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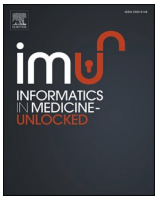

Determination of molecular signatures and pathways common to brain tissues of autism spectrum disorder: Insights from comprehensive bioinformatics approach

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ABSTRACT

Autism spectrum disorder (ASD) is a collection of neurological disabilities marked by difficulties with behavior, speech, language, and interaction. It is a complicated and behaviorally defined static disorder of the developing brain. Recently it has become a serious concern across the world. The goal of this project was to use bioinformatics tools and network biology to uncover the molecular signatures and pathways of ASD. We investigated brain transcriptomics gene expression datasets and determined 47 dysregulated differentially expressed common genes. Several kinds of crucial neurodegeneration-related molecular mechanisms in the signaling structures were determined as a result of these investigations. We implemented gene set enrichment analysis (GSEA) using bimolecular pathways and gene ontology (GO) terms to determine the role of these differentially expressed genes (DEGs), as well as protein-protein interactions (PPI), transcriptional factor interactions, and post-transcriptional factor interactions. PPI network collected the top ten hub genes including KIT, PIN1, GATA1, GRIN2A, PBX2, BLK, ATP6V1B1, TCF7L1, TRAF1, and HSPG2. The PPI network also revealed the existence of two sub-networks. Moreover, several transcription factors (NFIC, USF2, TFAP2A, RELA, FOXL1, GATA2, YY1, FOXC1, NFKB1, and E2F1) and post-transcription factors (mir-335-5p, mir-26b-5p, mir-124-3p, mir-192-5p, mir-1-3p, mir-215-5p, mir-6825-5p, mir-146a-5p, mir-8485, and mir-93-5p) were found throughout this study. Some drug-like molecules were also predicted that might have a beneficial effect against ASD. We detected potentially novel links between pathogenic conditions in ASD patient's brain tissues. This work offers molecular biomarkers at the gene expression level and protein bases that could aid in a better understanding of molecular pathways, as well as potential pharmacological approaches and therapies for developing effective ASD treatments.

1. Introduction

Autism spectrum disorder (ASD) is a term that has been accustomed to characterize a collection of initial socialization deformities and repeated neural activities that are linked to both a significant hereditary component and external factors. Kanner was the first who defined autism in 1943 through the study of 11 youngsters with comparable their odd behaviors [1–3]. The "American Psychiatric Association" changed the word 'Autistic' to Autism Spectrum Disorders (ASD) in 2013 [4]. ASD is a complicated mental condition indicated by problems in three areas: socializing, interaction, and confined as well as repetitious activity [5]. It has a significant genetic component with a complicated inheritance pattern [6–8]. ASD is four times as prevalent in men than it is in women (1 out of 34 men vs. 1 out of 144 women) [9]. Children with ASD may develop normally for the first few months or even years of their lives, but later on, they may become reclusive, aggressive, or lose language abilities that they had previously acquired [10,11]. Generally, the brain shapes and organization of autistic children differ from

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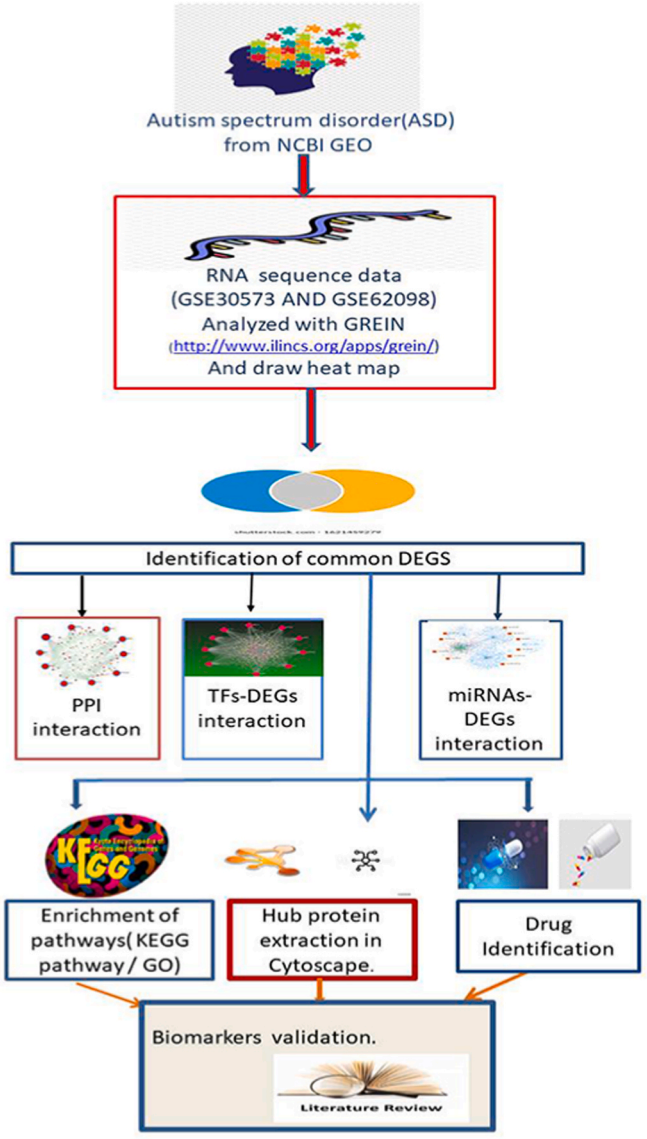


Fig. 1. This workflow depicts the whole work of this study.

neurotypical children [12]. According to the experts, autistic children have an overgrowth of synapses or linkages throughout brain cells, and this overgrowth is caused by a disruption in the usual trimming mechanism that happens throughout brain growth [13].

At present, ASD is predicted to affect 1 out of every 160 children all over the world [14,15]. In Bangladesh, Bangabandhu Sheikh Mujib Medical University (BSMMU) recently verified that about 2 out of every 1000 children in Bangladesh is a victim of ASD [16]. Furthermore, one in fifty-nine American children was identified with ASD according to the CDC (centers for disease control) [17]. Since the 1960s, the number of diagnoses has risen substantially, possibly due to improvements in diagnostic procedures [18]. The rate of diagnoses of ASD has become more than in the last two decades [19]. The autism rights movement advocates the notion of neurodiversity, which sees autism as a normal variety of the brain rather than a disease that can be treated.

A mixture of hereditary and environmental variables has been linked to autism. Though no confirmed reasons for ASD have been identified, it is commonly thought that ASD is caused by anomalies in the central nervous system. Genetic mutations may enhance the risk of ASD [20]. ASD in children can be recognized by many symptoms. Generally, ASD symptoms are visible by the age of two [21–23]. These symptoms are including less eye contact, facial expression, a paucity of responsiveness towards their name, apathy about their caretakers, repetitive motions (waving their hands, twisting fingers, or swaying their body), not talking as much as other youngsters, repeating the same sentences, etc., [24,25]. Moreover, people who are diagnosed with ASD can cause self-harm, injure themselves by hitting their head on solid objects or punching their arms or clawing their skin [26–28]. Hence people with ASD always need special care. They should be kept under observation.

Besides, there are several problems and challenges in diagnosing ASD and caring for an ASD patient. Only 10% to 20% of ASD patients with comparable pathogenic variants might be detected at various degrees of the spectrum. Detection is restricted in terms of sensitivity and specificity [29]. Moreover, it is a costly process to take care of an ASD patient. It is now associated with a significant financial strain. In 2015, the cost of caring for persons having ASD was 268 billion in the USA, according to statistics [30]. By 2025, this figure is anticipated to rise to 461 billion [31].

