

# Weighted Gene Correlation Network Analysis Applied to Identify the Immune Cell-related Hub Genes in ANCA Nephritis

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## Research Article

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# Abstract

**Background:** Antineutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) is the most common reason caused rapidly progressive glomerulonephritis worldwide. But the molecular mechanisms of ANCA - associated nephritis (AAN) have not been thoroughly expounded. So that we aim to seek the potential molecular pathogenesis of AAN by bioinformatic.

**Result:** Finally, four hub genes, PBK, CEP55, CCNB1 and BUB1B, were identified. These four hub genes was verified higher in AAN than normal.

**Conclusion:** Those four genes identified by integrated bioinformatics analysis may play a critical role in AAN. May offering a new insights and potential therapeutic to the AAN

## Background

The essential feature of antineutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) is the systemic necrotizing vasculitis, often involving the whole body blood vessels, which can develop at any age[1]. Three times more risk to death in AAV patients than normal people[2]. AAV is the commonest reason to generate rapidly progressing glomerulonephritis worldwide, with rapidly progress and poor renal prognosis [3]. About 80% AAV patients occurs kidney damage during the disease phase[4]. Despite advances in medical treatment, about 20 to 30 percent of patients still developed to kidney failure, with unfavourable prognosis and **inferior quality** of daily life [5]. In ANCA - associated nephritis (AAN) the main pathological are mainly divided into four, such as focal type (more than 50% normal glomerulus), sclerosing type (more than 50% global sclerosing glomerulus), crescent type (more than 50% glomerulus crescent type) and mixed type (no obvious lesion type). The most common pathological type was crescent nephritis [3,6]. There have been reports that extracellular glomerular myeloperoxidase (MPO) deposition and antigen-specific T and B cells activating, leading to crescent nephritis finally in AAN patients[7].

However, the pathophysiological mechanism of AAN remains unclear, and increasing evidence shows that immune cells play a vital role in AAN [7,8]. As the disease of AAN progresses, neutrophils and macrophages accumulate in the glomerulus[9]. Neutrophils infiltrate most prominently in the necrotic glomerulus, whereas macrophages aggregate most prominently in the crescent body[9]. Experimental results showed that there were antigen-specific effector CD4<sup>+</sup> T cells in peripheral blood during the acute infection stage of AAV, and the depletion of CD4<sup>+</sup> T cells alleviated the progression of the disease [10]. As report, the B cells, plasma cells and Treg cells in circulating blood of AAV patients were higher than those in healthy controls [11]. Monocytes have also been shown to accumulate in crescents of necrotizing glomerulonephritis [12]. However, the mechanism by which immune inflammatory cells infiltrate renal tissue has not been fully elucidated [13]. The molecular mechanisms of immune cells and AAN need to be further studied for the sake of elucidating the pathophysiological mechanism of AAN pathogenesis and provide guidance for the search for potential therapeutic targets.

WGCNA is an effective method to analyse the complex relationship between gene and phenotype[14]. In many diseases, it was used to screen for key genes, such as tumor[15,16], immunity[17], kidney disease[18]. Mining hub genes in specific modules greatly reduced the scope of screening genes, and finally screened key genes or markers related to phenotypes, improving the precise location of genes related to key traits [19]. In this paper, the co-expression network of immune cells and AAN genes was constructed based on WGCNA algorithm, and then enrichment and PPI analysis were conducted on the selected modules to screen out hub genes for further verification. This study is expected to provide new perspectives and potential quality targets for the therapy of AAN.

## Results

### Immune cell expression

Based on the microarray gene expression data set of the GSE104948, CiberSort was used to estimate the relative expression of 22 subtypes immune cells. (Figure 1). Remove low expression of immune cells from the sample, because the expression of these immune cells was not expressed in these samples. Pearson correlation analysis was performed on the remaining immune cells (Figure 2). There was significant positive correlation between T cells CD4 memory resting and T cells CD8 ( $r=0.84, p<0.001$ ) B cells resting was significantly positively correlated with B cell activated ( $r=0.92, p<0.001$ ), while NK cell activated was significantly positively correlated with NK cell resting ( $r = 0.86, p <0.001$ ).

### Construct the WGCNA network

The genes with the top 5000 differences before expression were selected for WGCNA analysis. A scale-free  $R^2 > 0.9$  co-expression network was constructed with soft threshold power  $\beta = 16$ . Eight modules, including green, magenta, blue, grey, greenyellow, royalblue, grey60, midnightblue were identified by the hierarchical cluster average linkage method (Figure 3).

