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TOKAT GAZİOSMANPAŞA UNIVERSITY

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ANALYSIS OF WASP GENE EXPRESSION IN PTERYGIUM

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Master Thesis

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ETHICAL CONTRACT

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MAHDI AL-ZANDI

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ABSTRACT

Analysis WASP Gene Expression in Pterygium. Tokat Gaziosmanpaşa University, Health Sciences Institute, Department of Medical Biology, MSc. Thesis, Tokat.

Pterygium is a benign growth of the conjunctive tissue that covers the sclera that is proliferative and causes intrusive ocular surface and wide matrix remodeling.

The WASP gene is located on the p arm (short arm) of the X chromosome at locations Xp11.4→ p11.21 and encodes a 502 amino acid sequence. The WASP gene comprises 12 exons, and the protein has a molecular weight of about 65 kD.

The WASP gene has multiple distinct domains, including a GTPase, GBD, PH domain, cofilin, and verprolin homology domain, SH3 binding motifs with a proline-rich locus, and the C-terminal acidic region, which plays a role in actin cytoskeleton remodeling by beginning Arp2/3. The WASP gene appears to play a function in signal transduction as well as cytoskeletal remodeling.

Actin restructuring, platelet formation/removal, cell-cell contact, actin polymerization, cell trafficking and motility, immunological synapse arrangement, and neutrophil formation/release are all linked to WASP.

The goal of this research was to see if the WASP gene was expressed in pterygium.Tissues were removed throughout surgery with the patient's agreement and knowledge. By collecting cDNA from the tissue levels established by real-time PCR, we employ the WASP gene expression technique. WASP gene expression levels were determined to be statistically significant higher in tissues with pterygium compared to the control group ($p < 0.005$), based on our findings that the increase in WASP gene expression is hypothesized to be linked to the formation and development of pterygium.

According to qRT-PCR data, WASP gene expression is pterygium tissue compared to healthy conjunctive tissue (1.9100.367-foldchange, p=0.0001).

Key Words: Pterygium, Real-Time PCR, WASP

ÖZET

Analiz Pterjiyumda WASP Gen Ekspresyonu, Tıbbi Biyoloji Anabilim Dalı, Sağlık Bilimleri Enstitüsü, Gaziosmanpaşa Üniversitesi, MSc. Tez, Tokat. Pterjiyum, sklera üzerinde uzanan konjonktif dokunun iyi huylu, kontrolsüz büyümesidir, pterjium, inflamatuar, proliferatif ve invaziv oküler yüzey ve kapsamlı matriks yeniden şekillenmesi ile karakterize edilir.

WASP geni X kromozomunun kısa (p) kolundadır. Xp11.4 → p11.21, 1823 baz çifti içerir ve bir 502 amino asit proteinini kodlar. WASP geni 12 eksona sahiptir, bu proteinin moleküler ağırlığı yaklaşık 65kD'dir.

WASP geni, bir guanozin trifosfataz (GTPaz) bağlanma alanı (GBD), bir pleckstrin homoloji (PH) alanı, bir verprolin ve bir cofilin homoloji alanı, SH3 bağlama motifleri ile prolin açısından zengin bir bölge dahil olmak üzere birkaç benzersiz alan içerir, ve Arp2/3 'ü aktive ederek aktin hücre iskeletinin yeniden düzenlenmesinde rol oynayan C-terminal asidik bölge WASP geninin, sinyal iletiminde ve hücre iskeletinin yeniden düzenlenmesinde kritik roller oynadığı görülmektedir. WASP, aktin yeniden organizasyonu, trombosit üretimi/uzaklaştırılması, hücre-hücre etkileşimi, aktin polimerizasyonu, hücre trafiği ve motilite, immünolojik sinaps oluşumu, fagositoz ve nötrofil üretimi/salımı ile ilgilidir.

Bu araştırmanın amacı, WASP geninin pterjium ekspresyon dokusundaki Düzeyini belirlemektir. Gerçek zamanlı PCR ile dokulardan cDNA sentezi ile, Wasp gen ekspresyon düzeyi tespit edildi olabileceği değişimin çalışma sonuçlarımız ile, WASP gen ekspresyon düzeylerinin pterjiyumlu dokularda kontrol grubuna göre istatistiksel olarak anlamlı derecede yüksek olduğu belirlendi ($p < 0{,}005$).

qRT-PCR verilerine göre WASP gen ekspresyonu sağlıklı konjonktif dokuya göre pterjiyum dokusudur (1.9100.367 kat değişim, p=0.0001).

Anahtar Kelimeler: Pterjium, Real-Time PCR, WASP

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ABBREVIATION AND SYMBOL LIST

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.
SNPs	:	single nucleotide polymorphisms.
PCNA	:	proliferation cell nuclear antigen.
DNMT3b	:	DNA methyltransferase 3b.
COX2	:	Cyclooxygenase-2.
NFkb	:	nuclear factor kb.
PEDF	:	pigment epithelium-derived factor.

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 an inflammatory and proliferative development originating from stem cells, it is characterizing by inflammatory, proliferative, and invasive ocular surface and broad matrix renovating, including the annihilation of (BL) Bowman layer (Ye et al., 2004; Di et al., 2006), further, it is related with astigmatism and obstructed or obscured vision. 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).

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).

In addition, the significance of heredity, rural older adults associated with hours spent under sunshine per day (Romano et al., 2016; Jiao et al., 2014), Heparin-binding epidermal development factor-like growth factor and UV light (Nolan et al., 2003), Intracellular modifications of cholesterol (Peiretti et al., 2007), Glucose-6-phosphate-dehydrogenase deficiency (Peiretti., 2007), raising cellular proliferation levels and cellular apoptosis low levels (Liang et al., 2011; Ozturk et al., 2017), Overexpression of ANG (Angiogenin) (Kim et al., 2013), chronic irritation by dust (Nuhoglu et al., 2013).

