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Research Article

Analysis of Gene Network and Application of Barabási–Albert Model to Find out the Core Genes Involved in Glaucoma

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Summary

Worldwide Glaucoma is affecting over 60 million people. It is a neurodegenerative ocular disorder. Here we have analysed the gene network of Glaucoma from microarray data to understand the molecular mechanisms as a whole. Barabási–Albert model has been used here in gene network analysis to identify the core communities and validated our finding with existing published data. Few new marker genes have been proposed for Glaucomatous neuropathy.

Abstract

Background

Glaucoma is an ocular disorder and also a neurodegenerative in character. Due to the rapid development of microarray technologies huge gene expression data is available to correlate with this disease phenotype. In Series GSE13534, it has been reported that ECM and pro-fibrotic gene expression are up-regulated compared with normal lamina cribrosa cells and have been proposed as pathological characteristic. Instead of a single gene a systems biology network-based approach has been adopted to investigate the pathological gene network.

Methods

Multiple genes, those contribute to a common disorder, tend to display high co-expression levels, exhibit expression as a group in a synchronized manner. In general gene regulatory network has been proposed to have a scale free nature by Barabási–Albert model. Here the co-expressed gene expression network has been constructed with the microarray data of Glaucoma using same model. Functional enrichment analysis of the set of genes with Gene Ontology and Pathway analysis helps to correlate and validate with the already existing published data.

Results

The nodes, which are densely connected internally, can be easily grouped into communities and the analysis of community structure in a complex network helps to simplify and makes easy to understand the network. The community, which is having more number of genes, are termed as core community. In the disease network total 51 genes such as, TUBA1C, VEGFB, and many more are interacting as in the core part where in the core part of the normal network the number of genes is only 35. Functional enrichment and Gene Ontology analysis tells about the main pathways like haemostasis, Gap Junction, Dilated Cardio-myopathy and Focal adhesion are responsible for Glaucoma. After detail analysis we have established the model protocol and predicted few marker genes like TUBA1C, VEGFB, CFL1P2, CFL1 and TMSB4X for glaucomatous optic neuropathy. PAOG is silent killer; these marker genes will help to identify the disease at the beginning stage.

Keywords: Glaucoma; Optic Neuropathy; Gene Network; Barabási–Albert Model; Marker Gene.

Introduction

Glaucoma is a neurodegenerative ocular disorder. It is affecting over 60 million people worldwide and is the primary cause of irreversible blindness. There are four different kinds of Glaucoma, Primary Open Angle Glaucoma (POAG), Angle Closure Glaucoma (ACG), Normal Tension Glaucoma (NTG) and Secondary Glaucoma (SG). ACG is not very common and sudden eye pain is the symptom, data for ACG is very rare. NTG is inherited disease and features are similar to POAG. SG is those that develop as secondary complication to, other conditions like diabetes, eye surgery, eye trauma or tumours. Primary open-angle glaucoma (POAG) is the most common kind of Glaucoma and gene expression data for POAG is accessible in GEO database. [1-4]. Normal and glaucomatous lamina cribrosa cells gene expression data of human have been taken here for studies. POAG comes with no pain and early diagnosis is the key point for treatment of POAG, so aim of the study to detect potential biomarkers. It has been characterised by the presence of glaucomatous optic neuropathy without an identifiable secondary cause. It has been shown that the reason behind the substantial amount of POAG has genetic predispositions and epigenetic risk factors. There are several glaucoma-causing

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genes like myocilin (MYOC), optineurin (OPTN) and WD-repeat domain 36 have been discovered. [5-6].

With the rapid development of microarray technologies and exponential increase in computational power, the wealth of gene expression data is gathered to correlate with common human diseases [7-8]. The microarray data (GSE13534) which has been taken here for studies is gene expression data of human normal and glaucomatous lamina cribrosa cells. It has been reported that ECM and pro-fibrotic gene expression are up-regulated in glaucoma compared with normal lamina cribrosa cells. One or two genes are not enough for pathological characteristic. The co-expressed gene network (CGN) analysis can be developed with the microarray data, which are then used to identify the co-expressed gene sets (i.e., modules) related to a specific disease phenotype [9-10]. Gene network means protein -protein networks make pathways and the knowledge of pathway-map makes it easy to interpret molecular mechanisms underlying these different networks which make a system as a whole. The study of these complex, epistatic interactions among multiple gene or protein expression attributes is crucial in explaining portions of the genotype-to-phenotype associations. Functional enrichment analysis of the modules with Gene Ontology can give subsequently co-functional gene clusters. A co-functional cluster is usually composed of a hub gene and multiple neighbours can have multiple biological and clinical applications. The implications of cellular interconnectedness on disease progression could lead to identification of disease genes and disease pathways, which, in turn, could offer better and more accurate biomarkers and targets for drug development [11-16].

The Barabási–Albert model introduced in 1998 explained the power-law degree distribution of networks by considering two main ingredients: growth and preferential attachment [17,18]. In the discussion of graph theory properties of cellular network, Barabasi has explained about the disease gene network where the nodes represent genes, and the edges link genes which are associated with the same disorder. In the disease gene network genes that contribute to the same disorder tend to be correlated in many other ways as well. They have an increased tendency to be expressed together in specific tissues, they typically display high co-expression levels, and, in many cases, they share common cellular and functional characteristics, as annotated in the Gene Ontology [19].

Our aim here is to present an overview of methodological frame work to make gene network of Glaucoma disease. The genes were clustered into different groups by barabasi community analysis. The community, which is having more number of genes, are termed as core community. The genes present in core community in Glaucoma network were identified. The complex mechanism of Glaucoma had been simplified into few pathways or set of pathways and their importance has been validated with already existing published experimental data [20]. The gene enrichment analysis of functionally related gene groups followed by GO analysis infers the main biological processes involved in Glaucoma.

