RESEARCH

Molecular associations of Primary Open-Angle Glaucoma with potential comorbid diseases (POAG-associome)

✍Olga V Saik[1], Natalia A Konovalova[2], Pavel S Demenkov[1], Timofey V Ivanisenko[1,3], Evgeny D Petrovskiy[1], Nikita V Ivanisenko[1,3], Dinara E Ivanoshchuk[1], Maria N Ponomareva[2], Olga S Konovalova[2], Inna N. Lavrik[1,4], Nikolay A Kolchanov[1], Vladimir A Ivanisenko[1]

[1] The Federal Research Center Institute of Cytology and Genetics, The Siberian Branch of the Russian Academy of Sciences
Prospekt Lavrentyeva 10, 630090, Novosibirsk, Russia
[2] Federal State Budgetary Educational Institution of Higher Education "Tyumen State Medical University" of the Ministry of Healthcare of the Russian Federation
Odessa street, 54, 625023, Tyumen, Russia
[3] Novosibirsk State University
Pirogova street 2, 630090, Novosibirsk, Russia
[4] Department of Translational Inflammation, Institute of Experimental Internal Medicine Otto von Guericke University, Leipziger Straße 44, D-39120, Magdeburg, Germany
✍saik@bionet.nsc.ru

ABSTRACT

Glaucoma is the leading cause of irreversible vision loss, which is caused by death of the retinal ganglion cells. Currently, glaucoma affects over 60 million people worldwide with primary open-angle glaucoma (POAG) being one of the most common forms of the disease. Despite the large amount of research devoted to glaucoma, molecular and genetic mechanisms of its development are still poorly understood. Thus, the aim of the present study was prediction of new potentially comorbid diseases of POAG, based on analysis of associative gene networks describing disease-disease interactions. Application of enrichment analysis to associative networks, constructed with the ANDSystem for 31 diseases that are comorbid to POAG according to the literature data, revealed that 10 diseases had a statistically significant overlap of proteins/genes with the POAG associative network (p-value < 0.01). Comparison of POAG with over 4000 diseases with the aid of the ANDSystem showed that there was a statistically significant overrepresentation of proteins/genes in the POAG associative network for more than 100 diseases. Analysis of Gene Ontology (GO) biological processes showed the importance of apoptosis-related and endothelium-related processes for the formation of comorbid conditions of POAG with cancer and cardiovascular diseases, among others.

Keywords: primary open-angle glaucoma, POAG, comorbid diseases, apoptosis, endothelial dysfunction, ANDSystem, gene networks

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RESUMEN

Asociaciones moleculares del glaucoma primario de ángulo abierto (POAG) con enfermedades comórbidas potenciales (asocioma de POAG). El glaucoma es la principal causa de la pérdida irreversible de la visión, que ocurre por la muerte de las células ganglionares de la retina. El glaucoma afecta a 60 millones de personas en el mundo y la forma conocida como glaucoma primario de ángulo abierto (POAG) es una de las más frecuentes. A pesar de los abundantes estudios sobre el glaucoma, aun se comprenden poco los mecanismos moleculares y genéticos que motivan su desarrollo. Por tales razones, el propósito de este estudio consistió en predecir cuáles son las enfermedades que muestran comorbilidad con el POAG, mediante el análisis de las redes de asociación de genes que describen las interacciones entre enfermedades. Diez enfermedades mostraron un solapamiento significativo de proteínas y genes con la red asociativa del POAG (p < 0.01). Ello fue posible tras el análisis de enriquecimiento de redes asociativas, construidas con el empleo del sistema ANDSystem, a partir de 31 enfermedades para las que se había descrito comorbilidad con el POAG en la literatura científica. La comparación del POAG con 4000 enfermedades, con la ayuda del sistema ANDSystem, mostró una representación incrementada significativa de proteínas y genes en la red asociativa de POAG para más de 100 enfermedades. El análisis de procesos biológicos en la Ontología de Genes (GO) permitió determinar la importancia de los procesos vinculados a la apoptosis y al endotelio, para la formación de condiciones de comorbilidad del POAG con el cáncer y las enfermedades cardiovasculares, entre otras.