WASP gene is encoded for a multifunctional protein with key roles in many activities. Working along with a range of adaptor molecules or kinases , WASP is involved in cytoplasmic motioning (Kheirkhah et al., 2013), platelet production/removal, cell-cell interaction, actin polymerization, cell trafficking, phagocytosis, and neutrophil creation/release, signal transduction and rearrangement of the cytoskeleton (W Hirst et al., 2003). locomotion, immune synapse formation, and apoptosis (Dushku et al., 1999), lipid pontoons following TCR(T-cell receptor) incitement, and synapses of immunology between antigen-introducing cells and T cells (Zhou et al., 2016), There are variances in way WASP quality is expressed in different organs, such as the broad expression in the spleen, then the appendix, and other tissue.

Our research was investigate the expression of the WASP 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 WASP gene expression technique.

From the data that we had obtained, WASP 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 frequent ocular surface chronic illness that is only seen in humans. Ultraviolet openness is a well-known aetiological element in the disease's etiology (Di Girolamo et al., 2004), pterygia is a pinkish three-sided molded tissue development on the cornea, pterygium means ‘‘wing,’’ from the Greek pterygos, pterygium was defined centuries ago by Hippocrates (Pujol et al., 2014).

The invasion of altered ocular surface tissues into the typical cornea is known as pterygium. Pterygium is a skin disorder that is harmless, uncontrolled growth of connective tissue overlaying the sclera (Liu et al., 2013). 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.

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. The relative roles of the limbal basal epithelial stem cell epithelial cells, and pterygium fibroblasts in the development of this disorder (James et al., 2002).

In addition, It's also been hypothesized that pterygium formation and maintenance are dependent on a familial hereditary. The effect of hereditary predisposition in pterygium maturation was investigated by Anguria et al., who hypothesized that pterygium inheritance be autosomal dominant. To demonstrate the role of heredity in pterygium pathology and to strengthen the postulated mechanism of inheritance (Cárdenas et al., 2016).

Normally, the disease is asymptomatic, but vision is impaired when it progresses into the pupil area of the cornea (Figure 2.1), On the corneal anterior to the pterygium, a pigment-iron line (Stocker line) can be visible (Levinger et al., 2020). Pterygiums are fibrovascular tissue masses that form from outside the cornea that frequently invade the optic axis. In many severe cases, pterygium can produce unequal corneal astigmatism, corneal stromal scar, and vision impairment (Chen et al., 2015).

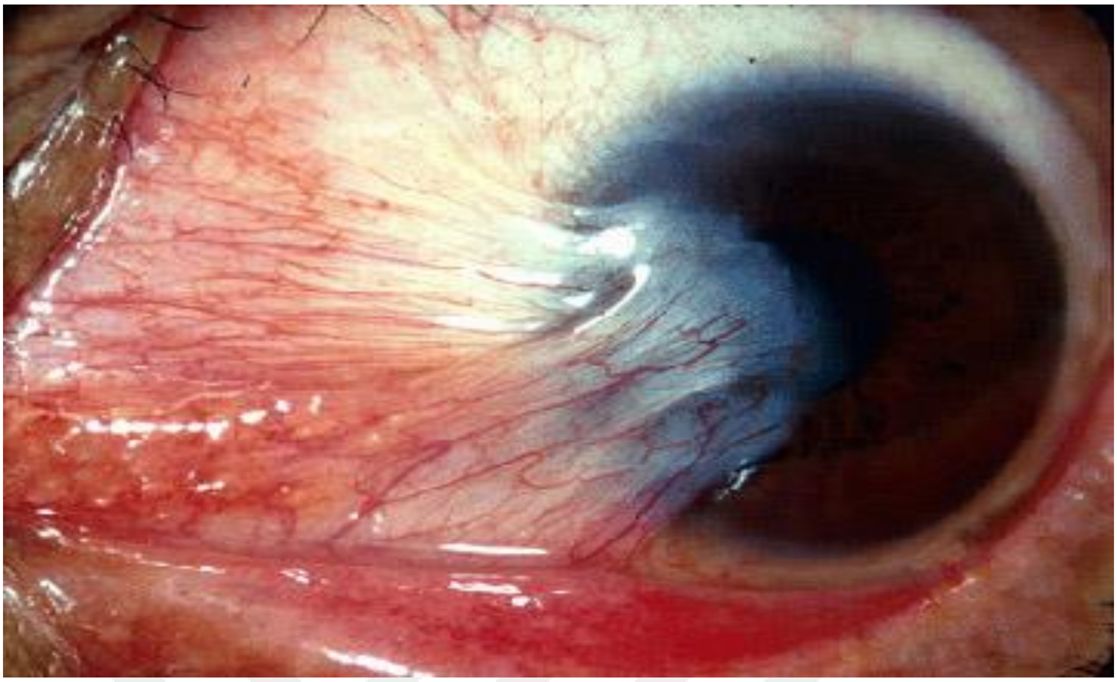

Figure 2.1. pterygium growing onto the cornea.

The pterygium, which is a peripheral corneal opacity, causes corneal light scattering, which raises retinal background illumination and reduces contrast sensitivity (Malik et al., 2014). Pterygium has been linked to inflammation, viral infection, oxidative stress, DNA methylation, inflammatory mediators, extracellular matrix modulators, apoptotic and oncogenic proteins, loss of heterozygozity, microsatellite instability, lymphangiogenesis, epithelial-mesenchymal cell transition, and changes in cholesterol metabolism (Wanzeler et al, 2019).

