Original Article

Investigation of Genes Associated with Primary Open-Angle Glaucoma (POAG) Using Expression Profile Analysis

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Abstract

Background: Glaucoma is recognized as one of the most common causes of global blindness observed in various types, such as primary open-angle glaucoma (POAG). This condition is characterized by progressive optic neuropathy, leading to the damage of optic nerve fibers. With no symptoms at the beginning, glaucoma results in decreased vision and eventually blindness over several years. Early treatment can prevent the progression of the disease.
Material and Methods: The researchers performed a study to evaluate differential gene expression in normal control and POAG cases. A total of 179 DEGs were discovered with 60 up-regulated and 119 down-regulated genes. After the selection of DEGs, the protein-protein interaction network was constructed. The result of GO enrichment showed the DEGs were involved in antioxidant activity, haptoglobin binding, and oxygen carrier activity. Then four modules of the primary protein network were obtained using a STRING database, using the K-means method. Next, gene ontology analysis and Kyoto encyclopedia of genes and genomes pathway enrichment were performed for four modules.
Results: The results showed that the selected module (Yellow module) is highly related to glaucoma pathogenesis genes. Among the genes identified in this module are TYRP1, FMOD, OGN, PAX6, COL8A2, HLA-DPA1, and HLA-DMB, which may be involved in the direct development of glaucoma.
Conclusion: Using integrated bioinformatical analysis, the researchers identified DEGs candidate genes and pathways involved in glaucoma, which improved our understanding of the cause and underlying molecular events. These candidate genes and pathways could be therapeutic targets for glaucoma.
Keywords: Glaucoma; Pathogenesis Genes; Primary Open-Angle Glaucoma; Protein-Protein Interaction Network; String.

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Introduction

Glaucoma is the second leading cause of blindness worldwide, affecting approximately 60 million people. Glaucoma is more common among men than women [1, 2]. Glaucoma is more common among adults. Generally, people at risk for glaucoma include those over 40 years of age, a family history of glaucoma, myopia or farsightedness, poor eyesight, diabetes, and steroid medications such as prednisone. Taking certain medications to treat bladder or seizures, Injury in the eye area, thinner-than-normal cornea, hypertension, heart disease, sickle cell anemia (sickle cell anemia), and high eye pressure [3]. Glaucoma is caused by a sharp increase in the pressure within the vitreous or the substance inside the eye, followed by leakage of fluid out of tiny pores around the iris. In fact, increased Intraocular pressure (IOP) is the cause of glaucoma in many people [4]. However, even a significant reduction in IOP could not prevent glaucoma progression in several clinical cases [5]. Glaucoma-associated cell death is primarily due to apoptosis caused by oxidative stress through mitochondrial damage, inflammation, disorder, endothelial dysfunction, and hypoxia [6]. In general, glaucoma is not preventable. However, if it is diagnosed early and treated appropriately, the vast majority of patients may retain beneficial visual function for life. Therefore, to prevent glaucoma, early diagnosis and treatment should be emphasized.

Glaucoma is categorized into the following types: a) Open-angle glaucoma: it is the most common type of glaucoma that exhibits no symptoms until advanced levels. This is because the peripheral part of the visual field is damaged first. Since the loss of central vision occurs later when the disease has progressed, affected individuals do not notice the early visual disturbance. As the disease progresses, central vision is lost as well and leads to blindness. b) Closed-angle glaucoma: less than 10% of glaucoma cases are in the United States, but more than half of all glaucoma cases are in other countries, especially in Asia. c) Congenital glaucoma: congenital glaucoma in infants and toddlers is characterized by symptoms of tearing, closing the eyes in contact with light, or fear of light and shrinking the eyelids [4].

It is reported that the cornera of the eye sometimes converts large and cloudy. In glaucoma, the forward and back eye sections are changed, and plain harm may be noticed in the trabecular meshwork [3]. Oxidative stress is deliberated to be accountable for the molecular harm in the forward cavity. Primary open-angle glaucoma (POAG) is the common glaucoma type, accounting for 60–70% of all glaucoma [4].

In some cases of POAG, affected individuals have mutations in the coding region of the myocilin (MYOC) gene. The frequency and nature of these mutations vary in patients with different ethnicity and age groups [3]. Therefore, early diagnosis is essential since it could help prevent the progression of POAG. Biomarker discovery could be a helpful approach in early disease diagnosis and prediction of disease incidence [7].

Innovation in systems biology has brought us a variety of computational tools and analytical means [8-10]. Proposing of the novel panels for early detection of diseases, drug discovery, analyzing signaling networks and metabolomics pathways, and repurposing drugs are attractive subjects in systems biology studies [11-16]. However, the shortage of online data is the main obstacle in the application of the high throughputs approach to ophthalmic.

The present study analyzed microarray transcriptome data from tissue samples of

patients with POAG and a normal group. Bioinformatics analysis were used to categorize the functional annotation and construct the protein-protein interaction (PPI) network related to glaucoma, to evaluate glaucoma pathogenesis and identity the volunteer genes for glaucoma.

Material and methods

Data collection

Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo) was screened using the keyword "glaucoma," and the researchers selected the GSE27276 dataset (platform GPL2507 Sentrix Human-6 Expression Bead Chip) Trabecular Meshwork tissues [17] with a total of 36 samples including 17 patients and 19 normal controls.

Data preprocessing and DEGs analysis

The researchers used the R software ("Limma" package from the Bioconductor project) to identify differentially expressed genes between normal and POAG samples. In order to adjust the p-values, Benjamini and Hochberg's false discovery rate method was used. Fold change of the gene expression between the control and patient was calculated for each gene. Genes with adjusted p-value less than 0.05 and |log fold change| > 1 were selected as differentially expressed, and then the DEGs were grouped as up-regulated and down-regulated DEGs.