Palabras clave: glaucoma primario de ángulo abierto primario, POAG, enfermedades comórbidas, apoptosis, disfunción endotelial, ANDSystem, redes de genes

*I*ntroduction

Glaucoma is a neurodegenerative disease characterized by the progressive loss of retinal ganglion cells, followed by a distinctive visual field constriction, and eventually by loss of vision [1]. In most cases, glaucoma is caused by an increase in intraocular pressure, although it may also develop under normal eye physiology, and primary open-angle glaucoma (POAG) is one of the commonest [2]. Therefore, current therapies for the disease are directed at reducing intraocular pressure; methods include laser or surgical

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intervention, and drug exposure. Unfortunately, these therapies are unable to stop the disease process completely and allow only to slow down the loss of eye function [1-4].

The presence of comorbid conditions contribute to hinder the diagnosis of many diseases, and can also lead to a decrease in treatment effectiveness, even with the correct diagnosis. Comorbidity is defined as the manifestation of an additional clinical condition that exists or arises in the background of a present illness [5]. It is a complex combination of several diseases, which tend to coexist in patients more frequently than it would be expected for random chance [6]. Thereby, predicting potential comorbid diseases is an urgent task for modern medicine. Moreover, the analysis of the molecular mechanisms of comorbid conditions can provide insight on the pathogenesis of the disease and a basis to search for new therapeutic targets [7].

Currently, reconstruction and analysis of molecular genetic networks is a widely used approach to study the molecular mechanisms of complex biological functioning and interaction processes, including the formation of pathological conditions [6-9]. To understand the mechanisms of comorbidity, it is important to identify the so-called pleiotropic genes, which are simultaneously involved in the development of several different pathological conditions [7]. Obviously, the degree of interaction between the molecular genetic networks associated with different diseases can play an important role in the formation of comorbid conditions. In particular, Lee *et al*. [10] showed that genes associated with comorbid diseases were frequently involved in the same metabolic process. Previously, using the example of asthma (BA) and tuberculosis (TB), we showed that the degree of intersection (according to Jaccard and meet/min indexes) of networks associated with comorbid diseases is significantly higher than that for the random pairs of diseases [11]. We performed a similar analysis to Glotov *et al*., which showed a significantly closer relationship between pre-eclampsia, diabetes mellitus, gestational diabetes and obesity, compared with random pairs of diseases [12].

Therefore, this work was aimed to study of the interaction between the POAG and 4711 other diseases, by searching for potentially comorbid diseases. To achieve this goal, we used the ANDSystem and earlier developed techniques of reconstructing gene networks associated with a disease [13]. Analysis of overrepresentation of genes from the associative gene network of POAG in the gene networks of other diseases allowed us to predict 107 potential comorbid diseases with POAG diseases (p-value < 0.01). Of them, 10 were confirmed in the literature as having possible comorbidity with glaucoma, and the remaining 97 diseases can be referred to as new potential comorbid diseases with POAG. Results on the role of genes involved in endothelium-related GO biological processes in the POAG associated gene network may serve as evidence in favour of the assumption that peripheral vascular endothelial dysfunction may be related to glaucoma progression [15].

Materials and methods

To generate a list of human genes associated with POAG and 4711 other diseases, a previously developed ANDSystem tool was used, which comprises a knowledge base of interactions and associations between molecular genetic entities, as well as diseases, biological processes, and other parameters [13]. It was created via automatic extraction of information from scientific publications and factual databases.

The Gene Ontology (Gene Ontology Consortium, GO; http://geneontology.org/) overrepresentation analysis was performed using the DAVID resource (https://david-d.ncifcrf.gov/summary.jsp) [16]. The list of human genes/proteins related to the GO category «Apoptotic process» was created by searching genes for the keyword «apoptotic process».

The list of human proteins/genes associated with the endothelium was constructed in two steps. First, 153 GO biological processes were selected with keywords «endothelium» or «endothelial» in the title. Next, the subset of 403 genes/proteins related to these GO biological processes and having the GO annotations for human was selected.

