CHAPTER 8

The role of neuroinflammation pathomechanism on autism spectrum disorder and unraveling potential biomarkers for early detection

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8.1. Abstract

Autism spectrum disorder (ASD) is a complex neurological and developmental condition characterized by a broad range of symptoms. Numerous studies have implicated multi-genetic and epigenetic factors in the etiology of ASD, and efforts have been made to identify potential biomarkers. However, the quest for potent and precise biomarkers for the detection and diagnosis of ASD remains an unmet challenge. In this study, a comprehensive bioinformatic analysis of gene expression profiles from ASD patients was conducted to identify common genes that serve as potential and reliable biomarkers for early diagnosis of ASD, particularly from accessible tissues. The analysis of brain datasets revealed 581 differentially expressed genes (DEGs), with functional enrichment implicating processes such as positive regulation of cytokine production, response to bacterial molecules, and involvement in the TNF signaling pathway.

In contrast, the blood dataset exhibited sixty significant DEGs associated with inflammatory responses, including chemokine response and the interaction of cytokines and cytokine receptors. Remarkably, eight common genes were identified between the two datasets. Among these common DEGs, *UPB1*, *CXCL1*, *CXCL10*, *CSF1*, *CCL2*, and *IL1B* were identified as novel genes associated with ASD, while *FFAR2* and *WWC2-AS2* were novel genes not previously linked to ASD. The common genes were enriched in the TNF signaling pathway. The findings revealed the relevance of immunity dysregulation in the neurodevelopmental of ASD. Additionally, these common genes hold promise as prospective biomarkers for early detection from accessible tissues and represent potential targets for future pharmacological interventions in ASD.

8.2. Introduction

Autism spectrum disorder (ASD) is a category of neurodevelopmental diseases that influence the development of neurological pathways, presenting a diverse array of manifestations, including impairment in communication, stereotypical behavior patterns, speech troubles, and social interaction deficits [1]. ASD has been exhibited an

imbalanced gender distribution, with boys showing a fourfold higher susceptibility than girls [2].

Heterogeneity and diversity in manifestations of ASD support the complex genetic and environmental etiology of ASD [3]. One of the largest studies to date, including more than two million children, concluded that 45–56% of ASD are inheritable [4]. Multiple studies have identified both frequent and rare variants, revealing the complexity of ASD disorder [5-7]. Moreover, accumulating studies have supported the involvement of numerous genetic variations in synaptopathies and neuroinflammation as pathomechanisms in ASD [8-10]. Than and his colleagues analyzed the composition of cerebrospinal fluid (CSF), and their findings revealed elevated levels of four inflammatory cytokines (TNF-α, IL-4, and IL-21) that play fundamental roles as inflammatory mediators driving the neuro-immune system in ASD patients [11]. In the same context, an investigation of the whole blood for detecting markers revealed three genes, namely *NMUR1*, *HMGB3*, and *PTPRN2*, were differently expressed and associated with ASD. Subsequent analysis of their ASD-associated signature highlighted the dysregulation of immune-inflammatory pathways [12]. Meta-analysis of postmortem brain tissue demonstrated 235 new differentially expressed genes (DEGs) not detected in previous studies. Gene ontology (GO) enrichment analysis identified the upregulation of genes involved in inflammatory immune response pathways, including TNF signaling [13]. In the same context, samples from Whole-exome sequence data of approximately 35,584, including 11,986 with ASD, were analyzed. It found 102 genes implicated in ASD risk, and 30 novel critical genes were reported [7].

Even though numerous studies have been conducted to define gene expression alterations from brain tissues in ASD, they have successfully predicted cellular and molecular processes that might have critical roles in the neurological development of ASD [6, 13-15]. Nevertheless, the genes may not possess predictive value if they are not expressed or modified in accessible tissues. Conversely, many high-risk genes were detected in various obtainable tissues, such as blood [12], saliva [16], and placenta [14], without confirming their crucial roles in the brain of ASD patients. Therefore, in the current study, two datasets from peripheral blood and post-mortem brain (PMB) tissue transcriptomes of ASD samples were used to identify the common genes as susceptible biomarkers for ASD diagnosis using accessible tissue. In this regard, DEGs and

functional enrichment analyses were performed separately to determine the top robust DEGs, GO, and biological pathways for each dataset to shed light on the putative processes underlying the etiology of ASD. Additionally, the overlap between studied DEGs lists was carried out to identify the common genes and their molecular biological pathways enriched within as candidate biomarkers with the benefits of early diagnosis in a consistent and reliable pathogenesis which may facilitate diagnostic precisions and therapeutic opinions.

8.3. Material and methodology

8.3.1. Acquisition of the transcriptomic Datasets

A transcriptomic database acquired RNA-Seq datasets of human ASD patients from Gene Expression Omnibus (GEO). The datasets were obtained from the GSE178205 dataset of frozen human brain samples [17] and GSE64018 [18], consisting of 47 samples (25 ASD, 22 typically developing control (TD)).

Furthermore, the subgroup dataset was nominated from the GSE140702 dataset of cultured peripheral blood monocyte samples [19]. This subgroup included TD control children (N = 23) (19 male, 4 female) and ASD patients (N = 25) (autism disorder AD (13 male, 4 female), Pervasive Developmental Disorder- Not Otherwise Specified(PDD-NOS) patients (6 male, 2 female)).

8.3.2. Differentially expressed genes analysis of two datasets

DESeq2 package [20] was applied to determine differentially expressed genes when comparing control versus ASD samples within the two datasets. Filtering criteria were implemented to identify statistically significant genes in the brain and blood datasets separately, employing P-value < 0.01 and -1 > log2FoldChange > 1 to mark upregulated and downregulated genes.

8.3.3. Functional annotation analysis

8.3.3.1. Gene ontology and enriched pathway analysis

Gene over-representation analysis was carried out independently on the two datasets using the ClusterProfiler R package [21] to identify cellular components (CC), biological processes (BP), and molecular functions (MF). Enrichment for Gene Ontology (GO) terms and pathways was determined based on a significance threshold of P-value < 0.01.

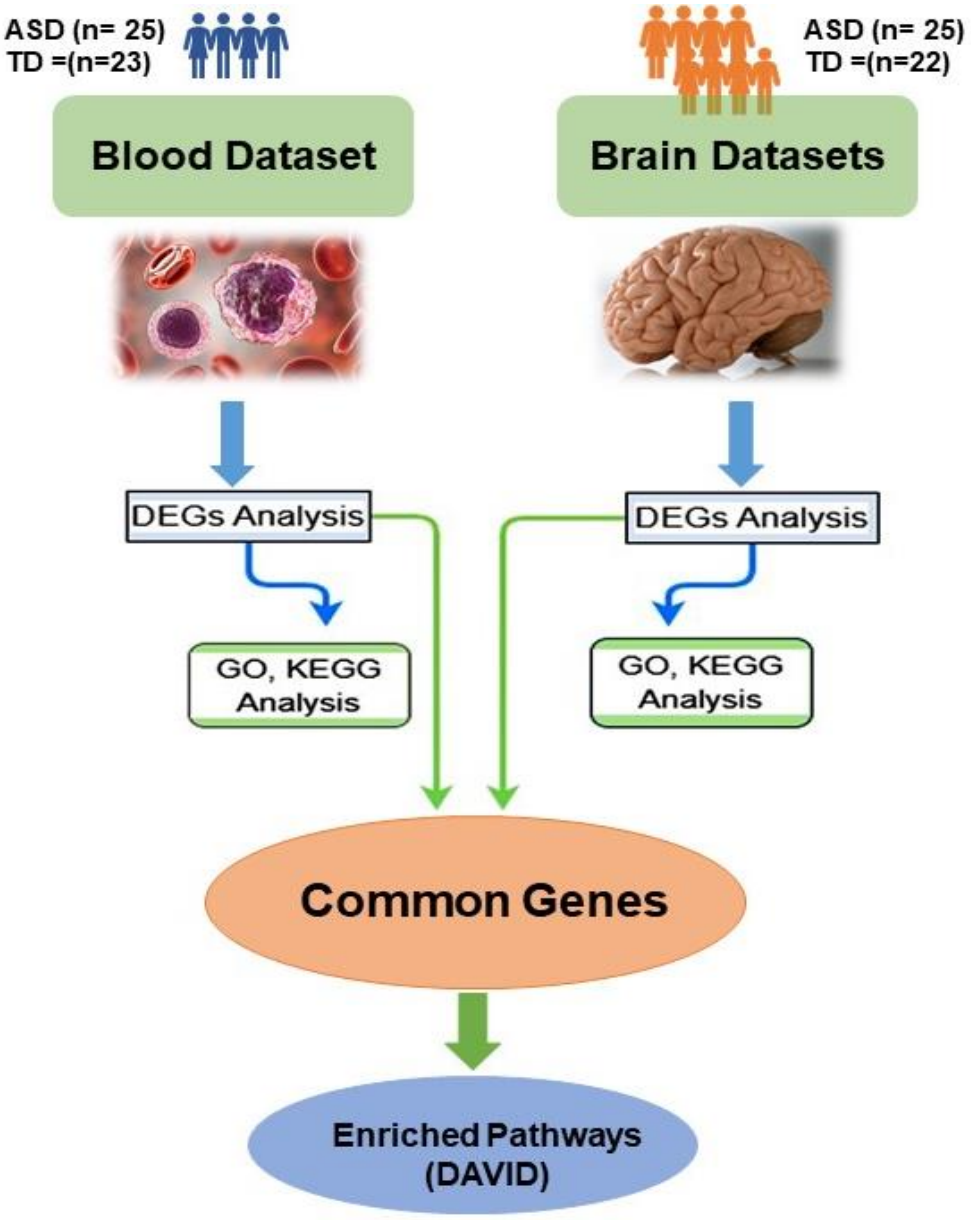
Additionally, the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was performed using the ClusterProfiler package.

8.3.4. Comparison of the DEGs between the two datasets in silico

Utilizing a Venn diagram, common DEGs between differentially expressed genes from the PMB tissue datasets and DEGs from the blood dataset were identified, taking into account a significance threshold of *P*-value < 0.01. ClusterProfiler R package was applied.

