International Journal Dental and Medical Sciences Research
Volume 6, Issue 5, Sep - Oct 2024 pp 386-396 www.ijdmsrjournal.com ISSN: 2582-6018

Exploring the Protein Interactions between the Human Gut and Autism Spectrum Disorder: Functional Characteristics and Network Analysis

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Submitted: 10-10-2024 Accepted: 20-10-2024

ABSTRACT
Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by challenges in social communication, restricted interests, and repetitive behaviors. In recent years, growing evidence has suggested a significant link between ASD and gastrointestinal (GI) disturbances, leading researchers to explore the intricate relationship between the gut and the brain in individuals with autism. The gut-brain axis, a bidirectional communication system between the central and enteric nervous systems, has emerged as a crucial factor in understanding this relationship. Of particular interest is the role of proteins in the gut and the brain, and how their interactions may contribute to or be affected by ASD. This study aimed to identify and analyze protein interactions and their molecular functions in the autism spectrum disorder (ASD)-gut relationship. Using a protein interaction network approach, we sought to uncover key pathways and mechanisms underlying the connection between ASD and gastrointestinal processes. The constructed network revealed new interactions. These included HTR1A in ASD interacting with GRM4 and CRHR1 in the gut and FPR2 in ASD interacting with FPR1 in the gut respectively. Also, the results showed ACTC1 in ASD which interacts with GSN, MYH10, and TTN in the gut. It was also suggested that FZD5 ASD is a protein candidate in interaction with FZD4 and FZD8 in the gut. positive regulation of intracellular signal transduction, second messenger mediated signaling, cell-cell signaling, multicellular organismal process, response to chemical, negative regulation of the biological process, cellular ion hemostasis, G-protein coupled receptor activity, response to extra stimuli are the main molecular roles of the protein network involved in ASD-gut contact. The results of the illness association research showed that deficiencies in the ASD-GUT relationship are also reflected in other disease networks, including metabolic, cardiovascular disease, and pharmacogenomics. We were able to determine the primary molecular roles and genes associated with illness in the ASD-gut interface protein by examining the network. Confirming the relevance of these newly compute-resolved interactions and the genetic connections between anomalies in ASD-gut interactions and the related disease will require more experimental research.

Abbreviations:ASD:Autism spectrum disorder; GI: gastrointestinal; PPIs: protein-protein interactions; HGNC: HUGO Gene Nomenclature Committee;PINs: protein interaction networks; GAD: Genetic Association Database; DAVID: Database for Annotation Visualization and Integrated Discovery; MS: mass spectrometry; STRING: Search Tool for the Retrieval of Interacting Genes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes,

I. INTRODUCTION

Autism spectrum disorder (ASD) is a type of condition characterized by difficulties, in communication and the occurrence of repetitive behaviors or interests. It impacts 2.3% of children aged 8 and 2.2% of adults in the United States[1]. Recent advances in genetic technology have highlighted the importance of genetic and metabolic factors in Autism Spectrum Disorder (ASD). Mental health problems affect a significant number of people worldwide due to various factors. However, in Iraq, there's a notable absence of concrete data on this issue. For the last 40 years, Iraq has been through extended periods of conflict and terrorism. Despite these challenging circumstances, there's been little scientific investigation into mental health patterns and associated factors in the Iraqi context[2]. Genetic syndromes or chromosomal abnormalities are now identified in as many as 40% of ASD cases. These can take various forms, including minor DNA deletions or duplications, disorders affecting single genes, or specific genetic variants. Furthermore, metabolic issues, particularly those involving mitochondrial function, have been linked to ASD.

This growing understanding of the genetic and metabolic underpinnings of ASD is contributing to a more comprehensive view of the

disorder's origins and potential treatment approaches[3, 4]. Individuals with ASD often experience gastrointestinal (GI) issues such as constipation, diarrhea, reflux, bloating, and abdominal pain, with varying frequency among patients[5, 6]. The gut microbiota significantly influences neuropsychological functions and intestinal balance through the microbiota-gut-brain axis. Research indicates that individuals with ASD typically have a lower ratio of Bacteroidetes to Firmicutes and other microbiome imbalances, potentially linked to factors such as digestive enzyme deficiencies, carbohydrate malabsorption, selective eating habits, bacterial toxins, serotonin metabolism, and inflammation. To alleviate GI symptoms, various approaches such as antimicrobials, probiotics, prebiotics, fecal microbiota transplantation, and dietary changes are available[7].