ASD is the world's most rapidly increasing developmental impairment. Early diagnostic and therapeutic innovations have been proven in studies to improve autistic people's long-term problems. Many therapies have been developed for Autistic children. According to research, the use of complementary and alternative medicine (CAM) for autistic symptoms in children was very common [32]. CAM was frequently viewed as "organic," with none of the negative side effects associated with traditional medical therapies [32,33]. CAM treatments are provided to 2%–50% of ASD children in the United States, according to estimates [33]. However, adults and children are becoming more familiar with complementary and alternative medicine (CAM) day by day. Moreover, electrophysiological biomarkers indicate medical progression for autism treatment. Electrophysiological biomarkers have been used in observational studies to determine the spatiotemporal anomalies in children and adults with ASD, along with newborns at risk for having ASD [34]. According to the researchers, many people with ASD are fascinated and driven by computers and computer-assisted learning can address a variety of academic and support needs, including emotion detection, communication, and social contact [35]. Effective computing technology can detect ASD person's emotional states and provide appropriate psychological treatment.

Though there is a plethora of scientific evidence demonstrating the over-representation of several conditions with ASD and a variety of treatments for this problem, there is still a lack of understanding of the mechanisms and molecular pathways that underpin ASD [36]. As a result, the number of ASD patients grows at an alarming pace year after year [37]. Nowadays, it has become a source of great concern among doctors. New technologies have a lot of promises in terms of offering unique and personalized solutions [38]. But still, there is a need for additional intervention research that can benefit persons with autism, their caretakers, and educators.

In our ongoing study, we wanted to find molecular signatures and pathways at transcriptional and post-transcriptional stages so that we can learn more about the pathogenic processes that cause ASD and find possible biomarkers for early detection. By knowing the processes underlying multiple signaling mechanisms in the genesis of autism might aid in the discovery of novel targeted therapies and the development of new pharmacological treatments. Here, we used statistical approaches to analyze transcriptome data set linked to ASD to predict hub genes, gene set variability, and other pathological changes using differentially expressed genes (DEGs). Furthermore, we also confirmed the interaction of proteins with one another, the transcriptional and post-transcriptional processes, as well as the selection of small pharmacological molecules.

2. Materials and methods

The schematic diagram in Fig. 1 depicts the whole method of the integrated systems biology and analytical technique to discover molecular markers and pathways in brain tissues of ASD.

Table 1
Overview and the quantitative measurements of datasets used in this study.

SL No	GEO accession	GEO Platform Sample	Sample Size	Control	Case	Number of DEGs		
						Up	Down	Total
1.	GSE30573	GPL9115	6	3	3	2142	1543	3685
2.	GSE62098	GPL11154	12	6	6	577	93	670

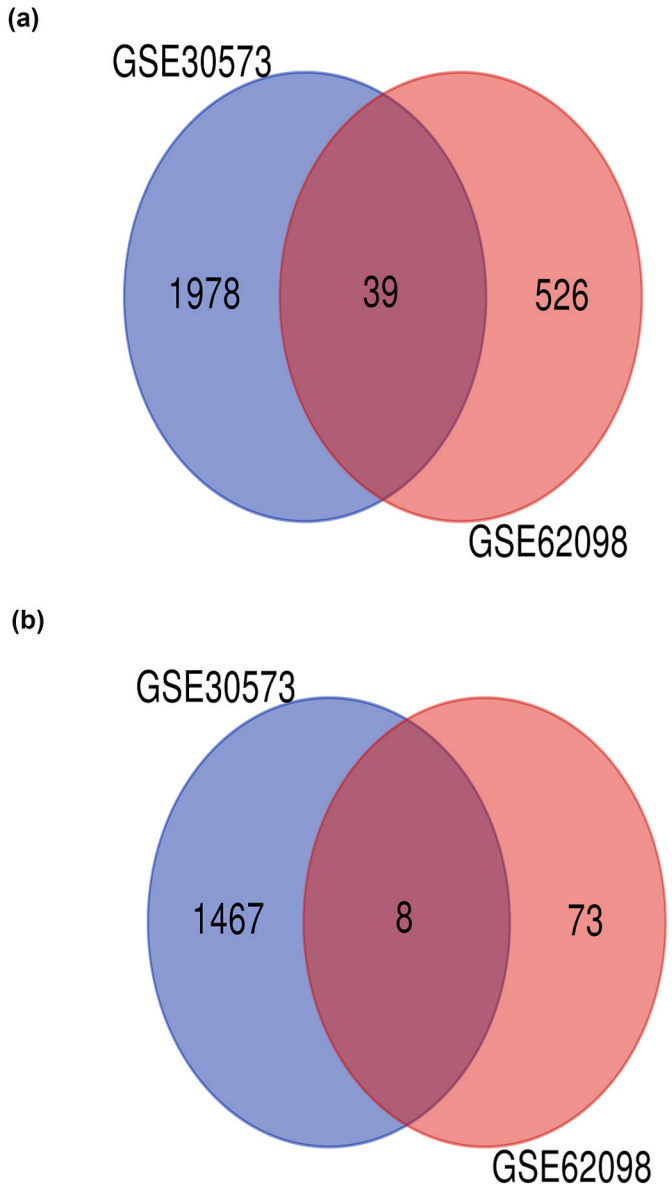


Fig. 2. Identification of common DEGs. (a) Upregulatory common DEGs. (b) Downregulatory common DEGs.

2.1. Data collection

We attempted to obtain some datasets relevant to ASD at the start of our research. We sought relevant datasets in the GEO (Gene Expression Omnibus) collection of the NCBI (National Center for Biotechnology Information)[1] [39,40]. The following keywords were used to search these datasets: "Autistic spectrum disease," "Blood," "Brain," and "*Homo sapiens*". From there, we collected two RNA sequence datasets, GSE30573 [41] and GSE62098 [42]. In the GSE30573 dataset, samples were collected from brain region BA41 (temporal cortex), brain region BA09 (frontal cortex) and brain region BA22 (temporal cortex) and in the GSE62098 dataset, samples were collected from frozen postmortem brain. The GSE30573 dataset had 6 samples (3 cases, 3 controls), whereas the GSE62098 dataset had 12 samples (6 cases, 6 controls). Then, examined these datasets and identified differentially expressed genes (DEGs) that were common in both datasets. These DEGs were used for the next processes.

2.2. Screening of DEGs

To bring out the significant DEGs, GSE30573 and GSE62098 datasets were analyzed through a statistical tool, GREIN[2] [43]. We adjusted p-value < 0.05 and $|\log F\ C| > 1.0$ and $|\log F\ C| < -1.0$ (large scale Fold change) to find statistically significant DEGs, applying Benjamini–Hochberg (BH) technique [44]. For the resultant DEGs we carried out a Venn analysis,[3] a bioinformatics web tool to compare two datasets and uncover the shared DEGs [45,46].

2.3. Functional enrichment of gene sets

Using the online bioinformatics tool Enrichr,[4] the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway, Reactome pathway, and the Gene Ontology (GO) of the DEGs were annotated. Enrichr is a major platform with several distinct genetic engineering libraries to examine gene enhancement across the whole genome [47]. GO is vital for bioinformatics to attempt to unify the representation of gene and gene product properties across all species as well as functional enrichment (biological process, cellular component, and molecular activities) [48,49]. It's a common method for studying the functional aspects of large-scale transcription or genomic data. To understand cellular metabolism, the KEGG pathway, and the Reactome pathway are usually applied [50].

