Neuropsychiatric Disease and Treatment

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Exploration of Epigenetic Mechanisms and Biomarkers Among Patients with Very-Late-Onset Schizophrenia-Like Psychosis

Yansha Gan[1,*], Weihua Yue[2,*], JiaoJiao Sun[1], DanTing Yang[1], ChunXia Fang[1], Zhenhe Zhou[1], JiaJun Yin[1], Hongliang Zhou[3]

[1]The Affiliated Mental Health Center of Jiangnan University, Wuxi, Jiangsu, 214151, People's Republic of China; [2]National Clinical Research Center for Mental Disorders, Peking University Sixth Hospital, Beijing, 100191, People's Republic of China; [3]Department of Psychology, The Affiliated Hospital of Jiangnan University, Wuxi City, Jiangsu, 214100, People's Republic of China

*These authors contributed equally to this work

Correspondence: JiaJun Yin, The Affiliated Mental Health Center of Jiangnan University, Wuxi, Jiangsu, 214151, People's Republic of China, Email yinjiajun@jiangnan.edu.cn; Hongliang Zhou, Department of Psychology, The Affiliated Hospital of Jiangnan University, No. 200, Huihe Road, Binhu District, Wuxi City, Jiangsu Province, People's Republic of China, Email Hongliangzh2022@hotmail.com

Objective: This study aimed to identify DNA methylation patterns associated with Very Late-Onset Schizophrenia-like Psychosis (VLOSLP) and to develop methylation-based biomarkers that differentiate VLOSLP from Schizophrenia (SCZ) and Alzheimer's Disease (AD).

Methods: We analyzed methylation microarray datasets (n = 1218) from SCZ and AD patients obtained from the GEO database. We then collected blood samples from VLOSLP patients and age-matched healthy controls (n = 80) at the Wuxi Mental Health Center for methylation microarray profiling and bisulfite sequencing validation. Differential methylation analysis and Gene Ontology (GO) enrichment analysis identified candidate loci. We prioritized key methylation sites through integrated analysis of methylation quantitative trait loci (meQTL), linkage disequilibrium (LD) patterns, and blood-brain methylation correlations. Machine learning algorithms generated diagnostic models, with classification performance evaluated using Area Under the Curve (AUC) metrics.

Results: Analysis revealed distinct DNA methylation signatures in VLOSLP patients compared to controls. The *GNB5* gene exhibited shared epigenetic modifications across SCZ, AD, and VLOSLP, suggesting a common pathogenic mechanism. The diagnostic model discriminating AD from VLOSLP demonstrated high accuracy, achieving an AUC of 1.0 in the training set and 0.958 in the test set (95% CI: 0.875–1.000). The AD versus SCZ classification model showed similar robustness, with AUCs of 0.995 and 0.955 in training and test sets, respectively (95% CI: 0.926–0.983). The SCZ versus VLOSLP model achieved perfect discrimination (AUC = 1.0) in both training and test sets, with substantial clinical utility. Additional analyses suggested distinct molecular subtypes within VLOSLP.

Conclusion: Specific DNA methylation alterations in VLOSLP are identified as potential diagnostic biomarkers. These findings may contribute to the development of molecular diagnostic tools, though further validation in larger, independent cohorts is warranted.

Keywords: very late-onset schizophrenia-like psychosis, epigenetics schizophrenia, Alzheimer's disease, biomarkers

Introduction

Very-late-onset schizophrenia-like psychosis (VLOSLP) represents a distinct psychiatric condition that manifests initially after age 60.[1] This disorder constitutes approximately 15% of geriatric psychiatric presentations and associates with elevated mortality rates[2] and increased dementia incidence.[3,4] VLOSLP exhibits a phenomenological profile that differentiates it from early-onset schizophrenia, characterized predominantly by delusional positive symptoms while maintaining relative cognitive preservation.[5] Despite these distinctive clinical features, the underlying biological mechanisms remain incompletely understood, creating substantial challenges for accurate diagnosis and therapeutic intervention.

Received: 23 December 2024
Accepted: 17 April 2025
Published: 22 April 2025

DNA methylation and other epigenetic modifications appear to mediate critical pathogenic processes in psychiatric disorders.[6] Research has demonstrated characteristic epigenetic alterations in both schizophrenia (SCZ)[7–9] and Alzheimer's disease[10–13] (AD). However, investigators have not yet systematically examined the epigenetic profile of VLOSLP. The distinctive clinical presentation and age-specific onset of VLOSLP suggest that understanding its epigenetic architecture may illuminate disease mechanisms and identify potential diagnostic biomarkers.

This investigation presents the first comprehensive analysis of genome-wide DNA methylation patterns in VLOSLP patients, incorporating comparative analyses with SCZ and AD cohorts. The research aims to elucidate VLOSLP-specific epigenetic signatures and their potential diagnostic and therapeutic implications. Figure 1 presents the methodological framework for this investigation.

Materials and Methods

Subjects

We retrieved datasets from the National Center for Biotechnology Information (NCBI) GEO database (https://www.ncbi.nlm.nih.gov/geo; accessed December 2023) using the keywords *Schizophrenia* and *Alzheimer's Disease*. The inclusion criteria for dataset selection were as follows: (1) Study design: Methylation profiling by array, with a minimum coverage

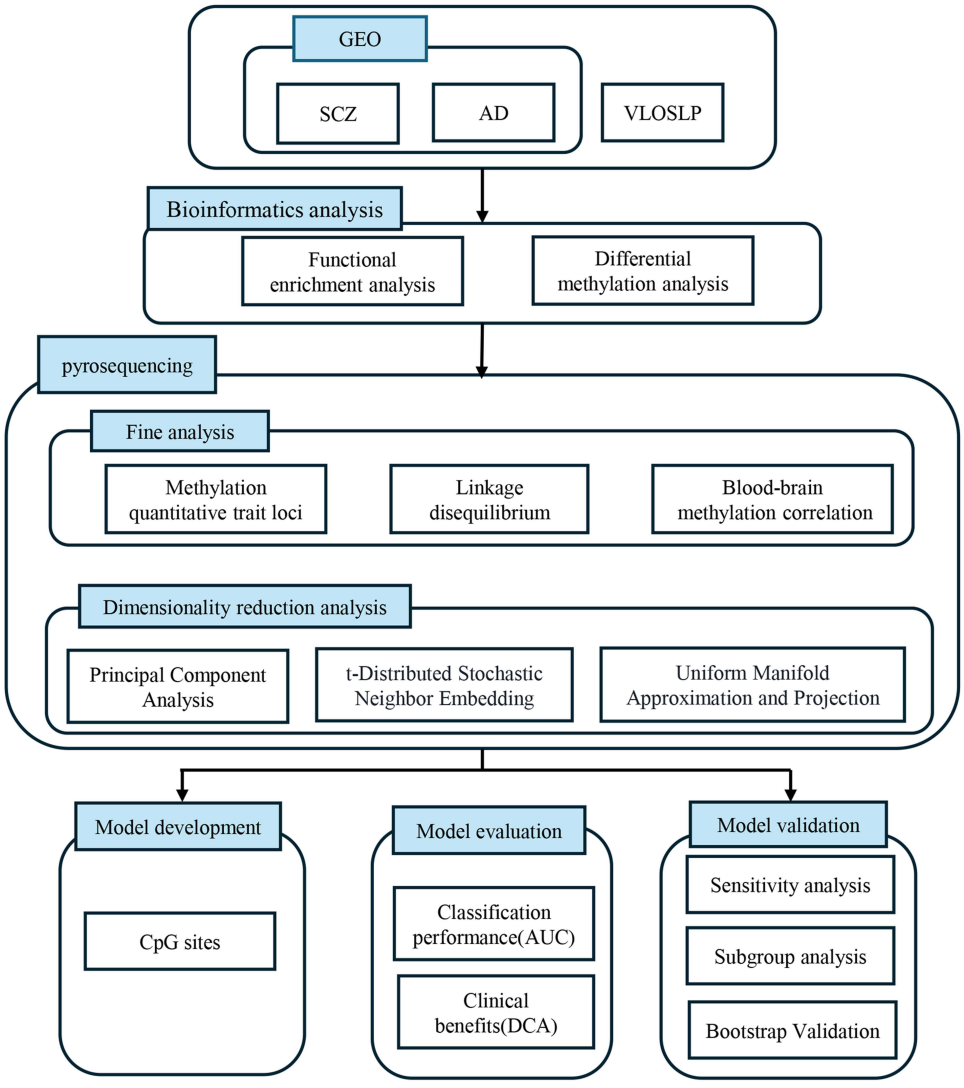

Figure 1 Research flow diagram. The flow diagrams illustrate the research design.

of 450,000 probes; (2) Specimen type: Post-mortem brain tissue or peripheral blood samples from *Homo sapiens*; and (3) Sample size: Each dataset must include at least 10 samples from either schizophrenia (SCZ) or Alzheimer's disease (AD) patients, along with a comparable number of control samples.[14]

Based on these criteria, six datasets were included in the final analysis: three derived from peripheral blood samples (GSE41169:[15] $n_{SCZ} = 62$, $n_{HC} = 33$; GSE153712:[16] $n_{AD} = 255$, $n_{HC} = 471$; GSE157252:[17] $n_{SCZ} = 41$, $n_{HC} = 50$) and three from post-mortem brain tissue samples (GSE61107:[18] $n_{SCZ} = 23$, $n_{HC} = 25$; GSE66351:[19] $n_{AD} = 106$, $n_{HC} = 84$; GSE112179:[20] $n_{SCZ} = 35$, $n_{HC} = 33$). In total, the study encompassed 161 SCZ patients, 361 AD patients, and 696 healthy controls. Detailed inclusion and exclusion criteria for each dataset are described in the respective publications.

