Research Article

Hub Gene Screening Associated with Early Glaucoma: An Integrated Bioinformatics Analysis

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Background. Primary open-angle glaucoma (POAG) is the most common type of glaucoma. The potential influence of some DEGs on the progression of POAG was still incomplete. In this study, we integrated transcriptome data with clinical data to investigate the relationship between them in POAG patients.

Methods. The gene expression profile (GSE27276) from Gene Expression Omnibus (GEO) was used to identify DEGs. The LIMMA package of R was used to identify the DEGs (Diboun et al., 2006). The adjusted P values (adj P value) were calculated instead to avoid the appearance of false-positive results. Genes with $|\log_2 \text{fold change (FC)}| > 1$ and adj P value $< 0.01$ were taken as DEGs between PH and PC samples. GO (Gene Ontology) function and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analyses of the DEGs were performed. Protein-protein interactions (PPIs) of the DEGs were constructed.

Results. A total of 182 DEGs were identified through our analysis, of which 119 genes were upregulated and 63 genes were downregulated. GO enrichment analysis illustrated that these DEGs were mostly enriched into haptoglobin binding, antioxidant activity, and organic acid binding. KEGG enrichment analysis illustrated that these DEGs were mostly enriched into Staphylococcus aureus infection. The most significant module was identified by MCODE consists of 8 DEGs, and BCL11A is the seeded gene. The second most significant module consists of 5 DEGs, and IL1RN is the seeded gene.

Conclusion. Our results demonstrate the potential influence of some DEGs on the progression of POAG, providing a comprehensive bioinformatics analysis of the pathogenesis, which may contribute to future investigation into the molecular mechanisms and biomarkers.

1. Introduction

Primary open-angle glaucoma (POAG) is the most common type of glaucoma, accounting for 60%-70% of all glaucoma, which usually affects both eyes but is not necessarily symmetrical [1]. The morbidity of POAG increased fast and threatened the health and life of the population with population growth and aging [2, 3]. Many different abnormalities have been noted on histopathological examination of the drainage angle in patients with POAG [4]. These include narrowed intertrabecular spaces, thickened basement membranes, fused trabecular beams, reduction in trabecular endothelial cells, reduction in actin filaments, narrowing of collector channels, foreign material accumulation, scleral spur thickening, and closure of Schlemm’s canal. POAG patients often find themselves with this disease when it has entered the middle and late stage of the disease, so if early detection and treatment can be achieved, the retina and optic nerve can be protected to a large extent, and the existence of effective vision of patients can be prolonged [5]. Visual loss from glaucoma is irreversible, and therefore, prevention is a key strategy to preventing morbidity from this condition.

Its pathogenesis is often related to genetic factors [6, 7]. At present, it is mainly believed that some structural changes in outflow channel of the aqueous humor caused by some factors could result in unobstructed outflow of the aqueous humor and increase of intraocular pressure, but there is basically no stenosis or obstruction in the structure of the atrial angle [8]. Intraocular pressure (IOP) is considered the most important risk factor for the development of POAG and remains the only known modifiable risk factor. Population
studies have shown increased prevalence of glaucoma with increasing IOP [9]. The prevalence of POAG increases with age, even after compensating for the association between age and IOP [10]. Several studies have shown POAG to be more prevalent in people of African-Caribbean descent compared with Caucasians. Not only is POAG more prevalent in black race, its onset is earlier, and disease progression has been shown to be faster and more refractory to treatment [11]. Myopia has also been shown to be a risk factor for POAG in several studies [12].

POAG is treated with medication of first choice, namely, eye drops. Drugs that reduce the generation of aqueous humor fluid and accelerate the outflow of aqueous humor fluid can be selected [13, 14]. If a combination of drugs does not achieve the desired IOP, a combination formulation may be used. If drugs do not work, selective laser trabeculoplasty is an option [15]. Glaucoma surgery is the last option if the visual field progression cannot be suppressed by drugs or lasers. No matter drugs, laser, surgical treatment are to make the IOP drop to the visual field injury no longer progress level [16]. According to the etiology and inducement of POAG, the key preventive measure is to regularly monitor intraocular pressure, maintain a good attitude, and pay attention to systemic diseases [17, 18]. POAG has a genetic tendency and is generally considered to be polygenic [19]. Therefore, the family history of primary open-angle glaucoma is the most dangerous factor. People with family genetic history should go to the hospital in time for early screening of POAG.