2.4. Protein-protein interaction analysis and hub protein extraction

Protein-protein interaction (PPI) refers to the interactions between proteins in a biological process. Proteins enter a cell with a comparable affliction, which is produced by a protein-protein network. The assessment and study of the PPI network and its functions are necessary for understanding and gaining insights into cellular machinery activities. It is a fundamental goal in cellular and system biological studies [51,52]. In this study, the PPI network of proteins encoded by DEGs was built, using the STRING database [53]. In the STRING intercom, a median confidence score of 600 was used. NetworkAnalyst was used to do the topological analysis [54]. The PPI network was formed by nodes, edges, and connections with the nearly entangled nodes being referred to as hub genes. Then, we used the CytoHubba plugin [55] in Cytoscape software[5] [56] to visualize the target network. The degree method was used to discover the hub Genes. CytoHubba provides 11 methods for topological analysis of networks from different perspectives. Thus, the

[1] https://www.ncbi.nlm.nih.gov/geo/.

[2] http://www.ilincs.org/apps/grein/.

[3] http://bioinformatics.psb.ugent.be/webtools/Venn/.

[4] https://maayanlab.cloud/Enrichr.

[5] https://cytoscape.org/.

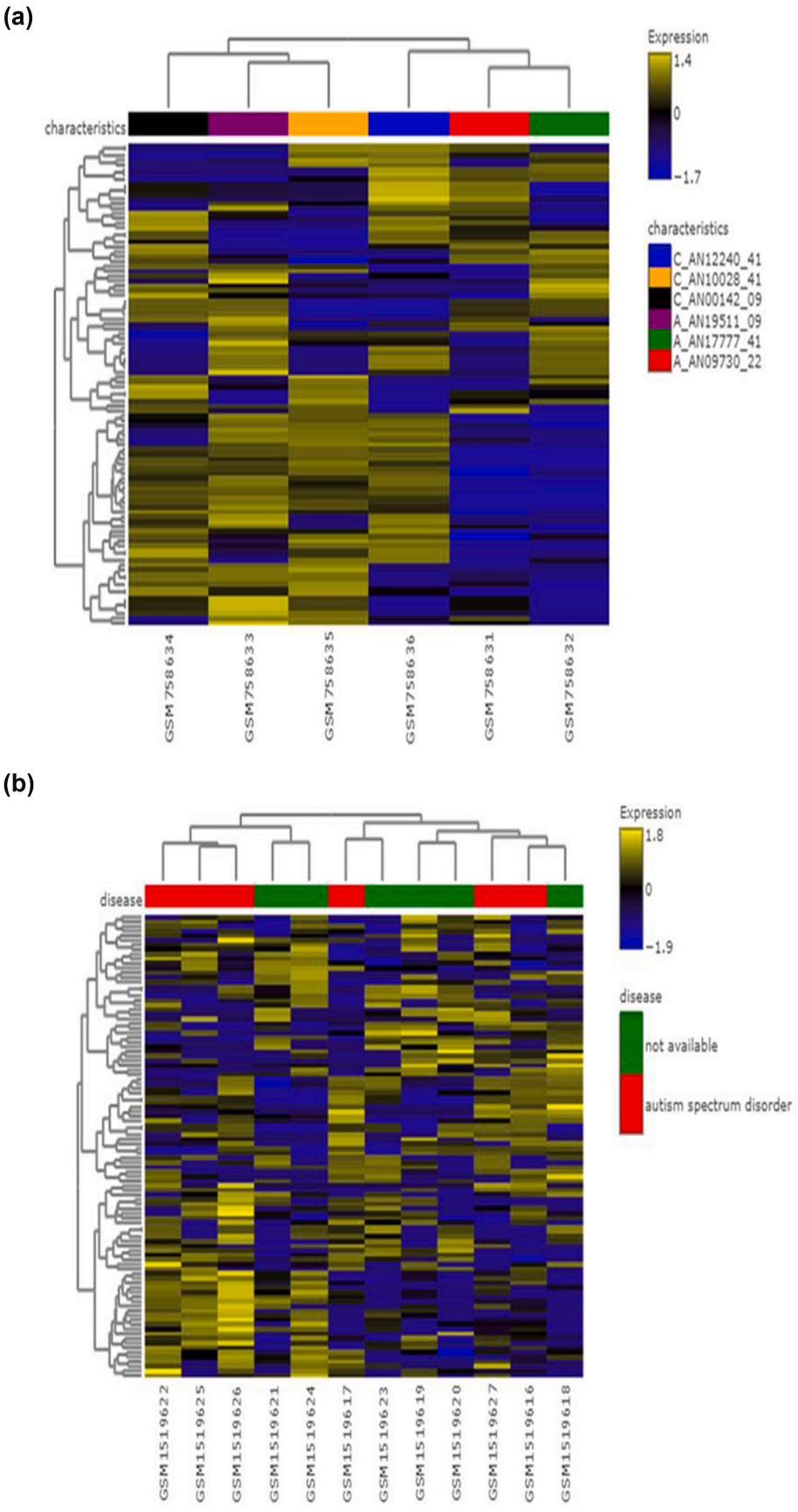


Fig. 3. Hierarchical heat map clustering of gene expression. (a) GSE30573 (b) GSE62098. In these graphs, upregulated gene expressions are denoted by yellow, downregulated gene expressions are denoted by blue, and insignificant gene expressions are denoted by black. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
The functional enrichment analysis to uncover the most important GO terms of DEGS.

Category	GO ID	GO Terms	p-Value
GO Biological process	GO:0071357	cellular response to type I interferon	2.85×10^{-05}
	GO:0060337	type I interferon signaling pathway	2.85×10^{-05}
	GO:1903363	negative regulation of cellular protein catabolic process	8.63×10^{-05}
	GO:0048568	embryonic organ development	3.45×10^{-04}
	GO:0003181	atrioventricular valve morphogenesis	6.55×10^{-04}
	GO:0060317	cardiac epithelial to mesenchymal transition	7.66×10^{-04}
	GO:0061515	myeloid cell development	7.66×10^{-04}
	GO:0032803	regulation of low-density lipoprotein particle receptor catabolic process	8.18×10^{-04}
	GO:0043062	extracellular structure organization	8.65×10^{-04}
	GO:0045229	external encapsulating structure organization	8.91×10^{-04}
	GO:0005887	integral component of plasma membrane	7.75×10^{-04}
GO Cellular Process	GO:0042612	MHC class I protein complex	2.85×10^{-05}
	GO:0008076	voltage-gated potassium channel complex	4.517×10^{-03}
	GO:0034705	potassium channel complex	6.248×10^{-03}
	GO:0044295	axonal growth cone	6.248×10^{-03}
	GO:0032279	asymmetric synapse	6.248×10^{-03}
	GO:0030670	phagocytic vesicle membrane	8.066×10^{-03}
	GO:0062023	collagen-containing extracellular matrix	8.477×10^{-03}
	GO:0014069	postsynaptic density	8.852×10^{-03}
	GO:0031901	early endosome membrane	1.215×10^{-02}
	GO:0005251	delayed rectifier potassium channel activity	2.26×10^{-04}
GO Molecular Function	GO:0022843	voltage-gated cation channel activity	2.68×10^{-04}
	GO:0005249	voltage-gated potassium channel activity	9.38×10^{-04}
	GO:0004936	alpha-adrenergic receptor activity	1.219×10^{-03}
	GO:0005267	potassium channel activity	9.774×10^{-03}
	GO:0005261	cation channel activity	1.2578×10^{-02}
	GO:0050750	low-density lipoprotein particle receptor binding	1.857×10^{-02}
	GO:0070325	lipoprotein particle receptor binding	2.6931×10^{-02}
	GO:0005546	phosphatidylinositol-4,5-bisphosphate binding	3.1483×10^{-02}
	GO:0001540	amyloid-beta binding	3.7072×10^{-02}

top ten hub genes from the PPI network were recognized. By using the MCODE plugin [57] in Cytoscape, the top two PPI network modules were discovered. The enrichment studies were used to further examine and describe the top two modules of the PPI network.

