Investigation of *ARHGEF12* Single Nucleotide Polymorphism in Hypercholesterolemia and Primary Open Angle Glaucoma

ARHGEF12 Tek Nükleotid Polimorfizminin Hiperkolesterolemi ve Primer Açık Açılı Glokomda Araştırılması

Derya Yaman[1], Tamer Takmaz[2], Selin Akad Dinçer[3], Feride İffet Şahin[3]

[1] Kağızman State Hospital, Department of Ophthalmology, Kars, Turkey
[2] Ankara City Hospital, Department of Ophthalmology, Ankara, Turkey
[3] Başkent University Faculty of Medicine, Department of Medical Genetics, Ankara, Turkey

ABSTRACT

Objective: To investigate the effect of single nucleotide polymorphism rs58073046 A>G within the *ARHGEF12* gene in patients with hypercholesterolemia and primary open angle glaucoma.
Methods: Blood samples of 20 patients with high serum cholesterol and primary open angle glaucoma (Group 1), 20 sex and age matched healthy subjects (Group 2) as controls were enrolled to the study. The *ARHGEF12* gene polymorphism was determined by polymerase chain reaction and DNA sequence analysis. The data were assessed by descriptive statics and Fisher exact x^2 test.
Results: The homozygous wild type genotype (AA) was identified in 95 % of Group 1 versus 100 % of Group 2. The homozygous mutant genotype (GG), presented the highest prevelance in Group 1 (5%), although the difference was not statistically significant between groups (p=0.5).
Conclusion: This is the first study to identify the role of *ARHGEF12* gene variant in the risk of hypercholesterolemia and POAG. Our results showed that there is no association between rs58073046 A>G polymorphism and disease development.

Key Words: *ARHGEF12*, DNA sequence, hypercholesterolemia, primary open angle glaucoma, polymorphism, rs58073046

Received: 03.08.2020 **Accepted:** 07.02.2020

ÖZET

Amaç: Hiperkolesterolemili ve primer açık açılı glokomlu hastalarda *ARHGEF12* genindeki rs58073046 A> G tek nükleotid polimorfizminin etkisini araştırmak.
Yöntem: Yüksek serum kolesterolü ve primer açık açılı glokomu olan 20 hastanın (Grup 1) ve kontrol grubu olarak cinsiyet ile yaş uyumlu 20 sağlıklı olgunun (Grup 2) kan örnekleri çalışmaya dahil edildi. *ARHGEF12* gen polimorfizmi, polimeraz zincir reaksiyonu ve DNA sekans analizi ile belirlendi. Veriler tanımlayıcı statik ve Fisher exact x2 testi ile değerlendirildi.
Bulgular: Homozigot yabani tip genotip (AA) Grup 2' nin tamamına karşı, Grup 1'in % 95'inde tanımlandı. Homozigot mutant genotip (GG), Grup 1'de en yüksek prevalansı (% 5) sunarken, fark istatistiksel olarak gruplar arasında anlamlı değildi (p = 0.5).
Sonuç: Bu, *ARHGEF12* gen varyantının hiperkolesterolemi ve POAG riskindeki rolünü tanımlayan ilk çalışmadır. Sonuçlarımız, rs58073046 A> G polimorfizmi ile hastalık gelişimi arasında bir ilişki olmadığını göstermiştir.

Anahtar Sözcükler: *ARHGEF12*, DNA sekansı, hiperkolesterolemi, primer açık açılı glokom, polimorfizm, rs58073046

Geliş Tarihi: 03.08.2020 **Kabul Tarihi:** 07.02.2020

The study was previously presented as a poster at the congress of V. Erciyes Tıp Genetik Günleri,2020.
ORCID IDs: D.Y.0000-0002-6425-9105, T.T. 0000-0001-9494-8756, S.A.D. 0000-0002-3808-7004, F.İ.Ş.: 0000-0001-7308-9673