The gut microbiota and its byproducts can influence various bodily functions through three main routes: the vagal, immune, and biochemical pathways. The vagus nerve serves as the primary communication channel between the brain and the gut. When gut microbes or their metabolites activate intestinal nerve endings, the signal travels to the nucleus tractus solitarius, and then on to the thalamus, amygdala, hypothalamus, and periaqueductal grey. Electrical stimulation of the vagus nerve by microbes has been shown to change neurotransmitter levels, including serotonin, glutamate, and GABA, in both mouse and human brains. Interestingly, studies using mouse models have found that cutting the vagus nerve can help reduce depression caused by immune system problems, highlighting the importance of this pathway[8]. Studying proteins and their interactions with other proteins leads to numerous new discoveries that contribute to our understanding of disease formation[9]. Because of this potential, researchers have been extensively studying possible biomarkers for this inherited condition. They're using various scientific approaches, including: Genomics (studying genes), Proteomics (studying proteins) Metabolomics (studying metabolites). These different methods aim to uncover the biological signs that might indicate ASD or help explain its underlying mechanisms[10]**.**

II. MATERIALS AND METHODS

2.1 Initial collection of ASD-associated proteins and GI-related proteins

Autism spectrum disorder (ASD) is a condition affecting brain development that has a significant genetic component. Studies involving families and twins have shown that genetics is a major factor in causing ASD. Research has found that both inherited mutations (passed down from parents) and new mutations (occurring for the first time in an individual) contribute to how autism develops. These genetic changes play important roles in the complex processes that lead to ASD[11, 12].To identify genes linked to autism spectrum disorder (ASD), researchers use AutismKB 2.0, a comprehensive database of genetic information related to ASD. This resource contains A total of 1,379 genes associated with ASD 1,280 non-syndromic genes (not related to other syndromes) and 99 syndromic genes (associated with other syndromes). Information on various genetic changes, including copy number variations, insertions, deletions, and linkage regions. A core dataset of 488 genes, these genes have high confidence scores.[11].

To gather information on genes related to gut function, I accessed the HGNC (HUGO Gene Nomenclature Committee) database. The HGNC is a widely recognized resource that provides unique and standardized names and symbols for human genes. Using relevant keywords and gene ontology terms associated with gastrointestinal processes, I queried the database to retrieve a comprehensive list of gut-related genes. This included genes involved in digestive functions, intestinal barrier maintenance, nutrient absorption, and gut motility. The HGNC database provided not only the approved gene symbols and names but also additional information such as gene locations, alternative names, and links to other genomic resources. This approach ensured that I obtained a reliable and up-to-date set of gut genes for further analysis in my research[13].

2.2 Construction of the protein-protein interaction(PPI) network

The initial step in constructing the protein-protein interaction (PPI) network involved identifying interactions among the collected proteins using the STRING database. Various identifyrs and accession numbers were utilized to query proteins across different databases. The resulting data, available in TEXT and PSI-MI formats, were filtered to include only high-confidence PPIs (STRING scores > 0.700).[14].These filtered data were then integrated and imported into Cytoscape 3.7.1. to generate the protein interaction network (PIN). This process was applied separately to autism spectrum disorder (ASD) and gut-related (GUT) proteins, creating distinct ASD and GUT PINs. The two networks were then compared, and their union (overlapping)

network was computed using Cytoscape's network modification plugin[15].This methodology allowed for a focused analysis of potential protein interactions relevant to the ASD-GUT relationship, emphasizing proteins with characteristics suggesting involvement in cellular communication or signaling**.**

2.3 Possible ASD- GL protein interactions

We divided the proteins identified from previous databases into two groups: ASD protein nodes and GL protein nodes. This categorization allowed us to examine the interactions and connections between these two groups.For our study, we employed the GeneMANIA database (http://genemania.org/) to predict protein-protein interactions (PPIs) and identify functional relationships among the collected proteins. GeneMANIA utilizes various functional association data to detect PPIs, including protein and genetic relationships, pathways, co-expression, co-localization, physical interaction, predicted interactions, and protein domain similarity. This approach enabled us to predict PPIs based on multiple characteristics by determining the interactions between relevant proteins using GeneMANIA's set of features. We used GeneMANIA to create several databases based on these features.

