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# Functional modules analysis based on protein-protein network analysis in ankylosing spondylitis

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**Abstract. – BACKGROUND: Ankylosing spondylitis (AS) is a chronic inflammatory arthritis involving primarily the sacroiliac joints and the axial skeleton that can lead to bone resorption and bone formation, ultimately resulting in ankylosis. However, our understanding of the genetic mechanisms of AS are far from being clear.**

**AIM: In this study, we identified the differentially expressed genes (DEGs) in AS samples compared with healthy control, and mapped these DEGs to the protein-protein interaction network.**

**MATERIALS AND METHODS: We applied a statistical approach of MCODE to cluster proteins in the network. Six functional modules were identified in our network.**

**CONCLUSIONS: Functional studies of these 6 modules suggest that the DEGs may play roles in both the inflammatory environment and bone and cartilage effects. We anticipate the results from this study will provide the groundwork for the understanding of AS pathogenesis.**

*Key Words:*
Protein-protein interaction network, Functional modules.

## Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory arthritis involving primarily the sacroiliac joints and the axial skeleton that can lead to bone resorption and bone formation, ultimately resulting in ankylosis[1,2]. The AS can cause inflammation or injury to other joints away from the spine, as well as to other organs, such as the eyes, heart, lungs, and kidneys. Early in 30 years ago, the strong association between human leukocyte antigen B27 (HLA-B27) and susceptibility to AS was reported. However, HLA-B27 accounts only for about 5% of the genetic risk in AS[3]. Non-HLA-B27 genetic and environmental factors have an important role in the development and progression of this disease.

Genome-wide transcriptome analysis using expression arrays recently has gained popularity as a means to acquire insight into disease pathogenesis, molecular classification, and identification of biomarkers for progression or treatment response. In recent years, genome-wide association studies have identified several other non-HLA susceptibility genes such as IL23R, ARTS1 and ERAP1 in AS[4-6]. However, the current understanding of the pathogenesis of this disorder is limited.

Cellular processes are coordinately carried out by groups of proteins interacting with each other in functional modules, and the modular structure of complex networks is critical to function[7-9]. Network clusters are defined as sub-groups of protein-protein interactions (PPI) network whose proteins are closely linked and work together for the same cellular process. Proteins within each cluster are relatively homogeneous in function, and the clusters are relatively independent of each other[1]. Identifying such functional modules in PPI networks is essential for understanding the functions, organization of biological systems.

In this study, we identified the functional modules in AS by integrating the protein-protein interactions and gene expression profiles. The availability and integration of high-throughput gene expression data and the genome-wide protein-protein interaction may shed new lights on AS study.

## Data and Methods

### Microarray Data

The transcription profile GSE30122 of ankylosing spondylitis was downloaded from GEO (http://www.ncbi.nlm.nih.gov/geo/), a public func-

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tional genomics data repository, which is based on the Illumina Inc. GPL6947 platform data (Illumina Human HT-12 whole-genome expression BeadChips). Total 32 chips were available for further analysis, including a total of 16 active AS patients, classified according to the New York criteria[11], and 16 gender- and age matched controls.

### Protein-Protein Interaction (PPI) Data

The Human Protein Reference Database (HPRD)[12] is a protein database accessible through the internet. The Biological General Repository for Interaction Datasets (BioGRID)[13] is a curated biological database of protein-protein and genetic interactions. Total 326,119 unique PPI pairs were collected in which 39,240 pairs are from HPRD and 379,426 pairs are from BioGRID. We constructed an ensemble protein-protein interaction network by integrating the PPI data collected from the two above PPI databases in human.

### Differentially Expressed Genes (DEGs) Analysis

For the GSE25101 dataset, the *t*-test method was used to identify DEGs. The raw expression datasets from all conditions were processed into expression estimates using the RMA (robust multiarray averaging) method with the default settings implemented in Bioconductor, and then construct the linear model. To circumvent the multi-test problem which might induce too much false positive results, the BH method[14] was used to adjust the raw *p*-values into false discovery rate (FDR). The genes only with FDR less than 0.05 and fold change value larger than 1.5 or less than 0.67 were selected as DEGs.

### Functional Module Network Construction

MCODE (Molecular Complex Detection) effectively finds densely connected regions of a molecular interaction network, many of which correspond to known molecular complexes, based solely on connectivity data[15]. MCODE detects protein complexes that are with the highest quality, in terms of the function and localization similarity of proteins within predicted complexes. We used MCODE to identify the functional modules with the number of nodes larger than 5.