In the present study, the differential expression of critical genes plays a key role in the mechanism of common development of the POAG and will affect therapy as well as the efficacy of medicine. Recent genome-wide studies have identified lots of novel loci associated with POAG. For example, the mutations myocilin (MYOC), optineurin (OPTN), and TANK-binding kinase 1 (TBK1) may cause POAG that is inherited as a Mendelian trait. The relationship between differentially expressed genes (DEGs) and the progression of POAG still demanded to be explained. The sharing of transcriptome data and the development of new bioinformatics analysis tools have enabled us to integrate transcriptome data with clinical data to investigate the relationship between them. This can help us understand the development of POAG from both perspectives and provide a new perspective for the prevention and treatment of the disease.

2. Material and Methods

2.1. Data. The gene expression profiles (GSE27276), which are composed of 13 controls and 15 primary open-angle glaucoma (POAG) cases, were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) and exploited as discovery datasets to identify differentially expressed genes (DEGs). This study compared genome-wide expression profiles of individuals with and without POAG.

Of these cases, six controls and one POAG cases had the expression performed from both left and right eyes. One technical replicate was done between two cases.

2.2. Identification of DEGs. The LIMMA package of R was used to identify the DEGs [20]. The adjusted $P$ values (adj $P$ value) were calculated instead to avoid the appearance of false-positive results. Genes with $|\log_2$ fold change (FC)| larger than 1 and adj $P$ value $< 0.01$ were taken as DEGs between PIH and PC samples. The relevant immune genes were searched in IMMPORT (https://www.immport.org/resources) to find potential immunotherapy targets.

2.3. GO and KEGG Enrichment Analyses. GO (Gene Ontology) function and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analyses of the DEGs were performed using clusterProfiler and pathview packages of R, which are designed for automating the process of biological-term classification and the enrichment analysis of gene clusters [21].

2.4. PPI Network Construction. Protein-protein interactions (PPIs) of the DEGs were constructed based on the Search Tool for the Retrieval of Interacting Genes (STRING; http://string.embl.de/) with the confidence score $\geq 0.9$ [22]. Subsequently, the PPI network was visualized by means of Cytoscape software (version 3.5.1). Furthermore, the plugin of Molecular Complex Detection (MCODE) [23] in Cytoscape software was applied to explore the significant modules in the PPI network with default settings.

2.5. Statistical Analysis. Statistical calculations were carried out using SPSS statistical software (SPSS Inc., USA). For multiple comparisons, data were analyzed via analysis of variance (ANOVA) with the Tukey-Kramer Multiple Comparisons Test. $P$ values $< 0.05$ were considered significant.

3. Results

3.1. Differentially Expressed Genes (DEGs). The gene expression profiles (GSE27276) were used to identify DEGs, which are composed of 13 controls and 15 primary open-angle glaucoma (POAG) cases. A total of 182 DEGs were identified through our analysis, of which 119 genes were upregulated and 63 genes were downregulated (Figures 1 and 2). Of those 182 DEGs, 36 DEGs were identified as immune-related genes (Table 1). Their functions can be classified as
antigen processing and presentation, antimicrobials, BCR signaling pathway, cytokines, cytokine receptors, interleukins, interleukin receptor, natural killer cell cytotoxicity, TCR signaling pathway, TGFβ family member, and TNF family member receptors. Of the 36 immune-related genes, 22 DEGs were upregulated, including CHP2, CSF3, DEFB1, FABP5, FAM3B, FAM3D, GDF15, IL1RN, IL20RB, LCN2, MASP1, MTNR1A, NAMPT, S100A11, S100A12, S100A14, S100A2, S100A8, S100A9, SAA2, SERPINA3, and SLPI. Of the 36 immune-related genes, 14 DEGs were downregulated, including CCN2, CD74, CLEC11A, GPHA2, GRP, HLA-DMB, HLA-DPA1, MCHR1, OGN, PTGDS, TNFRSF25, TPM2, TYROBP, and VIM.