The gene networks associated with POAG were reconstructed by using the ANDSystem. Betweenness centrality for each node of the analyzed associative gene network was calculated with the *igraph* package implemented in the R programming language [17]. Prediction of comorbid diseases of POAG was based on an evaluation of the statistical significance of overrepresentation of genes associated with POAG, among the list of genes associated with the disease under analysis. The statistical significance of genes overrepresentation was calculated by using the hypergeometric distribution, adjusted for multiple comparisons (FDR and Bonferroni correction), with the *stats.hypergeom.sf* function of the *SciPy* package implemented in the Python programming language and the *p.adjust* function of the stats package implemented in R [18, 19].

To generate a list of comorbid diseases of glaucoma found in the literature, abstracts containing the keywords “glaucoma” and “comorbidity” were found in PubMed. After manual inspection of the papers retrieved, a list of diseases explicitly mentioned as being comorbid with respect to glaucoma was extracted.

Results and discussion

Analysis of the molecular and genetic mechanisms underlying POAG pathogenesis

POAG is one of the basic, most common forms of glaucoma. Glaucoma is characterized by a persistent increase in intraocular pressure, accompanied by a progressive loss of retinal ganglion cells. Nevertheless, ganglion cell death mechanism still has not been studied in detail, but it was assumed that apoptosis plays a key role in this process [14]. Unfortunately, modern methods for treating glaucoma and POAG do not completely stop the disease progression and only slowdown the loss of eye function [1-4].

In order to search for the molecular and genetic mechanisms underlying the development of POAG, we formed a list of 96 genes associated with glaucoma modality, according to the ANDSystem. Analysis of GO biological processes revealed that the most overrepresented processes (p-value < 0.05) were those related to the extracellular matrix, immune system, circulatory system, regulation of apoptotic

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process, response to hypoxia, response to drug, and others (Table 1).

It is known that the extracellular matrix plays an important role in the formation and functioning of tissues. Violation of the extracellular matrix causes loss of elasticity and integrity of tissues [20, 21] and may serve as one of the reasons for dysfunctions in intraocular fluid outflow.

Another process, endothelial dysfunction, is studied as one of the potential mechanisms of POAG [15]. Endothelium lines the walls of blood vessels and plays an important role in the circulatory system. Particularly, the vascular endothelium is involved in the maintenance of vascular homeostasis and regulation of vascular tone [15]. In fact, dysfunction of endothelium is considered an important factor for development of systemic diseases such as atherosclerosis, hypertension, heart failure and others [22]. Previous works discuss on the role of vascular endothelial cell dysfunction in glaucoma patients [23-26]. Our results were consistent with those evidences since «blood coagulation» and «angiogenesis» processes were found among the most overrepresented GO Biological Processes. Unexpectedly, the categories that are directly related to the endothelium were not present among the significant GO biological processes. There were 153 biological processes identified containing the word 'endothelium' in the title. According to the number of genes involved in these processes, ranging from 1 to 104 (median of 4), they are still poorly investigated. Therefore, one of the reasons why endothelial processes were not statistically significant can be their insufficient annotation.

Connections between the immune system, apoptosis and glaucoma have been widely discussed [27-29]. Recent studies show that glaucoma is not purely degenerative, but also involves inflammatory and immune elements [27] and could include autoimmune processes [28, 29].

Similarly, apoptosis has been pointed out as significant for the destruction of retinal ganglion cells in glaucoma [14]. Apoptosis, the programmed cell death regulated by specific signals, drives cells to undergo organized cellular organelles degradation by activated proteolytic caspases (Cas). Additionally, apoptosis-inducing factors can activate Cas-independent apoptotic pathways. Moreover, it is known that unregulated apoptotic cell death is involved in some neurodegenerative diseases [30, 31].

