

Transcriptome-wide association study identifies susceptibility genes for rheumatoid arthritis

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Abstract

Objective

To identify rheumatoid arthritis (RA) associated susceptibility genes and pathways through integrating genome-wide association study (GWAS) and gene expression profile data.

Methods

A transcriptome-wide association study (TWAS) was conducted by the FUSION software for RA considering EBV-transformed lymphocytes (EL), transformed fibroblasts (TF), peripheral blood (NBL) and whole blood (YBL). GWAS summary data was driven from a large-scale GWAS, involving 5,539 autoantibody-positive RA patients and 20,169 controls. The TWAS-identified genes were further validated using the mRNA expression profiles and made a functional exploration.

Results

TWAS identified 692 genes with P_{TWAS} values < 0.05 for RA. CRIPAK ($P^{\text{EL}} = 0.01293$, $P^{\text{TF}} = 0.00038$, $P^{\text{NBL}} = 0.02839$, $P^{\text{YBL}} = 0.0978$), MUT ($P^{\text{EL}} = 0.00377$, $P^{\text{TF}} = 0.00076$, $P^{\text{NBL}} = 0.00778$, $P^{\text{YBL}} = 0.00096$), FOXRED1 ($P^{\text{EL}} = 0.03834$, $P^{\text{TF}} = 0.01120$, $P^{\text{NBL}} = 0.01280$, $P^{\text{YBL}} = 0.00583$) and EBPL ($P^{\text{EL}} = 0.00806$, $P^{\text{TF}} = 0.03761$, $P^{\text{NBL}} = 0.03540$, $P^{\text{YBL}} = 0.04254$) were collectively expressed in all the four tissues/cells. 18 genes, including ANXA5, AP4B1, ATIC ($P_{\text{TWAS}} = 0.0113$, down-regulated expression), C12orf65, CMAH, PDHB, RUNX3 ($P_{\text{TWAS}} = 0.0346$, down-regulated expression), SBF1, SH2B3, STK38, TMEM43, XPNPEP1, KIAA1530, NUFIP2, PPP2R3C, RAB24, STX6, TLR5 ($P_{\text{TWAS}} = 0.04665$, up-regulated expression), were validated with integrative analysis of TWAS and mRNA expression profiles. TWAS-identified genes functionally involved in endomembrane system organization, endoplasmic reticulum organization, regulation of cytokine production, TNF signaling pathway, etc.

Conclusion

We identified multiple candidate genes and pathways, providing novel clues for the genetic mechanism of RA.

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease primarily affecting the joints, even probably leading to accumulating joint damage and irreversible disability. Epidemiological studies in RA show that it affects up to 0.5%-1% of the general adult population worldwide. Approximately two thirds of cases are characterized by rheumatoid factor or autoantibodies that target various molecules including modified self-epitopes [1]. Some strong genetic components are known to be involved in the development of RA. Twins and family studies offered a strong suggestion that the risk of RA increased in

individuals with an RA family history by shared genetic factors [2–4]. The recognition of key genetic components and mechanism will led to the better understanding of the pathogenesis of RA.

Genome-wide association study (GWAS) represents a powerful approach of understanding of the genetic basis of many complex traits in common human diseases. Specially, it was proved extremely well-suited to the identification of common single nucleotide polymorphisms (SNPs) -based variants with modest to large effects on phenotype. GWAS with fine mapping, candidate gene approaches and a meta-analysis of GWAS has identified ~ 100 loci across the genome harbouring RA susceptibility variants [5]. However, the specific biological mechanisms and functional consequences of many genetic variants identified by GWAS remain largely unknown, in particular their role on disease severity; that is to say, GWAS approach is likely to miss expression-trait associations of small effect.

Gene expression is an intermediate phenotype between genetic variant and traits underlying disease susceptibility. Many genetic variants devote their effects on complex traits by modulating gene expression [6]. Unfortunately, large-scale expression-trait associations are hampered by specimen availability and cost, as well as intrinsic factor, small effects. Consequently, transcriptome-wide association study (TWAS) was developed to address these problems, which integrates gene expression with large-scale GWAS [7]. It uses a small set of individuals with both genotype and gene expression data as a reference panel to identify significant expression-trait associations. Through extensive simulations of available GWAS data, TWAS identified candidate genes associated with, schizophrenia [8], calcific aortic valve stenosis [9], nonobstructive azoospermia [10], inflammatory biologic age [11] and other complex traits [12, 13]. Meanwhile, the genetic susceptibility variants associated with RA commonly map to enhancer regions [14], which can regulate one or more genes at distant locations in a cell-type-specific manner. Thus, the improved understanding of gene regulation relationship defining which genes are important in which cell types is vital for the predisposition to RA.

