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Research on RNA methylation regulators in schizophrenia

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Abstract:

Background:

Emerging evidence indicates that RNA N6-methyladenosine (m6A) methylation regulators are crucial in neurodevelopment and psychiatric disorders. This study hypothesizes that abnormal expression of m6A regulators contributes to schizophrenia (SCZ) pathogenesis and could serve as candidate biomarkers for schizophrenia, though their clinical utility requires further validation.

Methods:

Using transcriptomic data from 106 SCZ patients and 96 controls from the GEO database, differential expression analysis identified key m6A regulators linked to SCZ. A random forest model was applied for feature selection, and gene interactions were examined via Pearson correlation and multivariate linear regression. SCZ was classified into subtypes using unsupervised consensus clustering, further characterized by immune cell infiltration screens via ssGSEA. Candidate gene expression levels were validated by quantitative PCR (qPCR) in peripheral blood from 60 SCZ patients and 47 matched controls.

Results:

Six m6A regulators showed significant differential expression in SCZ (all $P < 0.05$): *FTO*, *RBM15B*, *YTHDF1*, and *ALKBH5* were downregulated, whereas *RBM15* and *YTHDF3* were upregulated. The random forest model showed better diagnostic performance than the SVM model. Clustering identified subtypes with distinct immune signatures, indicating heterogeneity. qPCR confirmed reduced *FTO* expression in SCZ patient blood samples ($P < 0.01$).

Conclusions:

Dysregulated m6A regulators, particularly *FTO*, are highly associated with SCZ and distinct peripheral immune infiltration patterns. *FTO* emerges as a promising blood-based candidate biomarker for patient stratification. However, as altered expression of m6A regulators does not directly prove altered m6A modification, these findings primarily represent transcriptomic associations, and further validation in independent cohorts is needed to establish direct clinical utility.

Keywords: Schizophrenia, N6-methyladenosine (m6A) RNA methylation, biomarker, immune

infiltration

1. Introduction

1.1 Schizophrenia

Schizophrenia (SCZ) is a chronic, multifactorial psychiatric disorder with a global prevalence of approximately 1%[1]. It typically emerges in adolescence and is characterized by positive symptoms, negative symptoms, and cognitive deficits—the latter being a core and independent domain, affecting over 85% of patients and significantly impairing prognosis and social functioning[2]. Onset occurs before age 19 in 39% of males and 23% of females[3]. SCZ is associated with a 15-year reduction in life expectancy, and a suicide risk of 5%–10%, with first-episode patients having a 22-fold higher suicide risk within the first year of hospitalization[4]. Despite antipsychotics being the mainstay of treatment, therapeutic efficacy remains limited, and diagnosis largely depends on subjective clinical assessment. Reliable biomarkers from peripheral fluids such as blood or cerebrospinal fluid are lacking in psychiatric practice. Thus, elucidating the molecular mechanisms of SCZ and identifying objective biomarkers are essential for improving early diagnosis and therapeutic strategies

1.2 Genetic Landscape of Schizophrenia

Schizophrenia (SCZ) is a highly heritable polygenic psychiatric disorder, with heritability estimates ranging from 60% to 83%[5]. Beyond genetic predisposition, environmental factors across developmental stages—such as prenatal infections[6], winter or spring birth[7], migration[8], malnutrition, urbanization and adverse life experiences[9] all contribute to disease risk[10, 11]. Epigenetic modifications play a pivotal role in mediating gene–environment interactions, shaping gene expression in response to environmental stimuli[12]. Genome-wide association studies (GWAS) have identified numerous non-coding variants associated with SCZ, which may regulate gene expression via mechanisms such as expression quantitative trait loci (eQTL), alternative splicing, DNA methylation, histone modifications, non-coding RNAs, and chromatin remodeling[13]. Common risk loci include *BCL11B*, *CACNA1C*, *RERE*, *FOXP1*, *MYT1L*, and *SLC39A8*, with functions related to neurodevelopment, synaptic transmission, and immune regulation. Case-control studies in various human tissues further support the role of epigenetic dysregulation in the pathophysiology of SCZ[14].

1.3 Overview of RNA Methylation in Neuropsychiatric Disorders

Among the major epigenetic mechanisms—DNA methylation, histone modification, and RNA methylation—N6-methyladenosine (m6A) is the most prevalent internal modification of eukaryotic mRNA, accounting for ~80% of all RNA methylation events[15]. m6A is particularly enriched in the central nervous system, with increasing abundance from embryonic to adult stages, suggesting its critical role in brain development. Functionally, m6A modulates RNA splicing, export, stability, and translation, thereby fine-tuning gene expression and contributing to processes such as neurodevelopment, immune response, stem cell differentiation, and tumorigenesis. The m6A modification is dynamically regulated by three classes of proteins: “writers” (e.g., *METTL3*, *METTL14*, *ZC3H13*), “erasers” (e.g., *FTO*, *ALKBH5*), and “readers” (e.g., *YTHDF1*, *YTHDF3*), which together maintain the methylation balance and regulate transcriptomic fate. Emerging

evidence highlights the involvement of disrupted m6A dynamics in various psychiatric conditions, including SCZ, underscoring its potential as a biomarker and therapeutic target[10]. Despite the growing recognition of m6A dysregulation in neuropsychiatric disorders, previous investigations have predominantly relied on postmortem brain tissue. While biologically informative, this approach lacks translational utility for early, non-invasive clinical assessment. Furthermore, the interplay between m6A transcriptomic signatures and systemic immune dysregulation in the peripheral blood of SCZ patients remains largely uncharacterized. To bridge this translational gap, the present study investigates the associative relationship between m6A regulators and peripheral immunophenotypes. By identifying non-invasive candidate biomarkers, we aim to reflect systemic pathophysiological mechanisms and provide a foundation for future functional validation.

2. Materials and Methods

2.1 Identification of m6A Regulatory Genes

Based on a comprehensive literature review in PubMed using the keywords “RNA methylation”, “m6A”, “epigenetics”, and “N6-methyladenosine RNA methylation”, we curated a panel of 21 well-characterized m6A regulatory genes. These include methyltransferases (“writers”), demethylases (“erasers”), and m6A-binding proteins (“readers”) that have been widely validated in previous transcriptomic and epitranscriptomic studies. The selected genes—such as *METTL3*, *WTAP*, *RBM15*, *RBM15B*, *FTO*, *ALKBH5*, *YTHDF1*, *YTHDF3*, among others—were further analyzed to investigate their expression profiles and diagnostic potential in SCZ.

2.2 Differential Expression Analysis

Gene expression data from the GEO dataset GSE38484 (platform GPL6947, Illumina HumanHT-12 V3.0 Expression BeadChip) were utilized for transcriptome-wide analysis. The dataset comprises 106 patients with schizophrenia (SCZ), diagnosed according to DSM-5 criteria, and 96 healthy controls, with a mean patient age of 55 years. Expression differences of m6A regulatory genes between groups were analyzed using the “limma” R package. Significance thresholds were set at “ $P < 0.05$ and $|\log_2FC| \geq 1$ ”, Statistically significant genes were visualized using boxplots and heatmaps generated using ggplot2 and heatmap packages.

2.3 Correlation Analysis of Differential m6A Regulators

To elucidate potential synergistic or antagonistic regulatory networks among the differentially expressed m6A regulators, Pearson correlation coefficients were computed for all pairwise comparisons. To establish a robust threshold for initial feature selection during this exploratory phase, significant co-expression was defined by a nominal P-value < 0.05 and an absolute correlation coefficient ($|r|$) > 0.3 . This defined criterion was strategically selected to capture biologically meaningful interactions—encompassing both positive and inverse correlations—for subsequent downstream validation.

2.4 Construction of Random Forest and Support Vector Machine Models

To identify m6A regulators with high diagnostic value and prevent overfitting, machine learning models, including Random Forest (RF) and Support Vector Machine (SVM), were developed using the “randomForest” and “e1071” packages in R. To ensure computational reproducibility, a fixed

random seed was set prior to analysis. The dataset was randomly partitioned into an independent training set (70%) and a testing set (30%). Model training and feature selection were performed exclusively on the training set using a 10-fold cross-validation strategy to strictly avoid data leakage. For the RF model, parameter optimization was guided by the out-of-bag (OOB) error estimate; the optimal number of decision trees was set to 500, as the OOB error rate stabilized at this threshold (Figure 3C). The diagnostic performance of the finalized models was subsequently evaluated on the independent testing set using Receiver Operating Characteristic (ROC) curve analysis.