### Gene Ontology Analysis

DAVID (The Database for Annotation, Visualization and Integrated Discovery) consists of an integrated biological knowledgebase and analytic tools aimed at systematically extracting biological meaning from large gene/protein lists[16]. To explore whether genes in each target group share a common biological function, we searched for over-representation in gene ontology (GO) categories. We inputted each group of genes into DAVID for GO term enrichment analysis. The GO terms only with the count larger than 2 and FDR less than 0.01 were selected.

### Pathway Enrichment Analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis based on a system biology approach (http://vortex.cs.wayne.edu/) was used for pathway level analysis the count larger than 2 and FDR less than 0.01.

## Results

### Differentially Expressed Genes in Ankylosing Spondylitis

For dataset GSE 25101, FDR less than 0.05 was chosen as cut-off criterion. The DEGs with fold change value larger than 1.5 were defined as up-regulated genes, and the fold change value less than 0.67 were defined as down-regulated threshold. Finally, we got 71 DEGs in AS samples: 56 out of the 71 genes were up-regulated genes and the remaining 15 genes were down-regulated genes.

### Functional Network Construction in AS

We obtained the PPI interaction data from two databases, HPRD and BioGRID. Total 326119 unique PPI pairs were used to construct a protein-protein interaction (PPI) network by integrating above PPI databases in human. Then, we mapped all the 71 DEGs to the PPI network, and only kept the interactive DEGs and their nearest neighbor genes. Finally, we got a PPI network with 456 nodes and 2367 edges (Figure 1). Total 52 DEGs were included in this network and the remaining 19 DEGs were excluded because they don't interact with other genes.

In order to identify the functional neighborhoods from this PPI network, we employed a statistical approach of MCODE to cluster proteins. We got a total of 9 modules in this network, 6 of which have more than 5 nodes (Figure 2).

### Functional Evaluation of the Modules

To functionally classify these 6 modules, we used the online biological classification tool DAVID and observed significant enrichment of