In this study, we conducted cell/tissue related TWAS for RA based on GWAS dataset and gene expression from EBV-transformed lymphocytes (EL), transformed fibroblasts (TF), peripheral blood (NBL) and whole blood (YBL). We subsequently reevaluated the expression of the TWAS-identified genes and made a functional exploration. This is the first time that TWAS is applied to a large-scale GWAS data to detect susceptibility genes associated with RA.

Methods

GWAS summary data of RA

A recent large-scale genome-wide association study meta-analysis of RA was used here [15]. Briefly, the genome-wide summary data was collected from six GWAS collections, 5539 cases and 20169 controls in total, per-collection: Brigham Rheumatoid Arthritis Sequential Study (483 cases, 1449 controls), Canada (589 cases, 1472 controls), Epidemiological Investigation of Rheumatoid Arthritis (1173 cases, 1089 controls), North American Rheumatoid Arthritis Consortium I (867 cases, 1041 controls) and III (902

cases, 4510 controls), and Wellcome Trust Case Control Consortium (1525 cases, 10608 controls); typed at 2,556,272 SNPs. Genotyping was conducted using commercial platforms, such as Affymetrix 6.0 array and Illumina 550 K array. RA cases either met the 1987 American College of Rheumatology criteria for diagnosis of rheumatoid arthritis or were diagnosed by board-certified rheumatologists, with the limitation of anti-cyclic citrullinated peptide (anti-CCP) positive or rheumatoid factor (RF) positive. Detailed information of cohorts, genotyping, imputation, meta-analysis and quality control approaches can be found in the published studies [15–20].

TWAS

FUSION software was applied to the RA GWAS summary data for cell/tissue related TWAS analysis, including EL, TF, NBL and YBL. TWAS analysis used pre-computed gene expression weights together with disease GWAS summary statistics to calculate the association of every gene to disease. The genetic values of expression were computed one probe set at a time using SNP genotyping data located 500 kb on either sides of the gene boundary. For this study, the gene expression weights of EL, TF, NBL and YBL were driven from the FUSION website (<http://gusevlab.org/projects/fusion/>).

Validating TWAS results by genome-wide mRNA expression profiles of RA

The expression data of RA were downloaded from Gene Expression Omnibus (GEO) DataSets (<https://www.ncbi.nlm.nih.gov/sites/GDSbrowser?acc=GDS3794>) and corresponding reference [21]. In the study, a complete genome-wide transcript profiling of peripheral blood mononuclear cells (PBMCs) from 18 RA patients and 15 age and sex-matched controls were collected by the Illumina Human-6v2 Expression BeadChips. Differential analysis per gene was performed with one-way analysis of variance (ANOVA) and *P* values were adjusted to control the False Discovery Rate (FDR, 5%).

Functional Exploration

The significant genes identified by TWAS were further made a functional exploration that included Gene Ontology (GO), pathway analysis and PPI network construction using an online analysis tool of Gene Annotation & Analysis Resource, Metascape (<http://metascape.org>). GO analysis was based on Fisher's exact test and calculation of *P* values. Pathway analysis was performed for differentially expressed genes based on database. The construction of PPI network and associated module analysis was based on GO enrichment analysis using the plugin Molecular Complex Detection (MCODE). MCODE algorithm was then applied to this network to identify neighborhoods where proteins are densely connected.

Results

TWAS analysis results of RA

Using the GWAS summary data, TWAS identified 8403 genes in total, and 1440, 4224, 2410, 4628 genes for EL, TF, NBL, YBL, respectively. In the genes list, there were 692 significant genes with TWAS *P* values <

0.05 in total (Fig. 1), including 82 genes for EL, 257 genes for TF, 182 genes for NBL and 317 genes for YBL. Different tissues or cells have their own gene expression profile. In order to find out the most representative genes, we carried out overlap analysis of genes in different tissues/cells. The Venn diagram (Fig. 2) showed that the number of genes expressed in one or more tissues/cells. For example, there were 82 TWAS-identified significant genes associated with RA in EL; there were 32 significant genes in both EL and TF; there were 5 significant genes in EL, TF and YBL; there were 4 significant genes in EL, TF, YBL and NBL. The four novel TWAS-significant RA susceptibility genes identified in all four tissues/cells were CRIPAK, MUT, FOXRED1 and EBPL, which were located on Chromosome 4, 6, 11 and 13, respectively. Table 1 presented the more and detailed information of the four genes, including heritability of genes (HSQ), rsID of the most significant GWAS SNP in locus (BEST.GWAS.ID), number of SNPs in the locus (NSNP) and TWAS P value (P_{TWAS}).