### Construct the interrelationship between module and immune cell type

Correlation analysis was conducted between each module and the immune cell type selected above, including B cells native, B cells memory, plasma cells, T cells CD8, T cells CD4 memory resting, T cell follicular helper, T cell regulatory Tregs, NK cell resting, NK cell activated, Macrophages M1, Macrophages M2, Dendritic cells resting, Dendritic cells activated, Mast cells activated, Neutrophils. The results showed that the greenyellow module was positively correlated with T cells CD4 memory resting ( $r = 0.46, p = 0.003$ ), while the Blue module was negatively correlated with T cells CD4 memory resting ( $r = -0.5, p <0.001$ ) (Figure 4)

## Enrichment analysis

Genes in greenyellow block were selected for GO and KEGG functional enrichment analysis, and biological effects were studied using David Online tool (Figure 5). The most enrichment biological process including innate immune response, inflammatory response, mitotic nuclear division, leukocyte migration, cell division. Additional, plasma membrane, cytosol, membrane, extracellular exosome, integral component of plasma membrane were significant enriched in cellular components. For Molecular Function, the significant enriched were protein binding, ATP binding, receptor activity, protein kinase binding, microtubule binding. KEGG pathway analysis were mainly enriched in tuberculosis, phagosome, natural killer cell mediated cytotoxicity, osteoclast differentiation, staphylococcus aureus infection

## PPI Network identification of hub genes

String database was used to analyses the genes in the greenyellow module to assess the interactions. Finally, 99 nodes and 1193 edges were identified from network (Figure 6). The PPI network was then processed using CytoHubba MCC to identify the top ten genes.

## Hub gene validation

GSE109108 data set was used to detect the hub genes. The results showed that PBK, CEP55, CCNB1 and BUB1B were elevated in AAN patient (Figure 7). In addition, we combined Nephroseq database to ascertain the level of this hub gene between AAN and normal controls. The results also showed that PBK, CEP55, CCNB1 and BUB1B were significantly higher in AAN than in healthy group (Figure 8).

## Discussion

AAV is an autoimmune disease in which PR3(proteinase3) and MPO are the main **autologous antigens** in the cytoplasm of antineutrophil, leading to destructive inflammation of vessel throughout the body[9]. Extensive crescent formation with glomerular necrosis is characteristic of renal involvement[25]. It often leads to kidney failure in a short time. Despite numerous reports, the etiology and molecular mechanism pathogeny of AAN is not yet fully clarified. Therefore, it is critical to strengthen the research on the etiology and physiological mechanism of AAN, reveal the potential causes of the pathogenesis of AAN, and explore possible therapeutic targets.

In this study, data sets were extracted from GES104948 downloaded from the GEO database and Cibersort was used to estimate the composition of immune cells. Then the module with the strongest correlation with immune cell type was determined by WGCNA method. 8 modules were selected finally, and the greenyellow module has the highest relationship with T cells CD4 memory resting cells. Then, the selected modules were analyzed for gene enrichment. GO enrichment results showed that genes of selected modules were mainly enriched in innate immune response and inflammatory response. KEGG

pathway analysis showed that the gene was mainly enriched in innate immune response and inflammatory response pathways. Through protein interaction network analysis and Cytoscape's CytoHubba, the top 10 hub genes with the highest expression were finally screened. Finally, the expressions of PBK, CEP55, CCNB1 and BUB1B showed significant differences in AAN. The potential relationship between these four genes and AAN has not been thoroughly studied and deserves further research. Finally, the gene expression was verified by Nephroseq database. The genes expression levels of PBK, CEP55, CCNB1 and BUB1B in AAN patients were higher than those in normal, and were negatively correlated with [serum creatinine](#), which was consistent with bioinformatics analysis

PBK, also known as T lymphocyte kinase (TOPK), is a novel mitotic serine/threonine protein kinase[26]. It is involved in a multiple of biological functions, such as cell proliferation and transformation, cell cycle regulation, tumorigenesis and anti-apoptotic effects[27,28]. TOPK/PBK reduces UV-induced inflammation by enhancing the stability of MKP1 and thereby negatively regulating the activity of P38[27]. Some studies have found that PBK mutation may be related to the occurrence of kidney stones [29]. TOPK/PBK expression can be detected in a variety of malignancies and is associated with aggressive tumor phenotypes, which is considered as a potential target [30,31], and also regarded to be a transfer-promoting kinase in cancer metastasis[32].

