Heliyon 10 (2024) e25191

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

System biology approaches to identify hub genes linked with ECM organization and inflammatory signaling pathways in schizophrenia pathogenesis

Piplu Bhuiyan [a,b,**], Zhaochu Sun [a,***], Md Arif Khan [b,c], Md Arju Hossain [d], Md Habibur Rahman [e], Yanning Qian [a,*]

[a] Department of Anesthesiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu, People's Republic of China
[b] Department of Biotechnology and Genetic Engineering, Faculty of Life Science, University of Development Alternative, Dhaka, 1209, Bangladesh
[c] Bio-Bio-1 Bioinformatics Research Foundation, Dhaka, Bangladesh
[d] Department of Microbiology, Primeasia University, Banani, Dhaka 1213, Bangladesh
[e] Department of Computer Science and Engineering, Faculty of Engineering and Technology, Islamic University, Kushtia-7003, Bangladesh

ARTICLE INFO

Keywords:
Schizophrenia (SZ)
Bioinformatics
System biology
Extracellular matrix organization (EMC)
Inflammatory signaling pathway

ABSTRACT

Schizophrenia (SZ) is a chronic and devastating mental illness that affects around 20 million individuals worldwide. Cognitive deficits and structural and functional changes of the brain, abnormalities of brain ECM components, chronic neuroinflammation, and devastating clinical manifestation during SZ are likely etiological factors shown by affected individuals. However, the pathophysiological events associated with multiple regulatory pathways involved in the brain of this complex disorder are still unclear. This study aimed to develop a pipeline based on bioinformatics and systems biology approaches for identifying potential therapeutic targets involving possible biological mechanisms from SZ patients and healthy volunteers. About 420 overlapping differentially expressed genes (DEGs) from three RNA-seq datasets were identified. Gene ontology (GO), and pathways analysis showed several biological mechanisms enriched by the commonly shared DEGs, including extracellular matrix organization (ECM) organization, collagen fibril organization, integrin signaling pathway, inflammation mediated by chemokines and cytokines signaling pathway, and GABA-B receptor II and IL4 mediated signaling. Besides, 15 hub genes (FN1, COL1A1, COL3A1, COL1A2, COL5A1, COL2A1, COL6A2, COL6A3, MMP2, THBS1, DCN, LUM, HLA-A, HLA-C, and FBN1) were discovered by comprehensive analysis, which was mainly involved in the ECM organization and inflammatory signaling pathway. Furthermore, the miRNA target of the hub genes was analyzed with the random-forest-based approach software miRTarBase. In addition, the transcriptional factors and protein kinases regulating overlapping DEGs in SZ, namely, SUZ12, EZH2, TRIM28, TP53, EGR1, CSNK2A1, GSK3B, CDK1, and MAPK14, were also identified. The results point to a new understanding that the hub genes (fibronectin 1, collagen, matrix metalloproteinase-2, and lumican) in the ECM organization and inflammatory signaling pathways may be involved in the SZ occurrence and pathogenesis.

* Corresponding author.
** Corresponding author. Department of Anesthesiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu, People's Republic of China.
*** Department of Anesthesiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu, People's Republic of China.
E-mail addresses: bhuiyanneuroscience@gmail.com (P. Bhuiyan), zhchsun@njmu.edu.cn (Z. Sun), yanningqian@njmu.edu.cn (Y. Qian).

https://doi.org/10.1016/j.heliyon.2024.e25191
Received 2 January 2023; Received in revised form 18 December 2023; Accepted 22 January 2024
Available online 26 January 2024

1. Introduction

Schizophrenia (SZ) affects around 2.5 % of the global population and is one of the top 10 causes of disability globally [1]. SZ is a neurodevelopmental condition that appears early but is compensated for until the average age of clinical onset in young adulthood [2,3]. The growing prevalence of SZ illness is a severe global problem for the scientific community to overcome. The scientific community has undertaken efforts in recent decades to develop effective therapeutics for the devastating pathophysiology of SZ. In genome-wide association and gene-expression profiling, investigations into the impacts of SZ pathogenesis observed that numerous genes connected to the immune system, cytoskeletal development, and neuroinflammation had been implicated [4,5]. However, the precise pathological mechanisms that promote SZ progression are often ambiguous and poorly understood. The influence of environmental, genetic, neurodevelopmental, immunological, and neuroinflammatory factors on the onset and course of SZ has led to several theories to explain the pathophysiological mechanisms underpinning SZ [6–8].

The neuroinflammatory process has been linked to SZ-associated genes that have a variety of functions in the brain [9]. Accumulating recent evidences reported that the cytotoxic consequences of chronic microglial activation might promote the progression of SZ and enhance secondary neuronal degeneration, reduced neurogenesis, and synaptic dysfunction [10–12]. A growing body of research suggests that microglia play a novel role in regulating the ECM remodeling during normal brain homeostasis, and these activities may, in turn, become dysfunctional in SZ [13,14]. The ECM and interstitial environment are essential regulators of neuroinflammation and the immune response, just as they are for glial cells-mediated scar formation [15]. Damage-associated molecular patterns (DAMPs) are structural components of the ECM and products of its degradation that stimulate or inhibit microglial reactivity by signaling through pattern recognition receptors (e.g., Toll-like receptors) [13,16,17]. For example, in vitro culturing of microglia on a CSPG substrate induces microglial activation, proliferation, and expression of IGF-1, MMP-2, and MMP-9, whereas pharmacological inhibition of CSPG production with xyloside after spinal cord injury alters neuroinflammation and cytokine production differently depending on treatment timing [18]. It has been reported that repeated social defeat caused an increase in biomarkers for EMC remodeling and blood-brain barrier (BBB) leakage, thus resulting in enhanced BBB permeability associated with microglial phagocytosis [19,20].

MMPs are a family of structurally similar proteolytic enzymes that remodeling of the ECM to keep synaptic functioning and BBB intact [21]. MMP-mediated ECM dysfunction plays a crucial role in SZ pathophysiology [22]. Dysregulation of MMPs by neuroinflammation causes ECM abnormalities, which change neuronal processes such as synaptic plasticity and BBB disruption directly or indirectly [22,23]. Growth factors, cytokines including TNF-α, and IL-1β, chemical agents, physical stress, and, most crucially, cell-to-cell or cell-to-ECM interaction are all known to trigger MMP genes [24]. This study suggested that TNF-α, and IL-1β induce transcription of MMP-3 and MMP-9, which may be involved in neuroinflammation. MMP-9 can also be activated by other proteases (MMP-3) and free radicals (nitric oxide, which works by *N*-nitrosylation) [25]. MMPs have a role in the degradation of CSPGs, a key component of ECM [26]. Decreased levels of CSPGs have been discovered in the brains of SZ patients [27]. Alterations complicate the pathophysiology of SZ in MMP expression caused by glial cells [22]. MMP hydrolyzes ECM, which is involved in neural control, neurotransmitter signaling, and synaptic plasticity [28]. In the schizophrenic brain, upregulation of MMPs and an imbalance between MMP and TIMP are linked to various ECM abnormalities [22]. MMP expression can be upregulated by oxidative stress and neuroinflammation, leading to tissue degradation, neuronal death, and ECM abnormalities in SZ [29,30].

This is why well-established bioinformatics methods and analytical approaches were employed to investigate the therapeutic targets as well as molecular signaling pathways that could play a critical role in the SZ pathogenesis. Firstly, we identified shared overlapping DEGs using RNA sequencing data from SZ patients and healthy controls. Secondly, GO and functional enrichment pathway analyses of shared overlapping genes has investigated to uncover the significant biological mechanisms and signaling pathways. Then, the PPI network was used to identify the hub genes which are impacting the pathophysiology of SZ patients. Moreover, this work was predicted transcriptional factors and PKs that may be involved in the SZ pathogenesis. In addition, the miRNA-gene regulatory network of the selected hub genes was predicted by miRNA databases. Finally, this study suggested that the hub genes (FN1, Collagen, MMP-2, and Lumican) dysregulation linked with EMC organization and inflammatory signaling pathways may be involved in the SZ occurrence and pathogenesis.

2. Materials and methods

2.1. Literature search and datasets collection

In order to collect the RNA sequencing and transcriptomes gene expression raw data samples, this work was used to search two public repositories GEO RNA-seq database including NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) [31] and GREIN [32]. The keyword "Schizophrenia" extracted three RNA-seq datasets with accession numbers GSE92874 (4 controls and 4 cases), GSE63738 (6 controls and 6 cases), and GSE121376 (28 controls and 28 cases). In order to acquire RNA-seq datasets, the criteria were used to follow inclusion: (1) Original experiments studies were conducted for screening RNA expression levels between control and schizophrenia, (2) the RNA-seq datasets were identified only for the organism "*Homo sapiens*" (3) RNA-seq raw data samples were deposited in the NCBI GEO for publicly accessible use, (4) high throughput sequencing Illumina 2500 and 6000 platforms for extracting RNA-seq, and (5) RNA-seq datasets submitted in the year range between 2007 and 2021. The search excluded datasets obtained from the healthy control and SZ patients according to earlier criteria.

2.2. RNA-seq data processing and identification of common DEGs

The GREIN (http://www.ilincs.org/apps/grein/)[32] web application was used to identify DEGs from three RNA-seq datasets. GREIN employed a negative binomial generalized linear model executed in edgeR to find DEGs between control and SZ patients. Furthermore, using Bioinformatics & Evolutionary Genomics Venn diagram web application tool like jVenn (https://jvenn.toulouse.inrae.fr/app/index.html) [33] was identified common significant DEGs from all three RNA-seq datasets (GSE92874, GSE63738, and GSE121376).

2.3. GO and signaling pathway enrichment analyses of common DEGs

GO and pathway enrichment studies were used to determine the biological characteristics of mutual DEGs in SZ. GO enrichment analysis was performed on FunRich bioinformatics software [34]. Different statistically significant results for BP, MF, and CC were identified with a p-value <0.01.

Then, to determine the molecular signaling pathways functional enrichment analysis, Fisher's exact test employed the combined DEGs using EnrichR (https://maayanlab.cloud/Enrichr/) [35] (A comprehensive gene enrichment analysis web-based tool). Then, using different datasets suggested putative signaling pathways and gene annotation ontologies related to common DEGs. Four databases were utilized in this computational approach to investigate biological signaling pathways of commonly shared DEGs in SZ, namely: Reactome 2016, BioPlanet 2019, Panther 2016, and NCI Nature 2016. We chose the top-listed pathways depending on the standard criteria of a *P*-value of <0.01.

