# Screening of underlying genetic biomarkers for ankylosing spondylitis

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**Abstract.** Genetic biomarkers for the diagnosis of ankylosing spondylitis (AS) remain unreported except for human leukocyte antigen B27 (HLA-B27). Therefore, the aim of the present study was to screen the differentially expressed genes (DEGs), and those that also possess differential single nucleotide polymorphism (SNP) loci in the whole blood of AS patients compared with healthy controls by integrating two mRNA expression profiles (GSE73754 and GSE25101) and SNP microarray data (GSE39428) collected from the Gene Expression Omnibus (GEO). Using the t-test, 1,056 and 1,073 DEGs were identified in the GSE73754 and GSE25101 datasets, respectively. Among them, 234 DEGs were found to be shared in both datasets, which were subsequently overlapped with 122 differential SNPs of genes in the GSE39428 dataset, resulting in identification of two common genes [eukaryotic translation elongation factor 1 epsilon 1 (*EEF1E1*) and serpin family A member 1 (*SERPINA1*)]. Their expression levels were significantly upregulated and the average expression log R ratios of SNP sites in these genes were significantly higher in AS patients than those in controls. Function enrichment analysis revealed that *EEF1E1* was involved in AS by influencing the aminoacyl-tRNA biosynthesis, while *SERPINA1* may be associated with AS by participating in platelet degranulation. However, only the genotype and allele frequencies of SNPs (rs7763907 and rs7751386) in *EEF1E1* between AS and controls were significantly different between AS and the controls, but not *SERPINA1*. These findings suggest that *EEF1E1* may be an underlying genetic biomarker for the diagnosis of AS.

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## Introduction

Ankylosing spondylitis (AS) is a common inflammatory rheumatic disease, with an estimated prevalence (per 10,000) of 23.8 in Europe, 16.7 in Asia, 31.9 in North America, 10.2 in Latin America and 7.4 in Africa (1). AS mainly affects the spine and sacroiliac joints in the pelvis to cause low back pain, stiffness and functional disability, which seriously influence the quality of life of patients and impose a heavy economic burden on both family and society (2). Therefore, there is a need for the timely diagnosis and effective treatment of AS.

Although the pathogenesis remains not clearly defined, accumulating evidence has suggested that AS is highly heritable. Human leukocyte antigen (HLA)-B27, a class I surface antigen encoded by B locus in the major histocompatibility complex (MHC) on the short (p) arm of chromosome 6, is one of the convincing genetic factors associated with AS (3). HLA-B27 was reported to be present in 94.3% of patients with AS, but only 9.34% in organ donors (4). The expression of *HLA-B27* was found to be significantly higher in patients with AS than that in healthy subjects (5). Meta-analyses indicated that *HLA-B27* genetic polymorphism B2704 and B2702 may be risk factors, while B2703, B2706, B2707, B2727, B2729 and B2747 may be protective factors for AS (6,7). *HLA-B27*-positive patients had a significantly younger age at symptom onset, more uveitis, and a higher frequency of peripheral and hip joint involvement than HLA-B27-negative patients (7,8). Thus, *HLA-B27* has been the most commonly used biomarker for the diagnosis of AS (9). However, twin and family studies suggest that *HLA-B27* only can explain less than 30% of the overall risk for AS (10,11), meaning there are other genes related with the genetic disorder of AS. Recently, scholars have also aimed to investigate other inflammatory biomarkers for AS, including interleukin (*IL*)-8 (12), tumor necrosis factor (*TNF*)-α (13), C-reactive protein (*hsCRP*) (14) and C-C motif chemokine 11 (*CCL11*) (15), but studies that have focused on the genetic biomarkers are limited (16,17).

The aim of the present study was to integrate the microarray data of mRNA and the single nucleotide polymorphism (SNP) expression profile in whole blood of AS patients and healthy controls to screen for differentially expressed genes (DEGs), and those that also possess differential SNP loci,

which has not been previously performed. These SNP-related DEGs may be crucial genetic biomarkers for AS.

## Materials and methods

*Microarray data*. Three microarray datasets under accession nos. GSE73754 (18), GSE25101 (19) and GSE39428 (20,21) were downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). GSE73754 (platform: GPL10558; Illumina HumanHT-12 V4.0 expression BeadChip) detected the gene expression profile in whole blood samples from 52 AS and 20 healthy controls; GSE25101 (platform: GPL6947; Illumina HumanHT-12 V3.0 expression BeadChip) compared the gene expression profile in whole blood samples between 16 AS and 20 healthy controls; and GSE39428 (GPL15779; Illumina custom human SNP VeraCode microarray) analyzed the SNPs in 384 genes of 51 AS and 163 healthy controls.

*Data normalization*. For the two expression data from the Illumina platform, the TXT. data were downloaded and preprocessed using the Linear Models for Microarray data (LIMMA) method (22) (version 3.34.0; http://www.bioconductor.org/packages/release/bioc/html/limma.html) in the Bioconductor R package (version 3.4.1; http://www.R-project.org/), including base-2 logarithmic (log2) transformation and quantile normalization. The SNP signal spectrum in the GSE39428 dataset was preprocessed using hidden Markov model (HMM)-based program PennCNV (23) (version 1.0.4; http://penncnv.openbioinformatics.org/en/latest/), including the following steps: i) the signal intensity of the A and B alleles in each SNP were extracted and quantile normalized using the quantile method; ii) the normalize_affy_geno_cluster.pl procedure in the PennCNV package was used to calculate the Log R ratio (LRR) and B allele frequency (BAF) in each SNP, resulting in the generation of baf. files; the kcolumn.pl procedure in the PennCNV package was utilized to split the baf. files to signal intensity of single sample; the copy number variation (CNV) was detected using the detect_cnv.pl procedure in the PennCNV package.

*Differential analysis of mRNAs and SNPs*. The DEGs between control and AS in the GSE73754 and GSE25101 datasets were identified using the LIMMA method (22) based on the t-test where statistical significance was set to |logFC(fold change)| >0.263 and Benjamini and Hochberg adjusted (24) false discovery rate (FDR) <0.05. Hierarchical clustering heatmap illustrating the expression intensity and direction of the common DEGs in two mRNA datasets was constructed using the pheatmap R package (version 1.0.8; https://cran.r-project.org/web/packages/pheatmap) based on Euclidean distance. The differential SNPs were screened by comparing the LRR between AS and controls by using the Student's t-test. The genotype and allele frequencies of SNPs in DEGs between AS and controls were also compared using the Chi-square test (or Fisher's exact test), with P-value <0.05 set as the threshold value.

