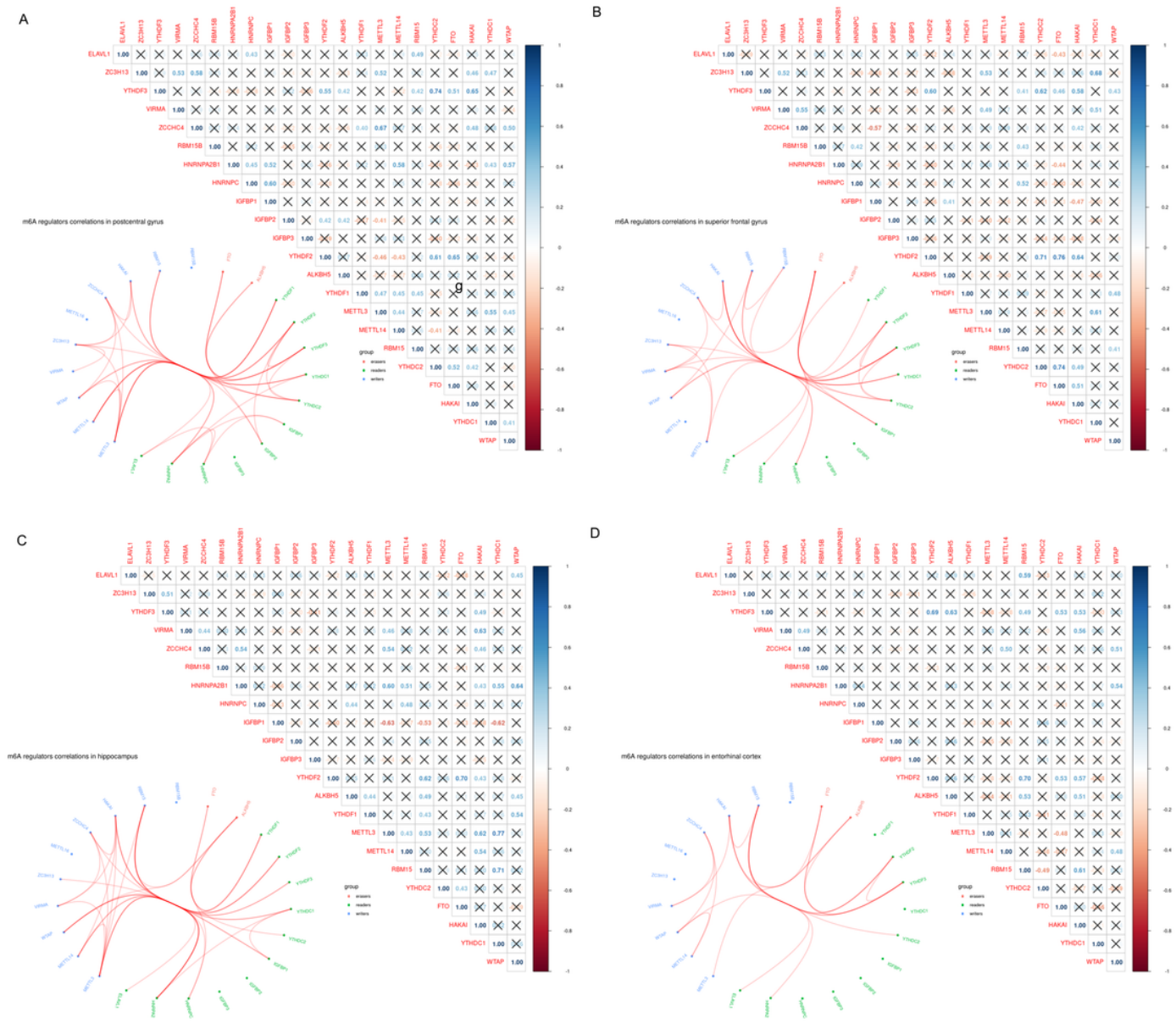

Figure 3

Correlations between regulators. (A) The PG region, (B) the SFG region, (C) the HP region, (D) and the EC region. For each region, the correlation heatmaps above show the significantly correlated regulators (p value ≤0.005), and hierarchical edge bundling shows the correlation between m6A writers, readers, and erasers.

