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Research article

Genomic Insights into Rheumatoid Arthritis through computational Profiling for Hub Genes

Sushma Thandal Anantharaman, Daniel Alex Anand, Athista Manoharan and Swetha Sunkar*

Department of Bioinformatics, Sathyabama Institute of Science and Technology, Chennai – 600119, India

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Abstract

Rheumatoid arthritis (RA) is a complex autoimmune disorder predominantly affecting joints, with its etiology and response to treatment still not fully understood despite extensive historical documentation. This study aims to shed light on the differentially expressed genes (DEGs) associated with RA progression, potentially identifying new drug targets and management strategies. This study analyzed gene expression data (GSE193193) from the Gene Expression Omnibus (GEO) database, identifying 3672 significant DEGs out of 36107 initially retrieved genes. Among these, 283 genes were up-regulated and 360 were down-regulated. Gene enrichment analysis was performed to uncover relevant gene ontology terms and pathways. Subsequently, network construction and analysis, along with hub gene prediction using Cytoscape's MCODE and CytoHubba plugins, were conducted. Key genes identified in this study include HBB, ALAS2, GATA1, AHSP, HBG1, HBG2, HBD, KLF1, SLC4A1, EPB42, ZMYND10, DNAJC7, HYDIN, LRRC6, FN1, NCAM1, FASLG, CTCF, SMAD4, and STAT1. These genes are implicated not only in RA but also in other diseases, presenting them as potential therapeutic targets. Additionally, three transcription factors (GATA1, NFKB1, and RELA) and one miRNA (has-mir-27a-3p) were identified as key regulators of these hub genes. In conclusion, this study not only enhances our understanding of the molecular mechanisms underlying RA but also identifies several critical DEGs and regulatory factors that could serve as promising targets for therapeutic intervention. The identification of these genes and regulatory elements paves the way for the development of targeted treatments, which could significantly improve disease management and patient outcomes. Future research focusing on these identified targets may lead to innovative strategies for combating RA and potentially other autoimmune disorders, thereby offering new hope to patients affected by these conditions.

Keywords: rheumatoid arthritis; differential gene expression; bioinformatics

*Corresponding author: E-mail: swetha.biotech@sathyabama.ac.in
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1. Introduction

Rheumatoid arthritis (RA) is a complex autoimmune disease primarily affecting joints, characterized by the immune system's erroneous attack on the body's joints, leading to inflammation, thickening of the joint linings, and subsequent swelling and pain. This condition predominantly affects small peripheral joints initially and can progress to involve proximal joints if left untreated, commonly impacting the joints of the hands, feet, wrists, elbows, knees, and ankles (Bullock et al., 2018). Individuals with RA typically experience morning stiffness in their affected joints lasting for more than 30 min (Tanaka, 2020). Extra-articular manifestations are also prevalent, with rheumatoid nodules and interstitial lung disease (ILD) being notable complications (Chauhan et al., 2022).

The global incidence of RA has significantly increased from 567,462.89 cases in 1990 to 1,074,390.80 cases in 2019, with an age-standardized rate (ASR) of 13 per 100,000. RA affects about 0.5% to 1.0% of the adult population in developed countries (Gabriel & Michaud, 2009), with women being two to three times more likely than men to develop the condition (Crowson et al., 2011). RA can occur at any age but predominantly affects individuals between 25 and 50 years old (Cassotta et al., 2020). The etiology of RA involves a combination of genetic factors and autoantibodies. Aberrations in the adaptive immune system led to the development of autoantibodies, such as rheumatoid factors (RFs) and anti-modified protein antibodies (AMPA) (Scherer et al., 2020). Patients with seropositive RA inherit approximately 40% to 65% of the disease from their ancestors, while those with seronegative RA inherit about 20% (Kłodziński & Wisłowska, 2018; Chauhan et al., 2022). Approximately 80% of RA patients have RF, and 60% to 70% have antibodies to cyclic citrullinated peptide (CCP), though RF can indicate other conditions as well. Specific HLA-DRB1 alleles, particularly HLA-DRB104, HLA-DRB101, and HLA-DRB1*10, are associated with RA, with 80% of ACPA-positive RA patients carrying the "shared epitope" sequence coded by these alleles (Derksen et al., 2017; Espina et al., 2019).

Autoantibodies and autoimmunity can be present before clinical symptoms of RA appear, though not all patients with autoantibodies develop noticeable symptoms. In some cases, autoimmunity leads to immune-mediated inflammation, primarily in the synovium (Chauhan et al., 2022). Individuals with RA show an increased presence of autoreactive T and B lymphocytes in their synovial tissues. Normally, T cells exhibit tolerance to autoantigens, but disruption of this tolerance activates autoreactive T cells, which in turn stimulate B cells to produce autoantibodies (Tanaka, 2020). Pro-inflammatory cytokines such as TNF-α and IL-1 are critical in chronic joint inflammation and subsequent bone and cartilage damage, as evidenced by research using animal models (Lubberts & van den Berg, 2013).