2.5. Identification of transcriptional factors and post-transcriptional factors

A transcription factor (TF) is a protein that binds to a specific gene and regulates the pace at which genetic information is transcribed. Connections between transcriptional regulatory proteins and their gene products are described by transcriptional regulatory circuits [58,59]. Post-transcriptional factors or miRNAs are tiny internal RNA molecules, present in mammals, trees, and certain viruses around [60,61]. They can lower the stabilization and translational activities of messenger RNAs (mRNAs) with completely or substantially similar sequences to regulate post-transcriptional gene expressions [60,62]. These TFs and miRNAs may influence relevant DEGs. So, to investigate the TFs interactions and miRNAs interactions among DEGs, we used the JASPR [63] and miRtarbase database [64] respectively in NetworkAnalyst [54].

2.6. Candidate drugs identification

Based on the DEGs of ASD, we identified therapeutic targets utilizing the drug signature database (DSigDB) [65] by using Enrichr. DSigDB is a worldwide database for identifying targeted messages. It is a novel gene set resource that plays an important role in the analysis of new genes that connects drug molecules with their target genes. DSigDB presently has 22527 gene sets, 17389 distinct chemicals, and 19531 genes [65]. DSigDB gene sets eventually merge with GSEA software, allowing for the linking of transcriptional activation with drugs for therapeutic applications and research programs. Differentially expressed genes are associated with the drug signature database. These drugs might have inhibitory properties against ASD [66].

2.7. Verification of biomarkers

Biomarkers comprise components or processes which are statistically detectable and medically verified to indicate descriptive, preventive, as well as a potential role with a standard and disordered physical situation [67]. To confirm our potential biomarkers, we performed a literature-based analysis. We have gone through ASD-related literature that supports the findings of our study. These literature analyses can make a valuable contribution to our identified potential biomarkers, which we've uncovered.

3. Results

3.1. DEGs identification

We retrieved two high-performance sequencing RNA datasets from the NCBI-GEO database. The GSE30573 dataset revealed 3685 DEGs that included 2142 upregulated and 1543 downregulated DEGs and the GSE62098 dataset revealed 670 DEGs including 577 upregulated DEGs and 93 downregulated DEGs (Table 1). We used the Venn diagram tool to find common differentially expressed genes between two datasets. A total of 47 common DEGs (39 upregulated and 8 downregulated) were figured out (Fig. 2). Furthermore, we found 713 DEGs when we stratified our study into the temporal and frontal cortex of the brain from the GSE30573 dataset. Among them, 196 genes were upregulated DEGs and 517 genes were downregulated DEGs (S1). Here, Fig. 3 shows the heat map cluster of the top 100 upregulated genes and downregulated genes. Table 1 represents the datasets and quantitative measurements of this study. We have stratified the analysis into the temporal cortex and frontal cortex to see the differences in the differentially expressed genes which is shown in Table A in the supplementary file.

3.2. Identification of gene ontology and pathway enrichment

Enrichr was used to find out the GO terms and pathway enrichment analysis to determine the biological relevance and enriched pathways associated with this present study. GO analysis was used to determine the molecular functions, biological roles, and cellular activities of the DEGs. Table 2 displays the list of top ten keywords in the categories of biological processes, molecular activities, and cellular functions. We

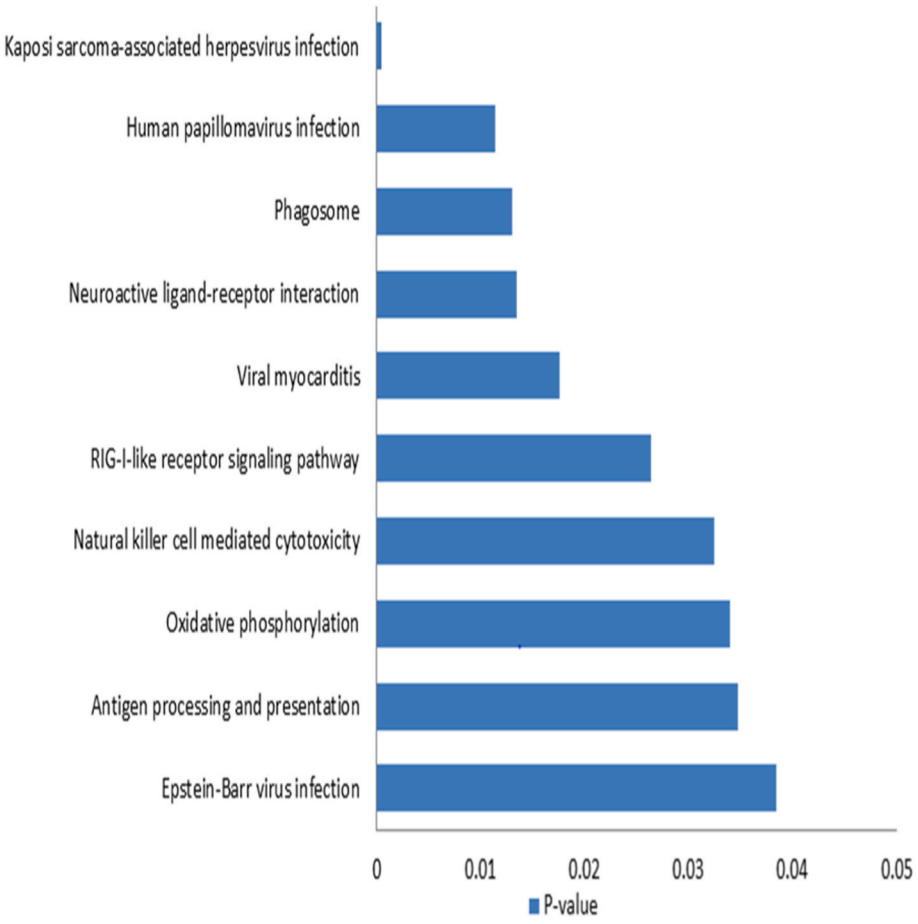


Fig. 4. Significantly enriched KEGG pathway.

Table 3
Significantly enriched Reactome pathway.

Reactome pathway	*P*-value
Interferon alpha/beta signaling *Homo sapiens* R-HSA-909733	3.70×10^{-05}
Interferon Signaling *Homo sapiens* R-HSA-913531	4.57×10^{-04}
Neuronal System *Homo sapiens* R-HSA-112316	4.71×10^{-04}
Voltage gated Potassium channels *Homo sapiens* R-HSA-1296072	6.33×10^{-04}
Antigen Presentation: Folding, assembly and peptide loading of class I MHC *Homo sapiens* R-HSA-983170	1.49×10^{-03}
Potassium Channels *Homo sapiens* R-HSA-1296071	2.17×10^{-03}
Interaction between L1 and Ankyrins *Homo sapiens* R-HSA-445095	2.31×10^{-03}
Adrenoceptors *Homo sapiens* R-HSA-390696	2.87×10^{-03}
ER-Phagosome pathway *Homo sapiens* R-HSA-1236974	2.97×10^{-03}

also used functional enrichment analysis to find molecular pathways that were enriched by the common DEGs. From the KEGG pathway, we identified that the significant pathways were mostly connected to Epstein-Barr virus infection, antigen processing, and oxidative phosphorylation and Reactome pathways were mainly connected with interferon signaling and neuronal system. Fig. 4 represents the KEGG pathway and Table 3 represents the significantly enriched Reactome pathway.

