• Basic Research •

NMI, POLR3G and APIP are the key molecules connecting glaucoma with high intraocular pressure: a clue for early diagnostic biomarker candidates

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Abstract

● **AIM:** To understand the molecular connectivity between the intraocular pressure (IOP) and glaucoma which will provide possible clues for biomarker candidates.

● **METHODS:** The current study uncovers the important genes connecting IOP with the core functional modules of glaucoma. An integrated analysis was performed using glaucoma and IOP microarray datasets to screen for differentially expressed genes (DEGs) in both conditions. To the selected DEGs, the protein interaction network was constructed and dissected to determine the core functional clusters of glaucoma. For the clusters, the connectivity of IOP DEGs was determined. Further, enrichment analyses were performed to assess the functional annotation and potential pathways of the crucial clusters.

● **RESULTS:** The gene expression analysis of glaucoma and IOP with normal control showed that 408 DEGs (277 glaucoma and 131 IOP genes) were discovered from two GEO datasets. The 290 DEGs of glaucoma were extended to form a network containing 1495 proteins with 9462 edges. Using ClusterONE, the network was dissected to have 12 clusters. Among them, three clusters were linked with three IOP DEGs [N-Myc and STAT Interactor (NMI), POLR3G (RNA Polymerase III Subunit G), and APAF1-interacting protein (APIP)]. In the clusters, ontology analysis revealed that RNA processing and transport, p53 class mediators resulting in cell cycle arrest, cellular response to cytokine stimulus, regulation of phosphorylation, regulation of type I interferon production, DNA deamination, and cellular response to hypoxia were significantly enriched to be implicated in the development of glaucoma. Finally, NMI, POLR3G, and APIP may have roles that were noticed altered in glaucoma and IOP conditions.

● **CONCLUSION:** Our findings could help to discover new potential biomarkers, elucidate the underlying pathophysiology, and identify new therapeutic targets for glaucoma.

● **KEYWORDS:** glaucoma; high intraocular pressure; biomarkers; gene expression; protein interaction; molecular pathways

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INTRODUCTION

Glaucoma is one of the most common causes of blindness worldwide[1]. A diagnosis of glaucoma has been made based on the optic nerve examinations[2]. Diagnosis is difficult since most of the patients with progressing glaucoma have minor or no symptoms[3]. Nonetheless, glaucoma visual damage is progressive and irreversible; early detection and subsequent therapy can reduce disease development[4]. Notably, high intraocular pressure (IOP) is one of the major risk factors for glaucoma[5]. In addition to age, gender, ethnicity, and IOP, there are other molecular risk factors that include oxidative stress, systemic ocular vascular factors, and autoimmune components that contribute to the disease[6-7]. Drugs, lasers, and surgery all aim at lowering IOP to eliminate the main risk factor for glaucoma progression. Although the current glaucoma treatment strategies are useful, early detection will be beneficial for patients with glaucoma.

The recent development of omics technology produces enormous molecular data that provides molecular entities for complex diseases. However, these data required the implementation of computational procedures to retrieve meaningful information on pathogenesis[8]. Particularly, systems biology is an integrated computational approach

that merges multiple molecular entities, such as genomics, proteomics, and metabolomics, from a single biological system or disease condition to provide predictive models. These prediction models frequently express core disease mechanisms by dissecting the network into multiple clusters. Such systems biological approaches are driven to comprehend the pathogenesis and predict biomarkers as well as the drug targets that may guide us to prevent or treat human disease[8-9].

In principle, the pathogenesis of glaucoma is complex and is mediated by multiple risk factors and, more precisely, by the involvement of high IOP, which triggers glaucoma[5]. Assessing the connectivity between IOP and glaucoma may provide an opportunity to understand the disease mechanism, diagnosis, progression, and therapeutic effectiveness. The development of biomarkers for glaucoma is anticipated to make it simpler to examine the ocular matrix that provides the underlying molecular mechanisms of disease progression and pharmacological therapy. Micro- and macromolecules have been used as molecular biomarkers. Currently, a thorough ophthalmology examination can be given to suspect patients who have a family history of early diagnosis of glaucoma[10]. Despite several investigations into aqueous humor in animal models[11-12], a few studies have proposed using biomarkers or estimating the glaucoma risk in humans. Recently, it has been proposed that nitric oxide levels are helpful in the diagnosis of glaucoma[10,13-14]. Similarly, patients with glaucoma have reported elevated levels of antibodies against Sjögren's syndrome-related antigens[15-16]. However, few of the suggested biomarkers were nonspecific enough to discriminate against glaucoma. For instance, nitric oxide is an oxidative stress marker that is critically altered in most inflammatory conditions. Therefore, biomarkers are needed to be sensitive and specific in detecting glaucoma.

Herein, we investigated the gene expression datasets to identify effective candidate biomarkers for glaucoma diagnosis. Using the gene expression dataset, the critical genes of glaucoma influenced by the IOP were determined such as APAF1 interacting protein (*APIP*; a negative regulator of ischemic injury), N-Myc and STAT interactor (*NMI*; acts as a signalling pathway regulator involved in innate immune system response), and RNA polymerase III subunit G (*POLR3G*; enables chromatin binding activity and involved in positive regulation of innate immune response). Further incorporating these critical genes into a systems biological approach that uses a series of computational analyses to demonstrate possible biomarker candidates for glaucoma based on the IOP risk factor.

