University of Sistan and Baluchestan

Journal of Epigenetics

Online ISSN: 2676 - 4350

Homepage: https://jep.usb.ac.ir

Iranian Genetics Society

Analysis of DRD1 gene expression pattern in patients with glaucoma

Mojtaba Reyhani[1], Mohammad Arish[2], Ali-Reza Maleki[2], Shahram Banaie[2], Dor Mohammad Kordi Tamandani[3*]

[1] Department of Biology, Sistan and Baluchistan University, Iran. E-mail: 73.reyhani@gmail.com

[2] Department of Ophthalmology, Al-Zahra Eye Hospital, Zahedan University of Medical Sciences, Zahedan, Iran. E-mail: arish.mohammed@gmail.com, A_R_maleki@yahoo.com, shahrambanaie@gmail.com.

[3] *Corresponding author*, Department of Biology, University of Sistan and Baluchestan, Zahedan, Iran. E-mail: dor_kordi@science.usb.ac.ir, Tel.: +989153781534.

ARTICLE INFO

Article type:
Research Article

Article history:
Received: 27 November 2021
Revised: 12 July 2022
Accepted: 05 August 2022

Keywords:
Glaucoma,
dopamine receptor D1A,
Gene Expression, Receptors,
Dopamine,
Real-Time Polymerase Chain Reaction.

A B S T R A C T

Background: Glaucoma is a term used to describe a group of diseases that progressively weaken the optic nerve and cause vision loss and eventually blindness. Glaucoma is one of the most common and controversial eye diseases. Dopamine, a neurotransmitter in the eye, regulates ciliary blood flow and aqueous humour production, which affects intraocular pressure and glaucoma. Based on previous studies on dopamine receptor ligands, it was concluded that the effect of dopamine on fluid production is stimulated by dopamine receptor 1 (D1) activation. Expression and translation of the DRD1 gene cause the production of the D1 receptor. Based on this, it was hypothesized that changes in DRD1 gene expression may play a role in the development of glaucoma. The aim of this study was to analyze DRD1 gene expression in patients with glaucoma. Materials and Methods: In this study, a statistical population of 40 people including 20 patients and 20 healthy individuals was used to analyze the effect of *DRD1* gene expression on patients with glaucoma. After RNA extraction and cDNA synthesis, the Real-time quantitative PCR (qRT-PCR) technique was used and its data were analyzed by SPSS software and Mann-Whitney statistical method. Results: The results of this study indicate a significant difference (P = 0.015) in the expression of the *DRD1* gene in patients with glaucoma compared to the control group.

Introduction

Genes encode proteins and proteins dictate cell function. The human genome encodes instructions for the regulation of gene expression, which varies both across cell types and across individuals. Regulation of gene expression can have a profound effect on cellular structure and function. Gene expression and regulation are the basis of cell development and differentiation, and any change in it may lead to various diseases and disorders.

Glaucoma, a progressive degenerative condition, is a group of optic neuropathies characterized by progressive degeneration of retinal ganglion cells (RGCs) in the inner retina and loss of their axons in the optic nerve, leading to irreversible blindness (Kwon, Song, et al., 2020). Glaucoma is mostly asymptomatic until late in the disease when visual problems arise. Vision loss from glaucoma cannot be recovered (Arora, Karun S., et al., 2012). Ophthalmologists use different comprehensive examinations such as ophthalmoscopy, tonometry, perimetry,

DOI: 10.22111/jep.2022.40753.1035

Publisher: University of Sistan and Baluchestan

How to Cite: Reyhani, M., Arish, M., Maleki, A., Banaie, S., Kordi-Tamandani, D. (2022). Analysis of DRD1 gene expression pattern in patients with glaucoma. Journal of Epigenetics, 3(2), 15-20. DOI: 10.22111/jep.2022.40753.1035

gonioscopy, and pachymetry to diagnose glaucoma (Juneja, Mamta, et al., 2020). Symptoms are usually not apparent until substantial irreversible damage has occurred. Therefore, it is important to facilitate early diagnosis to prevent vision loss. Approximately half of those affected remain undiagnosed, suggesting that current screening strategies lack efficacy (Souzeau, Emmanuelle, et al., 2017).

