TC
TOKAT GAZİOSMANPAŞA UNIVERSITY

GRADUATE SCHOOL INSTİTUTE

ANALYSIS OF ACTR2 GENE EXPRESSION IN PTERYGIUM

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Master Thesis

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TOKAT - 2022

The defense examination of the thesis study titled “Analysis ACTR2 gene expression in pterygium ‘ is prepared by SORAN OMAR ABDULLAH was held on - 08/02/2022 it has been accepted as a Master Thesis at Tokat Gaziosmanpaşa University Graduate school Institute Department of MEDICAL BIOLOGY .

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CONFIRMATION

08/ 02/ 2022

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(08 / 02/ 2022)

SORAN ABDULLAH

ACKNOWLEDGMENTS

I might want to thank my regarded manager – Prof. Dr. H. Ömer ATEŞ, for his significant oversight, backing, and tutelage throughout my MSc degree. My appreciation stretches out to the Institute of Health Sciences, for the subsidizing freedom to attempt my investigations at the Department of Medical Biology Department, TOKAT GAZİOSMANPAŞA UNIVERSITY. Moreover, I might want to offer thanks and for their mentorship to me Prof Asist. Nevin KARAKUŞ, Dr.Nihan BOZKURT, for their cherished help which was truly compelling in forming my examination techniques and studying my results.

Numerous individuals have aided me in various ways throughout my work on my thesis, and I am grateful to them all, particularly the following:

Mohammed was the one who was responsible for receiving my complaints and doubts and was always able to support me.

Mehdi and Mohammed, who put up with my inept handling of paperwork and documentation and had a calm and courteous demeanor throughout.

All employees in the operation rooms at tb fakülte, particularly those in the molecular laboratory and thesis guide service, for their understanding, patience, and assistance during the method's development.

My colleagues and friends in Turkey and Iraq, who listened to my concerns, uncertainties, and accompanied me as my thesis developed.

This study was supported by Tokat Gaziosmanpaşa University Scientific Research Projects Unit and TUBITAK.

(Project No: 2019/110) (Tokat Gaziosmanpaşa University)

Project No: 215S692 (TUBITAK)

ABSTRACT

Analysis of ACTR2 gene expression in pterygium

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Pterygium is a benign, uncontrolled growth of conjunctival tissue extending over the sclera. Pterygium is characterized by inflammatory, proliferative, invasive ocular surface and extensive matrix remodeling. Pterygium is a benign growth of conjunctival tissue lining the sclera that is proliferative and causes invasive ocular surface and extensive matrix remodeling. the ACTR2 gene is located on the p arm (short arm) of autosomes (body chromosome(s)) at locations 2p14 encoding the 399 amino acid sequence. The ACTR2 gene consists of 10 exons and consists of 3798 base pairs. ACTR2 encodes Actin-Associated Protein 2 (ARP2), is involved in the formation of branched actin networks in the cytoplasm, important for cell shape. It provides force for cell motility and takes part in cell proliferation. loss of ACTR2 or the presence of abnormal transcripts can result in aberrant cellular proliferation by the activity of ACTR2. In this study, it was aimed to determine the ACTR2 gene expression, which may cause abnormal benign tissue growth from the conjunctiva (mucous membrane covering the eye) to the cornea. It depends on cell adhesion, motility, and deformability resulting from quantitative changes and rearrangement of the actin-related protein. Our aim in this study is to determine the expression level of ACTR2 gene in pterygium tissue. qRT-PCR analysis was performed for expression of ACTR2 gene at RNA level in pterygium tissue. According to the data obtained at the end of the study, ACTR2 gene expression levels were found to be statistically significantly higher in tissues with pterygium compared to the control group ($p < 0.005$) ($p=0.005$).

Key Words: Expression**;** Pterygium; Real-Time PCR; ACTR2

ÖZET

Pterjiyumda ACTR2 Gen Ekspresyonu,

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Pterjiyum, sklera üzerine uzanan konjonktival dokunun iyi huylu, kontrolsüz büyümesidir, Pterjiyum, inflamatuar, proliferatif, invaziv oküler yüzey ve kapsamlı matriks yeniden şekillenmesi ile karakterize edilir. Pterjiyum, proliferatif olan ve invaziv oküler yüzeye ve geniş matriks yeniden şekillenmesine neden olan sklerayı kaplayan konjonktif dokunun iyi huylu bir büyümesidir. ACTR2 geni, 399 amino asit dizisini kodlayan 2p14 lokasyonlarında otozomların (vücut kromozom(lar)) p kolunda (kısa kol) bulunur. ACTR2 geni 10 ekzondan oluşur ve 3798 baz çiftinden oluşur. ACTR2, Aktin İlişkili Protein 2'yi (ARP2) kodlar, sitoplazmada dallı aktin ağlarının oluşumunda rol oynar, hücre şekli için önemlidir. Hücre hareketliliği için kuvvet sağlar ve hücre proliferasyonunda yer alır. ACTR2 kaybı veya anormal transkriptlerin varlığı, ACTR2'nin aktivitesi ile anormal hücresel proliferasyona neden olabilir. Bu çalışmada, konjonktivadan (gözü kaplayan mukus zarı) korneaya doğru anormal iyi huylu doku büyümesine neden olabilecek ACTR2 gen ekspresyonunun belirlenmesi amaçlanmıştır. Aktin ile ilgili proteinin kantitatif değişiklikleri ve yeniden düzenlenmesinden kaynaklanan hücre yapışmasına, hareketliliğine ve deforme olabilirliğine bağlıdır. Bu araştırmadaki amacımız, pterjiyum dokusundaki, ACTR2 geninin ekspresyon düzeyini belirlemektir. Pterjiyum dokusunda ACTR2 genin RNA düzeyinde ekspreyonu için qRT-PCR analizi yapıldı. Çalışma sonucunda elde edilen verilere göre, ACTR2 gen ekspresyon düzeylerinin pterjiyumlu dokularda kontrol grubuna göre istatistiksel olarak anlamlı derecede yüksek olduğu belirlendi ($p < 0,005$) ($p=0.005$).

Anahtar Kelimeler: Pterjiyum, qRT-PCR, ACTR2

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ABBREVIATION AND SYMBOL LIST

UOZ	:	Uninvolved optical zone
GTPase	:	Guanosine triphosphatase.
GBD	:	GTPase binding domain.
PH	:	pleckstrin homology.
NPFs	:	Nucleation-promoting factors.
VCA	:	verprolin-homology, cofilin-homology, and acidic.
BL	:	Bowman's layer.
HPV	:	human papillomavirus
ANG	:	Angiogenin.
TCR	:	T-cell receptor.
UV	:	Ultraviolet.
UVR	:	Ultraviolet radiation.
CSF	:	contrast sensitivity function.
VA	:	visual acuity.
EPO	:	Erythropoietin.
EPOR	:	EPO receptor.
ECM	:	stands for extracellular matrix.
BaP	:	pollutant benzo (a) pyrene.
BPDE	:	BaP-diol –epoxide.
PECs	:	primary pterygium cells.
LOH	:	loss of heterozygosity.
CMV	:	stands for Cytomegalovirus.
HPV	:	stands for human papillomavirus.
HSV	:	stands for herpes simplex virus.
MI	:	microsatellite instability.

TGM-2 : Transglutaminase-2.
MMC : mitomycin C.
TF : tissue factor.
EVE : everolimus.
PF : pterygial fibroblast.
BER : basic excisional repair system.
NER : nucleotide excision repair.
hODG1 : human 8-oxoguanine DNA glycosylase.
TdT : terminal deoxynucleotide transferase.
FR : free radicals.
SOD : superoxide dismutase.
CAT : Catalase.
MDA : Malone dialdehyde.
G6PD : Glucose-6-phosphate-dehydrogenase.
NO : nitric oxide.
PPP : pentose phosphate.
PF : pterygium fibroblasts.
NCF : normal conjunctival fibroblasts.
CDK : climatic droplet keratopathy.
ER : endoplasmic reticulum.
ARP : actin-related protein.
WH1 : Wiskott homology 1.
ARD : actin regulatory domain.
AR : acid Region.
WIP : Wiskott-Aldrich Interactive Protein.
EGF : epidermal growth factor receptor.
PRR : proline-rich region.
PKC : protein kinase C.
TCR : T lymphocyte receptor complex.
GBD : G protein-binding domain.

1. INTRODUCTION

Pterygium is derived from the ancient Greek words (pteryx) for wing and v (pterygion) for fin. Pterygium is a condition in which a triangular piece of the bulbar conjunctiva encroaches on the cornea1, frequently within the intrapalpebral fissure and most often from the nasal side (Sarkar et al., 2021).

and is a common ocular surface disease in subtropical countries and is a triangle fibrovascular subepithelial ingrowth of degenerative bulbar conjunctival tissue onto the cornea that occurs over the limbus (Yue et al., 2019)., This disorder affects about 8-13% of the population around the world (Bai et al., 2010), degenerative and hyperplastic lesions whose etiology and pathogenesis are not fully understood (Chui et al., 2008).

It is most frequently found in patients who have lived in hot areas and may be a reaction to UV exposure as well as other variables such as persistent surface dryness. Histologically, a pterygium shows elastotic degenerative changes in vascularized sub-epithelial stromal collagen. Pterygia encroach onto the cornea and invade the Bowman layer (Sarkar et al., 2021).

The actual cause of pterygium is uncertain, pterygia is a benign uncontrollable outgrowth of the conjunctive tissue, numerous factors have been characterized, these components incorporate tumor silencer gene p53 and different genes related with DNA repair, viral diseases hav been exhibited to be a danger factor in populations (Liu et al., 2013).

Additionally, epidemiological evidence indicates that persistent sun exposure without shielding from ultraviolet (UV) radiation plays a critical role ,. Throughout the bulbar conjunctiva, surface alterations have been described. The most advanced changes occur directly over the pterygium surface, confirming that pterygium is a surface condition of the eye. (Kim et al., 2013),

Intracellular modifications of cholesterol, Glucose-6-phosphate- dehydrogenase deficiency, raising cellular proliferation levels and cellular apoptosis low levels (Liang et al., 2011; Ozturk et al., 2017), Overexpression of ANG (Angiogenin) chronic irritation by dust (Nuhoglu et al., 2013).

the actin superfamily's Arp2 and Arp3 proteins are anticipated to bind ATP via a conserved nucleotide-binding domain In response to intracellular signals, the Arp2/3 complex, a seven-subunit protein complex containing Arp2 and Arp3, two actin-related proteins, induces the production of actin filament networks (Dayel et al., 2001).

ACTR 2/3 complex encoded for a protein with key roles in many activities. Additionally, the Arp2/3 complex plays a role in the cytoplasmic cytoskeleton by promoting nuclear actin polymerization (Yoo et al., 2007).

The pure complex is sufficient to initiate ActA-dependent actin polymerization at pterygium , and is necessary for the creation and movement of the actin tail. Actin-related proteins (ARPs) from the Arp2 and Arp3 subfamilies make up two of the complex's components (Dayel et al., 2001).

Our research was investigate the expression of the ACTR2 gene in pterygium. Tissues were removed during surgery with the consent and knowledge of the patient. By collecting cDNA from the first group of tissue levels established by real-time PCR, we employ the ACTR2 gene expression technique.

From the data that we had obtained, ACTR2 gene expression levels were found to be increased statistically significant in tissues with pterygium compared to the control group.

2. GENERAL INFORMATION

Pterygium is a common eye disease that has been diagnosed and treated in Greece, the Arabian Peninsula, and China for more than three thousand years. Even back then, there was a lot of debate over the most effective treatment. Even 3000 years later, we're having the same debates over how to cure pterygium. (Chan et al., 2015)

To put it another way: Pterygium is a condition when the conjunctiva grows abnormally and transgresses the cornea's surface, concealing the pupil while obstructing vision. (Chan et al., 2015) ..

Its name, pterygium, means "wing," and it develops as the fibro vascular tissue that starts in the conjunctiva bulbar moves toward the cornea. When it occurs on the cornea, it seems as though there are three triangular wings or an arrow point, and it can slowly extend into the patient's central vision (Chan et al., 2015 , Misha et al., 2019).

Pterygium is more common among those who spend the most of their time in market situations owing to occupational concerns and are exposed to ultraviolet (UV) radiation and dust. It has the highest rate of abundancy in tropical regions, with a prevalence of 22 percent (Singh et al., 2017) .

Pterygium is a skin disorder that is harmless, and often found inside the palpebral fissure, and occurs on the right-hand side of eye’s nasal in 90% of cases. uncontrolled growth of connective tissue overlaying the sclera (Liu et al., 2013).

Based on the size of the UOZ (uninvolved optical zone), pterygium is categorized into three groups: UOZ > 9 mm, UOZ = 7-8.9 mm, and UOZ = 5-6.9 mm. The prevalence of pterygium has been estimated to range from 0.3 to 37.46 percent worldwide (Zare et al., 2010) .

Redness, irritation, foreign body sensation, reduced vision, and ocular discomfort are the most common clinical manifestations. Based on the location of the pterygium head invasion on the cornea, the severity of pterygium can be categorized as grade . Pterygium may be caused by genetics as well as other environmental elements such as wind, dry air, dust, chemicals, air pollution, geographic location, and hereditary factors (Kassie Alemayehu et al., 2020).

Some people's pterygiums grow slowly throughout their lifetimes, while others stop developing after a particular time. A pterygium can occasionally grow large enough to cover the entire pupil of the eye (James et al., 2002). , a disfiguring condition that can lead to blindness and is a major public health concern in rural regions. It thrives in the warm, windy, and dry temperatures of Africa's tropical and sub-tropical "pterygium belt," which includes Ethiopia (Zare et al., 2010).

The pterygium tissue consists of an underlying fibrovascular component and a superficial conjunctival epithelial layer, with features indicative of both impaired growth and a degenerative process(Cárdenas et al., 2016).

It develops gradually over time, moving from the limbus to the cornea's center. It can occur on both the nasal and temporal sides in about 1-2 percent of cases. Size, pace of growth, fleshiness, and vision impairment6 have all been used to form classifications. Typically, both eyes are afflicted, with the dominant eye's lesion coming first (Yasemi et al., 2017).

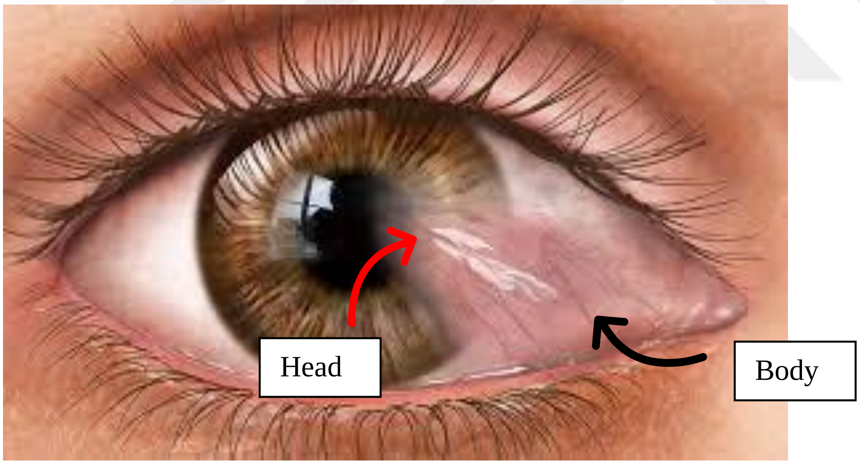


Figure 2.1 Anatomically, Pterygia is classified into two subtypes: head and body.