MATERIALS AND METHODS

Sources of Data The gene expression data containing glaucoma, IOP, and normal healthy controls were searched in the NCBI Gene Expression Omnibus (GEO) repository, which houses numerous curated gene expression data, including original series and platform records. Despite the availability of several gene expression profiles, only the data sets that match the following inclusion criteria were selected: 1) expression datasets containing a minimum of three samples in each group to perform statistical analysis; 2) datasets associated with glaucoma and ocular hypertension; 3) gene expression profiling in glaucomatous optic nerve heads. The study found the GSE45570 data, which contained glaucoma and ocular hypertension derived from optic nerve heads (ONH), to be appropriate. The dataset contained a significant number of samples in each group: six glaucoma samples, six IOP samples, and six normal samples that were executed using the GPL5175 Affymetrix Human Exon 1.0 ST Array platform. The GPL5175, employing *in situ* oligonucleotide technology, has a total of 316 919 spots in the array design category of the transcript cluster, presenting their unique identifiers for each exon.

Expression Analysis The Limma R package was utilized to analyze the collected datasets to determine the differentially expressed genes (DEGs) between the groups. In our study, variations in gene expression were determined between SET-A: glaucoma and normal and SET-B: IOP and normal. The statistical significance of DEGs was set at $P<0.05$ while screening both SET-A and B.

Protein Interaction Network In order to generate a protein interaction network, we use STRINGApp of Cytoscape software. The STRINGApp facilitates the functional associations between proteins from diverse sources, including STRING, STITCH, DISEASES, and PubMed text, into the Cytoscape platform. This allowed us to recover all of the possible links that could exist between the DEGs of glaucoma. The protein interaction arrived as a result of high-throughput research as well as information from biomolecular databases and published studies, which are used by STRING[17].

Cluster Analysis and Linking Hypertension Next, the glaucoma protein-protein interation (PPI) data was dissected using clustering with overlap neighborhood expansion (ClusterONE) to derive core functional clusters of closely linked proteins from the network[18-19]. ClusterONE was found to have a minimum density of >0.05 with a minimum of four nodes. Glaucoma clusters with a *P*-value of less than 0.05 were identified. Further, the glaucoma clusters linking the IOP were determined by identifying the shared proteins between clusters and the IOP differential expression gene set (SET-B), which aids in determining the glaucoma core clusters associated with the IOP.

Cluster Pathways The Enricher (http://biit.cs.ut.ee/gprofiler/) program was used to examine the functional profiles of proteins in selected clusters. The protein function was

determined based on the gene ontology function, which includes biological process (BP), molecular function (MF), and pathway enrichment. For gene ontology, we inputted the proteins of the clusters into the Enricher tool to determine their functions. Likewise, the molecular pathways associated with the clusters were determined using the Reactome database. The significance of the clusters associated with the ontology and pathways was set at a statistical significance of *P*-value <0.005.

RESULTS

According to differential gene expression analysis, the 294 and 137 exons were differentially expressed in the glaucoma and IOP, respectively. These exons were mapped with the GPL5175 platform as a reference to identify the gene symbol corresponding to the probes. In glaucoma, 294 probes were attributed to 277 genes. Similarly, 137 probes in the IOP condition were matched with 131 genes. Among 277 glaucoma DEGs, 168 were up-regulated and 109 were down-regulated. Similarly, in 131 IOP DEGs, 77 were up-regulated and 54 were down-regulated. Further, the 277 DEGs in glaucoma were subjected to a protein interaction network.

Protein Interaction Network The glaucoma protein network was built by obtaining all potential relationships between the seed proteins, with one node added as an external interacting neighbour. A total of 277 important genes retrieved from the expression analysis were queried in STRING, a cytoscape plug-in. Outliers like self-loops and duplicated edges were removed from the constructed protein network. A complex network was achieved, containing 1495 proteins with 9462 interconnected edges. To evaluate the topological properties of the glaucoma network, the protein network was subjected to a network analysis module, which showed topology characteristics such as network density of 0.0007, characteristic path of 3.530, network centralization of 0.08, and clustering coefficient of 0.120.

Glaucoma Cluster Connects with the Intraocular Pressure Next, the network was dissected into multiple clusters that represent the core functional modules of the entire network. Notably, 12 clusters were identified based on the density and degree node filters using the ClusterONE algorithm. Additionally, IOP association with the glaucoma clusters was determined by looking for the presence of IOP DEGs in the glaucoma clusters. A total of three glaucoma clusters were identified with the presence of three IOP DEGs, namely *NMI*, *POLR3G*, and *APIP*. Further, these clusters were enriched using Enricher tools to determine the functional role of these clusters.

