

# Genetic Characteristics of Systematic Juvenile Idiopathic Arthritis and The Bioinformatics Basis for Treatment

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## Research

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# Abstract

## Objective

Systemic Juvenile Idiopathic Arthritis (sJIA) is a distinctive subtype of Juvenile Idiopathic Arthritis (JIA). The pathogenesis of sJIA is still unclear with the treatment options limited. Although previous bioinformatics analyses have identified some genetic factors underlying sJIA, these studies were mostly single center with a small sample size and the results were often inconsistent. Herein, we combined two datasets of GSE20307 and GSE21521 and select the matrix of patients diagnosed as sJIA in it for further analysis.

## Methods

The GSE20307 and GSE21521 matrixs downloaded from the Gene Expression Omnibus (GEO) were analyzed using online-tool GEO2R, Venny, Metascape, STRING, and Cytoscape to identify differentially expressed genes (DEGs), enrichment pathways, protein-protein interaction (PPI), main Module and hub genes between sJIA individuals and healthy controls.

## Results

A total of 289 overlapping genes (consisting of 41 downregulated genes and 248 upregulated genes) were identified. Hub genes were primarily related to erythropoiesis. And the KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis of overlapping DEGs were mainly involved in Malaria and non-small cell lung cancer. Besides, DEGs in main module were involved in ubiquitin mediated proteolysis.

## Conclusions

our study suggests that the erythropoiesis signature indeed exists in sJIA similar to previous reports. And combining previous research and our results, we provide a basis for the application of proteasome inhibitors, hydroxychloroquine and kinase inhibitors in patients with sJIA from the perspective of bioinformatics.

## Introduction

As a distinctive subtype of Juvenile idiopathic arthritis (JIA), systemic juvenile idiopathic arthritis (sJIA) is recognized as a multisystem inflammatory syndrome extraarticular, and it accounts for 10%-20 % of JIA. The clinical manifestations of sJIA include fever, rash, arthritis, generalized lymphadenopathy, hepatosplenomegaly and polyserositis[1]. More seriously, a potential fatal feature associated with sJIA is macrophage activation syndrome, which can affect one-third sJIA, and characterized by the excessive activation of well-differentiated macrophages, resulting in high fever, hepatosplenomegaly, cytopenias,

and intravascular coagulation. Additionally, 50% of patients continue to suffer from active arthritis after being diagnosed with sJIA [2]. Notably, sJIA has a great impact on children's physiology as well as psychology and seriously affects their quality of life. Therefore, in order to better understand and treat the disease, extensive research has been conducted on the field and exceptional results have been reported [3–5]. Presently, the consensus state that unlike other JIA, sJIA is characteristic as autoinflammatory rather than autoimmune condition, and sJIA is more like an innate immune response dysregulation rather than adaptive immune disorder [6, 7]. In the pathogenetic process of sJIA, monocytes and neutrophils rather than lymphocytes occupy an important position [8], and the pro-inflammatory cytokines such as interleukins 1, 6, and 18 (IL-1, IL-6, IL-18), and tumor necrosis factor (TNF) were observed as the predominant cytokines. Some researchers also believe that the sJIA is a systemic immune inflammatory reactive disease triggered by infection in people with genetic predisposition [9]. Nonetheless, information regarding the pathogenesis of sJIA is still scarce despite the extensive effort that has been made and this makes surveillance as well as treatment of the disease rather challenging.

Bioinformatics analysis is as a reliable way to analyze disease-related pathways, finding biomarkers and predicting therapeutic targets for diseases from the genomics and transcriptomics perspective. Therefore, it has been increasingly used by researchers over the recent years, especially in cancers and autoimmune diseases [10]. Notably, genetic predisposition to sJIA was previously identified through peripheral blood genomic analysis [11, 12]. However, these studies were usually single center with a small sample size, and the results were often inconsistent.

Thus, in this study, we attempted to comprehensive analysis two public datasets of GEO to seek potential pathogenic pathways and suspected virulence genes, especially of sJIA by bioinformatic analysis. The sJIA and healthy controls in GSE20307 and GSE21521 were selected in our study, and genomics in these two datasets were both detected from peripheral blood mononuclear cells (PBMC) and based on the same platform, which can make the results much more reliable. Our study aimed at identifying genetic characteristics and providing treatment basis for sJIA from a bioinformatic perspective.

## **Materials And Methods**

### **Gene expression profile data**

The two gene expression profile datasets (GSE20307 and GSE21521) were downloaded from the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo>). The gene expression profiles of the two datasets were both obtained from PBMCs and were both based on the GPL570 platform ([HG-U133\_Plus\_2] Affymetrix Human Genome U133 Plus 2.0 Array). The profiles of sJIA and healthy controls were selected for the study. Therefore, in GSE20307, 20 sJIA and 50 healthy controls were involved while in GSE21521, 18 sJIA and 29 controls were selected.

### **Overlapping DEG identification**

GEO2R is an interactive online tool (<http://www.ncbi.nlm.nih.gov/geo/geo2r/>) and was used to screen for DEGs between sJIA and healthy controls, with the cut-off criteria of  $|\log_2FC| > 0.5$  and the adj.  $P < 0.05$ . Additionally, an online tool Venny (Version 2.1, available online: <http://bioinfogp.cnb.csic.es/tools/venny/index.html>) was used to obtain the overlapping DEGs in the two datasets.

## Functional and pathway analysis of DEGs

Gene Ontology (GO) terms[10] and KEGG[11] (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analyses are two main approaches used in bioinformatics to explore potential biological functions. GO terms include Cellular Component (CC), Molecular Function (MF) and Biological Process (BP). In addition, metaspice which is a convenient and up to date online gene annotation and analysis tool was used for analysis of the overlapping DEGs. Each gene of DEGs was studied for its pathway and process enrichment score for statistical significance in each biological process. Only terms with  $P < 0.01$ , a minimum count of 3 and an enrichment factor  $> 1.5$  were considered be significant. Genes were also clustered according to their pathways and the results visualized using bar charts.