The importance of genes for the functioning of certain biological processes may also be evaluated based on the value of their centrality in the gene networks describing them [32, 33]. For the analysis of the centrality of genes associated with POAG, an associative gene network of POAG was reconstructed using the ANDSystem [13]. The resulting network contained 1289 links of 14 different types of interactions presented in ANDSystem, the most significant being: expression regulation, activity regulation, transport regulation, co-expression, protein-protein interactions, among others. The betweenness centrality (BC) value was calculated for each of the nodes of the network. Noteworthy, the average centrality of nodes corresponding to proteins (BC = 113.46), was approximately twice the average centrality for the nodes corresponding to genes (BC = 64.81). This is primarily due to the fact that there is more data present in the literature on the intermolecular interactions in which proteins may be involved, compared to the regulatory interactions of genes. For instance, the p53 protein was ranked first in the list of genes, which were ordered descending by their betweenness centrality. At the same time, TP53 gene was located in third place, which also corresponds to a high degree of node centrality. It is known that p53 is a hub and plays an important role in many biological processes including apoptosis, so its high centrality was quite expected.

Table 1. The most overrepresented Gene Ontology (GO) biological processes (p-value adjusted FDR < 0.05) of genes associated with primary open-angle glaucoma (POAG)

GO term	p-value	Fold enrichment	Hypergeometric distribution		
			Bonferroni	Benjamini	FDR
GO:0030198~extracellular matrix organization	2.70×10^{-10}	9.80	3.66×10^{-7}	3.66×10^{-7}	4.43×10^{-7}
GO:0022617~extracellular matrix disassembly	6.62×10^{-9}	16.88	8.96×10^{-6}	4.48×10^{-6}	1.09×10^{-5}
GO:0032757~positive regulation of interleukin-8 production	1.80×10^{-7}	45.03	2.44×10^{-4}	8.12×10^{-5}	2.95×10^{-4}
GO:0010628~positive regulation of gene expression	1.82×10^{-7}	9.61	2.46×10^{-4}	6.16×10^{-5}	2.99×10^{-4}
GO:0007596~blood coagulation	6.79×10^{-7}	5.77	9.19×10^{-4}	1.84×10^{-4}	1.00×10^{-3}
GO:0030168~platelet activation	1.08×10^{-6}	9.34	1.00×10^{-3}	2.43×10^{-4}	2.00×10^{-3}
GO:0001525~angiogenesis	1.21×10^{-6}	9.21	2.00×10^{-3}	2.33×10^{-4}	2.00×10^{-3}
GO:0071548~response to dexamethasone	2.21×10^{-6}	135.08	3.00×10^{-3}	3.74×10^{-4}	4.00×10^{-3}
GO:0045944~positive regulation of transcription from RNA polymerase II promoter	1.40×10^{-5}	3.57	1.90×10^{-2}	2.00×10^{-3}	2.30×10^{-2}
GO:0043065~positive regulation of apoptotic process	1.46×10^{-5}	6.78	2.00×10^{-2}	2.00×10^{-3}	2.40×10^{-2}
GO:0001666~response to hypoxia	2.56×10^{-5}	9.11	3.40×10^{-2}	3.00×10^{-3}	4.20×10^{-2}
GO:0042493~response to drug	2.97×10^{-5}	6.20	3.90×10^{-2}	3.00×10^{-3}	4.90×10^{-2}

FDR: False discovery rate.

Besides that, genes involved in the GO category «apoptotic process», had a significantly higher average value of betweenness centrality (174.73) than the average of all the vertices of the network (88.84), with p-value < 10^{-4}. In this connection, apoptosis can be considered one of the central biological processes in the POAG network. Surprising for us was the fact that the nodes ranked by highest centrality and following the p53 protein corresponded to the proteins tumor necrosis factor (TNF), interleukin 6 (IL6), fibronectin protein (FN1) and caveolin 1 (CAV1), which are involved in the endothelium-related GO biological process. This is also consistent with the idea of the role of endothelial dysfunction in the development of POAG.

A large number of links present in the POAG gene network made it difficult to illustrate its comprehensive graphical representation, so we built a subnet, which included only regulatory connections, for the purpose of network visualization (Figure 1). Such connections are of particular interest in the