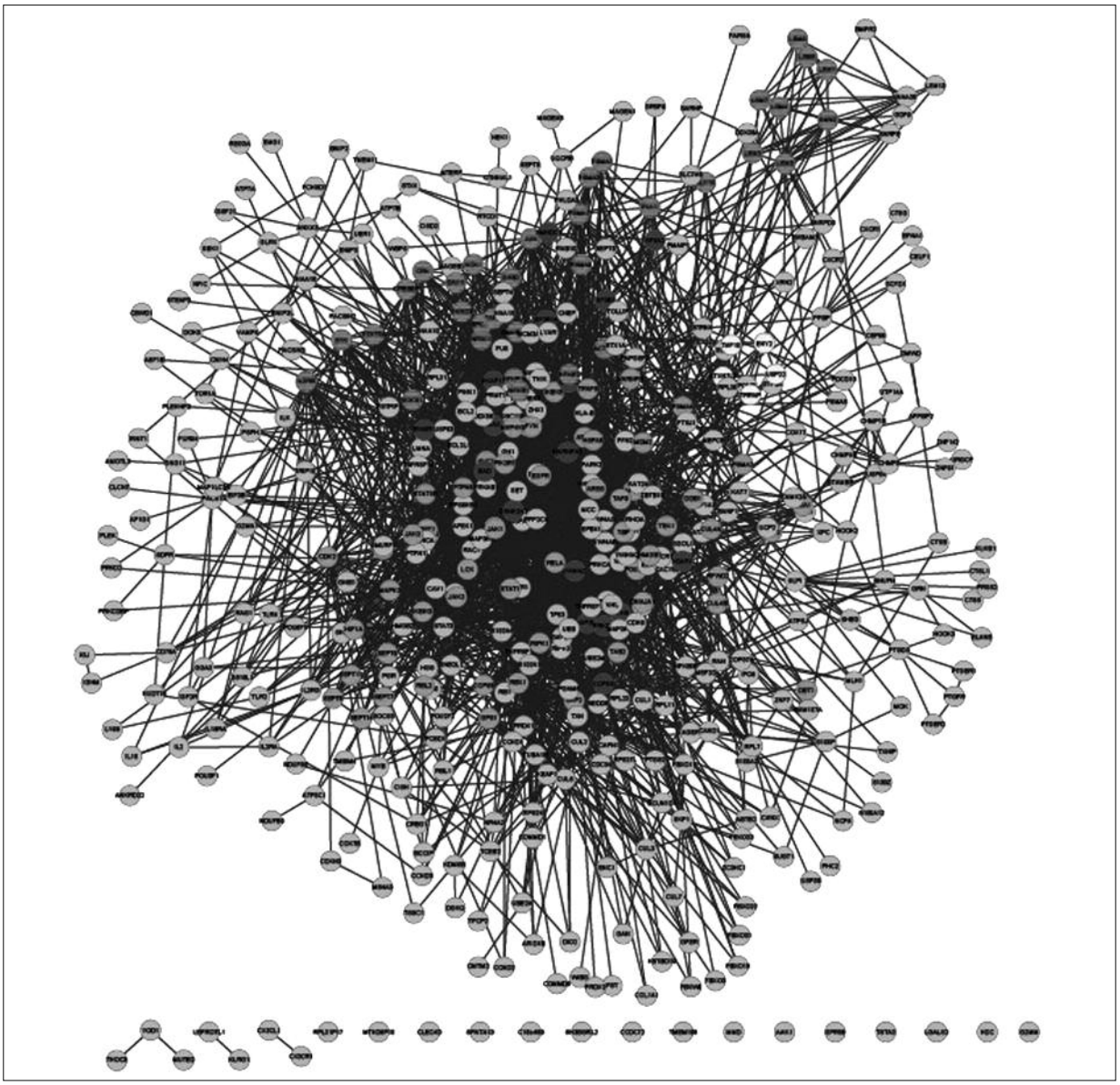

**Figure 1.** The PPI network of DEGs. The nodes represent proteins and edges represent pair-wise interactions. The pink nodes represent the neighbor genes of the interactive DEGs. The other color nodes represent the DEGs.

these genes in multiple GO categories (Table I). Total 12 GO terms were enriched in cluster 1. The most significant enrichment was the GO category of regulation of cellular protein metabolic process with FDR = 5.57E-07. The other significant GO categories included positive regulation of macromolecule metabolic process (FDR = 3.81E-06) and regulation of protein modification process (FDR = 2.79E-05). Total 10 GO terms were enriched in cluster 2. The most significant enrichment was the GO category of modification-dependent protein catabolic process with FDR = 4.77E-05. Total 9 GO terms were enriched in cluster 3, with the most significant enriched GO category of histone deubiquitination (FDR = 5.26E-10). There are only one enriched GO category in cluster 4, cluster 5 and cluster 6, respectively. The cluster 4 was enriched in GO category of cell cycle with FDR = 0.001251. The cluster 5 was enriched in GO category of RNA splicing with FDR = 0.005943. The cluster 5 was enriched in GO category of cell morphogenesis with FDR = 0.009722.

To gain further insights into the function of genes in our network, we performed pathway enrichment analysis using the KEGG pathways (Table II). Expect for cluster 3 and cluster 6, the