2.4. Construction of protein-protein interaction network and screening of hub genes

To further determine the functional interactions between these common DEGs, the protein interaction network was mapped using experimentally validated interactome database STRING (https://string-db.org/) (Version 11.5) [36]. Studies were also limited to "*Homo sapiens*" with a high confidence level of >0.7 and we selected a medium confidence level 0.4. Then, using the Cytoscape software (https://cytoscape.org/) (Version 3.9.0) [37], import a tab-separated values (TSV) file to visualize a protein-protein interaction network, where the number of nodes denotes proteins, and the edges indicate their interactions. Based on degree methods with a high interconnected score, the CytoHubba plugin [38] of the Cytoscape program was used to identify the top 15 hub genes. Based on the length of the shortest paths, CytoHubba plugin offers 11 topological analysis methods, such as Degree, Maximum Neighbourhood Component, and Closeness etc. Higher degrees values are more likely to be essential proteins, and the degree of a protein correlates with the gene's essentiality [39]. Using Cytoscape software was also able to identify the hub genes interaction network.

2.5. Identification of a transcription factor linked to the protein network

Transcription factors (TFs) play an essential role in several biological pathways by acting in the extensive protein complexes network generated by a protein-protein interaction network that initiates and controls genetic code transcription. Based on the hypergeometric p-value, the X2K is a web-based algorithm bioinformatics tool (https://maayanlab.cloud/X2K/) [40] (regulatory networks platform) from the ChIP-seq experiments (ChEA) database (http://amp.pharm.mssm.edu/lib/chea.jsp) [41] to determine the top transcriptional factors. The Genes2Networks (G2N) algorithm (https://maayanlab.cloud/G2N/) [42] was used to identify transcriptional factors by identifying proteins that interact physically with these transcription factors. This tool aids scientists in filtering transcription factors with protein network complex connections to better understand the cell signaling cascade.

2.6. KEA determines the relationship between kinases, proteins, and transcription factors

PTKs are enzymes that turn on the cell of phosphorylated targeted proteins to control signaling proteins dynamically. The KEA module of X2K [40] has been used to identify protein kinase. KEA [43] is a command-line program that can match shared DEGs lists of mammalian proteins to the PTKs expected to phosphorylate them. The upstream signaling network depended on a regulatory kinase-substrate interaction within the extended subnetwork incorporating protein kinases, protein-protein interactions, and transcription factors with phosphorylation using the HPRD (https://www.hprd.org/), PhosphoSite (https://www.phosphosite.org/homeAction.action), phospho. ELM (http://phospho.elm.eu.org/), NetworKIN (https://networkin.info/), and Kinexus (https://www.kinexus.ca/) databases.

2.7. Analysis of microRNA-hub genes regulatory network

The microRNA-hub gene interaction network identified the regulatory molecules which could be implicated in SZ pathogenesis. Based on miRTarBase database (https://mirtarbase.cuhk.edu.cn/) plugging [44], the miRNA-hub gene regulatory networks were revealed using the NetworkAnalyst (https://www.networkanalyst.ca/) [45] website, and then Cytoscape constructed the miRNA-hub genes network. Then, the determination of miRNA-hub genes enrichment analysis depended on the adjusted p-value of 0.05 and interaction threshold 3 using MIENTURNET (http://userver.bio.uniroma1.it/apps/mienturnet/) [46] interactive web tool. Further, the miRTarBase database determined the predicted miRNA targets with set an inclusion threshold criteria minimum ≥ 5 interactions and an adjusted p-value of 0.05. In order to ensure that the reported interactions are statistically significant, a minimum number of interactions (≥ 5) is necessary. Setting a minimal threshold can aid in filtering out false positives because a limited number of

interactions might be more susceptible to random chance [44].

2.8. Identification of potential biological signaling pathways

To identify the potential biological pathway connected with common DEGs, the FunRich (version 3.1.4) interactive bioinformatics tool [34] (http://www.funrich.org) was employed against the human genomic and proteomic database as integrated with the FunRich background.

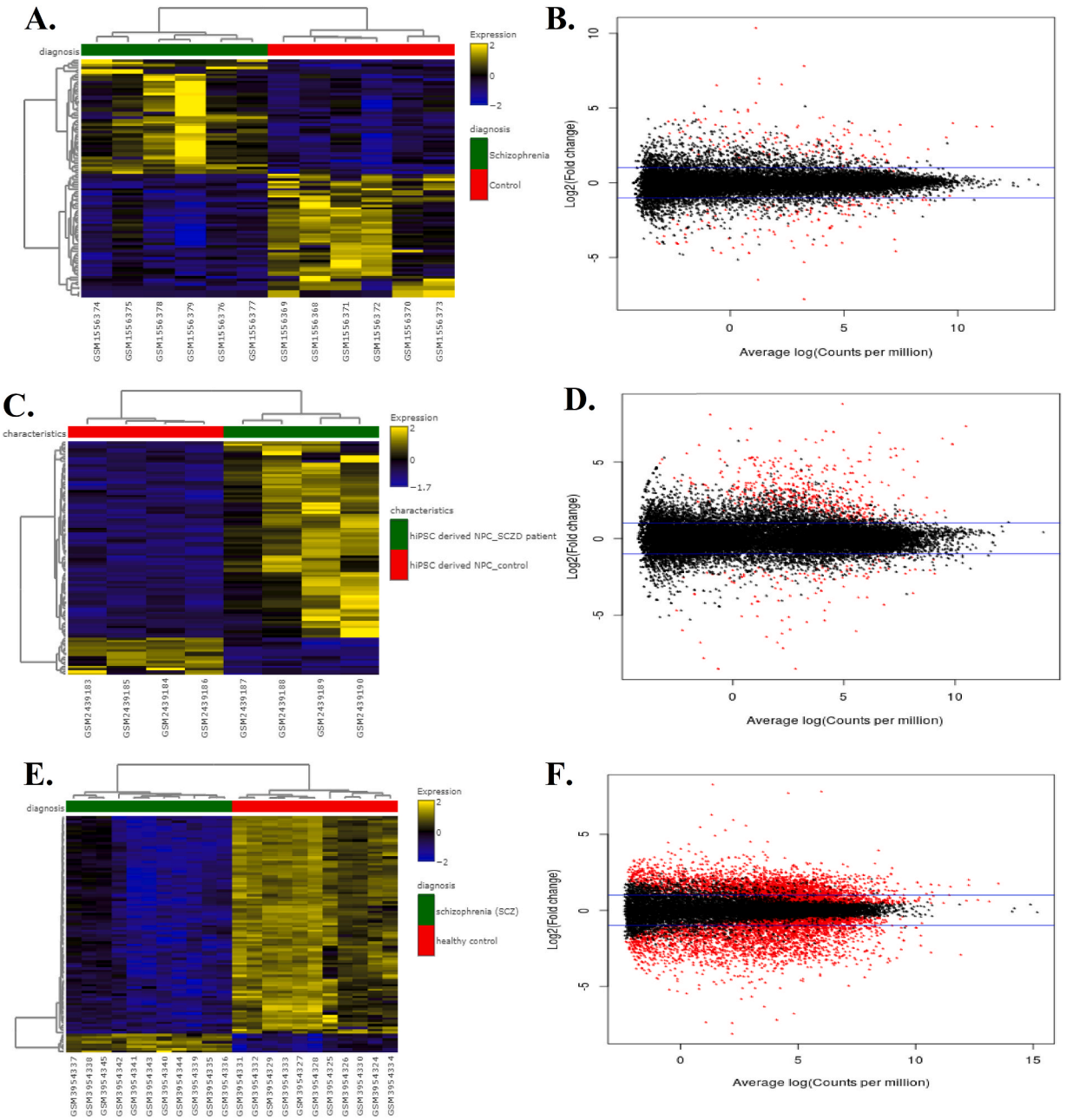

Fig. 1. Identification of common shared overlapping DEGs between SZ and healthy among three RNA-seq datasets. A, C, E are Interactive Heat map of the top 100 DEGs among three GEO datasets. B, D, F are MA plots of DEGs in healthy and SZ in the GSE63738, GSE92874, and GSE121376 datasets.

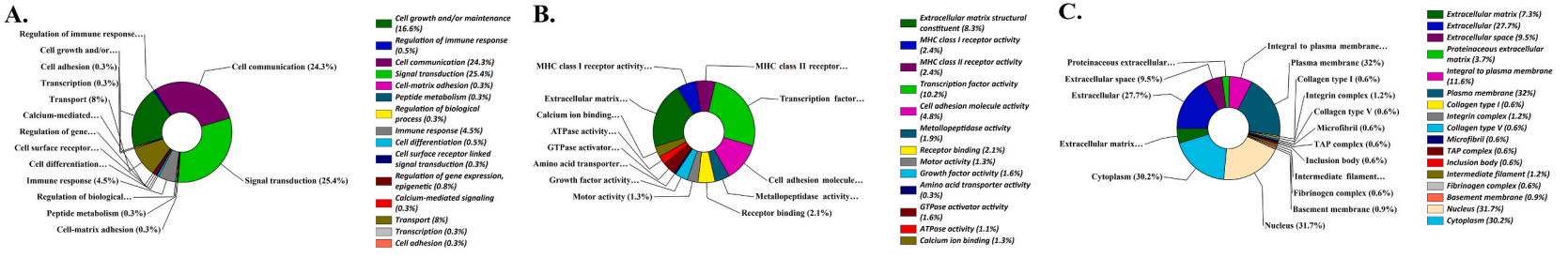

Fig. 2. GO enrichment analysis of commonly found DEGs in SZ. A) Biological process, B) Molecular function, (C) Cellular component.

2.9. Utilizing hub genes to infer the network biology of EMC architecture

Network biology approaches identified scored-based hub genes and EMC organization-associated interaction through data integration and quality control with several-fold more interactions (>500,000) network biology approach. The InWeb_InBioMap databases (https://www.lagelab.org/resources/) [47] was utilized with a relevance score cut-off of 0.85 to design hub genes associated with EMC organization and integrin cell surface interactions network pathway. The InWeb_InBioMap database has a cut-off value of 0.85 meaning that interactions with a relevance score of 0.85 or above are significant and are probably high-confidence protein-protein interactions. A relevance score of 0.85 is employed in this instance as a threshold to eliminate weaker (<0.45) or less dependable relationships and focused on those with higher (>1) indications of biological significance [48]. Finally, hub genes are also screened by sharing EMC organization, and integrin cell surface interactions networks link using these same databases.

2.10. Design EMC-Receptor interaction complex pathway

ShinyGO v0.741 (http://bioinformatics.sdstate.edu/go74/) [49] was used to create a diagram of the EMC-receptor interaction based on KEGG pathway databases. A threshold of *P*-value of 0.05 and the organism *Homo sapiens* were used to evaluate the significance level of KEGG pathway enrichment for drawing this diagram.