8.3.5. The Database for Annotation, Visualization, and Integrated Discovery (DAVID)

A functional profile was constructed by employing genes that exhibited differential expression in both datasets through the DAVID server. The meta-analysis workflow conducted to identify common differentially expressed genes is illustrated in (**Scheme. 8.1**).



Scheme 8.1 *The flowchart shows the meta-analysis workflow. ASD: autism spectrum disorder, TD: typically developing, DEGs: differentially expressed genes, GO: gene ontology, KEGG: kyoto encyclopedia of genes and genomes, DAVID: database for annotation, visualization, and integrated Discovery.*

8.4. Results

8.4.1. Identification of DEGs

The comprehensive analysis of differential expression revealed a total of 581 DEGs in the ASD brain dataset, meeting the criteria of *P* -value < 0.01 (**Table 8.1**). Among these, 520 genes exhibited significant upregulation with cut-off Log2FC >1, whereas 61 genes displayed downregulation with cut-off Log2FC < -1 (**Figure 8.1A**). Hierarchical clustering analysis underscored a distinct DEGs profile, highlighting the notable differences between ASD patients and control subjects. In the blood dataset analysis, significant DEGs between ASD and typically developing controls were identified using the *P*-value < 0.01 and 1 < Log2FC < -1 criteria, resulting in a curated list of 60 genes. Notably, six of these genes were novel namely (*SYNPO2*, *WWC2-AS2*, *CNIH3*, *RN7SL239P*, *KRT86*, and *TRIB3*) (**Table 8.2**) with 20 genes downregulated and 40 genes upregulated. The volcano plot represented the DEGs in (**Figure 8.1B**).

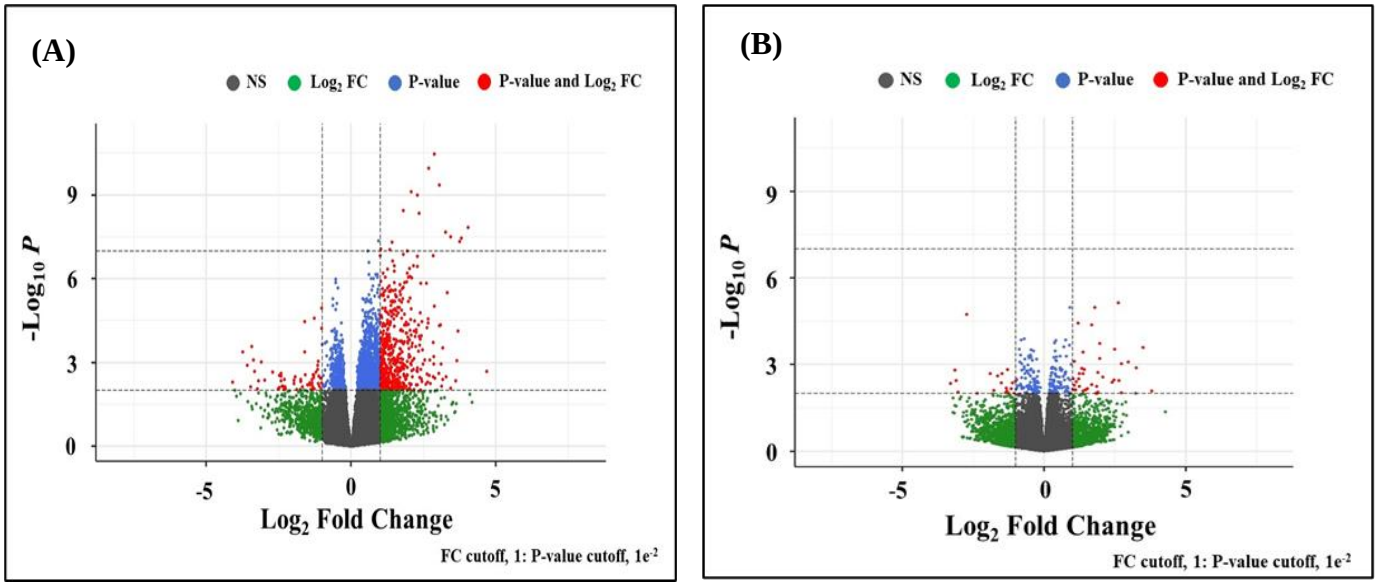


Figure 8.1. Differential expression genes using the Volcano plot. *(**A**) The brain datasets show 581 significant genes (P-value < 0.01 and 1 < Log2FC < -1 as thresholds) in red dots left. (**B**) The blood monocytes dataset showed 60 DEGs in red dots.*

Table 8.1. The differential expressed genes DEGs of brain datasets (-1 >Log2FoldChange > 1, *P*-value < 0.01)

Symbol	Entrez Gene ID	log2FoldChange	*P*-value	*P*-adj
HSPA6	3310	5.761707	5.87E-14	1.21E-09
HSPB1	3315	2.746181	8.49E-13	5.45E-09
SERPINH1	871	3.279011	1.06E-12	5.45E-09
BAG3	9531	2.892517	5.47E-13	5.45E-09
HSPA1A	3303	2.875769	3.50E-11	1.45E-07
HMOX1	3162	2.688162	1.13E-10	3.89E-07
RRAD	6236	3.046311	4.46E-10	1.31E-06
DEPP1	11067	2.070287	7.74E-10	2.00E-06
DNAJB1	3337	2.285098	1.05E-09	2.40E-06
NFKB2	4791	1.799041	3.70E-09	7.63E-06
CDKN1A	1026	2.353722	4.50E-09	8.45E-06
CSF3	1440	4.037722	1.48E-08	2.54E-05
SOCS3	9021	3.27043	2.17E-08	3.44E-05
NPAS4	266743	3.431592	3.11E-08	4.59E-05
SERPINA3	12	3.809963	3.57E-08	4.91E-05
LINC02138	400558	3.75165	4.61E-08	5.60E-05
BST2	684	1.411501	4.95E-08	5.68E-05
CSF1	1435	1.340807	9.09E-08	9.38E-05
ZC3HAV1	56829	1.01853	8.82E-08	9.38E-05
MAFF	23764	1.939069	1.02E-07	9.82E-05
CCN1	3491	1.810254	1.33E-07	0.00012
MKNK2	2872	1.015318	1.48E-07	0.000126
TNFRSF12A	51330	2.845228	1.53E-07	0.000126
GADD45B	4616	2.299832	1.63E-07	0.00013
CEBPD	1052	1.440674	2.38E-07	0.000182
FPR1	2357	2.152125	3.55E-07	0.000243
PPP1R15A	23645	1.489687	3.45E-07	0.000243
GBP1	2633	2.289502	3.64E-07	0.000243

SPHK1	8877	2.020066	4.32E-07	0.000279
HAP1	9001	1.263774	4.64E-07	0.00029
GADD45G	10912	1.503308	5.32E-07	0.000323
EIF4EBP1	1978	1.101979	6.34E-07	0.000373
FOSB	2354	1.946419	6.50E-07	0.000373
GBP2	2634	2.032393	8.37E-07	0.000432
ICAM1	3383	1.977718	8.97E-07	0.000434
SPI1	6688	1.190007	8.80E-07	0.000434
INHBA	3624	2.099578	9.60E-07	0.00045
CHI3L1	1116	1.925294	1.29E-06	0.000531
MSN	4478	1.029658	1.24E-06	0.000531
VASP	7408	1.022996	1.28E-06	0.000531
TNFAIP3	7128	1.501024	1.36E-06	0.000541
CD14	929	1.840842	1.36E-06	0.000541
SPP1	6696	2.595834	1.43E-06	0.000547
CD44	960	2.414603	1.46E-06	0.000547
TYMP	1890	1.698211	1.65E-06	0.000609
IRF1	3659	1.328726	1.81E-06	0.000643
C5AR1	728	1.807005	2.10E-06	0.000719
RHBDF2	79651	1.254428	2.14E-06	0.000719
FOS	2353	1.764481	2.26E-06	0.00074
PIM1	5292	1.080763	2.34E-06	0.000755
FAM166C	339778	1.539864	2.54E-06	0.000781
OAS3	4940	1.372264	2.50E-06	0.000781
DDIT4	54541	1.186545	2.68E-06	0.000801
NUPR1	26471	1.460617	2.74E-06	0.000808
NQO1	1728	1.328235	2.90E-06	0.000825
MX2	4600	1.356871	2.84E-06	0.000825
HELZ2	85441	1.687884	2.92E-06	0.000825
TNFRSF1A	7132	1.127309	3.17E-06	0.000884
SFN	2810	3.330597	3.23E-06	0.000888
ADM	133	1.711917	3.83E-06	0.000995

PARP14	54625	1.116163	3.90E-06	0.000995
PYGL	5836	1.033585	3.74E-06	0.000995
C1QC	714	1.534452	3.81E-06	0.000995
PLSCR1	5359	1.089636	4.10E-06	0.001032
FERMT3	83706	1.576382	4.44E-06	0.001063
ATOH8	84913	1.274708	4.48E-06	0.001063
SLC16A3	9123	1.557673	4.43E-06	0.001063
HTRA3	94031	2.037349	5.03E-06	0.00118
CD93	22918	1.792218	5.16E-06	0.001184
PLIN2	123	1.16446	5.86E-06	0.001272
ATF3	467	1.676841	6.05E-06	0.001301
C11orf96	387763	1.43349	6.18E-06	0.001302
MMP14	4323	1.5091	6.63E-06	0.001355
SCIN	85477	1.982044	6.61E-06	0.001355
PLK5	126520	1.747492	6.90E-06	0.001356
HIC1	3090	1.439835	6.82E-06	0.001356
CHST3	9469	1.375813	6.73E-06	0.001356
TNFRSF1B	7133	1.379135	8.94E-06	0.001709
PELATON	1.01E+08	2.867703	9.49E-06	0.00178
PLAAT4	5920	1.613809	1.07E-05	0.001985
MIR3619	1.01E+08	1.669635	1.10E-05	0.002019
LINC02217	1.02E+08	-1.01323	1.14E-05	0.002071
IFI16	3428	1.086368	1.14E-05	0.002071
DTX3L	151636	1.179406	1.17E-05	0.002108
OSGIN1	29948	1.512382	1.21E-05	0.002149
AMN	81693	2.072347	1.23E-05	0.002149
RDH10	157506	1.75251	1.33E-05	0.002293
GEM	2669	1.447218	1.37E-05	0.002297
PTPN6	5777	1.186311	1.40E-05	0.002297
THBD	7056	1.363866	1.40E-05	0.002297
KCNE4	23704	1.561336	1.44E-05	0.002345
JUNB	3726	1.234335	1.61E-05	0.002532

