Original Article
http://mjiri.iums.ac.ir
Medical Journal of the Islamic Republic of Iran (MJIRI)

Med J Islam Repub Iran. 2025 (27 Dec);39.165. https://doi.org/10.47176/mjiri.39.165

Peripheral IL-6/IL-17/NF-κB1 and IL-10 Signaling in Children with Autism Spectrum Disorder: Integrative Transcriptomic Analysis and qRT-PCR Validation

Yoosra Najim Abed Al-Saadi[1]*, Zainab Basim Mohammed[1], Mithal Abdulkareem Abdoun[1], Ali Mahmoudi[2]*

Received: 13 Aug 2025 Published: 27 Dec 2025

Abstract

Background: Autism spectrum disorder (ASD) is associated with immune and inflammatory dysregulation. However, the molecular networks linking peripheral immune signatures to neuroinflammatory processes remain poorly understood. This study aimed to explore inflammation-related molecular pathways in ASD through integrated transcriptomic network analysis and to validate key cytokine genes (IL6, IL10, IL17, NF-κB1) using quantitative real-time polymerase chain reaction (qRT-PCR).

Methods: This was an integrative computational-experimental study. We analyzed 4 gene expression Omnibus (GEO) transcriptomic datasets (GSE18123, GSE111176, GSE87847, GSE6575), constructed protein-protein interaction (PPI) networks, and identified inflammation-related modules. Selected inflammatory genes (IL6, IL10, IL17, NF-KB1) were validated by qRT-PCR in peripheral blood samples from ASD (n = 15) and healthy controls (n = 5). Statistical analyses were conducted in R. Data normality was assessed using the Shapiro-Wilk test, and normally distributed variables were compared using t-tests.

Results: Integration of datasets revealed core differentially expressed genes (DEGs) and a connected PPI network (26 nodes, 88 edges), with hub genes such as PUM1, TRRAP, ILF3, INO80, and PTBP1. Functional enrichment indicated cytokine-mediated signaling, leukocyte activation, and neuroinflammation processes. Network analysis highlighted central regulators linking chromatin remodeling, ribonucleic acid (RNA) processing, and immune signaling. qRT-PCR confirmed dysregulation of IL6 (fold change ≈ 12.8, $P = 0.049$), IL17 (≈ 21.3, $P = 0.048$), NF-KB1 (≈ 42.4, $P = 0.039$), and IL10 (≈ 0.101, $P = 0.038$).

Conclusion: The findings suggest an IL-6/IL-17/NF-κB1–centric proinflammatory axis and reduced IL-10–mediated regulation in ASD, implicating peripheral immune activation and transcriptional regulators in neuroinflammatory processes. The identified hub genes and pathways may serve as biomarkers and therapeutic targets for an inflammation-associated ASD subtype. Limitations include small qRT-PCR sample size and lack of protein-level validation; future studies should explore longitudinal and multiomics approaches.

Keywords: Autism Spectrum Disorder, Neuroinflammation, Inflammatory Factors, Protein–Protein Interaction, Transcriptomics, Computational Biology

Conflicts of Interest: *None declared*
Funding: *This work was supported by Ibn Sina University of Medical and Pharmaceutical Sciences.*

This work has been published under CC BY-NC-SA 4.0 license.
Copyright© Iran University of Medical Sciences

Cite this article as: Najim Abed Al-Saadi Y, Basim Mohammed Z, Abdulkareem Abdoun M, Mahmoudi A. Peripheral IL-6/IL-17/NF-κB1 and IL-10 Signaling in Children with Autism Spectrum Disorder: Integrative Transcriptomic Analysis and qRT-PCR Validation. *Med J Islam Repub Iran.* 2025 (27 Dec);39:165. https://doi.org/10.47176/mjiri.39.165

Corresponding author: *Dr Ali Mahmoudi, Alimahmoudi68@yahoo.com*
Dr Yoosra Nagem. Abed Al-Saadi, najimyusra0@gmail.com

1. Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq
2. Department of Basic Medical Sciences, Faculty of Medicine, Abadan University of Medical Sciences, Abadan, Iran

↑What is "already known" in this topic:
Immune dysregulation and inflammatory pathways, particularly IL-6, IL-17, and NF-κB signaling, have been implicated in the pathophysiology of autism spectrum disorder (ASD). Previous studies suggest altered cytokine levels and immune gene expression in ASD, but findings remain inconsistent and often lack validation across independent datasets.

→What this article adds:
This study integrates multiple transcriptomic datasets with experimental qRT-PCR validation, identifying consistent dysregulation of IL-6/IL-17/NF-κB and IL-10 pathways in children with ASD. The combined bioinformatic and experimental approach enhances evidence for peripheral immune signatures as potential biomarkers and mechanistic contributors in ASD.

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]

[DOI: 10.47176/mjiri.39.165]

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition first described by Kanner in 1943 and is now characterized by persistent deficits in social communication and interaction, along with restricted, repetitive behaviors [1]. The prevalence of ASD has significantly increased in recent decades, affecting approximately 0.7% to 1% of the population [2,3], with improved diagnosis accounting for only part of this rise. Twin and family studies suggest that around 50% to 55% of ASD risk is genetic [4], indicating a high level of heritability. However, various environmental and epigenetic factors also play a role, such as prenatal infections, toxins, gut dysbiosis, and maternal autoimmune conditions [4,5]. ASD is now understood as a multifactorial disorder, where genetic predisposition interacts with immune, metabolic, and environmental factors during development to contribute to ASD phenotypes [4,5]. Due to the diverse causes, ASD presentations can vary widely, but one common emerging feature in many cases is immune and inflammatory dysregulation.

Growing evidence suggests that those with ASD experience chronic, low-grade inflammation and immune dysfunction [1,3]. Studies have consistently found elevated levels of proinflammatory cytokines and chemokines—eg, interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), interleukin-17 (IL-17)—and reduced levels of anti-inflammatory mediators—particularly interleukin-10 (IL-10)—in the peripheral blood of children with ASD [6-8]. A meta-analysis by Zhao et al confirmed a strong association between elevated IL-6 levels in the blood and ASD [9]. These cytokine abnormalities are often correlated with the severity of symptoms, with higher IL-6 or TNF-α levels corresponding to more pronounced social or behavioral impairments [8,10]. Additionally, ASD has been linked to widespread immune activation, as evidenced by activated microglia and astroglia, as well as markers of neuroinflammation in various brain regions [11-13]. Overall, these findings suggest that ASD is characterized by a proinflammatory state in both the body and brain.

The development of autism involves a combination of genetic susceptibility and environmental factors, with growing evidence pointing to dysregulated inflammation as a crucial factor in its progression [1]. Key immune molecules, such as IL-6, IL-17, TNF-α, and NF-κB1, have been consistently linked to autism in both clinical and animal studies, whereas anti-inflammatory factors, such as IL-10, are deficient [7,14,15]. This study aimed to use computational network and pathway analysis to explore inflammation-related genes and pathways in autism. Specifically, we focus on genes such as IL6, IL10, IL17, and NF-κB1, as well as their interactions, to identify key inflammatory pathways that may contribute to autism. By mapping autism-associated genes onto immune and neuroinflammatory pathways, we aimed to elucidate how dysregulated inflammation contributes to autism and identify candidate genes for further clinical investigation. Our goal was to integrate the vast amount of immune-related data on autism to develop comprehensive models that could uncover new therapeutic targets within inflammatory signaling networks.

Methods

Differential Expression Analysis

Data Acquisition

Four publicly available gene expression datasets (GSE18123, GSE111176, GSE87847, and GSE6575) were obtained from the Gene Expression Omnibus (GEO, NCBI) using the GEOquery (Version 2.76.0) package in R (Version 4.5) (Table 1). Raw series matrix files and platform annotation files were downloaded to ensure precise probe-to-gene mapping.

Preprocessing

Each dataset was processed individually to maintain dataset-specific normalization and minimize interference from batch effects. Raw expression values were $\log_2$-transformed for variance stabilization. Probe identifiers were mapped to official gene symbols using platform annotation data, excluding probes without valid annotations or that mapped to multiple genes. For genes with multiple probes, the probe with the highest average expression was retained. Samples of poor quality and nonbiological controls were excluded based on experimental annotations.