The only currently approved treatment is aimed at lowering intraocular pressure (IOP) (Wentz, Scott M., et al., 2012). The reduction of IOP is obtained by eye drops or systemic application of glaucoma medications. These include carbonic anhydrase inhibitors, betablockers, cholinergic agonists, a2-adrenoceptor agonists, and prostaglandins (Rizzo, Maria Ida, et al., 2017).

Glaucoma subgroups are defined as ‘open-angle or ‘closed-a depending on the position of the ocular lens and iris relative to the trabecular meshwork. Open-angle glaucoma is further divided into subgroups defined by the ocular features that characterize them. For example, exfoliation syndrome (XFS) and related glaucoma (XFG) are defined by the accumulation of a characteristic fibrillar material on the ocular lens and trabecular meshwork (Wiggs, Janey L., and Louis R. Pasquale., 2017). Primary open-angle glaucoma (POAG), the most common type, is hereditary. Patients with POAG have glaucoma despite anatomically normal ocular structures including open angles. Like other forms of glaucoma, IOP elevation is an important risk factor for POAG, however, up to one-third of POAG patients with optic nerve degeneration have IOP in the normal range, defining normal-tension glaucoma (NTG) POAG subgroup. (Rizzo, Maria Ida, et al., 2017).

Glaucoma does not result from a single pathological mechanism, but rather a combination of pathways that are influenced by genes, age, and environment (Doucette, Lance P., et al., 2015). Elevated intraocular pressure (IOP) is a major risk factor for the development and progression of glaucoma. It is well known that intraocular pressure is not a fixed value but fluctuates over time (Kim, Ji Hyun, and Joseph Caprioli., 2018). The normal range for IOP is 10 – 20 mm Hg and is maintained at this level throughout life and between the sexes, though there is some diurnal and seasonal variation. IOP is normally regulated by changes in the volume of the aqueous humour. in the chronic setting, raised IOP may cause nerve damage at the optic nerve head leading to visual field loss. The next risk factor is the optic nerve head, which is considered to be the primary site of axonal injury in glaucoma (Wostyn, Peter., 2019).

Glaucoma risk increases with age. As a consequence, glaucoma can be expected to be associated with other age-related diseases. A Bayesian meta-analysis found that men were more likely to have Open-Angle Glaucoma with the reservation that gender influence depends on the definition of glaucoma (McMonnies, Charles W., 2017). Other risk factors include a Family history of glaucoma, thin cornea, High myopia, Diabetes, Eye surgery or injury, High blood pressure, and use of corticosteroids.

POAG is genetically complex. Linkage studies have identified over 20 genomic regions likely to contain POAG-related genes. Recent genome-wide association studies (GWAS) have identified several genetic risk factors for POAG overall, including single-nucleotide polymorphisms (SNPs) located in the *CAV1/CAV2* intergenic region, and in the genomic regions containing the *TMCO1* and *CDKN2BAS* genes in a study of glaucoma patients with advanced optic nerve disease (Wiggs, Janey L., et al., 2012).

G protein-coupled receptors (GPCRs), such as dopamine receptors, are cell membrane proteins with a seven-transmembrane structure that triggers signals within the cells, activates or inhibits specific effectors to induce cellular responses, and regulates many functions (Wang, Chen-Xi, et al., 2020).

There are five subtypes of dopamine receptors, D1, D2, D3, D4, and D5. The dopamine receptor subtypes are divided into two major subclasses: types 1 and 5 are similar in structure and drug sensitivity, and these two receptors are referred to as the "D1like" group or class of receptors. Dopamine receptor types 2, 3, and 4 are called the "D2like" group. Activation of the D1 receptor, through its association with adenylate cyclase, produces the cyclic adenosine monophosphate (cAMP) (Ayano, G. J. J. M. D. T., 2016). cAMP activates protein kinase A, followed by phosphorylation of intracellular proteins, including dopamine- and AMP-regulated phosphoprotein (DARPP-32). While phosphorylation at Thr-34 by PKA converts

DARPP-32 into a potent inhibitor of protein phosphatase 1 (PP1), phosphorylation at Thr-75 transforms DARPP-32 into an inhibitor of PKA. PP1 is involved in regulating the activity of a large number of phosphoproteins, including voltage-dependent sodium and calcium channels, the electrogenic pump Na^+, K^+-ATPase, and neurotransmitter receptors. DARPP-32-mediated inhibition of PP1 increases the phosphorylation of neurotransmitter receptors and ion channels crucial for synaptic function and plasticity (Belkhiri, Abbes, Shoumin Zhu, and Wael El-Rifai., 2016). In addition, inhibition of PP-1 increases cAMP-response element-binding protein (CREB) phosphorylation. CREB a ubiquitous transcription factor is a key molecule for learning and memory and a core component of the molecular switch that converts short-term to long-term memory (Cho, Kwangmin, et al., 2015).