Table 1
The TWAS-significant RA susceptibility genes in all four tissues/cells.

Tissue/Cell	Gene	CHR	HSQ	BEST.GWAS.ID	NSNP	P_{TWAS}
EL	CRIPAK	4	0.488	rs3755963	326	0.01293
EL	MUT	6	0.475	rs6458697	353	0.00377
EL	FOXRED1	11	0.604	rs602735	634	0.03834
EL	EBPL	13	0.410	rs1198329	391	0.00806
TF	CRIPAK	4	0.353	rs3755963	326	0.00038
TF	MUT	6	0.513	rs6458697	353	0.00076
TF	FOXRED1	11	0.390	rs602735	634	0.01120
TF	EBPL	13	0.287	rs1198329	391	0.03761
NBL	CRIPAK	4	0.134	rs3755963	333	0.02839
NBL	MUT	6	0.033	rs6458697	357	0.00778
NBL	FOXRED1	11	0.029	rs602735	650	0.01280
NBL	EBPL	13	0.073	rs1198329	401	0.03540
YBL	CRIPAK	4	0.345	rs3755963	301	0.00978
YBL	MUT	6	0.084	rs6458697	357	0.00096
YBL	FOXRED1	11	0.222	rs602735	641	0.00583
YBL	EBPL	13	0.521	rs1198329	400	0.04254

Functional Exploration Of The Twas-identified Genes Associated With Ra

Pathway and process enrichment analysis was carried out with the following ontology sources: KEGG Pathway, GO Biological Processes, GO Molecular Functions, Reactome Gene Sets, Canonical Pathways and CORUM. The total 654 TWAS-identified genes in the four tissues/cells were successfully submitted to Metascape performing GO enrichment analysis. TWAS-identified genes were annotated with an enrichment of biological processes and KEGG pathways involved in endomembrane system organization, endoplasmic reticulum organization, regulation of cytokine production, TNF signaling pathway and so on (Fig. 3A). The significant terms were then hierarchically clustered, selected a subset of representative terms and converted them into a network layout (Fig. 3B).

A protein-protein interaction (PPI) network of the TWAS-identified genes was constructed, and module analysis was conducted using the plugin Molecular Complex Detection (MCODE). The PPI network was constructed based on 1122 GO terms (Fig. 4A). The top three GO terms were regulation of TP53 activity through phosphorylation, retrograde transport at the trans-golgi-network, regulation of TP53 activity. The significant modules from the PPI network formed 9 MCODE clusters with a class of genes (Fig. 4B), for example, MCODE1, MCODE3, and MCODE5 were characterized by MAPK (mitogen activated kinase-like protein) family genes, ZNF (zinc finger protein) family genes, and NDUF (NADH ubiquinone oxidoreductase subunit) family genes, respectively.

Discussion

TWAS is a creative and valuable analysis method that can integrate genetic variation with gene expression to identify genes whose cis-regulated expression is associated with complex traits. It captures heterogeneous signals better than individual SNPs or cis-eQTLs and focuses prediction on the genetic component of expression that avoids confounding from environmental differences caused by the trait that may influence expression. What's more, TWAS avoids tissue acquisition challenges that may pose the greatest hurdle for producing larger datasets. Thus, TWAS has been widely applied to yield mechanistic disease insights, yet the first time for RA in this study.

RA is a systemic disease and a variety of immunological events occur not only joints but also outside the joint at mucosal surfaces and primary lymphoid tissues, especially synovium. Thus, various types of tissues and cells will be attacked by the disease, including synovium, cartilage, bone, fibroblasts, adipocytes, macrophage, immune cells and so on. In this work, we conducted cell/tissue related TWAS for RA. TWAS identified total 674 genes with transcriptome-wide-significant associations with RA in four tissues/cells. CRIPAK, MUT, FOXRED1 and EBPL, which were collectively expressed in all the four tissues/cells, were novel genes associating with RA. Consistent with the result of TWAS, eighteen genes, ANXA5, AP4B1, ATIC, C12orf65, CMAH, PDHB, RUNX3, SBF1, SH2B3, STK38, TMEM43, XPNPEP1, KIAA1530, NUFIP2, PPP2R3C, RAB24, STX6, TLR5, have been reported differently expressed in peripheral blood mononuclear cells of RA patients.