*PPI (protein-protein interaction) network construction*. The interaction pairs of the common DEGs were retrieved from the STRING 10.0 (Search Tool for the Retrieval of Interacting Genes; http://string db.org/) database (25) and then the PPI network was visualized using the Cytoscape software (version 3.6.1; www.cytoscape.org/) (26). Four topological characteristics of the genes in the PPI network, including degree [the number of edges (interactions) of a node (protein)], betweenness centrality (BC, the number of shortest paths that run through a node), closeness centrality (CC, the average length of the shortest paths between one node and any other node in the network) and average path length (APL, the average of distances between all pairs of nodes), were calculated using the CytoNCA plugin in Cytoscape software (http://apps.cytoscape.org/apps/cytonca) (27), the overlapped genes of the top 35 in four parameters were suggested as crucial genes.

To identify functionally related and highly interconnected clusters from the PPI network, module analysis was carried out by using the Molecular Complex Detection (MCODE) plugin of Cytoscape software under the followed parameters: Degree cutoff =2, Node score cutoff =0.2 and K-core =2 (ftp://ftp.mshri.on.ca/pub/BIND/Tools/MCODE) (28).

*Function enrichment analysis*. The underlying functions of common DEGs between two mRNA datasets, genes in the PPI and modules enrichment analyses were predicted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) online tool (version 6.8; http://david.abcc.ncifcrf.gov). P<0.05 was chosen as the threshold to determine the significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms which were visualized using R language.

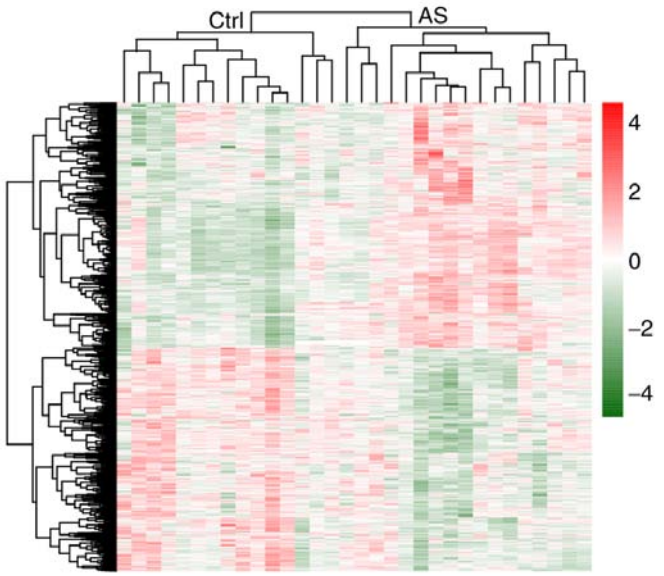


Figure 1. Hierarchical clustering heat map analysis of the common differentially expressed genes in two mRNA expression profile datasets (GSE73754 and GSE25101). AS, ankylosing spondylitis; Ctrl, control. Red indicates high expression; green indicates low expression.

## Results

*Identification of DEGs*. Based on the threshold (FDR <0.05 and |logFC| >0.263), a total of 1,056 and 1,073 DEGs were identified between AS and controls for GSE73754 and GSE25101 datasets, respectively. After comparison analysis,

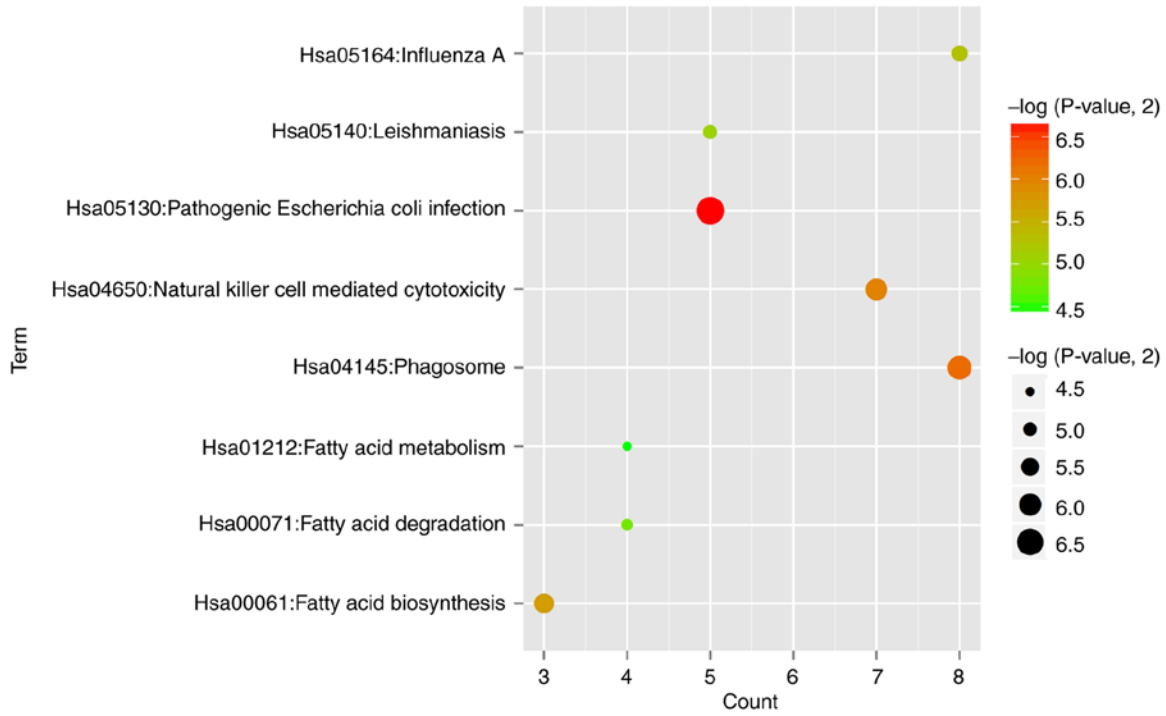


Figure 2. KEGG pathways enrichment for the common differentially expressed genes in two mRNA expression profile datasets (GSE73754 and GSE25101). The horizontal axis is the count of genes that are enriched in the pathways; the vertical axis indicates the KEGG pathways; the circle indicates the level of significance (P-value). KEGG, Kyoto Encyclopedia of Genes and Genomes.

105 upregulated and 129 downregulated DEGs were found to be shared in both two datasets. The hierarchical clustering heatmap suggested that these 234 common DEGs could well distinguish AS from control samples (Fig. 1).

*Function enrichment analysis for the common DEGs*. DAVID database was used to predict the underlying functions of the common DEGs. The results showed that 8 significant KEGG pathways (Fig. 2) were enriched, such as hsa05130:Pathogenic *Escherichia coli* infection (*TLR4*, toll like receptor 4) and hsa04145:Phagosome (*TLR4*) (Table I). In addition, 23 significant GO biological process (BP) terms including GO:0006418~tRNA aminoacylation for protein translation (*EEF1E1*, eukaryotic translation elongation factor 1 epsilon 1; *YARS*, tyrosyl-tRNA synthetase), GO:0051092~positive regulation of NF-κB transcription factor activity (*TLR4*), GO:0050776~regulation of immune response (*KLRD1*, killer cell lectin like receptor D1), and GO:0032715~negative regulation of interleukin-6 production (*TLR4*); 6 significant GO molecular function (MF) terms, consisting of GO:0005515~protein binding (*SERPINA1*, serpin family A member 1; *TLR4*); and 6 significant GO molecular function (MF), such as GO:0005515~protein binding (*SERPINA1*, *EEF1E1*); 26 GO cell component (CC) terms, including GO:0070062~extracellular exosome (*SERPINA1*, *EEF1E1*), GO:0005737~cytoplasm (*EEF1E1*) and GO:0005829~cytosol (*EEF1E1*); were enriched (Fig. 3 and Table I).

