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استكشاف الشبكات الوظيفية ومسارات KEGG للجينات والبروتينات المرتبطة بمرض طيف التوحد: دراسة معلوماتية بايولوجية

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المستخلص:

اضطراب طيف التوحد (ASD) هو خلل في النمو العصبي للدماغ ينشأ نتيجة عدة اسباب جينية وبيئية. هذه الدراسة تركز على استخدام أدوات المعلوماتية الحيوية (Bioinformatics)مثل (ENRICHRو STRING) لتحديد الشبكات الوظيفية بالإضافة إلى مسارات KEGG المرتبطة بثمانية جينات لها علاقة بوظائف الدماغ و لها أدوار أساسية في اضطراب طيف التوحد. هذه الجينات تتمثل ب SLC6A4, ITGB3, DISC1, AVPR1a, SHANK3, RPL10, RELN, DYX1c1. اقترحت المعلومات الوظيفية الجينية GO تحليلا للمكونات الخلوية والوظائف الجزيئية والعمليات البيولوجية بالإضافة إلى المستقبلات التي تشبه مستقبلاتASD . باستخدام تحليل مسار KEGG، اكتشفنا مسارات متعددة ترتبط بشكل كبير باضطراب طيف التوحد، بما في ذلك دورة الحويصلة المشبكية والمشبك الجلوتاماتي المعروف بدوره الأساسي في التشابك العصبي، والنقل العصبي، بالإضافة إلى تفاعل مستقبلات ECM التي تلعب دورًا رئيسيًا في النمو العصبي، والنقل العصبي، والمرونة التشابكية. حدد تحليل STRING شبكة معقدة من التفاعلات بين الجينات المختارة. تعمق هذه النتائج فهمنا للآليات الجزيئية التي تسبب اضطراب طيف التوحد وتقترح أهدافًا مستقبلية للتطوير العلاجي.

Exploring Functional Networks and KEGG Pathways of Autism-Associated Genes and Proteins: A Bioinformatics Study

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Abstract:

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Autism Spectrum Disorder (ASD) is a neuro-developmental disorder of the brain that arises due to numerous genetic and environmental interplay. In our study, we utilized bioinformatics tools (Enrichr and STRING) to find the functional networks as well as KEGG pathways associated with eight brain-related genes (RELN, SLC6A4, ITGB3, AVPR1a, DISC1, SHANK3, RPL10, and DYX1c1) that have essential roles in ASD. GO enrichment analyses identified cellular components, molecular functions, and biological processes in addition to those associated with ASD, such as receptor binding. Using KEGG pathway enrichment analysis, we identified multiple pathways that are highly associated with ASD, including the synaptic vesicle cycle and glutamatergic synapse, which are known for their essential roles in neurodevelopment (synaptic function), neurotransmission, and ECM-receptor interaction, which plays a key role in neurodevelopment, neurotransmission, and synaptic plasticity. STRING analysis identified an intricate network of interactions among the chosen genes. These findings deepen our understanding of the molecular mechanisms that cause the ASD disorder and suggest potential targets for future investigation and therapeutic development.

Keywords: Autism; KEGG; Gene Ontology, ENRICHR; STRING

Introduction

Autism Spectrum Disorder (ASD), the intricate neurodevelopmental syndrome, is characterized by a distinct behavioral and cognitive deficit represented by social interaction defects, communication, and repetitive activities (Khalifeh et al., 2016; Lord et al., 2020; Muammar & Alnusayri, 2021). ASD's occurrence has been growing over the last few years, and in the USA, one in 44 children is born with it (Walensky et al., 2021).

The pathophysiology of ASD is complex, and the exact causes of the disease remain unknown. However, research suggests a possible interaction between environmental and genetic variables. Environmental factors are represented by earlier exposure to particular chemicals, old parental age, and problems during pregnancy or delivery (Park et al., 2016; Sauer et al., n.d.), (Karimi et al., 2017; Modabbernia et al., 2017). Genetic factors, on the other hand, are denoted by numerous genes, and changes in these genes may raise the

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٢٠٢٤م/١٤٤٦هـ

chance of developing the ASD (Genovese & Butler, 2023; Rylaarsdam & Guemez-Gamboa, 2019a, 2019b). However, researchers continue to study the interplay between genetic and environmental influences to better understand the condition's roots, as they have not identified a single cause. Early diagnosis and intervention for children with ASD is essential, as they can significantly improve outcomes by treating progressive difficulties as early as they appear. Children with ASD can get an advantage from early intervention programs such as speech, occupational, and behavioral therapy since they help them to develop important skills, communicate better, and participate in more social situations. These treatments are largely fruitful in supporting autistic people in realizing their potentials as early as they start (Geschwind, 2009; Okoye et al., 2023).