In our analysis, we considered PPIs occurring on the same metabolic KEGG map as true positives, while those on different maps were not. STRING, the tool we used, fits the sigmoidal correlation between the raw score and the fraction of PPIs on the same KEGG map to the hill equation to derive a confidence score. These STRING-derived scores correspond to the probability of finding the PPI within the same KEGG pathway or map[16].

STRING employs a score combiner based on the product of probabilities, using the following formula:

$S = 1 - \prod(i=1 \text{ to } N) (1 - Si)$

In this formula:Si represents the probability score for each individual database ,S is the final combined score ,N is the total number of databases being integrated. After combining all the scores, STRING rescales the combined scores into a confidence range from 0.0 to 1.0. These rescaled scores are interpreted as follows, 0.0 to 0.400: Low confidence, 0.400 to 0.700: Medium confidence and Above 0.700: High confidence. For our analysis, we selected only the high-confidence PPIs, which are those with scores above 0.700. This approach ensures that we focus on the most reliable protein-protein interactions in our study[14].

2.4 Biological function analysis

The ClueGO component of Cytoscape, a bioinformatics tool, is used to analyze the molecular functions of the network of interacting genes associated with ASD and GL proteins [17]. ClueGO is a Cytoscape plugin that enhances the interpretation of the biological functions of gene clusters or comparisons between gene clusters. The analysis utilized several databases, including Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), BioCarta, REACTOME, and WikiPathways, to examine and visualize the group functions of the leukemia-associated gene networks in detail[18].

2.5Functional Network and Disease Correlations

The functional network was constructed from the protein-protein interaction (PPI) network of ASD and GL proteins using the ClueGO Cytoscape plugin. This functional network can be useful in developing new treatment options for disorders involving the proteins in this network. Additionally, understanding the complex and critical roles associated with these proteins is important. The disease genes related to the interactions among ASD and GL proteins were identified using the DAVID bioinformatics tool and the Genetic Association Database (GAD)[19, 20].

III. RESULTS AND DISCUSSION

3.1 Construction of the PPI network

Using Cytoscape version 3.7.1[15].The researchers loaded all the collected proteins to construct these networks. The resulting ASD protein network consists of 168 protein nodes with 341 interactions between them. In comparison, the GL protein network comprises 131 protein nodes and 187 interactions. These networks are visually represented in Figure 3.1.

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Volume 6, Issue 5, Sep - Oct 2024 pp 386-396 www.ijdmsrjournal.com ISSN: 2582-6018

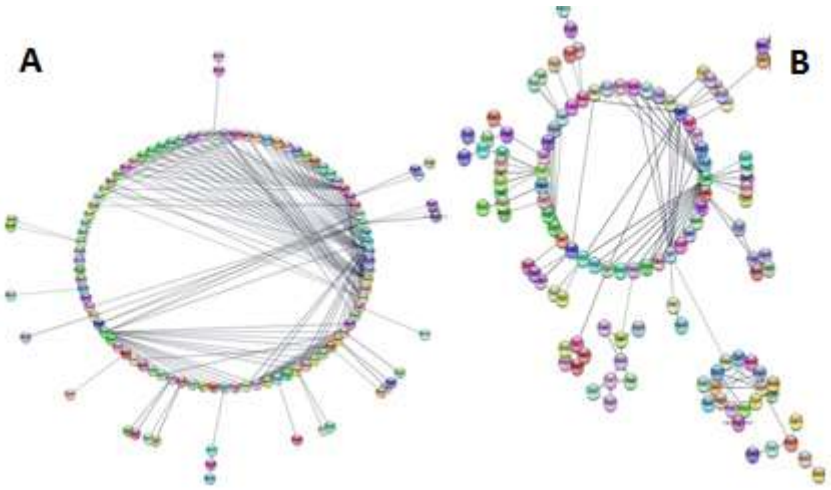

Figure 3.1 A) The network of ASD-associated proteins; B) the network of GUT-associated proteins