A total of 80 (44 VLOSLP patients and 36 matched health controls) participants from the Wuxi Mental Health Center, affiliated with Jiangnan University, were included in the final analysis. Six individuals were excluded from the study: four declined to participate, and two did not meet the inclusion criteria, which are detailed below. All participants provided complete medical histories, underwent physical examinations, and completed laboratory testing. Informed consent was obtained from each participant following a thorough explanation of the study's purpose and procedures. The research was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and received approval from the Ethics Committee of the Wuxi Mental Health Center (Ethics No. WXMHCIRB2023LLky001). The specific inclusion and exclusion criteria are: The inclusion criteria were: 1) Age 60–85 years, Han ethnicity, right-handed; 2) VLOSLP diagnosis according to international expert consensus criteria,[21] with initial psychiatric symptoms (delusions and hallucinations) onset after 60 year-old, confirmed by two senior psychiatrists. 3) No serious physical illness as well as normal laboratory results for past three months. 4) Cognitive screening criteria: Initial phase (first two participants): Mini-Mental State Examination (MMSE) score ≥24 points; Formal study phase (all participants): Montreal Cognitive Assessment (MoCA) score ≥ 26 points; 5) Capable of providing informed consent. The exclusion criteria: 1) DSM-5 Axis I disorders: organic mental disorders, primary emotional disorders, dementia, consciousness disturbances, delirium, memory impairments, or substance-induced mental disorders; 2) Unstable medical conditions in acute phase (diabetes, thyroid disorders, hypertension, cardiovascular diseases); 3) were treated with electroconvulsive therapy during the last month; 4) had abnormal liver or renal function; 5) had QTc prolongation, a history of congenital QTc prolongation, or recent (ie, within the past 6 months) myocardial infarction; 6) Has a history of previous treatment with antipsychotic medications, antidepressant agents, and mood stabilizing drugs; 7) The patient or their legally designated guardian is unable to provide signed consent on the informed consent form.

Genome-Wide DNA Methylation Profiling

Our study examined whole-genome DNA methylation patterns of peripheral blood using the methylation EPIC v2.0 BeadChip platform in a cohort comprising 5 VLOSLP patients and 3 matched healthy controls. Blood collection occurred at 0600 hours on enrollment day following informed consent protocols. We obtained 5 mL fasting blood samples from the antecubital vein and stored them in EDTA-coated tubes at −80°C.

DNA extraction from peripheral blood samples utilized the DNeasy Blood and Tissue Kit (Qiagen Cat. No. 69504). Nanodrop 2000 spectrophotometry (Thermo, Beijing, China) enabled DNA purity and concentration assessments. We processed 500 ng of DNA from each sample through bisulfite conversion using EZ DNA Methylation Kits (Zymo Research, Tustin, CA, USA). The processed samples underwent analysis via the Infinium Methylation EPIC 2.0 BeadChip platform according to manufacturer specifications (Illumina, Hayward, CA, USA). This platform enables quantitative analysis of over 935,000 CpG sites across the genome at single-nucleotide resolution.

Shanghai Bohao Biotechnology Co., Ltd. conducted all laboratory procedures, including DNA extraction, quality control assessment, bisulfite conversion, and BeadChip-specific protocols (amplification, labeling, hybridization, washing, and scanning) under standardized conditions.

Data Preprocessing and Analysis

To ensure the reliability and precision of our data analysis, each dataset was independently preprocessed and analyzed. After downloading the raw data, we utilized the ChAMP package[22] in R software for preliminary processing.

Specifically, we employed the champ.load () function to import and screen the data using the following parameters: methValue was set to "B" for beta values, autoimpute was enabled to automatically impute missing values, and filterDetP was enabled with a detection p-value threshold (detPcut) of 0.01 to exclude probes with detection p-values exceeding this limit. Probes with bead counts below 3% in at least 10% of the samples were removed, along with non-CpG probes, probes with multiplex mapping, and probes located on sex chromosomes (X and Y) or associated with single nucleotide polymorphisms (SNPs).

After initial filtering, stringent quality control was performed using the champ.QC () function. To normalize the beta value matrix and correct for biases between type I and type II probes, we applied the BMIQ normalization method through the champ.norm () function. To mitigate batch effects, we implemented the champ.runCombat () function for batch correction. Additional preprocessing parameters included the exclusion of probes based on bead count cutoffs (beadCutoff set to 0.05) and the adjustment for population-specific genetic variations (population parameter set to "EAS").

Differential Methylation Analysis

Following the preprocessing of genomic DNA methylation microarray data, we utilized the DMP () function from the ChAMP package in R to identify differentially methylated positions (DMPs). To determine the statistical significance of the detected DMPs, we applied the following criteria: a p-value < 0.05, an absolute beta value difference (|β difference|) > 0.1,[23] and false discovery rate (FDR) correction using the Benjamini-Hochberg procedure, with the adjusted p-value threshold set at < 0.05.

Gene Functional Enrichment Analysis

To gain a comprehensive understanding of the biological functions associated with the differentially methylated sites in our dataset, we employed the DAVID database (https://david.ncifcrf.gov). Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were subsequently performed on the genes containing these differentially methylated sites. Entries with a p-value < 0.05 were considered significantly enriched. This approach aimed to elucidate the functional roles and biological pathways influenced by these genes.

Bisulfite Pyrosequencing

To validate the reliability of array-based methylation measurements, we performed technical replication using bisulfite pyrosequencing on candidate CpG sites. Genomic DNA from an independent cohort of 44 VLOSLP patients and 36 matched healthy controls was bisulfite-converted using the EZ DNA Methylation-Gold Kit (Zymo Research) following manufacturer protocols.

Correlation Analysis of DNA Methylation in Multiple Tissues

Recognizing tissue specificity as a defining feature of DNA methylation, we utilized the Blood Brain DNA Methylation Comparison Tool (https://epigenetics.essex.ac.uk/bloodbrain/) to assess the concordance of methylation levels at differentially methylated loci between peripheral blood and selected brain regions. Comparative analyses were performed to evaluate methylation levels in peripheral blood relative to four brain regions: the prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG), and cerebellum (CER). A p-value < 0.05 was considered indicative of a statistically significant correlation.

The Methylation Quantitative Trait Loci Analysis

To explore the interplay between alleles and methylation sites, we extracted data from two well-established methylation quantitative trait loci (meQTL) databases. Specifically, we utilized the meQTL Database (https://epicmeqtl.kcl.ac.uk) to perform a detailed analysis of meQTL associations in the acquired dataset. Our investigation focused exclusively on cis-meQTLs, defining statistically significant single nucleotide polymorphism (SNP)-CpG site pairs as those with a p-value $\leq 1 \times 10^{-8}$.

Analysis of Linkage Disequilibrium

In our study, we utilized the Linkage Disequilibrium Score Regression (LDSC) tool (https://github.com/bulik/ldsc) to calculate linkage disequilibrium (LD) scores for single nucleotide polymorphisms (SNPs) within our dataset. The East

Asian cohort from the Thousand Genomes Project served as the reference population for these computations. SNPs were classified as being in linkage disequilibrium if their $R^2 > 0.5$ or $D' > 0.5$. To further elucidate the observed LD patterns, we employed the LDmatrix tool to generate graphical representations of the linkage disequilibrium structure.

Dimensionality Reduction Analysis

We utilized three advanced dimensionality reduction techniques to extract latent features indicative of mental illness risk: Principal Component Analysis (PCA), t-distributed Stochastic Neighbor Embedding (*t*-SNE), and Uniform Manifold Approximation and Projection (UMAP). PCA was performed using the prcomp () function within the R statistical environment. The t-SNE algorithm was implemented via the R t-SNE () function provided by the corresponding R package (https://github.com/jkrijthe/Rtsne), while UMAP was executed using the UMAP () function from the dedicated R package (https://github.com/tkonopka/umap). To ensure methodological consistency and result reliability, all datasets were preprocessed with centering and scaling using the preprocess () function from the caret package in R. This preprocessing step enabled a rigorous assessment of methylation profiles and clinical characteristics using these dimensionality reduction techniques.

Machine-Learning Model Development

Using the R software and the caret package (https://github.com/topepo/caret), we incorporated the observed differentially methylated positions (DMPs) and chip type as independent variables, with disease classification as the dependent variable. The dataset of methylated samples was divided into training and testing subsets in a 1:1 ratio. Data in the training set were centered, scaled, and transformed to follow a Gaussian distribution using the preprocess () function from the caret package prior to model development. To address potential underfitting or overfitting, we employed either ten-fold cross-validation or leave-one-out validation during the training phase.