Materials and Methods

The data set used for the network construction was retrieved from the gene expression omnibus (GEO). The dataset (GSE13534) consists of [HG-U133A] Affymetrix Human Genome U133A Array surveying comparison between human normal and glaucomatous lamina cribrosa cells. Total number of genes, present in the Microarray chip is 22283 [21].

Identification of Co-Expressed Genes

This study involves co-expressed genes in Glaucoma and Primary Open angle Glaucoma indicating responsible molecular mechanisms in the disease process. Five candidate genes involved in Glaucoma were selected. Based on the availability of specific probe sets in the HG-U133A microarray chip and co expression of at least two candidate genes together were selected. Ultimately 210 co expressed genes including 10 candidate genes involved in general Glaucoma were selected using the gene Recommender package [22] of the Bio conductor. In particular, the package ranks genes according to the strength of the correlation of the set of query genes over the experiments for which the query genes behave in a similar fashion. The query genes, which are chosen, are intended to be genes known to be involved in a process of interest. Since genes which are co-expressed are more likely to be functionally related and involved in or related to the process of interest.

Generation of Mutual Information Network

Mutual information networks are a subcategory of network inference methods which infer a link between a couple of nodes if it has a high score based on mutual information [23].

Mutual information network inference proceeds in two steps. The first step is the computation of the mutual information matrix (MIM), a square matrix whose *i, j*-th element

$$MIM_{ij} = I(X_i; X_j) \quad (1)$$

is the mutual information between X_i and X_j, where $X_i \in X$, $i = 1,...,n$, is a discrete random variable denoting the expression level of the i th gene. The second step is the computation of an edge score for each pair of nodes by an inference algorithm that takes the MIM matrix as input.

MRNET (23) infers a network using the maximum relevance/ minimum redundancy (MRMR) feature selection method [24,25]. The idea consists in performing a series of supervised MRMR gene selection procedures where each gene in turn plays the role of the target output.

The mutual information matrix was estimated using minuet (Mutual Information Network Inference) package of the Bio conductor available in the R language and environment [26]. Since mutual information is a non-linear measure of dependency, it measures a natural generalization of correlation. The MRNET algorithm was applied to infer the weighted adjacency matrix of the network.

Graph Generation

Minuet returns a matrix which is the weighted adjacency matrix

of the network. The weights range from 0 to 1 and can be seen as a confidence measure on the presence of the arcs. In order to display the network, R graph viz was loaded and use the following command" plot (as (returned matrix,"graph NEL")" was used. Then it is converted to igraph object which is then used for Barabási–Albert algorithm. The scale-free graphs according to the Barabási–Albert model were used to generate graph. It is a very simple stochastic algorithm for building a graph. The highly connected hub genes may play crucial roles in a network. It is a discrete time step model and in each time step a single vertex is added.

In the first step a single vertex and zero edges are used to start. Then in each time step one vertex is added and the new vertex initiates some edges to old vertices. The probability that an old vertex is chosen is given by P[i] ~ k[i]^alpha + a where k[i] is the in-degree of vertex i in the current time step (more precisely the number of adjacent edges of i which were not initiated by i itself) and alpha and are parameters given by the power and zero. Appeal arguments. The barabasi community detection program has been used to detect different community in the network which utmost important in hub genes detections [27,28].

Functional Enrichment and GO Analysis

The Database for Annotation, Visualisation and Integrated Discovery (DAVID) v6.7 is web accessible programs which uses EASE a customizable software application for rapid biological interpretation of gene lists [29].

It integrates almost all major and well-known public bioinformatics resources centralized by the DAVID Gene Concept [30]. It performs three basic functions. The first is theme discovery, defined as the identification of terms or phrases that describe a statistically significant number of genes in the list with respect to the number of genes described by the term or phrase in the population of genes from which the list derived. The second is customizable linking to online tools, and the third is creation of descriptive annotation tables.

As the members of two independent groups can fall into one of two mutually exclusive categories, Fisher Exact test is used to determine whether the proportions of those falling into each category differs by group. DAVID classify large gene list into functional related gene groups, rank the importance of the discovered gene groups and summarize the major biology of the discovered gene groups. DAVID also identifies the Gene Ontology terms.

DAVID tools have been used to functional enrichment analysis of the core gene communities in the disease and normal cells. It was used to identify important pathways which are built by core genes of glaucoma network.

Table-I: Enriched pathway (KEGG) for the core genes of disease and normal network of Glaucoma by DAVID enrichment analysis.

Sample	Pathway Name (term)	No of genes	Percentage of genes involved	P-Value
Disease Sample	Ribosome: hsa03010	14	**37.8%**	**3.7E-17**
	Pathogenic Escherichia Coli Infection: hsa05130	5	**13.5%**	**1.8E-4**
	Vibrio Choleri Infection: hsa05110	3	**8.1%**	**3.3E-2**
	Gap Junction hsa04540	3	**8.1%**	**7.5E-2**
	Dilated Cardiomyopathy hsa05414	3	**8.1%**	**8.0E-2**
	Focal adhesion hsa04510	4	**10.8%**	**8.1E-2**
	Regulation of actin cyto skeleton hsa04810	4	**10.8%**	**9.5E-2**
Normal Sample	Ribosome	9		

Table –II: Enriched pathway (REACTOME) for the core genes of disease and normal network of Glaucoma by DAVID enrichment analysis.