GO and KEGG pathway enrichment analyses with Toppgene

Gene ontology consists of three groups: biological processes (BP), cellular components (CC), and molecular function (MF). Cellular features include different parts of a cell or intercellular environment, molecular function indicating the activities of a gene at the molecular level, and biological processes, operations, or a set of molecular events with a specific beginning and end. KEGG (https://www.genome.jp/kegg) is a knowledge base for systematically analyzing gene function in gene networks or proteins. The PATHWAY database is the major component of KEGG, which consists of biochemical pathways as graphic diagrams [18]. Functional enrichment and KEGG pathway analysis were performed in module genes using the Toppgene database (https://toppgene.cchmc.org/). Toppgene site (http://toppgene.cchmc.org; this website is free and open to all users) is a one-stop portal for (I) gene list functional enrichment, (II) candidate gene prioritization using either functional annotations or network analysis, and (III) identification and prioritization of novel disease candidate genes in the interactome [19].

Protein-protein interaction network, GO and KEGG analysis for modules genes

Differentially expressed genes with an adjusted p-value of less than 0.5 and |log fold change| > 1 were selected. Then DEGs were used to construct the protein-protein interaction network using STRING (https://string-db.org). STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a biological database and web resource of known and predicted protein-protein interactions in molecular biology. Then, the STRING database and K-means method (with a confidence level of 0.7) were used to cluster the modules. Finally, the network of each module was drawn separately in the Cytoscape (https://cytoscape.org) software. GO enrichment analysis of the modules genes was performed using Toppgene.

Results

Clinical Features of Glaucoma Cases and Nonglaucomatous Controls

The researchers used the GSE27276 dataset to

Table 1: Clinical Characteristics of Patients Having POAG with Surgical TM Samples

Case No.	sex	Ethnicity	Age at Diagnosis	Eyes Assayed, n
1	M	C	72	1
2	M	AA	67	1
3	M	C	55	1
4	F	C	86	1
5	F	C	73	1
6	M	C	65	1
7	M	AA	61	1
8	M	AA	40	1
9	F	AA	82	1
10	F	AA	69	1
11	F	C	60	1
12	F	C	61	1
13	F	C	79	1
14	F	C	54	1
15	F	C	71	2
Average:	Male:60		Female:70.5	
Variance:	Male:128.8		Female:115.77	
Total Average:	66.33		Total Variance:	140.8

AA, African American; C, Caucasian.

identify differentially expressed genes between POAG and normal samples in this study. This dataset included 35 Trabecular Meshwork tissues from 15 patients with POAG and 13 healthy controls (Table 1 is provided for POAG, and Table 2 is for normal people). Including TM in both left and right eyes were taken from 6 controls and one case POAG. The clinical features of these 15 patients are listed in Table 1 (6 men and 9 women). There were five African-American POAG and 10 Caucasian POAG. They were 40 to 86 years old at the time of diagnosis. All patients with POAG had bilateral disease. As much as possible, Trabecular Meshwork was prepared from all eyes. There was only one trabeculectomy patient with Trabecular Meshwork in both eyes. It was later determined that the patient had an MYOC Q368X mutation in his eye. Trabecular networks were also obtained from 13 non-glaucomatous donors (5 males and 8 females) (Table 2). There were three African-American donors and 10 Caucasians. The age of death of eye donors was 48 to 94. The cause of death varied in non-glaucomatous individuals, but cancer was most common (in 7 of 13 patients).

Data preprocessing and Identification of differentially expressed genes (DEGs)

GSE27276 dataset obtained from GEO were successfully preprocessed (Table 1), and 19219 gene expression matrixes were finally obtained after statistical analysis. The researchers selected differential gene

Table 2: Clinical Characteristics of Non glaucomatous Control Subjects

Control	sex	Ethnicity	Age at Diagnosis	Eyes Assayed, n
1	F	C	67	1
2	M	C	64	1
3	F	AA	55	1
4	F	C	94	1
5	M	C	64	1
6	M	AA	49	1
7	F	C	72	1
8	F	C	48	2
9	M	C	67	2
10	F	C	53	2
11	M	C	59	2
12	F	C	59	2
13	F	AA	61	2
Average:	Male:60.6		Female:63.62	
Variance:	Male:50.3		Female:209.125	
Total Average:	62.46		Total Variance:	141.103

AA, African American; C, Caucasian.

expression in glaucoma and normal samples according to the adjusted p-value less than 0.05 and |log FC| > 1. One hundred seventy-nine differentially expressed genes were found between POAG and normal samples which were 60 up-regulated and 119 down-regulated genes (Supplementary Table 1).

Functional analysis and pathway enrichment of DEGs

According to the results obtained from Toppgene, for up-regulated and down-regulated genes, the GO and pathway analyses were performed. The Toppgene GO terms in biological processes, cellular components, and molecular function for DEGs are listed in figure 1 (Supplementary Table 2). The results showed that the most significant GO terms for DEGs in molecular function, biological processes, and Cellular components were antioxidant activity, gas transport, haptoglobin-hemoglobin complex, and hemoglobin complex, respectively.

The KEGG pathway analysis showed that the DEGs in patients with POAG were significantly enriched in antigen processing and presentation, tyrosine metabolism, and cell adhesion molecules (CAMs). The result of the KEGG analysis is listed in figure 2 (Supplementary Table 3).

