Heliyon 10 (2024) e30020

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Identification of SOCS3 and PTGS2 as new biomarkers for the diagnosis of gout by cross-species comprehensive analysis

Jie Peng [a,b,e,1], Yawen Gu [a,b,1], Jiang Liu [c,1], Hao Yi [c], Dong Ruan [a,b,d], Haoyu Huang [c], Yuan Shu [b], Zhen Zong [c], Rui Wu [a,**], Hui Li [a,*]

[a] Department of Rheumatology and Immunology, the First Affiliated Hospital, Jiangxi Medical College, Nanchang University, 330006, Nanchang, China
[b] The Second Clinical Medical College, Jiangxi Medical College, Nanchang University, 330006, Nanchang, China
[c] Department of Gastrointestinal Surgery, the Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, 1 MinDe Road, 330006, Nanchang, China
[d] Department of Rehabilitation Medicine, the Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, 1 Minde Road, 330006, Nanchang, China
[e] Department of Sports Medicine, Huashan Hospital, Fudan University, 200040, Shanghai, China

ARTICLE INFO

Keywords:
Cross-species
Gout
Biomarkers
Diagnostic
SOCS3
PTGS2

ABSTRACT

Background: Gout is the most common inflammatory arthritis in adults. Gout is an arthritic disease caused by the deposition of monosodium urate crystal (MSU) in the joints, which can lead to acute inflammation and damage adjacent tissue. Hyperuricemia is the main risk factor for MSU crystal deposition and gout. With the increasing burden of gout disease, the identification of potential biomarkers and novel targets for diagnosis is urgently needed.

Methods: For the analysis of this subject paper, we downloaded the human gout data set GSE160170 and the gout mouse model data set GSE190138 from the GEO database. To obtain the differentially expressed genes (DEGs), we intersected the two data sets. Using the cytohubba algorithm, we identified the key genes and enriched them through GO and KEGG. The gene expression trends of three subgroups (normal control group, intermittent gout group and acute gout attack group) were analyzed by Series Test of Cluster (STC) analysis, and the key genes were screened out, and the diagnostic effect was verified by ROC curve. The expression of key genes in dorsal root nerve and spinal cord of gout mice was analyzed. Finally, the clinical samples of normal control group, hyperuricemia group, intermittent gout group and acute gout attack group were collected, and the expression of key genes at protein level was verified by ELISA.

Result: We obtained 59 co-upregulated and 28 co-downregulated genes by comparing the DEGs between gout mouse model data set and human gout data set. 7 hub DEGs(IL1B, IL10, NLRP3, SOCS3, PTGS2) were screened out via Cytohubba algorithm. The results of both GO and KEGG enrichment analyses indicate that 7 hub genes play a significant role in regulating the inflammatory response, cytokine production in immune response, and the TNF signaling pathway. The most representative hub genes SOCS3 and PTGS2 were screened out by Series Test of Cluster, and

* Corresponding author. Department of Rheumatology and Immunology, the First Affiliated Hospital of Nanchang University, 330006, Nanchang, China.
** Corresponding author. Department of Rheumatology and Immunology, the First Affiliated Hospital of Nanchang University, 330006, Nanchang, China.
E-mail addresses: ndyfy00400@ncu.edu.cn (R. Wu), ndyfy04408@ncu.edu.cn (H. Li).
[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e30020
Received 3 May 2023; Received in revised form 18 April 2024; Accepted 18 April 2024
Available online 23 April 2024

ROC analysis results showed the AUC values were both up to 1.000. In addition, we found that PTGS2 expression was significantly elevated in the dorsal root ganglia and spinal cord in monosodium urate(MSU)-induced gout mouse model. The ELISA results revealed that the expression of SOCS3 and PTGS2 was notably higher in the acute gout attack and intermittent gout groups compared to the normal control group. This difference was statistically significant, indicating a clear distinction between the groups.

Conclusion: Through cross-species comprehensive analysis and experimental verification, SOCS3 and PTGS2 were proved to be new biomarkers for diagnosing gout and predicting disease progression.

1. Introduction

Gout is a common disease caused by the deposition of monosodium urate crystals in joints and nonjoint structures [1]. People with acute gout attacks experience severe foot pain, injury, and disability [2], which often manifests as intense unbearable pain in the joints [3]. The new classification standard contains at least 1 case of swelling, pain, or tenderness of peripheral joints or cysts [4]. Depending on the study population, gout affects <1 %–6.8 % of the population, and this number is increasing [5]. Male sex, advanced age, obesity, diet, drug use, and genetics are all risk factors for gout [6]. As a major risk factor for gout, hyperuricaemia is usually defined as a blood uric acid level of no less than 0.42 mmol/L (7 mg/dL) [1]. However, the presence of hyperuricaemia alone should not be diagnosed as gout. Approximately 20 % of Americans have asymptomatic hyperuricaemia [7]. A large proportion of patients with hyperuricaemia have never had a gout attack, and some patients with gout may have uric acid levels within the normal range [8].

The four stages of gout include asymptomatic hyperuricaemia, acute gouty arthritis, intercritical gout, and chronic tophaceous gout [9]. ABCG2 regulates serum uric acid levels through physiologically important mechanisms involved in renal and extrarenal urate excretion [10]. ABCG2 dysfunction leads to an overload of urate excretion. The concentration of urate in the blood increases, as does the formation of uric acid crystals. Acute gout symptoms are caused by an inflammatory response to monosodium urate crystals. Martinon et al. reported that MSU can produce active interleukin 1b and interleukin 18 through activated NALP3 [11]. The inhibition of GLUT1-mediated glucose uptake suppresses MSU crystal-induced NLRP3 activation and IL-1β production [12]. POP1 inhibits NLRP3 inflammasome activation by interfering with key interactions between NLRP3 and ASC in the inflammasome complex [13]. In addition, IL-33/ST2 can mediate pain hypersensitivity and inflammation in a mouse model of gout [14]. IL-37 promotes the noninflammatory phagocytic activity of macrophages and may be a potentially valuable option for the treatment of patients with chronic gout [15].

Experts suggest the presence of monosodium urate crystals in synovial fluid or gout aspirates as the gold standard for diagnosis [16]. It is difficult to perform sodium urate crystal analysis by collecting synovial fluid or gout aspirate because the examination is invasive and requires specialized personnel and technology. To better predict the onset of gout and reduce misdiagnosis and missed diagnoses of gout, we identified novel diagnostic and therapeutic markers for gout across species via bioinformatics analysis. Furthermore, we gathered general information and blood biochemistry samples from both gout patients and individuals without this condition. Using ELISA, we analyzed and compared the expression of key gene regulators in individuals with gout. The present study provides new directions for identifying new biomarkers for gout.

2. Methodology

2.1. Dataset collection

The keyword “gout” was used to search the microarray datasets from the GEO database (https://www.ncbi.nlm.nih.gov/), which was used to investigate the gene expression profile of gout. We chose microarray datasets containing both samples from patients with gout and healthy controls. Finally, we included two datasets for follow-up research, namely, GSE160170 (3 human acute gout samples, 3 human intermittent gout samples, and 6 normal samples) and GSE190138 (18 before- and after-induced mouse gout models, including 6 mouse spinal cord-sourced samples, 6 mouse dorsal root ganglia-sourced samples, and 6 mouse ankle joint-sourced samples). The complete dataset information is shown in Table 1.

Table 1
Datasets used in the present study.

GEO ID	Platform	Organism	Organization	Sample			Country	Year	Author
				Control	Case				
GSE160170	GPL21827	Homo sapiens	blood	6	3 (IG)	3 (AG)	China	2020	Daifei Dai
GSE190138	GPL10558	Mus musculus	ankle joint	3	3		China	2021	Yi Zhang
			spinal cord	3	3				
			dorsal root ganglion	3	3				

2.2. Cross-species differential expression analysis

The GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) and "limma" R packages were used to screen out differentially expressed genes (DEGs) in the GSE160170 and GSE190138 datasets. The GEOquery package was used to read the data, and the "limma" package was used to calculate the multiple differential expression. The DEGs in both datasets were selected using the ggplot2 package in the volcano diagram. We set criteria including a p value < 0.05 and a $|\log FC| > 1$ as the threshold values. Ultimately, to identify cross-species DEGs more accurately, a Venn diagram was drawn via the Venn tool (http://vip.sangerbox.com) to obtain overlapping DEGs that were upregulated or downregulated in both human and mouse gout samples for further analysis.

2.3. Functional enrichment analysis

Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were conducted for exploring the enrichment pathways of gout-associated genes [17,18]. FDR<0.01 and $P < 0.05$ was set as the criteria of GO (BP/CC/MF) analysis, and the results were described in aspects including biological processes (BPs), molecular functions (MFs), and cellular components (CCs). KEGG analysis was performed using "clusterProfile" package of R 4.0.

2.4. Construction of the protein–protein interaction network

In this study, we obtained 59 coupregulated and 28 codownregulated DEGs after cross-species differential expression analysis. Subsequently, the STRING database (http://string-db.org) was used to construct a predicted protein–protein interaction (PPI) network for evaluating the interactions among these DEGs. The key modules were then visualized using the MCODE plugin in Cytoscape (http://www.cytoscape.org). Next, 7 algorithms in the CytoHubba plugin were utilized to further analyze the 15 gout-related genes among the 87 DEGs; thus, 7 key genes were obtained. Finally, gout-related hub genes were obtained after we took the intersection of the key modules and 7 key genes, and the GeneMANIA website (http://genemania.org) was used to conduct coexpression analysis.

