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Constitutional risk factors for the development of glaucoma and cataracts in the Europioid population of Russia

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ABSTRACT

Aim. To identify endogenous risk factors for the development of glaucoma and cataracts based on the results of a comparative analysis of the nature of complex genetic trait distribution, including variants of genes for a number of cytokines and receptors for them, metalloproteinases, and their tissue inhibitors included in the genome of patients.

Materials and methods. The study included 501 people of the Caucasian race born and living in the Siberian region of Russia. They were divided into three groups of patients – patients with primary open-angle glaucoma (POAG) (n = 99), patients with senile cataract (n = 100), and the control group (n = 302) without ophthalmic pathology. Genotyping of the analyzed polymorphic loci was carried out by real-time PCR using the SYBRGreen I dye and TaqMan probes and by restriction fragment length polymorphism (RFLP) for different polymorphisms.

Results. The results of the study on the frequency of the analyzed genetic traits among patients with POAG compared to the control group showed the presence of combined genetic traits. The frequency of their detection in POAG was high and characterized by the two-digit value of the odds ratio, high values of specificity (99–100%), and high diagnostic coefficient. A direct comparison of the distribution of two ensembles of genes which protein products are involved in the extracellular matrix remodeling revealed a significant number of genetic traits characteristic of both diseases. This indicates significant differences in the implementation of the genetic predisposition to their development.

Conclusion. The data obtained indicate the possibility of developing reliable laboratory criteria (riskometers) for predicting predisposition to the development of POAG and early diagnosis at the stage of preclinical manifestations.

Keywords: primary open-angle glaucoma, cataract, extracellular matrix, *TGFB1 – TGFBR2*, MMP – TIMP, immunogenetics

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Conformity with the principles of ethics. All patients signed an informed consent to surgery, blood sampling, and the use of research data for scientific purposes. The study was approved by the Ethics Committee at the Research Institute of Clinical and Experimental Lymphology (Protocol No. 177 of 02.02.2003) and the Bioethics Committee at the Novosibirsk branch of S. Fyodorov Eye Microsurgery Federal State Institution (Protocol No. 2 of 2.09.2018).

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Конституциональные факторы риска первичной открытоугольной глаукомы и катаракты у европеоидного населения России

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РЕЗЮМЕ

Цель: выявление эндогенных факторов риска развития глаукомы и катаракты по результатам сравнительного анализа характера распределения комплексных генетических признаков, включающих в себя варианты генов ряда цитокинов и рецепторов к ним, металлопротеиназ и их тканевых ингибиторов, входящих в геном пациентов.

Материалы и методы. Обследован 501 человек европеоидного происхождения, родившихся и проживающих в сибирском регионе России, разделенных на три группы пациентов – с первичной открытоугольной глаукомой (ПОУГ) (n = 99), со старческой катарактой (n = 100), контрольная группа (n = 302) без офтальмопатологии. Генотипирование анализируемых полиморфных позиций осуществляли методами реал-тайм ПЦР с использованием интеркалирующего красителя SYBRGreen I, TaqMan зондов и методом рестриктного анализа длин продуктов амплификации (ПДРФ-анализ) – для разных полиморфных генов.

Результаты. Результаты исследования частот встречаемости анализируемых генетических признаков среди пациентов с ПОУГ относительно данных контрольной группы показали наличие комбинированных генетических признаков, частота выявления которых при ПОУГ высока и характеризуется двухзначными показателями отношения шансов, высокими значениями показателей специфичности 99–100% и высокими значениями величины диагностического коэффициента. Прямое сравнение характера распределения двух ансамблей генов, белковые продукты которых участвуют в процессах ремоделирования внеклеточного матрикса, выявило значительное количество генетических признаков, характерных как для одного, так и для другого заболевания, что свидетельствует о значительных различиях в реализации генетической предрасположенности к их развитию.

Заключение. Полученные данные свидетельствуют о принципиальной возможности разработки достоверных лабораторных критериев (рискометров) прогноза предрасположенности к развитию ПОУГ и ранней диагностики на стадии доклинических проявлений.

Ключевые слова: первичная открытоугольная глаукома, катаракта, внеклеточный матрикс, *TGFB1 – TGFBR2*, MMP – TIMP, иммуногенетика

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Исследование проводилось в рамках государственного задания НИИКЭЛ – филиал ИЦиГ СО РАН FWNR-2022-0009 (регистрационный номер 122022800132-1) и НМИЦ «МНТК "Микрохирургия глаза" им. акад. С.Н. Федорова» «Разработка комплексной системы диагностики и мониторинга больных первичной открытоугольной глаукомы с оценкой состояния зрительных функций и эффективности лечения (медикаментозное, лазерное, хирургическое)» (регистрационный номер № 121072800028-3).