Dopamine (DA) is a major neurotransmitter in the modulation of neural adaptations to light in the retina of mammals (Hirasawa, Hajime, Massimo Contini, and Elio Raviola., 2015). It has been reported that DA might change RGC spiking by regulating retinal neuronal circuits. In addition, DA might also modulate RGC activity directly (Yin, Ning, et al., 2020). During studies on dopaminergic signaling in the retina have found that dopamine modulates ciliary blood flow and aqueous production in a dose-dependent manner with a significant decrease of IOP. These findings have an important impact on glaucoma. earlier studies on the dopamine receptor have shown that dopamine has a more complex effect on aqueous production. It has been proposed that aqueous production is stimulated by the activation of D1R and inhibited by the activation of D2R (Bucolo, Claudio, et al., 2019).

D2R-like and specifically D3R agonists were found to be effective in decreasing IOP (Bucolo, Claudio, et al., 2018).

DA1 receptors seem to be more expressed by the ciliary body and the outflow pathway of aqueous humor. The administration of DA 1-selective agonists stimulates the production of aqueous humor, increasing IOP, therefore, the risk to develop glaucoma (Pescosolido, Nicola, et al., 2013).

In this study, the expression of the dopamine D1 receptor gene (*DRD1*) in glaucoma patients was evaluated using Real-time quantitative PCR (qRT-PCR).

Materials and methods:

Study subjects

The patient's group consisted of 20 glaucoma patients with the mean age of 56 years who were referred to Alzahra Eye Hospital, Zahedan, Iran, between 2019 and 2020 were enrolled. The control group included 20 individuals and an average of 60 years old. Patient consent for all samples was obtained according to the Declaration of Helsinki principles. The pathological information of all patients was obtained from the Pathology Department of this academic Hospital. Tissue samples were obtained from the trabecular meshwork and were collected in cell preparation tubes containing 1ml RNA later. Demographic information of statistical population is given in Table1.

Table 1- General demographic and clinical data of study participants.

		Control	Case
Gender	males	10	13
	females	10	7
Age	Mean±Std.deviation	60.7±12.4	55.9±18.7
	Minimum	23	3
	Maximum	90	79
TOP	Mean±Std.deviation	14.1±1.5	32.3±9.3
	Minimum	12	14
	Maximum	16	46

Total RNA extraction and cDNA synthesis

Total RNA was extracted using the Total RNA Extraction kit (ParsTous, Iran) according to the manufacturer's instructions. The light absorbance at 260, 280, 230 nm and A260/A280, A260/A230 ratios were measured using Spectrophotometer. The RNA samples were treated by reverse transcription using Easy cDNA Synthesis Kit (ParsTous, Mashhad, Iran). The primers for the *DRD1* receptor gene, and 18s rRNA gene as housekeeping gene were designed based on GenBank sequences using Gene Runner software and similar primers have been used for conventional PCR and real-time PCR (table 2).

Primer specificity was theoretically checked by BLAST database search against nucleotide reference NCBI database and experimentally

verified by the positive control amplification. To confirm the presence of the *DRD1* gene in Trabecular meshwork tissue, a common PCR technique was carried out.

Table 2- The Features of Primers used in this study.