3.3. Development of the PPI network and determination of hub genes

We operated STRING at NetworkAnalyst to find PPI networks for both up and down-regulated genes to evaluate the PPI among DEGs. The PPI network is a scale-free architecture made up of a few highly linked proteins known as hub genes. KIT, PIN1, GATA1, GRIN2A, PBX2, BLK, ATP6V1B1, TCF7L1, TRAF1, and HSPG2 were identified as hub genes by topological analysis (Fig. 5). Among these hub genes, KIT, GRIN2A, BLK, and TRAF1 had been strongly associated with ASD, which we explained with relevant references in the biomarkers validation section. These hub genes might be used as biomarkers, which could lead to novel treatment approaches for the illnesses being studied. Table 4 represents the degree of the top ten hub genes.

In addition, the modular structure of the PPI network revealed the existence of two strongly linked modules (Fig. 6), according to the research. There were three nodes and three edges in these two modules. These two modules can assist us in comprehending their proximity and connection.

3.4. Determination of regulatory biomolecules

We used a network-based method to detect significant alterations at the transcriptional and post-transcriptional levels. These transcriptional and post-transcriptional regulatory networks of corresponding DEGs uncovered the interactions between transcriptional factors (TFs) and post-transcriptional factors (miRNAs). Fig. 7 represents the TFs-DEGs network. This network revealed the top ten transcriptional factors which were associated with ASD. They were NFIC, USF2, TFAP2A, RELA, FOXL1, GATA2, YY1, FOXC1, NFKB1, and E2F1.

Furthermore, Fig. 8 shows the miRNAs-DEGs interactions network.

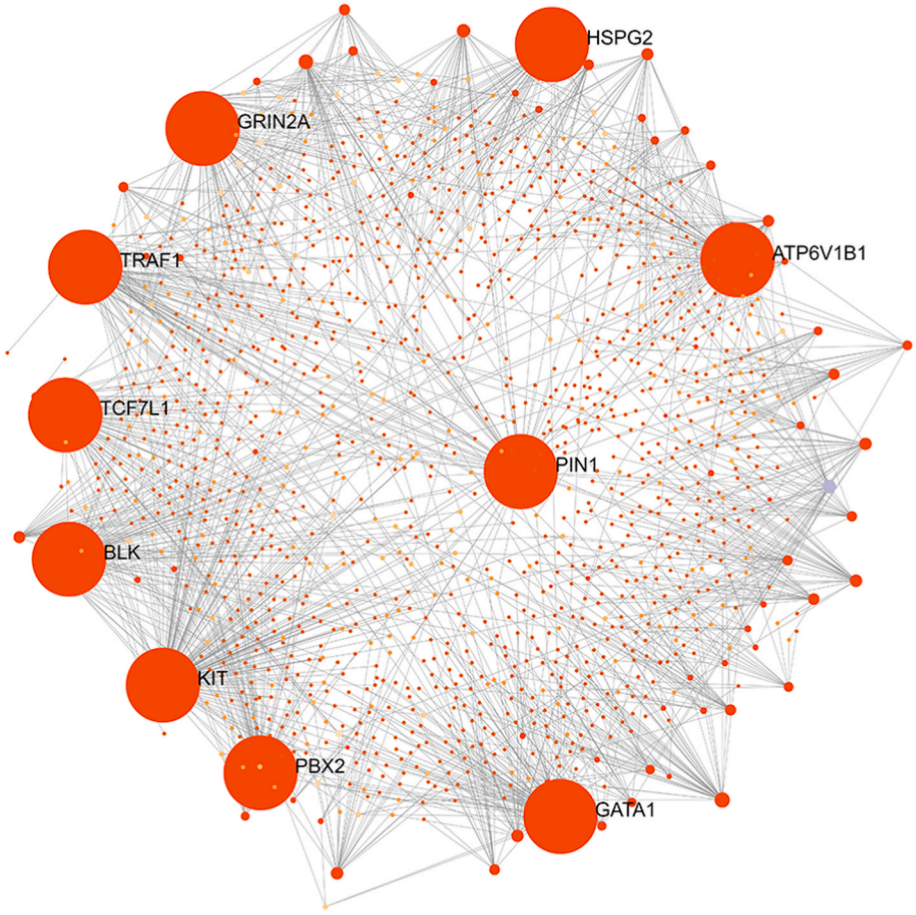


Fig. 5. PPI network of DEGs. In the figure, DEGs are shown by the nodes, while interactions among genes are shown by the edges. The highlighted nodes are indicating the top 10 hub genes.

Table 4
The ten putative HUB genes of the PPI network in topological attributes

Rank	Hub genes	Full form	p-Value	Degree	Regulation
1	KIT	Proto-Oncogene, Receptor Tyrosine Kinase	5.67×10^{-04}	132	Down
2	PIN1	Peptidylprolyl Cis/Trans Isomerase, NIMA-Interacting 1	2.86×10^{-02}	68	Down
3	GATA1	GATA Binding Protein 1, Erythroid transcription factor	8.41×10^{-03}	61	Up
4	GRIN2A	Glutamate Inotropic Receptor NMDA Type Subunit 2 A)	1.96×10^{-04}	59	Down
5	PBX2	Pre-B-cell leukemia transcription factor 2	1.76×10^{-07}	55	Up
6	BLK	Proto-Oncogene, Src Family Tyrosine Kinase	1.27×10^{-02}	52	Up
7	TRAF1	TNF receptor-associated factor 1	1.92×10^{-03}	51	Up
8	ATP6V1B1	ATPase H + Transporting V1 Subunit B1	1.42×10^{-03}	51	Up
9	TCF7L1	Transcription factor 7-like 1	1.17×10^{-2}	51	Up
10	HSPG2	Heparan sulfate proteoglycan 2	4.6×10^{-3}	43	Up

The top ten miRNAs were mir-335-5p, mir-26b-5p, mir-124-3p, mir-192-5p, mir-1-3p, mir-215-5p, mir-6825-5p, mir-146a-5p, mir-8485, and mir-93-5p.

3.5. Identification of candidate drugs and small molecules

This study revealed some candidate drug molecules. We used the DsigDB database to figure out the molecular interactions between drug-like molecules and DEGs. Based on their p-value, the top ten chemical compounds were retrieved. This allowed us to suggest viable medicines and pharmacological targets. Table 5 represents candidate drug compounds along with their chemical formula and chemical structure.

