Original Article

Novel Potential Drugs for Therapy of Age-Related Macular Degeneration Using Protein-Protein Interaction Network (PPI) Analysis

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Abstract

Background: Age-related macular degeneration (AMD) is a common cause of blindness in older people. If diagnosed early, its progression in humans can be prevented.
Material and Methods: To understand of AMD pathogenesis, this study was carried out to investigate differential gene expression in AMD and normal samples. Here, Differentially Expressed Genes (DEGs) with P value of less than 0.01 were selected to construct the Protein- Protein interaction Network (PPI) using STRING web tool and visualized by Cytoscape software. Next, four PPI modules were discovered from the network. Then, the GO and pathway enrichment analyses were carried out on the modules' genes. Drug- gene interactions were obtained for modules' genes and reconstructed as a single drug- gene network.
Results: Bevacisumab, Degzamethazone and Pegaptanib, as the most potent therapeutic candidate drugs and previously mentioned as a therapy for AMD, had interaction with the genes associated with AMD. The other candidate drugs are Docetaxel, Cisplutin, Carboplatin, Methotrexate, Bexarotene, Raloxifene Hydrochloride, Acitretine, Adapalene, and Doxorubicine, some of which were previously discovered to be efficient against cancer. They had two gene targets in different modules.
Conclusion: Computational tools are efficient for therapeutic goals, experimental validation of findings as well as testing of drug toxicity are critical for better treatment. Drugs proposed in this study might promote future studies on AMD.
Keywords: Age-Related Macular Degeneration; Differential Gene Expression; Drug- Gene Network; Protein-Protein Interaction.

Article Notes: Received: -Jun. 7, 2019; Received in revised form: Jul. 2, 2019; Accepted: Jul. 16, 2019; Available Online: Oct. 1, 2019.

How to cite this article: Abedi Z, MotieGhader H, Maghsoudloo M, Sheikh Beig Goharrizi MA, Masoudi-Nejad A, Shojaei A. Novel Potential Drugs for Therapy of Age-Related Macular Degeneration Using Protein-Protein Interaction Network (PPI) Analysis. Journal of Ophthalmic and Optometric Sciences . 2019;3(4): 11-23.

Introduction

Age-related macular degeneration (AMD) is a serious macular disorder and an important cause of blindness [1]. Main symptomatic presentations of AMD include progressive visual damage and loss of central vision [2]. Marked by multifactorial etiology, AMD is regarded as a multifactorial disorder caused by interactions between diverse genetic, metabolic and environmental factors [3]. About 30 to 50 million people are affected by AMD worldwide [4]. There are two types of AMD, including dry and wet [5]. The dry type is more common as it affects about 90 % of patients. The wet type is usually associated with more severe vision loss. The wet AMD is the severe form of the disease causing blindness while the dry form is the milder variant characterized by geographic atrophy [6]. The pathologic mechanisms behind AMD are still unknown. However, it is suggested that the dry type may be caused by aging and thinning of the macular tissue, deposition of pigments in the macula, or a combination of the two. In the wet type, new blood vessels grow under the retina, leading to fluid leakage and hemorrhaging [7]. This further causes the death of retinal cells and causes blind spots in central vision. However, the mechanism of AMD remains poorly understood. Besides, very few studies have been conducted on AMD [8].

Although intensive studies have been performed on AMD, limited therapeutic drugs are available to treat AMD. Some drugs such as Ranibizumab, Bevacizumab, and Aflibercept have an anti-VEGFA effect. These drugs are effective reducing choroidal neovascularization in AMD. Injection of these drugs into the vitreous is a suitable treatment for wet AMD [9].

Early AMD diagnosis is essential to help prevent disease progression and develop new and effective therapeutic options. In this regard, finding novel AMD related genes could be beneficial. Besides, investigating related pathways could improve our understanding of AMD development.

Innovation in systems biology has brought us a variety of computational tools and analytical means [10-13]. 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 [14-17].

Currently, limited effective drugs target AMD and experimental drug discovery approaches that are considered costly and time-consuming. Therefore, in the present study, computational methods were used including, differential gene expression and drug-gene network analyses to identify new potential drugs targeting AMD.

