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ORIGINAL ARTICLE *free on line*

Differences in gene expression in lymphocytes of patients with high-tension, PEX, and normal-tension glaucoma and in healthy subjects

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Purpose: *Differences in the gene expression of leukocytes between patients with normal-tension glaucoma (NTG) and controls have been described. This study was performed in order to detect the differences in gene expression in peripheral lymphocytes in patients with primary open-angle glaucoma (POAG), patients with pseudoexfoliation glaucoma (PEX), and patients with NTG, and in healthy subjects.*
Methods: *Ten patients with POAG, 11 patients with PEX, 10 patients with NTG, and 42 sex- and age-matched healthy persons were recruited. All study subjects were Caucasian. Twenty-two preselected genes were chosen and their expression in blood lymphocytes was quantified by real-time PCR. First, a univariate comparison among all groups was performed using the nonparametric Friedman test. Second, an L1 penalized logistic regression was performed.*
Results: *Using the Friedman test to compare the 4 groups, 9 genes showed a different expression ($p<0.05$). Comparing the controls vs patients with POAG, 8 genes were differently expressed ($p<0.05$). Comparing patients with PEX vs controls, 9 genes were significantly different ($p\leq0.05$). The statistical analysis of patients with NTG vs controls showed a difference in gene expression of 7 genes ($p\leq0.05$). All these genes were upregulated in the glaucoma groups compared with the controls. The genes RhoGDI and RAR showed the most significant statistical difference in the L1-penalized logistic regression. The genes overexpressed in POAG/PEX differed from the ones in NTG.*
Conclusions: *In this masked study among the preselected 22 genes, several genes are overexpressed in the blood lymphocytes of Caucasian patients with glaucoma compared with the controls. The genes upregulated in POAG/PEX differed from the ones in NTG.*

Keywords: *Gene expression, Lymphocytes, Glaucoma, PCR, RAR, RhoGDI*

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INTRODUCTION

Glaucoma is diagnosed based on optic nerve damage. Pathophysiologically, the different mechanisms leading to this damage have been discussed. Depending on their activity, cells adapt their gene expression. Quantification of gene expression allows a conclusion about the activity of the corresponding cells. In human patients with glaucoma and in animals with experimental glaucoma, gene expression is altered in the optic nerve head (ONH), particularly in the astrocytes (1, 2).

In humans, ONH tissue is only accessible postmortem for histologic analysis (3). Blood, however, is easily accessible in humans. Seemingly uninvolved cells like the blood lymphocytes might provide us indirectly with information about certain diseases. This is surprising as no lymphocyte

infiltration has occurred in the optic nerve or the lateral geniculate nucleus as in glaucoma. But this does not exclude an information transfer from the optic nerve to the lymphocytes, for example, via vascular endothelial cells into circulating blood lymphocytes.

Indeed, in neurodegenerative diseases such as Alzheimer disease (4) or in schizophrenia (5) the blood lymphocytes change their gene expression. To some extent, this has been observed in cases of glaucoma (6, 7). The change of profile of autoantibodies in glaucoma confirms an information transfer between the eye and/or visual pathways and lymphocytes (8, 9).

The present study is neither a genetic nor an immunologic study. The purpose was not to detect gene polymorphisms or gene mutations. We only quantified gene expression of preselected genes in blood lymphocytes. Although this preselection was to some extent based on previous studies and on our pathophysiologic concept, it was more or less arbitrarily done. It was not the purpose to test all potential genes and to compare them. We only tested the hypothesis that circulating lymphocytes express certain genes differently in different groups of patients. It was not the purpose to exclude differences in other genes. We examined lymphocytes because they are easily accessible in humans and not because they are immune cells. Glaucoma is a continuum in which the risk factors play a quantitatively different role. Nevertheless, arbitrarily defined limits (e.g., of intraocular pressure [IOP]) are used to classify the subtypes of glaucoma. These subtypes seem useful for studies to detect possible differences in pathophysiology.