*PPI network*. After mapping the DEGs to the STRING database, 356 interaction pairs were obtained which were used for constructing the PPI network where 154 nodes (64 upregulated and 88 downregulated) were included (Fig. 4). By calculating degree, BC, CC and APL, and comparing genes ranked as the top 30, *HDAC1* (histone deacetylase 1), *YARS*, *EPRS* (glutamyl-prolyl-tRNA synthetase), *APEX1* (apurinic/apyrimidinic endodeoxyribonuclease 1), *ACTG1* (actin γ 1), MDH2 (malate dehydrogenase 2), *TNF* (tumor necrosis factor), *CCT3* (chaperonin containing TCP1 subunit 3), *TLR4* (Toll-like receptor 4), *TUBB* (tubulin β class I), *FCGR2A* (Fc fragment of IgG receptor IIa), *KLRD1* (killer cell lectin-like receptor D1) and *FASN* (fatty acid synthase) were found to be shared by these 4 topological characteristics, suggesting they were hub genes for AS (Tables II and III).

Subsequently, four functionally related and highly interconnected modules were screened (Fig. 5). The genes in module 1 were associated with aminoacyl-tRNA biosynthesis (*YARS*) (Fig. 5A); the genes in module 2 were related with natural killer cell mediated cytotoxicity (*KLRD1*) and immune response (*KLRD1*) (Fig. 5B); the genes in module 3 were relevant with metabolic pathways (*EPRS*) (Fig. 5C); and the genes in module 4 were enriched in GO terms of platelet degranulation (*SERPINA1*) (Fig. 5D) (Table IV).

*Integration of SNP microarray and expression profile data*. The LRR of each SNP for 384 genes in AS and control samples was computed. The LRR in most samples were lower than 1, indicating the presence of copy number deletions. Subsequently, the statistical difference in LRR of each SNP between AS and control samples were determined by Student's t-test, with 122 differential SNP identified. After overlapping the genes having differential SNP with the DEGs, two common genes (*EEF1E1* and *SERPINA1*) were obtained. *SERPINA1* was upregulated in AS (Fig. 6A) and the average expression LRR of the rs6575424 polymorphism in AS samples was

Table I. Function enrichment for the differentially expressed genes between patients with ankylosing spondylitis and controls.

| Category | Term | P-value | Genes |
|---|---|---|---|
| KEGG_PATHWAY | hsa05130:Pathogenic *Escherichia coli* infection | 9.97E-03 | *ACTG1*, *TUBB*, *EZR*, *TLR4*, *TUBA1B* |
| KEGG_PATHWAY | hsa04145:Phagosome | 1.36E-02 | *ACTG1*, *TUBB*, *NCF4*, *TLR4*, *FCGR2A*, *M6PR*, *TUBA1B*, *HLA-DRA* |
| KEGG_PATHWAY | hsa04650:Natural killer cell mediated cytotoxicity | 1.58E-02 | *IFNAR2*, *TNFSF10*, *TNF*, *CD247*, *KLRD1*, *SH2D1B*, *HCST* |
| KEGG_PATHWAY | hsa00061:Fatty acid biosynthesis | 1.90E-02 | *ACSL1*, *FASN*, *ACSL4* |
| KEGG_PATHWAY | hsa05164:Influenza A | 2.56E-02 | *ACTG1*, *IFNAR2*, *TNFSF10*, *TNF*, *MAP2K4*, *TLR4*, *IVNS1ABP*, *HLA-DRA* |
| KEGG_PATHWAY | hsa05140:Leishmaniasis | 3.01E-02 | *TNF*, *NCF4*, *TLR4*, *FCGR2A*, *HLA-DRA* |
| KEGG_PATHWAY | hsa00071:Fatty acid degradation | 3.63E-02 | *ACSL1*, *ECHS1*, *ACSL4*, *ALDH9A1* |
| KEGG_PATHWAY | hsa01212:Fatty acid metabolism | 4.52E-02 | *ACSL1*, *FASN*, *ECHS1*, *ACSL4* |
| GOTERM_BP_ DIRECT | GO:0007166~cell surface receptor signaling pathway | 5.44E-05 | *CD8A*, *CD247*, *EVL*, *BIRC2*, *ADGRG1*, *IFNAR2*, *TNFSF10*, *ADRB2*, *KLRG1*, *NUP62*, *TDP2*, *CD81*, *CDA*, *KLRD1* |
| GOTERM_BP_ DIRECT | GO:0006418~tRNA aminoacylation for protein translation | 1.67E-03 | *YARS*, *EEF1E1*, *AARS*, *EPRS*, *QARS* |
| GOTERM_BP_ DIRECT | GO:0043123~positive regulation of I-kappaB kinase/NF-kappaB signaling | 4.76E-03 | *CARD11*, *TNFSF10*, *TNF*, *NUP62*, *PINK1*, *CXXC5*, *BIRC2*, *S100A12* |
| GOTERM_BP_ DIRECT | GO:0051092~positive regulation of NF-kappaB transcription factor activity | 7.34E-03 | *CARD11*, *IRAK3*, *NLRC4*, *TNF*, *PRKCH*, *TLR4*, *S100A12* |
| GOTERM BP_DIRECT_ | GO:0050776~regulation of immune response | 8.11E-03 | *CARD11*, *CD96*, *CD8A*, *CD247*, *CD81*, *KLRD1*, *SH2D1B*, *HCST* |
| GOTERM_ BP_DIRECT | GO:2001240~negative regulation of extrinsic apoptotic signaling pathway in absence of ligand | 1.17E-02 | *TNF*, *ZC3HC1*, *MCL1*, *CX3CR1* |
| GOTERM_BP_ DIRECT | GO:0030890~positive regulation of B cell proliferation | 1.35E-02 | *CARD11*, *CD81*, *TLR4*, *ADA* |
| GOTERM_BP_ DIRECT | GO:2000377~regulation of reactive oxygen species metabolic process | 2.66E-02 | *TNF*, *PINK1*, *BIRC2* |
| GOTERM_BP_ DIRECT | GO:0071353~cellular response to interleukin-4 | 3.74E-02 | *XBP1*, *FASN*, *TUBA1B* |
| GOTERM_BP_ DIRECT | GO:0032715~negative regulation of interleukin-6 production | 4.96E-02 | *IRAK3*, *TNF*, *TLR4* |
| GOTERM_MF_ DIRECT | GO:0005515~protein binding | 3.50E-10 | *PDLIM7*, *PPP2R5A*, *TLR1*, *CNOT2*, *TLR4*, *RNF216*, *CCT3*, *ARID1A*, *TGFA*, *SERPINA1* |
| GOTERM_MF_ DIRECT | GO:0044822~poly(A) RNA binding | 1.11E-04 | *ABCF1*, *CCT3*, *ZNF207*, *EXOSC10*, *HNRNPM*, *EZR*, *FASN*, *APEX1*, *YARS*, *MDH2* |
| GOTERM_MF_ DIRECT | GO:0005524~ATP binding | 5.01E-03 | *ABCF1*, *PINK1*, *MAP4K1*, *QARS*, *CCT3*, *TRIB1*, *ACTG1*, *EPRS*, *ADK*, *EIF4A1* |
| GOTERM_MF_ DIRECT | GO:0042288~MHC class I protein binding | 2.42E-02 | *TUBB*, *CD8A*, *ATP5A1* |
| GOTERM_MF_ DIRECT | GO:0031625~ubiquitin protein ligase binding | 3.16E-02 | *ACTG1*, *RPA2*, *TUBB*, *XBP1*, *SLC25A5*, *RALB*, *PINK1*, *TUBA1B*, *TRIB1* |
| GOTERM_MF_ DIRECT | GO:0047485~protein N-terminus binding | 3.59E-02 | *RPA2*, *HDAC1*, *BIRC2*, *GLRX*, *FEZ1* |
| GOTERM_CC_ DIRECT | GO:0070062~extracellular exosome | 3.20E-06 | *HIST2H2AA3*, *CAPZA2*, *PTGS1*, *CCT3*, *PDHB*, *RTN3*, *ACTG1*, *N4BP2L2*, *CCNY*, *LILRA5* |