Gene name	Nucleotide sequences	Annealing temperature (°C)	Primer length	Product length
DRD1	F: GACCTTGTCTGTACTCATCTCCT	57	23	118
	R: GTCACAGTTGTCTATGGTCTCAG	57	23	
18s rRNA	F: GTAACCCGTTGAACCCCATT	57	20	112
	R: CCATCCAATCGGTAGTAGCG	57	20	

Real-time quantitative PCR (qRT-PCR)

Real-time quantitative PCR (qRT-PCR) was performed based on the SYBR Green method (StepOnePlus™48, Applied Biosystem, USA). The total volume for the qPCR reaction was 20 mL containing 10 ml of RealQ Plus 2x Master Mix Green (Ampliqon, Denmark). The thermal profile was 15min pre-incubated at 95 °C, followed by (30s at 95 °C, 30 s at primer annealing temperature, 30 s at 72 °C) 40 cycles. Melting curve analysis ramping from 57 °C to 97 °C was done at the end of the reaction to confirm the qPCR results specificity. The mRNA expression levels of DRD1 was normalized to 18s rRNA. The sequences of the primers used for conventional PCR and real-time PCR in listed in Table 2.

Statistical analysis of data

The correlation between the changes in dopamine receptor gene expression and the items such as the age of patients and IOP amount was assessed by SPSS software (version 26.0). ΔCt values (which have a symmetrical theoretical distribution) obtained from Real-time quantitative PCR (qRT-PCR) were recalculated into relative copy number values.

Obtained results were not normally distributed (Kolmogorov-Smirnov test) and therefore nonparametric statistical tests were used for analyzing the results (Mann-Whitney U test). The relationship between the expression of *DRD1* genes and the risk of Glaucoma was determined by the Mann-Whitney U test. In the current study, the p-value less than 0.05 ($P< 0.05$) was considered statistically significant.

Results

In this work, we evaluated the influence of dopamine D1 receptor expression on patients with glaucoma. In this study, the RNA of 20 primaries open-angle glaucoma and 20 normal conjunctiva tissues was extracted and modified for the assessment of expression of the *DRD1* gene by real-time quantitative reverse transcriptase-polymerase chain reaction. The mRNA expression level results showed statistically significant differences ($P<0.05$) between cases and healthy controls for the *DRD1*. Statistically significant associations were found for *DRD1* ($P = 0.015$).

Table 3- comparison of relative gene expression for *DRD1* between patients with glaucoma and healthy control.

Gene name		N	Mean±Std. deviation	Range	P-value
DRD1	Case	20	3.01±9.33	15.13	**0.015**
	Control	20	2.06±4.02	41.07	

Discussion

According to the NCBI database, the *DRD1* gene is located on the long arm of chromosome 5 (5q35.2). This gene encodes the dopamine receptor 1 (D1). The D1 receptor stimulates adenylyl cyclase and activates c-AMP-dep protein kinases. D1 receptors regulate nerve growth and differentiation and mediate some behavioral responses. The expression of this gene may be associated with the development of glaucoma.

Although much scientific progress has been made in recent years, the genetic basis of POAG is still not fully understood, and more research is needed to identify new genes and pathways affecting glaucoma that could facilitate diagnostic and therapeutic strategies. Various molecules can regulate IOP, including dopaminergic pathways.

According to a 2000 study by Carlo Cavallotti et al, the possibility that dopamine receptor D1 plays a role in controlling uveoscleral tissue functions is suggested (Cavallotti, Carlo, et al., 1999).

From the studies in 2013 carried out on animals and humans, discussed previously, we can conclude that DA2 and DA3 agonists have an important influence on the modulation of IOP, with significant implications for the management of patients affected by GL (Pescosolido, Nicola, et al., 2013).

These authors studied the effects on the IOP of D1 receptors and demonstrated that the blockade of D1 receptors induces an IOP-lowering action.

In a 1998 study by C.Prünte et al. on dopaminergic effects on IOhe results suggest that both D1 and D2 receptors each play an independent role in the regulation of IOP in rabbits. The authors studied the effects on the IOP of D1 receptors and demonstrated that the blockade of D1 receptors induces an IOP-lowering action. Thus, simultaneous blockade of D1 receptors and stimulation of D2 receptors may provide a new pharmacological approach for the treatment of ocular hypertension frequently associated with glaucoma (Prünte, C., et al., 1997).

A 2017 study was examined on the effects of different lighting exposures on the expression of dopamine receptors in rat retinal precursor cells. These cells were exposed to darkness and high light and then total RNA was isolated and the expression of dopamine receptors was measured by Real-time quantitative PCR (qRT-PCR). Immunofluorescence microscopy showed increased transcription of the *DRD1* gene in light. These results suggest that dopamine receptors in the retinal cells might actively respond to the environmental lighting to act as an important player in the activation of the dopaminergic system in the ocular structures relevant to the lighting-induced pathogenic development of myopia (Ke, Yan, et al., 2017).