The treatment of RA primarily involves the early initiation of disease-modifying antirheumatic drugs (DMARDs) to achieve minimal disease activity or remission. Commonly used DMARDs include methotrexate, hydroxychloroquine, sulfasalazine, and leflunomide. Biologic DMARDs, such as TNF inhibitors (etanercept, infliximab), IL-6 inhibitors (tocilizumab), T-cell co-stimulation inhibitors (abatacept), and B-cell depleting monoclonal antibodies (rituximab), are also utilized. Targeted synthetic DMARDs like Janus kinase (JAK) inhibitors (tofacitinib, baricitinib, upadacitinib) provide additional treatment options. NSAIDs help alleviate joint inflammation and pain, while corticosteroids are often administered temporarily in newly diagnosed patients with high disease activity. Despite these treatments, long-term management remains challenging, with many patients requiring continuous medication to maintain disease control (Chauhan et al., 2022). Early and aggressive treatment strategies can lead to improved outcomes, reduced joint damage, and

better quality of life for patients. Disease activity is commonly assessed using indices such as the SDAI, CDAI, and DAS28, which guide treatment decisions and adjustments (Tanaka, 2020).

Despite advances in treatment, the etiology of RA is still not fully understood, necessitating further research. RA's genetic components and the role of autoantibodies, such as rheumatoid factors (RFs) and anti-modified protein antibodies (AMPA), are key areas of interest. Genetic risk factors, including specific HLA-DRB1 alleles, are implicated in RA, with these alleles coding for a sequence known as the "shared epitope," present in a significant proportion of RA patients. Understanding the genetic and molecular basis of RA is crucial for developing targeted therapies and improving patient outcomes.

Previous work on RA genomics using computational approaches has provided valuable insights into the genetic and molecular mechanisms underlying the disease. Given the complexity of rheumatoid arthritis (RA) and the challenges in understanding its etiology and response to treatment, this study aimed to elucidate the differentially expressed genes (DEGs) associated with RA progression, potentially revealing new drug targets and management strategies using a comprehensive bioinformatics approach utilizing gene expression datasets via functional enrichment and network analysis.

2. Materials and Methods

2.1 Retrieval of GEO dataset

The dataset used for analysis was freely available and was retrieved from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The keyword ‘Rheumatoid arthritis’ was used to search in GEO datasets and after a careful review, the gene expression profile GSE193193 was selected from the resulting datasets (Fan et al., 2022). The dataset chosen was based on the platform GPL24676, Illumina NovaSeq 6000 (Homo sapiens).

2.2 Processing of data for differentially expressed genes

The selected dataset is a curated dataset that comprises the Differentially Expressed Genes (DEGS). The analysis of DEGs involved several critical steps. Initially, raw RNA-seq data underwent preprocessing to eliminate low-quality reads and adapter sequences, followed by quality control checks to ensure data integrity. Normalization was performed using the trimmed mean of M-values (TMM) method to account for differences in sequencing depth and RNA composition across samples. From the total set of DEGs, significant genes were filtered based on the adjusted probability value (p value) which is less than 0.05. From the significant genes, up-regulated and down-regulated genes were detected and categorized based on the cut-off criteria of log fold change (log2fc) values such as log2fc >1.5 and <-1.5, respectively. These DEGs were analyzed further for their functional enrichment.

2.3 Analysis of differentially expressed genes (DEGs) for functional enrichment and pathway identification

DEGs identified as up-regulated and down-regulated were subjected to functional and enrichment analysis using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) tool. The process begins with the user inputting a list of gene identifiers, which DAVID maps to a common gene set. Users can specify a custom background set or use the default genomic background to control for biases. DAVID then groups genes into

clusters based on shared functional annotations, utilizing the kappa statistic to identify functionally related groups. For Gene Ontology (GO) analysis, DAVID identifies overrepresented GO terms among the input genes using the modified Fisher's Exact Test (EASE score) to calculate the significance of enrichment and derives enrichment scores to represent the relative importance of each term. Pathway analysis maps genes to known biological pathways, such as KEGG and REACTOME, performing enrichment analysis to highlight significant pathways involved in the gene list. The results are visualized through various charts and tables, providing a comprehensive overview of the functional roles of the genes, thereby integrating multiple sources of annotation data for a thorough understanding of the biological context of DEGs (Huang et al., 2009). This tool provides insights into Gene Ontology (GO) terms, including Molecular Function (MF), Biological Process (BP), and Cellular Component (CC), as well as pathway information sourced from databases like KEGG and Reactome. Pathway analysis revealed the involvement of DEGs in various pathways related to cancer, diseases, drugs, and chemical substances.