Resulting from our previous study, we have chosen 22 different genes that potentially may be involved in the pathophysiology of the IOP increase or in the pathophysiology of glaucomatous damage. The justification of this selection of the gene pattern is provided in the context of our previous issue-related publications to which we refer here (10-12).

MATERIALS AND METHODS

Study subjects

Ten patients with primary open-angle glaucoma (POAG), 11 patients with pseudoexfoliation glaucoma (PEX), 10 patients with normal-tension glaucoma (NTG), and 42 sex- and age-matched healthy Caucasian patients were recruited at the University Eye Clinic in Basel, Switzerland. Care was taken to select cases without prior participation in scientific studies.

Consecutive glaucoma patients from the outpatient glaucoma department, University Hospital Basel, Switzerland, were included. All had a clearly established diagnosis of glaucoma with obvious changes of the optic disc, repeated demonstration of visual field defects, and at least 2 diurnal tension curves. All patients were under treatment; the groups were not controlled for previous treatment or duration of disease, but the patients did not have intercurrent conditions that may have influenced the results, such as inflammatory diseases or infections. Age- and sex-matched healthy volunteers were recruited. Ethical approval was obtained from the local medical ethics committee, and written informed consent was received from all subjects before entry into the study. The study was designed and conducted in accordance with the tenets of the Declaration of Helsinki.

Patients with glaucoma had to meet the following inclusion criteria according to the guidelines of the European Glaucoma Society: 1) treated IOP ≤21 mm Hg, 2) visual field changes in either side in at least 3 successive perimetric tests (Octopus G1 program: MD ≤–7 dB), 3) glaucomatous optic nerve cupping, 4) open angles on gonioscopy, and 5) the absence of alternative causes of optic neuropathy. In high-tension glaucoma (HTG) and PEX patients, the highest measured IOP was above 21 mm Hg; in NTG patients, the highest measured IOP was equal to or below 21 mm Hg measured at diurnal IOP curves. Unilateral or bilateral PEX was diagnosed by the presence of PEX material in the anterior segment.

Patients with glaucoma and healthy subjects with any of the following criteria were excluded: history of other ocular or systemic disease (e.g., diabetes mellitus), smoking, drug, or alcohol abuse, ocular trauma, ocular infection, or inflammation.

The laboratory evaluations were done without any knowledge of the diagnosis. In turn, the statistical analysis was done without knowing the genes. The code for the diagnosis was revealed after all measurements were done. The code for the genes was revealed after the statistical analysis was completed.

Lymphocytes isolation

Blood samples anticoagulated with heparin were collected from patients. Lymphocytes (including monocytes and stem cells) were separated using Ficoll-Hypaque gradients

(Histopaque 1077, Sigma, Buchs, Switzerland) as described previously (13). Then, 2 mL of hitopaque was placed into 10 mL of sterile centrifuge tubes and 5 mL of diluted blood sample were carefully layered onto each histopaque gradient. Gradients were centrifuged at 475 *g* at 20°C for 15 minutes. The lymphocytes bands were removed from interface between plasma and the histopaque layers of each tube and collected into one 50-mL tube. The total volume was brought to 50 mL with cold Dulbecco Modified Eagle Medium (DMEM, GibcoBRL, Basel, Switzerland). The cell suspension was washed 3 times with DMEM and the total number of cells was determined. Cells were finally suspended in balanced saline solution and aliquoted into Eppendorf tubes at 10^7 cells/tube. After centrifugation, cell pellets were dried and stored at –70°C until mRNA isolation.

Reverse transcriptase PCR

In order to detect the expression of the target genes in lymphocytes and optimize the reaction conditions for real-time quantitative polymerase chain reaction (RT-QPCR), reverse-transcriptase PCR (RT-PCR) was performed using template-specific primer sets that have been created and optimized in series of parallel experiments. cDNA synthesis was performed using an iScript™ cDNA Synthesis Kit (Bio-Rad, Philadelphia, Pennsylvania, USA). The PCR reactions were hot-started at 95°C for 5 minutes before adding 1.5 units of Taq Polymerase (Red-Hot®, ABgene, Newcastle, UK) at the annealing temperature of 56°C, followed by polymerization at 72°C for 1 minute. Amplification was carried out in a Perkin-Elmer DNA Thermal Cycler TC480 for 45 cycles (denaturation for 45 seconds at 95°C, annealing for 45 seconds at 56°C, and polymerization at 72°C for 30 seconds), followed by a final 7-minute extension at 72°C. Negative controls without DNA as well as positive controls with a sequenced template were performed for each set of PCR experiments.