2.5 Nomogram Model Development

A diagnostic nomogram was constructed based on the expression levels of key m6A regulators, utilizing the “RMS” package in R. Calibration curves were employed to evaluate the agreement between predicted probabilities and actual outcomes. Additionally, decision curve analysis (DCA) was used to evaluate the potential net benefit of the model across a range of decision thresholds.

2.6 Molecular Subtyping of SCZ Based on m6A Regulators

Unsupervised consensus clustering was performed using the “Consensus Cluster Plus” R package to stratify SCZ patients into molecular subtypes based on the expression profiles of differentially expressed m6A regulators. Cluster stability and internal consistency were evaluated to ensure robust and reliable classification.

2.7 Immune Correlation Analysis of Characteristic m6A Regulators

Single-sample Gene Set Enrichment Analysis (ssGSEA) was employed to estimate enrichment scores for 22 immune cell signatures in each SCZ sample. Pearson correlation analysis was then conducted to examine the relationships between key m6A regulators and immune cell infiltration, providing insights into the immunoregulatory roles of these genes in SCZ.

2.8 Identification of m6A-Related Gene Clusters and Immune Infiltration Patterns

Based on the expression of differentially expressed m6A regulators, consensus clustering was applied to identify distinct m6A-related gene clusters. Immune infiltration profiles were analyzed across clusters to uncover m6A regulator expression patterns potentially linked to SCZ immunopathology.

3. Experimental Validation

3.1 Participant Characteristics

This study enrolled 60 patients with schizophrenia (SCZ) and 47 healthy controls recruited from The First Affiliated Hospital of Kunming Medical University and Yuxi Second People's Hospital between January 2022 and December 2022. The research protocol complied with the Declaration of Helsinki and was approved by the Ethics Committee of The First Affiliated Hospital of Kunming Medical University (Approval No.: (2022) Lun Shen L No. 284). Written informed consent was obtained from all participants or their legal guardians, as appropriate. Inclusion criteria: (1) Diagnosis of schizophrenia confirmed according to the International Classification of Diseases, 10th edition (ICD-10); (2) Age between 18 and 59 years; (3) PANSS total score ≥ 70 ; (4) Education level of at least primary school, fluent in Chinese, able to understand the assessment materials, and

capable of completing the scales; (5) Patients or their guardians provided written informed consent. Exclusion criteria: (1) Presence of organic brain diseases or severe physical illnesses that impair cooperation; (2) Substance dependence or abuse (including alcohol, cannabis, opioids, cocaine, etc.); (3) Intellectual disability; (4) Severe agitation, mutism, suicidal tendencies, or other conditions posing risks or impeding cooperation; (5) Recent (within six months) or ongoing electroconvulsive therapy (ECT); (6) Pregnant, planning to conceive, or breastfeeding women. Individuals with a history of traumatic brain injury or unstable clinical status were also excluded.

The severity of psychiatric symptoms was assessed using the Positive and Negative Syndrome Scale (PANSS) [16]. The PANSS is a previously published standardized instrument; no questionnaire or scale was developed specifically for this study. A PANSS total score ≥ 70 at enrollment was required for study eligibility. At a separate clinical assessment time point, the mean PANSS total score was 54.5 ± 8.2 . During the medication-stable phase, 5–8 mL of peripheral blood was collected via cubital venipuncture. All patients were receiving stable maintenance doses of atypical antipsychotics (e.g., risperidone, olanzapine) at the time of sample collection. Red blood cells were lysed using a red blood cell lysis buffer (in a 3:1 volume ratio), and leukocytes were isolated by centrifugation. The resulting white blood cell pellets were suspended in 1 mL TRIzol reagent (LIFE 15596-026) and stored at -80°C until RNA extraction. All procedures involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University. Written informed consent was obtained from all participants or their legal guardians, as appropriate.

3.2 RNA Extraction and Quantitative PCR (qPCR)

Total RNA was extracted from leukocytes using TRIzol reagent (Life Technologies, USA), followed by phase separation with chloroform. RNA concentration and purity were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Samples with an A260/A280 ratio between 1.8 and 2.0 were considered acceptable for downstream analyses. Subsequently, 1 μg of total RNA was reverse-transcribed into cDNA using the HiScript III All-in-one RT SuperMix for qPCR (Vazyme Biotech Co., Ltd., China), according to the manufacturer's instructions.

Quantitative real-time PCR (qPCR) was conducted using Taq Pro Universal SYBR qPCR Master Mix (Vazyme Biotech, China) to assess the expression levels of *RBM15*, *RBM15B*, *YTHDF1*, *YTHDF3*, *FTO*, and *ALKBH5*, with ACTB as the internal control. Relative gene expression was normalized to ACTB and calculated using the $2^{-\Delta C_t}$ method, where $\Delta C_t = (C_{t_{\text{target}}} - C_{t_{\text{ACTB}}})$ (Table 1).

Gene	Primer Sequence (5' → 3')
<i>ALKBH5</i>	F: CACACGGCCTCAGGACATCAAG
	R: CGGTTCTCTTCCTTGCCATCTC
<i>FTO</i>	F: ACGGACAAGATGAAGTGGACATT
	R: CTCAGGGCCTTCACAGCTATAA

<i>RBM15</i>	F: TCAGCCTCTCCCAAACCTCTG R: CCCTGCTACTTTGATGCGTC
<i>RBM15B</i>	F: ACCTGGTCTCCTACTTGAAACAG R: TCTAGCTTGCCCAATGTCCTTAG
<i>YTHDF1</i>	F: TGACTTTGAGCCCTACCTTACTG R: CGTAGGTGGTGAGGTATGGAATC
<i>YTHDF3</i>	F: GCTCCTAAGGCCCATGTTCTATC R: TGGATCTGACATTGGTGATAGC
<i>ACTB</i>	F: GGCCAACCGCGAGAAGATGAC R: GGATAGCACAGCCTGGATAGCAAC

Table 1. Primer sequences used for qPCR analysis of m6A regulatory genes and the ACTB housekeeping gene.

4. Statistical Analysis

All statistical analyses were executed using R software (version 4.0.3). The assumption of normality for continuous variables was assessed via the Shapiro-Wilk test, and the homogeneity of variances was evaluated using Levene's test. Accordingly, Student's t-test was used for normally distributed continuous variables when the assumption of equal variances was met; otherwise, Welch's t-test was applied. The Wilcoxon rank-sum test was used for non-normally distributed continuous variables. Given the two-stage discovery-validation design of this study, the initial bioinformatic analysis of the GSE38484 dataset served strictly as an exploratory screening phase. Consequently, nominal P-values < 0.05 were considered statistically significant without multiple testing adjustments. This approach was adopted to minimize Type II errors and preserve a broad pool of putative candidate genes for rigorous empirical evaluation in the subsequent independent clinical cohort.

5. Results

5.1 Differential Expression of m6A Regulators in SCZ

Analysis of 21 m6A regulatory genes identified six with significant dysregulation in peripheral blood of SCZ patients. Expression of *RBM15* and *YTHDF3* was markedly increased, whereas *RBM15B*, *YTHDF1*, *FTO*, and *ALKBH5* were significantly decreased (Figure 1A–B). These findings highlight a subset of m⁶A regulators potentially implicated in SCZ pathophysiology.