CEP55 (centrosome protein) is a vital component in cell cycle progression and plays a critical role in the final stage of cytokinesis, regulating the physical separation of two daughter cells[33]. Recent reports suggest that CEP55 is overexpressed in a large number of tumors and is associated with poor prognosis[34]. Overexpression of CEP55 can up-regulate PI3K/AKT signaling pathway to promote tumor migration and invasion[35]

CCNB1, a cyclin, is a regulatory subunit of cyclin-dependent kinase 1 (CDK1), which promotes cell mitotic division[36]. CCNB1 was related to cell proliferation and differentiation, and inhibition of CCNB1 expression could inhibit the proliferation of gonorrhea and spermatogonia and promote apoptosis[37]. When CCNB1 is abnormally expressed, the immune system interprets the abnormally expressed CCNB1R as a tumor antigen and activates humoral and cellular immunity[38].

BUB1B, also known as mitotic checkpoint serine/threonine kinase B, is a family of spindle assembly checkpoint (SAC) proteins[39]. Loss of spindle detection sites and a doubling of severe chromosome separation defects proved to be associated with BUB1B inactivation[40]. BUB1B mutated, resulting in reduced BUB1B expression, increased brittleness of antioxidant stress, and premature ovarian failure[41]. In a variety of cancers, malignant cell proliferation and poor clinical prognosis are closely related to the overexpression of BUB1B[42-44].

Our study has some limitations. Firstly, our study is based on the GEO public database with a small sample size. More clinical samples are needed to validate our study. Second, in this article, the correlation between selection genes and AAN has not been reported in literature. It is necessary to clearly validate the functional importance and mechanistic role of these genes in this pathological setting.

## Conclusions

In conclusion, the results of these bioinformatics analyses and hub genes afford a new sight to the mechanisms of AAN pathogenesis. Further research is needed elucidate the regulatory character of these genes, determining the values as clinical biomarkers or therapeutic targets.

## Methods

### Data acquisition and processing

Dataset GSE104948 was downloaded from GEO database(<https://www.ncbi.nlm.nih.gov/geo/>) GPL22945 platform including 22 AAN patients and 18 healthy controls. R package "WGCNA" was used to structure the co-expression network among the genes ranking the first 5000 median absolute deviation in dataset GSE104948. A series of matrix file data GSE108109 was used, including AAN (n=15) and healthy controls group (n= 6), for subsequent model validation.

### Immune cell correlation

Based on the gene expression matrix of the GSE104948 dataset Cibersort(<https://cibersort.stanford.edu/>) algorithm was designed to estimate the abundances of the immune cells composition. The algorithm can estimate the relative expression levels of 22 immune cell types [20].

### WGCNA

The co-expression network of differential genes and immune cells was constructed [19]. An appropriate soft threshold power  $\beta=16$  was determined and a scale-free  $R^2=0.95$  were selected. Then cluster DEGs into modules and transform adjacency matrix into topological overlap matrix (TOM). After a height cutoff of 0.25, similar modules were merged, with a minimum of 30 genes per module and a threshold of 0.25 for module merging. The relative expression levels of each module and immune cells were calculated. Those with the highest correlation were selected for further analyses.

### Function of enrichment

Gene ontology (GO) and KEGG were performed using DAVID(version6.8, <https://david.ncifcrf.gov/>)[21]. To reveal biological processes , cellular components, molecular functions, and pathways associated with genes in the above modules [22], with a  $p < 0.05$  were significantly enriched.

### PPI network and identify hub gene

The genes in the selected module were imported into the interactive gene retrieval tool STRING database(<https://cn.string-db.org/>)[23] to generate a protein-protein interaction (PPI) network identifying the interaction genes. The results downloaded from the STRING database are then visualized using Cytoscape software. Use Cytoscape software plug-in CytoHubba(v3.8.2, <http://cytoscape.org>) [24] to identify hub genes. Hub gene expression was validated by dataset GSE109018.

Nephroseq(<http://v5.nephroseq.org/>) was used to verify the relationship between hub gene and clinical features

## Statistics analysis

GraphPad Prism 7.0 software and R were used to draw graphics and perform statistical analysis. Unpaired Students T-test was used to compare gene expression levels in different group.  $P < 0.05$  was considered statistically significant.

## Abbreviations

ANCA antineutrophil cytoplasmic antibody

AAV antineutrophil cytoplasmic antibody associated vasculitis

AAN ANCA - associated nephritis

MPO myeloperoxidase

Proteinase3 PR3

TOPK T lymphocyte kinase activated killer cell-derived protein kinase

CDK1 cyclin-dependent kinase 1

SAC spindle assembly checkpoint

TOM topological overlap matrix

PPI protein-protein interaction

## Declarations

## Authors' contributions

DZ and JJ designed the experiments. DZ wrote the paper. ,WFH and JSZ analyzed the data and prepared figures. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

## Availability of supporting data

Data is available at NCBI GEO: , GSE109108.