Model construction was performed using the XGBoost algorithm.[24,25] Key hyperparameters were optimized via a random search approach, including the number of trees (n estimators), maximum tree depth (max depth), minimum samples required for a split (min samples split), minimum samples required at a leaf node (min samples leaf), learning rate (eta), regularization parameter (C), feature sampling rate per tree (colsample bytree), kernel coefficient (gamma), sample sampling rate per tree (subsample), and the number of boosting iterations (n rounds).

The classification performance of the predictive models was evaluated using the area under the curve (AUC). Clinical utility was further assessed via decision curve analysis, implemented using the rmda package in R (https://github.com/mdbrown/rmda).

Statistical Analysis

Statistical analyses were performed using SPSS version 26.0. Continuous variables were analyzed using *t*-tests, ANOVA, or Kruskal–Wallis tests, depending on their distribution. Categorical variables were assessed with chi-square tests, while Pearson correlation was employed for evaluating relationships between continuous variables. A statistical power analysis was conducted using G*Power 3.1.9.2 software. Based on preliminary experimental data, we set $\alpha = 0.05$, $\beta = 0.1$ (Type II error rate of 10%), and estimated an effect size of 0.79. The analysis determined that a sample size of 70 participants provided sufficient power ($1-\beta = 0.90$) for all statistical tests. To account for multiple comparisons, we applied false discovery rate (FDR) correction using the Benjamini-Hochberg method, with a threshold of $q = 0.05$.

Results

Overview of Whole Genome DNA Methylation

Our genome-wide DNA methylation analysis identified significant differential methylation sites across 5 VLOSLP patients compared to 3 healthy control samples. The analysis revealed 2213 hypermethylated and 3274 hypomethylated sites (Figure 2A), suggesting distinct epigenetic modification patterns between experimental groups. Functional domain analysis of hypermethylated sites demonstrated their distribution across genomic regions: 296 sites (63%) within promoter regions, 32 sites (7%) in 5' untranslated regions (5'UTR), 13 sites (3%) in 3' untranslated regions (3'UTR),

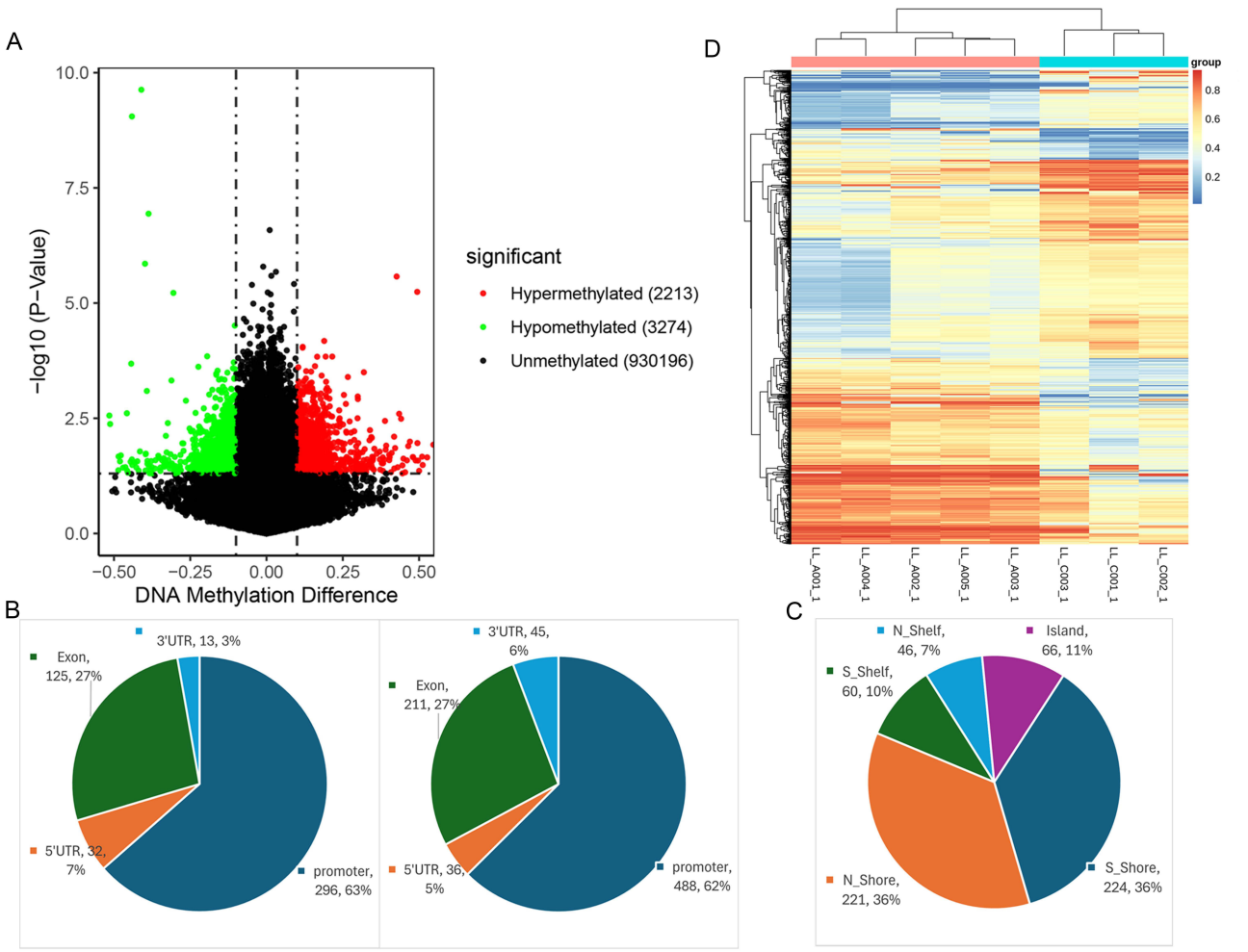

Figure 2 Overview of Whole Genome DNA Methylation. (**A**) Volcano plot illustrating the analysis of differential methylation sites in VLOSLP. (**B**), left: Functional domain analysis of hypermethylated sites (**B**), right: Functional domain analysis of hypomethylated sites. (**C**) Analysis of differential methylation sites in the promoter region. (**D**) Hierarchical clustering analysis of VLOSLP and healthy group.

and 125 sites (27%) within exonic regions. Similarly, hypomethylated sites exhibited a comparable distribution pattern: 488 sites (62%) in promoter regions, 36 sites (5%) in 5'UTR, 45 sites (6%) in 3'UTR, and 211 sites (27%) in exonic regions (Figure 2B).

Further analysis of the 1246 differentially methylated sites in promoter regions revealed specific patterns of CpG-related features. The distribution comprised 11% CpG islands, while flanking regions showed distinct patterns: North (N) and South (S) shores each represented 36%, and N and S shelves constituted 7% and 10%, respectively (Figure 2C). Hierarchical clustering analysis revealed substantial similarities among samples within their respective groups. Notably, the disease group samples exhibited higher methylation pattern homogeneity and clustered distinctly from control group samples (Figure 2D). Additional details are provided in Supplementary Figure S1.

Specific Differentially Methylated Sites of VLOSLP

This investigation examined genome-wide DNA methylation patterns across four distinct groups: patients with VLOSLP (5 cases), healthy controls (696 cases), individuals with SCZ (161 cases), and patients with AD (361 cases). Subsequent gene mapping analysis of these differentially methylated sites, visualized through Venn diagram analysis (Figure 3A), identified 983 uniquely differentially methylated genes, suggesting distinct epigenetic signatures associated with VLOSLP.

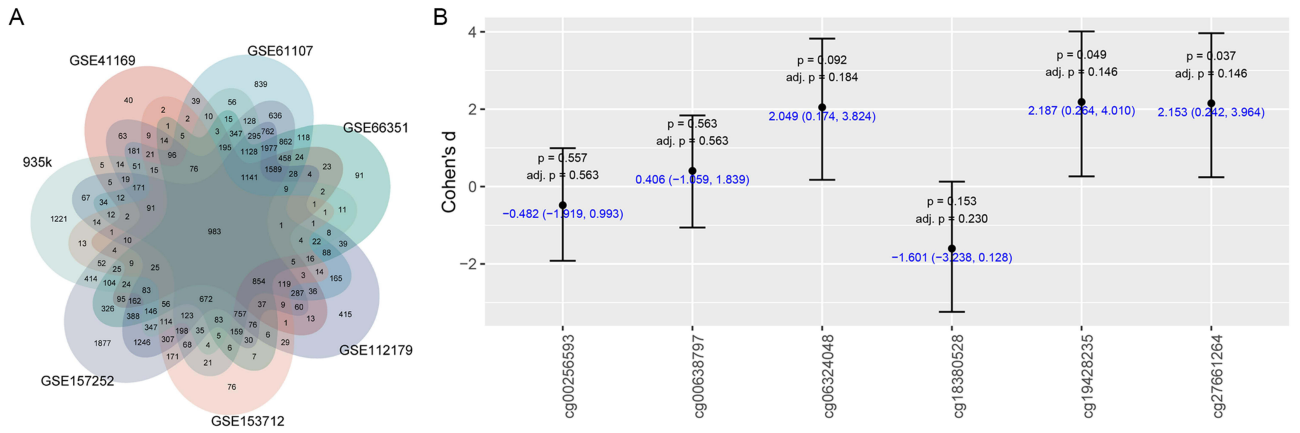

Figure 3 Specific differential methylation site analysis. (**A**) Venn diagram illustrating the overlap of differentially expressed genes across datasets, revealing 983 genes commonly altered with the intersecting regions highlighting the shared differentially expressed genes among the datasets. (**B**) Analysis of Specific Differential Methylation Sites in VLOSLP.