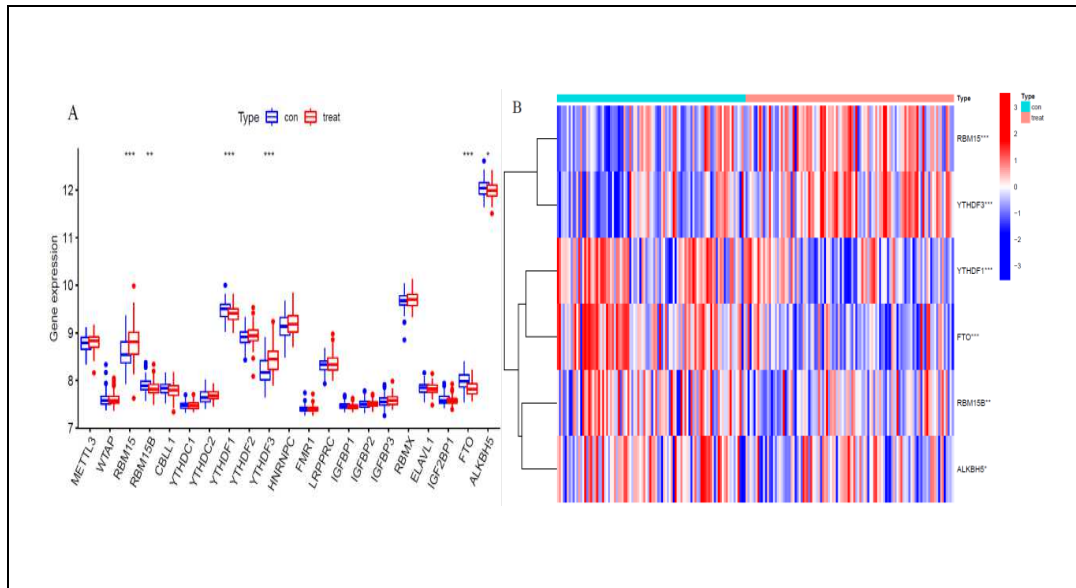


Figure 1. Expression profiles of m6A regulators in SCZ. (A) Bar plot showing differential expression of 21 m6A regulators between SCZ and healthy controls (HC). (B) Heatmap of significantly dysregulated m6A regulators in SCZ patients.

5.2 Interplay between m6A “Writers” and “Erasers”

Homeostatic balance of m6A marks relies on coordinated activity of writer and eraser enzymes. Linear regression revealed positive correlations between ALKBH5 and the writers CBL1, METTL3, and RBM15B (Figures 2A–C). Conversely, FTO was negatively correlated with RBM15 (Figure 2D), indicating coordinated expression patterns among m6A regulators in SCZ.

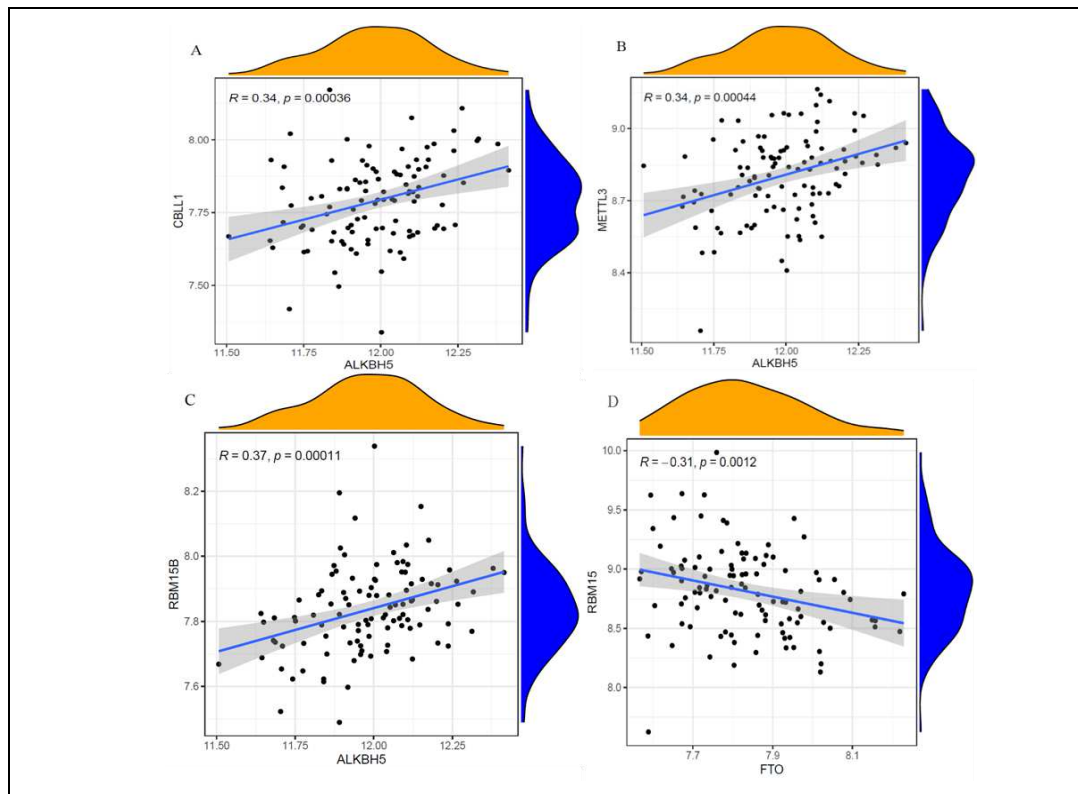


Figure 2. Correlation analysis of methylation regulators in SCZ. (A–C) Positive correlations between the demethylase *ALKBH5* and the writers *CBL1*, *METTL3*, and *RBM15B*. (D) Negative correlation between the demethylase *FTO* and the writer *RBM15*.

5.3 Random Forest Model Optimizes Diagnostic Accuracy

Single-gene biomarkers lacked adequate sensitivity; therefore, ensemble learning was applied. A Random Forest (RF) model outperformed a Support Vector Machine (SVM) classifier, demonstrating lower residuals (Figure 3A–B) and minimal error at 500 decision trees (Figure 3C). Variable importance ranking underscored YTHDF3 and ALKBH5 as principal discriminators based on the Mean Decrease Gini index (Figure 3D), providing a prioritized gene set for further validation.

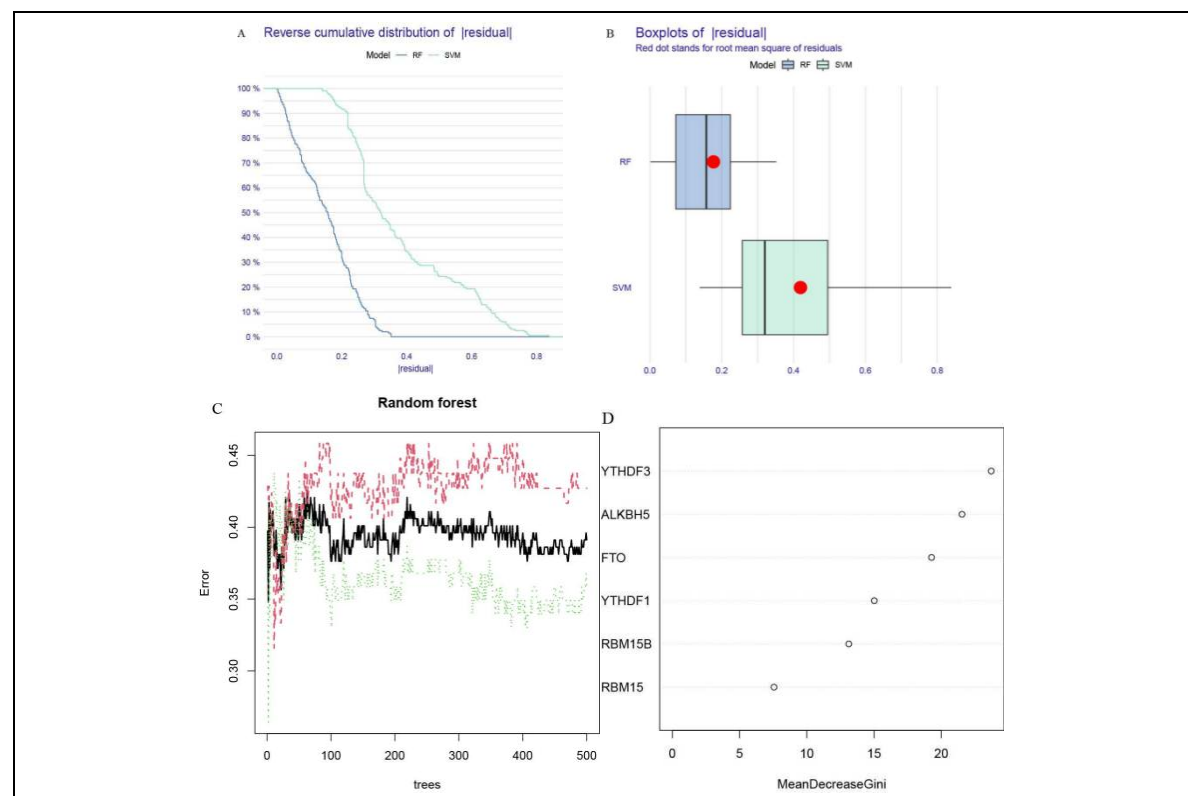


Figure 3. Construction of the random forest (RF) diagnostic model. (A) Reverse cumulative distribution of residuals comparing RF and SVM models. (B) Boxplot of residuals for RF and SVM models. (C) Error rate of RF model across different numbers of decision trees. (D) Variable importance of six m6A regulators based on Mean Decrease Gini index.

