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COMPREHENSIVE BIOINFORMATICS AND NETWORK ANALYSIS OF GLAUCOMA-ASSOCIATED GENES REVEALS KEY MOLECULAR PATHWAYS

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Abstract:

Glaucoma is a chronic neurodegenerative eye disease characterized by progressive optic nerve damage and irreversible vision loss. Elevated intraocular pressure is a major risk factor; however, disease progression may occur even under controlled conditions, indicating the involvement of genetic, molecular, and environmental factors. Current treatments primarily reduce intraocular pressure but do not prevent neuronal degeneration. A systems biology approach was employed to identify gene–disease associations using DisGeNET. Protein–protein interaction and gene network analyses were performed using STRING and GeneMANIA, respectively. Functional enrichment analysis was conducted using Enrichr, and pathway mapping was carried out using Reactome to elucidate underlying biological processes. The analysis revealed a highly interconnected network of glaucoma-associated genes involved in apoptosis, oxidative stress, neuroinflammation, extracellular matrix remodelling, and vascular dysfunction. These findings suggest that glaucoma arises from complex disruptions in neuronal survival and cellular homeostasis, highlighting the potential of bioinformatics approaches in identifying therapeutic targets and supporting precision medicine.

Keywords: *Glaucoma, Neurodegeneration, Bioinformatics, Gene Network Analysis, Apoptosis, Oxidative Stress, Neuroinflammation, Precision Medicine.*

Introduction

Glaucoma is mainly characterized by progressive degeneration of retinal ganglion cells and the optic nerve, leading to irreversible loss of visual field and blindness. The most common form of the condition is primary open-angle glaucoma; often undetected in the early stages, the symptoms develop gradually and appear only after significant

damage has been done to the optic nerve. While elevated intraocular pressure is an essential, modifiable risk factor, it has been demonstrated that the disease process may progress independently of the treatment of intraocular pressure, suggesting the role of further molecular and cellular processes. Genetic factors influence susceptibility and progression of glaucoma (4). Many genes, including MYOC, OPTN, CYP1B1, FOXC1, LTBP2, TMCO1, CDKN2B-AS1, and PITX2, have all had associations with inherited and sporadic forms of glaucoma (5). Indeed, these genes control numerous biological processes: extracellular matrix remodeling, oxidative stress response, neuroprotection, immune regulation, and vascular homeostasis (6). Changes in these genes may impact trabecular meshwork function, retinal ganglion cell survival, and optic nerve integrity, thus resulting in disease development and progression (7).

Current treatments of glaucoma primarily aim to alleviate intraocular pressure via pharmacological therapy as well as surgical procedure (8). Although such treatments slow the movement of the disease, they do not have a direct effect toward preventing neuronal degeneration (9), and can be accompanied by side effects and lower patient compliance. The presence of optic nerve damage even in patients with typical intraocular pressure provides a strong rationale for intervention targeting neurodegeneration as well as the molecular cascades underlying glaucoma (10). The advancement of genomics and high-throughput technologies have produced substantial bodies of biological data that have to be computed more efficiently to make sense of them (11). Bioinformatics tools facilitate a systematic search for disease-inclined genes, characterize biological networks, and predict potential intervention targets more effectively than classical experimental methods (12). Network-based analyses are particularly useful in identifying main regulatory or hub genes controlling various aspects of the biology pathways-which might be promising for drug discovery (13).

Bioinformatic tools such as GeneMANIA can visualize networks of gene interactions for coexpression and physical interactions (14). Enrichr integrates multiple biological databases for functional enrichment and pathway analysis, while STRING serves to identify protein–protein interaction and network connectivity, and DisGeNET offers a curated gene–disease relationship (15). In addition, Reactome facilitates pathway mapping to investigate underlying biological processes. These tools support a more effective process for studying disease mechanisms and speeding up translational research. Here, thirty genes linked with glaucoma were investigated by GeneMANIA, Enrichr, Reactome, STRING and DisGeNET to study the molecular mechanisms of glaucoma, as well as identify suitable targets to treat potential disease.

Materials and Methods

DisGeNET analysis

DisGeNET is a publicly accessible bioinformatics tool that aggregates evidence to show associations between disease and related genes from curated databases, genome-wide association studies (GWAS), animal models, and scientific literature (15). Its evidence-based associations, combined with confidence scores representing the strength of the association between genes and diseases, are reported to be robust (15). Genes that were selected for further analysis were genes for which the DisGeNET score was ≥ 0.45. A total of 30 genes were shortlisted based on relevance and evidence score.