As genetic studies progress, researchers have identified numerous genes and pathways that influence the development of ASDs, highlighting the intricate molecular networks involved. The reelin gene (RELN), Solute Carrier Family 6 Member 4 (SLC6A4), integrin beta 3 (ITGB3), arginine vasopressin receptor 1A (AVPR1a), disrupted schizophrenia 1 (DISC1), SH3 and multiple ankyrin repeat domains (SHANK3), ribosomal protein L10 (RPL10), and dyslexia candidate gene (DYX1C1) are among the brain-related genes that play key roles in the occurrence of the ASD [15]. These genes contribute to an array of biological activities, such as regulating signal transduction, synaptic transmission, brain development, and synaptic potentiation. All of these genes are critical for proper brain growth and function. ASD's complexity depends on the interactions between these genes and the larger molecular networks in which they contribute (Sauer et al., n.d.).

Bioinformatics is one of the most effective tools for analyzing the functional networks and pathways connected to genes involved in disease (Tang et al., 2023; Wei et al., 2022). Various genomic and proteomic data integrate in bioinformatics methods to understand the molecular mechanisms behind ASD (Talli et al., 2022; Tang et al., 2023). Particularly, the Kyoto Encyclopedia of Genes and Genomes (KEGGpathways) offers crucial insights into the biochemical pathways and signaling networks that these

genes and their protein products share. This work aims to examine the functional networks and KEGG pathways linked with the genes AVPR1a, DISC1, DYX1C1, ITGB3, SLC6A4, RELN, RPL10, and SHANK3 that have been associated with ASD. To better understand the molecular pathways behind ASD, we will investigate the interactions between these genes and the proteins they encode through bioinformatics techniques. This paper aims to reveal putative biological processes and signaling cascades that contribute to the pathophysiology of ASD by aligning these genes to certain KEGG pathways. Comprehending these pathways provides an insight into novel ideas for therapy targets and ASD intervention techniques.

Methodologies

1. Gene Assortment

In this study, 8 autism spectrum disorder (ASD) -related genes: RELN, SLC6A4, ITGB3, AVPR1a, DISC1, SHANK3, RPL10, and DYX1C were designated. By reviewing the literature, these genes were previously selected as brain-related that have important role on the prevalence of Autism (Lopes Cardoso & Almeida, 2019; Yonan et al., n.d.).

2. Functional Enrichment Analysis

To perform the functional enrichment analysis, Enrichr database server (https://maayanlab.cloud/Enrichr/) was used. We retrieved enriched terms into Gene Ontology (GO) Categories such as Cellular Component (CC), Molecular Function (MF), and Biological Process (BP). The KEGG (Kyoto Encyclopedia of Genes and Genomes) via Enrichr tool was utilized to conduct pathway enrichment analysis. This tool allows to crosslink genes data with diverse databases such as Gene Ontology or Reactome.

3. Network Analysis of Protein-Protein Interaction (PPI)

The network of protein-protein interaction (PPI) was shaped by STRING database v.11 (https://string-db.org/) (Abdal Rhida, 2024). The selected genes group was uploaded to the STRING database. Interaction sources included databases, experimental data, gene fusion, co-expression, co-occurrence, and neighborhood.

Results and Discussion

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In this study, we conducted a comprehensive analysis of the functional network and KEGG pathways of eight genes (RELN, SLC6A4, ITGB3, AVPR1a, DISC1, SHANK3, RPL10, and DYX1C1) associated with autism, using bioinformatics tools such as Enrichr and STRING databases.

The functional enrichment analysis accomplished using the Enrichr platform revealed a number of significant Gene Ontology (GO) terms and pathways linked with the selected gene set. The Gene Ontology (GO) terms analysis addressed the Cellular Components (CC), Molecular Functions (MF), and Biological Processes (BP) to better understand the roles these genes could play in neurodevelopmental processes and any potential contributions these genes might have in (ASD).

