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Original Article

Systematic post-translational modification genome wide identifies therapeutic targets for Alzheimer's disease: evidence from multi-cohort analysis

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ABSTRACT

Background: The rapid increase in the incidence of Alzheimer's disease (AD) has raised concerns, given its profound effects on both society and the economy. Despite extensive research efforts in this area, there are no existing treatments that have the ability to change the progression of the disease.
Methods: To identify the distinct subtypes of AD, consensus clustering was employed. Following this, module genes were identified through the implementation of WGCNA. In addition, the investigation included the identification of hub genes through the application of machine learning. Ultimately, a thorough analysis was performed utilizing a methodical strategy to perform post-translational modification (PTM) genome wide.
Results: GO and KEGG analyses were conducted by examining of 21 different types of PTMs, revealing that the majority of these genes play key roles in the PTM pathways, as well as AD-related pathways. Correlation analysis revealed that these PTM were significantly correlated with gamma secretase activity, beta secretase activity, amyloid-beta 42, clinical dementia rating, Braak stage, plaque, and neurofibrillary tangle. Then, two distinct subtypes of PTM were identified, each characterized by unique clinical characteristic. By utilizing machine learning, we developed an PTM.score, and has shown impressive predictive capabilities for AD when tested against various datasets (brain AUC: 0.859, blood AUC: 0.898), indicating its potential utility in clinical settings for risk stratification and therapeutic decision-making. Moreover, our investigation led to the identification of two genes (TRIM47 and LNX1) that may represent potential drug targets for AD (brain tissues or blood samples). Research further indicated a potential correlation between TRIM47 and concentrations of CSF Aβ (OR 1.068 (1.029–1.108)), CSF p-tau (OR 1.315 (1.136–1.524)), and total hippocampal (OR 1.176 (1.058–1.307)).
Conclusions: The findings from this study not only enhance our comprehension of the underlying mechanisms of AD but also serve to inform and direct future initiatives in drug discovery. By focusing on TRIM47, the work paves the way for identifying innovative therapeutic strategies.

1. Introduction

Alzheimer's disease (AD) has become the fifth leading cause of death globally [1,2]. Among individuals aged 65 and older, the prevalence of AD is approximately 3.21 %. With increasing age, particularly in those aged 85 and above, the prevalence of AD rises sharply, reaching as high as 30 %–40 % [3]. However, in stark contrast, the diagnosis and treatment rates for AD remain low [4]. The etiology and pathogenesis of AD are not yet fully understood, so clinical diagnosis still relies on exclusion. Studies have shown that pathological changes associated with AD begin to manifest in the brain 10 to 20 years before symptoms appear. This finding indicates that a "window period" of over a decade exists between the onset of pathological changes and the emergence of symptoms. Early screening and intervention during this period are most effective. Currently, the primary therapeutic agents for AD in clinical practice fall into two major categories: the first is cholinesterase inhibitors, and the second is N-methyl-d-aspartic acid receptor (NMDA) antagonists [5,6]. However, due to the complex nature of AD, which is

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influenced by multiple factors, currently available drugs can only delay disease progression and cannot reverse or halt its advancement. Thus, it is essential to explore and identify further key genes, along with understanding the mechanisms that govern their functions.

Pathological changes in AD include β-amyloid (Aβ) plaques, neurofibrillary tangles (abnormal Tau protein aggregates), synaptic dysfunction, neuronal loss, and brain atrophy [7]. Tau protein participates in regulating neuronal functions such as signal transduction, neurodevelopment, and neural plasticity through mechanisms including mRNA alternative splicing and post-translational modifications [8]. Tau protein undergoes multiple types of post-translational modifications (PTM), but these modifications produce differing biological effects [9]. For instance, while excessive phosphorylation of tau initially exerts a protective role, its overall biological effect is detrimental. Modification types with negative effects also include glycosylation, glycation, acetylation, nitration, and SUMOylation. Targeted therapies for pathological tau protein have become a current research focus. Fyn is a tyrosine kinase that becomes hyperactivated during AD pathogenesis, potentially leading to tau protein phosphorylation at tyrosine 18, formation of toxic Aβ oligomers, and elevated levels of cellular prions [10]. The small-molecule anticancer drug AZD0530, a Fyn inhibitor, demonstrated activity in suppressing tau protein aggregation in transgenic mouse models. A Phase 1b clinical trial demonstrated that AZD0530 is highly safe and well tolerated in patients with mild to moderate AD [11]. Abnormal post-translational modifications of β-amyloid precursor protein (APP) are key factors affecting APP metabolism and leading to the pathological process of Aβ deposition. These modifications regulate APP metabolic processes such as cleavage and degradation [12]. Research has found that HRD1 protein levels are significantly reduced in the cerebral cortex of AD patients, leading to APP accumulation. HRD1 can bind to APP and promote its ubiquitination and proteasome-dependent degradation, thereby reducing Aβ production [13]. The C-terminal region of APP contains multiple ubiquitinoylation sites. Ubiquitinoylation is involved in APP degradation, which requires the ubiquitin-proteasome system (UPS). Impairment of the UPS increases the pathological accumulation of toxic proteins, affecting the degradation of both APP and Aβ [14]. Simultaneously, Aβ inhibits proteasome activity, thereby impairing the multivesicular body (MVB) sorting pathway, creating a vicious cycle between Aβ deposition and UPS dysfunction [15]. In summary, post-translational modifications of APP and Tau play a crucial role in the deposition process of Aβ and Tau. Research into the associated regulatory mechanisms will provide a solid basis for selecting drug targets in AD.

Despite significant progress in the field, a thorough understanding of the relationship between PTM and AD continues to be a challenge. The intricate connection between PTM and changes in clinical characteristics associated with AD necessitates further detailed investigation to clarify these associations. To address these existing knowledge gaps, we undertook an integrative approach by analyzing 21 distinct types of PTM. Our goal was to uncover potential biomarkers that could enhance the diagnosis and understanding of AD. By utilizing advanced machine learning algorithms, we developed an innovative metric known as PTM. score aimed at forecasting efficacy in AD patients. Our research demonstrated that PTM.score exhibits remarkable effectiveness in predicting clinical outcomes for individuals diagnosed with AD. In the context of a genome-wide analysis, TRIM47 has been identified as a critical molecular component that plays a significant role in the pathogenesis of AD. This discovery marks TRIM47 as a factor that had not been previously recognized in relation to AD, highlighting its importance in the disease's underlying mechanisms. Consequently, TRIM47 represents a promising new target for therapeutic interventions aimed at treating this debilitating condition.

2. Materials and methods

2.1. Data gathering

Firstly, eight brain multicenter datasets transcriptome data from GSE106241 [16], GSE28146 [17], GSE118553 [18], GSE48350 [19], GSE122063 [20], GSE5281 [21], GSE132903 [22], and GSE84422 [23] datasets were downloaded. Furthermore, we also assembled a collection of 3 blood multicenter datasets (GSE63060 [24], GSE63061 [24], and GSE85426). In cases where multiple probes corresponded to a single gene, the expression level for that gene was determined by averaging the expression values of the various probes. To eliminate batch effects between different batches within the same dataset and platform, the removeBatchEffect function from the 'Limma' package in R software will be utilized. When performing joint analyses of data from different datasets or platforms, common gene symbols will first be extracted, and the different datasets or platforms will be labeled as distinct batches. Based on this, the removeBatchEffect function will be applied again to remove batch effects [25]. Furthermore, PTM related genes were systematically curated through comprehensive literature examination (Supplementary Table 1).

2.2. Consensus clustering

Cluster analysis is an analysis method that groups similar samples into a group through static classification, such that samples in different groups have differences, and samples in the same group have the same attributes. We used "ConsensusClusterPlus" to identify subtypes by PTM expression profiles [26]. PCA was used to evaluate the distribution differences patients in different subtypes.