Material and Methods

Dataset and preprocessing

The expression data were selected from the National Center for Biotechnology Information Gene expression Omnibus (GEO) using GSE29801 accession number. The platform of the chip analyzer was GPL4133 (Agilent-014850 Whole Human Genome Microarray 4x44K G4112F (Feature Number version)). The data contain mRNA expression profiles in two different tissues.

The non-gene transcripts were removed from the original file before the preprocessing of the dataset. Then, using the “Limma” R package from the Bioconductor project to identify differentially expressed genes between normal and AMD samples. In order to adjust the P values, Benjamini and Hochberg’s false discovery rate method

was used. The genes were mapped to their related IDs, and ID-less genes were removed. Genes with P value < 0.01 were considered for further analyses. 1,218 genes remained after preprocessing and removing outliers (Supplementary file S1). In the original paper of this dataset, in order to enhance data quality, the gene expression dataset was filtered for the putative contaminants. Therefore, it was decided to use all DEGs for further analysis. Using this gene list, we constructed the PPI network by applying the STRING database (version 11. 0), and further analysis was performed. This dataset contains 142 female and 151 male samples.

Functional annotation and pathway enrichment analysis of the differential expressed genes

In order to identify the biological mechanisms of differential expressed genes, the functional enrichment analysis was performed. DAVID bioinformatics tool was used for Functional enrichment of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analyses [18, 19].

Protein– protein interaction (PPI) network

Differentially expressed genes with a P value of less than 0.01 were selected to construct the protein-protein interaction network using STRING database v.11.0. Then, Cytoscape v.3.8.2 was used for visualizing data. Different algorithms were used for performing cluster analysis by clusterViz. In line with the aim of this study, the fast agglomerate edge clustering (FAG-EC) algorithm was selected. First, the edge clustering coefficient for each edge in the network was calculated by algorithm. According to clustering coefficients, sorting of edges were performed in a non- increasing way. Protein complexes were detected based on the bottomup condensing of the hierarchical clustering algorithm. In order to analyze large protein networks, the FAG-EC algorithm is useful because of its low complexity and fast computational power [20]. The researchers selected these parameters in this algorithm: DefinitionWay: Strong, In/OutThreshold: 10, Overlapped: True. Finally we found four different PPI clusters (i.e., PPI complexes). Figure 2 is provided these three PPI modules, and supplementary file S2 shown the list of genes for every cluster (module). The top four modules were selected for enrichment analysis of containing genes regarding gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Go and KEGG pathways analysis were performed via DAVID v.6.8.

Drug- gene network construction

After selecting the three most significant subnetworks, the drug-gene network for extracted genes. The findings could be used to suggest candidate potential candidate drugs for AMD. The Drug Gene Interaction Database (DGIdb) was used to suggest potential candidate drugs. This database has accumulated drug-gene interaction data from six different databases (My Cancer Genome 39, TALC 40, TEND 41, PharmGKB 42, TTD43, and DrugBank 44).

Results

Differentially expressed (DE) genes in Age-related Macular Degeneration (AMD) and Normal

To obtain a list of genes involved in the pathogenesis of AMD, we analyzed the GSE29801 microarray dataset. Table 1 shows the properties of this dataset. The Table 2 shows the average and variance of age on gender in different groups.

When comparing gene expression levels in AMD compared with normal, we found 1,218 significant DE genes (p-value < 0.01)

(Supplementary file S1). The average of expression was used for identical gene symbols.

Table 1: Number of data samples according to their gender.

Gender	AMD	Normal
Male	57	88
Female	85	63
Total	142	151

Table 2: Average and standard deviation of age based on gender in different groups

	Female	Male	Total
AMD	83/75 ± 8/56	80/54 ± 8/97	81/05 ± 10/59
Normal	65/19 ± 23/11	62/05 ± 23/34	63/36 ± 23/22

Enrichment analysis

DAVID database was used for extracting the important pathways and functional enrichment. We selected the pathways with P value < 0.01 as significant on the studied gene list. The most significant pathways for the gene list were systemic lupus erythematosus, alcoholism, viral carcinogenesis, pathway in cancer, hypertrophic cardiomyophathy (HCM), arginine and proline metabolism and dilated cardiomyopathy. Functional enrichment showed the involvement of genes in protein binding, unfolded protein binding, protein heterodimerization activity, chaperone binding, cadherin binding involved cell-cell adhesion, poly (A) RNA binding and zinc ion binding. The important pathways and functional enrichment are provided in supplementary file S2.