3. Results

3.1. Identification of overlapping common DEGs

This study collected the gene expression profiles of three RNA-seq datasets (GSE92874, GSE63738, and GSE121376) from the NCBI GEO database to identify and investigate differentially expressed gene expression patterns that may impact SZ development. Firstly, the DEGs were obtained by analyzing three RNA-seq datasets using the GREIN interactive web platform. A total of 3462 (2431 upregulated and 1031 downregulated genes), 1772 (990 upregulated and 782 downregulated genes), and 5643 (2887 upregulated and 2756 downregulated genes) deregulated DEGs were identified from the GSE92874, GSE63738, and GSE121376 respectively based on the criteria of p-value <0.05 and log2FC ≥ 1 or ≤ -1. Fig. 1A, C, E illustrates an interactive heatmap of the top 100 upregulated and downregulated genes. Fig. 1 (B, D, F) shows all the DEGs with a log2FC versus the – log10 (p-value) between the SZ patients and healthy groups in a MA plot, and positive Log2FC represents upregulated genes, whereas negative Log2FC indicates downregulated genes. Four hundred twenty overlapping DEGs were identified using Venn diagrams among, GSE92874, GSE63738, and GSE121376 datasets, including 290 upregulated genes and 130 downregulated genes (Fig. S1). Then, using bioinformatics and machine learning tools were employed to analyze the functional annotation of genes, enrichment analysis of interaction networks, and molecular and biological pathways using those commonly deregulated DEGs.

3.2. Gene ontological analysis of overlapping DEGs

The GO is a comprehensive conceptual model for describing the functional characteristics of DEGs. GO analyzed the functional annotation of overlapping DEGs into three functional categories: BP, MF, and CC. In the aspect of BP, the overlapping DEGs were enriched in cell communication, and signal transduction etc. (Fig. 2A). In terms of MF (Fig. 2B), the commonly shared DEGs were mainly involved in EMC structural constitute, and MHC receptor class I receptor activity etc. For the CC category, overlapping expressed genes were significantly enriched in extracellular, and EMC pathways etc. (Fig. 2C). These findings suggest that EMC-related processes are essential in the SZ development mechanisms.

3.3. Identification of significant signaling pathway enrichment analysis

Using web-based bioinformatics tools like EnrichR, including four pathways databases such as Reactome, Panther, BioPlanets, and NCI nature, performed gene set enrichment analysis of overlapping DEGs to find critical signaling pathways that may relate to SZ pathogenesis. The top 15 signaling pathways were chosen based on the significance of the adjusted p-value of less than 0.01 as a criterion for pathway analysis. Critical biological pathways were identified which may be implicated in SZ using the Reactome 2016 pathway database, including EMC organization, and collagen biosynthesis and modifying enzymes etc. (Fig. S2A). Panther 2016 pathway revealed integrin signaling pathway, and ionotropic glutamate receptor pathway etc. (Fig. S2B). Results from the BioPlanet 2019 pathway database identified the most significant biological function associated with signaling pathways, including beta-1 integrin cell surface interactions, TGF-beta regulation of EMC, and EMC organization etc. (Fig. S2C). The pathway analysis of the NCI 2016 nature database revealed beta1 integrin cell surface interactions, and syndecan-1-mediated signaling events etc. (Fig. S2D). We showed pathway enrichment analysis, gene name, adjusted p-value, and combined score (Supplementary Tables 1A, 1B, 1C, 1D). These pathway enrichment analysis findings suggest that EMC and integrin cell surface contacts may have played a role in the progression of SZ.

3.4. Protein-protein interaction network and hub protein identification

The PPIs network of DEGs was generated using the STRING database (version 11.5), with combined scores more significant than 0.7

(interaction score: high confidence), and visualized using Cytoscape software, with 183 nodes and 384 edges (Fig. S3A). When we selected a medium confidence level 0.4 as an interaction score, we found 280 nodes and 687 edges. Besides, by setting up highest confidence level 0.9 and low confidence level 0.150 as an interaction score, we only found 40 nodes and 123 edges and 389 nodes and 1000 edges, respectively. Finally, in our study, we selected a medium confidence level 0.4 as exact value to test network with reported interaction score in different confidence level. Furthermore, the PPIs network is employed for hub protein discovery, which may aid in identifying a therapeutic biomarker for the disease comorbidities.

To analyze hub protein across the PPIs network, MNC, degree, closeness and MCC approaches employed the Cytoscape plugin CytoHubba, which generated the Hub proteins network. The PPI networks selected the top 15 hub proteins using the techniques above. The therapeutic targets were determined SZ pathogenesis and progression by identifying the top 15 hub proteins (FN1, COL1A1, COL3A1, COL1A2, COL5A1, COL2A1, COL6A2, COL6A3, MMP2, THBS1, DCN, LUM, HLA-A, HLA-C, and FBN1), which have significantly higher degree interactions (Fig. 3). The found hub proteins are potential biomarkers that might lead to new SZ therapeutic targets and play critical roles in EMC construction during SZ development.

3.5. Analysis of transcription factors

Transcription factor enrichment analysis (TFEA) was performed based on the hypergeometric p-value and selected the top 20 TF candidates (Fig. 4A). SUZ12, EZH2, IRF8, TP63, TRIM28, TP53, FOSL2, and EGR1 etc. The Genes2Networks (G2N) method is employed to find proteins that physically interact with PPIs and TFs to investigate their relationships. Based on the degree of the nodes, the regulatory network of linked TFs and their functionally and physically interacting proteins was depicted (Fig. 4B).

3.6. Identification of protein kinase with upstream regulatory network

Using the KEA module of X2K, the most critical PTKs linked with SZ were identified to examine possible neurotherapeutic kinase targets. The results of KEA revealed that CSNK2A1, GSK3B, CDK1, MAPK14, and ATM, etc., are the most critical PTKs in intracellular signaling pathways related to SZ (Fig. 5A). Finally, several human protein reference databases constructed a regulatory kinase-substrate network in which active PTKs phosphorylate substrates inside the enlarged subnetwork of transcription factors and intermediary proteins (Fig. 5B).

3.7. Prediction of miRNAs targets with hub genes

Using network analyst, the miRNA-hub gene regulatory network associated with the development of SZ was constructed. According to the miRNA-hub gene-targeted network, the network included hsa-miR-29c-3p, hsa-miR-29b-3p, hsa-miR-29a-3p, and hsa-miR-143-3p, etc. which had targeted relationships with the hub genes (Fig. 6A). Finally, using mirTarBase employed to identify the miRNA-target enrichment analysis result based on significant adjusted p-value and target hub genes interaction (Fig. 6B).

The FunRich software examined possible biological pathways related to SZ (Fig. 7). These findings revealed that SZ's major potential biological pathways had been compromised of integrin family cell surface interactions, VEGFR3 signaling in lymphatic

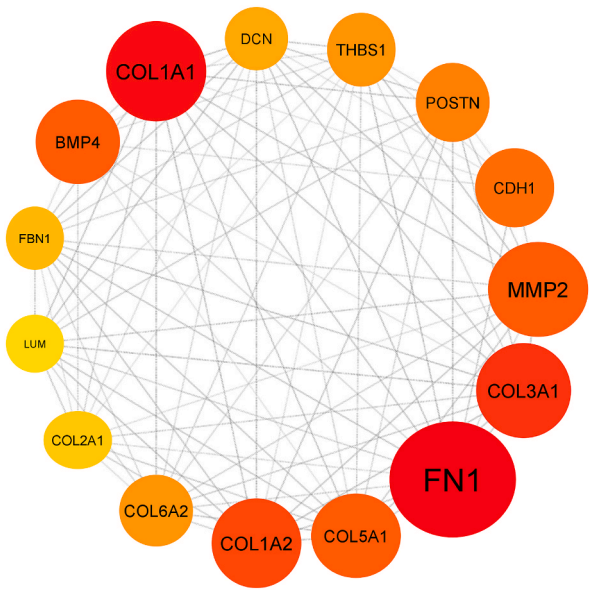

Fig. 3. Construction of protein-protein interactions network and hub gene identification using Cytohubba plugin. Interactomics analysis of Hub genes interaction networks. Larger to smaller circles represent higher to lower degrees of ranking.

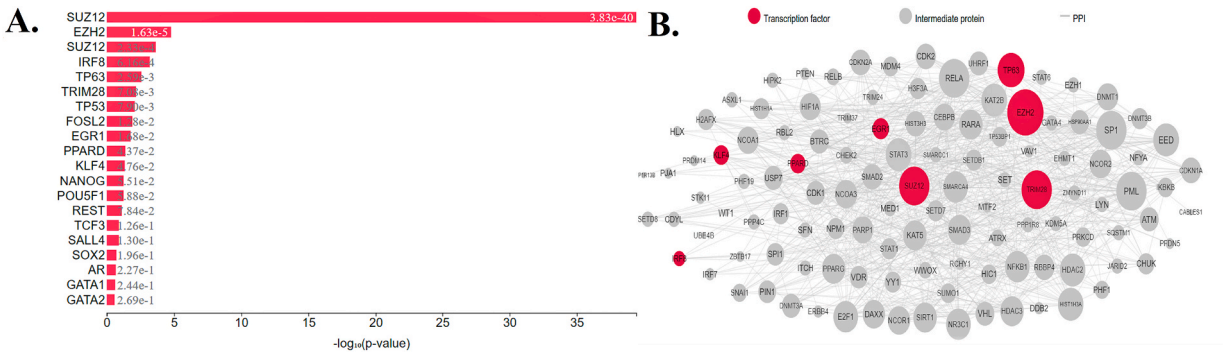

Fig. 4. Transcriptional factors analysis of common DEGs. A) Identification of top 20 transcription factors based on hypergeometric p-value, B) Interaction between transcription factors with known protein-protein network by using the G2N method. TFs were represented by red nodes, whereas grey nodes represented proteins. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