2.3. DEGs screening and enrichment analysis

Consequently, differential expression genes (DEGs) were identified based on a significance threshold set at FDR <0.01, ensuring that the findings were statistically significant and minimizing the likelihood of false positives. To further understand the possible biological functions and mechanisms of the key genes in AD, we used the 'clusterProfiler' package for GO and KEGG of the screened key genes [27]. During the analysis process, we retained the enriched items according to the significance level ($P < 0.05$) to ensure the reliability and accuracy of the results.

2.4. Screening key genes based on weighted gene co-expression network analysis (WGCNA)

We use the "WGCNA" package to screen out the key genes related to different subtypes in AD. First, we selected the genes with 25 % high variance, and the correlation matrix was constructed and the weighted adjacency matrix was generated by quantifying the correlation between each gene the cell phenotype through the gene expression spectrum. Second, the power exponent β and the scale-free topological fitting index R^2=0.85 were automatically calculated the function pickSoftThreshold and the function softConnectivity, respectively, and the soft threshold parameter was set so that the subsequent network construction was more consistent with the scale-free characteristics. Further, the topological overlap matrix was generated, the correlation degree between genes was calculated, and the hierarchical clustering tree of genes was obtained according to this. Finally, the clustering was divided, and different gene co-expression modules were obtained, the function verboseScatterplot was used to the correlation between gene expression and trait relatedness and gene expression and module relatedness, and the genes with GS value (>0.80) and high MM value (>0.85) were defined as the threshold for identifying key genes in the. Finally, the module most related to each subtype and the most important key genes in the module were obtained [28].

2.5. Identification of PTM.score

The GSE118553 dataset served as the foundational dataset for the construction of the risk model, while the datasets GSE106241, GSE28146, GSE48350, GSE122063, GSE5281, GSE132903, and GSE84422 were employed to validate the findings within the cohort. The methodology for generating the signature proceeded through several key steps: First, DEGs, PTM, and WGCNA were conducted among the various subtypes. This initial analysis aimed to discern the significant genetic variations that may influence prognosis and therapeutic approaches. Next, a comprehensive integration of ten distinct machine learning algorithms was executed. This step was crucial for exploring different methodologies and determining which algorithms might provide the best predictive performance in the context of the model being developed. Finally, the AUC-index for dataset was calculated.

2.6. Evaluation and analysis of immune microenvironment

7 algorithms were used to analyze the immune microenvironment for AD. The distribution of cell types was depicted using the 'pheatmap' R package in molecular subtype and high PTM.score and low PTM.score groups [29]. In addition, we the differences in the expression levels of immunomolecules in high PTM.score and low PTM.score groups.

2.7. eQTL datasets

The blood eQTL consists of an extensive dataset that captures the cis-expression quantitative trait loci (cis-eQTLs), offering a rich foundation for exploring genetic influences on gene expression. The findings we present are both reliable and scientifically valid with FDR$<$0.05. In addition to this, we also collected crucial allele frequency data to further reinforce our conclusions. In our investigation, we maintained a focus on significant eQTLs, adhering to the same FDR threshold of less than 0.05 as applied in the blood eQTL analysis. Additionally, we ensured that all pertinent single nucleotide polymorphism (SNP) information was collected to substantiate the outcomes of our research, thereby providing a robust framework for understanding the genetic factors influencing gene expression in these specific contexts [30,31].

2.8. GWAS dataset

Gene expression served as the exposure variable, while the primary outcome of interest was the presence of Alzheimer's disease. To minimize potential bias from weak instrumental variables and to ensure the robustness of the results, specific thresholds were applied for screening SNPs. The criteria included a threshold of r^2 values less than 0.001, a kilobase distance (kb) of less than 10,000, and a p-value threshold set at less than 5×10^{-8}. Additionally, the heterogeneity of the instrumental variables was assessed through the use of the MR-Egger method, as referenced in previous literature. A two-sample MR study was conducted. This approach allows researchers to explore the potential influence of genetic factors on the development of this neurological condition [32].

2.9. Statistics

R version 4.2.0 was the tool used for all bioinformatics analyses. We used the Kruskal-Wallis test for non-parametric data and the Wilcoxon test for comparisons between two independent samples and multiple samples, respectively. One-way ANOVA and *t*-tests were applied to parametric data. A p-value of less than 0.05 was deemed statistically significant for every analysis.

3. Results

3.1. Identification of molecular subtypes of AD

In the examination of 21 different types of PTMs, there was a noteworthy variation in the number of associated genes. The gene counts fluctuated significantly, with some PTMs linked to as few as 2 genes, while others were associated with as many as 426 genes, as illustrated in Fig. 1A. This range highlights the diverse genetic landscape related to various PTMs. Subsequently, GO and KEGG analyses were conducted, revealing that the majority of these genes play key roles in the PTM pathways, as well as AD-related pathways. The results of these analyses are presented in Figs. 1B and 1C, underscoring the significant involvement of these genes in critical biological processes related to PTMs and AD. Correlation analysis revealed that these PTM were significantly correlated with gamma secretase activity, beta secretase activity, amyloid-beta 42, clinical dementia rating, Braak stage, plaque, and neurofibrillary tangle (Fig. 1D-K). This finding suggests that the PTMs could serve as a valuable indicator of patient prognosis, highlighting the potential of these modifications in influencing clinical outcomes in AD.

Then, consensus clusters were generated for PTM profiles by employing clustering techniques, which allowed for a detailed analysis of 21 different types of PTMs. The analysis revealed that optimal clustering of the data was achieved when the number of clusters, denoted as k, was set to 2, as illustrated in Fig. 2A-H. Then, based on the mRNA expression of PTM-related genes, PCA effectively illustrated the differences between the two identified subtypes. Following the identification of DEGs across the two clusters, a total of 1441 shared DEGs were recognized (Supplementary Table 2). Further analysis demonstrated that DEGs were predominantly enriched in various AD-related pathways (glutamatergic synapse, cognition, GABA receptor activity, asymmetric synapse) (Fig. 2I and 2J). This suggests that these DEGs might be pivotal in the progression and development of AD.

3.2. Clinical characteristic in molecular subtypes of AD

Then, our findings indicated that patients categorized within PTM.cluster.A exhibited significantly higher levels of gamma-secretase activity, beta-secretase activity, and amyloid-beta 42 in comparison to those individuals classified in PTM.cluster.B (Figs. 3A-D). The findings derived from the GSE84422 dataset indicated a significant difference between the two clusters of patients. Specifically, those categorized in PTM.cluster.A exhibited elevated levels of Braak staging, which is a histopathological grading system used to assess the progression of neurofibrillary tangles associated with AD. Additionally, patients in PTM.cluster.A demonstrated a greater accumulation of plaques, another hallmark characteristic of neurodegenerative conditions. Furthermore, these individuals also received a higher clinical dementia rating, suggesting more severe cognitive impairment relative to their counterparts in PTM.cluster.B (Figs. 3E-I). This distinction underscores the varying degrees of neurodegenerative pathology present in the two patient populations. Moreover, patients in PTM.cluster.A was significantly older compared to those in other clusters (Figs. 3J). This finding underscores the potential impact of age as an important factor in the characteristics of this group. Furthermore, the analysis also uncovered a significant variation in gender distribution, suggesting that gender may play a role in the composition and perhaps the underlying biology of this patient group (Figs. 3K). In addition to age, our study highlighted a notable difference in genetic predispositions, indicating a potential link between this genetic marker and the characteristics of patients (Figs. 3L). This finding suggests that the clustering based on PTMs could serve as a valuable indicator of patient prognosis, highlighting the potential of these modifications in influencing clinical outcomes in AD.

