Clinical and Experimental Health Sciences

# Increased Expression of the *Actin-Related Protein 2 (ACTR2)* Gene in Pterygium

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**Received:** 22.02.2023 **Accepted:** 24.06.2024

**ABSTRACT**

**Objective:** Pterygium is a fibrovascular conjunctival degeneration whose pathogenesis remains unclear, although many risk factors have been identified. In our study, we purposed to find the level of *Actin Related Protein 2 (ACTR2)* gene expression in healthy conjunctiva tissues and pterygium to increase our understanding of the pathogenesis of pterygium.

**Methods:** The study included 27 patients who underwent pterygium excision. *ACTR2* mRNA expression level in healthy conjunctiva tissues and pterygium were determined by the Real-Time PCR method.

**Results:** According to the results we obtained, *ACTR2* gene expression was increased in 74% (20/27) of our cases, while *ACTR2* gene expression was decreased in 26% (7/27). *ACTR2* mRNA expression was detected to be remarkably higher in pterygium in proportion to conjunctiva tissue ($p < 0.05$).

**Conclusion:** Our findings show that the *ACTR2* gene and cell migration mechanism may play a role in the development of pterygium. However, supplementary research is requirement to determine the efficacy of the *ACTR2* gene in pterygium disease and to better understand the relationship of the *ACTR2* gene with pterygium.

**Keyword:** Actin cytoskeleton, *ACTR2*, Cell migration, Expression, Pterygium

## 1. INTRODUCTION

Pterygium is an ocular surface disease caused by abnormal growth of the conjunctiva and defined by conjunctivalization, inflammation, and connective tissue remodeling (1,2). Although the pathogenesis of pterygium is not clearly known, many risk factors such as Ultraviolet (UV), sex, age increase, inflammatory mediators, viral infections, epithelial-mesenchymal cell transition, apoptotic and oncogenic proteins, oxidative stress, lymph angiogenesis, and DNA methylation play a role in the pathogenesis of pterygium (3,4).

Pterygium is a benign disease with neoplastic-like features such as proliferation, angiogenesis, cell migration, and recurrence (5). In many recent studies, data supporting that pterygium may be a neoplastic condition has been obtained. For example, it has been shown that the *Mitogen-activated protein kinase (MAPK*) signaling pathway, which is active in all cancer types, is also active in pterygium tissue (6). A significant relationship was found between *Kirsten rat sarcoma viral oncogene (K-RAS)*, one of the most frequently observed proto-oncogenes in tumor proliferation, and pterygium. A correlation between postoperative recurrence and younger age has been demonstrated (7). Tumor-associated genes *P63* and *P16* were determined to be remarkably higher in the pterygium epithelial tissue compared to normal conjunctival tissue (8). *P53* is a tumor suppressor protein responsible for regulating cell cycle arrest, and mutations in *P53* are used as the most common genetic markers in human neoplasms (6,9). It has been reported that the function and expression of *P53* in pterygium tissues are irregular and mutant *P53* has been shown to be overexpressed in pterygium tissues (6,10). Although many genes, risk factors, and molecular mechanisms associated with pterygium have been identified, its pathogenesis still remains unclear (3,11).



*DOI: 10.33808/clinexphealthsci.1254942*

The best available treatment option is excision with conjunctival autograft due to the low recurrence rate (12). However, recurrence usually occurs in surgically resected pterygium and post-relapse dysplasia is observed (11,13). Therefore, the identification of mechanisms that activate or support the proliferation and cell migration of pterygium is important for the treatment and prevention of primary pterygium and recurrent pterygium (5).

The actin cytoskeleton *(ARP2/3 complex)* is a dynamic complex with well-known functions in cell morphogenesis, cell migration, inflammation, cell division, and signal transduction (14,15). Actin-Related Protein 2 *(ACTR2-ARP2)* is a member of the *ARP2/3 complex*, which consists of seven proteins. *ACTR2*-mediated actin regulation is thought to drive lamellipodia generation and act as a control center for actin-based cell migration (16). Cell migration, which is a fundamental cellular process, can promote intravascular proliferation and metastasis of cancer cells by using microtubule and actin dynamics (17). Thus, the *ARP2/3 complex* has been associated with promoting the migration and invasion of diverse cancers, and ARP subunits have been shown to be abnormally expressed in tumors (18,19).

Considering this information, we think that there may be a relationship between *ACTR2*, which plays a central role in actin-based cell migration, and pterygium, which is thought to be a tumor precursor. However, there is no publication documenting the relationship between the *ACTR2* and pterygium proliferation, migration, or invasion. In our study, we purposed to determine the *ACTR2* gene expression in pterygium and healthy conjunctival tissues and to reveal the role of actin-based cell migration in the pathogenesis of pterygium.