The majority of pterygium is seen on the nasal side, however double-head pterygium is widespread in the “pterygium belt” region, which lies between 30 N and 30 S of the equator. Many techniques have been established to improve the long-term success of pterygium surgery. Autografts of the conjunctiva (CAU), the limbal-conjunctiva (LCAU), mitomycin C (MMC), and the amniotic membrane (AMT) [4–6, respectively] (AMT) are all often utilized adjuvants in the literature (Chan et al., 2015).

2.1. Epidemiology

Pterygium is a widespread condition that affects people in various parts of the world. is a condition characterized by uncontrolled fibrovascular proliferation of the conjunctiva of the eye that occurs more frequently in places near the equator. It has a high recurrence rate, which results in more aggressive and difficult to treat recurrences. Numerous therapy approaches have been offered, each with distinct drawbacks (Gupta et al., 2018).

This condition is more common in the "pterygium belt" of 37 degrees north and south latitudes around the equator, where prolonged exposure to sunlight is more common. Prevalence rates have ranged from 0.3% to 29% in these areas (Ang et al., 2007).

It is more prevalent in rural regions of the country (Nangia et al., 2013). In Barbados, 23.4 percent of the black population and 23.7 percent of the mixed Black and White population have pterygium. (Luthra et al., 2001).

And 10.2 percent of the population is White. Other countries have varying prevalence rates, such as 10.1 percent in Singapore, 30.8 percent in Japan, 14.49 percent in a Tibetan population in China, and 17.9 percent in an elderly Mongolian population in Henan County, China. 2022 (ANG et al., 2021).

provides demographic data that may be used to directly compare racial disparities in prevalence and risk variables. Malays had a higher prevalence of pterygium than Indians and Chinese, even after adjusting for known confounding variables such as age, gender, and employment., While Indians have darker complexion than Malays and Chinese, this may not necessarily be a risk factor for pterygium, as previously suggested (Luthra et al., 2001).

Because men spend more time working outside than women do, pterygium is more common in males (Ang et al., 2012).

A pterygium was discovered in 798 eyes (8.47±0.29%) of 608 (12.9±10.49%) subjects. 190 patients had bilateral pterygia (4.0 percent of study population). Pterygia prevalence increased from 6.7±0.8 percent in the 30-39 age group to 13.5±1.2 percent in the 50-59 age group and 25±32.1 percent in the 70-79 age group (Nangia et al., 2013).

TABLE 2.1: Pterygium prevalence in various studies summarized (Saw et al., 1999).

Country	Sample size	Age (years)	Prevalence (%).
Solomon Islands	512	1 to 89	0.3
South Africa (Transkei)	1,519	all ages	0.5
Denmark (Copenhagen)	810	all ages	0.7
Australia	64314 Aborigines 40,799 non-Aborigines	all ages groups	3.4 in Aborigines 1.1 in non-Aborigines
Greenland	659	all ages	8.6
Australia (New South Wales)	287	10 and above	6.9
Jordan	127	10 and above	12

It was shown that rates of pterygium were greater in nations with hot, dusty, and dry climates, especially those located between 37 degrees north and south of the equator.

Tthe Male sawmill employees in Thailand had a prevalence rate of 27 percent, compared to 2 percent in white male sawmill workers in British Columbia. Pterygium is more common as people get older, according to several research. The most prevalent onset age appears to be in one's 20s and 30s (Detels R. et al., 1967).

It's possible that a 'pterygium belt' exists where UV radiation is most intense., It's possible that sunlight exposure is linked to the development of pterygium, which explains this intriguing geographic variation. Ringland Anderson was the first to notice that pterygium operations were most common in Australia's northern regions. (Karai et al., 1984).

Dimitry conducted a survey of pterygium operations from the main hospital in each state in the United States of America in 1937 (Saw et al., 1999).

2.2. Etiology

Certain characteristics of pterygium suggest tumor-like characteristics. Pterygium, for example, can recur aggressively following surgical excision, and treatment modalities are similar to those used to treat neoplasia, including wide excision, adjunctive radiotherapy, and antimitotic chemotherapy. Although the specific cause of pterygium is uncertain Ultraviolet light, which is a known etiologic factor in skin cancers, has also been proven to be a significant etiologic factor in the prevalence of pterygium in epidemiological studies (Tan et al., 1997).

Thus, wearing UV B-absorbing wrap-around sunglasses and wide-brimmed hats when going outside has been advised (Chan et al., 2002).

Much attention has recently been paid to the possibility of viral oncogenes involvement in the multistage process of pterygium formation. Until now, Human papillomaviruses (HPV) and Epstein-Barr virus (EBV) have both been examined as putative pterygium causal agents., According to the findings, a direct link between HHV-6 and the development of pterygium is less likely, implying that other etiologic agents must be involved in the disease's multistep process (Misha et al., 2019).

The PPI network of pterygium has the highest concentration of FN1.
Pterygia development and recurrence both raise FN1 levels. FN1 expression was observed to be elevated in pterygium in this study (Yue et al., 2019).

The current study demonstrates that widowed participants who developed pterygium were older. The explanation for this may be related to aging, as the people in this study were older. Despite ongoing dispute, numerous writers have shown that pterygium is a degenerative (aging) condition of the conjunctival tissue that results in vascular expansion (Kassie Alemayehu et al., 2020).

Cohort and case-control studies with precise estimations of the major risk variables for pterygia might shed additional insight on the pterygia causes. According to current evidence from case-control studies, UV B radiation is the primary risk factor for pterygium development. Solar radiation is the primary source of light that reaches the eye (albedo) (Chan et al., 2002).

The progression of pterygia formation has been determined. Pterygium is most often found on the nasal side, however it can also be found on the temporal side. The influence of UV radiation in the pathophysiology of pterygium has been described; this role includes the modification of fibroblasts and limbal stem cells, both of which relate to pterygium activation, as well as the inductive reasoning of various pro-inflammatory cytokines, growth regulators, and matrix metalloproteinases, all of which aid pterygium advancement (Zhou et al., 2016).

Induced growth factors include growth factors such as basic fibroblast growth factor (bFGF), transforming growth factor (TGF), platelet-derived growth factor (PDGF), VEGF, and connective tissue-derived growth factor (CTGF) (HB-EGF) by ultraviolet light, even if the exposure is brief (Kria et al., 1996; Nolan et al., 2003).

Growth factors are released by fibroblasts, endothelial cells, and inflammatory cells in response to oxidative stress. The conjunctival epithelium is also stimulated by these growth factors. But in pterygia, growth factor proteins enhance vigorous proliferation of fibroblasts while at the same time slowing down the mitotic process in controls. Overexpression of these proteins in pterygia does not appear to be the cause of vigorous fibroblast mitosis, according to this finding (Anguria et al., 2014).

Several studies have been conducted to determine the molecular mechanisms that underlie the growth and proliferation of pterygium disease. revealing the roles of viruses, oxidative stress, DNA methylation, the cellular epithelial-mesenchymal transition, apoptotic and oncogenic proteins, inflammatory mediators, loss of heterozygosis (Wanzeler et al., 2019)

2.3. Risk Factors Affecting Pterygium Formation

2.3.1. Pterygia pathogenesis may be influenced by genetic and hereditary factors.

For pterygium to develop, it must be passed on through the generations. Family history, which suggests genetics, was linked to the development of pterygiums, regardless of the use of conventional eye medications or the presence of an unstable tear film. Having pterygium-affected relatives identified, which implicates heredity, was linked to pterygium incidence, confirming familial pterygium occurrence. Pterygium patients and unaffected people were exposed to the same amount of sunshine, indicating that family incidence is caused by genetics rather than environment (Anguria et al., 2014).

Genetically deficient or damaged limbal stem cells with altered matrix metalloproteinases been proven to have a substantial involvement in the etiology of pterygium1. 139 (Dua et al., 2000; Dushku et al., 2001).

genetics may have a role is that pterygium is related with numerous syndromes such as the multiple pterygium syndrome (autosomal dominant or recessive) (autosomal dominant or recessive). the relevance of genetic variables in pterygium onset is still uncertain 152 The chances ratio for a family history of pterygium was 4.75 (Saw et al., 1999).

The combined family of pterygium probands had a 9:7 ratio of unaffected to pterygium-affected members., This implies digenic inheritance (the most straightforward kind of multifactorial inheritance (Savage et al., 2002).

The genotypes of affected and unaffected progeny are shown in Table 1. Active alleles are denoted by the letters A and B, whereas inactive alleles are denoted by the letters a and b. The expected genotypes of a pterygium patient are shown in bold font. Only two letters occur in the gametes of the parents, indicating expected alleles. AaBb and AaBb are the genotypes of the two parents. Each letter, whether capital or lowercase, indicates a different gene. This table indicates that at least one kind of gene in pterygium instances has both alleles inactive (determinant gene) (Anguria et al., 2014).

Table 2.2 Depicted genotypes of affected and unaffected offspring (Anguria et al., 2014)

Genotype	AB	Ab	AB	ab
AB	AABB	AABb	AaBB	AaBb
Ab	AAbB	AAbb	AabB	Aabb
aB	aAbB	AABb	aaBB	aaBb
ab	aAbB	aAbb	aabB	aabb

This table indicates that at least one kind of gene in pterygium instances has both alleles inactive (determinant gene).

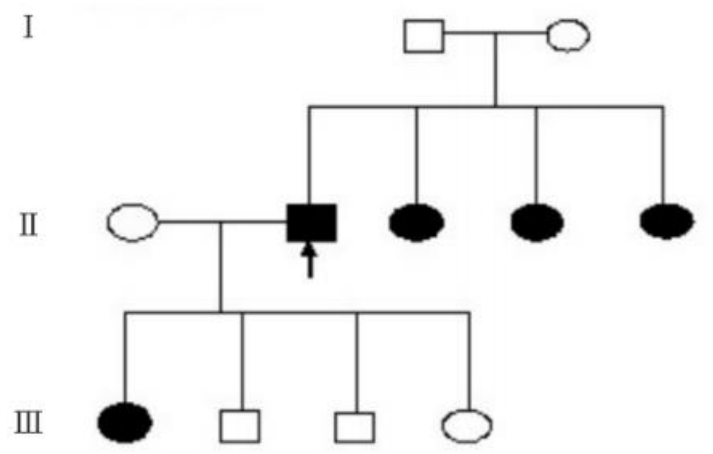


Figure 2.2. Depicts a pedigree of a pterygium proband (Anguria et al., 2014).

The unaffected females are depicted by the oval empty drawings, whereas the afflicted females are depicted by the shaded oval drawings., Unaffected males are represented by rectangular empty drawings, whereas affected males are represented by colored rectangle drawings. The proband is shown by the arrow. Autosomal dominant with partial penetrance in the first generation is likely because two generations were impacted (Zhang et al., 1987).

This could be because only the second and third generations had pterygium patients due to autosomal recessive inheritance, where the first generation and the proband's spouse were both carriers for this gene mutation (Li et al., 2011).

sex-based inheritance may have been the mechanism of transmission as the male proband may have acquired a recessive gene from his carrier mother and passed it on to his daughter's mother, who was also a carrier for the disease, Mendelian inheritance is unlikely in pterygium incidence since this lineage does not reveal a consistent Mendelian pattern (Anguria et al., 2014).

The altered genes could be implicated in pterygium disease and could be employed as pterygium therapy targets (Yue et al., 2019) .

Between pterygium and a healthy individual, researchers found 557 DEGs. FN1, KPNB1, DDB1, NF2, and BUB3 have high degrees in the PPI network. It was discovered that transcription factors influenced the expression of genes as far down the gene chain as SSH1, PRSS23, LRP5L, MEOX1, RBM14, and ABCA1. the qRT-PCR results revealed significant upregulation of FN1, PRSS23, ABCA1, KRT6A, ECT2 and SPARC in pterygium and non-significant upregulation of MEOX1 and MMP3 in pterygium (Yue et al., 2019).

Abnormal expression in the epithelium of the pterygium and the superior bulbar conjunctiva was detected in three of the eight eyes with pterygium. P53 expression anomalies in the epithelium of primary and recurrent pterygium specimens indicate that pterygium is a growth disorder rather than a degeneration (Tan et al., 1997).

2.3.2. P53 Mutant in Pterygium

The p53 gene is a notable tumor suppressor that upregulates cycle, DNA repair, cell differentiation, and apoptosis; p53 is a well-known cell stress regulator that helps to organize signals from a number of different cell stressors. Most well genetic marker of individual neoplastic progression is p53 mutations, and p53 mutations are most well-known hereditary marker of human neoplastic development, Immunohistochemistry reveals uneven amounts of p53 expression in pterygium samples (Liu et al., 2013).

Other research has discovered aberrant p53 tumor suppressor gene expression in pterygium, implying a link19 (Feng et al., 2017).

Patients with pterygium had aberrant expressions in the epithelium and superior bulbar conjunctiva, which were seen in three of eight eyes studied. In primary and recurrent pterygium specimens, there is abnormal p53 expression in the epithelium, which shows that pterygium is a growth problem rather than a degenerative condition (Tan et al., 1997).

Abnormal p53 expression was seen in seven out of the thirteen pterygia samples examined (54 percent). There was no discernible difference between the two groups. All four pterygia (50%) without recurrence and five pterygia (55.5%) with recurrence were positive in this study. The control specimens had no abnormal staining. Findings from this study imply that Pterygoid epithelium may be a result of excessive cell growth, rather than an indication of degenerative disease. Recurrence and aberrant p53 expression do not appear to be linked. (Weinstein et al., 2002).

The aberrant p53 expression was seen in seven out of the thirteen pterygia samples (54 percent). There was not a single distinction. When looking at normal conjunctiva, primary and recurrent human pterygium, researchers discovered that p53 expression differed between the two compartments: epithelial and fibrous. There was no p53 expression in any of the three normal conjunctiva samples tested, whether they were epithelial or connective tissue. Human pterygium had a higher percentage of p53-positive cells in the epithelial compartment than in the stromal compartment. When it comes to primary and recurrent pterygium, p53 positivity was found in the basal and suprabasal epithelial cell nuclei, with the latter being more prevalent (Figure 2.3A, B) (Cimpean et al., 2013).

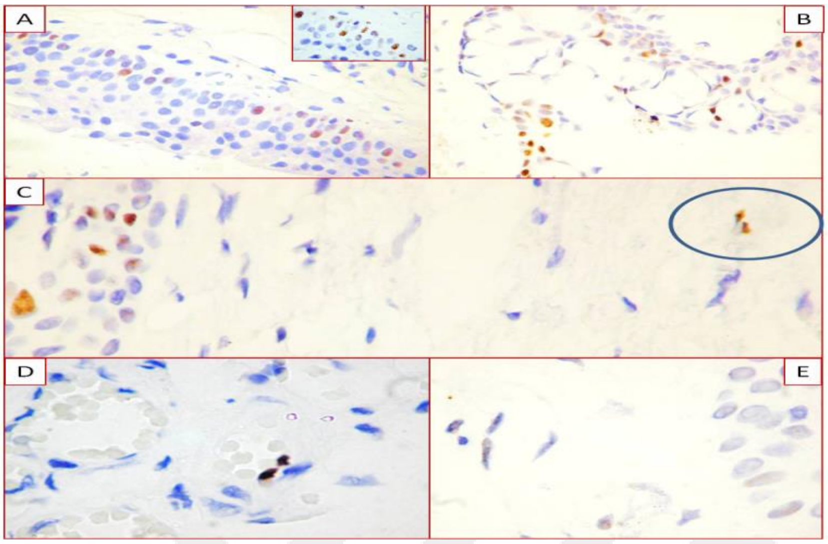


Figure 2.3. P53 gene expression in human pterygium the human pterygium possesses a basal and parabasal p53-positive cell distribution (A, B). The fibrovascular compartment of the human pterygium is filled with p53-positive cells (C). The epithelial compartment's basal layer has an enhanced density of p53-positive cells, whereas the stromal component lacks p53 expression. The p53-positive blood vessel endothelial cells (D) have a higher intensity than the lymphatic vessel endothelial cells (L) (E).