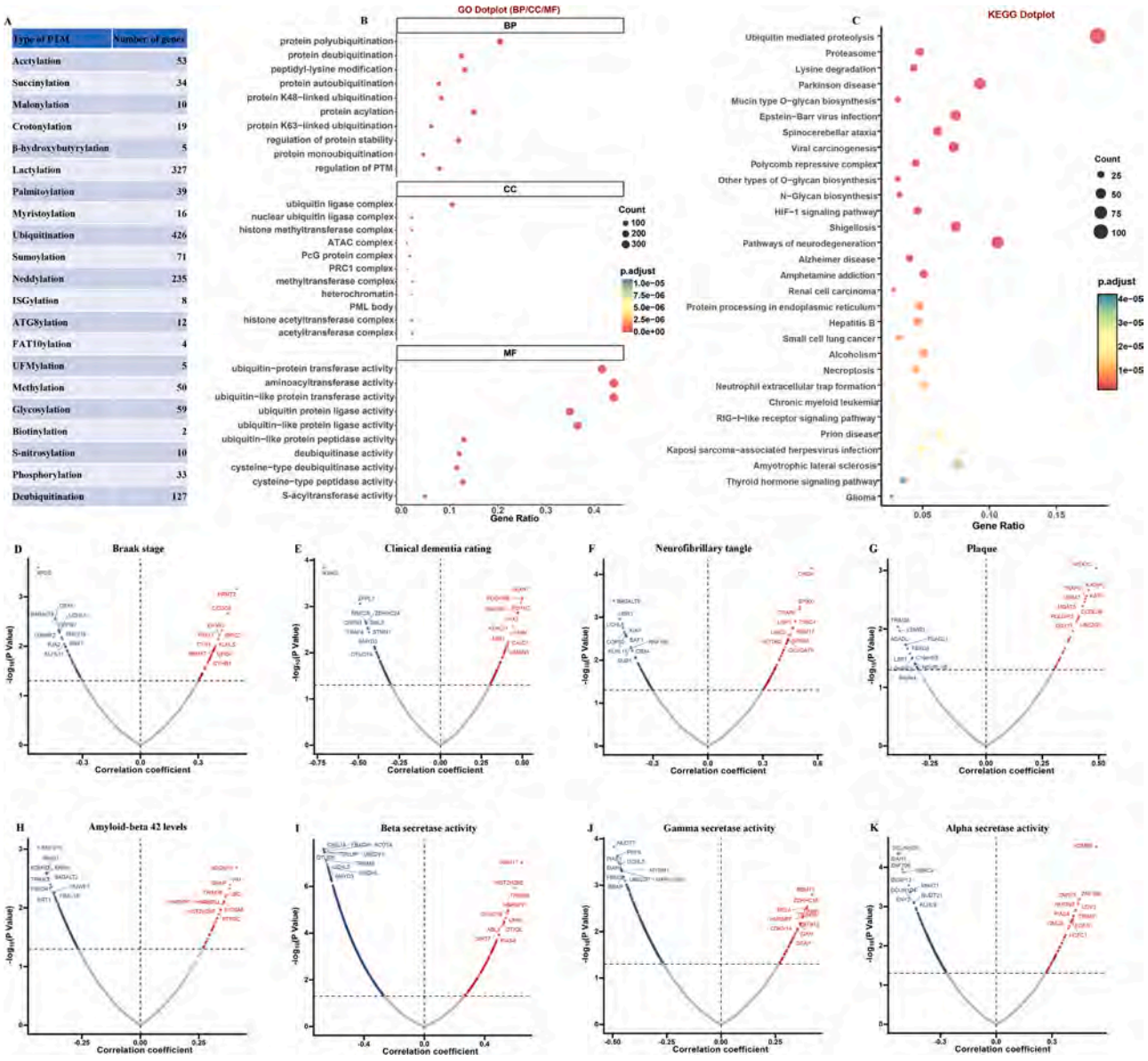

Fig. 1. Correlation of 21 different types of post-translational modification with AD. (A) The 21 types of PTMs used in this study. (B) GO and (C) KEGG enrichment analysis on PTMs. (D-K) Correlation analysis between the expression of PTMs and gamma secretase activity, beta secretase activity, amyloid-beta 42, clinical dementia rating, Braak stage, plaque, and neurofibrillary tangle.

3.3. *WGCNA analysis*

To uncover the significant module genes linked to the progression of AD, WGCNA was utilized. The analysis indicated that a β value of 3 yielded a high determination coefficient (R^2=0.85), signifying a robust relationship among the genes (Figs. 4A). Subsequently, a dendrogram of gene modules was generated by assessing the differences between these modules, leading to the identification of a total of 38 distinct modules (Figs. 4B). The analysis revealed that the blue module exhibited a negative correlation with PTM.cluster.A (r=−0.53, $P < 0.001$, Fig. 4C). Additionally, GO and KEGG pathway analyses highlighted that the key module genes were predominantly enriched in AD-related biological processes (learning, neurotransmitter secretion, neuron to neuron synapse, and Alzheimer disease) (Figs. 4D and 4E). Collectively, these findings suggest that the identified key module genes are likely to play significant roles in the development of AD. Finally, a specific selection was made of the 36 overlapping genes found among the DEGs and prognosis genes identified between the two subtypes and the key module genes and PTM genes (Supplementary figures 1). These genes will be the focus of further investigation to enhance our understanding of their contributions to the AD.

3.4. *Construction of PTM.score*

It was particularly fascinating to note that the primary model identified in our analysis was XGBoost. This hybrid model exhibited remarkable performance metrics, attaining an outstanding average AUC index of 0.859 for brain tissues and 0.898 for blood samples (Figs. 5A-C). AUC values for the PTM.score in all datasets were significantly high, with values recorded at 0.841 for GSE118553, 0.993 for GSE106241, 0.856 for GSE122063, 1.000 for GSE132903, 0.624 for GSE28146, 0.904 for GSE48350, 0.797 for GSE5281, 0.855 for GSE84422, 0.989 for GSE63060, 0.989 for GSE63061, and 0.800 for GSE85426. This notable result not only highlights the model's effectiveness in assessing risks but also reinforces its reliability as a tool for such evaluations. The high AUC index value indicates that the model has a strong capacity to accurately