5.4 Nomogram Model Based on Six m6A Regulators

A nomogram incorporating the six differentially expressed m6A regulators was constructed to distinguish SCZ cases from healthy controls (Figure 4A). *FTO* contributed most substantially to total points. Calibration analysis demonstrated close alignment between predicted and observed outcomes (Figure 4B), while decision curve and clinical impact analyses revealed meaningful net benefit across risk thresholds (Figure 4C–D). These results support the potential discriminative value of the nomogram.

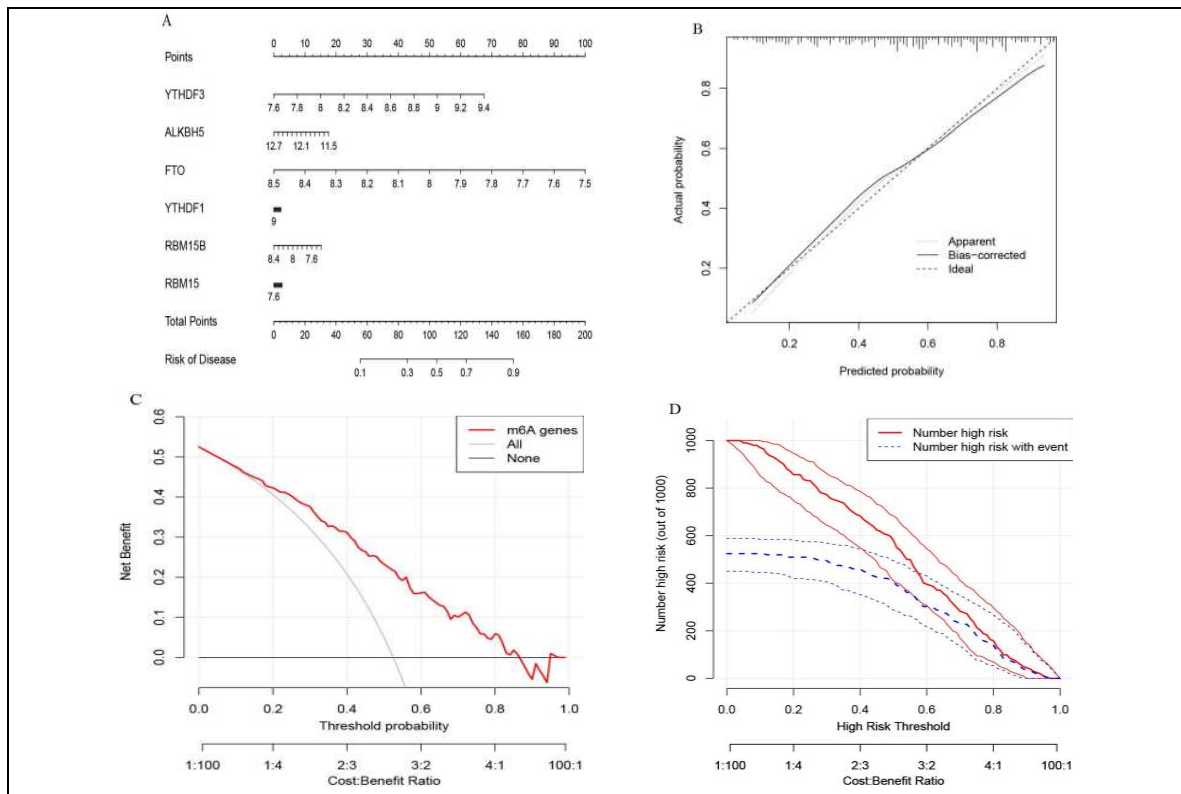
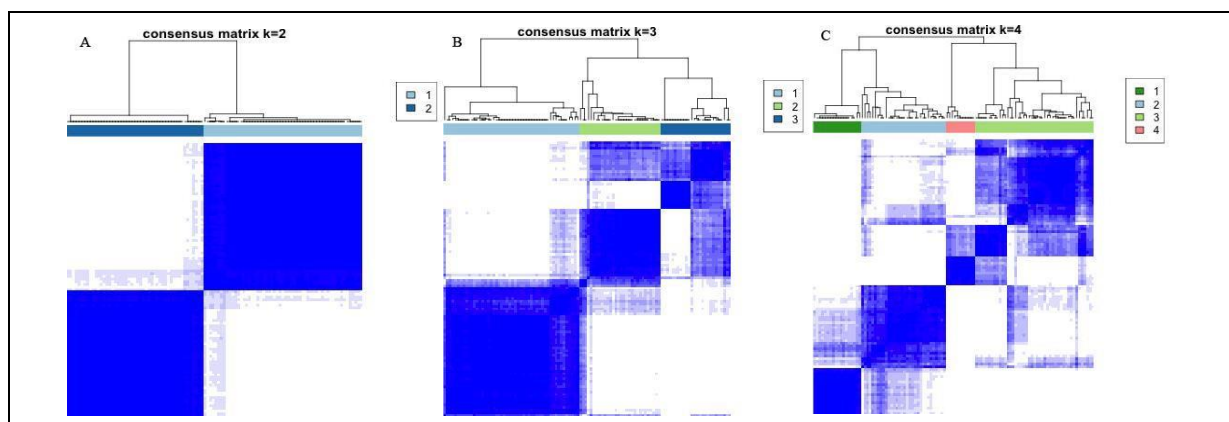


Figure 4. Construction and validation of the nomogram model. (A) Nomogram based on six m6A regulators for distinguishing SCZ cases from healthy controls. (B) Calibration curve evaluating model fit. (C) Decision curve analysis (DCA) indicating clinical benefit. (D) Clinical impact curve assessing model utility.

5.5 Molecular Subtyping Based on m6A Profiles

Consensus clustering of SCZ samples defined two robust molecular subtypes—Cluster A and Cluster B—at $K = 2$ (Figure 5A–E). Cluster A was characterized by upregulation of *RBM15B*, *YTHDF1*, *ALKBH5*, and *FTO*, whereas *RBM15* and *YTHDF3* were downregulated (Figure 5F–G). Subtype-specific transcriptomic differences (Figure 5H) underscore heterogeneous m6A regulator expression patterns that may reflect distinct disease mechanisms.