The fact that sunlight itself may be an important risk factor for certain ocular diseases is increasingly accepted, and pterygium has long been included among them (Moran et al, 1984). Although race has already been investigated as a health risk for pterygium development, the precise process is unknown, and the link between race and pterygium recurring is even less understood (Campagna et al., 2018).

The environmental, demographic, and lifestyle variables are the three types of Pterygium risk factors. The primary demographic risk factors for acquiring pterygium are exposure to sunshine, outdoor occupation, male gender, older age, and residing in rural areas. The most common environmental risk factor is exposure to sunshine

(Rezvan et al., 2018). Pterygium is found all around the world, but it is more common in the equatorial "pterygium belt." Pterygium that causes uneven astigmatism, restricted eye movement, or an unattractive esthetic appearance is usually treated surgically (Prajna et al., 2016).

According to experience, the pterygium is formed by an abnormal limbal basal epithelial stem cell going to Bowman's layer and triggering its collapse. The clear corneal epithelial cells, which we named pterygium cells, contained these aberrant limbal cells prior to the pterygium. This demonstrated that in order to prevent a pterygium from returning, a large region of presumably normal epithelium must also be eliminated (Anguria et al., 2013).

2.1. Epidemiology

Cameron looked at the worldwide spread of pterygium and discovered that it was more common in dusty, dry, and hot environments. The illness was predominantly seen between 37° north and south equatorial, implying that latitude played a role.

Cameron thought ultraviolet irradiation to be a significant element in the genesis of pterygium, which varies with latitude. Bowman's membrane is damaged by UV light, and the subconjunctival connective tissue thickens and hyperplasia occurs. This condition's occurrence is also influenced by the quantity of time spent in different climates. Although there is no definite link between pterygia and humidity, it is more common among outdoor workers and men. (Droutsas et al., 2010; Hacıoğlu et al., 2017).

Pterygium is more common in men than in women, according to research, which is line with the observations of this meta-analysis (around two times). According to the findings of a meta-analysis, the occurrence of pterygium was higher in rural areas than in urban areas (Liu et al., 2013). Although it has been established that inheritance plays a major role in pterygium formation. Low penetrance is the most common hereditary component, however it looks that the actual lesion is really not inherited, but rather the eye's inclination to respond to environmental stimulation in this way (HILL et al., 1989).

2.2. Etiology

Although the specific cause of pterygium is uncertain, there is growing evidence that chronic UV exposure is a contributing factor (Hacolu et al., 2017). 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).

The new grading of fibrovascular redness of pterygium may well be assessed from digital pictures and demonstrate good connection with CSF (contrast sensitivity function) and VA (visual acuity). In some situations, pterygium causes flatness of the cornea towards the apex, and visual acuity may be harmed as a result of pterygium's direct invasion of the visual axis of astigmatism (Avisar et al). (visual acuity) (Malik et al., 2014; Hilmi et al., 2017; Malik et al., 2014).

There have been a number of studies carried out to determine the molecular mechanisms underlying the growth and proliferation of ptrygium disease, revealing the roles of viruses, oxidative stress, DNA methylation, the cellular epithelial-mesenchymal transition, apoptotic and oncogenic proteins, inflammatory mediators, loss of heterozygosia.

Pterygium size was discovered to be related to postoperative alterations in corneal refractive power by Tomidokoro et al. A large pterygium covering the pupil (Light rays reaching the pupil are either absorbed directly by tissue within the eye or absorbed after diffused reflections inside the eye, part of which does not exit the constricted pupil, causing the pupil to look black.), critical changes in corneal structure, and deformations of Bowman's membrane (Figure 2.2) were thought to require longer time to restore cornea (Nejima et al., 2015; Bozkurt et al., 2020).