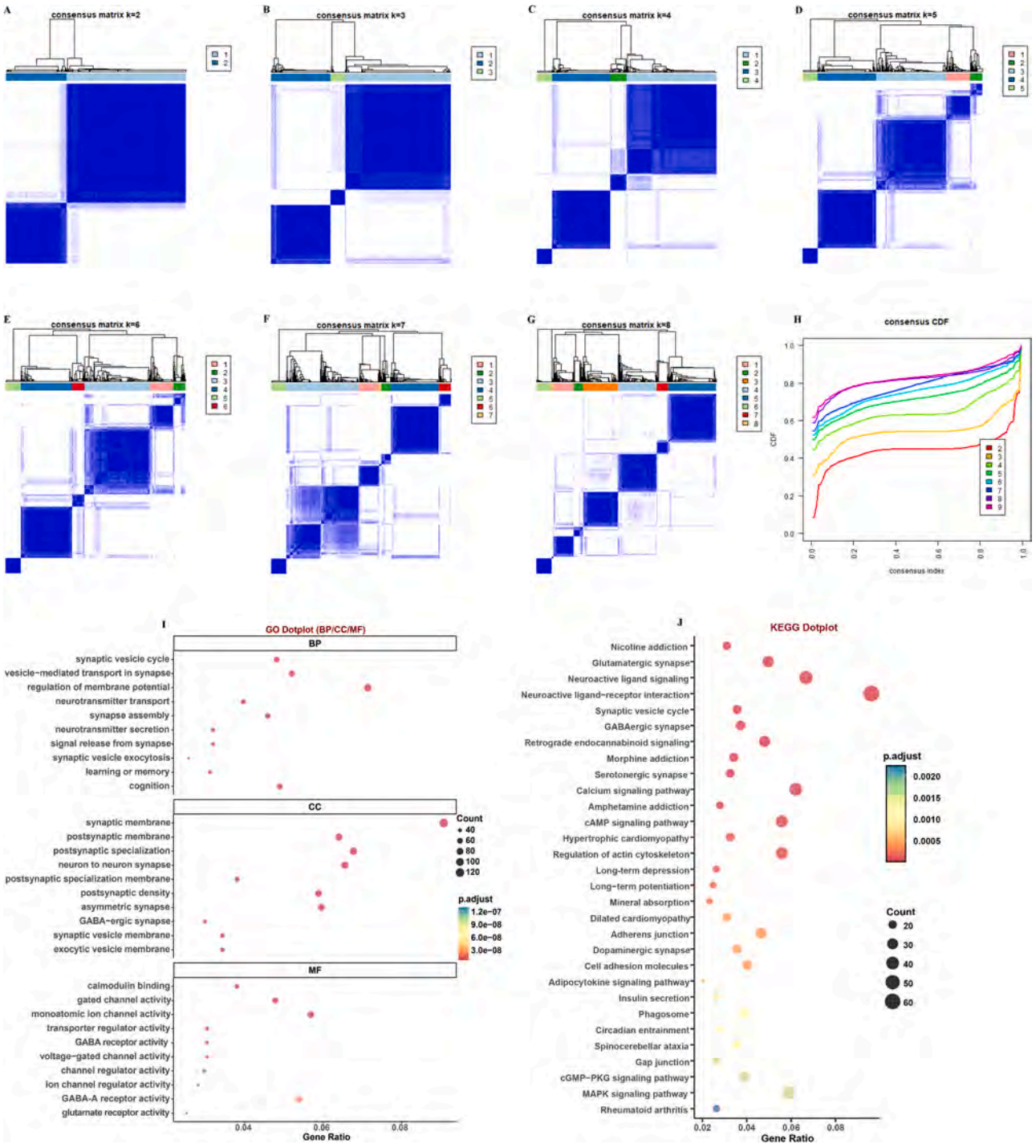

Fig. 2. Identification of molecular subtypes of AD. (A-H) Consensus clustering utilizing PTM genes. GO (I) and KEGG (J) enrichment analysis on the DEGs between the 2 clusters.

predict outcomes, making it an important asset in the field of risk assessment.

3.5. Immune cell infiltration

Following the identification of DEGs across high and low PTM.score, a total of 6578 shared DEGs were recognized (Supplementary Table 3). Further analysis demonstrated that DEGs were predominantly enriched in various AD-related pathways (Fig. 6A and 6B). This suggests that these DEGs might be pivotal in the progression and development of AD.

Utilizing the ESTIMATE algorithm, we found significant differences in immune metrics between two distinct groups identified as high and low PTM.score. Specifically, the high PTM.score group exhibited elevated scores in terms of immune cell infiltration when compared to low PTM.score (Fig. 6C). This finding suggests that the biological environment associated with high PTM.score is more conducive to immune engagement. Furthermore, an in-depth examination of the immune landscape, assessed through the lens of seven distinct algorithms. The analysis indicated that the high PTM.score group contained a richer variety and higher abundance of immune cells (Fig. 6C). This emphasizes the potential for a more active immune response in individuals within high PTM.score group. In addition to the differences in immune

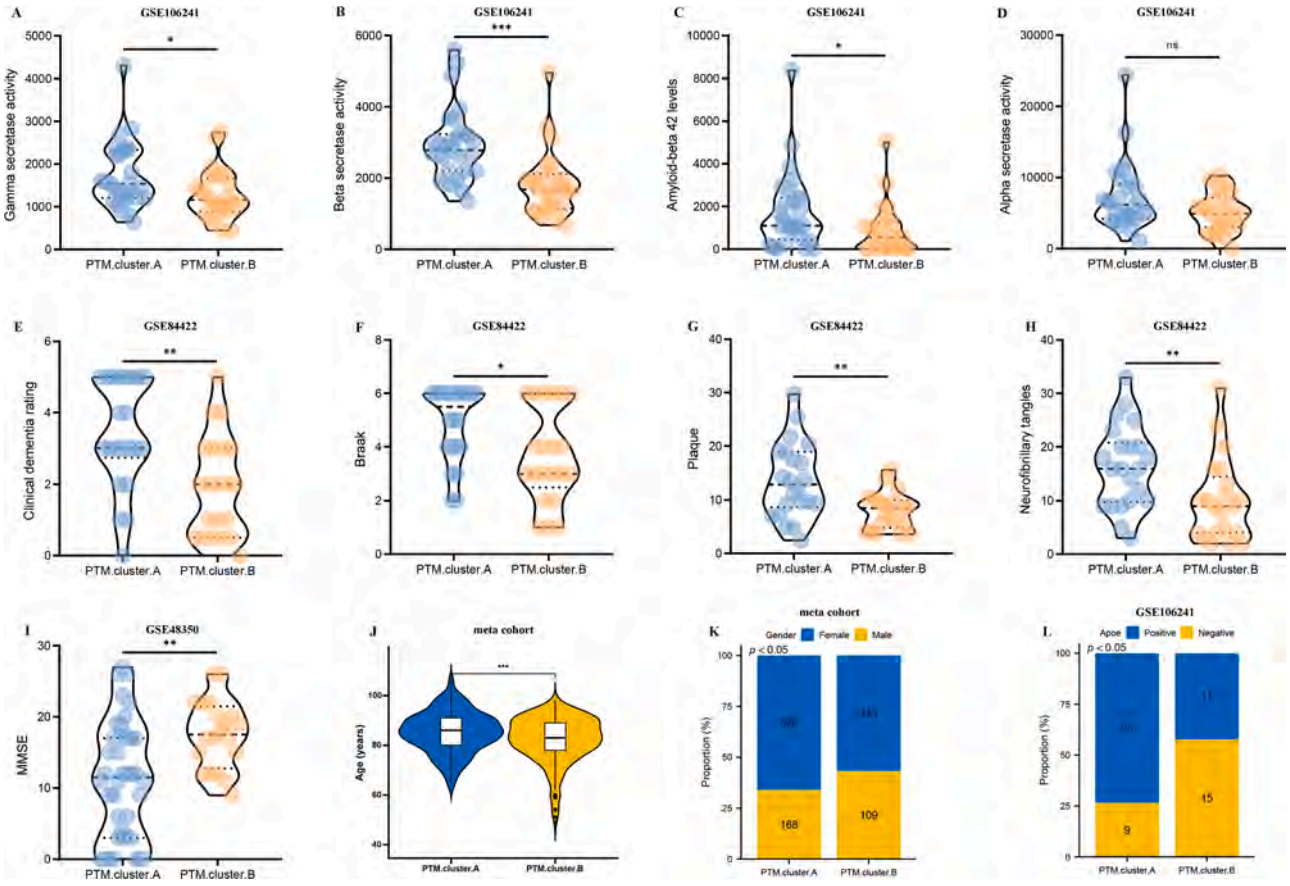

Fig. 3. Clinical of the AD subtypes. Comparison of gamma-secretase activity (A), beta-secretase activity (B), amyloid−beta 42 levels (C), alpha secretase activity (D) between two AD subtypes in GSE106241. Comparison of Braak (E), plaque (F), NFT (G), clinical dementia rating (H), between two AD subtypes in GSE84422. Comparison of MMSE (I) between two AD subtypes in GSE48350. (J) Comparison of age between two AD subtypes in meta cohort. (K) Proportion of sex between two AD subtypes in meta cohort. (L) Proportion of APOE between two AD subtypes in GSE106241.

cell populations, the levels of immune modulators were also found to be significantly greater in the high PTM.score group. This could imply that high PTM.score group is enriched with factors that enhance immune activity and regulation, further supporting the notion of a robust immune system in meta cohort (Fig. 6D). In summary, the findings suggest that patients belonging to high PTM.score group demonstrate a significantly greater immune infiltration and an abundance of immune modulators. These characteristics may collectively contribute to the advancement of AD, indicating a potential area for further research and therapeutic targeting.