Cysteine rich PAK1 inhibitor (CRIPAK) is an endogenous inhibitor of p21-activated protein kinase 1 (PAK1), which interacts with Pak1 through the N-terminal regulatory and inhibited enhancement of estrogen receptor transactivation. The decrease in gene expression of CRIPAK could act to promote accumulation of phosphorylated myosin light chain and its stimulation of actomyosin ATPase activity in laser-captured serotonin neurons from macaques treated with ovarian hormones [22]. There are few reports about the role and mechanism of CRIPAK in diseases, especially no reports in RA. However, PAK1 has been extensively studied. PAK1, a potential mediator of Rac1/Cdc42 signaling pathway, is involved in regulating the migration, invasion, proliferation, and inflammation of fibroblast-like synoviocytes from rheumatoid arthritis patients [23, 24]. These studies indirectly support the potential role of CRIPAK in rheumatoid arthritis.

Methylmalonyl-CoA mutase (MUT) encodes the mitochondrial enzyme methylmalonyl Coenzyme A mutase. In humans, the gene encoded enzyme catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA, while this enzyme may have different functions in other species. FAD dependent oxidoreductase domain containing 1 (FOXRED1) encoded protein that is localized to the mitochondria and whose function is involved in assembly, stability and/or correct functioning of complex I [25, 26]. Numerous processes involved in mitochondrial function are related to RA. For example, oxidative stress impairs energy metabolism in primary cells and synovial tissue of RA patients [27]; the interaction of abnormal cellular metabolism, mitochondrial dysfunction, hypoxia and the proinflammatory signaling pathways in synovial cells is contributed to synovial invasiveness of RA [28]; rare/low-frequency variants of the mitochondria respiratory chain-related proteins were aggregated RA patients [29]; etc. Meanwhile, The top three GO terms in MCODE 5 of PPI network were complex I biogenesis, NADH dehydrogenase complex assembly and mitochondrial respiratory chain complex I assembly. Therefore, we predict the two genes may play roles in the pathology of RA via affecting mitochondrial function.

EBPL is an emopamil-binding protein (EBP) like protein. EBP is a high-affinity binding protein for [H-3] emopamil and belongs to the family of so-called sigma receptors. Mutations disrupted EBP impair cholesterol biosynthesis and cause X-chromosomal dominant chondrodysplasia punctate. The EBPL mRNA was expressed ubiquitously and most abundant in liver, lung and kidney. However, EBPL has a yet-to-be-discovered function [30].

In the common RA associated gene list identified by TWAS, we should paid attention to ATIC, RUNX3 and TLR5. 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC) encodes a bifunctional protein, which catalyzes the last two steps of the de novo purine biosynthetic pathway. ATIC plays crucial role in the mechanisms underlying methotrexate's anti-inflammatory and antiproliferative effects. ATIC missense variant and gene polymorphism affects response to methotrexate treatment in RA patients [31–33]. Runt related transcription factor 3 (RUNX3) encodes a member of the runt domain-containing family of transcription factors and involves on T-cell development, T-cells polarization and T cell selection [34, 35]. RA is characterized by the presence of activated T lymphocytes. It is indicated that RUNX3 may play roles on the mechanisms of T cell activation in RA. Toll like receptor 5 (TLR5) encodes a member of TLR family that plays an essential role in pathogen recognition and innate

immune responses activation. TLR5 agonist, flagellin, can promote monocyte infiltration and osteoclast maturation directly through myeloid TLR5 ligation and indirectly via TNF-alpha production from RA and mouse cells [36]. Angiogenesis in RA is fostered directly by TLR5 ligation and indirectly through interleukin-17 induction [37]. TLR5 is the bridge that interconnects formation of new blood vessels with maturation of joint osteoclasts, thereby accelerating the bone destruction process in RA [38]. There have other researches indicate that TLR5 is involved on RA inflammation, bone destruction and angiogenesis; thus, TLR5 is a critical element and target for RA mechanism.