The enriched GO terms in the Cellular Components (CC) category included neuron projection (GO:0043005) especially for RELN, SHANK3, and SLC6A4, asymmetric synapse (GO:0032279), and postsynaptic density (GO:0014069) for both of DISC1 and SHANK3 (Table 1). These results come in agreement with what have been experimentally evidenced by (Jossin, 2020; Kirkpatrick et al., 2006; Tao-Cheng et al., 2016; Wang et al., 2024). These terms propose that the selected genes are largely associated with synaptic structures, highlighting their roles in neuronal wiring and signaling, which are vital processes often disrupted in ASD (Figure 1).

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٢٠٢٤م/١٤٤٦هـ

Table1. The top 10 GO Cellular Component with significant p-values and q-values forComponent

term	p-value	q-value	overlap genes
Glutamatergic Synapse (GO:0098978)	0.000414	0.009577	[ITGB3, DISC1]
Cell Projection Membrane (GO:0031253)	0.000749	0.009577	[ITGB3, SHANK3]
Neuron Projection (GO:0043005)	0.001084	0.009577	[RELN, SHANK3, SLC6A4]
Asymmetric Synapse (GO:0032279)	0.001197	0.009577	[DISC1, SHANK3]
Postsynaptic Density (GO:0014069)	0.001539	0.009849	[DISC1, SHANK3]
Filopodium Membrane (GO:0031527)	0.004392	0.023425	[ITGB3]
Microvillus Membrane (GO:0031528)	0.005189	0.023721	[ITGB3]
Platelet Alpha Granule Membrane (GO:0031092)	0.006383	0.024243	[ITGB3]
GABA-ergic Synapse (GO:0098982)	0.007576	0.024243	[DISC1]
Smooth Endoplasmic Reticulum (GO:0005790)	0.007576	0.024243	[RPL10]

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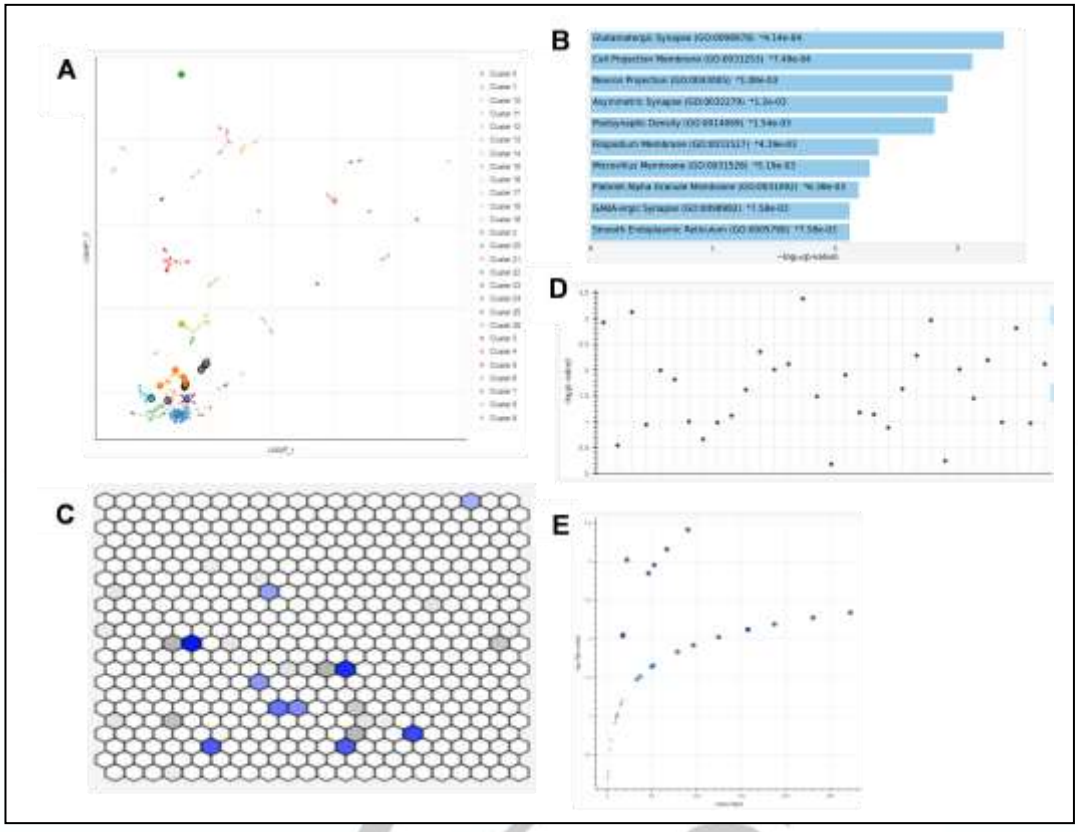