Sample	Pathway Name (term)	\No of genes Involved	Percentage of genes involved	P-Value
	REACT_17015:Metabolism of proteins	19	51.35135	6.82E-15
	REACT_1762:3' -UTR-mediated translational regulation	15	40.54054	4.37E-14
	REACT_6167:Influenza Infection	15	40.54054	8.72E-12
	REACT_71:Gene Expression	16	43.24324	4.27E-08
	REACT_604:Hemostasis	7	18.91892	0.014921567
Normal Sample	REACT_17015:Metabolism of proteins	11	40.74074	1.21E-08
	REACT_1762:3' -UTR-mediated translational regulation	9	33.33333	1.54E-08
	REACT_6167:Influenza Infection	9	33.33333	2.97E-07
	REACT_71:Gene Expression	9	33.33333	1.23E-04

Results

Identification of Co-Expressed Genes

The 10 candidate genes were picked up from the published literatures which are MYOC, TGFB1, TGFB2, TGFA, OPTN, CYP1B1, ESM1, VEGFC, PDGFA and LTBP 2. MYOC encodes the protein myocilin, which is believed to have a role in cytoskeleton function. MYOC is expressed in many ocular tissues. Optineurin (OPTN) may also function in cellular morphogenesis and membrane trafficking, vesicle trafficking, and transcription activation, Optineurin may play a role in normal-tension glaucoma and adult-onset primary open angle glaucoma. Mutations in MYOC, OPTN, CYP1B1 and LTBP 2 have been found in glaucoma. Transforming growth factor-β2 (TGF-β2) is found in increasing amounts in aqueous humour and reactive optic nerve astrocytes of patients with primary open-angle glaucoma (POAG) [31]. Elevated transforming growth factor β1 in plasma of primary open-angle glaucoma patients. [32]. In glaucoma, A common finding was the differential regulation of genes involved in inflammation and immunity, including the complement system and the cytokines transforming growth factor β (TGFβ) and tumor necrosis factor α (TNFα) [33]. Vascular endothelial growth factor C (VEGFC) and platelet-derived growth factor A (PDGFA) were markedly reduced in the lamina cribrosa of the ONHs of donors with POAG [34]. The co-expressed gene list of 210 genes including the candidate genes were generated using gene Recommender. The genes that were selected by gene Recommender in disease samples and normal samples are different. 193 genes were common among both the network. 17 genes were unique for disease cells and some other 17 genes were unique for normal cells (gene list are provided in the Supplementary material). Pictorially it is shown in a Venn diagram (Fig 1).

Graph Generation and Community Analysis

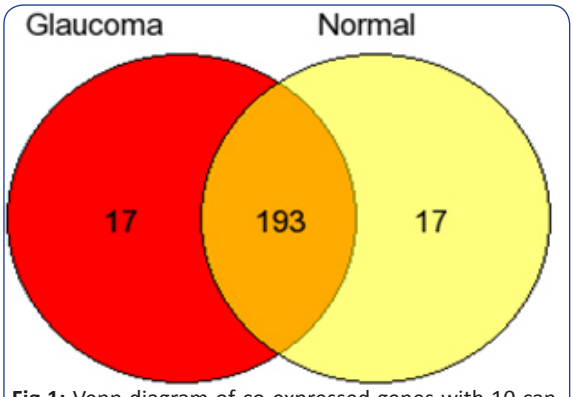


Fig 1: Venn diagram of co-expressed genes with 10 candidate genes in Glaucoma (red) and Normal (yellow)Cell. 193 genes are common to each other and 17 genes are unique to Glaucoma samples and different 17 genes are unique to Normal Samples. The gene list has been provided in the Supplementary material -I

After that using minuet and i graph the final graphs had been generated. The "Barabasi game" function was used which generates a simple graph starting from one node and adding more nodes and links based on a preset level of preferential attachment (how much new actors would prefer to form links to the more popular nodes in the network). The disease network is shown in Fig 2. The disease network is different from the normal cell network. Total 51 genes are interacting in the core of the disease network where in normal network the number of genes in the core part is only 35. In the normal cells the other genes are equally distributed in the surrounded space of the core part where in disease network a bias has been observed.

The barabasi community method was employed to identify different communities in the network. Total 6 communities were identified in each graph. Core community are shown in gray colour. Here we have analysed only the core community in more detail. (Detail gene list for each community is provided in the Supplementary material 2).

Functional and Pathway Enrichment Analysis using DAVID

The core proteins in the Glaucoma network and normal Network have been studied by Database for Annotation, Visualization and Integrated Discovery (DAVID) pathway enrichment analysis and then compared with normal healthy cell network to predict the most probable pathways involved in the disease process. The analysis was done with two different pathway database, one is KEGG pathway [35,36] and other is REACTOME pathway [37]. This analysis resulted in 7 enriched pathways in disease network where in normal network had only one enriched KEGG pathway that ribosomal pathway. Ribosomes-the primary macromolecular machines responsible for translating the genetic code into proteins-are complexes of precisely folded RNA and proteins. Impairment of the process also may lead to disease. While we are getting only 9 genes in normal network, in disease network the number increases to 14. If we exclude microbial infection pathways Gap Junction, Dilated Cardiomyopathy, Focal adhesion, Regulation of actin cytoskeleton are important in core part of the disease network.

Eye lens epithelial cells express the gap junction proteins or connexins. Different connexins form gap junction channels and hemi channels with different properties. Previous experimental studies shows that under conditions of elevated intraocular pressure (IOP), in glaucoma astrocytes may lose Gap Junction intercellular communication which alters the homeostasis of RGC axons, adopting the reactive phenotype and contribute to glaucomatous neuropathy [38]. Dilated cardiomyopathy is somewhat correlated with glaucoma. A recent study shows that Chronic Heart Failure is associated with lower ocular perfusion pressure and glaucomatous optic nerve head changes [39].

