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ORIGINAL RESEARCH

The Role of Pyroptosis-Related Gene Signature and Immune Infiltration in Juvenile Dermatomyositis

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Objective: Juvenile dermatomyositis (JDM) is a complex autoimmune disease, and its pathogenesis remains poorly understood. Building upon previous research on the immunological and inflammatory aspects of JDM, this study aims to investigate the role of pyroptosis in the pathogenesis of JDM using a comprehensive bioinformatics approach.

Methods: Two microarray datasets (GSE3307 and GSE11971) were obtained from the Gene Expression Omnibus database, and a list of 62 pyroptosis-related genes was compiled. Differential gene expression analysis and machine learning analysis were performed to identity the hub pyroptosis-related differentially expressed genes (PR-DEGs). Functional enrichment analysis, immune cell infiltration analysis, gene set variation analysis (GSVA), and gene set enrichment analysis (GSEA) were performed to elucidate the potential roles of PR-DEGs in JDM pathogenesis.

Results: A total of 2526 common DEGs were identified, among which 12 PR-DEGs were identified, with CASP1, IRF1, NOD2, and PYCARD identified as hub PR-DEGs. These genes were involved in cytokine production, inflammasome activity, necroptosis, NOD-like receptor signaling, and TNF signaling. Immune infiltration analysis showed increased pro-inflammatory immune cell infiltration in JDM patients, with PR-DEGs positively correlated with various immune cell types. GSVA and GSEA analyses demonstrated the involvement of PR-DEGs in multiple inflammation and immunity-related pathways, with the NOD-like receptor signaling pathway playing a central role.

Conclusion: This study highlights the crucial role of pyroptosis in the pathogenesis of JDM, with the identified PR-DEGs potentially contributing to disease development and progression by regulating key inflammatory and immune-related pathways.

Keywords: pyroptosis, JDM, immune infiltration analysis, machine learning, diagnostic

Introduction

Juvenile dermatomyositis (JDM) is a severe autoimmune disorder characterized by chronic inflammation of the skin and muscles, resulting in characteristic skin rashes and progressive muscle weakness in children.[1] Previous studies have provided valuable insights, indicating that dysregulated immune responses and aberrant inflammatory processes are pivotal in the development and progression of JDM.[2,3] In the early stages, cytokines and interferons drive an inflammatory process leading to small vessel vasculitis.[4] In later stages, non-inflammatory mechanisms, including luminal occlusion and capillary dropout, contribute to vessel destruction and surrounding tissue necrosis.[4] Muscle biopsy reveals perivascular mononuclear cell infiltration, myofiber necrosis, and perifascicular myocyte atrophy,[5] with infiltrating inflammatory cells mainly consisting of B cells, $CD4^+$ helper T cells, and macrophages.[6] However, the exact molecular

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mechanisms underlying these pathological changes in JDM remain elusive, highlighting the need for a better understanding of the disease pathogenesis.

Pyroptosis, a novel form of inflammatory programmed cell death, has recently emerged as a key player in the pathogenesis of various autoimmune diseases.[7] This distinct form of programmed cell death is characterized by the formation of membrane pores, a process that is mediated by the N-terminal domains of the gasdermin protein family. Activated caspases frequently cleave these gasdermin proteins, leading to cell swelling, rupture, and the release of inflammatory mediators, such as IL-1β, IL-18, and cytoplasmic contents.[8,9] The primary pathways involved in pyroptosis include the caspase-1-mediated pathway, the caspase-4/5/11-mediated pathway, the caspase-3-mediated pathway, and the caspase-independent pathway.[10] Classical inflammasomes, including NLRP3, AIM2, and P2X7-NLRP3, serve as essential switches for pyroptosis by activating caspases and play critical roles in pyroptosis associated with autoimmune diseases.[11] The excessive activation of pyroptosis has been implicated in the pathogenesis of autoimmune diseases by disrupting immune system homeostasis and promoting autoimmunity through the sustained release of abundant inflammatory factors.[12] Pyroptosis has been demonstrated to play significant roles in various autoimmune disorders, such as systemic lupus erythematosus, rheumatoid arthritis, and dermatomyositis.[12]

In this study, we aimed to investigate the role of pyroptosis in JDM using a comprehensive bioinformatics approach. By analyzing gene expression profiles from JDM patients and healthy controls, we sought to identify pyroptosis-related differentially expressed genes (PR-DEGs) and elucidate their potential roles in modulating inflammatory and immune responses in JDM.

Methods

Data Acquisition

Two microarray datasets, GSE3307[13] and GSE11971,[14] were retrieved from the Gene Expression Omnibus (GEO) database, both sequenced using the GPL96 platform. The GSE3307 dataset consisted of 16 control and 21 disease samples, while GSE11971 comprised 4 control and 19 disease samples. Furthermore, a list of 62 pyroptosis-related genes (PRGs) was compiled from the Gene Ontology (GO) term "c5.go.symbols.gmt", the Reactome term "c2.cp.reactome.v2022.1.Hs.symbols.gmt" from the Molecular Signatures Database (MSigDB), and a previous literature report.[15]

Differential Gene Expression Analysis

Background correction, normalization, and batch effect removal were performed using the "limma" package in R. Differentially expressed genes (DEGs) were identified based on an adjusted p-value < 0.05 and $|\log2 \text{ fold change}| > 0$. The results were visualized using volcano plots and heatmaps. The common DEGs from both datasets were intersected with the PRGs to identify PR-DEGs, which were illustrated using Venn diagrams.

Functional Enrichment Analysis

GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted on DEGs and PR-DEGs using the "clusterProfiler" package in R, with an adjusted p-value < 0.05. The results were visualized using bubble plots and chord diagrams.

Expression Validation and Correlation Analysis

The expression levels of PR-DEGs were examined in both datasets, and statistical differences were calculated using the Wilcoxon rank-sum test. To facilitate further analysis, the datasets were merged using the "sva" package. Spearman correlation analysis was performed to evaluate the correlations among PR-DEGs in the JDM group.

Machine Learning Analysis

Three machine learning approaches were employed to identify the most important PR-DEGs. Lasso regression analysis was performed using the "glmnet" package in R, with the optimal regularization parameter determined by cross-validation. Genes with non-zero coefficients were considered important features. Support Vector Machine-Recursive Feature Elimination (SVM-RFE) analysis was conducted using the "e1071" package, while random forest analysis was

carried out using the "randomForest" package. The intersection of important features from the three machine learning methods was identified as the hub PR-DEGs and visualized using a Venn diagram.

Immune Cell Infiltration Analysis

The single-sample gene set enrichment analysis (ssGSEA) method in the "GSVA" package was utilized to assess immune cell infiltration levels. Boxplots were generated using the "ggpubr" package to display differences in ssGSEA scores between groups, which were evaluated using the Wilcoxon rank-sum test. Spearman correlation test was used to calculate the correlation coefficients and p-values between gene expression and ssGSEA scores of each immune cell type, as well as between different immune cell types in the JDM group. The results were visualized using correlation heatmaps.

Gene Set Variation Analysis (GSVA)

GSVA was performed using Hallmark gene sets from the MSigDB to assess pathway activity variation in the JDM group. The "GSVA" package was employed to calculate enrichment scores for each sample. Samples were then divided into high and low expression groups based on the median expression of the gene of interest. Enrichment scores for these groups were compared using *t*-tests, and bar plots of the t-values were generated using the "ggpubr" package.

Gene Set Enrichment Analysis (GSEA)

GSEA was performed using KEGG gene sets from the MSigDB to identify pathways significantly enriched with the PR-DEGs. Samples were divided into high and low expression groups based on the median expression of the gene of interest. Differential expression between these groups was calculated, producing a ranked list of log2 fold changes. The "clusterProfiler" package was then used to perform GSEA on the ranked gene list, identifying significantly enriched pathways with a p-value < 0.05.

Results

Identification of DEGs

Batch effect removal was performed on the GSE3307 and GSE11971 datasets (Figure 1A–D). In the GSE3307 dataset, a total of 6,132 DEGs were identified, consisting of 2,647 upregulated and 3,485 downregulated genes (Figure 1E and F). Likewise, the GSE11971 dataset revealed 4,156 DEGs, with 2,013 upregulated and 2,143 downregulated genes (Figure 1G and H). The substantial number of DEGs in both datasets underscores the profound alterations in gene expression patterns associated with the disease state.