## 2. METHODS

### *2.1. Subjects*

Twenty-seven volunteers who applied to Tokat Gaziosmanpasa University Faculty of Medicine, Department of Ophthalmology, and were diagnosed with pterygium were included in the study. While forming the study groups, pterygium tissues taken during the surgical operation were used as the patient group, and conjunctival tissues from the same eye of the same patients were used as the control group. Necessary permission was obtained for the study by the Clinical Research Ethics Committee of Tokat Gaziosmanpasa University Faculty of Medicine (approval number 19 KAEK-024). Written informed ethical consent was obtained from all participants before the study. The Declaration of Helsinki was complied with while conducting the study. Tokat Gaziosmanpasa University Scientific Research Projects (project number 2019/110) helped to finance our study.

Patients without glaucoma, corneal disease, and uveitis were added to the project. The pterygium diagnosis of the patients added in our study was confirmed histopathologically. Information such as age, gender, disease history, and family history was recorded using the hospital information system. Tissues were obtained from 16 right and 11 left eyes. In the power analysis performed with G. Power 3.1.9.6 Statistical Software, the minimum sample size in the groups was determined as 20.

### *2.2. Identifying ACTR2 Gene Expression*

Following the manufacturer's instructions, total RNA isolation from conjunctival and pterygium tissues were carried out (Thermo, USA). Reverse Transcription Polymerase Chain Reaction method cDNA synthesis (GeneAll, Korea) was performed using isolated total RNAs. The cDNA concentrations were detected with the Qubit dsDNA Assay Kit (Invitrogen, USA). The quantity of cDNA required for PCR was calculated separately for each sample. The *ACTR2* expression level was detected utilizing SYBR Green-based Real-Time PCR (qRT-PCR) (Applied Biosystem StepOnePlus). The PCR reaction (total volume 20 µL) included 2X SYBR Green Master Mix (10 µL), cDNA (3 µL), primers (1 µL), 1X ROX dye (0.4 µL), Nuclease Free Water (NFW) (4.6 µL), and NFW was used as the negative control. The PCR program consisted of 2 minutes (1 cycle) at 50 °C, followed by 40 cycles at 95 °C for 10 minutes, at 95 °C for 15 seconds, and at 55 °C for 1 minute. *ACTR-2* gene expression level was normalized with the Actin-Beta *(ACTB)* housekeeping gene and the relative expression was detected by the $2^{-\Delta\Delta Ct}$ value (20).

### *2.3. Statistical Analysis*

SPSS software version 16.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. Values are given as Mean ± SD. T-test was used to determine *ACTR2* mRNA expression in pterygium and healthy conjunctival tissues. p values less than 0.05 were considered significant. Fold change was determined according to the range of 0.9 – 1.1. Accordingly, the *ACTR2* gene expression is decreased in pterygium tissue at values below 0.9. If it is in the range of 0.9–1.1, it does not change compared to normal conjunctival tissue. *ACTR2* gene expression at values greater than 1.1 was interpreted as increased in pterygium tissues (21).

## 3. RESULTS

The patients in the study were 33% (9) female and 67 % (18) male, with an age range of 43 – 78 years and a mean age ± SD 58± 8.43. According to the qRT-PCR analysis results *ACTR2* gene expression level increased by 74% (20/27) and decreased by 26% (7/27) in all pterygium tissues. When these data were analyzed, it was found that *ACTR2* gene expression increased (2.70±0.520) in pterygium tissue compared to normal conjunctival tissues. Fold change is shown in Figure 1. A range of 0.9-1.1 was used in the evaluation of the data. The increase in *ACTR2* mRNA expression level was statistically significant in pterygium tissue (p=0.005). *ACTR2* expression level is shown in Table 1.

***Table 1.*** *Expression level of ACTR2*

| | *ACTR2* (mean ± SD) | *p* value |
|---|---|---|
| Pterygium tissue (n= 27) | 2.70±0.520 | * *p*=0.005 |
| Conjunctiva tissue (n=27) | 1 | |

*Abbreviations: SD, standard deviation, *= p < 0.05*

## 4. DISCUSSION

Pterygium is a conjunctival vascular growth that occurs on the cornea, causing symptoms such as redness, double vision, blurred vision and itching. Many risk factors have been identified and UV rays are shown as the strongest risk factor (22). However, its pathogenesis has not yet been clarified. Although pterygium is considered a degenerative disease and a benign tumor, it is thought that it should be considered a neoplasia due to its tumor-like properties (23). The observation of cell migration, cell proliferation, and local angiogenesis in the development of pterygium indicates uncontrolled cell proliferation (6). In addition, the fact that the pterygium shows common features with tumors such as hyperplasia, corneal invasion, uncontrolled cell proliferation, dysplasia, and recurrence after resection supports the view that it may be a premalignant tissue (6,23).

ARP2/3 complex play a role in many cellular processes such as cell activation, cellular movement, intercellular interactions, cytokinesis, vesicular trafficking, signal transduction, phagocytosis, adhesion, and mechanical processes (24,25,26). The function and dysregulation of *ACTR2* one of the two main actin-related proteins in the ARP2/3 complex have been associated with many diseases and cancer types in recent years (25). For example, Essential Thrombocytosis (ET), a myeloproliferative neoplasm, is characterized by abnormal proliferation of megakaryocytes and platelets. In this disease, low *ACTR2* expression ($p < 0.05$) has been reported to have significant prognostic value (27).