Furthermore, the instances of hyperplastic epithelium where more than 70% of the epithelial cell nuclei were positive for thymine dimers had the greatest density of p53 positive nuclei. It was observed in the fibroblast-like cells of the fibrovascular compartment (Figure 4C) as well as in the endothelial cells of the blood and lymphatic arteries only in two of the eight instances (Figure 4D.E). The presence and location of p53 did not vary between primary and recurrent plerygium. (Cimpean et al., 2013).

Plasmid p53 is substantially expressed in plerygium10, according to a study. Pterygium may have a dormant form of the p53 protein, according to this theory. In this way, activating p53 in pterygium may result in pterygium going into apoptosis (Cao et al.,2018).

2.3.3. UV Radiation

Sun exposure has been related to the development of pterygia, since there is a direct correlation between UVR exposure and an increased risk of developing pterygium. Direct phototoxic effects on cellular DNA and the production of reactive oxygen species (ROS), which damage cellular DNA, proteins, and lipids, are two mechanisms through which this type of radiation has the ability to injure cells and change tissues. This type of radiation (Marchetti et al 2011; Balci et al., 2011).

Because the cornea is able to absorb most of the shorter wavelengths (less than 300 nm), scientists believe these are the most physiologically active. The ocular surface is immediately affected by UV-B light with wavelengths ranging from 280 to 315 nm (Threlfall et al., 1999).

The exact molecular process by which UV radiation causes this disease is still being researched, and numerous potential solutions have been presented. The key causative elements for the development of pterygium, as well as the affected molecular pathways, are depicted in Figure 1 in connection to the stage of pterygium growth (Cárdenas et al., 2016).

Pterygium-promoting mediators can be induced by UV radiation, which activates many signaling pathways. The formation of free radicals, which attack and deactivate numerous macromolecules, has a molecular link to UV light, and free radicals may in turn be deactivated by tear film proteins. (Liu et al., 2013).

Pterygia can occur as a result of UV-B and UV-B-driven abnormal expression of MMPs and KRT24, according to some research. Could lead to new treatment and preventative strategies for pterygia, thanks to this discovery (Shibata et al., 2020).

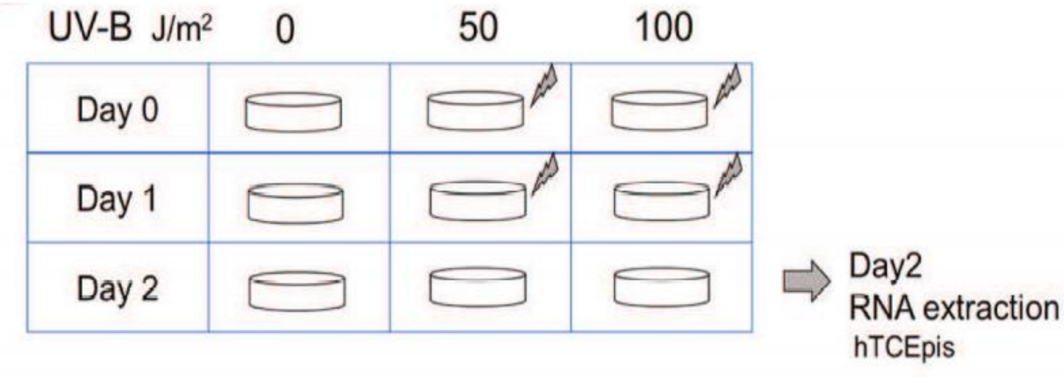


Figure 2.4: shows a way for doing culture experiments. Every day, UV-B was irradiated at different intensities (A: 0, 50, 100, 200 J/m2) or (B: 0, 50, 100 J/m2), with RNA extraction on the second and/or fourth days (Shibata et al., 2020).

Experiments with UV-B irradiation were conducted on the HConFs, HCEpCs, and HPFs in 1X phosphate-buffered saline (PBS) for 2 and/or 4 days, respectively (Fig. 2A). Afterwards, a new medium was installed. To create ultraviolet-B light, a 15-W UVB light source (312 nm) and a UV light meter were utilized (UVP, Upland, CA). Two days of UV-B exposure at 0, 50, or 100 J/m2 were used on hTCEpis in 1 mL of 1X PBS (Fig. 2B). The experiment was repeated three times for each kind of cell (Shibata et al., 2020).

Contrary to popular belief, the eyes do not have much protection from light that enters from the side or below (the albedo effect of the snow, water and sand. have investigated the potential influence of this scattered UV light on the nasal limbus, which could cause the death of corneal epithelial stem cells (Coroneo et al,.1991 ; Di Girolamo et all., 1999).

2.3.4. dry eyes

Drying and a proclivity for new growth could be caused by irregularities in the tear film. As a result, cell proliferation may ensue, leading to the creation of pterygia. However, the evidence for dry eyes as a cause of pterygia is restricted to a few studies. The marginal tear strip was shown to be disrupted in aborigines with pterygium compared to aborigines without pterygium (Zare et al., 2010).

A case-control study in a Johannesburg hospital found no link between dry eyes in Bantu people and pterygium (Ang et al., 2007).

There was a total of 43 cases and 23 controls studied. Those with pterygium had a considerably shorter break-up period of the tear film as compared to patients with normal eyes in India. Schirmer test results from 60 individuals with unilateral pterygia were inconclusive, according to Biedner et al. There was no comparison with a 'control' group in this research (Zare et al., 2010).

2.3.5. Viral factor

In recent years, the theory has gained favor. Certain authors have postulated a viral pathogenesis involving HSV and HPV18 To find out if herpes simplex virus (HSV) and human papilloma virus (HPV) were present in pterygia and phenotypically normal conjunctiva, and if there was a link between viral presence and clinical data, this study was conducted, according to the authors (Detorrakis et al., 2001).

Oncogenic viral infections, P53 mutations, and loss of heterozygosity (LOH) are all factors in pterygium. An expanding corneoscleral limbus lesion Preliminary results show that pterygia samples have been discovered to include oncogenic viruses such as HPV, CMV and HSV (Detorakis et al., 2000; Cárdenas-Cant et al., 2016).

Fifty examples of pterygia and their associated conjunctiva were obtained. Each patient had a personal and family history recorded. The detection and type of HSV and HPV viruses were done by polymerase chain reaction amplification of viral genomes (Detorakis et al., 2001).

In 11 (22% of the cases), HPV-18 (type 18) was found in 12 (24% of the cases), and in 3 (6% of the cases) of pterygia, both HSV-1 and HPV-18 were detected. No conjunctival specimens were positive for HSV, however four tested positive for HPV (8 percent). Postoperative conjunctivitis and recurrence in patients with concomitant HSV and HPV diagnoses was significantly greater.

Since HSV is not found in conjunctival tissues, this supports a more direct link between HSV and pterygium. Pterygium may be a neoplastic condition, given the presence of cancer-causing viruses like HSV and HPV. Correlations between surgical recurrence, conjunctivitis, and concurrent HPV and HSV results imply a potential viral cooperation modifying the clinical presentation of pterygium (Detorakis et al., 2001).

2.3.6. Sunlight

Pterygium is not caused by sunlight exposure alone. Pterygiums were not associated with excessive exposure to sunshine, which is consistent with recent studies. All pterygium samples include chronic inflammatory cells, and MMPs are suppressed in both pterygium and controls. (Anguria et al., 2013).

Verify that all pterygium patients and controls have been exposed to sunshine. There was no correlation between the degree of infiltration and sun exposure in pterygia with collagen breakdown (an indicator of long-term UV exposure). Individuals with a predisposition to pterygium development may be harmed by any amount of sunlight, no matter how bright. (Anguria et al., 2012).

This might be accomplished by causing oxidative stress on the ocular surface. Sunlight may also be a cause for pterygium recurrence following excision (Anguria et al., 2014).

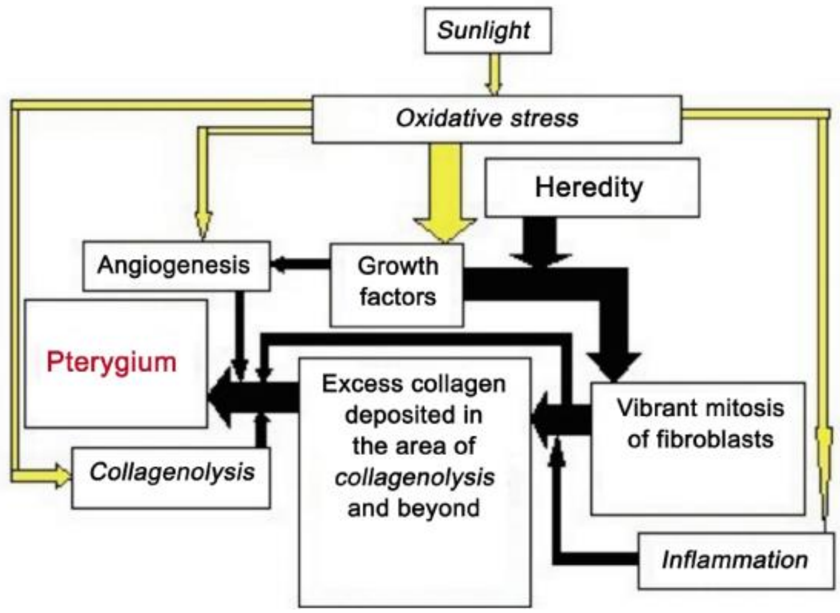


Figure 2.5. Proposed model of pterygium development. (Anguria et al., 2014)

The Growth of Pterygium Figure 2 depicts the suggested model of pterygium development, which is a flow chart depicting how genetics and sunlight exposure impact pterygium growth. Growth factor synthesis, angiogenesis, chronic inflammation, and collagenolytic are all induced by sunlight and oxidative stress (Anguria et al., 2014).

2.4. Molecular Basis of Pterygium Development

In addition to epigenetics and cell proliferation factors, inflammation mediators, angiogenic and lymphangiogenic factors, immunologic processes and the epithelial-mesenchymal transition, pterygium etiology has been connected to a variety of other variables. Just a few examples. Figure 2.6 roughly depicts the factors associated with pterygiums (Cárdenas-Cantú et al., 2016).

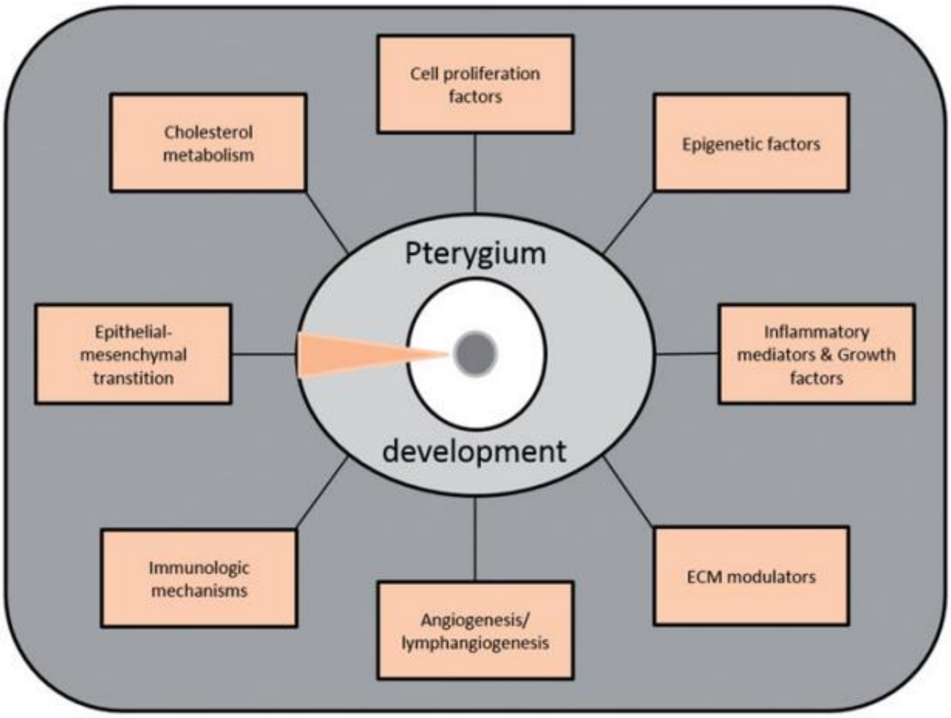


Figure 2.6: Pterygium Developmental Molecular Mechanisms (Cárdenas-Cantú et al., 2016).

It is important to get a better understanding of the molecular processes that underpin its etiology. Until date, significant progress has been made in this area. Several molecular pathways linked to cellular processes involved in pterygium development and progression have been identified, offering insight into possible biomarkers. Nonetheless, epithelial cells and fibroblasts may be extracted from pterygia tissue samples (Shahraki et al., 2021).

2.4.1. Angiogenesis in Pterygium

Angiogenin (ANG) was first discovered as an angiogenic tumor factor, but its biologic action has lately been expanded to include cell proliferation stimulation. When pterygium is seen as a tumorigenic mimicry (Sadagopan et al 2012).

In order to find out the expression levels of the ANG gene, immunohistochemistry, RT-PCR, and Western blotting were used on tissues from the pterygium and conjunctiva. The recurrence, growth activity, pterygium body translucency, and vascularity of pterygia phenotypes were employed as four grading criteria. ANG levels in pterygia and normal conjunctiva were compared when pterygia phenotypes were identified. It was also determined that the amount of a-smooth muscle actin (a-SMA) was associated with an increased proliferative capacity in the fibroblast. (Chui et al., 2007).

Pterygium stroma immunohistochemistry showed that ANG was highly expressed in all four severe phenotypes (recurrence, active growth, thick body, and significant arterialization), particularly in the surrounding tissue. Cultured pterygia fibroblasts with a severe phenotypic had greater levels of ANG expression than those with normal conjunctiva or no phenotype at all (Kim et al., 2013).

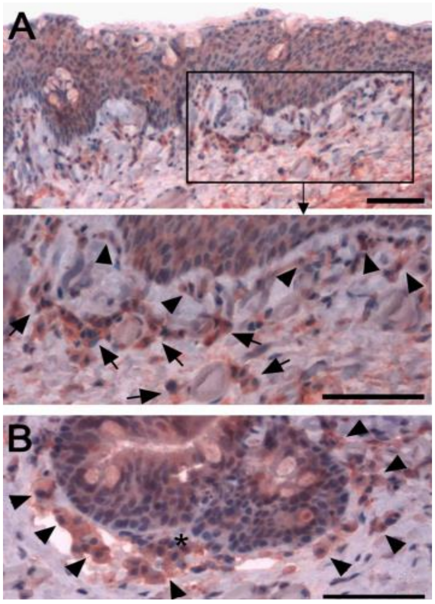


Figure 2.7: ANG expression in two pterygium
and right). (A and B) The stroma's subepithelial fibrovascular layer and the perivascular area both include ANG-stained cells. The stroma and basal layer of the epithelium express ANG in a manner that is very comparable (see asterisk in [B]) and is discussed just once. (Kim et al., 2013).

2.4.2. Pterygium Cells' Proliferation and Migration

A pterygium is a wing-shaped conjunctival fibrovascular growth that develops over the cornea. Pterygium is a condition marked by abnormal basal epithelial cell growth, neovascularization, and invasion of the neighboring corneal epithelium. Although numerous attempts have been made to identify probable pathogenic pathways, the etiology of this illness remains unknown (Cárdenas et al., 2016).
Pterygium incidence may be influenced by inflammation and fibrovascular proliferation (Anguria et al., 2014).