3.6. Clinical characteristics of PTM.score

Our findings indicated that patients categorized within high PTM.score exhibited significantly higher levels of gamma-secretase activity, beta-secretase activity, and amyloid-beta 42 in comparison to those individuals classified in low PTM.score group (Figs. 7A-D). The findings derived from the GSE84422 dataset indicated a significant difference between the two clusters of patients. Specifically, those categorized in high PTM.score exhibited elevated levels of Braak staging, which is a histopathological grading system used to assess the progression of neurofibrillary tangles associated with AD. Additionally, patients in high PTM.score demonstrated a greater accumulation of plaques, another hallmark characteristic of neurodegenerative conditions. Furthermore, these individuals also received a higher clinical dementia rating, suggesting more severe cognitive impairment relative to their counterparts in low PTM.score group (Figs. 7E-I). This distinction underscores the varying degrees of neurodegenerative pathology present in the two patient populations. Our meta-cohort analysis revealed that patients in high PTM.score was significantly older compared to those in other clusters (Figs. 7J). This finding underscores the potential impact of age as an important factor in the characteristics of this group. Furthermore, the analysis suggested that gender may play a role in the composition and perhaps the underlying biology of this patient group (Figs. 7K). In addition, our study highlighted a notable difference in genetic predispositions, indicating a potential link between this genetic marker and the characteristics of patients (Fig. 7L).

3.7. MR analysis

Next, our analysis revealed 13 genes that could potentially be targeted for drug development in the context of AD, as identified across various datasets focused on both brain and blood samples. Among these genes, two previously known TRIM47 and LNX1 genes demonstrated statistically significant associations in both blood (TRIM47 OR 1.074 (1.021–1.129), LNX1 OR 0.915 (0.853 – 0.981)) and brain tissues (TRIM47 OR 1.093 (1.024–1.307), LNX1 OR 0.955 (0.919 – 0.993)). Furthermore, these genes were found to have a notable impact on the risk factors associated with the development of AD, indicating their relevance in potential therapeutic strategies.

In our examination of prior reviews, we focused on identifying the biochemical and neuroimaging markers associated with AD. Due to the limited availability of GWAS data specifically pertaining to AD markers, our investigation yielded a select group of markers that possessed adequate data for analysis. Our research indicates a potential link between TRIM47 in both blood and brain tissue and the total/right/left hippocampal volume, CSF Aβ, and CSF p-tau. This finding suggests that TRIM47 may play a role in the structural integrity of this vital brain

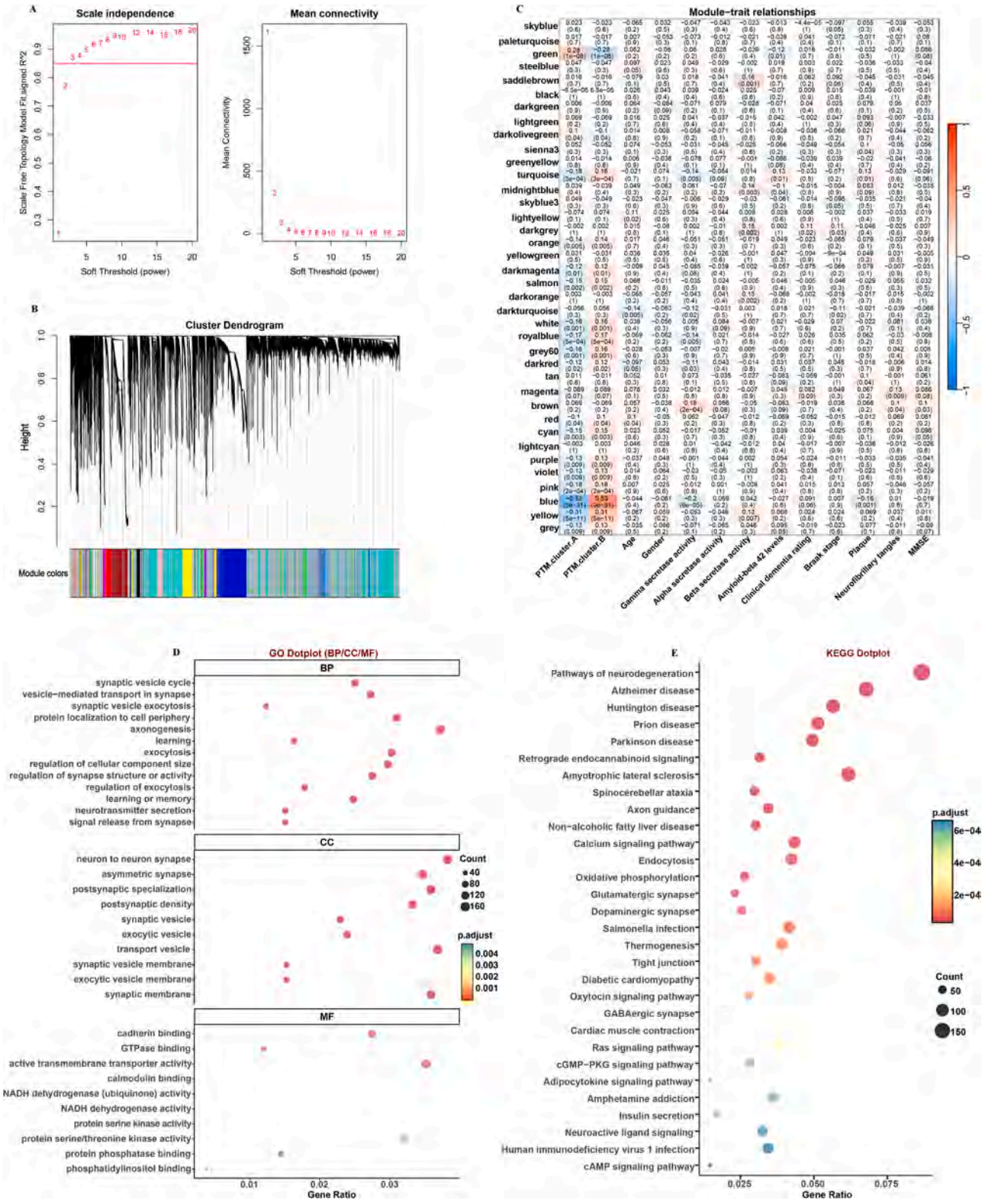

Fig. 4. WGCNA analysis. (A) The sample similarity of each subgroup was assessed by calculating the Silhoutte score. (B) Identification of co-expression gene modules. (C) Correlation analysis between module eigengenes and clinical traits. (D) GO and (E) KEGG enrichment analysis on the key module genes.

region, which is known to be involved in memory and cognitive function. However, it is important to note that we did not observe any significant associations between three previously identified PTM genes (LNX1) and the AD biomarkers that were analyzed in our study. This lack of correlation prompts further investigation into the specific roles of these genes in the context of AD and highlights the complexity of the genetic factors that may influence the disease and its markers (Fig. 8).