Differential expression analysis was conducted using the limma (Version 3.64.3) package with a linear modeling framework and empirical Bayes moderation. Contrasts were defined between the ASD and the neurotypical control groups. P values were adjusted for multiple comparisons using the Benjamini-Hochberg method, with an adjusted p-value (FDR) < 0.05 considered statistically significant. Genes with $|\log_2 \text{fold change}| > 0$ were classified as differentially expressed genes (DEGs), and further categorized as upregulated or downregulated. Volcano plots were generated to visualize $\log_2$ fold changes against $-\log_{10}$ adjusted P values. Heatmaps of the top 50 DEGs were created using z-score normalized expression values, grouped by hierarchical clustering (Euclidean distance, complete linkage) using pheatmap (Version 1.0.13).

Table 1. Summary of Key Characteristics of GEO Datasets used in Autism Spectrum Disorder Transcriptomic Studies

GEO Series	Tissue / Source	Platform(s)	Number of samples
GSE18123	Blood	GPL570 (Affymetrix Human Genome U133 Plus 2.0), GPL6244 (Affymetrix HuGene-1_0_ST)	285
GSE6575	Blood	GPL570 (Affymetrix Human Genome U133 Plus 2.0)	56
GSE111176	Blood	GPL10558 Illumina HumanHT-12 v4.0 BeadChip	288
GSE87847	Blood	GPL18281 Illumina HumanHT-12 WG-DASL v4.0 R2 BeadChip	93

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]

[DOI: 10.47176/mjiri.39.165]

Overlap and Core Gene Analysis

DEGs intersections were analyzed separately for upregulated and downregulated gene sets in different datasets. pSet plots generated using the UpSetR package were used to visualize complex intersections, whereas Venn diagrams generated using the VennDiagram package (version 1.7.3) were used to illustrate overlaps among 2 to 4 data sets. Core genes shared across all datasets were identified through set intersection (Reduce (intersect, ...). Pairwise overlaps were quantified and visualized with bar plots using ggplot2 (Version 4.0.0).

Protein-Protein Interaction Network Construction

Data Preparation

Protein-protein interaction (PPI) data were obtained from the STRING database (Version 12.0) in TSV format (date: 10.01.2025).

Network Construction and Characterization

An undirected weighted PPI network was constructed using the igraph (Version 2.1.4) package. Network topology metrics, such as the number of nodes, edges, average degree, diameter, average path length, and clustering coefficient, were calculated to characterize the network structure.

Hub Gene Identification

Node centrality measures (degree, betweenness, and closeness centrality) were computed for all genes. Genes were ranked based on each centrality metric, and the top-ranked genes were identified as hub genes due to their significant network influence.

Cell–type–specific Expression Patterns

To investigate the expression patterns of the identified hub genes across various cell types, we used the GTEx Portal's single-cell multi-gene query tool (https://www.gtexportal.org). Each gene was uploaded and examined across the available single-cell ribonucleic acid (RNA) sequencing datasets. The expression levels were represented as log-normalized counts per 10,000 reads (ln[CP10k+1]) across different tissues and cell populations. This methodology facilitated comparison of the transcriptional activity of the hub genes across tissue- and lineage-specific cellular contexts.

Functional Enrichment Analysis

Functional enrichment analysis of network genes was conducted using the clusterProfiler (Version 4.16.0) package. Gene ontology (GO) enrichment focused on the biological process category, with an emphasis on inflammation-related terms. Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome pathway enrichment analyses were performed to identify enriched biological pathways among DEGs, with a focus on inflammation and neuroinflammation. A subnetwork of genes related to neuroinflammation was extracted from the full PPI network and GO. KEGG enrichment analyses were repeated specifically for this subnetwork to identify neuroinflammation-relevant biological processes and pathways.

Sample Collection and Preparation

Peripheral blood samples were collected from patients diagnosed with ASD and healthy controls. The ASD diagnosis was based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, criteria and was confirmed through clinical assessment and, when applicable, standardized diagnostic tools. Controls were matched for age, sex, and socioeconomic status.

Blood was collected in EDTA tubes and processed within 2 hours to preserve RNA integrity. Peripheral blood mononuclear cells (PBMCs) were isolated, and total RNA was extracted using Trizol reagent (Invitrogen). RNA concentration and purity were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific), and integrity was confirmed by gel electrophoresis.

cDNA Synthesis and Quantitative PCR

cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) in 20 µL reactions containing 1 µg of total RNA, random primers, and reverse transcriptase. The thermal cycling conditions were 25 °C for 10 min, 37°C for 120 min, and 85°C for 5 min to inactivate the enzyme. Also, qPCR was then conducted to quantify the expression of IL-6, IL-10, IL-17, and NF-κB1, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference gene. The primers were designed using Gene Runner 6.0 software. The primer sequences are as follows: IL-6 (Forward: 5′-ACTCACCTCTTCAGAACGAATTG-3, Reverse: 5′-CCATCTTTGGAAGGTTCAGGTTG-3), IL-10 (Forward: 5′-TGAATTCCCTGGGTGAGAAG-3, Reverse: CTCTTCACCTCCTCCACTGC-3), IL-17 (Forward: 5′-ACGATGACTCCTGGGAAGACC-3, Reverse: 5′-GGGATTGTGATTCCTGCCTTC-3), NF-κB1 (Forward: 5′-TGGCGCAGAAATTAGGTCTGG-3, Reverse: :5′-GATCACTTCAATTGCTTCGGTGTA-3), GAPDH (Forward: 5′-GTCTCCTCTGACTTCAA-3′, Reverse: 5′-ACCACCCTGTTGCTGTA-3′).

Each 20 µL reaction contained 2 µL of cDNA, 0.3 µM primers, 6 µL of water, and 10 µL of SYBR Green Master Mix (Applied Biosystems). The qPCR thermal program started with an initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. Melt curve analysis was conducted to confirm amplification specificity. Ct values for each gene were recorded, and ΔCt values were calculated by subtracting the Ct value of GAPDH from the target gene. Group means were compared by calculating ΔΔCt (ΔCt_ASD – ΔCt_Healthy), and fold changes in gene expression were determined using the 2^(–ΔΔCt) method. Biological interpretation considered fold changes >1 as upregulation and <1 as downregulation.

Statistical Analyses

Statistical analyses were performed in R utilizing the tidyverse, ggpubr, rstatix, boot, and lmerTest packages. Raw Ct values were entered manually, and samples were

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]

[DOI: 10.47176/mjiri.39.165]

classified as either ASD (n = 15) or healthy (n = 5). The normality of data for each gene within each group was evaluated using the Shapiro-Wilk test. Given the normal distribution, t-tests were employed for analysis. Bootstrapping with 10,000 resamples was used to compute 95% confidence intervals for fold-change estimates, thereby providing robust assessments of differential gene expression between the groups.

Results

Differential Expression Patterns in ASD

We analyzed 4 independent gene expression datasets related to ASD (GSE18123, GSE111176, GSE87847, GSE6575) to identify DEGs between patients with ASD and neurotypical controls.

Volcano Plot Analysis

Volcano plots were used to visualize the distribution of $\log_2$ fold change ($\log_2$FC) versus statistical significance ($-\log_{10}$ FDR) for each dataset (Figure 1).

Significant DEGs were identified based on FDR <0.05 and $|\log_2 FC| > 0$, with upregulated genes shown in red and downregulated genes in blue.

The top 10 most statistically significant genes in each dataset were highlighted for clarity.

Distinct dysregulation patterns were observed across da-

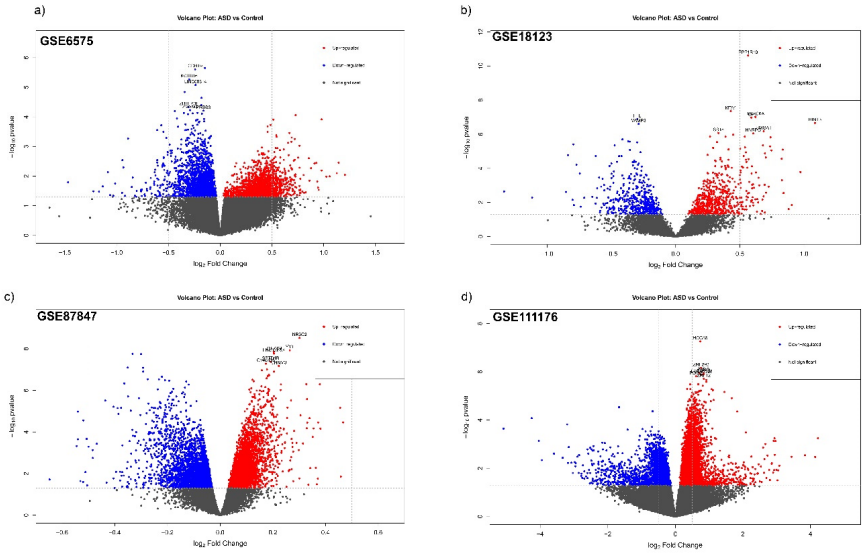

Figure 1. Volcano plots for each dataset. Red = upregulated; blue = downregulated; labeled points = top 10 most significant DEGs.