No prior study has examined its effect on conjunctival/pterygial cell proliferation. Two factors have been explored in relation to cell proliferation: loss of heterozygosity (LOH) and microsatellite instability (MI). LOH is connected with cancer and cell proliferation because it usually arises in the absence of tumor suppressor gene activity, whereas MI is a symptom of a dysfunctional DNA mismatch repair system (Cárdenas-Cantú et al., 2016).

Cell growth was slowed by 39-fold when 50 mM Nutlin was applied. Pterygium cell death was 95 percent and 28 percent following treatment with 2 mM and 50 mM of Nutlin, respectively, but conjunctival cell death was only 28 percent after treatment with MMC. At a fatal dosage of 50% for pterygium cells, 95% of conjunctival cells survived Nutlin therapy, whereas only 63% survived MMC treatment. P21 expression was not observed in pterygium cells treated with MMC, but was found after Nutlin treatment in Figure 2.8 (Cao et al.,2018).

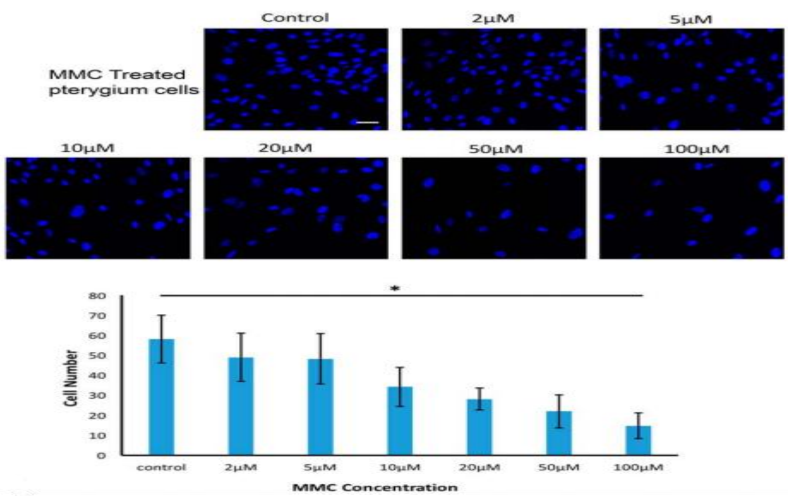


FIGURE 2.8: In the pterygium and conjunctiva, MMC inhibited cell growth and migration. Proliferation of pterygium cells was dramatically inhibited after MMC treatment, and this was accompanied by an increase in MMC concentration. (Cao et al.,2018).

2.4.3. Epigenetic Factors

Infection, recurrence, and cell proliferation are all features of Pterygium. When compared to conjunctiva that is healthy, Ki-67, PCNA and erythropoietin were overexpressed in the pterygium's epithelial cells; these proteins are linked with higher proliferative activity, low levels of cell apoptosis (Bcl-2 and p53), and malignancy (Hsp90) (Turan et al., 2020).

These proteins have been reported to be elevated in many cancer types, underlying neoplastic diseases, and tumor proliferative activity,95–97 and have been used to measure tumor proliferative activity (Weinstein et al., 2002; Cimpean et al., 2013).

In many cancers, DNA methylation patterns alter dramatically, and this has prompted a variety of studies on pterygium methylation patterns due to the similarities between the two conditions., Cell adhesion and matrix remodelling genes (MMP-2 hypomethylation) have been associated to pterygium invasion (TGM-2 and CD24 hypermethylation) (Shimmura et al., 2000).

DNA methyltransferase 3b (DNMT3b) overexpression in pterygium has also been associated to increased methylation of the tumor suppressor gene p16, according to the research. (Tasai et al., 2005).

These epigenetic changes are an alternative to DNA mutations, which can result in unregulated expression or repression of key genes involved in pterygium development., Although no such research has been done, oxidative damage induced by UV radiation might be to blame for these aberrant methylation patterns (Weinstein et al., 2002).

2.4.4. Growth Factors and Chronic inflammation is promoter of pterygium enlargement

Factors of growth30 Angiogenic and lymphangiogenic genes are stimulated by viruses, epigenetic abnormalities, the epithelial-mesenchymal transition, immune and anti-apoptotic processes, and other factors. Inflammation cascades4 and bone marrow recruitment, as well as alterations in cholesterol metabolism, are being disrupted (Song et al., 2005; Lee JK et al 2007).

A wide range of growth factors have been investigated because of the cell proliferation that occurs during pterygium development. Table 2.2 has a comprehensive list of all of these compounds, as well as a brief explanation of their involvement as pathogenic agents. (Cárdenas et al., 2016).

TABLE 2.2: Growth factors and their function in the pathogenesis of pterygium. The molecular foundation (Cárdenas et al., 2016).

Growth factor	**Abnormality found**	**Potential pathogenic role**
BFGF	Up-regulated after UVB radiation	Promotes cell proliferation
PDGF	Overexpression	Promotes cell proliferation
TGF-β	Overexpression	Promotes cell proliferation and inflammation
HB-EGF	Up-regulated after UVB radiation	Promotes cell migration
PEDF	Down-regulated	Promotes angiogenesis
VEGF	Up-regulated after UVB radiation	Promotes angiogenesis
IGFBP-2	Overexpression	Promotes cell proliferation
Stem cell factor	Overexpression	Promotes angiogenesis
CTGF	Overexpression	Promotes connective tissue remodeling

The heparin-binding epidermal growth factor (HB-EGF) has been shown to increase cell migration in pterygium (Komango et al., 2004).

Exposure to UV-B radiation has been reported to enhance the expression of basic fibroblast growth factor (bFGF),120, VEGF, and HB-EF. (Komango et al., 2004).

Pterygium macrophages have been discovered to contain VEGF and COX2, suggesting a function in angiogenesis, collagen deposition, and perhaps pterygium growth (Iwaya et al., 2007).

Pigment epithelium-derived factor (PEDF)128 and IGFBP-2130 have been shown to be reduced in patients with pterygia. A decrease in PEDF, an angiogenic inhibitor, might result in angiogenesis. Stem cell factor expression levels have risen (Wu et al., 2013).

Researchers have discovered many proteins in pterygium that are related to platelet-derived growth factor (PDGF) and connective tissue growth factor (CTGF) (TGF-b) (Shibata et al., 2020).

Inflammatory or degenerative, a pterygium (from the Greek word pterygos, which meaning "wing") is a condition. an increase in size Pterygium samples with infiltrating inflammatory cells are indicative of inflammation (Saw et al., 1999).

It's possible, but not likely, that inflammation can be used as an indicator of pterygium size because the count in all cases was very low in all sizes of pterygium, regardless of how large the pterygium was. The degree of inflammatory cell infiltration may be a better indicator of inflammation than the size of the pterygium. (Sakoonwatanyoo et al., 2004).

It is possible that pterygium development can be sped up by inflammation, independent of its intensity. Epithelial mesenchymal transition is unlikely to be the cause of pterygium since it need extremely high levels of inflammation in order to take place (Dushku et al., 1997).

Pterygium samples and controls with decreased MMP activity are almost certainly connected to inflammation (Sakoonwatanyoo et al., 2004).

TGF is activated during inflammation, which encourages fibroblasts to produce collagen., Furthermore, TGF suppresses MMPs. Collagen produced in the laboratory is deposited randomly, resulting in the opaqueness of previously clear tissues. Collagen is what gives pterygia their fleshiness and the cap (Thoft et al., 1997).

Inhibiting MMPs reduces collagenolysis, indicating that the collagen degradation seen in the majority of pterygia and controls was not caused by MMPs., Transforming growth factor-beta induces the production of VEGF (Kwok et al., 1994).

which increases neovascularization; hence, inflammation enhances pterygium neovascularization as well. Numerous studies have been published demonstrating the presence of inflammatory cells and abnormal HLA-DR expression (Chan et al., 2002).

2.4.5. Pterygium Immunological Mechanisms

Cytokines and growth factors; viral infections; genetic predisposition; anti-apoptotic mechanisms; extracellular matrix remodeling and immunological mechanisms have all been linked to the pathogenesis of Pterygium (Pinkerton et al., 1984).

Pterygium may also be caused by an immunologic process, as tiny lymphocytes and plasma cells infiltrate the pterygium. (Saw et al., 1999).

Immunofluorescence labeling may be used to confirm the presence of both MMPS and MMPS in explanted pterygium tissue fibroblasts (Kim et al., 2017).

Pterygium Fibroblasts are inhibited Based on the results of immunohistochemistry. MMP-3 and MMP-13 were highly expressed by fibroblasts migrating from the pterygium explant culture. By day 3, the cultivated conjunctiva fibroblasts had begun to move out of the sample. Just before staining, both conjunctiva and pterygium specimens were taken out of the eye. Few migrating cells expressing MMP-3 and MMP-13 were found by immunofluorescence labeling in conjunctiva tissue (Kim et al., 2017).

To check for abnormal p53 expression, researchers employed an anti-human p53 mouse monoclonal antibody. pAb 240 was utilized on eight eyes, six of which had initial pterygium and two of which had recurrent pterygium (Tan et al., 1997).

Repeated microtrauma in combination with poorly understood humoral (immunoglobulin A llgAL, IgM, and IgG) and cellular immunological processes (lymphocytes, plasma cells and mast cells). immunologic effects, perhaps by a type I mechanism involving stromal IgE accumulation. (Detorakis et al., 2001).

2.4.6. Extracellular Matrix Modulators

The extracellular matrix (ECM) is an acellular biomaterial that surrounds and supports all cells. The collagens, proteoglycans, and glycoproteins that make up ECM play an important function in the regulation of cellular differentiation and transformation; it also acts as a framework for correct conjunctival development (Anderson et al., 2017).

Extracellular matrices are well-organized networks made up of cells and non-cellular components found in all tissues and organs (ECMs). ECMs not only act as physical scaffolds for cells, but they also regulate a variety of cellular activities such as growth, migration, differentiation, survival, homeostasis, and morphogenesis (Theocharis et al., 2016).

Epigenetic changes in gene expression are well-known to have a role in the progression of human malignancies (Riau et al., 2011).

Proteoglycans/glycosaminoglycans, elastin, fibronectin, laminin, and other glycoproteins comprise the EMC, which is a non-cellular three-dimensional macromolecular network. (Theocharis et al., 2016).

Cells of various kinds interact with the ECM, a dynamic network of macromolecules with diverse physical and metabolic characteristics (Gattazzo et al.,2014).

ECM signals are transduced into cells via cell surface receptors, 171 Proliferation, adhesion, and other essential cell features are all influenced by this dynamic entity, which is of critical relevance. Maintaining proper homeostasis depends on the processes of migration, polarity, differentiation, and the survival of apoptotic cells. (Yue., 2014., Gattazzo et al.,2014).

The highest degree of FN1 was discovered in the PPI network of pterygium. It's a part of the extracellular matrix, and it's been found to be up-regulated in the whole retina of people who have had significant nerve damage., Pterygia development and recurrence are also linked to an increase in FN1., The ECM3 attachment of cells is a crucial event that regulates their survival, proliferation, and migration. Detachment of normal epithelial cells, (Mizutani et al., 2002).

TGM-2, MMP-2, and CD24 transcript and protein expression were altered in regions of aberrant DNA methylation, and suppression of methylation in cultured cells increased the expression of these genes. Dysregulation of these genes, which are involved in cell adhesion and matrix remodeling, could result in fibroblastic and neovascular alterations, as well as the creation of pterygium., Transglutaminase 2 (TGM-2) protein and location, as well as transcript levels of matrix metalloproteinase 2 (MMP2) and CD24 in pterygium compared to conjunctiva (Riau et al., 2011).

The proliferative cells are able to access numerous growth factors and cytokines as a result of this process, and so overcome the collagen barriers that prevent them from migrating, The action of numerous proteinases is primarily responsible for the modification of the extracellular matrix, many MMPs, particularly MMP-1, MMP-2, MMP-3, MMP-7, and MMP-9, were been demonstrated to have higher expression levels in the pterygium, notably in the head, an important region of migration and proliferation(Cárdenas-Cantú et al., 2016).

MMP-8 and MMP-13 expression has indeed been linked to pterygium etiology. TGM-2, that promotes extracellular matrix rebuilding, and MMP-2 both show aberrant DNA methylation, showing that the methylation status of essential extracellular matrix genes is linked to pterygium etiology. TIMPs (MMP inhibitors) were identified in abundance in pterygium specimens (Cárdenas-Cantú et al., 2016; Tsai et al., 2020).

2.4.7. Epithelial-Mesenchymal Transition

Cellular abnormalities such the epithelial-mesenchymal transition (EMT) to a myofibroblast phenotype have been related to the development of pterygium. (Riau et al., 2011). changing their phenotypic to fibroblastic cells under morphogenic pressure from wounded tissue is called epithelial- mesenchymal transition (EMT) , EMT is a well-characterized process that occurs during vertebrate development and is also found in adults during tissue healing and the earliest stages of cancer metastasis (Kato et al., 2007) .

When pterygium recurs, it shares characteristics with other malignant cells, particularly when it crosses the epithelium to the mesenchymal junction (EMT) (epithelial-mesenchymal transition) Various procedures, such like conjunctival autografts, amniotic membrane, radiation therapy, and mitomycin C, have been used (MMC), have demonstrated potential suppression of pterygium recurrence, additional study on molecular pathways is needed to give adjuvant therapy delivered after surgery (Meshkani et al., 2019).

Extracellular signals, such as ligand-dependent signals from soluble growth factors including TGF-B, FGF family and hepatocyte growth factors, can promote EMT in pterygium. These signals include both cell-to-cell and cell-to-extracellular matrix interactions, such as collagen and hyaluronic acid37 (Turan et al., 2020).

Exact signaling pathways leading to EMT after ligand activation are difficult to identify. Downregulation of E-cadherin is a significant final common mechanism. When the Snail and the Slug are phosphorylated on Ser38, they enter the nucleus and block the transcription of genes. Pterygium's development is clearly aided by EMT, but the specific upstream events that trigger Snail activation in PECS remain elusive (Gonzalez et al., 2014).

The observed cellular alterations might be the result of an abnormal wound healing response. There's a chance that MMP-7, as well as other effectors, are upregulated as a result of genome mutations, and there's also a chance that chronic inflammation is to blame. To connect UV radiation and/or inflammation to the activation of EMT in the etiology of pterygium, more research is needed (Eger et al., 2005).

2.5. DNA Repair in Pterygium

Endogenous stress (for example, during replication) and external stress (for example, when exposed to UV radiation) are constant threats to cells, both of which can result in DNA damage. Cells contain an arsenal of repair proteins that engage the proper repair pathway or, if damage is irreversible, cause cell cycle arrest and/or death to preserve genomic integrity. This article series delves into the pathways that detect and repair various forms of DNA damage, revealing novel DNA damage response regulatory mechanisms as well as the clinical consequences of disturbed DNA repair (Falet et al., 2002)

Epidemiological research shows that ultraviolet (UV) irradiation has the most significant role in the etiology of pterygia. More and more researchers now believe that pterygium is a form of uncontrolled cell proliferation caused by UV radiation, similar to that of a tumor, as evidenced by elevated levels of the tumor suppressor protein, p53, and a p53 gene mutation in the epithelium. (Tsai et al., 2005; tan et al., 1997).

Gene mutation and uncontrolled cell growth can occur as a result of UV irradiation. The DNA repair mechanism is able to restore most of the damaged DNA. More than 70 genes in humans are involved in five primary DNA repair pathways: direct repair, base excision repair (BER), nucleotide excision repair (NER) mismatch repair, and double strand break repair (DSBR). The NER and BER systems are two of the most important repair mechanisms for UV-induced DNA damage (Ichihashi et al., 2003).