PPI network and clustering analysis

The PPI network of 1,218 genes were extracted from the String database (Figure 1). Supplementary file S3 provides the details of the PPI network. In order to find highly interacting protein modules, clustering analysis was performed. Then, four protein modules were found which are shown in Figure 2. (Additional information about these modules is supplied in supplementary file S4).

Functional annotation and pathway enrichment analysis of PPI modules

The DAVID was used to study the underlying biological functions of genes in gene clusters (19, 21). The results of GO enrichment are shown in supplementary file S5. Based on this file, the most significant terms for PPI modules 1- 4 were a structural constituent of ribosome, poly (A) RNA binding, RNA binding, protein heterodimerization activity, DNA binding, histone binding, unfolded protein binding, ATP binding, transcription factor activity, zinc ion binding, steroid hormone receptor activity, chromatin binding, retinoic acid receptor activity, respectively.

KEGG pathway enrichment analysis was performed for PPI-module 1-4. Ribosome, systemic lupus erythematosus, alcoholism, viral carcinogenesis, prostate cancer, strogen signaling, protein processing in the endoplasmic reticulum, and pathway in cancer were the significant pathways for all three PPI modules which are listed in supplementary file S5.

Gene regulator drugs detection

The DGIdb was used to detect drugs that aim module genes. For this purpose, by importing modules' genes into DGIdb and limiting drugs to permitted drugs, drug-gene interactions were acquired for modules' genes.

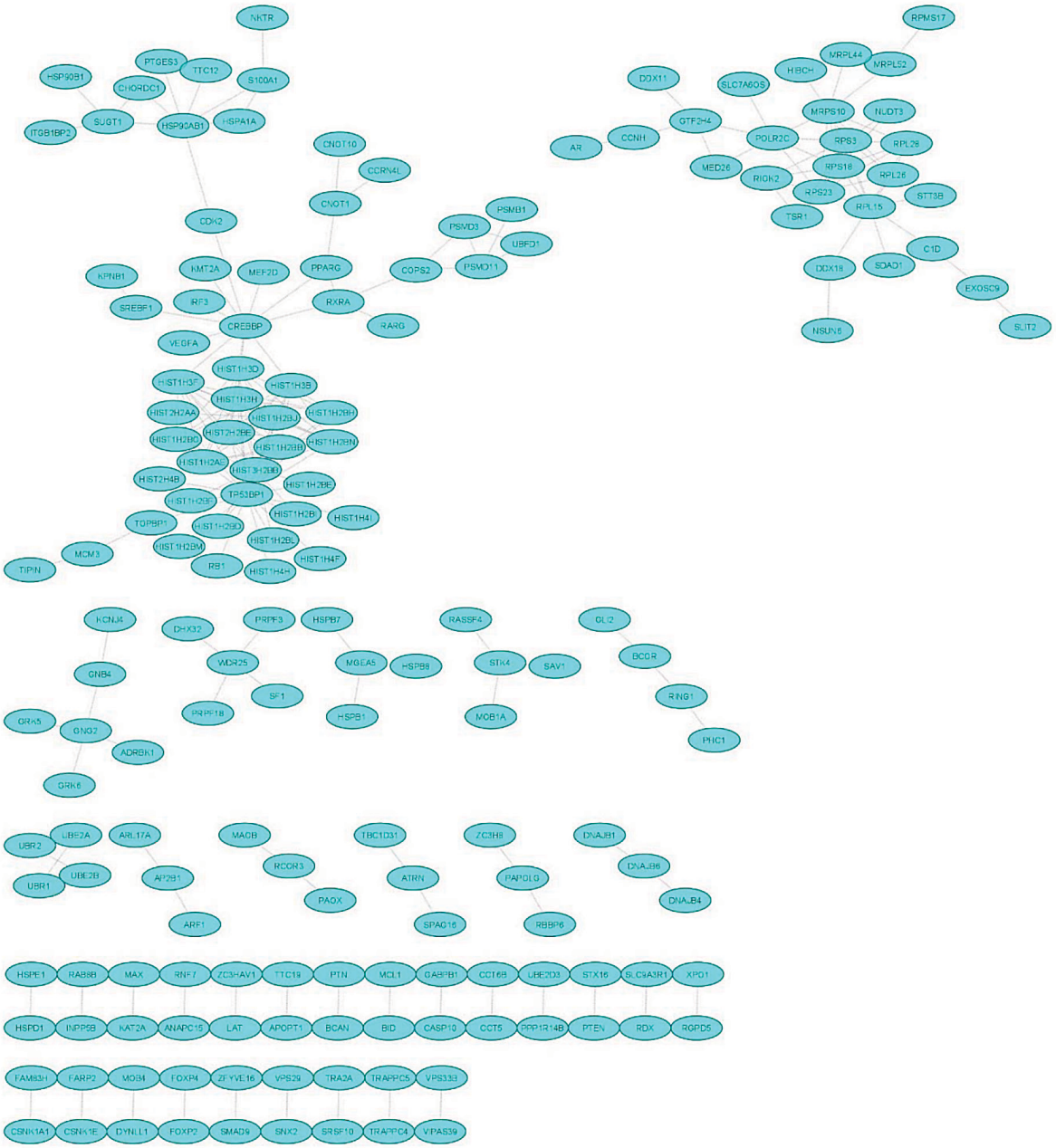
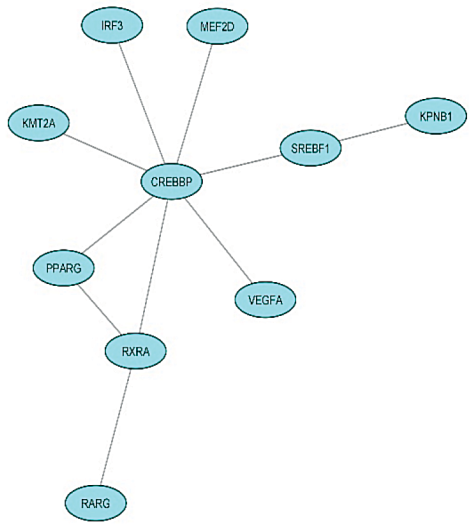