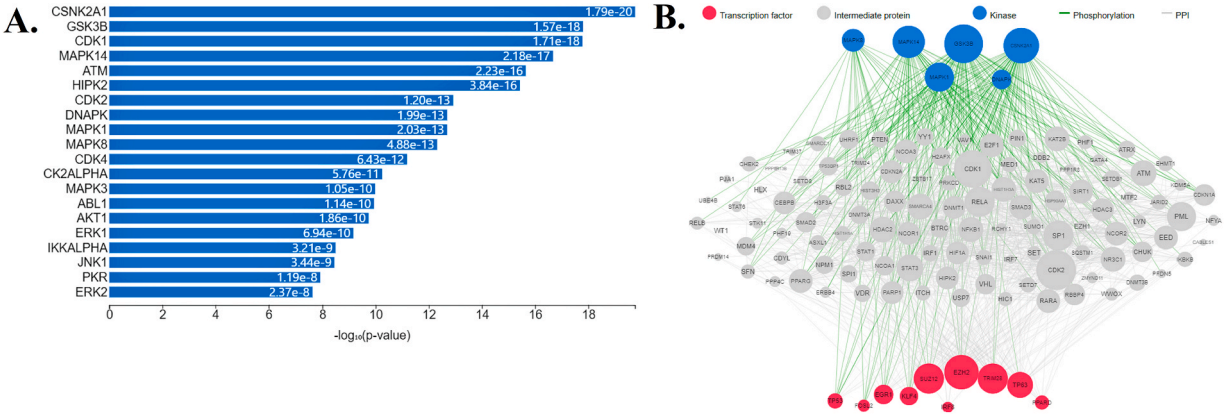

Fig. 5. Kinase enrichment analysis of common DEGS with transcription factors and protein-protein interaction network. A) Recognition of top 20 transcription factors from commonly DEGs in schizophrenia. B) Upstream pathway involvement transcription factors to kinases through known protein-protein interactions of commonly DEGs. Blue nodes represent protein kinase, grey nodes illustrate intermediate protein, and red nodes describe transcription factors. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

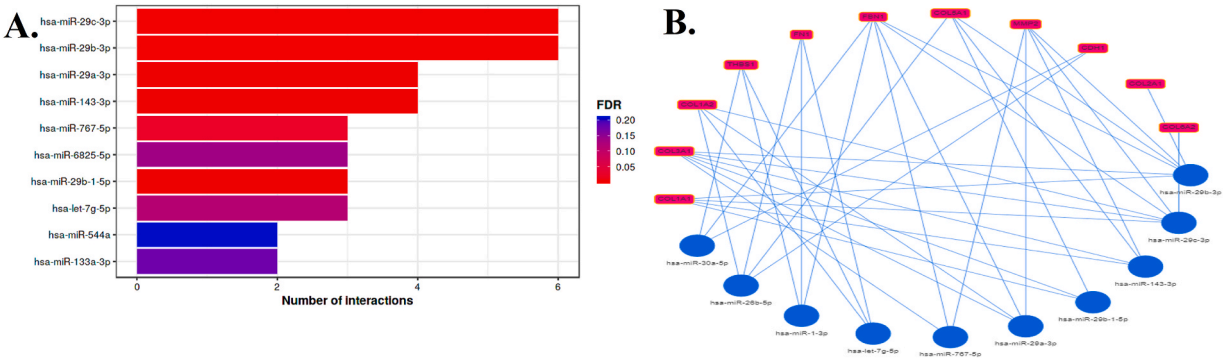

Fig. 6. Hub Genes and miRNA targets interaction network using Network Analyst. A) miRNA-target Enrichment analysis result using MitarBase, B) Network of miRNA and hub genes specific targets by miTarbase. Violate color represents hub genes and blue circles describe miRNA. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

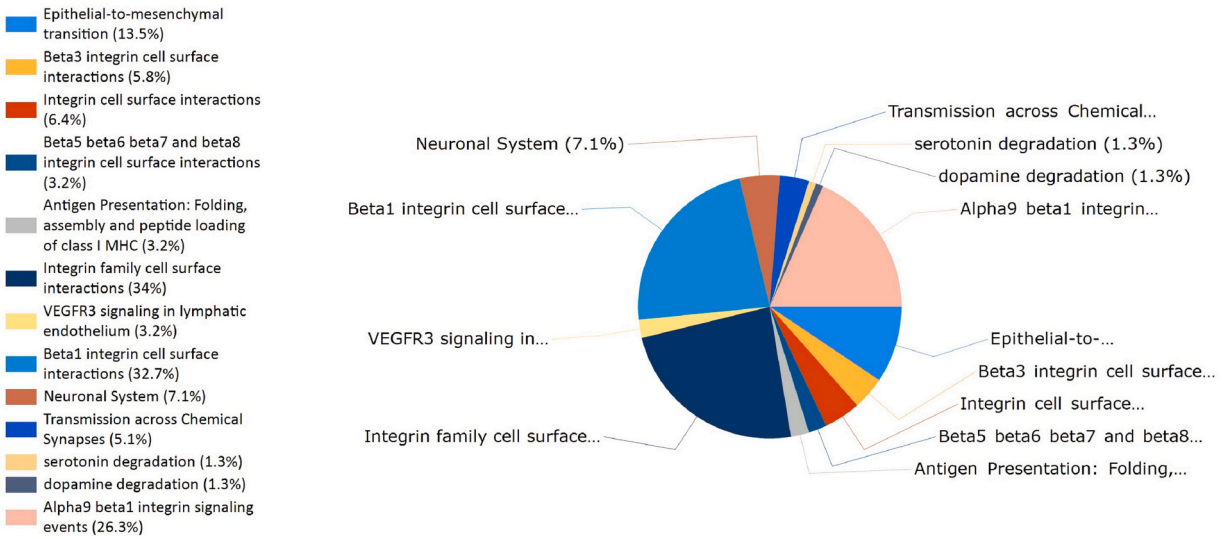

Fig. 7. Identification of potential biological pathways associated with SZ pathogenesis. 420 DEGs overlapping genes shared multiple biological mechanisms may be involved in the disease pathology of SZ.

endothelium, dopamine, and serotonin degradation pathway, and transmission across chemical synapses etc. According to the biological pathways, analysis results might be helpful for future studies to investigate the involvement the SZ pathogenesis.

3.8. Interconnection of hub genes associated with EMC organization and integrin cell surface interactions mechanism

Connectome analysis observed a network of signaling molecules, receptors, plasma membrane-bound transcription factors, kinases, enzymes, and structural proteins interconnected with hub genes to determine the potential biological signal of EMC organization and integrin cell surface interactions network pathway. Fig. 8A shows that FBN1-"governs as a signaling molecule" interacts with MMP-2/13/8/12, regulates structural protein networks, and maintains plasma membrane stability. Similarly, MIA, itself also a signaling molecule, is tightly bound with EMCs proteins such as FN1, THBS1, COL5A1, COL3A1, COL1A2, COL2A1, COL2A1, COL5A1, and COL6A3, thus may be modulated integrin activity. Then, the hub genes association with shared EMC organization and integrin cell surface interactions network pathway was determined. Our hub genes identification result described that EMC organization shared their interaction with integrin cell surface molecules and their signaling pathways (Fig. 8B). However, it needs to be confirmed and experimentally validated this computational analysis results. Finally, hub genes used overlapping DEGs of SZ to create a schematic diagram of the EMC-receptor interaction complex pathway based on the KEGG database. In Fig. 8C, we tried to identify how EMC-associated genes interact with integrin receptor and proteoglycan molecules based on the KEGG pathway analysis by

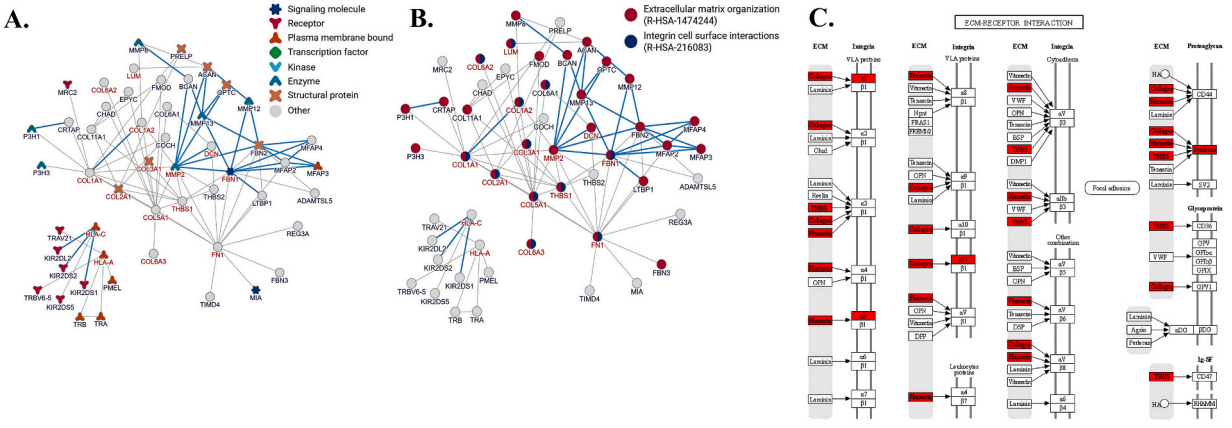

Fig. 8. Hub genes association with extracellular matrix organization and integrin cell surface interactions. A) Hub genes interaction related to ECM-integrin signaling pathway with signaling molecules, receptors and structural proteins, B) ECM-integrin signaling pathway with sharing hub gene interactions, C) EMC-Receptor interaction complex pathway using KEGG database. Red color represent common DEGs present in SZ. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

bioinformatics method. We observed that fibronectin, collagen, and THBS interact with integrin dimer (α1, α2, α3, α8, αV, αIIb, β1, and β3), thus resulting in maintaining their cell adhesion and signal strength.

3.9. Identified targets validation with literature review

According to GO and functional enrichment analysis, the commonly shared overlapping dysregulated genes were mainly implicated in EMC organization, signal transduction, and ECM-receptor interaction process. These suggested results might be crucial in the progression of SZ. ECM molecules govern GABAergic function, and neuronal migration etc. [50]. Previously, it has been reported that altered ECM organization has been associated with the pathophysiology of SZ and neurodegenerative diseases [51,52]. A small peptide generated from a collagen protein may protect the brain from SZ by stimulating the formation of neural connections [53]. These studies suggested that abnormalities of collagen fibril organization may be linked to the pathogenesis of SZ. Evidence reported that signal transduction anomalies caused by changes in the kinase activity network are the foundation of SZ fundamental symptoms [54]. The results of functional enrichment analysis were consistent with earlier research. Pathway enrichment analysis was used to identify the top 15 pathways associated with the pathogenesis of SZ. Previously, it has been reported that SZ patient-derived cells are sensitive to ECM proteins that bind integrin receptors, thus resulting in increases in focal adhesion number and size in response to ECM proteins and alterations in cell shape and cytoskeleton [55]. Cadherins have a critical role in developing brain circuitry and mature synaptic function [56], hence genes encoding members of the cadherin superfamily play a vital role in the pathophysiology of neuropsychiatric illnesses. In the mammalian brain, the cadherin signaling system regulates adhesion molecules that are important in cell-cell contact [57]. The TGF-beta signaling pathway is crucial for the use-dependent control of GABAA synaptic transmission and dendritic homeostasis; moreover, a disruption in the excitatory-inhibitory balance in the hippocampal network may induce psychiatric-like behavior [58]. In human mental illnesses like SZ, the TGF-β signaling pathway components have been altered in the hippocampus [58]. Syndecan signaling from the ECM to various cytoplasmic components and their distribution to different membrane compartments rely on oligomerization; hence, the syndecan homodimer is a crucial functional unit [59]. However, future research needs to investigate the bidirectional link between Syndecan signaling and ECM organization in the pathophysiology of SZ. Mounting studies have reported that inflammatory signaling pathways have a significant role in depression and SZ, and chronic inflammation is linked to immunological dysregulation in SZ [60–62].