ADAMTS9	56999	1.387952	1.61E-05	0.002532
COL18A1	80781	1.248728	1.62E-05	0.002532
CXCL10	3627	2.387652	1.68E-05	0.002565
C1QB	713	1.621971	1.68E-05	0.002565
EPS8L1	54869	1.843859	1.85E-05	0.00275
NMB	4828	1.293399	1.87E-05	0.002752
PLAUR	5329	1.471298	1.92E-05	0.002787
ONECUT1	3175	1.57517	2.07E-05	0.002992
FLNA	2316	1.010096	2.13E-05	0.003056
SLC15A3	51296	1.044035	2.15E-05	0.003061
IFITM3	10410	1.390748	2.29E-05	0.003171
NES	10763	1.061736	2.47E-05	0.00335
MSR1	4481	1.747428	2.59E-05	0.003477
PVALB	5816	-1.27382	2.69E-05	0.003563
LOC101929076	1.02E+08	2.66195	2.83E-05	0.003624
C4B	721	1.481683	2.78E-05	0.003624
LRRC32	2615	1.262908	3.06E-05	0.00383
SLC11A1	6556	2.066839	3.03E-05	0.00383
KISS1R	84634	2.191113	3.23E-05	0.003992
GABRA6	2559	-1.58925	3.43E-05	0.004191
HSPA1B	3304	2.442323	3.48E-05	0.004225
THBS1	7057	1.349217	3.58E-05	0.004268
COL4A2	1284	1.010457	3.64E-05	0.004308
FOSL1	8061	1.902364	3.70E-05	0.004342
LINC01686	284648	2.652634	3.90E-05	0.004469
H2AC11	8969	1.353433	3.96E-05	0.004513
PDLIM4	8572	1.247213	4.01E-05	0.004524
KCTD11	147040	1.119401	4.08E-05	0.004552
YBX3	8531	1.288823	4.11E-05	0.004556
ARID5A	10865	1.101296	4.21E-05	0.004643
ATP8B3	148229	1.978929	4.38E-05	0.004731
LGALS9	3965	1.345381	4.52E-05	0.004809

RDH10-AS1	1.02E+08	1.911925	4.58E-05	0.004851
FSCN2	25794	1.836721	4.62E-05	0.004869
ADGRG3	222487	2.332311	4.71E-05	0.004935
AEBP1	165	1.173331	4.82E-05	0.004948
FLNC	2318	1.184242	4.81E-05	0.004948
LCP1	3936	1.172683	4.85E-05	0.00495
RNF223	401934	3.053645	4.87E-05	0.00495
GJA4	2701	1.085325	5.04E-05	0.005073
S100A11	6282	1.486716	5.09E-05	0.005083
CHI3L2	1117	2.61392	5.77E-05	0.005562
FLT3	2322	-1.01402	6.23E-05	0.005869
APOL6	80830	1.279393	6.21E-05	0.005869
STUB1-DT	1.05E+08	2.260003	6.69E-05	0.006167
NGFR	4804	1.734427	6.91E-05	0.006283
APOL1	8542	1.257136	6.90E-05	0.006283
CD163	9332	2.030501	7.02E-05	0.006348
APOBR	55911	1.701579	7.17E-05	0.006437
MIR4525	1.01E+08	2.183198	7.41E-05	0.006532
PTGER1	5731	1.795517	7.51E-05	0.006542
PXN	5829	1.124314	7.46E-05	0.006542
KDM6B	23135	1.036946	8.64E-05	0.007133
RGS1	5996	2.247389	8.64E-05	0.007133
COX6B2	125965	1.918802	8.73E-05	0.007147
CXCL2	2920	1.893837	9.06E-05	0.007364
STAB1	23166	1.214234	9.44E-05	0.007527
MYC	4609	1.110292	9.95E-05	0.007807
GLI2	2736	1.318774	0.000101	0.007926
S100A10	6281	1.269785	0.000105	0.008108
PRKAG3	53632	2.078557	0.000108	0.008293
MT2A	4502	1.267522	0.00011	0.008338
GPR25	2848	2.424925	0.000111	0.008348
NKX2-8	26257	2.347817	0.000116	0.008632

CD164L2	388611	2.223197	0.000121	0.008923
SECTM1	6398	1.955898	0.000122	0.008946
C1R	715	1.070093	0.000128	0.009188
PARD6G-AS1	1E+08	1.217851	0.000131	0.009321
ACTA1	58	1.331674	0.000135	0.009519
GFAP	2670	1.269316	0.000145	0.010046
MYOD1	4654	2.388949	0.000145	0.010046
CRYAB	1410	1.14584	0.000146	0.010102
TGIF1	7050	1.19874	0.000147	0.010102
CASZ1	54897	1.483343	0.000147	0.010111
HSD3B7	80270	1.046229	0.000152	0.010377
PDE6G	5148	2.015065	0.000155	0.010437
PLAU	5328	1.5526	0.000154	0.010437
MC1R	4157	1.199393	0.000169	0.01091
TGFB3	7043	1.235542	0.000168	0.01091
PLEKHG4B	153478	1.746974	0.000175	0.010965
GADD45A	1647	1.027938	0.000172	0.010965
ITGA5	3678	1.1593	0.000174	0.010965
SRGN	5552	1.191171	0.00017	0.010965
RGS16	6004	1.330635	0.000172	0.010965
HASPIN	83903	1.969129	0.000172	0.010965
SIX5	147912	1.692135	0.000178	0.010992
ARHGEF16	27237	1.599003	0.000177	0.010992
IL4R	3566	1.189951	0.000179	0.011024
FIBCD1	84929	1.185489	0.000186	0.011291
LRRC3-DT	1.01E+08	1.843629	0.000189	0.011316
CENPM	79019	1.749863	0.000189	0.011316
ITGB2	3689	1.109521	0.000195	0.01144
IL21R	50615	1.817709	0.000195	0.01144
TRIM56	81844	1.236432	0.000199	0.011655
AOC1	26	2.11674	0.000206	0.011944
ZFP36	7538	1.968652	0.00021	0.012049

TMC8	147138	1.221872	0.000213	0.012146
JARID2-AS1	1.01E+08	1.537192	0.000219	0.012352
GDF15	9518	1.805335	0.00022	0.012352
MTRNR2L8	1E+08	2.367801	0.000237	0.012752
CDCP1	64866	1.395614	0.000237	0.012752
TGM2	7052	1.111706	0.000236	0.012752
FAM157A	728262	1.156561	0.000239	0.012752
ADAT3	113179	1.397216	0.000245	0.012866
TENT5A	55603	1.079824	0.000245	0.012866
UCP2	7351	1.285435	0.000245	0.012866
LINC01799	1.01E+08	-3.42367	0.000264	0.013531
SERPINA1	5265	1.496322	0.000265	0.013531
PTGS2	5743	1.365405	0.000267	0.013559
S100A9	6280	2.091003	0.000273	0.013757
FOXI3	344167	2.229069	0.000278	0.013874
RAB20	55647	1.151827	0.000279	0.013895
GBP3	2635	1.203617	0.000293	0.014354
TRIP10	9322	1.158444	0.000294	0.014394
ODF3B	440836	1.398597	0.0003	0.014635
APLNR	187	1.344786	0.000315	0.01496
TPRX1	284355	3.160382	0.000311	0.01496
SMTN	6525	1.000043	0.000315	0.01496
PIK3AP1	118788	1.033297	0.000318	0.014965
SPDYC	387778	2.001067	0.000317	0.014965
FAM167B	84734	1.690383	0.000316	0.014965
LRG1	116844	1.699128	0.000331	0.015039
TOR4A	54863	1.305638	0.000326	0.015039
ENGASE	64772	1.010838	0.000336	0.015039
TYROBP	7305	1.132782	0.000335	0.015039
IFITM1	8519	1.297772	0.000333	0.015039
TLR2	7097	1.131961	0.000359	0.01586
SSTR5-AS1	146336	2.476533	0.000367	0.016088

SBNO2	22904	1.102491	0.000371	0.01621
C1QA	712	1.145656	0.000378	0.016392
TNC	3371	1.463355	0.00038	0.016433
PRSS42P	339906	2.330458	0.00039	0.016824
APOLD1	81575	1.090781	0.000412	0.017479
MIR3976HG	645355	-1.59725	0.000424	0.017807
F2RL3	9002	1.766242	0.000426	0.017863
COL4A1	1282	1.110451	0.00045	0.018551
MT1L	4500	1.440857	0.000454	0.018655
ADAMTS1	9510	1.009682	0.000455	0.018664
TNFSF9	8744	1.185339	0.000458	0.018698
EMP3	2014	1.192011	0.000476	0.01915
NLRC5	84166	1.360701	0.000479	0.019155
PPP1R13L	10848	1.136402	0.000482	0.019165
NR4A1	3164	1.359261	0.000496	0.019629
FOXJ1	2302	1.695987	0.000502	0.019643
TCF15	6939	1.332408	0.000522	0.020173
PLSCR3	57048	1.998938	0.000525	0.020214
ND3	4537	1.404757	0.000529	0.020284
IFI44L	10964	1.28398	0.000539	0.020515
CLDN6	9074	1.65119	0.000538	0.020515
TDRD10	126668	1.347944	0.000556	0.021024
KIF1C	10749	1.106094	0.000566	0.021134
PNP	4860	1.041275	0.000597	0.021885
TEKT4	150483	2.04352	0.00061	0.022212
HBB	3043	1.609588	0.000612	0.02224
MVP	9961	1.083501	0.000661	0.023434
MMEL1	79258	1.994029	0.000674	0.023675
MIR4516	1.01E+08	1.467787	0.000684	0.023921
TSSK6	83983	1.043211	0.000714	0.024396
SAMD11	148398	1.734106	0.000727	0.024665
HBM	3042	2.166348	0.000748	0.02514