Соответствие принципам этики. Все пациенты подписали информированное согласие на проведение

операции, забор крови, а также использование данных исследования в научных целях. Исследование одобрено этическим комитетом НИИКЭЛ – филиал ИЦиГ СО РАН (протокол № 177 от 02.02.2003) и комитетом по биомедицинской этике Новосибирского филиала НМИЦ «МНТК "Микрохирургия глаза" им. акад. С.Н. Федорова» (протокол № 2 от 2.09.2018).

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INTRODUCTION

The extracellular matrix (ECM) is a well-organized 3-dimensional architectural network that plays an important structural and functional role in the organization and remodeling of tissues, as well as in the regulation of cellular processes [1]. The building blocks of these ultrastructures are collagens, proteoglycans and glycosaminoglycans, elastin and elastic fibers, laminins, fibronectin, and other proteins / glycoproteins [2]. ECM provides interaction between cells in organs and tissues, coordinating multiple commands for the transmission of intracellular and intercellular signals. As a consequence, ECM affects morphogenesis, development, and homeostasis of tissues through regulation of cellular physiology, growth, proliferation, differentiation, and adhesion. ECM undergoes intensive remodeling under pathological conditions, playing a key role in the progression of many diseases, including ophthalmic pathologies [3, 4].

Like most complexly organized physiological systems, the functional state of ECM is largely determined by genetic factors, the most important of which are structures of polymorphic sites in regulatory regions of genes of both ultrastructural ECM components and humoral factors affecting its activity (growth factors, cytokines, chemokines, matrix metalloproteinases, their tissue inhibitors, etc.). Combinations of structural variants in regulatory regions of these genes, usually located in promoter regions, determine the intensity of protein expression and the level of synthesis by producer cells [5]. These parameters define the concept of "quantitative trait loci", which are attracting increasing attention of researchers in the field of medical genetics [6]. Given the undoubtedly polygenic nature of a person's genetic predisposition to development of most diseases, studies on the association of pathological processes not so much with single candidate genes as with functionally related complexes of polymorphic genotypes is of the greatest interest.

The aim of the study was to identify endogenous risk factors for the development of glaucoma and cataracts based on the results of a comparative analysis of the nature of complex genetic trait distribution, including variants of genes for a number of cytokines and receptors for them, metalloproteinases, and their tissue inhibitors included in the genome of patients.

MATERIALS AND METHODS

The study was carried out in accordance with the principles of the Declaration of Helsinki "Ethical Principles of Medical Research Involving Human Subjects", the Federal Law of the Russian Federation No. 323 FZ of 21.11.2011 "On principles of health preservation of citizens of the Russian Federation", and requirements of the Federal Law No 152-FZ of 27.07.2006 (ed. of 21.07.2014) "On personal data" (with amendments that came into effect on 01.09.2015). The study included 501 people of the Caucasian race born and living in the Siberian region of Russia.

Following the ophthalmic examination (determination of visual acuity, binocular indirect ophthalmoscopy, perimetry, echoophthalmography, optical coherence tomography, measurement of intraocular pressure), they were divided into three groups of patients. Group 1 included 99 patients with a verified diagnosis of stage II (advanced) primary open-angle glaucoma (POAG) (ICD-10 code H40.1), 52 (52.53%) males and 47 (47.47%) females. The average age of patients in this group was 62.8 ± 4.3 years.

Group 2 encompassed 100 patients with senile (uncomplicated) cataract, 81 (81%) females and 19 (19%) males, 63.5 ± 0.4 years old. Exclusion criteria were inflammatory eye diseases, diabetic retinopathy, neovascular glaucoma, uveitis, hemophthalmos, verified autoimmune diseases and tumors, as well as diabetes mellitus without ocular complications. The control group (similar in age and ethnic composition to groups 1 and 2) included 302 people without ophthalmic pathology.