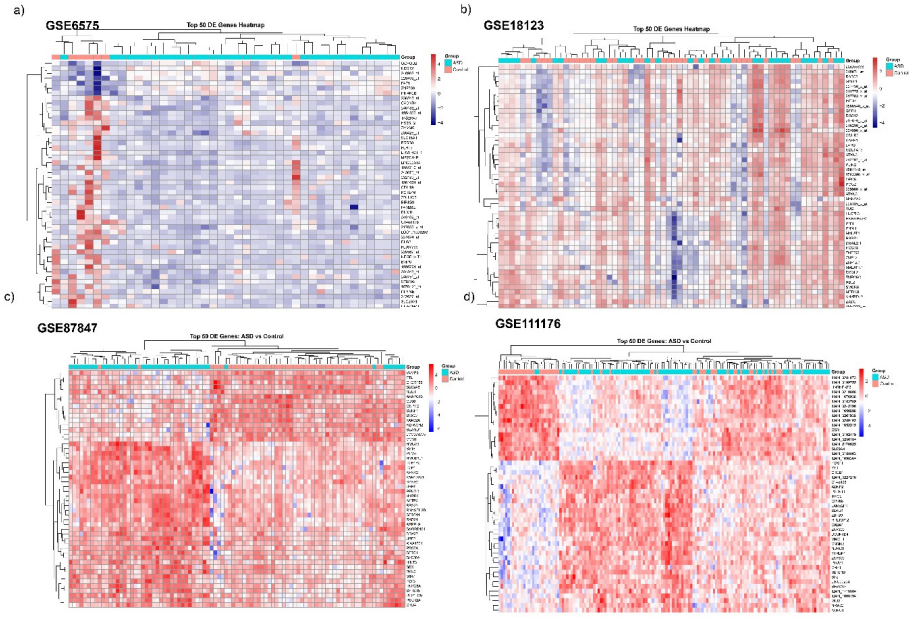

Figure 2. Heatmaps of the top 50 DEGs per dataset. Rows = genes; columns = samples. Clustering performed using Euclidean distance, complete linkage.

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]

[DOI: 10.47176/mjiri.39.165]

tasets, indicating potential tissue-specific and platform-specific influences.

Heatmap Visualization

Hierarchical clustering of the top 50 DEGs in each dataset demonstrated clear separation between ASD and control groups (Figure 2).

Consistent expression patterns were observed within groups, while differences between datasets reflected a combination of biological variability and technical factors.

Overlap Analysis of Differentially Expressed Genes

Across the 4 ASD datasets, a total of 7932 genes were significantly upregulated (FDR <0.05), with 7909 unique genes identified. The dataset with the largest number of upregulated genes was GSE18123 (2904), while GSE87847 had the fewest (510). Only 23 genes were consistently upregulated across all datasets. These genes include SMARCC2, ILF3, SLC35E1, SEC16A, DDX27, EIF2S3, WDR82, TRRAP, CCNY, RDH11, GNPTAB, PUM1, CRTAP, RBBP4, SMARCA2, INO80, SON, GLG1, RBM17, PTBP1, FAM193A, YWHAG, and LUC7L.

For downregulated genes, a total of 5360 were identified across the datasets, with no gene shared by all four datasets (Figures 3 and 4).

Network Topology

The PPI network consisted of 26 nodes and 88 edges, representing a moderately sized and biologically meaning-

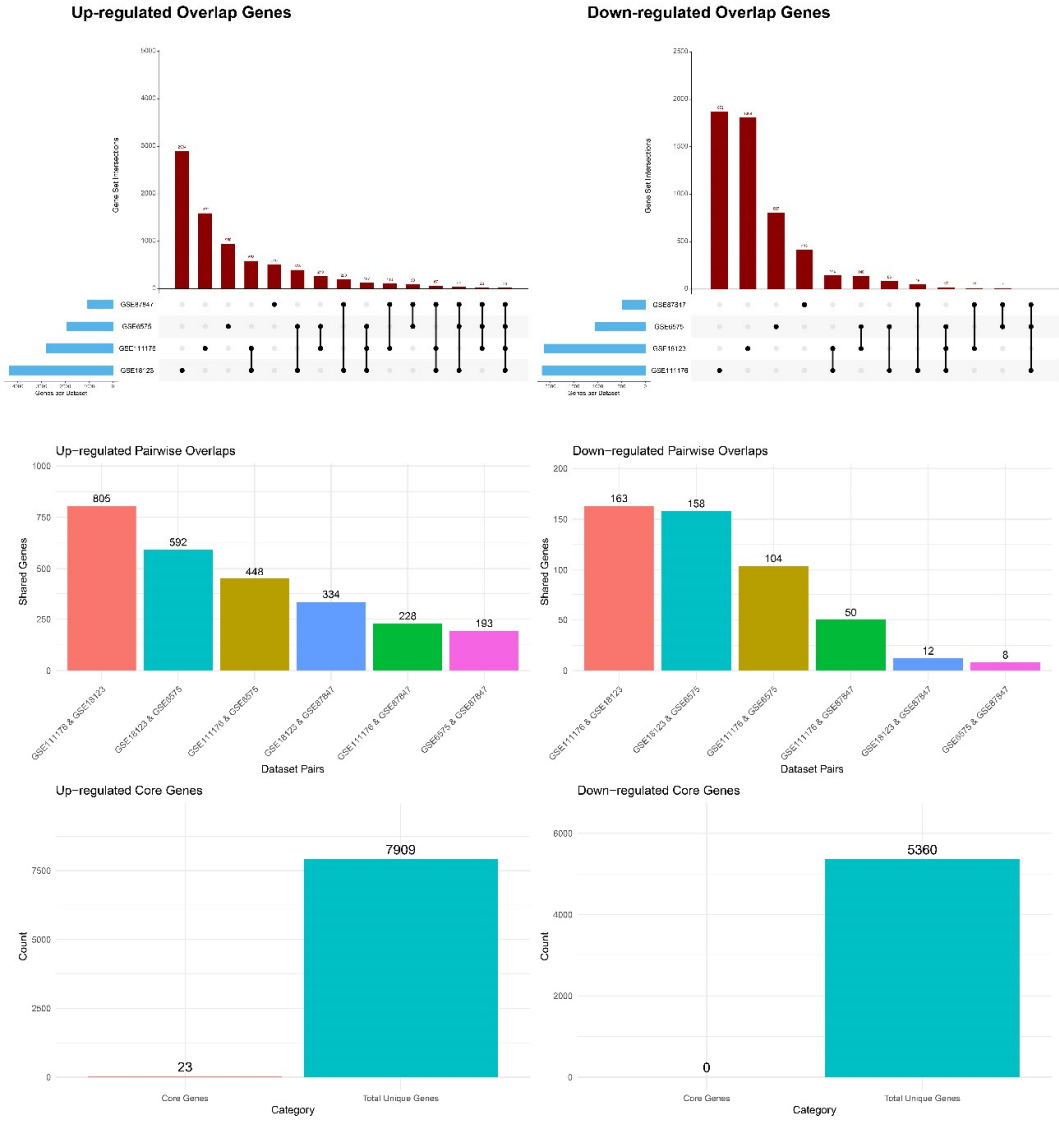

Figure 3. UpSet plots of DEG overlaps across datasets. Left = upregulated; right = downregulated. Bars = intersection size; dots = datasets in intersection.