PPI network analysis for DEGs

The protein-protein interaction network was extracted for 179 genes from the STRING database (Figure 3). Finally, to find important protein modules, based on the STRING database and with high confidence [0.7, with 68 nodes] were screened out for the construction

Table 3: Important diseases obtained from genes involved in glaucoma pathogenesis using a Toppgene database

	ID	Name	pValue	FDR B&H	FDR B&Y	Bonferroni	Genes from Input	Genes in Annotation
1	C0344559	Irido-corneo-trabecular dysgenesis (disorder)	1.400E-6	1.553E-3	1.191E-2	1.683E-3	3	23
2	C0017601	Glaucoma	6.633E-6	1.553E-3	1.191E-2	7.973E-3	6	511
3	C0010034	Corneal Diseases	8.333E-6	1.553E-3	1.191E-2	1.002E-2	3	41
4	cv:C0010036	Corneal dystrophy	9.102E-6	1.553E-3	1.191E-2	1.094E-2	2	5
5	C1562689	Congenital hereditary endothelial dystrophy	9.102E-6	1.553E-3	1.191E-2	1.094E-2	2	5
6	cv:CN296457	Corneal endothelial dystrophy	9.102E-6	1.553E-3	1.191E-2	1.094E-2	2	5
7	C0016781	Fuchs Endothelial Dystrophy	9.102E-6	1.553E-3	1.191E-2	1.094E-2	2	5
8	C0010036	Corneal dystrophy	1.033E-5	1.553E-3	1.191E-2	1.242E-2	3	44
9	C1857800	CORNEAL DYSTROPHY, FUCHS ENDOTHELIAL, 2	1.364E-5	1.640E-3	1.258E-2	1.640E-2	2	6
10	C0544008	Chandler syndrome	1.364E-5	1.640E-3	1.258E-2	1.640E-2	2	6
11	C1857569	CORNEAL ENDOTHELIAL DYSTROPHY 2	3.269E-5	3.572E-3	2.740E-2	3.929E-2	2	9
12	C1852555	CORNEAL ENDOTHELIAL DYSTROPHY 1, AUTOSOMAL DOMINANT	5.982E-5	5.992E-3	4.596E-2	7.191E-2	2	12
13	C0339284	Polymorphous corneal dystrophy	8.239E-5	7.618E-3	5.842E-2	9.903E-2	2	14
14	C0016781	Fuchs Endothelial Dystrophy	8.909E-5	7.649E-3	5.866E-2	1.071E-1	3	90
15	C0004903	Beckwith-Wiedemann Syndrome	1.449E-4	1.161E-2	8.902E-2	1.741E-1	3	106
16	C0020597	Hypobetalipoproteinemias	1.714E-4	1.242E-2	9.528E-2	2.060E-1	2	20
17	C0078917	Albinism, Ocular	2.279E-4	1.242E-2	9.528E-2	2.739E-1	2	23
18	C0242852	Proliferative vitreoretinopathy	3.360E-4	1.242E-2	9.528E-2	4.039E-1	3	141
19	C0038454	Cerebrovascular accident	3.843E-4	1.242E-2	9.528E-2	4.619E-1	7	1515
20	C0205647	Follicular adenoma	4.187E-4	1.242E-2	9.528E-2	5.033E-1	3	152