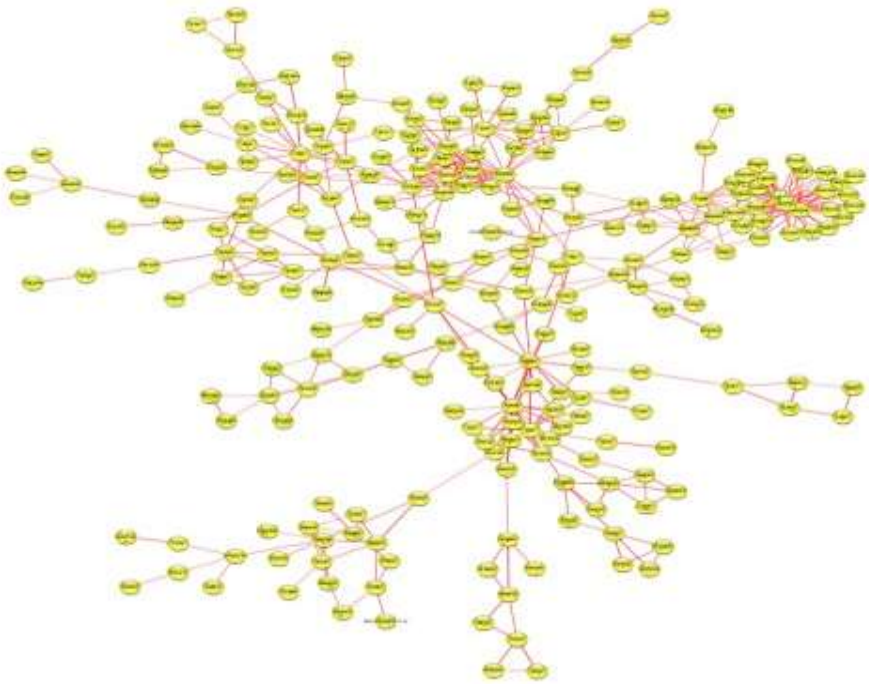

Figure 3.2 The gene union interaction network of the ASD- GUT network (created by Cytoscape)

3.2 Extraction ASD-GL interaction network

After merging the networks, we focused on extracting proteins that possess both a signal sequence and a transmembrane domain. This refined extraction resulted in a protein network comprising 299 nodes and 528 interactions, as illustrated in Figure 3.2.We then categorized the proteins identified through mass spectrometry (MS) in the initial phase of our methodology into two distinct groups: the ASD protein group and the GL protein group.

Some of the interactions we identified between ASD and GL proteins have been previously documented and verified in human studies. These established connections support the validity of our findings and provide a foundation for exploring the potential significance of the novel interactions we've uncovered. This overlap between our results and existing research underscores the relevance of our work in the context of ASD-GL relationships in humans.

We hypothesize that the patient's phenotype is caused by either haploinsufficiency of GRIA2 or a GLRB/GRIA2 fusion gene resulting in a protein with dominant-negative effects. Glutamate receptors are typically located in the

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dendritic regions of synapses. Mutations in genes encoding glutamate receptor subunits have been linked to intellectual disability (ID), including GRIN2A, GRIN2B, GRIA3, and GRIK2.

GRIA2 encodes the GLUR2 subunit of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and is predominantly expressed in brain tissue. The GLUR2 subunit plays a crucial role in regulating key biophysical properties of AMPA receptors. It is also involved in cognitive processes such as learning and memory, as well as synaptic plasticity[21]. KIF1B and DYNC1H1, genes that encode molecular motor proteins, are known to be directly involved in axonal transport. While these two genes play crucial roles, emerging evidence suggests that a broader range of intracellular trafficking and mitochondrial dynamics may be impaired in inherited neurological disorders. This dysfunction could serve as a common thread linking various inherited neurological disorderscausing genes that previously seemed unrelated[22].