Figure 1. GO Cellular Component. **A.** Scatterplot of terms in the gene ontology Cellular Component gene set library. Points represent terms in the library. **B.** Bar chart of the top 10 enriched terms from GO Cellular Component gene set library. **C.** Hexagonal canvas plot of terms from GO Cellular Component gene set library. Each hexagon represents a single term. **D.** Manhattan plot of terms from GO Cellular Component gene set library..**E.** Volcano plot of terms from the GO Cellular Component gene

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٢٠٢٤م/١٤٤٦هـ

Biological Processes (BP) revealed enrichment in processes like synaptic signaling (GO:1900273) and Synaptic Transmission, Glutamatergic (GO:0051968) specifically for both RELN and SHANK3, neurodevelopment (GO:0007420) for RELN, SHANK3, and SLC6A4. According to [31], RELN absence or damage in its signaling cascade causes neurodevelopmental defects which are linked with ataxia, intellectual disability, autism, and several psychiatric disorders. These results are consistent with what has been experimentally shown by (Arons et al., 2016; Huang et al., 2019; Joly-Amado et al., 2023; Murphy & Moya, 2011). RELN and SHANK3 have shown Biological Processes in Regulating Dendritic Spine Morphogenesis (GO:0061001) (Table 2). According to (Dansie & Ethell, 2011; Knuesel, 2010), mutations in RELN have been reported to be a more likely reason of autism and schizophrenia. Additionally, research has shown that mutations in SHANK3 related with autism make modifications to dendritic spine morphology via actin-dependent mechanism (Durand et al., 2012; Grabrucker et al., 2011; Roussignol et al., 2005) (Figure 2).

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٢٠٢٤م/١٤٤٦هـ

Table 2. The top 10 GO Biological Process with significant p-values and q-values

term	p-value	q-value	overlap genes
Positive Regulation of Signal Transduction (GO:0009967)	0.000002	0.000417	[RELN, ITGB3, DISC1, SHANK3]
Brain Morphogenesis (GO:0048854)	0.000008	0.000780	[SHANK3, SLC6A4]
Positive Regulation of Long-Term Synaptic Potentiation (GO:1900273)	0.000013	0.000860	[RELN, SHANK3]
Positive Regulation of Dendritic Spine Development (GO:0060999)	0.000029	0.000934	[RELN, SHANK3]
Brain Development (GO:0007420)	0.000032	0.000934	[RELN, SHANK3, SLC6A4]
Positive Regulation of Synaptic Transmission, Glutamatergic (GO:0051968)	0.000032	0.000934	[RELN, SHANK3]
Positive Regulation of Excitatory Postsynaptic Potential (GO:2000463)	0.000032	0.000934	[RELN, SHANK3]
Regulation Of AMPA Receptor Activity (GO:2000311)	0.000038	0.000976	[RELN, SHANK3]
Regulation Of Dendritic Spine Morphogenesis (GO:0061001)	0.000061	0.001194	[RELN, SHANK3]
Modulation Of Excitatory Postsynaptic Potential (GO:0098815)	0.000065	0.001194	[RELN, SHANK3]