FN1 mutations may increase SZ susceptibility, suggesting that this gene is an SZ-susceptible gene [63]. Moreover, in patients with SZ, FN1 gene expression levels involved complement pathway activators (C1qA) and mediators (C3 or C4) enhanced in the midbrain parenchyma near dopamine cell bodies, especially in individuals with a high inflammatory biotype [64]. This study also discovered that peripheral macrophages were significantly increased in the midbrain of SZ cases related to high inflammatory potential. However, it remains unclear how FN1 triggered inflammatory signaling pathways in the progression of SZ pathogenesis. Evidence reported that the collagen chain gene (COL1A1) expression might be associated with the EMC ligands, thus resulting in the inactivation of DDR1 with a time-dependent decrease in the SZ patients [65]. In addition, evidence reported that COL1A1 and COL1A2 genes expression levels were significantly decreased in patients with SZ compared to control [66]. One of the most increased genes, COL3A1, is detected, which encodes the pro-1 chains of type III collagen and exhibits a de novo mutation in SZ patients [67]. COL5A1 may be associated with genetic variations of psychotic experiences in SZ [68]. In distinct brain areas, the expression patterns of collagen family genes (COL1A1, COL3A1, COL1A2, COL5A1, COL2A1, COL6A2, COL6A3) encoding proteins that are fundamental components of ECM [69]. Chronic agonistic interactions may cause abnormal collagen genes expression, which might indicate specific ECM abnormalities in the brain areas of mice with different social experiences [70]. MMP-2 levels were significantly increased in the CSF of SZ patients, thus may be connected to neuro-inflammation in the brain [71]. This study postulated that the pathophysiology of SZ seems to be influenced by state-dependent changes in MMP-2 and activation of MMP-2, -7, and -10 cascades. A clinical study reported that THBS1 gene might have a critical role in the development of SZ patients [72]. The involvement of the THBS1 gene in SZ pathogenesis will need to be investigated further in the future.

This investigation revealed that transcriptional factors (including SUZ12, EZH2, TRIM28, TP53, and EGR1) are associated with the disease progression and development of SZ pathogenesis. SUZ12 binds a location 1.5 kb downstream of the GAD1 TSS, and the H3K27me3 mark is enhanced at the GAD1 promoter region in the prefrontal cortex of individuals with SZ, along with a significant number of the discovered DMRs within the GAD1 regulatory network [73,74]. EZH2 may play a role in SZ risk at two different times: during development, when it causes neurodevelopmental abnormalities, and during adulthood, when it causes abnormal expression reactivation [75]. TRIM28 suppresses ERVs in the gene regulatory network that governs gene expression of protein-coding transcripts essential for brain development [76]. An essential link between TP53 and SZ was discovered by haplotype analysis [77]. These findings suggest that TP53 may have a role in SZ etiology. Consequently, transcriptional factors might be useful to develop potential therapeutic targets for SZ. Identification of PKs such as CSNK2A1, GSK3B, CDK1, and MAPK14 were reported to have a critical role in the pathogenesis of SZ [78,79].

4. Discussion

This work integrated three RNA sequencing data from SZ patients using bioinformatics and systems biology techniques to uncover possible therapeutic targets for SZ prevention. Firstly, this study identified 420 DEGs with statistically significant overlap in SZ, with 290 upregulated and 130 downregulated genes. The multi-step integrative bioinformatics methods used to look at the functional annotation of commonly shared DEGs, GO enrichment pathway analysis, interactomics analysis, upstream regulatory molecules, kinase enrichment analysis, miRNA-hub genes regulation network, biological signaling pathways analysis, as well as possible potential therapeutic targets.

Based on the pathway enrichment analysis results, overlapping genes were enriched in ECM remodeling, inflammation mediated by chemokines and cytokines signaling pathway, and GABA-B receptor II and IL4 mediated signaling. Numerous studies have reported that dysfunction of ECM remodeling contributes to pathological features of SZ, such as disruption of synapses connectivity, change of neuronal migration and axonal guidance, and abnormalities of neurotransmission, including GABAergic, glutamatergic, dopaminergic systems as well as neuroinflammatory process [80–82]. Given that many cognitive symptoms of SZ are thought to be linked to a mismatch between inhibitory GABA and excitatory glutamate neurotransmission in the dorsolateral prefrontal cortex. SZ cognitive deficits may be partly caused by MCHTAP expression or function dysregulation that disrupts GABAergic/glutamatergic balance [83–85]. ECM proteins are involved in neuronal cell migration, axonal growth, myelination, and the formation and maintenance of the neuromuscular junction at the BBB [86]. The ECM-integrin signaling pathway may regulate cell adhesion and signaling, endosomal trafficking, CSK dynamics, and gene expression at all stages of nervous system development, maintenance, degeneration, and regeneration [87,88]. Alterations of the GABAergic system have been implicated in the pathology of SZ [89]. In SZ, the GluRl and GluR2 subunits of kainic acid/amino-3-hydroxy5-methyl-4-isoxazole propionic acid (KA/AMPA) glutamate receptors are attenuated in the hippocampus [90]. Due to the loss of GABAergic hippocampal interneurons, $GABA_A$ receptors are increased, and GABA-uptake sites are diminished [91,92]. Furthermore, serotonergic 5-HT1A and 5-HT2 receptors are upregulated in the hippocampus in SZ, although 5-HT uptake sites remain constant [93]. Reduced GABA release and uptake at synaptic terminals, decreased expression of the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD), altered expression of GABAA receptors, and a reduction in axon cartridges of GABAergic chandelier neurons have all been reported as anomalies of GABAergic interneurons [94]. Multiple GABA-related proteins, including GAD65, SYNPR, DBI, GAT3, SN1, and CPT1A, were dramatically downregulated in adolescence [95]. To maintain normal GABA homeostasis, these molecules participate in the GABA cycle, which includes GABA synthesis, release, re-uptake, and replenishment. Dysregulation of the GABAergic system is mainly affected in the prefrontal cortex, thalamus, and hippocampus during childhood and adolescence [96].

Though complicated biological mechanisms but linked, hypotheses for SZ pathology include GABAergic system and EMC remodeling hypotheses are being proposed here. Abnormalities in the ECM would affect GABAergic synaptic transmission, which is essential in the pathophysiology of neuropsychiatric disorders, including SZ [97]. It has been reported that the enhanced and persistent GABA activity that balances the reduced density of parvalbumin neurons might be connected to changes in the ECM mediated by gene-environment interactions [98,99]. However, it remains unexplored how ECM-associated proteins, including FN1, Collagen, MMP-2, and Lumican, consequently affect GABAergic system function during childhood. Is an altered epigenetic regulation of gene expression the molecular mechanism mediating transcription controlling pathways, thus resulting in a significant impact on the downstream pathways of ECM remodeling and GABAergic system in SZ?

The bidirectional communication between the major histocompatibility complex (MHC) and the GABAergic system is hugely complicated during brain development and maintaining synaptic plasticity. Neurite outgrowth, synaptic transmission, the development and maintenance of cortical connections, activity-dependent refinement in the visual system, and long-term and homeostatic plasticity are all regulated by MHC I molecules [100]. The balance of excitation to inhibition (E/I balance) on cortical neurons is controlled by MHCI, which affects glutamatergic vs. GABAergic synapses differently [101]. Because of the MHC I molecule's capacity to influence glutamatergic and GABAergic synapse density in both directions, it is in a prime position to contribute to alterations in neural circuitry throughout brain development that might explain prodromal symptoms and enhance susceptibility to the second hit in SZ [102]. It has been demonstrated that MHC I regulates synapse density in the developing brain in an age-dependent manner [103,104]. MHC I levels are affected by genetic variations, gene expression alterations, or immunological dysregulation, and as in SZ in humans, synapse density should also be affected [100,105]. Further studies need to be clarified how MHC and GABAergic systems interact after a chronic immune-dysregulated state in offspring, thus causing SZ.

In our study, MMP-2 identified as hub gene based on bioinformatics analysis which may be play a critical role in the pathogenesis of SZ. MMPs are zinc-dependent endopeptidases that play a key role in BBB integrity and brain disorders [106]. Recent studies have reported that MMP-9 is linked to the language and fluency aspects of cognition, and it raises the likelihood of cognitive impairment in SZ [107]. MMP-9, an extracellular network protease involved in glutamatergic signaling pathway, may have a role in SZ pathogenesis [108]. MMP-9 is involved in the maturation of inhibitory neurons that contain the calcium-binding protein parvalbumin, the embryonic production of the specialized extracellular matrix structure perineuronal net, synaptic pruning, and myelination, all of which are considered to be impaired in SZ [109]. On the flip side, MMP-9 has a role in the brain development as well as ability to control synaptogenesis, axonal pathfinding and myelination [110]. Clinical study demonstrated that upregulated MMP-9 expression levels are directly linked with cognitive impairment in SZ patients [111]. It has been reported that over-activation of the MMP9/RAGE pathway causes redox dysregulation and neuro-inflammation, which leads to an inhibitory/excitatory imbalance in SZ pathophysiology [112]. An increase levels of MMP-2 and MMP-9 have been observed in the pathophysiology of neuropsychiatric diseases [113]. The extracellular matrix is degraded by MMP proteins, which govern vascular remodeling [114]. Extracellular components such as collagen, gelatin, and laminin are cleaved by MMP-1 [115]. MMP-1 can also activate other MMPs, including as MMP2 and MMP9, which together can lower the expression of tight junction proteins such TJP1, OCLN, and CLDN5 [116]. MMP-2/-9 is capable of degrading tight junction proteins and basement membranes, two BBB components, directly, resulting in enhanced BBB permeability [117]. Microglial activation has been shown to degrade BBB by releasing MMP-2/-9. α–synuclein increased MMP-9 expression and gene transcripts in primary microglia from rats in a dose-dependent manner [118]. The link between MMP-2 and MMP-9 might also be assumed to have a potential biological mechanism of SZ pathogenesis. Therefore, we can postulate that MMP-2 and MMP-9 may have therapeutic importance in improvement of SZ disease progression and pathology.