3.6. Potential biomarkers verification

We did literature research to verify our suggested possible goals in our research. Our investigation included ten hub genes (KIT, PIN1, GATA1, GRIN2A, PBX2, BLK, TCF7L1, TRAF1, and HSPG2) which were connected with ASD (Table 4). The dysregulation of these genes can cause the severe neurodevelopmental disorder. KIT mutation has been shown to cause intellectual disability [68]. It has been also shown as a candidate gene for the lack of communication in ASD [59]. A. J. Kilsby et al. [58] and A. Ben'ıtez-Burraco et al. [69] identified the KIT gene as a biomarker in their research that is associated with our study. Mutations in the PIN1 gene can cause neural diseases. Many findings show that a decrease of PIN1 activity may rise to synaptic plasticity depletion in the development of Alzheimer's disease [70]. Mutations in the GATA1 can cause down syndrome [71,72] and research studies indicated ASD, behavioral, and mental problems are all common in persons with Down

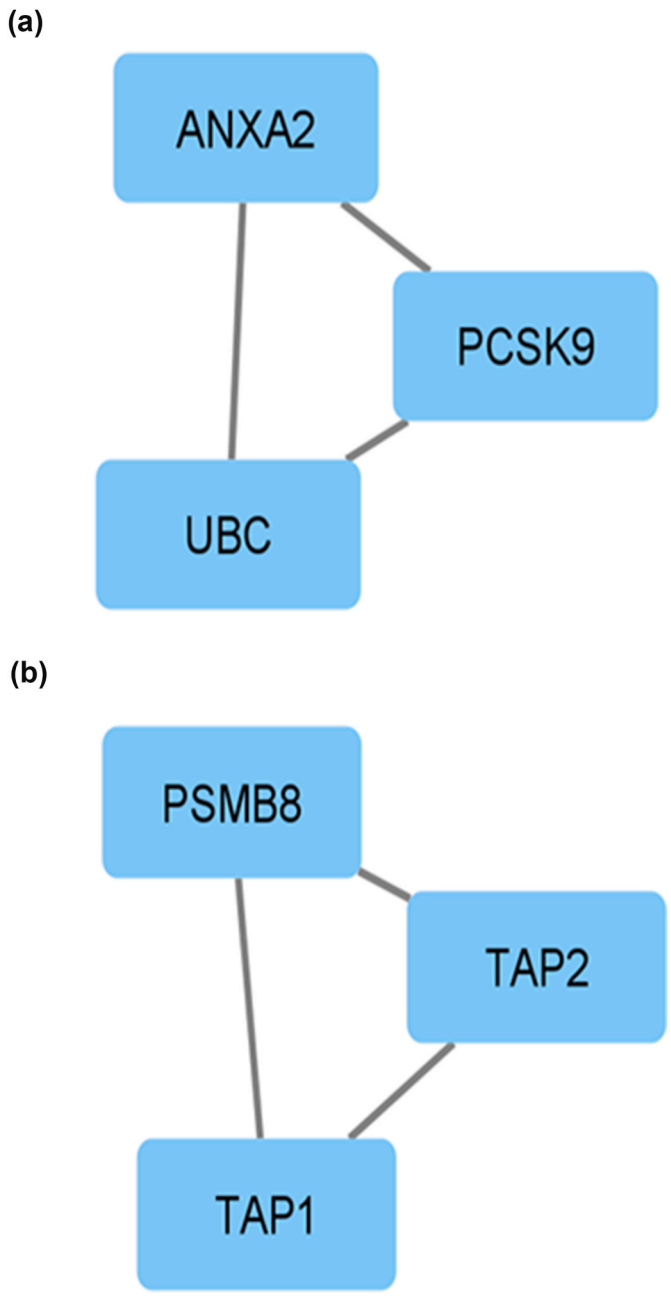


Fig. 6. Top two modules (a, b) of PPI network. The DEGs are represented by the nodes, while the connections between two Genes are shown by the edges.

syndrome [73]. So indirectly, the GATA1 gene may control ASD. GRIN2A has been proven as a candidate gene for ASD [74,75]. Mutations in the GRIN2A gene are the reason for learning disabilities and cognitive problems [76,77]. BLK has been linked to the processes of ASD genesis in the cross-tissue study [78]. C. Rodriguez-Fontenla and A. J. T. p. Carracedo proved BLK as an ASD biomarker that is associated with our study [78]. The functionality of PBX2 in ASD has yet to be discovered. Furthermore, dysregulation of TCF7L1 and FATP6V1B2 gene is linked with intellectual impairments, but ATP6V1B2 is yet to be proof of its association with Autism [79]. TRAF1 is a protein complex that participates in a variety of signal transduction and physiological functions [80]. M. R. Rahman et al. Identified TRAF1 as a biomarker of ASD in their research [81]. It is linked with our present study. The previous study suggests that the HSPG2 factor is associated with Alzheimer's disease, although its function in ASD is still unclear [82]. Table 6 represents only those biomarkers that were associated with previous ASD-related literature as well as associated with our current study.

4. Discussions

ASD is a complicated and medically diverse condition distinguished by a wide range of symptoms. Doctors, educators, and support organizations all around the world have documented substantial growth in the number of children diagnosed with ASD. Despite extensive study, molecular signatures and pathways of this disorder remained unclear, hence the number of ASD sufferers is expanding bit by bit [83]. While ASD mostly impacts the operation of the brain, notably its effects on social functionality and comprehension, we do not know the full extent to which other organisms and processes are affected by it. The published studies have revealed extensive alterations both in systemic and intracellular immune systems of children with autism [36]. ASD brain samples show evidence of severe, continuing inflammation, as well as changes in immunological transmission of gene pathways. Furthermore, several genetic investigations have found a connection between autism and genes involved in both the neurological and immunological systems [84]. Both systems can be affected by the changes in these pathways. To discover these pathways and molecular signatures that might serve as possible treatment targets or biomarkers for ASD, we analyzed regulatory patterns, molecular key pathways, PPI interactions, TFs-DEGs interactions, miRNAs-DEGs interactions to look at differential gene expression of this disorder.

We worked with differentially expressed genes which were common between the two datasets. From there, we identified some substantial GO terms in the biological process including cellular response to type I interferon, negative regulation of cellular protein, and atrioventricular valve morphogenesis. These were the top GO terms. The synthesis of type I interferon is a frequent cellular response to viral infections and connects to a common, heterodimeric cellular receptor and triggers the production of antibodies through signaling pathways [85,86]. Then, negative regulation of cellular protein can appear under different types of physiological complications [87]. The atrioventricular valves divide atria and ventricles from each other and control blood flow during the heart pump [88]. Then in the section of cellular component, we determined the major GO terms which were an integral component of the plasma membrane and MHC class I protein complex. Here, plasma membrane functions like power and metabolic capacitor [89], and MHC class I protein complex functions effectively in the immune response [90]. Moreover, the most important molecular activities in the DEGs were delayed rectifier potassium channel activities, activities of voltage-gated cation channels, and alpha-adrenergic receptor activity. Mutations in the potassium channel might play a pivotal part in ASD etiology [91]. It is proven that genes associated with voltage-gated cation channel activity have been linked to different types of neurodevelopmental disorder [92]. Furthermore, alpha-adrenergic receptors are commonly utilized as a stimulant to boost the stimulant's effectiveness [93]. It controls the neurotransmission process and central nervous system by engaging and stimulating norepinephrine and epinephrine hormones [94].