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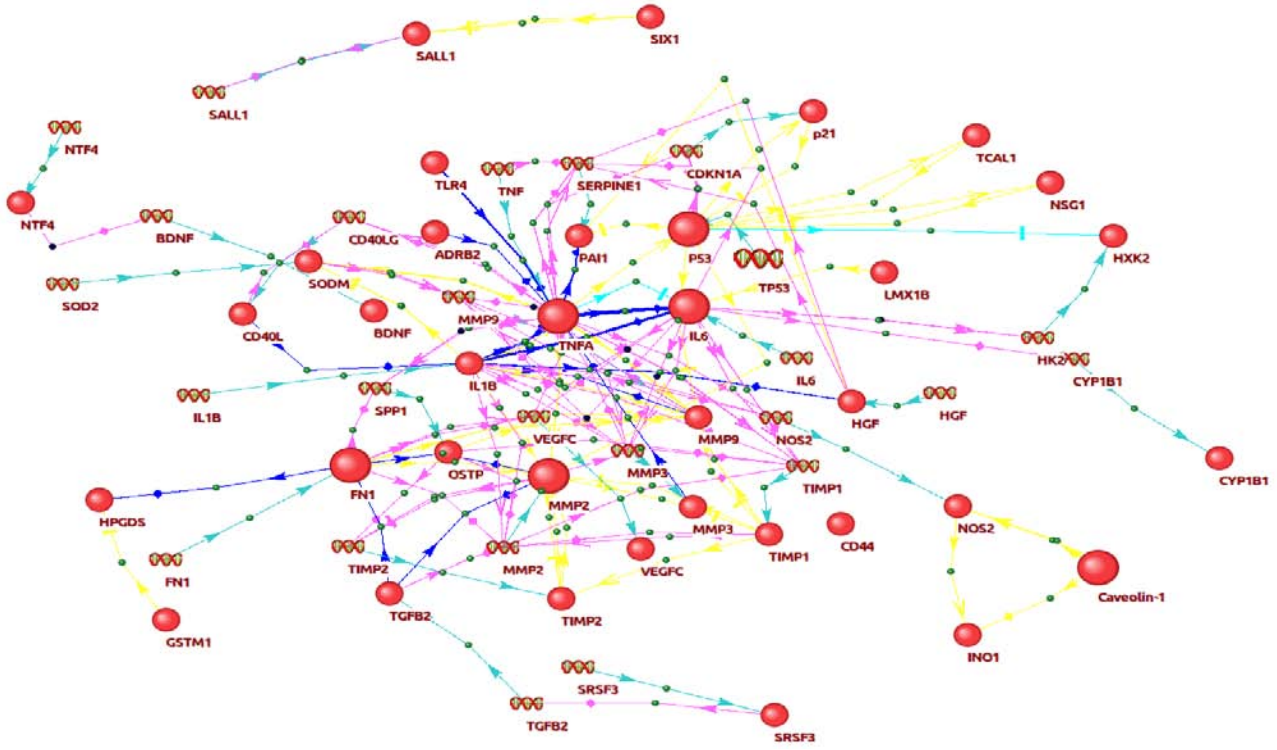

Figure 1. Associative regulatory network of Primary Open-Angle Glaucoma (POAG). Proteins and genes with high centrality (over 450) in the entire POAG network are shown with larger nodes. Yellow arrows indicate the "activity regulation" interaction type. Light blue arrows show the "regulation of degradation" and "expression" types. Pink arrows stand for the "regulation of the expression" type. Dark blue arrows correspond to the "regulation of transport" type.

analysis of molecular genetic networks, in spite of their exclusion by most existing systems.

As shown in figure 1, 16 out of the 36 proteins are involved in the regulation of expression. For instance, the protein IL-6 enhances the expression of the genes of matrix metallopeptidases 3 and 13 (Mmp3 and Mmp13, respectively), and TNF-alpha protein induces expression the expression of the inducible nitric oxide synthase (iNOS) in various cell types. Twenty-four proteins were involved in the regulation of protein activity, including p21 protein which blocks p53 functions in stem cells; and TNF protein which increases proapoptotic p53 levels in foetal membranes. Fourteen proteins take part on the regulation of transport/release, including the Toll-like receptor 4 protein (TLR4), a pathogen-associated molecular pattern receptor which mediates silica-induced TNF-alpha release from macrophages [34], and TGF-beta2, which can induce the sustained release of MMP-2 [35]. It was found that p53 protein participates in the highest number of different types of regulatory relations, including associations with two genes (TP53 and the Cyclin Dependent Kinase Inhibitor 1A (CDKN1A)) and eight proteins (p21, plasminogen activator inhibitor-1 (PAI1), TNF-alpha, MMP2, MMP9, hexokinase 2 (HXK2), neuron specific gene family member 1 (NSG1) and the Transcription elongation factor A protein-like 1 (TCAL1)) with the following types of connections: expression regulation, activity upregulation, activity regulation, activity downregulation, degradation downregulation, expression downregulation, and expression.