To noted, we identified six VLOSLP-specific sites (Figure 3B). Statistical analysis revealed varying effect sizes and confidence intervals for these sites: cg06324048 (effect size: 2.05 [95% CI: 0.174–3.824]; cg27661264 (effect size: 2.15 [95% CI: 0.24–3.96]; cg19428235 (effect size: 2.19 [95% CI: 0.26–4.01]; cg18380528 (effect size: −1.60 [95% CI: −3.24–0.13]; cg00638797 (effect size: 0.406 [95% CI: −1.06–1.84]; cg00256593 (effect size: −0.482 [95% CI: −1.92–0.99]).

Functional Enrichment Analysis

Gene Ontology (GO) functional enrichment analysis revealed distinct biological focuses in the datasets. The SCZ dataset (Figure 4A) was primarily enriched for processes related to glutamate synapse function and neuron cell body structure. In contrast, the AD dataset (Figure 4B) demonstrated enrichment in synapse activity, glutamate synapse, neuron cell body, nervous system development, axonogenesis, and neuronal protrusion growth.

In the independent VLOSLP dataset, functional enrichment analysis of the identified differentially methylated positions (DMPs) highlighted significant involvement in key biological processes essential to nervous system function. These included G protein-coupled receptor activity, protein tyrosine kinase activator activity, and the regulation of neurotransmitter secretion and phagocytosis mediated by calcium ions (Figure 4C).

KEGG pathway enrichment analysis indicated that the identified DMPs were significantly enriched in pathways such as adrenergic signaling in cardiomyocytes, cAMP signaling, cGMP-PKG signaling, linoleic acid metabolism, arachidonic acid metabolism, and cocaine addiction signaling pathways (Figure 4D).

Furthermore, disease-associated gene network analysis revealed notable interactions involving *GNB5, GNAS, GNB1, GNAI1*, and *ADCYAP1R1*, forming a robust interaction network (Figure 4E).

Bisulfite Pyrosequencing Validation

To validate our genome-wide methylation findings, we conducted bisulfite pyrosequencing on seven neurologically relevant CpG sites selected by effect size magnitude (effect size>0.4): (*GNB1* (cg16645714), *GNAI1* (cg19428235), *RYR2* (cg18380528), *GNAS* (cg06324048, cg27661264), *GNB5* (cg00638797), *ADCYAP1R1* (cg00256593). The analysis revealed locus-specific methylation alterations in VLOSLP patients compared to healthy controls. A statistically significant hypermethylation was observed at the *GNB5* cg00638797 site ($\Delta\beta$ = +14.7%, $p < 0.001$, Mann–Whitney U) (Figure 5). In contrast, the remaining sites showed no significant intergroup differences: *GNB1* (cg16645714, $p = 0.753$); *GNAI1* (cg19428235, $p = 0.881$); *RYR2* (cg18380528, $p = 0.097$); *GNAS* (cg06324048, $p = 0.498$); *GNAS* (cg27661264, $p = 0.958$); *ADCYAP1R1* (cg00256593, $p = 0.923$), as shown in Supplementary Table S1.

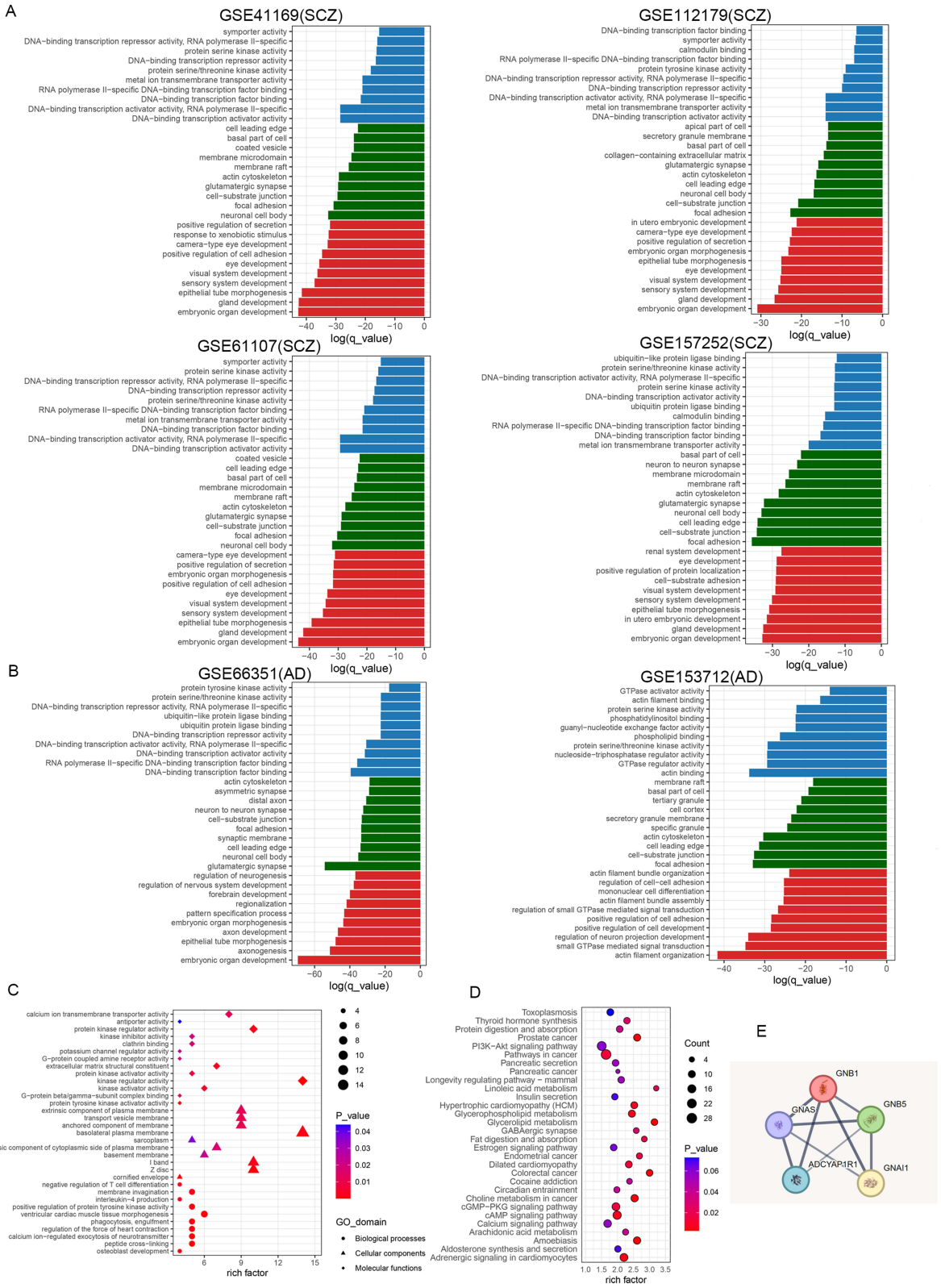

Figure 4 GO and KEGG enrichment analysis. (**A**) GO functional enrichment analysis of SCZ. (**B**) GO functional enrichment analysis of AD. The red column denotes Biological Processes (BP), the green signifies Cellular Components (CC), while the blue column is indicative of Molecular Functions (MF). (**C**) GO functional enrichment analysis of VLOSLP. (**D**) KEGG pathway enrichment analysis of VLOSLP. (**E**) Network analysis of GNB5 related diseases.

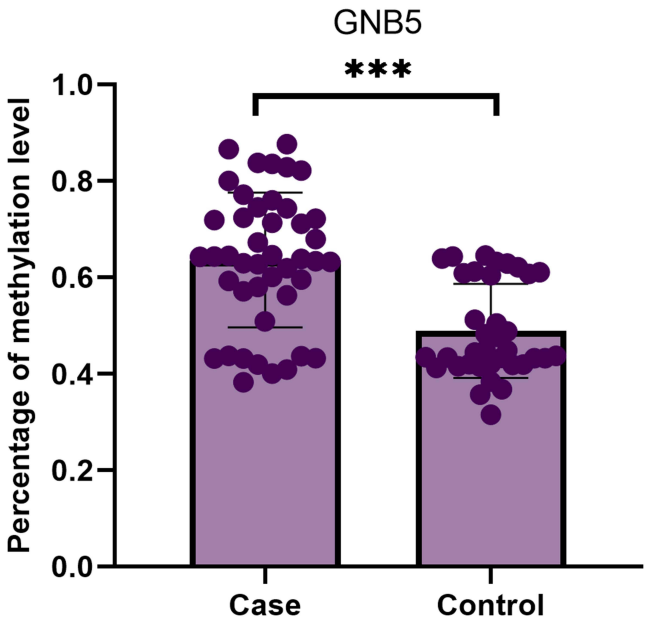

Figure 5 Pyrophosphate verification of *GNB5*. Asterisks denote statistical significance: ***$p < 0.001$.