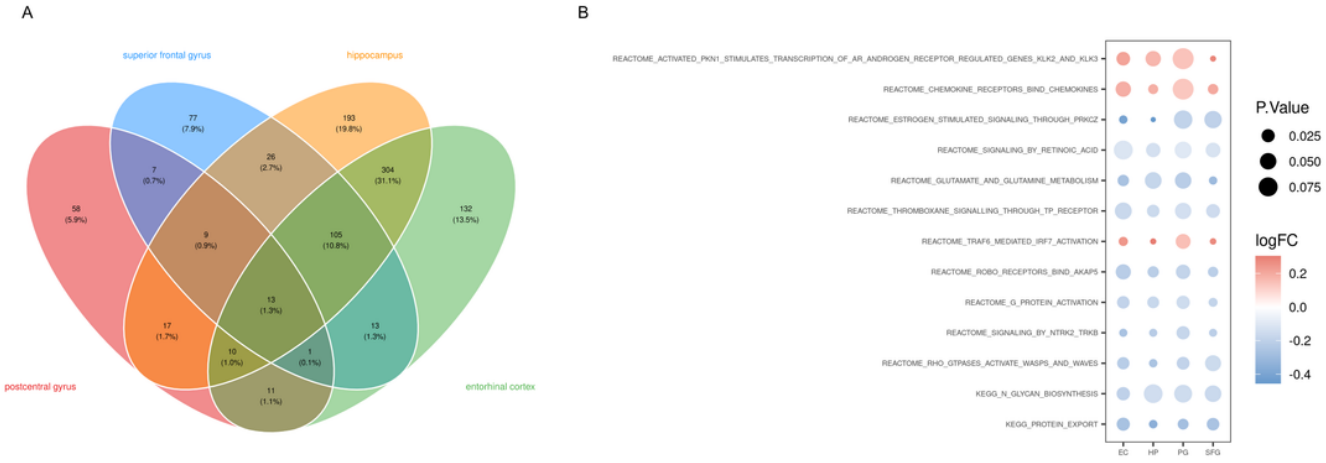

Figure 4

Pathways related to AD-related m6A regulators. (A) Venn diagram of pathways that have activation state changes and are related to AD-related regulators among 4 brain regions. (B) Correlation between m6A regulators and pathways that have changes in activities among 4 brain regions.