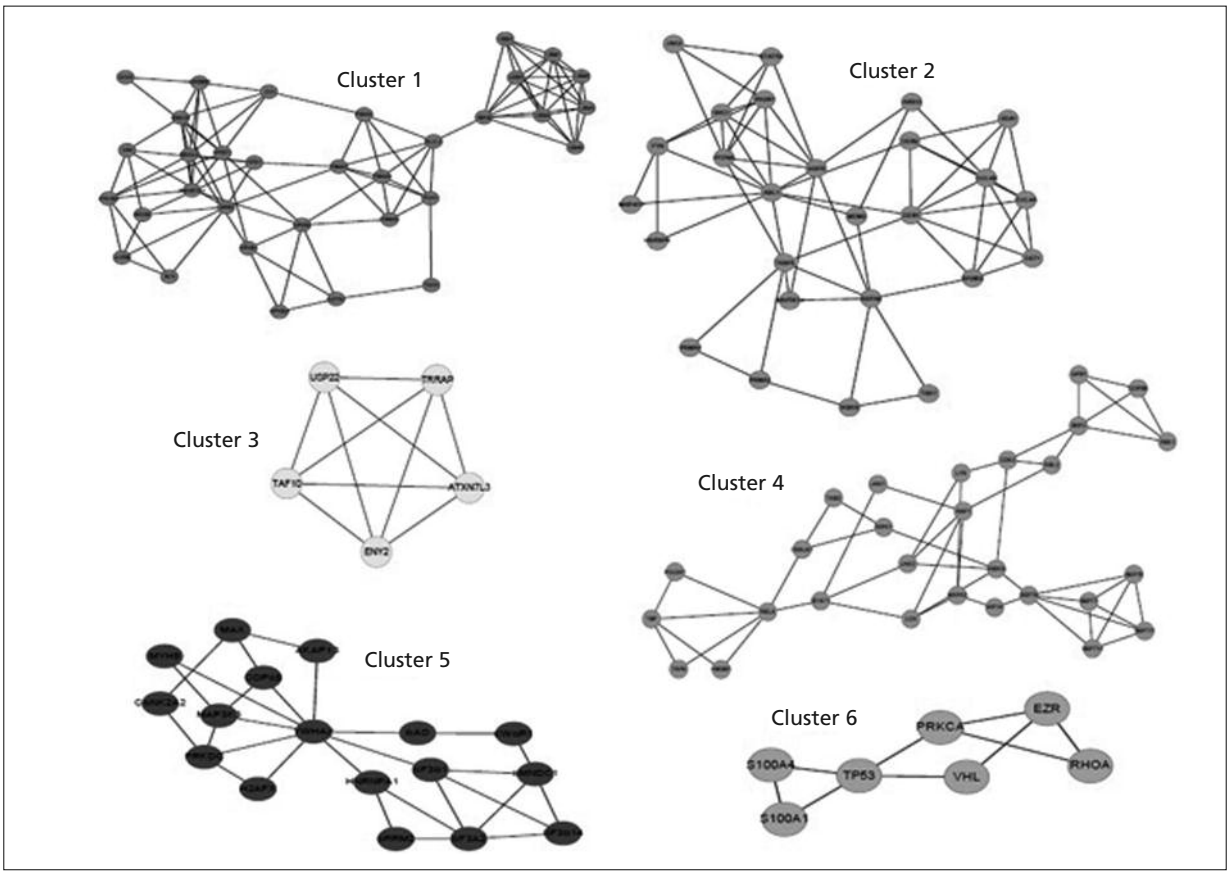


**Figure 2.** The functional modules in the PPI network.

remaining clusters were enriched in several KEGG pathways. The most significant enriched pathways in cluster 1 is RNA degradation with FDR = 3.66E-05. The most significant enriched pathways in cluster 2 is ubiquitin mediated proteolysis with FDR = 6.35E-05. The most significant enriched pathways in cluster 4 is Pathways in cancer with FDR = 1.12E-04. Only one pathway was enriched in cluster 5, which is spliceosome with FDR = 0.004717.

## Discussion

Ankylosing spondylitis (AS) is a disease that affects young people, who generally present at around 26 years of age. Although there are several popular hypothesis of ovarian cancer pathogenesis[17-20], the genetic mechanisms of AS are far from being clear. In this study, we obtained a protein interaction network of DEGs, by combining the DEGs in AS samples compared with control samples and the pair-wise interactions between proteins. Further, we study the properties of this network to identify functional modules: sets of proteins that together are involved in a biological process.

We identified 71 differentially expressed genes between AS patients and healthy controls. Among them, 56 were up regulated and 15 were down-regulated. Hub genes are believed to play major roles in a highly interacted network. In this study, we have defined many highly connected hub genes in each module network. Further data mining revealed that some of these genes have well-documented inflammatory roles or an action on bone/cartilage metabolism. For example, the gene NR3C1 in cluster 1 is essential for many inflammatory and immune responses. NR3C1, also known as GR (glucocorticoid receptor), is expressed in almost every cell in the body and involved in inflammatory responses, cellular proliferation, and differentiation in target tissues. The gene breast cancer gene 1 (BRCA1) produces a protein which plays a role in transcription, DNA repair of double-stranded breaks ubiquitination, transcriptional regulation as well as other functions[21,22]. Research suggests that BRCA1 proteins