Cluster Glaucoma Enrichment The results of the GO enrichment analysis of ten clusters having no involvement of IOP genes that demonstrate the association with biological processes such as RNA polymerase II regulation (GO:0045944), deubiquitination (GO:0016579), cellular response to cytokine stimulus (GO:0071345), regulation of beta receptor signalling pathway (GO:0017015), nucleic acid-templated transcription (GO:1903507), cell population proliferation (GO:0042127), metabolic process (GO:0010604), programmed cell death (GO:0043067), and nuclear factor (NF)-kappaB transcription factor activity (GO:0051092; Figure 1). Similarly, these clusters contribute to molecular function, including ubiquitin-like protein ligase binding (GO:0044389), kinase (GO:0019900), DNA (GO:0003677), I-SMAD (GO:0070411), histone deacetylase (GO:0042826), nuclear receptor (GO:0016922), RNA (GO:0003723), cadherin (GO:0045296), sequence-specific DNA (GO:0043565), protease (GO:0002020) binding, histone acetyltransferase activity (GO:0004402), thioesterase binding (GO:0031996), TNF receptor binding (GO:0005164), translation initiation factor activity (GO:0003743), and NF-kappaB binding (GO:0051059; Figure 2). Finally, the cluster proteins were investigated, which showed involvement in a variety of molecular pathways, as demonstrated in Figure 3.

Clusters of Glaucoma with Intraocular Pressure The ontological analysis of the glaucoma cluster (Cluster 1: *TAB2*, *KIF5B*, *SKIL*, *CALM1*, *NFKBIE*, *YEATS4*, *RIPK2*, *BRCA1*, *ACTL6A*, *MEPCE*, *RBBP4*, *DDX5*, *CSNK2A1*, *HSP90AB1*, *RBL1*, *TUBA3D*, *VPS72*, *CSNK2A2*, *RELB*, *XRCC6*, *TAB1*, *KIF5C*, *PIAS3*, *HSPA5*, *CALM2*, *RPLP0*, *ZNHIT1*, *NR3C1*, *ACTR6*, *TUBA3C*, *HDAC1*, *TSG101*, *CALM3*, *MDM2*, *NMI*; Cluster 2: *APIP*, *CASP3*, *PLA2G4A*, *VIM*, *NIF3L1*; Cluster 3: *POLR3C*, *POLR3H*, *POLR3D*, *POLR3G*) containing IOP genes (*NMI, POLR3G*, and *APIP*) showed association with biological processes such as chromatin remodelling (GO:0043044), type I interferon production (GO:0032481), phosphoprotein phosphatase activity (GO:0032516), interferon-beta production (GO:0032648), phosphatase activity (GO:0010922), innate immune response (GO:0045088), cyclic-nucleotide phosphodiesterase activity (GO:0051342) calcium-release channel activity (GO:0060316), p53 mediator signal transduction (GO:1901796), cell communication by electrical coupling (GO:1901844), calcium-release channel activity (GO:0060315), substantia nigra development (GO:0021762), cell communication by electrical coupling (GO:0010649), cell cycle process (GO:0010564), cytokine production (GO:0001819), and NF-kappaB signalling (GO:0007249; Figure 4). In terms of molecular function, it is strongly associated with disordered domain-specific binding (GO:0097718), adenylate cyclase binding (GO:0008179), titin binding (GO:0031432), protein phosphatase activator activity (GO:0072542), nucleosomal DNA binding (GO:0031492), kinase activator activity (GO:0043539), calcium channel

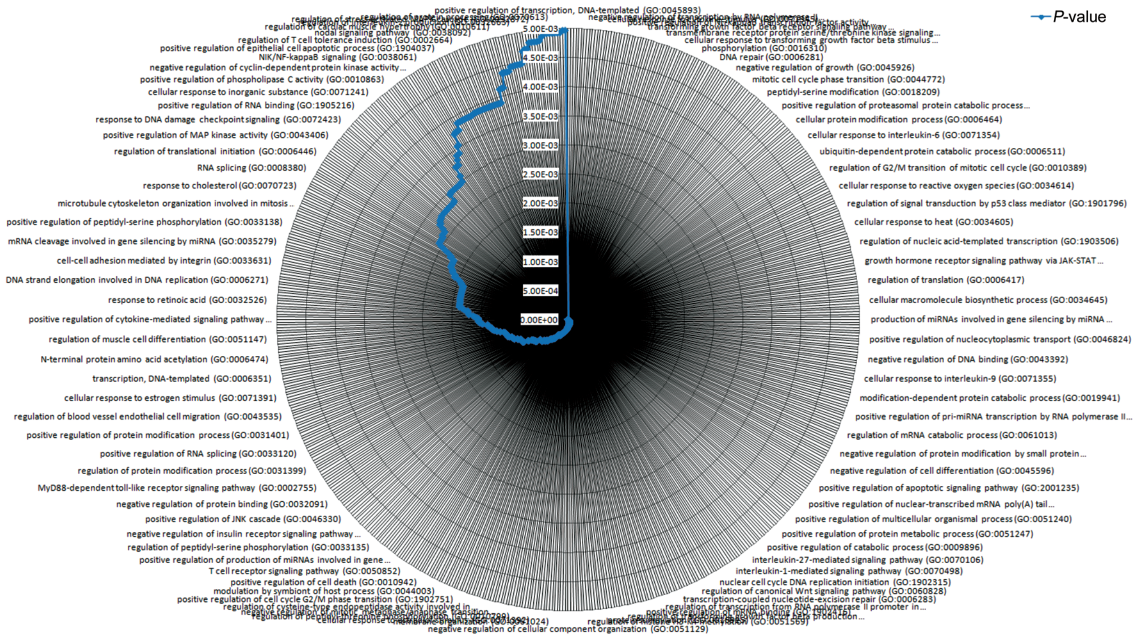

Figure 1 Glaucoma clusters proteins contributing the biological process that describes the glaucoma functional mechanism.