However, there are some limitations and drawbacks of this present study. We utilized several bioinformatics methods, network biology approaches and free online tools for functional analyses. Firstly, the bioinformatics methods and network biology approaches

have certain limitations, such as the fact that it only considers the number of genes or DEGs value. In addition, an artificial threshold was required to get the genes of interest or differential expression. Furthermore, it often employs the most significant genes while ignoring others with no significant alterations, which may result in the loss of genes with lower significance but a more critical function, resulting in lowered detection sensitivity. Secondly, RNA-seq data were received from the GEO database, not conducted by the authors. Thirdly, only a small number of samples were used in our work to generate the transcriptome data, whereas a larger number of samples might yield a significant number of concordant genes. To find a suitable balance between finding statistically significant genes and limiting false positives rates, most convenient statistical method was not used. For pathway analysis, in the EnrichR online tool, we have not found the count number of gene and logFC value of each pathway. Finally, the predicted results should be validated by experimental data.

5. Conclusion

The main objective of this study is to investigate potential therapeutic targets in the development of SZ pathogenesis using bioinformatics and network biology approaches. The pathway enrichment analysis provided most significant ECM organization pathways. The crucial hub genes were identified that may be therapeutically targeted for SZ pathogenesis, including fibronectin 1, collagen, matrix metalloproteinase-2, and lumican. Protein kinases, including CSNK2A1, GSK3B, CDK1, and MAPK14, as well as transcriptional factors such as TP53, EGR1, SUZ12, and TP63, play a significant role in the development of SZ in intracellular pathways.

Funding

This study was supported by the grants from the National Natural Science Foundation of China (No 81671387).

Availability of data and materials

The Gene Expression Omnibus (GEO) database from NCBI (https://www.ncbi.nlm.nih.gov/geo/) was used to access the GSE92874, GSE63738, and GSE121376 datasets.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Piplu Bhuiyan: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Formal analysis, Data curation, Conceptualization. **Zhaochu Sun:** Visualization, Validation, Software, Methodology, Formal analysis. **Md Arif Khan:** Writing – review & editing, Software, Methodology. **Md Arju Hossain:** Writing – review & editing, Visualization, Validation, Software, Resources. **Md Habibur Rahman:** Visualization, Validation, Software, Formal analysis. **Yanning Qian:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Piplu Bhuiyan reports financial support, administrative support, and statistical analysis were provided by University of Development Alternative. Yanning Qian reports financial support, administrative support, and statistical analysis were provided by The First Affiliated Hospital With Nanjing Medical University. Piplu Bhuiyan reports a relationship with University of Development Alternative that includes: non-financial support. The authors declare that there is no conflict of interest regarding this study. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25191.

References

[1] W.W. Fleischhacker, C. Arango, P. Arteel, T.R. Barnes, W. Carpenter, K. Duckworth, et al., Schizophrenia-time to commit to policy change, Schizophr. Bull. 40 (Suppl_3) (2014) S165–S194.
[2] M.J. Owen, M.C. O'Donovan, Schizophrenia and the neurodevelopmental continuum: evidence from genomics, World Psychiatr. 16 (3) (2017) 227–235.
[3] M.J. Owen, M.C. O'Donovan, A. Thapar, N. Craddock, Neurodevelopmental hypothesis of schizophrenia, Br. J. Psychiatry 198 (3) (2011) 173–175.

[4] R. Radhakrishnan, M. Kaser, S. Guloksuz, The link between the immune system, environment, and psychosis, Schizophr. Bull. 43 (4) (2017) 693–697.
[5] M.R. Bennett, Schizophrenia: susceptibility genes, dendritic-spine pathology and gray matter loss, Prog. Neurobiol. 95 (3) (2011) 275–300.
[6] M. Reale, E. Costantini, N.H. Greig, Cytokine imbalance in schizophrenia. From research to clinic: potential implications for treatment, Front. Psychiatr. 12 (2021) 536257.
[7] J. Vilain J, A.M. Galliot, J. Durand-Roger, M. Leboyer, P.M. Llorca, F. Schürhoff, A. Szöke, Environmental risk factors for schizophrenia: a review, L'encephale. 39 (1) (2012) 19–28.
[8] M.G. Henriksen, J. Nordgaard, L.B. Jansson, Genetics of schizophrenia: overview of methods, findings and limitations, Front. Hum. Neurosci. 11 (2017) 322.
[9] A.L. Comer, M. Carrier, M.È. Tremblay, A. Cruz-Martín, The inflamed brain in schizophrenia: the convergence of genetic and environmental risk factors that lead to uncontrolled neuroinflammation, Front. Cell. Neurosci. 14 (2020) 274.
[10] R. Gober, M. Ardalan, S. Shiadeh, L. Duque, S.P. Garamszegi, M. Ascona, et al., Microglia activation in postmortem brains with schizophrenia demonstrates distinct morphological changes between brain regions, Brain Pathol. 32 (1) (2022) e13003.
[11] E. Parellada, P. Gassó, Glutamate and microglia activation as a driver of dendritic apoptosis: a core pathophysiological mechanism to understand schizophrenia, Transl. Psychiatry 11 (1) (2021) 271.
[12] A. Monji, Y. Mizoguchi, Neuroinflammation in late-onset schizophrenia: viewing from the standpoint of the microglia hypothesis, Neuropsychobiology 81 (2) (2022) 98–103.
[13] J.D. Crapser, M.A. Arreola, K.I. Tsourmas, K.N. Green, Microglia as hackers of the matrix: sculpting synapses and the extracellular space, Cell. Mol. Immunol. 18 (11) (2021) 2472–2488.
[14] P.T. Nguyen, L.C. Dorman, S. Pan, I.D. Vainchtein, R.T. Han, H. Nakao-Inoue, et al., Microglial remodeling of the extracellular matrix promotes synapse plasticity, Cell 182 (2) (2020) 388–403.e15.
[15] E. Dzyubenko, D. Manrique-Castano, C. Kleinschnitz, A. Faissner, D.M. Hermann, Role of immune responses for extracellular matrix remodeling in the ischemic brain, Therapeutic Adv. Neurol. Disord. 11 (2018) 1756286418818092.
[16] S. Ghorbani, V.W. Yong, The extracellular matrix as modifier of neuroinflammation and remyelination in multiple sclerosis, Brain 144 (7) (2021) 1958–1973.
[17] A.D. Gaudet, P.G. Popovich, Extracellular matrix regulation of inflammation in the healthy and injured spinal cord, Exp. Neurol. 258 (2014) 24–34.
[18] A. Rolls, R. Shechter, A. London, Y. Segev, J. Jacob-Hirsch, N. Amariglio, et al., Two faces of chondroitin sulfate proteoglycan in spinal cord repair: a role in microglia/macrophage activation, PLoS Med. 5 (8) (2008) e171.
[19] A.M. Stankiewicz, J. Goscik, A. Majewska, A.H. Swiergiel, G.R. Juszczak, The effect of acute and chronic social stress on the hippocampal transcriptome in mice, PLoS One 10 (2015) e0142195.
[20] M.L. Lehmann, T.K. Weigel, H.A. Cooper, A.G. Elkahloun, S.L. Kigar, M. Herkenham, M. Decoding microglia responses to psychosocial stress reveals blood-brain barrier breakdown that may drive stress susceptibility, Sci. Rep. 8 (2018) 11240.
[21] R.G. Rempe, A.M.S. Hartz, B. Bauer, Matrix metalloproteinases in the brain and blood-brain barrier: versatile breakers and makers, Journal of cerebral blood flow and metabolism, J. Cereb. Blood Flow Metab. 36 (9) (2016) 1481–1507.
[22] K. Chopra, A. Baveja, A. Kuhad, MMPs: a novel drug target for schizophrenia, Expert Opin. Ther. Targets 19 (1) (2015) 77–85.
[23] G.A. Cabral-Pacheco, I. Garza-Veloz, C. Castruita-De la Rosa, J.M. Ramirez-Acuña, B.A. Perez-Romero, J.F. Guerrero-Rodriguez, et al., The roles of matrix metalloproteinases and their inhibitors in human diseases, Int. J. Mol. Sci. 21 (24) (2020) 9739.
[24] Y.S. Kim, T.H. Joh, Matrix metalloproteinases, new insights into the understanding of neurodegenerative disorders, Biomol. Ther. 20 (2) (2012) 133–143.
[25] G.A. Rosenberg, Matrix metalloproteinases and their multiple roles in neurodegenerative diseases, Lancet Neurol. 8 (2) (2009) 205–216.
[26] S. Haylock-Jacobs, M.B. Keough, L. Lau, V.W. Yong, Chondroitin sulphate proteoglycans: extracellular matrix proteins that regulate immunity of the central nervous system, Autoimmun. Rev. 10 (12) (2011) 766–772.
[27] M.K. Sethi, J. Zaia, Extracellular matrix proteomics in schizophrenia and Alzheimer's disease, Anal. Bioanal. Chem. 409 (2) (2017) 379–394.
[28] H. Fujioka, Y. Dairyo, K. Yasunaga, K. Emoto, Neural functions of matrix metalloproteinases: plasticity, neurogenesis, and disease, Biochem. Res. Int. 2012 (2012) 789083.
[29] S. Devanarayanan, H. Nandeesha, S. Kattimani, S. Sarkar, Relationship between matrix metalloproteinase-9 and oxidative stress in drug-free male schizophrenia: a case control study, Clin. Chem. Lab. Med. 54 (3) (2016) 447–452.
[30] C. De Luca, M. Papa, Matrix metalloproteinases, neural extracellular matrix, and central nervous system pathology, Pro. Mol. Biol. Tran. Sci. 148 (2017) 167–202.
[31] T. Barrett, T.O. Suzek, D.B. Troup, S.E. Wilhite, W.C. Ngau, P. Ledoux, et al., NCBI GEO: mining millions of expression profiles—database and tools, Nucleic Acids Res. 1 (33) (2005) D562–D566.
[32] N.A. Mahi, M.F. Najafabadi, M. Pilarczyk, M. Kouril, M. Medvedovic, GREIN: an interactive web platform for Re-analyzing GEO RNA-seq data, Sci. Rep. 9 (1) (2019) 7580.
[33] P. Bardou, J. Mariette, F. Escudié, C. Djemiel, C. Klopp, jvenn: an interactive Venn diagram viewer, BMC Bioinf. 15 (1) (2014) 1–7.
[34] M. Pathan, S. Keerthikumar, C.S. Ang, L. Gangoda, C.Y. Quek, N.A. Williamson, et al., FunRich: an open access standalone functional enrichment and interaction network analysis tool, Proteomics 15 (15) (2015) 2597–2601.
[35] Z. Xie, A. Bailey, M.V. Kuleshov, D. Clarke, J.E. Evangelista, S.L. Jenkins, et al., Gene set knowledge discovery with enrichr, Curr. Proteonomics 1 (3) (2021) e90.
[36] D. Szklarczyk, R. Kirsch, M. Koutrouli, K. Nastou, F. Mehryary, R. Hachilif, et al., The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest, Nucl. Aci. Res. Jan 6 (51) (2023) VD638–V646.
[37] P. Shannon, A. Markiel, O. Ozier, N.S. Baliga, J.T. Wang, D. Ramage, et al., Cytoscape: a software environment for integrated models of biomolecular interaction networks, Genet. Res. 13 (11) (2003) 2498–2504.
[38] C.H. Chin, S.H. Chen, H.H. Wu, C.W. Ho, M.T. Ko, C.Y. Lin, cytoHubba: identifying hub objects and sub-networks from complex interactome, BMC Syst. Biol. 8 (4) (2014) 1–7.
[39] C.H. Chin, S.H. Chen, H.H. Wu, C.W. Ho, M.T. Ko, C.Y. Lin, cyto-Hubba, A Cytoscape plug-in for hub object analysis in network biology, in: 20th International Conference on Genome Informatics, 2009.
[40] D. Clarke, M.V. Kuleshov, B.M. Schilder, D. Torre, M.E. Duffy, A.B. Keenan, et al., eXpression2Kinases (X2K) Web: linking expression signatures to upstream cell signaling networks, Nucleic Acids Res. 46 (W1) (2018). W171–W179.
[41] A. Lachmann, H. Xu, J. Krishnan, S.I. Berger, A.R. Mazloom, A. Ma'ayan, ChEA, Transcription factor regulation inferred from integrating genome-wide ChIP-X experiments, Bioinformatics 26 (19) (2010) 2438–2444.
[42] S.I. Berger, J.M. Posner, A. Ma'ayan, Genes2Networks: connecting lists of gene symbols using mammalian protein interactions databases. BMC bioinformatics, 8 (372) (2007).
[43] A. Lachmann, A. Ma'ayan, KEA, Kinase enrichment analysis, Bioinformatics 25 (5) (2009) 684–686.
[44] H.Y. Huang, Y.C. Lin, J. Li, K.Y. Huang, S. Shrestha, H.C. Hong, et al., miRTarBase 2020: updates to the experimentally validated microRNA–target interaction database, Nucleic Acids Res. 8 (48) (2020) D148–D154.
[45] J. Xia, M.J. Benner, R.E. Hancock, NetworkAnalyst-integrative approaches for protein–protein interaction network analysis and visual exploration, Nucleic Acids Res. 1 (42) (2014) W167–W174.
[46] V. Licursi, F. Conte, G. Fiscon, P. Paci, MIENTURNET: an interactive web tool for microRNA-target enrichment and network-based analysis, BMC Bioinf. 20 (1) (2019) 545.
[47] T. Li, R. Wernersson, R.B. Hansen, H. Horn, J. Mercer, G. Slodkowicz, et al., A scored human protein-protein interaction network to catalyze genomic interpretation, Nat. Metab. 14 (1) (2017) 61–64.
[48] M. Wu, K. Fang, W. Wang, W. Lin, L. Guo, J. Wang, Identification of key genes and pathways for Alzheimer's disease via combined analysis of genome-wide expression profiling in the hippocampus, Biophysics Rep 5 (2019) 98–109.