X-ray repairs Both the NER system's XPA and XPD genes, as well as cross complementary I (XRCCI), a prominent BER system gene, are associated with the Xeroderma pigmentosum (XPA/XPD) gene family. Gene polymorphisms are thought to affect the host's ability to repair DNA damage and, as a result, modify cancer risk (Wu X et al., 2003 ; Xing DY et al., 2001)

Since both genetics and UV exposure are implicated in the development of pterygium, DNA repair enzyme polymorphisms XRCC1 and XPA, which are linked to pterygium, have recently been found using the single nucleotide polymorphism (SNP) method—and since these polymorphisms influence the host's capacity to eliminate DNA damage, it seems sense that there is a relationship between the two. (Xing et al., 2001)

In XRCC1, four polymorphism sites were investigated: codons 107, 194, 280, and 399. The majority of investigations on the codon 194 polymorphism—an arginine (Arg) to Tryptophan (Trp) conversion (CT, Arg194Trp)—found that the T allele was associated with a lower risk of cancer. The codon 399 polymorphism—a change from arginine (Arg) to glutamine (Gln) (GA, Arg399Gln)—has never been linked to cancer formation or poor DNA repair. Only a few modest investigations have determined that the less prevalent codon 280 polymorphism and its link to cancer are debatable (Goode et al., 2002)

There have been no studies linking the codon 107 polymorphism to the development of malignancies. A23G and codon 228 (G709A, Arg228Gln) were identified as polymorphism sites in XPA [29]. Several variants in XPD have been discovered, however the XPD codon 751 polymorphism (A, Lys751Gln) has been shown to impair DNA repair proficiency [18,20]. Pterygium development and XRCC1 codon 107, 194, 280, and 399 polymorphisms; XPA A23G; XPA codon 228; and XPD codon 751 polymorphisms were investigated in a case-control study (Chiang et al., 2010).

Double strand DNA (dsDNA) repair is mediated by two mechanisms: NHEJ and homologous recombination (HR). The most serious DNA lesions are double strand DNA breaks (DSBs), which if left untreated can result in death, mutations, or uncontrolled cell proliferation. Unlike single-strand DNA breaks, DSB are not induced by direct UV radiation exposure (SSB). They can, however, occur from two SSB nascent in the excision repair process lying close to one other (Batty et al., 2000; Park et al., 2002).

Ku70 is the only recombinational repair gene associated with pterygium that has been identified so far. Pterygium development is linked to the Ku70 promoter T-991C polymorphism, according to Tsai and colleagues7. The scientists showed that DSB plays a significant role in pterygium development and that repair of such damages in pterygium can be insufficient, which is linked to gene alterations (Chiang et al., 2010)

2.6. Apoptosis and Apoptosis-Related Gene Expression Levels in Pterygium

Pterygium's pathogenesis has yet to be determined. Recent research has revealed similarities between pterygium and neoplasia, raising the notion that pterygium is a neoplastic-like growth condition. The most well-known etiologic factor is UV light, which causes conjunctival degeneration. Pterygium, on the other hand, has lately been discovered to have various tumor-like features. The p53 gene is a frequent neoplasia marker that regulates cell cycle, differentiation, and death. (Weinstein et al., 2002).

By Ke and colleagues in 1972, apoptosis as a unique kind of cell death was established. Necrosis, on the other hand, is an active process. It takes both energy and a coordinated gene expression cascade to induce cell death. The expression of genes that promote or prevent apoptosis is thought to have a role in the progression down an apoptotic route. Genes such as-bel-2 and bel-xl are thought to be apoptosis inhibitors.

However, genes that induce apoptosis include other-geHES, like as Bcl-2, Bax, Bcl- x, Bak, and BelXs. Bel-2 and Bax are known to form heterodimers, and it is thought that the ratio of bel-2 to bax influences the destiny of the cell. (Oltvai et al., 1993 : Yang et al., 1995).

Bel-2, which inhibits apoptosis, binds to bax if it is in excess and the cell goes along an apoptotic route. All of the apoptosis-promoting Bax is bound, and the cell is protected against cell death when Bcl-2 levels are high. Interestingly, the p53 tumor suppressor gene regulates transcription of both of these genes, and p53 decreases bel-2 expression while increasing bax expression (Tan et al., 2000).

Pterygia specimens showed that apoptotic cells were concentrated in the epithelial layer's basal layer, which is located very next to the fibrovascular support layer of the epithelia. The apoptosis-inhibiting protein bel-2, as well as p53 and bax, were found to be highly expressed in these cells. There was no BCL-2 expression in normal conjunctival samples, and apoptotic cells were seen across the whole expression. the epithelium layer and high bax concentrations (Kerr et al, 1972).

2.7. Pterygium, loss of heterozygosity (LOH), and instability of microsatellites (MI)

Pterygia's genetic changes have been explored recently. Microsatellite markers have revealed a significant prevalence of LOH in pterygia, particularly in the 9p, 9q, and 17q regions. In the context of 'Knudson's two-hit theory,' the presence of LOH is a critical indicator of TSGS, and by screening matched blood and tumor samples with markers spread over the genome, we may identify potential places for TSGS (Freireich EJ et al., 1995).

There is a strong correlation between LOH in chromosomal regions 9p, 17q and P16 (9p21) and P53 (17pl3.1) and the development and progression of neoplasia in these regions. This is consistent with the fact that candidate TSGS, such as P16 (9p21) and P53 (17pl3.1), play a universal role in the development of cancer (Spandidos et al., 1997; Detorakis et al.,1998).

Using matched tumor and blood samples to compare microsatellite markers, the presence of several alleles in the tumor can be discovered, a discovery known as "microsatellite instability" (MI). A mutational route in oncogenesis has been proposed to explain MI, in which a failure to perform precise replication or post-replication error correction might destabilize oncogenic or TSGS (such as ras, P53 or Rb) and eventually lead to cancer formation. (Loeb., 1994; Jass JR 1999).

MI incidence in pterygia was shown to be much lower than LOH incidence, which is intriguing. Since the LOH pathway (shown by LOH) appears to be extremely active in the pathogenesis of pterygium, it is possible that the ‘mutator pathway (indicated by MI) may not play a significant role. (Detorakis et al., 2000).

A microsatellite study indicated another genetic modification in pterygium DNA, with microsatellite instability corresponding to a high mutation rate., Microsatellite instability indicates a disruption of the genome, which can affect other genes, resulting in dysregulation in cells with these mutations, the latter of which may be linked to pterygium-induced fibroblast proliferation. It would be interesting to investigate mutations in DNA repair genes in pterygiums with myocardial infarction and determine if similar mutations are also found in the patients' germline (Spandidos et al., 1997)

2.8. Pterygium Etiopathogenesis and the Role of Oxidative Mechanisms

Intracellular RF (Free Radical) causes oxidative damage, undamaged is acting on macromolecules such as proteins, lipids, and nucleic agents, FRs that are examined for levels such as superoxide anion (O2-), hydrogen peroxide, and others, Phototoxicity is a direct effect of UV radiation, while free radicals are an indirect effect (FR). Superoxide dismutase, catalase, and glutathione are enzymes that detoxify peroxynitrite and superoxide).

Homeostasis is improved when external or endogenous oxidative stress is present, and RF denatures many internal intracellular interactions such as nucleic functions, proteins, and lipids. If Malone dialdehyde (MDA) has been identified as a result of oxidative fat breakdown, and if the loss in its major chains becomes unsaturated, if there is oxidative stress, MDA is utilized as a leading sign for lipid peroxidation (Balci et al., 2011).

This suggests that oxidative activity will also play a significant role in pterygium pathogenesis, which is produced by ultraviolet radiation in these circumstances. To better understand the function of oxidative behavior in the etiology of primary or recurring pterygium, more research is needed (Uçakhan et al., 2009).

2.9. ACTR2 (Actin-related protein 2)

Using sequence homology to typical actin, the actin-related proteins (Arps) have recently been found, and it has been demonstrated that several Arps are definitely associated with cytoskeletal activities (Machesky et al., 1999; Pollard et al., 2000).

To govern actin dynamics, the Arp2/3 complex appears to play an important role in the formation of filaments, the encapsulation of the pointed ends of filaments, and the linking of filaments into networks (Mullins et al., 1998; Weeds and Yeoh, 2001 This complex must be positioned on the plasma membrane's cytoplasmic face in order to work with other regulatory proteins in the development189 of protrusive forces (Goldmann et al., 2002).

Alternative name: Actin-like protein 2; Other entities represented in this entry:

arp2/3 complex, included. Although the precise function of this gene is unknown, the protein it encodes is known to be a significant component of the ARP2/3 complex. The Arp2/3 complex is responsible for the creation of branching actin networks in the cytoplasm, which act as a driving force for cell motility (Welch et al., 1997).

It appears to come into touch with the daughter actin filament's pointed end (PubMed:9000076). Lamellipodia development in podocytes is a downstream effect of AVIL and PLCE1 regulation (Rao et al.,2017).

Apart from its cytoplasmic cytoskeleton function Additionally, the Arp2/3 complex regulates gene transcription and DNA repair by promoting actin polymerization in the nucleus, where it plays a key function (Yoo et al., 2007; Schrank2018).

By boosting nuclear actin polymerization, the Arp2/3 complex facilitates repair of DNA damage by encouraging the movement of double-strand breaks (DSBs), Because of its role in assembling and protruding lamellipodial actin, this complex is found on the cell's surface. We were able to identify two distinct transcript variants that encode this gene's two isoforms. (Schrank et al., 2018).

ARP2 are synonyms for this gene. cytogenetic location: 2p14 ; Genomic coordinates (GRCh38): 2:65,227,830-65,271,252 ACTR2 (Actin Related Protein 2) is a gene that codes for a protein. Wiskott-Aldrich Syndrome and Myopathy, Centronuclear, 2 are two diseases connected with ACTR2. Among the pathways to which it is connected are the RhoGDI Pathway and TCR Signaling (Qiagen). Actin filament binding and structural constituent of the cytoskeleton are two Gene Ontology (GO) annotations associated with this gene. ACTB is a significant paralog of this gene. (Sun et al., 2011).

The Wiskott-Aldrich syndrome gene is made up of a collection of proteins with similar domain structures that are involved in signaling transmission from cell surface receptors to the actin cytoskeleton.

The ACTR2 gene does have 10 exons and is found on the chromosome number 2 short arm (p) locations 2p14, it has 14,474 base pairs (Figure 2.9).

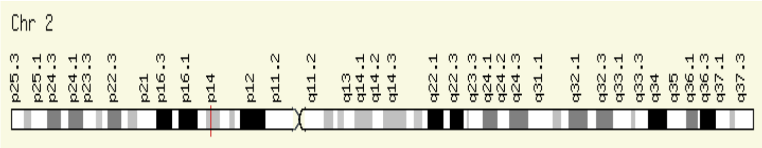


Figure 2.9: ACTR2 gene location on chromosome 2.

An amino acid sequence of 399 is encoded by the ACTR2. Arp2/3 is an important, evolutionarily conserved seven-subunit protein complex that, following stimulation by members of the WASP family, facilitates the assembly of the actin cytoskeleton in the cytoplasm. (Yoo et al., 2007).

Many fundamental biological activities, such as endocytosis and cell motility, depend on the cell's ability to rapidly assemble actin filaments. Actin assembly is mediated in part by the Arp2/3 complex. There are two proteins (Arp2 and Arp3) closely similar to actin in sequence and shape, as well as five additional proteins that are present in humans. (Pollard et al., 2003; Welch et al 1997)

A new branch of F actin can be formed by the Arp2/3 complex, which can kick-start actin polymerization. (Yoo et al., 2007).

The Arp2/3 complex is required for lamellipodia formation, as it promotes nucleation primarily at the leading edge (as opposed to regions further away from the front). Although it is not found in the shafts of filopodia, it is known to be involved in the beginning and growth of these structures. Invadopodium shafts, on the other hand, include the Arp2/3 complex. Additionally, it has been discovered near the base of invadopodium, but not at the points (Schoumacher et al., 2010).

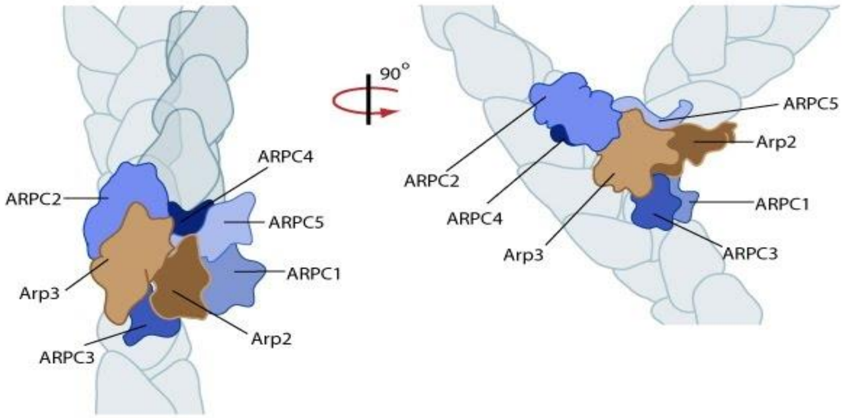


FIGURE 2.10: By attaching to the side of the mother filament and attracting actin monomers, the Arp2/3 complex generates a new branching filament. The Arp2/3 complex components remain at the filament's pointed end (Yoo et al., 2007).

The Wiskott-Aldrich syndrome protein (WASP)2 family, includes WASP, activates the Arp2/3 complex. Scar/WA VE (Wasp family verprolin homologous protein) and N-WASP are two such proteins. Activation of the Arp2/3 complex is facilitated by the interaction of the V domain with actin monomer and the affiliation of the CA domain with the Arp2/3 complex to stimulate filament production in these proteins. (Higgs, et al., 1999; Panchal et al., 2003)

A critical role in cytoplasmic actin filament nucleation and organization, the Arp2/3 complex has yet to be explored in relation to its location and function in the nucleus. (Yoo et al., 2007).

Table 2.3: This gene has 5 transcripts (splice variants), 115 orthologues, 2 paralogues and is a member of 1 Ensemble protein family (Yoo et al., 2007).

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt
ACTR2-201	ENST00000260641.9	3861	394aa	Protein coding	CCDS1881	P61160
ACTR2-202	ENST00000377982.8	2685	399aa	Protein coding	CCDS46307	P61160
ACTR2-205	ENST00000542850.2	1429	339aa	Protein coding	-	F5H6T1
ACTR2-204	ENST00000476840.5	587	53aa	Nonsense mediated decay	-	A0A1B0GWD0
ACTR2-203	ENST00000471552.5	554	65aa	Nonsense mediated decay	-	A0A1B0GWH0

In the Arp2/3 complex (actin-related protein 2/3 complex), there are six subunits: Arp2, Arp3, Arpc1, Arpc2, and Arpc3. Actin filament assembly is started when the complex attaches to an existing actin filament. In order to initiate the formation of a new filament, two actin-related proteins, ARP2 and ARP3, need to be linked to the mother filament by five other proteins. (Rouiller et al., 2008).

Many cellular activities are regulated by the Arp2/3 complex. Cell migration and adhesion are disrupted in many species when RNAI or inhibitory antibodies block the complex's activities. These processes include endocytosis and mitotic polarity establishment Interactions with nucleation-promoting factors are required for Arp2/3 complex participation in the production of new branching actin filaments (NPFS). WASP, N-WASP, WAVEI, WAVE2, WAVE3, and the newly discovered compounds, WASH. WHAMM. and JMY. comprise the NPFS family. Cdc42 and Rac activate the NPFS, while Abpl Panl and cortactin activate the Arp2/3 complex, according to recent research. (Sun et al., 2011).