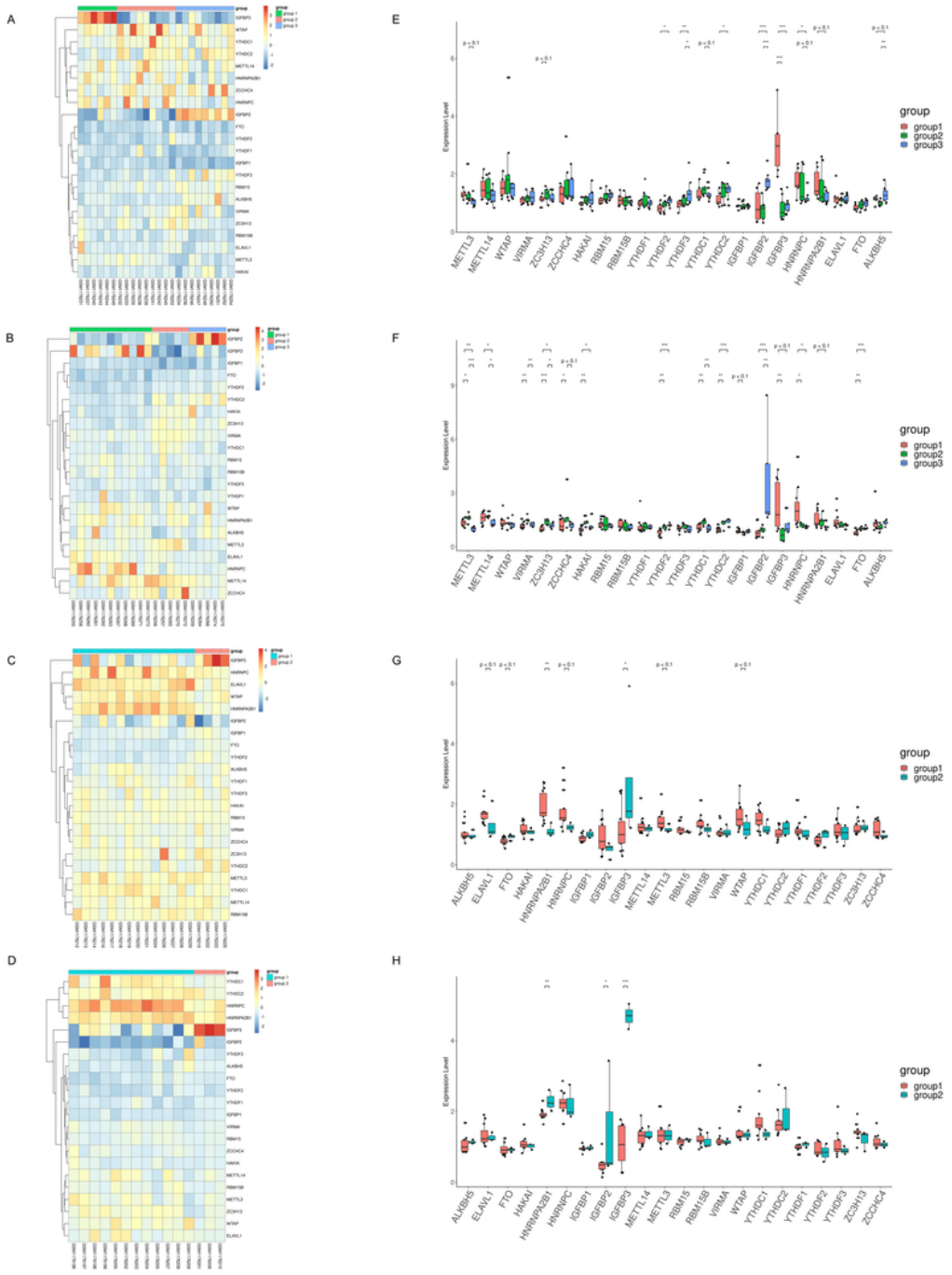

Figure 5

Identification of different modification patterns in AD samples among four brain regions. (A) Heatmaps of consensus clustering results based on m6A regulator profiles in the PG region, (B) the SFG region, (C) the HP region, (D) and the EC region. (E) The m6A regulators that have significant changes among different modification patterns ($p \leq 0.1$) in the PG region, (F) the SFG region, (G) the HP region, and (H) the EC region.