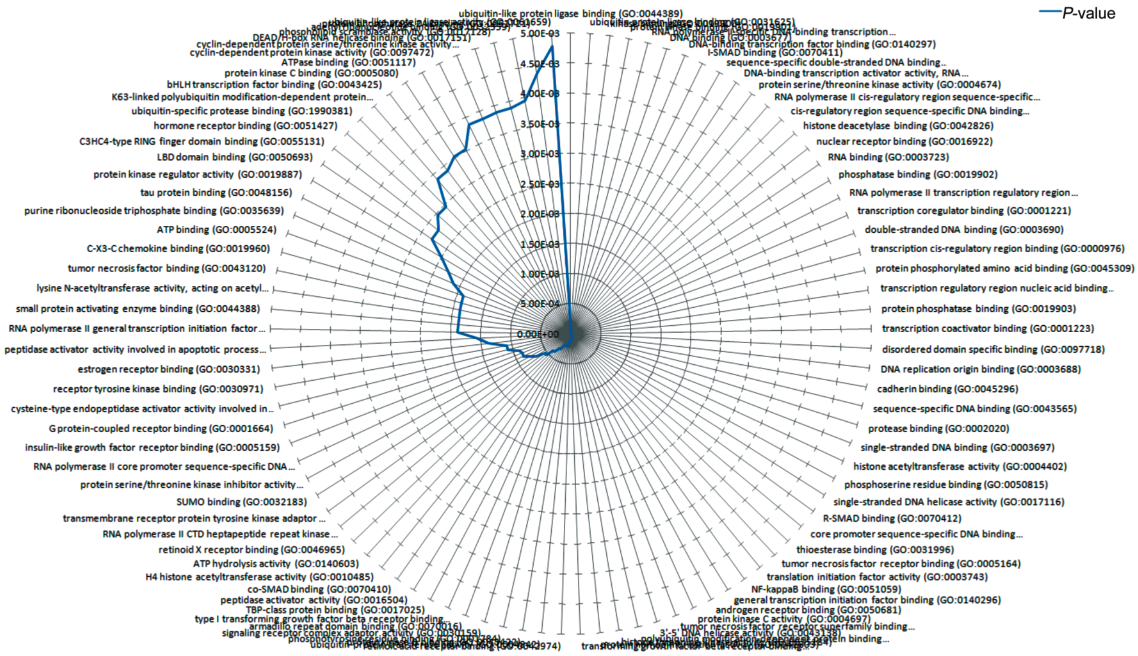

Figure 2 Plot representing the molecular function attributed by the 12 glaucoma clusters.

activity (GO:0019855), ubiquitin ligase binding (GO:0031625), kinase activator activity (GO:0030295; Figure 5). Subsequently, the molecular pathway analysis showed involvement in cellular responses to stress, transport of mature transcripts to RNA polymerase, neurodegenerative disease pathway, cellular senescence, C-type lectin receptor signalling pathway, neurotrophin signalling pathway, dopaminergic synapse, cytosolic DNA-sensing pathway, NF-kappaB signalling pathway, amphetamine addiction, glioma, and pertussis (Figure 6).

Expression of Three Crucial Genes The gene expression of *NMI*, *POLR3G*, and *APIP* was assessed from the GSE45570 microarray datasets of glaucoma and ocular hypertension derived from ONH. The *NMI* and *POLR3G* were noticed to be upregulated in glaucoma and IOP. Whereas APIP was downregulated in glaucoma and IOP conditions, suggesting its crucial role in glaucoma and ocular hypertension (Table 1).

DISCUSSION

Glaucoma is the main cause of visual impairment, which has a negative impact on quality of life and places a financial, social, and medical burden. Glaucoma is frequently brought on by trabecular meshwork and aqueous outflow system dysfunction, which result in excessive intraocular aqueous fluid build-up and IOP[5]. Therefore, it is crucial to understand the molecular interphase of high IOP leading to glaucoma to detect and/