Address for Correspondence / Yazışma Adresi: Derya Yaman, MD Kağızman State Hospital, Department of Ophthalmology, Kars, Turkey E-mail: deryayaman89@hotmail.com

doi:http://dx.doi.org/10.12996/gmj.2020.142

INTRODUCTION

Primary open angle glaucoma (POAG) is one of the most prevalent type of optic neuropathy leading to irreversible visual loss (1), and usually caused by a building of pressure in the eye. Genome wide association studies (GWAS) have identified multiple loci for introcular pressure (IOP) homeostasis (2-6). One such downstream target is the *ARHGEF12* gene, a member of the Rho guanine nucleotide exchange factors (RhoGEFs) (7).

The *ARHGEF12* gene which is involved in activation of the GTP-dependent RhoA activity, is located on the long (q) arm of chromosome 11 at position 23.3. RhoA pathways coordinate cell skeletal dynamics, tissue remodelling and plasticity of trabecular meshwork (TM) (7-9). Although, the clinical importance is still limited in GWAS, the single nucleotide polymorphism (SNP) rs58073046 on chromosome 11, a variant in the first intronic region of the *ARHGEF12* gene, has been reported to be significantly correlated with conventinal aqueous outflow pathway and IOP levels (7). Interestingly, *ARHGEF12* is also central for cholesterol efflux capacity via stabilization of ABCA1 protein which is involved in lipid metabolism (10).

Furthermore, research indicates the positively correlation between the high serum level of lipid parameters and glaucomatous optic neuropathy (11-13).

There are various data about the effects of statins on glaucomatous neurotoxicity, which are medications used to lower cholesterol in patients with hyperlipidemia (11,12).

In view of this, we aimed to investigate a possible association of the SNP rs58073046 on patients with hypercholesterolemia and POAG.

METHODS

In this study, the patients were divided into 2 groups: Group 1, consisting of 20 patients receiving primary open angle glaucoma treatment with statin use for hypercholesterolemia, and Group 2, consisting of 20 age and sex- matched healty controls living in the same region. The participants were enrolled after verbal and written informed consent. The study was approved by the Ethic Committee of Baskent University, and carried out compatible with the Declaration of Helsinki. The diagnosis of POAG was based on biomicroscopic, gonioscopic examination and visual field test. Patients who had a history of ocular surgery before the diagnosis of POAG were excluded. The genomic DNA was extracted from peripheral blood using the commercial genomic DNA Purification Kit (Invitrogen®, USA). The SNP rs58073046 was detected using PCR- DNA Sequence Analyzer (Applied Biosystems 3500). The primer sequences are listed in Table 1.

Table 1: Primer sequences of genetic polymorphism

Genetic Polymorphism	Primer Sequences
rs58073046	F 5'ATACTTTTCAGATGCATCCAAATTG 3'
(*ARHGEF12*)	R 5' TGACTCAGCAATTCTACTCTGAGATG 3'

Statistics

The data were analyzed by SPSS 18 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used for the demographic characteristics. Quantitative results are shown as means ± standard deviation (SD). The frequency of the genotypes in patients and controls was compared using Fisher's exact and chi-square test, and $p<0.05$ was considered significant.

RESULTS

There were no significant differences between the groups in terms of age and gender. The mean age was 56.65 ± 2.75 years (range 52-61 years) in Group 1, and 56.90 ± 4.01 years (range 50-64) in Group 2 (p=0.79). The gender distribution was similar for both groups (p=1.0, Table 2).The clinical data among groups was shown in table 2.

Table 2: Comparison of clinical data between groups
P values were calculated by chi-square and Fisher's exact test.