Arp2/3, Cdc42, and Rac activators have been found to be essential for oocyte polarization, spindle formation, and migration during meiosis in mouse oocytes. An oocyte's meiotic maturation is aided by the Arp2/3 complex, which was the focus of the current study's investigation. That the complex is truly engaged in this mechanism and the ensuing asymmetrie division was shown by its unique expression pattern and its suppression by a particular inhibitor and RNA (Sun et al., 2011).

The protein family act as promoter for the nucleation of the highly expressed Arp2/3 complex is a WASP protein , which leads to actin filament branching. The development of lamellae and filopodia, endocytosis and/or phagocytosis in the plasma membrane, and the generation of charged vesicles via organelles such as the Golgi, the endoplasmic reticulum (ER), and the Endo lysosomal network are all regulated by Arp2/3 actin.

The activation of the actin-related protein (ARP2/3cytoplasmic) is required for WASP nucleation-promoting factors to reprogrammed gene transcription. Because WASP has been discovered to contain functional nuclear localization and exit sequences, its transcriptional actions are largely governed by its nuclear entry and escape via nuclear pores.

WASP is a nucleation-promoting factor (NPF) known for its cytoplasmic role in the production of F-actin via the actin-linked protein (ARP) 2/3, a cortical cytoskeleton regulatory . Although WASP expression is defined in hematopoietic cells, its absence causes a variety of abnormalities in lymphocytes, dendritic cells, myeloid cells, and megakaryocytes. Aberrant morphological and functional aspects are not usually associated with difficulties related to the F-actin cytoskeleton in WASP- deficient T lymphocytes, natural killer cells, and giant cells of a mouse or human, as well as cells harboring a WASP mutant removed by VCA (Sadhukhan et al., 2014).

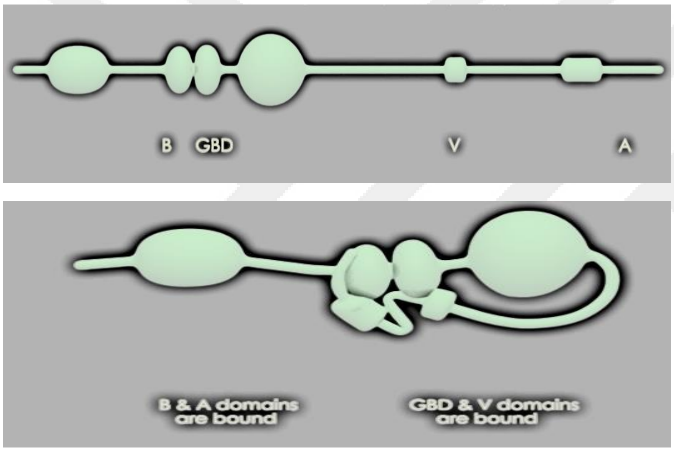
Although WASP without a structural CRIB domain normally localizes in clathrinid pits and activates the Arp2/3 complex during endocytosis, pharmacological inhibition of Rac has really no effect on WASP localization or activation at these sites. As a result, small GTPase-WASP interactions are more complicated than previously believed: WASP functions are controlled by Rac, but Active Rac helps prevent them from interacting with one another via the CRIB motif (Amato et al., 2019).

2.9.1. Activation mechanisms of the ACTR 2 and 3 complex

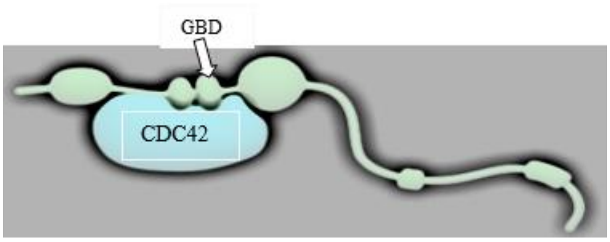
The Arp 2/3 complex comprise 7 protein sub unit, Both Arp2 and Arp 3 display a similar homology to actin., Arp 2/3 complex is activated by wiskott – Aldrich syndrome protein (WASp), Arp 2/3 complex mediate actin nucleation,

WASp exists in an auto-inhibited state witch B with A domain are bound and GBD with V domains are bound (figure A), until it is activated by cdc42 which binds to the GBD, displacing the VCA domains (Figure B) The VCA domain interact with ARP2/3 Complex,

ARP2/3 will not interact with auto-inhibited WASP ,. The VCA domains of activated WASp bind to ARP2 and ARP3 .



A.



B.

Figure 2.11 WASp in an auto-inhibited state witch B with A domain are bound and GBD with V domains are bound (A), Auto-inhabited WASp is activated by CDC42 which binds to the GBD domain (B) , The VCA domains of activated WASp bind to ARP2 and ARP3 .

This will induce conformational change in each of the 7 sub unit., activated WASp also bind to G-Actin . Inactivated ARP 2/3 complex will not associate with actin filament.

a weak association will form between WASP bound Arp2/3 complex and actin filaments; this will be strengthened by additional interactions between WASP-G-actin & the Arp 2/3 complex Figure 2.12:

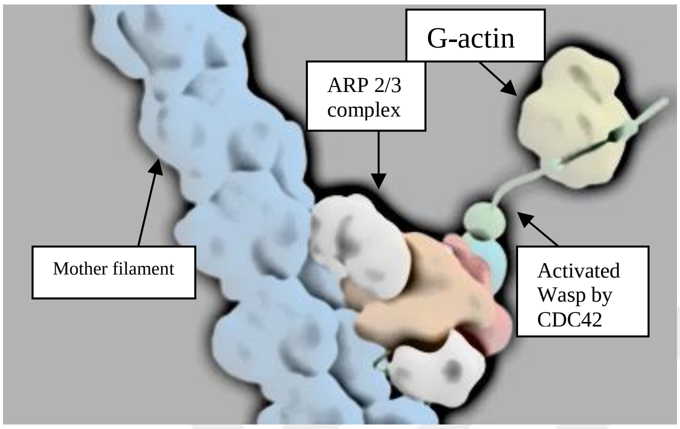


Figure 2.11 :- modifications anchor the Arp2/3 complex to the mother filament and enable the complex to interact with G-actin. By polymerization, the freshly nucleated daughter filament will continue to expand.

Small Cdc42 and PtdIns(4.5)P2 can modulate WASP and N-WASP functions. Activated Cdc42 (arrow 1) induces a conformational shift in WASP/N-WASP upon receptor contact. The binding of PtdIns (4,5) P2 amplifies the impact of Cdc42 and may also target WASP proteins to the plasma membrane. Actin filament construction and cytoskeleton remodeling are induced by WASP proteins that attach to the Arp2/3 complex and increase its actin-nucleation activity, which is activated by WASP activation When referring to proteins, abbreviations like WHI/PH (WASP homology domain1/pleckstrin homology), CRIB (Cdc42/Rac binding), and W (G-actin-binding domain) are frequently used (acidic domain). Figure 2.13 (Sechi et al., 2000).

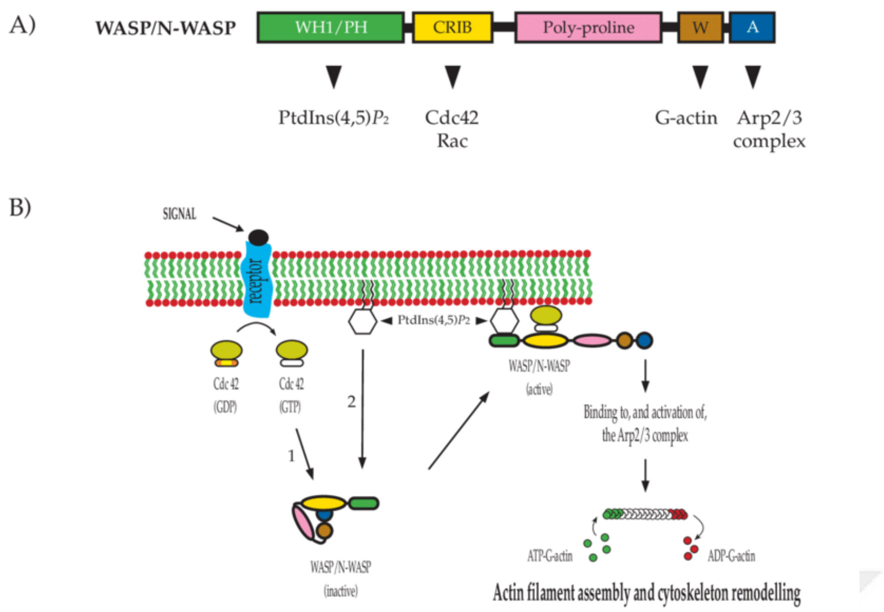


Figure 2.13. (A) S representation of the functional domains of WASP/N-WASP and their ligands. (B) Modulation of WASP/N-WASP function by small Cdc42 and PtdIns(4,5)P2. (Sechi et al., 2000).

Actin cytoskeleton reorganizes quickly in response to morphological characteristics and cell mobility, being signal-regulated molecular switches that trigger actin polymerization, Cdc42, a small Rho family GTPase that interacts in the philosophy, has functional and physical connections with WASP and N WASP. But on the other hand, it has been established that the WAVE/Scar proteins are linked to Rac, a Rho family protein that governs membrane turbulence. At the C-terminus of all WASP proteins is a VCA domain that activates the Arp2/3 complex, allowing actin polymerization to germinate. Unexpected activities of WASP proteins in multicellular animals have just recently been discovered through analysis of model organisms (Miki et al., 2003).

Cdc42, a Rho family GTPase, can govern the actin cytoskeleton by activating WASP family members. This activation promotes self-inhibitory interaction between both the GTPase binding domain and the carboxy-terminal area of WASP proteins (figure 2.9). Organic co-solvents or the C terminal region induce the structure of WASP's GTPase-binding domain (Kim et al., 2000).

Intramolecular interaction with the GTPase binding domain deactivates C- terminal residues that control the Arp2/3-actin nucleation complex in the self-inhibited complex. "Inherently unstructured peptides, such as the WASP GTPase-binding domain , may even be induced in functional states and diverse structural states, depending on the circumstances, yhey are a component of the ringless GTP-GDP dependent family that is independent of GTP-GDP, and the vegetations operate as molecular switches in the signaling processes that bind surface receptors to intracellular mechanisms, and they govern a wide array of cellular activities, from growth to differentiation (Miki et al., 2003; Kim et al., 2000).

Signaling that regulates the cytoskeleton, gene expression, a cell cycle symbol, and tumor metastasis is comparable to that of the Ras Rho-like subfamily GTPase. The primary members of the group include Cdc42, Rac, and Rho, which have a range of competing actin structures in cells, as well as filopodia and lamellipodia in stress fibers (Miki et al., 2003; Kurisu et al., 2010).

Cdc42 and RAC promote actin polymerization from scratch, whereas Rho mostly operates by bundling preexisting actin structures, elements of the Wiskott- Aldrich syndrome protein (WASP) family can block this interaction. Mutations in the prototypical member of group, WASP, produce the Wiskott Aldrich syndrome, is a childhood illness defined by actin cytoskeletal abnormalities in hematopoietic cells, resulting in thrombocytopenia, eczema, and immunodeficiency (Miki et al., 2003)

Arp2/3 complex connects WASP proteins with the cytoskeleton. The shape and dynamics of actin filament networks at the cell's anterior edge are controlled by an actin nucleus configuration. WASP, Highly Expressed Neuronal WASP (N-WASP), other Scar/WAVE proteins, and Bee1p/Las17p proteins all have a proline-rich central section followed by the VCA terminal section (Verprolin homology region (VHR), cofilin homology region (CHR), and acid region (AR)). The VCA region interacts with the Arp2/3 complex and actin, resulting in increased actin nucleation and rapid production of new actin filaments by the Arp2/3 complex (Kim et al., 2000).

Because the amino ends of WASP proteins differ from their carboxyl termini, members of the Arp2/3 family can be activated in response to distinct upstream signals. The WASP and N-WASP’s N-termini consist of 16-residue sequence known as the CRIB (for interactive Cdc42/Rac binding) motif, which is seen on many other Cdc42 and Rac effectors.

N-WASP and WASP bind to Cdc42, which is stimulated through the GTPase Binding Domain (GBD), which is made up of the CRIB motif and other sequences. The structural of the Cdc42-WASP-GBD complex was revealed, revealing that the conserved CRIB residues at the N-terminus create a linear epitope that binds to GTPase's b2 strand, whereas the C-terminal residues in ac b & ac propellers fold winding against switch I and II (Hagemann et al., 1999; Kurisu et al., 2010).

The GBD can also link to N-C-terminal WASP's VCA region, causing the protein's Arp2/3 stimulatory function to be autoinhibited. Cdc42 binding changes the structure of N-WASP, causing intramolecular interactions to break down and enhanced Arp2/3-mediated actin polymerization. Cdc42 activates N-WASP, which governs the activity of the Arp2/3 complex and, as a result, the actin dynamics required for the creation of filopodia. According to the researchers, the WASP GBD, like N-WASp, could bind its own VCA domain in a fashion that is competitive with Cdc42.

2.9.2. Mutations in the ACTR2 gene and phenotypic

Various researchers have separately documented a link between clinical complex proteins related to actin. Actin organization is regulated by the ARP2/3 complex, which contains the main subunits 2 and 3. (Mathur et al., 2003).

Arps were initially discovered in yeast but have since been discovered in amoebas and human cells. Arp1, or dynactin, forms a short filament with four new proteins and the p150 attached microtubule motor protein, which is required for microtubule structure and perhaps membrane trafficking., Similarly, Arp2 and Arp3 form a complex with five additional proteins that binds to actin in vitro and is immunofluorescent co-localized with the actin cytoskeleton in cells (Machesky et al., 1997).

The role of the Arp2/3 complex in mammalian cells is crucial because it may govern lamellipodial expansion and actin-based motility (Machesky et al., 1997).

However, the ARP2/3 complex, which is recognized as a versatile regulator of the actin cytoskeleton, is known to bind with five other new subunits, including ARP2 and ARP3 (Machesky et al., 1999).

The ARP2/3 complex is found on actin filaments in cells where dynamic actin assembly is taking place, according to research. promotes the nucleation of actin, which is necessary for organelle and pathogenic organism movement to be driven by actin polymerization. (Welch et al., 1997).

membrane fusion, as well as endocytose processes. It has been shown that in budding yeast, the loss of ARP2/3 complex subunits leads in sluggish development and a deficiency in cortical actin patches in the absence of ARP2/3 complex subunits (Mathur et al., 2003).

There was a decrease in mitochondrial mobility due to mutations in ARPC5 (an Arc16p homolog). Conditions defective in these genes exhibit problems in fission yeast's cortical actin cytoskeleton when Arp2, Arp3, or sop2 (Arc40p homolog) are present (Balasubramanian et al., 1996; McCollum et al., 1996).

Loss-of-function mutations in the Arpc1 and Arp3 genes in Drosophila reveal that the Complex is necessary for ring canal expansion, which is a risky step in oogenesis (Hudson et al., 2002).

ARP2, ARP3, ARPC2, ARPC3, and ARPC4 comprise the mammalian Arp2/3 complex. ARPCIA and ARPCIB, as well as ARPCSA and ARPC5B, each have one molecule. Human Ch.7 contains ARPCIA and ARPCIB in tandem. Both WD40 domain repeats anticipated to form a b-propellerfold3 are found in the isoforms they express, with 68 percent amino acid sequence identity15. Pancreatic cancer cells' migration and invasion may be regulated by ARPCIA. (Hudson and Cooley, 2002).

