Abstract. – OBJECTIVE: Emerging studies have suggested a strong link between Crohn’s disease (CD) and IgA nephropathy (IgAN), but the underlying pathogenesis remains unclear. This led us to explore the common pathogenic genes for the two diseases by originally applying a bio-informatic method.

MATERIALS AND METHODS: The CD and IgAN datasets were downloaded from the Gene Expression Omnibus (GEO) database. The common differentially expressed genes (DEGs) of the two diseases were identified. GO and KEGG enrichment analyses for the common DEGs were further performed. Then, PPI networks were constructed to identify the hub genes. Afterwards, the receiver operating characteristic (ROC) curves were constructed to assess the diagnostic value of the hub genes. Finally, the immune infiltrations in the samples were analyzed and the correlation of the hub genes with the immune infiltration was studied.

RESULTS: 47 common DEGs were identified between CD and IgAN with the threshold of p-value < 0.05 and log2FC > 1. The top 5 GO terms and 5 KEGG pathways were displayed, and the top 10 hub genes were selected. The diagnostic value of these hub genes was evaluated by calculating the area under the ROC curves. Among the hub genes, CXCL2 was not only identified as the common hub gene, but also with the highest diagnostic value. Finally, CXCL2 was verified to be crucially correlated with the immune infiltration in the samples of CD and IgAN.

CONCLUSIONS: Our study identified critical pathogenic genes commonly responsible for the pathogenesis of CD and IgAN, which provided novel biomarkers and promising therapeutic targets for the two diseases. Further experimental and clinical research are needed to verify our results.

Key Words: Crohn’s disease, IgA nephropathy, Bioinformatics, Differentially expressed gene, Hub gene, Immune infiltration.
was significantly higher in IBD patients than that in non-IBD patients\textsuperscript{2}. These findings indicate that the common pathogenesis may be shared between CD and IgAN. Finding new biomarkers that can predict the coexistence of these two diseases is crucial, since renal puncture and biopsy are invasive manipulations with a certain risk of bleeding. Thus, exploring the common pathogenesis, as well as the sensitive and specific biomarkers of CD and IgAN, is of great significance.

Here in our study, we used the microarray data from the Gene Expression Omnibus (GEO) database trying to explore the underlying pathogenic genes and diagnostic biomarkers of the concomitant CD and IgAN by bioinformatics analysis. The results will provide new targets for further research, as well as for future clinical diagnosis and treatment.

Materials and Methods

Data Acquisition

We searched the gene expression profiles on the GEO database (https://www.ncbi.nlm.nih.gov/geo/), which is a free open-access database, with the key words “Crohn’s disease” or “IgA nephropathy”. The inclusion criteria for selecting the datasets were set as follows: (a) the samples were from homo sapiens; (b) the study type was expression profiling by array; (c) the dataset was a comparison between patients with CD or IgAN and healthy controls; (d) the tissues detected were of the same type (for example, in our work, the samples of CD were all from the ileum, and the samples of IgAN were from the glomerulus); (e) the sample size was at least 10; (f) the number of differentially expressed genes (DEGs) was higher than 100. In this way, two microarray datasets of CD patients and normal controls (GSE102133, GSE75214), as well as one microarray dataset of IgAN patients and healthy controls (GSE93798), were enrolled in our study. The GSE102133 dataset contained the gene expression profiles of ileal mucosal biopsies from 65 CD patients and 12 normal controls, and the GSE75214 dataset contained the gene expression profiles of mucosal biopsies of terminal ileum from 67 CD patients and 11 controls. Both datasets corresponded to the same platform GPL6244. Meanwhile, the gene expression profiles of 20 IgAN patients and 22 controls were included in the GSE93798 dataset. Since all the data were downloaded from the public database on the internet, neither ethics committee approval nor informed consent of patients was required in our research.

Identification of DEGs in CD and IgAN

First, we used the Linear Models for Microarray Data (LIMMA) package for R to identify the DEGs between CD patients and their normal controls, as well as IgAN patients and their healthy controls\textsuperscript{15}. The filtering criteria were set as $p$-value $< 0.05$ and $|\log_2 FC| > 1$. Then, we employed the online analysis tool Venn (http://bioinformatics.psb.ugent.be/webtools/Venn/) to gain the intersection genes from the two sets of DEGs that came from the two disease groups.