Functional Enrichment Analysis of DEGs

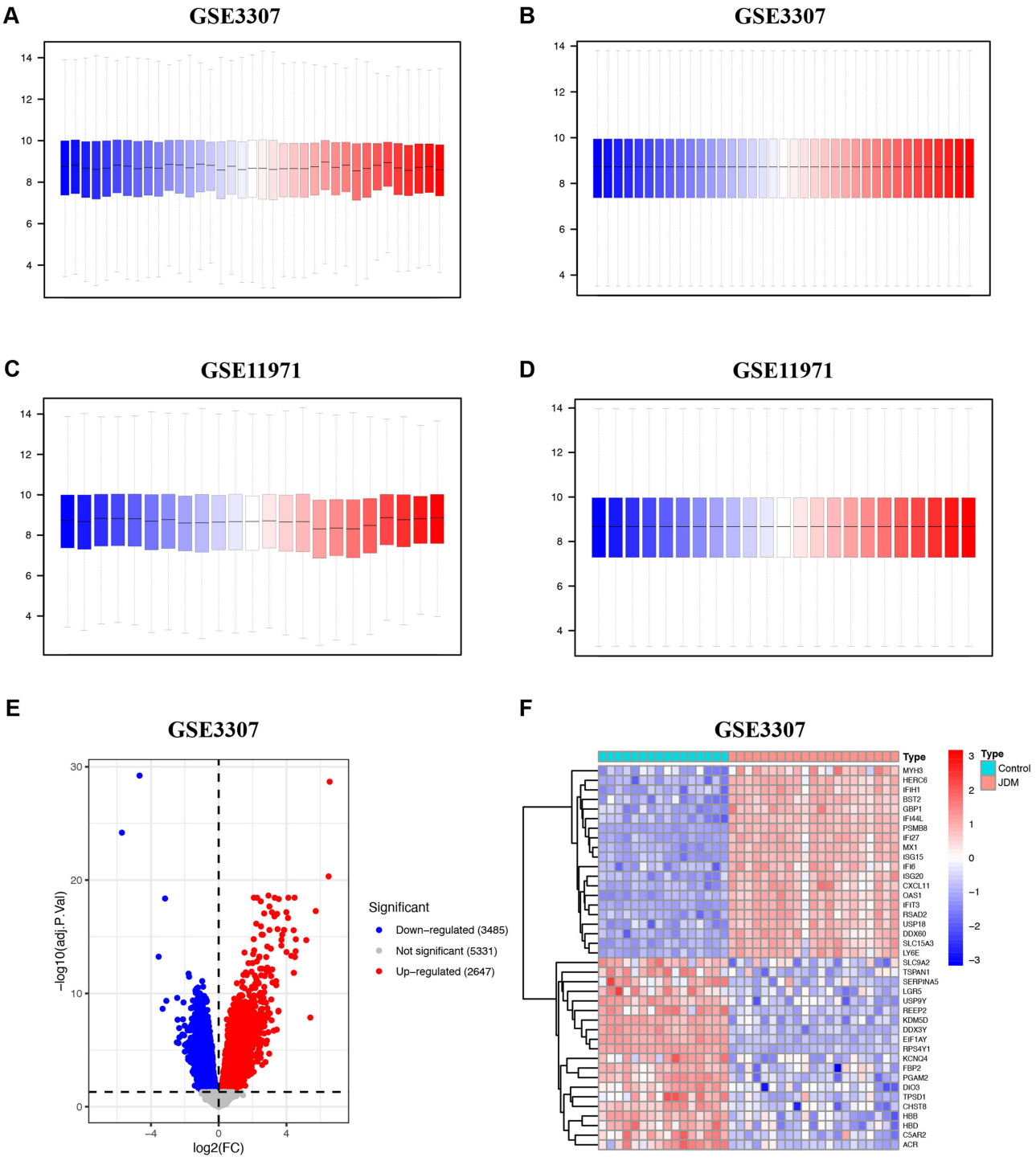
The intersection of DEGs from both datasets yielded 2,526 common DEGs (Figure 2A). GO analysis of common DEGs revealed enrichment in immune response, viral infection, and regulation of viral processes (Figure 2B). KEGG of common DEGs analysis highlighted the involvement of pathways such as diabetic cardiomyopathy, chemical carcinogenesis, viral infections, lipid and atherosclerosis, non-alcoholic fatty liver disease, NOD-like receptor signaling, oxidative phosphorylation, natural killer (NK) cell-mediated cytotoxicity, and antigen processing and presentation (Figure 2C), implicating these DEGs in the pathogenesis of JDM.

Identification and Functional Analysis of PR-DEGs

The intersection of common DEGs and pyroptosis-related genes yielded 12 PR-DEGs (Figure 3A). Among these PR-DEGs, BAK1, PYCARD, CASP1, CASP8, NOD2, IRF1, IL18, AIM2, and HMGB1 were upregulated, while APIP, CHMP2B, and CHMP6 were downregulated in JDM patients compared to controls (Figure 3B and C). To further elucidate the functional implications of these PR-DEGs, GO and KEGG analyses were performed. The results revealed that the PR-DEGs were involved in various biological processes and pathways, including cytokine production, inflammasome activity, necroptosis, NOD-like receptor signaling, and tumor necrosis factor (TNF) signaling (Figure 4A–C).

These findings suggest that the identified PR-DEGs play crucial roles in the regulation of inflammatory responses and cell death mechanisms in JDM pathogenesis.

To minimize the potential impact of batch effects on the analysis, the datasets were merged, and batch effect removal was performed (Figure 4D and E). Spearman correlation analysis was then conducted to investigate the relationships among the PR-DEGs in the JDM group. The results showed positive correlations among BAK1, PYCARD, CASP1, CASP8, NOD2, IRF1, IL18, AIM2, and HMGB1, indicating their coordinated expression and potential functional



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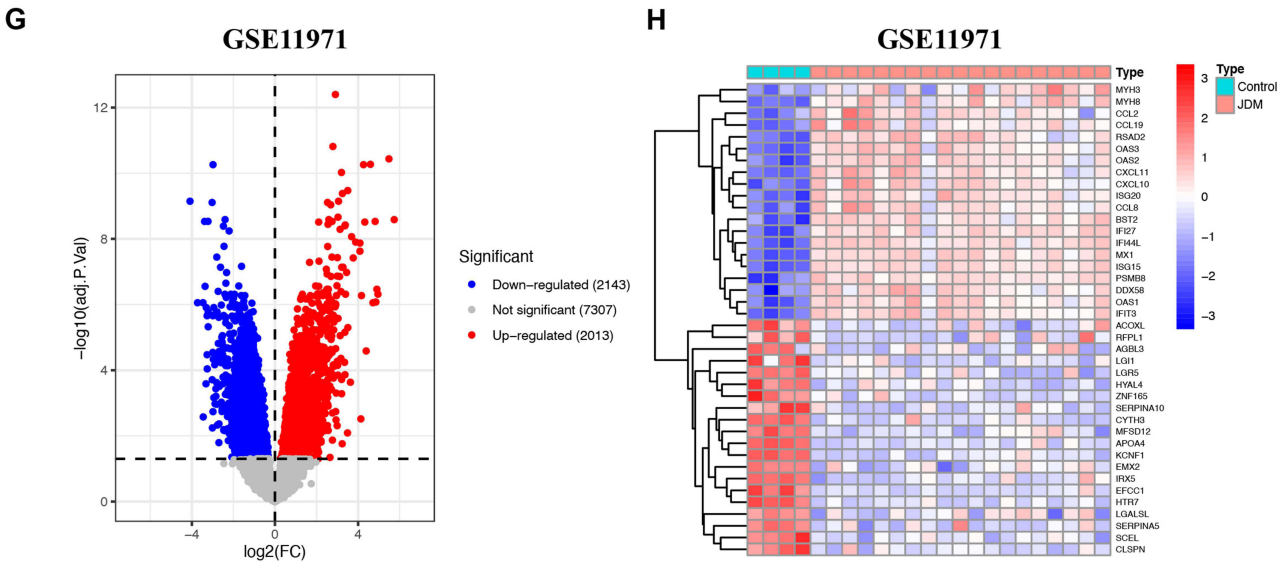

Figure 1 Identification of differentially expressed genes (DEGs) in juvenile dermatomyositis (JDM) patients. (**A** and **B**) Box plots of normalized expression data in the GSE3307 dataset before (**A**) and after (**B**) batch effect removal, respectively. (**C** and **D**) Box plots of normalized expression data in the GSE11971 dataset before (**C**) and after (**D**) batch effect removal, respectively. (**E**) Volcano plot of DEGs in the GSE3307 dataset. (**F**) Heatmap of DEGs in the GSE3307 dataset. (**G**) Volcano plot of DEGs in the GSE11971 dataset. (**H**) Heatmap of DEGs in the GSE11971 dataset.

interactions in JDM. In contrast, APIP, CHMP2B, and CHMP6 exhibited negative correlations with the other PR-DEGs, suggesting their potential antagonistic roles in the pyroptosis-related processes (Figure 4F).