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]

[DOI: 10.47176/mjiri.39.165]

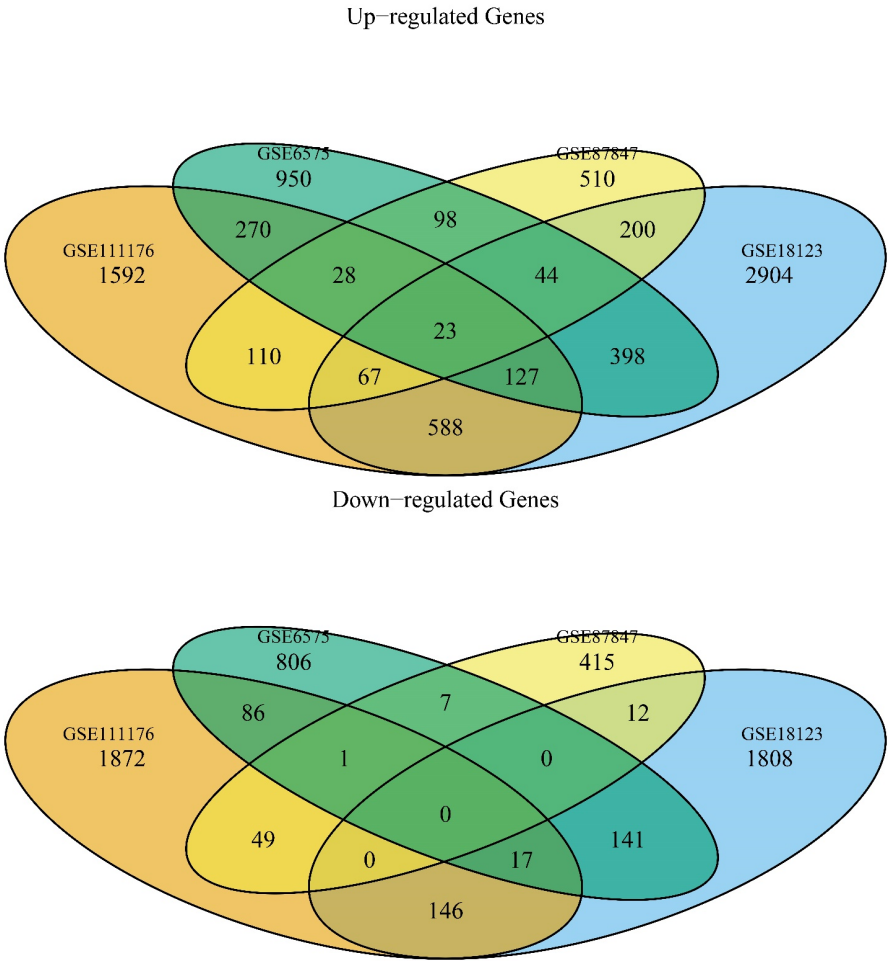

Figure 4. Venn diagrams of core DEGs. Top = upregulated genes (n = 23); bottom = downregulated genes (n = 0). Colors indicate datasets.

ful gene-gene interaction landscape. The average degree of the network was 6.8, indicating that each node was connected to approximately 7 other nodes. The network diameter was 4, indicating a relatively small maximum shortest path between any 2 genes, and the average path length was 1.98, showing efficient connectivity between nodes. The clustering coefficient of 0.506 indicated a moderate level of local clustering, commonly seen in biological networks where genes with related functions form interconnected modules. Overall, these features suggest a well-connected and biologically plausible network architecture with potential for coordinated functional regulation.

Identification of Hub Genes

Hub gene analysis identified several highly connected and central nodes in the network. Pumilio RNA-Binding Family Member 1 (PUM1) was identified as the top hub gene, with a degree of 15 and a betweenness centrality of 36.31, highlighting its crucial role in facilitating communication within the network. Other significant hub genes included TRRAP (TRRAP Complex Subunit) (TRRAP) (degree: 12, betweenness: 39.07), interleukin enhancer binding factor 3 (ILF3) (degree: 11, betweenness: 29.74), INO80 complex subunit (INO80) (degree: 11, betweenness: 20.74), and polypyrimidine tract binding protein 1 (PTBP1) (degree: 11, betweenness: 31.67). These genes are positioned centrally in the network, indicating their potential importance in maintaining network stability and regulating biological responses, particularly in the context of ASD-related processes. The visualization of the PPI network (Figure 5) revealed distinct clusters associated with inflammation- and neuroinflammation-related genes, with hub genes occupying central, highly connected positions.

Inflammation-Related Functional Enrichment

GO enrichment analysis revealed strong associations between network genes and immune-related biological processes. Significant GO terms included positive regulation of lymphocyte activation ($P = 7.10 \times 10^{-5}$), positive regulation of leukocyte activation ($P = 1.13 \times 10^{-4}$), regulation of chemokine production ($P = 3.56 \times 10^{-4}$), and inflammatory response to wounding ($P = 6.74 \times 10^{-4}$). Reactome pathway analysis further highlighted enrichment in cytokine-mediated signaling, particularly Interleukin-4 and IL-13 signaling ($P = 7.52 \times 10^{-4}$) and IL-10 signaling ($P = 2.81 \times 10^{-3}$) (Figure 6). These findings emphasize the cen-

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]

[DOI: 10.47176/mjiri.39.165]

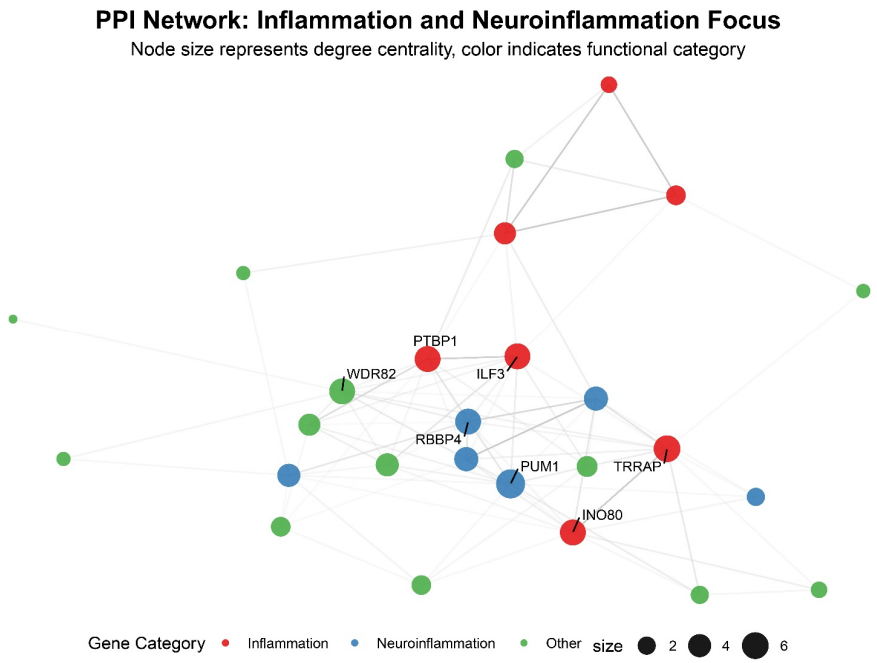

Figure 5. PPI network of ASD-associated genes. Nodes represent genes, and edges represent experimentally validated or predicted protein–protein interactions. Node size is proportional to degree centrality, with hub genes labeled. Color clusters indicate modules enriched for inflammation and neuroinflammation-related functions.

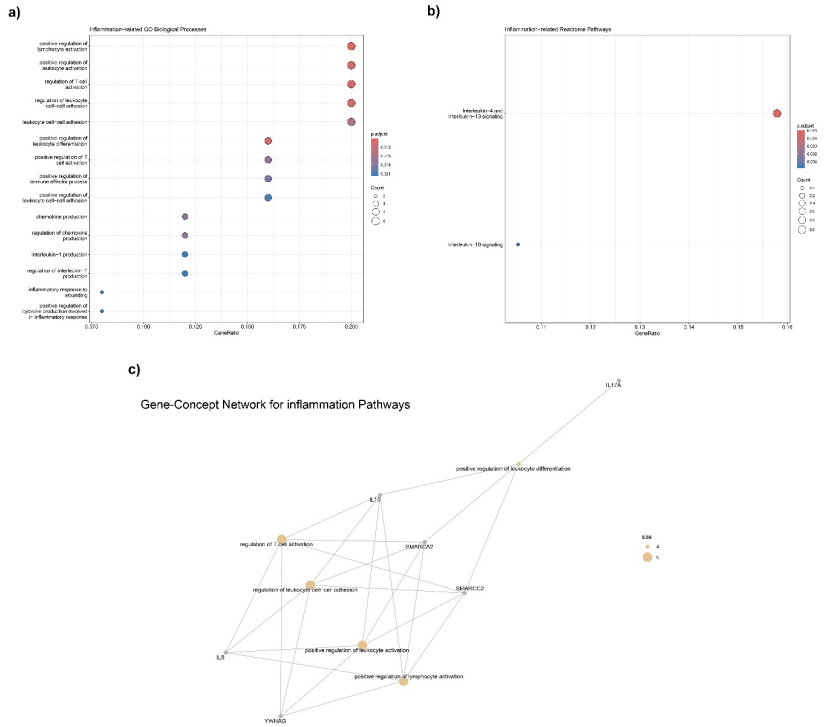

Figure 6. Functional enrichment results for PPI network genes in inflammation. (a) Dot plot showing top gene ontology biological processes enriched in the network (b) Dot plot showing top Reactome pathway enriched in the network. Dot size corresponds to the number of genes involved, and color represents adjusted p-values. (c) Gene concept network illustrating the relationships between enriched terms (blue nodes) and associated genes (red nodes), with emphasis on immune and neuroinflammatory pathways.

tral role of immune modulation and inflammatory signaling in the identified gene network.