Functional Enrichment Analyses for the Common DEGs

To investigate the biological functions and signaling pathways affected by the common DEGs, the Gene Ontology (GO) enrichment analyses – including biological process (BP), molecular function (MF) and cellular component (CC) – as well as the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted by using the R package of clusterProfiler Version 4.0.2\textsuperscript{16}. $p$-value $< 0.05$ was regarded as statistically significant.

Construction of Protein-Protein Interaction (PPI) Network and Identification of the Hub Genes

To explore the interactions among the proteins encoded by the DEGs, we constructed PPI networks via the Search Tool for the Retrieval of Interacting Genes (STRING), Version 11.5\textsuperscript{17}. The minimum required interaction score was set as 0.400, which was considered statistically significant. The results were visualized on Cytoscape, version 3.8.0\textsuperscript{18}. Nodes stood for the proteins, and lines represented the predicted interactions between proteins. Furthermore, the hub genes were explored by calculating the topological feature of each protein in the PPI networks with the cytoHubba plugin through maximal clique centrality (MCC) methods\textsuperscript{19}. Meanwhile, the Molecular Complex Detection (MCODE), another plugin of Cytoscape, was also applied to screen the key molecular modules from the PPI network.

Construction of Receiver Operating Characteristic (ROC) Curves

By using the R package of pROC Version 1.17.0.1\textsuperscript{20}, we constructed the ROC curves and then calculated the area under curve (AUC) of the
Identification of common key genes associated with Crohn’s Disease and IgA nephropathy

corresponding hub genes, which represented their diagnostic efficiency. \( p \)-value < 0.05 was considered statistically significant.

Correlation of the Hub Gene with Immune Infiltration

R package of Gene Set Variation Analyses (GSVA) Version 1.40.1 was used to calculate the abundance of immune cells, according to the expression of reference gene within the gene set\(^{21}\). 28 types of immune cells were enrolled in our study, including activated B cells, activated CD4\(^+\) T cells, activated CD8\(^+\) T cells, activated dendritic cells, CD56\(^{bright}\) natural killer cells, CD56\(^{dim}\) natural killer cells, central memory CD4\(^+\) T cells, central memory CD8\(^+\) T cells, effector memory CD4\(^+\) T cells, effector memory CD8\(^+\) T cells, eosinophils, Gamma delta T cells, immature B cells, immature dendritic cells, macrophages, mast cells, MDSCs, memory B cells, monocytes, natural killer cells, natural killer T cells, neutrophils, plasmacytoid dendritic cells, regulatory T cells, T follicular helper cells, Th1 cells, Th17 cells and Th2 cells. The immune scores of each sample were calculated by method of single-sample gene set enrichment analysis (ssGSEA). Finally, we evaluated the relationships between the hub gene and infiltrated immune cells with Spearman correlation analysis.

Results

Analysis of DEGs in CD and IgAN

We downloaded two CD-related datasets (GSE102133 and GSE75214), which belonged to the same sequencing platform, and the emerged dataset contained 132 CD patient samples and 23 control samples. Meanwhile, the IgAN dataset (GSE93798) was enrolled in our study, which contained 20 IgAN patient samples and 22 control samples. The detailed information of these datasets is shown in Table I. The flow chart of our study is presented in Figure 1. The heatmaps and volcano plots showing the gene expression profiles of CD and IgAN are separately presented in Supplementary Figures 1 and 2 and Figure 2. As shown in Figure 3, 342 (204 upregulated, 138 downregulated) and 347 (104 upregulated, 243 downregulated) DEGs were found between patients and their corresponding controls, respectively. Moreover, 47 common DEGs of the two diseases were obtained by using the Venn Diagram, and their detailed information is shown in Table II.

