Proceedings

**RHO Gene Polymorphisms in Patients with Pterygium †**

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**Abstract:** Pterygium is one of the most common ocular surface diseases, and characterized by inflammatory infiltrates, proliferation, fibrosis, angiogenesis, and extracellular matrix breakdown. We investigated the association of polymorphisms in the RHO genes RHOA, RHOB, RHOC, RHOD, and RND3 (RHOE). The results of this study demonstrate for the first time the association of RHO genes with the pterygium. We displayed that the RHO gene polymorphisms were significantly associated with pterygium in the Turkish population.

**Keywords:** polymorphism; pterygium; Rho proteins

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1. **Introduction**

Pterygium, one of the most common eye disorders, is the growth of subepithelial fibrovascular tissue starting from the bulbar conjunctiva onto the cornea [1]. It is characterized by an altered basal epithelial cell proliferation, vascularization, and invasion of the adjacent corneal epithelium. Independent risk factors include age, male sex, rural life, and exposure to sun or ultraviolet (UV) light [2]. The pathogenesis of this disease remains unclear, but evidences suggest that UV light is a major contributor in the formation of pterygia [3]. Genetic and environmental factors are important in the etiology of pterygium. Genetic factors are thought to have a role in the development of pterygium, but the most of the molecular mechanisms leading to pterygium are still unknown. Pterygium formation impairs vision, limits eye movements, and can cause eye irritation, foreign body sensation, and dryness. Currently, surgery is the only effective treatment for pterygium, but recurrences are common [4]. Rho proteins are members of the Ras superfamily of small GTPases that play critical roles in many cellular processes including actin dynamics, vesicular trafficking, gene transcription, cytoskeletal reorganization, cell-cycle progression, differentiation, adhesion, and migration [5]. These proteins also contribute to pathological processes such as cancer cell migration, invasion, metastasis, fibrosis, inflammation, angiogenesis, and wound repair. Rho proteins consist of a family of 20 intracellular signaling molecules including RhoA, RhoB, RhoC, RhoD, and RhoE [6].
Involvement of the genetic variants of \( RHO \) genes in pterygium development has not been examined yet. Therefore, the purpose of the present study was to investigate possible associations between \( RHO \) gene polymorphisms and pterygium in a Turkish population.

2. Experimental Procedure

A total of 379 patients, diagnosed as pterygium at the Ophthalmology Clinic of the Gaziantep University Hospital and 324 unrelated healthy controls, were included to this study. Routine ophthalmologic evaluations were performed on all subjects. A control group of age- and sex-matched individuals was chosen randomly from a sample of patients admitted to the ophthalmology outpatient department for routine ophthalmic examinations, refractive errors, blepharitis, conjunctivitis, burning, itching, presbyopia, or senile cataracts. This study was approved by the local Ethics Committee. Peripheral venous blood samples (5 mL) were obtained and collected in sterile siliconized vacutainer tubes with 2 mg/mL disodium ethylenediaminetetraacetic acid. Genomic DNA was extracted from whole peripheral blood samples with salting-out method and stored at \(-20^\circ C\) until analysis [7]. Genomic DNAs were analyzed in all patients and controls using the dynamic array system (Fluidigm, South San Francisco, CA, USA) as previously described [8]. A 96.96 dynamic array on the BioMark HD system (Fluidigm, South San Francisco, CA, USA) was used for detection of polymorphism. The Digital PCR Analysis software (Fluidigm, South San Francisco, CA, USA) was used to process the data after the reaction. In the present study, 15 single nucleotide polymorphisms (SNP) were assayed. For calculation of the significance of differences in allele frequencies, the Chi-square test (with Yate’s correction) or Fisher’s exact test was used. A statistical comparison of two groups was performed with unpaired Student t-test. The original significance level was set at a p value of 0.05. Bonferroni correction for multiple testing was used for polymorphism studies, and a P value of <0.0033 (0.05/15) was considered statistically significant.