STRING analysis

STRING is an online bioinformatics database used to research known and predicted protein–protein interactions. These interactions can take on direct physical interactions, or indirect functional associations essential for

revealing biological processes (16). To build a protein–protein interaction network, a high-confidence interaction score (>0.7) was applied.

GeneMANIA analysis

GeneMANIA combines data from lab experimentation, published articles and simulations to create a network depicting how genes are correlated. Genes that are closely related to the input list are presented when they are stronger, so stronger relationships are weighted higher (14). That means choosing genes associated with glaucoma and then using network tools to identify their interactions and key hub genes. This provides insights into their role in common pathways and the way their disruption drives glaucoma.

Enrichr analysis

Enrichr compares the input gene list to several gene-set libraries (KEGG pathways, Reactome pathways, Gene Ontology terms). The analysis computes statistical scores and p-values to ascertain which pathways or functions most strongly correspond to the input genes (17). Gene support of 3 to 6 is the results provided in the analysis. High counts (5–6) of genes are important hub genes and low counts (3–4) are supporting genes. This means that these genes cooperate and their loss might lead to glaucoma.

Reactome analysis

Reactome maps a list of the genes or proteins on top of a curated database of biological pathways. It identifies pathways that are significantly associated with the input list and notes their functions in biological processes (18). The workflow directly maps genes to pathways, identifying over-represented pathways by p-value and FDR. It identifies major processes such as gene regulation and cellular stress, extending its value by identifying the most important mechanisms in disease.

Results

DisGeNET

Table 1: Thirty genes associated with glaucoma were comprehensively analyzed

Gene Symbol	Gene Description	Number of Diseases Associated	No. of Variants Associated	Score
MYOC	Myocilin	145	237	1.0
BDNF	Brain derived neurotrophic factor	1487	108	0.95
TGFB2	Transforming growth factor beta 2	1924	488	0.9
FOXC1	Forkhead box C1	370	321	0.85
LTBP2	Latent transforming growth factor beta binding protein 2	301	329	0.85
CDKN2B	Cyclin dependent kinase inhibitor 2B	636	32	0.85
ARHGEF12	Rho guanine nucleotide exchange factor 12	72	215	0.85
CNTF	Ciliary neurotrophic factor	195	2	0.85
OPTN	Optineurin	203	233	0.8
WDR36	WD repeat domain 36	45	121	0.8
SLC4A4	Solute carrier family 4 member 4	165	198	0.8

ANGPT1	Angiopoietin 1	570	131	0.8
CDKN2A	Cyclin dependent kinase inhibitor 2A	1965	667	0.7
HLA-DRB1	Major histocompatibility complex, class II, DR beta 1	1483	121	0.7
EPO	Erythropoietin	907	17	0.65
LOXL1	Lysyl oxidase like 1	216	97	0.6
LMX1B	LIM homeobox transcription factor 1 beta	424	345	0.6
HLA-DQB1	Major histocompatibility complex, class II, DQ beta 1	1048	504	0.6
PRPF8	Pre-mRNA processing factor 8	118	181	0.55
TDRD7	Tudor domain containing 7	81	95	0.55
CYP1B1	Cytochrome P450 family 1 subfamily B member 1	837	257	0.5
VEGFA	Vascular endothelial growth factor A	2992	49	0.5
MMP9	Matrix metallopeptidase 9	2099	65	0.5
MYLK	Myosin light chain kinase	359	1147	0.5
LINGO1	Leucine rich repeat and Ig domain containing 1	87	80	0.5
PITX2	Paired like homeodomain 2	481	171	0.45
EDN1	Endothelin 1	1376	30	0.45
CDKN2B-AS1	CDKN2B antisense RNA 1	448	488	0.45
TMCO1	Transmembrane and coiled-coil domains 1	194	64	0.45
GAS7	Growth arrest specific 7	69	109	0.45

The DisGeNET analysis of the top 30 glaucoma-associated genes demonstrates their strong involvement in multiple human diseases, highlighting their importance in glaucoma pathogenesis (15) (Table 1). Key genes such as MYOC, BDNF, TGFB2, FOXC1, and OPTN show high association scores, indicating strong scientific evidence linking them to glaucoma and related ocular disorders (4). The large number of disease-associated variants suggests that genetic alterations in these genes can significantly influence eye development, intraocular pressure regulation, and optic nerve integrity (19).