GO and KEGG Enrichment Pathway Analysis

Then, we performed GO functional enrichment and KEGG pathway analyses of the 47 common DEGs between CD and IgAN and displayed the top 5 pathways with the minimum \( p \)-value. As shown in Figure 4, changes in GO biological processes mainly included inflammatory or immune reactions (e.g., response to molecule of bacterial origin, response to lipopolysaccharide, leukocytes chemotaxis, myeloid leukocytes migration) and response to zinc ion. Changes in cellular component were notably focused on enrichment of apical plasma membrane, apical part of cell, cluster of actin-based cell projections, brush border and brush border membrane. Moreover, changes in the molecular function were significant in CXCR chemokine receptor binding, as well as the transporter activity (i.e., solute: cation symporter activity, solute: sodium symporter activity, organic acid: sodium symporter activity, vitamin transmembrane transporter activity). In particu-
lar, changes in the KEGG pathway were mostly enriched in immune-related pathways (TNF signaling pathway, IL-17 signaling pathway), viral protein interaction with cytokine and cytokine receptor, AGE-RAGE signaling pathway in diabetic complications, as well as African trypanosomiasis.

**PPI Network Analysis and Hub Gene Identification**

To distinguish the hub genes from the common DEGs of the two diseases, the PPI network was constructed. As shown in Figure 5B, interleukin-1 beta (IL-1B), matrix metalloproteinase-2 (MMP2), indoleamine 2,3-dioxygenase 1 (IDO1), selectin E (SELE), C-X-C motif chemokine receptor 2 (CXCR2), serpin family E member 1 (SERPINE1), C-X-C motif chemokine ligand 2, 10, 11 (CXCL2, 10, 11) and suppressor of cytokine signaling 3 (SOCS3) were identified as the hub genes of the concomitant CD and IgAN by MCC method. In the meantime, Figure 5A shows that a significant module was obtained from the PPI network of common DEGs by using MCODE, including 7 nodes representing IL1B, CXCR2, CXCL2, 10, 11, SELE and SERPINE1, as consistent with the previous results obtained by MCC method. We also constructed PPI networks to obtain the hub genes of CD and IgAN. The results, which are presented in Figure 5C-D and Table III, suggested that there were four intersection genes shared by the three sets of hub genes, including CXCL2, 10, 11 and CXCR2.

**Validation of the Diagnostic Value of the Hub Genes**

Then, we constructed ROC curves to validate the diagnostic value of the 10 hub genes obtained from the PPI network on the common DEGs. As shown in Figure 6, the corresponding AUC for IL-1B, MMP2, IDO1, SELE, CXCR2, SERPINE1, CXCL2, 10, 11 and SOCS3 in CD patients and normal controls were 0.929, 0.851, 0.937, 0.866, 0.917, 0.910, 0.903, 0.903, 0.900 and 0.907, respectively. The AUC for the hub genes were 0.850, 0.980, 0.816, 0.718, 0.884, 0.859, 0.964, 0.784, 0.818 and 0.955, respectively, in IgAN patients and their controls. From the above results, we found the AUCs of CXCL2 and SOCS3 were higher than 0.9 in both disease groups, indicating that these two hub genes possessed the greatest value in diagnosing the concomitant CD and IgAN.

**The Correlation of CXCL2 and Immune Infiltration in CD and IgAN**

According to previous studies, immunity is crucially involved in the pathogenesis of CD and IgAN. Thus, we analyzed the quantified infiltration of 28 types of immune cells in the samples of the two disease groups. As depicted in Figure 7, the infiltration of activated CD4+ T cells, activated dendritic cells, CD56bright natural killer cells, cen-
Identification of common key genes associated with Crohn’s Disease and IgA nephropathy

Central memory CD4+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells, eosinophils, Gamma delta T cells, immature dendritic cells, macrophages, mast cells, MDSC, monocytes, natural killer cells, natural killer T cells, neutrophils, plasmacytoid dendritic cells, regulatory T cells, T follicular helper cells, type 1 T helper cells, type 17 T helper cells and type 2 T helper cells in the samples of CD patients was significantly different from the normal controls. Similarly, there also existed statistical differences in the infiltration of activated B cells, activated dendritic cells, CD56bright natural killer cells, central memory CD4 T cells, effector memory CD8 T cells, eosinophils, immature B cells, mast cells, MDSC, memory B cells, monocytes, natural killer cells, natural killer T cells, neutrophils, plasmacytoid dendritic cells, regulatory T cells, T follicular helper cells, Th1 and Th17 cells between IgAN patients and their healthy controls.