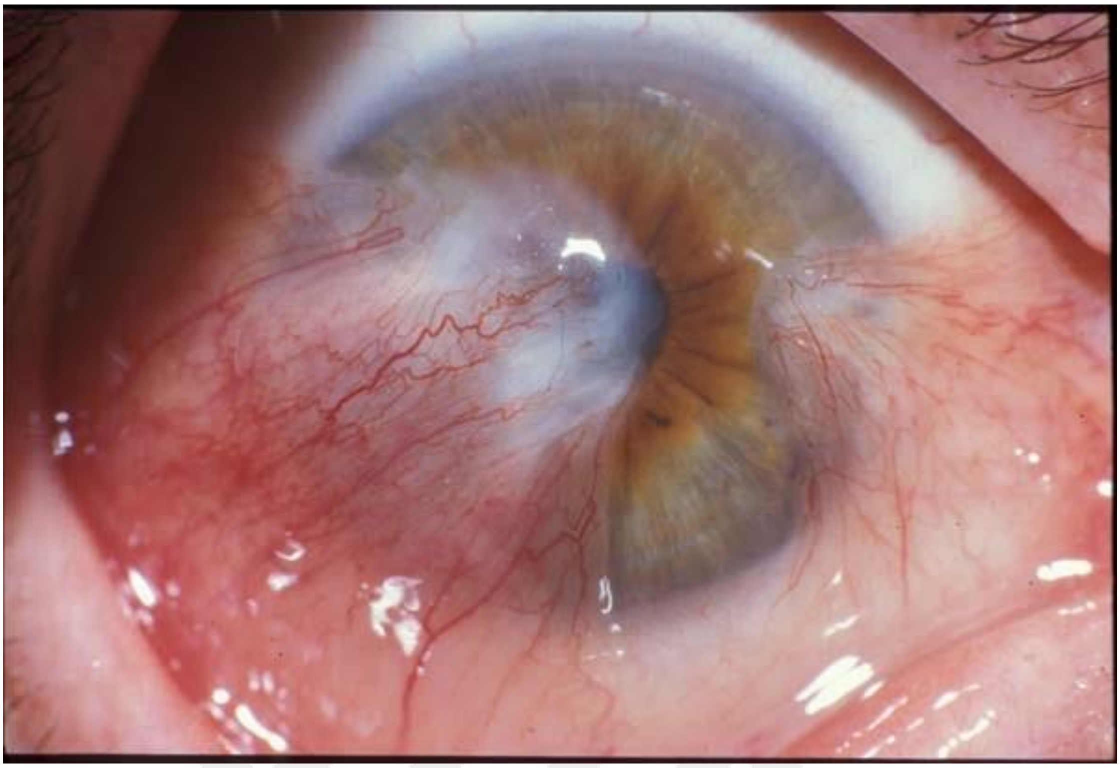

Figure 2.2: The pterygium is approximately covered with the pupil of the eye.

Proteinases that degrade ECM are more abundant in pterygia. MMPs (matrix metalloproteins) are capable of degrading practically all components of the ECM and have been implicated in a variety of normal and pathological biological processes, including embryogenesis, angiogenesis, wound healing, and cancer. MMP-9, MMP-8, MMP-7, MMP-3, MMP-2, MMP-1, and MMP-14 have all been demonstrated to be upregulated by Pterygium (Seet et al., 2012; Tsai et al., 2020).

Because erythropoietin (EPO) drives physiological functions such as cell proliferation, migration, and angiogenesis in EPO receptor (EPOR)-expressing vascular endothelium and tumor cells, the EPO-independent EPOR pathway has been believed to play a role in cell proliferation and angiogenesis in human pterygium.

One of the main assumptions is that environmental variables like UV radiation and HPV infection cause pterygium to develop in people who have a genetic susceptibility. For the condition to manifest clinically, environmental and genetic factors may interact in a variety of ways (Figure 2.3) (Bozkurt., 2020; Liu et al., 2013).

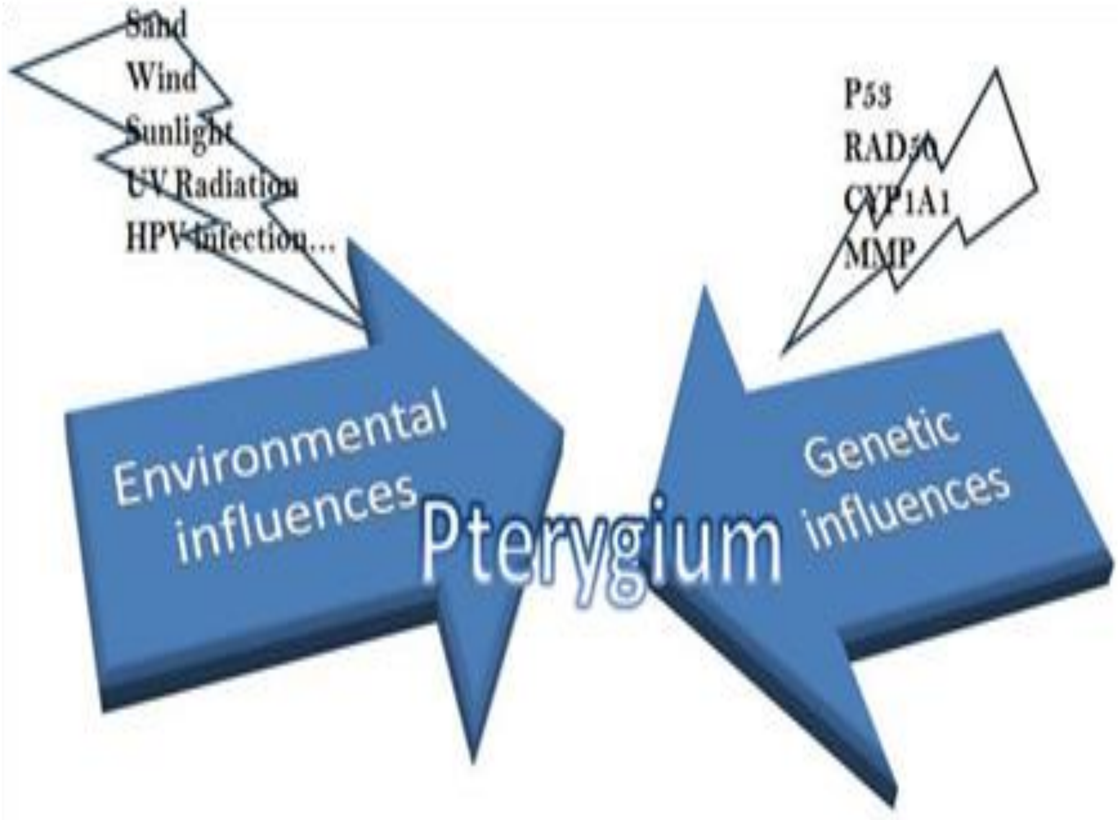

Figure 2.3: Pterygium pathogenesis is influenced by the interaction of environmental and genetic variables.

2.3. Factors Affecting Pterygium Formation

2.3.1. UV Radiation

Pterygium is characterized by angiogenesis, proliferation, extracellular matrix (ECM) breakdown, Since a clear correlation has been demonstrated between sunlight exposure and a higher occurrence of pterygium, UVR and inflammatory infiltrates have been linked to the development of pterygium (Chao et al., 2013; Cárdenas-Cantú et al., 2016).