Machine Learning Analysis

To further identify the most important PR-DEGs in JDM, three machine learning approaches were employed. Lasso regression analysis was performed, which identified 9 important genes (Figure 5A). SVM-RFE was conducted, revealing 4 important genes (Figure 5B). Lastly, random forest analysis was carried out, which identified 10 important genes (Figure 5C). Notably, CASP1, IRF1, NOD2, and PYCARD were consistently identified as hub PR-DEGs by all three machine learning methods (Figure 5D).

GSVA

GSVA analysis revealed that CASP1 (Figure 6A), PYCARD (Figure 6B), NOD2 (Figure 6C), and IRF1 (Figure 6D) exhibit distinct expression patterns across various Hallmark pathways in JDM. In the high expression groups of CASP1, PYCARD, and NOD2 several pathways were commonly upregulated, including spermatogenesis, KRAS signaling (downregulated), Hedgehog signaling, and Notch signaling. However, in the high expression groups of all four genes (PYCARD, NOD2, IRF1, and CASP1), multiple inflammation and immune-related pathways were consistently down-regulated, such as allograft rejection, interferon-alpha response, interferon-gamma response, inflammatory response, TNF-alpha signaling via NF-κB, IL-6/JAK/STAT3 signaling, complement, and IL-2/STAT5 signaling. These findings indicate that these pathways may serve as common mechanisms through which PR-DEGs regulate JDM progression.

GSEA and Visualization of the NOD-Like Receptor Signaling Pathway

GSEA further investigated the potential mechanisms of PR-DEGs in JDM. The high expression groups of CASP1 (Figure 7A), PYCARD (Figure 7B), NOD2 (Figure 7C), and IRF1 (Figure 7D), were significantly enriched in multiple inflammation and immunity-related KEGG pathways. Notably, the NOD-like receptor signaling pathway was enriched across all four PR-DEGs high expression groups, highlighting its central role in pyroptosis-mediated JDM progression. The co-enrichment of chemokine signaling pathways, cytokine-cytokine receptor interactions, and Leishmania infection across multiple PR-DEGs high expression groups indicates their involvement in shared regulatory mechanisms in JDM

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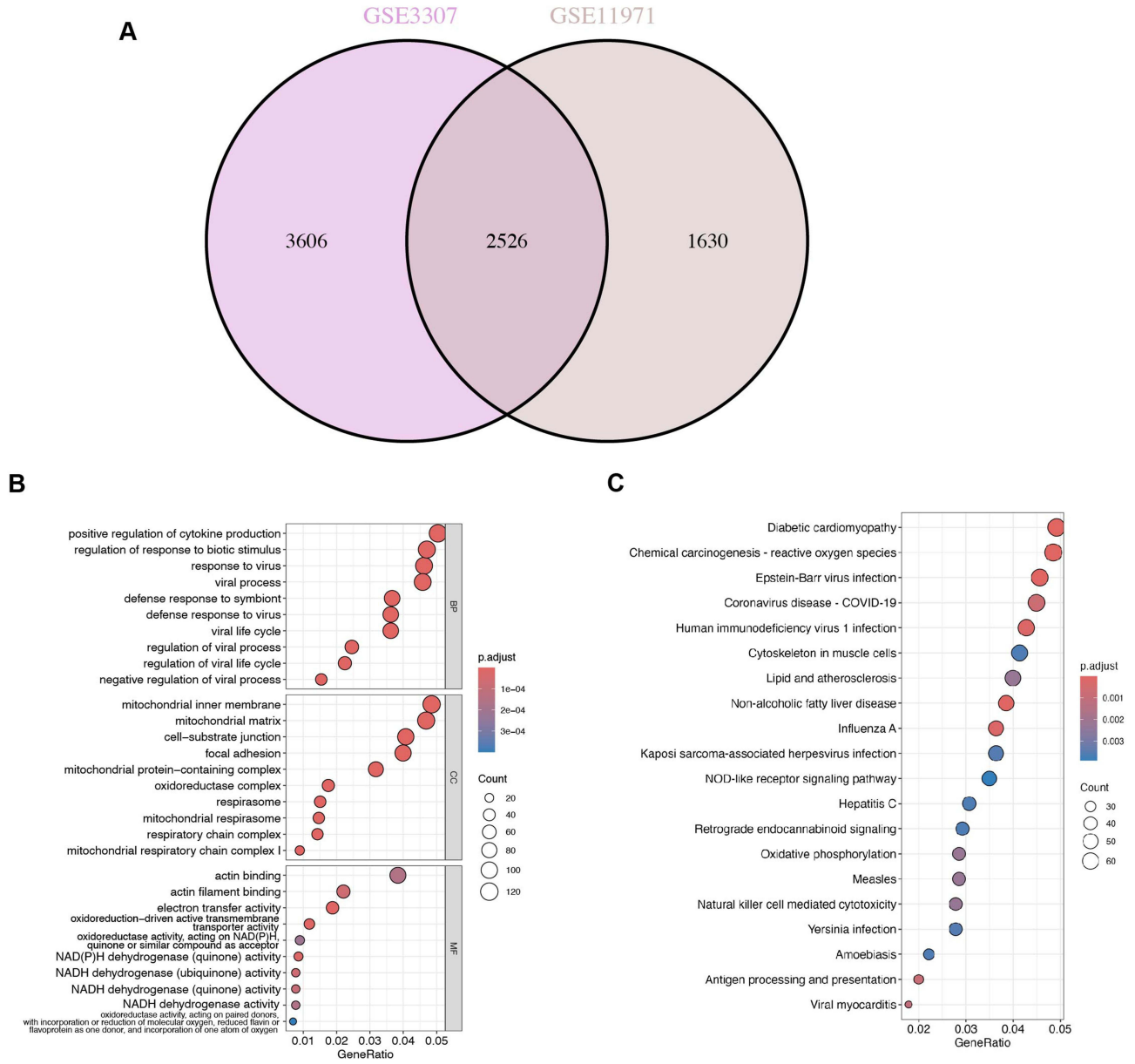

Figure 2 Functional enrichment analysis of common DEGs across datasets. (**A**) Venn diagram showing the intersection of DEGs from GSE3307 and GSE11971 datasets. (**B**) Gene Ontology (GO) analysis of common DEGs. (**C**) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of common DEGs. GO terms and KEGG pathways are ranked by gene ratio from high to low.

pathogenesis. Moreover, the enrichment of cell adhesion molecules and hematopoietic cell lineage in PYCARD and CASP1 high expression groups further emphasizes their roles in modulating immune cell recruitment and differentiation in JDM.

To better illustrate the involvement of the hub PR-DEGs in the NOD-like receptor signaling pathway, we performed a KEGG pathway visualization with the expression of CASP1, PYCARD, NOD2, and IRF1 (Figure 7E). The schematic diagram clearly shows the significant upregulation of CASP1, PYCARD, and NOD2 in JDM patients compared to healthy controls, suggesting their potential impact on the activation of the NOD-like receptor signaling cascade and the subsequent inflammatory and autoimmune responses in JDM.