JOBS
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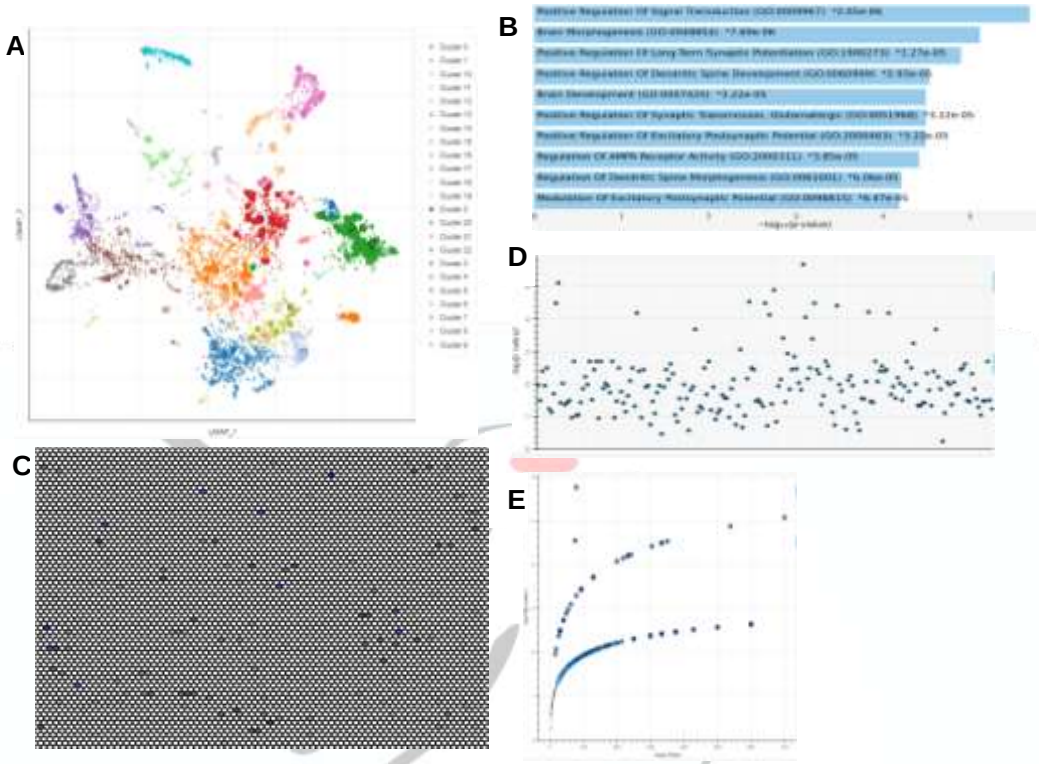

Figure 2. GO Biological Process. **A.** Scatterplot of terms in the gene ontology Biological Process gene set library. Points represent terms in the library. **B.** Bar chart of the top 10 enriched terms from GO Biological Process gene set library. **C.** Hexagonal canvas plot of terms from GO Biological Process gene set library. Each hexagon represents a single term. **D.** Manhattan plot of terms from GO Biological Process gene set library. **E.** Volcano plot of terms from the GO Biological Process gene set library. Each point denotes a single term.

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٢٠٢٤م/١٤٤٦هـ

Molecular Functions (MF) analysis showed significant terms such as receptor binding Platelet-Derived Growth Factor Receptor Binding (GO:0005161) for ITGB3 which has been involved in platelet disorder (Ross et al., 2021), ion channel activity Sodium: Chloride Symporter Activity (GO:0015378) for SLC6A4, and G-protein coupled receptor activity such as Glutamate Receptor Binding (GO:0035255) for SHANK3 (Table 3). These findings line up with what has been experimentally demonstrated by [24], [25] These functions are suggestive of the genes' contribution in neurotransmission and signaling pathways, more supporting their importance to ASD (Figure 3).

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٢٠٢٤م/١٤٤٦هـ

Table 3. The top 10 GO Molecular Function with significant p-values and q-values

term	p-value	q-value	overlap genes
Sodium Ion Binding (GO:0031402)	0.002398	0.014357	[SLC6A4]
Vascular Endothelial Growth Factor Receptor 2 Binding (GO:0043184)	0.003196	0.014357	[ITGB3]
Monoamine Transmembrane Transporter Activity (GO:0008504)	0.004392	0.014357	[SLC6A4]
Ionotropic Glutamate Receptor Binding (GO:0035255)	0.004392	0.014357	[SHANK3]
Insulin-Like Growth Factor I Binding (GO:0031994)	0.004791	0.014357	[ITGB3]
Vascular Endothelial Growth Factor Receptor Binding (GO:0005172)	0.004791	0.014357	[ITGB3]
Platelet-Derived Growth Factor Receptor Binding (GO:0005161)	0.004791	0.014357	[ITGB3]
Sodium: Chloride Symporter Activity (GO:0015378)	0.005189	0.014357	[SLC6A4]
Alkali Metal Ion Binding (GO:0031420)	0.005587	0.014357	[SLC6A4]
Insulin-Like Growth Factor Binding (GO:0005520)	0.005985	0.014357	[ITGB3]

KEGG pathway analysis recognized several pathways significantly associated with the selected genes. Specifically, Table 4 enriched pathways related to glutamatergic synapse, synaptic vesicle cycle, and ECM-receptor interaction. These pathways are commonly involved in synaptic transmission and neuronal connection, making them vital for precise neurodevelopment and implicated in the ASD's pathophysiology. The significance of these pathways ($p < 0.05$) underlines the latent role of these genes in taking part in the ASD by disturbances of these important pathways (Figure 4).