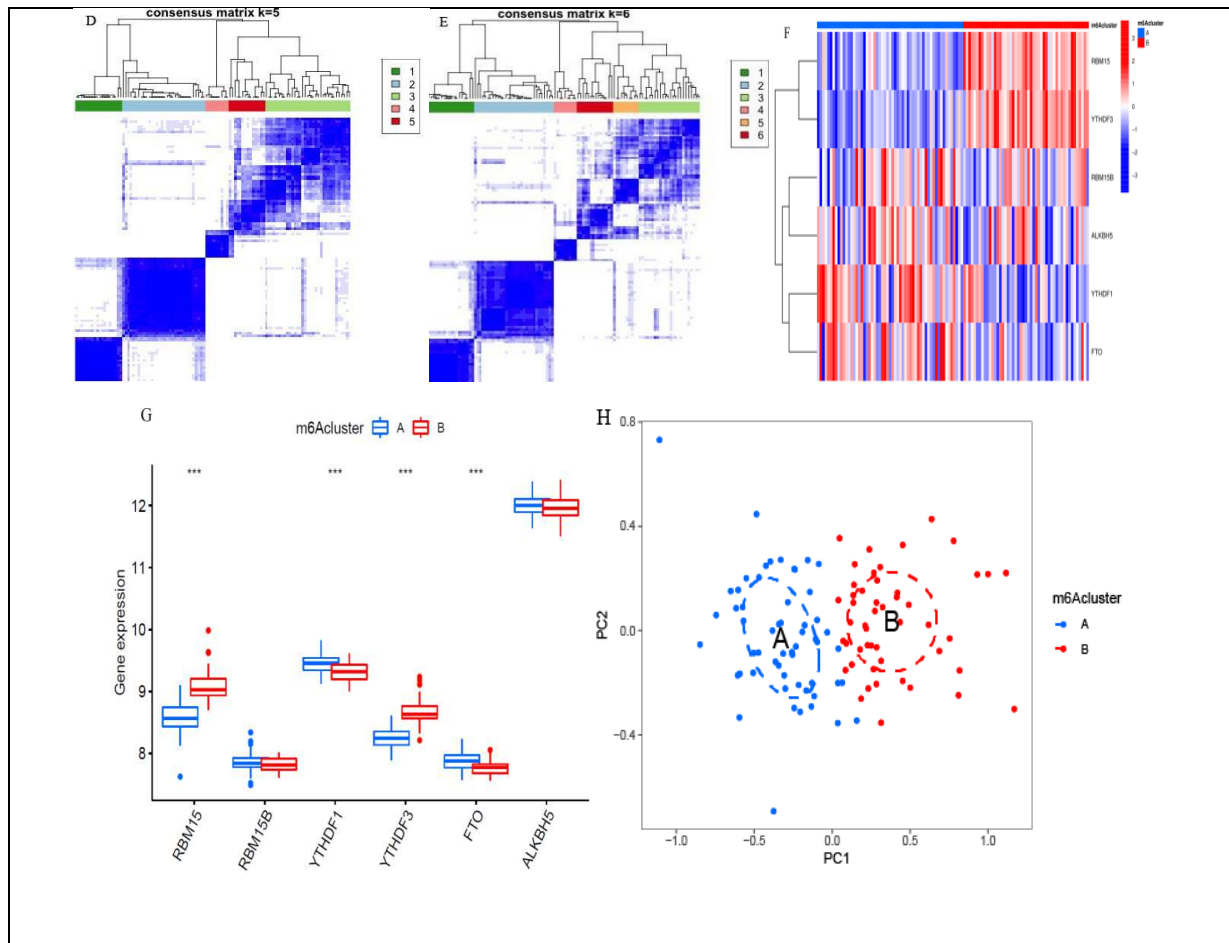


Figure 5. m6A-based molecular subtyping of SCZ. (A–E) Consensus matrices for $k = 2$ to 6 clusters. Optimal clustering was achieved at $k = 2$. (F) Heatmap of six key m6A regulators in Clusters A and B. (G) Histogram showing differential expression of the six m6A regulators between clusters. (H) Transcriptomic differences between the two m6A subtypes (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

5.6 m6A Regulators Correlate with Immune Infiltration

Single-sample GSEA estimated enrichment scores for 22 immune cell signatures in SCZ blood and revealed that RBM15 displayed the strongest positive correlations ($r > 0.5$, $p < 0.001$) with activated CD4⁺ T cells, CD8⁺ T cells, and Th1 cells, coupled with negative association in monocytes ($p < 0.01$; Figure 6A). Comparative analysis between Clusters A and B showed diminished infiltration of activated T cell subsets and $\gamma\delta$ T cells in Cluster A, in contrast to enrichment of NK cells, monocytes, and T follicular helper cells; Cluster B exhibited the inverse pattern (Figure 6B). Comparison of ssGSEA enrichment scores between groups with high and low RBM15 expression revealed differences in several immune cell signatures (Figure 6C), further supporting an association between RBM15 expression and peripheral immune profiles in SCZ.