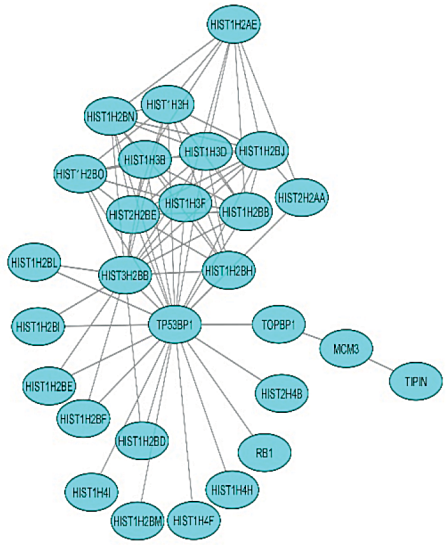
Figure 1: PPI: Protein-protein interaction. Every node corresponds to a protein, and edges show experimentally validated physical interactions

After finding drug-gene interactions for all modules, the total drug-gene interactions were used to reconstruct a drug-gene network. Based on DGIdb database results, 112 drugs were identified. Though, there were no drug for several genes in PPI modules. Finally, in order to visualize the drug –gene network, the Cytoscape (Version 3.6) was applied (Figure 3).

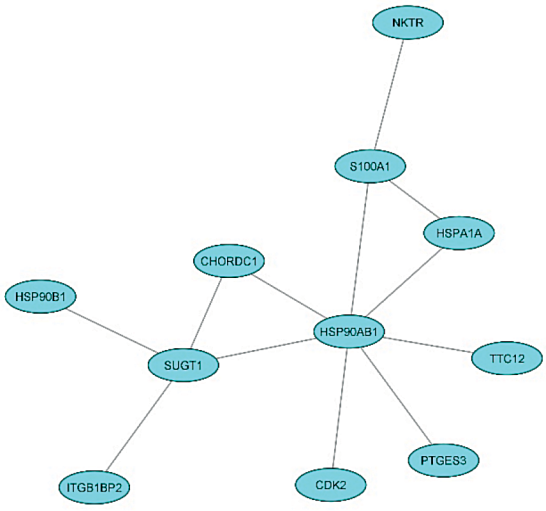
Some genes have more than one drug in the drug-gene network. Also, some drugs regulate two genes. Thus, these drugs are more important in the drug-gene network. The drugs such as Bevasizumab, Dexamethazone, Methotrexate, Acetretine, Cisplatine, Raloxifine Hydrochloride, Docetaxel, Carboplatine, Bexarotene, Acitretine, Adapalene, and Doxorubicin have two interactions with the