The trabecular meshwork (TM) is located in the anterior segment of the eye and is responsible for regulating the out flow of aqueous humour. Most of the outflow resistance is thought to be from the

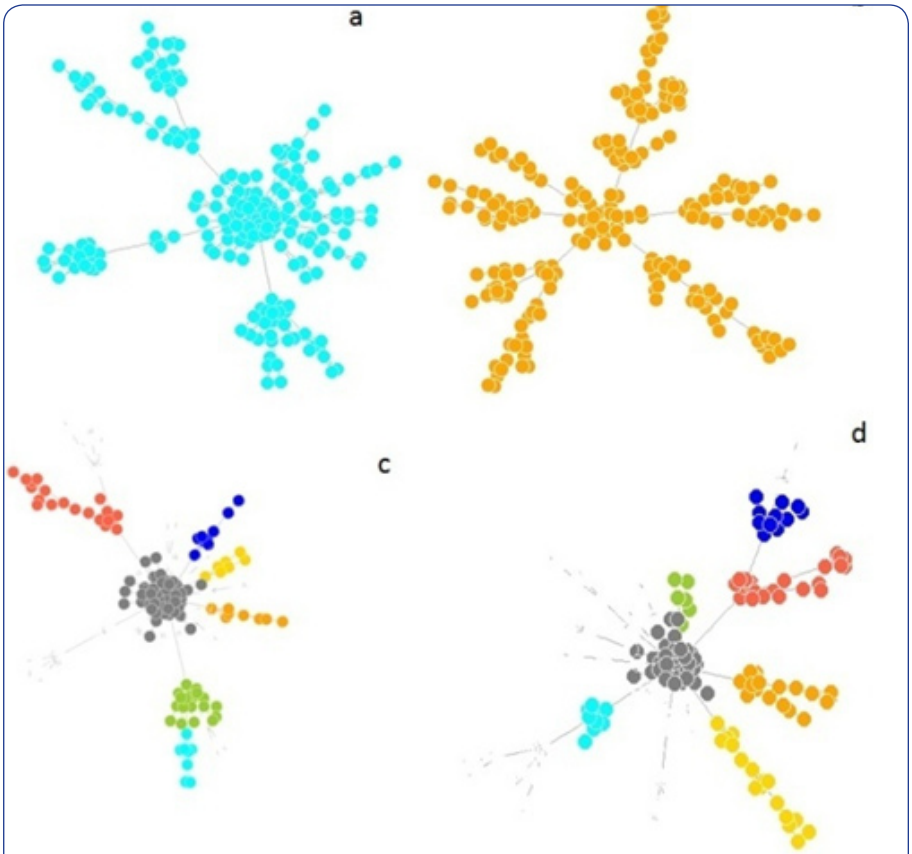


Fig 2: Gene networks in (a) glaucoma and (b) normal cells. Barabasi community analysis in (c) glaucoma and (d) normal cells. 7 different communities are shown in different color. The number of genes in the core group of disease cell is 51 and the same of normal cell is 35 is shown in gray color. 2nd to 7th community are shown in red,golden,green,blue,cyan and orange color. The detail about the gene list is provided in Supplementary material II.

extracellular matrix (ECM) of the juxtacanalicular region, the deepest portion of the TM, and from the inner wall basement membrane of Schlemm's canal. Focal adhesion Kinase mediates cross-talk between integrin-based focal adhesions and intercellular adherens junctions to regulate endothelial barrier function of SC inner wall cells. Thus The Focal adhesion pathway plays import role in intraocular pressure regulation and dysregulation in glaucoma. Actin cytoskeleton is also related to focal adhesion [40].

In case of REACTOME pathway we found Haemostasis is unique for Disease network. (Table I and II). In basal blood flow (BF) of the optic nerve head (ONH) increases in early stage of glaucoma, means altered hemodynamic is associated with glaucoma [41].

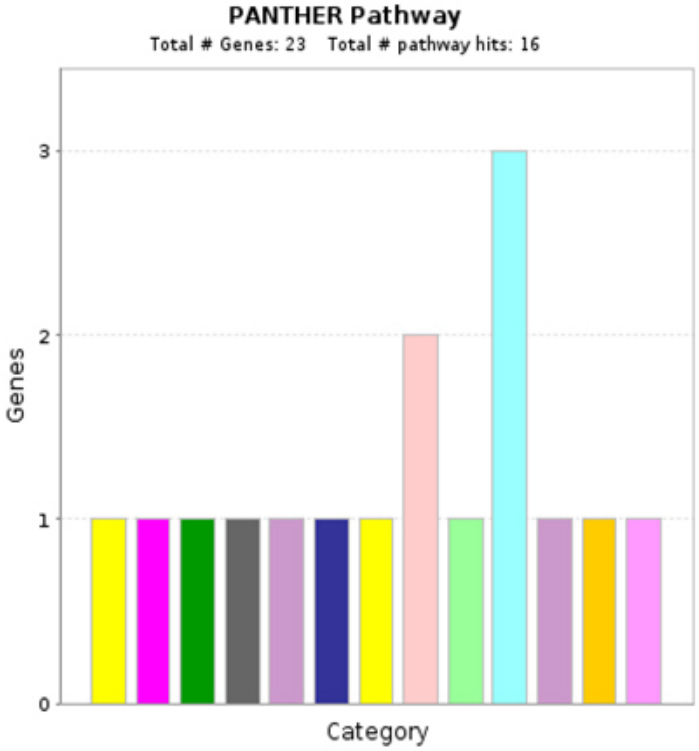
We have also done PANTHER pathway analysis, pathway names along with the genes names are shown in the Figure 4.The common pathways are shown in orange font. The genes names are shown in bracket. Which shows that Cytoskeletal regulation by Rho GTPase is common in both the cells but the genes CFL1 is unique to disease cell. There is a significance role of RhoA/Rho kinase signalling in regulation of TM cell plasticity, fibrogenic activity and myofibroblast activation, eventually in the pathobiology of elevated intraocular pressure in glaucoma patients. (Ref). CFL1 may act as a important biomarker for Glaucoma. Gene ontology has been displayed in Fig 4.