The secretory function of dopamine was determined by studies performed by Richard Stone et al. On the non-pigmented epithelium of the ciliary body of animals and humans. DARPP-32 acts as a third messenger to mediate some of the physiological effects of dopamine at the D-1 receptor. Dopamine is involved in aqueous humor production by regulating the phosphorylation of DARPP-32 (Stone, RICHARD A., et al., 1986).

Karnezis, Tom A, et al. examined the effects of norepinephrine, angiotensin II and fenoldopam, a selective D1 receptor agonist, on intraocular pressure, in eight healthy human volunteers. Data suggest that DA1 receptor activation can modulate intraocular pressure (Karnezis, Tom A., et al., 1988). The analysis of DRD1 gene expression patient with glaucoma has been studied for the first time worldwide in this study. According to the studies that have been done in the past and according to the current study, the importance of the role of this gene has been determined to a large extent, but to confirm the current results, it is recommended to perform a test at a large statistical level.

Conclusion

Based on the results of previous studies, and considering that a study on the relationship between *DRD1* gene expression and glaucoma has not been performed in humans or any organisms, the results of this experiment cannot be compared. However, previous studies have shown that IOP levels increase during dopamine D1 receptor activation by its associated agonists, leading to glaucoma. Also, according to the molecular pathway previously mentioned, by increasing the amount of cAMP due to stimulation of D1 receptors, the amount of aqueous humor flow increases by activating Na^+ / K^+ channels and affects the intraocular pressure. As a result, our studies, which show a significant association between DRD1 gene expression and glaucoma expression, are valid. However, it is better to do more studies in this field with the larger statistical community.

Acknowledgments

This research was financially supported by the Sistan and Baluchistan University in Iran. This study was carried out with the assistance of Mrs. Parnian Zare Dastjerdi as well as with the cooperation of Alzahra Eye Hospital. Hereby we appreciate their valuable contribution to this survey.

References

Kwon, S., Kim, S. H., Khang, D., & Lee, J. Y. (2020). Potential therapeutic usage of nanomedicine for glaucoma treatment. Int. J. Nanomed, 15, 5745.

Arora, K. S., Jefferys, J. L., Maul, E. A., & Quigley, H. A. (2012). Choroidal thickness change after water drinking is greater in angle closure than in open-angle eyes. IOVS, 53(10), 6393-6402.

Juneja, M., Thakur, S., Wani, A., Uniyal, A., Thakur, N., & Jindal, P. (2020). DC-Gnet for detection of glaucoma in retinal fundus imaging. Mach Vis Appl, 31, 1-14.

Souzeau, E., Tram, K. H., Witney, M., Ruddle, J. B., Graham, S. L., Healey, P. R., & Craig, J. E. (2017). Myocilin predictive genetic testing for primary open-angle glaucoma leads to the early identification of at-risk individuals. OPHTDG, 124(3), 303-309.

Wentz, S. M., Kim, N. J., Wang, J., Amireskandari, A., Siesky, B., & Harris, A. (2014). Novel therapies for open-angle glaucoma. F1000 Med. Rep, 6.

Rizzo, M. I., Greco, A., De Virgilio, A., Gallo, A., Taverniti, L., Fusconi, M. & De Vincentiis, M. (2017). Glaucoma: recent advances in the involvement of autoimmunity. Immunol. Res., 65(1), 207-217.

Wiggs, J. L., & Pasquale, L. R. (2017). Genetics of glaucoma. Human molecular genetics, 26(R1), R21-R27.

Doucette, L. P., Rasnitsyn, A., Seifi, M., & Walter, M. A. (2015). The interactions of genes, age, and environment in glaucoma pathogenesis. Surv. Ophthalmol., 60(4), 310-326.

Kim, J. H., & Caprioli, J. (2018). Intraocular pressure fluctuation: is it important? JOVR, 13(2), 170.

Wostyn, P. (2019). Glaucoma is a dangerous interplay between ocular fluid and cerebrospinal fluid. Med. Hypotheses, 127, 97-99.