4. Discussion

Bioinformatics analysis has rapidly advanced over recent decades and been applied to numerous diseases, uncovering novel biomarkers

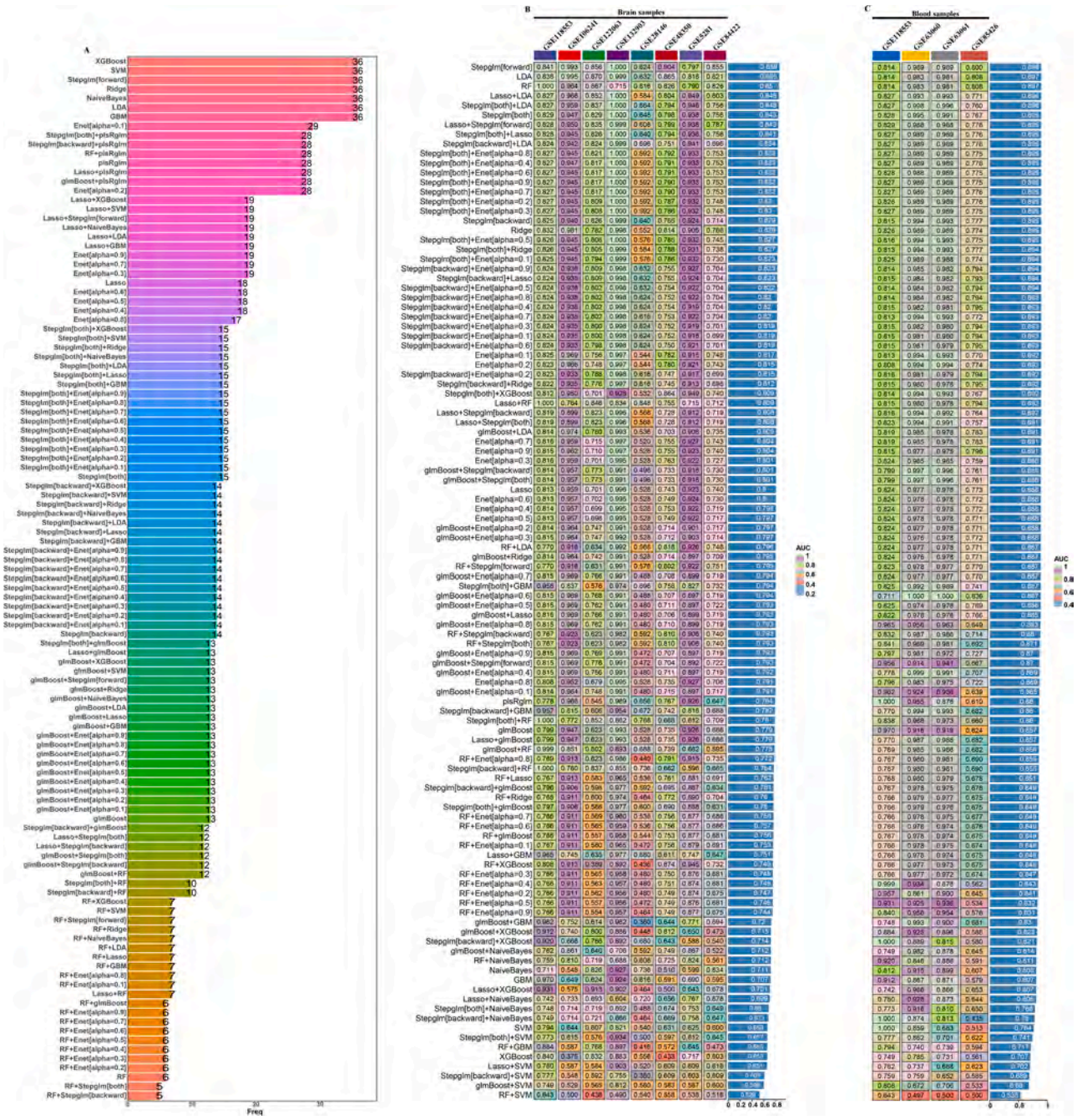

Fig. 5. Identification of hub genes by machine learning. (A) The most valuable overlapping genes based on the multiple algorithms. Hub genes were identified by a total of 113 combinations of machine learning algorithms in brain tissues (B) or blood samples (C).

for diagnosis and treatment while revealing the complex pathogenic mechanisms underlying certain conditions. However, previous studies on AD have typically relied on single datasets. This research combines and analyzes 8 multicenter datasets collected from the GEO database, identifying AD-associated subtypes. Subsequently, a series of subtype-based integrated analyses were conducted, including DEG, GO, and KEGG enrichment analyses, WGCNA analysis, and machine learning analysis. The final key genes were validated using other GEO datasets. Finally, MR analysis was performed on the key genes. Collectively, these efforts identified potential AD candidate biomarkers and therapeutic targets, providing valuable insights into the molecular mechanisms underlying the pathogenesis and progression of AD.

First, GO and KEGG pathway enrichment analysis revealed that DEGs between subtypes were predominantly enriched in synaptic function, with key module genes predominantly associated with learning and memory, processes closely linked to AD pathogenesis. Additionally, studies indicate that the MAPK signaling pathway is activated in susceptible brain regions of AD patients and contributes to disease progression, suggesting MAPKs as potential therapeutic targets for AD [33, 34]. Furthermore, GO enrichment analysis indicates that DEGs participate in diverse biological processes and exhibit distinct molecular functions. Collectively, Alterations in biological processes, cellular components, molecular functions, and pathways likely contribute significantly to the development and progression of AD. These changes are interconnected and can influence one another, ultimately leading to the neurodegenerative symptom's characteristic of this condition. Understanding the intricate relationships between these various factors is essential for gaining insight into the mechanisms underlying AD and