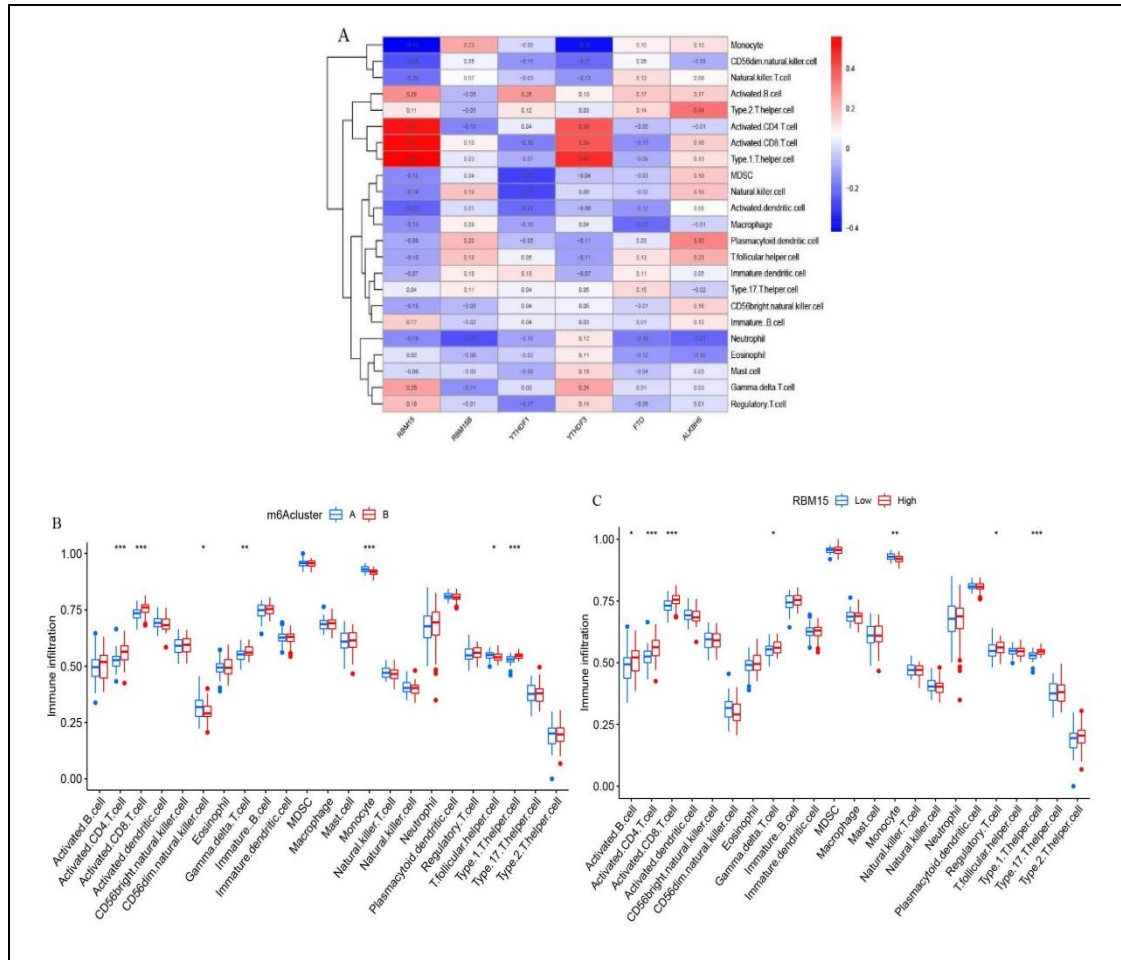


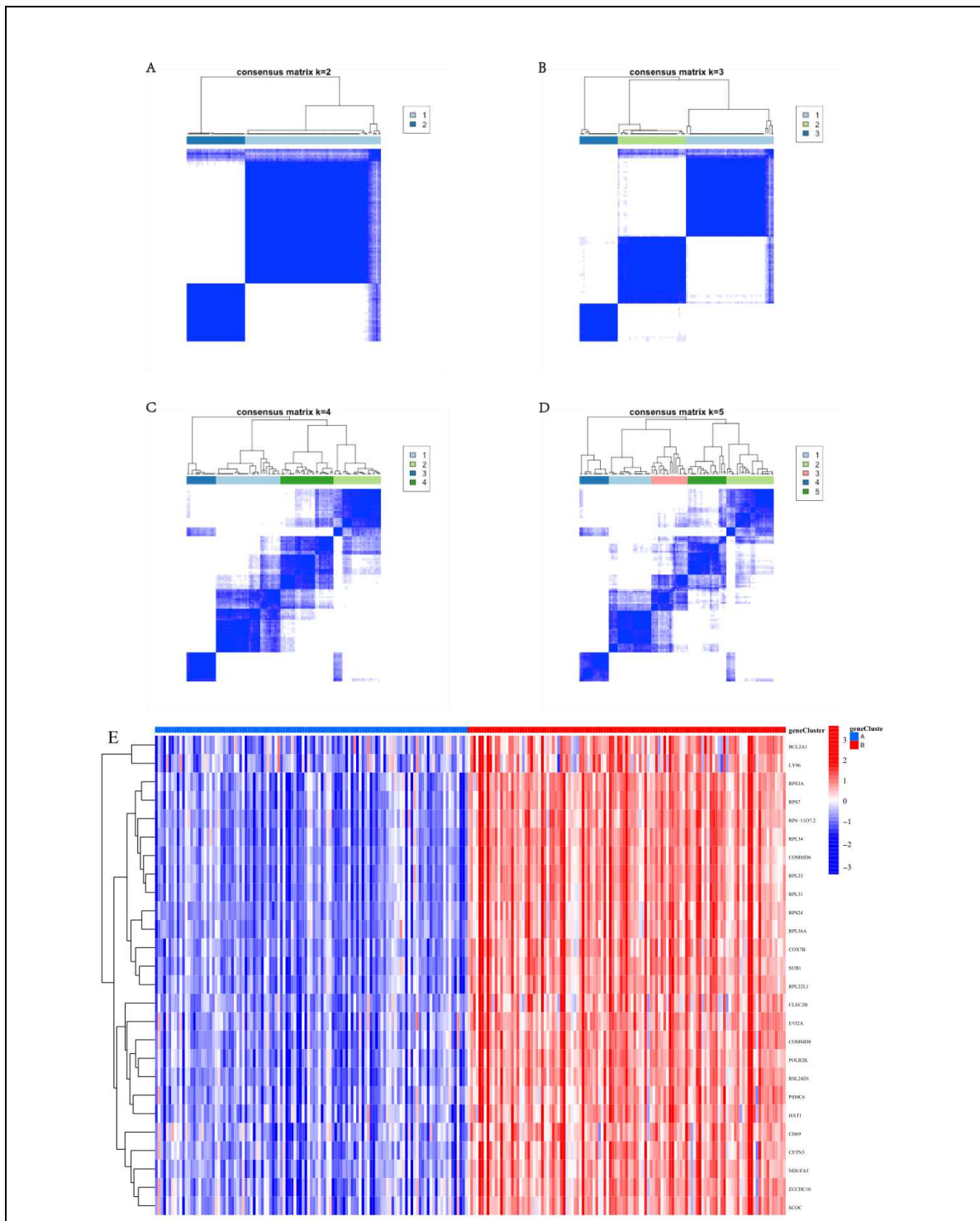
Figure 6. Immunological profiling associated with m6A regulators. (A) ssGSEA analysis showing correlations between m6A regulators and immune cell infiltration. RBM15 displayed strong associations with activated CD4⁺ T cells, CD8⁺ T cells, and Th1 cells. (B) Comparison of immune cell abundance between Clusters A and B. (C) Comparison of ssGSEA enrichment scores between groups with high and low RBM15 expression.

5.7 Identification and Evaluation of Distinct m6A-Related Expression Patterns

Using consensus clustering based on the expression profiles of key m6A regulators, SCZ patients were stratified into two distinct gene clusters (Figure 7A–D). The optimal clustering was achieved at $K = 2$. Expression profiles of 26 differentially expressed m6A-related genes across the two gene clusters are shown (Figure 7E). Specifically, *ALKBH5*, *FTO*, and *YTHDF1* were highly expressed in gene Cluster A, while *RBM15* and *YTHDF3* were downregulated (Figure 7F).

Immune infiltration analysis between the two gene clusters revealed notable differences. In gene Cluster A, higher abundances were observed for activated dendritic cells, macrophages, monocytes, natural killer T cells, NK cells, plasmacytoid dendritic cells, and follicular helper T cells. Conversely, activated B cells, CD4⁺ T cells, CD8⁺ T cells, $\gamma\delta$ T cells, and Th1 cells were less abundant (Figure 7G). These immune imbalances were consistent with those observed between the previously defined m6A regulator expression-based clusters, supporting the reliability of clustering analysis in molecular subtyping of SCZ.

Finally, m6A scores were compared between m6A gene clusters and m6A regulator-based clusters (Figure 7H, I), and the relationships among m6A clusters, gene clusters, and m6A score groups were visualized using a Sankey diagram (Figure 7J).



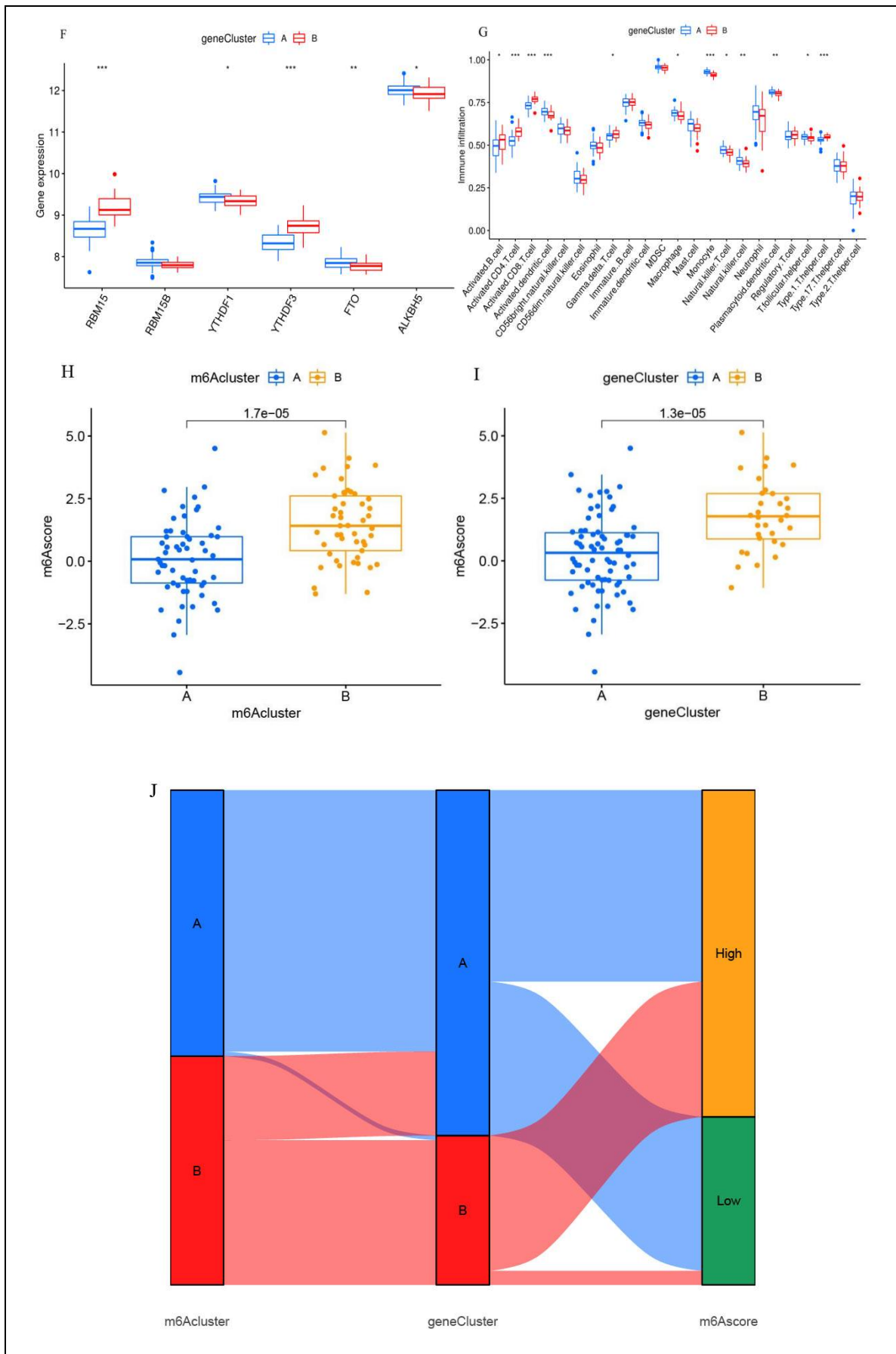


Figure 7. Consensus clustering and immunological characteristics of 26 m6A-related differentially

expressed genes in SCZ. (A–D) Consensus matrices for $k = 2-5$ based on the expression of 26 m6A-related DEGs. (E) Heatmap of 26 m6A-related gene expression profiles. (F) Boxplot showing the expression levels of key m6A regulators between gene Cluster A and B. (G) Differences in immune cell infiltration between the two gene clusters. (H) m6A score comparison between m6A clusters. (I) m6A score comparison between gene Cluster A and B. (J) Sankey diagram illustrating the relationships among m6A clusters, gene clusters, and m6A scores.