The specificity of each PCR amplification was controlled using the site-specific restriction analysis of target PCR products. The amplification products underwent an extraction from the agarose gel using a DNA isolation kit (DNA Gel Extraction Kit, Fermentas, Lithuania) before digestion. They were digested in a final volume of 50 µL with 20 units of each restriction endonuclease for 2 hours, according to conditions specified by the manufacturer (Fermentas, Lithuania), and they were imaged after electrophoresis (MWG-Biotech, Germany).

Real-time quantitative PCR

In order to profile precisely the changes in an expression of target genes, RT-QPCR was used. SYBR® Green I (Molecular Probes, Eugene, Oregon, USA) was utilized as the intercalation dye and fluorescent reporter molecule detecting the accumulation of the amplified double-stranded product in the iCycler iQIM Detection System (Bio-Rad), according to the protocol supplied by the manufacturer. A total of 50 ng of the synthesized cDNAs (see RT-PCR) were used for each real-time PCR analysis. The reaction mixtures had the same contents as those for RT-PCR, with the exception of Red-Hot® polymerase (ABgene), which was substituted for Thermoprime Plus DNA Polymerase (Thermo Fisher Scientific, ABgene product line) in order to avoid color signal disturbances. The same amplification program has been used in qualitative RT-PCR and quantitative real-time PCR analyses. The algorithm of the iCycler iQIM Detection System normalizes the reporter signal (non-intercalated SYBR® Green I) into a passive reference and multiplies the SD of the background signal in the first few cycles by a default factor of 10 to determine a threshold. The cycle at which this baseline level is exceeded is defined as a threshold cycle (C_t), which depends on the initial template copy number and is proportional to the log of the starting amount of nucleic acid. By subtracting the difference of the C_t values of a target gene from those of the housekeeping one (β-actin), the data were normalized. The relative levels were calculated for each sample based on the differences in C_t values.

Statistical methods

In a first step, univariate comparisons among all 4 study groups were performed using the nonparametric Friedman test. However, these comparisons were considered as purely exploratory. p Values are adjusted for multiple comparisons using false discovery rate control (FDR).

In a second step, L1 penalized logistic regression was applied predicting the study groups. This is a common accepted tool for gene selection; therefore, this method is considered as decisive. Details are described in Park and Hastie (14).

The method constrains the sum of the absolute values of the coefficients to a parameter λ (regularization). Generally, L1 regularization provides better results than traditional methods as forward or backward stepwise regression,

especially in situations where the number of predictors is large compared with the number of subjects.

Generating a coefficient path dependent on all values of λ makes it possible to identify the order in which the genes enter the model. To choose the model with the best predictive performance, the parameter λ with the smallest prediction error based on the log likelihood was selected.

The prediction error is calculated by means of a 10-fold crossvalidation (14). Prior to evaluation, all expression values were log-transformed. All values below quantification (BLQ) were set to half BLQ, and missing values were imputed using an algorithm described by Hastie et al (15).

Descriptive statistics of expression levels across study groups are presented in Table V. A p value <0.05 is considered significant (after adjusting for FDR).

All evaluations were done using the package "glmpath" implemented in the statistical software *R* (a language and environment for statistical computing) (16).

RESULTS

Since the purpose of this study was to determine relative differences among study groups, the remainder of the results are confined to descriptions of the level of significance between groups (Tab. I).

As a major finding, differences in gene expression between glaucoma patients and controls and between glaucoma subgroups were found (Tabs II, III).