### 3.2. Functional Enrichment Analysis of DEGs

GO enrichment analysis illustrated that these DEGs were enriched in several terms (Figure 3), including haptoglobin binding, antioxidant activity, organic acid binding, oxygen carrier activity, peroxidase activity, oxidoreductase activity, calcium-dependent protein binding, MAP kinase phosphatase activity, fatty acid binding, oxygen binding, protein tyrosine/threonine phosphatase activity, protein tyrosine/threonine phosphatase activity, RAGE receptor binding, insulin-like growth factor binding, extracellular matrix structural constituent, MAP kinase tyrosine/serine/threonine phosphatase activity, long-chain fatty acid binding, intermediate filament binding, molecular carrier activity, monocarboxylic acid binding, protein serine phosphatase activity, protein threonine phosphatase activity, structural constituent of muscle, serine-type endopeptidase activity, extracellular matrix structural constituent conferring compression resistance, growth factor binding, serine-type peptidase activity, serine hydrolase activity, protein serine/threonine phosphatase activity, and protein tyrosine phosphatase activity. KEGG enrichment analysis illustrated that these DEGs were enriched in several pathways (Figure 4). The top 10 most enriched pathways were *Staphylococcus aureus* infection, estrogen signaling pathway, tyrosine metabolism, IL-17 signaling pathway, malaria, toxoplasmosis, African trypanosomiasis, mineral absorption, phenylalanine metabolism, and histidine metabolism.