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## Consent for publication

Not applicable.

## Ethics approval and consent to participate

Not applicable.

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Not applicable

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## Figures

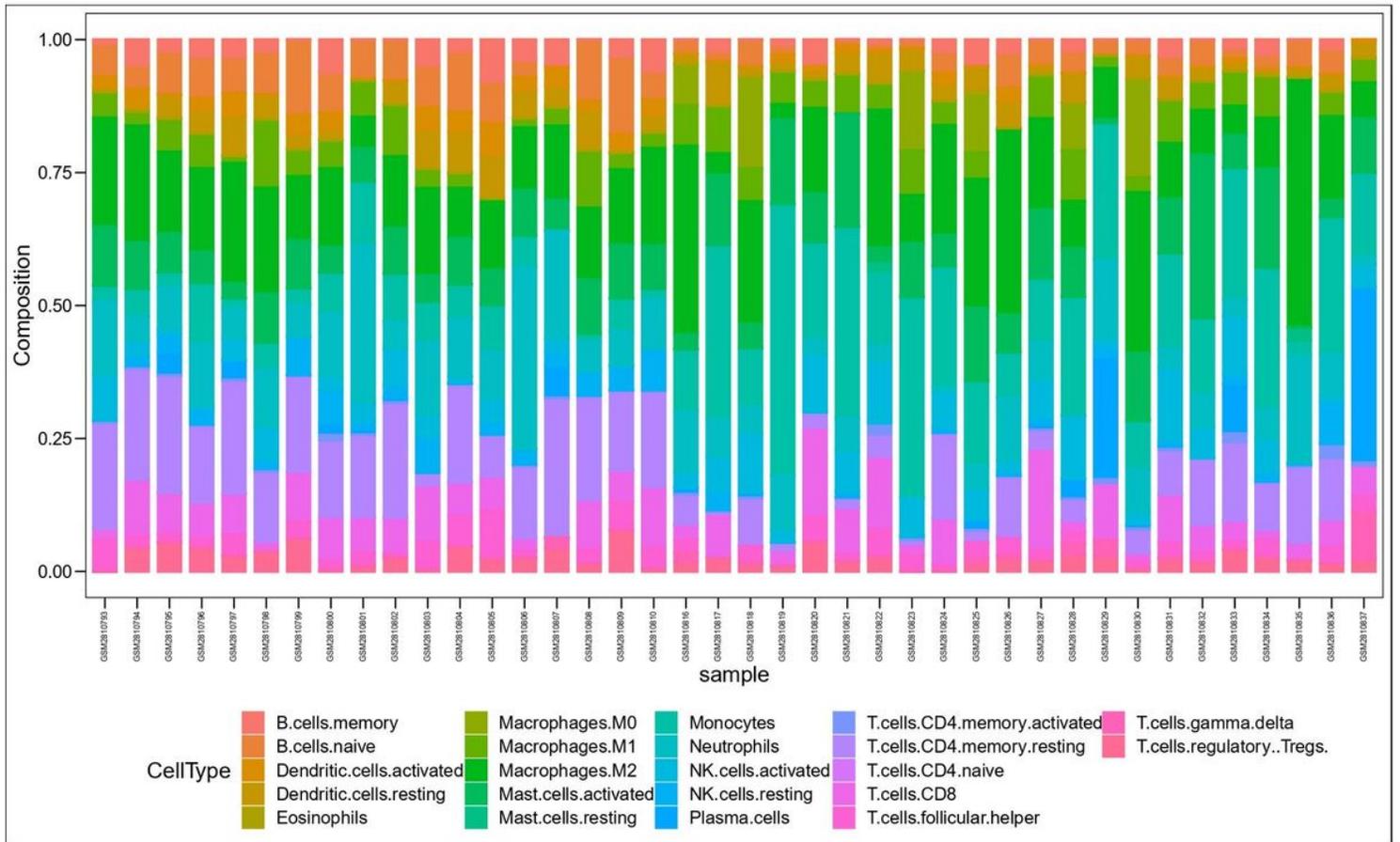
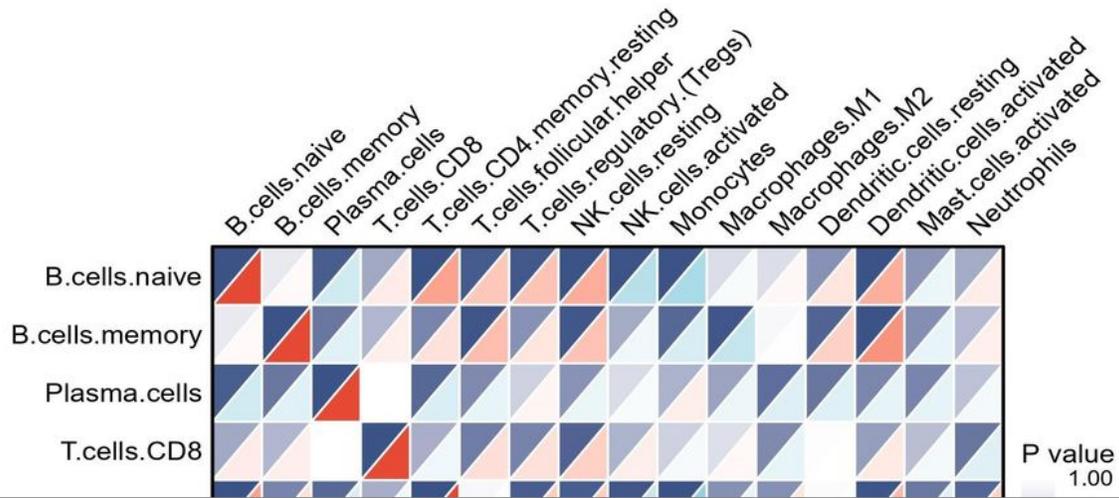