2.5. Series test of clusters

Based on the acute gout, intermittent gout, and control samples from the GSE160170 datasets, principal component analysis (PCA) and association analysis were performed to further verify the necessity and accuracy of exploring acute gout and intermittent gout, respectively. Next, a series test of cluster (STC) was conducted to extensively explore key clusters closely associated with gout [19]. Through further analysis of the STC data, we were able to identify the most significant hub genes involved in the progression of gout. To verify our findings, we investigated the expression levels of these hub genes, SOCS3 and PTGS2, in both human and mouse samples from the gout and control groups. The detailed results are shown with box plots drawn with the ggplot2 package. Moreover, the "pROC" package was used to construct a receiver operating characteristic (ROC) curve prediction model to determine the clinical value of SOCS3 and PTGS2 [20].

2.6. Immune cell infiltration analysis

The ssGSEA score was calculated to evaluate the immune infiltration level in the GSE160170 dataset, in which the expression scores of 28 types of immune cells were generated [21]. Notably, the expression score was based on the expression levels of immune cell-specific marker genes and was obtained from a previous article [22]. The ssGSEA was conducted with the GSVA package of R 4.0. Moreover, Pearson correlation analyses were conducted among 28 immune cells and between the 7 hub genes and 28 immune cells.

2.7. Construction and validation of diagnostic prediction model

To identify important diagnostic factors, we utilized a machine learning algorithm to predict the status of the disease. Specifically, we employed the least absolute shrinkage and selection operator (LASSO) algorithm, which incorporates regularization to improve the accuracy of predictions through regression analysis. We first applied univariate Cox regression analysis to detect diagnosis-related genes among the gout-related genes with a log-rank $P < 0.01$. The LASSO regression algorithm was carried out using the "glmnet" package in R to identify the genes significantly associated with the discrimination of gout patients from normal individuals. ROC analysis was employed to evaluate the model's accuracy and thus assess the clinical diagnostic value of the hub genes.

2.8. Validations and explorations of PTGS2 and SOSC3 expression in multiple tissues with mice gout model

ROC analysis was performed to evaluate the clinical value of PTGS2 and SOSC3 using mice gout and control samples. In addition, we conducted expression validations of PTGS2 and SOSC3 in various tissues using the GSE190138 dataset.

2.9. Collection of samples

We collected blood samples from 40 patients (10 cases in the acute gout attack group, 10 cases in the intermittent gout group, 10 cases in the hyperuricemia group, and 10 cases in the normal control group) from the First Affiliated Hospital of Nanchang University.

The Medical Research Ethics Committee of the First Affiliated Hospital of Nanchang University approved the study, and all patients provided their informed consent by signing the necessary form. The specific demographic characteristics of the samples can be shown in Table S1 and Fig. S1.

2.10. Enzyme-linked immunosorbent assay (ELISA)

The ELISA kit (Jiangsu enzyme Industries Co, Ltd, China) (SOSC3, Cat No. 2H-KMLJh315354; PTGS2, Cat No. 2H-KMLJh315369) was utilized to detect PTGS2 and SOSC3 levels in serum samples extracted from blood samples of 10 hyperuricemia gout patients, 10 high intermittent gout patients, 10 acute gout patients, and 10 healthy controls. Microplate reader was adopted to detect the optical density (OD) value, after which the PTGS2 and SOSC3 levels in human serum samples were calculated via a standard curve.

2.11. Statistical analysis

In this study, all statistical analysis was performed via R version 4.0, which followed two-sided, and p-values of <0.05 were considered as statistically significant differences.

3. Results

3.1. Cross-species differential expression analysis

The flow chart of this study is shown in Fig. 1. We screened 980 DEGs (402 upregulated genes and 578 downregulated genes) in human gout samples and 2880 DEGs (1543 upregulated genes and 1337) in mouse gout samples compared with those in the healthy control group (Fig. 2 A–B). Fifty-nine coupregulated and 28 codownregulated genes were obtained as the most related DEGs after we overlapped the above results, as shown in the Venn diagram (Fig. 2 C).

Additionally, GO/KEGG analysis was employed to explore the associations between 87 DEGs and correlated pathways. The results indicated that in the GO (MF) analysis, the top 3 correlated pathways were “response to external stimulus”, “response to molecule of bacteria origin” and “response to lipopolysaccharide”; in the GO (CCs) analysis, the top 3 correlated pathways were “plasma membrane part”, “integral component of plasma membrane” and “intrinsic component of plasma membrane”; and in the GO (BP) analysis, the top 3 correlated pathways were “receptor ligand activity”, “receptor regulator activity” and “cytokine activity” (Fig. 2 D). KEGG analysis revealed that the DEGs were correlated with pathways related to “TNF signalling pathway”, “IL-17 signalling pathway” and

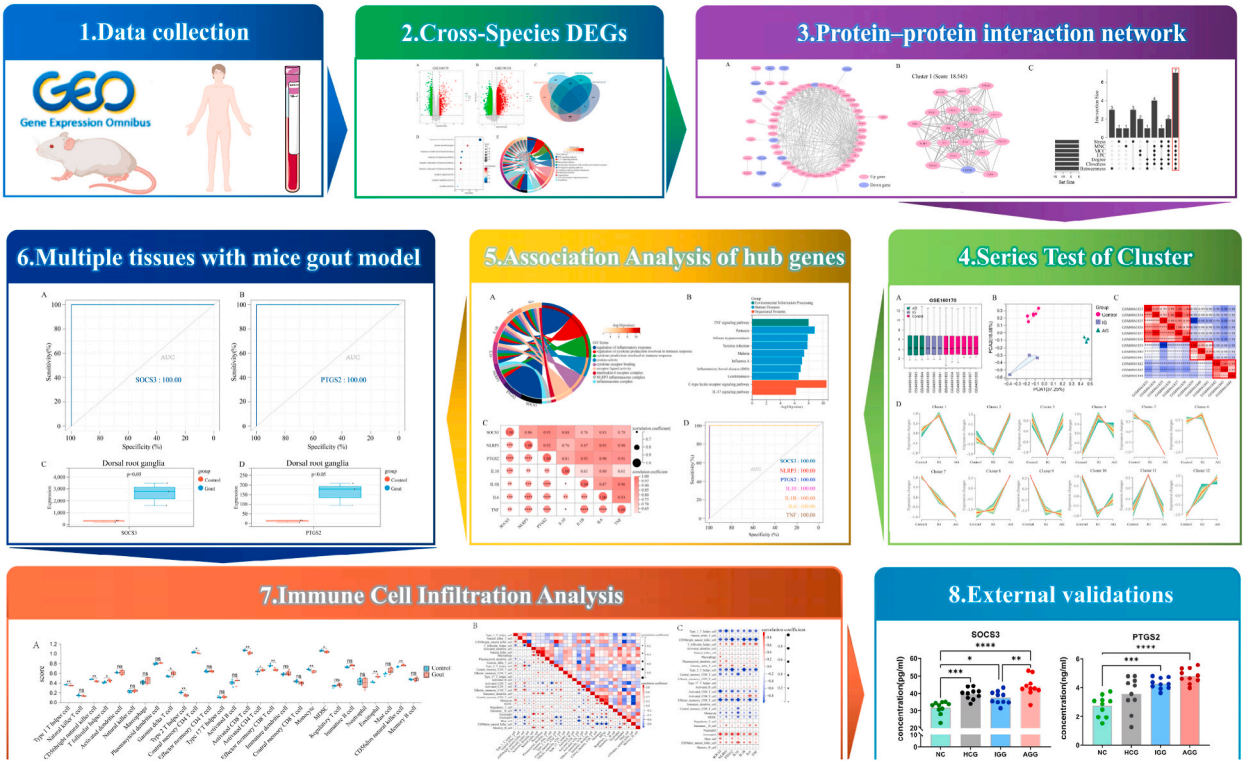

Fig. 1. Flow chart.

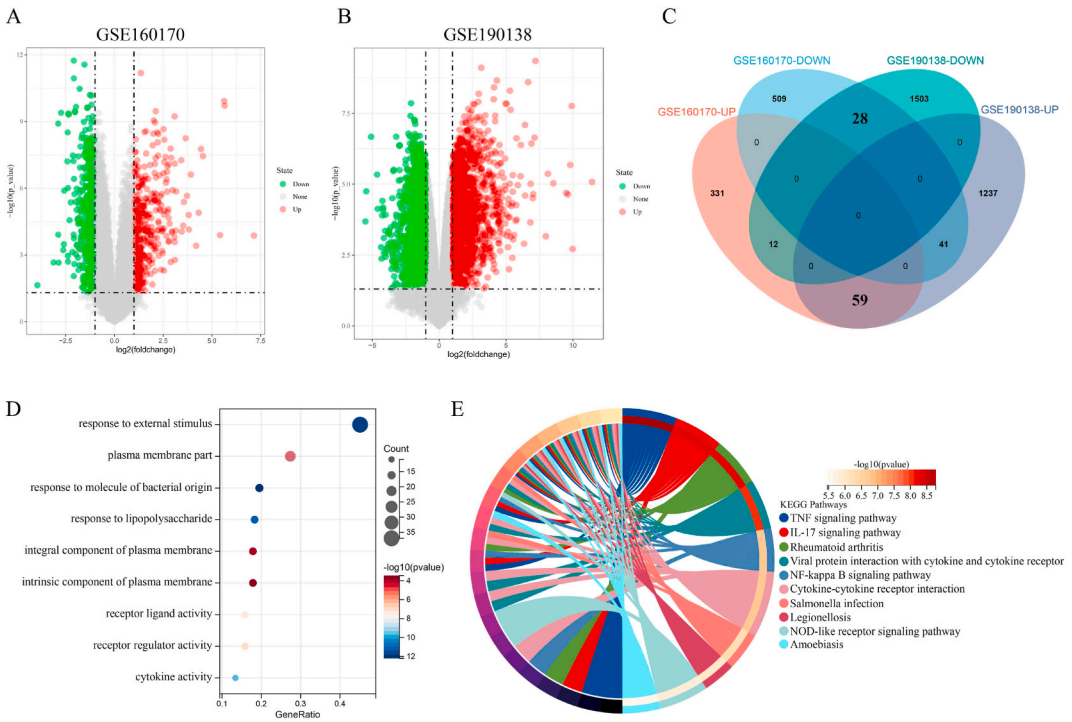

Fig. 2. Identification of DEGs. (**A**, **B**): Volcano plots of upregulated DEGs in both human gout samples (GSE160170) and mice gout samples (GSE190138). The green and red colors respectively represent that compared to control groups, the gene expression state in gout group was down and up. (**C**): Venn diagram of co-DEGs in both human and mice gout samples. (**D**): Heatmap of co-DEGs and correlated pathways by GO analysis. (**E**): Heatmap of co-DEGs and correlated pathways by KEGG analysis. P value of <0.05 and a FDR of <0.25 were considered statistically significant. The top 10 KEGG pathways were shown based on enrichment score (−log10 [p-value]). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

"rheumatoid arthritis" (Fig. 2 E). In summary, the results revealed close relationships between these DEGs and immune response and cellular metabolic processes.