2.4 Construction of protein-protein interaction (PPI) network

The PPI networks of the DEGs were predicted and constructed using an online tool STRING (https://string-db.org/) for retrieving the interacting genes/proteins (Szklarczyk et al., 2015). The interactions in STRING are derived from several sources, including direct (physical) and indirect (functional) associations. STRING utilizes a combination of text mining, experimental data, computational prediction methods, and public databases to score and compile these interactions. The network is constructed by assigning confidence scores to each interaction based on the evidence supporting it. High-confidence interactions are used to build a detailed network, providing insights into how proteins may collaborate to perform biological functions. The PPI network generated by STRING aids in identifying key proteins, understanding their interactions, and exploring their potential roles in disease mechanisms. Based on the STRING tool, the PPI networks of the DEGs were constructed separately as up-regulated and down-regulated gene networks

2.5 Screening of PPI network for modules and hub genes analysis

Cytoscape version 3.9.1, an open-source software, was used for visualizing the networks and integrated networks to find their molecular interactions and associated biological pathways. Subsequently, the PPI network retrieved from the STRING database was exported to Cytoscape and visualized for further analysis (Shannon et al., 2003). MCODE (Molecular Complex Detection) uses a graph-theoretical approach to find densely connected regions in large protein interaction networks, which are often indicative of functional modules or protein complexes. The algorithm assigns a score to each node (protein) based on the density of its neighborhood and then iteratively identifies and scores clusters. Higher scores indicate regions of higher connectivity, suggesting a potential biological significance. Additionally, the CytoHubba plugin was used to identify hub genes within the PPI network. CytoHubba integrates 12 distinct topological algorithms to rank nodes (proteins) based on various centrality measures, such as Degree, Betweenness, Closeness, and others. This multi-algorithm approach provides a robust identification of hub genes, which are proteins that play central roles in the network and are likely crucial for maintaining the network's integrity and function. By analyzing the top-ranked nodes, CytoHubba helps in pinpointing key regulatory proteins that might serve as potential biomarkers or therapeutic targets in the context of the studied disease. Combining the

results from MCODE and CytoHubba allows for a comprehensive understanding of the PPI network, highlighting both densely interconnected clusters and pivotal hub genes that could be integral to disease mechanisms. This dual approach enhances the reliability of identifying significant proteins and protein complexes, providing deeper insights into the molecular underpinnings of the disease under investigation.

2.6 Gene-disease association analysis

DisGeNET, an online database housing comprehensive collections of genes and variants linked to human diseases, was utilized to explore the connection between hub genes and rheumatoid arthritis. It employs a systematic approach to curate and annotate gene-disease associations, incorporating data from literature mining, genome-wide association studies (GWAS), and other curated databases.

2.7 Identification of miRNAs targeting the hub genes

miRNet 2.0, a miRNA-centric network visual analytics platform was used to discover the miRNAs and transcription factors targeting the hub genes (Fan et al., 2016). This tool employs a combination of network-based and sequence-based algorithms to elucidate microRNA (miRNA) interactions and their regulatory roles. The relationship between transcription factors and the hub genes were analyzed using the TRRUST (Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining) database in miRNet. This integrates natural language processing techniques with curated literature data to extract transcriptional regulatory relationships. It systematically analyzes vast amounts of text from scientific literature to identify and annotate regulatory interactions between transcription factors (TFs) and their target genes. By parsing and extracting relevant information from sentences, TRRUST constructs a comprehensive regulatory network that elucidates the complex regulatory mechanisms governing gene expression.

3. Results and Discussion

3.1 Data acquisition and analysis of DEGs

In this study, the RNA sequencing dataset GSE193193, containing 5 rheumatoid arthritis (RA) samples and 10 control samples, was obtained from the GEO database and analyzed to screen the differentially expressed genes (DEGs) in rheumatoid arthritis (Shannon, 2003). Out of 36,107 DEGs found in the dataset, 3,672 genes were significant based on the p-value < 0.05. Among these, 283 genes were significantly up-regulated and 360 genes were significantly down-regulated, based on log2fc values > 1.5 and <-1.5, respectively. A comparable methodology was employed in a study aimed at screening variously expressed lncRNAs implicated in regulating the intrinsic apoptosis pathway in colorectal cancer (Akbari et al., 2020), as well as in another study focused on identifying hub genes linked to human osteoarthritis cartilage (Sunkar et al., 2022). A logarithmic value within the range of 1.1 to 1.5 is commonly regarded as a sensible threshold option. The up-regulated and down-regulated p-values and log2fc values are provided in Table 1.

Table 1. List of differentially expressed genes

(a) List of up-regulated genes:

gene_name	log2(fc)	pval	Regulation
RNU11	4.58	0.00	up
HBD	9.56	0.00	up
CA1	7.78	0.00	up
ALAS2	7.69	0.00	up
HBG1	6.10	0.00	up
HBA1	6.15	0.00	up
SAMD14	2.32	0.00	up
SLC4A1	6.37	0.00	up
HBB	6.68	0.00	up
HBA2	5.78	0.00	up
SELENBP1	6.60	0.00	up
OSBP2	4.49	0.00	up
AHSP	9.37	0.00	up
MYL4	8.00	0.00	up
SNCA	4.35	0.00	up
HBG2	6.40	0.00	up
KRT1	6.32	0.00	up
RUNDC3A	6.88	0.00	up
SLC6A8	5.34	0.00	up

Table 1. List of differentially expressed genes (continued)