On the other hand, we also identified KEGG and Reactome pathways. Pathway studies demonstrate how the organism reacts to its intrinsic changes. Pathway analysis is a model approach for illustrating the interplay of multiple illnesses via fundamental molecular or biological mechanisms. To find out a systematic investigation of genetic functions, the KEGG pathway database can hold a higher level of functional information [95]. Again, the Reactome pathway connects human proteins to their molecular activities. It enables the creation of a resource that serves as both a repository for living organisms as well as a technique for identifying unanticipated functional connections in datasets like gene regulation surveys [96]. The KEGG pathway analysis revealed the majority of the pathways were associated with Epstein-Barr virus infection, oxidative phosphorylation, and natural killer cell-mediated cytotoxicity. The Epstein-Barr virus is a human pathogen. It is one of the most

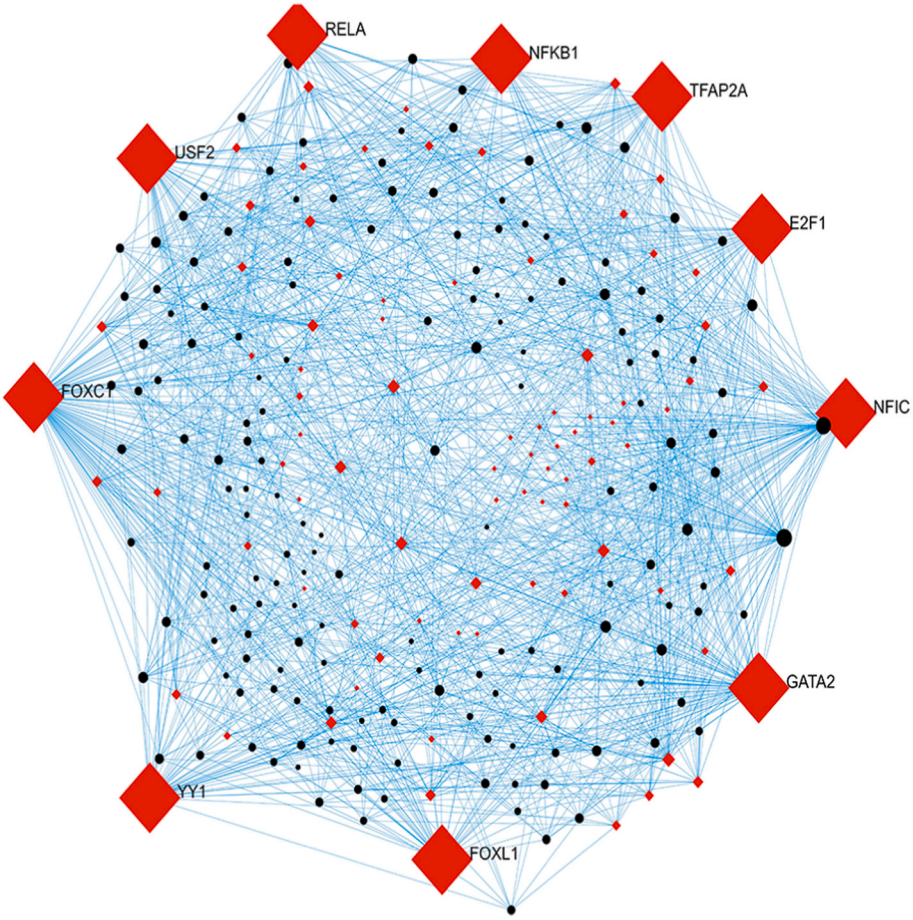


Fig. 7. The regulatory network of TFs-DEGs interactions. In the figure, DEGs are shown by the black nodes, while TFs are shown by the red nodes. The highlighted nodes are indicating the top 10 TFs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

prevalent viruses. This viral infection can cause cerebellitis encephalitis, radiculopathy, meningitis, and other brain disorders [97]. Then, oxidative phosphorylation deficiencies in people can cause significant developmental issues [98]. Natural killer cells perform an essential role in the immunological regulation of the human body. They have a great effect on brain regulation. Immunological dysfunction like inappropriate immune responses or autoimmunity can cause neurodegenerative problems, particularly in the initial phases of brain growth and development [99]. On the other hand, the Reactome pathway is mostly associated with interferon-alpha/beta signaling. The major role of interferon-alpha/beta signaling is to inform the cell when it is infected with a virus [100]. Analysis of the PPI network is a potential method for determining the disease's underlying processes [101]. As a result, we looked at the protein intercom to find hub genes. We have already discussed these hub genes and their dysregulation in the biomarkers validation part. As these hub genes play a fundamental role in the pathogenic mechanisms of ASD, we recommend more basic experimental studies to uncover the probable relevance of these genes. The regulatory biomolecules (TFs and miRNAs) were also discovered. As a result, biomolecule alternation may give crucial information about ASD gene expression. Regulatory transcription factors (NFIC, USF2, TFAP2A, RELA, FOXL1, GATA2, YY1, FOXC1, NFKB1, and E2F1) might be linked to ASD and central cellular processes. NFIC, FOXL1, and FOXC1 dysregulation were connected with ASD [81]. Here, FOXC1 duplication or deletion has been linked to cerebellar malformation, indicating that mutations in the FOXC1 are involved in neurodevelopmental disorders [102]. GATA2 regulates GABAergic neuron growth, motility, and neuron-specific gene expression [103]. In development and neurological disorders, YY1, E2F1, and USF2 were overrepresented [104–106]. Mutations of these transcription factors can cause neurodevelopmental disorder. TFAP2A transcription controls neural crest patterning and mutation of TFAP2A causes neuroblastoma [107]. However, this factor is not associated with any brain disorder. An analysis found that NFKB1 provides an important role in the progression of treatment-resistant schizophrenia in the Chinese Han community [108]. According to literature reviews, the function of RELA was not linked with ASD but in our current study, we marked RELA as a new ASD-related regulatory transcription factor. As in 70% of the central nervous system, miRNAs are present, so dysregulation of miRNAs might be used as biomarkers [109]. mir-335-5p, mir-26b-5p, mir-124-3p, mir-192-5p, mir-1-3p, mir-215-5p, mir-6825-5p, mir-146a-5p, mir-8485, and mir93-5p were the first ten miRNAs which were visualized in miRNA-DEGs network. Mutations in the mir-335-5p link with a serious neurodevelopmental Rett syndrome [110]. mir-26b-5p was identified as a potential biomarker in neurological disorder [111]. mir-93-5p was scientifically proven ASD-related miRNA [112]. miR-1-3p was proven as a potential biomarker in amyotrophic lateral sclerosis/motor neuron disease [113]. mir-124-3p and mir-192-5p were not linked to ASD according to other literature but in our study, we found that mir-124-3p and mir-192-5p were related to ASD. mir-146a-5p controls the growth and activities of cancer cells [114] and mir-215-5p acts as a tumor suppressor [115]. However, they were not related to any neurodevelopmental disorder.