RAR gene in NTG and *RhoGDI* gene in POAG/PEX were the statistically most differently expressed genes as calculated in the penalized logistic regression. *ICAM*, *P2Y*, *MMP-9*, and *MT1-MMP* have also been selected to be different using the penalized logistic regression (Tab. IV).

Interestingly, all genes that were significantly different in one of the 3 glaucoma groups compared with the controls were upregulated (Tab. V). Genes that were less expressed in one glaucoma group compared with the controls did not differ significantly (definitions of abbreviations, see Tab. VI).

TABLE I - DIFFERENCES AMONG THE 4 GROUPS BASED ON THE FRIEDMAN TESTS: COMPARING ALL GROUPS

Gene	p Value	Adjusted p value (FDR)
P2Y	<0.001	<0.001
RhoGDI	<0.001	<0.001
MT1-MMP	<0.001	<0.001
RhoC	0.002	0.011
XIAP	0.003	0.011
RAR	0.003	0.011
Na/Ca channel	0.009	0.028
ICAM	0.016	0.044
TIMP1	0.039	0.095
LxRec	0.078	0.158
Thioredoxin 2	0.079	0.158
ENOS	0.104	0.191
MMP-9	0.129	0.211
LyGDI	0.138	0.211
XAPC7	0.14	0.211
RhoA	0.227	0.308
TIMP2	0.238	0.308
RhoB	0.296	0.362
MDR3	0.367	0.425
XPGC	0.403	0.443
Cytochrome	0.486	0.509
ITBG	0.918	0.918

FDR = false discovery rate.

DISCUSSION

In this study, we investigated the differences in the gene expression of the blood lymphocytes in HTG, NTG, and PEX patients compared with normal controls. Previously, differences in the gene expression of leukocytes among NTG patients with primary vascular dysregulation and controls has been described (6) using the method of gene hunting to screen a broad spectrum of genes. The results pointed toward genes involved in apoptosis and reperfusion damage.

In the present masked study, we investigated 22 potentially interesting genes. The gene expressions were analyzed first individually and then by logistical regression in order to avoid false-positive results.

TABLE II - DIFFERENCES AMONG THE 4 GROUPS BASED ON THE FRIEDMAN TESTS: COMPARING THE CONTROL GROUP VS POAG, PEX, NTG

Gene	p Value	Adjusted p value (FDR)	Gene	p Value	Adjusted p value (FDR)	Gene	p Value	Adjusted p value (FDR)
POAG			PEX			NTG		
P2Y	<0.001	<0.001	*P2Y*	<0.001	<0.001	*RAR*	0.005	0.066
RhoGDI	0.001	0.011	*MT1-MMP*	<0.001	<0.001	*ICAM*	0.006	0.066
RhoC	0.004	0.029	*RhoGDI*	<0.001	<0.001	*XIAP*	0.011	0.077
Na/Ca channel	0.012	0.066	*RhoC*	0.004	0.022	*MT1-MMP*	0.014	0.077
TIMP1	0.019	0.082	*XIAP*	0.005	0.022	*XAPC7*	0.027	0.117
ICAM	0.023	0.082	*RAR*	0.006	0.022	*Thioredoxin 2*	0.032	0.117
XIAP	0.026	0.082	*Na/Ca channel*	0.007	0.022	*TIMP1*	0.044	0.138
MT1-MMP	0.044	0.121	*ENOS*	0.02	0.055	*LxRec*	0.105	0.289
TIMP2	0.066	0.161	*LxRec*	0.039	0.095	*LyGDI*	0.134	0.297
Thioredoxin 2	0.077	0.169	*MMP-9*	0.051	0.112	*MMP-9*	0.145	0.297
RhoA	0.104	0.208	*RhoB*	0.141	0.28	*Na/Ca channel*	0.159	0.297
MMP-9	0.186	0.34	*LyGDI*	0.153	0.28	*RhoGDI*	0.162	0.297
MDR3	0.201	0.34	*Cytochrome*	0.177	0.3	*RhoA*	0.186	0.315
RhoB	0.265	0.404	*MDR3*	0.223	0.35	*MDR3*	0.266	0.418
XAPC7	0.301	0.404	*RhoA*	0.249	0.365	*TIMP2*	0.287	0.419
LyGDI	0.312	0.404	*XPGC*	0.268	0.369	*RhoC*	0.305	0.419
ENOS	0.312	0.404	*TIMP2*	0.351	0.454	*Cytochrome*	0.473	0.612
RAR	0.335	0.409	*XAPC7*	0.404	0.494	*RhoB*	0.504	0.616
XPGC	0.359	0.416	*ITBG*	0.583	0.675	*XPGC*	0.561	0.65
Cytochrome	0.634	0.697	*Thioredoxin 2*	0.775	0.824	*ENOS*	0.767	0.844
ITBG	0.763	0.799	*ICAM*	0.787	0.824	*ITBG*	0.853	0.876
LxRec	0.825	0.825	*TIMP1*	0.956	0.956	*P2Y*	0.876	0.876