## Construction of the PPI network, identification of modular analysis and significant candidate genes

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, <https://string-db.org/>) was used to construct a Protein-Protein Interaction (PPI) network of the overlapping DEGs with a combined score  $> 0.4$  as the threshold for statistically significant interaction. Then Cytoscape (version 3.4.0; <http://www.Cytoscape.org>) was used to visualize the PPI network and the plug-in application named Molecular Complex Detection (MCODE) [12] used to further identify important molecules in the PPI network[13]. The recognition criteria were MCODE scores  $> 5$ , degree cut-off = 2, node score cut-off = 0.2, Max depth = 100 and k-score = 2. Additionally, Hub genes in the PPI network were filtered using cytoHubba based on the connectivity node degree analysis. The nodes with a high degree were identified as hub genes, which might be key candidate genes in the pathogenesis of the disease.

## Results

### Identification of DEGs in sJIA

The sJIA and healthy controls in GSE20307 and GSE21521 were involved in our studies for they based on the same platform and the gene profiles were both obtained from PBMC. A total of 559, and 322 DEGs were identified from GSE20307 and GSE21521 respectively. A total of 289 genes (consisting of 41 downregulated genes and 248 upregulated genes) overlapped among the two datasets and was shown in the Venn diagram (Figure 1), and the details were showed in table 1.

### Enrichment analysis of the overlapping DEGs

The online tool of metaspice was utilized to analyze the GO and KEGG pathway enrichment

of overlapping DEGs. For GO term analysis (Figure 2), the overlapping DEGs were primarily enriched in myeloid leukocyte activation, myeloid cell differentiation, reactive oxygen species metabolic process, hydrogen peroxide catabolic process, porphyrin-containing compound metabolic process for biological processes (BP) (Figure 2a). The molecular function (MF) (Figure 2b) terms were mainly enriched in hemoglobin binding, organic acid binding, haptoglobin binding, cofactor transmembrane transporter activity, protein kinase inhibitor activity. And enriched cell component (CC) (Figure 2c) of DEGs significantly involved in specific granule, specific granule lumen, spectrin-associated cytoskeleton, haptoglobin-hemoglobin complex, mitochondrial outer membrane. In addition, the KEGG pathway enrichment analysis (Figure 2d) showed that the overlapping DEGs were mainly involved in Malaria (hsa05144), Non-small cell lung cancer (hsa05223), Mitophagy - animal (hsa04137), Transcriptional misregulation in cancer (05202), Porphyrin and chlorophyll metabolism (hsa00860).

### **Construction of the Protein-protein Interaction (PPI) network, Module Analysis and Identification of Hub genes**

A PPI network of the overlapping DEGs was constructed on the STRING website and visualized with Cytoscape, where nodes that were less connected to the entire network were deleted. Consequently, a total of 203 nodes and 622 edges were constructed in the PPI network (Figure 3). Most of the genes in the network were up-regulated. In addition, four modules (Figure. 4) from the complex PPI network were selected for MCODE analysis and the most significant module included 28 nodes and 123 edges (Figure 4a). KEGG pathway enrichment analysis revealed that the genes in this module were involved in Ubiquitin mediated proteolysis. Moreover, the top 10 genes with the highest degrees in the PPI network were identified using a plug-in tool (cytohubba) in Cytoscape (Figure 5). They were as follows; EPB42 (Erythrocyte Membrane Protein Band 4.2), SLC4A1 (Solute Carrier Family 4 Member 1 (Diego Blood Group)), ALAS2 (5'-Aminolevulinate Synthase 2), MMP9 (Matrix Metalloproteinase 9), EGF (Epidermal Growth Factor), AHSP (Alpha Hemoglobin Stabilizing Protein), FBXO7 (F-Box Protein 7), FECH (Ferrochelatase), KLF1 (Kruppel Like Factor 1) and BCL2L1 (BCL2 Like 1). All of them were involved in module 1.

## **Discussion**

sJIA poses a threat to the physical health of young people as it can affect all systems in the body. Unfortunately, the pathogenesis and pathological process of the disease remain largely unclear with the treatment rather challenge. Notably, the recent development of high-throughput technologies has led to a better understanding of the pathogenesis of various diseases [14]. And more genes have been discovered and validated by high-throughput sequencing. Our studies combination of two cohort profile datasets and integrated bioinformatics methods and ultimately identify some suspected genes and pathways in the pathogenesis of sJIA. Besides, our results revealed an important module in sJIA, and KEGG pathway analysis of which revealed that the genes involved were associated with ubiquitin mediated proteolysis. What's more, the top 10 hub genes identified in the PPI network were all present in this module, suggesting that ubiquitin mediated proteolysis was an important process in the pathogenesis in sJIA.

Among the DEGs, 10 hub genes were selected, 8 of which were related to erythropoiesis. These included genes encoding both adult and fetal hemoglobin as well as those coding for structural proteins in the red blood cells along with proteins and enzymes on the cell surface, similar to previous studies[15–17]. Hinze, C.H et al. reported that an erythropoiesis signature was present in sJIA with anemia but not in other JIA subtypes with anemia [18]. Additional research also showed that the erythropoiesis signature was quite special in active sJIA (with fever) but not in the inactive form of the disease (without fever)[15, 19]. Moreover, Hinze, C.H et al. showed that the index of the erythropoiesis signature decreased with the improvement of sJIA [19]. Further research by Fall et.al through flow cytometry, also revealed that sJIA patients had precursor cells expansion, with a higher proportion of CD34<sup>+</sup> and CD15<sup>+</sup>CD16<sup>-</sup> immature PBMC subgroups[16]. This was consistent with the BP terms enriched from GO analysis in this study. The results from the current study also revealed the activation of myeloid in sJIA. Generally, the findings corroborated with those from previous studies in showing that there is indeed an increase in erythropoiesis genes and myeloid activation in sJIA.