All patients underwent an immunogenetic examination at the Laboratory for Clinical Immunogenetics of the Research Institute of Clinical and Experimental Lymphology, a branch of ICIG SB RAS. Genotyping was carried out by real-time PCR using SYBRGreen I dye (Litech, Russia) for rs1800629, rs361525, rs1800630, rs1143627, rs2243250, rs1800872, rs1800896 and TaqMan probes for rs1800795, rs243865, and rs3918242 (Syntol, Russia); by restriction fragment length polymorphism (RFLP) for rs4073 [7], rs4898 [8], rs8179090 [9], rs3025058 [10], rs1800469 [11], Gene ID 7046, D50683, L07594 [12].

The comparison group was analyzed by 11 polymorphic sites (Table 1). Patients with glaucoma and cataract were examined for 8 cytokine genes: *IL8- A251T* (rs4073), *IL17A- A197G* (rs227593), *TGFB- C509T* (rs1800469), *TGFBRI, TGFBR II, TGFBR III* receptor genes (Gene ID 7046, D50683, L07594, respectively), metalloproteinase inhibitor genes *TIMP1 -C372T* (rs4898), *TIMP2 -G 418C* (rs8179090).

In the statistical analysis of the results of genetic studies, we calculated the frequency of genotypes and their combinations, the odds ratio (OR) and the 95% confidence interval (CI) [13]. The distribution of genotypes was tested by the Hardy – Weinberg equilibrium.

To evaluate the results obtained, in addition to the common methods of statistical processing, we used computational methods of bioinformatics based on the probability theory of pattern recognition, based on the Bayes theorem (inverse probability theorem or hypothesis theorem) and the modified Wald's sequential probability test – a heterogeneous sequential pattern recognition procedure that allows to determine the diagnostic value of variables by calculating diagnostic coefficients (DCs) [14]. DC is a decimal logarithm of ratios of smoothed variables multiplied by 10. DC is represented by positive or negative numbers. At the same time, the greater the value of the DC, the more differential diagnostic information it carries. DCs of each found genetic trait are summed up, and when the limit values (threshold) are reached, the probability of the presence or absence of one of the alternative diseases (conditions) is established.

When calculating the integral characteristics of genetic traits as diagnostic and prognostic criteria, in addition to calculating DC, the specificity of the biomarker (Sp) was calculated as the probability of a truly negative proportion [15].

The differences in the genotype frequencies were determined by the two-tailed Fisher's exact test with 2 x 2 contingency tables. Statistical processing of the obtained results was performed using IBM SPSS Statistics 23 software package (USA). The differences were considered statistically significant at $p < 0.01$. The critical significance level in multiple comparisons was assumed with account of the Bonferroni correction [16, 17].

RESULTS

The comparison of the distribution of single and complex genetic traits in groups of patients with POAG and cataract with similar data from the reference group of healthy individuals without signs of ophthalmic diseases was carried out in 11 polymorphic sites (Table 1).

Table 1

Polymorphic sites of the studied genes in groups of patients with POAG and cataract and individuals without ophthalmic pathology

	Parameters	Polymorphic site	Locus (chromosome)	Reference sequence number
Cytokine genes				
1	*TNFA*	-238 G/A	6q21.3	rs361525
2	*TNFA*	-308 G/A	6q21.3	rs1800629
3	*TNFA*	-863 C/A	6q21.3	rs1800630
4	*IL1 B*	-31 C/T	2q14.2	rs1143627
5	*IL4*	-590 C/T	5q31.1	rs2243250
6	*IL6*	-174 G/C	7p21.	rs1800795
7	*IL10*	-592 C/A	1q31–q32	rs1800872
8	*IL10*	-1082 A/G	1q31–32	rs1800896
Metalloproteinase genes				
9	*MMP2*	-1306 C/T	16q12.2	rs243865
10	*MMP3*	-1171 5A/6A	11q22.3	rs3025058
11	*MMP9*	-1562 C/T	20q13	rs3918242

The results of the study of the POAG patients are presented in Table 2. During the initial analysis of the significance of differences between healthy individuals and POAG patients using the two- tailed Fisher's exact test, we revealed variables whose frequency significantly differed towards both an increase and a decrease in the POAG group with the significance of differences $p < 0.01$.

To obtain more significant values, which can be transferred into clinical practice to develop additional laboratory early diagnostic and prognostic criteria, we used the Bonferroni correction. It was used as a way to eliminate the effect of multiple comparisons that occurs when it is necessary to build a family of statistical conclusions, which avoids false conclusions about the presence of differences between groups, whereas in fact

the null hypothesis about the absence of differences is true. The application of this approach made it possible to select traits whose frequency differed most significantly in the group of POAG patients from the distribution of similar genetic traits in a sufficiently significant healthy group while maintaining the significance level of differences $p < 0.01$.