Neuroinflammation-Specific Enrichment

In the context of ASD's neurological aspects, a subanalysis focusing on neuroinflammation was conducted. GO enrichment analysis revealed processes such as negative

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]

[DOI: 10.47176/mjiri.39.165]

regulation of the oxidative stress-induced neuron intrinsic apoptotic signaling pathway ($P = 0.0104$), positive regulation of neuroinflammatory response ($P = 0.0133$), and regulation of microglial cell activation ($P = 0.0155$). Subnetwork analysis identified key neuroinflammatory genes, including IL-6, IL-10, SMARCA2, and SMARCC2, that mediate microglial activation, cytokine signaling, and neuronal stress responses. This subnetwork underscores the potential involvement of inflammatory pathways in ASD neuropathology. Enrichment plots (Figure 7), comprising dot plots and gene-concept networks, show strong enrichment of immune and neuroinflammatory pathways within the network. These findings collectively emphasize the intersection of immune regulation and neuroinflammation in the molecular landscape of ASD.

Single-Cell Expression Profiling Across GTEx

Single-cell expression profiling across GTEx tissues has demonstrated that PUM1, TRRAP, ILF3, INO80, and PTBP1 are highly expressed regulatory genes, exhibiting notable enrichment in both epithelial and stromal compartments. Within the epithelial subsets, luminal epithelial cells derived from breast and prostate tissues, along with squamous and basal epithelial cells from the esophageal mucosa, displayed pronounced expression levels of PTBP1, ILF3, and PUM. Fibroblasts and smooth muscle myocytes, particularly those from the esophageal muscularis and cardiac tissues, exhibited elevated levels of TRRAP and INO80. Endothelial and immune cell populations, including T cells, macrophages, and dendritic cells, showed moderate yet consistent expression of all 5 genes, underscoring their role in maintaining cellular homeostasis across lineages (Figure 8).

Demographic and Clinical Characteristics

Table 2 presents the demographic and clinical characteristics of the study participants. The ASD group (n = 15) had a mean age of 4.9 ± 2.5 years, which was younger than that of the control group (n = 5), with a mean age of 7.2 ± 3.3 years. Both groups had an identical male pre-

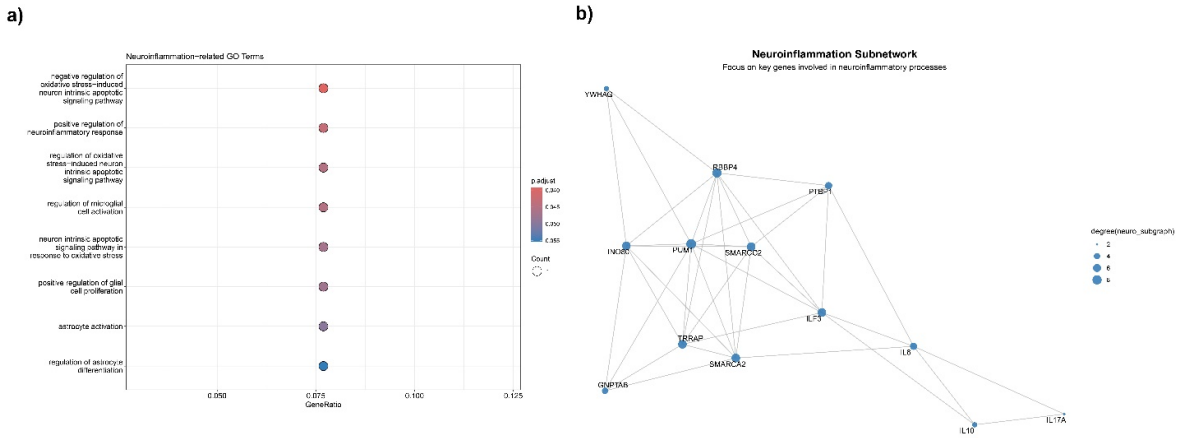

Figure 7. Functional enrichment results for PPI network genes in neuroinflammation
(a) Dot plot showing the top gene ontology biological processes enriched in the network. Dot size corresponds to the number of genes involved, and color represents adjusted P values. (b) Gene-concept network illustrating the relationships between enriched terms (blue nodes) and associated genes (red nodes), emphasizing immune and neuroinflammatory pathways.

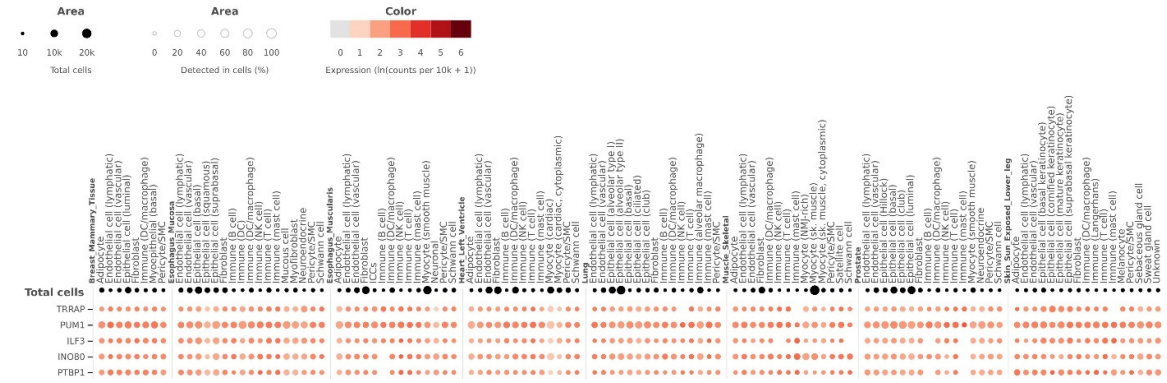

Figure 8. Single-cell expression profiles of PUM1, TRRAP, ILF3, INO80, and PTBP1 across multiple human tissues from the GTEx database. The heatmap illustrates normalized expression levels (ln[CP10k+1]) across distinct cell populations.

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]

[DOI: 10.47176/mjiri.39.165]

Table 2. Demographic and Clinical Characteristics of Study Participants

Variable	ASD Group (n=15)	Control Group (n=5)
Child's age, years	4.9 ± 2.5	7.2 ± 3.3
Male sex	12 (80%)	4 (80%)
Parental consanguinity	8 (53%)	3 (60%)
ASD severity		
- Mild	8 (53%)	0 (NA%)
- Moderate	7 (47%)	0 (NA%)
Functional speech	4 (27%)	5 (100%)
Adequate eye contact	6 (40%)	2 (50%)
Self-care ability	3 (21%)	2 (40%)
Hand dominance		
- Left	5 (36%)	3 (60%)
- Right	9 (64%)	2 (40%)
Hyperactivity present	5 (33%)	2 (40%)
Hyperactivity level		
- Mild	4 (80%)	2 (100%)
- Severe	1 (20%)	0 (0%)
Comorbidities	14 (100%)	5 (100%)
Father's age, years	37.7 ± 6.6	38.6 ± 5.4
Mother's age, years	33.5 ± 5.8	31.2 ± 5.7

NA: Not applicable (ASD severity does not apply to control participants).
*Percentages are calculated based on available data; in some cases, the denominator was smaller due to missing clinical information.

dominance (80%). Parental consanguinity was reported in over half of participants in each group (53% in ASD vs 60% in controls).

All children in the ASD group were clinically classified as having either mild (53%) or moderate (47%) ASD, whereas no control participants had an ASD diagnosis. Functional speech was reported in 27% of children with ASD, compared with 100% in controls. Adequate eye contact was documented in 40% of ASD cases and 50% of controls, while self-care ability was noted in 21% of ASD participants and 40% of controls.