### 3.3. Protein-Protein Interaction Network

STRING was used to construct the PPI network, and the most significant modules in the PPI network were identified in Cytoscape software. The regulatory network is complex (Figure 5), and the top 5 DEGs with the highest degrees are LCN2, HP, KRT19, CDH2, and KRT5 (Figure 6). The most significant
| Symbol  | ID      | Name                                      | Synonyms                                      | Chromosome | Category                                      |
|---------|---------|-------------------------------------------|-----------------------------------------------|------------|-----------------------------------------------|
| CCN2    | 1490    | Cellular communication network factor 2   | CTGF|HCS24|IGFBP8|NOV2                                          | 6          | Cytokines                                     |
| CD74    | 972     | CD74 molecule                              | DHLAG|HLADG|Il|Ia-GAMMA|p33                                           | 5          | Antigen_processing_and_presentation           |
| CHP2    | 63928   | Calcineurin-like EF-hand protein 2        |                                              | 16         | BCRSignalingPathway, NaturalKiller_cell_cytotoxicity, TCRSignalingPathway |
| CLEC11A | 6320    | C-type lectin domain-containing 11 A      | CLECSF3|LSLCL|P47|SCGF                                          | 19         | Cytokines                                     |
| CSF3    | 1440    | Colony-stimulating factor 3               | C17orf3|CSF3OS|CSF                                           | 17         | Cytokines                                     |
| DEFB1   | 1672    | Defensin beta 1                           | BD1|DEFB-1|DEFB101|HBDD1                                        | 8          | Antimicrobials, chemokines, cytokines        |
| FABP5   | 2171    | Fatty acid-binding protein 5              | E-FABP|EFABP|KFABP|PA-FABP|PAFABP                                      | 8          | Antimicrobials                                |
| FAM3B   | 54097   | FAM3 metabolism regulating signaling molecule B | 2-21|C21orf1|C21orf76|ORF9|PANDER|PRE44                                    | 21         | Cytokines                                     |
| FAM3D   | 131177  | FAM3 metabolism regulating signaling molecule D | EF7|OIT1                                        | 3          | Cytokines                                     |
| GDF15   | 9518    | Growth differentiation factor 15          | GDF-15|MIC-1|MIC1|NAG-1|PDF|PLAB|PTGFB                                       | 19         | Antimicrobials, cytokines, TGFb_family_member |
| GPHA2   | 170589  | Glycoprotein hormone subunit alpha 2      | A2|GPA2|ZSG51                                       | 11         | Cytokines                                     |
| GRP     | 2922    | Gastrin-releasing peptide                 | BN|GRP-10|preproGRP|proGRP                                         | 18         | Cytokines                                     |
| HLA-DMB | 3109    | Major histocompatibility complex, class II, DM beta | D6S221|RING7                                       | 6          | Antigen_processing_and_presentation           |
| HLA-DPA1| 3113    | Major histocompatibility complex, class II, DP alpha 1 | DP(W3)|DP(W4)|DP1A|HLA-DP1A|HLA-DPB1|HLADP|HLASB|PLT1                                       | 6          | Antigen_processing_and_presentation           |
| IL1RN   | 3557    | Interleukin 1 receptor antagonist         | DIRA|CIL-1RA|IL-1RNL|IL-1ra|IL-1ra3|IL1F3|ILRA|IRAP|MVCD4                                    | 2          | Cytokines, interleukins                       |
| IL20RB  | 53833   | Interleukin 20 receptor subunit beta      | DIRS1|FNDC6|IL-20R2                                    | 3          | Cytokine_receptors, interleukins_receptor    |
| LCN2    | 3934    | Lipocalin 2                               | 24p3|MSF1|INGAL|p25                                        | 9          | Antimicrobials                                |
| MASP1   | 5648    | Mannan-binding lectin                serine peptidase 1 | 3MC1|CRARF|CRARF1|MAP1|MASP|MASP3|MAP44|PRSS5|RaRF                                    | 3          | Antimicrobials                                |
| MCHR1   | 2847    | Melanin-concentrating hormone receptor 1 | GPR24|MCH-1R|MCH1R|SLC-1|SLC1                                        | 22         | Cytokine_receptors                           |
| MTNR1A  | 4543    | Melatonin receptor 1A                    | MEL-1A-R|MT1                                     | 4          | Cytokine_receptors                           |
| NAMPT   | 10135   | Nicotinamide phosphoribosyltransferase    | 1110035014Rik|PB|PB1|PB2|PB4|PY|VISFATIN                                         | 7          | Cytokines                                     |
| OGN     | 4969    | Osteoglycin                               | OG|OS|SLR3A                                        | 9          | Cytokines                                     |
| PTGDS   | 5730    | Prostaglandin D2 synthase                | L-PGDS|LPGDS|PDS|PG2|PGD2|PGDS|PGDS2                                      | 9          | Antimicrobials, cytokine_receptors           |
| Symbol  | ID    | Name                                      | Synonyms                                            | Chromosome | Category                          |
|---------|-------|-------------------------------------------|-----------------------------------------------------|------------|-----------------------------------|
| S100A11 | 6282  | S100 calcium-binding protein A11          | HEL-S-43|MLN70|S100C                             | 1          | Antimicrobials                    |
| S100A12 | 6283  | S100 calcium-binding protein A12          | CAAF1|CAGC|CGRP|ENRAGE|MRP-6|MRP6|p6                                | 1          | Antimicrobials                    |
| S100A14 | 57402 | S100 calcium-binding protein A14          | BCMP84|S100A15                                | 1          | Antimicrobials                    |
| S100A2  | 6273  | S100 calcium-binding protein A2           | CAN19|S100L                                  | 1          | Antimicrobials                    |
| S100A8  | 6279  | S100 calcium-binding protein A8           | 60B8AG|CAGA|CFAG|CGLA|CP-10|L1Ag|MA387|MIF|MRP8|NIF|P8                                | 1          | Antimicrobials                    |
| S100A9  | 6280  | S100 calcium-binding protein A9           | 60B8AG|CAGB|CFAG|CGLB|L1AG|LIAG|MAC387|MIF|MRP14|NIF|P14                               | 1          | Antimicrobials                    |
| SAA2    | 6289  | Serum amyloid A2                          | SAA|SAA1                                   | 11         | Chemokines, cytokines             |
| SERPINA3| 12    | Serpin family A member 3                  | AACT|ACT|GG24|GIG25                              | 14         | Antimicrobials                    |
| SLPI    | 6590  | Secretory leukocyte peptidase inhibitor   | ALK1|ALP|BLPI|HUSI|HUSI-I|MPI|WAP4|WFDC4                              | 20         | Antimicrobials                    |
| TNFRSF25| 8718  | TNF receptor superfamily member 25        | 3|DDR3|DR3|GEF720|LARD|PLEK|HGS|TNFRSF12|TR3|TRAMP|WSL1|WSL-LR                             | 1          | Cytokine_receptors, TNF_family_members_receptors |
| TPM2    | 7169  | Tropomyosin 2                             | AMCD1|DA1|DA2B|DA2B4|HEL-S-273|NEM4|TMSB                               | 9          | Antimicrobials                    |
| TYROBP  | 7305  | Transmembrane immune signaling adaptor TYROBP | DAP12|KARAP|PLOSL|PLOSL1                          | 19         | Natural_Killer_cell_cytotoxicity  |
| VIM     | 7431  | Vimentin                                  | —                                    | 10         | Antimicrobals                      |
A module was identified by MCODE with 8 nodes and 54 edges (Table 2). The module consists of 8 DEGs, including HP, HBG2, HBD, HBB, HBG1, HBA1, HBA2, and BCL11A. Of the 8 DEGs, BCL11A is the seeded gene. The average degree of the 8 DEGs is 6.75 and the average score is 5.84. They are enriched into two KEGG pathways, including African trypanosomiasis and malaria. The second most significant module was identified by MCODE with 5 nodes and 20 edges (Table 3). This module consists of 5 DEGs, including IL1RN, LCN2, S100A8, S100A12, and S100A9. Of the 5 DEGs, IL1RN is the seeded gene. The average degree of the 5 DEGs is 4, and the average score is 3.78. They are enriched into two KEGG pathways, including IL-17 signaling pathway and cytokine-cytokine receptor interaction.
4. Discussion