FDR = false discovery rate; NTG = normal-tension glaucoma; PEX = pseudoexfoliation glaucoma; POAG = primary open-angle glaucoma.

There are differences among glaucoma patients and controls and there are also differences among glaucoma subgroups. The altered gene expression pattern as detected in the penalized logistic regression may point towards well-known molecular pathways affected in glaucoma.

The statistically most significant upregulated genes were *RAR* in the case of NTG and *RhoGDI* in the case of POAG/PEX.

The *RAR* gene (RAR-related orphan receptor C) that is overexpressed in NTG encodes for a nuclear receptor that interacts with coactivators and corepressors to positively or negatively regulate the transcription of target genes, and it is involved in a wide range of processes. There is evidence that *RAR* is involved, on the one hand, in modulation of immune response and, on the other, on apoptosis. Both an altered immune response (17) and an increased

TABLE III - DIFFERENCES AMONG THE 4 GROUPS BASED ON THE FRIEDMAN TESTS: COMPARING POAG VS NTG

Gene	p Value	Adjusted p value (FDR)	POAG vs NTG
P2Y	0.034	1	POAG > NTG
RAR	0.041	1	POAG < NTG
LxRec	0.050	1	POAG < NTG
RhoC	0.078	1	POAG > NTG
RhoGDI	0.110	1	POAG > NTG
XAPC7	0.153	1	POAG < NTG
LyGDI	0.177	1	POAG > NTG
XPGC	0.325	1	POAG > NTG
RhoB	0.347	1	POAG > NTG
RhoA	0.348	1	POAG > NTG
Cytochrome	0.391	1	POAG < NTG
TIMP1	0.413	1	POAG > NTG
MT1-MMP	0.461	1	POAG < NTG
XIAP	0.487	1	POAG < NTG
TIMP2	0.513	1	POAG > NTG
Thioredoxin 2	0.592	1	POAG > NTG
MMP-9	0.624	1	POAG > NTG
Na/Ca channel	0.653	1	POAG > NTG
ENOS	0.807	1	POAG > NTG
ITBG	0.903	1	POAG < NTG
ICAM	0.932	1	POAG > NTG
MDR3	0.935	1	POAG < NTG

FDR = false discovery rate; NTG = normal-tension glaucoma; POAG = primary open-angle glaucoma.

TABLE IV - DIFFERENCES AMONG THE 4 GROUPS BASED ON THE PENALIZED LOGISTIC REGRESSION: THE FOLLOWING GENES WERE SIGNIFICANTLY DIFFERENT, EVEN IN THE PENALIZED LOGISTIC REGRESSION[a]

	Genes
Control–all glaucoma	*RhoGDI, P2Y*
Control–POAG	*ICAM*
Control–NTG	*RAR, ICAM*
Control–PEX	*RhoGDI, P2Y, MMP-9, MT1-MMP*

NTG = normal-tension glaucoma; PEX = pseudoexfoliation glaucoma; POAG = primary open-angle glaucoma.
[a] The genes *RhoGDI* and *RAR* showed the most statistically significant difference in the L1-penalized logistic regression. This is why these genes are briefly described in the Discussion.

apoptosis (18) might be involved in the pathophysiology of NTG.