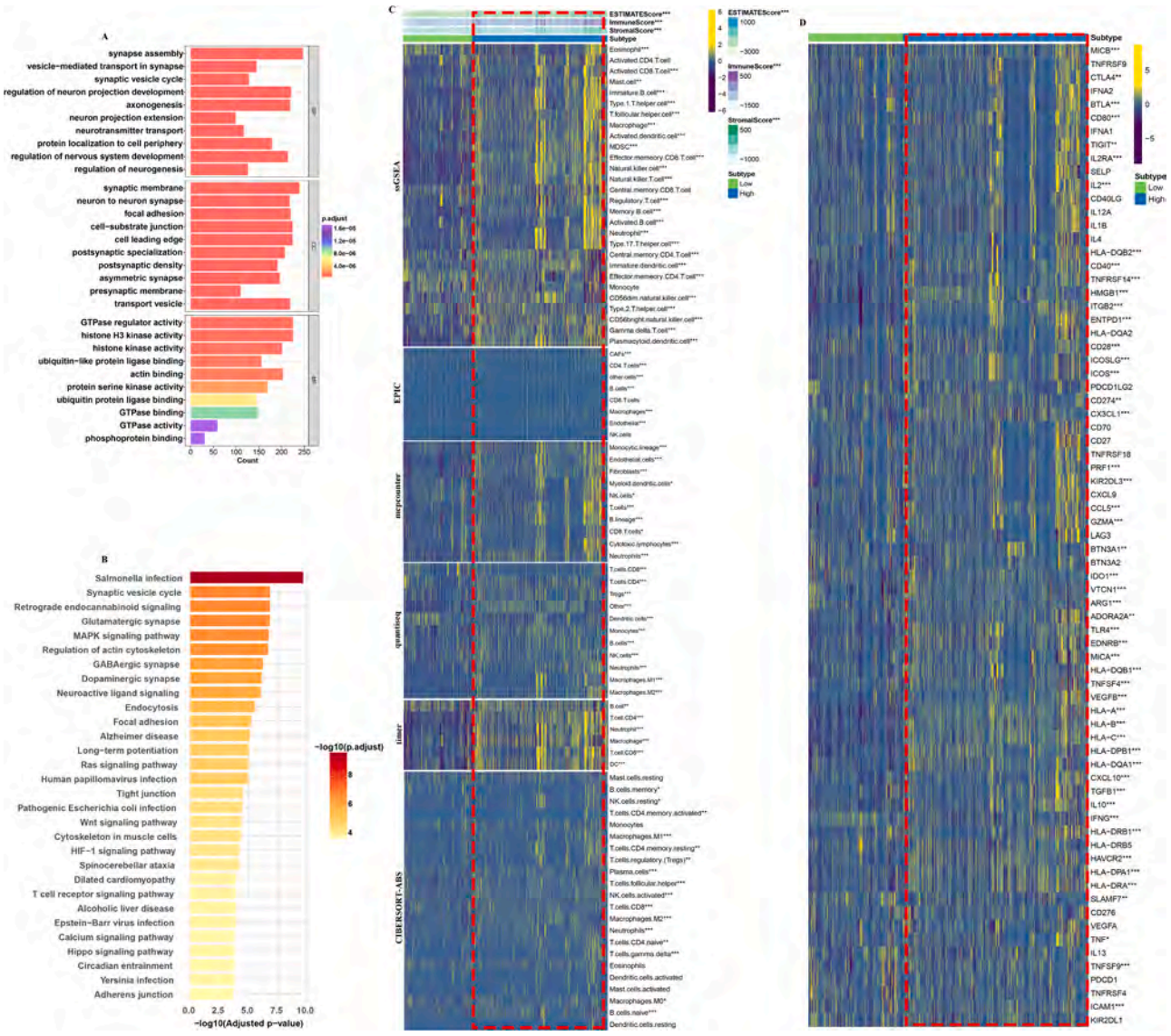

Fig. 6. Immune cell infiltration of high/low PTM.score. GO (I) and KEGG (J) enrichment analysis on the DEGs between the high/low PTM.score. (C) Immune cell infiltration difference between high/low PTM.score. (D) The immune modulator molecules expression between high/low PTM.score.

could be pivotal in identifying potential targets for therapeutic intervention.

Previous studies have demonstrated that the immune system plays a crucial role in the pathogenesis and progression of AD [35,36]. As effector cells of the innate immune system within the CNS, microglia perform immune surveillance, recognizing exogenous and endogenous CNS injuries and initiating immune responses. They provide nutritional support to neuronal cell bodies and axons, thereby ensuring brain tissue repair and maintaining cerebral homeostasis [37,38]. During AD progression, microglia can phagocytose amyloid beta and clear damaged neurons. However, they may also exhibit pro-inflammatory activity while simultaneously impairing their own clearance mechanisms [39]. Neutrophils represent the initial line of defense by being the first type of immune cells to be attracted to areas experiencing inflammation. Their primary role in host defense is multifaceted, as they engage in critical activities such as phagocytosis, where they engulf and digest pathogens. Additionally, neutrophils contribute to the immune response through degranulation, a process wherein they release antimicrobial substances stored in their granules to combat infections. Furthermore, they can form neutrophil extracellular traps, which are web-like structures composed of DNA and proteins that trap and neutralize microbial invaders. These combined functions underscore the vital importance of neutrophils in the body's immune response to harmful stimuli. Infiltrating neutrophils have been detected in brain tissue from AD patients and AD transgenic animal models [40,41]. In two AD mouse models (5xFAD and 3xTg-AD mice), neutropenia induced by Ly-6G or LY-6C antibody treatment significantly reduced amyloid burden and microglial activation. It also improved mouse performance in the Y-maze spontaneous alternation task and conditioned fear test, indicating that neutrophils promote pathological progression in AD [40]. This is consistent with our results, patients categorized with a PTM.cluster.A and high PTM.score group displayed a immune cell infiltration that was more actively engaged, which corresponded with a poor clinical characteristic.

Then, by integrating different categories of genes, including DEGs, module genes, and PTM-related genes, 36 hub genes were identified (RNF175, PRMT8, UCHL1, GABARAPL1, UBE2V2, UBE2E2, ZDHHC23, LNX1, SMYD2, B4GALT6, HECW1, PELI3, ACACB, RNFT2, USP11, TRIM37, TRIM36, RNF41, UCHL5, B3GNT1, ZDHHC22, GOT2, DTX3L, LONRF2, RFPL2, OXCT1, HERC1, OTUD7B, RNF128, PDGFRB, RNF135, UHRF1, TRIM47, OGDHL, ZNRF3, and S100A8). Among them, research by Gong et al. revealed that UCHL1 protein levels are reduced in the

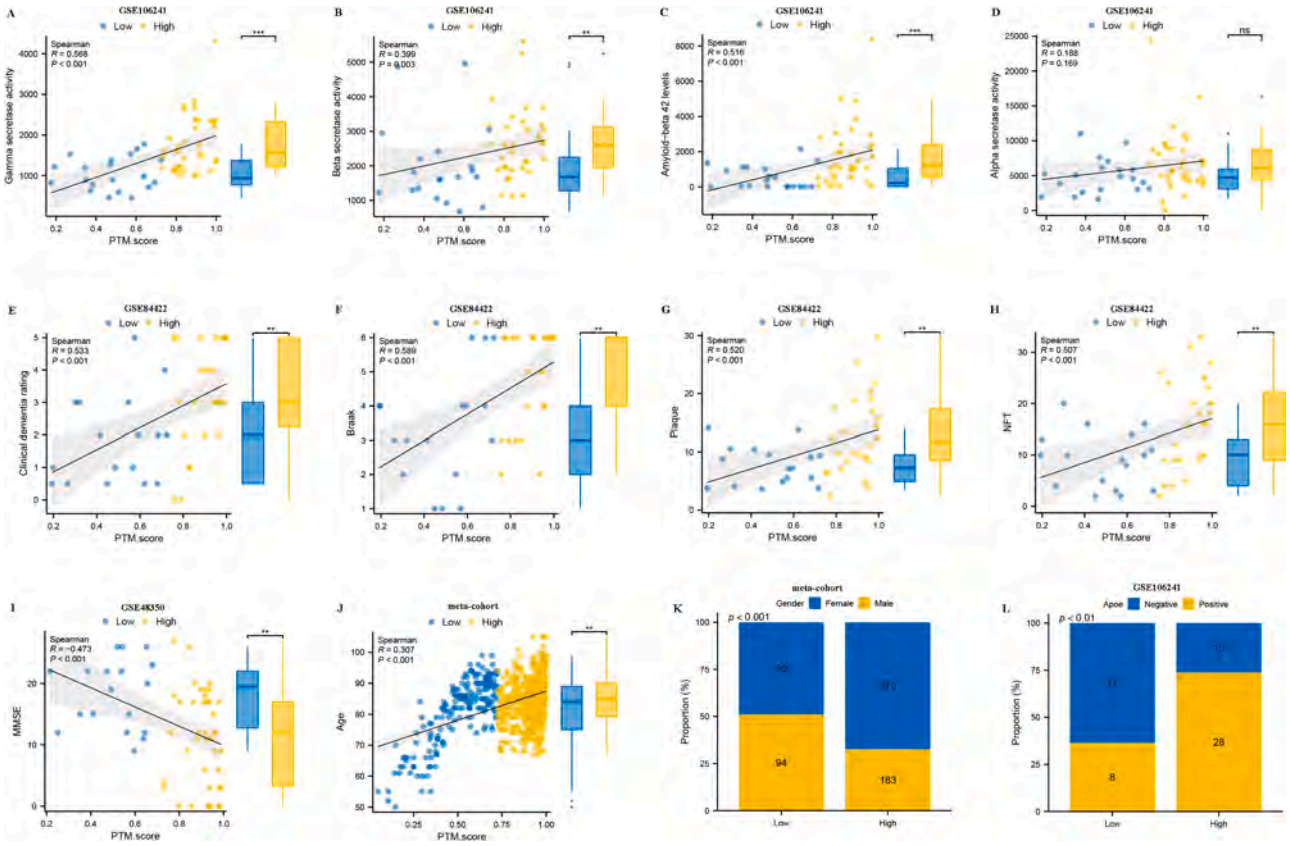

Fig. 7. Clinical characteristics of PTM.score. (A-L) Analysis of differences of clinical characteristics between high/low PTM.score.

APP/PS1 transgenic mouse model of AD [42]. Furthermore, the study found that downregulation of UCHL1 promotes tau protein phosphorylation, while increased degradation of UCHL1 protein was observed to cause greater neuronal damage [42]. In the frontal cortex of AD patients, UCHL1 is the primary protein affected by oxidative damage, with oxidatively modified UCHL1 exhibiting a 20–60 % loss of hydrolase activity. Studies on AD have revealed that exogenous supplementation of wild-type UCHL1 protein can restore damaged synaptic function and learning capacity in APP/PS1 transgenic AD mouse models [43].