As part of complex genetic traits, whose frequency is significantly changed among POAG patients, both variants of cytokine genes with proinflammatory and anti-inflammatory activity and variants of matrix metalloproteinase genes are identified. Among the cytokine genes for these traits, variants of the *TNFA* and *IL 10* genes are most often detected, and among the metalloproteinase genes, variants of the *MMP2* gene are commonly detected. Variants of the *IL1B* and *MMP3* genes were not included in any of the complexes that significantly differ in the frequency of occurrence in the groups. The traits themselves are complex and include from two to six loci, variously combined and associated with different gene expression levels.

Among the complex genetic traits most closely associated with the development of POAG, the following complexes are distinguished: IL6-174:IL4-590:IL10-592:MMP2-1306:MMP9-1562, TNF-238:IL6-174:IL4-590:IL10-592:MMP2-1306, TNF-308:IL6-174:IL4-590:IL10-592:MMP2-1306, and *TNF-308:IL4-590, TNF-308:TNF-238.* Their prognostic value, according to the DC, exceeds 11.0, which corresponds to the reliability of the prognostic conclusion of over 95%. The combined genetic traits TNF-238:IL4-590:IL10-1082, IL10-1082:MMP2-1306:MMP9-1562, and TNF-308:IL10-1082:MMP2-1306 were practically not detected among patients with POAG, which indicates their probable protective value.

The comparative analysis on the frequency of occurrence of genetic traits in POAG patients and healthy individuals revealed formally similar, but different in content results. Thus, the analysis of the significance of differences according to the two-tailed Fisher's exact test revealed a significant group of 844 traits. The application of the Bonferroni correction as a way to eliminate the effect of multiple comparisons made it possible to select the traits whose frequency most significantly differed in the group of POAG patients as opposed to healthy individuals ($p < 0.01$). Among the complex genetic traits associated with the development of cataract, traits containing various variants of the *TNF* gene in all three studied sites prevailed. However, the *A* or *AA* variant in the position -308, associated with an increased ability of cells to produce this proinflammatory cytokine, was predominant (Table 3).

Table 2

The distribution frequency of the analyzed genetic traits in patients with POAG and healthy individuals

Polymorphic site	Genotypes	POAG, %	Control, %	OR	95% CI	Sp	Dc	*p*_cor
TNF-308	AA	7.07	0.66	11.41	2.33–55.90	99.34	10.3	0.0033
TNF-308:TNF-238	AA-GG	6.06	0.34	18.84	2.24–158.50	99.66	12.5	0.0091
TNF-308:IL4-590	AA-CC	7.07	0,34	22.37	2.72–184.21	99.66	13.2	0.0024
IL10-592:MMP2-1306	CA-TC	26.26	7.89	4.16	2.15–8.02	92.11	5.2	0.0008
TNF-308:IL10-592:MMP2-1306	GG-CA-TC	19.19	5.70	3.93	1.85–8.32	94.30	5.3	0.0080
TNF-238:IL10-592:MMP2-1306	GG-CA-TC	24.24	7.05	4.22	2.13–8.37	92.95	5.4	0.0015
IL6-174:IL10-592:MMP2-1306	GC-CA-TC	17.35	3.07	6.63	2.65–16.57	96.93	7.5	0.0023
IL4-590:IL10-592:MMP2-1306	CC-CA-TC	17.17	3.51	5.70	2.37–13.71	96.49	6.9	0.0022
TNF-308:IL6-174:IL10-592:MMP2-1306	GG-GC-CA-TC	14.29	2.63	6.17	2.29–16.58	97.37	7.3	0.0092
TNF-238:IL6-174:IL10-592:MMP2-1306	GG-GC-CA-TC	16.33	3.08	6.13	2.43–1.44	96.92	7.2	0.0038
IL6-174:IL4-590:IL10-592:MMP2-1306	GC-CC-CA-TC	12.24	0.88	15.77	3.46–71.91	99.12	11.4	0.0053
IL6-174:IL10-592:MMP2-1306:MMP9-1562	GC-CA-TC-CC	13.27	2.22	6.73	2.33–19.45	97.78	7.8	0.0098
TNF-238:IL6-174:IL4-590:IL10-592:MMP2-1306	GG-GC-CC-CA-TC	11.22	0.88	14.22	3.09–65.48	99.12	11.1	0.0073
IL6-174:IL4-590:IL10-592:MMP2-1306:MMP9-1562	GC-CC-CA-TC-CC	11.22	0.44	28.32	3.60–222.67	99.56	14.0	0.0090

Note (in all tables): *p*_cor - adjusted *p* value in the two-tailed Fisher's exact test (Bonferroni correction).