The *RhoGDI* (RhoGDP dissociation inhibitor) gene codes for a regulator family involved in the negative modulation of the activity of the Rho family members and their translocation between the cytosol and the membrane (19). The Rho family members mainly regulate the organization of the actin cytoskeleton. Their function is broad. The upregulation of *RhoGDI* in POAG and PEX could point toward inflammation potentially induced by reactive oxygen species (ROS). In fact, the assumption that ROS are involved in glaucoma is widely accepted (20). However, it could also point to an altered geometry of the trabecular meshwork or to counterregulation in order to improve the aqueous outflow or to protect the neurons from damage.

P2Y purinreceptor plays a role in the regulation of movements of leukocytes from the blood into the tissue and is involved in the genesis of pathologic inflammation and edema (21). Especially remarkable is its influence on leukotrienes. Enhanced concentrations have been found, e.g., in CSF of patients with multiple sclerosis (22).

ICAM is thought to be involved in the adhesion and transendothelial migration of leukocytes, leading, e.g., to an increased blood–brain barrier permeability (23) observed also in glaucoma pathology (24). Inhibition of the interaction of the leukocytes with blood vessel endothelium via antibodies has been shown to be neuroprotective and reduce ischemia–reperfusion damage (25). *ICAM* has been found to be upregulated in trabecular meshwork cells from postmortem glaucomatous eyes (26). Metalloproteinases contribute to tissue remodeling and degradation of tissue observed in glaucoma (6).

Inflammation, reperfusion damage, or remodeling are widely believed to play a major role in glaucoma. The results of this study can neither confirm nor disprove these theories. The meaning of the findings has to be further investigated.

TABLE V - DESCRIPTIVE STATISTICS OF EXPRESSION LEVELS: MEAN (SD)

Gene	Controls (n = 42)	HTG (n = 10)	NTG (n = 9)	PEX (n = 11)
P2Y	0.00289 (0.00575)	0.00541 (0.00188)	0.00475 (0.00353)	0.00784 (0.00302)
ICAM	0.00019 (0.00004)	0.01131 (0.00881)	0.00582 (0.00014)	— (—)
Thioredoxin 2	0.07204 (0.05320)	0.04118 (0.02434)	0.04217 (0.02713)	0.05942 (0.04944)
XPGC	0.32981 (0.77476)	0.03513 (0.03120)	0.12035 (0.22536)	0.08433 (0.04202)
XAPC7	0.10236 (0.10183)	0.08571 (0.02112)	0.12136 (0.05767)	0.11942 (0.05277)
LyGDI	0.27802 (0.13415)	0.35151 (0.18205)	0.23521 (0.07500)	0.32886 (0.15922)
RhoB	0.00469 (0.00328)	0.00742 (0.00565)	0.00360 (0.00118)	0.00768 (0.00648)
RhoC	0.04532 (0.07133)	0.05693 (0.02500)	0.14721 (0.36117)	0.20623 (0.55084)
RhoGDI	0.17094 (0.05497)	0.27861 (0.11672)	0.20196 (0.06497)	0.41324 (0.25960)
RhoA	5.16730 (20.68035)	3.40337 (6.07818)	0.46539 (0.22481)	1.93422 (4.36035)
ITBG	0.07481 (0.03511)	0.07532 (0.03267)	0.07434 (0.03129)	0.06698 (0.02540)
ENOS	0.00400 (0.00763)	0.00360 (0.00459)	0.00280 (0.00266)	0.00473 (0.00301)
Na/Ca channel	0.00285 (0.00417)	0.00394 (0.00246)	0.01101 (0.01426)	0.00915 (0.01215)
MDR3	0.00068 (0.00126)	0.00056 (0.00026)	0.00058 (0.00034)	0.00078 (0.00083)
Cytochrome	0.00385 (0.00364)	0.00291 (0.00123)	0.00347 (0.00156)	0.00363 (0.00144)
RAR	0.00069 (0.00048)	0.00082 (0.00043)	0.00183 (0.00147)	0.00122 (0.00060)
LxRec	0.03687 (0.07887)	0.01882 (0.02232)	0.10576 (0.18261)	0.07921 (0.17942)
XIAP	0.00110 (0.00110)	0.00185 (0.00171)	0.00173 (0.00072)	0.00202 (0.00157)
TIMP1	0.01127 (0.01285)	0.01729 (0.00955)	0.01353 (0.00485)	0.01164 (0.00914)
TIMP2	0.00779 (0.00857)	0.01054 (0.00484)	0.00829 (0.00279)	0.00868 (0.00461)
MMP-9	0.00082 (0.00126)	0.00038 (0.00034)	0.00056 (0.00070)	0.00034 (0.00022)
MT1-MMP	0.00023 (0.00013)	0.00030 (0.00015)	0.00040 (0.00024)	0.00051 (0.00046)