3.3 New protein interaction in human ASD- GL proteins

Using the STRING database, we calculated a 'combined score' to forecast interactions between the identified groups. This analysis, conducted with high confidence (0.700), revealed previously unreported connections between Autism Spectrum Disorder (ASD) and (GL) proteins in human protein-protein interactions (PPIs). Our findings uncovered a total of 15 protein interactions, with 11 of these being novel. These results are summarized in Table 1. For example, HTR1A interact with GRM4 and CRHR1. The HTR1A gene is implicated in several mental health disorders, including anxiety, depression, and suicidal tendencies. Its functioning impacts serotonin signaling in the brain through two mechanisms: reduced activity of HTR1A autoreceptors results in increased serotonin transmission. At the same time, decreased function of HTR1A receptors on target neurons leads to diminished effects of neural signaling. Studies have shown that lower expression of the HTR1A gene correlates with enhanced stress reactivity and increased anxiety-like behaviors, highlighting its significance in regulating mood and stress responses[23].

Table 1. The new interactions between ASD and GUT proteins that are extracted from the merged network

ASD proteins	GUT proteins	Interaction detection method	Combined-score
HTR1A	GRM4	Co-Expression	0.760
HTR1A	CRHR1	Co-Expression	0.637
FPR2	FPR1	Cooccurrence Across Genomes	0.984
EPB41L1	SLC12A5	Co-Expression	0.472
FZD5	FZD4	Co-Expression	0.553
FZD5	FZD8	Cooccurrence Across Genomes	0.690
ACTC1	GSN	Co-Expression	0.583
ACTC1	MYH10	Co-Expression	0.754
ACTC1	TTN	Co-Expression	0.833
GPC5	EXT1	Association in Curated Databases	0.625
DYNC1H1	KIF1A	Co-Expression	0.570

The CRHR1 receptor plays a key role in promoting intestinal damage and inflammation. By activating CRHR1, it triggers a cascade of harmful effects on the gut, including increasing gut permeability, altering the structure and morphology of the intestines, and disrupting the intestinal microbiome. This leads to the development of intestinal injuries, particularly during the neonatal period when the risk of conditions like necrotizing enterocolitis is elevated. However, selectively blocking or inhibiting the CRHR1 receptor could potentially prevent the onset of these intestinal injuries and instead support the repair and healing of the gut. Targeting CRHR1 appears to be a promising therapeutic approach for protecting the delicate intestinal system, especially in vulnerable newborns[24].

While, the GRM4 have role in gut health, inflammation, and its interactions with the gut-brain axis. The second new protein interaction is

FPR2 interact with FPR1, The protein FPR2 regulates inflammatory responses in the body. When activated, FPR2 sets off a series of reactions inside cells, activating various kinases (enzymes that modify other proteins). This involvement in inflammatory processes suggests that FPR2 could be a biological marker for tracking inflammation in Autism Spectrum Disorder (ASD)[25]. While, FPR1 has a detrimental impact on celiac disease, a common autoimmune condition that affects the digestive system, which contributes to the disease process by encouraging neutrophils (a type of white blood cell) to move into the gut, triggering inflammation. This migration occurs in response to gliadin, which is the component of gluten that causes problems for people with celiac disease. By promoting this inflammatory response, FPR1 exacerbates the symptoms and progression of celiac disease, making it a potential target for new treatment strategies[26].

From new intaeraction also ACTC1gene which interact with GSN, MYH10 and TTN.Our research indicates a potential link between the ACTC1 gene and autism spectrum disorder (ASD). While this specific connection is novel, it aligns with existing studies by Jessica X. Chong et al., which show a correlation between neurodevelopmental disorders like ASD and increased rates of congenital heart disease. This finding may offer new insights into the genetic factors underlying ASD[27]. The GSN gene encodes gelsolin, a protein crucial for actin remodeling, cell movement, and shape. It's implicated in various health conditions, including those affecting gut health.Gelsolin contributes to gut health through itsroles in actin dynamics, inflammation control, and epithelial cell repair[28]. The MYH10 gene encodes myosin heavy chain 10, a protein crucial for muscle contraction and cell motility. It contributes to gut health by maintaining epithelial integrity and managing inflammation[29].