With TWAS in this study, we found that the mRNA expression of some genes in human tissues/cells can be affected by SNPs, further associating with RA susceptibility. For example, four genes (CRIPAK, MUT, FOXRED1 and EBPL) in four distinct loci (rs3755963, rs6458697, rs602735 and rs1198329) were associated with RA susceptibility. We carried out a TWAS strategy to pinpoint RA associated genes; both genomics and transcriptomics were combined, and cis-heritable genes were explored and evaluated efficiently. The study provides a potential functional mechanism of how genetic variants on chromosome may increase RA susceptibility.

Conclusions

In summary, TWAS study identifies novel and common susceptibility genes for rheumatoid arthritis. Beyond specific mechanistic findings for RA, this work outlines a systematic approach to identify functional mediators of complex disease.

Abbreviations

RA: Rheumatoid arthritis; GWAS:Genome-wide association study; TWAS:Transcriptome-wide association study; EL:EBV-transformed lymphocytes; TF:Transformed fibroblasts; NBL:Peripheral blood; YBL:Whole blood; SNPs:Single nucleotide polymorphisms; anti-CCP:Anti-cyclic citrullinated peptide; RF:Rheumatoid factor; GEO:Gene expression omnibus; PBMCs:Peripheral blood mononuclear cells; ANOVA:Analysis of variance; FDR:False discovery rate; GO:Gene ontology; MCODE:Molecular complex detection; PPI:Protein-protein interaction; MAPK:Mitogen activated kinase-like protein; ZNF:Zinc finger protein; NDUF:NADH ubiquinone oxidoreductase subunit; CRIPAK:Cysteine rich PAK1 inhibitor; PAK1:P21-activated protein kinase 1; MUT:Methylmalonyl-CoA mutase; FOXRED1:Oxidoreductase domain containing 1; EBP:Emopamil-binding protein; RUNX3:Transcription factor 3; TLR5:Toll like receptor 5.

Declarations

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Authors' contributions

All authors were involved in drafting the article or revising it critically for important intellectual content and all authors approved the final version to be published. Prof. Feng Zhang has full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Cuiyan Wu, Feng Zhang and Xiong Guo are involved on the study conception and design. Ping Li, Bolun Cheng, Sijia Tan, Peilin Li, Wenyu Li, Huan Liu, Feng'e Zhang, Sen Wang, Yujing Ning and Yan Wen are contributed to the acquisition of data. Cuiyan Wu drafted the manuscript.

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Availability of data

The gene expression datasets generated during the study are available in the FUSION website (<http://gusevlab.org/projects/fusion/>).

Ethics approval and consent to participate

The institutional review board at each site approved the study, and informed consent was obtained from all the participants.

Consent for publication

Not applicable.

Conflicting interests

None declared.