Figure1.  
The expression of 22 kinds immunity cells of each sample in GSE10908 dataset.

Figure 1

Please See image above for figure legend.



**Figure 2**

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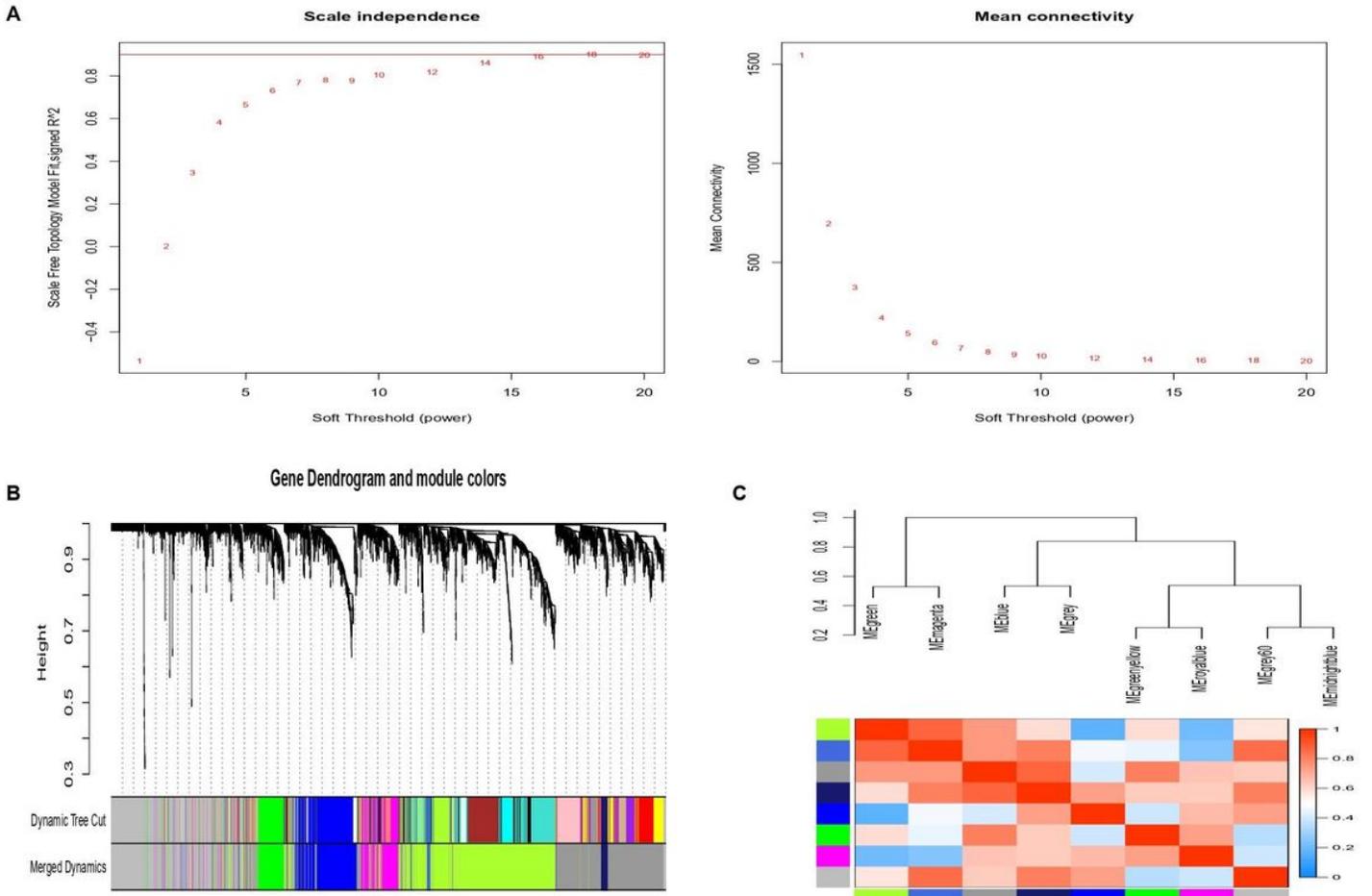