Prediction of diseases comorbid to POAG

In this study, we revealed potential comorbid diseases of POAG. We suggest that comorbid diseases are more closely related to each other at the molecular genetic level and have a higher number of common genes than random pairs of diseases. It is known that bronchial asthma (BA) and tuberculosis (TB) are dystrophic diseases [11]. In Bragina *et al*. [11], using the example of BA and TB, we have shown that the number of genes in the intersection of gene networks specific to BA and TB is significantly higher than the number of genes in the intersections of gene networks of randomly selected pairs of diseases. This pattern was maintained not only for the absolute number of genes, but also for the percentage of genes included in the intersection, relative to the total number of genes of the two networks, which was shown by Jaccard's and meet/min indices.

An analysis was performed on the interaction between POAG and 4711 other diseases by considering the number of common genes to them. The overrepresentation of genes associated with POAG was further estimated among the list of genes associated with the analyzed diseases. The statistically significant relationships between a pair of diseases were defined by assessing the overrepresentation of genes associated with one disease, among the genes

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associated with other diseases of the analyzed pair. This approach was used to determine the connectivity between POAG and the 4711 diseases, and revealed the diseases significantly associated with POAG, according to the presence of common genes associated with both diseases simultaneously. Up to 107 diseases were found for which the overrepresentation of genes was significant with Bonferroni correction (p-value < 0.01). Among the most significant diseases (p-value < 10^{-9}) identified were: endometriosis, hepatitis, ovarian neoplasms, diabetic retinopathy, coronary artery disease and urinary bladder neoplasms.

In the next step, we searched for comorbid diseases of glaucoma known in the literature. Using keywords "glaucoma" and "comorbidity", a corpus of PubMed abstracts was formed and subjected to manual analysis, aimed at identifying the list of diseases for which the authors mention their comorbidity with respect to glaucoma. The literature search revealed 31 such diseases (Table 2). The results indicated that 10 of the 31 comorbid diseases for glaucoma found in the literature were significantly overrepresented with a p-value less than 0.01.

Table 2. Diseases comorbid to primary open-angle glaucoma (POAG), as reported in the scientific literature

Known comorbid disease to POAG	Genes common with POAG	Genes associated with the disease	CBGE Bonferroni-corrected p-value	Publications
Anxiety	3	55	1	[36]
Asthma	25	594	1×10^{-4}	[37]
Cataract	9	142	0.2127	[38]
Chronic obstructive pulmonary disease	1	8	1	[37]
Congestive heart failure	21	389	2.919×10^{-5}	[37]
Deficiency anaemias	11	206	0.1563	[37]
Dementia	8	206	1	[37, 39]
Depression	11	128	1.6×10^{-3}	[36, 37]
Diabetes mellitus	14	251	8×10^{-3}	[37, 40-42]
Diabetic retinopathy	16	98	1.3647×10^{-10}	[43]
Epilepsy	13	321	0.5415	[37]
Headaches	3	17	1	[37, 44]
Hepatitis B	8	104	1	[37]
Hyperlipidaemia	6	57	0.2300	[37]
Hypertension	30	568	1.3029×10^{-8}	[37, 41]
Hypothyroidism	1	81	1	[37, 45]
Ischaemic heart disease	9	121	0.0597	[37]
Liver diseases	7	106	1	[37]
Macular degeneration	12	146	7×10^{-4}	[43, 46]
Migraines	14	157	2.3439×10^{-5}	[37]
Myopia	6	90	1	[47]
Neurologic disorders	1	70	1	[37]
Peptic ulcers	3	37	1	[37]
Peripheral vascular disorders	4	30	1	[37]
Psychosis	4	123	1	[37]
Renal failure	18	274	1.8975×10^{-5}	[37]
Rheumatoid arthritis	34	710	4.8017×10^{-9}	[37]
Sleep disorder	4	47	1	[36]
Stroke	1	22	1	[37, 48]
Systemic lupus erythematosus	17	437	8.08×10^{-2}	[37]
Tuberculosis	14	339	0.2410	[37]

CBGE: connectivity by gene enrichment, corresponding to the overrepresentation of genes associated with POAG among the list of genes associated with the analyzed diseases.