Dry and hot weather, Environmental factors such as a dusty environment, as well as the duration of stay in these conditions, all help the development of pterygium (Hacıoğlu et al., 2017). According to Tan et al, pterygia that develops in young people is primarily reliant on the degree of exposure to weather conditions (it is more common in outdoor workers) and it may have increased UV exposure as a result of variable lifestyle activities, This is linked to the higher risks of postoperative recurrence in this age range (Nuhoglu et ai., 2013).

The ocular surface is affected by UV-B radiation wavelengths ranging from (280 315 nm) (in these ranges has created substantial evidence of neoplasia and hyperplasia) (Cárdenas-Cant et al., 2016; Cameron et al., 1983). Because of its ability to generate oxidative stress, UV-B radiation is known as the primary cause of pterygium development. The chemical 8-hydroxy-20-deoxyguanosine (8-OHdG) was identified in the epithelium of the primary pterygium's head epithelium as a sign of oxidative stress with a great mutagenesis potential (Cárdenas-Cant et al., 2016).

In the basal section of the epithelium from primordial pterygium, clusters of cells with a high percentage of thymine dimers (the conversion of C to T and CC to TT during DNA replication) have been found. Furthermore, the number of basal cells is reduced in recurring pterygium, most likely due to a defective DNA repair process (Cárdenas-Cant et al., 2016; Bradley et al., 2010). In 45 percent of instances, UV-A is absorbed by the cornea, and the effect of UV-A on pterygium were also studied.

Following UV-A exposure, Chao et al. observed mRNA levels and uPA (urokinase-type plasminogen activator) activity in pterygium fibroblasts. This protease (matrix metalloproteinases) is responsible for tissue disintegration and tumor cell invasion by activating MMPs (Figure 2.4) (Cárdenas-Cant and colleagues, 2016).

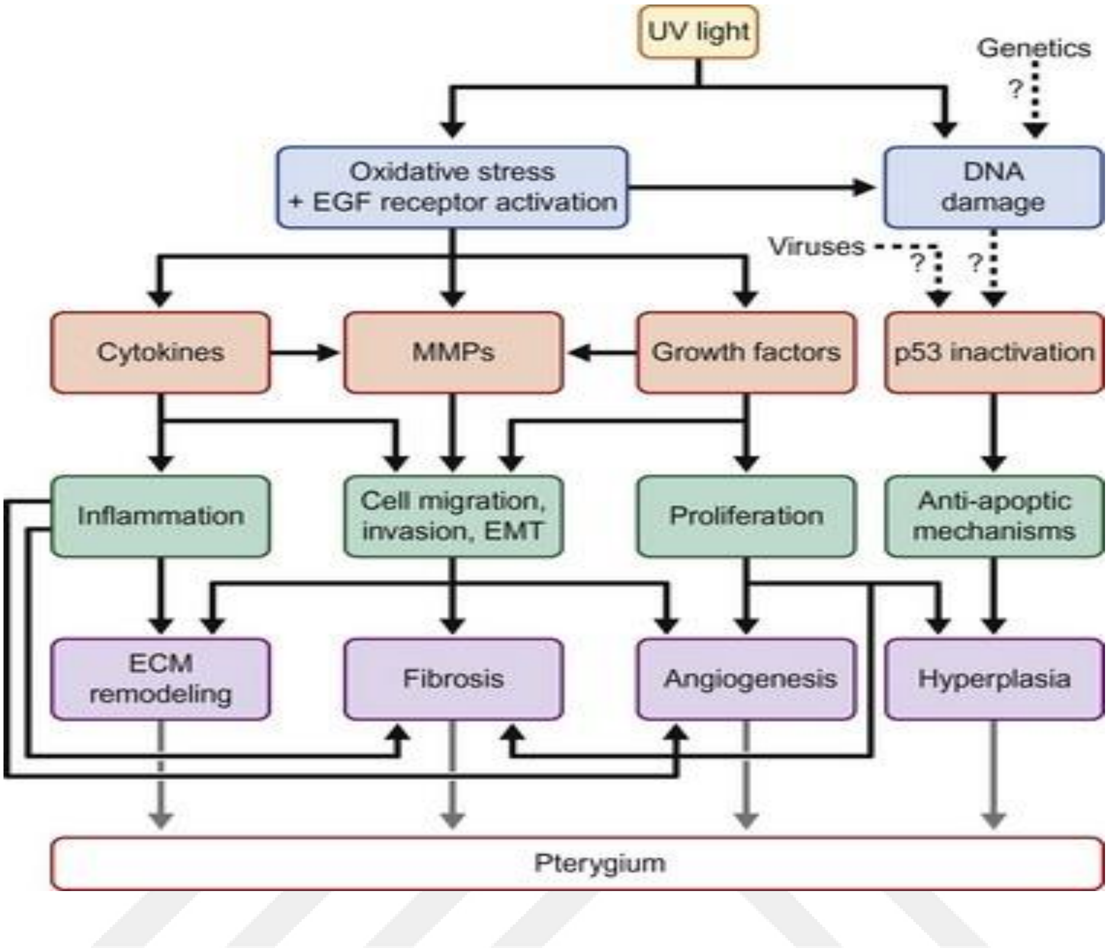

Figure 2.4: The effect of UV on the pterygium through several mechanisms.

The importance of UVA in the process of UV radiation-induced illness was missed in early study. UVA has recently been shown to harm a variety of cellular macromolecules, which has the potential to induce skin cancer, including melanoma. UVB radiation damages cellular DNA directly, whereas UVA radiation induces considerable oxidative stress in cells (Cárdenas-Cantú et al., 2016).