(b) List of down-regulated genes:

gene_name	log2(fc)	pval	regulation
FAT4	-2.33	0.00	down
AP000654	-3.22	0.00	down
KLRC4	-2.43	0.00	down
PCDHGB7	-3.18	0.00	down
LINC00299	-1.77	0.00	down
GNLY	-1.88	0.00	down
AC034102	-2.46	0.00	down
KLRC3	-2.62	0.00	down
OR2L6P	-3.25	0.00	down
AC243829	-2.44	0.00	down
KLRC2	-3.05	0.00	down
ENPP5	-1.79	0.00	down
OR2L2	-3.97	0.00	down
AC006033	-1.72	0.00	down
BNC2	-2.16	0.00	down
GPR141BP	-2.11	0.00	down
AL365475	-1.92	0.00	down
LINC00893	-1.72	0.00	down
MIR181A2HG	-2.58	0.00	down

3.2 Functional enrichment of DEGs

Functional annotation of DEGs was conducted using the DAVID online software, assessing gene ontology terms (biological process, molecular function, cellular component) and pathway enrichment (KEGG, REACTOME) for both up-regulated and down-regulated genes (Ma et al., 2021).

3.2.1 Up-regulated genes

Analysis of biological processes (BP) revealed that up-regulated genes were primarily associated with innate immune response, negative regulation of apoptotic process, and cell

adhesion. In terms of molecular function (MF), these genes were notably enriched in identical protein binding and zinc ion binding. Regarding cellular component (CC), up-regulated genes were predominantly associated with the plasma membrane, cytosol, and extracellular exosome. KEGG pathway analysis indicated their involvement in transcriptional mis-regulation in cancer and phagosomes, while REACTOME pathways highlighted roles in the immune system and innate immune system (Yap et al., 2018). These results clearly indicate the importance of the innate immune system in RA development and progression.

3.2.2 Down-regulated genes

Down-regulated genes showed enrichment in biological processes related to cell adhesion, nervous system development, and homophilic cell adhesion via plasma membrane adhesion molecules. Molecular function analysis revealed significant enrichment in calcium ion binding, while cellular component analysis linked these genes with the plasma membrane and integral components of the membrane. KEGG pathway analysis identified involvement in antigen processing and presentation and natural killer cell-mediated cytotoxicity, while REACTOME pathways highlighted roles in signal transduction and developmental biology (Zhu et al., 2023). These findings are consistent with reports suggesting the critical role of signal transduction pathways in RA pathogenesis.

The enrichment analysis revealed significant associations between DEGs and specific biological processes and pathways, shedding light on their potential involvement in RA pathophysiology. The up-regulated genes underscore the dysregulation of immune responses and apoptotic processes, consistent with the inflammatory nature of RA. Conversely, the down-regulated genes suggest alterations in cell adhesion and signalling pathways, reflecting disruptions in tissue homeostasis and immune regulation observed in RA. These findings provide valuable insights into the molecular mechanisms underlying RA pathogenesis and offer potential targets for therapeutic intervention.

3.3 PPI network construction

PPI networks were constructed separately for up-regulated and down-regulated genes using the STRING database and the results are provided in Figure 1. The PPI network for up-regulated genes comprised 251 nodes and 647 edges, with an average node degree of 5.16 and an average local clustering coefficient of 0.416 with enrichment value <1.0e-16. Conversely, the PPI network for down-regulated genes consisted of 232 nodes and 199 edges, with an average node degree of 1.72 and an average local clustering coefficient of 0.387.

The construction of PPI networks offers insights into the molecular interactions underlying RA pathogenesis. A higher average node degree within the PPI network for up-regulated genes may indicate increased connectivity between proteins in that network and their involvement in several interactions. This heightened connectivity may reflect the dysregulation of signalling pathways and biological processes associated with RA pathophysiology. Conversely, the lower average node degree for the PPI network for down-regulated genes might mean less connectivity among proteins in that network, indicating reduced functional interaction among proteins. Disruption of protein-protein interactions might be a factor in altered cellular processes and pathways leading to RA.

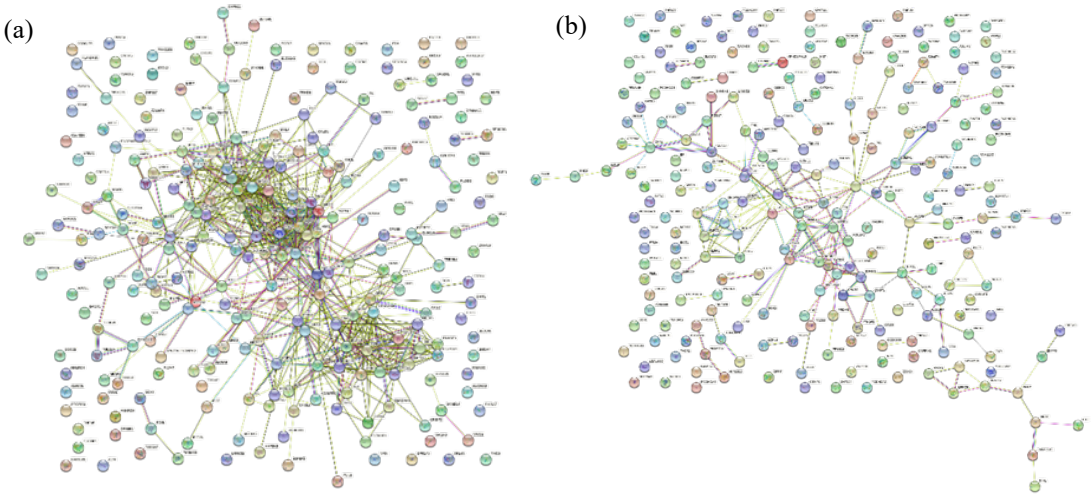

Figure 1. PPI network constructed using STRING database (a) Up-regulated genes network (b) Down-regulated genes network