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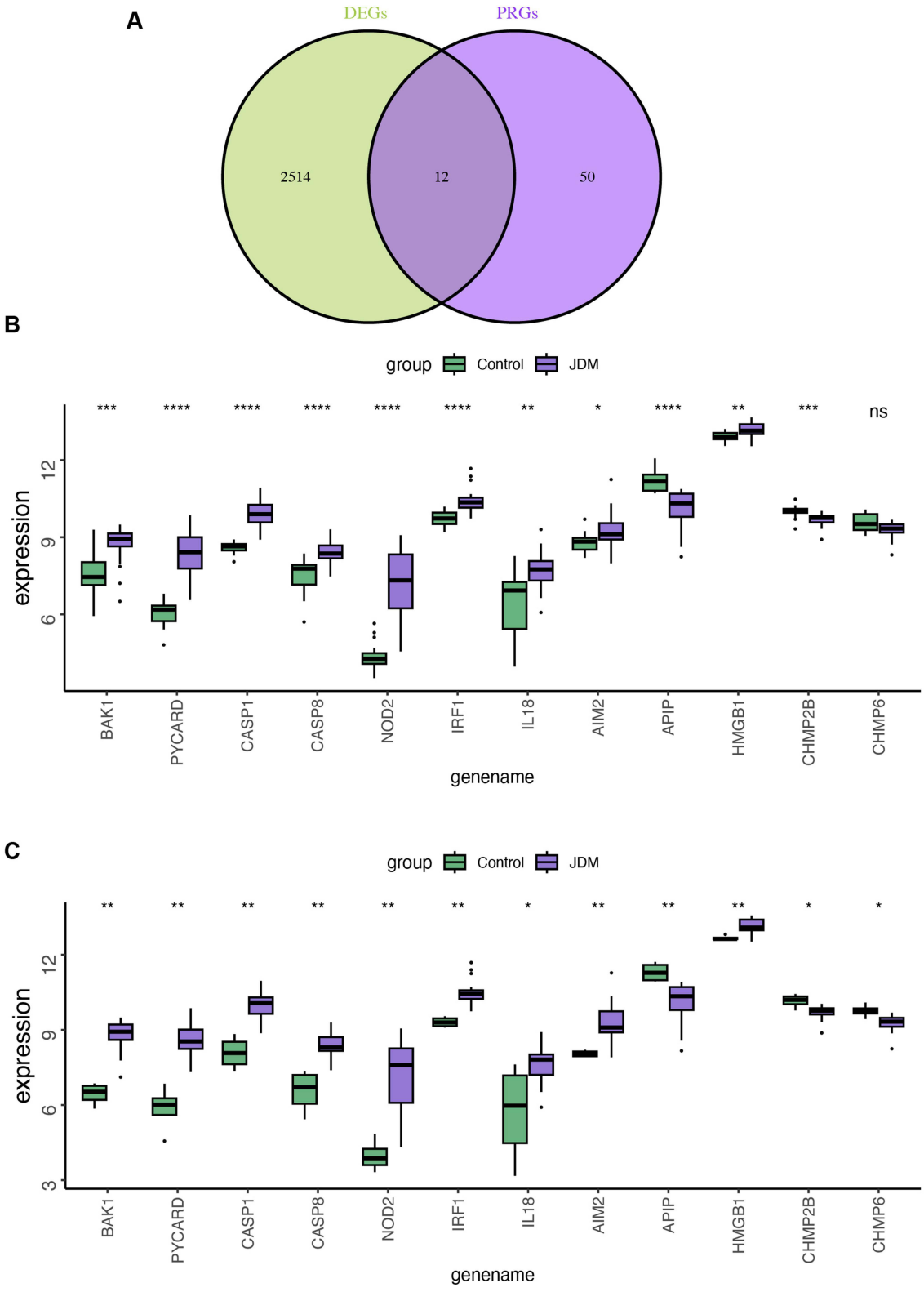

Figure 3 Identification of pyroptosis-related DEGs (PR-DEGs). (**A**) Venn diagram illustrating the intersection of common DEGs and pyroptosis-related genes. (**B** and **C**) Expression levels of PR-DEGs in the GSE3307 (**B**) and GSE11971 (**C**) datasets. Statistical significance is indicated as follows: ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001.

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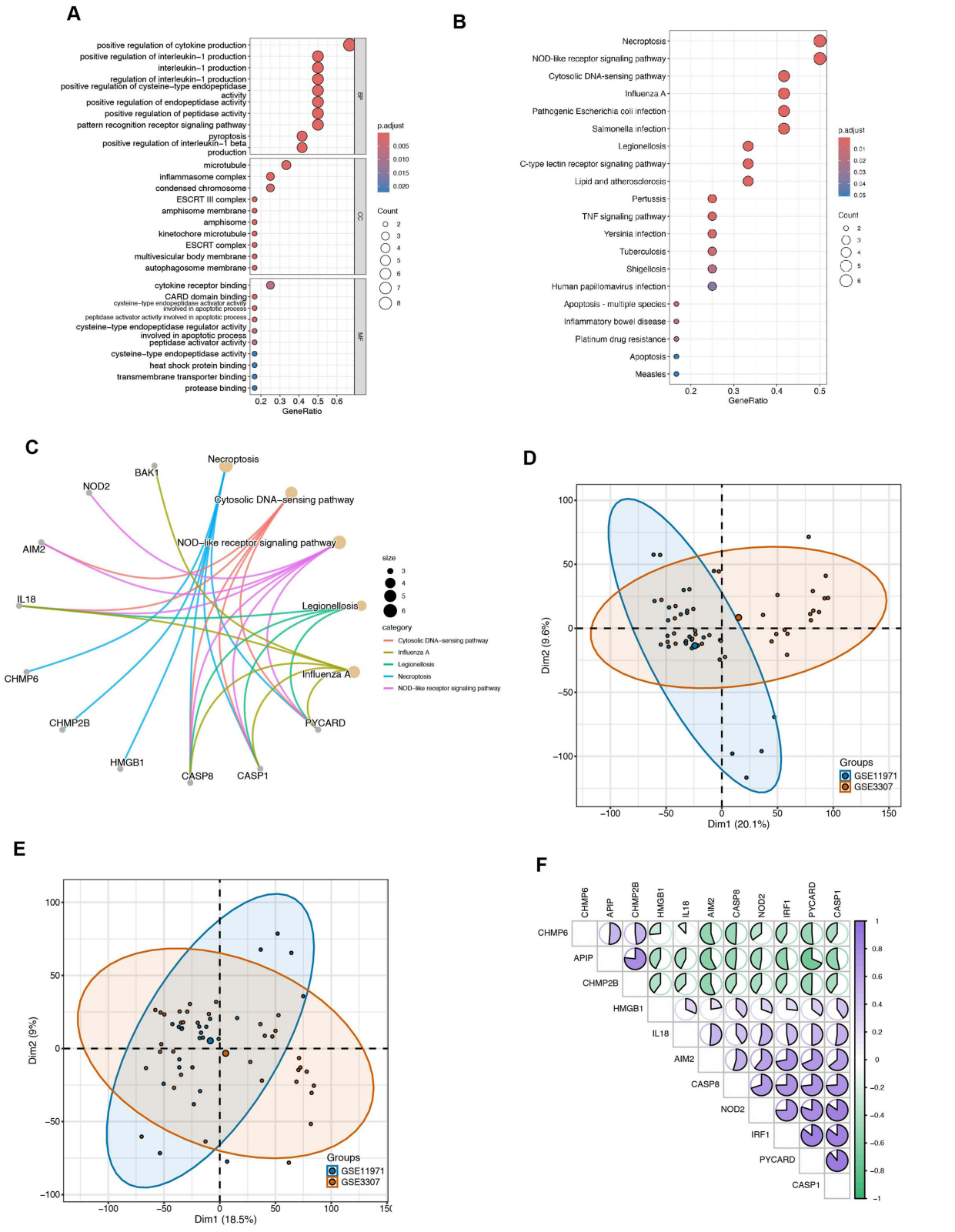

Figure 4 Functional analysis and correlation of PR-DEGs. (**A**) GO analysis of PR-DEGs. (**B** and **C**) KEGG pathway analysis of PR-DEGs. (**C**) Chord diagram illustrating the relationships between PR-DEGs and significant KEGG pathways. (**D**) PCA plot of the merged dataset before batch effect removal. (**E**) PCA plot of the merged dataset after batch effect removal. (**F**) Spearman correlation analysis of PR-DEGs in the merged dataset. GO terms and KEGG pathways are ranked by gene ratio from high to low.