3.2. Identification of hub genes via a protein–protein interaction (PPI) network

Fifty-nine coupregulated and 28 codownregulated DEGs were used to establish a PPI network via Cytoscape. Most of the 87 proteins were closely correlated with each other (Fig. 3 A). The key gene cluster was selected using the MCODE plugin of Cytoscape, and most of the DEGs in the key module were upregulated (Fig. 3 B). Next, taking the intersection of the results of 7 mainstream algorithms, the 7 hub DEGs, namely, IL1B, IL10, NLRP3, SOCS3, and PTGS2, were further screened out from the 15 top gout-related genes of the 87 DEGs via Cytohubba (Fig. 3 C). Notably, we conducted a coexpression network analysis between DEGs in the key module and the 7 hub DEGs via GeneMANIA (Fig. 3 D). SOCS3 and PTGS2 were closely correlated with TNFAIP3, HBEGF, and ICAM-1, which have been reported to play essential roles in gout occurrence and progression [23–25]. In conclusion, we examined the expression levels of seven hub DEGs in both the gouty and control groups. Our findings indicate that the expression levels of these seven hub genes were significantly elevated in gout samples compared to control samples (Fig. 3 E).

3.3. Functional enrichment analysis (and association analysis of hub genes)

GO/KEGG analysis was applied to investigate the correlations between the 7 hub genes and correlated pathways. The results showed that in GO(MFs) analysis, the top 3 correlated pathways were "regulation of inflammatory response", " response of cytokine production involved in immune response" and "cytokine production involved in immune response", and in GO (CCs) analysis, the top 3 correlated pathways were "interleukin-6 receptor complex", "NLRP3 inflammasome complex" and "inflammasome complex", and in GO (BPs) analysis, the top 3 correlated pathways were "cytokine receptor binding ", "receptor ligand activity" and "cytokine activity" (Fig. 4 A). KEGG analysis revealed that the DEGs were correlated with pathways related to "pertussis", "C-type lectin receptor signalling pathway" and "TNF signalling pathway" (Fig. 4 B). Next, association analysis was conducted and revealed that there were significant positive mutual correlations among the 7 hub genes (Fig. 4 D). Furthermore, we assessed the predictive value of the hub genes by analysing their performance in distinguishing between human gout and control samples. This was achieved by plotting the ROC curve, and our findings indicate that the AUC values of these genes were as follows: 1.000 (SOCS3), 1.000 (NLRP3), 1.000

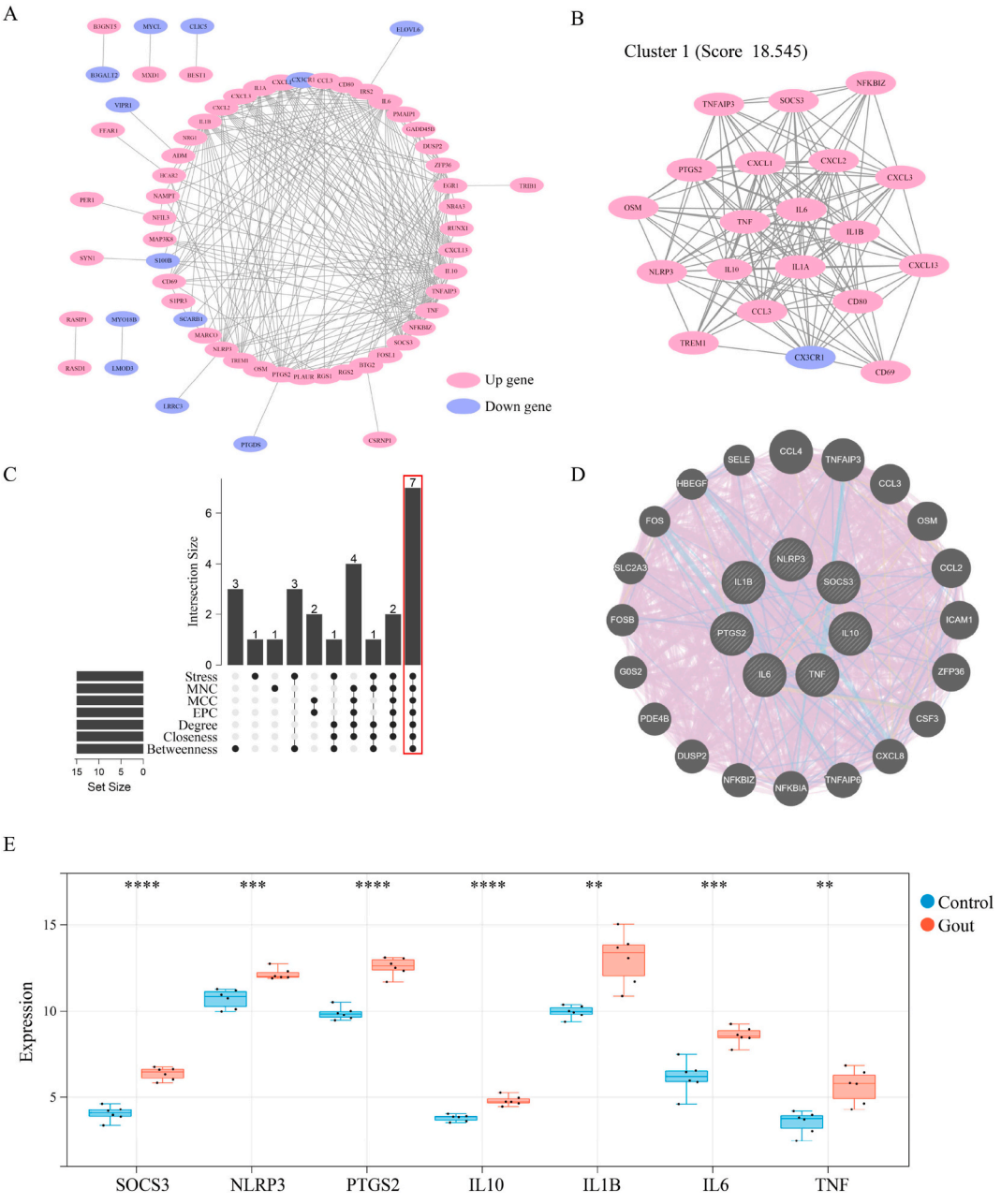

Fig. 3. PPI (Protein-protein interaction) network and identification of key genes. (**A**): PPI network of co-DEGs in both human and mice gout samples. (**B**) The details of genes clustered in one module was exhibited by MCODE. (**C**) An Upset plot displaying the intersections of key genes searched for by seven algorithms in the CytoHubba plugin. (**D**): The co-expression analysis of the seven hub genes by GENEMANIA plugin. (**E**): Expressions of seven hub genes in human gout samples (GSE160170).

(PTGS2), 1.000 (IL10), 1.000 (IL1B), 1.000 (IL6), and 1.000 (TNF) (Fig. 4 C). The results demonstrated that the 7 hub genes all had excellent diagnostic value. The LASSO analysis results indicate that SOCS3, PTGS2, and IL10 genes play crucial roles in gout (Fig. 4 E, F).

3.4. Series test of cluster

The expression data from the GSE160170 dataset were used to construct an overall cohort, which was classified into acute gout, intermittent gout, and healthy control groups, and the results are shown with a box plot (Fig. 5 A). As shown in the figure, after normalization, the expression boxplots of the samples were almost the same, which implied that they could be used for subsequent