In the present study, the DEGs between controls and primary open-angle glaucoma (POAG) patients were explored. A total of 182 DEGs were identified through our analysis, of which 119 genes were upregulated and 63 genes were downregulated. Of the 36 immune-related genes, 22 DEGs were upregulated and 14 DEGs were downregulated. Their functions can be classified as antigen processing and presentation, antimicrobials, BCR signaling pathway, cytokines, cytokine receptors, interleukins, interleukin receptor, natural killer cell cytotoxicity, TCR signaling pathway, TGFβ family member, and TNF family member receptors. GO enrichment analysis illustrated that these 182 DEGs were mostly enriched into haptoglobin binding, antioxidant activity, and organic acid binding. KEGG enrichment analysis illustrated that these 182 DEGs were mostly enriched into Staphylococcus aureus infection. Haptoglobin is an acute phase reactive protein [24]. Antioxidant activity is usually by preventing the diffusion stage of oxidation chain reactions [25]. This study is meaningful since transcriptome data was integrated to investigate the potential pathogenesis of DEGs between controls and primary open-angle glaucoma (POAG) patients. This study provides a reference for understanding the pathogenesis value of DEGs and formulating reasonable clinical diagnosis and treatment.

The top 5 DEGs with the highest degrees in the protein-protein network are LCN2, HP, KRT19, CDH2, and KRT5. The gene LCN2 encodes a protein that belongs to the lipocalin family. Members of this family transport small hydrophobic molecules such as lipids, steroid hormones, and retinoids [26]. The gene HP encodes a preproprotein, which is processed to yield both alpha and beta chains, which subsequently combine as a tetramer to produce haptoglobin [27]. The gene CDH2 encodes a classical cadherin and member of the keratin family. The keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells and are subdivided into cytokeratins and hair keratins [28]. The gene CDH2 encodes a classical cadherin and member of the cadherin superfamily. Alternative splicing results
in multiple transcript variants, at least one of which encodes a preproprotein proteolytically processed to generate a calcium-dependent cell adhesion molecule and glycoprotein [29]. The protein encoded by this gene KRT5 is a member of the keratin gene family. The type II cytokeratins consist of basic or neutral proteins which are arranged in pairs of heterotypic keratin chains coexpressed during differentiation of simple and stratified epithelial tissues [30].

The most significant module was identified by MCODE with 8 nodes and 54 edges. This module consists of 8 DEGs, including HP, HBG2, HBD, HBB, HBG1, HBA1, HBA2, and BCL11A. Of the 8 DEGs, BCL11A is the seeded gene. This gene BCL11A encodes a C2H2 type zinc-finger protein by its similarity to the mouse Bcl11a/Evi9 protein [31]. The corresponding mouse gene is a common site of retroviral integration in myeloid leukemia and may function as a leukemia disease gene, in part, through its interaction with BCL6. During hematopoietic cell differentiation, this gene is downregulated. It is possibly involved in lymphoma pathogenesis since translocations associated with B cell malignancies also deregulate its expression [32]. The second most significant module was identified by MCODE with 5 nodes and 20 edges. This module consists of 5 DEGs, including IL1RN, LCN2, S100A8, S100A12, and S100A9. Of the 5 DEGs, IL1RN is the seeded gene. The protein encoded by this gene IL1RN is a member of the interleukin 1 cytokine family. This protein inhibits the activities of interleukin 1, alpha (IL1A), and interleukin 1, beta (IL1B), and modulates a variety of interleukin 1-related immune and inflammatory responses, particularly in the acute phase of infection and inflammation [33, 34].

Some limitations should be acknowledged. First, only one dataset was included in the analysis, without considering the impact of population heterogeneity in different countries on the results. Second, the lack of verifiable datasets in this study limits the extrapolation of research results. Third, this study is only for the reanalysis of existing data and lacks the support and verification of experimental data. In conclusion, our results provide a comprehensive bioinformatics analysis between controls and POAG patients, which could contribute to the understanding of the development of POAG and prevention and treatment of the disease.

5. Conclusion

This study demonstrated the potential influence of some DEGs on the progression of POAG, providing a comprehensive bioinformatics analysis of the pathogenesis, which may contribute to future investigation into the molecular mechanisms and biomarkers.

Data Availability

The data used to support this study is available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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