Figure 2. Identification of the DEGs in CD and IgAN. A. The volcano plot showing the DEGs in CD. B. The volcano plot showing the DEGs in IgAN. DEGs: differentially expressed genes; CD: Crohn’s disease; IgAN: immunoglobulin A nephropathy.
Since CXCL2 was not only identified as one of the four intersection genes shared by the three sets of hub genes, but it also represents one of the hub genes with the highest diagnostic value in the concomitant CD and IgAN, we further studied the relationship of CXCL2 and the immune cell infiltration in the two disease groups. As shown in Figure 8, CXCL2 was mostly related with the infiltration of neutrophils, activated dendritic cells, immature dendritic cells, Th2 cells, plasmacytoid dendritic cells, effector memory CD8+ T cells, Th17 cells, myeloid-derived suppressor cells, activated CD4+ T cells, regulatory T cells and mast cells in the samples of CD patients and their controls. In addition, in the samples of IgAN patients and normal controls, CXCL2 was positively related to the infiltration of eosinophils, memory B cells, neutrophils, activated CD4+ T cells, mast cells, Th17 cells and negatively related with effector memory CD8+ T cells, activated B cells, immature B cells, etc. These results suggested CXCL2 had strong correlations with most of the immune cells that differently infiltrated between the patients and controls, indicating that CXCL2 was crucially involved in the immune infiltration, thus playing a key role in the pathogenesis of the two diseases.

### Discussion

Crohn’s disease is a chronic, relapsing and incurable inflammatory bowel disease with variable symptoms and sometimes developing extraintestinal manifestations which can affect skin, joints, eyes, liver, blood vessels and kidneys. Recently, emerging clinical case reports have indicated a strong link between CD and IgAN. Although it is now widely accepted that the immunity dysregulation resulting from the complexed interplay between genetic susceptibility and environmental factors contributes to the occurrence and development of CD and IgAN, the specific pathogenesis still remains unclear. At present, there are three main hypotheses regarding the mechanisms...
underlying the pathogenesis of CD and IgAN. Firstly, the systemic absorption of IgA at the inflammatory sites of the intestinal mucosa of CD patients is thought to be associated with the occurrence of IgAN. Secondly, the two diseases may share some common pathogenic genetic factors, such as human leukocyte antigen (HLA)-DR1. Thirdly, abnormal T-helper lymphocytes may be a factor contributing to the onset of these two diseases. Nevertheless, the above hypotheses still need further experimental confirmation.

As we know, genetic research on the common mechanisms of CD and IgAN still lack. Herein, our study has been the first to explore the possible common pathogenic genes in these two diseases. As a result, we found 47 common DEGs between CD and IgAN by using data from the GEO datasets, and further performed the GO and KEGG enrichment analyses. The results showed that the changes in GO biological processes mainly included inflammatory or immune reactions, such as response to molecule of bacterial origin, response to lipopolysaccharide, leukocytes chemotaxis and myeloid leukocytes migration. Moreover, the changes in the KEGG pathway were mostly enriched in immune-related pathways (TNF signaling pathway, IL-17 signaling pathway), viral protein interactions with cytokine and cytokine receptors, as well as AGE-RAGE signaling pathways in diabetic complications. The above results indicated that the common DEGs of the two diseases were closely related to inflammatory and immune responses, providing further supports for the current mainstream opinion that both CD and IgAN are immune-related diseases.