The simultaneous increase in the erythropoiesis signature and precursor cell activation in sJIA may be due to the increase of inflammatory cytokines [15]. It was previously reported that there was an increase in the erythropoiesis signature in adult patients with rheumatoid arthritis and anemia[20], in which IL-6 plays an important role. Therefore, the erythropoiesis observed in sJIA may have similar pathogenic mechanisms[21]. IL-6 has an important effect on bone marrow hematopoiesis and hyper-IL-6 plays a regulatory role in the differentiation of myeloid and erythroid progenitor cells derived from human cord blood. IL-6 can directly stimulate glycoprotein (gp) 130 (the membrane-anchored signal transducing receptor component of IL-6), effectively stimulating the *in vitro* expansion of human CD34 stem cells/progenitor cells and promoting erythropoiesis [22]. Moreover, the administration of IL-6 was reported to stimulate multi-lineage hematopoietic function and accelerate recovery from radiation-induced hematopoietic hypofunction. Furthermore, IL-6 has a strong promoting effect on other aspects of hematopoiesis [23]. For example, it can induce the expression of hepcidin in liver cells, reduce the absorption of iron in the intestine and induce the expression of ferritin in monocytes/macrophages [24]. Therefore, serum iron is retained in the periphery and the available iron reaching the hematopoietic area is reduced. This may be the reason behind the increased production of red blood cells. In addition, the increased expression of erythropoiesis genes may be related to the hemophagocytic syndrome in sJIA. It was previously shown that two-thirds of sJIA patients have macrophage polarization and hemophagocytic syndrome[25]. In the presence of the hematopoietic syndrome, a large number of blood cells are destroyed and erythrocytes are renewed, potentially leading to the expression of the secondary erythropoiesis signature. In this study, the results of MF analysis showed that enrichment of hemoglobin and haptoglobin binding may be another indication of an increase in not only the erythropoiesis signature but also erythrocyte precursors.

Another finding of our study is that the modular analysis results of PPI show that the main modules are related to ubiquitin-mediated proteolysis (UPS). UPS is another important way of protein degradation in eukaryotes besides the autophagy-lysosome pathway. It was first reported in reticulocytes and studies

have found that it is more active in erythrocyte precursor cells [26]. UPS can program the degradation of pre-existing cellular proteins in the terminally differentiated erythroid precursors and reshape their proteome hence simplifying the cellular proteome of mature erythrocytes. This is essential in maintaining the normal function of red blood cells. Furthermore, Grune et al. showed that the proteasome in erythrocytes plays a key role in the degradation of oxidized hemoglobin [27]. Further research by Hanash et al.'s on reticulocytes and normal red blood cell lysates also showed that early erythrocyte precursors had a greater ability to reduce excess alpha chains compared to mature erythrocyte [28]. Additional studies also reported that the UPS system is a protective mechanism against hemoglobinopathies as it can degrade unstable globins, especially in thalassemia [29]. Therefore, activation of the ubiquitin proteasome system in sJIA further suggested that there was indeed an increase in the expression of erythropoiesis genes, an increase erythrocyte precursor cells and possible abnormal hemoglobin chains.

In any case, the important role of the proteasome in immune diseases has been paid increasing attention. Existing evidence suggests that UPS is increased in rheumatoid arthritis, systemic lupus erythematosus and other autoimmune inflammatory diseases. It was reported that the constituent subunits of the proteasome are replaced by inducible subunits ( $\beta 1i/LMP2$ ;  $\beta 2i/Mecl-1$ ;  $\beta 5i/LMP7$ ) during inflammation [30], leading to the formation of the Immunoproteasome (IP). IP is related to various biological processes of autoimmune diseases such as MHC (major histocompatibility complex)-mediated antigen presentation, B cell maturation and antibody secretion, Th1 and Th17 differentiation, production of inflammatory cytokines and macrophage polarization [31]. Moreover, the most important link between UPS and inflammation is NF- $\kappa$ B [32], which is the main regulator of many inflammatory cytokine genes and whose activation is mediated by UPS. UPS can regulate the degradation of I $\kappa$ B and control the activity of NF- $\kappa$ B, hence regulating the secretion of NF- $\kappa$ B-related inflammatory cytokines (including TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-10). Moreover, related studies showed that inhibiting UPS can interfere with the antigen presentation role of T cells, inhibit the production of Th17 cells, reduce the quantity and quality of autoantibodies [33]. Thus, UPS inhibitors such as Bortezomib (BTZ) have shown their effectiveness in the treatment of autoimmune diseases in human and animal studies [34, 35]. In short, combined with those previous researches and the results of our study, we believe that UPS inhibitors may be an effective alternative option to sJIA.

The KEGG pathway enrichment analysis also showed that Malaria and Non-small cell lung cancer were enriched in sJIA. We believe that the enrichment of malaria pathway provides the basis for the application of hydroxychloroquine in sJIA. Hydroxychloroquine is an effective anti-malarial drug that has also proven to be effective in the control of rheumatic diseases [36]. Studies have showed that hydroxychloroquine can inhibit the antigen presentation function of the autophagy lysosome pathway by destroying membrane stability, interfering with the activity of lysosomes and damaging the maturation of lysosomes and autophagosomes [37]. The drug also directly and indirectly inhibits the toll-like receptor signaling pathways and reduces the production of cytokines mediated by macrophages [38]. Moreover, it was reported that hydroxychloroquine can inhibit T and B cell receptor calcium signaling [39], reduce the expression of CD154 in T cells [40], therefore inhibiting T cell proliferation and immunoglobulin production. Therefore, based on the previous studies and those obtained from KEGG enrichment analysis

herein, hydroxychloroquine may be more applicable in the management of sJIA given that the malaria pathway was enriched in the disease.