	Group 1 (n=20)	Group 2 (n=20)	p value*
Mean Age (± SD) (years)	56.65 ± 2.75 (52-61)	56.90 ± 4.01 (50-64)	0.79
Sex (n)(Female/ Male)	9/11	10/10	1.0
Mean IOP (± SD) (mm Hg)	17.76 ± 2.85 (14-24)	15.74 ± 1.90 (12-19)	0.42
Mean Serum Cholesterol Levels (mg/dl)	222.14 ± 24.16 (160-270)	167.58 ± 20.86 (130-200)	**<0.001***

The homozygous mutant genotype (GG) was only found in one patient (5%) of Group 1, whereas homozygous wild typegenotype (AA) was present in 20 subjects (100%) of Group 2 (Table 3, Figure 1).

There was no significant association between homozygous mutant genotype (GG) and the risk of hyperlipidemia and POAG (p=0.5). None of the subjects were seen to be heterozygous for the SNP rs58073046.

Table 3: Allele and genotype frequencies of SNP rs58073046 in the *ARHGEF12* gene.

	Group 1(n=20)	Group 2 (n=20)	p value*
Genotype			
AA	19	20	0.5
GG	1	0	
Allele			
A	38	40	0.48
G	2	0	

P values were calculated based on the chi-square test and Fisher's exact test.