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Figures

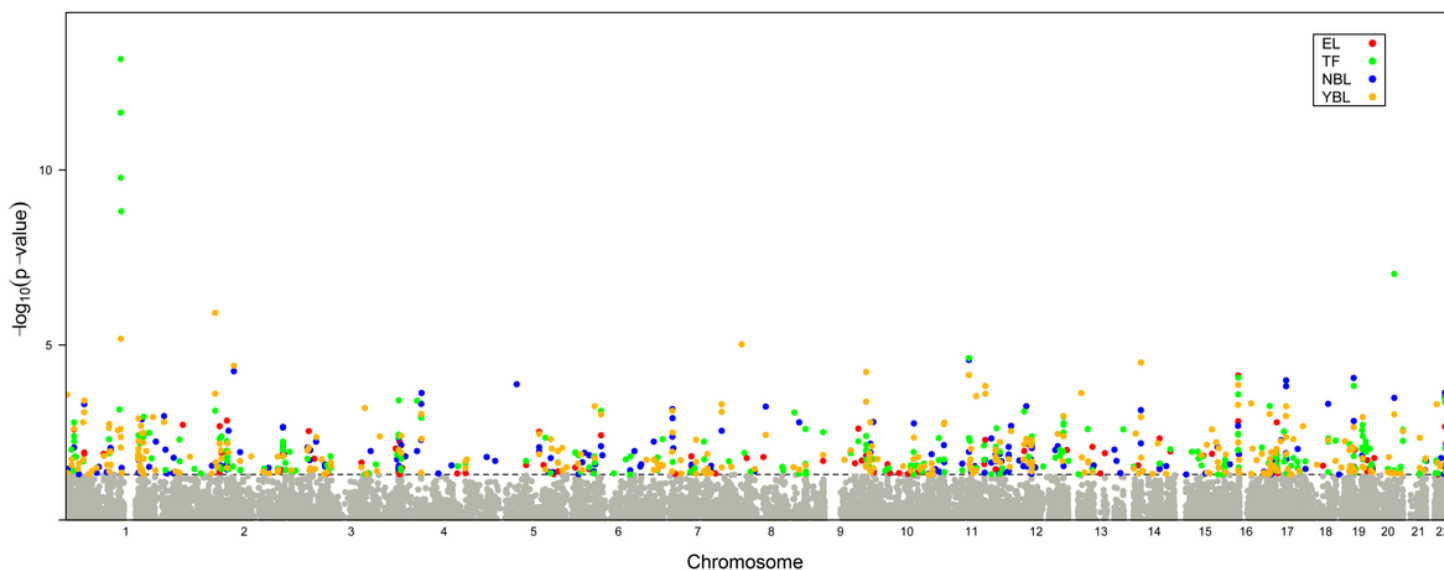


Figure 1

Manhattan plot of TWAS-identified genes and significantly expressed genes associated with RA (colorful points). Each point represents a single gene, with physical position (chromosome localization) plotted on the x axis and $-\log_{10}(P \text{ value})$ of association between gene and RA plotted on the y axis. The significant genes in different tissues/cells are highlighted with different colors (red, EL; green, TF; blu, NBL; yellow, YBL; grey, all).