Table I. Continued.

| Category | Term | P-value | Genes |
|---|---|---|---|
| GOTERM_CC_ DIRECT | GO:0005737~cytoplasm | 1.87E-05 | *ABCF1, C9ORF72, E2F3, PDLIM7, AGTPBP1, PPP2R5A, PTGS1, CNOT2, PINK1, SHOC2* |
| GOTERM_CC_ DIRECT | GO:0005829~cytosol | 1.39E-04 | *ABCF1, AGTPBP1, CAPZA2, CNOT2, PINK1, DPH2, RNF216, QARS, ARHGAP17, CCT3* |
| GOTERM_CC_ DIRECT | GO:0030529~intracellular ribonucleoprotein complex | 2.73E-04 | *ZFP36L2, HNRNPM, NUP62, CSNK1E, RPL22, SNRPB, EPRS, DYRK2, HNRNPR* |
| GOTERM_CC_DIRECT | GO:0016020~membrane | 1.212E-03 | *ABCF1, KCNJ15, GNAI3, TNF, MCL1, PPP2R5A, CAPZA2, TLR1, CD247, CNOT2* |

KEGG, Kyoto encyclopedia of Genes and Genomes; GO, Gene Ontology; BP, biological process; MF, molecular function; CC, cell component.

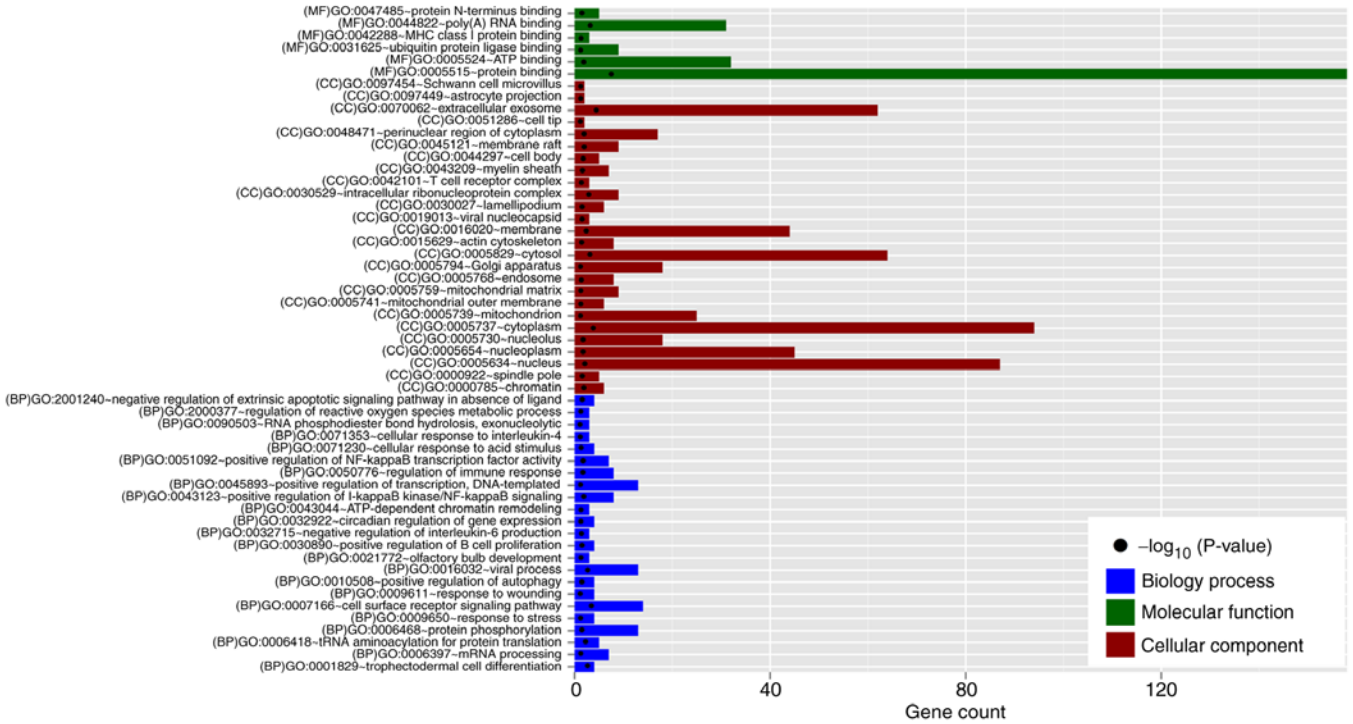


Figure 3. GO terms for the common differentially expressed genes in the two mRNA expression profile datasets (GSE73754 and GSE25101). The horizontal axis displays the count of genes that are enriched in the GO term; the vertical axis lists the GO terms. The circle indicates the level of significance (P-value). GO, Gene Ontology.

significantly higher than that in the controls (0.05 vs. -0.14, P=6.57E-07) (Fig. 6B); *EEF1E1* was also upregulated in AS (Fig. 6A) and the average expression LRRs of rs7763907 (-4.88 vs. -5.91, P=0.048), rs9328453 (0.07 vs. -0.12, P=3.69E-05) (Fig. 6B), rs7751386 (-0.85 vs. -1.49, P=2.52E-04), and rs12660697 (0.08 vs. -0.02, P=0.02) polymorphisms in AS samples were significantly higher than that in controls.