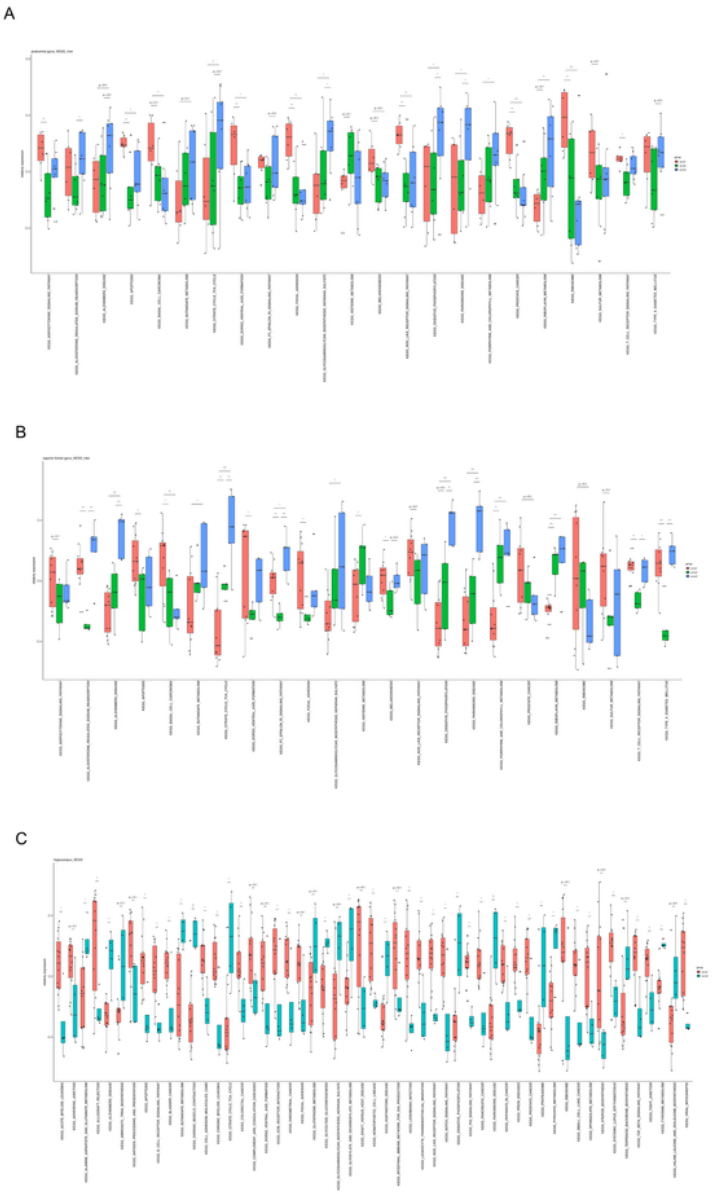

Figure 6

Pathways related to m6A modification patterns. (A) Pathways that have changes in GSVA scores and have similar alterations ($p \leq 0.1$) among different m6A modification patterns between the PG region and (B) the SFG region. (C) Pathways that have differences ($p \leq 0.1$) in GSVA scores between different m6A modification patterns in the HP region.

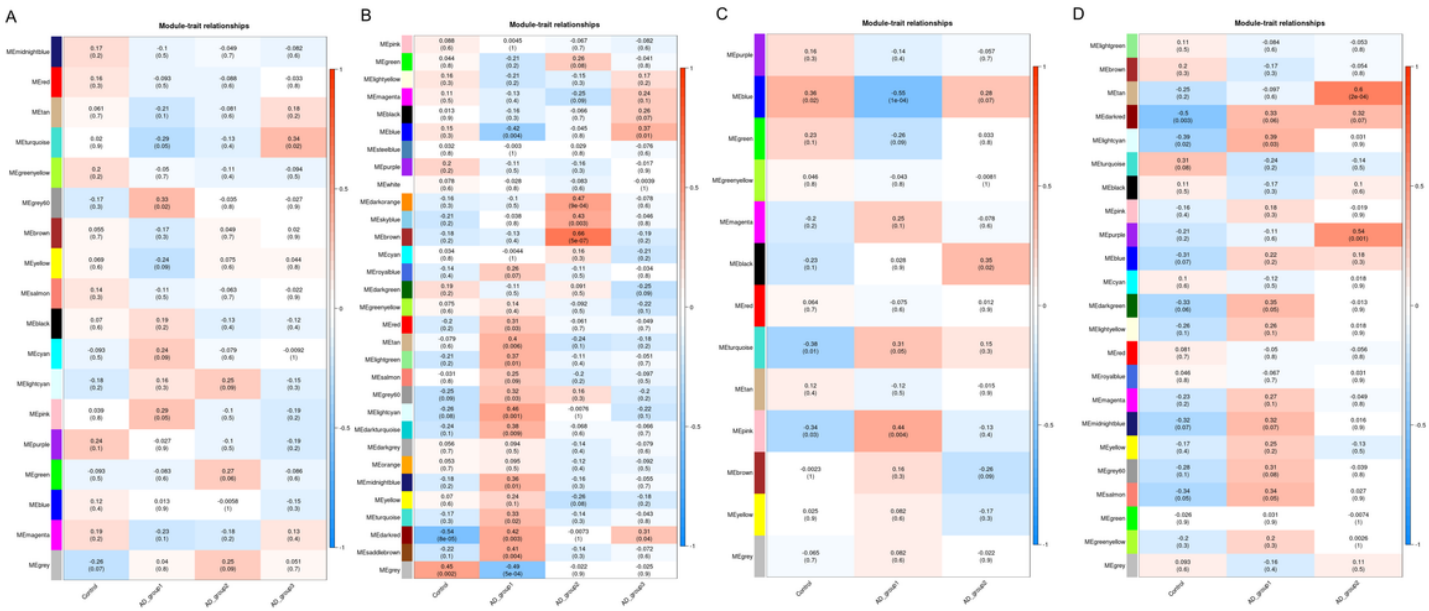

Figure 7

Screening of key regulators and their targets. (A) Heatmap of relationships between modules and phenotypes (AD group is divided into different modification patterns) in the WGCNA network in the PG region, (B) the SFG region, (C) the HP region, (D) and the EC region. In each column, the figures above show the correlation between module and phenotype, and the figures below show the p value of the correlation.