PPI_module-3



PPI_module-4



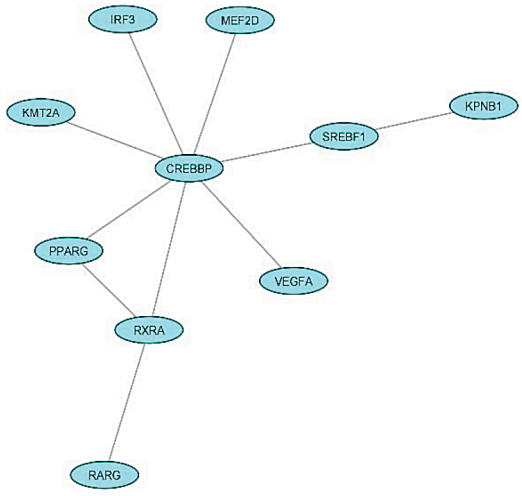


Figure 2: Obtained PPI modules from PPI networks. Note. Protein-protein interaction. The blue round ellipse represent each module genes

genes associated with AMD. The mentioned drugs can be repurposed for treating AMD. Supplementary file S6 shows more details.

Discussion

In order to find more effective drugs for AMD and better understand the pathogenesis of this disease, this integrated analysis was performed between AMD patients and normal groups. 1,218 genes across the studies were consistently differentially expressed in AMD and normal with P value < 0.01. The analysis of biological pathways suggest that the DE genes are significantly associated with systemic lupus erythematosus, alcoholism, viral carcinogenesis, pathway in cancer, hypertrophic cardiomyophathy (HCM), arginine and proline metabolism, and dilated cardiomyopathy of the signaling pathways. Arginine and proline metabolism