Specifically, the GO biological process analysis suggested that bacterial infection and the lipopolysaccharide, which is an endotoxin of the gram-negative bacteria, might be pathogenic factors for CD and IgAN. According to the literature, the intestinal microbial composition has already been proved to be related with the onset of IBD. The microbiota dysbiosis with pathogen overgrowth may impair mucosal barrier function, leading to increased bacterial translocation and absorption of lipopolysaccharide,
Figure 5. PPI network construction and the hub genes in different colors. A, PPI network of all the common DEGs and the key molecular module obtained by MCODE. B, PPI network of the top 10 hub genes obtained from the common DEGs by MCC method. C, PPI network of the top 10 hub genes from the DEGs of CD by MCC method. D, PPI network of the top 10 hub genes from the DEGs of IgAN by MCC method. E, Venn diagram of intersecting common hub genes. PPI: protein-protein interaction; DEGs: differentially expressed genes; MCODE: molecular complex detection; MCC: maximal clique centrality; CD: Crohn’s disease; IgAN: immunoglobulin A nephropathy.
Identification of common key genes associated with Crohn's Disease and IgA nephropathy

contributing to the inflammation of the gut\textsuperscript{31,32}. Additionally, alterations in the intestinal bacterial composition have also been found in IgAN patients, indicating a possible role of the microbiota dysbiosis and pathogen infection in the pathogenesis of IgAN\textsuperscript{31}. Consistently, a previous study\textsuperscript{33} reported a case of concomitant occurrence of IgAN and CD with a history of Helicobacter pylori infection. This study does bring up the interesting association of the concomitant CD and IgAN with the history of bacterial infection, though whether they are truly related to each other deserves further investigation.

Furthermore, the KEGG pathway analysis showed that the common DEGs were mostly enriched in TNF signaling pathways and IL-17 signaling pathways. TNF is a critical cytokine involved in cell survival, apoptosis, inflammation and immunity\textsuperscript{34}. TNF-alpha has been widely recognized to play a key role in the development of CD, and anti-TNF-alpha has become the most successful treatment of CD in clinical practice, so far\textsuperscript{28}. Also, TNF-alpha released from human mesangial cells and podocytes has been proved to mediate the development of interstitial damage in IgAN\textsuperscript{35}. These facts indicated that TNF might be a common pathogenic factor for the two diseases. IL-17 is an important proinflammatory cytokine which has been the focus of intensive studies because of its critical role in inflammatory and immune disorders\textsuperscript{36}. In a previous case, IL-17 was measured by immunostaining in both renal and colon tissues from the patient suffering from rapidly progressive IgAN concurrent with exacerbation of CD. Results showed that IL-17 was positive in both tissues, but negative in renal tissues from a patient only with primary IgAN, indicating that IL-17 activation might also be involved in the common pathogenesis of these two diseases\textsuperscript{12}. In conclusion, inflammation and immunity play a key role in the common pathogenesis of CD and IgAN.

Then, we constructed PPI networks to identify the hub genes and verified their diagnostic value through the ROC curves. We noticed that CXCL2 was a common hub gene that simultaneously possessed the highest diagnostic value. Chemokines are a kind of cytokine or signaling protein secreted by cells\textsuperscript{37}. They have the ability to induce targeted chemotaxis of nearby reactive cells\textsuperscript{38}. They contain four subfamilies: CXC, CC, CX3C and XC\textsuperscript{39}. CXCL2, also known as macrophage inflammatory protein (MIP)-2, belongs to the CXC subfamily\textsuperscript{40}. It is secreted by a variety of cell types, including monocytes, macrophages, endothelial cells and hepatocytes, in response to infections or injuries\textsuperscript{41}. It mainly affects the recruitment of polymorphonuclear leukocytes into sites of injury or infection, thereby regulating immune and inflammatory responses\textsuperscript{41}. Hence, we analyzed the association of CXCL2 with the immune cell infiltration in the two diseases. Indeed, the results suggested that CXCL2 was crucially involved in the immune infiltration, thereby participating in the pathogenesis of the two diseases.