Urokinase-type plasminogen (uPA) expression and secretion were shown to be elevated in pterygium and associated fibroblasts recently. Following the formation of the pterygium, the expression of uPA by a pterygium increased rapidly (Chao et al., 2013).

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). Weinstein and colleagues discovered that the tumor suppressor p53 regulates one of the most significant identified checkpoints in cell growth (but not degenerative damage), whose involvement in pterygium improvement has been widely investigated, and that there is no link between aberrant p53 expression and recurrences (Nuhoglu et al., 2013).

Another tumor suppressor protein, p16, has been found to be down-regulated in pterygium, most likely because to hypermethylation of its promoter region, in any event, mutations in the oncogene KI-Ras codon 12 have been discovered, pterygium has been observed to have a considerable number of them (Cárdenas-Cant et al., 2016). The occurrence of p53 mutations in pterygia's limbal epithelium, limbal cancers, and the majority of pingueculae shows that p53 mutations occurred early in these cells' development (Dushku et al., 1997).

Other mechanisms that can contribute to the decrease of p53 function have been identified and mutations in the gene, such as the interaction of cellular or viral gene products with p53 protein, which results in p53 inactivation or fast destruction, The abnormal expression of factors that govern p53 expression, as well as the silencing of p53 gene transcription through abnormal expression of factors that determine p53 expression, silences p53 gene transcription. In these cells, the reasons of p53 gene silencing and p53 expression loss are currently being investigated (Reisman et al., 2004; Tsai et al., 2005).

One of the photooxidative DNA products, 8 hydroxydeoxyguanosine (8-OHdG), is thought to be a delicate and long-lasting biomarker for detecting DNA damage. Recent research on tissues exposed to oxidative stress stimuli has shown higher levels of 8-OHdG and the protein p53 (Balci et al., 2011).

Because p53 and vascular endothelial growth factor (VEGF) have been linked to tumor vascularization, p53 and VEGF proteins were discovered in main and recurrent pterygium fibroblast, vascular endothelial cells, and epithelial cells. However, the expression of p53 and VEGF in primary pterygium found to differ significantly. Despite the fact that p53 immunoreactivity in repeated pterygiums was weak to moderate, but VEGF immunoreactivity was robust, p53 and VEGF expression were weak to negative in normal conjunctiva (Cárdenas-Cant et al., 2016). The etiopathogenesis of pterygium is complicated by proliferation and antiapoptotic processes (Turan et al., 2020).

2.3.3. Viral Infections

P53 mutation, loss of heterozygozygozity (LOH), and the presence of oncogenic viral infections are all involved in pterygium. It is a corneoscleral limbus lesion that grows in size, Oncogenic viruses like cytomegalovirus (CMV), human papillomavirus (HPV), as well as herpes simplex virus (HSV) have been found in pterygia samples (Detorakis et al., 2000; Cárdenas-Cant et al., 2016).

According to studies, the most prevalent HPV strains in pterygium tests were found in Turkish, Japanese, and Ecuadorian patients. The HPV produces proteins that inactivate p53, resulting de chromosomal instability and an increased risk of cancer (0 percent , 4.8 percent, and 21 percent, respectively), while Italian and Brazilian patients have been reported to have the highest predominance (100%, 58.3%, and 50%, respectively) (Cárdenas-Cantú et al., 2016).

They also discovered that the production of HPV E6 oncoprotein is linked to p53 inactivation in pterygium. indicating that p53 inactivation by a high-risk HPV E6 oncoprotein may play a key role in HPV-mediated pterygium pathogenesis. However, it may have a synergistic function in the multi-stage growth process. Detorakis et al. looked into the prevalence of HSV and HPV in pterygium and uncovered a possible viral collaboration that influences the pterygium's clinical characteristics (Liu et.al., 2013).

HPV has been implicated in the formation of pterygium in several investigations. However, the prevalence of HPV in pterygia varies widely by geographic region, ranging from 0% to 100%. Pterygium is thought to be caused by adenoviruses, but other factors may play a role in developing of pterygia (Kelishadi et al., 2015). HSV recognition was achieved by polymerase chain reaction amplification of viral sequences to analyze the presence of herpes simplex virus infection (HSV) in pterygia and explore the probable association between HSV and pterygia. HSV was found in 5% of pterygia cases, with no conjunctival control showing HSV (Hsiao et al., 2010).

2.4. Molecular Basis of Pterygium Development

Pterygium pathogenesis is mostly linked to the presence of UV light, but the exact methods by which it develops are unknown. Several studies have attempted to decipher the mechanisms at the molecular underpinning Proliferation and maturation of pterygium, demonstrating the importance of numerous components including such cell proliferation factors and cytokines.

Lymphangiogenic factors, growth factors, inflammatory mediators, and angiogenic, immunological processes, cholesterol metabolism alterations, lymphangiogenesis, cell epithelial-mesenchymal transitions, and extracellular matrix modulators are all involved in pterygium growth.
The identification of a molecular foundation for pterygium brings up newly therapeutic possibilities for treating and preventing disease (Figure 2.5) (Cárdenas-Cantú et al., 2016).