Many of the identified genes are involved in critical biological processes including neuroprotection (BDNF, CNTF, EPO), extracellular matrix remodelling (MMP9, LOXL1), immune response (HLA-DRB1, HLA-DQB1), and vascular regulation (VEGFA, ANGPT1, EDN1) (6). High confidence scores (close to 1) further validate the robustness of these gene–disease associations (15). Overall, this analysis highlights essential genes that contribute to the molecular mechanisms of glaucoma and provides valuable targets for understanding disease progression and developing therapeutic strategies (13).

STRING

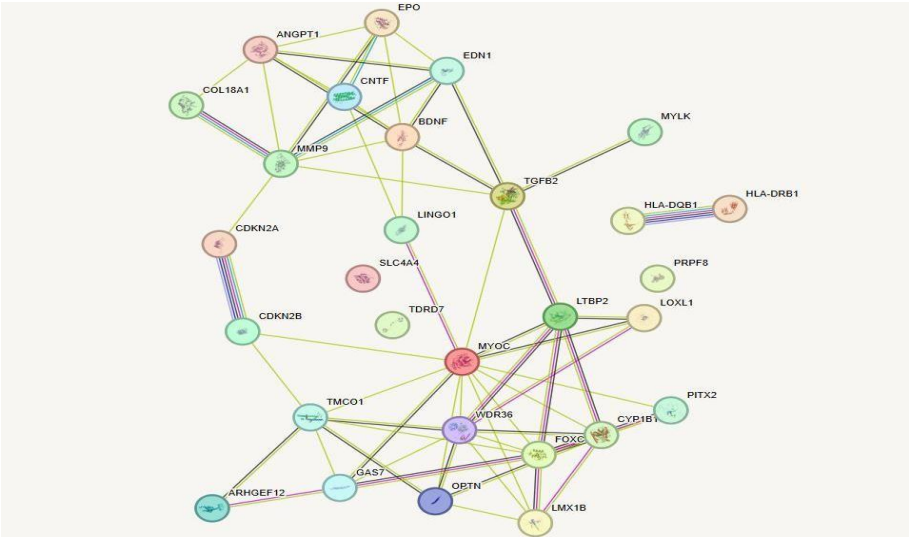


Figure 1: STRING protein–protein interaction network connectivity among glaucoma genes

Table 2: Key Biological Themes and Representative GO Processes in Glaucoma

Theme	**Representative GO Biological Process Terms**
Retinal neurodegeneration	Neuron death, axon degeneration, regulation of neuron apoptotic process
Oxidative stress	Response to oxidative stress, regulation of reactive oxygen species metabolic process
Neuroinflammation	Glial cell activation, inflammatory response, cytokine-mediated signalling
Extracellular matrix remodelling	Extracellular matrix organization, collagen fibril organization
Vascular dysfunction & hypoxia	Response to hypoxia, regulation of blood vessel diameter, nitric oxide biosynthesis

The STRING protein–protein interaction network shows that the chosen glaucoma-associated genes are highly interrelated and represent, according to the theory, coordinated pathways instead of functioning independently (16) (Figure 1). The dense interaction pattern highlights critical hub genes involved in optic nerve function, retinal ganglion cell survival, and regulation of intraocular pressure (7). Numerous interactions are mediated by neuroprotection, extracellular matrix organization, vascular regulation, and immune response, highlighting their activity in preserving normal ocular physiology (6). Gene Ontology (GO) enrichment studies demonstrate that these genes are primarily engaged in biological processes, including those associated with neuron survival, oxidative stress response, regulation of apoptosis, and tissue remodelling, which are key elements of glaucoma progression (20) (Table 2). They are also highly enriched in pathways involved in synaptic signalling and axonal integrity, both of which are key to communication mechanisms between retinal neurons (7). Various genes participate in eye development and formation of the optic nerve, and are critical in the visual system development and maintenance (1).

GeneMANIA

The interaction network has shown that the analysed glaucoma-associated genes form a highly linked and coordinated system, suggesting that they work in concert along mutual biological pathways rather than individually (16) (Figure 2a and 2b). MYOC, TGFB2, FOXC1, OPTN, CYP1B1, VEGFA, and LOXL1 genes are at the center of the network as hub nodes, suggesting their main regulatory functions (13).