LHB	3972	1.630834	0.000748	0.02514
CD300A	11314	1.074829	0.000764	0.025304
SOCS1	8651	1.788552	0.000763	0.025304
TRARG1	286753	1.529783	0.000766	0.025322
PLP2	5355	1.291133	0.000779	0.025424
PLEKHA4	57664	1.415673	0.00078	0.025424
LAPTM5	7805	1.071379	0.000779	0.025424
DNASE1L2	1775	1.141355	0.000784	0.025527
WWTR1	25937	1.015353	0.000805	0.025978
TREM2	54209	1.120219	0.000805	0.025978
TM4SF1-AS1	1.01E+08	-3.36441	0.000826	0.026484
IRX1	79192	2.5444	0.00083	0.026522
CYP21A1P	1590	1.611097	0.000836	0.026604
VSIG4	11326	1.376054	0.00088	0.027684
FCER1G	2207	1.526436	0.000882	0.02771
LINC02192	1.01E+08	-1.13801	0.000903	0.028129
ITPRIP	85450	1.060705	0.0009	0.028129
CPAMD8	27151	1.09147	0.000913	0.028214
TM4SF1	4071	1.095555	0.00093	0.028487
LDLRAD2	401944	1.951368	0.000932	0.028495
ENTPD8	377841	1.493886	0.000938	0.028614
GCNT3	9245	1.607383	0.000974	0.029277
CP	1356	1.565941	0.000998	0.029651
FGF18	8817	1.164056	0.001002	0.029714
GDF5	8200	1.731794	0.001015	0.029971
SLC6A12-AS1	1.02E+08	1.703735	0.001034	0.030408
LINC01588	283551	1.341779	0.001077	0.031052
CLEC18B	497190	1.545066	0.001107	0.031615
LINC01224	1.04E+08	1.452484	0.001114	0.03164
PRSS40A	150527	2.85804	0.001117	0.03164
CAPS	828	1.058605	0.001116	0.03164
LINC01354	1.01E+08	1.038977	0.00115	0.031955

TTLL10	254173	2.480889	0.001143	0.031955
ANGPTL4	51129	1.169107	0.00114	0.031955
LINC01143	1.04E+08	3.10912	0.001164	0.032167
SCARNA17	677769	1.057557	0.001161	0.032167
MSLN	10232	1.51505	0.001181	0.032383
MIR661	724031	1.828246	0.001188	0.032423
NOS3	4846	1.068102	0.001241	0.03344
SMPX	23676	-1.1548	0.001277	0.033967
CABP4	57010	1.229218	0.001284	0.034115
SELE	6401	1.872371	0.001304	0.034418
CCDC33	80125	1.744076	0.001429	0.036437
LINC01554	202299	1.383167	0.001457	0.036918
DNMT3L	29947	1.792066	0.001491	0.037477
CYP27C1	339761	1.3919	0.001491	0.037477
GPR4	2828	1.391017	0.0015	0.037576
CLDN19	149461	1.951732	0.001525	0.037823
MLKL	197259	1.264725	0.001524	0.037823
IL4I1	259307	1.582605	0.001538	0.037823
C9orf50	375759	1.008132	0.001523	0.037823
CT66	1.01E+08	1.750894	0.001564	0.03814
ELF4	2000	1.0827	0.001568	0.03814
GNG8	94235	1.659157	0.001567	0.03814
UPB1	51733	1.192704	0.001617	0.038904
TEX11	56159	1.782515	0.001637	0.039086
PDPN	10630	1.102655	0.001689	0.039798
KIAA0040	9674	1.011151	0.001701	0.039869
CNN2	1265	1.069162	0.001711	0.039905
C5orf60	285679	1.830569	0.001716	0.039969
CH25H	9023	1.238515	0.001739	0.040205
PSG5	5673	-1.14458	0.001747	0.040267
AATBC	284837	1.056306	0.001757	0.040392
MAMDC4	158056	1.110201	0.001806	0.041033

GRK1	6011	1.124643	0.001812	0.041042
SLC1A7	6512	1.331661	0.001836	0.041354
OR2B11	127623	1.732429	0.001846	0.041407
TMPRSS13	84000	1.544543	0.001888	0.042173
MBOAT4	619373	1.351221	0.001931	0.042426
RNF122	79845	1.116363	0.00193	0.042426
C14orf178	283579	1.504854	0.001959	0.04289
LCN8	138307	1.359008	0.001977	0.043132
MFSD6L	162387	2.1021	0.001993	0.043312
EMP1	2012	1.691395	0.002006	0.043411
OTOP3	347741	2.263111	0.00202	0.043444
SELENOV	348303	1.615439	0.002078	0.044289
GZMM	3004	1.535053	0.002089	0.044415
FCGR3A	2214	1.036038	0.0021	0.044453
MIR149	406941	1.539452	0.002125	0.044531
MROH6	642475	1.127593	0.002119	0.044531
SERPINE1	5054	1.669014	0.002149	0.044935
TSPAN16	26526	1.186533	0.002157	0.044938
LRRC71	149499	1.354011	0.002162	0.044987
CILP2	148113	1.249929	0.002249	0.046071
LINC02418	1E+08	-1.21436	0.002272	0.046248
MMP25	64386	1.314055	0.002439	0.048635
RNA5S13	1E+08	3.025204	0.002481	0.049195
ASCL2	430	1.395338	0.002487	0.049204
BATF2	116071	1.198966	0.002527	0.049539
PAX2	5076	1.265916	0.00254	0.049702
SEMG2	6407	-2.41159	0.002564	0.049967
PRIMA1	145270	1.170896	0.002598	0.050196
HIPK2	28996	1.070251	0.002631	0.050427
ITGB4	3691	1.029559	0.002652	0.050777
CXCL3	2921	1.828203	0.002672	0.05107
MTRNR2L1	1E+08	2.632282	0.002687	0.051203

HM13-AS1	1.01E+08	1.480581	0.002719	0.051394
IL1B	3553	1.471316	0.002726	0.051484
RAPSN	5913	1.932399	0.002734	0.051489
NFE2	4778	1.614367	0.002746	0.051622
PRICKLE3	4007	1.047567	0.002757	0.051643
SPINT1	6692	1.157864	0.002755	0.051643
GUCA1C	9626	-1.58509	0.00278	0.051925
CARD9	64170	1.095892	0.002802	0.052076
LINC00924	145820	-1.2958	0.002811	0.052079
SYNPR-AS1	1.01E+08	-1.30538	0.002855	0.052508
CFAP45	25790	1.413027	0.002857	0.052508
TCIRG1	10312	1.009494	0.002915	0.05324
RAB43	339122	1.08007	0.002919	0.05328
RBM47	54502	1.027081	0.002994	0.054066
CPT1B	1375	1.297586	0.003008	0.054267
TNNC1	7134	1.50506	0.003155	0.056187
OR5B12	390191	-2.41363	0.003173	0.056457
SNORD114-17	767595	-1.12127	0.003202	0.05685
KRT40	125115	-1.92244	0.003285	0.057447
CXCL1	2919	1.858962	0.003282	0.057447
LINC02709	440028	1.088645	0.003274	0.057447
BCL3	602	1.153026	0.003317	0.057916
CABP2	51475	2.83977	0.003371	0.058376
LAIR1	3903	1.292494	0.003405	0.058813
MMP11	4320	1.239467	0.003474	0.059609
VTRNA1-3	56662	1.684531	0.003488	0.05969
HOXB7	3217	2.370539	0.003505	0.059933
SPATA12	353324	1.443686	0.00351	0.059933
EGR4	1961	1.037684	0.00352	0.059997
SPINK6	404203	-1.2844	0.003533	0.060075
CERKL	375298	-1.0625	0.003613	0.060783
CFAP73	387885	1.158718	0.00362	0.06085

H19	283120	1.052814	0.003631	0.060983
APOC1	341	1.06314	0.003647	0.061107
PKD1P6	353511	1.035885	0.003646	0.061107
TK1	7083	1.062786	0.003674	0.061363
HCST	10870	1.058886	0.003705	0.061528
AKAIN1	642597	-1.02583	0.00374	0.061846
OVOL3	728361	1.224566	0.003762	0.061917
WWC2-AS2	152641	1.304611	0.003791	0.062206
C2CD4A	145741	1.067767	0.00382	0.062387
AQP5	362	1.169627	0.003862	0.06267
USP17L19	1E+08	1.615847	0.003887	0.06302
NAT16	375607	1.069305	0.003895	0.063111
PPP1R18	170954	1.143954	0.003911	0.063232
TIMP1	7076	1.067739	0.003929	0.063336
CTRB2	440387	1.905965	0.003938	0.06336
CFAP99	402160	1.218486	0.004021	0.064234
CTF1	1489	1.08676	0.004044	0.064368
FOXP4-AS1	1.01E+08	1.57093	0.004083	0.064772
LINC01876	1.02E+08	-1.74019	0.004097	0.064894
LINC01478	1.02E+08	-1.34512	0.004108	0.065018
SLC12A3	6559	1.539515	0.004121	0.065143
PIRT	644139	1.283811	0.004137	0.065281
NTN3	4917	1.006471	0.00416	0.065502
TNFRSF8	943	1.878377	0.004183	0.065708
LOC101927727	1.02E+08	1.164057	0.00422	0.066082
LRRC18	474354	1.274474	0.004359	0.067441
OAS2	4939	1.062914	0.004401	0.067616
OR13C4	138804	-1.97392	0.004456	0.068174
SIGLEC1	6614	1.307587	0.004459	0.068174
LINC00528	200298	1.501346	0.004534	0.068892
GDNF	2668	1.347511	0.004547	0.068952
KCNQ1	3784	1.033129	0.00471	0.070393