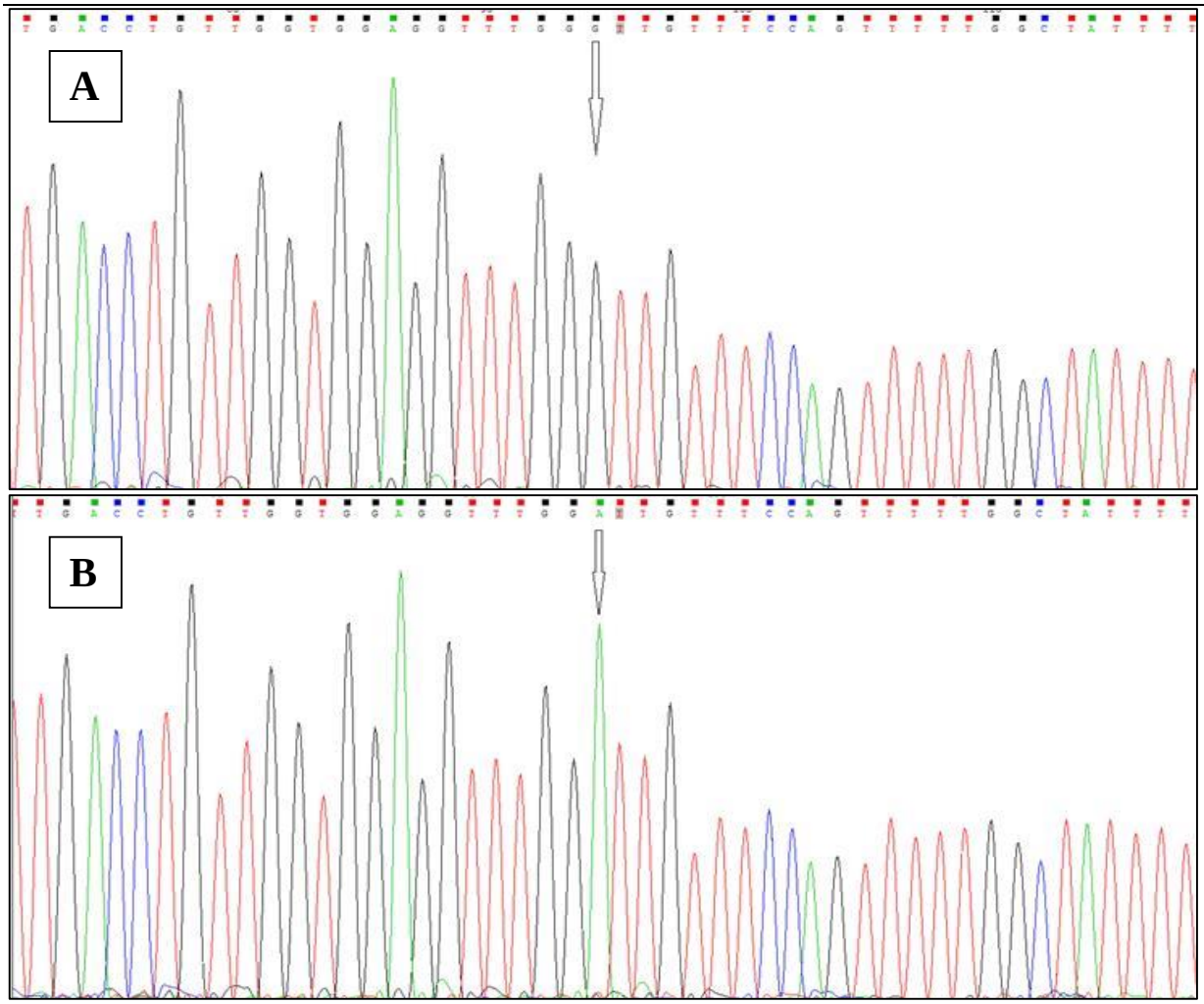

Figure 1: Electropherograms of mutant and wt sequence for rs58073046. **A)** Electropherogram homozygous mutant (GG) genotype, **B)** Electropherogram for wt (AA) genotype.

DISCUSSION

Recent decades have seen a profound transformation in the understanding of the complex pathophysiology of glaucoma, with the evolution of new treatment modalities that move beyond purely IOP control to try to mitigate vascular and extracellular matrix changes that increase aqueous outflow resistance (14-17).

One of the currently researched treatment target is *ARHGEF12* induced RhoA/ROCK pathway, which is highly expressed in the iridocorneal angle components, retina and optic nerve (7,18,19). The *ARHGEF12* gene plays a crucial role in activation of the RhoA and ROCK pathway which can modulate stress fiber reorientation responses of TM and glaucomatous neurotoxicity (20-24). However, there are limited numbers of studies related to the variants in the *ARHGEF12* gene in human diseases (25-29). Springelkamp and collagues reported that *ARHGEF12* has been suggestively associated with IOP homeostasis (P=1.87x10-8 for rs rs58073046) (7) .

In addition, the association of hyperlipidemia and glaucoma has been crucial in recent times. Ye and colleagues found that high serum lipid parameters are assoiated with blood viscosity and high episcleral venous pressure (30). Also, several human studies have reported the clinical importance of RhoA/ROCK inhibition by statins in glaucoma prognosis (11, 31, 32).

However, in this case-control study, we have shown that the rs58073046 A>G polymorphism within the *ARHGEF12* gene was not associated with the risk of POAG and hyperlipidemia. We mainly identified wild type genotype (AA) in patients with hyperlipidemia and POAG, indicating this polymorphism has a very low minor allele frequency (7). An explanation may be that the allele and genotype frequency affect from ethnic difference.

This study has strengths and limitations. Of particular strength was the careful diagnosis of subjects, the strict criteria for healthy controls. Also, to our knowledge, this is the first study to investigate the relationship of the *ARHGEF12* gene polymorphism with the risk of hypercholesterolemia and POAG. The limitations of the present study include small sample size. Additionally, there may be a selectian bias due to the clinic-based case- control study.

In conclusion, we found no association between the SNP rs58073046 and disease profile. Future genetic studies in larger groups are also required to clarify the role of the *ARHGEF12* gene in POAG and hyperlipidemia.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Kwon YH, Fingert JH. Kuehn, MH, Alward,WL. Primary open-angle glaucoma. N. Engl. J. Med. 2009;360:1113–1124.

2. Hysi PG, Cheng CY, Springelkamp H, Macgregor S, Bailey JN, Wojciechowski R, et al. Genome-wide analysis of multi-ancestry cohorts identifies new loci influencing intraocular pressure and susceptibility to glaucoma. Nat Genet. 2014; 46: 1126-1130.