Moreover, the results from KEGG analysis suggested that non-small cell lung cancer (NSCLC) related pathways were involved in the pathogenesis of sJIA. It is noteworthy that rheumatoid arthritis and cancer have certain aspects in common. Both cases have an increase in inflammatory cytokines (including IL-6, IL-23, TNF- $\alpha$ , IL-1 and IL-17) and the proliferation of related cells. On the one hand, previous studies showed that the above-mentioned cytokines play an important role in the occurrence and development of autoimmune inflammatory diseases as well as in non-small cell lung cancer [41]. On the other hand, recent reports indicate that some kinase inhibitors used for the treatment of NSCLC also have some extent of efficacy against rheumatic diseases[42]. Animal studies revealed that the Protein Tyrosine Kinase inhibitors (PTKs); Imatinib and Nilotinib, can regulate the processing of the antigen peptide proteasome and inhibit the function of T cells thus alleviating the effects of collagen-induced rheumatoid arthritis in mice [43]. Besides, the Epidermal Growth Factor Receptor (EGFR) kinase inhibitors; Erlotinib and Gefitinib were shown to be able to treat non-cancer-related TNF- $\alpha$ -mediated inflammatory autoimmune diseases [44]. In short, kinase inhibitors for non-small cell lung cancer may also be effective in the treatment of sJIA due to the pathway of NSCLC, which provides a potential alternative for the management of sJIA.

## Conclusion

This study comprehensively analyzed the datasets of the two centers and combined with previous research reports, revealing that there is indeed increased erythropoiesis in sJIA. In addition, through our study, we provide a basis for the application of proteasome inhibitors, hydroxychloroquine and kinase inhibitors in patients with sJIA from the perspective of bioinformatics.

## Abbreviations

sJIA  
Systemic Juvenile Idiopathic Arthritis  
GEO  
Gene Expression Omnibus  
DEGs  
Differentially Expressed Genes  
PPI  
Protein-protein Interaction  
KEGG  
Kyoto Encyclopedia of Genes and Genomes  
TNF  
Tumor Necrosis Factor  
PBMC

Peripheral Blood Mononuclear Cells

GO

Gene Ontology

CC

Cellular Component

MF

Molecular Function

BP

Biological Process

MCODE

Molecular Complex Detection

UPS

Ubiquitin-mediated Proteolysis

MHC

Major Histocompatibility Complex

BTZ

Bortezomib

NSCLC

Non-small Cell lung Cancer

EGFR

Epidermal Growth Factor Receptor

## **Declarations**

### **Ethics approval and consent to participate**

Not applicable.

### **Consent for publication**

Not applicable.

### **Availability of data and materials**

The data used for analysis in this study are available from the Gene Expression Omnibus database ([www.ncbi.nlm.nih.gov/geo](http://www.ncbi.nlm.nih.gov/geo)) freely.

### **Competing interests**

The authors declare that they have no competing interests

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This study had no funding supported.

## Authors' contributions

Conceptualization: Jibo Wang; Writing - original draft preparation and data analysis: Wenping Liu; Data collection: Dawei Wen; Writing - review and editing: Ziyi Liu; Supervision: Kunyu Wang. All authors read and approved the final manuscript.

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## Tables

Table 1. 289 overlapping genes of GSE20307 and GSE21521.