Correlation Analysis of Methylation Levels Between Peripheral Blood and Brain Tissue

In VLOSLP peripheral blood, the methylation status of cg13934625 demonstrated a significant association with the prefrontal cortex ($p = 0.000331$) and the entorhinal cortex ($p = 0.0025$), as shown in Figure 6A. Similarly, the methylation level of cg11871050 was significantly correlated with the frontal lobe ($p = 0.0394$), entorhinal lobe ($p = 0.016$), superior temporal lobe ($p = 0.0126$), and cerebellum ($p = 0.0408$), as illustrated in Figure 6B. For cg00638797, a strong correlation was observed with the frontal lobe ($p = 0.00091$), entorhinal lobe ($p = 0.0249$), superior temporal lobe ($p = 0.000696$), and cerebellum ($p = 4.2\times10^{-16}$), as depicted in Figure 6C. Additional correlations are provided in Supplementary Figure S2.

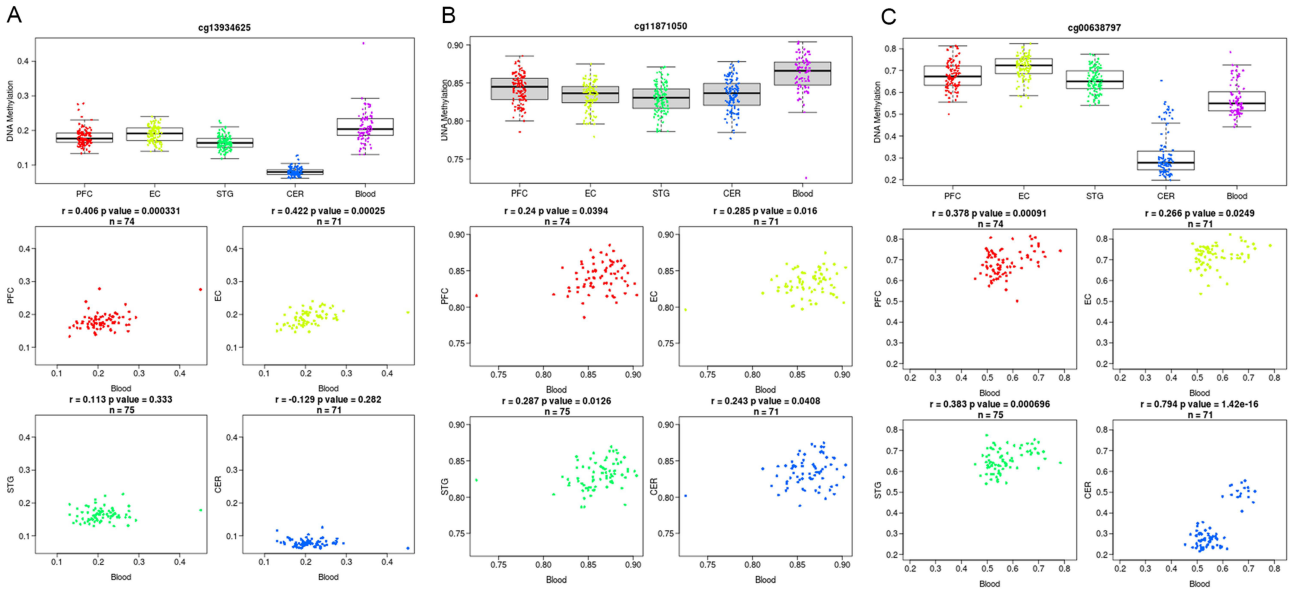

Figure 6 Correlation of *GNB5*-linked methylation patterns in blood and brain tissue. (**A**–**C**) show methylation for cg13934625, cg11871050, and. cg00638797.

https://doi.org/10.2147/NDT.S513992

Genetic-Epigenetic Interaction Analysis

Analysis of genetic-epigenetic interactions within the *GNB5* gene in VLOSLP patients, specifically examining the relationship between differential methylation sites and methylome quantitative trait loci (mQTL), revealed 286 significant single nucleotide polymorphism (SNP)-CpG pairs functioning as mQTLs across 349 differentially methylated regions (see Supplementary Table S2).

Linkage disequilibrium analysis, conducted with reference to East Asian population allele frequencies, indicates potential associations between the *GNB5*-meQTL and multiple neurobiological phenotypes. These associations encompass structural brain characteristics (including volumetric variations in specific brain regions), calcium homeostasis, and white matter integrity, with particular emphasis on the posterior cingulate cortex. Moreover, the *GNB5*-meQTL appears to correlate with pharmacological response to antipsychotic agents and may contribute to the pathophysiology of severe depressive disorder (comprehensive data presented in Supplementary Table S3).

Development, Validation, and Evaluation of the Predictive Model

We developed a diagnostic classification model predicated on DNA methylation patterns at five specific CpG sites (cg13934625, cg14120436, cg19366178, cg19747632, cg20557935) within the *GNB5* gene to differentiate among SCZ, AD, and VLOSLP. The model's classification performance underwent evaluation using both training and test datasets (Figure 7A and B). In the binary classification of AD versus VLOSLP (n train = 184(n_{AD} = 181; n_{VLOSLP} = 3), n test = 182(n_{AD} = 180; n_{VLOSLP} = 2)), the model demonstrated an area under the curve (AUC) of 1.0 in the training set and 0.958 (95% CI: 0.875–1.000) in the test set. The AD versus SCZ binary classification (n train = 264(n_{AD} = 181; n_{SCZ} = 83), n test = 263(n_{AD} = 180; n_{SCZ} = 83)) yielded an AUC of 0.995 in the training set and 0.955 (95% CI: 0.926–0.983) in the test set. For SCZ versus VLOSLP differentiation (n train = 84(n_{SCZ} = 81; n_{VLOSLP} = 3), n test = 82 (n_{SCZ} = 80; n_{VLOSLP} = 2)), the model achieved an AUC of 1.0 in both training and test sets.

Decision curve analysis (Figure 7C) indicates substantial net benefits in the test set for all three binary classification models (AD vs SCZ, AD vs VLOSLP, and SCZ vs VLOSLP). These findings suggest that *GNB5* methylation patterns may serve as potential biomarkers for distinguishing among these neuropsychiatric conditions, though further validation studies are warranted.

The outcomes of the sensitivity assessment, subgroup examination, and Bootstrap testing indicate a high degree of robustness for the model. Details are provided in Supplementary Figure S3.

Dimensionality reduction analysis revealed associations between *GNB5* methylation profiles and specific clinical parameters. Notably, variations in language performance scores on the Montreal Cognitive Assessment (MoCA) and Activities of Daily Living (ADL) scores, stratified by *GNB5* methylation levels, appear to contribute to the identification of VLOSLP subtypes (Figure 7D).

Discussion

This study identified significant DNA methylation changes in the blood of patients with VLOSLP, primarily affecting gene promoter regions. Our enrichment analysis suggests that pathways related to neurotransmitter regulation, inflammation, and cognitive function may play central roles in VLOSLP pathogenesis. Notably, we observed a strong genetic-epigenetic interaction involving the *GNB5* gene,[26] which is critical for brain structure and has been implicated in various psychiatric disorders. By leveraging the methylation status of *GNB5*, we developed and validated a predictive model that demonstrated excellent classification performance and clinical utility. Moreover, we identified distinct VLOSLP subtypes with unique clinical features, emphasizing the disorder's heterogeneity.