Furthermore, the genotype and allele frequencies of SNPs in *EEF1E1* and *SERPINA1* between AS and controls were compared using the Chi-square (or Fisher's exact) test. The results showed there were significant differences in the genotype and allele frequencies of rs7763907 between AS and control samples. The genotype frequency of rs7751386 between AS and control samples was also significantly differential. These findings suggest that these two polymorphic sites of the *EEF1E1* gene may be associated with the susceptibility to acquire AS (Table V).

## Discussion

In the present study, two crucial genes (*EEF1E1* and *SERPINA1*) were identified for the diagnosis of ankylosing spondylitis (AS) by analyzing two mRNA expression profile datasets and one single nucleotide polymorphism (SNP) dataset. Their expression levels were significantly upregulated and the average expression LRRs of SNP sites in these genes were significantly higher in AS patients that those in the controls. *EEF1E1* was involved in AS by influencing aminoacyl-tRNA biosynthesis, while *SERPINA1* may be associated with AS by participating in platelet degranulation.

Table II. Topological characteristics.

A, Degree

| Genes | Value |
|---|---|
| *TNF* | 24 |
| *EPRS* | 19 |
| *ACTG1* | 17 |
| *YARS* | 16 |
| *TLR4* | 14 |
| *HDAC1* | 14 |
| *CCT7* | 14 |
| *NOP56* | 13 |
| *MDH2* | 13 |
| *IMP3* | 12 |
| *CCT3* | 12 |
| *EIF4A1* | 12 |
| *ATP5A1* | 11 |
| *POLR1C* | 11 |
| *GNAI3* | 10 |
| *CS* | 10 |
| *ATIC* | 10 |
| *APEX1* | 9 |
| *NOP2* | 9 |
| *SNRPB* | 9 |
| *CD247* | 9 |
| *KLRD1* | 9 |
| *DDX47* | 8 |
| *AARS* | 8 |
| *MCL1* | 8 |
| *SRSF5* | 8 |
| *TUBB* | 8 |
| *FASN* | 8 |
| *FCGR2A* | 7 |

B, Closeness centrality

| Genes | Value |
|---|---|
| *RNF126* | 1.0000 |
| *KLHL2* | 1.0000 |
| *FBXO21* | 1.0000 |
| *GPBP1* | 1.0000 |
| *PLEKHF1* | 1.0000 |
| *RTN3* | 1.0000 |
| *RRAGD* | 1.0000 |
| *TNF* | 0.4000 |
| *HDAC1* | 0.3946 |
| *ACTG1* | 0.3852 |
| *CCT3* | 0.3605 |
| *YARS* | 0.3596 |
| *EPRS* | 0.3570 |
| *ALDH9A1* | 0.3510 |
| *FCGR2A* | 0.3427 |
| *MDH2* | 0.3387 |

Table II. Continued.

B, Closeness centrality

| Genes | Value |
|---|---|
| *APEX1* | 0.3349 |
| *ADA* | 0.3333 |
| *FASN* | 0.3318 |
| *CS* | 0.3288 |
| *ATIC* | 0.3281 |
| *TLR4* | 0.3274 |
| *EZR* | 0.3244 |
| *DDB1* | 0.3237 |
| *CCT7* | 0.3230 |
| *AARS* | 0.3223 |
| *KLRD1* | 0.3216 |
| *TUBB* | 0.3216 |
| *CYB5R4* | 0.3216 |

C, Betweenness centrality

| Genes | Value |
|---|---|
| *TNF* | 0.2996 |
| *ACTG1* | 0.2278 |
| *HDAC1* | 0.1883 |
| *YARS* | 0.0824 |
| *EPRS* | 0.0783 |
| *TLR4* | 0.0753 |
| *GNAI3* | 0.0745 |
| *CD247* | 0.0743 |
| *APEX1* | 0.0688 |
| *RALB* | 0.0622 |
| *ALDH9A1* | 0.0559 |
| *EIF4A1* | 0.0558 |
| *ADA* | 0.0508 |
| *FASN* | 0.0507 |
| *KLRD1* | 0.0499 |
| *TUBB* | 0.0491 |
| *FCGR2A* | 0.0430 |
| *MDH2* | 0.0382 |
| *EZR* | 0.0360 |
| *CYB5R4* | 0.0359 |
| *CCT3* | 0.0337 |
| *PRKCH* | 0.0316 |
| *MCL1* | 0.0315 |
| *NUP214* | 0.0289 |
| *HIST2H2AA3* | 0.0288 |
| *SERPINA1* | 0.0286 |
| *TDP2* | 0.0282 |
| *SHOC2* | 0.0274 |
| *MAP4K1* | 0.0273 |

Table II. Continued.

D, Average path length

| Genes | Value |
|---|---|
| *RNF126* | 1.0000 |
| *KLHL2* | 1.0000 |
| *FBXO21* | 1.0000 |
| *GPBP1* | 1.0000 |
| *PLEKHF1* | 1.0000 |
| *RTN3* | 1.0000 |
| *RRAGD* | 1.0000 |
| *TNF* | 2.5000 |
| *HDAC1* | 2.5342 |
| *ACTG1* | 2.5959 |
| *CCT3* | 2.7740 |
| *YARS* | 2.7808 |
| *EPRS* | 2.8014 |
| *ALDH9A1* | 2.8493 |
| *FCGR2A* | 2.9178 |
| *MDH2* | 2.9521 |
| *APEX1* | 2.9863 |
| *ADA* | 3.0000 |
| *FASN* | 3.0137 |
| *CS* | 3.0411 |
| *ATIC* | 3.0479 |
| *TLR4* | 3.0548 |
| *EZR* | 3.0822 |
| *DDB1* | 3.0890 |
| *CCT7* | 3.0959 |
| *AARS* | 3.1027 |
| *KLRD1* | 3.1096 |
| *TUBB* | 3.1096 |
| *CYB5R4* | 3.1096 |

Table III. Overlapping DEGs according to topological features (degree, closeness centrality, betweenness centrality and average path length).

| Common genes | Expression |
|---|---|
| *HDAC1* | Down |
| *YARS* | Down |
| *EPRS* | Down |
| *APEX1* | Down |
| *ACTG1* | Down |
| *MDH2* | Down |
| *TNF* | Down |
| *CCT3* | Down |
| *TLR4* | Up |
| *TUBB* | Down |
| *FCGR2A* | Up |
| *KLRD1* | Down |
| *FASN* | Down |