DYNLT4	343521	1.545828	0.00477	0.071041
DPY19L3-DT	400684	1.265545	0.004796	0.071264
C19orf67	646457	1.144443	0.004789	0.071264
ZAP70	7535	1.137074	0.004808	0.071396
SLC23A3	151295	1.310699	0.004818	0.071441
PRAM1	84106	1.092004	0.004835	0.071615
CATSPER1	117144	1.669311	0.004862	0.07188
MTRNR2L2	1E+08	2.44574	0.004954	0.072387
C2CD4B	388125	1.580573	0.004963	0.072387
OR2G6	391211	-1.74366	0.004964	0.072387
LRRC25	126364	1.090599	0.005128	0.073654
SMIM1	388588	1.399279	0.005135	0.073702
HAMP	57817	1.226888	0.005242	0.074519
DPEP3	64180	1.227312	0.005348	0.075292
DPT	1805	-1.00655	0.005405	0.075737
IRGM	345611	1.313883	0.005398	0.075737
GCOM1	145781	-1.4212	0.005439	0.075927
DUOXA1	90527	1.318874	0.00544	0.075927
SULT1C2P1	151234	-1.35423	0.005482	0.076195
STC1	6781	1.324434	0.005513	0.076376
PLA2G4E	123745	1.390556	0.005522	0.076449
SAG	6295	1.396071	0.005534	0.076461
OVOL1	5017	1.451633	0.005587	0.076836
LINC02875	388407	1.598003	0.005661	0.07743
CRTAM	56253	-1.13991	0.005678	0.077524
UBE2C	11065	1.535035	0.005739	0.077683
FFAR2	2867	2.028285	0.005731	0.077683
LRRC26	389816	1.832309	0.005739	0.077683
FOXF1	2294	1.292522	0.005759	0.077847
COX2	4513	1.237483	0.005776	0.077927
ADGRE1	2015	1.577064	0.00584	0.078109
SH2D6	284948	1.095818	0.005838	0.078109

ANXA2	302	1.087795	0.005828	0.078109
MYO18B	84700	1.017854	0.005802	0.078109
KRTAP5-1	387264	1.39696	0.005894	0.078543
TLR9	54106	1.686645	0.005894	0.078543
SERPINA4	5267	2.075329	0.005999	0.079414
TNFRSF10D	8793	1.040265	0.006218	0.081065
PLAC8	51316	1.189626	0.00623	0.081129
CEBPE	1053	2.050308	0.006304	0.081698
LINC00347	338864	-1.50337	0.006353	0.082158
CLRN3	119467	-1.43141	0.006462	0.082954
CA12	771	1.136338	0.006458	0.082954
CACTIN-AS1	404665	1.436747	0.0065	0.083281
C5orf64-AS1	1.01E+08	1.181087	0.006509	0.083295
TPI1P2	286016	1.003028	0.006508	0.083295
TNFSF14	8740	1.414925	0.006554	0.083404
TLCD2	727910	1.085945	0.006634	0.08406
LBX2	85474	1.579046	0.006735	0.084976
FXYD3	5349	1.014006	0.006767	0.08522
MAFA	389692	1.831581	0.006806	0.085554
PAQR6	79957	1.028522	0.006836	0.085781
ADORA2A	135	1.585538	0.006852	0.085813
SPOCD1	90853	1.695552	0.006856	0.085813
TMEM210	1.01E+08	1.600959	0.006958	0.086493
BCAR4	400500	1.629018	0.00698	0.08654
OR52E6	390078	-2.35868	0.006992	0.08658
LMO7DN	729420	-1.33128	0.007033	0.086672
ZNRF4	148066	1.656437	0.007052	0.086769
WNT3A	89780	1.894303	0.007104	0.087022
LINC01793	1.02E+08	-1.08822	0.007134	0.087189
PCDHB1	29930	1.15345	0.007183	0.087624
MUC2	4583	1.58002	0.007325	0.088464
DOK7	285489	1.006896	0.007334	0.088481

LOC102724421	1.03E+08	-1.6757	0.007375	0.08866
C11orf91	1E+08	1.505707	0.007409	0.08896
HCAR3	8843	1.692873	0.007479	0.089492
GTSE1	51512	1.497741	0.00751	0.089705
TEAD4	7004	1.292904	0.007525	0.089779
SSMEM1	136263	-2.2774	0.007541	0.089926
LINC01426	1.01E+08	1.434712	0.007606	0.090357
LOC105371899	1.05E+08	1.118145	0.007645	0.090357
TEPP	374739	1.253275	0.007651	0.090357
SLC22A11	55867	1.749717	0.007593	0.090357
OPTC	26254	1.242466	0.007805	0.091597
H1-5	3009	1.607219	0.007967	0.09239
IRF9	10379	1.15315	0.008063	0.093039
PBK	55872	1.650466	0.008066	0.093039
TLX1	3195	-2.48117	0.008113	0.093089
DPRXP4	503645	1.389419	0.008114	0.093089
IL22RA1	58985	1.843308	0.008254	0.093693
MIR3680-1	1.01E+08	1.041817	0.008319	0.094195
CCL2	6347	1.782402	0.00835	0.094488
LINC01659	1.02E+08	1.291491	0.008564	0.095598
RNA5S11	1E+08	3.443435	0.008598	0.095621
LILRB3	11025	1.268371	0.008577	0.095621
MS4A12	54860	-2.2667	0.008711	0.09605
OSM	5008	1.488639	0.008739	0.096258
SIGLEC12	89858	1.737658	0.008815	0.096816
GALNT4	8693	1.364773	0.008896	0.097361
PRG4	10216	-1.03291	0.00896	0.097936
CD1D	912	1.159017	0.008963	0.097936
LINC02728	1.02E+08	-1.26149	0.008991	0.098047
MISP	126353	1.280916	0.00904	0.098309
GALNT5	11227	-1.32272	0.009085	0.098515
CTRC	11330	1.223551	0.009097	0.098515

AGTR2	186	-1.8679	0.009214	0.099259
LINC01922	1.02E+08	2.183066	0.009235	0.099308
GDPD4	220032	1.272959	0.009238	0.099308
LINC01618	152578	-1.7553	0.009294	0.09981
RAB42	115273	1.119902	0.009344	0.100142
REG3G	130120	-2.32413	0.009343	0.100142
FOXL1	2300	1.06727	0.009415	0.100581
CXCL8	3576	1.322078	0.009509	0.101268
ADGRF4	221393	-1.38129	0.009643	0.102123
FSCN3	29999	1.592507	0.009849	0.102985
CYP19A1	1588	1.450437	0.009887	0.103068
CLDN7	1366	1.047682	0.009902	0.103073
LINC00163	727699	1.651412	0.009956	0.103466
SNORD115-39	1E+08	1.086384	0.009711	NA
RBPMS-AS1	1E+08	1.759077	0.009141	NA
MIR1915	1E+08	2.286234	0.001604	NA
MIR4316	1E+08	2.574339	0.005938	NA
MIR3181	1E+08	1.951211	0.009556	NA
MIR3197	1E+08	3.689881	7.49E-05	NA
MIR3909	1.01E+08	1.949571	0.002912	NA
LINC01718	1.01E+08	3.61376	0.00472	NA
MIR4466	1.01E+08	2.039839	0.009263	NA
MIR4449	1.01E+08	2.121267	0.003325	NA
MIR4479	1.01E+08	1.814136	0.007267	NA
LINC00492	1.01E+08	-3.33268	0.002103	NA
ZFX-AS1	1.01E+08	1.376862	0.008966	NA
LINC00358	1.01E+08	-3.74364	0.000421	NA
COL4A2-AS1	1.01E+08	2.058727	0.001099	NA
LINC01216	1.01E+08	-3.10369	0.000991	NA
LINC02402	1.02E+08	3.170078	0.004008	NA
LINC01708	1.02E+08	-3.23468	0.008736	NA
PLA2G4E-AS1	1.02E+08	1.821313	0.002986	NA

LOC101929536	1.02E+08	1.796051	0.001065	NA
LINC01058	1.04E+08	2.10803	0.000885	NA
CELP	1057	2.293706	0.00211	NA
OR5I1	10798	-3.58564	0.001286	NA
CGB2	114336	2.410039	0.009757	NA
OR6N1	128372	2.639826	0.005067	NA
SCP2D1	140856	-3.19232	0.004576	NA
TRAV8-1	28685	-2.52074	0.009049	NA
TRAV1-1	28693	-4.09129	0.005246	NA
IGKV6D-21	28870	-3.46924	0.00774	NA
HOXA9	3205	4.681392	0.002067	NA
KCNQ1-AS1	338653	3.097168	4.62E-05	NA
OR52L1	338751	-1.96947	0.005797	NA
SYCN	342898	2.697877	0.003001	NA
LILRA5	353514	2.726068	0.000817	NA
OR52A5	390054	-2.37944	0.007009	NA
OR6M1	390261	-2.42269	0.004549	NA
OR7G2	390882	-2.98857	0.004272	NA
CIBAR1P2	403315	2.01028	0.008492	NA
MIR132	406921	2.827598	0.000195	NA
MIR212	406994	2.075189	0.001237	NA
RNASE13	440163	2.861922	0.000607	NA
CYMP-AS1	440602	1.969082	0.00967	NA
ATOH1	474	2.7333	0.005595	NA
PPDPFL	492307	1.786662	0.005376	NA
PRLH	51052	3.645554	0.000853	NA
DUSP21	63904	2.888804	0.003727	NA
FABP9	646480	-2.70819	0.002269	NA
SPRR2F	6705	3.128219	0.007173	NA
SNORD62B	692093	1.44991	0.004328	NA
MIR602	693187	2.39804	0.009844	NA
ARL14	80117	2.420292	0.00384	NA

MPIG6B	80739	1.534788	0.007776	NA
OR2B2	81697	-2.47542	0.002937	NA
MIA	8190	2.152159	0.001749	NA
H3-4	8290	1.690598	0.007093	NA
C15orf48	84419	2.26261	0.006564	NA
GPR174	84636	-2.29749	0.004062	NA
MBD3L1	85509	2.299661	0.006439	NA
UCN2	90226	2.405504	0.00181	NA
FTMT	94033	3.277739	0.003322	NA

Table 8.2. The differential expression genes DEGs of blood dataset (*P*-value < 0.01, -1 > Log2FC > +1), * Novel genes, NA no symbol ID