The observed DNA methylation alterations primarily affected promoter regions of genes involved in neurotransmitter pathways. As DNA methylation modulates gene expression, increased methylation levels may downregulate genes associated with the synthesis, release, and metabolism of neurotransmitters,[27] thereby affecting the balance of the neurotransmitter system. For instance, the methylation status of *GNB5* is closely related to VLOSLP occurrence. Studies have shown that *GNB5* is significantly enriched in the brain, particularly in the hippocampus and striatum[28]

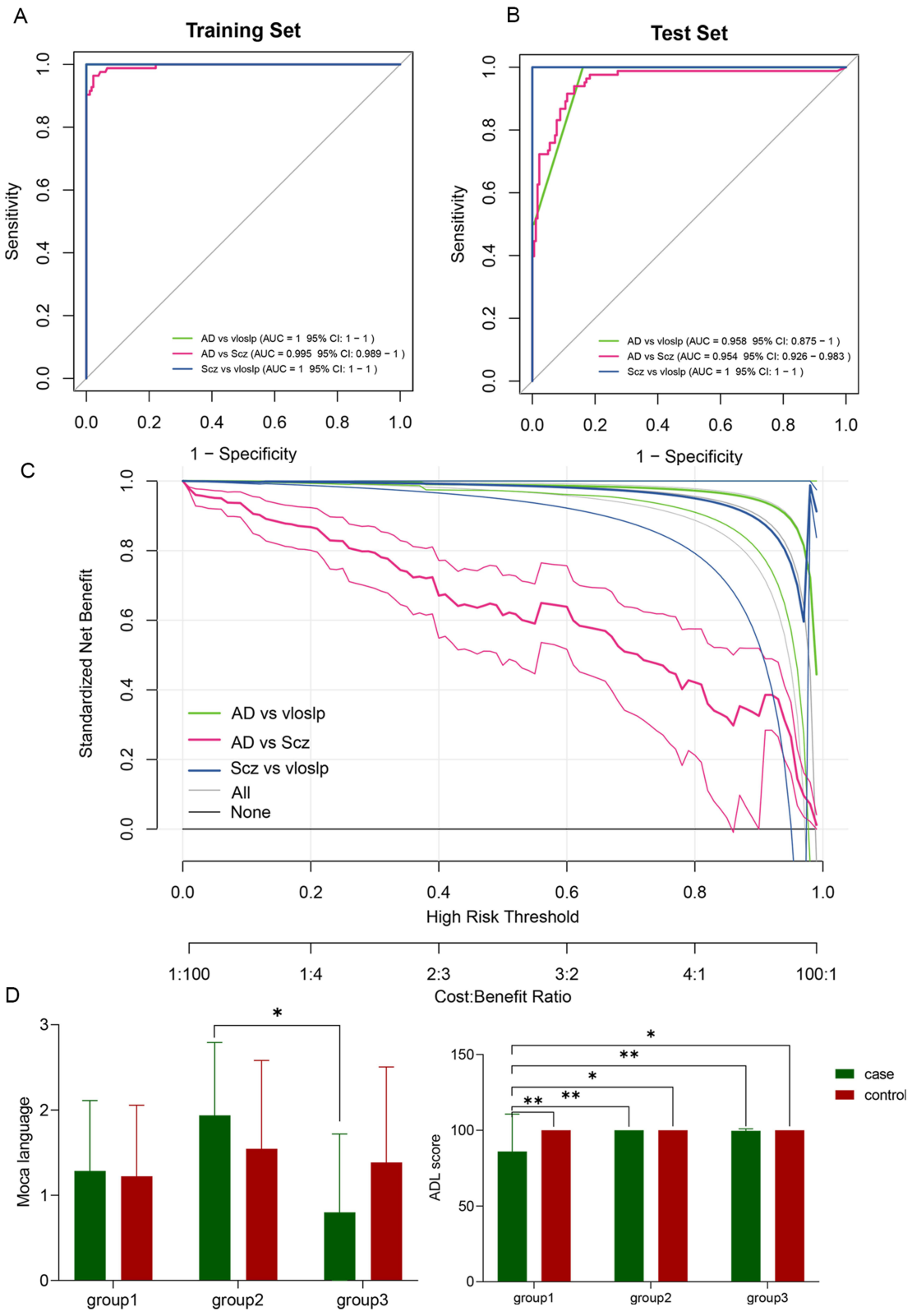

Figure 7 Performance evaluation of predictive models. (**A**) Performance of the training set model. (**B**) Test set model performance. AUC. Area under the curve. (**C**) Decision Curve Evaluation for Identifying Distinctive Features of Three Models in the Test Data. The green curve shows the AD vs VLOSLP binary classification model, the red curve shows the AD vs SCZ binary classification model, and the blue curve shows the SCZ vs VLOSLP binary classification model. (**D**) Dimensionality reduction analysis of *GNB5* methylation profile, Montreal Cognitive Assessment (MoCA) language function, and Activities of Daily Living (ADL). Error bars show data variability (standard error or confidence intervals).
Note: Asterisks denote statistical significance: *p < 0.05, **p < 0.01.

https://doi.org/10.2147/NDT.S513992

and is integral to neurotransmitter signal transduction.[29] Altered *GNB5* methylation may thus contribute to neurotransmitter dysfunction and the pathogenesis of VLOSLP.

Our study revealed distinct neurotransmitter pathway-specific methylation alterations that significantly differ from findings in SCZ epigenetic research. Previous genome-wide methylation studies of SCZ primarily identified methylation abnormalities in glutamatergic receptors (*GRM3*,[30] *GRIN2A*[31]) and dopamine receptor *DRD2*,[32] whereas our findings demonstrate predominant methylation changes in *GNB5*, a key gene in the G protein signaling transduction pathway. This discrepancy suggests that VLOSLP may involve different regulatory targets compared to typical SCZ. Specifically, VLOSLP appears to predominantly involve G protein-mediated transmembrane signaling transduction, while typical SCZ more frequently associates with receptor functional impairments.

Comparative analysis with AD revealed that although both conditions exhibit epigenetic modifications in cognition-related pathways, AD characteristic methylation loci predominantly cluster in amyloid precursor protein metabolism[33,34] (*ANK1, APP, PSEN1*) and tau phosphorylation regulation[35] (*MAPT*). In contrast, the *GNB5* methylation signature identified in our study may reflect synaptic plasticity impairments - a potential VLOSLP-specific pathological mechanism.

Besides, the methylation characteristics of VLOSLP demonstrate a distinct pattern from late-onset major depressive disorder (MDD) and anxiety disorders. In MDD, where elevated methylation of neuroplasticity-related genes (*BDNF*) and stress-responsive loci (*NR3C1*) is linked to depression risk.[36] What's more, generalized anxiety disorder[37,38] (GAD), shows methylation changes at neuroinflammation-associated CpG sites (*RIOK3* cg21515243, *PSMB4* cg01334186). However, VLOSLP predominantly involves G protein-coupled receptor (*GNB5*) and calcium-mediated synaptic plasticity pathways.

Notably, the epigenetic dysregulation observed in VLOSLP exhibits pathway-level convergence with neurodegenerative disorders beyond AD. This is particularly evident in GPCR signaling pathways — as evidenced by the involvement of inhibin-mediated GPCR modulation in frontotemporal dementia pathogenesis.[39] Furthermore, calcium signaling alterations in VLOSLP show functional parallels to neurodegenerative mechanisms, such as the calcium dyshomeostasis observed in striatal neurons in Huntington's disease,[40] though these disorders diverge in their specific molecular targets within shared calcium-regulated neurotransmitter pathways.

Aging-related epigenetic changes appear to play a key role in VLOSLP. Epigenetic modifications accumulate with age,[41] influencing gene expression and potentially leading to cognitive decline[42] and neuropsychiatric symptoms.[43] In this study, DNA methylation alterations associated with VLOSLP suggest that age-related epigenetic remodeling may underlie the disorder's late onset. Shifts in methylation patterns of neurotransmitter-related genes might compromise cognitive function and promote neuropsychiatric manifestations as individuals grow older.

Epigenetic regulation, including DNA methylation, significantly affects neurocognitive processes. It can impair neuronal metabolism, synaptic regulation, neurogenesis, and neuroplasticity,[44,45] all of which are vital for maintaining cognitive function. Consistent with previous findings,[5] our MoCA assessments revealed cognitive deficits in VLOSLP patients, aligning with the observed methylation alterations at specific loci. These changes may further disturb neurotransmitter equilibrium, exacerbating cognitive dysfunction.

From a clinical perspective, our findings underscore the potential of DNA methylation markers as biological tools for diagnosis, treatment planning, and prognosis.[46] In this study, DNA methylation markers exhibited high performance (AUC: 0.958 to 1) for diagnosing VLOSLP and can be integrated with existing clinical scales for more accurate early screening. Additionally, they show promise for predicting drug responses and monitoring treatment efficacy. Identification of prognosis-related methylation sites could guide early interventions, inform personalized treatment strategies, and support dynamic monitoring of disease progression and recurrence risk.

Although numerous epigenetic studies in SCZ focus on late adolescence or early adulthood,[47] most emphasize isolated loci, non-coding RNAs, epigenetic aging, or telomere shortening.[8] There remains a paucity of empirical research specifically examining first-episode SCZ in individuals aged 40 or above, or 60 and older, with existing studies often limited to clinical follow-up or cross-sectional comparisons.[5] A PubMed search (2000–August 2024) for "late-onset schizophrenia-like psychosis" yielded 95 publications, of which only 3 were clinical trials focused solely on antipsychotic treatments.[48–50] By conducting a whole-genome DNA methylation study on VLOSLP, we address this gap,

demonstrating unique epigenetic signatures and highlighting the importance of neurotransmitter-, inflammatory-, and cognition-related pathways in disease pathology.

VLOSLP presents particular diagnostic challenges, especially when distinguishing it from other age-related neurodegenerative conditions such as AD, frontotemporal dementia, and Lewy body de entia.[51,52] Cognitive impairments in SCZ and AD overlap across domains including attention, memory, and language.[53] Our predictive model, based on five CpG sites within *GNB5*, effectively differentiated VLOSLP from AD, providing a novel biomarker that could refine differential diagnosis and deepen our understanding of cognitive dysfunction across these conditions.