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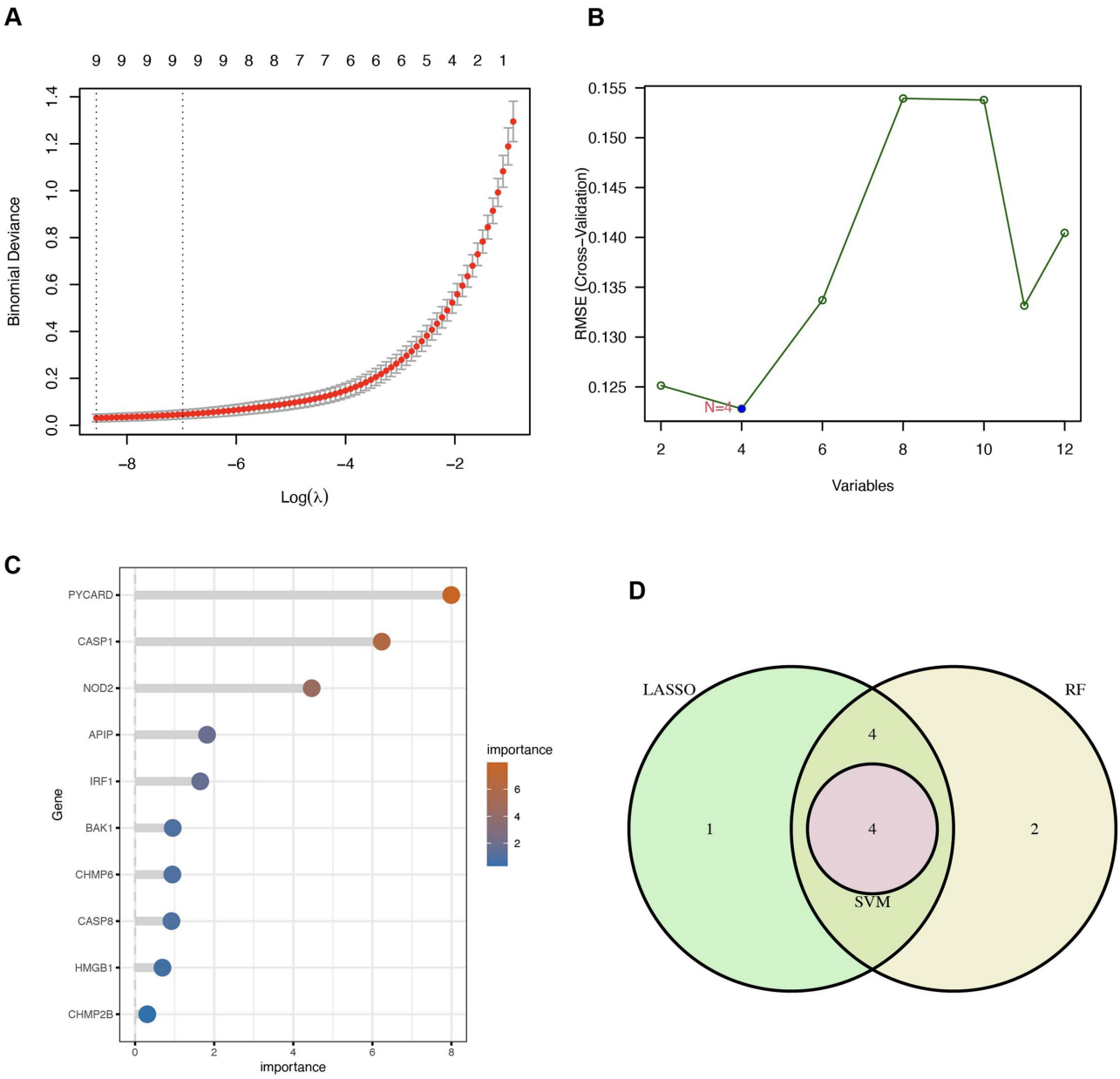

Figure 5 Machine learning analysis for identifying hub PR-DEGs. (**A**) Lasso regression analysis. (**B**) Support Vector Machine-Recursive Feature Elimination (SVM-RFE) analysis. (**C**) Random forest (RF) analysis. (**D**) Venn diagram showing the intersection of important features from the three machine learning methods.

Immune Infiltration Analysis

Immune infiltration analysis revealed significantly higher single-sample ssGSEA scores for various immune cell types in the JDM group compared to controls, indicating heightened pro-inflammatory immune cell infiltration in JDM patients (Figure 8A). Significant positive correlations were observed among different immune cell types in JDM patients (Figure 8B). Furthermore, PR-DEGs, including CASP1 (Figure 9A), PYCARD (Figure 9B), NOD2 (Figure 9C), and IRF1 (Figure 9D), exhibited significant positive correlations with several immune cell types, such as activated T cells, macrophages, dendritic cells, and NK cells. These findings suggest that PR-DEGs may influence the pathogenesis of JDM by modulating immune cell infiltration and activation, contributing to an enhanced inflammatory response.

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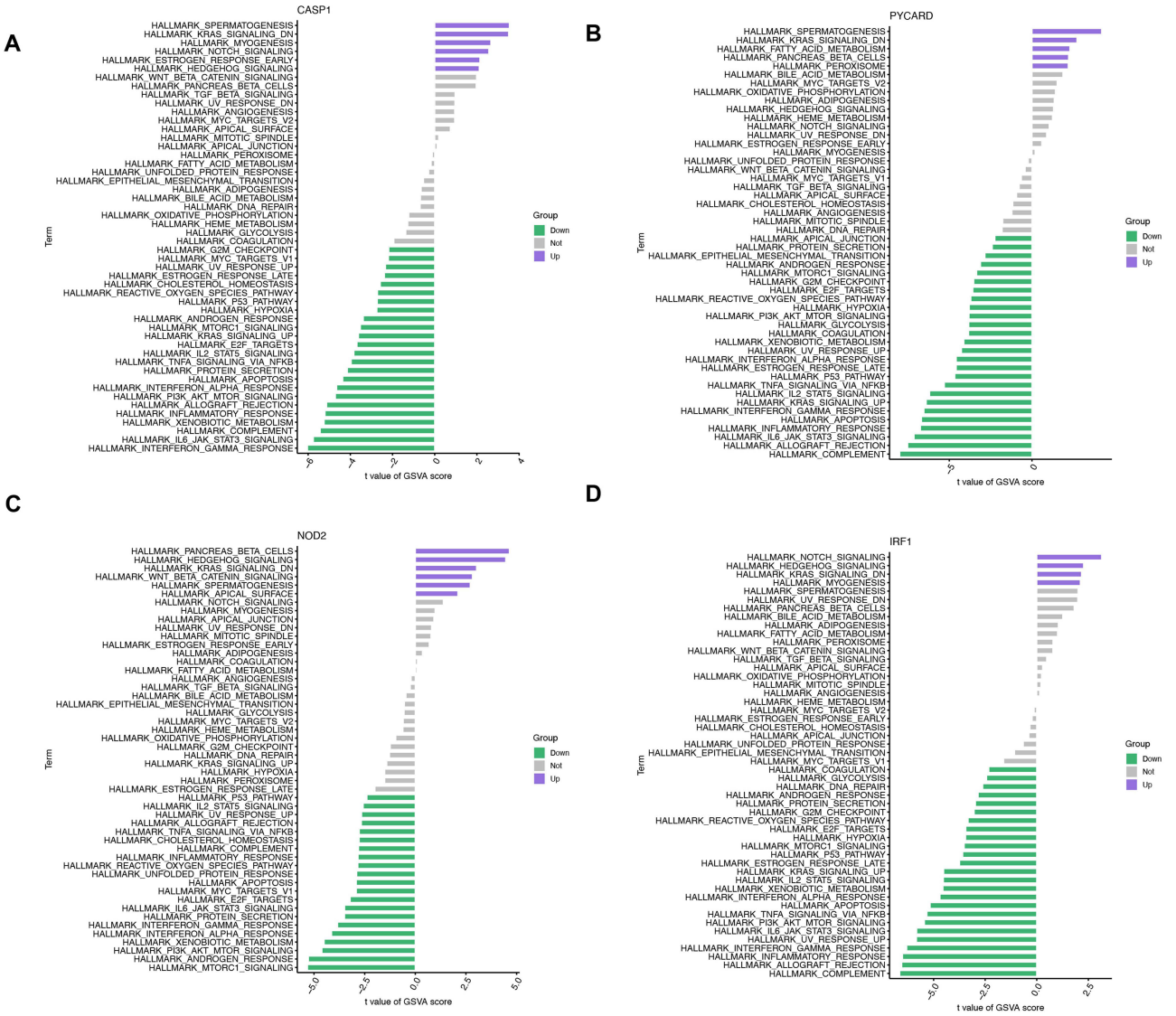