McMonnies, C. W. (2017). Glaucoma history and risk factors. J. Optom., 10(2), 71-78.

Wiggs, J. L., Yaspan, B. L., Hauser, M. A., Kang, J. H., Allingham, R. R., Olson, L. M., ... & Haines, J. L. (2012). Common variants at 9p21 and 8q22 are associated with increased susceptibility to optic nerve degeneration in glaucoma. PLoS Genet., 8(4), e1002654.

Wang, C. X., Ge, X. Y., Wang, M. Y., Ma, T., Zhang, Y., & Lin, Y. (2020). Dopamine D1 receptor-mediated activation of the ERK signaling pathway is involved in the osteogenic differentiation of bone mesenchymal stem cells. Stem Cell Res. Ther., 11(1), 1-13.

Ayano, G. J. J. M. D. T. (2016). Dopamine: receptors, functions, synthesis, pathways, locations and mental disorders: a review of literatur. J Ment Disord Treat, 2(120), 2.

Belkhiri, A., Zhu, S., & El-Rifai, W. (2016). DARPP-32: from neurotransmission to cancer. Oncotarget, 7(14), 17631.

Cho, K., Cho, M. H., Seo, J. H., Peak, J., Kong, K. H., Yoon, S. Y., & Kim, D. H. (2015). Calpain-mediated cleavage of DARPP-32 in A lzheimer's deisease. Aging cell, 14(5), 878-886.

Hirasawa, H., Contini, M., & Raviola, E. (2015). Extrasynaptic release of GABA and dopamine by retinal dopaminergic neurons. Philos. Trans. R. Soc. Lond., B, Biol. Sci. PHILOS T R SOC B, 370(1672), 20140186.

Yin, N., Yang, Y. L., Cheng, S., Wang, H. N., Hu, X., Miao, Y., & Wang, Z. (2020). Dopamine D2 receptor-mediated modulation of rat retinal ganglion cell excitability. Neurosci. Bull., 36(3), 230-242.

Bucolo, C., Leggio, G. M., Drago, F., & Salomone, S. (2019). Dopamine outside the brain: The eye, cardiovascular system, and endocrine pancreas. Pharmacol. Ther., 203, 107392.

Bucolo, C., Platania, C., Drago, F., Bonfiglio, V., Reibaldi, M., Avitabile, T., & Uva, M. (2018). Novel therapeutics in glaucoma management. *Curr. Neuropharmacol.*, 16(7), 978-992.

Pescosolido, N., Parisi, F., Russo, P., Buomprisco, G., & Nebbioso, M. (2013). Role of dopaminergic receptors in glaucomatous disease modulation. Biomed Res. Int., 2013.

Cavallotti, C., Pescosolido, N., Pescosolido, V., & Iannetti, G. (1999). Determination of dopamine D1 receptors in the human uveo scleral tissue by light microscope autoradiography. Int. Ophthalmol., 23(3), 171-179.

Pescosolido, N., Parisi, F., Russo, P., Buomprisco, G., & Nebbioso, M. (2013). Role of dopaminergic receptors in glaucomatous disease modulation. Biomed Res. Int., 2013.

Prünte, C., Nuttli, I., Markstein, R., & Kohler, C. (1997). Effects of dopamine D-1 and D-2 receptors on intraocular pressure in conscious rabbits. J. Neural Transm. Suppl., 104(2-3), 111-123.

Ke, Y., Li, W., Tan, Z., & Yang, Z. (2017). Induction of dopamine D1 and D5 receptors in R28 cells by light exposures. Biochem. Biophys. Res. Commun., 486(3), 686-692.

Stone, R. A., Laties, A. M., Hemmings Jr, H. C., Ouimet, C. C., & Greengard, P. A. U. L. (1986). DARPP-32 in the ciliary epithelium of the eye: a neurotransmitter-regulated phosphoprotein of brain localizes to secretory cells. J. Histochem. Cytochem., 34(11), 1465-1468.

Karnezis, T. A., Murphy, M. B., Weber, R. R., Nelson, K. S., Tripathi, B. J., & Tripathi, R. C. (1988). Effects of selective dopamine-1 receptor activation on intraocular pressure in man. Exp. Eye Res., 47(5), 689-697.