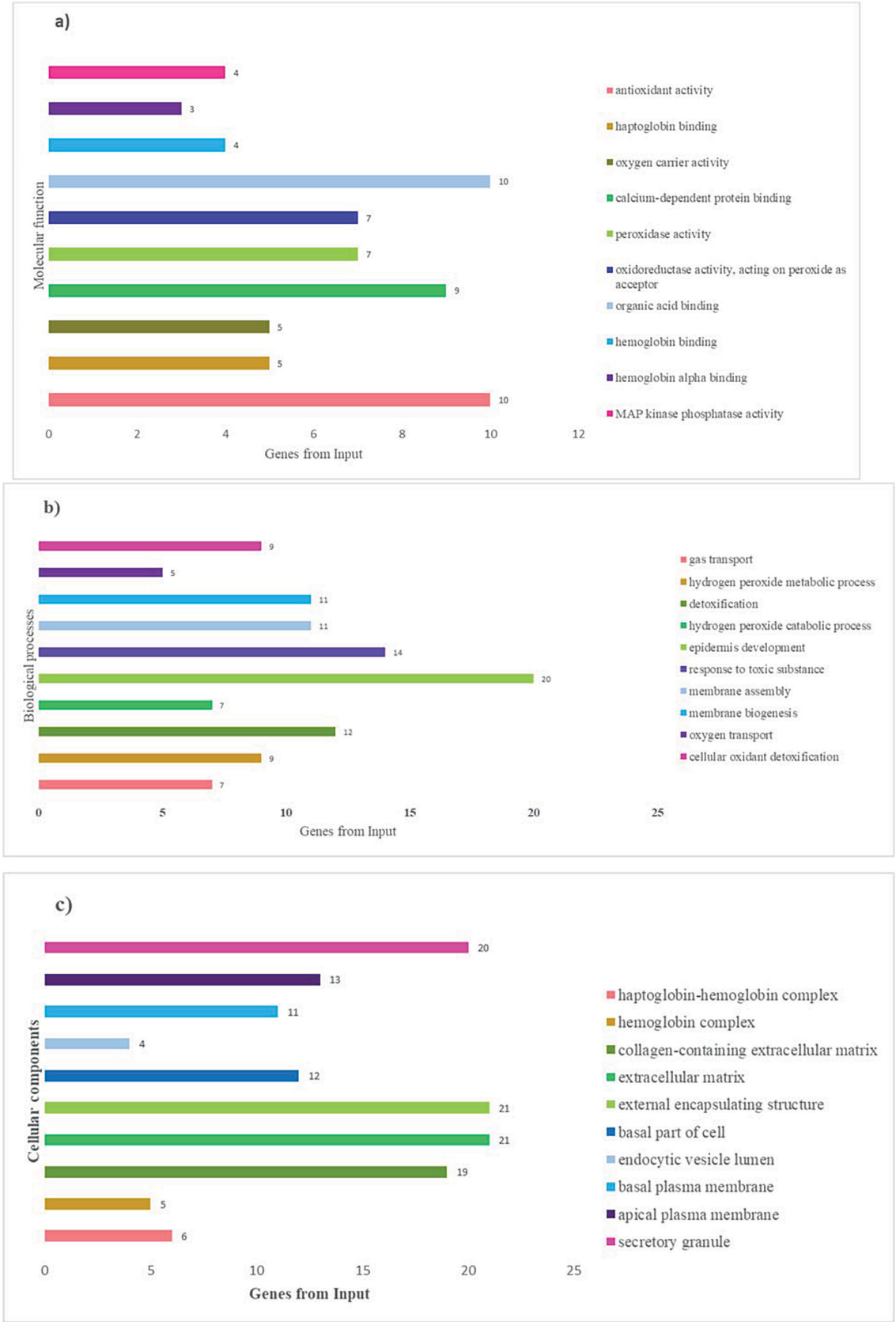

Figure 1: Gene ontology functional enrichment of differentially expressed genes

a) Molecular Function b) Biological Processes c) Cellular Components

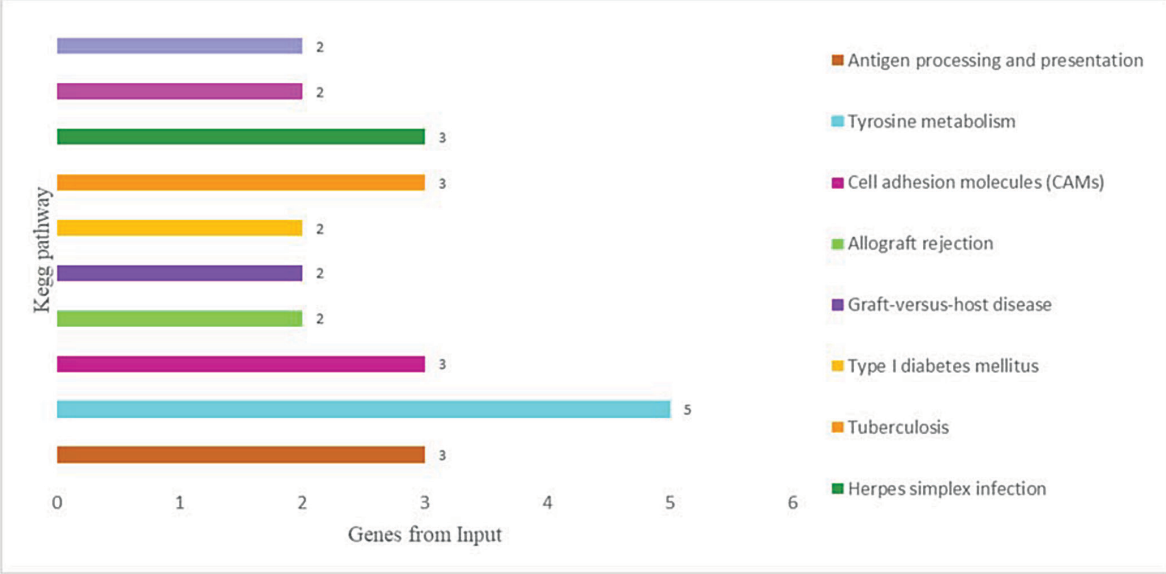

Figure 2: KEGG pathway analysis of differentially expressed genes in glaucoma

of the PPI network (Figure 4).

Gene Ontology and KEGG pathway analysis were performed for the genes of these four modules. The second module (yellow) was most associated with glaucoma pathogenesis genes. The results of GO enrichment for the second module showed that the biological processes were mostly in the antigen processing and presentation of exogenous peptide antigen via MHC class II. Also, the result of molecular function showed that the modules genes enriched to MHC class II protein binding, MHC class II protein complex binding, and extracellular matrix structural constituent conferring compression resistance and Cellular components of modules genes were in the MHC class II protein complex and MHC protein complex (Figure 5) (Supplementary Table 4). KEGG analysis showed that the modules genes were involved in Antigen processing, Type I diabetes mellitus, and Cell adhesion molecules (CAMs) (Figure 6) (Supplementary Table 5).

Based on the aim of the study, to identify pathogenic genes in glaucoma, most pathogenic genes were classified in the second module. There are 20 genes as potentially vital genes in the PPI network, including TYRP, FMOD, OGN, PAX6, LGALS1, CDH2, COL8A2, SLC4A11, GRP, MCHR1, MTNR1A, AP1M2, HCAR3, CD74, HLA-DPA1, KLC3, HLA-DMB, TNNT3, MT2A, DEFB1. Moreover, according to the Toppgene database, diseases related to the second module genes were analyzed. The results showed that all of these genes were potentially involved in the development of glaucoma or eye diseases (Table 3).