Finally, TRIM47 could play a crucial role in the disease's progression and might be a valuable biomarker for assessing. The TRIM protein family comprises over 70 RING-domain-containing proteins that primarily function as E3 ligases in the ubiquitination process. TRIM proteins are essential in the regulation of a variety of cellular physiological activities. These proteins significantly influence mRNA localization, which is critical for the proper expression of genes, ensuring that the mRNA reaches the appropriate cellular locations for translation [44]. Additionally, TRIM proteins are involved in autophagy, the process by which cells degrade and recycle cellular components, thereby maintaining cellular homeostasis [45]. Moreover, TRIM proteins have a role in programmed cell death pathways, such as proptosis and apoptosis, which are vital for normal development and the elimination of damaged cells. They also participate in the regulation of the cell cycle and mitosis, ensuring that cells divide correctly and that genetic material is accurately distributed to daughter cells. In response to DNA damage, TRIM proteins help initiate repair mechanisms, thereby safeguarding genomic stability. Furthermore, these proteins are implicated in the cellular response to viral infections, where they can modulate the immune response and enhance the clearance of pathogens. TRIM proteins also activate various immune pathways and can influence inflammatory processes, contributing to the body's response to infection and injury. Overall, the multifunctional roles of TRIM proteins underscore their importance in maintaining cellular integrity and responding to environmental challenges. TRIM proteins play a crucial role in the regulation of essential proteins within various signaling pathways. They achieve this by functioning as ubiquitinated effectors and ubiquitin-like modifiers, which are integral in maintaining cellular homeostasis and modulating key biological processes [46]. Additionally, the TRIM family of proteins has garnered attention for their potential as biomarkers for early detection and diagnosis in cancer [47]. Researchers have actively evaluated these proteins to assess their utility in identifying malignancies at earlier stages, thereby improving diagnostic accuracy and patient outcomes.

Our findings should be interpreted in light of several limitations. The expression of key genes remains to be validated across various biological levels, including mRNA, cellular, and protein levels. To establish a comprehensive understanding, further in vivo and in vitro experiments are essential. These additional investigations will help elucidate the signaling pathways that may be involved with TRIM47 in the context of AD. Moreover, while there is a significant correlation between elevated TRIM47 expression and poor prognosis in AD, the nature of its relationship with specific clinical and pathological parameters, such as gender, age, ethnicity, smoking history, and the stage of the disease, has yet to be clarified. This gap in understanding signifies the need for more detailed research. Lastly, to bolster the reliability and generalizability of the findings, it is crucial to expand the sample size and to involve multiple research centers in future studies. Such measures will enhance the credibility of the results and contribute to a more robust assessment of these important factors in AD research.

In summary, a thorough examination was conducted to identify possible drug targets within PTM associated with AD using integrated bioinformatics approaches. This research offers genetic information that highlights the possible therapeutic advantages of focusing on TRIM47 for the treatment of AD. Consequently, a detailed investigation of TRIM47 in the context of AD enhances our understanding of the disease's pathogenesis and suggests effective treatment strategies.

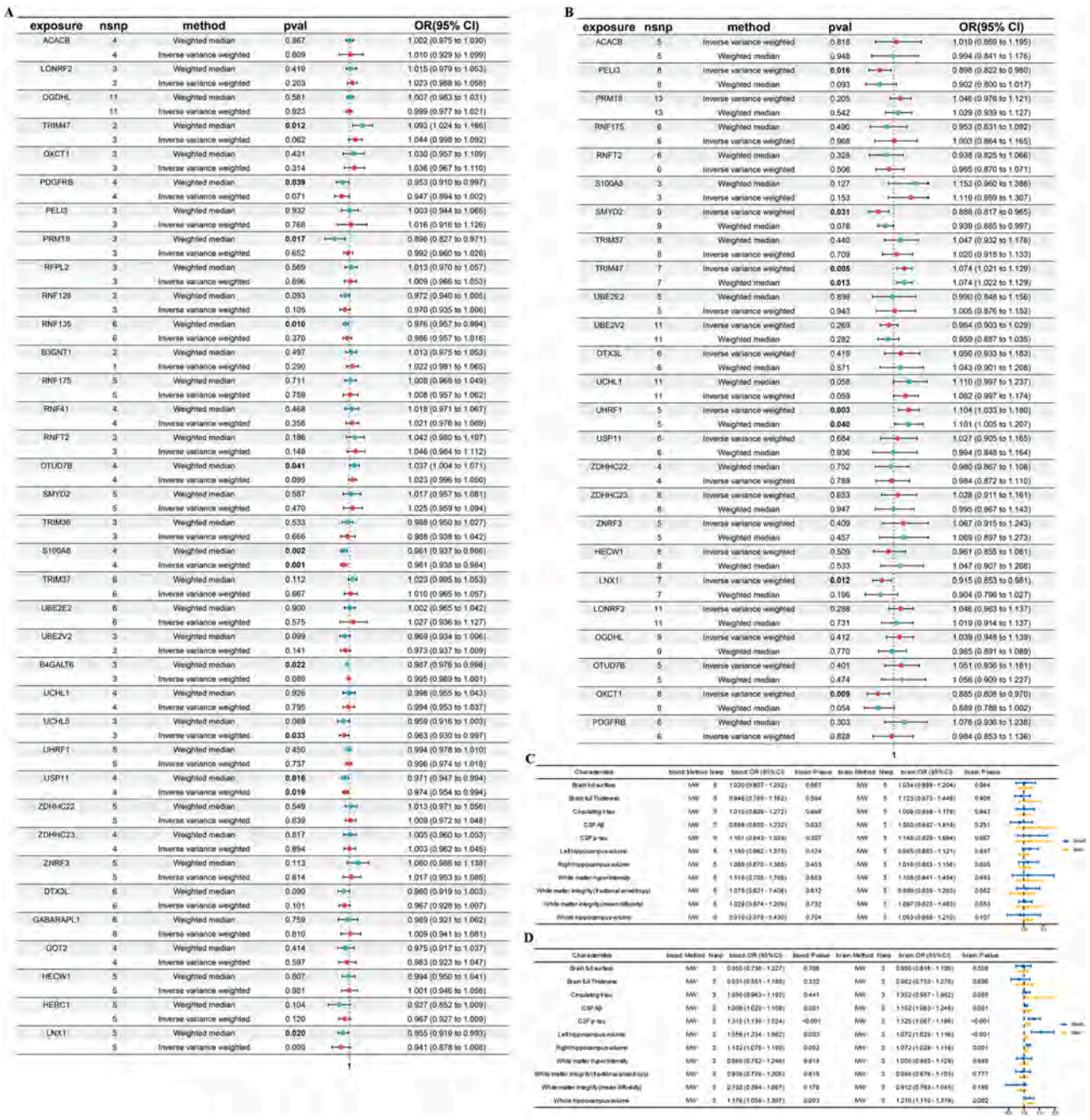

Fig. 8. Identification of hub genes in AD by MR. (A) Forest plot for MR results between brain eQTL and AD. (B) Forest plot for the MR result between blood eQTL and AD. MR results of TRIM47 (C) and LNX1 (D) and AD markers outcome in IVW method.

CRediT authorship contribution statement

Xiaoming Wang: Methodology, Formal analysis, Data curation. **Yuancheng Liu:** Validation, Software, Data curation. **Juncai Fu:** Validation, Formal analysis. **Yizhao Li:** Software, Methodology. **Mengying Zhao:** Validation, Software. **Qing Tian:** Writing – original draft, Validation.

Declaration of competing interest

The authors report no conflicts of interest in this work.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tjpad.2025.100422.

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