**Table I.** Go enrichment analysis of the clusters.

| Class | Term | Description | Count | *p*-value | FDR |
|---|---|---|---|---|---|
| Cluster 1 | GO:0032268 | Regulation of cellular protein metabolic process | 13 | 3.56E-10 | 5.57E-07 |
| Cluster 1 | GO:0010604 | Positive regulation of macromolecule metabolic process | 15 | 2.43E-09 | 3.81E-06 |
| Cluster 1 | GO:0031399 | Regulation of protein modification process | 10 | 1.78E-08 | 2.79E-05 |
| Cluster 1 | GO:0032270 | Positive regulation of cellular protein metabolic process | 8 | 9.58E-07 | 0.0015 |
| Cluster 1 | GO:0051247 | Positive regulation of protein metabolic process | 8 | 1.27E-06 | 0.001988 |
| Cluster 1 | GO:0031398 | Positive regulation of protein ubiquitination | 6 | 1.44E-06 | 0.002262 |
| Cluster 1 | GO:0031396 | Regulation of protein ubiquitination | 6 | 3.43E-06 | 0.005371 |
| Cluster 1 | GO:0032269 | Negative regulation of cellular protein metabolic process | 7 | 3.47E-06 | 0.005434 |
| Cluster 1 | GO:0008380 | RNA splicing | 8 | 3.58E-06 | 0.005609 |
| Cluster 1 | GO:0031401 | Positive regulation of protein modification process | 7 | 4.33E-06 | 0.006775 |
| Cluster 1 | GO:0051248 | Negative regulation of protein metabolic process | 7 | 4.33E-06 | 0.006775 |
| Cluster 1 | GO:0010605 | Negative regulation of macromolecule metabolic process | 11 | 4.51E-06 | 0.007059 |
| Cluster 2 | GO:0019941 | Modification-dependent protein catabolic process | 11 | 3.21E-08 | 4.77E-05 |
| Cluster 2 | GO:0043632 | Modification-dependent macromolecule catabolic process | 11 | 3.21E-08 | 4.77E-05 |
| Cluster 2 | GO:0051603 | Proteolysis involved in cellular protein catabolic process | 11 | 4.89E-08 | 7.26E-05 |
| Cluster 2 | GO:0044257 | Cellular protein catabolic process | 11 | 5.12E-08 | 7.61E-05 |
| Cluster 2 | GO:0030163 | Protein catabolic process | 11 | 6.86E-08 | 1.02E-04 |
| Cluster 2 | GO:0007243 | Protein kinase cascade | 9 | 2.09E-07 | 3.10E-04 |
| Cluster 2 | GO:0044265 | Cellular macromolecule catabolic process | 11 | 2.88E-07 | 4.28E-04 |
| Cluster 2 | GO:0009057 | Macromolecule catabolic process | 11 | 5.74E-07 | 8.52E-04 |
| Cluster 2 | GO:0006793 | Phosphorus metabolic process | 11 | 4.27E-06 | 0.006332 |
| Cluster 2 | GO:0006796 | Phosphate metabolic process | 11 | 4.27E-06 | 0.006332 |
| Cluster 3 | GO:0016578 | Histone deubiquitination | 5 | 5.13E-13 | 5.26E-10 |
| Cluster 3 | GO:0016579 | Protein deubiquitination | 5 | 1.07E-11 | 1.10E-08 |
| Cluster 3 | GO:0070646 | Protein modification by small protein removal | 5 | 1.70E-11 | 1.75E-08 |
| Cluster 3 | GO:0016570 | Histone modification | 5 | 6.30E-09 | 6.46E-06 |
| Cluster 3 | GO:0016569 | Covalent chromatin modification | 5 | 7.18E-09 | 7.36E-06 |
| Cluster 3 | GO:0070647 | Protein modification by small protein conjugation or removal | 5 | 1.89E-08 | 1.93E-05 |
| Cluster 3 | GO:0016568 | Chromatin modification | 5 | 1.65E-07 | 1.69E-04 |
| Cluster 3 | GO:0006325 | Chromatin organization | 5 | 6.00E-07 | 6.16E-04 |
| Cluster 3 | GO:0051276 | Chromosome organization | 5 | 1.63E-06 | 0.001674 |
| Cluster 4 | GO:0007049 | Cell cycle | 10 | 8.28E-06 | 0.001251 |
| Cluster 5 | GO:0008380 | RNA splicing | 6 | 1.42E-05 | 0.005943 |
| Cluster 6 | GO:0000902 | Cell morphogenesis | 5 | 6.78E-06 | 0.009722 |