Table 3

The distribution frequency of the analyzed genetic traits in patients with cataract and healthy individuals

Polymorphic site	Alleles/Genotypes	Cataract, %	Control, %	OR	95% CI	Sp	DC	*p*_cor
IL1B-31	T	50.50	64.46	0.56	0.41–0.78	49.50	–1.1	0.0012
IL1B-31	C	49.50	35.54	1.78	1.28–2.46	64.46	1.4	0.0012
TNF-308	AA	12.00	0.66	20.45	4.49–93.12	99.34	12.6	0.0003
IL1B-31	CC	28.00	13.59	2.47	1.42–4.29	86.41	3.1	0.0057
TNF-863:TNF-308	CC-AA	11.00	0.67	18.42	4.01–84.64	99.33	12.2	0.0007
TNF-863:MMP2-1306	CA-TT	9.00	0.87	11.32	2.40–53.42	99.13	10.2	0.0045
TNF-308:TNF-238	AA-GG	12.00	0.34	39.82	5.11–310.52	99.66	15.5	0.0007
TNF-308:IL1B-31	AA-TC	7.00	0.35	21.53	2.61–177.27	99.65	13.0	0.0036
TNF-308:IL6-174	AA-GC	6.00	0.00	21.81	2.65–179.55	100.00	15.8	0.0018
TNF-308:MMP9-1562	AA-CC	8.00	0.71	12.09	2.52–57.94	99.29	10.5	0.0040
IL6-174:IL10-1082	GC-GG	3.00	16.47	0.16	0.05–0.53	97.00	–7.4	0.0054
TNF-863:TNF-308:TNF-238	CC-AA-GG	11.00	0.34	36.09	4.60–283.40	99.66	15.1	0.0013
TNF-863:IL6-174:IL10-1082	CC-GC-GG	1.00	12.35	0.07	0.01–0.54	99.00	–10.9	0.0088
TNF-863:MMP2-1306:MMP9-1562	CA-TT-CC	8.00	0.44	19.65	2.42–159.36	99.56	12.6	0.0088
TNF-308:TNF-238:IL1B-31	AA-GG-TC	7.00	0.36	21.08	2.56–173.56	99.64	12.9	0.0085
TNF-308:TNF-238:IL6-174	AA-GG-GC	6.00	0.00	21.29	2.59–175.31	100.00	15.7	0.0054
TNF-308:TNF-238:MMP9-1562	AA-GG-CC	8.00	0.36	23.91	2.95–193.77	99.64	13.4	0.0013
IL6-174:IL4-590:IL10-1082	GC-CC-GG	0.00	10.06	0.08	0.01–0.64	100.00	–13.2	0.0100

In this group of patients, a significantly higher proportion of genetic traits closely associated with the development of the disease was found. Thus, of the traits whose frequency is significantly increased in cataract, 12 are characterized by a two-digit DC, 99–100% specificity, and a two-digit OR.

The following traits have the maximum prognostic value: TNF-308:TNF-238, TNF-863:TNF-308:TNF-238, TNF-308:TNF-238:MMP9-1562, TNF-308:IL6-174, TNF-308: IL1B-31, TNF-308:TNF-238:IL6-174, and TNF-308:TNF-238:IL1B-31. At the same time, their composition in all cases includes TNFA -308 AA. Complex genetic traits with high protective value were also identified: IL6-174:IL 4-590:IL 10-1082, and TNF-863:IL6-174:IL10-1082.

Obtaining data on the similarities and differences in the distribution of single and complex genetic traits among POAG and cataract patients prompted us to investigate these results in more detail. IL17A-197 A/G, TGFB-509 C/T, and IL8 -251 A/T genes were added to the comparative analysis of the distribution of genetic traits in both groups of patients due to data on the active involvement of their protein products in the regulation of inflammatory processes and remodeling of the ECM. Genes of tissue metalloproteinase inhibitors TIMP1-372 C/T, TIMP2-418 G/C, constituting a single regulatory complex with *MMP*, and TGFB receptor genes: *TGFBRI, TGFBR II* and *TGFBR III* were also added to the analysis (Table 4).