In another study, increased expression of *ACTR2* and *ACTR3* was associated with the stage of malignancy in colon cancer cells and stromal cells around the tumor (28).

Zhang et al. (29) observed that *ACTR2* expression was higher in gastric cancer tissues compared to normal gastric tissues, and *ACTR2* supported both cell proliferation and invasion. Moreover, high *ACTR2* expression has been associated with the aggressive behaviors observed in gastric cancer such as poor prognosis, tumor size, advanced tumor stage, and lymph node invasion.

Silencing *ARPC2* in breast cancer has been observed to result in a significant reduction in the invasion of breast cancer cells (30). Furthermore, high *ARPC2* expression level has been associated with low quality of life of breast cancer patients (31).

Huang et al. (19) investigated the possible association between members of the ARP2/3 complex and hepatocellular carcinoma (HCC) and reported significant upregulation of ARP2/3 complex subunits (especially *ARPC2, ACTR3*, and *ARPC5*) and correlated it with poor prognosis. Therefore, they reported that *ARPC2, ACTR3*, and *ARPC5* could be used as a biomarker and promising molecular targets for HCC therapy in the future.

Chen and Jiang (25) investigated the roles of *ACTR2* in diffuse large B-cell lymphoma (DLBCL) and found that upregulation of *ACTR2* exacerbates DLBCL malignancy by activating Wnt Signaling. All of these studies emphasize the importance of the ARP2/3 complex in the migration and invasion of many cancer cell types.

According to the literature, there is no study investigating the possible relationship between ARP2/3 complex and pterygium. However, the relationship between cell migration-related genes such as *Spermidine/Spermine N1-Acetyltransferase 1 (SAT1)* or *Calgranulin B (S100A9)* and pterygium has been investigated. In particular, pterygium migration was reduced when IPENSpm (SAT1 inhibitor) was administered. As a result, it was thought that the spread of pterygium could be prevented by using cell migration inhibitors and could be used as new treatment options (32).

Our research is the first to investigate the effects of the *ARP2/3 complex (ACTR2)* on cell migration in pterygium. According to our study, *ACTR2* gene expression in pterygium, which is thought to be a tumor analog, was significantly increased in pterygium tissues compared to normal conjunctival tissues (p=0.005). Our research results suggest that *ACTR2* may play a role in the pathogenesis of pterygium. However, although our study shows that *ACTR2* affects the migration of pterygium cells, experiments such as Western Blot, RNA interference (RNAi) and sequencing for this mutation are needed to define the function of *ACTR2* in pterygium formation and recurrence.

## 5. CONCLUSION

Surgical excision seems to be the best treatment option since the pathogenesis of pterygium remains unclear and effective treatments other than surgery are not sufficient. However, more effective treatment options are needed due to the astigmatism, and recurrence rates that can be observed after excision. Cell migration inhibitors affecting the *ACTR2* and perhaps other members of the *ARP2/3 complex* may provide new opportunities for therapeutic approaches to block or reduce the spread of pterygium.

***Acknowledgements***:
***Funding:*** *The study was funded by Tokat Gaziosmanpasa University Scientific Research Projects (project number 2019/110).*
***Conflict of Interest****: All authors declared that there is no conflict of interest.*
***Ethics Committee Approval:*** *This study was approved by Ethics Committee of Tokat Gaziosmanpasa University Faculty of Medicine, Clinical Research Ethics Committee, (Number 19-KAEK-024.) Written informed ethical consent was obtained from all participants included in the study. The research was conducted per the Declaration of Helsinki. This study was prepared based on the master's thesis titled "Analysis of ACTR2 gene expression in pterygium" conducted under the supervision of Omer ATES.*
*Peer-review: Externally peer-reviewed.*

***Author Contributions: (Initials only)***

*Research idea: KŞ, ÖA, NB,*

*Design of the study: KŞ, ÖA, NB, SŞ*

*Acquisition of data for the study: SOA, ÖA, HDD, RT*

*Analysis of data for the study: SOA, KŞ, NB, SŞ*

*Interpretation of data for the study: SOA, KŞ, ÖA, NB, SŞ, RT*

*Drafting the manuscript: SOA, KŞ, ÖA*

*Revising it critically for important intellectual content: KŞ, ÖA, HDD, RT, NB*

*Final approval of the version to be published: SOA, KŞ, ÖA, NB, SŞ, HDD, RT*

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**How to cite this article:** Abdullah Omar S, Şahin K, Ateş Ö, Bozkurt N, Şavkın S, Demir DH, Tetikçok R. Increased Expression of the Actin-Related Protein 2 (ACTR2) Gene in Pterygium. Clin Exp Health Sci 2024; 14: 631-635. DOI: 10.33808/clinexphealthsci.1254942