The STRING database constructed the PPI network, which provided clear insights into the interactions between the designated genes and their encoded proteins. The network analysis displayed a high degree of connectivity, especially among the proteins encoded by RELN, SLC6A4, ITGB3, and DISC1, which are well-documented for their roles in synaptic function and neurodevelopment (Figure 5). The STRING analysis revealed a network

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٢٠٢٤م/ ١٤٤٦هـ

with eight nodes and eight edges, resulting in an average node degree of two and an average local clustering coefficient of 0.562. The observed number of edges significantly exceeded the expected number (1 edge), with a PPI enrichment p-value of 4.78e-07, verifying that the network has significantly more connections than expected by chance.

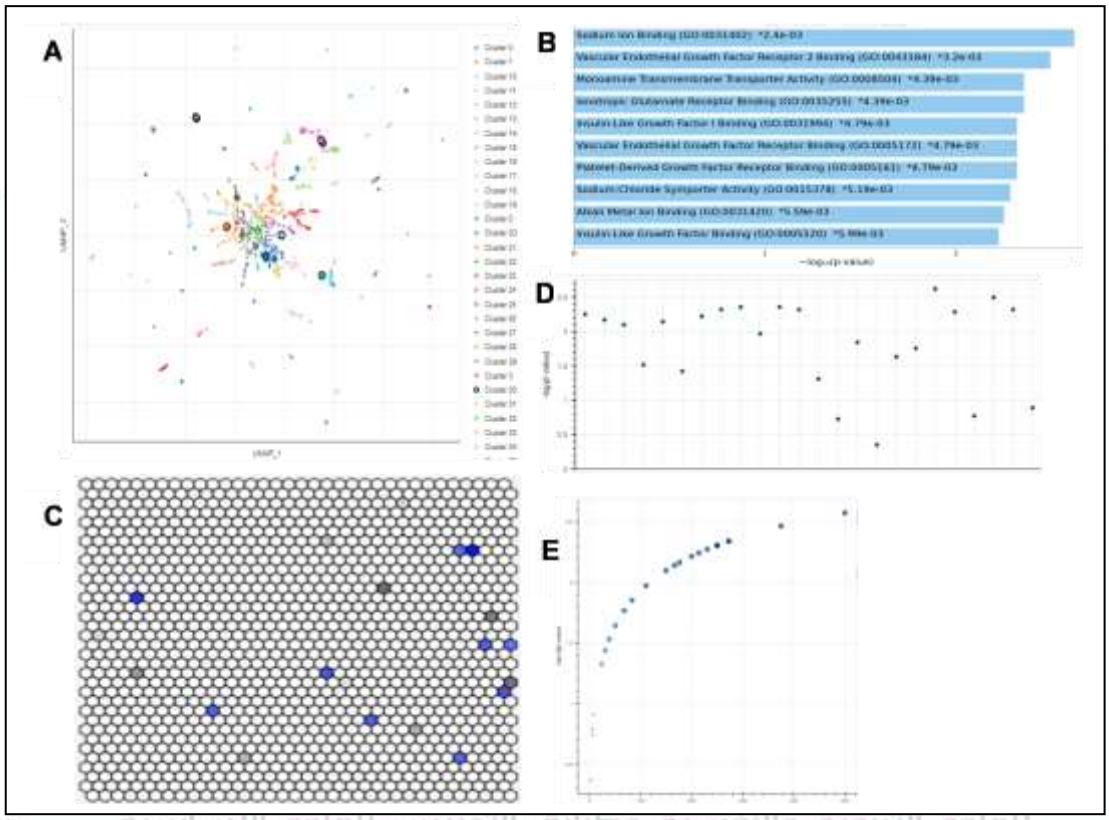

Figure 3. GO Molecular Function. **A.** Scatterplot of terms in the gene ontology Molecular Function gene set library. Points represent terms in the library. **B.** Bar chart of the top 10 enriched terms from GO Molecular Function gene set library. **C.** Hexagonal canvas plot of terms from GO Molecular Function gene set library. Each hexagon represents a single term. **D.** Manhattan plot of terms from GO Molecular Function gene set library. **E.** Volcano plot of terms from the GO Molecular Function gene set library. Each point denotes a single term.