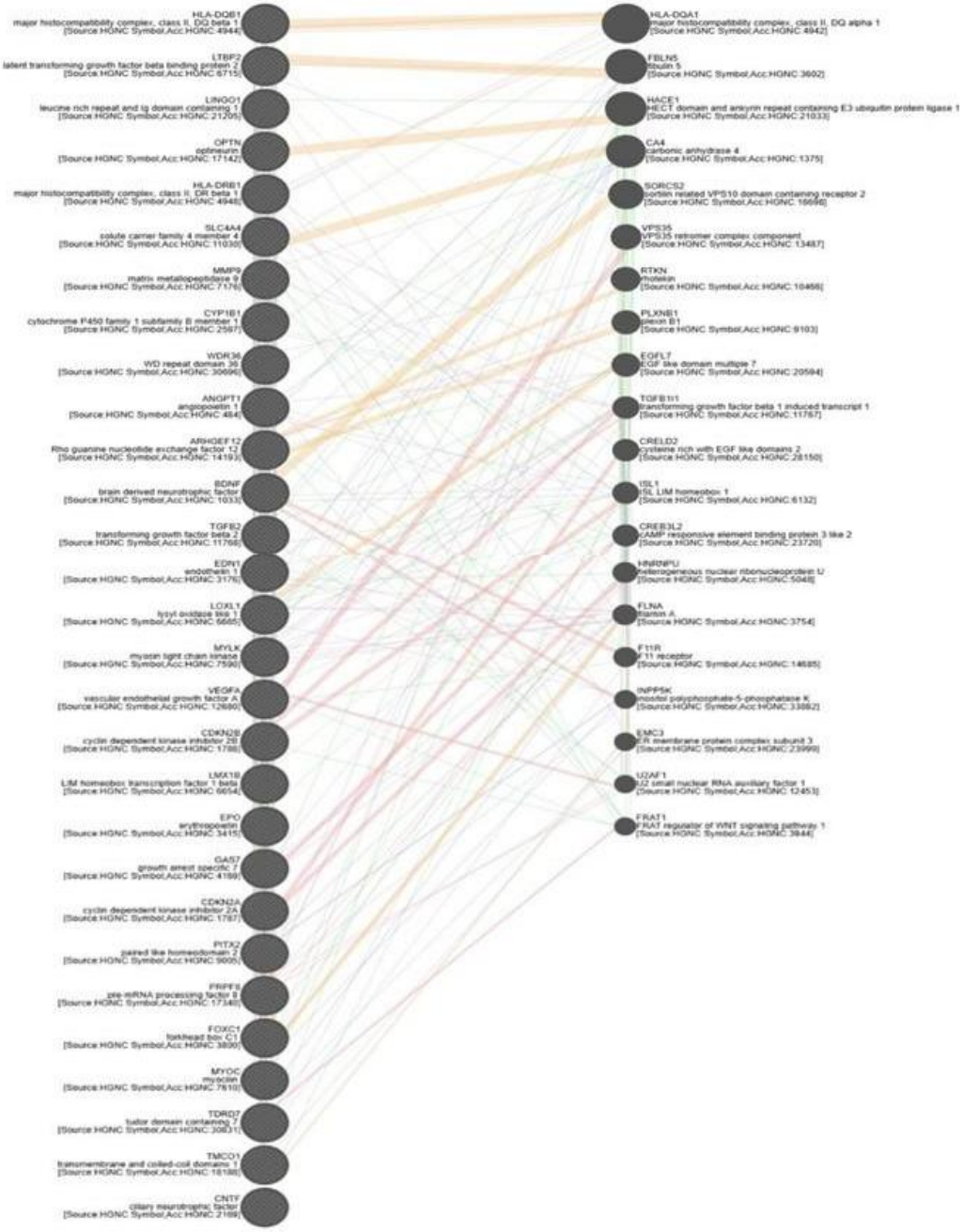


Figure 2a: GeneMANIA network showing functional associations between input glaucoma-associated genes (left panel) and predicted related genes (right panel). The connections represent co-expression, physical interactions, shared pathways, and genetic interactions, highlighting the expansion of the gene network based on functional similarity.