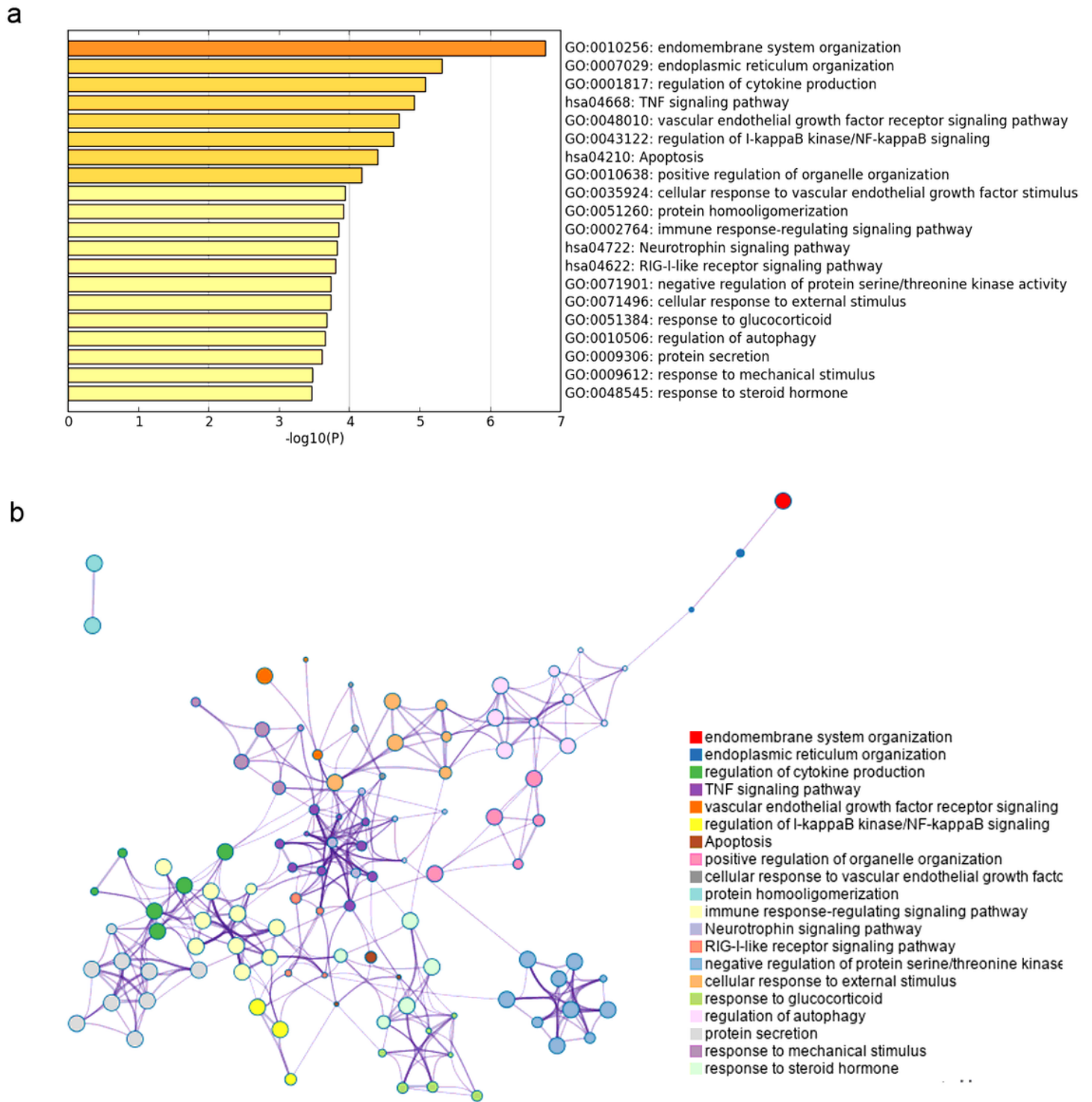


Figure 2

Venn diagram reveals the overlap of TWAS-significant genes in different tissues/cells. Purple, EL; yellow, TF; green, NBL; pink, YBL.

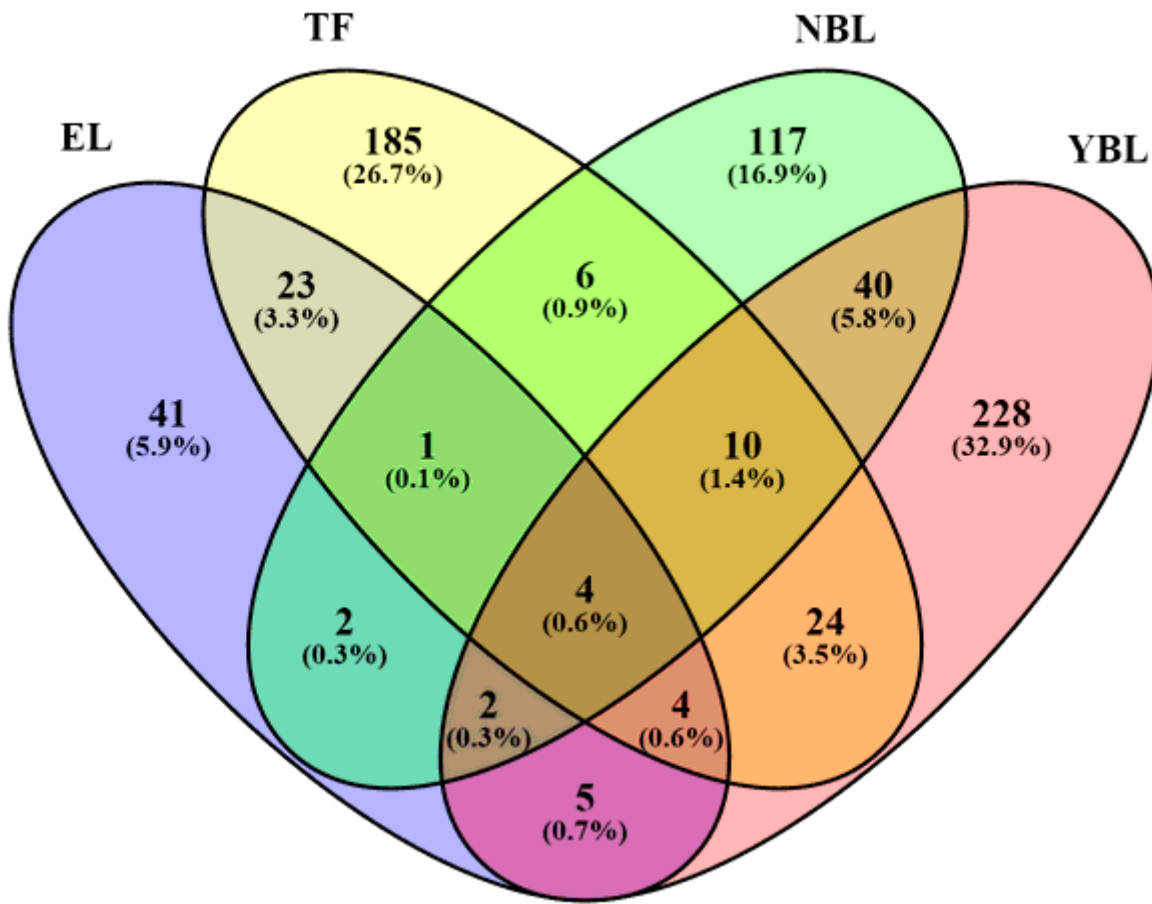


Figure 3

Gene ontology (GO) term analysis of differentially expressed genes. (A), Heatmap of enriched GO terms and (B). The network layout of representative GO terms under hierarchical clustering. In the network, each circle node represents a term, where its size is proportional to the number of input genes fall into that term, and its color represent its cluster identity (i.e., nodes of the same color belong to the same cluster). Terms with a similarity score > 0.3 are linked by an edge (the thickness of the edge represents the similarity score).