Discussion

Glaucoma is the second leading cause of blindness in the world. Nearly seventy million people in the world and about two percent of people over the age of forty suffer from this illness. There is no definitive treatment for this disease, and there are treatments available to control and prevent blindness due to it. Therefore, early diagnosis, appropriate treatment, and regular follow-up of patients are of special importance (20). This study tried to identify the genes that play an important role in glaucoma pathogenesis precisely. Also,

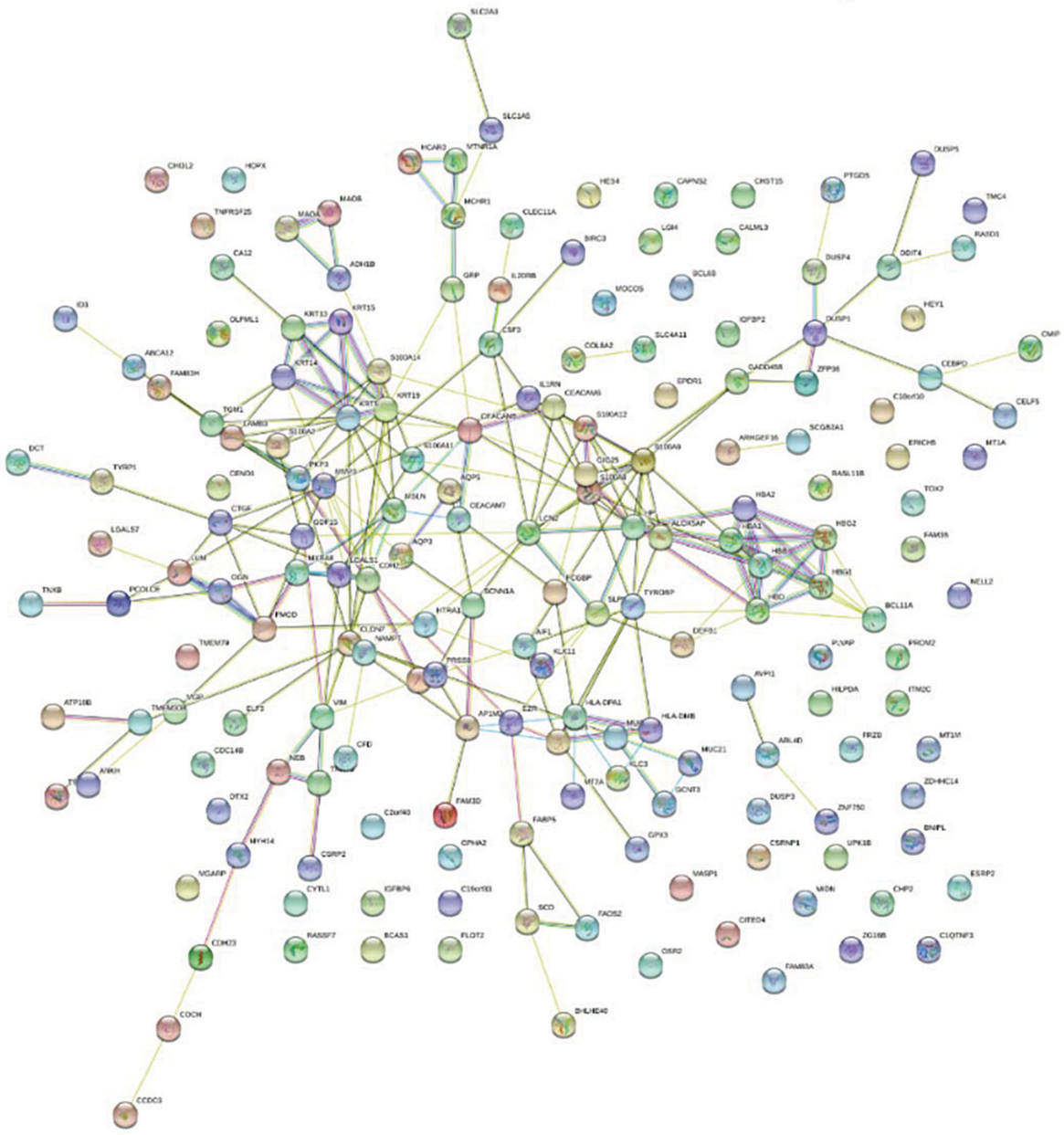

Figure 3: Protein-protein interaction network for 179 differentially expressed genes

an integrated analysis was performed using the data obtained from the GEO database. STRING, Toppgene, and R tools were used to analyze DEGs. One hundred seventy-nine differential expression genes were obtained (60 up-regulated and 119 down-regulated). This study tried to identify the genes that play an important role in glaucoma pathogenesis precisely. Also, an integrated analysis was performed using the data obtained from the GEO database. STRING, Toppgene, and R tools were used to analyze DEGs. One hundred seventy-nine differential expression genes were obtained (60 up-regulated and 119 down-regulated). Gene ontology DEGs showed that most of the pathways were related to antioxidant activity, haptoglobin binding, and peroxidase activity. These pathways are used to inhibit reactive oxygen species at destructive levels. Peroxidases are enzymes