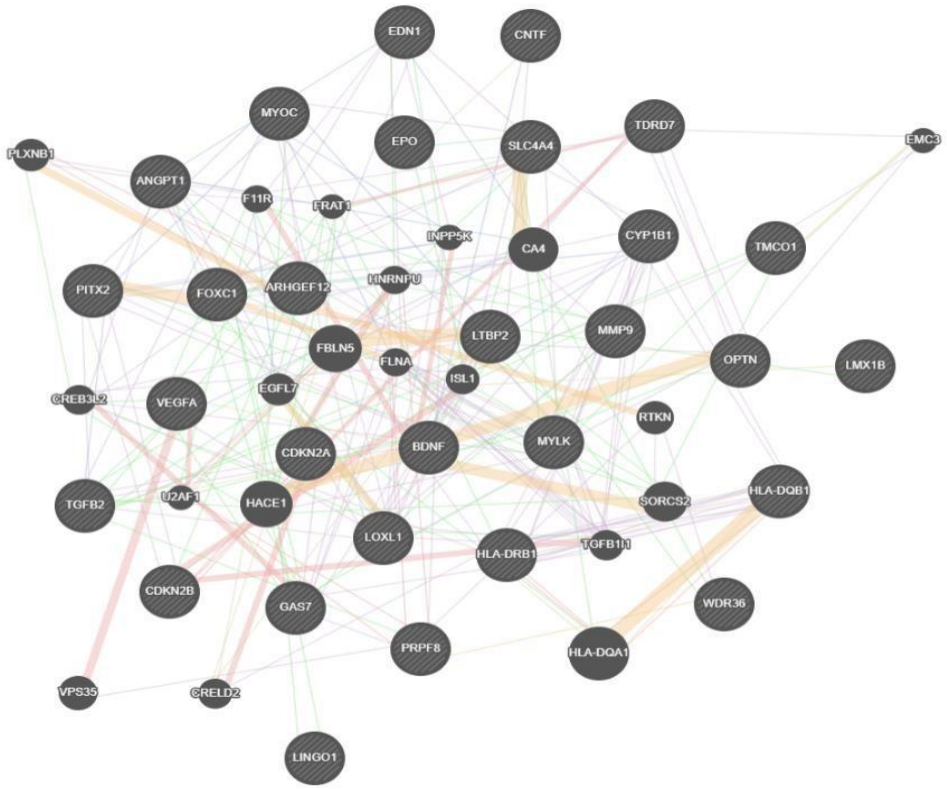


Figure 2b: Integrated GeneMANIA interaction network illustrating the complex connectivity among glaucoma-associated genes and their predicted partners. The dense network highlights key hub genes and their interactions, reflecting coordinated involvement in biological pathways relevant to glaucoma pathogenesis.

The vast majority of interactions are in line with optic nerve signalling, retinal ganglion cell survival, extracellular matrix remodelling, and regulation of intraocular pressure (7). Many genes are also involved in eye development, vascular regulation, and immune response, which are essential for maintaining normal ocular function (26). This illustrates that these genes assist in maintaining normal visual signal transmission and optic nerve integrity (1). Any disruption of this interaction network might contribute to the progressive deterioration of the optic nerve and vision loss seen in glaucoma (21).

Enrichr

KEGG 2021 Human pathways enrichment assessment reveals that the included glaucoma-associated genes participate in ocular development, optic nerve function, and retinal ganglion cell survival (1). There are many genes involved with cellular signalling, stress response, and neuroprotection in the eye, including MYOC, TGFB2, OPTN, CYP1B1, VEGFA, BDNF, and LOXL1 (4) (Figure 3a and 3b). Numerous enriched pathways are associated with extracellular matrix organization, TGF-β signalling, apoptosis, and vascular regulation, involved with intraocular pressure and normal optic nerve physiology (22). Some pathways are also associated with gene regulation and immune response, which shows that the genes contribute to cellular homeostasis in ocular tissues (6). Together, these genes work as part of a multistep series to preserve normal visual function and optic nerve

integrity, and disruption of these pathways can result in optic nerve damage and progressive vision loss, which are characteristic features of glaucoma (10).