Involvement of apoptosis and endothelium-related GO biological processes in interactions between POAG and comorbid diseases

The literature extensively discusses the contribution of apoptosis and endothelial dysfunction to glaucoma development [14, 15, 23-26]. Our analysis of overrepresented biological processes for genes associated with POAG, showed that apoptosis is among the top of the list of significantly processes. Also, it is known that, blood coagulation and angiogenesis, which were found in our top list as well, are related to different endothelial dysfunctions, and disturbances in apoptosis and endothelial function are important factors in pathogenesis of many diseases [22, 30, 31]. Therefore, we were interested in analysing the overrepresentation of genes involved in apoptosis and endothelial dysfunction among genes associated simultaneously with both POAG and potentially comorbid diseases.

For that purpose, in the first step, 107 diseases from the ANDSystem were selected which had statistically significant connection with POAG by associated networks overlap (p-value < 0.01). We considered such diseases as potentially comorbid to POAG. Further, for each of the 107 diseases, a list of genes associated with both POAG and the analyzed diseases was formed, with apoptosis significantly overrepresented in this list of genes (Bonferroni-corrected p-value < 0.01) for 18 diseases. They included: cancer (colorectal neoplasms, prostatic neoplasms and breast neoplasms), cardiovascular diseases (cardiovascular diseases, myocardial infarction, hypertension, heart failure, atherosclerosis), including those found in the literature as potential comorbid diseases for glaucoma (hypertension and diabetic retinopathy).

Regarding endothelial dysfunction, endothelium-related GO biological processes were significantly overrepresented in 27 of the 107 diseases (Bonferroni-corrected p-value < 0.01), among them: cardiovascular (hypertension, heart failure, pre-eclampsia, aortic aneurysm, abdominal, myocardial infarction, atherosclerosis), kidney diseases (glomerulonephritis, renal insufficiency, glomerulonephritis, membranous, diabetic nephropathies) and cancer (prostatic neoplasms, ovarian neoplasms, carcinogenesis, pancreatic neoplasms, urinary bladder neoplasms, breast neoplasms, leukaemia).

To illustrate the interactions between POAG and potential comorbid diseases at the molecular genetic level, we selected diabetic retinopathy (Figure 2). In the literature, some authors consider diabetic retinopathy as comorbid in relation to POAG [43]. The associative gene network, reconstructed with the ANDSystem, describes the interaction between POAG and diabetic retinopathy for 165 genes and 162 proteins with 5437 interactions. The "regulation of expression" interaction type was chosen for a graphical representation of the network, since it is the best one for the representation of regulatory crosstalk between networks. The network is divided into three parts: the left side corresponds to the genes/proteins associated only with diabetic retinopathy, the central part corresponds to the genes/proteins associated with both diabetic retinopathy and POAG, and the right side is associated only with POAG. As shown in figure 2, the regulatory interactions between genes associated with diabetic retinopathy and genes associated with POAG mainly pass through the central part of the network. In other words, genes simultaneously associated with both diseases