Discussion

In this network analysis we have found lose Gap intercellular communication, regulation of actin cytoskeleton, Haemostasis are the important pathways in the Glaucoma disease and also there is a correlation of dilated cardiomyopathy with glaucoma. The role of these pathways from existing knowledge also correlates with our findings. Cytoskeleton regulation is predicted as crucial as it came in both pathway analysis, KEGG pathway and PANTHER pathway. It can be predicted target for Glaucoma from our analysis where already published article says that selective inhibitor of Rho GTPase/ROCK pathway is a novel therapeutic approach for Glaucoma (24). Thus here we have validated our protocol for target identification.

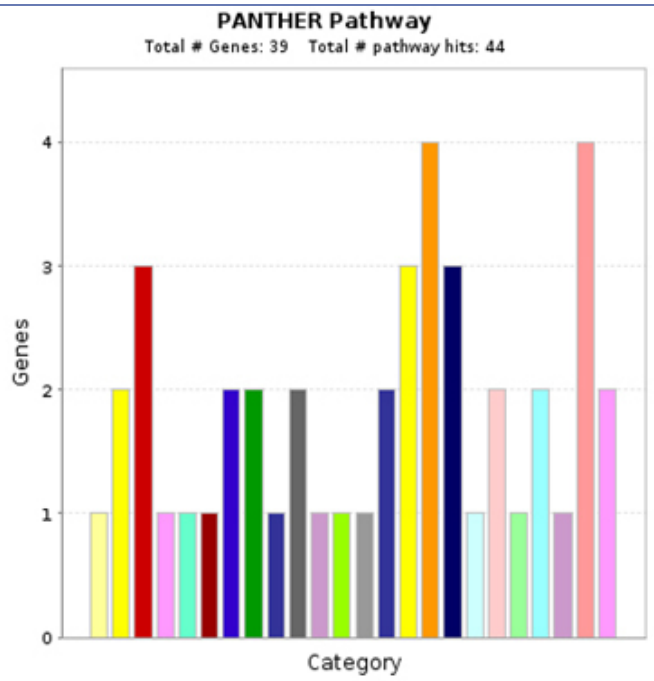
So the genes which we have found by network analysis may act as marker or target protein for glaucoma therapeutics in future.

Detailed investigation with KEGG pathway analysis shows that the genes other than ribosomal protein which are unique to disease network can act as Biomarker for glaucoma. Single biomarker is



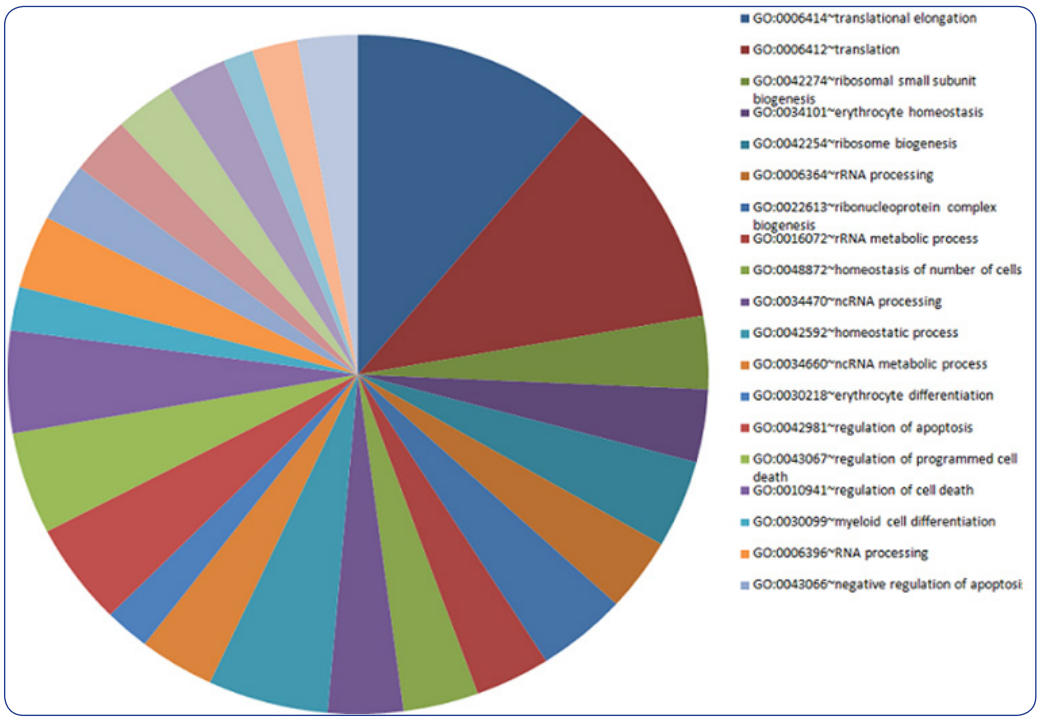
Alzheimer disease-presenilin pathway (P00004) (Genes: ACTB)
Apoptosis signaling pathway (P00006) (Genes : HSPA8)
Cadherin signaling pathway (P00012) (Genes: ACTB)
Cytoskeletal regulation by Rho GTPase (P00016) (Genes: ACTB)
Fructose galactose metabolism (P02744) (ALDOA)
Glycolysis (P00024) (ALDOA)
Gonadotropin-releasing hormone receptor pathway (P06664) (Genes:TUBA1B)
Huntington disease (P00029) (Genes: ACTB,OPTN)
Inflammation mediated by chemokine and cytokine signaling pathway (P00031)
Integrin signalling pathway (P00034) (Genes: COL1A1, FN1, ACTB)
Nicotinic acetylcholine receptor signaling pathway (P00044)(Genes: ACTB)
Parkinson disease (P00049) (genes: HSPA8)
Wnt signaling pathway (P00057) (Genes: ACTB)