The TTN gene, responsible for producing titin, is essential for muscle structure and function, particularly in cardiac and skeletal muscles. While its primary role is in muscular systems, emerging research suggests that TTN may also impact digestive health. This potential connection to gut wellness could be related to titin's involvement in muscle contractions, maintaining the integrity of epithelial tissues, and modulating inflammatory processes[30].From new born FZD5 interact with FZD4 and FZD8. The FZD5 gene encodes a protein that is part of the frizzled gene family, functioning as receptors for Wnt signaling molecules. These Wnt signaling pathways are instrumental in various developmental processes, with a particular emphasis on neurodevelopment. The importance of Wnt signaling extends to crucial aspects of brain formation, including the establishment of neuronal connections and the proper functioning of synapses. Given its significant role in these fundamental neurodevelopmental processes, it's not surprising that disruptions in the Wnt signaling pathway have been associated with various neurodevelopmental disorders, autism among them. This connection underscores the potential importance of FZD5 and related genes in the complex etiology of autism spectrum disorders[31].

The frizzled gene family members FZD4 and FZD8 play crucial roles in gut health through their involvement in Wnt signaling pathways. These genes contribute to maintaining intestinal homeostasis by regulating stem cell activity, cell proliferation, and differentiation in the gut epithelium. FZD4 is particularly important for preserving the intestinal barrier function by organizing tight junctions between epithelial cells, while FZD8 appears to be involved in modulating inflammatory responses in the gut[32]. DYNC1H1 interact with KIF1A as new interaction in this research. DYNC1H1 encodes a crucial component of dynein, a motor protein essential for neuronal transport. This gene is vital for moving cellular components within neurons, supporting proper neuronal development and connectivity. Mutations in DYNC1H1 can disrupt these processes, potentially contributing to the neurological differences seen in autism spectrum disorder (ASD). Studies have linked DYNC1H1 mutations to increased ASD risk, with effects potentially manifesting as the characteristic social, communicative, and behavioral challenges associated with autism[33].

The research Caroline S. Hirst, et al, on KIF1BP, a gene known to regulate axon growth and synaptic vesicle transport, reveals its broader significance in nervous system development.While not directly linked to autism, the study draws parallels between the neurobiological processes affected by KIF1BP and those underlying autism spectrum disorders. Both involve fundamental developmental processes such as axon extension and synapse formation, impacting central and peripheral neurons. This similarity suggests that studying KIF1BP could provide valuable insights into the mechanisms of autism and other neurodevelopmental disorders[34].

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Volume 6, Issue 5, Sep - Oct 2024 pp 386-396 www.ijdmsrjournal.com ISSN: 2582-6018

3.4 Molecular function analysis

The ClueGO tool, a plug-in for Cytoscape, was employed to examine the network of interactions between ASD and GUT. This analysis determined the primary molecular functional group involved in the protein network. The findings revealed that the majority of proteins are engaged in positive regulation of intracellula signal transduction, second messenger mediated signalling, cell- cell signalling, multicellular organisal process, response to chemical, negative regulation of biological process, cellular ion haemostasis, G-protein goupled receptor activity, response to extra stimulli. (Figure 4.1).positive regulation of intracellula signal transduction, second messenger mediated signalling, cell- cell signalling are all interestingly linked molecular activities.Signal transduction is a vital biological process, equally important as metabolism or self-replication in living organisms. It involves cells detecting and responding to external stimuli. When a cell receives information from its surroundings, it processes this input and converts it into internal cellular responses. For long-lasting changes, some of these signals must travel to the cell's nucleus. Once there, they trigger alterations in gene expression, allowing the cell to adapt its behavior or function based on the external information it receives[35].