EEF1E1, also known as aminoacyl-tRNA synthetase-interacting multifunctional protein 3 (AIMP3/p18), was initially found to encode an auxiliary component of the macromolecular aminoacyl-tRNA synthase complex that catalyzes the ligation of a specific amino acid to its compatible cognate tRNA to form an aminoacyl-tRNA to initiate protein translation (29,30). Thus, *EEF1E1* may be upregulated to promote the development of various types of cancer (31). However, recent studies indicate that EEF1E1 may also function as a tumor-suppressor (32,33) by upregulating the growth factor- or Ras-dependent induction of p53 (34,35). Cells with loss of *EEF1E1* were found to exhibit impaired p53 transactivity and genomic instability and thus were found to became susceptible to cell malignant transformation (34,36), while overexpression of *EEF1E1* induced cellular senescence phenotypes (37). It was also demonstrated that the p53 level was significantly higher in the peripheral blood supernatant of a rheumatoid arthritis (RA) group than the level in control groups and there was a positive correlation between p53 levels and the disease activity score in the RA group (38). In addition, in RA synovial tissues, 80% of p53-positive cells were found to be TUNEL-positive (39). These results indicate that upregulation of the p53 gene may result in chronic inflammation and apoptosis in RA patients. In addition, other members of the AIMP families, such as *AIMP1*, were also found to promote the expression of pro-inflammatory genes in monocytes/macrophages and dendritic cells (40) and induce cytokine (i.e. TNF-α)-dependent apoptosis (41). The antibody atliximab was reported to neutralize the expression of AIMP1 and then block the AIMP1-mediated production of inflammatory cytokines, ultimately attenuating collagen-induced arthritis (42). Accordingly, we speculate that *EEF1E1* may also be involved in inflammation of AS by upregulating p53 and pro-inflammatory cytokines. In line with this hypothesis, our results showed that *EEF1E1* was upregulated in the whole blood of AS patients compared with the control. Upregulation of *EEF1E1* may be attributed to genetic mutations (rs7763907 and rs7751386) since the LRR of AS was significantly higher than that of controls and the genotype and allele frequencies were significantly different. However, further experimental validation is needed as studies investigating the SNPs of *EEF1E1* are limited apart from the study of Liu *et al* that showed the number of risk alleles of rs12199241 in *AIMP3* to be significantly associated with high DNA damage level (43).

*SERPINA1* is a gene that encodes alpha-1-antitrypsin (AAT). It was found that the AAT concentration was higher in AS patients under active phase than the patients with remission/partial remission (44). In addition, the carboxyl terminal fragment of AAT was demonstrated to significantly induce the production of pro-inflammatory molecules (gelatinase B, monocyte chemoattractant protein-1 and IL-6) in human monocytes by interactions with the CD36 scavenger receptor and low density lipoprotein (LDL) receptor (45). These findings suggest that *SERPINA1* may be a potential biomarker for the diagnosis of AS and evaluation of the efficacy of treatment by influencing inflammation. In line with these studies, we also found that *SERPINA1* was upregulated in AS patients and it participated in GO terms

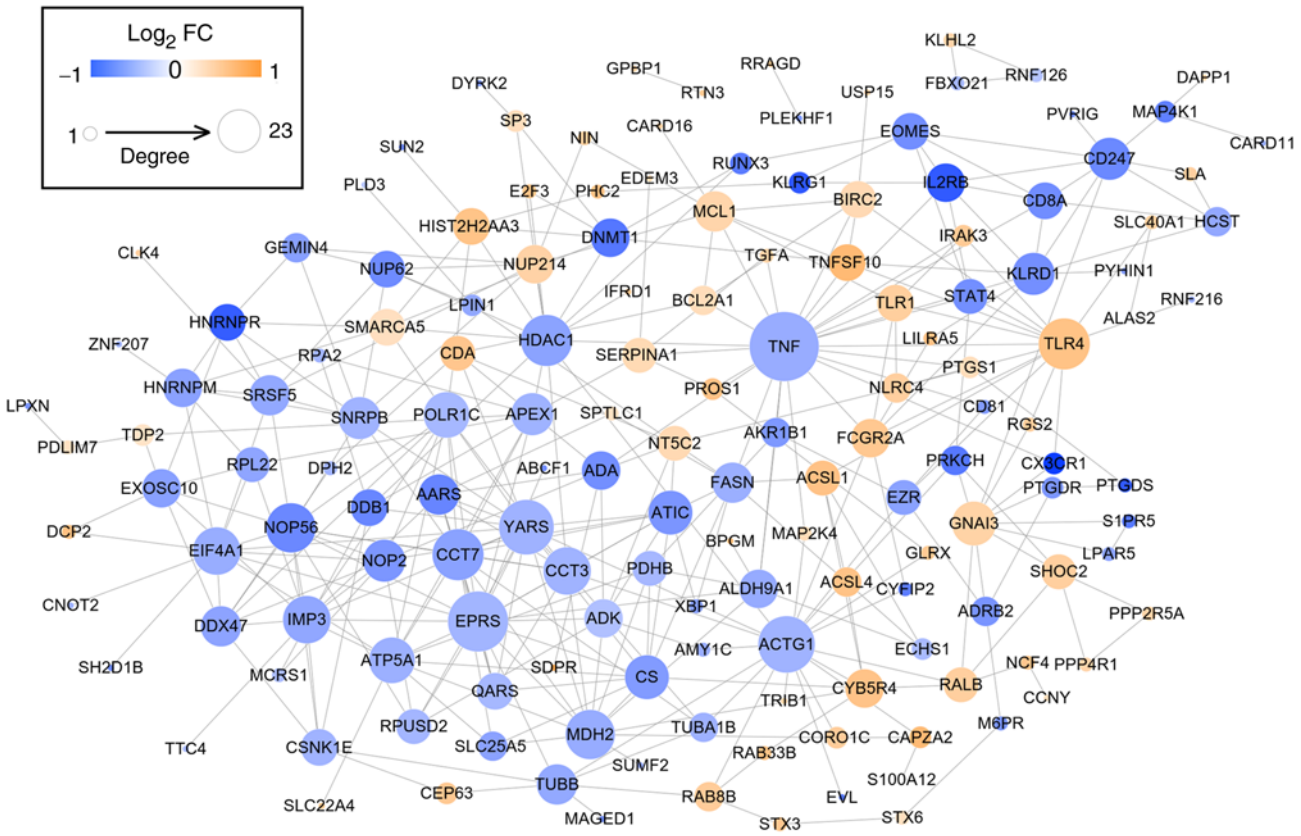


Figure 4. Protein-protein interaction network using the common differentially expressed genes in the two mRNA expression profile datasets (GSE73754 and GSE25101). The network is constructed using the interaction data from the STRING 10.0 database via the Cytoscape software. Orange, upregulated; blue, downregulated. The larger size of the node (protein) indicates a higher degree (interaction relationships). FC, fold change; STRING, Search Tool for the Retrieval of Interacting Genes.