5.8 Experimental Validation

A total of 60 schizophrenia (SCZ) patients were enrolled from The First Affiliated Hospital of Kunming Medical University and Yuxi Second People's Hospital. Their ages ranged from 18 to 59 years, with a mean age of 34 ± 13.05 years. Additionally, 47 healthy controls were included, with ages also between 18 and 59 years, and a mean age of 35.92 ± 11.18 years (Table 2). qPCR validation revealed that *FTO* expression was significantly downregulated in SCZ patients, consistent with the bioinformatic predictions (Figure 8). In contrast, although *RBM15* and *YTHDF3* also showed reduced expression in SCZ samples, these findings were not aligned with the initial computational analysis. Moreover, no statistically significant differences were observed in the expression of *RBM15B*, *ALKBH5*, and *YTHDF1* between SCZ patients and healthy controls (Table 3).

	HC	SCZ	t/x ²	P
Sex M/F	17:30	32:28	3.128	0.077
age	35.92 ± 11.18	34.00 ± 13.05	0.819	0.415
PANSS ^a	N/A	54.5 ± 8.2	-	-
Antipsychotic Medication	N/A	Stable atypical antipsychotics	-	-
RNA Purity (A260/280)	1.8 - 2.0 (QC passed)	1.8 - 2.0 (QC passed)	-	-

Table 2. Demographic and clinical characteristics of the study participants.

^a PANSS was assessed at a separate clinical time point and does not represent the score used to determine eligibility at enrollment.

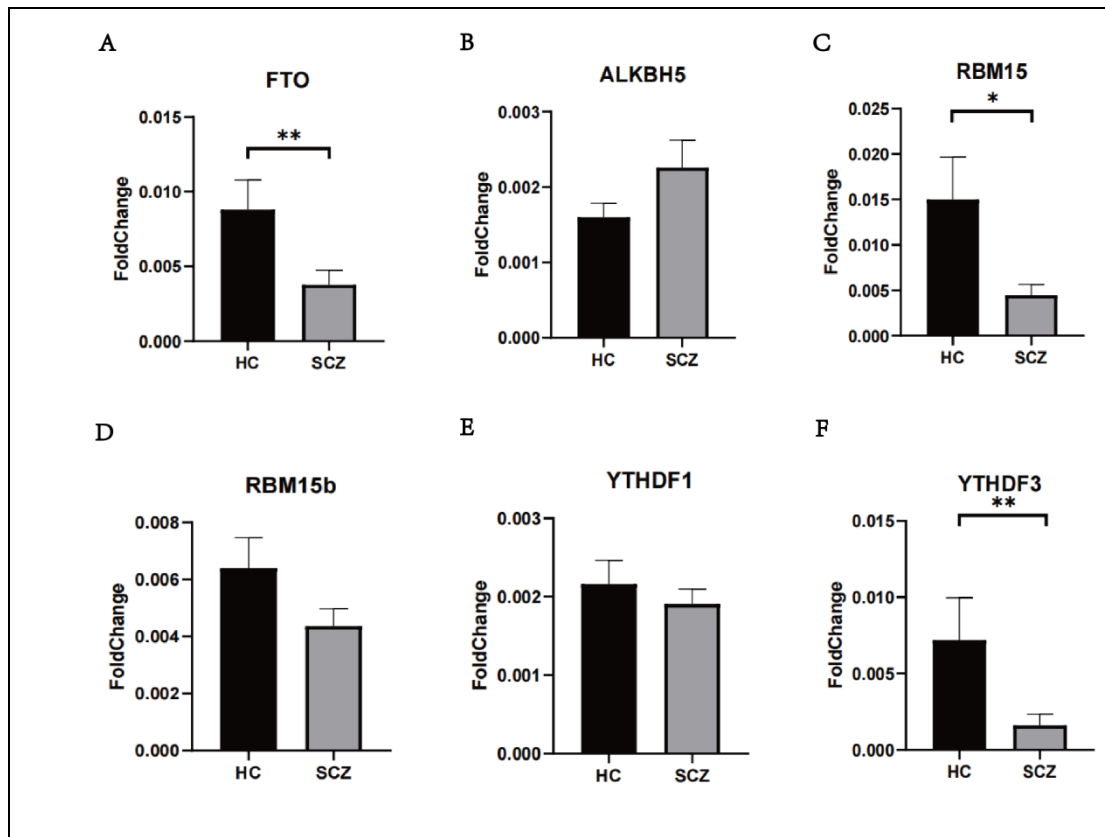


Figure 8. qPCR validation of key m6A regulators in SCZ. Relative expression was normalized to ACTB and calculated as $2^{-\Delta Ct}$.

	<i>ALKBH5</i>	<i>FTO</i>	<i>RBM15</i>	<i>RBM15B</i>	<i>YTHDF1</i>	<i>YTHDF3</i>
Bioinformatics	Low	Low	High	Low	Low	High
qPCR	/	Low	Low	/	/	Low

Table 3. Differential Expression of Key m6A Regulators.

Footnote: / indicates no statistically significant difference.

Discussion

Schizophrenia (SCZ) is a polygenic psychiatric disorder associated with severe somatic comorbidities, premature mortality, and a high socioeconomic burden. Currently, clinical diagnosis relies almost entirely on symptom-based assessments, as objective and reliable molecular biomarkers remain elusive [17, 18].

While genome-wide association studies (GWAS) have identified numerous CNS-related risk loci [19], genetic factors alone do not fully explain the disease variance. For instance, twin studies have shown incomplete concordance rates, highlighting the substantial contribution of environmental interactions and epigenetic modifications to SCZ pathogenesis [20, 21]. Consequently, characterizing these epigenetic changes is essential for understanding the underlying disease mechanisms and discovering novel targets for early intervention.

In this study, we investigated the expression patterns of m6A regulatory genes in peripheral blood.

Peripheral blood was selected because it is readily accessible for clinical sampling, and previous studies have reported schizophrenia-associated epigenetic alterations in peripheral tissues [22]. By integrating m6A expression profiles with inferred peripheral immune cell signatures, we stratified SCZ patients into distinct immune-related molecular subtypes, providing a new perspective on the biological heterogeneity of the disorder.

It is important to note that our bioinformatics analysis and ex vivo qPCR results represent cross-sectional associations. Therefore, the identified m6A regulators, particularly *FTO*, should be strictly viewed as candidate biomarkers that require rigorous longitudinal validation before clinical application. Based on these integrated results, the roles of specific m6A regulators in SCZ are discussed below:

1. *FTO*

FTO is a well-characterized N6-methyladenosine demethylase [23, 24] highly expressed in the central nervous system. In neurons and adult neural stem cells, it regulates energy homeostasis, dopaminergic signaling, and cognitive processes such as learning and memory [25-29]. Beyond its neurodevelopmental roles, altered *FTO* expression is closely linked to oxidative stress and systemic inflammation in various experimental models [30-32]. Because neuroinflammation and metabolic dysregulation are prominent clinical features of SCZ, our findings of decreased *FTO* expression alongside altered peripheral immune infiltration suggest a potential mechanistic link.

Previous studies have examined associations between *FTO* polymorphisms, including rs9939609 and rs8050136, and antipsychotic-related weight gain or metabolic abnormalities in patients with SCZ, although findings have been inconsistent across cohorts [33-38]. While previous research predominantly focused on these genetic variants, our study adds a transcriptomic perspective. We observed a consistent downregulation of *FTO* in medication-stabilized SCZ patients across both the bioinformatic discovery and independent qPCR validation cohorts.

As a demethylase, a reduction in *FTO* could theoretically lead to the hypermethylation of specific target transcripts, thereby disrupting downstream inflammatory or metabolic pathways. While the exact downstream RNA targets in SCZ remain to be identified, the cross-cohort stability of *FTO* makes it a promising blood-based biomarker candidate that warrants investigation in larger, prospective clinical studies.