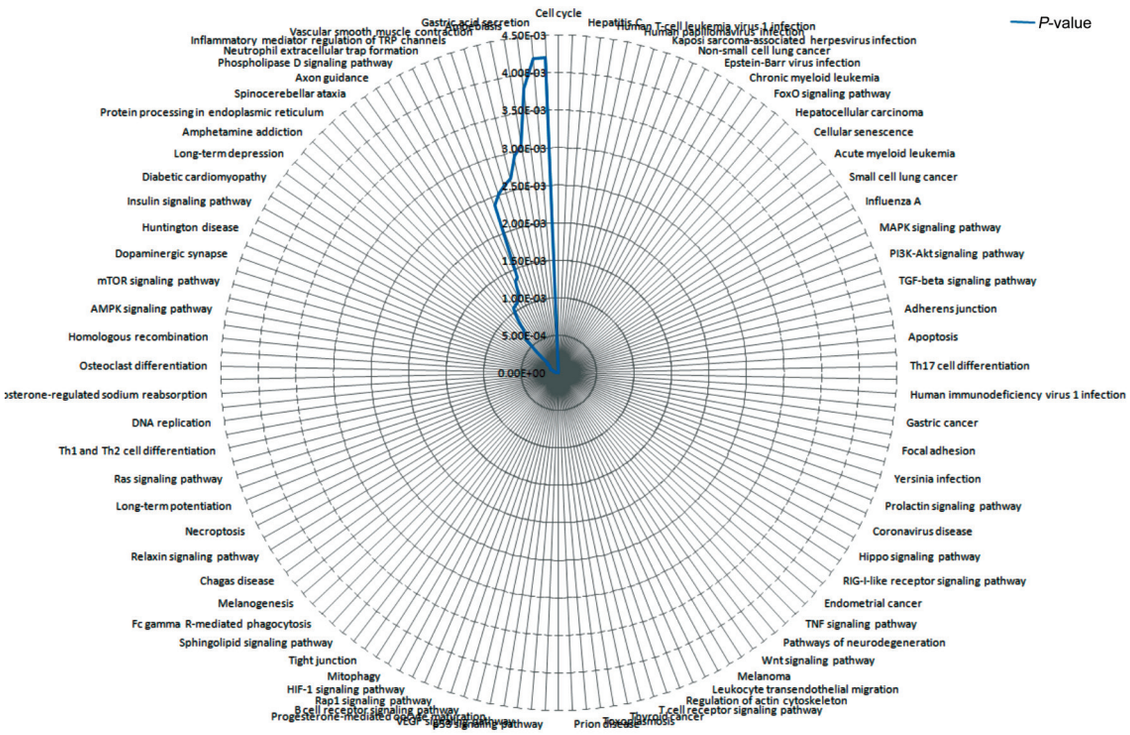

Figure 3 Glaucoma clusters proteins contributing the molecular pathways that describes the glaucoma functional mechanism.

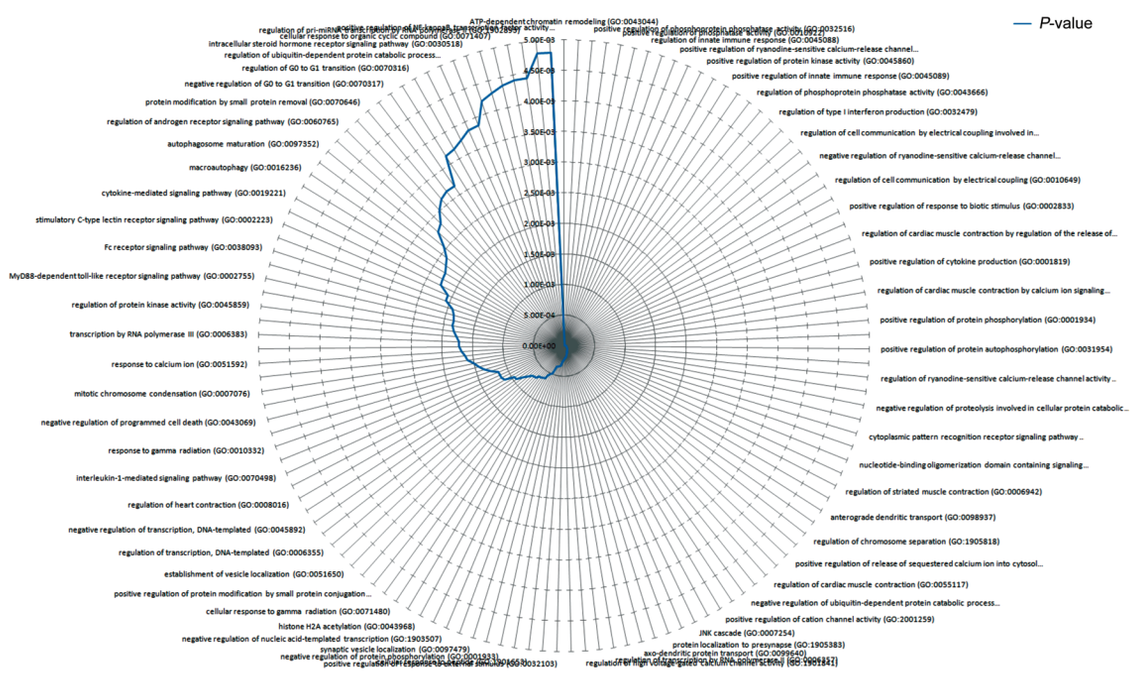

Figure 4 Glaucoma clusters with IOP proteins contributing molecular function with the *P*<0.005 IOP: Intraocular pressure.

Table 1 Comparative gene expression levels of *APIP*, *NMI*, and *POLR3G*

Gene symbol	Glaucoma			High IOP		
	logFC (normal *vs* glaucoma)	*P*	Regulation	logFC (normal *vs* IOP)	*P*	Regulation
APIP	-0.69	0.041	Down	-0.73	0.01	Down
NMI	0.40	0.03	Up	0.32	0.03	Up
POLR3G	0.42	0.03	Up	0.42	0.02	Up

APIP: APAF1 interacting protein; *NMI*: N-Myc and STAT interactor; *POLR3G*: RNA polymerase III subunit G; IOP: Intraocular pressure.

or treat this disease. Here, we implemented the systems biological approach to assess the IOP gene connectivity with the core functional module of the glaucoma network. Our series of investigations was initialized by screening DEGs in glaucoma and IOP compared to the control. At ONH, this initial screening reveals the critical genes that undergo