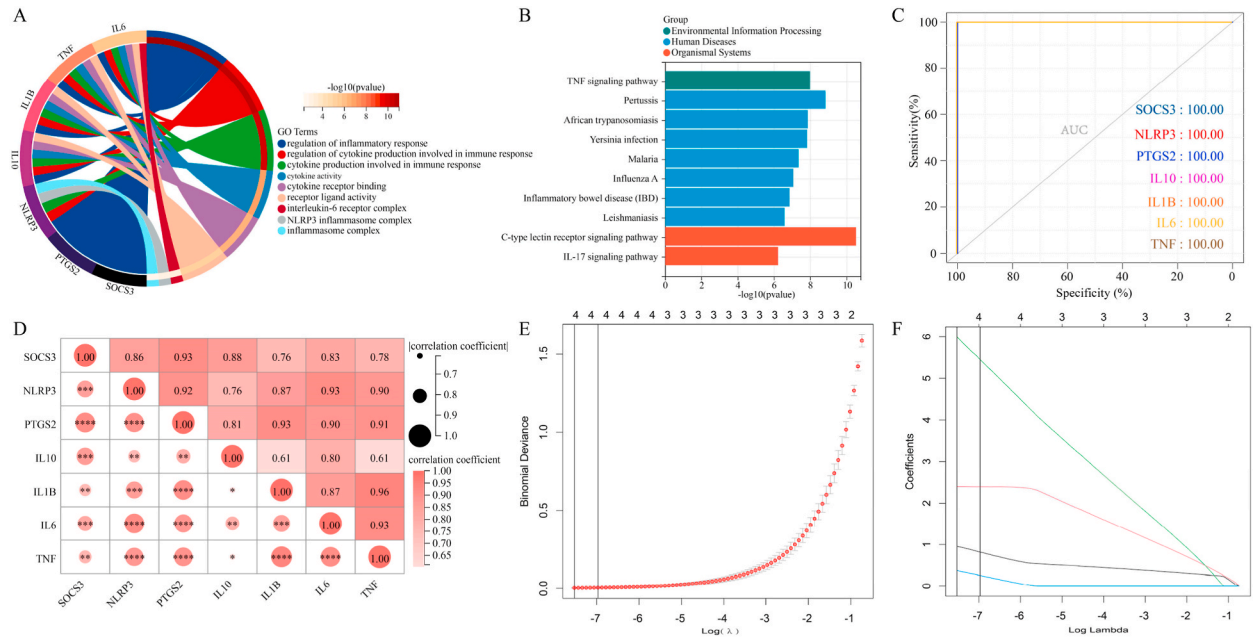

Fig. 4. Further exploration of key genes. (**A**) Gene Ontology (GO) enrichment results of seven key genes. P value of <0.05 and a FDR of <0.25 were considered statistically significant. (**B**) Bar chart of seven key genes by KEGG analysis. The top 10 KEGG pathways were shown based on enrichment score (−log10 [p-value]). (**C**) ROC analysis of seven key genes. (**D**) Heatmap for expression correlation analysis of seven key genes. (**E**) Selection of the optimal l value. (**F**) Least absolute shrinkage and selection operator (LASSO) regression of seven key genes.

analysis. Moreover, PCA and correlation analysis were also conducted for the GSE160170 datasets, and the results demonstrated significant differences among the acute gout, intermittent gout, and control samples, which indicated that investigating acute gout, intermittent gout, and healthy control samples separately was imperative and accurate (Fig. 5 B–C). Based on the above results, 7 gout-related hub genes were stratified into 12 clusters using the Series Test of Cluster ($P < 0.05$ as the criterion), which aimed to further screen out the most representative hub genes (Fig. 5 D). Cluster 12 (SOCS3 and PTGS2) was thus selected, and the detailed results are shown with box plots (Fig. 5 E-F). The results showed that SOCS3 expression significantly increased from the control group to the intermittent gout group and to the acute gout group. Although the difference in PTGS2 expression was not statistically significant, the overall trend of both hub genes increased. Notably, the results of our external experiment demonstrated that PTGS2 expression did not differ between intermittent gout and acute gout, which may provide a possible explanation and further verify the above findings.

3.5. *Validation and exploration of PTGS2 and SOSC3 expression in multiple tissues in a mouse gout model*

ROC analysis was also conducted to evaluate the clinical value of PTGS2 and SOSC3 in mouse gout and control samples, and the results showed that the AUC values of PTGS2 and SOSC3 were as high as 1.000, which indicated that the hub genes all had superior diagnostic performance (Fig. 6 A–B). Moreover, PTGS2 and SOSC3 expression in various tissues were validated using the GSE190138 dataset, which revealed that the PTGS2 and SOSC3 expression levels exhibited an overall gradually increasing trend from the control group to the gout group in both the dorsal root ganglia and the spinal cord, while the change in SOSC3 expression in the dorsal root ganglia was not statistically significant. These negative results might be ascribed to potential bias caused by inadequate samples or other limitations (Fig. 6 C–F). These results demonstrated that PTGS2 and SOSC3 expression was upregulated in distinct tissues of the mouse models and might play essential roles in gout pathogenesis and acute pain.

3.6. *Immune cell infiltration analysis*

The patterns of 28 immune cell subpopulations in patients with gout from the GSE160170 dataset were revealed via ssGSEA. The proportions of immune cells were distinct between the two groups. The results showed that the fraction of 10/28 immune cells varied distinctly between gout patients and healthy controls. Moreover, immune cells, including neutrophils, regulatory T cells, mast cells, gamma delta T cells, and macrophages, were significantly upregulated, and the fraction of 14/28 immune cells was significantly different (Fig. 7 A). Next, we conducted an association analysis of 28 immune cells and found that most of the 28 immune cells were positively correlated with each other (Fig. 7 B). Subsequently, we conducted an association analysis between 28 immune cells and 7 hub genes. The results of our study showed that seven hub genes were significantly positively correlated with Eosinophil cells but significantly negatively correlated with CD56 bright nature killer cells, type 2 T helper cells, and effector memory CD8 T cells. These findings suggest that these specific cells may play a crucial role in the development of gout (Fig. 7 C).

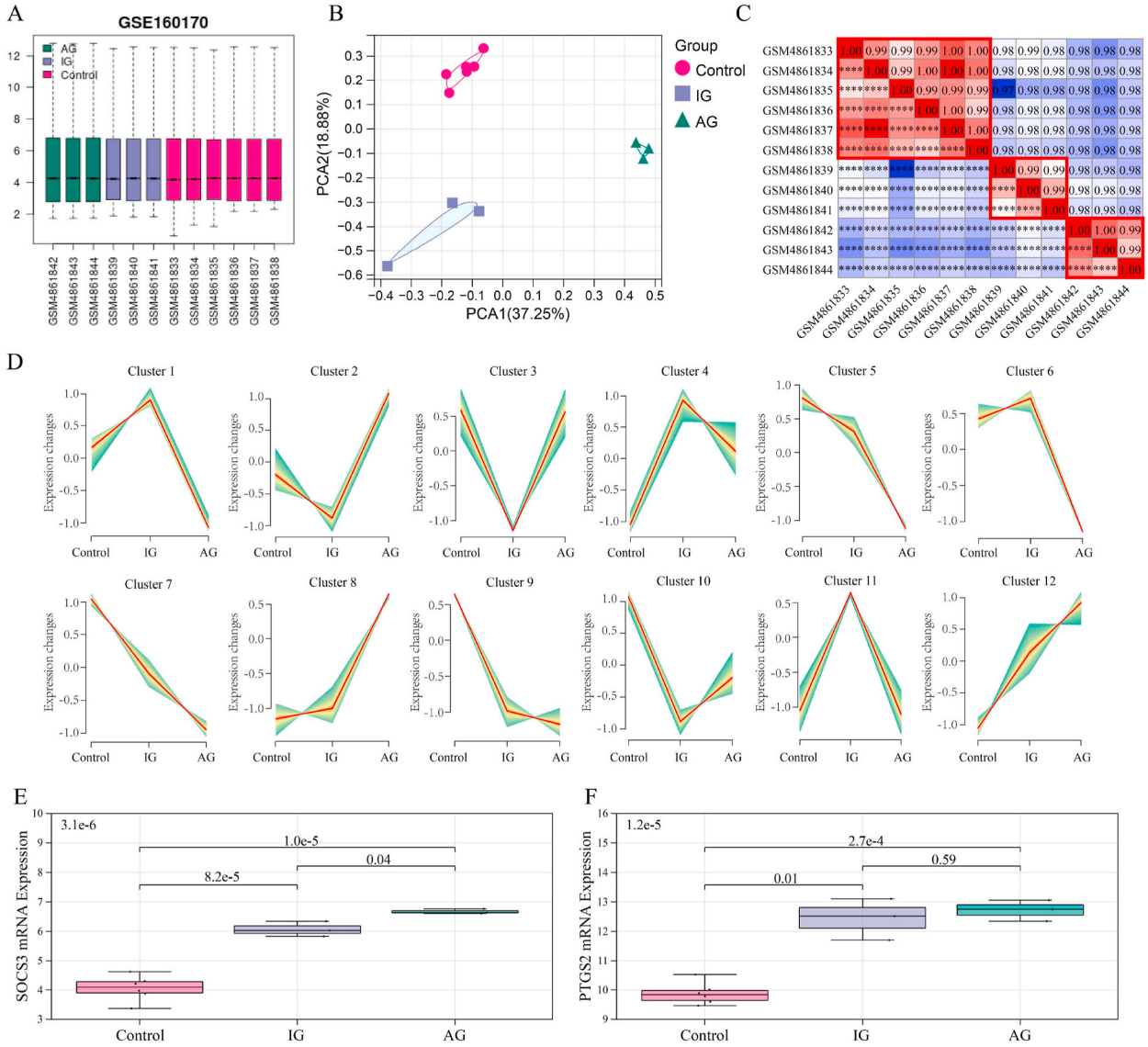

Fig. 5. Further exploration of subgroups of acute and chronic gout in human gout samples (GSE160170). (**A**) Sample box plots after standardization and normalization. (**B**) PCA analysis based on gene expression. (**C**) Correlation heatmap based on gene expression level. (**D**) Gene clustering trend chart for all genes. All genes are clustered into 12 categories based on gene expression. (**E**) Analysis of SOCS3 gene expression subgroups. (**F**) Analysis of PTGS2 gene expression subgroups.

3.7. External validations

We performed ROC analysis using real data collected from the First Affiliated Hospital of Nanchang University to evaluate the clinical diagnostic value of PTGS2 and SOSC3 in human gout and control samples. The results showed that the AUC value of SOSC3 was 0.930 and that of PTGS2 was 0.955, indicating that both SOSC3 and PTGS2 have superior diagnostic performance (Fig. 8 A–B). The ELISA results indicated that SOCS3 expression was significantly greater in the hyperuricaemia, intermittent gout, and acute gout attack groups than in the normal control group (Fig. 8 C). Notably, there was a significant difference in SOCS3 expression between the intermittent gout and acute gout groups. Our results also demonstrated that PTGS2 expression was significantly greater in the intermittent gout and acute gout groups than in the normal control group (Fig. 8 D). However, PTGS2 expression did not significantly differ between the hyperuricaemic gout group and the normal control group.