Fig 3a



5HT4 type receptor mediated signaling pathway (P04376) (GNAS)
Alzheimer disease-presenilin pathway (P00004) (FSTL1, ACTB)
B cell activation (P00010)(HGNC:1445,HGNC:1442, HGNC:1449 all (CALM1)
Beta1 adrenergic receptor signaling pathway (P04377)(GNAS)
Beta2 adrenergic receptor signaling pathway (P04378) (GNAS)
Beta3 adrenergic receptor signaling pathway (P04379) (GNAS)
CCKR signaling map (P06959) (RPS6, CALM1)
Cadherin signaling pathway (P00012) (FSTL1,ACTB)
Cortocotropin releasing factor receptor signaling pathway (P04380)(GNAS)
Cytoskeletal regulation by Rho GTPase (P00016) (ACTB, CFL1)
Dopamine receptor mediated signaling pathway (P05912) (GNAS)
Endothelin signaling pathway (P00019) (GNAS)
Enkephalin release (P05913) (GNAS)
Glycolysis (P00024) (GAPDH, PGK1)
Gonadotropin-releasing hormone receptor pathway (P06664) (GNAS, TUBA1C, TUBA1B)
Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway (P00026) (GNAS, 3 CALM1)
Heterotrimeric G-protein signaling pathway-rod outer segment phototransduction (P00028)(3CALM1)
Histamine H2 receptor mediated signaling pathway (P04386)(GNAS)
Huntington disease (P00029)(ACTB, GAPDH)
Inflammation mediated by chemokine and cytokine signaling pathway (P00031) (ACTB)
Integrin signalling pathway (P00034) (ACTB, COL1A2)
Nicotinic acetylcholine receptor signaling pathway (P00044) (HGNC:132,ACTB)

Fig 3: Panther Pathway in (a) Normal Cell and (b) Disease cell. The names of the coomon pathway are written in orange colour.



Term	RT	Genes	Count	%	P-Value	Benjamini
translational elongation	RT		16	40.0	3.4E-23	1.9E-20
translation	RT		16	40.0	2.9E-15	7.9E-13
ribosomal small subunit biogenesis	RT		5	12.5	1.5E-8	2.8E-6
erythrocyte homeostasis	RT		5	12.5	9.2E-6	1.3E-3
ribosome biogenesis	RT		6	15.0	1.9E-5	2.1E-3
rRNA processing	RT		5	12.5	1.1E-4	1.0E-2
ribonucleoprotein complex biogenesis	RT		6	15.0	1.2E-4	9.4E-3
rRNA metabolic process	RT		5	12.5	1.3E-4	8.9E-3
homeostasis of number of cells	RT		5	12.5	1.5E-4	9.3E-3
ncRNA processing	RT		5	12.5	1.6E-3	8.5E-2
ncRNA metabolic process	RT		5	12.5	3.5E-3	1.6E-1
homeostatic process	RT		8	20.0	3.8E-3	1.6E-1
erythrocyte differentiation	RT		3	7.5	6.1E-3	2.3E-1
response to calcium ion	RT		3	7.5	9.9E-3	3.2E-1
regulation of apoptosis	RT		7	17.5	2.1E-2	5.3E-1
regulation of programmed cell death	RT		7	17.5	2.2E-2	5.3E-1
regulation of cell death	RT		7	17.5	2.2E-2	5.1E-1
myeloid cell differentiation	RT		3	7.5	2.7E-2	5.6E-1
response to metal ion	RT		3	7.5	4.8E-2	7.6E-1
RNA processing	RT		5	12.5	6.1E-2	8.2E-1
negative regulation of apoptosis	RT		4	10.0	7.2E-2	8.6E-1
negative regulation of programmed cell death	RT		4	10.0	7.4E-2	8.5E-1
negative regulation of cell death	RT		4	10.0	7.5E-2	8.4E-1
cellular component morphogenesis	RT		4	10.0	9.4E-2	8.9E-1
Rho protein signal transduction	RT		2	5.0	9.9E-2	9.0E-1

Fig 4: Gene ontology of the core genes in the disease network

not enough for diagnosis purpose, the multiple genes together play role. So all the unique genes like FSTL1, GNAS, CD63, COL1A1, TMSB4X EEF1G VEGFB, CFL1, TUBA1B, TUBA1C etc in disease core are Biomarkers. Detail analysis of panther pathway also tells that the genes which are unique to disease cells are GNAS, CALM1, CFL1, FSTL1, TUBA1C, TUBA1B etc. VEGFB is a biomarker for cardiovaccular disease, GNAS is for Cancer and FSTL1 is a Biomarker for rheumatoid arthritis [42-44] but all these together is linked to Glaucomatous neuropathy. The different microarray data which have been reported for the proposal of these markers have to be reinvestigated with gene network model.

Our proposed markers genes for Glaucoma and their nearest neighbour were visualized by open source web based software PC Viz. The picture is shown in Fig 5.