[49] S.X. Ge, D. Jung, R. Yao, ShinyGO: a graphical gene-set enrichment tool for animals and plants, Bioinformatics 36 (8) (2020) 2628–2629.
[50] G.M. Khandaker, L. Cousins, J. Deakin, B.R. Lennox, R. Yolken, P.B. Jones, Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment, Lancet Psychiatr. 2 (3) (2015) 258–270.
[51] S. Berretta, Extracellular matrix abnormalities in schizophrenia, Neuropharmacology 62 (3) (2012) 1584–1597.
[52] J. Ma, C. Ma, J. Li, Y. Sun, F. Ye, K. Liu, H. Zhang, Extracellular matrix proteins involved in alzheimer's disease, Chemistry 26 (53) (2020) 12101–12110.
[53] T.M. Maynard, L. Sikich, J.A. Lieberman, A.S. LaMantia, Neural development, cell-cell signaling, and the "two-hit" hypothesis of schizophrenia, Schizophr. Bull. (2001) 457–476.
[54] J.L. McGuire, E.A. Depasquale, A.J. Funk, S.M. O'Donnovan, K. Hasselfeld, S. Marwaha, Abnormalities of signal transduction networks in chronic schizophreni, NPJ Schizophrenia 3 (1) (2017) 30.
[55] X. Wu, D.S. Reddy, Integrins as receptor targets for neurological disorders, Pharmacol. Ther. 134 (1) (2012) 68–81.
[56] C. Redies, N. Hertel, C.A. Hübner, Cadherins and neuropsychiatric disorders, Brain Res. 1470 (2012) 130–144.
[57] K. Punovuori, M. Malaguti, S. Lowell, Cadherins in early neural development, Cell. Mol. Life Sci. 78 (9) (2021) 4435–4450.
[58] M. Sun, J.C. Gewirtz, L. Bofenkamp, R.J. Wickham, H. Ge, M.B. O'Connor, Canonical TGF-beta signaling is required for the balance of excitatory/inhibitory transmission within the hippocampus and prepulse inhibition of acoustic startle, J. Neurosci. 30 (17) (2010) 6025–6035.
[59] C.M. Klass, J.R. Couchman, A. Woods, Control of extracellular matrix assembly by syndecan-2 proteoglycan, J. Cell Sci. 113 (Pt 3) (2000) 493–506.
[60] T. Feng, A. Tripathi, A. Pillai, Inflammatory pathways in psychiatric disorders: the case of schizophrenia and depression, Curr. Behav. Neurosci. Rep. 7 (3) (2020) 128–138.
[61] N. Müller, E. Weidinger, B. Leitner, M.J. Schwarz, The role of inflammation in schizophrenia, Front. Neurosci. 9 (2015) 372.
[62] N. Müller, M.J. Schwarz, A psychoneuroimmunological perspective to Emil Kraepelins dichotomy: schizophrenia and major depression as inflammatory CNS disorders, Eur. Arch. Psychiatr. Clin. Neurosci. 258 (Suppl 2) (2008) 97–106.
[63] K. Nakata, H. Ujike, A. Sakai, M. Takaki, T. Imamura, Y. Tanaka, S. Kuroda, Association study between the fibronectin gene and schizophrenia, Am. J. Med. Genet. Part B: Neuropsychiatric Gen. 116B (1) (2003) 41–44.
[64] T.D. Purves-Tyson, K. Robinson, A.M. Brown, D. Boerrigter, H.Q. Cai, C. Weissleder, et al., Increased macrophages and C1qA, C3, C4 transcripts in the midbrain of people with schizophrenia, Front. Immunol. 11 (2020) (2002).
[65] G. Muntané, M. Chillida, S. Aranda, A. Navarro, E. Vilella, Coexpression of the discoidin domain receptor 1 gene with oligodendrocyte-related and schizophrenia risk genes in the developing and adult human brain, Brain and Behav 11 (8) (2021) e2309.
[66] R. Xu, J. Liang, Y. Luo, X. Wan, K. Li, L. Qi, et al., Mass spectrometry identification of potential biomarker proteins in the 150-kD electrophoretic band in patients with schizophrenia, Medicine 97 (51) (2018) e13553.
[67] C. Huo, X. Liu, J. Zhao, T. Zhao, H. Huang, H. Ye, Abnormalities in behaviour, histology and prefrontal cortical gene expression profiles relevant to schizophrenia in embryonic day 17 MAM-Exposed C57BL/6 mice, Neuropharmacology 140 (2018) 287–301.
[68] S. Zammit, M. Hamshere, S. Dwyer, L. Georgiva, N. Timpson, V. Moskvina, et al., CA population-based study of genetic variation and psychotic experiences in adolescents, Schizophr. Bull. 40 (6) (2014) 1254–1262.
[69] J.K. Kular, S. Basu, R.I. Sharma, The extracellular matrix: structure, composition, age-related differences, tools for analysis and applications for tissue engineering, J. Tissue Eng. 5 (2014) 2041731414557112.
[70] D.A. Smagin, A.G. Galyamina, I.L. Kovalenko, V.N. Babenko, N.N. Kudryavtseva, Aberrant expression of collagen gene family in the brain regions of male mice with behavioral psychopathologies induced by chronic agonistic interactions, BioMed Res. Int. (2019) 7276389.
[71] W. Omori, K. Hattori, N. Kajitani, M.O. Tsuchioka, S. Boku, H. Kunugi, et al., Increased matrix metalloproteinases in cerebrospinal fluids of patients with major depressive disorder and schizophrenia, Int. J. Neuropsychopharmacol. 23 (11) (2020) 713–720.
[72] H.J. Park, S.K. Kim, J.W. Kim, W.S. Kang, J.H. Chung, Association of thrombospondin 1 gene with schizophrenia in Korean population, Mol. Biol. Rep. 39 (6) (2012) 6875–6880.
[73] H.S. Huang, A. Matevossian, C. Whittle, S.Y. Kim, A. Schumacher, S.P. Baker, S. Akbarian, Prefrontal dysfunction in schizophrenia involves mixed-lineage leukemia 1-regulated histone methylation at GABAergic gene promoters, Neuroscience 27 (42) (2007) 11254–11262.
[74] W.B. Ruzicka, S. Subburaju, F.M. Benes, Circuit and diagnosis-specific DNA methylation changes at γ-aminobutyric acid-related genes in postmortem human Hippocampus in schizophrenia and bipolar disorder, JAMA Psychiatr. 72 (6) (2015) 541–551.
[75] K.J. Billingsley, M. Manca, O. Gianfrancesco, D.A. Collier, H. Sharp, V.J. Bubb, J.P. Quinn, Regulatory characterisation of the schizophrenia-associated CACNA1C proximal promoter and the potential role for the transcription factor EZH2 in schizophrenia aetiology, Schizophrenia Res. 199 (2018) 168–175.
[76] P.L. Brattås, M.E. Jönsson, L. Fasching, J. Nelander Wahlestedt, M. Shahsavani, R. Falk, et al., TRIM28 controls a gene regulatory network based on endogenous retroviruses in human neural progenitor cells, Cell Rep. 18 (1) (2017) 1–11.
[77] X. Ni, J. Trakalo, J. Valente, M.H. Azevedo, M.T. Pato, C.N. Pato, J.L. Kennedy, Human p53 tumor suppressor gene (TP53) and schizophrenia: case-control and family studies, Neurosci. Lett. 388 (3) (2005) 173–178.
[78] E. Rees, H. Creeth, H.G. Hwu, W.J. Chen, M. Tsuang, S.J. Glatt, et al., Schizophrenia, autism spectrum disorders and developmental disorders share specific disruptive coding mutations, Nat. Commun. 12 (1) (2021) 5353.
[79] R.S. Jope, M.S. Roh, Glycogen synthase kinase-3 (GSK3) in 1034psychiatric diseases and therapeutic interventions, Curr. Drug Targets 7 (11) (2006) 1421–1434.
[80] D. Rodrigues-Amorim, T. Rivera-Baltanás, P. Fernández-Palleiro, M. Iglesias-Martínez-Almeida, L. Freiría-Martínez, C. Jarmardo-Rodriguez, et al., Changes in the brain extracellular matrix composition in schizophrenia: a pathophysiological dysregulation and a potential therapeutic target, Cell. Mol. Neurobiol. (2021) 1–2.
[81] H. Pantazopoulos, T.U. Woo, M.P. Lim, N. Lange, S. Berretta, Extracellular matrix-glial abnormalities in the amygdala and entorhinal cortex of subjects diagnosed with schizophrenia, Arch. Gen. Psychiatr. 67 (2) (2010) 155–166.
[82] G. Matuszko, S. Curreli, R. Kaushik, A. Becker, A. Dityatev, Extracellular matrix alterations in the ketamine model of schizophrenia, Neuroscience 350 (2017) 13–22.
[83] J. Su, J. Chen, K. Lippold, A. Monavarfeshani, G.L. Carrillo, R. Jenkins, M.A. Fox, Collagen-derived matricryptins promote inhibitory nerve terminal formation in the developing neocortex, J. Cell Biol. 212 (6) (2016) 721–736.
[84] D.A. Lewis, B. Moghaddam, Cognitive dysfunction in schizophrenia: convergence of gamma-aminobutyric acid and glutamate alterations, Arch. Neurol. 63 (10) (2006) 1372–1376.
[85] C. Kehrer, N. Maziashvili, T. Dugladze, T. Gloveli, Altered excitatory-inhibitory balance in the NMDA-hypofunction model of schizophrenia, Front. Mol. Neurosci. 1 (2008).
[86] K.M. Baeten, K. Akassoglou, Extracellular matrix and matrix receptors in blood-brain barrier formation and stroke, Devel. Neurobiol. 71 (11) (2011) 1018–1039.
[87] L.F. Reichardt, A. Prokop, Introduction: the role of extracellular matrix in nervous system development and maintenance, Devel. Neurobiol. 71 (11) (2011) 883–888.
[88] R.J. Khadilkar, K. Ho, B. Venkatesh, G. Tanentzapf, Integrins modulate extracellular matrix organization to control cell signaling during hematopoiesis, Curr. Biol. 30 (17) (2020) 3316–3329.e5.
[89] W.M. Bullock, K. Cardon, J. Bustillo, R.C. Roberts, N.I. Perrone-Bizzozero, Altered expression of genes involved in GABAergic transmission and neuromodulation of granule cell activity in the cerebellum of schizophrenia patients, Am. J. Psychiatr. 165 (12) (2008) 1594–1603.
[90] P.J. Harrison, D. McLaughlin, R.W. Kerwin, Decreased hippocampal expression of a glutamate receptor gene in schizophrenia, Lancet 337 (8739) (1991) 450–452.
[91] G.P. Reynolds, C. Czudek, H.B. Andrews, Deficit and hemispheric asymmetry of GABA uptake sites in the hippocampus in schizophrenia, Biol. Psychiatr. 27 (9) (1990) 1038–1044.