Importantly, the epigenetic patterns we identified in VLOSLP differ markedly from known aging-related epigenetic markers.[54] These findings suggest that aging-related epigenetic mechanisms might be central to VLOSLP pathogenesis. Recent epigenetic meta-analyses[55] further support the relevance of factors such as age, gender, disease duration, and polygenic risk in SCZ, reinforcing the complexity of epigenetic influences in late-onset presentations.

This study offers several innovations. First, we systematically characterized VLOSLP's DNA methylation landscape for the first time, identifying distinct epigenetic markers that differentiate VLOSLP from SCZ and AD. Second, we developed a DNA methylation analysis framework for elderly psychiatric disorders, integrating multi-omics strategies (genomics and epigenetics) with clinically useful predictive models. Theoretically, we proposed specific hypotheses regarding age-related epigenetic mechanisms in VLOSLP, establishing patterns of association between epigenetic changes and clinical phenotypes. Practically, we proposed diagnostic, therapeutic, and prognostic models based on epigenetic markers.

Despite these strengths, some limitations must be acknowledged. The relatively small sample size reduces statistical power, potentially explaining why certain VLOSLP-specific sites did not reach significance after correction. Larger cohorts are needed for validation. In addition, SCZ vs VLOSLP, AD vs VLOSLP classifiers need to be interpreted with caution and require external dataset validation. In addition, the prediction model includes blood and brain tissue samples, which may cause some confusion. Methodological constraints persist in DNA methylation testing, bioinformatics approaches, and phenotype-genotype association studies.[56] Confounding variables, including age, gender, medication use, comorbidities, and lifestyle, may influence our results. Additionally, the follow-up lacked and incomplete phenotypic data collection limit our capacity for deeper mechanistic understanding. Finally, our research is still in the conceptual validation stage, and the directionality of the observed associations is still unclear without functional experiments.

Future research should thus expand sample sizes, extend follow-up durations, incorporate functional validations, enrich clinical data, integrate multi-omics peripheral blood data, explore multi-class models, and apply multivariate analytical methods to mitigate confounders. Further explore the heterogeneity of VLOSLP subtypes. Through these steps, we can refine our understanding of VLOSLP's epigenetic underpinnings, ultimately improving diagnostic accuracy, treatment efficacy, and prognostic precision.

Conclusions

This study identifies novel DNA methylation alterations in VLOSLP, revealing distinct epigenetic biomarkers and a highly accurate diagnostic model (85%). These findings differentiate VLOSLP from other psychiatric disorders, implicate key pathways, and offer new targets for personalized treatment, advancing both diagnostic and therapeutic strategies.

Abbreviations

VLOSLP, Very-late-onset schizophrenia-like psychosis; SCZ, Schizophrenia; AD, Alzheimer's Disease; GO, Gene Ontology; meQTL, methylation quantitative trait loci; LD, linkage disequilibrium; SNPs, single nucleotide polymorphisms; DMPs, differentially methylated positions; PFC, the prefrontal cortex; EC, entorhinal cortex; STG, superior temporal gyrus; CER, cerebellum; PCA, Principal Component Analysis; t-SNE, t-distributed Stochastic Neighbor Embedding; UMAP, Uniform Manifold Approximation and Projection; AUC, the area under the curve; DMP, Differential methylation position; mQTL, methylome quantitative trait loci; MOCA, Montessori cognitive assessment; ADL, activities of daily living; 95% CI, 95%confidence interval; adj. *P*-val., adjusted *P*-value.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of The Wuxi Mental Health Center. Informed consent was obtained from all the subjects. The trial registration number was WXMHCIRB2023LLky001. All procedures carried out in studies conformed to the 1964 helsinki Declaration and its subsequent amendments or similar ethical standards.

Acknowledgments

We would like to thank the VLOSLP patients and healthy subjects for their cooperation in the study.

An unauthorized version of the Chinese MMSE was used by the study team without permission, however this has now been rectified with PAR. The MMSE is a copyrighted instrument and may not be used or reproduced in whole or in part, in any form or language, or by any means without written permission of PAR (www.parinc.com).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study is supported by Wuxi Municipal Health Commission Major Project (202107), Wuxi Taihu Talent Project (WXTTP2021), and Top Talent Support Program for young and middle-aged people of Wuxi Health Committee (BJ2023086).

Disclosure

All authors declare no conflicts of interest in this work.

References

1. Stafford J, Howard R, Dalman C, Kirkbride JB. The incidence of nonaffective, nonorganic psychotic disorders in older people: a population-based cohort study of 3 million people in Sweden. *Schizophr Bull*. 2019;45(5):1152–1160. doi:10.1093/schbul/sby147
2. Talaslahti T, Alanen H-M, Hakko H, et al. Patients with very-late-onset schizophrenia-like psychosis have higher mortality rates than elderly patients with earlier onset schizophrenia. *Int J Geriatr Psychiatry*. 2015;30(5):453–459. doi:10.1002/gps.4159
3. Stafford J, Dykxhoorn J, Sommerlad A, et al. Association between risk of dementia and very late-onset schizophrenia-like psychosis: a Swedish population-based cohort study. *Psychol Med*. 2023;53(3):750–758. doi:10.1017/S0033291721002099
4. Kodesh A, Goldberg Y, Rotstein A, et al. Risk of dementia and death in very-late-onset schizophrenia-like psychosis: a national cohort study. *Schizophr Res*. 2020;223:220–226. doi:10.1016/j.schres.2020.07.020
5. Suen YN, Wong SMY, Hui CLM, et al. Late-onset psychosis and very-late-onset-schizophrenia-like-psychosis: an updated systematic review. *Int Rev Psychiatry*. 2019;31(5–6):523–542. doi:10.1080/09540261.2019.1670624
6. Chen J, Zang Z, Braun U, et al. Association of a reproducible epigenetic risk profile for schizophrenia with brain methylation and function. *JAMA Psychiatry*. 2020;77(6):628–636. doi:10.1001/jamapsychiatry.2019.4792
7. Lockwood L, Miller B, Youssef NA. Epigenetics and first-episode psychosis: a systematic review. *Psychiatry Res*. 2022;307:114325. doi:10.1016/j.psychres.2021.114325
8. Srivastava A, Dada O, Qian J, et al. Epigenetics of schizophrenia. *Psychiatry Res*. 2021;305:114218. doi:10.1016/j.psychres.2021.114218
9. Hannon E, Dempster EL, Mansell G, et al. DNA methylation meta-analysis reveals cellular alterations in psychosis and markers of treatment-resistant schizophrenia. *Elife*. 2021;10:e58430. doi:10.7554/eLife.58430
10. Mendioroz M, Celarain N, Altuna M, et al. CRTC1 gene is differentially methylated in the human hippocampus in Alzheimer's disease. *Alzheimers Res Ther*. 2016;8(1):15. doi:10.1186/s13195-016-0183-0
11. Qazi TJ, Quan Z, Mir A, Qing H. Epigenetics in Alzheimer's disease: perspective of DNA methylation. *mol Neurobiol*. 2018;55(2):1026–1044. doi:10.1007/s12035-016-0357-6
12. Nikolac Perkovic M, Videtic Paska A, Konjevod M, et al. Epigenetics of Alzheimer's disease. *Biomolecules*. 2021;11(2):195. doi:10.3390/biom11020195
13. Coppedè F, Tannorella P, Stoccoro A, et al. Methylation analysis of DNA repair genes in Alzheimer's disease. *Mech Ageing Dev*. 2017;161:105–111. doi:10.1016/j.mad.2016.04.003
14. Li Z, Li X, Jin M, et al. Identification of potential biomarkers and their correlation with immune infiltration cells in schizophrenia using combinative bioinformatics strategy. *Psychiatry Res*. 2022;314:114658. doi:10.1016/j.psychres.2022.114658