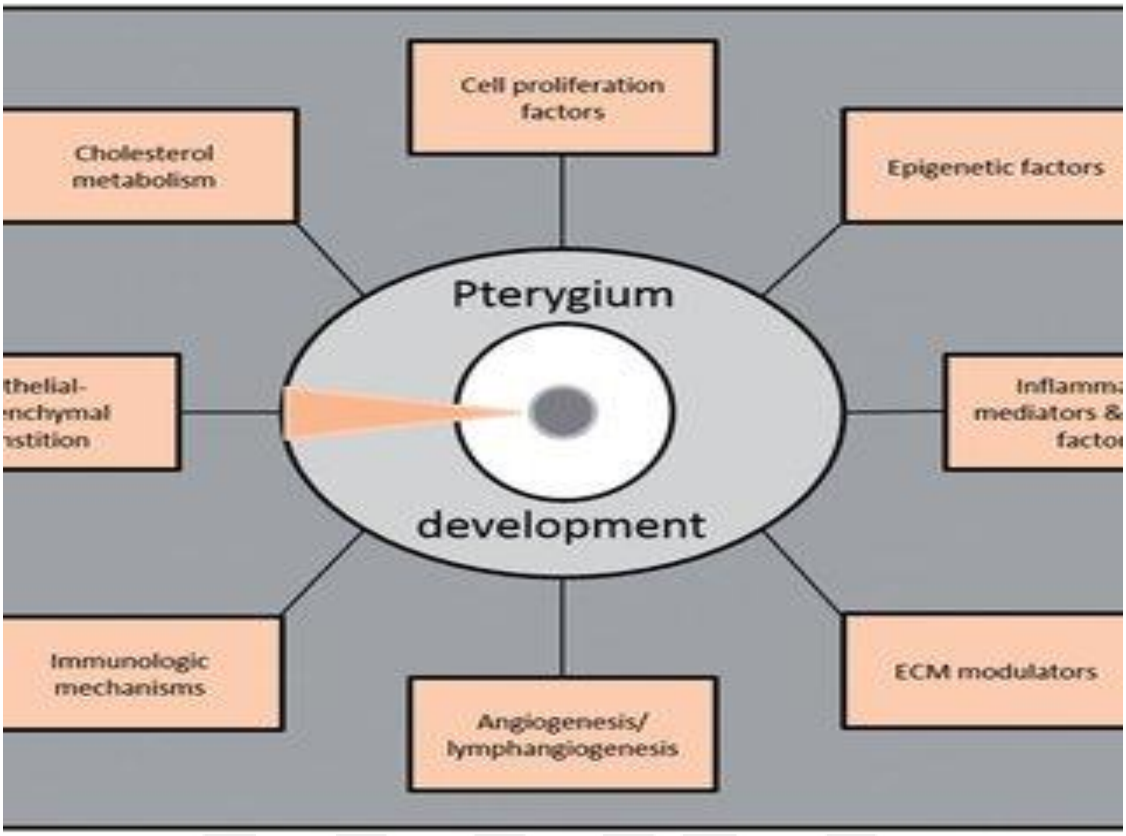

Figure 2.5: Pterygium Developmental Molecular Mechanisms (Cárdenas-Cantú et al., 2016).

2.4.1. Angiogenesis in Pterygium

Angiogenin (Angiogenin) overexpression in pterygium tissue fibroblasts is linked to active pterygium growth. With reviewing pterygium as a tumorigenic mimicry, elevated ANG (Angiogenin) expression in pterygium body fibroblasts was linked to active pterygium growth. Pterygium with a thick body Angiogenin was originally found as an angiogenic tumor agent, but its bioactivity has lately been broadened to encompass cell proliferation, implying that angiogenesis may play a role in pterygium pathogenesis (Peiretti et al., 2010; Kim et al., 2013).

Vascular micro density examination has revealed the presence of angiogenesis at the level of the pterygium, as well as the overexpression of the VEGF, which basically, in the proliferating designs of the pterygium, Scientific proof for this growth factor's harmful role in pterygium growth (Livezeanu et al., 2011). According to Wong et al., produces a pterygium angiogenesis factore, dspite the fact that they assumed that the angiogenesis factor was a substance created by autolysis, it's also plausible that vascularization is caused by related leukocytes.

Based on the identification of plasma cells, lymphocytes, and immunoglobulins within pterygium in recent articles, an immunologic explanation has been presented. IgG was discovered mainly in the stroma in locations corresponding to lymphocyte and plasma cell infiltration, while IgE was discovered in the epithelium, the stroma, and the space between such two issues, according to immunofluorescent tests. Examinations of normal conjunctiva with immunofluorescence revealed no (negative) IgE or IgG (HILL et al., 1989).

2.4.2. Proliferation and Migration of Pterygium Cells

Pterygia is a commonly diagnosed ocular surface disease that describes inflammatory infiltrates, proliferation, extracellular matrix breakdown, fibrosis, and angiogenesis. Despite the fact that pterygium is a neoplastic-like development problem, It isn't a cancer that has spread to other parts of the body. Pterygium development, on the other hand, comparable to cancer metastasis, needs cell migration and regional angiogenesis.

Metastasis, or the spread of cancer cells to organs other than the one where they began, is the main cause of cancer-related death. Within pterygium tissue, genes implicated in cell proliferation, also including mutant p53, have been found to be overexpressed (Liu et al., 2013). The tumor suppressor p53 regulates one of the most significant cell proliferation checkpoints, and p53 levels have been discovered to be higher in the pterygia basal epithelium. P16, a tumor suppressor protein, was found to be down-regulated in pterygium, most likely because to hypermethylation of its promoter region. There is no evidence that the oncogenic protein p63 is expressed differently in pterygium tissue and normal conjunctiva (Cárdenas-Cantú et al., 2016; Liu et al., 2013).

Loss of heterozygozygozity (LOH) and microsatellite instability(MI) are two factors that have been studied in relation to cell proliferation. Because it frequently occurs in the lack of tumor suppressor gene function, LOH is associated with cancer and cell proliferation, whereas MI is indication of a malfunctioning DNA mismatch repair system (Cárdenas-Cantú et al., 2016).