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٢٠٢٤م/١٤٤٦هـ

Table 4. The top 10 KEGG Human with significant p-values and q-values

term	p-value	q-value	overlap genes
ECM-receptor interaction	0.000527	0.015804	[RELN, ITGB3]
Focal adhesion	0.002704	0.040562	[RELN, ITGB3]
Human papillomavirus infection	0.007158	0.061138	[RELN, ITGB3]
PI3K-Akt signaling pathway	0.008152	0.061138	[RELN, ITGB3]
Arrhythmogenic right ventricular cardiomyopathy	0.030393	0.092235	[ITGB3]
Synaptic vesicle cycle	0.030783	0.092235	[SLC6A4]
Hypertrophic cardiomyopathy	0.035444	0.092235	[ITGB3]
Dilated cardiomyopathy	0.037767	0.092235	[ITGB3]
Hematopoietic cell lineage	0.038927	0.092235	[ITGB3]
Serotonergic synapse	0.044324	0.092235	[SLC6A4]

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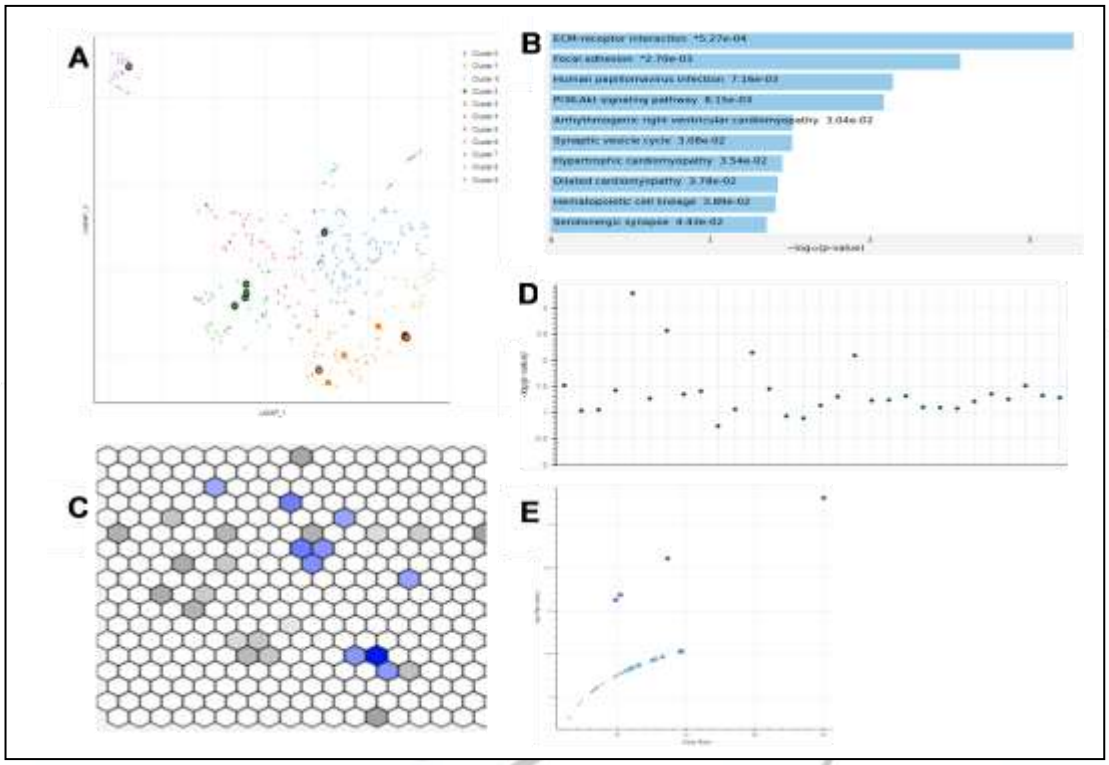

Figure 4. KEGG Pathway Human Gene Set Library. **A.** Scatterplot of terms in the KEGG Pathway human gene set library. Points represent terms in the library. **B.** Bar chart of the top 10 enriched terms from KEGG Pathway human gene set library. **C.** Hexagonal canvas plot of terms from KEGG Pathway humen gene set library. Each hexagon represents a single term. **D.** Manhattan plot of terms from KEGG Pathway human gene set library. **E.** Volcano plot of terms from the KEGG Pathway human gene set library. Each point denotes a single term.