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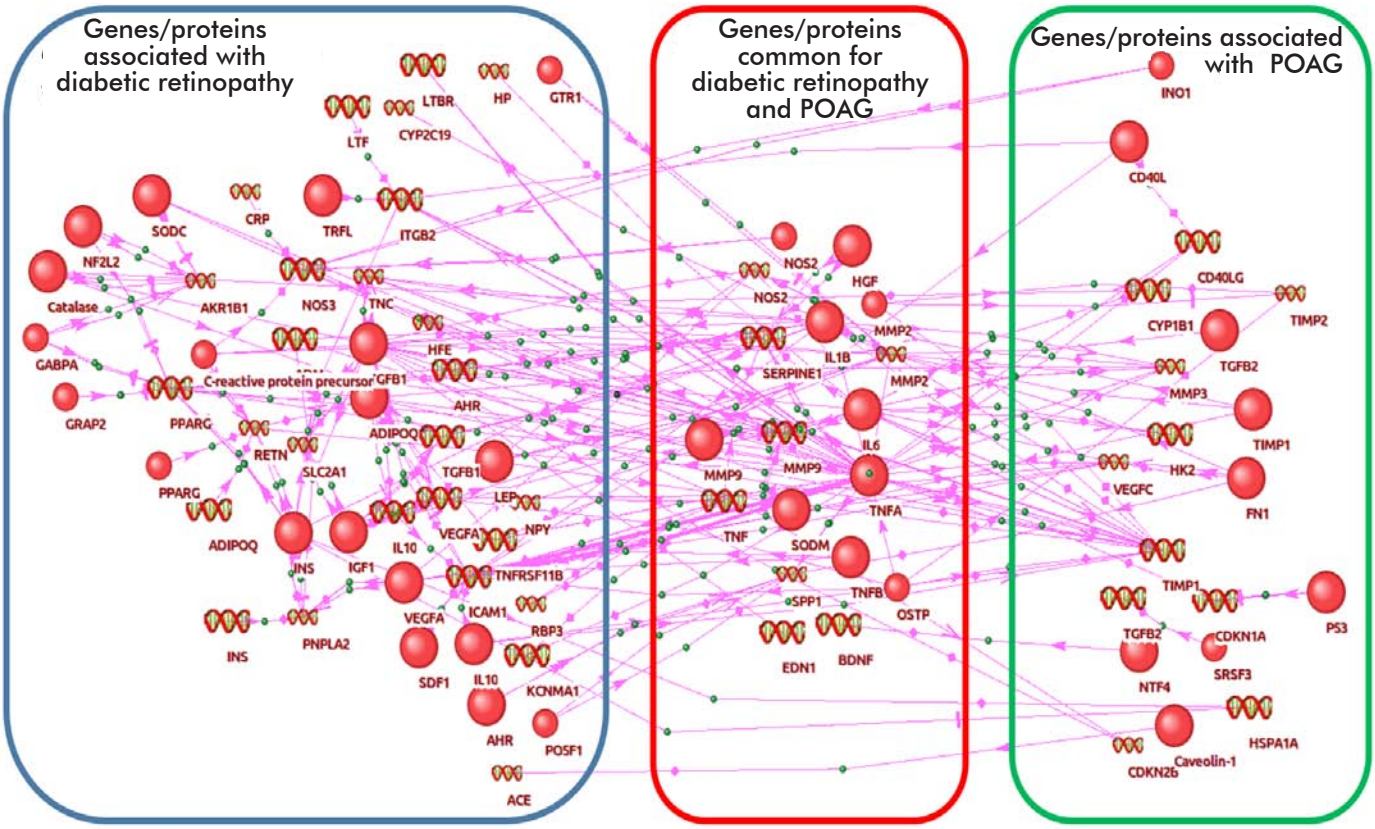

Figure 2. Associative regulatory network of Primary Open-Angle Glaucoma (POAG). Proteins and genes with high centrality (over 450) in the entire POAG network are shown with larger nodes. Yellow arrows indicate the "activity regulation" interaction type. Light blue arrows show the "regulation of degradation" and "expression" types. Pink arrows stand for the "regulation of the expression" type. Dark blue arrows correspond to the "regulation of transport" type.

can act as an interface in the interactions between gene networks for these diseases. This can be one of the reasons why comorbid diseases have common genes.

Particularly, it was found that the genes/proteins involved in apoptosis are distributed in approximately equal proportions among all three parts of the POAG/diabetic retinopathy regulatory network (Figure 2): 0.61, 0.72 and 0.7, respectively. It should be noted that apoptosis plays an important role in the development of both diabetic retinopathy [49, 50] and POAG [14].

In summary, an analysis of associative gene networks allowed us to predict 97 new potentially comorbid diseases of POAG. These data emphasise the roles of apoptosis and endothelial dysfunction in POAG comorbidity to other diseases.

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