[92] M.D. Simpson, P. Slater, J.F. Deakin, M.C. Royston, W.J. Skan, Reduced GABA uptake sites in the temporal lobe in schizophrenia, Neurosci. Lett. 107 (1–3) (1989) 211–215.
[93] J.N. Joyce, A. Shane, N. Lexow, A. Winokur, M.F. Casanova, J.E. Kleinman, Serotonin uptake sites and serotonin receptors are altered in the limbic system of schizophrenics, Neuropsychopharmacology 8 (4) (1993) 315–336.
[94] J.C. de Jonge, C.H. Vinkers, H.E. Hulshoff Pol, A. Marsman, GABAergic mechanisms in schizophrenia: linking postmortem and in vivo studies, Front. Psychiatr. 8 (2017) 118.
[95] X. Wang, Y. Hu, W. Liu, Y. Ma, X. Chen, T. Xue, D. Cui, Molecular Basis of GABA Hypofunction in Adolescent Schizophrenia-like Animals, Neural Plasticity, 2021 9983438.
[96] A. Caballero, K.Y. Tseng, GABAergic function as a limiting factor for prefrontal maturation during adolescence, Trends Neurosci. 39 (7) (2016) 441–448.
[97] M. Jahangir, J.S. Zhou, B. Lang, X.P. Wang, GABAergic system dysfunction and challenges in schizophrenia research, Front. Cell Dev. Biol. 9 (2021) 663854.
[98] L.S. Kegeles, X. Mao, A.D. Stanford, R. Girgis, N. Ojeil, X. Xu, et al., Elevated prefrontal cortex γ-aminobutyric acid and glutamate-glutamine levels in schizophrenia measured in vivo with proton magnetic resonance spectroscopy, Arch. Gen. Psychiatr. 69 (5) (2012) 449–459.
[99] T. Wang, A. S Sinha, T. Akita, Y. Yanagawa, A. Fukuda, Alterations of GABAergic neuron-associated extracellular matrix and synaptic responses in gad1-heterozygous mice subjected to prenatal stress, Front. Cell. Neurosci. 12 (2018) 284.
[100] B.M. Elmer, A.K. McAllister, Major histocompatibility complex class I proteins in brain development and plasticity, Trends Neurosci. 35 (11) (2012) 660–670.
[101] M.W. Glynn, B.M. Elmer, P.A. Garay, X.B. Liu, L.A. Needleman, F. El-Sabeawy, A.K. McAllister, MHCI negatively regulates synapse density during the establishment of cortical connections, Nat. Neurosci. 14 (4) (2011) 442–451.
[102] T.M. Maynard, L. Sikich, J.A. Lieberman, A.S. LaMantia, Neural development, cell-cell signaling, and the "two-hit" hypothesis of schizophrenia, Schizophr. Bull. 27 (3) (2001) 457–476.
[103] H. Neumann, A. Cavalié, D.E. Jenne, H. Wekerle, Induction of MHC class I genes in neurons, Science 269 (5223) (1995) 549–552.
[104] C.A. Goddard, D.A. Butts, C.J. Shatz, Regulation of CNS synapses by neuronal MHC class I, Pro, N. Aca. Sci. 104 (16) (2007) 6828–6833.
[105] M. Michel, M.J. Schmidt, K. Mirnics, Immune system gene dysregulation in autism and schizophrenia, Devel, Neurobiol 72 (10) (2012) 1277–1287.
[106] R.G. Rempe, A. Hartz, B. Bauer, Matrix metalloproteinases in the brain and blood-brain barrier: versatile breakers and makers, J. Cerebr. Blood Flow Metabol. 36 (9) (2016) 1481–1507.
[107] N. Keshri, H. Nandeesha, M. Rajappa, V. Menon, Matrix metalloproteinase-9 increases the risk of cognitive impairment in schizophrenia, Nor. J. Psychiatry. 75 (2) (2021) 130–134.
[108] G. Schoretsanitis, R. de Filippis, M. Ntogka, S. Leucht, C.U. Correll, J.M. Kane, Matrix metalloproteinase 9 blood alterations in patients with schizophrenia spectrum disorders: a systematic review and meta-analysis, Schizophr. Bull. 47 (4) (2021) 986–996.
[109] B. Bitanihirwe, T.W. Woo, A conceptualized model linking matrix metalloproteinase-9 to schizophrenia pathogenesis, Schizophrenia Res. 218 (2020) 28–35.
[110] S.M. Reinhard, K. Razak, I.M. Ethell, A delicate balance: role of MMP-9 in brain development and pathophysiology of neurodevelopmental disorders, Front. Cell. Neurosci. 9 (2015) 280.
[111] N. Kudo, H. Yamamori, T. Ishima, K. Nemoto, Y. Yasuda, M. Fujimoto, et al., Plasma levels of matrix metalloproteinase-9 (MMP-9) are associated with cognitive performance in patients with schizophrenia, Neuropsycho. Rep. 40 (2) (2020) 150–156.
[112] D. Dwir, B. Giangreco, L. Xin, L. Tenenbaum, J.H. Cabungcal, P. Steullet, et al., MMP9/RAGE pathway overactivation mediates redox dysregulation and neuroinflammation, leading to inhibitory/excitatory imbalance: a reverse translation study in schizophrenia patients, Mol. Psychiatr. 25 (11) (2020) 2889–2904.
[113] A. Beroun, S. Mitra, P. Michaluk, B. Pijet, M. Stefaniuk, L. Kaczmarek, MMPs in learning and memory and neuropsychiatric disorders, Cell. Mol. Life Sci. 76 (16) (2019) 3207–3228.
[114] Y. Suzuki, N. Nagai, K. Umemura, A review of the mechanisms of blood-brain barrier permeability by tissue-type plasminogen activator treatment for cerebral ischemia, Front. Cell. Neurosci. 10 (2016) 2.
[115] Y. Ma, R.P. Iyer, L.E. de Castro Brás, H. Toba, A. Yabluchanskiy, K.Y. Deleon-Pennell, et al., Cross talk between inflammation and extracellular matrix following myocardial infarction, Inflamm. Heart Fail. (2015) 67–79.
[116] R.G. Rempe, A. Hartz, E. Soldner, B.S. Sokola, S.R. Alluri, E.L. Abner, et al., Matrix metalloproteinase-mediated blood-brain barrier dysfunction in epilepsy, J. Neurosci. 38 (18) (2018) 4301–4315.
[117] C. Yang, K.E. Hawkins, S. Doré, E. Candelario-Jalil, Neuroinflammatory mechanisms of blood-brain barrier damage in ischemic stroke, Am. J. Physiol.: Cell Physiol. 316 (2) (2019) C135–C153.
[118] S.H. Joo, K.J. Kwon, J.W. Kim, M.R. Hasan, H.J. Lee, S.H. Han, C.Y. Shin, Regulation of matrix metalloproteinase-9 and tissue plasminogen activator activity by alpha-synuclein in rat primary glial cells, Neurosci. Lett. 469 (3) (2010) 352–356.