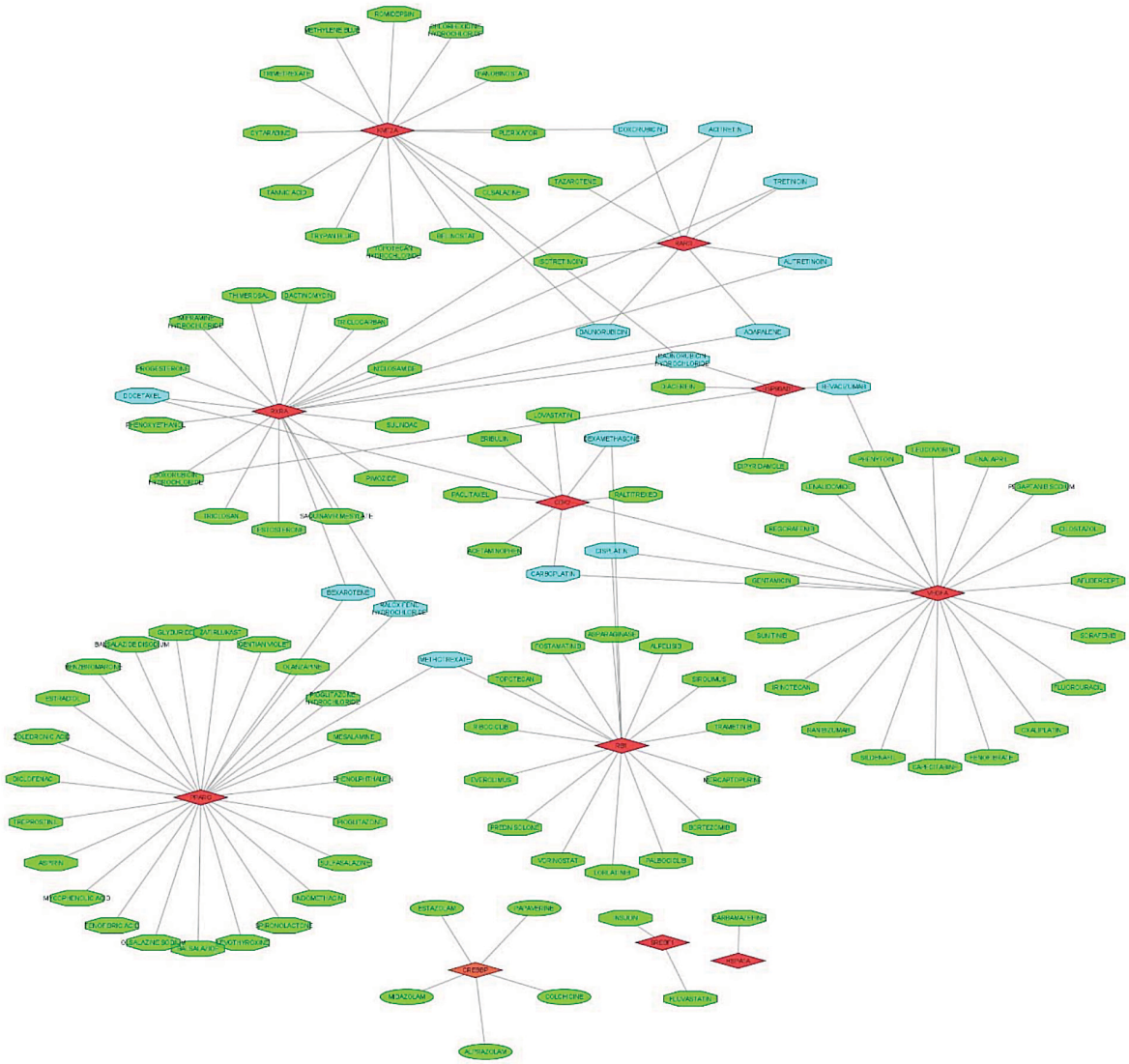


Figure 3: Drug-Gene Interaction Network. Note. The green octagon and red diamond nodes are drugs and PPI module genes, respectively. The higher degree of the drug node represents a blue octagon

pathway was reported in AMD [22]. Because of their high metabolic activity and the high polyunsaturated fatty acids (PUFAs) content, the eyes, especially the maculae, are susceptible to oxidative stress. The amino acid of proline has been reported to regulate the development of AMD. Oxidative stress may contribute to AMD's occurrence and/or progression [23]. Retinal Pigmented epithelial (RPE) cells were mainly affected by this stress in AMD [24]. The main nutrient for RPE cells is proline [25], and it can mediate metabolic communication between RPE cells and the retina [26]. By controlling mitochondrial function, proline plays an important role by making an alternative energy source during stress or hypoxia and an antioxidant for maintaining redox balance [25].

Also, many physiological processes in the body are controlled by protein dimerization controls. Homo-, hetero-, or oligomerization form of protein is made to regulate the

cellular processes. Any deregulation of these processes may result in a disease state [27]. Systemic lupus erythematosus (SLE) is the best-known disease-causing complement cascade activation. Diagnoses of systemic lupus erythematosus (SLE) is performed before the AMD diagnosis [28]. According to the results, the above pathways may have critical functions in AMD.

PPI network analysis was performed for differential expressed genes between AMD and normal samples. Then, for further investigation, four clusters of proteins were specified as the complexes of proteins, which are more associated with AMD. The central idea was to discover novel therapeutic candidate drugs to control gene regulation in AMD. After reconstructing a drug-gene network, Bevacizumab was one of the drugs related to two genes in the drug-gene network (VEGFA and HSP90AB1).