15. Horvath S, Zhang Y, Langfelder P, et al. Aging effects on DNA methylation modules in human brain and blood tissue. *Genome Biol*. 2012;13(10):R97. doi:10.1186/gb-2012-13-10-r97
16. Nabais MF, Laws SM, Lin T, et al. Meta-analysis of genome-wide DNA methylation identifies shared associations across neurodegenerative disorders. *Genome Biol*. 2021;22(1):90. doi:10.1186/s13059-021-02275-5
17. Piao Y-H, Cui Y, Rami FZ, et al. Methylome-wide association study of patients with recent-onset psychosis. *Clin Psychopharmacol Neurosci*. 2022;20(3):462–473. doi:10.9758/cpn.2022.20.3.462
18. Wockner LF, Noble EP, Lawford BR, et al. Genome-wide DNA methylation analysis of human brain tissue from schizophrenia patients. *Transl Psychiatry*. 2014;4(1):e339. doi:10.1038/tp.2013.111
19. Gasparoni G, Bultmann S, Lutsik P, et al. DNA methylation analysis on purified neurons and glia dissects age and Alzheimer's disease-specific changes in the human cortex. *Epigenet Chromatin*. 2018;11(1):41. doi:10.1186/s13072-018-0211-3
20. Pai S, Li P, Killinger B, et al. Differential methylation of enhancer at IGF2 is associated with abnormal dopamine synthesis in major psychosis. *Nat Commun*. 2019;10(1):2046. doi:10.1038/s41467-019-09786-7
21. Howard R, Rabins PV, Seeman MV, Jeste DV, Late-Onset TI. Late-onset schizophrenia and very-late-onset schizophrenia-like psychosis: an international consensus. The international late-onset schizophrenia group. *Am J Psychiatry*. 2000;157(2):172–178. doi:10.1176/appi.ajp.157.2.172
22. Tian Y, Morris TJ, Webster AP, et al. ChAMP: updated methylation analysis pipeline for Illumina BeadChips. *Bioinformatics*. 2017;33(24):3982–3984. doi:10.1093/bioinformatics/btx513
23. Bai Y, Xu J, Li D, et al. HepaClear, a blood-based panel combining novel methylated CpG sites and protein markers, for the detection of early-stage hepatocellular carcinoma. *Clin Clin Epigenet*. 2023;15(1):99. doi:10.1186/s13148-023-01508-7
24. Bentejac C, Csorgo A, Martınez-Munoz G. A comparative analysis of XGBoost.
25. Malik S, Harode R, Singh A. XGBoost: a deep dive into boosting.
26. Zhang J-H, Pandey M, Seigneur EM, et al. Knockout of G protein β5 impairs brain development and causes multiple neurologic abnormalities in mice. *J Neurochem*. 2011;119(3):544–554. doi:10.1111/j.1471-4159.2011.07457.x
27. Wu T, Cai W, Chen X. Epigenetic regulation of neurotransmitter signaling in neurological disorders. *Neurobiol Dis*. 2023;184:106232. doi:10.1016/j.nbd.2023.106232
28. Watson AJ, Katz A, Simon MI. A fifth member of the mammalian G-protein beta-subunit family. Expression in brain and activation of the beta 2 isotype of phospholipase C. *J Biol Chem*. 1994;269(35):22150–22156. doi:10.1016/S0021-9258(17)31768-4
29. Shamseldin HE, Masuho I, Alenizi A, et al. GNB5 mutation causes a novel neuropsychiatric disorder featuring attention deficit hyperactivity disorder, severely impaired language development and normal cognition. *Genome Biol*. 2016;17(1):195. doi:10.1186/s13059-016-1061-6
30. Saini SM, Mancuso SG, Mostaid MS, et al. Meta-analysis supports GWAS-implicated link between GRM3 and schizophrenia risk. *Transl Psychiatry*. 2017;7(8):e1196. doi:10.1038/tp.2017.172
31. Harrison PJ, Bannerman DM. GRIN2A (NR2A): a gene contributing to glutamatergic involvement in schizophrenia. *mol Psychiatry*. 2023;28(9):3568–3572. doi:10.1038/s41380-023-02265-y
32. Ovenden ES, McGregor NW, Emsley RA, Warnich L. DNA methylation and antipsychotic treatment mechanisms in schizophrenia: progress and future directions. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;81:38–49. doi:10.1016/j.pnpbp.2017.10.004
33. Palma-Gudiel H, Yu L, Huo Z, et al. Fine-mapping and replication of EWAS loci harboring putative epigenetic alterations associated with AD neuropathology in a large collection of human brain tissue samples. *Alzheimers Dement*. 2023;19(4):1216–1226. doi:10.1002/alz.12761
34. Martinez-Feduchi P, Jin P, Yao B. Epigenetic modifications of DNA and RNA in Alzheimer's disease. *Front Mol Neurosci*. 2024;17:1398026. doi:10.3389/fnmol.2024.1398026
35. Balmik AA, Chinnathambi S. Methylation as a key regulator of Tau aggregation and neuronal health in Alzheimer's disease. *Cell Commun Signal*. 2021;19(1):51. doi:10.1186/s12964-021-00732-z
36. The associations between DNA methylation and depression: a systematic review and meta-analysis - PubMed. Available from: https://pubmed.ncbi.nlm.nih.gov/36717033/. Accessed April 21, 2025.
37. Hettema JM, van den Oord EJ, Zhao M, et al. Methylome-wide association study of anxiety disorders. *mol Psychiatry*. 2023;28: 3484–3492.
38. Guo L, Ni Z, Wei G, et al. Epigenome-wide DNA methylation analysis of whole blood cells derived from patients with GAD and OCD in the Chinese Han population. *Transl Psychiatry*. 2022;12(1):465. doi:10.1038/s41398-022-02236-x
39. Kee TR, Khan SA, Neidhart MB, et al. The multifaceted functions of β-arrestins and their therapeutic potential in neurodegenerative diseases. *Exp Mol Med*. 2024;56(1):129–141. doi:10.1038/s12276-023-01144-4
40. Cherubini M, Lopez-Molina L, Gines S. Mitochondrial fission in Huntington's disease mouse striatum disrupts ER-mitochondria contacts leading to disturbances in Ca2+ efflux and Reactive Oxygen Species (ROS) homeostasis. *Neurobiol Dis*. 2020;136:104741. doi:10.1016/j.nbd.2020.104741
41. Seale K, Horvath S, Teschendorff A, Eynon N, Voisin S. Making sense of the ageing methylome. *Nat Rev Genet*. 2022;23(10):585–605. doi:10.1038/s41576-022-00477-6
42. Cui D, Xu X. DNA methyltransferases, DNA methylation, and age-associated cognitive function. *Int J mol Sci*. 2018;19(5):1315. doi:10.3390/ijms19051315
43. Rizzardi LF, Hickey PF, Rodriguez DiBlasi V, et al. Neuronal brain-region-specific DNA methylation and chromatin accessibility are associated with neuropsychiatric trait heritability. *Nat Neurosci*. 2019;22(2):307–316. doi:10.1038/s41593-018-0297-8
44. Kremer LPM, Cerrizuela S, El-Sammak H, et al. DNA methylation controls stemness of astrocytes in health and ischaemia. *Nature*. 2024;634(8033):415–423. doi:10.1038/s41586-024-07898-9
45. Ho NF, Tng JXJ, Wang M, et al. Plasticity of DNA methylation, functional brain connectivity and efficiency in cognitive remediation for schizophrenia. *J Psychiatr Res*. 2020;126:122–133. doi:10.1016/j.jpsychires.2020.03.013
46. Fischer CE. Biomarkers of neurodegeneration among patients with very late-onset schizophrenia-like psychosis: future implications. *Int Psychogeriatr*. 2024;36(1):24–27. doi:10.1017/S1041610222001181
47. Kiltschewskij DJ, Reay WR, Cairns MJ. Schizophrenia is associated with altered DNA methylation variance. *mol Psychiatry*. 2024. doi:10.1038/s41380-024-02749-5
48. Howard R, Cort E, Bradley R, et al. Amisulpride for very late-onset schizophrenia-like psychosis: the ATLAS three-arm RCT. *Health Technol Assess*. 2018;22(67):1–62. doi:10.3310/hta22670

49. Howard R, Cort E, Bradley R, et al. Antipsychotic treatment of very late-onset schizophrenia-like psychosis (ATLAS): a randomised, controlled, double-blind trial. *Lancet Psychiatry*. 2018;5(7):553–563. doi:10.1016/S2215-0366(18)30141-X
50. Miyaoka T, Wake R, Furuya M, et al. Yokukansan (TJ-54) for treatment of very-late-onset schizophrenia-like psychosis: an open-label study. *Phytomedicine*. 2013;20(7):654–658. doi:10.1016/j.phymed.2013.01.007
51. Satake Y, Kanemoto H, Taomoto D, et al. Characteristics of very late-onset schizophrenia-like psychosis classified with the biomarkers for Alzheimer's disease: a retrospective cross-sectional study. *Int Psychogeriatr*. 2024;36(1):64–77. doi:10.1017/S1041610222001132
52. Van Assche L, Van Aubel E, Van de Ven L, et al. The neuropsychological profile and phenomenology of late onset psychosis: a cross-sectional study on the differential diagnosis of very-late-onset schizophrenia-like psychosis, dementia with Lewy bodies and Alzheimer's type dementia with psychosis. *Arch Clin Neuropsychol*. 2019;34(2):183–199. doi:10.1093/arclin/acy034
53. Liu Y, Xiao X, Yang Y, et al. The risk of Alzheimer's disease and cognitive impairment characteristics in eight mental disorders: a UK Biobank observational study and Mendelian randomization analysis. *Alzheimers Dement*. 2024;20(7):4841–4853. doi:10.1002/alz.14049
54. Minteer C, Morselli M, Meer M, et al. Tick tock, tick tock: mouse culture and tissue aging captured by an epigenetic clock. *Aging Cell*. 2022;21(2):e13553. doi:10.1111/acel.13553
55. Ori APS, Olde loohuis LM, Guintivano J, et al. Meta-analysis of epigenetic aging in schizophrenia reveals multifaceted relationships with age, sex, illness duration, and polygenic risk. *Clin Clin Epigenet*. 2024;16(1):53. doi:10.1186/s13148-024-01660-8
56. Li Y. Modern epigenetics methods in biological research. *Methods*. 2021;187:104–113. doi:10.1016/j.ymeth.2020.06.022