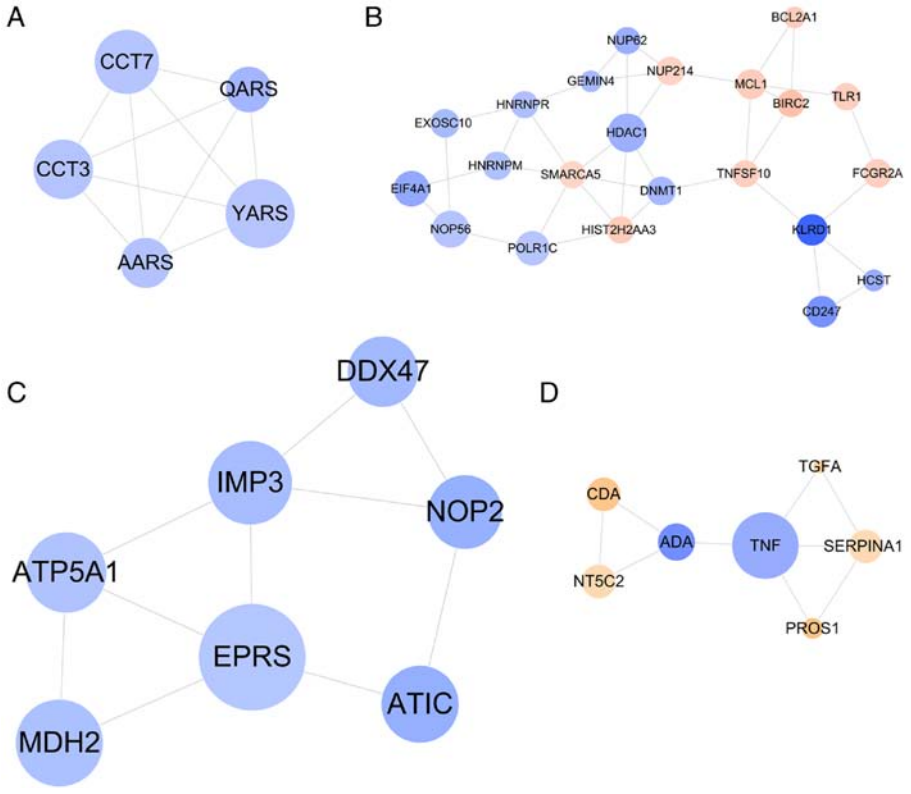


Figure 5. Modules extracted from the protein-protein interaction network. (A) Module 1; (B) Module 2; (C) Module 3; (D) Module 4. The modules are extracted using Molecular Complex Detection (MCODE) plugin of Cytoscape software. Orange, upregulated; blue, downregulated. The larger size of the node (protein) indicates the higher degree (interaction relationships).

Table IV. Function enrichment for genes in modules.

| | Category | Term | P-value | Genes |
|---|---|---|---|---|
| 1 | KEGG_PATHWAY | hsa00970:Aminoacyl-tRNA biosynthesis | 8.99E-05 | *YARS, AARS, QARS* |
| | GOTERM_BP_DIRECT | GO:0006418~tRNA aminoacylation for protein translation | 3.31E-05 | *YARS, AARS, QARS* |
| | GOTERM_BP_DIRECT | GO:0006457~protein folding | 6.76E-04 | *CCT7, AARS, CCT3* |
| | GOTERM_BP_DIRECT | GO:1904871~positive regulation of protein localization to Cajal body | 1.90E-03 | *CCT7, CCT3* |
| | GOTERM_BP_DIRECT | GO:1904874~positive regulation of telomerase RNA localization to Cajal body | 3.57E-03 | *CCT7, CCT3* |
| | GOTERM_BP_DIRECT | GO:0032212~positive regulation of telomere maintenance via telomerase | 7.60E-03 | *CCT7, CCT3* |
| | GOTERM_BP_DIRECT | GO:0007339~binding of sperm to zona pellucida | 8.31E-03 | *CCT7, CCT3* |
| | GOTERM_BP_DIRECT | GO:1901998~toxin transport | 8.55E-03 | *CCT7, CCT3* |
| | GOTERM_BP_DIRECT | GO:0050821~protein stabilization | 3.20E-02 | *CCT7, CCT3* |
| 2 | KEGG_PATHWAY | hsa04650:Natural killer cell mediated cytotoxicity | 4.23E-03 | *TNFSF10, CD247, KLRD1, HCST* |
| | KEGG_PATHWAY | hsa03013:RNA transport | 1.10E-02 | *NUP214, NUP62, EIF4A1, GEMIN4* |
| | GOTERM_BP_DIRECT | GO:0007166~cell surface receptor signaling pathway | 3.33E-04 | *TNFSF10, NUP62, CD247, BIRC2, KLRD1* |
| | GOTERM_BP_DIRECT | GO:0016032~viral process | 4.64E-04 | *NUP214, NUP62, HDAC1, CD247, EIF4A1* |
| | GOTERM_BP_DIRECT | GO:0043066~negative regulation of apoptotic process | 2.21E-03 | *MCL1, NUP62, HDAC1, BCL2A1, BIRC2* |
| | GOTERM_BP_DIRECT | GO:0043123~positive regulation of I-kappaB kinase/NF-kappaB signaling | 1.70E-02 | *TNFSF10, NUP62, BIRC2* |
| | GOTERM_BP_DIRECT | GO:0050776~regulation of immune response | 2.06E-02 | *CD247, KLRD1, HCST* |
| | GOTERM_BP_DIRECT | GO:0043044~ATP-dependent chromatin remodeling | 2.84E-02 | *HDAC1, SMARCA5* |
| | GOTERM_BP_DIRECT | GO:0006364~rRNA processing | 2.90E-02 | *EXOSC10, NOP56, GEMIN4* |
| | GOTERM_BP_DIRECT | GO:0006409~tRNA export from nucleus | 3.93E-02 | *NUP214, NUP62* |
| | GOTERM_BP_DIRECT | GO:0010827~regulation of glucose transport | 4.05E-02 | *NUP214, NUP62* |
| | GOTERM_BP_DIRECT | GO:0097192~extrinsic apoptotic signaling pathway in absence of ligand | 4.17E-02 | *MCL1, BCL2A1* |
| 3 | KEGG_PATHWAY | hsa01100:Metabolic pathways | 1.94E-02 | *ATIC, EPRS, ATP5A1, MDH2* |
| | GOTERM_BP_DIRECT | GO:0006888~ER to Golgi vesicle-mediated transport | 1.32E-03 | *TGFA, SERPINA1, PROS1* |
| | GOTERM_BP_DIRECT | GO:0048566~embryonic digestive tract development | 5.70E-03 | *TNF, ADA* |
| | GOTERM_BP_DIRECT | GO:0048208~COPII vesicle coating | 2.16E-02 | *TGFA, SERPINA1* |
| 4 | GOTERM_BP_DIRECT | GO:0002576~platelet degranulation | 3.62E-02 | *SERPINA1, PROS1* |
| | GOTERM_BP_DIRECT | GO:0000187~activation of MAPK activity | 3.76E-02 | *TNF, TGFA* |
| | GOTERM_BP_DIRECT | GO:0010951~negative regulation of endopeptidase activity | 4.25E-02 | *SERPINA1, PROS1* |

KEGG, Kyoto encyclopedia of Genes and Genomes; GO, Gene Ontology; BP, biological process.