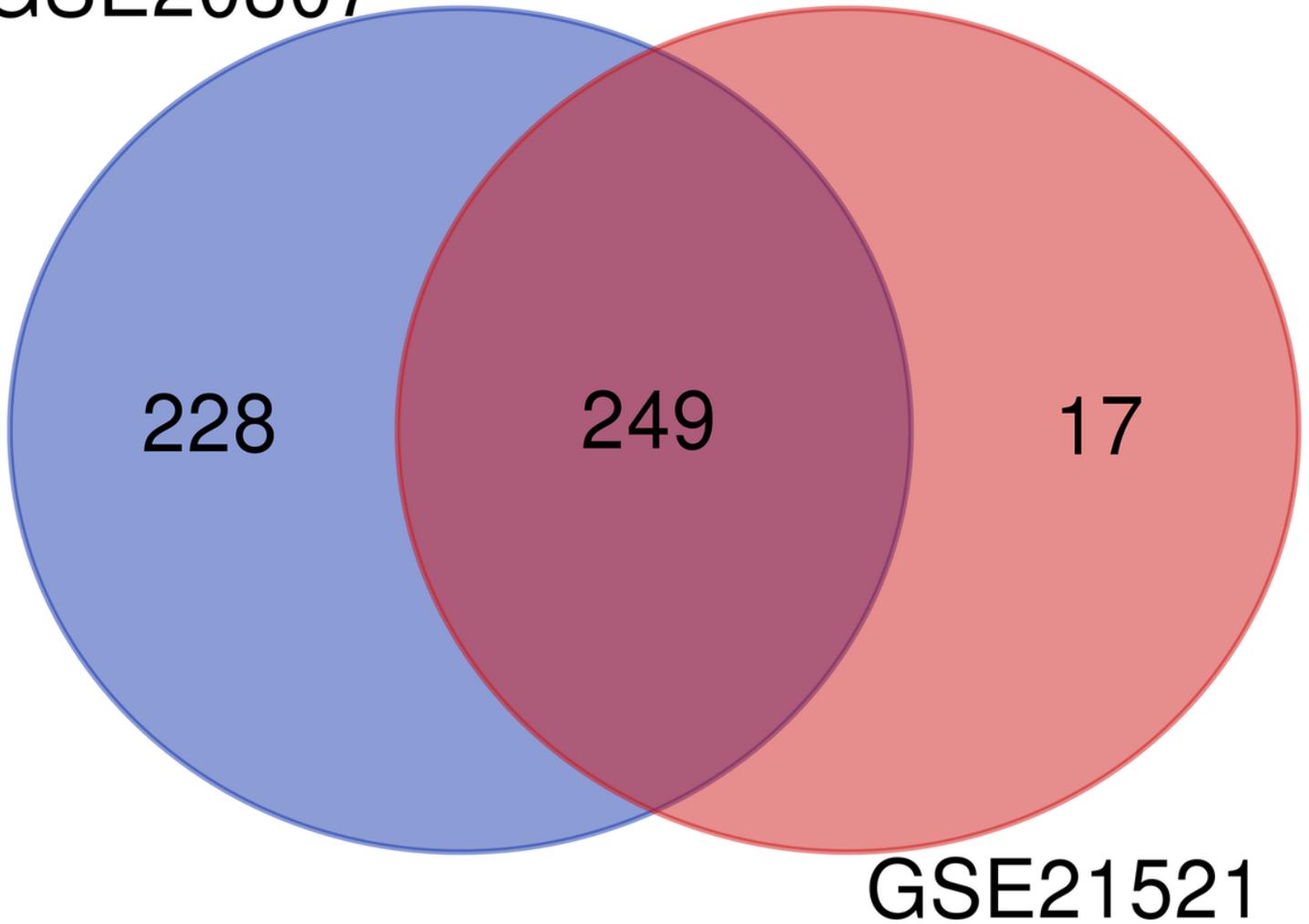
Group	Gene names
Upregulated (248)	HBA2 SLC2A3 SMIM24 CHPT1 PINK1 YIPF6 IQGAP3 FECH SLC4A1 WASF1 TNFAIP6 PDZK1IP1 MGAM EIF2AK1 NCF4 ARG2 MYL4 KLF1 MKI67 SLC1A3 KANK2 TSTA3 TTC25 NFE2 LOC643072 ABCG2 BPGM NPRL3 FHDC1 UBBP1 MAN1A1 TMOD1 SMIM5 S100P OSBP2 EPB41 SIAH2 BCL3 TMEM158 ASAP1 SLC25A37 FURIN HMGB3 NEDD4L MYL9 EGF SLC22A4 SESN3 CTNNA1 AQP9 XK IGF2BP2 TUBB2A AGO2 FAM20A SMOX GK CR1 HP MPO METTL7B ANKRD22 KDM7A RNF10 STRADB ITGA2B CR1L F5 PGLYRP1 MAP1LC3B MMP8 PLEK2 RAD23A UBBP2 BNIP3L LRG1 FAXDC2 CD177 RXRA HEMGN GUK1 CDC34 FAM210B MAP2K3 KRT1 ADM TMOD1 UBXLN6 MPP1 ANXA3 FPR2 FCGR1A GADD45A NARF ARHGEF12 PCGF5 MXD1 NFIX YOD1 TREML3P PPP3R1 ADGRG3 AGO2 LOC102724387 ARG1 RHD ASCC2 YBX3 MS4A4A TCP11L2 HBD DYSF UBALD2 TFDP1 MND1 FBXO9 PIP4K2A FAM46C MXI1 BBOF1 HIST1H2BD DMTN H2BFS FZD5 BEX3 DCUN1D1 GYPC GMPR GYPA FCGR1A EPB42 ORM1 LCN2 ALAS2 ELL2 ABCC13 R3HDM4 RETN CEACAM1 C7orf73 ALS2CR12 TNS1 PPM1A SNCA SLC2A3 TRIB1 HBQ1 SLC48A1 CLU SEC14L1 FOXO3 DCAF12 MKRN1 HBG1 SUCNR1 GLUL UHRF1 PLSCR1 RAP1GAP FKBP1B RIOK3 WNK1 HIST1H1C TOP2A CDC42BPA TREML1 HIST2H2AA3 GYPB HBBP1 SPTB CPEB4 RNASE2 TESC CA1 WDR26 NATD1 TAL1 BCL2L1 KCNJ15 ACSL1 E2F1 SOCS3 CYBRD1 NPLJAZF1 UBE2H SLC6A8 BMP2K FGFR1OP2 RNF123 MSI2 PLOD2 ISCA1 USP32 LRP10 RUNDC3A ANK1 TMCC2 HIST1H2BE ANKRD9 WBP2 GSPT1 SLC25A39 GPR146 BAG1 CHI3L1 TSPAN5 SELENBP1 DSC2 AHSP FBXO7 TP53INP2 JHDM1D-AS1 MBOAT2 CDKL1 FCGR1B CETP FLVCR2 BLVRB TRIM58 HBM GLRX5 HIST1H2BC MCEMP1 H1F0 HLX BCL2A1 CR1 RAB13 PBX1 HMBS ABCC4 SLC6A8 GPR84 NUDT4 FOXO3 RBM38 MBNL3 S100A9 CYSTM1 TRIM10 SOX6 ADIPOR1 MMP9 FKBP8 TFPI ZFAND3 E2F2 HP
Downregulated (41)	OLIG1 PRSS23 CCDC65 CEP126 AGAP1 MYBL1 BMPR1A SEZ6L KIF5C CAMTA1 CD160 KLRC4 LILRA4 DLG5 PTGDR ELOVL4 SIGLEC17P SH3BP4 GPM6B LDB2 PTCH1 KLRC3 CAMK2N1 PDGFD BNC2 KLRB1 ALDH1A1 PID1 PYHIN1 ADAMTS5 KLRC1 AUTS2 CACNA2D3 PPP2R2B SYTL2 PSMB2 TNFRSF21 CLEC4C LINC00996 KLRF1 PHLDB2 PDZD4

Top 10 hub genes with high degree of overlapping DEGs in sJIA.