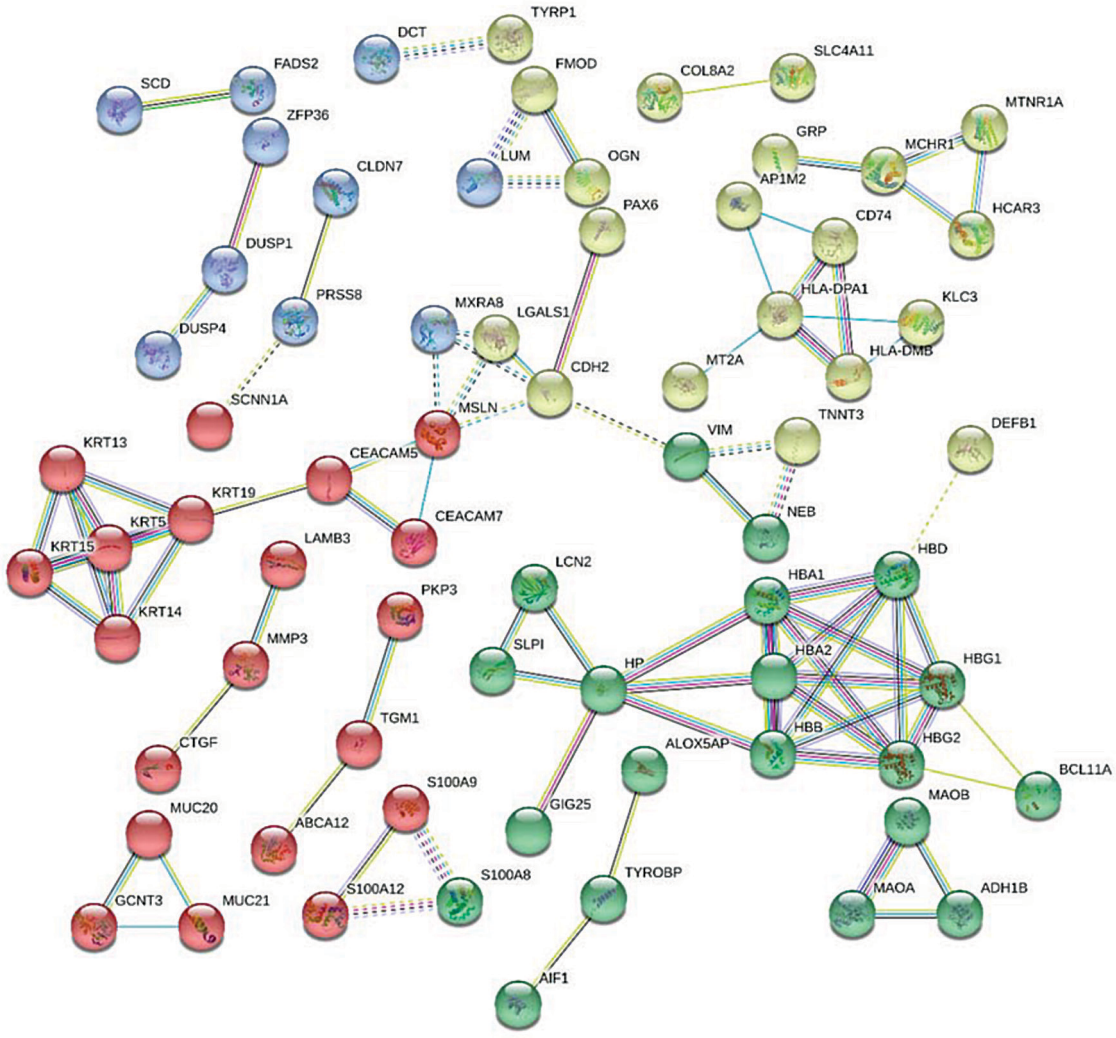

Figure 4: The sub-network module analyzed by STRING with the K-means method

Module 1 (Blue), Module 2 (Yellow), Module 3 (Red), and Module 4 (green)

that catalyze the redox reaction by reducing the free radical mechanism and converting several compounds into oxidized or polymeric products. Increased levels of aqueous humor Peroxidase enzymes may be linked with POAG. Furthermore, they may be useful antioxidant enzyme levels in aqueous humor of POAG patients as a result of glaucoma disease and not a cause [21].

The KEGG pathway obtained from DEGs included antigen processing and presentation pathways, Type I diabetes mellitus, and cell adhesion molecules (CAMs), which were highly associated with POAG disease. After drawing the PPI network by k-means method from STRING with a 0.7 confidence level, DEGs were classified into four modules (first module in the blue, second module in the yellow, third module in the red, and fourth module in green). The GO and the KEGG pathway were examined separately for each module, and the genes of each module were checked for disease with the Toppgene database. Of the four modules obtained, the second module (yellow), which was closest to ocular diseases and glaucoma, was discussed

Figure 5: Gene ontology functional enrichment the genes in the PPI Yellow module (module 2) a) Molecular Function b) Biological Processes c) Cellular Components

separately. In the second module, most of the signaling pathways were related to Antigen processing and presentation and Cell adhesion molecules (CAMs). Protein antigen during antigen processing and exhibition is ingested by an antigen-presenting cell (APC), partially digested into peptide fragments and then displayed on the level of the APC associated