Furthermore, we had identified candidate drug molecules. They were retinoic acid, pyrrolidine dithiocarbamate, silica, trichostatin A, cytarabine, zinc sulfate, 8-Bromo-cAMP, decitabine, valproic acid, guanidine hydrochloride. It has been proven that greater risk for ASD and mild ASD in a Chinese population was related to decreased serum retinoic acid [116]. Reduced CD38 transcription is upregulated by

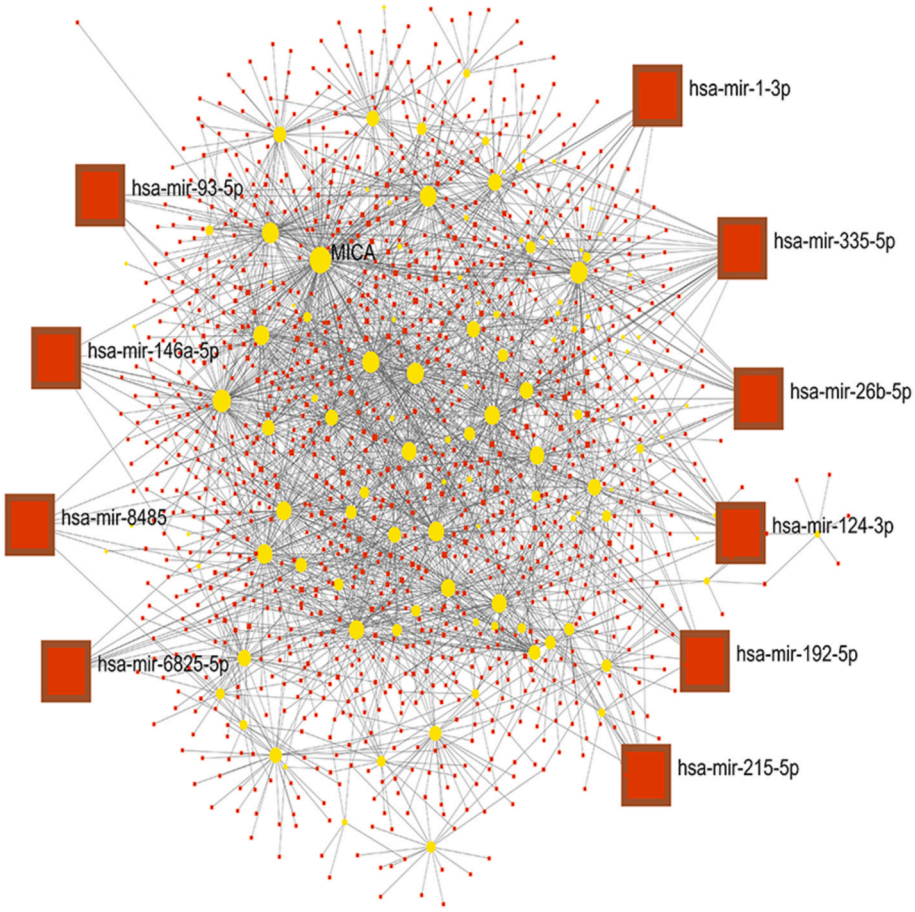


Fig. 8. The regulatory network of miRNAs-DEGs interactions. In the figure, DEGs are shown by the yellow nodes, while miRNAs are shown by the red nodes. The highlighted nodes are indicating the top 10 miRNAs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

all-trans retinoic acid in lymphoblastic cell lines from autism spectrum disorder patients [117]. So, retinoic acid can be considered a significant therapy for the treatment of ASD. Pyrrolidine dithiocarbamate diminishes surgical inflammation and cognitive impairments [118]. Cytarabine was identified as a novel drug for Alzheimer's disease [119]. Valproic acid (VPA) is a contraceptive and a behavioral stabilizer drug. Language impairments and the increase of ASD are related to it. Medical data suggested a connection between VPA and the development of ASD risks [120].

Our research different from previous research on ASD patients. It is believed that genes regulated in multiple tissues have a significant role in the transmission of complicated illnesses including ASD. Despite the strong hereditary basis of ASD, several dysfunctional neurodevelopmental processes could reappear in leukocytes on a regular basis, allowing postpartum studies in easily obtainable tissues [70,81]. Numerous organics in brain cells from ASD children are disrupted, including cell growth, maturation, and microtubule architecture, which is consistent with some dysfunctional mechanisms previously identified in pluripotent stem cells of people with ASD [81]. Furthermore, the examination of brain cells cannot reveal key facts on dysfunctional biological activities that happen in ASD blood cells regardless of the existence of congenital and genetic anomalies.

However, more investigations will be required to confirm these findings. We anticipate that our procedure can assist the researchers not just with their study, but also with early detection and directing suitable treatment measures.

5. Conclusion

One of the most prevalent neurodegenerative disorders in the world is ASD. It is a multifaceted disorder with a wide range of clinical and genetic variability. Approximately hundreds of genes have been discovered in recent decades that contribute to severe communication, social cognitive, and behavior impairments. In this study, we used a network-based method to identify important pathways and biomolecules in ASD to find common DEGs. These DEGs were used in pathway analysis and also used to uncover protein-protein interactions, transcription factors, post-transcriptional factors, and potential therapeutic compounds. We had collected some differentially expressed common genes. Between them, Several TFs and miRNAs have been discovered as candidate transcriptional and post-transcriptional factors of the DEGs. As a result, we identified some possible molecular signatures and pathways that are often dysregulated in ASD brain tissues. However, we hope that our observations will provide new insights on the molecular basis of ASD and aid in the identification of prospective medicines.

Author contributions

Sadia Afrin Bristy, A. M. Humyra Islam and Md Habibur Rahman contribute to conception and design. Sadia Afrin Bristy and K. M. Salim Andalib performed the computational analyses and wrote the draft manuscript; Umama Khan helped in the preparation of the tables and figures. Md. Abdul Awal and Md Habibur Rahman were involved in the preparation of the important intellectual content and critical revision;

Table 5
The DsigDB gene sets were used to identify the top ten medicines in drug target enrichment.

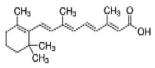
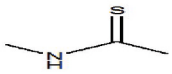
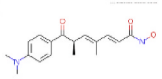
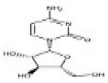
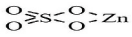
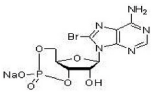
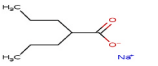
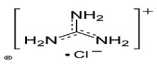
Candidate Drugs	*P*-Value	Chemical Formula	Chemical Structure
Retinoic acid CTD 00006918	4.89×10^{-04}	$C_{20}H_{28}O_2$	
Pyrrolidine dithiocarbamate CTD 00001021	0.001949895	$C_5H_9NS_2$	
Silica CTD 00006678	0.005023575	SiO_2	
trichostatin A CTD 00000660	0.00895069	$C_{17}H_{22}N_2O_3$	
cytarabine CTD 00005743	0.012590962	$C_9H_{13}N_3O_5$	
Zinc sulfate CTD 00007264	0.021665516	$ZnSO_4$	
8-Bromo-cAMP, Na CTD 00007044	0.028224152	$C_{10}H_{10}BrN_5NaO_6P$	
Decitabine CTD 00000750	0.045867237	$C_8H_{12}N_4O_4$	
VALPROIC ACID CTD 00006977	0.047008054	$C_8H_{16}O_2$	
Guanidine hydrochloride	0.048861168	$CH_5N_3 \cdot HCl$	

Table 6
Potential biomarkers validation through literature reviews.

Gene Name	ASD
KIT	[59,68]
GRIN2A	[74–77]
BLK	[78]
TRAF1	[81]

Md. Abdul Awal and Md Habibur Rahman supervised the whole study. All authors approved the final version for submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

(ASD) Autism Spectrum Disorder
(GSEA) Gene Set Enrichment Analysis
(DEGs) Differentially Expressed Genes
(GO) Gene Ontology
(CDC) Centers for Disease Control
(TFs) Transcription factors
(BSMMU) Bangabandhu Sheikh Mujib Medical University
(GEO) Gene Expression Omnibus
(GREIN) GEO RNA-seq experiments interactive navigator
(NCBI) National Center for Biotechnology Information
(KEGG) The Kyoto Encyclopedia of Genes and Genomes
(BH) Benjamini-Hochberg.
(PPI) Protein-Protein Interaction
(mRNAs) Messenger RNAs
(DSigDB) Drug Signature Database

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imu.2022.100871.

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