Gene symbol	Function	degree
EPB42	Erythrocyte membrane protein band 4.2 is an ATP-binding protein, it probably has a role in erythrocyte shape and mechanical property regulation. Gene Ontology (GO) annotations related to this gene include structural constituent of cytoskeleton and protein-glutamine gamma-glutamyltransferase activity.	29
SLC4A1	The protein encoded by this gene is part of the anion exchanger (AE) family and is expressed in the erythrocyte plasma membrane. Among its related pathways are <a href="#">transport of glucose and other sugars</a> , <a href="#">bile salts and organic acids</a> , <a href="#">metal ions and amine compounds</a> and <a href="#">Neuroscience</a> . Gene Ontology (GO) annotations related to this gene include protein homodimerization activity and transporter activity.	28
ALAS2	The product of this gene specifies an erythroid-specific mitochondrially located enzyme. Among its related pathways are <a href="#">Porphyrin and chlorophyll metabolism</a> and <a href="#">Metabolism</a> . Gene Ontology (GO) annotations related to this gene include pyridoxal phosphate binding and glycine binding.	28
MMP9	Among its related pathways are <a href="#">Regulation of Wnt-mediated beta catenin signaling and target gene transcription</a> and <a href="#">Transcriptional misregulation in cancer</a> . Gene Ontology (GO) annotations related to this gene include identical protein binding and metalloendopeptidase activity. Studies in rhesus monkeys suggest that the enzyme is involved in IL-8-induced mobilization of hematopoietic progenitor cells from bone marrow.	26
EGF	This gene encodes a member of the epidermal growth factor superfamily. Among its related pathways are <a href="#">DAG and IP3 signaling</a> and <a href="#">RET signaling</a> . Gene Ontology (GO) annotations related to this gene include calcium ion binding and epidermal growth factor receptor binding.	23
FBXO7	This gene encodes a member of the F-box protein family. The F-box proteins constitute one of the four subunits of the ubiquitin protein ligase complex called SCFs. The protein encoded by this gene belongs to the Fbxs class and it may play a role in regulation of hematopoiesis. Among its related pathways are <a href="#">Neuroscience</a> and <a href="#">Innate Immune System</a> . Gene Ontology (GO) annotations related to this gene include protein kinase binding and ubiquitin-protein transferase activity.	20
AHSP	This gene encodes a molecular chaperone which binds specifically to free alpha-globin and is involved in hemoglobin assembly. Diseases associated with AHSP include <a href="#">Beta-Thalassemia</a> and <a href="#">Thalassemia</a> . Gene Ontology (GO) annotations related to this gene include unfolded protein binding and hemoglobin binding.	19
FECH	The protein encoded by this gene is localized to the mitochondrion, where it catalyzes the insertion of the ferrous form of iron into protoporphyrin IX in the heme synthesis pathway. Mutations in this gene are associated with erythropoietic protoporphyria. Among its related pathways are <a href="#">Porphyrin and chlorophyll metabolism</a> and <a href="#">Metabolism</a> . Gene Ontology (GO) annotations related to this gene include iron ion binding and 2 iron, 2 sulfur cluster binding.	19
BCL2L1	The protein encoded by this gene belongs to the BCL-2 protein family. BCL-2 family members form hetero- or homodimers and act as anti- or pro-apoptotic regulators that are involved in a wide variety of cellular activities. Among its related pathways are <a href="#">Apoptosis Modulation and Signaling</a> and <a href="#">Nucleotide-binding domain, leucine rich repeat containing receptor (NLR) signaling pathways</a> . Gene Ontology (GO) annotations related to this gene include protein homodimerization activity and protein heterodimerization activity.	18
KLF1	This gene encodes a hematopoietic-specific transcription factor that induces high-level expression of adult beta-globin and other erythroid genes. Among its related pathways are <a href="#">Hematopoietic Stem Cell Differentiation</a> . Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and proximal promoter sequence-specific DNA binding.	18

## Figures

# GSE20307

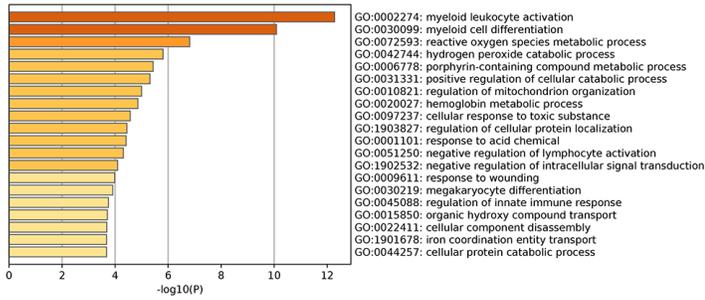


# GSE21521

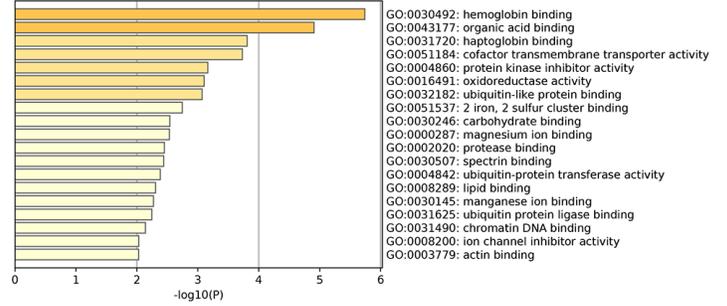
**Figure 1**

289 overlapping DEGs from the two datasets GSE21521 and GSE20307. Different color areas represented different datasets. The cross areas meant the overlapping DEGs. DEGs were identified with classical t test and statistically significant DEGs were defined with the cut-off criteria of  $|\log_2FC| > 0.5$  and the adj.  $P < 0.05$ . (DEGs, differentially expressed genes)