4. Discussion

Gout is a chronic disease characterized by local inflammation that results from the deposition of monosodium urate (MSU) crystals

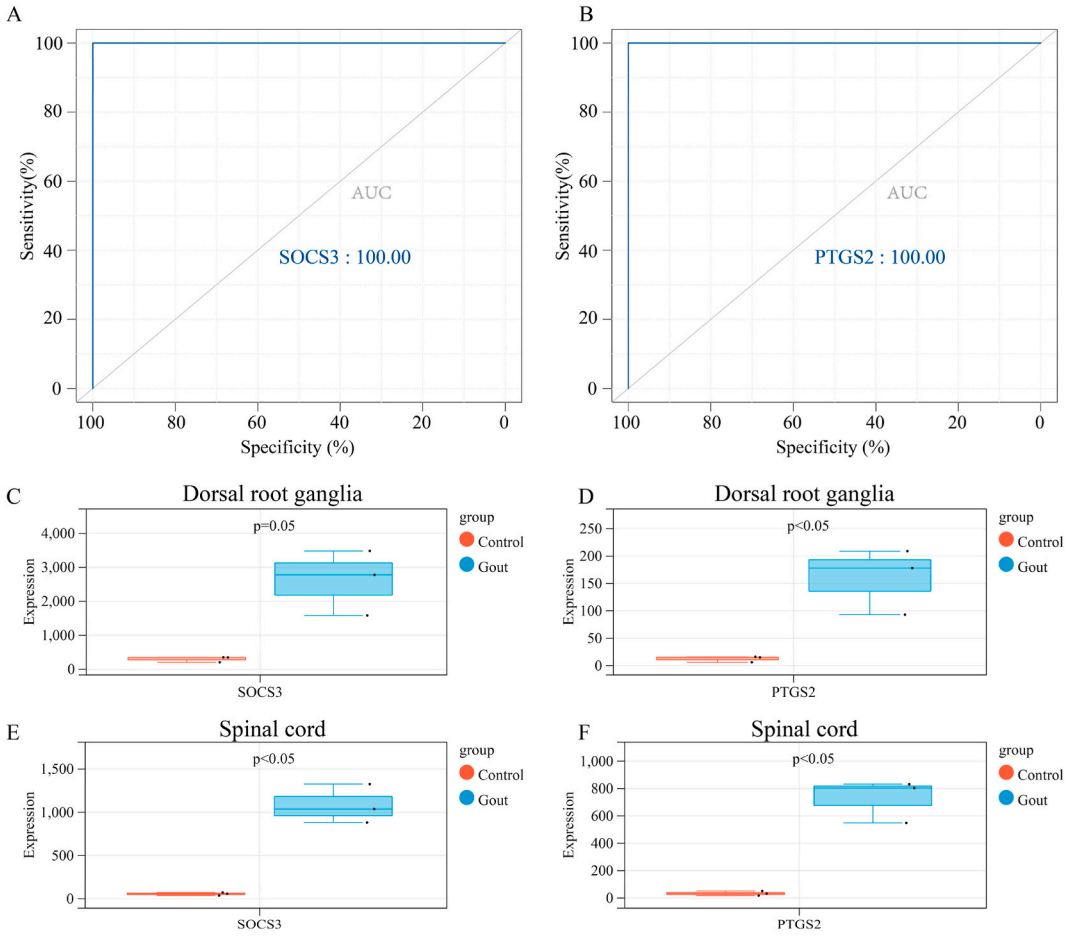

Fig. 6. Analysis of the expression of SOCS2 and PTGS2 genes in mice gout samples (GSE190138). (**A**) ROC analysis of SOCS2 in ankle joint samples. (**B**) ROC analysis of PTGS2 in ankle joint samples. (**C**,**D**) Expression of (**C**) SOCS3 and PTGS2 (**D**) in the dorsal root ganglion samples. (**E**,**F**) Expression of (**E**) SOCS3 and PTGS2 (**F**) in the spinal cord samples.

in joints or adjacent tissues. The primary risk factor for MSU crystal deposition and gout is elevated serumuric acid (hyperuricaemia). To conduct our research, we obtained data from datasets on patients with gout and datasets on gout mouse models from the GEO database, 59 upregulated DEGs and 28 down-regulated DEG were obtained by intersection, and 7 important genes by the Cytohubba algorithm, which included SOCS3, NLPR3, PTGS2, IL10, IL1β, IL6 and TNF-α. Enrichment analysis showed that these genes were involved mainly in the regulation of the inflammatory response and cytokine production involved in the immune response. The activation of NLRP3 has been shown to promote the release of IL-1β and other proinflammatory cytokines, accompanied by vasodilation and an increase in fine granulocytes near the crystal deposition site, which amplifies inflammation and is the key factor in the process of gout [26,27]. TNF-α and IL-6 are crucial factors in both the immune response and bone metabolism. They work to activate macrophages, facilitate antigen presentation, and regulate immunity through distinct mechanisms [28].

Prior research, as well as our own investigation, have highlighted the significance of inflammatory factors like IL-1βand IL-6 in the context of gout, serving as crucial diagnostic indicators. Yet, our Series Test of Cluster analysis reveals that only SOCS3 and PTGS2 exhibit a positive correlation with gout progression(Fig. 5 D). The diagnostic accuracy of SOCS3 and PTGS2, validated in clinical real data, reached 0.930 and 0.995, respectively (Fig. 8 A). These values are higher than the currently reported accuracy of inflammatory factors such as IL-1βand IL-6 [29]. Due to the broad expression of classical inflammatory factors in various immune and non-immune diseases, their lack of specificity makes it challenging to pinpoint a specific diagnosis. Therefore, we propose that SOCS3 and PTGS2 could serve as novel markers for the accurate diagnosis of gout. The ELISA method was utilized to analyze the expression levels of SOCS3 and PTGS2 in clinical samples(Fig. 8 C-D). The results indicated a significantly higher expression of these two genes in the IGG and AGG groups compared to the NC group. This suggests that SOCS3 and PTGS2 could potentially serve as key markers for differentiating gout patients from healthy individuals. Moreover, SOCS3 showed a statistical increasing trend (NC-IGG-AGG), indicating that further research of SOCS3 could provide valuable insights to distinguish between acute gout and intermittent gout.

SOCS3 belongs to the cytokine signal transduction inhibitor (SOCS) family and is the key negative feedback inhibitor of the JAK-STAT3 pathway. It directly inhibits JAK1, JAK2 and TYK2 by targeting the (glycine-glutamine-methionine) GQM motif, thus inhibiting

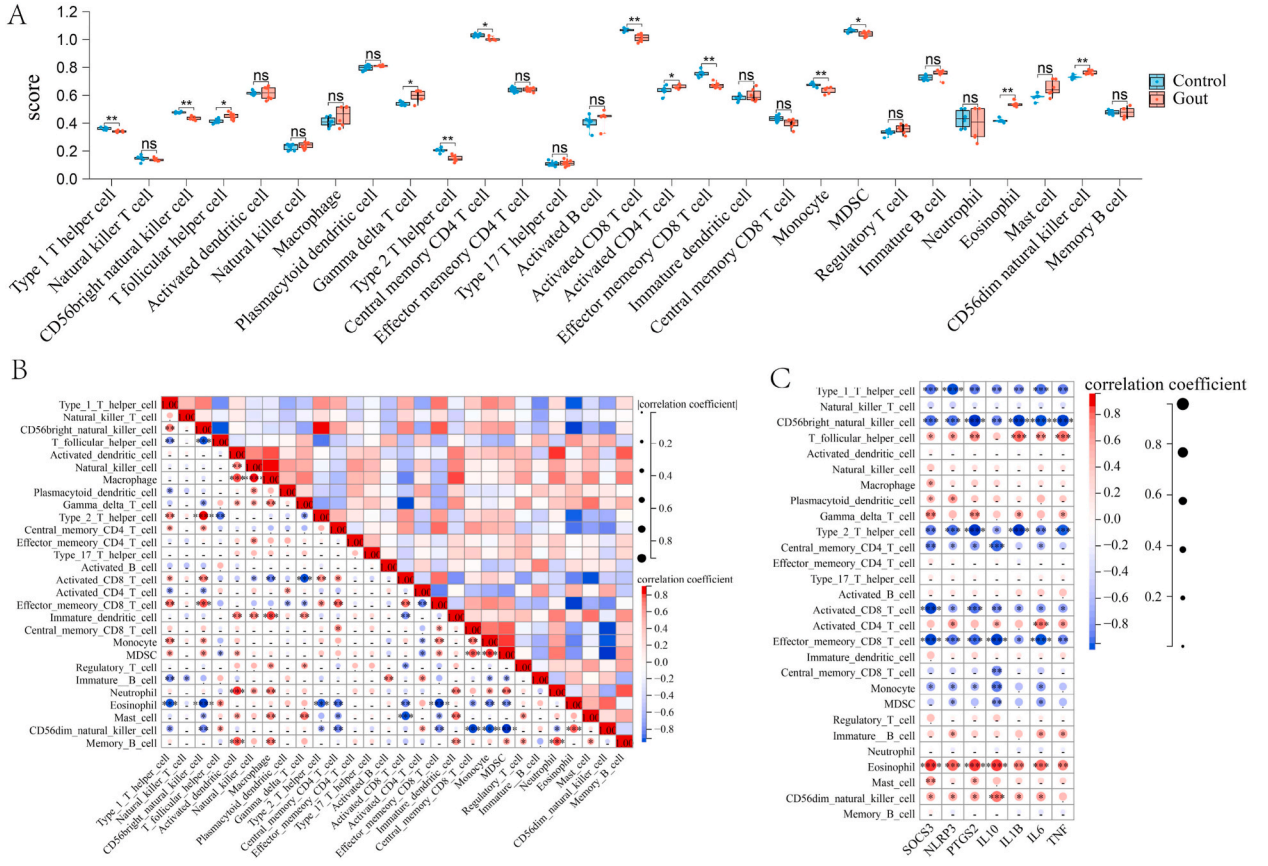

Fig. 7. Correlation between seven key genes and 28 immune cells. (**A**) Differential analysis of 28 types of immune cell infiltration between normal and disease samples. (**B**) Correlation heatmaps of 28 immune cells. (**C**) Correlation heatmap between key genes and 29 types of immune cells.