Another measure that shows the structural properties and organization of the network is the average local clustering coefficient, which indicates the density of connections among a protein's neighbours in the network. The high average local clustering coefficient in the PPI networks might suggest a presence of connected regions or modules in both networks. These modules may represent functional units or protein complexes involved in some biological processes relevant to RA (Bader & Hogue, 2003; Rees et al., 2010; Hu et al., 2017; Osterman et al., 2020). Overall, the development and analysis of PPI networks provide a systematic framework for exploring the molecular mechanisms underlying RA and identifying potential therapeutic targets. Further investigation of the proteins and specific interactions in these networks will help to understand their role in the pathogenesis and progression of RA.

3.4 Screening of PPI for modules and hub genes analysis

The PPI networks obtained from STRING were visualized in Cytoscape to identify hub genes and functional modules within the network. The MCODE plugin was utilized to identify clusters of closely interlinked nodes, representing potential functional modules or complexes (Figure 2) (Bader & Hogue, 2003).

3.4.1 Up-regulated genes

Among the seven clusters detected in the up-regulated gene network, a particularly significant cluster encompassed 13 nodes and 75 edges, boasting the highest cluster score of 12.500 (Bader & Hogue, 2003; Rees et al., 2010; Osterman et al., 2020; Yu et al., 2022). These clusters likely represent functional modules intricately involved in biological processes pertinent to RA pathogenesis. Subsequent CytoHubba analysis spotlighted 10 hub genes, including HBB, ALAS2, GATA1, AHSP, HBG1, HBG2, HBD, KLF1, SLC4A1, and EPB42, pivotal in driving disease processes (Osterman et al., 2020).

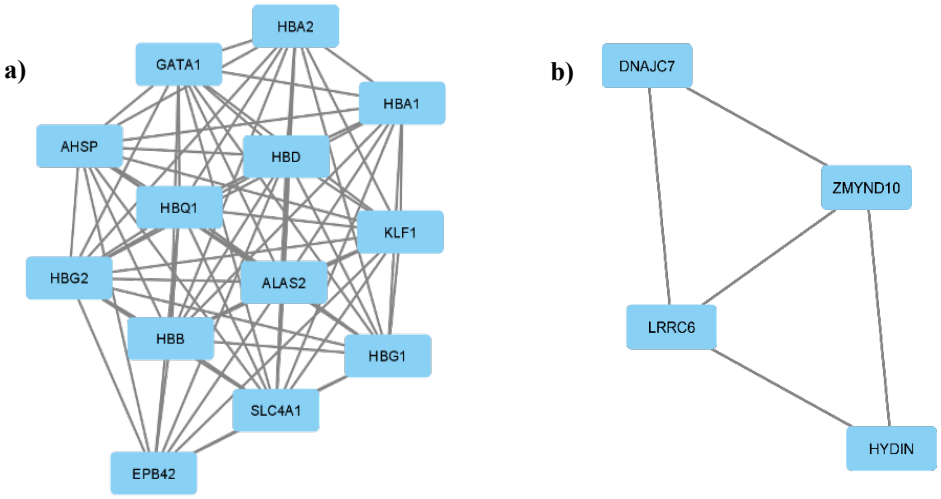

Figure 2. Module analysis using MCODE for cluster identification a) Up-regulated genes b) down-regulated genes

3.4.2 Down-regulated genes

Similarly, among the six clusters identified in the down-regulated gene network, a cluster with 4 nodes and 5 edges, boasting the highest cluster score of 3.333, was deemed noteworthy. CytoHubba analysis unveiled 10 hub genes, such as ZMYND10, DNAJC7, HYDIN, LRRC6, FN1, NCAM1, FASLG, CTCF, SMAD4, and STAT1, implicated in RA pathogenesis (Osterman et al., 2020).

These clusters often represent functional modules or complexes of proteins that collaborate in specific biological processes or pathways, aiding in the understanding of the network's functional organization. MCODE simplifies these networks by highlighting regions of high connectivity, facilitating the focus on biologically relevant subnetworks. This simplification enhances the interpretation of complex data (Bader & Hogue, 2003).