In terms of hand dominance, left-handedness was more common in the control group (60%) than in the ASD group (36%), whereas right-handedness was more prevalent in the ASD group (64% vs 40%). Hyperactivity was reported in 33% of ASD participants and 40% of controls, with mild hyperactivity being more common (80% in ASD and 100% in controls) than severe hyperactivity, which was only observed in one ASD participant. Comorbidities were present in all participants in both groups. The mean paternal age was similar between the groups (37.7 ± 6.6 years in ASD vs 38.6 ± 5.4 years in controls), as was the mean maternal age (33.5 ± 5.8 years in ASD vs 31.2 ± 5.7 years in controls).

Several data points were missing across variables, especially for functional measures (such as eye contact and self-care ability), behavioral assessments (such as hyperactivity level), and certain demographic indicators. These missing entries could have affected the thoroughness of group comparisons and should be taken into account when interpreting the results.

Quantitative Real-Time PCR Validation of Key Inflammatory Genes

qRT-PCR analysis was conducted to validate the expression patterns of selected inflammation- and neuroinflammation-related genes identified from the transcriptomic analysis. The results showed significant differential expression between the ASD and healthy control groups (Figure 9 and Table 3).

IL-6 was markedly upregulated in the ASD group, with a fold change of 12.8 (P = 0.049), consistent with its known proinflammatory role. Similarly, IL-17 showed a significant upregulation, with a fold change of 21.3 (P = 0.048), indicating increased Th17-mediated inflammatory responses in ASD. NF-KB1, a key transcription factor in immune activation pathways, was the most upregulated gene, with a fold change of 42.4 (P = 0.039), suggesting robust activation of downstream inflammatory cascades.

In contrast, IL-10, an anti-inflammatory cytokine, was significantly downregulated in the ASD group, with a fold change of 0.101 (P = 0.038), indicating a potential imbalance between pro- and anti-inflammatory signals.

Discussion

The PPI network analysis identified PUM1, TRRAP, ILF3, INO80, and PTBP1 as central hub genes with high connectivity in subnetworks enriched for immune-related and neuroinflammatory pathways. Functional enrichment analysis (gene ontology and Reactome) of the network revealed terms such as "immune response," "cytokine signaling," and "innate immune system," consistent with previous ASD transcriptome studies highlighting inflammatory signaling pathways in autism [16-18]. These hub genes play crucial roles in networks associated with immune signaling, potentially linking neuronal regulation to dysregulated neuroimmune processes in ASD. Further investigation of key inflammatory genes through qRT-PCR analysis showed significant dysregulation of inflammation- and neuroinflammation-related genes in individuals with ASD compared to healthy controls.

Notably, proinflammatory cytokines IL-6 and IL-17 exhibited substantial upregulation with fold changes of 12.8 and 21.3, respectively. The NF-κB1, a central regulator of immune activation, showed the highest upregulation with a fold change of 42.4 (P = 0.039). In contrast, the anti-inflammatory cytokine IL-10 was significantly downregulated (fold change = 0.101).

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]

[DOI: 10.47176/mjiri.39.165]

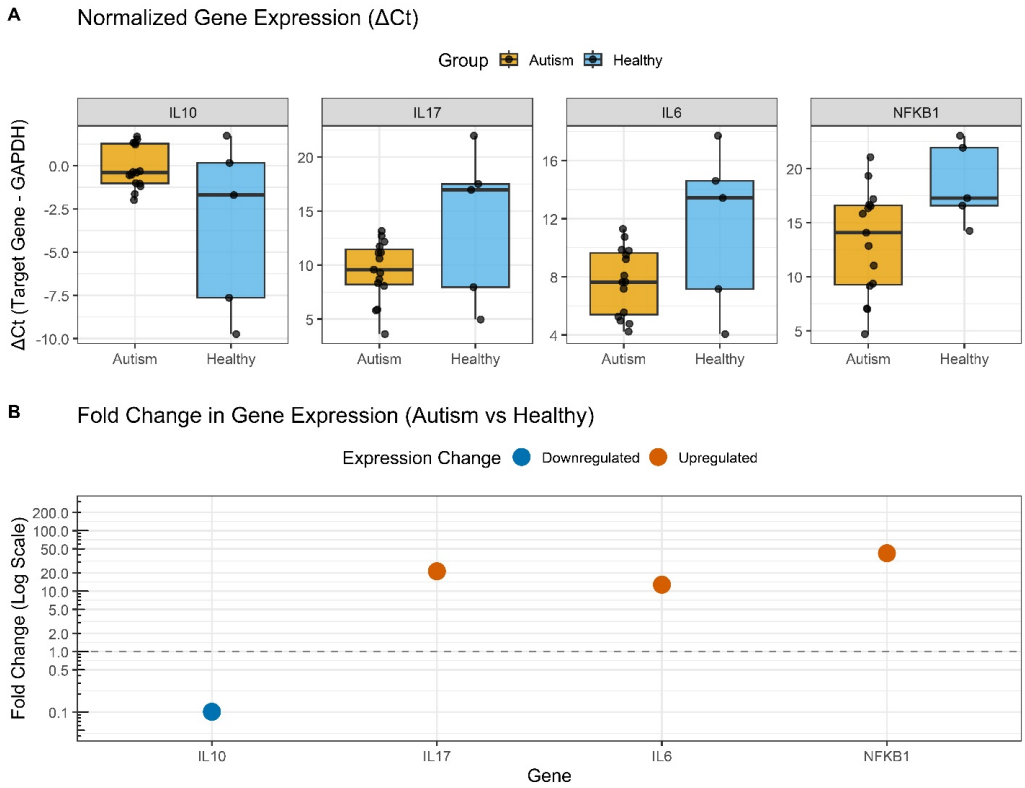

Figure 9. Differential expression of inflammation-related genes in ASD versus healthy controls by qRT-PCR.
(a) Bar plot of ΔCt values showing mean ± standard deviation for each gene. Lower ΔCt values correspond to higher transcript abundance. Upregulated genes (IL-6, IL-17, NF-KB1) in ASD samples are reflected by lower ΔCt compared to controls, whereas IL-10 shows higher ΔCt, indicating downregulation. (b) Dot plot of fold changes ($2^{-}\Delta\Delta Ct$) with 95% confidence intervals, illustrating the magnitude and direction of expression differences. Upregulated genes (IL-6, IL-17, NF-KB1) appear above the baseline (fold change > 1), while IL-10 is positioned below (fold change <1), indicating reduced expression in ASD. Significance was determined using Student's t-test ($P < 0.05$).

Table 3. Details of the statistical analysis results of real-time

Gene	Expression	Std. Error	95% C.I.	P(H1)	Result
IL6	12.77	0.145 - 680.550	0.017 - 6,905.431	0.049	UP
IL10	0.101	0.002 - 2.767	0.000 - 7.984	0.038	DOWN
IL17	33.825	0.075 - 5,668.436	0.006 - 122,457.650	0.048	UP
NF-κB1	42.381	0.884 - 3,872.334	0.042 - 80,490.958	0.039	UP

PUM1 is an RNA-binding translational repressor that regulates innate immunity by suppressing the cytosolic RNA sensor LGP2, which helps limit interferon and pro-inflammatory cytokine expression [19]. Loss of PUM1 function can lead to increased IL-6 and type I interferon responses, potentially exacerbating neuroinflammatory processes. Clinically, PUM1 haploinsufficiency is associated with severe neurodevelopmental syndromes [20]. While direct links to ASD are not established, its immune-regulatory role suggests it could contribute to cytokine imbalances in autism.

TRRAP, a scaffold protein in histone acetyltransferase complexes, has been implicated in syndromic neurodevelopmental disorders, including ASD, due to rare de novo missense variants [21]. In addition to its role in neuronal gene transcription, TRRAP and other chromatin remodelers can impact immune gene expression, suggesting that disruptions in TRRAP may affect both neuronal and immune regulatory pathways [22].

ILF3 (NF90/NFAR2) negatively regulates innate immune responses in dendritic cells, leading to increased cytokine production when knocked down [23]. ILF3 also plays a role in stress-responsive neuronal gene programs, suggesting a potential influence on microglial and neuronal responses in inflammatory conditions, which may impact neuroimmune dysregulation in ASD [24].

INO80, the ATPase of the INO80 chromatin-remodeling complex, is crucial for maintaining genome stability in neural progenitors [22]. While its direct immune function is limited, chromatin remodeling intersects with innate immunity through DNA damage signaling and transcriptional control of inflammatory genes. We propose that INO80 dysfunction may play a role in neuroinflammation associated with ASD by inducing genome instability and the release of danger-associated molecular patterns.