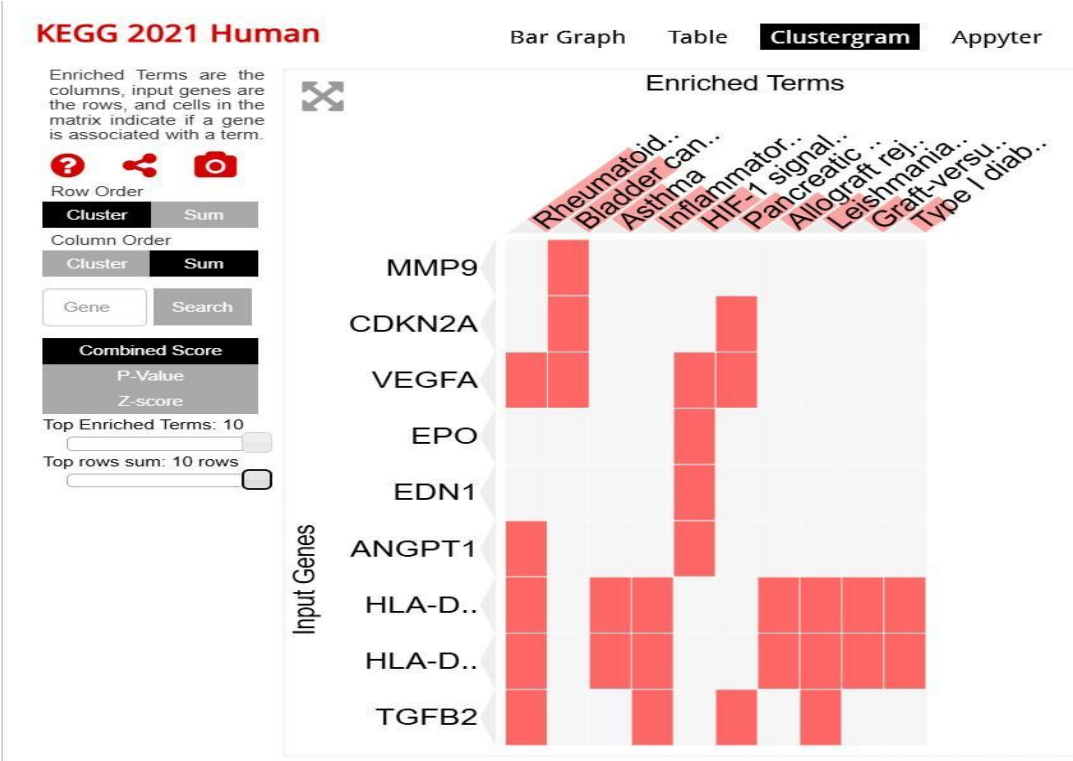


Figure 3a: Heatmap Representation of KEGG Pathway Enrichment in Glaucoma Genes

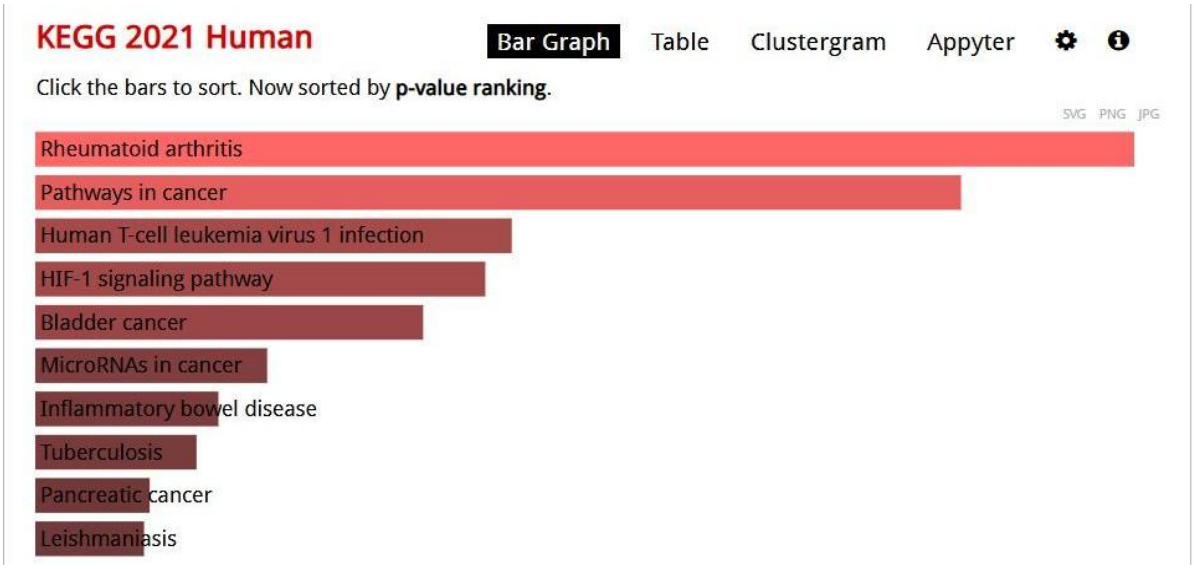


Figure 3b: Enrichr output showing enriched KEGG pathways and biological processes associated with glaucoma-related genes