the signal transduction of the JAK-STAT3 pathway [30] [31]. Our study revealed that the expression of SOCS3 increased at different stages of gout. Many previous studies have shown that IL-6 is the primary inducer of JAK-STAT3 activation [32]. When exposed to harmful MSU stimulation, many inflammatory mediators (IL-1β, TNF-α, and prostaglandin E) induce the secretion of IL-6, and IL-6 phosphorylates STAT3 to promote inflammation; thus, the negative feedback regulator SOCS3 significantly increases to maintain the strict regulation of the JAK-STAT pathway [32] [33]. A recent study also confirmed that the expression of CIS and SOCS3, which are SOCS family members, is upregulated in MSU crystal-stimulated macrophages and that the expression of extracellular anti-inflammatory molecules is significantly increased in gout synovial fluid [34]. The results of this study demonstrated that the overexpression of CIS can hinder the MSU crystal-induced production of proinflammatory factors, such as IL-1β and TNFα. Additionally, it enhances the transcriptional activation of the STAT3 and TGFβ1 promoters, leading to an increase in the production of the anti-inflammatory factor TGFβ1 [34]. This increase in TGFβ1 production may be associated with the spontaneous regression of acute gouty arthritis. Moreover, the PS-MerTK axis plays a role in limiting gout inflammation. MerTK activation induces SOCS3, which subsequently suppresses TLR-induced cytokine release, inhibiting IL-6 signalling [35][36]. Additionally, SOCS3 inhibits G-CSF signal transduction in neutrophils. In a murine acute gouty arthritis model, SOCS3 deficiency in haematopoietic and endothelial cells led to more severe joint inflammation and more lymphoid tissue neutrophils due to the production of G-CSF and IL-6 [37,38]. IL-37 can inhibit the expression of NLRP3 and enhance the expression of IL-1R8 and SOCS3 to carry out anti-inflammatory signal transduction. These results suggest that SOCS3 can regulate the aggregation of neutrophils and monocytes in gout-related inflammation to some extent [39]. Furthermore, researchers have discovered that SOCS-3 plays a crucial role in suppressing CD4 T lymphocytes. Our analysis of immune cells also revealed a significant negative correlation between the expression of SOCS-3 and the infiltration of various types of T lymphocytes. Combined with the findings of other studies demonstrating the relationship between gout and uncontrolled growth of T lymphocytes, it is possible that SOCS3 regulates T-cell activity by modulating the systemic level and response of individuals to IL-6 and various cytokines, thereby reducing the acute inflammatory response to gout [40][41]. Moreover, a recent study highlighted the important role of SOCS3 in analgesia. The opening of the KATP channel can have an analgesic effect through the upregulation of the Gas6/Axl/SOCS3 signalling pathway, which also reduces alveolar inflammation in ischaemia–reperfusion-induced acute lung injury [42,43]. IL-6 can induce the chemokine CCL2, which directly influences pain regulation through the JAK/STAT3 pathway [44]. Based on our findings and previous research, we hypothesize that the upregulation of SOCS3 in gout patients suppresses the downstream

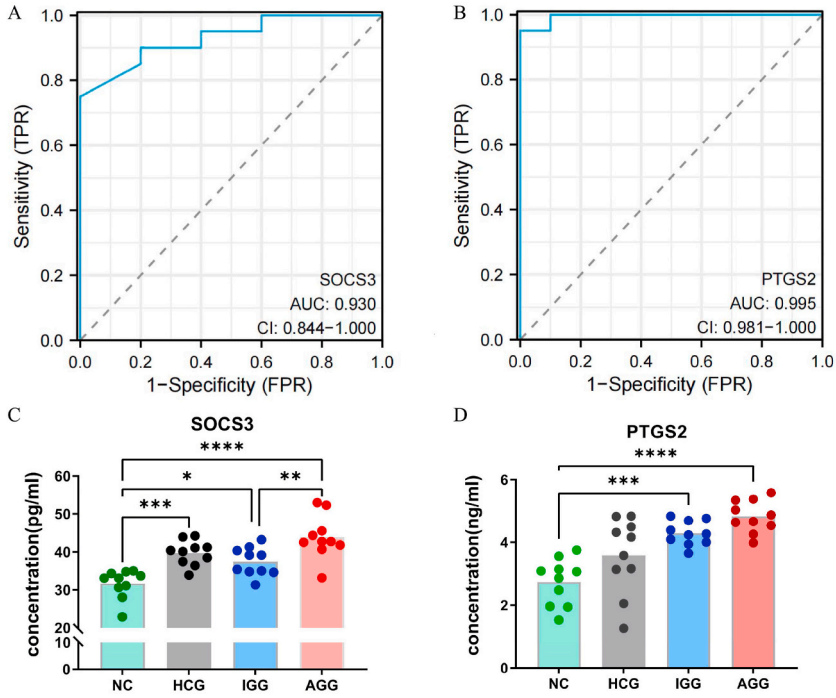

Fig. 8. Verification of the expression of SOCS3 and PTCS2 genes. *(A,B) ROC analysis shows the diagnostic value of SOCS3 and PTCS2. (C,D) Comparison of ROCS2 and PTGS2 expression between hyperuricemia, acute gout, intermittent gout and normal samples.* ***NC****, normal control;* ***HCG****, hyperuricemia group;* ***IGG****, intermittent gout group;* ***AGG****, acute gout attack group.*

signalling of the JAK2/STAT3 pathway, thereby blocking the direct impact of IL-6 and the chemokine CCL2 on pain [45][46]. This, to some extent, can lead to a reduction in inflammation and pain relief. Consequently, further investigations into SOCS3 as a potential diagnostic marker and therapeutic target for gout are warranted.

PTGS2, which encodes the COX-2 protein, is a key enzyme involved in the synthesis of prostaglandins (PGs) from arachidonic acid. COX is expressed on the lumen surface of the endoplasmic reticulum (ER) and the intima and adventitia of the nuclear envelope (NE). COX-1 can protect the PG to protect the gastric intima and normal renal function. COX-2 can induce the expression of proinflammatory cytokines and growth factors, which exist in the brain and spinal cord and participate in the nerve transmission of pain and fever, indicating that COX-2 plays an important role in the occurrence and development of inflammation and pain [47]. It has been reported that monosodium urate (MSU) crystals, the causative agent of gout, can specifically induce COX-2 overexpression in human monocytes [48]. In addition, COX-2 overexpression and prostaglandin production can also be detected in arthritis and inflammatory bowel disease [49]. In our study, the increase in PTGS2 expression in gout patients showed a trend similar to that described above, indicating that PTGS2 plays an important role in gout-related inflammation and pain and has the potential to become a diagnostic marker of gout. Phosphotyrosine-specific antibodies directed against COX2 phosphorylation sites can currently be produced, thus becoming a diagnostic tool capable of detecting COX-2 activity [49].

To further investigate the connection between SOCS3 and PTGS2 expression and pain in gout patients, we analyzed the expression of these genes in the dorsal root nerve and spinal cord using data from a gout mouse model. PTGS2 expression was significantly increased in a monosodium urate (MSU)-induced gout mouse model. SOCS3 expression exhibited a significant upwards trend, but this trend was not significant because of the small sample size. Previous studies have demonstrated that IL-1β hypersecretion caused by the NLPR3 inflammasome, PGE2, substance P and bradykinin plays important roles in gouty pain [50]. The synergistic effect of TRPV1 and TRPA1 has also been shown to be involved in pain processes, and MSU increases the expression of both receptors [50][51][52]. Our study confirmed that PTGS2 is closely related to the occurrence of pain in gout patients, and the SOCS3 gene still needs to be further studied to validate the findings.

The limitation of this study is the small sample size due to the limitations of race and geography. To address this limitation, we incorporated data from a gout mouse model for cross-analysis. Although the datasets come from different species with differences in gene transcription and physiology, it is important to note that more than 90 % of mouse and human genomes have conserved homologous regions. Approximately 80 % of mouse genes have a single identifiable orthologue in the human genome, and the expression profiles of these human and mouse orthologues are generally similar [53,54]. Previous studies have successfully used joint human–mouse cross-species analysis to identify pathogenic genes at lipid GWAS sites and discover new modifiers for the risk of schizophrenia [55][56][57]. Moving forward, validating the analysis results through the use of animal models (NHPs) that are more closely related to humans will be beneficial.

Currently, studies on the diagnostic markers SOCS3 and PTGS2 for gout are limited. We utilized bioinformatics and clinically

verified our samples to identify new and significant clinical diagnostic markers and potential treatment targets for gouty arthritis. This research is valuable for advancing our understanding of the disease pathogenesis and has the potential to simplify gout diagnosis.

5. Conclusion

As supported by comprehensive cross-species analysis and experimental verification, SOCS3 and PTGS2 could be used as new biomarkers for diagnosing gout and predicting disease progression, providing a new solution to the difficulties in the clinical diagnosis of gout. In the future, it is necessary to further explore the molecular mechanism of SOCS3 and PTGS2 to further confirm their role in the pathogenesis of gout.