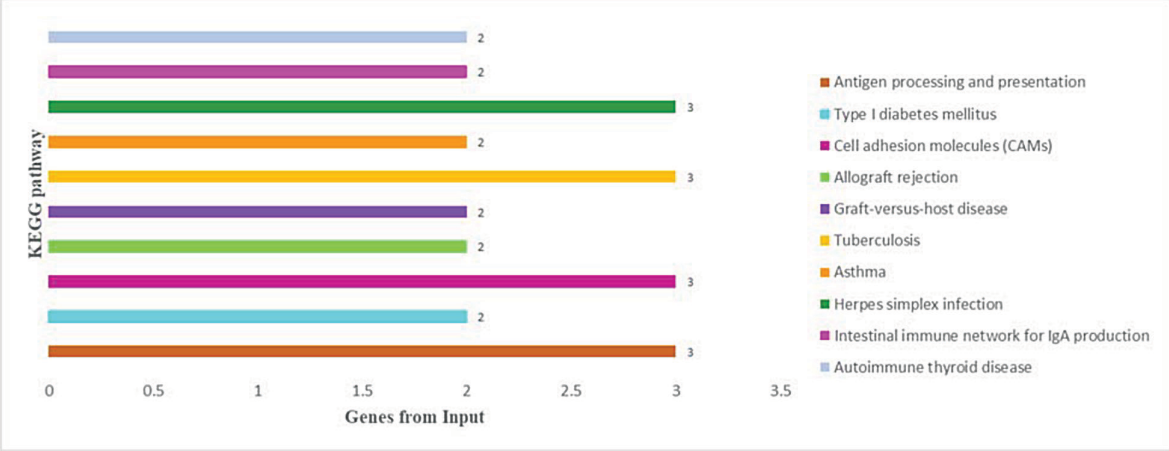

Figure 6: KEGG pathway analysis of the genes in the PPI module 2

with an antigen-presenting molecule such as MHC class I or MHC class II, for distinction by determine lymphocytes such as T cells. The results of Tezel et al. illustrated that glial cells subjected to ROS-producing complexes are potent T cell activation inducers that evaluated by enhanced T-cell sprinkle and cytokines proliferation. Their results also revealed that in furthermore to MHC class II molecules upregulation on glial cells, ROS function as an inducer molecules in antigen presentation. The results revealed that the attendance of ROS, incensed the antigen-presenting capacity of glial cells in the glaucomatous retina and optic nerve head can cause a stimulated immune response [22]. Cell adhesion molecules are a cell adhesion proteins subsection that situated on the cell outer complicated in linking with other cells or with the extracellular matrix in the process known cell adhesion. Molecules of cell adhesion facilitate cells stick to each other and their environs. The results of Bacon et al. revealed that encance cell adhesion molecules levels on the microvasculature and the features that control them are probable to be accountable for the influence of cells behavior their ligands and may preserve inflammation in the chronic figures of allergic eye disease [23]. Nearly all disease related to the second module were associated to POAG or eye diseases, especially glaucoma, which was contained in this group.

The up-regulated genes in the second module were FMOD, OGN, LGALS1, CDH2, COL8A2, SLC4A11, GRP, MCHR1, HLA-DPA1, HLA-DMB, among which the FMOD gene was significantly higher in patients with Glaucoma than it was in the control group. The FMOD (Fibromodulin) gene is a gene encoding protein, and related diseases include Pseudoachondroplasia and Myopia. The results obtained from this gene were consistent with the study of Feng et al. [24]. The OGN gene encodes a member of the small leucine-rich proteoglycan (SLRP) family of proteins. The encoded protein causes extrauterine bone formation along with a change in beta growth factor and may regulate osteoblast differentiation [16]. A study of Trabecular Meshwork among people with POAG and Trabecular Meshwork among healthy people showed different glycogen expressions. They also identified certain glycogenes, including OGN, whose differential expression in patient tissue could affect the course of the disease [25]. The researchers also observed differential expression of the COL8A2 gene, which is highly involved in glaucoma pathogenesis and

is highly involved in the biological function of ocular morphogenesis and ocular growth. The current study results were consistent with the results of previous studies [26]. Genes such as CDH2, GRP, HLA-DPA1, and HLA-DMB are mostly involved in the underlying diseases that cause glaucoma, including inflammation of the arteries, heart disease, dysfunction of the eye, and visual acuity [27, 28, 29].

The down-regulated genes in the second module included TYRP1, PAX6, MTNR1A, AP1M2, HCAR3, CD74, KLC3, TNNT3, MT2A, and DEFB1; most of which played an important role in glaucoma pathogenesis. The TYRP1 gene is responsible for making the enzyme tyrosinase-related protein 1. This enzyme is found in melanocytes, specialized cells that produce melanin pigment. Melanin is a substance that colors the skin, hair, and eyes [30]. Expression of this gene was reduced in patients with glaucoma. This gene plays an important role, and the normal activity of this gene and its constituent proteins are disrupted and reduced despite glaucoma. Pigmentary Glaucoma is a special form of Open-Angle Glaucoma seen in patients with pigmentation syndrome. The results of other studies [31] suggest that several factors, including other genetic factors, may be required to induce intraocular pressure and eventually color glaucoma in individuals with pigment dispersion characteristics. The human TYRP1 gene is an excellent candidate for a secondary genetic factor influencing glaucoma in patients with pigmentation syndrome. In addition to iris stromal atrophy and chromatic glaucoma in DBA / 2J mice, changes in the TYRP1 gene have been shown to affect the phenotypic expression of the genes responsible for pigment production. Mutations in TYRP1 may lead to qualitative or quantitative changes in iris pigment production that may directly or indirectly lead to increased intraocular pressure [32].

The PAX6 gene acts a key role in the tissue and organs formation in embryonic development. PAX6 gene's family members are also necessary to maintain the normal function of specified cells after birth. For performing these functions, PAX6 genes provide orders to produce proteins that bind to specific regions of DNA and assistance regulator the specific genes activity (expression). Founded on this function, PAX6 proteins are named transcription factors. Within embryonic progress, the PAX6 gene functions a vital role in tissues and organs formation during embryonic growth. It is thought that the PAX6 protein, in embryonic growth, activates the genes that involved in the eyes, pancreas, spinal cord (central nervous system), and brain formation. Furthermore, PAX6 protein regulates many prenatal eye development features. After birth, the PAX6 protein is probably controls the various genes expression in many eye constructions [33]; probably a factor as to why they are doing so poorly in early-stage glaucoma. The current study results are in line with the results of previous studies [17, 24]. The PAX6 gene plays an essential role in eye growth [34]. Decreased functional mutations cause Andrea's disease, one of the symptoms of which is an abnormal enlargement of the iris. This condition often affects both eyes. It can also affect the cornea, anterior chamber, lens, retina, and optic nerve. Approximately 50% of patients with ocular growth abnormalities caused by PAX6 mutations are also affected by early glaucoma [35]. The TNNT3 (troponin) gene is also a major player in calcium regulation of thin actin function and is essential for striated muscle contraction. This gene is specifically involved in joint contractions [36]. The (Defensin Beta 1) gene DEFB1 encodes