Symbol	**Ensembl ID**	**log2FoldChange**	**P-value**	**P-adj**
CXCL1	ENSG00000163739	2.611602717	0.00000742	0.089229768
TNFAIP6	ENSG00000123610	1.799753071	0.089229768	
TRIB3*	ENSG00000101255	-2.726146762	0.0000188	0.121223383
GCH1	ENSG00000131979	1.198998008	0.0000374	0.181947932
CCL2	ENSG00000108691	1.675389985	0.0000423	0.181947932
CCL23	ENSG00000274736	1.96012341	0.000183082	0.393657267
SYNPO2*	ENSG00000172403	3.50336981	0.000257933	0.439093898
IL1A	ENSG00000115008	2.486405818	0.000300338	0.439093898
SLC39A8	ENSG00000138821	1.368699557	0.000376551	0.462655263
CXCL10	ENSG00000169245	1.968878262	0.000628524	0.50734157
CCL3	ENSG00000277632	1.061085072	0.000772934	0.539006273
WWC2-AS2*	ENSG00000251359	2.961915035	0.000817072	0.552064475
UPB1	ENSG00000100024	2.728739755	0.00092418	0.593987671
NA	ENSG00000276758	3.253834459	0.001287987	0.65061141
ETV5	ENSG00000244405	1.321385714	0.001349342	0.65061141
TTC30A	ENSG00000197557	1.428187366	0.00146891	0.65061141
NA	ENSG00000210174	-1.291393539	0.0014902	0.65061141
RN7SL239P*	ENSG00000242999	-3.141583004	0.001573124	0.65061141

RPL10P9	ENSG00000233913	1.910385627	0.001864703	0.673109563
SERPINB2	ENSG00000197632	1.254455656	0.002032955	0.673109563
PLEKHA8P1	ENSG00000134297	-1.489533346	0.002084396	0.674070977
SYT17	ENSG00000103528	1.377078129	0.002094827	0.674070977
NA	ENSG00000280374	-1.908446479	0.002116105	0.674070977
PEX11G	ENSG00000104883	-1.64352962	0.002517743	0.751817899
NA	ENSG00000272841	2.085921021	0.00268939	0.76254564
NA	ENSG00000267034	1.02179119	0.003073523	0.825372624
TRMT61A	ENSG00000166166	1.230547487	0.003166882	0.825372624
NA	ENSG00000226632	2.474821561	0.0033634	0.835385097
IFI27	ENSG00000165949	2.654235993	0.003583636	0.855808299
KRT86*	ENSG00000170442	-3.099508734	0.003688596	0.855808299
PER3	ENSG00000049246	-1.008221097	0.00375306	0.856959661
SEMA3A	ENSG00000075213	1.145874269	0.003971855	0.86848977
NA	ENSG00000285554	2.392676774	0.004185527	0.878007923
NA	ENSG00000225447	-1.509240149	0.004280926	0.879560203
POLD4	ENSG00000175482	-1.111272808	0.004307395	0.879560203
CNIH3*	ENSG00000143786	-3.30000464	0.00454354	0.901787772
NA	ENSG00000256393	1.678411723	0.005080971	0.966239624
FFAR2	ENSG00000126262	1.07301739	0.005321545	0.966239624
TTC23	ENSG00000103852	1.237361265	0.005742232	0.966239624
TNIP3	ENSG00000050730	1.860231145	0.005759736	0.966239624
YPEL1	ENSG00000100027	-1.336695541	0.00649346	0.991386185
NA	ENSG00000210164	-1.165930877	0.00684723	0.997785732
WASH2P	ENSG00000146556	1.537272136	0.00692405	0.997785732
ABHD17C	ENSG00000136379	1.059222256	0.007425797	0.997785732
NA	ENSG00000260317	1.454725355	0.00748741	0.997785732
IL1B	ENSG00000125538	1.124032672	0.007627075	0.997785732
CCL8	ENSG00000108700	1.456913088	0.007700658	0.997785732
TSPAN15	ENSG00000099282	-1.78059737	0.007787389	0.997785732
CSF1	ENSG00000184371	-1.311989813	0.00811142	0.997785732
RAVER2	ENSG00000162437	3.81308529	0.008155484	0.997785732

ITGB8	ENSG00000105855	1.383004583	0.008239489	0.997785732
NA	ENSG00000262979	1.891203212	0.008905453	0.997785732
NA	ENSG00000275769	-3.039747183	0.009122883	0.997785732
NA	ENSG00000261618	2.738691309	0.009399018	0.997785732
CES1	ENSG00000198848	-1.351234248	0.00960455	0.997785732
TRGC2	ENSG00000227191	1.87219908	0.009757642	0.997785732
NA	ENSG00000279502	-2.358142683	0.009798447	0.997785732
ZNF79	ENSG00000196152	-1.257711983	0.009855672	0.997785732
FCRL1	ENSG00000163534	1.829359047	0.009868655	0.997785732
STN1	ENSG00000107960	-1.059537111	0.009956252	0.997785732

8.4.2. Visualization and Functional Interpreting of DEGs

The prospective molecular and biological mechanisms involved in the pathogenesis of ASD were decoded, subjecting significant DEGs to GO and KEGG analysis. As demonstrated in (**Figure 8.2A**), the GO analysis revealed that numerous BP in ASD brain datasets were prominently involved in the positive regulation of cytokine production, response to molecules of bacterial origin, and response to lipopolysaccharide. The key MF enriched by the DEGs encompassed receptor ligand activity and DNA-binding transcription activator activity, as depicted in **Figure 8.2B**. However, the DEGs primarily encoded the cellular components known as collagen-containing extracellular matrix and secretory granule lumen (**Figure 8.2C**). Furthermore, KEGG pathway enrichment analysis was accomplished for the nominally significant DEGs from the brain dataset; five pathways exhibited statistically meaningful enrichment with *P*-value < 0.01. The top three pathways included the TNF signaling pathway, NF-kappa B signaling pathway, and Malaria, as visualized in **Figure 8.2D**.

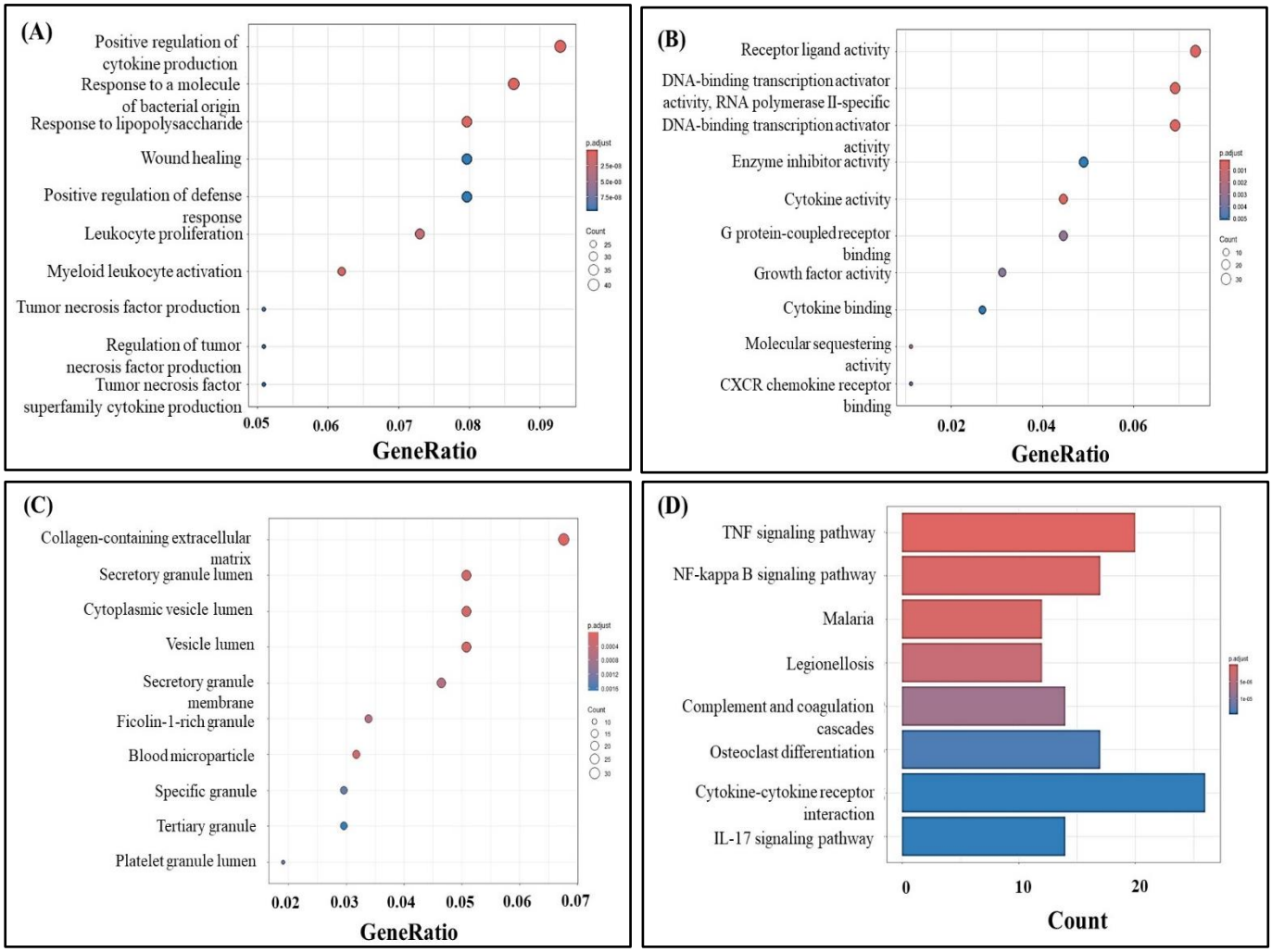


Figure 8.2. Enriched GO and KEGG analysis of brain dataset. *(**A**) points to the biological process category, (**B**) to cellular components, and (**C**) to molecular function. The Padj-value (FDR) is used to color-code the bars ranging from blue to red, and (**D**) the bar plot represents the KEGG pathways enriched by DEGs. Based on the FDR, the bars are colored from blue to red.*

In the same context, the KEGG pathways and GO terms engaged with the significant DEGs from the blood dataset were explored. The enriched BP related to the DEGs included leukocyte migration, granulocyte migration and leukocyte chemotaxis, as depicted in **Figure 8.3A**. In terms of MF, the DEGs list exhibited enrichment in receptor-ligand activity, cytokine activity, and cytokine receptor binding as illustrated in **Figure 8.3B**. In the context of the CC category, there was enrichment in the Cytokine-cytokine receptor interaction and viral protein interaction with cytokine and cytokine receptor.