Funding

This research was financially supported by "TCM scientific research project of Jiangxi Provincial Health and Family Planning Commission" (Grant Number: 2018B038).

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the First Affiliated Hospital of Nanchang University Medical Research Ethics Committee (No. Review [2023] No. CDYFYYLK (02–017)).

Informed consent statement

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data availability statement

The original data used in this project can be downloaded in the GEO (https://www.ncbi.nlm.nih.gov/geo/) website. Other data will be made available on request.

CRediT authorship contribution statement

Jie Peng: Writing – review & editing, Writing – original draft, Software, Methodology, Data curation. **Yawen Gu:** Writing – review & editing, Writing – original draft, Data curation. **Jiang Liu:** Validation, Resources, Methodology, Data curation. **Hao Yi:** Resources. **Dong Ruan:** Writing – original draft, Resources. **Haoyu Huang:** Writing – original draft, Resources, Project administration, Methodology. **Yuan Shu:** Visualization, Validation, Methodology. **Zhen Zong:** Software, Project administration, Funding acquisition, Data curation. **Rui Wu:** Writing – review & editing, Project administration. **Hui Li:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Data curation.

Declaration of competing interest

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

Acknowledgments

No applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30020.

References

[1] N. Dalbeth, A.L. Gosling, A. Gaffo, A. Abhishek, Gout, Lancet 397 (2021) 1843–1855.
[2] K. Rome, M. Frecklington, P. McNair, P. Gow, N. Dalbeth, Foot pain, impairment, and disability in patients with acute gout flares: a prospective observational study, Arthritis Care Res. 64 (2012) 384–388.
[3] J.A. Singh, I. Herbey, A. Bharat, J.E. Dinnella, S. Pullman-Mooar, S. Eisen, N. Ivankova, Gout self-management in african American veterans: a qualitative exploration of challenges and solutions from patients' perspectives, Arthritis Care Res. 69 (2017) 1724–1732.

[4] T. Neogi, T.L. Jansen, N. Dalbeth, J. Fransen, H.R. Schumacher, D. Berendsen, M. Brown, H. Choi, N.L. Edwards, H.J. Janssens, F. Liote, R.P. Naden, G. Nuki, A. Ogdie, F. Perez-Ruiz, K. Saag, J.A. Singh, J.S. Sundy, A.K. Tausche, J. Vazquez-Mellado, S.A. Yarows, W.J. Taylor, Gout classification criteria: an American college of rheumatology/European league against rheumatism collaborative initiative, Arthritis Rheumatol. 67 (2015) 2557–2568.
[5] M. Dehlin, L. Jacobsson, E. Roddy, Global epidemiology of gout: prevalence, incidence, treatment patterns and risk factors, Nat. Rev. Rheumatol. 16 (2020) 380–390.
[6] T. Neogi, Gout, Ann. Intern. Med. 165 (2016) ITC1–ITC16.
[7] L.A.B. Joosten, T.O. Crisan, P. Bjornstad, R.J. Johnson, Asymptomatic hyperuricaemia: a silent activator of the innate immune system, Nat. Rev. Rheumatol. 16 (2020) 75–86.
[8] W.Z. Zhang, Why does hyperuricemia not necessarily induce gout? Biomolecules 11 (2021).
[9] M.D. Harris, L.B. Siegel, J.A. Alloway, Gout and hyperuricemia, Am. Fam. Physician 59 (1999) 925–934.
[10] K. Fujita, K. Ichida, ABCG2 as a therapeutic target candidate for gout, Expert Opin. Ther. Targets 22 (2018) 123–129.
[11] F. Martinon, V. Petrilli, A. Mayor, A. Tardivel, J. Tschopp, Gout-associated uric acid crystals activate the NALP3 inflammasome, Nature 440 (2006) 237–241.
[12] F. Renaudin, L. Orliaguet, F. Castelli, F. Fenaille, A. Prignon, F. Alzaid, C. Combes, A. Delvaux, Y. Adimy, M. Cohen-Solal, P. Richette, T. Bardin, J.P. Riveline, N. Venteclef, F. Lioté, L. Campillo-Gimenez, H.K. Ea, Gout and pseudo-gout-related crystals promote GLUT1-mediated glycolysis that governs NLRP3 and interleukin-1β activation on macrophages, Ann. Rheum. Dis. 79 (2020) 1506–1514.
[13] L. de Almeida, S. Devi, M. Indramohan, Q.Q. Huang, R.A. Ratsimandresey, R.M. Pope, A. Dorfleutner, C. Stehlik, POP1 inhibits MSU-induced inflammasome activation and ameliorates gout, Front. Immunol. 13 (2022) 912069.
[14] C. Yin, B. Liu, Y. Li, X. Li, J. Wang, R. Chen, Y. Tai, Q. Shou, P. Wang, X. Shao, Y. Liang, H. Zhou, W. Mi, J. Fang, B. Liu, IL-33/ST2 induces neutrophil-dependent reactive oxygen species production and mediates gout pain, Theranostics 10 (2020) 12189–12203.
[15] L. Zhao, T. Zhao, X. Yang, L. Cao, R. Xu, J. Liu, C. Lin, Y. Yu, D. Xuan, X. Zhu, L. Liu, Y. Hua, C. Deng, W. Wan, H. Zou, Y. Xue, IL-37 blocks gouty inflammation by shaping macrophages into a non-inflammatory phagocytic phenotype, Rheumatology 61 (2022) 3841–3853.
[16] P. Richette, M. Doherty, E. Pascual, V. Barskova, F. Becce, J. Castaneda, M. Coyfish, S. Guillo, T. Jansen, H. Janssens, F. Lioté, C.D. Mallen, G. Nuki, F. Perez-Ruiz, J. Pimentao, L. Punzi, A. Pywell, A.K. So, A.K. Tausche, T. Uhlig, J. Zavada, W. Zhang, F. Tubach, T. Bardin, 2018 updated European League against Rheumatism evidence-based recommendations for the diagnosis of gout, Ann. Rheum. Dis. 79 (2020) 31–38.
[17] G. Yu, L. Wang, Y. Han, Q. He, clusterProfiler: an R package for comparing biological themes among gene clusters, OMICS A J. Integr. Biol. 16 (2012) 284–287.
[18] M. Kanehisa, S. Goto, KEGG: kyoto encyclopedia of genes and genomes, Nucleic Acids Res. 28 (2000) 27–30.
[19] J. Ernst, Z. Bar-Joseph, STEM: a tool for the analysis of short time series gene expression data, BMC Bioinf. 7 (2006) 191.
[20] R. Cao, I. López-de-Ullibarri, ROC curves for the statistical analysis of microarray data, Methods Mol. Biol. 1986 (2019) 245–253.
[21] A. Subramanian, P. Tamayo, V. Mootha, S. Mukherjee, B. Ebert, M. Gillette, A. Paulovich, S. Pomeroy, T. Golub, E. Lander, J. Mesirov, Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 15545–15550.
[22] G. Bindea, B. Mlecnik, M. Tosolini, A. Kirilovsky, M. Waldner, A. Obenauf, H. Angell, T. Fredriksen, L. Lafontaine, A. Berger, P. Bruneval, W. Fridman, C. Becker, F. Pagès, M. Speicher, Z. Trajanoski, J. Galon, Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer, Immunity 39 (2013) 782–795.
[23] X. Hui, Z. Li, L. Tang, J. Sun, X. Hou, J. Chen, Y. Peng, Z. Wu, H. Guo, A self-powered, highly embedded and sensitive tribo-label-sensor for the fast and stable label printer, Nano-Micro Lett. 15 (2022) 27.
[24] K. Qiu, T. Zeng, Y. Liao, J. Min, N. Zhang, M. Peng, W. Kong, L. Chen, Identification of inflammation-related biomarker pro-ADM for male patients with gout by comprehensive analysis, Front. Immunol. 12 (2021) 798719.
[25] S. Luo, C. Chin, L. Ho, W. Tseng, C. Kuo, J. Lai, Monosodium urate crystals induced ICAM-1 expression and cell-cell adhesion in renal mesangial cells: implications for the pathogenesis of gouty nephropathy, Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi 53 (2020) 23–32.
[26] A.K. So, F. Martinon, Inflammation in gout: mechanisms and therapeutic targets, Nat. Rev. Rheumatol. 13 (2017) 639–647.
[27] T. Salvatore, P.C. Pafundi, R. Galiero, K. Gjeloshi, F. Masini, C. Acierno, A. Di Martino, G. Albanese, M. Alfano, L. Rinaldi, F.C. Sasso, Metformin: a potential therapeutic tool for rheumatologists, Pharmaceuticals 13 (2020) 234.
[28] T. Wang, C. He, TNF-A and IL-6: the link between immune and bone system, Curr. Drug Targets 21 (2020) 213–227.
[29] H. Yu, W. Xue, H. Yu, H. Gu, L. Qin, A. Peng, Joint application of multiple inflammatory cytokines in diagnosis of gout flare, J. Inflamm. Res. 16 (2023) 1771–1782.
[30] J.J. Babon, N.J. Kershaw, J.M. Murphy, L.N. Varghese, A. Laktyushin, S.N. Young, I.S. Lucet, R.S. Norton, N.A. Nicola, Suppression of cytokine signaling by SOCS3: characterization of the mode of inhibition and the basis of its specificity, Immunity 36 (2012) 239–250.
[31] E. Dominguez, C. Rivat, B. Pommier, A. Mauborgne, M. Pohl, JAK/STAT3 pathway is activated in spinal cord microglia after peripheral nerve injury and contributes to neuropathic pain development in rat, J. Neurochem. 107 (2008) 50–60.
[32] D.E. Johnson, R.A. O'Keefe, J.R. Grandis, Targeting the IL-6/JAK/STAT3 signalling axis in cancer, Nat. Rev. Clin. Oncol. 15 (2018) 234–248.
[33] M. Suganuma, S. Okabe, M. Kurusu, N. Iida, S. Ohshima, Y. Saeki, T. Kishimoto, H. Fujiki, Discrete roles of cytokines, TNF-alpha, IL-1, IL-6 in tumor promotion and cell transformation, Int. J. Oncol. 20 (2002) 131–136.
[34] Y.H. Chen, S.C. Hsieh, W.Y. Chen, K.J. Li, C.H. Wu, P.C. Wu, C.Y. Tsai, C.L. Yu, Spontaneous resolution of acute gouty arthritis is associated with rapid induction of the anti-inflammatory factors TGFβ1, IL-10 and soluble TNF receptors and the intracellular cytokine negative regulators CIS and SOCS3, Ann. Rheum. Dis. 70 (2011) 1655–1663.
[35] A. Cumpelik, B. Ankli, D. Zecher, J.A. Schifferli, Neutrophil microvesicles resolve gout by inhibiting C5a-mediated priming of the inflammasome, Ann. Rheum. Dis. 75 (2016) 1236–1245.
[36] B.A. Croker, H. Kiu, S.E. Nicholson, SOCS regulation of the JAK/STAT signalling pathway, Semin. Cell Dev. Biol. 19 (2008) 414–422.
[37] B.A. Croker, D. Metcalf, L. Robb, W. Wei, S. Mifsud, L. DiRago, L.A. Cluse, K.D. Sutherland, L. Hartley, E. Williams, J.G. Zhang, D.J. Hilton, N.A. Nicola, W. S. Alexander, A.W. Roberts, SOCS3 is a critical physiological negative regulator of G-CSF signaling and emergency granulopoiesis, Immunity 20 (2004) 153–165.
[38] A. Kimura, I. Kinjyo, Y. Matsumura, H. Mori, R. Mashima, M. Harada, K.R. Chien, H. Yasukawa, A. Yoshimura, SOCS3 is a physiological negative regulator for granulopoiesis and granulocyte colony-stimulating factor receptor signaling, J. Biol. Chem. 279 (2004) 6905–6910.
[39] L. Liu, Y. Xue, Y. Zhu, D. Xuan, X. Yang, M. Liang, J. Wang, X. Zhu, J. Zhang, H. Zou, Interleukin 37 limits monosodium urate crystal-induced innate immune responses in human and murine models of gout, Arthritis Res. Ther. 18 (2016) 268.
[40] P.K. Wong, P.J. Egan, B.A. Croker, K. O'Donnell, N.A. Sims, S. Drake, H. Kiu, E.J. McManus, W.S. Alexander, A.W. Roberts, I.P. Wicks, SOCS-3 negatively regulates innate and adaptive immune mechanisms in acute IL-1-dependent inflammatory arthritis, J. Clin. Invest. 116 (2006) 1571–1581.
[41] Y. Huang, X. Wu, L. Gui, Y. Jiang, L. Tu, X. Li, B. Jiang, Y. Wang, X. Zheng, Q. Wei, Q. Li, J. Ou, Z. Chen, Y. Xie, Z. Lin, Z. Liao, L. Fang, M. Qiu, S. Cao, J. Gu, Age-specific imbalance of circulating tfh cell subsets and its association with gout-targeted kidney impairment, Front. Immunol. 11 (2020) 625458.
[42] C. Qian, Y. Fan, L. Zong, C. Miao, L.L. Ji, L. Wan, R. Jia, X. Qin, Y. Wang, Q. Wu, X.Y. Tao, L. Hao, L. Hu, W.T. Liu, Opening K(ATP) channels induces inflammatory tolerance and prevents chronic pain, Brain Behav. Immun. 107 (2023) 76–86.
[43] C.K. Peng, C.P. Wu, J.Y. Lin, S.C. Peng, C.H. Lee, K.L. Huang, C.H. Shen, Gas6/Axl signaling attenuates alveolar inflammation in ischemia-reperfusion-induced acute lung injury by up-regulating SOCS3-mediated pathway, PLoS One 14 (2019) e0219788.
[44] E. Dominguez, A. Mauborgne, J. Mallet, M. Desclaux, M. Pohl, SOCS3-mediated blockade of JAK/STAT3 signaling pathway reveals its major contribution to spinal cord neuroinflammation and mechanical allodynia after peripheral nerve injury, J. Neurosci. 30 (2010) 5754–5766.
[45] N. Meng, N.N. Ji, Z. Zhou, Y. Qian, Y. Tang, K. Yang, B. Chen, Y.M. Zhang, The role of SOCS3 in the hypothalamic paraventricular nucleus in rat model of inflammatory pain, J. Inflamm. 17 (2020) 12.
[46] X.M. Wang, M. Hamza, T.X. Wu, R.A. Dionne, Upregulation of IL-6, IL-8 and CCL2 gene expression after acute inflammation: correlation to clinical pain, Pain 142 (2009) 275–283.