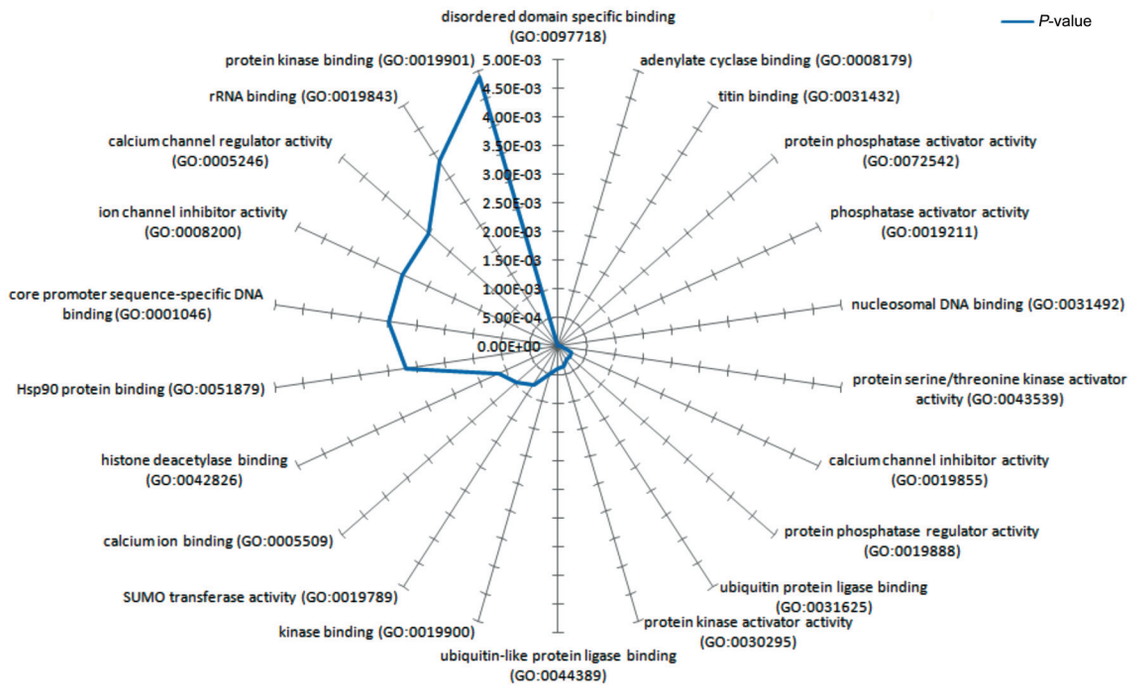

Figure 5 Plot representing the biological process attributed by the glaucoma clusters with IOP proteins connectivity IOP: Intraocular pressure.

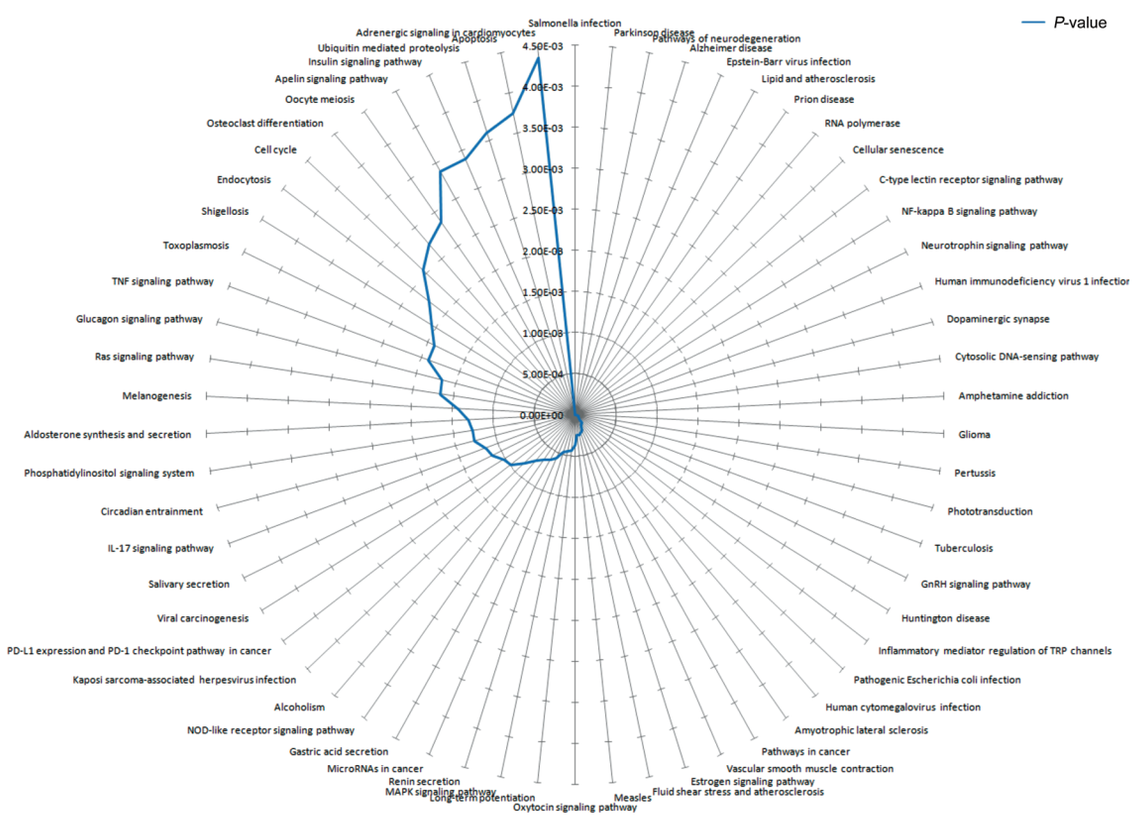

Figure 6 Glaucoma clusters with IOP proteins contributing the molecular pathways that describe the mechanism influenced by IOP in glaucoma IOP: Intraocular pressure.