Table 4

Distribution frequency of the analyzed genetic traits in patients with POAG and cataract

Polymorphic site	Alleles/Genotypes	POAG, %	Cataract, %	OR	95% CI	Sp	DC	*p*_cor
IL1B-31	T	64.65	50.50	1.79	1.20–2.68	49.50	1.1	0.0092
IL1B-31	C	35.35	49.50	0.56	0.37–0.83	64.65	–1.5	0.0092
TGFBR2	C	98.48	91.50	6.04	1.74–20.95	8.50	0.3	0.0040
TGFBR2	G	1.52	8.50	0.17	0.05–0.57	98.48	–7.5	0.0040
TGFBR2	CC	96.97	84.00	6.10	1.72–21.65	16.00	0.6	0.0084
IL1B-31:MMP2-1306	TT-TC	22.22	5.00	5.43	1.96–15.00	95.00	6.5	0.0036
TNF-238:IL1B-31:MMP2-1306	GG-TT-TC	22.22	5.00	5.43	1.96–15.00	95.00	6.5	0.0072
IL8-251:IL17-197:MMP9-1562	TA-GG-CC	21.21	4.08	6.33	2.08–19.21	95.92	7.2	0.0088
TNF-863:TNF-238:IL1B-31:IL4-590	*CC-GG-CC-CC*	1.01	16.00	0.05	0.01–0.41	98.99	–12.0	0.0060

Table 4 (continued)

Polymorphic site	Alleles/Genotypes	POAG, %	Cataract, %	OR	95% CI	Sp	DC	*p*_cor
TNF-863:TNF-238:IL1B-31:TIMP2-418	CC-GG-CC-GG	4.04	21.00	0.16	0.05–0.48	95.96	–7.2	0.0092
TNF-308:IL6-174:IL17-197:MMP2-1306	GG-GC-GG-TC	15.31	1.00	17.89	2.31–138.31	99.00	11.8	0.0051
IL6-174:IL17-197:MMP2-1306:TGFBR2	GC-GG-TC-CC	15.31	1.00	17.89	2.31–138.31	99.00	11.8	0.0037
IL8-251:IL17-197:MMP9-1562:TGFBR2	TA-GG-CC-CC	20.20	3.06	8.02	2.30–27.97	96.94	8.2	0.0066
TNF-308:IL6-174:IL17-197:MMP2-1306:TIMP2-418	GG-GC-GG-TC-GG	15.31	1.00	17.89	2.31–138.31	99.00	11.8	0.0060
TNF-308:IL6-174:IL17-197:MMP2-1306:TGFBR2	GG-GC-GG-TC-CC	14.29	0.00	17.82	2.31–137.72	100.00	14.7	0.0000
IL6-174:IL17-197:MMP2-1306:TIMP2-418:TGFBR2	GC-GG-TC-GG-CC	15.31	1.00	17.89	2.31–138.31	99.00	11.8	0.0047
TNF-308:IL6-174:IL10-592:IL17-197:TIMP2-418:TGFBR2	GG-GC-CA-GG-GG-CC	13.27	0.00	16.44	2.12–127.60	100.00	14.4	0.0067
TNF-308:IL6-174:IL17-197:MMP2-1306:TIMP2-418:TGFBR2	GG-GC-GG-TC-GG-CC	14.29	0.00	17.82	2.31–137.72	100.00	14.7	0.0000

When analyzing these results, three main aspects are of interest. Firstly, with the Bonferroni correction, the number of significantly different genetic traits increases significantly compared to the group of healthy individuals with a given level of the significance $p < 0.01$. Secondly, the composition of complex genetic traits includes a large number of variants of newly included genes, which confirms the correctness of their inclusion in the study. Thirdly, the number of highly significantly different traits increases significantly, which increases their prognostic value. We increased the significance level of the differences by five times from 0.05 to 0.01 in order to select the analyzed genetic traits suitable for possible transfer into clinical practice.

The predominant participation of *TNFA* gene variants in the formation of complex genetic traits differentiating comparable diseases is again of great interest when analyzing the results obtained. The results of the study showed a significant increase in the frequency of the T allele in the *IL1B* gene at -31T in the group of POAG patients ($p = 0.0046$), whereas in the study of patients of the Balkans' population, the protective role of *IL1B* rs16944 in the development of this disease was shown [18]. More significant differences between the groups were found in the study of the distribution of the *TGBR 2* gene. D50683 C was detected in more than 98% of POAG patients, which is significantly more common than in patients with cataract (OR = 6.04; $p = 0.002$).