Actin cytoskeletal abnormalities are caused by mutations in the arp2 gene. When compared to wild-type cells, the arp2-1 mutant arrests with randomly dispersed cortical actin patches that display somewhat reduced staining with anti-actin antibodies. Throughout the cell cycle in S. pombe, the distribution of actin patches varies, with actin patches concentrating at regions of growth and cell wall deposition (Morrell et al.,1999).

Arp2/3 deficiency causes embryonic death20, whilst its inhibition inside cells prevents lamellipodia generation and migration (Kahr et al., 2017).

The actin-related protein arp2 colocalizes with actin at the cortical actin patch and is encoded by the arp2 gene. Both fission and budding yeasts have been investigated for the Arp2/3 complex. Subunits of the complex have been found in cortical actin patches in yeasts, and temperature sensitive Arp3p alleles cause actin patch motility to be lost (Morrell et al., 1999).

Furthermore, an Arp2p mutant in S. cerevisiae appears to have an endocytosis deficiency but not an exocytosis problem (Moreau et al., 1996, 1997). The capacity of a mutant deficient in Sop2p (the p41 subunit) function to reduce the temperature-sensitive growth deficiency of the profilin mutant cdc3-124 was originally found in S. pombe. Similarly, a mutation in the Arp3p gene in S. pombe suppresses the mutant profilin, suggesting that profilin and the Arp2/3 complex interact (McCollum et al., 1996).

2.9.3. Actr2 gene in disease

Actin filament branching is dependent on the Arp2/3 complex, which contains two ARPC1 component isoforms, one of which, ARPCIB, is highly expressed in human blood cells.

In most tissues, Actr2 is expressed in the cytoplasm. The highest levels of expression can be seen in subsets of immune cells in a variety of organs. The most highly expressed complex components were ARPC3 and ARPC4, whereas ARPC1B and ARPC2 were expressed at modest levels. Cell migratory capacity was generally decreased when the ARP2/3 complex subunits were silenced. Silencing of ARPC4 decreased cell migration in all of the cell lines examined, with a notable impact on Hs700T and HPAFII migration (50 and 68 percent reduction, respectively, $p<0.001$ (Rauhala et al., 2013) .

TABLE2.4: Disease and trait annotations associated with variants in this ACTR2 gene:

Variant ID	Chr: bp	Alleles	Class	p-value	Phenotypes
rs6715876	2:65259794	T/C	SNP	1.00e-6	Calcific aortic valve stenosis
rs72822411	2:65272334	G/A/T	SNP	6.00e-9	Coronary Artery Disease
rs62139085	2:65280779	G/A	SNP	8.00e-7	Coronary Artery Disease
rs36010930	2:65289100	T/A/C	SNP	8.00e-12	Heel bone mineral density
rs1039766	2:65293011	C/T	SNP	2.00e-6, 3.00e-6	Lung adenocarcinoma; Lung Cancer
rs10186240	2:65350517	T/G	SNP	7.00e-6	Metabolite levels

3. MATERIALS (equipment) AND METHODS

3.1. Materials (equipment)

3.1.1. Sampling

This study included twenty-seven individuals who were diagnosed with pterygium and applied to Tokat Gaziosmanpaşa University Medical Faculty's Department of Eye Diseases. In the formation of the study groups, pterygium tissues taken during the operation as the patient group; healthy conjunctival tissues belonging to the same eye of the same patients were defined as the control group. The necessary permission for the study was obtained by the Clinical Research at Tokat Gaziosmanpaşa University's Faculty of Medicine by project number 2019/110. Tokat Gaziosmanpaşa University Scientific Research funded this research with project number 19. KAEK-024. The research was carried out in Medical Biology and Genetics laboratories.

3.1.2. Storing Tissues

With the permission and knowledge of the participants involved in the research, tissue samples taken during the surgery were quickly cut into desired pieces for further study. The residual (remaining) tissues were frozen at -80 degrees Celsius for RNA isolation, cDNA extraction, and gene expression analysis. The expression levels of the ACTR2 gene from cDNA isolated from tissues were assessed using real-time PCR.

3.1.3. Devices Used in the Study

Refrigerator (Arcelik, 570 465 MB, Turkey)
Shaker (Ika, Germany)
-20 ° C freezer (Arcelik, 2052DY, Turkey)
-80 ° C Deep Freezer (Nuaire, NU-9483E, USA)
Distilled Water Device (Elga-option Q7, UK)
Oven (Memmert, Germany)
Power Supply (Biorad, 1645070, USA)
Precision Balance (Kern ABT, WB0750631, Germany)
Homogenizer (Next Advance Storme 24, BBY24M-CE, USA)
Magnetic Stirrer (Velp Scientifica, F20520162, Italy)

Microwave (Arcelik, MD554, Turkey)

Autoclave (HMC, HV25, Germany)

Automatic pipettes (Gilson, France)

pH Meter (Hanna Instruments, HI2020W, USA)

Plate (Applied Biosystems, 4346907, England)

Plate adhesive (Applied Biosystems, 201405092, UK)

Qubit 2.0 Fluorometer (Invitrogen by life technologies, Australia)

Real-Time PCR (Applied Biosystems, UK)

Centrifuge (Hettich, D78532, Germany)

Refrigerated centrifuge (Hettich, Germany)

Vortex (Velp Scientifica, F20220176, Italy).

3.1.4. Chemical Materials Used, Kits and Kit Contents

cDNA synthesis kit (GeneAll, Hyperscript, First strand synthesis kit Catalog # 601-005, Korea)

Reverse Transcriptase Enzyme

10X RTase Reaction Solution

Mix 10 mM dNTP

RNase Inhibitor

Oligo (dT) 20

Random hexamer (primary)

Nuclease-free water

Ethanol (Sigma-Aldrich Catalog No: E7023, USA)

Qubit ssDNA Assay Kit (Invitrogen Catalog No: Q10212, USA)

Qubit RNA HS-Assay Kit (Invitrogen Catalog No: Q32852, USA)

Isolation of RNA kit (Thermo, Catalog No: 12183018A, USA)

Lysis Solution

Washing Solution 1

Washing Solution 2

RNase-free Water

RNaseZap (Thermo Catalog No: AM9780, USA)

Running Solution (ThermoFisher, NP0002, USA)

3.2. Method

The tissues taken during the surgery were kept at-80 ° C. The tissue pieces were used for RNA extraction and gene expression analysis.

3.2.1. RNA Isolation from Tissue

1-Using a homogenizer, homogenize pterygium specimens in 0.75 ml RiboExTM LS/50–100 mg of tissue.

2-Pipette or vortex 0.25 ml of the specimen into 0.75 ml RiboExTM LS.

3-Incubate the mixture for 5 minutes at room temperature.

4-Transfer the supernatant to a new tube after centrifuging at 12,000 x g for 10 minutes at 4°C.

5-0.75 ml RiboEx TM LS is mixed with 0.2 ml chloroform. Mix thoroughly for 15 seconds, then set aside for 2 minutes at room temperature.

6-Centrifuge for 15 minutes at 4°C at 12,000 x g, then transferred the aqueous phase to a new tube.

7-Incubate specimens for 10 min at room temperature.

8-Centrifuge at 12,000 x g for 10 minutes at 4°C then remove the supernatant.

9-Wash the RNA pellet with 1 mL of 75 percent ethanol. The RNA precipitated can be kept in a 75 percent ethanol solution at 4°C.

10-Centrifuge for 5 minutes at 7,500 x g at 4°C. Thoroughly remove the supernatant, ethanol, after that air-dry the RNA pellet for 5 minutes.

11-Incubate for 10 to 15 minutes at 56°C after dissolving RNA in DEPC-treated water or a 0.5 percent SDS solution.

3.2.2. cDNA Synthesis

To obtain optimum performance with the cDNA Synthesis Kit, make sure that RNA samples do not contain PCR inhibitor, reverse transcription inhibitor, RNase activity, and genomic DNA. Mix the kit components in a microtube in the recommended proportions below. Centrifuge the tube immediately to remove any air bubbles and spin down the contents. Place the test tube in the freezer until the reverse transcriptase reaction is complete.

TABLE 3.1. Reverse transcriptase reaction volume (20 µl)

For 20 µl Reverse Transcriptase Reaction	Volume
10X Reaction Buffer	2 microliters
dNTP mixture (2.5 mM each)	1 microliter
Randomized hexamer (50 µM)	2 microliters
Reverse Transcriptase (200 U/µl)	1 microliter
RNase Inhibitors	0.5 microliter
RNase free Water	3.5 microliters
RNA Template	10 microliters

TABLE 3.2. PCR program applied for cDNA synthesis

RT Steps	**Temp (°C)**	**Time**	**Cycle**
Step 1	25	10 minutes	1
Step 2	37	120 minutes	1
Step 3	85	5 minutes	1
Step 4	4	∞	1

3.2.3. qRT-PCR step

Real-time PCR, which enables the simultaneous observation of nucleic acid amplification, is a technique based on the measurement of a fluorescent signal that increases in proportion to the amount of DNA. In this method, the amount of amplification is determined by binding to the DNA helix and using fluorescent dyes (such as SYTO9, SYBR Green) or probe sequences that generate signals due to the degradation (Tsai et al., 2009).

TABLE.3.3. PCR reaction components (20 µl) The reaction cycle was set up on the Applied Biosystems Real Time PCR instrument as follow

Components	**Volume**
A.B.T.™ 2X qPCR SYBRGreen Master Mix (with ROX)	10 microliters
1X ROX Dye (50X)	0.4 microliter
Forward Primer	1 microliter
Reverse Primer	1 microliter
Template cDNA	3 microliters
Rnase-free Water	4.6 microliter

3.2.4. qRT-PCR program implemented

50 ° Celsius 20 sec

95 ° Celsius 10 min

95 ° Celsius 15 sec

55 ° C 1 min

Melt Curve: 95 ° Celsius 30 sec, 60 ° Celsius C 15 sec

40 cycles

3.2.5. Calculation of cDNA Concentration

The amount of cDNA was measured using the Qubit 2.0 device and the ssDNA Assay Kit from Qubit (Invitrogen, Q10212). The amount of cDNA to add to each assay was calculated to be 60 ng, and also the amount of cDNA to add to each sample was calculated independently.

3.2.6. RNA Quantification

For measuring the concentrations of RNA samples, Qubit 2.0 device and RNA HS-Assay kit (Q32852) were used.

4. RESULT

4.1. Result of working group

In this research, 27 patients with pterygium illness (18 men and 9 women) were sent to the Tokat Gaziosmanpaşa University Hospital's Ophthalmology Department. The patients in the study were 33 percent female and 67 percent male, with an age range of 43 – 78 years and an average age of 58± 8,43. Tokat Gaziosmanpaşa University Faculty of Medicine Medical Biology and Genetics laboratories were used to complete the entire investigation, depicts the gender distribution of the studied patients in Figure 4.1.

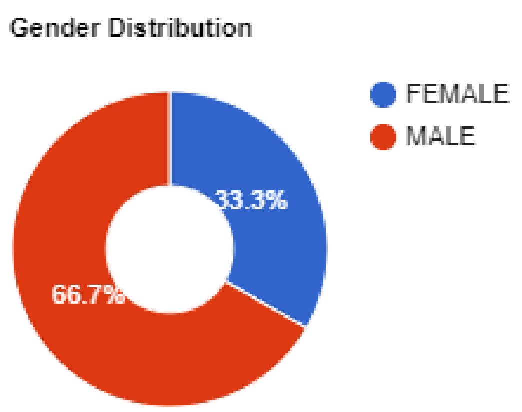


Figure 4.1: The Gender Distribution of the studied Patients

4.2. Results of quantitative real-time PCR (qRT-PCR) analysis

4.2.1. qRT-PCR image and evaluation of the ACTR2 gene

The quantitative Real-Time PCR method based on SYBR Green was used to evaluate the gene expression with ACYR2 mRNA, for ACTR2 and the gene preferred as a reference (Actin: ACTB), samples of cDNA (1/10: 1/100: 1/1000 and 1/10000) at different concentrations were prepared and serial dilution was made.

In the figures below, the amplification curve and melting peak of ACTB and ACTR2 genes observed as a result of qRT-PCR are given, respectively (Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5)

Figure 4.2: Gene amplification plot.

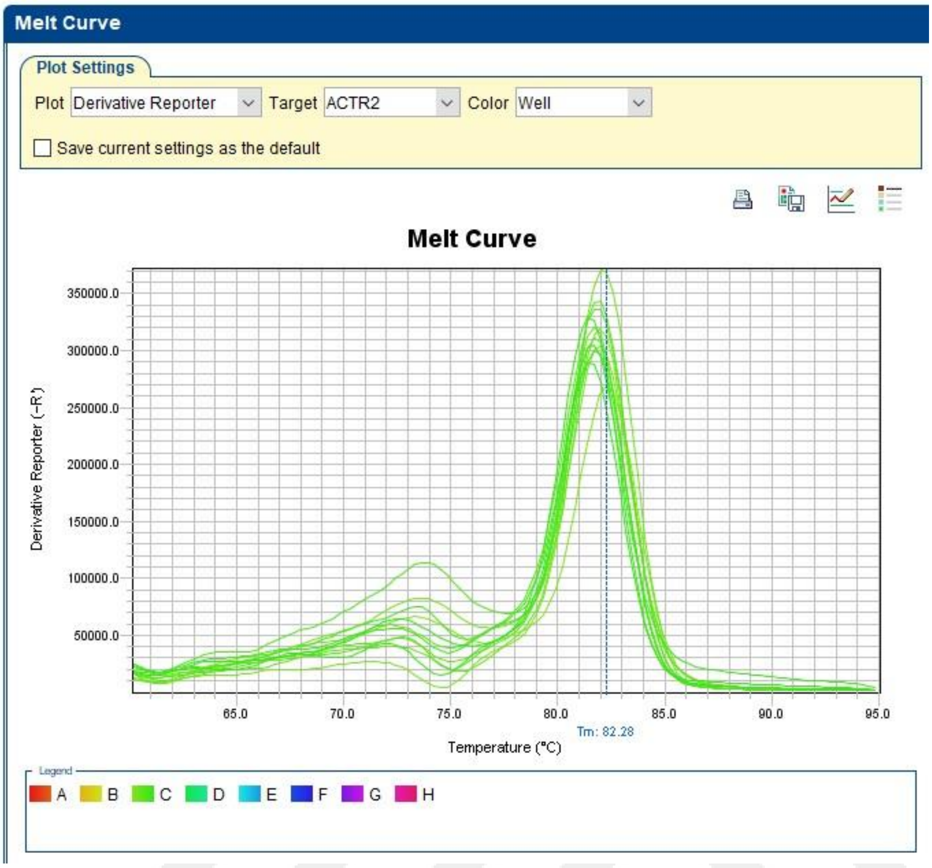


Figure 4.3: ACTR2 gene melt curve.