of platelet degranulation. Platelet-specific degranulation gene Munc13-4 knockout mice were shown to display a reduction in airway hyper-responsiveness and eosinophilic inflammation, indirectly confirming the pro-inflammatory roles of SERPINA1 in AS (46). Importantly, a study was conducted to use TaqMan method to genotype tag SNPs (rs2753934,

Table V. Genotype and allele frequency of SNP loci for SERPINA1 and EEF1E1.

| Genes | SNP | | Genotype | | | | Allele | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | AS | Control | P-value | | AS | Control | P-value |
| SERPINA1 | rs6575424 | AA | 9 | 12 | 0.077 | A | 25 | 79 | 0.665 |
| | | AB | 16 | 67 | | B | 42 | 151 | |
| | | BB | 26 | 84 | | | | | |
| EEF1E1 | rs7763907 | AB | 1 | 0 | <0.001 | A | 4 | 4 | 0.047 |
| | | BB | 13 | 5 | | B | 14 | 5 | |
| | | NC | 37 | 154 | | | | | |
| | | AA | 0 | 4 | | | | | |
| | rs9328453 | AB | 0 | 3 | 1.000 | A | 0 | 3 | 1.000 |
| | | BB | 51 | 163 | | B | 51 | 166 | |
| | rs7751386 | AA | 7 | 2 | <0.001 | A | 12 | 41 | 1.000 |
| | | AB | 5 | 39 | | B | 25 | 80 | |
| | | BB | 20 | 41 | | | | | |
| | | NC | 19 | 81 | | | | | |
| | rs12660697 | AA | 0 | 1 | 0.631 | A | 4 | 10 | 0.749 |
| | | AB | 4 | 9 | | B | 51 | 162 | |
| | | BB | 47 | 153 | | | | | |

SNP, single nucleotide polymorphism; AS, ankylosing spondylitis.

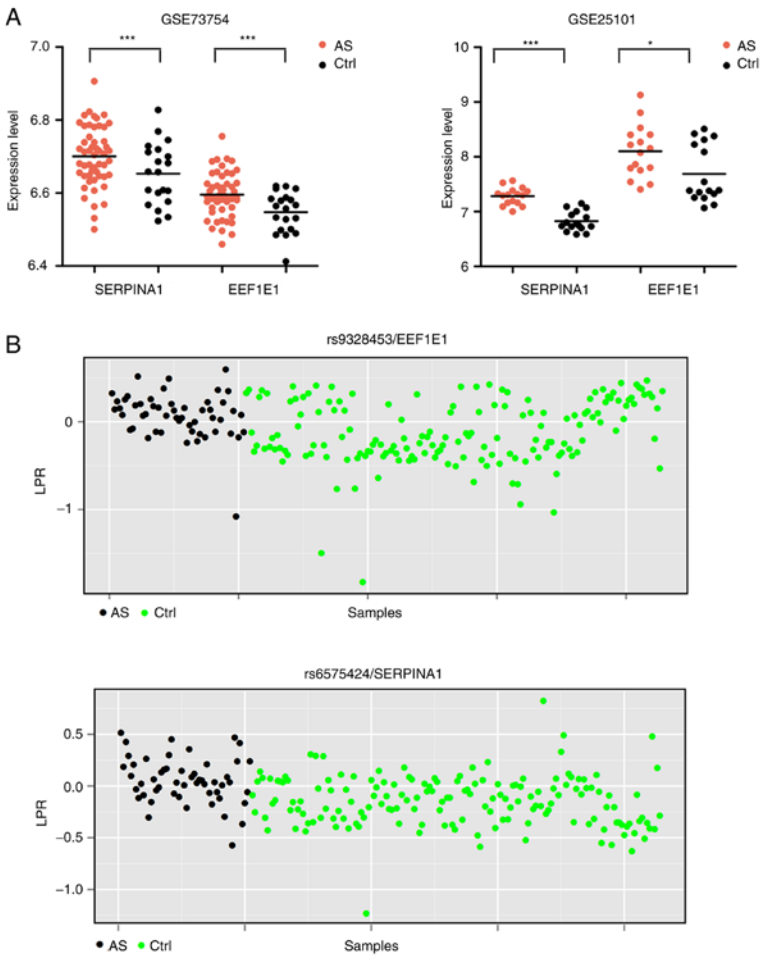


Figure 6. The expression levels and LRR of SNP loci of *EEF1E1* and *SERPINA1*. (A) The expression levels of *EEF1E1* and *SERPINA1* in blood samples of GSE73754 (AS: n=52; Ctrl: n=20) and GSE25101 (AS: n=16; Ctrl: n=20) datasets, respectively. $^{*}$P<0.05; $^{***}$P<0.001. (B) LRR of SNPs in blood samples of GSE39428 (AS: n=51; Ctrl: n=163). Only the most significant SNPs were displayed. The t-test was used to determine the difference in expression and LRR between AS and Ctrl. AS, ankylosing spondylitis; Ctrl, control; LRR, Log R ratios; SNP, single nucleotide polymorphism.

rs2749531 and rs6575424) in *SERPINA1* of 56 AS cases and 160 healthy controls. The results revealed an increased expression of AAT in synovial membranes of AS compared with control samples, but no significant association was observed between the AAT polymorphism and AS (47). This also seems to be in accordance with our results and indicates that SERPINA1 may not be a genetically related biomarker for AS.

However, there were some limitations to the present study. First, this study was only performed to preliminarily screen the potential genetic biomarkers for AS. Further experiments are necessary, including clinical confirmation of the association between the polymorphism of *EEF1E1* and *SERPINA1* and the risk of AS and patient prognosis; clinical validation of the expression of *EEF1E1* and *SERPINA1*; clinical (correlation analysis), *in vitro* (site-directed mutagenesis to construct the expression vector with different alleles, transfection of monocytes or osteoblasts followed by detection of cell proliferation, inflammatory factor release or mineralization) and *in vivo* (mutation knockout in animal models followed by assessment of histology and bone joint) verification of the association between gene polymorphisms and their expressions as well as corresponding phenotypic changes. Second, the SNP microarray used in this study only analyzed the SNPs in specific 384 genes, but not all the genes. Additional SNP discovery by deep sequencing with a larger sample size is essential to obtain more genetic biomarkers.

In conclusion, our findings preliminarily suggest that *EEF1E1* may be an underlying novel, important genetic biomarker for the diagnosis of AS. Its rs7763907 and rs7751386 polymorphisms may lead to its upregulated expression and then promote the transcription of p53 and pro-inflammatory cytokines, leading to the development of AS.

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**Availability of data and materials**

The microarray data GSE73754 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE73754), GSE25101 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE25101) and GSE39428 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE39428) were downloaded from the GEO database in NCBI.

**Authors' contributions**

XF was involved in the conception and design, analysis and interpretation of data and drafted the initial manuscript. BQ collected the data. LM and FM contributed to the interpretation of the data. BQ, LM and FM revised the manuscript critically for important intellectual content. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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