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

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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 x² 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 prevalence 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 çalışılmaya 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 1’in %95’inde tanımlandı. Homozigot mutant genotip (GG), Grup 1’in %5’inde tanımlandı. Homozigot mutant genotip (GG), Grup 1’de en yüksek prevalans (%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 Sözcüklər: ARHGEF12, DNA sekansi, hiperkolesterolemi, primer açık açılı glokom, polimorfizm, rs58073046

Geilş Tarihi: 03.08.2020 Kabul Tarihi: 07.02.2020
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 intratrocular 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 conventional 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).

Table 1: Primer sequences of genetic polymorphism

| Genetic Polymorphism | Primer Sequences |
|----------------------|-----------------|
| rs58073046 (ARHGEF12) | F 5’ATACTTTTCAGATGCATCCAAATTG 3’ |
|                       | 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.

Table 2: Comparison of clinical data between groups

|                      | 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).

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

| Genotype | Group 1(n=20) | Group 2 (n=20) | p value* |
|----------|---------------|----------------|----------|
| AA       | 19            | 20             | 0.5      |
| GG       | 1             | 0              |          |

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.

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.

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 healthy controls living in the same region. The participants were enrolled after verbal and written informed consent. The study was approved by the Ethnic 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.
**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 targets 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 colleagues reported that ARHGEF12 has been suggestively associated with IOP homeostasis (P=1.87x10^-8 for 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 associated 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 selection 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.

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