Figure 6 Gene Set Variation Analysis (GSVA) of hub PR-DEGs in JDM. (**A–D**) GSVA of Hallmark pathway enrichment scores for CASP1 (**A**), PYCARD (**B**), NOD2 (**C**), and IRF1 (**D**), displaying differences between high and low expression groups.

Discussion

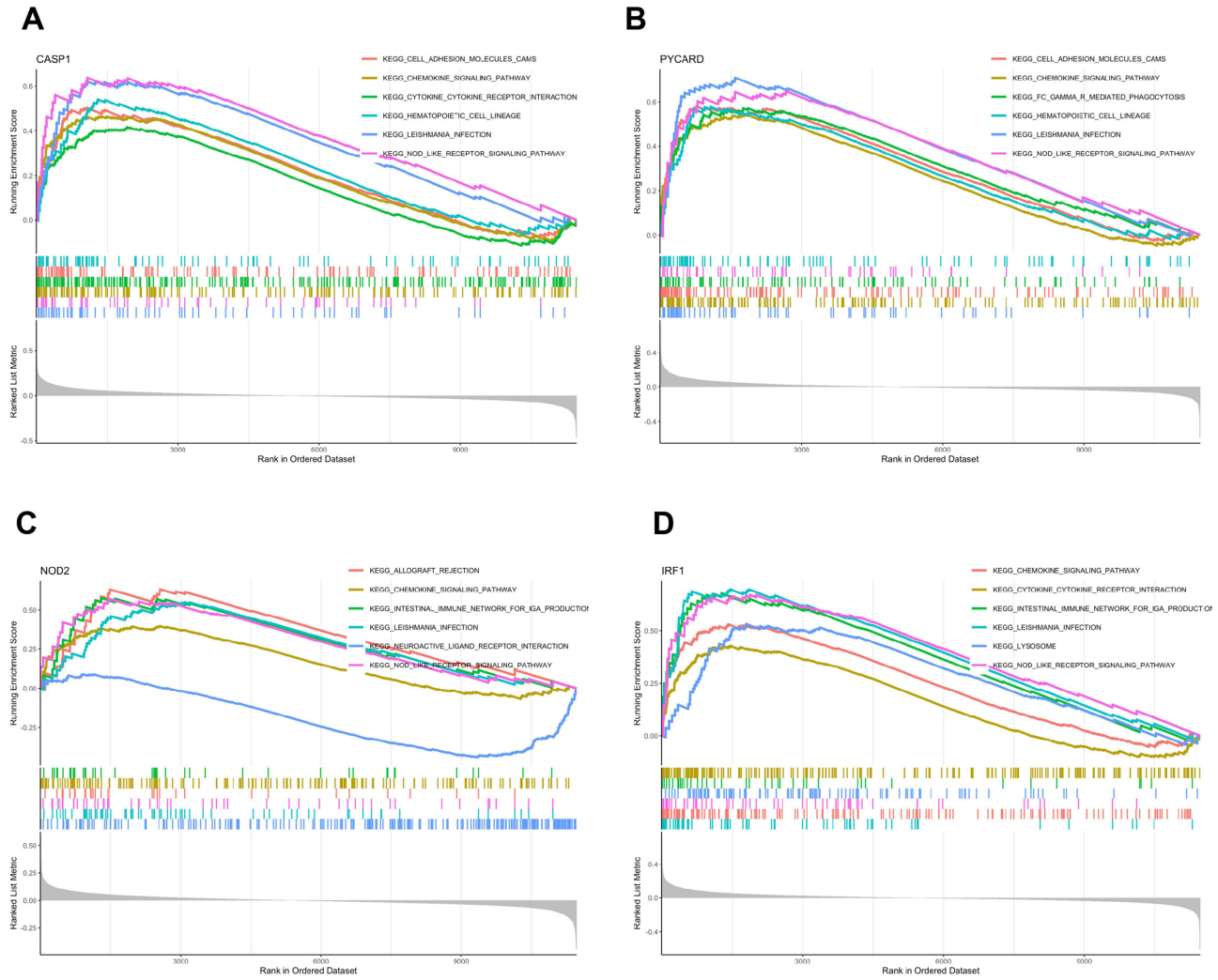
In this study, we identified 12 PR-DEGs, among which CASP1, IRF1, NOD2, and PYCARD were identified as the four hub PR-DEGs by machine learning algorithms, exhibiting significant upregulation in JDM patients. Functional enrichment analysis revealed that these PR-DEGs are involved in multiple inflammation and immune-related biological processes, such as cytokine production, inflammasome activity, and necroptosis. Concurrently, immune infiltration analysis demonstrated a significant increase in pro-inflammatory immune cells (eg, activated T cells and M1 macrophages) in JDM patients, with PR-DEG expression closely associated with immune cell infiltration.

Pyroptosis has been proven to participate in the pathological processes of various autoimmune diseases by regulating inflammation and immune responses.[12,16,17] Utilizing machine learning algorithms, we selected four hub PR-DEGs, CASP1, IRF1, NOD2, and PYCARD, which exhibited significantly elevated expression levels in JDM patients. CASP1 is a key component of inflammasomes, promoting inflammatory responses by cleaving and activating inflammatory factors such as IL-1β and IL-18.[18] Previous studies have reported upregulated CASP1 expression in autoimmune diseases like systemic lupus erythematosus.[19] IRF1 is a transcription factor that plays a crucial role in innate and adaptive immune responses, with increased expression observed in autoimmune diseases such as rheumatoid arthritis.[20,21] NOD2 is an

intracellular pattern recognition receptor that recognizes bacterial and viral components and activates inflammatory responses, closely associated with autoimmune diseases like inflammatory bowel disease.[22,23] PYCARD, also known as apoptosis-associated speck-like protein (ASC), is an essential component for NLRP3 inflammasome assembly, with mutations and aberrant expression linked to various autoinflammatory diseases.[24,25] Consistent with the functional enrichment analysis results, these hub PR-DEGs positively correlate with pathways related to inflammation, immunity, and cell death. Therefore, we speculate that the upregulation of these four hub PR-DEGs may lead to excessive inflammation and autoimmune responses in JDM patients by disrupting inflammation and immune-related pathways.

The GSEA results in our study reveal that the NOD-like receptor signaling pathway is significantly enriched in the high expression groups of CASP1, PYCARD, NOD2, and IRF1, highlighting its central role in the pathogenesis of JDM. NOD-like receptors are crucial intracellular pattern recognition receptors that detect various pathogen-associated molecular patterns and damage-associated molecular patterns, playing a key role in innate immune responses.[26] CASP1 and PYCARD, identified as hub PR-DEGs in our study, are essential components of the NLRP3 inflammasome, while NOD2 has also been shown to contribute to inflammatory responses by interacting with NLRP1 and NLRP3 to mediate inflammasome activation and caspase-1-dependent IL-1β secretion.[27] We therefore hypothesize that NOD2, CASP1, and PYCARD may exert synergistic effects in the pathogenesis of JDM through the NOD-like receptor signaling pathway.