2. *RBM15* and *RBM15B*

RBM15 and *RBM15B* are RNA-binding proteins belonging to the Spen family, functioning as regulators in key signaling cascades such as the Wnt and Notch pathways [39]. Biologically, they are essential for B-cell differentiation and the suppression of cellular proliferation within myeloid and megakaryocytic lineages [40]. Both proteins also participate in XIST-mediated X-chromosome inactivation, a fundamental epigenetic silencing mechanism. Research by Niu et al. demonstrated that *RBM15* modulates these hematopoietic functions via the oncogene c-Myc, a mechanism that impacts prognosis across various malignancies [41]. Despite these established roles in cellular and hematopoietic regulation, the specific involvement of *RBM15B* in SCZ remains unclear. In our analysis, *RBM15B* showed minimal correlation with peripheral immune-related pathways and did

not exhibit significant differential expression in the qPCR validation cohort. These findings suggest that while *RBM15* and *RBM15B* may interact within broader m6A regulatory networks, *RBM15B* alone currently lacks the robust cross-cohort stability required for an independent SCZ biomarker.

3. *YTHDF1* and *YTHDF3*

The human genome encodes multiple *YTH* domain-containing proteins that recognize m6A modifications [42]. In the cytoplasm, *YTHDF1*, *YTHDF2*, and *YTHDF3* collaboratively regulate mRNA metabolism: *YTHDF1* enhances the translation efficiency of targeted transcripts, *YTHDF2* promotes their degradation, and *YTHDF3* facilitates both processes [43]. *YTHDF1* acts as a critical reader for neuronal function. In the adult mouse hippocampus, *YTHDF1* responds to neuronal activity by promoting the protein synthesis necessary for learning and memory [44]. Mice lacking *YTHDF1* display severe defects in postsynaptic density, glutamate receptor abundance, and overall synaptic transmission, highlighting its necessity for memory formation [45].

Beyond neurobiology, extensive research highlights the roles of *YTHDF1* and *YTHDF3* in systemic immune regulation and disease progression. *YTHDF1* modulates neoantigen-specific immunity and drives cellular proliferation in diverse malignancies, including lung, colorectal, and breast cancers [46-48]. Similarly, *YTHDF3* governs complex cellular interactions, such as facilitating blood-brain barrier extravasation during brain metastasis and driving progression in triple-negative breast cancer [49, 50]. Although these readers possess powerful regulatory functions in both the brain and systemic environments, their baseline expression levels in peripheral blood remain poorly characterized. The reasons for the discrepant peripheral blood findings remain unclear and require further investigation. Future research assessing *YTHDF1* and *YTHDF3* directly in SCZ postmortem brain tissues is required to clarify their diagnostic utility.

4. *ALKBH5*

ALKBH5 is an m6A demethylase that regulates mRNA export, RNA metabolism, and the assembly of processing factors in nuclear speckles [51]. Unlike regulators with broad tissue distribution, *ALKBH5* is predominantly expressed in the testes, with minimal expression in the heart and brain. Molecular analyses confirm it is a core gene for spermatogenesis; *ALKBH5* deficiency in male mice increases m6A levels in mRNA and is associated with impaired spermatogenesis and infertility [51, 52].

In our study, the qPCR validation did not detect significant differential expression of *ALKBH5* in peripheral blood. Therefore, the lack of a significant difference in peripheral blood does not exclude a possible role for *ALKBH5* in other tissues or biological contexts relevant to SCZ. Future research relying on postmortem brain tissues or targeted multi-center cohorts is needed to clarify its direct mechanisms.

5. Validation Discrepancies

Our bioinformatics screening identified a six-gene m6A signature, but the ex vivo qPCR validation yielded mixed results. While *FTO* was consistently downregulated in both cohorts, *RBM15* and *YTHDF3* were significantly downregulated in our clinical samples, reversing the trend observed in the in silico data. The expression levels of *RBM15B*, *YTHDF1*, and *ALKBH5* did not reach statistical

significance in the validation cohort.

These discrepancies reflect the inherent challenges of translating public microarray data to clinical samples. Biologically, the cohorts exhibit distinct demographic and clinical profiles. The GSE38484 discovery cohort primarily consists of older patients (mean age ~55 years) with variable disease durations, whereas our validation cohort recruited younger, medication-stabilized individuals (mean age ~34 years). Peripheral blood is also a heterogeneous tissue; shifts in specific leukocyte subpopulations—such as those driven by systemic immune dysregulation—can readily obscure or invert bulk transcript signatures. Technically, microarray hybridization and targeted qPCR rely on different quantification methods with varying sensitivities. These combined factors may have contributed to the partial replication and underscore why *FTO*, the only gene demonstrating stable cross-cohort expression, is the most robust candidate for a peripheral biomarker.

6. Immune infiltration and epigenetics in SCZ

Peripheral immune dysregulation is a recognized core component of SCZ pathology. Clinical observations frequently document systemic imbalances in SCZ patients, encompassing alterations in T cell subsets, B cells, and natural killer cells. To quantify this systemic immune landscape, we applied the ssGSEA algorithm to our cohort.

The analysis demonstrated direct correlations between specific m6A regulators and distinct immune populations. Specifically, *RBM15* expression correlated strongly with activated T cells and Th1 cells. These results align with the hypothesis that m6A RNA modifications may modulate the systemic immune dysfunction seen in SCZ. While the cross-sectional nature of our data precludes causal inference, these findings suggest that m6A-related expression patterns may be associated with peripheral immune heterogeneity in SCZ and could inform future studies of molecular stratification.

Limitations

Several limitations should be acknowledged. First, the initial computational analysis relied on a single transcriptomic dataset (GSE38484), which may limit the generalizability of the findings across populations and platforms. Second, the qPCR validation was conducted in a relatively small peripheral blood cohort. Third, the cross-sectional design precludes causal inference and does not allow assessment of biomarker changes over time. Fourth, the initial transcriptomic screening relied on nominal P-values without correction for multiple testing, which may increase the risk of Type I error. In addition, potential confounding factors, including age, biological sex, and antipsychotic medication regimens, were not fully adjusted for. The lack of multi-omics and functional validation also limits mechanistic interpretation. Larger, independent, longitudinal cohorts with multivariable adjustment and integrated functional approaches are needed to validate these findings.

Conclusions

Six m6A regulatory genes were differentially expressed in the public schizophrenia dataset. In the independent qPCR cohort, *FTO*, *RBM15*, and *YTHDF3* showed significant expression differences, but only *FTO* changed in the same direction in both cohorts. Expression-based clusters also differed in their inferred immune cell signatures. These findings support further investigation of *FTO* as a

candidate peripheral blood biomarker for schizophrenia. However, regulator expression does not directly establish changes in m6A modification, and the diagnostic value of these findings requires validation in larger, independent cohorts.

Declarations

Abbreviations

SCZ: schizophrenia; HC: healthy controls; m6A: N6-methyladenosine; GEO: Gene Expression Omnibus; GWAS: genome-wide association studies; eQTL: expression quantitative trait loci; RF: random forest; SVM: support vector machine; OOB: out-of-bag; ROC: receiver operating characteristic; DCA: decision curve analysis; ssGSEA: single-sample gene set enrichment analysis; PANSS: Positive and Negative Syndrome Scale; ICD-10: International Classification of Diseases, 10th Revision; DSM-5: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; ECT: electroconvulsive therapy; qPCR: quantitative real-time polymerase chain reaction; CNS: central nervous system; NK: natural killer; DEGs: differentially expressed genes.

Ethics approval and consent to participate

The research protocol complied with the Declaration of Helsinki and was approved by the Ethics Committee of The First Affiliated Hospital of Kunming Medical University [Approval No.: (2022) Lun Shen L No. 284]. Written informed consent was obtained from all participants or their legal guardians, as appropriate.

Consent for publication

Not applicable.

Availability of data and materials

The publicly available transcriptomic dataset analyzed in this study is available in the Gene Expression Omnibus (GEO) under accession number GSE38484 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE38484>). The qPCR data generated during the current study are available from the corresponding authors on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

H.Z. and S.T. conceived and designed the study, performed the bioinformatics analyses, and drafted the manuscript. X.Y.L., M.J.L., and M.T.L. performed the qPCR validation experiments and sample processing. F.Y. and X.M.L. contributed to clinical data collection and quality control. Z.T. and F.L. supervised the study, acquired funding, and critically revised the manuscript. All authors read and

approved the final manuscript.

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