**Table II.** Pathway enrichment analysis of the clusters.

| Class | Term | | Count | *p*-value | FDR |
|---|---|---|---|---|---|
| Cluster 1 | hsa03018 | RNA degradation | 7 | 5.90E-07 | 3.66E-05 |
| Cluster 1 | hsa05220 | Chronic myeloid leukemia | 6 | 5.50E-05 | 0.001703 |
| Cluster 1 | hsa05200 | Pathways in cancer | 9 | 3.56E-04 | 0.007339 |
| Cluster 1 | hsa03040 | Spliceosome | 6 | 6.35E-04 | 0.009805 |
| Cluster 1 | hsa04662 | B cell receptor signaling pathway | 5 | 7.84E-04 | 0.00968 |
| Cluster 2 | hsa04120 | Ubiquitin mediated proteolysis | 8 | 1.08E-06 | 6.35E-05 |
| Cluster 2 | hsa04012 | ErbB signaling pathway | 6 | 2.73E-05 | 8.06E-04 |
| Cluster 2 | hsa05220 | Chronic myeloid leukemia | 5 | 2.61E-04 | 0.005123 |
| Cluster 4 | hsa05200 | Pathways in cancer | 10 | 5.02E-07 | 3.06E-05 |
| Cluster 4 | hsa05212 | Pancreatic cancer | 6 | 3.67E-06 | 1.12E-04 |
| Cluster 4 | hsa04620 | Toll-like receptor signaling pathway | 6 | 1.95E-05 | 3.97E-04 |
| Cluster 4 | hsa04062 | Chemokine signaling pathway | 7 | 2.93E-05 | 4.47E-04 |
| Cluster 4 | hsa04662 | B cell receptor signaling pathway | 5 | 1.14E-04 | 0.001393 |
| Cluster 4 | hsa05215 | Prostate cancer | 5 | 2.22E-04 | 0.002259 |
| Cluster 4 | hsa04660 | T cell receptor signaling pathway | 5 | 4.68E-04 | 0.004072 |
| Cluster 4 | hsa05221 | Acute myeloid leukemia | 4 | 0.001018 | 0.007734 |
| Cluster 4 | hsa04621 | NOD-like receptor signaling pathway | 4 | 0.001236 | 0.00835 |
| Cluster 5 | hsa03040 | Spliceosome | 5 | 1.53E-04 | 0.004717 |

regulate the activity of other genes and play a critical role in regulation of cell cycle checkpoints[23]. The gene CDK2 is a member of the cyclin-dependent kinase family of Ser/Thr protein kinases. It is a catalytic subunit of the cyclin-dependent kinase complex, whose activity is restricted to the G1-S phase of the cell cycle, and is essential for the G1/S transition. Cdk2 plays an critical role in cell proliferation[24].

Protein-protein interaction networks serve to carry out basic molecular activity in the cell. They are crucial for many biological functions[25]. Understanding the structure and function of these fundamental cellular networks is essential for study the pathogenesis of diseases. In this work, we identified six significant functional modules using a statistical approach of MCODE and explore the function of each module using the online biological classification tool DAVID. From Table I, we can find that several GO categories were enriched in each cluster. In fact, almost all of the significant GO categories can be divided into two groups: protein metabolism (cluster 1 and cluster 2) and cell cycle biological function (cluster 3, cluster 4 and cluster 5). Our finds is consist with previous study which suggests that spinal inflammation and new bone formation are characteristic of AS[26,27].

We also performed functional study to identify pathways that play a role in the pathogenesis of AS. From our result of Table II, we can find that 4 clusters were enriched in several KEGG pathways expect for cluster 3 and cluster 6. A number of these pathways have well-documented inflammatory roles or an action on immune response. For example, the nucleotide oligomerization domain (NOD)-like receptor signaling pathway (hsa04621), B cell receptor signaling pathway (hsa04662), T cell receptor signaling pathway (hsa04660) and so on.

NOD-like receptors may form oligomers that activate inflammatory caspases (e.g. caspase1), causing cleavage and activation of important inflammatory cytokines such as IL-1. NOD Like Receptors (NLRs) may also activate the NF-κ (nuclear factor-κβ) signaling pathway to induce production of inflammatory molecules[28,29]. Activation of T lymphocytes is a key event for an efficient response of the immune system. B cells are an important component of adaptive immunity. They produce and secrete millions of different antibody molecules, each of which recognizes a different (foreign) antigen. Our result also detected that Toll-like receptor signaling pathway (hsa04620) was enriched in cluster 4 which provide support for the importance of a pathogen-associated molecular pattern in the pathogenesis of AS. This find is in line with previous study[2].

## Conclusions

We identified 71 differentially expressed genes in AS samples and identified 6 functional modules by integrating these DEGs and the protein-protein interactions. Functional studies of these 6 modules suggest that the DEGs may play roles in both the inflammatory environment and bone and cartilage effects. Results from this study will provide the groundwork for the understanding of AS pathogenesis. Future investigations are needed to confirm some of the possible interactions suggested by this research.

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