Immune cell infiltration analysis further revealed significant changes in the immune cell composition of JDM patient tissues. Compared to normal controls, JDM patient tissues exhibited a marked increase in pro-inflammatory immune



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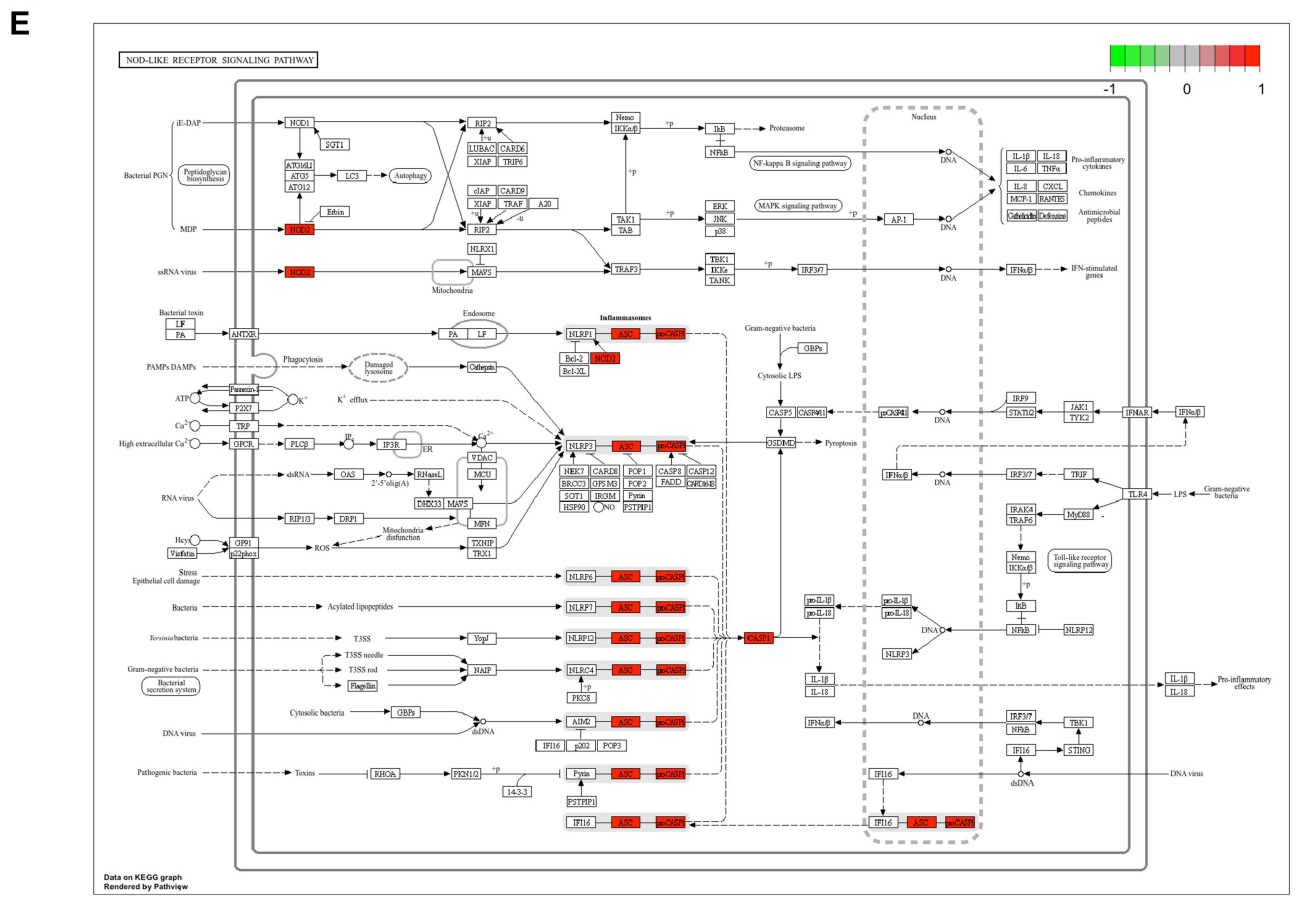

Figure 7 Gene Set Enrichment Analysis (GSEA) of hub PR-DEGs in JDM. (**A–D**) GSEA plots showing enriched KEGG pathways in high expression groups of CASP1 (**A**), PYCARD (**B**), NOD2 (**C**), and IRF1 (**D**). (**E**) Representative KEGG pathway diagram of the NOD-like receptor signaling pathway, with upregulated hub PR-DEGs highlighted in red.

cells, such as activated T cells, M1 macrophages, dendritic cells, and γδ T cells. These results are consistent with previous studies, suggesting the critical role of immune cells in the pathogenesis of JDM. Sag et al found that T cells, B cells, plasma cells, and macrophages constitute the inflammatory milieu in JDM muscle biopsies, with Th1 and Th17 T-helper cell subtypes being the main contributors.[28] Furthermore, they observed that IL-17 and IFN-γ positive cells were located in the perimysium, endomysium, and perivascular areas of the affected muscle tissues, suggesting their potential role in direct muscle injury.[28] Recently, Chen et al discovered that various cell types including monocytes and T cells in peripheral blood, as well as smooth muscle cells and fibroblasts in muscles of JDM patients, exhibit enhanced type I interferon responses, indicating their participation in autoimmune responses through the type I interferon pathway.[29]

Furthermore, correlation analysis in our study suggested that the expression of CASP1, IRF1, NOD2, and PYCARD is closely associated with the infiltration of various pro-inflammatory immune cells in JDM patients. This is in line with previous studies that have implicated these genes in the regulation of immune cell infiltration and activation in other autoimmune diseases. Caspase-1 has been demonstrated to facilitate the production of IL-1β and IL-18, thereby promoting IL-17 secretion by γδ T and CD4 T cells and contributing to the pathogenesis of autoimmune diseases.[30] Aberrant IRF1 activation in monocytes is associated with interferon signatures and disease severity in systemic lupus erythematosus, while IRF1-dependent IFN-β production driven by TNFR signaling in macrophages and endothelial cells contributes to the pathogenesis of inflammatory and autoimmune diseases.[31] NOD2 plays a critical role in the pathogenesis of autoimmune diseases by modulating innate and adaptive immune responses, particularly the activation of Th1 and Th17 pathways.[32] PYCARD is implicated in innate and adaptive immune responses in monocytes, macrophages, dendritic cells, neutrophils, and lymphocytes, where it facilitates inflammasome formation and function, and