alteration in each condition[3,8]. Assessing the gene expression change in ONH will directly reflect the pathological changes associated with glaucoma. Particularly, retinal nerve fibre layer damage is typically followed by alterations in the optic nerve head and significant changes in the visual field. This is due to the fact that the retinal nerve fibre layer (RNFL) is the layer that houses the nerve fibres of the retina[3,8]. Although the significant variation that exists in the normal morphology of the optic disc to the definitive glaucoma diagnosis is predicated on the morphology of the ONH, it depends on the observer's experience and is therefore quite subjective. Hence, assessing the gene expression changes will demonstrate the molecular

status of diseases, which helps in diagnosis and avoids subjective bias.

To identify differential genes between glaucoma and control, the protein interaction network was constructed. Nowadays, protein interaction is the main strategy to determine biological function. Proteins control cellular and molecular functions, which in turn determine an organism's state of health or disease. Additionally, they interact with other proteins and other kinds of molecules to perform functions. Choudhari *et al*[20] developed an online repository on interactions between protein-coding genes and trace elements related to glaucoma pathology. Recently, Ding *et al*[21] developed the glaucoma gene network to establish drug-regulated gene signatures to find the molecular indicators for drug-induced glaucoma. All of these studies gave significant information on the molecular mechanisms of glaucoma but did not explicitly address the role of risk factors in leading to the condition. In contrast, our study includes the critical IOP as one of the factors influencing the glaucoma network.

The identification of protein complexes or functional modules has emerged as a primary area of investigation within the field of systems biology. Numerous studies have shown that clustering of protein interaction networks is an efficient method to understand disease mechanisms[18-19]. Here, we dissected the synthesised glaucoma protein network into multiple clusters to identify the core functionality of the network. We identified twelve clusters with varying numbers of nodes and edges. On enrichment analysis, most of these clusters were associated with RNA synthesis, stability, processing, and metabolism, which reflected that RNA signalling was markedly altered in glaucoma. This was in agreement with the previous study[22]. Among 12, three crucial clusters were selected, containing three genes (*NMI*, *POLR3G*, and *APIP*) that were differentially expressed in IOP (Figure 6). Among these interlinked genes, *NMI* is involved in immune response[23], *POLR3G* is associated with transcription by RNA polymerase III[24], and *APIP* is linked with pyroptosis[25]. More particularly, the IOP belonging clusters were linked with RNA polymerase, neurodegenerative disease pathway, cellular senescence, C-type lectin receptor signalling pathway, NF-kappaB signalling pathway, neurotrophin signalling pathway, dopaminergic synapse, cytosolic DNA-sensing pathway, amphetamine addiction, glioma, and pertussis. The systematic process behind how these genes affect glaucoma is largely understood, despite the widespread reporting of similar molecular pathways in cancer, immunity, and neurodegeneration (Figures 3 and 6). Therefore, it is necessary to conduct further research to comprehend the functioning of these genes. However, our analysis of these genes revealed significant changes in gene expression in both glaucoma (SET-A) and IOP (SET-B). Both glaucoma and IOP showed upregulation of NMI and POLR3G. However, APIP was downregulated in both glaucoma and IOP. Hence, these genes are crucial and could guide us in detecting glaucoma. The study identifies a new group of genes that associated with glaucoma and IOP in comparison to Xie *et al*[13].

In conclusion, this study employed a series of systems biology techniques to identify the DEGs associated with glaucoma and IOP. The DEGs were used to form a network, which ultimately led to the identification of the essential genes *NMI*, *POLR3G*, and *APIP* that connect glaucoma and IOP. These genes have a strong correlation with the molecular causes of glaucoma, which is important in the progression of the disease. These genes exhibit similar changes in expression in both glaucoma and IOP. Further investigation is required to effectively employ these genes as biomarkers. As genetic data become more publicly accessible, it is anticipated that similar approaches will become increasingly popular for future investigations.

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Conflicts of Interest: Almarzouki N, None.

REFERENCES

1 Vashist P, Senjam SS, Gupta V, Gupta N, Shamanna BR, Wadhwani M, Shukla P, Manna S, Yadav S, Bharadwaj A. Blindness and visual impairment and their causes in India: results of a nationally representative survey. *PLoS One* 2022;17(7):e0271736.

2 Anton A, Nolivos K, Pazos M, *et al*. Diagnostic accuracy and detection rate of glaucoma screening with optic disk photos, optical coherence tomography images, and telemedicine. *J Clin Med* 2021;11(1):216.