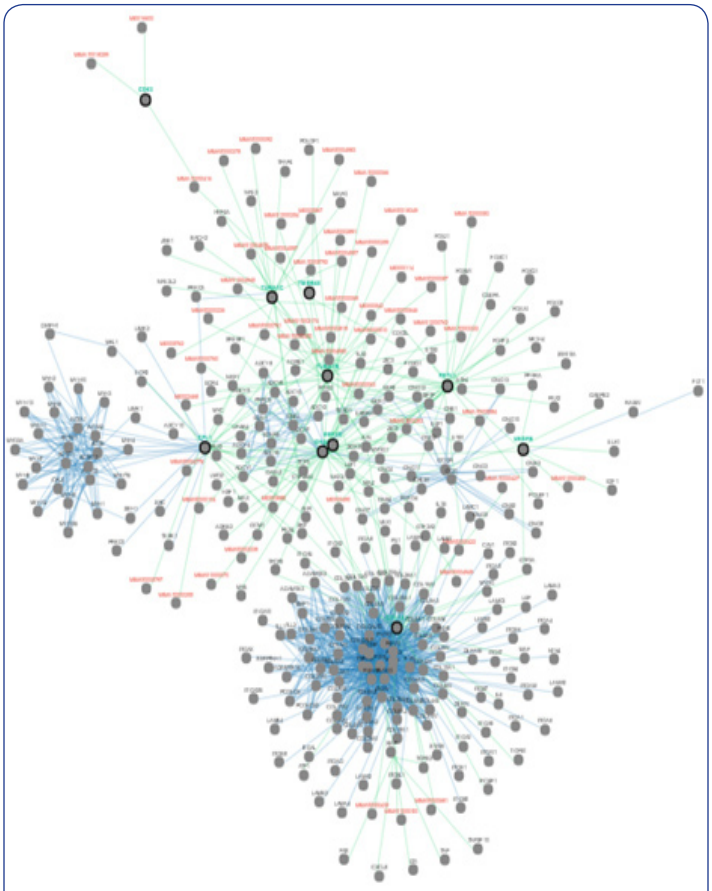


Fig 5: FSTL1, GNAS, CD63, COL1A1, TMSB4X EEF1G VEGFB, CFL1 , TUBA1B, TUBA1C and their network neighbourhood were shown by PCViz software.

Conclusion

Here we have looked into the Co-expressed gene network in Glaucoma and normal cell to understand a system and to differentiate between Glaucoma and Healthy cells. We predicted Rho GTPase is a target protein for Glaucoma. Except that another four pathways like haemostasis, Gap Junction, Dilated Cardiomyopathy, Focal adhesion is related to Glaucoma. We found barabasi community analysis can be applied to identify core community in gene network which leads us to get gene marker and those may be possible therapeutic targets. Ten genes (FSTL1, GNAS, CD63,COL1A1,TMSB4X EEF1G VEGFB, CFL1 , TUBA1B, TUBA1C) , which involved in enriched pathways like Gap Junction, Dilated Cardiomyopathy, Focal adhesion, Regulation of actin cytoskeleton may act as markers for Glaucoma.

References

1. Varma R, Lee PP, Goldberg I, Kotak S (2011) An assessment of the health and economic burdens of glaucoma. Am J Ophthalmol. 152(4):515–522.
2. Quigley HA, Broman AT (2006) The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol. 90(3):262–267.
3. Quigley HA (2011) Glaucoma. Lancet. 377(9774):1367–1377.
4. Fan BJ, Wiggs JL (2010) Glaucoma: genes, phenotypes, and new directions for therapy. J Clin Invest. 120(9): 3064–3072.
5. Kumar A, Basavaraj MG, Gupta SK, Qamar L, Ali AM et al. (2007) Role of *CYP1B1*, *MYOC*, *OPTN* and *OPTC* genes in adult-onset primary open-angle glaucoma: predominance of*CYP1B1* mutations in Indian patients. *Molecular Vision*. 13:667-676.
6. Monemi S, Spaeth G, DaSilva A, Popinchalk S, Ilitchev E, et al. (2005) Identification of a novel adult-onset primary open-angle glaucoma (POAG) gene on 5q22.1. Hum Mol Genet. 14(6):725–733.
7. Jakobs TC (2014) Differential gene expression in glaucoma. Cold Spring Harb Perspect Med. 4(7):20636.
8. Yang Z, Zack DJ (2011) What has gene expression profiling taught us about glaucoma? Exp Eye Res. 93(2):191-195.
9. YAN X, YUAN F, CHEN X, DONG C (2015) Bioinformatics analysis to identify the differentially expressed genes of glaucoma. Molecular Medicine Reports. 12(14):4829-4836.
10. Nickells RW, Pelzel HR (2015) Tools and resources for analyzing gene expression changes in glaucomatous neurodegeneration. Experimental eye research. 141:99-110.
11. Diao H, Li X, Hu S, Liu Y (2012) Gene Expression Profiling Combined with Bioinformatics Analysis Identify Biomarkers for Parkinson Disease. PLoS ONE 7(12): e52319.
12. Diao H, Li X, Hu S, Liu Y.(2012) Gene expression profiling combined with bioinformatics analysis identify biomarkers for Parkinson disease. PLoS One. 7(12):e52319. Epub 2012 Dec 28.
13. Zhang L, Guo Y, Li B, Qu J, Zang C, et al. (2013) Identification of biomarkers for hepatocellular carcinoma using network-based bioinformatics methods. Eur J Med Res. 1(18):35
14. Bhattacharya SK, Lee RK, Grus FH (2013) the Seventh ARVO/ Pfizer Ophthalmics Research Institute Conference Working Group. Molecular Biomarkers in Glaucoma. Investigative Ophthalmology & Visual Science. 54(1):121-131.
15. Nikolskaya T, Nikolsky Y, Serebryiskaya T, Zvereva S, Sviridov E, et al. (2009) Network analysis of human glaucomatous optic nerve head astrocytes. BMC Medical Genomics. 2(2):24.