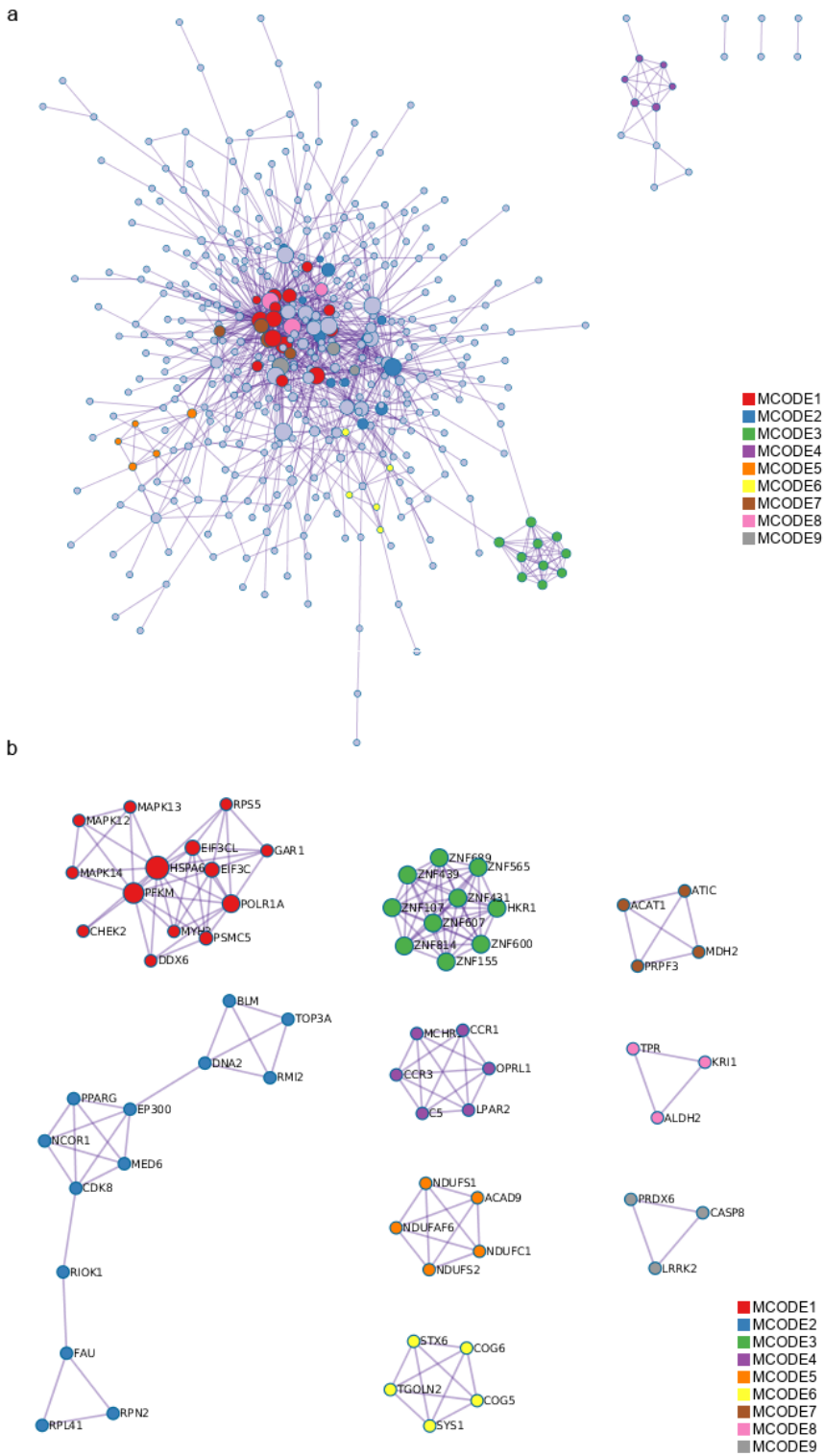


Figure 4

PPI network and the significant module. (A) PPI network of the TWAS-identified genes. (B) Significant modules of the PPI network.