3. Nag A, Venturini C, Small KS, International Glaucoma Genetics C, Young TL, Viswanathan AC, et al. A genome-wide association study of intra-ocular pressure suggests a novel association in the gene *FAM125B* in the TwinsUK cohort. Hum Mol Genet. 2014; 23: 3343-3348.

4. Chen F, Klein AP, Klein BEK, Lee KE, Truitt B,Klein R, et al. Exome array analysis identifies CAV1/2 as a susceptibility locus for intraocular pressure. Invest. Ophthalmol. Vis. Sci. 2014;56:544-551.
5. van Koolwijk LM, Ramdas WD, Ikram MK, Jansonius NM, Pasutto F, Hysi PG, et al. Common genetic determinants of intraocular pressure and primary open-angle glaucoma. PLoS Genet. 2012;8:e1002611.
6. Fingert JH. Primary open-angle glaucoma genes. Eye (Lond) 2011;25:587–595.
7. Springelkamp H, Iglesias AI, Cuellar-Partida G, Amin N, Burdon KP, van Leeuwen EM, et al. *ARHGEF12* influences the risk of glaucoma by increasing intraocular pressure. Hum Mol Genet. 2015 May 1;24(9):2689-99.
8. Kumar J, Epstein DL. Rho GTPase-mediated cytoskeletal organization in Schlemm's canal cells play a critical role in the regulation of aqueous humor outflow facility. J. Cell. Biochem., 2011;112:600–606.
9. Gasmi-Seabrook GM, Marshall CB, Cheung M, Kim B, Wang F, Jang YC, et al. Real- time NMR study of guanine nucleotide Exchange and activation of RhoA by PDZ-RhoGEF. J Biol Chem. 2010;285(8):5137-45.
10. Okuhira K, Fitzgerald ML, Tamehiro N, Ohoka N, Suzuki K, Sawada J, et al. Binding of PDZ-RhoGEF to ATP-binding cassette transporter. A1 (ABCA1) induces cholesterol efflux through RhoA activation and prevention of transporter degradation J. Biol.Chem. 2010; 285, 16369–16377.
11. Stein JD, Newman-Casey PA, Talwar N, Nan B, Richards JE, Musch DC. The relationship between statin use and open angle glaucoma. Ophthalmology. 2012;119:2074–2081.
12. Chen HY, Hsu SY, Chang YC, Lin CC, Sung FC, Chen WC, et al. Association between statin use and open-angle glaucoma in hyperlipidemia patients: a Taiwanese population-based case-control study. Medicine (Baltimore). 2015;94:e2018.
13. Wang S, Bao X. Hyperlipidemia, Blood Lipid Level, and the Risk of Glaucoma: A Meta-Analysis. Invest Ophthalmol Vis Sci. 2019 Mar 1;60(4):1028-1043.
14. Wiederholt M, Thieme H,Stumpff F. The regulation of trabecular meshwork and ciliary muscle contractility. Prog Retin Eye Res. 200;19(3):271-95.
15. Stamer WD, Acott TS. Current understanding of conventional outflow dysfunction in glaucoma. Curr Opin Ophthalmol. 2012;23(2):135-43.
16. Sacca SC, Gandolfi S, Bagnis A, Manni G, Damonte G, Traverso CE, et al. From DNA damage to functional changes of the trabecular meshwork in aging anf glaucoma. Ageing Res Rev. 2016;29:26-41.
17. Pertl L, Mossböck G, Wedrich A, Weger M, Königsbrügge O, Silbernagel G, et al. Triglycerides and open angle glaucoma-A meta-analysis with meta-regression. Sci Rep. 2017;7:7829–37.
18. Goldhagen B, Proia AD, Epstein DL, Rao PV. Elevated levels of RhoA in the optic nerve head of human eyes with glaucoma. J Glaucoma. 2012 Oct-Nov;21(8):530-8. doi: 10.1097/IJG.0b013e318241b83c.