16. Tezel GA (2013) Proteomics View of the Molecular Mechanisms and Biomarkers of Glaucomatous Neurodegeneration. *Progress in retinal and eye research*. 35:18-43.

17. R Albert, Barabási AL (2002) Statistical mechanics of complex networks. Reviews of Modern Physics 74:47-97.

18 Barabási AL, R Albert (1999) Emergence of scaling in random networks. Science 286(5439):509-512

19. MEJ Newman (2005) Contemporary Physics 46:323-351.

20. Boucher B, Jenna S (2013) Genetic interaction networks: better understand to better predict. Frontiers in Genetics. 2013;17(4):290.

21. Kirwan RP, Wordinger RJ, Clark AF, O'Brien CJ (2009) Differential global and extra-cellular matrix focused gene expression patterns between normal and glaucomatous human lamina cribrosa cells. *Mol Vis*. 15:76-88.

22. Hather GJ, Owen wcfAB, Speed TP (2005) *geneRecommender:* A gene recommender algorithm to identify genes coexpressed with a query set of genes. R package version 1.44.0.

23. Meyer PE, Kontos K, Lafitte F, Bontempi G (2007) Information-Theoretic Inference of Large Transcriptional Regulatory Networks. *EURASIP J Bioinform Syst Biol* . 79879.

24. Tourassi GD, Frederick ED, Markey MK, Floyd C E J (2001) Application of the mutual information criterion for feature selection in computer-aided diagnosis. *Medical Physics*. 28(12):2394–2402.

25. Peng H, Long F, Ding C (2005) Feature selection based on mutual information: criteria of max-dependency, max-relevance, and min-redundancy. IEEE Transactions on Pattern Analysis and Machine Intelligence 2005, 27(8):1226–1238.

26. Meyer PE, Lafitte F Bontempi G (2008). "MINET: An open source R/Bioconductor Package for Mutual Information based Network Inference." *BMC Bioinformatics*

27. Csardi G, Nepusz T (2006) The igraph software package for complex network research, InterJournal, Complex Systems 1695.

28. Barabasi AL, Albert R (1999). Emergence of scaling in random networks *Science*, 286(5439):509-512.

29. Huang da W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 4(1):44-57.

30. Huang da W, Sherman BT, Lempicki RA (2009). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 37(1):1-13.

31. Kanagavalli J, Pandaranayaka E, Krishnadas SR, Krishnaswamy S, Sundaresan P (2014) A review of genetic and structural understanding of the role of myocilin in primary open angle glaucoma. Indian J Ophthalmol. 52(4):271-280.

32. Huang X, Li M, Guo X, Li S, Xiao X, et al. (2014) Mutation analysis of seven known glaucoma-associated genes in Chinese patients with glaucoma. Invest Ophthalmol Vis Sci. 55(6):3594-3602.

33. Fuchshofer R, Tamm ER. (2012) The role of TGF-Î² in the pathogenesis of primary open-angle glaucoma. Cell Tissue Res. 2012 347(1):279-290.

34. Rudzinski MN, Chen L, Hernandez MR (2008) Antiangiogenic characteristics of astrocytes from optic nerve heads with primary open-angle glaucoma. Arch Ophthalmol. 126(5):679-685.

35. Kanehisa, M, Sato Y, Kawashima M, Furumichi M, Tanabe, M (2016) KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res. 44(D1):D457-D462.

36. Kanehisa M, Goto S (2000) KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 28(1):27-30.

37. Fabregat A, Sidiropoulos K, Garapati P, Gillespie M, Hausmann K, et al. (2014) The Reactome pathway Knowledgebase. Nucleic Acids Res. 44:D481-7. Epub 2015.

38. Malone P, Miao H, Parker A, Juarez S, Hernandez MR (2007) Pressure induces loss of gap junction communication and redistribution of connexin 43 in astrocytes. Glia. 55(10):1085-1098.

39. MeiraFreitas D, Melo LA , AlmeidaFreitas DB, Paranhos A. (2012) Glaucomatous optic nerve head alterations in patients with chronic heart failure. Clin Ophthalmol. 6:623-629.

40. Vranka JA, Kelley MJ, Acott TS, Keller KE (2015) Extracellular matrix in the trabecular meshwork: Intraocular pressure regulation and dysregulation in glaucoma. Exp Eye Res. 133:112–125.

41. Cull G, Burgoyne CF, Fortune B, Wang L (2013) Longitudinal hemodynamic changes within the optic nerve head in experimental glaucoma. Invest Ophthalmol Vis Sci. 54(6):4271-4277.

42. Devaux Y, Vausort M, Azuaje F, Vaillant M, Lair ML, et al. (2012) Low levels of vascular endothelial growth factor B predict left ventricular remodeling after acute myocardial infarction. J Card Fail.18(4):330-337.

43. Tominaga E, Tsuda H, Arao T, Nishimura S, Takano M, et al. (2010) Amplification of GNAS may be an independent, qualitative, and reproducible biomarker to predict progression-free survival in epithelial ovarian cancer. Gynecol Oncol. 118(2):160-166.

44. Wilson DC, Marinov AD, Blair HC, Bushnell DS, Thompson SD, et al. (2010) Follistatin-like protein 1 is a mesenchyme-derived inflammatory protein and may represent a biomarker for systemic-onset juvenile rheumatoid arthritis. Arthritis Rheum. 62(8):2510-2516.