In the context of disease, identifying dysregulated or central protein modules within the PPI network can aid in prioritizing potential drug targets. MCODE can pinpoint these key modules, enabling researchers to concentrate their efforts on understanding and targeting specific components of the network (Rees et al., 2010), thereby supporting the discovery of novel targets in RA. CytoHubba, an additional plugin within Cytoscape, integrates 12 distinct algorithms and provides a user-friendly interface for analyzing the topology of PPI networks. This tool was utilized to predict hub genes in the study. CytoHubba analysis was performed on the resulting clusters of MCODE and the entire PPI network separately. The resulting genes from each CytoHubba algorithm were compared, yielding a list of 8 common genes from the up-regulated gene network and 4 common genes from the down-regulated gene network (Figure 3), which were further compared with the results of MCODE. The same methodology was applied to the entire PPI network, and the results were compared with the previous CytoHubba findings. The analysis revealed a total of 20 hub genes, with 10 genes being up-regulated (HBB, ALAS2, GATA1, AHSP, HBG1, HBG2, HBD, KLF1, SLC4A1,

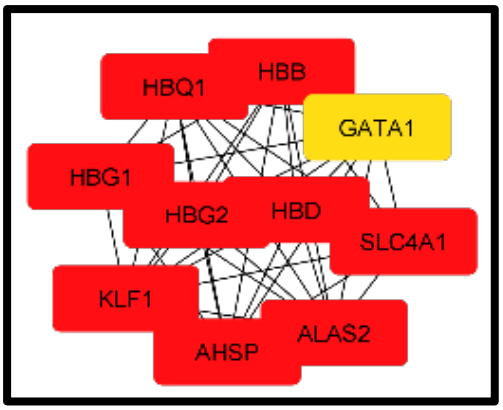

Figure 3. Common hub genes in up-regulated network from CytoHubba on MCODE

EPB42) and 10 genes being down-regulated (ZMYND10, DNAJC7, HYDIN, LRRC6, FN1, NCAM1, FASLG, CTCF, SMAD4, STAT1). These findings underscore the intricate interactions and significance of these genes in disease processes.

The identified hub genes, whether up-regulated or down-regulated, are likely instrumental in dysregulated biological processes fuelling RA progression. Targeting these hub genes holds promise for uncovering novel therapeutic avenues in RA management.

The integration of MCODE and CytoHubba analyses offers a holistic understanding of network topology and the identification of biologically relevant components. These findings underscore the intricate interplay among genes implicated in RA pathophysiology, offering valuable targets for therapeutic intervention. Further exploration of the functional roles and interactions of these hub genes within the PPI network is warranted to elucidate their precise contributions to RA pathogenesis.

3.5 Gene expression validation and disease association analysis

The study delved into the intricate relationship of the identified hub genes in rheumatoid arthritis, shedding light on their roles and potential implications in the disease's onset and progression. Haemoglobin subunit beta (HBB) is pivotal in oxygen transport and is also related to conditions like sickle cell anaemia and beta-thalassemia. Notably, HBB's role in musculoskeletal diseases like knee joint valgus deformity indirectly associates it with RA (Rees et al., 2010). ALAS2 is exclusively found in developing red blood cells, playing a role in heme formation during erythropoiesis. It is primarily linked to erythrocyte differentiation and homeostasis. GATA1, a transcriptional regulator, aids in erythroid development. It is directly involved in activating genes crucial to erythroid differentiation (Yu et al., 2002). Interestingly, GATA1 is associated with musculoskeletal and skin diseases, including RA, positioning it as a pivotal gene in RA's pathology.

AHSP protects free alpha-haemoglobin from precipitation, modulating states of alpha-haemoglobin excess such as beta-thalassemia. Haemoglobin subunit gamma-1 (HBG1) and haemoglobin subunit gamma-2 are essential for foetal haemoglobin, which usually gets replaced by adult haemoglobin post birth (Steinberg et al., 1997). Haemoglobin subunit delta (HBD) functions similarly to HBB, being key in oxygen transport. It is also associated with beta-thalassemia. Krueppel-like factor 1 (KLF1) is a transcription regulator of erythrocyte development, with a role in switching between foetal and adult globin. The

Solute Carrier family 4-member 1 (SLC4A1) gene helps in maintaining the body's correct acid levels. Mutations in this gene can lead to diseases impacting red cell membrane stability and kidney acid secretion. Erythrocyte membrane protein band 4.2 (EPB42) may regulate the association of protein 3 with ankyrin, influencing erythrocyte shape and mechanical properties. ZMYND10, a potential tumor suppressor, is crucial for dynein motor assembly. DNAJC7, another hub gene, encodes a member of the DNAJ heat shock protein 40 family, playing a role in cellular responses to stress.

HYDIN is essential for ciliary motility, mutations of which cause primary ciliary dyskinesia. LRRC6 is involved in dynein arm assembly, being crucial for the formation and motility of spermatozoal flagella (Horani & Ferkol, 2018). Fibronectin 1 (FN1) has multifaceted roles ranging from cell adhesion, and wound healing to maintenance of cell shape. Its involvement in musculoskeletal diseases, particularly RA, highlights its significance. Neural Cell Adhesion Molecule 1 (NCAM1) participates in cellular interactions crucial for development and differentiation processes. It contributes to the proliferation of T cells, B cells, and NK cells, which are essential for immune surveillance. The Fas ligand (FASLG) gene, belonging to the tumor necrosis factor superfamily, is pivotal in apoptosis and is associated with conditions such as systemic lupus erythematosus (Firestein et al., 2017). CCCTC-binding factor (CTCF) is a transcriptional regulator with potential links to invasive breast cancers, prostate cancers, and Wilms' tumours. The SMAD4 gene, part of the TGF-β pathway, regulates cell growth and division, playing a role in the development of many body systems. Notably, it is indirectly associated with RA's onset (van der Pouw Kraan et al., 2003; Massagué, 2012; Yoshida et al., 2012; Ivashkiv & Donlin, 2014).