The KEGG analysis findings of DEGs of blood showed notable enrichment pathways in cytokine-cytokine receptor interaction, chemokine signaling pathway, and viral protein interaction with cytokine and cytokine receptor (**Figure 8.4**).

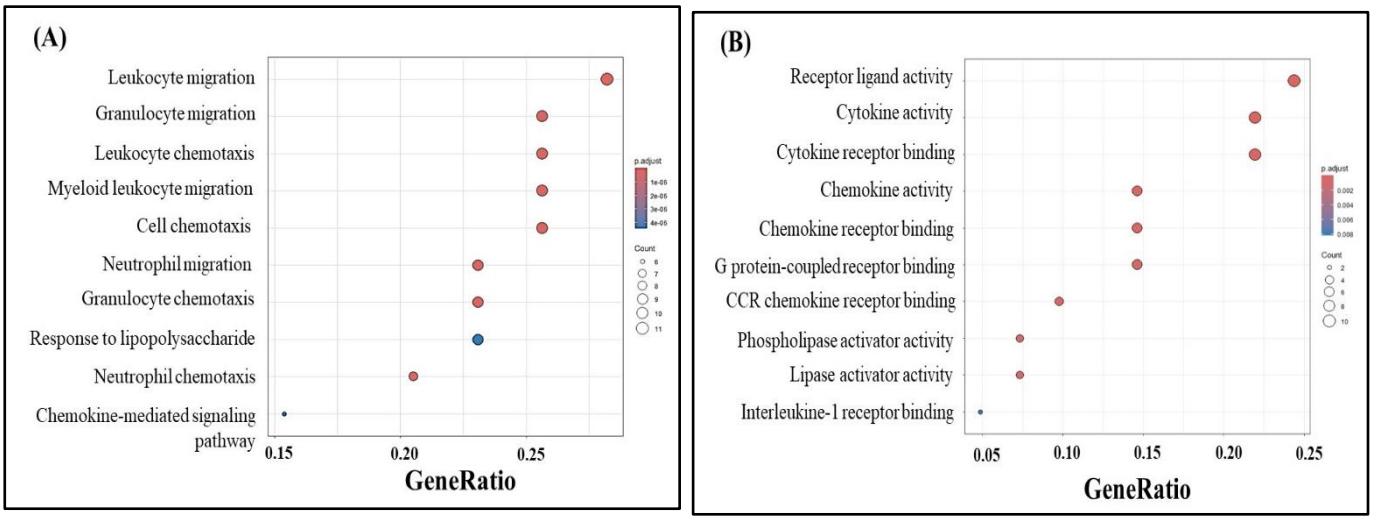


Figure 8.3. Enriched GO of the blood dataset. *(**A**) points to the biological process category and (**B**) to molecular function. The FDR is used to color the dots, which range from blue to red, and the count of genes determines the size of the dots.*

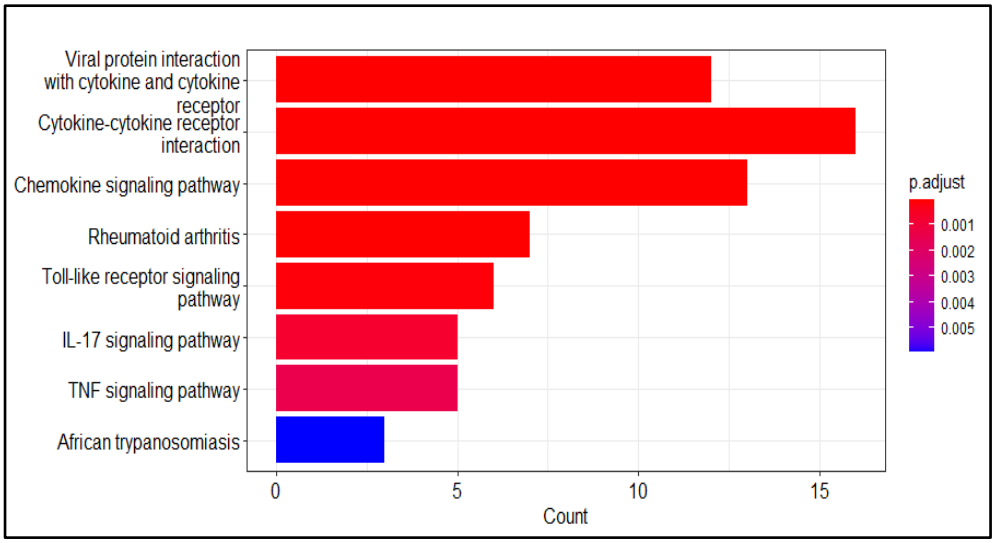


Figure 8.4. KEGG analysis of blood dataset. *The bar plot depicts the KEGG pathways enriched by DEGs (P-value 0.01). The Padj- value determines the color of the bars, which range from blue to red.*

8.4.3. Comparison DEGs between two datasets and Functional Annotation

The common genes identified in both datasets are presented in the Venn diagram (**Figure 8.5** and **Table 8.3**). A total of eight DEGs were discerned, potentially serving as pathogenic genes in ASD. Among these DEGs, *UPB1*, *CXCL1*, *CXCL10*, *CSF1*, *CCL2*, and *IL1B* demonstrated associations with previously detected ASD susceptibility genes. Intriguingly, no investigations have been conducted on these genes in the context of ASD within brain tissues. Notably, the remaining two genes (*FFAR2* and *WWC2-AS2*) are novel and have not been previously linked to ASD.

Based on the KEGG pathway analysis outcomes, the eight DEGs were predominantly enriched in the biological processes, including the TNF signaling pathway, cytokine-cytokine receptor interaction, and the IL-17 signaling pathway (**Table 8.4**).

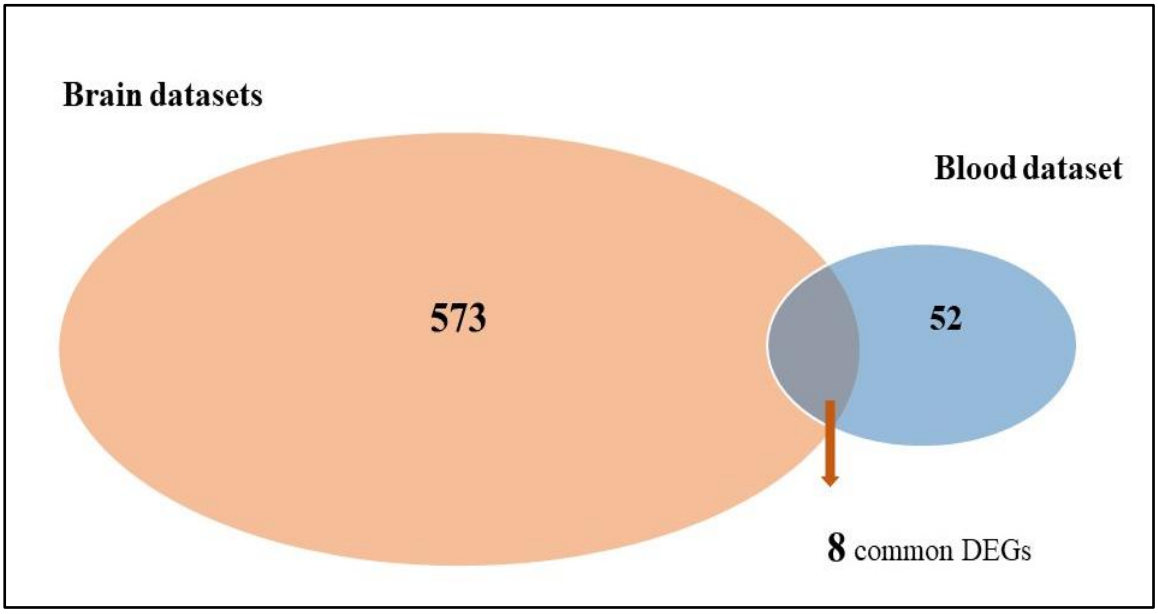


Figure 8.5. Venn map represents the eight common genes crossing among two datasets. *The orange circle shows the 573 DEGs of the brain dataset; the blue circle shows the 52 DEGs of the blood dataset, considering P-value < 0.01 threshold for both, and the purple region shows eight common genes between the two datasets.*

Table 8.3. Eight common genes are shared between the brain dataset and the blood dataset

Gene Symbol	Entrez ID	Function	Remark	Ref.
UPB1	51733	The enzyme βUP. Its role in the final step of pyrimidine breakdown produces two products, both of which are speculated to affect nervous system functions.		[22]
CXCL1	2919	It is one of the CXC subgroups of chemokines and is capable of chemoattraction to neutrophils.	High-functioning ASD groups showed increased *CXCL1* plasma levels.	[23]
CXCL10	3627	*CXCL10* induces immune cells, like natural killer cells, macrophages, and dendritic cells.	*CXCL10* levels in autistic patients' Cerebrospinal fluid were elevated compared to controls.	[24]
CCL2	6347	Is a member of chemokines superfamily involved in immunoregulatory and inflammatory processes	Elevated levels of *CCL2* in ASD subjects	[25, 26]

IL1B	3553	*IL1 β* interacting protein is abundant in lymph nodes.	ASD samples have been linked to heightened levels of inflammatory cytokines, including IL-1β in biological fluids	[27, 28]
CSF1	1435	is a hematopoietic growth factor included in the reproduction, differentiation, and survival of monocytes, macrophages	Mechanism mediated by CSF1-CSF1R signaling contributes to sociable behavior and motor function.	[29]
FFAR2	2867	free fatty acid receptor		
WWC2-AS2	152641	WWC2 Antisense Gene Protein 2		

2

Table 8.4. Significant enriched KEGG of eight common genes.