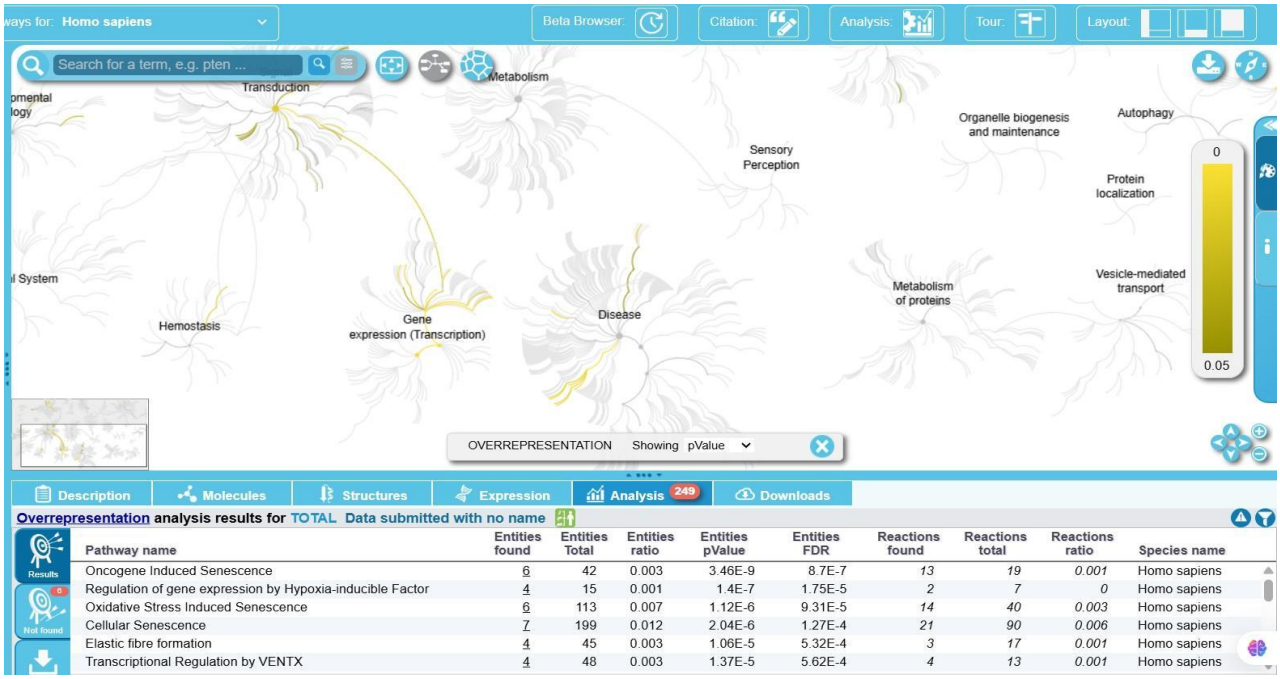
Table 3: Hub-genes identified by multi database Enrichment Analysis

Gene	Reactome Pathways	KEGG Human	Wiki Pathways	Elsevier	ARCHS4 Kinases	PPI Hub Protein	Count
MYOC	YES	YES	YES	YES	YES	YES	6
TGFB2	YES	YES	YES	YES	YES	YES	6
OPTN	YES	YES	YES	YES	NO	YES	5
CYP1B1	YES	YES	YES	YES	YES	NO	5
VEGFA	NO	YES	YES	YES	YES	YES	5
BDNF	NO	YES	YES	YES	NO	NO	4
LOXL1	YES	YES	YES	NO	YES	NO	4
FOXC1	YES	NO	YES	YES	NO	YES	4
LTBP2	YES	YES	YES	NO	NO	YES	4
MMP9	NO	YES	YES	YES	YES	NO	4
ANGPT1	NO	YES	YES	YES	NO	YES	4
EDN1	NO	YES	YES	YES	NO	NO	3
CDKN2A	YES	NO	YES	YES	NO	YES	4
HLA-DRB1	YES	NO	YES	YES	NO	NO	3