3 Schuster AK, Erb C, Hoffmann EM, Dietlein T, Pfeiffer N. The diagnosis and treatment of glaucoma. *Dtsch Arztebl Int* 2020;117(13):225-234.

4 Gosling D, Meyer JJ. Normal tension glaucoma. In: *StatPearls* 2022 Nov 21. StatPearls Publishing.

5 Sharfuddin Ahmed M, Ullah AY, Barman N, Ratan ZA, Mostafa S, Khaleque A, Kabir S, Khan MH, Haque MA. Risk factors associated with elevated intraocular pressure: a population-based study in a rural community of Bangladesh. *BMJ Open Ophthalmol* 2023;8(1):e001386.

6 Böhm EW, Buonfiglio F, Voigt AM, Bachmann P, Safi T, Pfeiffer N, Gericke A. Oxidative stress in the eye and its role in the pathophysiology of ocular diseases. *Redox Biol* 2023;68:102967.

7 Goodman D, Ness S. The role of oxidative stress in the aging eye. *Life* 2023;13(3):837.

8 Garzon M, Mainali S, Chacon MF, Azizzadeh-Roodpish S. A computational approach to biological pathogenicity. *Mol Genet Genomics* 2022;297(6):1741-1754.

9 Ahmed SS, Ahameethunisa AR, Santosh W, Chakravarthy S, Kumar S. Systems biological approach on neurological disorders: a novel molecular connectivity to aging and psychiatric diseases. *BMC Syst Biol* 2011;5:6.

10 Boehm N, Wolters D, Thiel U, Lossbrand U, Wiegel N, Pfeiffer N, Grus FH. New insights into autoantibody profiles from immune

privileged sites in the eye: a glaucoma study. *Brain Behav Immun* 2012;26(1):96-102.

11 Park HL, Kim SW, Kim JH, Park CK. Increased levels of synaptic proteins involved in synaptic plasticity after chronic intraocular pressure elevation and modulation by brain-derived neurotrophic factor in a glaucoma animal model. *Dis Model Mech* 2019;12(6):dmm037184.

12 Gye HJ, Kim JM, Yoo C, Shim SH, Won YS, Sung KC, Lee MY, Park KH. Relationship between high serum ferritin level and glaucoma in a South Korean population: the Kangbuk Samsung health study. *Br J Ophthalmol* 2016;100(12):1703-1707.

13 Xie RL, Nie HY, Xu YX. Identification of hub genes for glaucoma: a study based on bioinformatics analysis and experimental verification. *Int J Ophthalmol* 2023;16(7):1015-1025.

14 Zhang ZQ, Xie Z, Chen SY, Zhang X. Mitochondrial dysfunction in glaucomatous degeneration. *Int J Ophthalmol* 2023;16(5):811-823.

15 Wax MB, Tezel G, Saito I, Gupta RS, Harley JB, Li Z, Romano C. Anti-Ro/SS-a positivity and heat shock protein antibodies in patients with normal-pressure glaucoma. *Am J Ophthalmol* 1998;125(2):145-157.

16 Grus FH, Joachim SC, Bruns K, Lackner KJ, Pfeiffer N, Wax MB. Serum autoantibodies to alpha-fodrin are present in glaucoma patients from Germany and the United States. *Invest Ophthalmol Vis Sci* 2006;47(3):968-976.

17 Szklarczyk D, Gable AL, Lyon D, *et al*. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 2019; 47(D1):D607-D613.

18 Nepusz T, Yu HY, Paccanaro A. Detecting overlapping protein complexes in protein-protein interaction networks. *Nat Methods* 2012;9(5):471-472.

19 Wan FC, Cui YP, Wu JT, Wang JM,-Z Liu Q, Gao ZL. The PPI network and cluster ONE analysis to explain the mechanism of bladder cancer. *Eur Rev Med Pharmacol Sci* 2013;17(5):618-623.

20 Choudhari JK, Eberhardt M, Chatterjee T, Hohberger B, Vera J. Glaucoma-TrEl: a web-based interactive database to build evidence-based hypotheses on the role of trace elements in glaucoma. *BMC Res Notes* 2022;15(1):348.

21 Ding RF, Yu Q, Liu K, Du J, Yin HJ, Ji ZL. Gene network analyses unveil possible molecular basis underlying drug-induced glaucoma. *BMC Med Genomics* 2021;14(1):109.

22 Mochizuki H, Murphy CJ, Brandt JD, Kiuchi Y, Russell P. Altered stability of mRNAs associated with glaucoma progression in human trabecular meshwork cells following oxidative stress. *Invest Ophthalmol Vis Sci* 2012;53(4):1734-1741.

23 He T, Qiao YB, Yang Q, Chen J, Chen YY, Chen XK, Hao ZX, Lin MJ, Shao ZY, Wu P, Xu F. NMI: a potential biomarker for tumor prognosis and immunotherapy. *Front Pharmacol* 2022;13:1047463.

24 Lautré W, Richard E, Feugeas JP, Dumay-Odelot H, Teichmann M. The POLR3G subunit of human RNA polymerase III regulates tumorigenesis and metastasis in triple-negative breast cancer. *Cancers* 2022;14(23):5732.

25 Zhang BZ, Wang Z. A novel pyroptosis-regulated gene signature for predicting prognosis and immunotherapy response in hepatocellular carcinoma. *Front Mol Biosci* 2022;9:890215.