Recent research suggests that signal transduction pathways play a significant role in various cellular processes linked to autism spectrum disorders. These processes include gene expression, protein synthesis, neurotransmitter release and reception, modifications to gene activity without altering DNA sequences, and immune system responses. Understanding these connections could lead to the identification of specific molecular targets, potentially advancing the development of more effective treatments for autism[36]. A multicellular organisal process refers to any biological activity that takes place within a complex organism composed of multiple cells, which contributes to its overall functioning and survival. These processes are essential for maintaining the organism's life and well-being. From these process apoptosis, research indicates that the process of programmed cell death, may be implicated in autism spectrum disorders. Apoptosis typically plays a crucial role in normal tissue and organ development, maintaining a dynamic equilibrium by regularly replacing mature cells in various organs. However, in autism, this process appears to be disrupted at different developmental stages. In the developing brains of infants with autism, there is a deficiency in apoptosis, suggesting that fewer cells are eliminated when they should be. Conversely, in children and adolescents with autism, an excess of cells undergo apoptosis, indicating increased cell death. This imbalance in apoptotic processes at different stages of development could contribute to the neurological differences observed in individuals with autism, potentially affecting brain structure and function in ways that might explain some characteristics associated with the disorder[37].

Evidence suggests that autism spectrum disorders (ASD) are characterized by abnormalities in neuronal excitability, metabolism, and immune function. Consequently, treatments focused on restoring homeostasis may offer a broad-based solution for reducing core behavioral symptoms and comorbidities associated with ASD. This approach to maintaining balance should extend to the gestational period, as emerging research indicates that adverse immune or metabolic conditions during fetal development can increase autism risk. A comprehensive strategy for managing ASD may therefore involve combining efforts to promote neural, metabolic, and immune homeostasis with proven behavioral interventions. This integrated approach, addressing both physiological imbalances and behavioral symptoms, could potentially provide the most effective treatment and lead to improved long-term outcomes for individuals with autism spectrum disorders[38].

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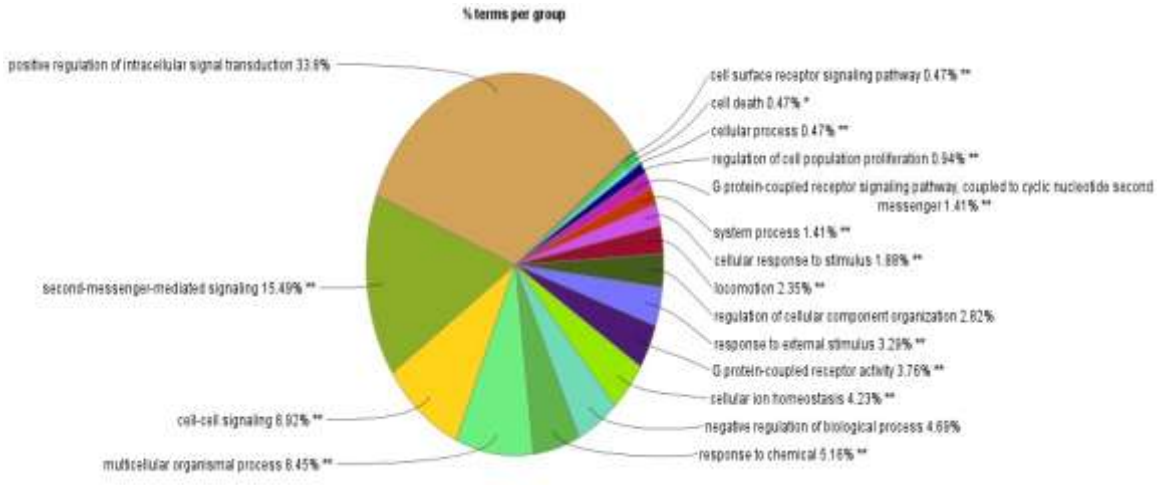

Figure 4.1 ClueGO analysis of ASD–GUT interaction protein network (Created by ClueGO plug-in in Cytoscape)

3.5 Database for Annotation Visualization and Integrated Discovery (David) analysis

The DAVID bioinformatics platform utilized the Genetic Association Database (GAD) to identify genes linked to diseases involving ASD-GUT interactions disorder. To determine significant disease associations, a stringent p-value threshold of 0.01 was applied using the Benjamini-Hochberg method for multiple testing correction. The results of thisanalysis are presented in Table 2, 116 genes of ASD-GUT PINs are involved in METABOLIC disease, 100 genes in CARDIOVASCULAR, and 90 in PHARMACOGENOMIC. Research suggests a significant connection between gut health and neurological conditions, including autism spectrum disorder (ASD). Disruptions in the balance of gut microbiota, known as dysbiosis, are thought to play a role in the onset or worsening of various neurological and psychiatric disorders. This occurs through alterations in the communication pathways of the microbiota-gut-brain axis. Clinical observations further support the link between gut health and ASD.