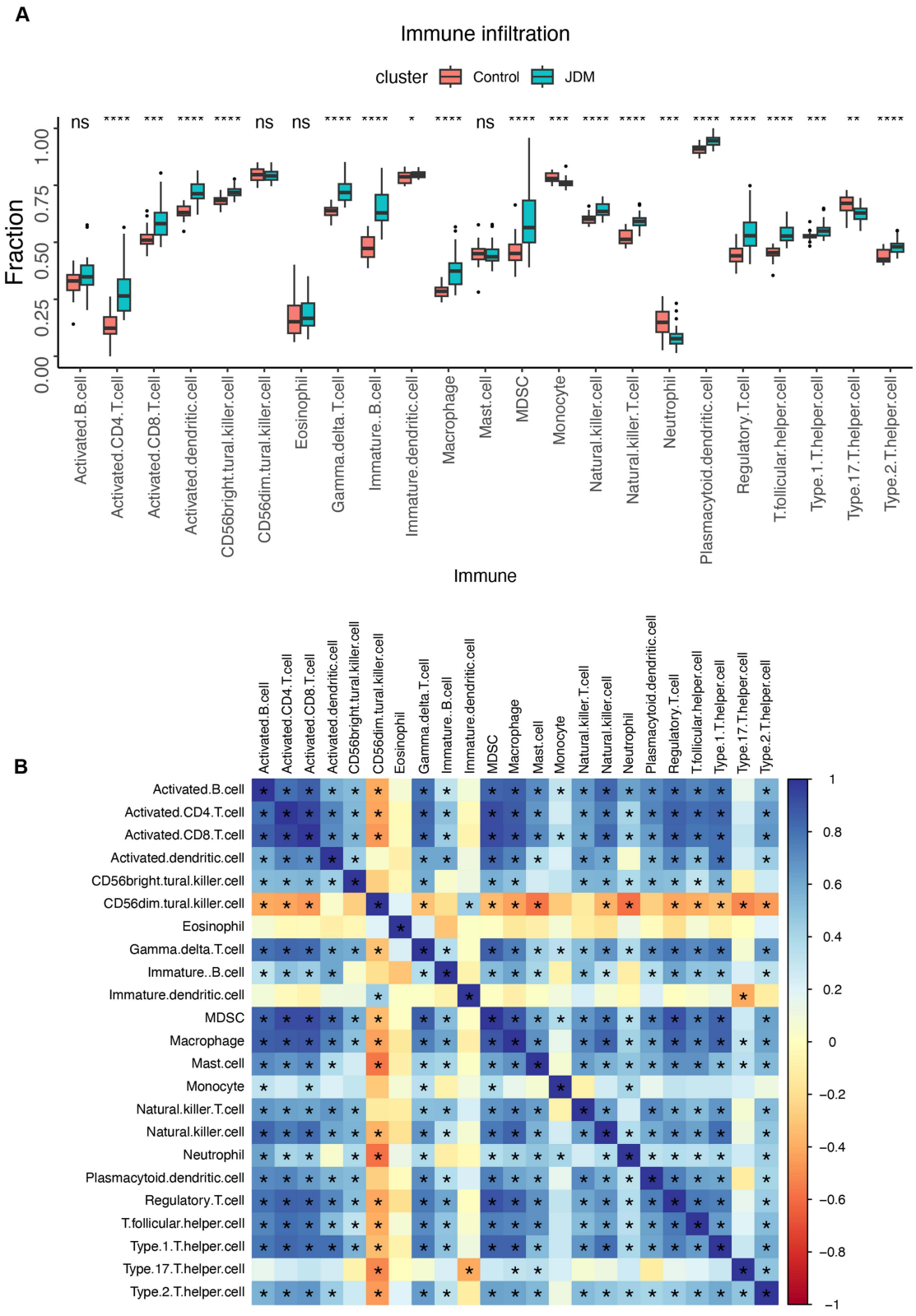

Figure 8 Immune cell infiltration analysis in JDM patients. (**A**) Boxplots displaying differences in single-sample Gene Set Enrichment Analysis (ssGSEA) scores between JDM and control groups. Statistical significance is indicated as follows: ns: p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001. (**B**) Correlation heatmap of immune signatures in the JDM group. Asterisks (*) indicate statistically significant correlations (p<0.05).

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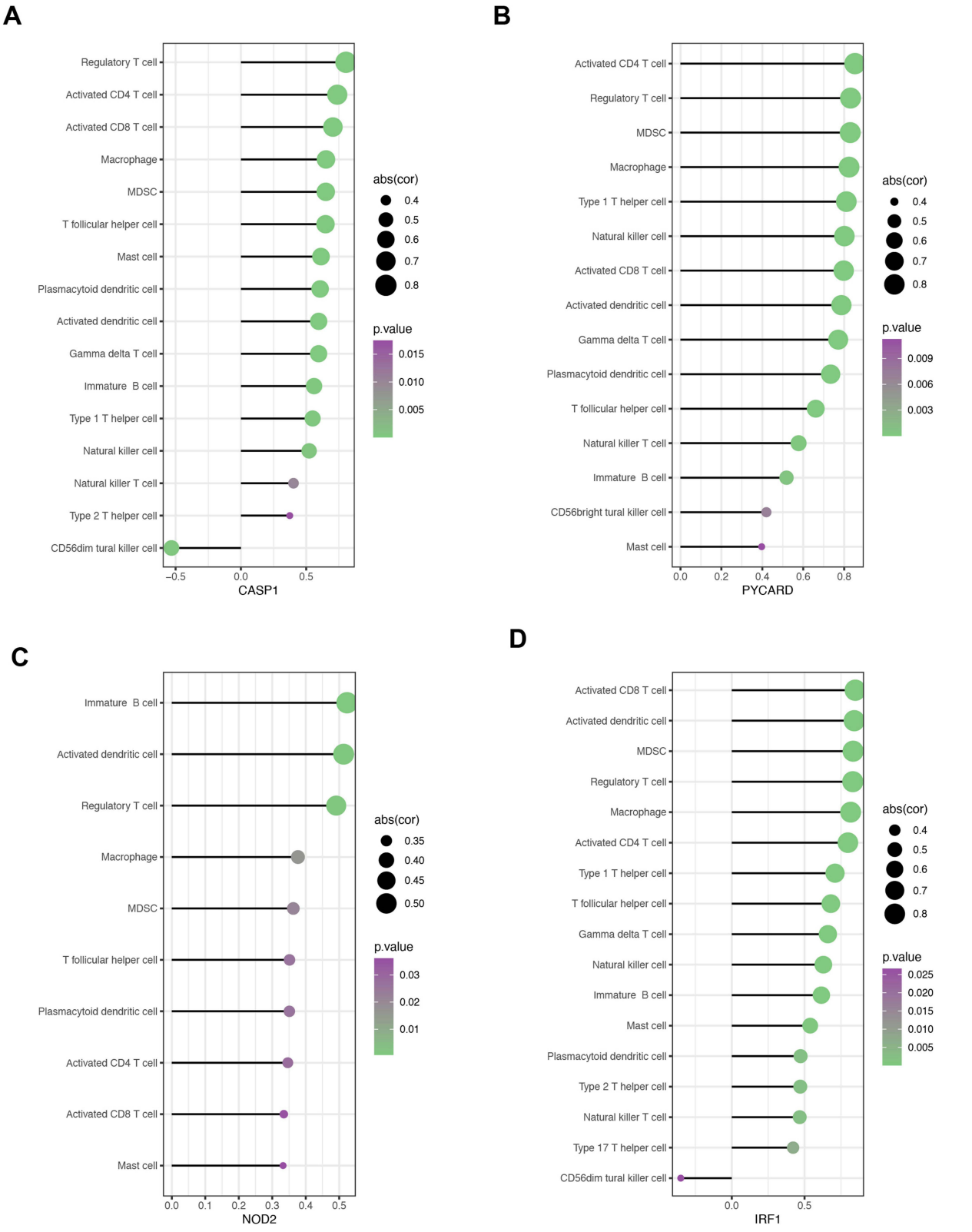

Figure 9 Correlation analysis between PR-DEGs and immune cell infiltration in JDM patients. (**A–D**) Correlation plots showing the relationship between the expression of CASP1 (**A**), PYCARD (**B**), NOD2 (**C**), and IRF1 (**D**) and ssGSEA scores of immune cell types.

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aberrant ASC speck release from these cells contributes to chronic inflammation in various autoimmune, autoinflammatory, neurodegenerative and infectious diseases.[33]

This study also has some limitations. Firstly, our analysis relies on gene expression data from two datasets, and the identified core PR-DEGs and their functions in JDM require experimental validation to confirm their biological significance. Secondly, the sample size of the datasets used in this study is relatively small, necessitating validation of our findings in larger cohorts. Despite these limitations, this study provides valuable insights into the role of pyroptosis-related genes and immune cell infiltration in the pathogenesis of JDM.

Conclusion

The hub PR-DEGs we identified are closely associated with abnormal immune cell infiltration in JDM patients and may play a crucial role in the immunopathological processes of JDM by regulating signaling pathways such as inflammasomes, interferons, and NF-κB, reshaping the immune microenvironment of patients and triggering excessive inflammation and autoimmune responses. These PR-DEGs hold promise as potential diagnostic markers and therapeutic targets for JDM, but still require further experimental and clinical research for validation.

Ethics Approval and Consent to Participate

The studies were approved by the Ethics Committee of the Central Hospital of Dalian University of Technology (Dalian Municipal Central Hospital, approval no. YN2024-164-01).

Disclosure

The authors declare no competing interests in this work.

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