PTBP1, a splicing regulator crucial for neuronal maturation, also plays a role in inflammation by promoting the senescence-associated secretory phenotype, leading to the secretion of IL-6 and IL-8 [25]. This dual function links def-

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]
[DOI: 10.47176/mjiri.39.165]

icits in synaptic development to the regulation of inflammatory cytokines, consistent with the combined neurodevelopmental and neuroinflammatory characteristics of ASD.

The enrichment of immune-related GO terms, such as cytokine-mediated signaling and response to interferon, highlights the functional convergence of these hub genes on immune activation pathways. PUM1 and ILF3 directly control the expression of antiviral and cytokine genes [19,23], while PTBP1 influences inflammatory secretions [25]. This is in line with transcriptomic studies that have shown increased interleukin and interferon signaling in ASD brain tissue [16].

Our discovery of elevated IL-6 expression is consistent with previous research linking IL-6 to ASD. Wei et al. found that increased IL-6 levels in the brains of transgenic mice led to autistic-like behaviors and synaptic imbalances [26]. Clinical studies have also shown elevated IL-6 in the serum, cerebrospinal fluid, and brain tissue of individuals with ASD [27-30]. For example, Nadeem et al. found higher IL-6 levels in B cells of children with ASD [28], while Hughes et al. observed increased IL-6 production in monocytes of ASD patients, correlating with specific behaviors [29]. The significant upregulation of IL-6 in our dataset (12.8-fold) may be due to mRNA sensitivity in qRT-PCR or tissue-specific differences compared to protein-level studies.

Our study showed a significant increase (21.3-fold) in IL-17 expression, consistent with Th17-mediated inflammation in ASD. Previous research by Nadeem et al. demonstrated dysregulated IL-6 receptor signaling, which promotes Th17 differentiation and IL-17A production in ASD CD4+ T cells [31]. Other studies have reported elevated Th17/Treg ratios, reduced FoxP3 expression, and decreased IL-10 production in ASD, suggesting a pro-inflammatory T helper response. IL-17 can compromise blood-brain barrier integrity and activate microglia, both relevant to ASD neuropathology [32].

Our findings show a significant increase (42.4-fold) in NF-κB1 upregulation, consistent with previous studies that reported elevated NF-κB1 DNA-binding activity in ASD peripheral blood mononuclear cells [33] and increased NF-κB1-associated transcripts in ASD brain tissue[34,35]. NF-κB1 is known to regulate the expression of IL-6 and IL-17, suggesting that its overactivation may contribute to the observed cytokine imbalance. Additionally, Honarmand Tamizkar et al demonstrated dysregulation of NF-κB1-associated long non-coding RNAs in ASD, highlighting the role of NF-κB1 signaling in transcriptional control in ASD-related immune dysfunction [35].

Inflammatory regulation in ASD. Nadeem et al. reported reduced IL-10 in B cells [28]. Ross et al found that a higher IL-6/IL-10 ratio correlated with more severe social deficits in 22q11.2 deletion syndrome with autistic features [36]. Jyonouchi et al also linked altered mitochondrial function to high IL-1β/IL-10 ratios, indicating a broader immune-metabolic interplay in ASD [32]. IL-10 deficiency can worsen Th17 responses, perpetuating inflammation.

Our study highlights the IL-6/IL. Although direct links to ASD are not established, its immune-regulatory role suggests it could contribute to the 17/NF-κB1 axis as a mechanistic cascade where NF-κB1 serves as a master regulator of inflammatory gene transcription, including IL-6, which promotes Th17 differentiation and IL-17 production [31,34]. This proinflammatory loop is further fueled by the downregulation of IL-10, which typically restrains NF-κB1 activity and Th17 expansion.

In the central nervous system (CNS), IL-6 can impact neurodevelopment by altering synaptic formation and the balance between excitatory and inhibitory signals [26]. IL-17 has been shown to trigger microglial activation and disrupt neuronal function. NF-κB1 signaling is activated not only in immune cells but also in glial cells and neurons in response to oxidative stress, mitochondrial dysfunction, and environmental triggers [32,34]. The interaction between mitochondria and the immune system, as described by Jyonouchi et al., may further enhance NF-κB1 activation and the expression of inflammatory cytokines, creating a harmful cycle [32].

Peripheral immune dysregulation can also contribute to neuroinflammation by increasing blood-brain barrier permeability, allowing cytokines and activated immune cells to enter the CNS. Inflammasome activation, as highlighted by Saresella et al, is another factor driving the release of IL-1β, IL-6, and IL-18, linking dysfunction in the gastrointestinal barrier to CNS inflammation [37]. Our discovery of NF-κB1 hyperactivation may partially explain these connections, as NF-κB1 is known to regulate inflammasome components at the transcriptional level.

Clinical Implications

Our findings, along with existing research, suggest that the expression patterns of IL-6, IL-17, NF-κB1, and IL-10 could serve as biomarkers for a pro-inflammatory subtype of ASD. This immune profiling could help in categorizing patients for targeted treatments. For instance, IL-6 inhibitors (such as tocilizumab) and inhibitors of the Th17 pathway are hypothesized to offer benefits in various neuroinflammatory disorders; however, their efficacy in ASD remains to be established.

Nutraceutical interventions, like luteolin supplementation, have demonstrated the ability to reduce IL-6 and TNF-α levels in ASD [27], indicating the potential for modulating these pathways in a clinical setting. NF-κB1 inhibitors, currently under study for autoimmune and inflammatory disorders, may also be relevant given the significant upregulation of NF-κB1 observed in our study. Additionally, IL-10 supplementation or strategies to enhance IL-10 production could help restore immune balance and alleviate neuroinflammatory processes. Apart from therapeutic implications, these cytokines and transcription factors could be valuable for early screening in high-risk populations, as immune abnormalities have been identified in young children and genetic syndromes linked to ASD [30,36].

However, our study has limitations, including a small sample size, the need for protein-level validation of cytokines (eg, ELISA or Western Blot), and the opportunity for broader pathway analysis. Future research should focus on longitudinal designs, multi-omics approaches, and experimental studies targeting the IL-6/IL-17/NF-κB1 axis

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]

[DOI: 10.47176/mjiri.39.165]

in ASD models. Clinical trials assessing immune-modulating agents and interventions to enhance gut barrier integrity are also advised. Biomarker discovery efforts should aim to predict ASD onset or progression for early intervention.

Conclusion

This study combines transcriptomic analysis and targeted qRT-PCR validation to identify an immune-driven molecular profile in ASD. The findings indicate an overactivation of the IL-6 → Th17 (IL-17) pathway regulated by NF-κB1, along with a deficiency of the anti-inflammatory cytokine IL-10. Key network hubs (PUM1, TRRAP, ILF3, INO80, PTBP1) suggest a link between chromatin remodeling, RNA-binding proteins, and inflammatory signaling in amplifying neuroimmune responses. Future research should focus on longitudinal studies, integrating multiple omics data, experimental manipulation of pathways in model systems, and clinical trials testing immunomodulatory interventions in ASD subgroups selected based on biomarkers.

Authors' Contributions

Yoosra N. Abed Al-Saadi contributed to the study's conceptualization and design, participated in methodology development, was involved in data collection, and contributed to the original draft. Zainab Basim Mohammed was responsible for data analysis and visualization and participated in the review and editing of the manuscript. Mithal Abdulkareem Abdoun was involved in the investigation, data validation, and potentially software development. Ali Mahmoudi, as the corresponding author, provided supervision, managed project administration, extensively reviewed and edited the manuscript, and may have secured funding for the project.

Ethical Considerations

The study was reviewed and approved by the Institutional Review Board Ethical Committee of Ibn Sina University of Medical and Pharmaceutical Sciences (Approval Code: ISU.15.1.24; Approval Date: 21 October 2024). Written informed consent was obtained from all participants, in the case of minors, from their parents and legal guardians before sample collection. The research was conducted in accordance with the principles of the Declaration of Helsinki.

Acknowledgment

The authors would like to thank all the participants for sharing their information. They also extend their appreciation to the staff and colleagues at Nabu Scientific Foundation, whose assistance in laboratory work and data analysis was invaluable.

Conflict of Interests

The authors declare that they have no competing interests.