Many children diagnosed with ASD frequently experience digestive issues and a variety of gastrointestinal symptoms. These can include abdominal discomfort, chronic constipation or diarrhea, and excessive gas. Such observations highlight the potential importance of gut health in the management and understanding of ASD, suggesting that addressing gastrointestinal issues could be an important aspect of comprehensive care for individuals with autism[39]. A recent meta-analysis has confirmed that individuals with autism spectrum disorder (ASD) are at an elevated risk for developing cardiometabolic diseases, including diabetes, dyslipidemia, and cardiovascular disease (CVD). This increased risk is particularly pronounced in children with autism, emphasizing the need to address these risk factors at an early age. The findings underscore the importance of early screening and intervention for cardiometabolic health issues in the autism population. Healthcare providers and caregivers should consider implementing preventive strategies and tailored interventions from a young age to mitigate these risks. This research highlights the necessity of a comprehensive approach to autism care that extends beyond managing core autism symptoms to include proactive monitoring and management of physical health risks, potentially improving long-term outcomes and quality of life for individuals with ASD[40].

Our sutdy finding 77 genes participate in Cancer, previous studies has revealed an elevated cancer risk among individuals with Autism Spectrum Disorder (ASD), particularly in those up to 30 years of age, when compared to individuals without ASD. While the exact causes of autistic disorders remain unknown, there are indications that abnormalities in tumor suppressor genes might play a role in some cases of autism development. These genetic irregularities could potentially explain the observed increased risk of cancer or other neoplasms in individuals with ASD. This finding suggests a possible link between the genetic factors contributing to autism and those influencing cancer susceptibility. The connection between ASD and cancer risk highlights the importance of comprehensive health monitoring for individuals with autism, especially in their early years and young adulthood. Further research is needed to fully understand the mechanisms behind this

association and to develop appropriate screening and preventive strategies for this population[41, 42].

Depended on the results from the DAVID analysis in Table 2

Category	Term	Count	%	P-Value
GAD_DISEASE_CLASS	PSYCH	81	28.0	2.0E-15
GAD_DISEASE_CLASS	PHARMACOGENOMIC	90	31.1	9.6E-12
GAD_DISEASE_CLASS	UNKNOWN	63	21.8	1.1E-10
GAD_DISEASE_CLASS	OTHER	58	20.1	1.1E-8
GAD_DISEASE_CLASS	NORMALVARIATION	26	9.0	1.6E-6
GAD_DISEASE_CLASS	NEUROLOGICAL	77	26.6	9.2E-6
GAD_DISEASE_CLASS	CARDIOVASCULAR	100	34.6	4.5E-5
GAD_DISEASE_CLASS	METABOLIC	116	40.1	1.1E-4
GAD_DISEASE_CLASS	HEMATOLOGICAL	45	15.6	1.9E-4
GAD_DISEASE_CLASS	IMMUNE	74	25.6	1.9E-4
GAD_DISEASE_CLASS	DEVELOPMENTAL	44	15.2	2.4E-4
GAD_DISEASE_CLASS	CANCER	77	26.6	3.2E-4
GAD_DISEASE_CLASS	RENAL	40	13.8	1.1E-3
GAD_DISEASE_CLASS	REPRODUCTION	30	10.4	1.3E-3

IV. CONCLUSION

The study revealed novel protein interactions between ASD and gut-related proteins, identifying key molecular functions and pathways involved in the ASD-gut relationship. The findings suggest that disruptions in this relationship may have broader implications for other diseases and pharmacogenomics. While these computational results provide valuable insights, further experimental research is needed to validate the newly identified interactions and their potential impact on ASD and related disorders. This work establishes a foundation for future investigations into the molecular mechanisms of the gut-brain axis in ASD and may guide the development of new therapeutic strategies.

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