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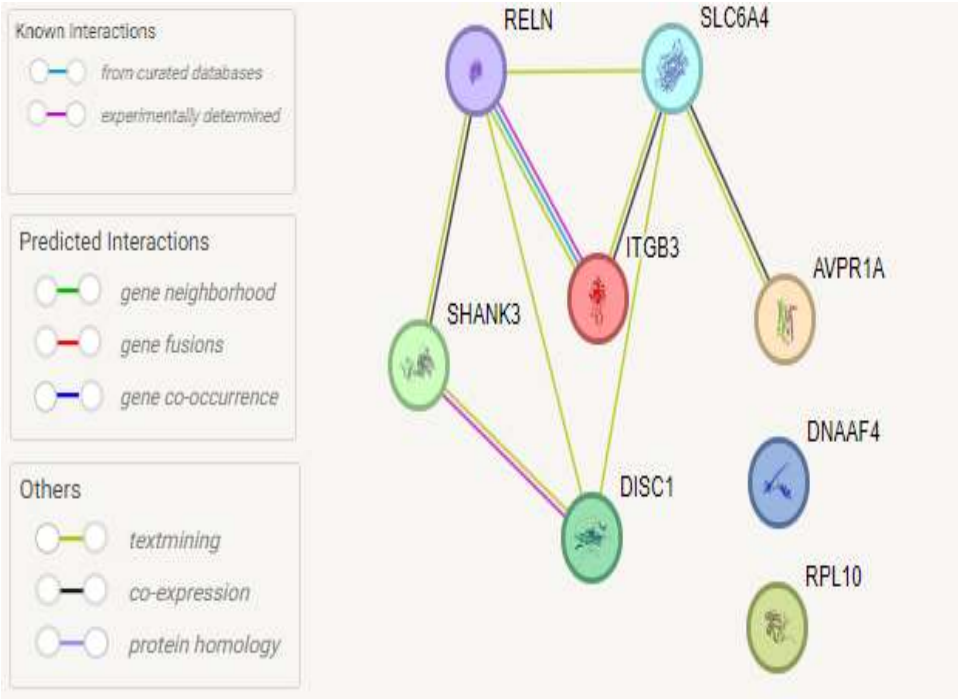

Figure 5. protein-protein interaction (PPI) network construct of brain-related genes involved in ASD.

Conclusion

In this study, we focused on RELN, SLC6A4, ITGB3, AVPR1a, DISC1, SHANK3, RPL10, and DYX1C1 to examine the functional network and KEGG pathways of genes and proteins linked with autism spectrum disorder (ASD). Our analysis showed momentous insights into the molecular mechanisms causing ASD.

We identified several pathways associated with ASD through KEGG pathway enrichment analysis using the Enrichr database. These pathways include the glutamatergic synapse, the synaptic vesicle cycle, and the ECM-receptor interaction. These pathways are important for neurotransmission, synaptic plasticity, and neurodevelopment, supporting the hypothesis that dysregulation in these areas subsidizes the pathophysiology of ASD.

The functional enrichment analysis found a number of significant Gene Ontology (GO) terms and pathways linked with the selected gene set. The enriched GO terms in the Cellular Components category included neuron projection, asymmetric synapse, and postsynaptic density, underscoring their key roles in neuronal connecting and signaling, which are vital processes often disrupted in ASD. The biological processes category, on the other hand, showed enrichment in processes like synaptic signaling, neurodevelopment, and regulation of neurotransmitter levels, which are vital in the ASD situation. The Molecular Functions analysis showcased significant terms like receptor binding factor, ion channel activity, and G-protein coupled receptor activity, implying their involvement in neurotransmission and signaling pathways and emphasizing their significance in ASD. The STRING analysis highlighted a complex network of connections among the selected genes, indicating that these genes share an interconnected web of signaling pathways and biological processes. RELN and SLC6A4 were identified as key hubs in the network, highlighting their dominant roles in synaptic function and neuronal signaling, both of which are important to standard cognitive and social behaviors. This study determined the efficacy of bioinformatics tools like Enrichr and STRING in detecting multifaceted functional networks and pathways linked with autism. The findings provided a deeper understanding of the molecular mechanisms underlying the condition and proposed promising approaches for further investigation and treatment progression. Recognizing the significance of RELN and SLC6A4 in autism as potential biomarkers or intervention targets requires further work.

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