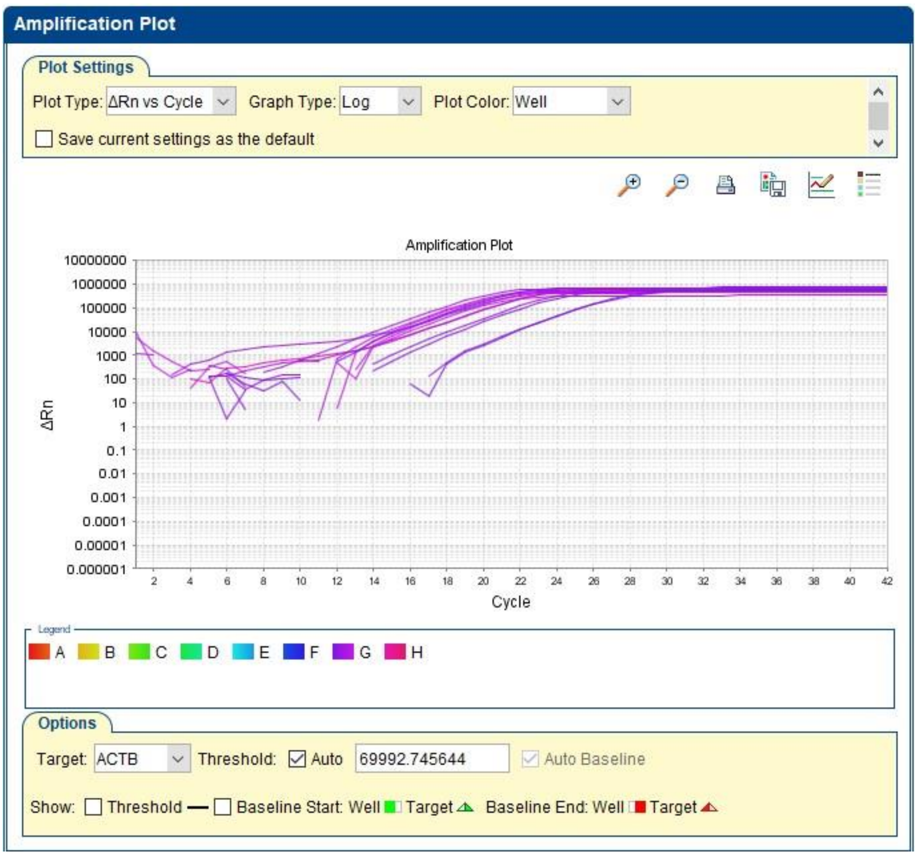


Figure 4.4: ACTB amplification plot.

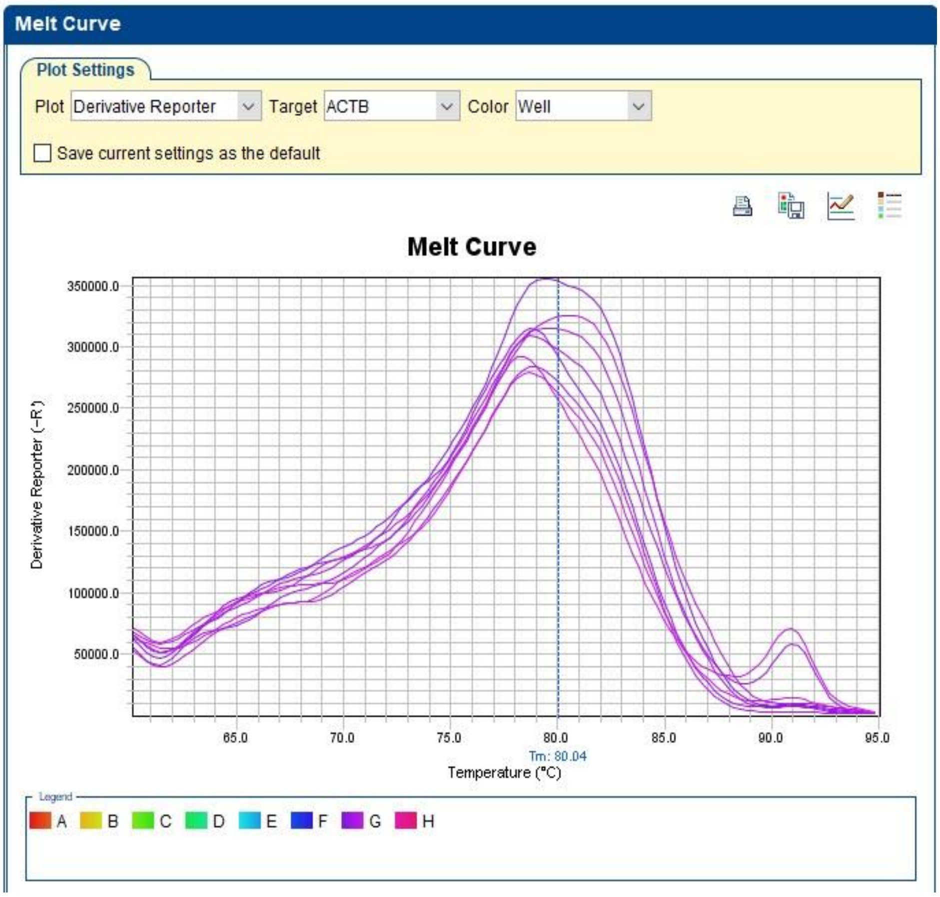


Figure 4.5: ACTB melt curve.

4.3. Statistical analysis of qRT-PCR findings of the ACTR2 mRNA gene

qRT-PCR analysis was performed for mRNA level expression of ACTR2 gene found in pterygium tissues and control conjunctiva tissues. The qRT-PCR results of ACTR2 and ACTB genes were quantified with step one plus software v2.3. The data belonging to the ACTR2 gene was normalized with the ACTB gene and the $2^{-\Delta\Delta Ct}$ formula was used to calculate the fold change. Statistical analysis was performed with the data obtained from this process. All data was given as mean±SEM. Analysis of the results was done according to the range of 0.9 – 1.1. At values less than 0.9, the expression level of the relevant gene is decreased in pterygium tissue compared to normal conjunctival tissue. If it is in the range of 0.9–1.1, it does not change compared to normal conjunctival tissue, and at values higher than 1.1, there is an increase in the expression of the gene compared to normal conjunctival tissue. (Schmittgen and Livak., 2008). Statistical analysis of expression data was performed using SPSS 16.0 software. A student's T test was used to examine the data's significance levels. If $p \leq 0.05$ is significant, the statistical significance threshold was accepted as 0.05; if $p > 0.05$, the evaluation that there is no difference.

4.3.1. T-Test

Analysis results

TABLE4.1: Statistical analysis of $2^{-\Delta\Delta Ct}$ values of ACTR2 gene and conjunctival tissues

Gene	Tissue	
	Pterygium tissue (N=27)	Normal Conjunctiva (N=27)
ACTR2 gene (AVG±SEM)	2.70±0,520	
P	0,005	

According to the qRT-PCR analysis results When the expression level of the ACTR2 gene was compared with pterygium tissues, it was found that it increased ACTR2 gene expression in the pterygium tissue (2.70±0.520). Statistically significant in terms of ACTR2 gene mRNA expression level (p=0,0001). When the expression level of the ACTR2 gene was compared with the pterygium and normal conjunctival tissues, it was determined that the pterygium tissue increased (2.70±0.520change) compared to the normal conjunctival tissue (Figure 4.6). In terms of mRNA expression level, the gene was found to have a statistically significant ($p \leq 0.05$). That means there is a significant difference was found between expression levels of ACTR2 gene in pterygia disorder tissues and healthy conjunctival tissue ($p \leq 0.005$).

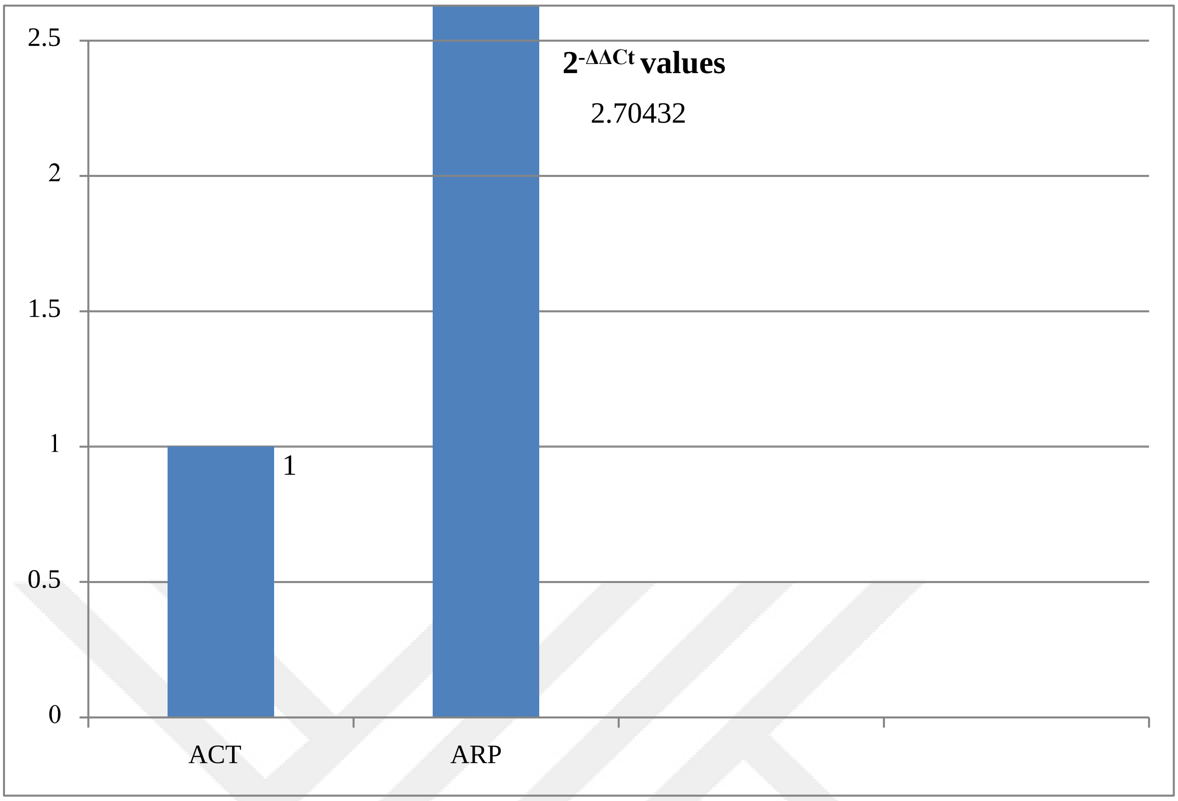


Figure: 4. 6. Average $2^{-\Delta\Delta Ct}$ values of ACTR2 and ACTB genes.

5. DISCUSSION

A pterygium is a wing-shaped conjunctival fibrovascular growth that develops over the cornea. 1 People who reside in locations with strong UV exposure are more likely to develop pterygium. Dusty, hot, dry, windy, and smoky conditions also contribute. 2 The majority of cases occur on the nasal side. (Hall et al., 2016)

UV radiation from sun light is a significant environmental component in pterygium growth. Despite the fact that pterygium is considered a degenerative lesion with elastic degeneration. According to recent findings, because it has numerous tumor-like characteristics, such like invasion of healthy tissue, higher frequency recurrence percentages post excision, & subsequent premalignant lesions, pterygium is considered a pre - malignant tissue (Bozkurt et al., 2020).

There may be redness, inflammation, blurring of vision, double vision, itching, and aesthetic concerns. (Hall et al., 2016)

Pterygium pathogenesis has been linked to a mutation in the p53 gene in numerous studies. As a consequence, pterygium is more likely to be considered a tumor caused by uncontrolled cell proliferation than a growth condition like neoplasia. The reality that pterygium is a benign neoplastic lesion with limited local invasion and no metastasis, as well as the fact that its cells show tumor-like genetic features, lends credence to the notion that it is a benign neoplastic lesion. (Weinstein et al., 2002).

To determine pterygium size, it is likely that the fraction of growth factors lacking inhibitory smad proteins (polygenic model) determines the pterygium's growth rate. According to the fraction of growth factors lacking inhibitory smad proteins in big pterygia, severity may be assessed.(Ganna et al., 2013).

FN1, KPNB1, DD B1, NF2, BUB3, PRSS23, MEOX1, ABCA1, KRT6A, SSH1, RBM14, and UPK1B are all DEGs that have a role in pterygium pathophysiology. (Yue ET AL., 2019)

Additionally, the immortalized human corneal epithelial cell lines, have ECT2 overexpression. A variety of eye illnesses can be attributed to MMPs. Pterygium, has been linked to MMPS abnormalities in the eye, as well as a variety of other conditions. Pterygium's etiology and development may be aided by MMP3, a member of the MMPS family. MMP3 was discovered to be up-regulated in pterygium (Seet et al., 2012; Di Girolamo et al., 2000).

Pterygium, a symptom of extracellular matrix disintegration, may be protected from by MMP3 inhibitors, it has been claimed. (Yue et al., 2019).
The fact that MMP3 was shown to be overexpressed in pterygium supports the theory that MMP3 plays a function in the disease process. In primary pterygium, SPARC, a matrix protein, plays a role in the epithelial-mesenchymal transition, tissue fibrosis, and angiogenesis. Aside from collagen formation and deposition, SPARC has been shown to be a well-known mediator. SPARC expression has been linked to abnormal extracellular matrix deposition in pterygium, according to one study (Bradshaw et al., 2009).

A large increase in BCL-2 expression with anti-apoptotic capabilities reveals that both cell proliferation and death involved in the pathophysiology of pterygium (Liang et al., 2011). By evaluating removed pterygium tissue and superior conjunctival tissue that was acquired by PCR, the expression of MiR-21 was determined. After pterygium surgery, human pterygium fibroblasts (HPFs) were collected and a primary culture was kept. A total of 58 patients with a unilateral pterygium issue were evaluated.

Relative to healthy conjunctival tissues, the pterygium tissue had a higher amount of miR-21. With the severity of the pterygium, the amount of miR-21 rises. By raising PTEN expression, the miR-21 inhibitor promoted death and inhibited proliferation in HPF cells, while the PTEN VO-Ohpic inhibitor trihydrate lowered p- AKT expression. (Li et al., 2018).

Cancer cells spread by invading local tissue and then traveling to various organs via through bloodstream or lymphatic system (metastasis). A multi-step process that causes to metastatic progression is known as a metastatic cascade. Cancer cells, particularly those of epithelial origin, diminish cadherin-based cell adhesion and it can destroy the underlying basement membrane at first, despite the fact that some cellular activities are altered throughout these processes. The cells then become movable in the extracellular stromal matrix (ECM) and show increased motility with high proteolytic activity, culminating in a pathological state called local invasion.

Understanding the mechanisms underpinning invasive progression, the first stage in metastasis, is crucial to understanding cancer-related death because cancer mortality is predominantly caused by progression to invasive and metastatic states, the actin cytoskeleton plays a vital role when tumor cells become invasive: its major function is to sustain intercellular mechanical adhesion (cell–cell adhesion) and to govern the morphology of the cells. An epithelial-mesenchymal transition (EMT) occurs in some benign cancers. (Kurisu et al., 2010).

It has been suggested that the Arp2 and 3 complex initiates actin polymerization. Actin filaments at the leading edges of moving cells are organized into stiff networks. (Mullins et al., 1998)

ACTR2 gene expression in primary pterygia was studied as part of this study to see if it is possible that pterygium is caused by uncontrolled cell proliferation rather than a degenerative disease.
There is currently no known function for the ACTR2 gene, although the protein encoded by this gene is a significant component of the ARP2/3 complex, which includes ARP2 and ARP3. Lamellipodial actin assembly and protrusion are critical to cell shape and motility, and this complex is positioned on the cell surface.

According to the findings obtained from the real-time PCR data, there was even a meaningful value between the gene and the disease from expression pain ($p \leq 0.005$).

When the pterygia and conjunctival tissues were compared, it was found that the gene expression level of the pterygium compared to the conjunctiva (1.910±0,367-Fold change, p=0,0001) significantly increased ($p \leq 0.005$),

The findings imply that the ACTR2 gene plays a role in pterygium disease, but additional research is needed to determine its efficacy and further understand the ACTR2 gene's association with pterygium. More research on the ACTR2 gene should be done in the future, using different approaches with more patients to achieve various results and better understand the ACTR2 gene's role in pterygium.

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