[47] J.R. Vane, Y.S. Bakhle, R.M. Botting, Cyclooxygenases 1 and 2, Annu. Rev. Pharmacol. Toxicol. 38 (1998) 97–120.
[48] M. Pouliot, M.J. James, S.R. McColl, P.H. Naccache, L.G. Cleland, Monosodium urate microcrystals induce cyclooxygenase-2 in human monocytes, Blood 91 (1998) 1769–1776.
[49] A. Alexanian, A. Sorokin, Cyclooxygenase 2: protein-protein interactions and posttranslational modifications, Physiol. Genom. 49 (2017) 667–681.
[50] R. Ramonda, F. Oliviero, P. Galozzi, P. Frallonardo, M. Lorenzin, A. Ortolan, A. Scanu, L. Punzi, Molecular mechanisms of pain in crystal-induced arthritis, Best Pract. Res. Clin. Rheumatol. 29 (2015) 98–110.
[51] C. Hoffmeister, M.A. Silva, M.F. Rossato, G. Trevisan, S.M. Oliveira, G.P. Guerra, C.R. Silva, J. Ferreira, Participation of the TRPV1 receptor in the development of acute gout attacks, Rheumatology 53 (2014) 240–249.
[52] G. Trevisan, C. Hoffmeister, M.F. Rossato, S.M. Oliveira, M.A. Silva, R.P. Ineu, G.P. Guerra, S. Materazzi, C. Fusi, R. Nassini, P. Geppetti, J. Ferreira, Transient receptor potential ankyrin 1 receptor stimulation by hydrogen peroxide is critical to trigger pain during monosodium urate-induced inflammation in rodents, Arthritis Rheum. 65 (2013) 2984–2995.
[53] R.H. Waterston, K. Lindblad-Toh, E. Birney, J. Rogers, J.F. Abril, P. Agarwal, R. Agarwala, R. Ainscough, M. Alexandersson, P. An, S.E. Antonarakis, J. Attwood, R. Baertsch, J. Bailey, K. Barlow, S. Beck, E. Berry, B. Birren, T. Bloom, P. Bork, M. Botcherby, N. Bray, M.R. Brent, D.G. Brown, S.D. Brown, C. Bult, J. Burton, J. Butler, R.D. Campbell, P. Carninci, S. Cawley, F. Chiaromonte, A.T. Chinwalla, D.M. Church, M. Clamp, C. Clee, F.S. Collins, L.L. Cook, R.R. Copley, A. Coulson, O. Couronne, J. Cuff, V. Curwen, T. Cutts, M. Daly, R. David, J. Davies, K.D. Delehaunty, J. Deri, E.T. Dermitzakis, C. Dewey, N.J. Dickens, M. Diekhans, S. Dodge, I. Dubchak, D.M. Dunn, S.R. Eddy, L. Elnitski, R.D. Emes, P. Eswara, E. Eyras, A. Felsenfeld, G.A. Fewell, P. Flicek, K. Foley, W. N. Frankel, L.A. Fulton, R.S. Fulton, T.S. Furey, D. Gage, R.A. Gibbs, G. Glusman, S. Gnerre, N. Goldman, L. Goodstadt, D. Grafham, T.A. Graves, E.D. Green, S. Gregory, R. Guigó, M. Guyer, R.C. Hardison, D. Haussler, Y. Hayashizaki, L.W. Hillier, A. Hinrichs, W. Hlavina, T. Holzer, F. Hsu, A. Hua, T. Hubbard, A. Hunt, I. Jackson, D.B. Jaffe, L.S. Johnson, M. Jones, T.A. Jones, A. Joy, M. Kamal, E.K. Karlsson, et al., Initial sequencing and comparative analysis of the mouse genome, Nature 420 (2002) 520–562.
[54] B.Y. Liao, J. Zhang, Evolutionary conservation of expression profiles between human and mouse orthologous genes, Mol. Biol. Evol. 23 (2006) 530–540.
[55] J.A. Votava, B.W. Parks, Cross-species data integration to prioritize causal genes in lipid metabolism, Curr. Opin. Lipidol. 32 (2021) 141–146.
[56] Z. Li, J.A. Votava, G.J.M. Zajac, J.N. Nguyen, F.B. Leyva Jaimes, S.M. Ly, J.A. Brinkman, M. De Giorgi, S. Kaul, C.L. Green, S.L. St Clair, S.L. Belisle, J.M. Rios, D. W. Nelson, M.G. Sorci-Thomas, W.R. Lagor, D.W. Lamming, C.L. Eric Yen, B.W. Parks, Integrating mouse and human genetic data to move beyond GWAS and identify causal genes in cholesterol metabolism, Cell Metabol. 31 (2020) 741–754.e5.
[57] D.G. Ashbrook, S. Cahill, R. Hager, A cross-species systems genetics analysis links APBB1IP as a candidate for schizophrenia and prepulse inhibition, Front. Behav. Neurosci. 13 (2019) 266.