19. Wang J, Liu X, Zhong Y. Rho/Rho-associated kinase pathway in glaucoma (Review). Int J Oncol. 2013 Nov;43(5):1357-67. doi: 10.3892/ijo.2013.2100. Epub 2013 Sep 16.
20. Ren R, Li G, Le TD, Kopczynski C, Stamer WD, Gong H. Netarsudil Increases Outflow Facility in Human Eyes Through Multiple Mechanisms. Invest Ophthalmol Vis Sci. 2016 Nov 1;57(14):6197-6209. doi: 10.1167/iovs.16-20189.
21. Sturdivant JM, Royalty SM, Lin CW, Moore LA, Yingling JD, Laethem CL,et al. Discovery of the ROCK inhibitor netarsudil for the treatment of open-angle glaucoma. Bioorg Med Chem Lett. 2016 May 15;26(10):2475-2480.
22. Song J, Deng PF, Stinnett SS, Epstein DL, Rao PV. Effects of cholesterol-lowering statins on the aqueous humor outflow pathway. Investigative ophthalmology & visual science. 2005; 46:2424–2432.
23. Iden S, Collard JG. Crosstalk between small GTPases and polarity proteins in cell polarization. Nat. Rev. Mol. Cell Biol. 2008; 9, 846–859.
24. Iglesias AI, Springelkamp H, Ramdas WD, Klaver CC, Willemsen R, van Duijn CM. Genes, pathways, and animal models in primary open-angle glaucoma. Eye (Lond). 2015 Oct;29(10):1285-98. doi: 10.1038/eye.2015.160. Epub 2015 Aug 28.
25. Holzapfel C, Klopp N, Grallert H, Huth C, Gieger C, Meisinger C, et al. Genetic variants in the leukemia-associated Rho guanine nucleotide exchange factor (*ARHGEF12*) gene are not associated with T2DM and related parameters in Caucasians (KORA study). Eur J Endocrinol. 2007 Sep;157(3):R1-5.
26. Mabuchi F, Mabuchi N, Sakurada Y, Yoneyama S, Kashiwagi K, Iijima H, et al. Additive effects of genetic variants associated with intraocular pressure in primary open-angle glaucoma. PLoS One. 2017 Aug 23;12(8):e0183709. doi: 10.1371/journal.pone.0183709. eCollection 2017.
27. Kovacs P, Stumvoll M, Bogardus C, Hanson RL, Baier LJ. A functional Tyr1306Cys variant in LARG is associated with increased insulin action in vivo. Diabetes. 2006 May;55(5):1497-503.
28. Xie Y, Gao L, Xu C, Chu L, Gao L, Wu R, et al. *ARHGEF12* regulates erythropoiesis and is involved in erythroid regeneration after chemotherapy in acute lymphoblastic leukemia patients. Haematologica. 2019 Aug 29. pii: haematol.2018.210286. doi: 10.3324/haematol.2018.210286.
29. Dirse V, Bertasiute A, Gineikiene E, Zvirblis T, Dambrauskiene R, Gerbutavicius R, et al. A population-based single nucleotide polymorphism array analysis of genomic aberrations in younger adult acute lymphoblastic leukemia patients. Genes Chromosomes Cancer. 2015 May;54(5):326-33.
30. Ye S, Chang Y, Kim CW, Kwon MJ, Choi Y, Ahn J, et al. Intraocular pressure and coronary artery calcification in asymptomatic men and women. Br J Ophthalmol. 2015;99:932–6.
31. Rasoulinejad SA, Kasiri A, Montazeri M, Rashidi N, Montazeri M, Hedayati H. The association between primary open angle glaucoma and clustered components of metabolic syndrome. Open Ophthalmol J. 2015;9:149–55.
32. Marcus MW, Muskens RP, Ramdas WD, Wolfs RC, De Jong PT, Vingerling JRet al. Cholesterollowering drugs and incident open-angle glaucoma: a population-based cohort study. PLoS One. 2012;7:e29724