There is a rapid decrease in retinal thickness to normal or near-normal levels after intravitreal bevacizumab injection. It can improve the visual acuity in eyes with choroidal neovascularization (CNV) due to AMD [29]. Bevacizumab (Avastin; Genentech, South San Francisco, CA, USA) is used to treat metastatic carcinoma of the colon and rectum. This drug is a recombinant humanized monoclonal antibody against all VEGF-A isoforms. [30]. Furthermore, it has shown considerable promise in improving vision [31, 32]. Angiogenesis and vascular permeability occur in AMD, which vascular endothelial growth factor (VEGF) is a potentially stimulating factor for it. VEGF is a major pathogenic factor for wet AMD [33], which can induce macular eduma (ME) via promoting angiogenesis and vascular permeability. HSP90AB1 is the coding gene for the heat shock protein 90 (Hsp90) isoforms, which is a multitask chaperone involved in the activation and stability of proteins that function in immunity, signaling and, protein trafficking [34]. Hsp90 increasing occurs in RPE of AMD, and the the increses's measure was directly proportional to AMD severity. [35]. Higher Hsp90 levels which were reported in AMD-RPE cells were compared with control-RPE cells by Hytti et al. (2021) [36]. To achieve a certain result on the role of Hsp90 inhibition in attenuation of disease-related phenotypes in AMD-RPE cells, more studies are required. Regarding the role of Hsp90 in the induction of inflammation, a higher level of this protein in AMD-RPE genes further backs up persistent chronic inflammation in these cells [36]. Therefore, it is suggested that more experimental studies be performed on this drug to determine the results of the effect on the mentioned genes.

The most potent therapeutic candidate drug was Dexamethasone. Dexamethasone intravitreal implant has been approved for the treatment of macular oedema secondary to retinal vein occlusions (RVOs), noninfectious uveitis, and diabetic macular oedema. [37, 38]. The dexamethasone intravitreal implant combined with anti-VEGF therapy was reported to that be a feasible option for those patients who show an incomplete response to anti-VEGF monotherapy [39]. Based on the findings of the current study, another drug, Pegaptanib, was the other anti-angiogenic medicine for the treatment of neovascular (wet) age-related macular degeneration (AMD). By selectively binding with VEGF165, it can inhibit both the growth of blood vessels and vascular leakage. The Food and Drug Administration in the United States approved Pegaptanib as

the therapy for the treatment of all subtypes of neovascular AMD in 2004. [40].

Choroidal neovascularization (CNV) inhibition is induced by intraocular injection of doxorubicin (doxo) [41]. Doxorubicin (DOX) was previously discovered to be efficient against cancers such as leukemia, breast cancer, ovarian cancer, lymphomas, and glioblastomas. Doxorubicin (DOX) is an anthracycline antibiotic. It is reported that intravitreal injection of DOX caused the improvement of choroidal neovascularization in mice [42]. Doxorubicin has been confirmed to prevent the overexpression of VEGFA [34].

Methotrexate is an inhibitory drug that affects various enzymes in folic acid metabolic pathways, which can prevent the synthesis of metabolites such as thymidylates, purines, and methionine [43]. Inflammatory [44], angiogenic [45], and proliferative [46] are created processes during AMD [44]. MTX can inhibit these processes [47], and properly another therapy for CNV due to AMD.

In a study to investigate the safety and combined intravitreal bevacizumab and methotrexate effect on choroidal neovascularization in age-related macular degeneration (AMD), Soheilian et al. [48] revealed that the increase of intravitreal methotrexate to bevacizumab was safe in 7 patients. Also, they offered that the therapeutic effect may enhance the relapse of neovascularization in AMD and may decrease the fibrous component development and disciform scar constitution [48].

The candidate drugs such as Docetaxel, Cisplatine, Carboplatine, Methotrexate, Bexarotene, Raloxifine Hydrochloride, Acetretine, Adapalene and Doxorubicin some of which were previously discovered to be efficient against cancer, had two gene targets in different modules. Programmed cell death (apoptosis) and blood vessel growth (angiogenesis) are two hallmarks of cancer [41]. Also, these hallmarks were observed in AMD [37]. So, drugs used to treat cancer due to two hallmarks of cancer in a drug-gene network are available.

Conclusion

In present study, by comparing AMD with normal samples, 1,218 DEGs were acquired. To discovery related genes with AMD, the protein-protein interaction network was applied. Next, a drug-gene interaction network was reconstructed for PPI modules' genes. All results in this study were obtained by the computational methods and the limitation of experimental resources, so mentioned drugs need to valid in vitro and in vivo examinations, experimental and clinical investigations. Furthermore, this workflow only explored drug efficacy. Therefore, validation of safety of repurposed drugs for AMD requires clinical trials. Although computational tools are efficient for therapeutic goals, experimental validation of findings as well as testing of drug toxicity are critical for better treatment. Drugs proposed in this study might promote future studies on AMD.

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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.