Signal transducer and activator of transcription 1 (STAT1) is responsible for coordinating cellular reactions to interferons and various cytokines (Ivashkiv & Donlin, 2014). It is directly linked to RA, with studies supporting its elevated expression in RA synovium. Several hub genes, including EPB42, HBB, SLC4A1, and KLF1, are related to hereditary hemolytic anaemia. Others, such as GATA1, are tied to conditions like X-linked dyserythropoietic anaemia. Intriguingly, genes like STAT1, FASLG, FN1, NCAM1, and GATA1 are directly associated with RA, as supported by various studies (Fan et al., 2016).

The hub genes identified were further scrutinized to establish a direct connection with rheumatoid arthritis using the DisGeNET database. The outcomes revealed that the STAT1, FASLG, FN1, NCAM1, and GATA1 genes were directly associated with RA (Table 2). Supporting evidence for the involvement of the STAT1 gene in RA includes a study by Yoshida et al. (2012) which noted significantly higher expression levels of signal transducer and activator of transcription 1 in the synovium of RA compared to osteoarthritis. Additionally, van der Pouw Kraan et al. (2003) demonstrated the prominent role of activated STAT1 pathways in rheumatoid tissues. Zhang et al. (2019) suggested that increased expression of IL7R and STAT1 in synovial tissues might be linked to RA, and Wang et al. (2018) found that E2F2 directly regulates STAT1 pathways, exacerbating the inflammatory phenotype in RA synovial fibroblasts. Lee et al. (2007) conducted a study genotyping 67 single nucleotide polymorphisms within the STAT1 and STAT4 regions in Korean patients with RA, further supporting the association.

Regarding the FASLG gene, an association between FASL rs763110 polymorphisms and RA susceptibility in Asians through meta-analysis (Lee et al., 2015) was revealed. A study found that polymorphisms in the FasL gene, related to apoptosis, may increase genetic susceptibility to RA in the Turkish population (Yıldır et al., 2013). For the FN1 gene, Yan et al. (2013) suggested that PADI4 in the RA synovium may contribute to cartilage destruction through the citrullination of FN. Silva et al. (2009) proposed that

Table 2. Gene-disease association

Gene Symbol	Gene Name	Gene ID	Disease Class	Semantic Type	N.genes d	N.SNPs d	Score	Functions	Other Diseases Associated
STAT1	signal transducer and activator of transcription 1	6772	Skin and Connective Tissue Diseases; Musculoskeletal Diseases; Immune System Diseases	Disease or Syndrome	2723	2387	0.4	cellular response to interleukin-6,response to interleukin-6	Precursor T-Cell Lymphoblastic Leukemia-Lymphoma; Immunodeficiency 31B (IMD31B);Immunodeficiency 31A (IMD31A); Immunodeficiency 31C (IMD31C)
FASLG	Fas ligand	356	Skin and Connective Tissue Diseases; Musculoskeletal Diseases; Immune System Diseases	Disease or Syndrome	2723	2387	0.34	tumor necrosis factor receptorbinding, protein binding, negative regulation of transcription from RNA polymerase II promoter, cellular response to interferon-gamma	Autoimmune lymphoproliferative syndrome 1A(ALPS1A); systemic lupus erythematosus (SLE)
FN1	Fibronectin 1	2335	Skin and Connective Tissue Diseases; Musculoskeletal Diseases; Immune System Diseases	Disease or Syndrome	2723	2387	0.1	cell adhesion and migration processes includingembryogenesis, wound healing,blood coagulation, host defense, and metastasis	Glomerulopathy with fibronectin deposits 2 (GFND2); Spondylometaphyseal dysplasia; corner fracture type (SMDCF); Miscarriage; Spontaneous abortion; Early Pregnancy Loss; Abortion, Tubal
NCAM1	neural cell adhesion molecule 1	4684	Skin and Connective Tissue Diseases; Musculoskeletal Diseases; Immune System Diseases	Disease or Syndrome	2723	2387	0.03	Involved in various cellular interactions, both between cells and with the extracellular matrix, throughout developmental processes and differentiation. It contributes significantly to nervous system development by regulating	Miscarriage; Spontaneous abortion; EarlyPregnancy Loss; Abortion, Tubal

Table 2. Gene-disease association (continued)