HTG = high-tension glaucoma; NTG = normal-tension glaucoma; PEX = pseudoexfoliation glaucoma.

Limitations of the study

Our study may harbor 2 important limitations. First, the analyzed genes were preselected. The results do not allow extrapolation to other genes. We also cannot exclude that by increasing the sample size, more genes would have been significantly differently expressed. Second, the results are based on a cohort of Caucasian patients and cannot necessarily be applied to other populations.

Further studies may elucidate how far these results can be used for the understanding of pathophysiology and for diagnostic purposes.

TABLE VI - GENES: ABBREVIATIONS

Abbreviation	Name	Gene family	Genbank Acc
P2Y	Purinreceptor 7	G-protein-coupled purinreceptor family	U41070
MT1-MMP	Matrix metallopeptidase 14 (membrane-inserted)	Metalloproteinase	U41078
RhoGDI	RhoGDP dissociation inhibitor (GDI) beta	Inhibitor of RhoGTPases	X69549
RhoC	Ras homolog gene family, member C	Serine/threonine kinase family	X06821
XIAP	X-linked inhibitor of apoptosis	Apoptosis inhibitor	U45880
RAR	RAR-related orphan receptor C	Transcription activators and repressors, orphan, nuclear receptors	U16997
Na/Ca channel	Solute carrier family 8 (sodium/calcium exchanger), member 1	Symporter/antiporter family	M91368
LxRec	Nuclear orphan receptor LXR-alpha	Orphan	U22662
MMP-9	Type IV collagenase	Metalloproteinase	J05070
RhoB	Ras homolog gene family, member B	Serine/threonine kinase family	NM_004040
LyGDI	RhoGDP dissociation inhibitor (GDI) beta	Inhibitor of RhoGTPases	L20688
Cytochrome	P450 (cytochrome) oxidoreductase	Xenobiotic metabolism	S90469
MDR3	Membrane glycoprotein P (MDR3)	Xenobiotic transporters, drug resistance proteins, ABC transporters	M23234
RhoA	Ras homolog gene family, member A	Serine/threonine kinase family	NM_001664
XPGC	Xeroderma pigmentosum gene C	DNA-repair gene	X71342
TIMP2	Tissue inhibitor of metalloproteinase 2	Inhibitors of proteases	NM_003255
XAPC7	20S proteosome a-subunit	Proteosome proteins	AF022815
ITBG	Leukocyte adhesion protein beta subunit	Cell-cell adhesion receptors	M15395
Thioredoxin 2	ATL-derived factor/thiredoxin	Redox control, oxido-reductases	X77584
ICAM-1	Intercellular adhesion molecule-1	Matrix adhesion receptor	J03132
TIMP1	Tissue inhibitor of metalloproteinase 1	Inhibitors of proteases	NM_003254

CONCLUSIONS

This study shows that there are differences in gene expression in blood lymphocytes among glaucoma patients and controls and among glaucoma subgroups. How far these findings can be interpreted in the context of pathophysiology or be used for diagnostic purposes needs further investigation.

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