Figure3

The result of the weighted genes correlation analysis. (A)The soft-power  $\beta$  was confirmed. (B)The gene dendrogram and module colors. (C)The cluster color modules, red means positive correlation and blue means negative correlation.

### Figure 3

Please See image above for figure legend.

Module-trait relationships

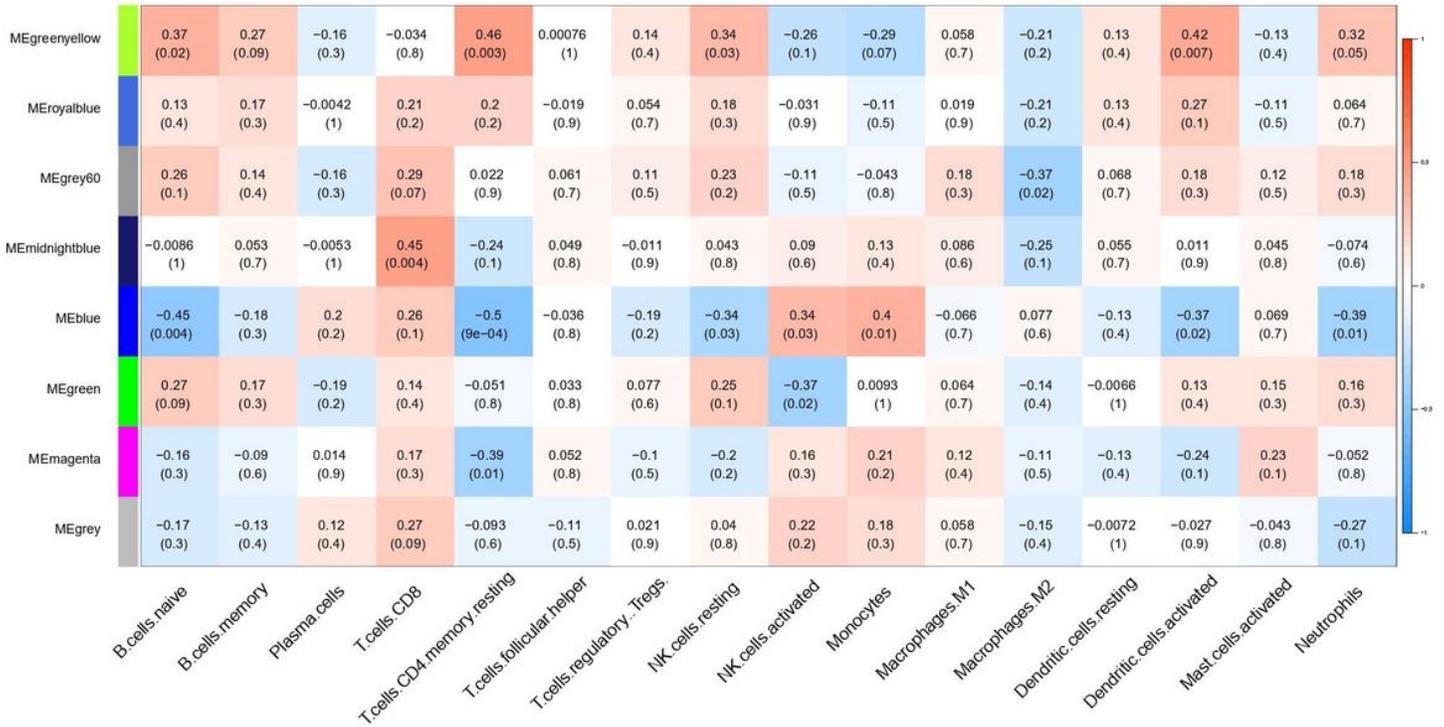
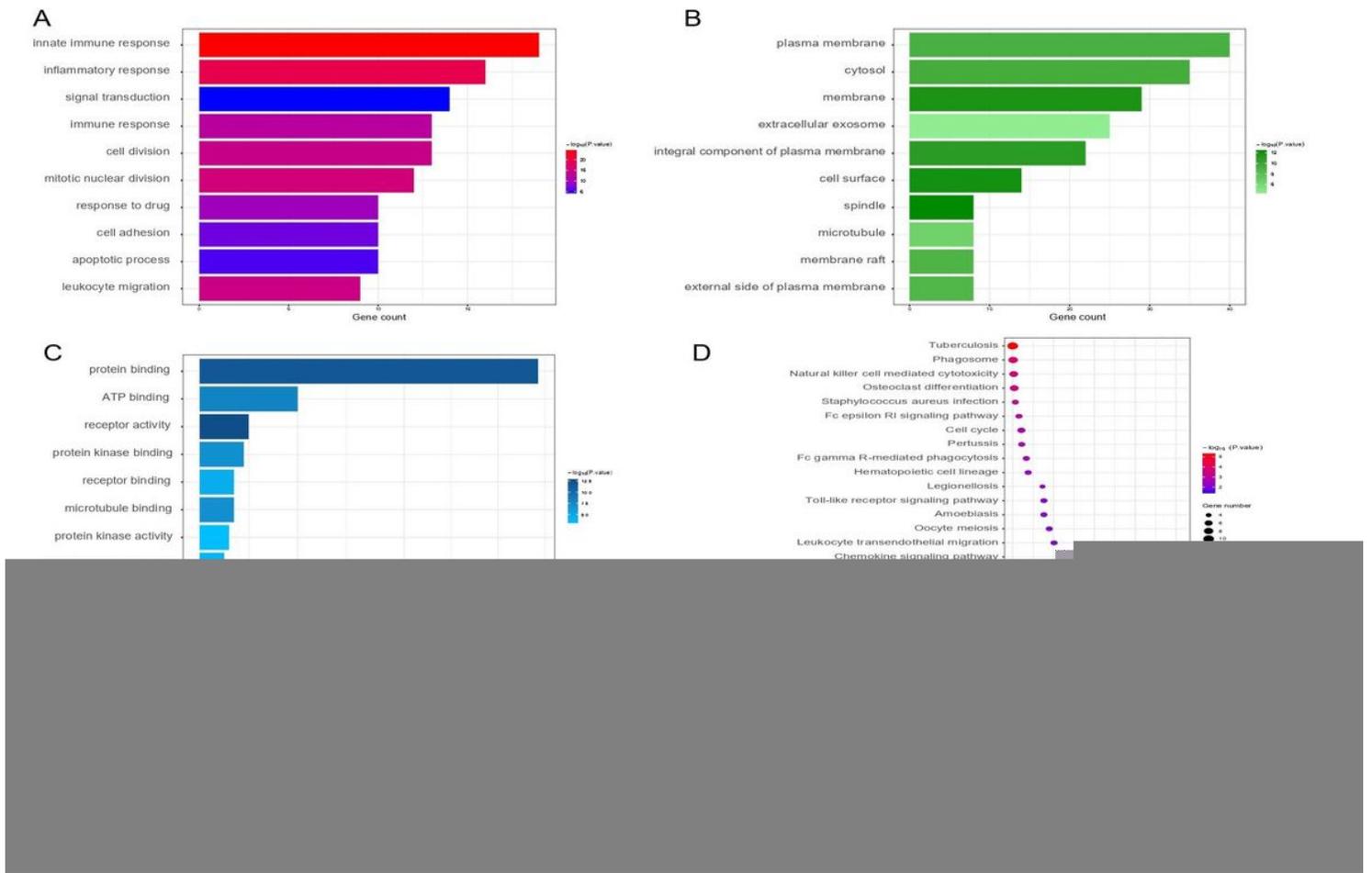


Figure4

The correlation between module and immunity cells. Red means positive correlation, blue means negative correlation.