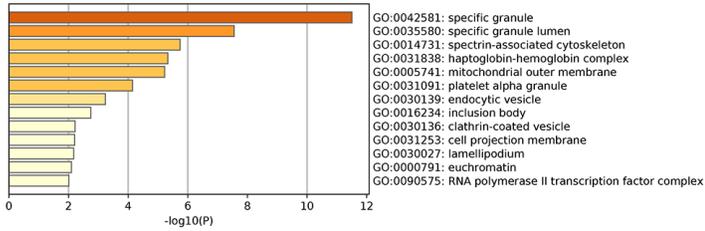
a. BP



b. MF



c. CC



d. KEGG

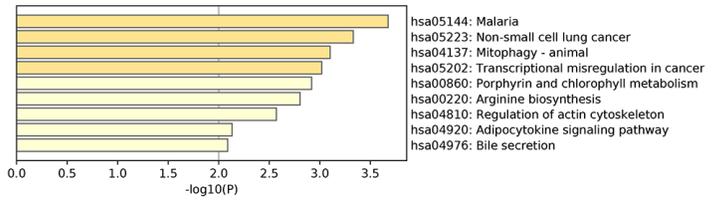
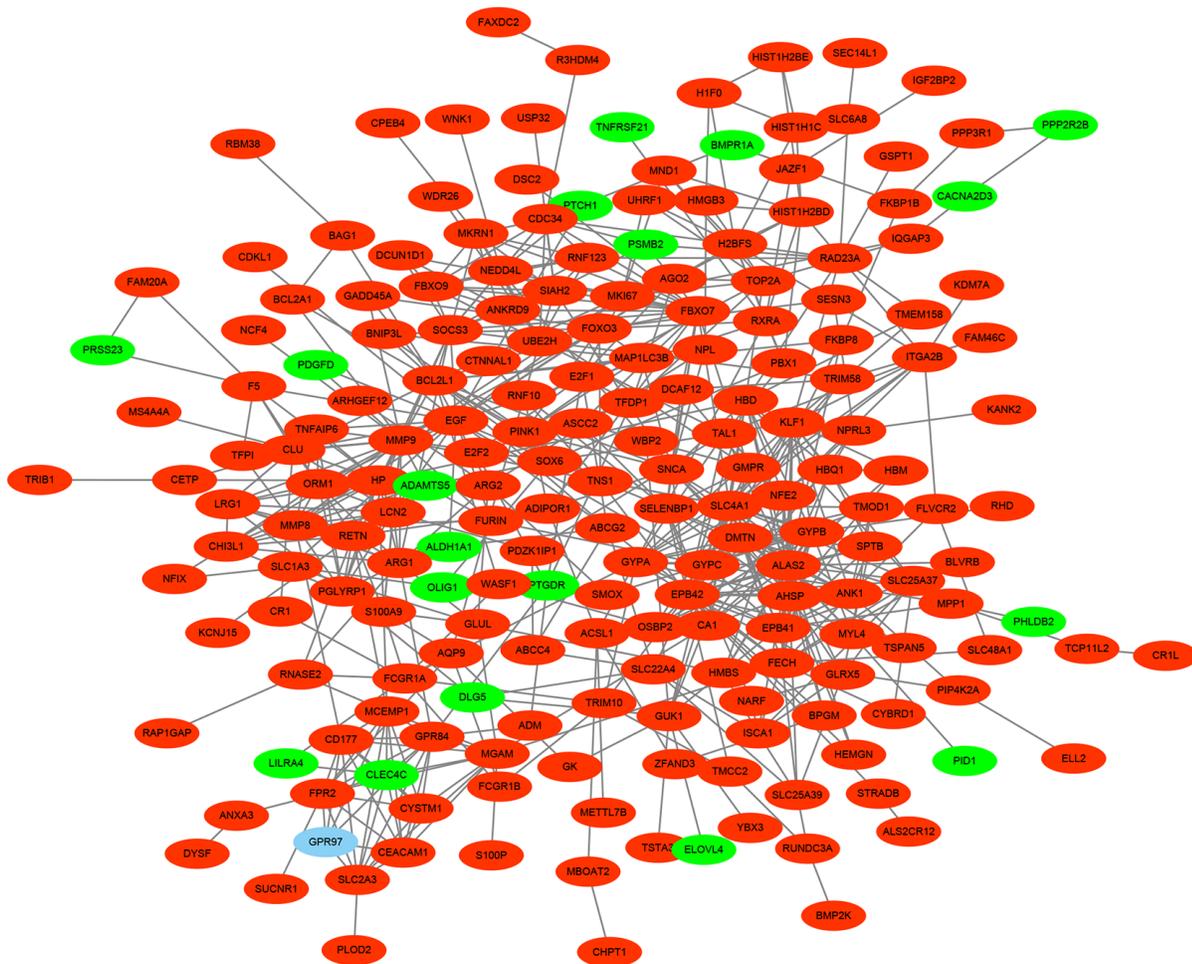