3. Results

The mean age and gender of the patients (50.9 ± 12.4 years, 51.5% female) and control (52.1 ± 14.7 years, 51.9% female) groups were similar (\( p > 0.05 \)). Table 1 shows the distributions of genotypes and alleles between the case and control groups. There were marked associations for the genotype frequencies of the \( RHOA \) gene rs6784820 polymorphism between the patients and the control group. Both genotype and allele frequencies of \( RHOB \) gene rs2602160, rs62121967, rs1541350 SNPs, \( RHO \) gene rs2306937 and rs11102522 SNPs, \( RHOD \) gene rs61891303 and rs7112925 SNPs, and \( RND3 \) (\( RHOE \)) gene rs13418763 and rs1441982 SNPs were significantly associated with pterygium. There were no marked associations with the other 5 \( RHO \) polymorphisms (\( RhoA \) gene rs2177268, rs2878298 SNPs, \( RhoC \) gene rs2999156 SNP, \( RhoD \) gene rs3923903 SNP, \( RhoE \) gene rs1528428 SNP) studied (Table 1, \( p > 0.0033 \)).

| Gene SNP  | Genotype/Allele | Controls | Pterygium | \( n \) | \( p \) |
|-----------|-----------------|----------|-----------|------|------|
| \( RHOA \) rs2177268 | TT/TA/AA       | 165/118/28 | 210/139/30 | 311 | 0.7834 |
| \( RHOA \) rs6784820 | AA/AG/GG      | 55/205/51  | 34/284/61  | 311 | 0.0024 |
| \( RHOB \) rs2602160 | AA/AG/GG      | 115/123/72 | 153/186/37 | 310 | 0.0001 |
| \( RHOB \) rs62121967 | GG/GT/TT      | 311/0/13   | 379/0/0   | 324 | <0.0001 |
| \( RHO \) rs1541350 | CC/CA/AA      | 307/120/0  | 377/0/0   | 319 | <0.0001 |
4. Discussion

This is the first study to examine the association of the RHO gene polymorphisms with the risk of developing pterygium. In this case-control study, we showed that the RHO gene polymorphisms were significantly associated with pterygium in the Turkish population. Therefore, RHO gene variants could be a risk factor for developing pterygium, and may increase susceptibility to pterygium in Turkish patients.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bradley, J.C.; Yang, W.; Bradley, R.H.; Reid, T.W.; Schwab, I.R. The science of pterygia. *Br. J. Ophthalmol.* 2010, 94, 815–820, doi:10.1136/bjo.2008.151852.

2. McCarty, C.A.; Fu, C.L.; Taylor, H.R. Epidemiology of pterygium in Victoria, Australia. *Br. J. Ophthalmol.* 2000, 84, 289–292.

3. Cárdenas-Cantú, E.; Zavala, J.; Valenziuela, J.; Valdez-Garcia, J.E. Molecular basis of pterygium development. *Semin. Ophthalmol.* 2016, 31, 567–583, doi:10.1080/08820538.2014.971822.

4. Clearfield, E.; Muthappan, V.; Wang, X.; Kuo, I.C. Conjunctival autograft for pterygium. *Cochrane Database Syst. Rev.* 2016, 2, CD011349, doi:10.1002/14651858.CD011349.pub2.

5. Parri, M.; Chiarugi, P. Rac and Rho GTPases in cancer cell motility control. *Cell. Commun. Signal.* 2010, 8, 23, doi:10.1186/1478-811X-8-23.

6. Vega, F.M.; Ridley, A.J. Rho GTPases in cancer cell biology. *FEBS Lett.* 2008, 582, 2093–2101, doi:10.1016/j.febslet.2008.04.039.

7. Miller, S.A.; Dykes, D.D.; Polesk, H.F. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988, 16, 1215.

8. Saracaloglu, A.; Demiryurek, S.; Okumus, S.; Oztuzcu, S.; Bozgeyik, I.; Coskun, E.; Aksoy, U.; Kaydu, E.; Erbagci, I.; Güler, B.; et al. Toward novel diagnostics for primary open-angle glaucoma? An association study of polymorphic variation in Ras homolog family member (A, B, C, D) genes RHOA, RHOB, RHOC, and RHOD. *OMICS* 2016, 20, 290–295, doi:10.1089/omi.2016.0031.