Figure 4

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**Figure 5**

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**Figure 6**

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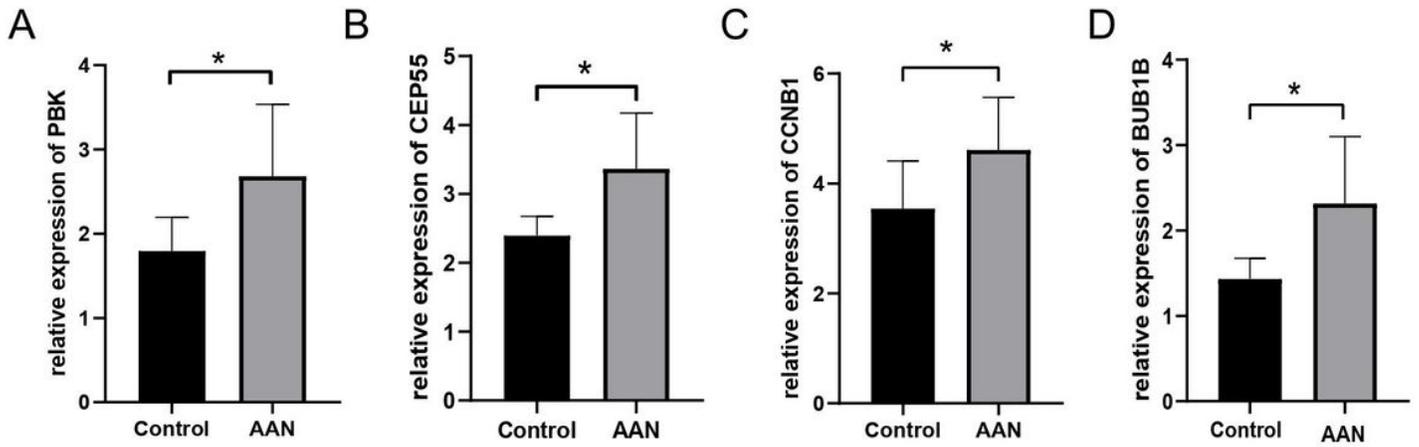


Figure7  
The hub gene expression in GSE109108. (A-D) The relative expression of PBK, CEP55, CCNB1, BUB1B in AAN and normal control. \*P<0.05

## Figure 7

Please See image above for figure legend.

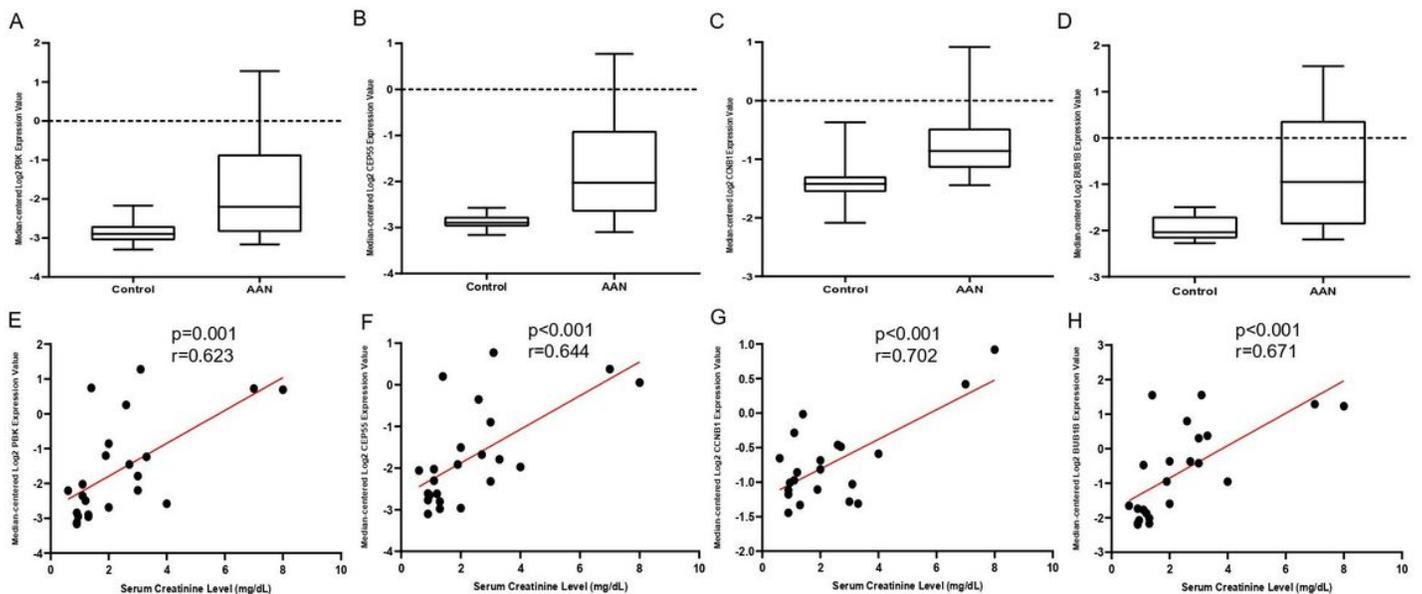


Figure8  
The hub genes relative expression and correlation in Nephroseq database. (A-D) The relative expression of PBK, CEP55,CCNB1,BUB1B. (E-D) The correlation between PBK, CEP55,CCNB1,BUB1B and serum creatinine. P<0.05 means significantly

## Figure 8

Please See image above for figure legend.