It indicates that many glaucoma-associated genes are supported by different biological databases, supporting strong and reliable evidence of their involvement in glaucoma-related pathways (11) (Table 3). Genes (MYOC, TGFB2, OPTN, CYP1B1) are present in most databases indicating their utility as essential regulatory and hub proteins (4). Other genes (VEGFA, BDNF, LOXL1) are mainly involved in vascular regulation, neuroprotection, and extracellular matrix remodelling (6). The regular appearance of these genes in pathway and protein-protein interaction databases indicates that they cooperate in preserving optic nerve integrity and pressure control in the eye (16). Mutations of these pathways may be an aspect of progression to glaucoma and loss of vision (10).

Reactome

The figure 4 presents the glaucoma-related genes clustering into several gene clusters, which indicates they have common biological roles (13). This cluster represents functionally related genes linked to common pathways associated with optic nerve health, retinal ganglion cell survival, and regulation of intraocular pressure (7). Many clusters are also tightly connected, indicating that related genes that regulate neuroprotection, oxidative stress response, extracellular matrix remodelling, and vascular regulation are closely linked (20). The branching pattern indicates that certain gene groups are more closely related than others, which reflects the complex and coordinated molecular mechanisms underlying glaucoma progression (3).



Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
Oncogene Induced Senescence	6	42	0.003	3.46E-9	8.7E-7	13	19	0.001	Homo sapiens
Regulation of gene expression by Hypoxia-inducible Factor	4	15	0.001	1.4E-7	1.75E-5	2	7	0	Homo sapiens
Oxidative Stress Induced Senescence	6	113	0.007	1.12E-6	9.31E-5	14	40	0.003	Homo sapiens
Cellular Senescence	7	199	0.012	2.04E-6	1.27E-4	21	90	0.006	Homo sapiens
Elastic fibre formation	4	45	0.003	1.06E-5	5.32E-4	3	17	0.001	Homo sapiens
Transcriptional Regulation by VENTX	4	48	0.003	1.37E-5	5.62E-4	4	13	0.001	Homo sapiens

Figure 4: Reactome pathway clustering coordinated molecular mechanisms underlying glaucoma progression

Discussion

Bioinformatics analysis has confirmed the strong functional connectivity among genes related to glaucoma with the hub genes TGFB2, VEGFA, MMP9, BDNF, and ANGPT1 being central elements in neuroprotection, extracellular matrix remodelling and vascular regulation (6). GeneMANIA method successfully highlighted other genes and predicted interactions with related genes, which gave a wider network picture (14). STRING supplemented this by highlighting the protein–protein interactions of nodes and identifying highly interrelated nodes validating that hub genes were identified (16). Enrichr and Reactome pathway analyses provided detailed functional and pathway enrichment, emphasizing pathways such as apoptosis, TGF-β signalling, oxidative stress, and neuroinflammation (17). Compared with single-tool analyses, combining several platforms enabled cross validation of major genes and pathways, thus facilitating confidence in outputs (11). Initial gene–disease associations were obtained through DisGeNET, but the tools that followed would provide functional and network-based insights that would demonstrate that glaucoma pathogenesis is driven by intricate and coordinated gene networks, not isolated genes (15). All these tools had complementary strengths: GeneMANIA for gene relationships, STRING for interaction confidence, Enrichr for pathway enrichment and Reactome for curated biological context (13). However, this study is limited by its reliance on in silico analysis and publicly available databases. Experimental validation is required to confirm the functional roles of the identified genes and pathways.

Conclusion

As highlighted by the current cohort, glaucoma is a complex interplay amongst several genes associated with optic nerve integrity, vascular function, neuroinflammation, and extracellular matrix remodelling. By uniting DisGeNET, GeneMANIA, STRING, Enrichr, and Reactome, this study revealed the important hub genes and their functional

pathways and interactions. More importantly, multi-tool analysis strengthened the reliability of findings and elucidated targeted avenues for therapeutic intervention, reinforcing a multidimensional molecular strategy for understanding glaucoma progression and enabling early-stage drug discovery. These findings provide a foundation for future experimental validation and targeted therapeutic development.

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