The same pattern was revealed for the homozygous *TGFBR2 CC* genotype. To date, there has been no data on the effect of *TGBR 2* gene polymorphism on the expression of its protein products, however, the available data on a significant increase in the content of the TGFß2RII protein in the trabecular network of POAG patients suggest the presence of such a link in the development of fibrotic processes in POAG [19]. Significant changes in the level of TGFß isoforms in the intraocular fluid of POAG patients were described by us earlier [20].

A comparison of the distribution of gene variants involved in the ECM remodeling revealed significant differences between the groups of patients. The number of genetic traits, which presence in the patient's genome is significantly associated with the development of POAG increases, which is characterized by two-digit values of OR in the range from 16.44 to 17.89, the DC of up to 14.7, and specificity of 99–100%. Twelve such complex genetic traits are presented in Table 4. There are also genetic traits, whose frequency is significantly increased in patients with cataract.

The data obtained in the digital format are clearly illustrated in Figure, which presents the patterns of frequency distribution of the studied genetic traits in both diseases in a diagram.

The figure clearly demonstrates pronounced differences in the frequency of occurrence of complex genetic traits, although a zone of repeated combinations is visible in the central part of the figure, which probably reflects the presence of common links in the pathogenesis of eye diseases associated with inflammation, fibrosis, and ECM remodeling.

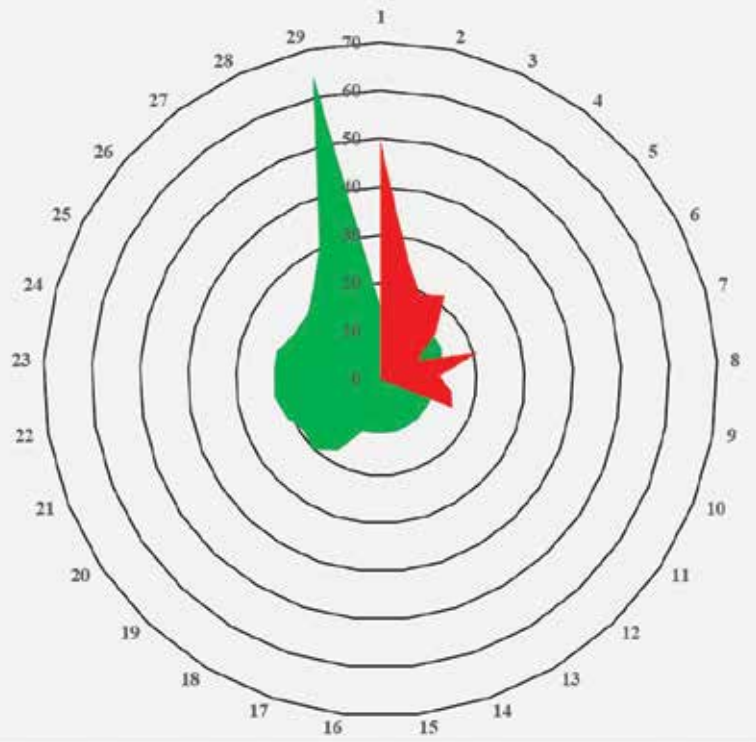

Figure. Graphical representation of differences in the frequencies of combinations of polymorphic variants in the studied genes in POAG and cataract: the numbers and scale of the radial axes correspond to the numerical values in Table 4.

DISCUSSION

We began the analysis of the distribution of polymorphic gene variants by comparing data of patients with POAG with similar data in the control group, which included 302 individuals of the Caucasian race born and permanently living in Russia. The results of the study showed significant deviations in the distribution of the studied traits both for a number of variants of individual genes and for their combinations. The analysis of single-nucleotide polymorphism (SNP) distribution revealed an increase in IL10-1082 A and IL10-592 A among patients with POAG, with a corresponding relative decrease in alternative *G* and *C* variants, which is confirmed by changes in the frequencies of the corresponding genotypes. The -1082 G/A polymorphism in the promoter region is associated with higher IL-10 production in the presence of the *G* allele relative to the *A* allele in the same position [21]. These data may indirectly indicate that among patients with POAG, alleles of the *IL-10* gene associated with low production of the corresponding cytokine with anti-inflammatory activity synthesized by Th2 cells and suppressing the production of cytokines by Th1 cells are more common.