Gene Symbol	Gene Name	Gene ID	Disease Class	Semantic Type	N.genes d	N.SNPs d	Score	Functions	Other Diseases Associated
								neurogenesis, promoting neurite outgrowth, and facilitating cell migration. Additionally, it participates in the expansion of T lymphocytes, B lymphocytes, and natural killer (NK) cells, crucial for immune surveillance. Furthermore, it plays a pivotal role in signal transduction mechanisms by interacting with fibroblast growth factor receptors, N-cadherin, and other extracellular matrix components. These interactions trigger signaling cascades involving FYN-focal adhesion kinase (FAK), mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K)	
GATA1	GATA binding protein 1	2623	Skin and Connective Tissue Diseases; Musculoskeletal Diseases; Immune System Diseases	Disease or Syndrome	2723	2387	0.01	coagulation, erythrocyte differentiation, erythrocyte homeostasis, hemostasis, homeostasis of number of cells, myeloid cell development, myeloid cell differentiation, myeloid cell homeostasis	X-linked dyserythropoietic anemia and thrombocytopenia (XDAT); Thrombocytopeniawith beta-thalassemia, X-linked (XLTT); Anemia without thrombocytopenia, X-linked (XLAWT); Hemolytic anemia due to elevated adenosine deaminase (HAEADA); Diamond-Blackfan anemia

fibronectin fragments stimulate mediators of matrix and cartilage destruction in RA, providing insights into the functional role of HLA-DRB1 in RA pathogenesis. Five citrullinated residues in fibronectin from RA synovial fluid were identified (van Beers et al., 2012), while researchers found associations between synovial fibronectin fragmentation and domain expressions in RA progression (Przybysz et al., 2007).

The NCAM1 gene's association with RA is supported by another study demonstrating the accumulation of CD56 (bright) natural killer cell subsets with immunoregulatory properties in tissue sites of inflammation, including the synovial membrane in RA patients (Conigliaro et al., 2011). Finally, the GATA1 gene's association with RA is backed by Liu et al. (2018), highlighting the crucial involvement of synovial GATA1 in the progression and aggravation of RA through its induction of NOS2 transcription.

The study revealed a set of 20 hub genes, with 10 up-regulated and 10 down-regulated genes, which play diverse roles ranging from erythropoiesis and cell adhesion to immune regulation and apoptosis. Notably, genes such as STAT1, FASLG, FN1, NCAM1, and GATA1 emerged as key players directly associated with RA. These findings were corroborated through extensive literature review and analysis using the DisGeNET database. For instance, STAT1, a pivotal regulator of cytokine responses, exhibited elevated expression levels in RA synovium, implicating its role in driving the inflammatory cascade characteristic of RA. Similarly, genes like FASLG, FN1, NCAM1, and GATA1 demonstrated associations with various aspects of RA pathogenesis, including apoptosis dysregulation, immune cell activation, and cartilage degradation.

Overall, the comprehensive validation and disease association analysis have deepened our understanding of the molecular mechanisms underlying RA. The identification of these hub genes provides valuable insights into potential therapeutic targets and avenues for further research aimed at finding the complexities of RA and developing targeted interventions to mitigate its impact.

3.6 Identification of miRNAs targeting the hub genes

The study utilized miRNet 2.0 database to identify miRNAs and transcription factors targeting the hub genes. Out of the 20 submitted genes, AHSP and ZMYND10 were not mapped in the interaction database. The resulting network comprised 18 genes, 70 transcription factors, and 402 miRNAs, totaling 660 edges. Applying a degree filter, less significant nodes were eliminated, revealing a refined network with 18 genes, 103 miRNAs, and 10 transcription factors connected by 301 edges. Interestingly, the network highlighted GATA1 and KLF1 as hub genes functioning both as transcription factors and genes.

Among the 70 transcription factors, GATA1, NFKB1, and RELA targeted 6 hub genes. Among the 402 miRNAs, hsa-mir-27a-3p was identified to target 10 hub genes. This suggests the potential significance of hsa-mir-27a-3p and the transcription factors GATA1, NFKB1, and RELA in the pathogenesis and development of rheumatoid arthritis, possibly regulating gene expression in RA. However, the hypothesis requires validation through further in-vitro and in-vivo research.

Prior studies have indicated the up-regulation of miR-27a-3p in TGF-β1-treated human lung fibroblasts and in fibroblasts from mice with experimental pulmonary fibrosis, suggesting its involvement in Smad2/3-dependent pathways (Cui et al., 2016). Additionally, MIR-27a has been found to regulate the TGF-β signaling pathway by targeting SMAD2 and SMAD4 in lung cancer (Chae et al., 2017). These findings add context to the potential role of hsa-mir-27a-3p in the regulatory network associated with RA pathogenesis.

4. Conclusions

Our study focuses on rheumatoid arthritis, a serious ailment that affects people all over the world. According to the findings of this research, it can be inferred that five specific genes exhibit greater significance, namely STAT1, FN1, FASLG, NCAM1, and GATA1. A few of these genes are also associated with other diseases such as Mental disorders like bipolar disorder, anaemia, respiratory disorders and other autoimmune disorders. In addition, miRNA, has-mir-27a-3p and three transcription factors, GATA1, NFKB1, and RELA were predicted to play a significant role in controlling the gene expression of the hub genes. Therefore, this study indicates that the identified hub genes and miRNAs might provide new concepts for developing diagnosis and serve as a platform for developing therapeutics against rheumatoid arthritis.

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6. Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ORCID

Daniel Alex Anand https://orcid.org/0000-0002-5842-5365

Athista Manoharan https://orcid.org/0000-0003-0966-7971

Swetha Sunkar https://orcid.org/0000-0001-9103-5070

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