KEGG Pathways	**Count**	**Genes**	***P*-Value**
TNF signaling pathway	5	*CCL2, CXCL10, CXCL1, CSF1, IL1B*	4.2E-7
Cytokine-cytokine receptor interaction	5	*CCL2, CXCL10, CXCL1, CSF1, IL1B*	1.9E-5
IL-17 signaling pathway	4	*CCL2, CXCL10, CXCL1, IL1B*	2.4E-5
Rheumatoid arthritis	4	*CCL2, CXCL1, CSF1, IL1B*	2.4E-5

8.5. Discussion:

Early clinical prediction of ASD, together with targeted interventions, has been an area of interest, and several studies have been conducted to determine biomarkers with disease-predictive potential [6, 14, 15]. Nonetheless, the potent and accurate biomarkers for detecting and diagnosing ASD are an unmet challenge. In the present study, we have investigated the DEGs in the brain and blood tissues of ASD patients. The results of the brain datasets showed 581 DEGs. Of these, 520 genes were considered significantly expressed upregulated genes. The top three significant upregulated genes included immune response genes, namely, *HSPA6*, *CSF3*, and *SERPINA3*. *CSF3* gene encodes interleukin-6 (IL-6). The results obtained from children diagnosed with ASD revealed augmented production of IL-6 in lipopolysaccharide (LPS)-stimulated monocytes. Notably, this heightened IL-6 production exhibited a correlation with the manifestation of restricted and repetitive behaviors, thereby implicating an association between excessive inflammation stemming from innate immune responses and aberrant behavioral patterns in ASD [19, 30]. Choi et al. [31] pointed out that the production of IL-17 in maternal inflammation induces IL-6, interfering with fetal neurological development and causing ASD. Previous papers have suggested a link between IL-6 and ASD. Nevertheless, the precise nature of this relationship and its role in ASD pathogenesis are still areas of active investigation. Furthermore, *HSPA6* was unveiled to be a dysregulated gene correlated with microglia in the post-mortem brains of neuropsychiatric patients [32]. Similarly, comparative transcriptome investigation highlighted *HSPA6* as a significant upregulation gene related to immune system dysregulation in ASD subjects [33]. Prior research has documented heightened levels of *SERPINA3* and proinflammatory cytokines in the cerebral regions of individuals with schizophrenia [34]. Furthermore, *SERPINA3* and heat shock proteins have been linked to

neuroinflammation [35]. Conversely, mice with a knock-in of *SERPINA3* demonstrated improved cognitive capacities in behavioral tasks. Notably, the upregulation of *SERPINA3* resulted in increased neocortical folding, augmented neuronal abundance, and improved cognitive performance [36]. Conversely, the *TRAV1-1* gene, which was among the downregulated genes, encodes an alpha chain in the V region of the T cell receptor variable domain that participates in antigen recognition. T cells play roles in dysregulation immunity in ASD subjects [37].

The meta-analysis of the blood dataset revealed 60 DEGs. *RAVER2* gene was the highest upregulated, whereas *CNIH3* and *TRIB3* were downregulated significant DEGs. *CNIH3* is a member of the cornichon family of transmembrane proteins that play a role in the regulation of α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor [38]. *CNIH3* is involved in synaptic plasticity, learning, and memory [39]. Cheng et al. [40] reported that alterations or deficits in AMPAR lead to specific neurological disorders like ASD and epilepsy. Our findings align with previously mentioned studies [40, 41], indicating that *CNIH3* could be used as a susceptible gene linked to ASD. However, further research is needed to fully understand the role of *CNIH3* and its potential as a therapeutic target for neurological disorders.

Zhang et al. [42] noticed that higher levels of the *TRIB3* gene could inhibit kainic acid (KA), which is known to cause neuronal apoptosis and seizures. Meanwhile, lower levels of TRIB3 have been shown to protect against KA-induced neuronal apoptosis, leading to the conclusion that *TRIB3* could be a promising therapeutic target for epilepsy recovery. Conversely, in the current study, *TRIB3* was downregulated in ASD patients. Therefore, further investigations are needed to understand the molecular function of *TRIB3* in ASD, especially considering reports that suggest approximately 21.5% of ASD patients also have epilepsy [43, 44].

RAVER2 is an RNA-binding protein [45]. The function of *RAVER2* in neurodevelopmental diseases is not well known because of its restricted transcription pattern and absence of expression in brain cell lines [46]. However, the gene-wise analysis revealed a genome-wide meaningful correlation between *RAVER2* and the capacity to recognize feelings in ASD patients [47]. A recent study has been applied to predict relevant risk genes associated with Parkinson's disease (PD) and revealed that

RAVER2 was an upregulated DEG [48]. Therefore, it shows that *RAVER2* may affect ASD pathogenesis; thus, more investigation is required to detect its biological pathways.

Functional and enrichment analysis of the brain dataset revealed the positive regulation of cytokine production and response to lipopolysaccharide. According to epidemiological statistics, maternal immune activation may contribute to the emergence of ASD [49, 50]. It is noteworthy that LPS, a Gram-negative bacteria cellular membrane constituent, can trigger a pro-inflammatory cascade, primarily through stimulated macrophage/microglial cells [51]. As a result, it evokes many modifications in the inflammatory responses, contributing to a detrimental influence on the maturation of synapses [52]. Numerous studies have documented that prenatal intake of LPS caused immunological dysfunction [53, 54] as well as behavioral dysfunctions in mice, including communicative difficulties, social deficits, and stereotypical behavior [55-57]. Taken together, chronic neuroinflammation emerges as a pivotal contributor to the development of ASD [58, 59] by causing an overproduction of proinflammatory cytokines, resulting in severe pathophysiological alterations and neurobehavioral issues [9, 60-62].

An enrichment analysis of the blood dataset showed significantly enriched pathways, including responses to chemokine and cytokine-cytokine receptor interaction pathways. The DEGs implicated in previous pathways were *CCL2*, *CCL23*, *CCL3*, *CXCL1*, and *CXCL10*. Case-control research from peripheral blood demonstrated that the concentration of *CCL2* was significantly high in ASD children [25]. Chen et al. [26] revealed significantly increased serum levels of CCL3 in ASD model mice [63]. In the same context, *CCL5* has been linked to inflammatory responses in the brain [64]. Likewise, levels of CCL2, CCL3, and CCL5 peripheral blood were observed to be higher in ASD samples [25, 26]. These chemokines have been implicated in a lack of attention and hyperactivity behaviors, neuroinflammation, and cerebral infarction [25, 65]. Consequently, these proteins might have a potential role in neuroinflammation and affect ASD pathogenesis.

The autism transcriptomic datasets in brain and blood cells are bound to vary; the expression of genes in blood might co-regulate with genes associated with central nervous system (CNS) pathogenesis, making them highly valuable biomarkers. However, transcriptomic adjustments in the brains of autistic subjects might be the most crucial components of CNS pathogenesis; nevertheless, they may not possess predictive value if

these genes are not expressed or modified in obtainable peripheral tissue. Consequently, our approach was to detect the common genes between the brain and the blood datasets as robust candidate biomarkers in ASD patients. The eight common genes were *UPB1*, *CXCL1*, *CXCL10*, *CSF1*, *IL1B*, *CCL2*, *FFAR2* and *WWC2-AS2*. A previous study assessed 60 growth factors and cytokines levels. They found an array of changes in interleukins and CXC chemokines, such as CXCL10, which indicates a dysregulated immune response in pregnant mothers and in the cord blood of newborns later diagnosed with ASD [66]. Vargas et al. [24] noticed an increase in the level of *CXCL10* in the CSF of the ASD group compared to the healthy controls. In the same context, Osborne et al. [67] indicated an increase in the *CXCL10* serum level due to early-life inflammatory responses, which could be potential reasons for autism disorder. In contrast, a previous study reported increased *CXCL1* plasma levels in high-functioning ASD groups [23].

On the other hand, the *UPB1* gene encodes a beta-ureidopropionase (βUP) enzyme, which is implied in the pyrimidine breakdown process' last stage. βUP enzyme transforms N-carbamyl-beta-aminoisobutyric acid into N-carbamyl-beta-alanine and beta-aminoisobutyric acid, which in turn broken down into beta-alanine, ammonia, and carbon dioxide. Both of the products are presumed to have functions in the nervous system. Beta-aminoisobutyric acid induces leptin protein production, which protects nerve cells from injury due to toxins, inflammatory processes, etc. [68]. Beta-alanine is engaged in signal pathways in neuronal synapses and controls the neurotransmitter dopamine level [69]. Dopamine influences the alteration of behavior, cognition, awareness, sleep, verbal memory, acquiring knowledge, inspiration, voluntary movement, and punishment and rewards [68]. Dichter et al. [70] highlighted the impact of dopamine deficits on the specific brain area and concluded that the impairments could cause autistic-like behavior. In the same context, individuals with βUP deficiency are diagnosed in the early years of life with clinical features such as verbal deficit, mental disabilities, growth failure, and seizures [22]. *CSF1* signaling has been shown to be necessary for the survival, migration, and activation of microglia in the cerebellum. Therefore, the deficiency of *CSF1* reduced the number of microglia in the cerebellum, leading to impaired motor function, learning, memory, and social interaction [29]. However, the molecular function of these genes and their roles in ASD pathogenesis are unknown well. *FFAR2*, as a free fatty acid receptor, plays a pivotal role in the regulation of the immune system by influencing the expansion, development, and function of T

cells as well as the local intestine dendritic cell population [71]. Additionally, *FFAR2* knockout mice lacking the short-chain fatty acid (SCFA) receptor, exhibited notable morphological, functional, and developmental modifications in microglial cells [72]. Currently, there is a lack of studies establishing a link between *FFAR2* and ASD. Further investigations are required.

The KEGG enrichment pathways of the common genes were implicated in the TNF signaling and cytokine-cytokine receptor interaction pathways, which aligned with the findings of prior studies, suggesting the linkage between these pathways and the development of ASD [13, 30].

8.6. Conclusion

In conclusion, the current study sought to identify high-risk genes in both brain and blood datasets from individuals with ASD. These DEGs caused a cascade of immune activation abnormalities, elevated cytokine production, and ultimately manifested in abnormal alterations in molecular signaling pathways and neuronal synapses, potentially contributing to the pathogenesis of ASD. However, further experimental validation is essential to elucidate the biological functions of these DEGs in the pathophysiology of ASD. Meanwhile, our approach identified eight common genes, suggesting these genes can be potential biomarkers for the early detection of ASD from available tissues and represent prospective targets for future pharmacological interventions in ASD. Nevertheless, extensive research with larger sample sizes is warranted to validate these findings and enable accurate early diagnosis of ASD.

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