a protein. One of the diseases associated with DEFB1 is endophthalmitis, related to intraocular infection. Endophthalmitis is a severe inflammation of the tissues inside the eye that is usually caused by an infection with fungi (e.g., Candida, Aspergillus) or bacteria (e.g., Staphylococcus species, Streptococcus species, Gram-negative bacteria). Among the ways related to it, we can mention the innate immune system and defense. The association of this gene has not been directly observed in glaucoma, but intraocular infection has been observed in patients who have undergone trabeculectomy [37].

In this study, the researchers also identified the MTNR1A (melatonin 1A receptor) gene, which may be associated with a decreased expression of this gene, which is commonly associated with glaucoma. Studies have shown that the MTNR1A gene is associated with a variety of diseases, including coronary artery disease and Type 2 diabetes [38]. Type 2 diabetes is a multi-genetic disease, so not just one gene causes it, ; hundreds of genes together can increase a person's susceptibility to the disease, so it can be inferred that each gene contributes very little to the disease [38]. Findings from several studies in recent years show that diabetics are more likely to develop glaucoma than previously thought [39, 40, 41, 42]. Melatonin is a hormone produced by the pineal gland, retina, gastrointestinal tract, and several other organs and secreted by the pineal gland [43]. Also, involved in the production of this hormone, the genes act as a protective factor against chronic diseases, gastrointestinal diseases, and metabolic and behavioral disorders. The MTNR1A gene is one of the two forms of melatonin encoding the primary hormone secreted by the pineal gland. It regulates various physiological functions and nerves and endocrine glands in mammals [38].

The HCAR3 gene is one of the key genes for coronary atherosclerotic heart disease (CHD), which is used as a biomarker gene, and its low expression increases the risk of atherosclerosis [44]. HCAR3 is a hydroxycarboxylic acid receptor involved in the metabolism of fatty acids [45]. Human HCAR3 is activated by MAPK cascades, which significantly increases ATP production by increasing the oxidation of free fatty acids under conditions such as diabetic ketoacidosis and fasting [46]. Oxidation of free fatty acids is critical in myocardial metabolism. Myocardial free fatty acid metabolism in CHD patients with high HCAR3 may be better than in patients with low levels and affect the symptoms of CHD. On the other hand, retinal artery occlusion is caused by a blood clot or narrowing of the retinal blood vessels; in this condition, blood flow to the retina is cut off and, if left untreated, can cause vision loss. The study results by Song et al. indicate that the risk of atherosclerosis in patients with glaucoma is higher than in patients with other ocular diseases, and in patients with atherosclerosis compared with patients with high blood hypertension or more general population [47].

The MT2A gene is one of the Metallothionein's (MTs) genes. MTs are a large family of proteins that play a variety of roles, including binding toxic metals, inhibition of free radicals, and oxidative stress. Mammalian metallothionein-2A (MT2A) has received much attention due to its vital pathophysiological role in antioxidant, anti-apoptotic, detoxifying, anti-inflammatory, immune defense, cell proliferation, cell proliferation differentiation, and angiogenesis [48, 49, 50]. Since this gene acts as a scavenger of free radicals to protect cells and tissues from oxidative stress, the role of oxidative stress in the development of glaucoma [51] is important. Oxidative damage has been shown to play a major

role in eye diseases such as cataracts and macular degeneration [52]. Increasing empirical evidence suggests that oxidative stress plays an important role in the pathogenesis of the leading cause of irreversible blindness, primary open-angle glaucoma (POAG) [53]. Although increased intraocular pressure (IOP) is a major risk factor for POAG, other factors affecting the eye play an important role, including increased glutamate levels, changes in nitric oxide (NO) metabolism [54], vascular changes [55], and oxidative damage. It has oxygen [54] from active species.

Conclusion

Further knowledge of the molecular and pathogenetic mechanisms of POAG is an essential prerequisite for implementing effective measures for the prevention and treatment of this disease. Although the molecular mechanism of pathogenicity of wide-angle glaucoma is not yet fully understood, studies interpret this pathology as a multifactorial disease in which oxidative damage is an important pathogenic pathway that stimulates trabecular meshwork and leads to ocular hypertension. In addition, considering the genes that play an important role in the pathogenesis of glaucoma in this study, oxidative damage seems to play an important role in the development and maintenance of optic nerve and ganglion retinal cells. Among the influential genes that may be involved in the direct occurrence of glaucoma and identified in this study were TYRP1, FMOD, OGN, PAX6, COL8A2, HLA-DPA1, and HLA-DMB, and genes that may be indirectly involved. They play an important role in developing underlying diseases such as diabetes, hypertension, and heart disease, including MTNR1A, HCAR3, CD74, MT2A, and DEFB1. These results may help clarify potential new biomarkers, reveal the underlying pathogenesis, and set new therapeutic targets for glaucoma treatment. Of course, more experiments are needed to confirm the results of this study.

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Footnotes and Financial Disclosures

Conflict of interest

The authors have no conflict of interest with the subject matter of the present manuscript.