It is worth noting that complex genetic traits closely associated with POAG, as a rule, include gene variants with both proinflammatory and anti-inflammatory activity.

The analysis of the obtained data showed that among patients with cataract, the frequency of occurrence of the *A* allele and *AA* genotype in TNF-308 was increased. It was previously shown that the *A* allele is associated with an increased level of production of this proinflammatory cytokine [22]. There are only isolated meta-analysis data on a weak association of the development of POAG with the *A* allele and the AA genotype of the *TNFA* – 308G/A (OR 1.6–1.7), more pronounced in Mongoloids. None of the other polymorphisms was significantly associated with the risk of POAG [23].

In our study, no isolated association of any of the three polymorphic variants of this gene with POAG or cataract was noted. However, in the composition of significantly associated complex genetic traits, various genotypes of this gene were repeatedly identified. In contrast to the group of patients with POAG, among patients with cataract we found an increase in the frequency of the *C* variant and the homozygous CC genotype in the *ILB1 -31* gene. At the same time,

in the group of patients with POAG, an increase in the frequency of *IL10-1082 A* was revealed with a corresponding relative decrease in alternative variants *G*, which indicates the spread of the variant associated with a low ability of cells to produce IL-10 among patients with cataract.

The addition of IL17A-197 A/G, TGFB C/T, and IL8 -251 A/T cytokine genes, TIMP1-372 C/T, TIMP2-418 G/C tissue metalloproteinase inhibitor genes, which form a single regulatory complex with *MMP*, and TGFB receptor genes *TGFBRI, TGFBR II,* and *TGFBR III* more fully characterizes the state of ECM in comparable diseases.

Products of these genes are involved in the processes of vital activity in cells producing structural elements of the ECM, in the regulation of elasticity, looseness or stiffness of the ECM, in angiogenesis and lymphangiogenesis, in tissue fluid exchange, in the homeostatic nature of its composition, in cell migration, apoptosis, etc. Practically, the analysis includes two ensembles of genes – the genes of cytokines and their receptors, as well as the genes of metalloproteinases and their tissue inhibitors. Along with the possible practical application of the results obtained for prognostic purposes, comparing the nature of the distribution of genes involved in the ECM remodeling is also of fundamental importance, allowing to identify general and specific traits underlying a genetic predisposition to the development of eye diseases of various genesis.

Although the genes analyzed in this study have only partially been included in the genes responsible for remodeling of human eye tissue structures in glaucoma so far [24], we believe that the high degree of association of their variant complexes with eye diseases of various genesis makes their analysis promising for further research. Previously, the association of POAG with TIMP polymorphisms in Mongoloids [25] and MMP-TIMP polymorphisms in Caucasians [26] was shown.

CONCLUSION

The comparative analysis of the results of the study on the frequency of the analyzed genetic traits, including cytokine and metalloproteinase genes, in patients with POAG and healthy individuals showed the presence of complex genetic traits, whose frequency is extremely high among POAG patients, which is characterized by two-digit OR, high specificity (99–100%), and high DC.

Alternative results were obtained by us in a comparative analysis of the frequency of genetic traits in groups of patients with cataract and healthy individuals. The application of the Bonferroni correction as a way to eliminate the effect of multiple comparisons made it possible to select the traits whose distribution frequency most significantly differed in the group of patients with cataract compared to the distribution of similar genetic traits in the control group of 302 healthy individuals while maintaining the given $p < 0.01$.

The direct comparison of the distribution of two gene ensembles whose products are involved in the processes of ECM remodeling – genes of cytokines and receptors to some of them (*TGFB1 – TGFBR2*), as well as genes of metalloproteinases and their tissue inhibitors (MMP – TIMP) revealed a significant number of genetic traits characteristic of both diseases, which indicates significant differences in the implementation of genetic predisposition to their development.

The received data indicate a fundamental possibility of developing reliable laboratory criteria (riskometers) for early diagnosis and prognosis of predisposition to the development of POAG and cataract before the development of clinical and laboratory signs of the disease at a young age.

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Konenkov V.I. – conception and design, critical revision of the manuscript for important intellectual content, drafting of the article. Shevchenko A.V. – carrying out of the laboratory studies, analysis and statistical processing of the data, drafting of the article. Prokofiev V.F. – analysis and statistical processing of the data, drafting of the article. Chernykh V.V. – conception and design, critical revision of the manuscript for important intellectual content. Trunov A.V. – justification of the manuscript, design, selection of patients for the study, drafting of the article.

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