There were a few limitations in our study. The biggest defect might be the representativeness of our datasets downloaded from GEO database. Only two CD datasets and one IgAN dataset were enrolled in our research; therefore, our results may not necessarily well represent the results obtained from other datasets. On the

<table>
<thead>
<tr>
<th>Rank</th>
<th>Name</th>
<th>Score</th>
<th>Name</th>
<th>Score</th>
<th>Name</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IL1B</td>
<td>195</td>
<td>CXCL8</td>
<td>2.484163704284E+13</td>
<td>JUN</td>
<td>4284572</td>
</tr>
<tr>
<td>2</td>
<td>CXCL10</td>
<td>186</td>
<td>CXCL1</td>
<td>2.4865782025742E+13</td>
<td>FOX</td>
<td>3990892</td>
</tr>
<tr>
<td>3</td>
<td>SELE</td>
<td>168</td>
<td>CXCL10</td>
<td>2.188857239634E+13</td>
<td>CXCL10</td>
<td>3862542</td>
</tr>
<tr>
<td>4</td>
<td>CXCR2</td>
<td>144</td>
<td>CXCL5</td>
<td>2.15245882656E+13</td>
<td>CXCR2</td>
<td>3733102</td>
</tr>
<tr>
<td>5</td>
<td>CXCL2</td>
<td>132</td>
<td>CXCL2</td>
<td>2.1499167796201E+13</td>
<td>CCL4</td>
<td>3660080</td>
</tr>
<tr>
<td>6</td>
<td>CXCL11</td>
<td>126</td>
<td>CCL20</td>
<td>2.134232275536E+13</td>
<td>CASR</td>
<td>3649576</td>
</tr>
<tr>
<td>7</td>
<td>MMP2</td>
<td>50</td>
<td>CXCR2</td>
<td>2.114361688416E+13</td>
<td>CX3CR1</td>
<td>3648588</td>
</tr>
<tr>
<td>8</td>
<td>SERPINE1</td>
<td>27</td>
<td>CXCL9</td>
<td>2.11321691384E+13</td>
<td>CXCL2</td>
<td>3643098</td>
</tr>
<tr>
<td>9</td>
<td>SCOS3</td>
<td>18</td>
<td>CXCL11</td>
<td>2.1122613762504E+13</td>
<td>CXCL11</td>
<td>3640368</td>
</tr>
<tr>
<td>10</td>
<td>IDO1</td>
<td>12</td>
<td>CXCR1</td>
<td>2.103547864449E+13</td>
<td>FPR3</td>
<td>3629958</td>
</tr>
</tbody>
</table>

\textsuperscript{1}MCC: maximal clique centrality  
\textsuperscript{2}CD: Crohn’s disease  
\textsuperscript{3}IgAN: IgA nephropathy

Table III. Top 10 hub genes ranked by MCC method.
Figure 6. Diagnostic value of the top 10 hub genes from the common DEGs with ROC curves in CD (A) and IgAN (B). DEGs: differentially expressed genes; ROC: receiver operating characteristic; CD: Crohn’s disease; IgAN: immunoglobulin A nephropathy.
Figure 7. Analyses of infiltration of 28 types of immune cells in the tissues from patients with CD (A) and IgAN (B). CD: Crohn’s disease; IgAN: immunoglobulin A nephropathy.
Figure 8. The Spearman correlation analyses of CXCL2 with immune infiltration in CD (A) and IgAN (B). CXCL2: C-X-C motif chemokine ligand 2; CD: Crohn’s disease; IgAN: immunoglobulin A nephropathy.
other hand, it is a great pity that we did not further verify our bioinformatics analyses through experiments. Hence, further investigations are still needed to explore the common pathogenetic mechanisms underlying the concomitant CD and IgAN.

Conclusions

We found 47 common DEGs between CD and IgAN. These genes are crucially involved in inflammatory and immune responses. CXCL2 is the common hub gene and has the greatest diagnostic value. It is also strongly correlated with the immune infiltration in the two diseases. Our results provide some novel and common pathogenic genes that have the potential to become an effective breakthrough for diagnosis and treatment of the concomitant CD and IgAN. However, the exact common pathogenesis shared by these two diseases still needs more in-depth studies.

Funding

This work was supported by the National Natural Science Foundation of China (82000245 to Yaping Wang).

Conflict of Interest

The authors declare that they have no conflicts of interest.

References