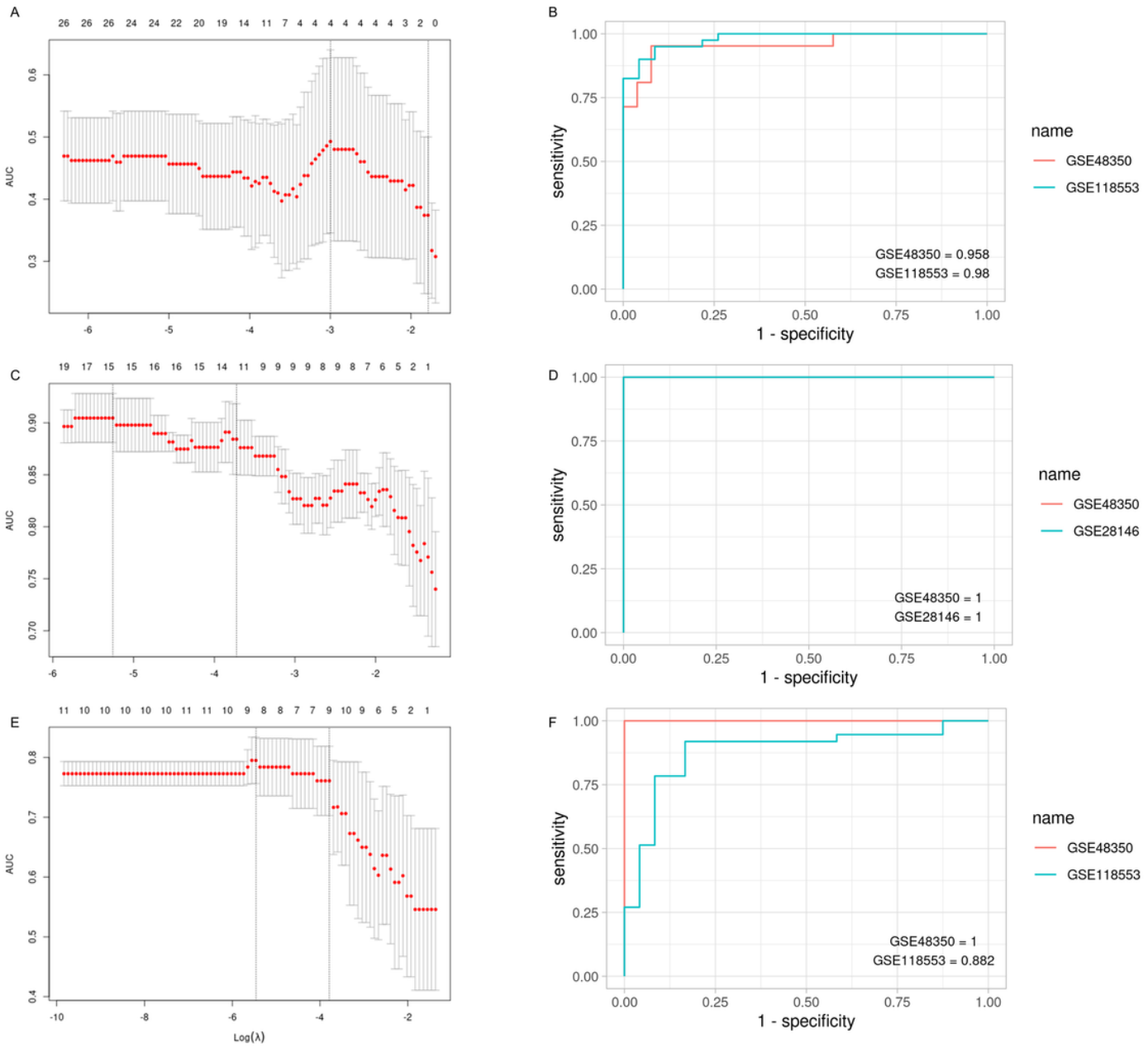

Figure 8

Construction of diagnostic models in each brain region using key m6A regulators and their target genes. (A) LASSO punished logistic regression, showing the relation between λ and AUC in the SFG region. (B) The AUC of the final model in the test dataset and validation dataset. The same results are shown in (C-D) the HP region and (E-F) the EC region.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuppFig1.tif
- SuppFig2.tif
- SuppFig3.tif
- SuppFig4.tif
- SuppFig5.tif
- SuppFig6.tif
- Supplementarytable1.xlsx
- Supplementarytable2.xlsx
- Supplementarytable3.xlsx
- Supplementarytable4.xlsx
- Supplementarytable5.xlsx
- Supplementarytable6.xlsx
- Supplementarytable7.xlsx
- Supplementarytable8.xlsx
- Supplementarytable9.xlsx