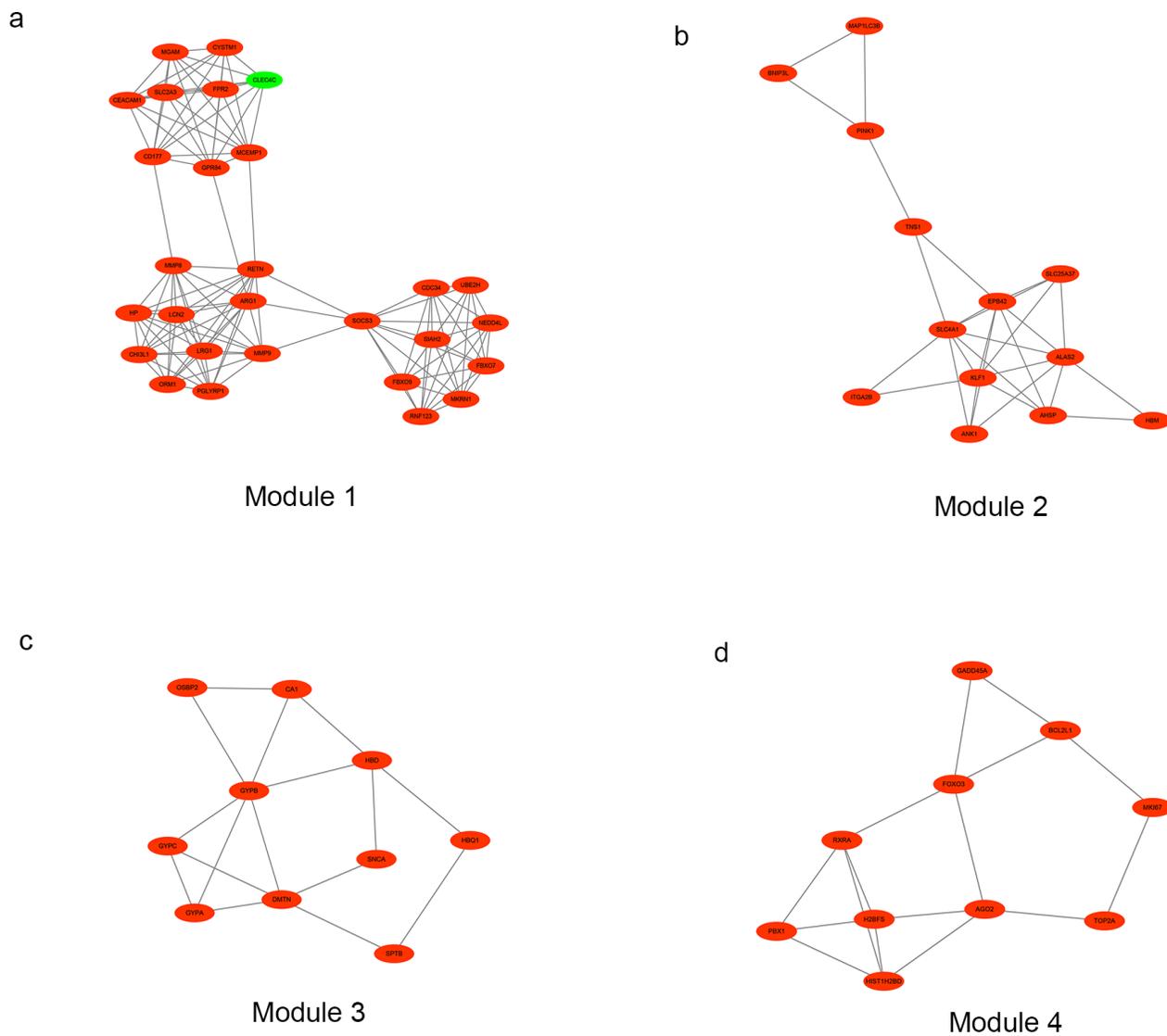
Figure 2

Enrichment analysis of the overlapping DEGs in sJIA. GO analysis under the category of (a) biological processes, (b) molecular functions, and (c) cellular components. (d) KEGG pathway enrichment analysis of overlapping DEGs. (GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; sJIA, Systemic Juvenile Idiopathic Arthritis.)



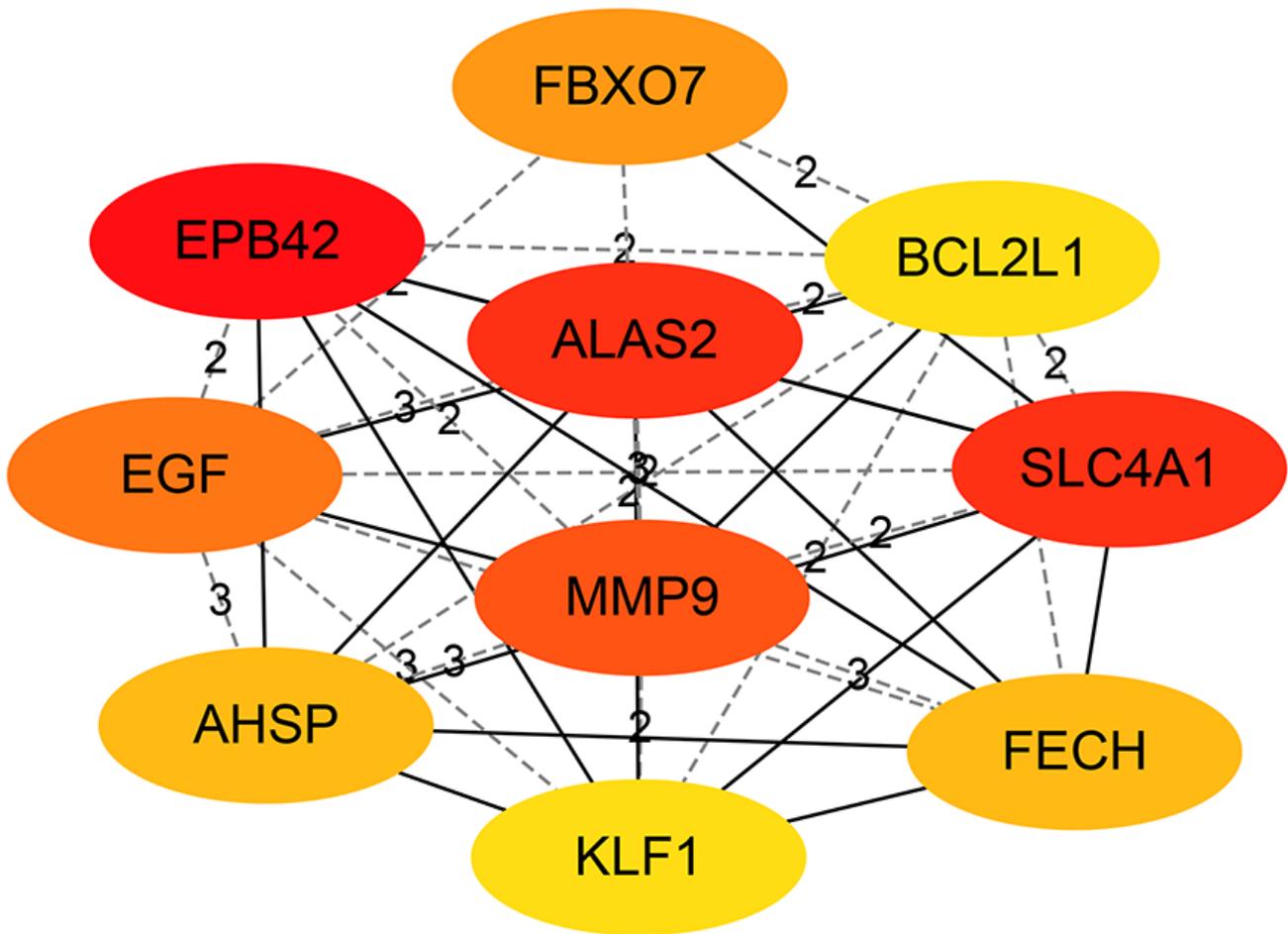
**Figure 3**

Protein-protein interaction (PPI) network of overlapping DEGs in sJIA, with 203 nodes and 622 edges. Red nodes represent upregulated genes while Green nodes represent downregulated genes. (DEG, differentially expressed gene; sJIA, Systematic Juvenile Idiopathic Arthritis.)



**Figure 4**

Four main modules in PPI network of overlapping DEGs in sJIA. Modular analysis using MCODE analysis in Cytoscape. The recognition criteria were MCODE scores > 5, degree cut-of = 2, node score cut-off= 0.2, Max depth = 100 and k-score = 2. (PPI, Protein-protein interaction; MCODE, Molecular Complex Detection)



**Figure 5**

Top 10 hub genes and cointeraction in the PPI network constructed by cytoHubba of Cytoscape based on a degree score. Color scale represents highly of degree scores.