References

1. Siniscalco D, Schultz S, Brigida AL, Antonucci N. Inflammation and Neuro-Immune Dysregulations in Autism Spectrum Disorders. Pharmaceuticals. 2018;11(2):56.
2. Chen Y, Du X, Zhang X, Li F, Yuan S, Wang W, et al. Research trends of inflammation in autism spectrum disorders: a bibliometric analysis. Front Immunol. 2025;16.
3. Prata J, Santos SG, Almeida MI, Coelho R, Barbosa MA. Bridging Autism Spectrum Disorders and Schizophrenia through inflammation and biomarkers - pre-clinical and clinical investigations. J Neuroinflammation. 2017;14(1):179.
4. Meltzer A, Van de Water J. The Role of the Immune System in Autism Spectrum Disorder. Neuropsychopharmacology. 2017;42(1):284-98.
5. Roe K. Autism Spectrum Disorder Initiation by Inflammation-Facilitated Neurotoxin Transport. Neurochem Res. 2022;47(5):1150-65.
6. Eftekharian MM, Ghafouri-Fard S, Noroozi R, Omrani MD, Arsang-Jang S, Ganji M, et al. Cytokine profile in autistic patients. Cytokine. 2018;108:120-6.
7. Bryn V, Aass HC, Skjeldal OH, Isaksen J, Saugstad OD, Ormstad H. Cytokine Profile in Autism Spectrum Disorders in Children. J Mol Neurosci. 2017;61(1):1-7.
8. Inga Jácome MC, Morales Chacòn LM, Vera Cuesta H, Maragoto Rizo C, Whilby Santiesteban M, Ramos Hernandez L, et al. Peripheral Inflammatory Markers Contributing to Comorbidities in Autism. Behav Sci (Basel). 2016;6(4).
9. Zhao H, Zhang H, Liu S, Luo W, Jiang Y, Gao J. Association of Peripheral Blood Levels of Cytokines With Autism Spectrum Disorder: A Meta-Analysis. fpsyt. 2021;12:670200.
10. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun. 2011;25(1):40-5.
11. Wei H, Alberts I, Li X. Brain IL-6 and autism. Neuroscience. 2013;252:320-5.
12. Wei H, Mori S, Hua K, Li X. Alteration of brain volume in IL-6 overexpressing mice related to autism. Int J Dev Neurosci. 2012;30(7):554-9.
13. Wei H, Chadman KK, McCloskey DP, Sheikh AM, Malik M, Brown WT, et al. Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. Biochim Biophys Acta. 2012;1822(6):831-42.
14. Jyonouchi H, Geng L, Davidow AL. Cytokine profiles by peripheral blood monocytes are associated with changes in behavioral symptoms following immune insults in a subset of ASD subjects: an inflammatory subtype? J Neuroinflammation. 2014;11:187.
15. Wei H, Alberts I, Li X. Brain IL-6 and autism. Neuroscience. 2013;252:320-5.
16. Alshammery S, Patel S, Jones HF, Han VX, Gloss BS, Gold WA, et al. Common targetable inflammatory pathways in brain transcriptome of autism spectrum disorders and Tourette syndrome. Front Neurosci. 2022;Volume 16 - 2022.
17. Brigida AL, Schultz S, Cascone M, Antonucci N, Siniscalco D. Endocannabinod Signal Dysregulation in Autism Spectrum Disorders: A Correlation Link between Inflammatory State and Neuro-Immune Alterations. Int J Mol Sci. 2017;18(7).
18. Brocker C, Thompson D, Matsumoto A, Nebert DW, Vasiliou V. Evolutionary divergence and functions of the human interleukin (IL) gene family. Hum Genomics. 2010;5(1):30-55.
19. Liu Y, Qu L, Liu Y, Roizman B, Zhou GG. PUM1 is a biphasic negative regulator of innate immunity genes by suppressing LGP2. Proc Natl Acad Sci U S A. 2017;114(33):E6902-e11.
20. Ayhan F, Konopka G. Regulatory genes and pathways disrupted in autism spectrum disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2019;89:57-64.
21. Cogné B, Ehresmann S, Beauregard-Lacroix E, Rousseau J, Besnard T, Garcia T, et al. Missense Variants in the Histone Acetyltransferase Complex Component Gene TRRAP Cause Autism and Syndromic Intellectual Disability. Am J Hum Genet. 2019;104(3):530-41.
22. Keil JM, Doyle DZ, Qalieh A, Lam MM, Funk OH, Qalieh Y, et al. Symmetric neural progenitor divisions require chromatin-mediated homologous recombination DNA repair

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]

[DOI: 10.47176/mjiri.39.165]

by Ino80. Nat Commun. 2020;11(1):3839.
23. Nazitto R, Amon LM, Mast FD, Aitchison JD, Aderem A, Johnson JS, et al. ILF3 Is a Negative Transcriptional Regulator of Innate Immune Responses and Myeloid Dendritic Cell Maturation. J Immunol. 2021;206(12):2949-65.
24. Yamashita A, Shichino Y, Fujii K, Koshidaka Y, Adachi M, Sasagawa E, et al. ILF3 prion-like domain regulates gene expression and fear memory under chronic stress. iScience. 2023;26(3):106229.
25. Georgilis A, Klotz S, Hanley CJ, Herranz N, Weirich B, Morancho B, et al. PTBP1-Mediated Alternative Splicing Regulates the Inflammatory Secretome and the Pro-tumorigenic Effects of Senescent Cells. Cancer Cell. 2018;34(1):85-102.e9.
26. Wei H, Chadman KK, McCloskey DP, Sheikh AM, Malik M, Brown WT, et al. Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. Biochim Biophys Acta - Mol Basis Dis. 2012;1822(6):831-42.
27. Tsilioni I, Taliou A, Francis K, Theoharides TC. Children with autism spectrum disorders, who improved with a luteolin-containing dietary formulation, show reduced serum levels of TNF and IL-6. Transl Psychiatry. 2015;5(9):e647-e.
28. Nadeem A, Ahmad SF, Al-Harbi NO, Al-Ayadhi LY, Sarawi W, Attia SM, et al. Imbalance in pro-inflammatory and anti-inflammatory cytokines milieu in B cells of children with autism. Mol Immunol. 2022;141:297-304.
29. Hughes HK, Onore CE, Careaga M, Rogers SJ, Ashwood P. Increased Monocyte Production of IL-6 after Toll-like Receptor Activation in Children with Autism Spectrum Disorder (ASD) Is Associated with Repetitive and Restricted Behaviors. Brain Sci. 2022; 12(2).
30. Anastasescu CM, Gheorman V, Stoicanescu E-C, Popescu F, Gheorman V, Udriștoiu I. Immunological Biomarkers in Autism Spectrum Disorder: The Role of TNF-Alpha and Dependent Trends in Serum IL-6 and CXCL8. Life. 2024;14(9):1201.
31. Nadeem A, Ahmad SF, Attia SM, Al-Ayadhi LY, Al-Harbi NO, Bakheet SA. Dysregulation in IL-6 receptors is associated with upregulated IL-17A related signaling in CD4+ T cells of children with autism. Prog Neuropsychopharmacol Biol Psychiatry. 2020;97:109783.
32. Jyonouchi H, Geng L, Rose S, Bennuri SC, Frye RE. Variations in Mitochondrial Respiration Differ in IL-1ß/IL-10 Ratio Based Subgroups in Autism Spectrum Disorders. Front Psychiatry. 2019;Volume 10 - 2019.
33. Naik US, Gangadharan C, Abbagani K, Nagalla B, Dasari N, Manna SK. A Study of Nuclear Transcription Factor-Kappa B in Childhood Autism. PLOS ONE. 2011;6(5):e19488.
34. Liao X, Li Y. Nuclear Factor Kappa B in Autism Spectrum Disorder: A Systematic Review. Pharmacol Res. 2020;159:104918.
35. Honarmand Tamizkar K, Badrlou E, Aslani T, Brand S, Arsang-Jang S, Ghafouri-Fard S, et al. Dysregulation of NF-κB-Associated LncRNAs in Autism Spectrum Disorder. Front Mol Neurosci. 2021;Volume 14 - 2021.
36. Ross HE, Guo Y, Coleman K, Ousley O, Miller AH. Association of IL-12p70 and IL-6:IL-10 ratio with autism-related behaviors in 22q11.2 deletion syndrome: A preliminary report. Brain Behav Immun. 2013;31:76-81.
37. Saresella M, Piancone F, Marventano I, Zoppis M, Hernis A, Zanette M, et al. Multiple inflammasome complexes are activated in autistic spectrum disorders. Brain Behav Immun. 2016;57:125-33.

[Downloaded from mjiri.iums.ac.ir on 2026-01-31]

[DOI: 10.47176/mjiri.39.165]