2.4.3. Epigenetic Factors

Studies based on specific nucleotide polymorphisms (SNPs), polymerase chain reaction (PCR), and immunohistochemistry study of pterygium patient samples have advanced our understanding of the genetics of pterygium. Pterygium-relevant genes, according the research, are involved in a number of biological processes, including DNA regulaion, cell proliferation, migration, and angiogenesis. These genes' mutations or polymorphisms are linked to changes in their functionality, which are involved in the formation of pterygiums (Liu et al., 2013).

Cell proliferation, invasion, and recurrence following resection, as well as proteins linked to enhanced proliferative activity, are all characteristics of pterygium (Ki-67, proliferation cell nuclear antigen [PCNA], and erythropoietin), In compared to typical conjunctiva, the somatic cell layers of pterygium has been identified to be overexpressed with and malignancy, with low rates of cellular apoptosis (Bcl-2 and p53) (Hsp90).

Emotional anomalies in DNA methylation patterns are widespread in many cancer forms, and based on the similarity between cancer and pterygium, some research has been done on pterygium methylation patterns as well. Changes in DNA methylation of genes involved in matrix remodeling (MMP-2 hypomethylation) and cell adhesion are linked to pterygium invasion (TGM-2 and CD24 hypermethylation) (Cárdenas-Cantú et al., 2016).

2.4.4. Growth Factors and Inflammatory Mediators

Greater rates of interleukin 1 (IL-1), 6 (IL-6), and 8 (IL-8) should be found in considerable quantities irrespective of the source to be regarded key factors in the genesis of pterygium (IL-8). After UV-B irradiation, tumor necrosis factor-alpha (TNF) was also found in the pterygium. In this condition, the enzyme cyclooxygenase-2 (COX2) is overexpressed, due to an increase in prostaglandin products and inflammatory cascades.

The nuclear factor kb (NFkb) pathway was discovered to be active in human pterygium tissue, with an elevation in phospholipase D, an enzyme related in inflammation and cell differentiation (Cárdenas-Cant et al.2016). Itdiscovered that cystatin C, a cysteine proteinase inhibitor, is highly produced in this disease and is identified in tears more frequently in people with the sickness. The canonical and non-canonical NF-kb pathways (related to clinical and late inflammatory responses, respectively) are active in pterygium or may be involved in a variety of bioactivities, including inflammatory cell migration into the tissue. Each of these inflammatory mediators is likely produced as a result of pterygium formation (Cárdenas-Cant et al., 2016).

In pterygium, cell migration has been reported to be stimulated by heparin-binding epidermis growth factor (HB-EGF). UV-B radiation has been shown to upregulate vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and hepatocyte growth factor (HB-EGF). VEGF has been linked to COX2 in macrophages in this context (Cárdenas-Cant et al., 2016). In pterygium, PEDF (pigment epithelium-derived factor) was found to be lower and insulin-like growth factor-binding protein 2 was found to be higher (IGFBP-2).

A decrease in PEDF, which is an angiogenic inhibitor, could result in angiogenesis. Stem cell factor, platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), and transforming growth factor-beta (TGF-b) all have higher levels of expression.

In the stroma of severe pterygium, the up-regulator of organ repair, stromal-derived factor-1 (SDF-1), as well as its receptor (CXCR-4), have recently been discovered to be overexpressed (Bianchi et al., 2012). Many studies of SDF-1-derived signaling as a potential approach for determining pterygium severity may aid in the creation of an anti-SDF-1 drug that decreases pterygium tissue fibrosis (Cárdenas-Cant et al., 2016; Kim et al., 2016).

2.4.5. Pterygium Immunological Mechanisms

The pterygium is really an invasion of a wing of changed ocular surface tissue into the ordinary regular cornea; the head is slightly raised, and the globe is only location where the globe is securely attached is where the globe is firmly connected. E-cadherin and beta-catenin as well as immunologically enriched in pterygium tissue and thus are assumed to be important in epithelial growth and adhesion (Kase et al., 2007).

To investigate the involvement of immunological systems in pterygium associated to proliferative activity, immunohistochemical expression of human leukocyte antigen (HLA-DR-DR isotype), CD8, CD4, CD68, S1OO, PCNA, and Ki-67 was done. In the pterygium, high amounts of PCNA and Ki-67 expression were seen in the same locations as the HLA-DR antigen, furthermore, abnormal HLA-DR antigen expression appears to be linked to the lesion's growth fraction (Altan-Yaycioglu et al., 2013).

The etiology and genesis of pterygium have been associated to elevated rates of immunological activities markers such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and human leukocyte antigen DR (HLA-DR). In the pterygium, plasma cells, mast cells, lymphocytes, CD4 +, CD8 + T cells, and dendritic cells all expanded. Because the degree of the inflammation is dependent on the amount of antigen, macrophage-type cells were scattered irregularly throughout the pterygium tissue (Cárdenas-Cant et al., 2016).

2.4.6. Extracellular Matrix Modulators

Pterygium is an inflammatory and proliferative development of limbal epithelial stem cells that causes cornea tissue invasions with severe matrix modification, involving the breakdown of Bowman's layer cpllaps (BL). 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, and have been described in more detail.

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)

According to expression patterns of MMP and TIMP at the pterygium progression margin in specimens from the human eye and tears from patients with this illness, Those enzymes may well be responsible for the breakdown of BL, and their various expression modalities suggests that each of them plays a unique function in pterygium pathogenesis.

Pterygial epithelial cells, corneal stromal fibroblasts, and pterygial fibroblasts that migrated between the epithelium and the BL to the pterygium's anterior margin all expressed collagenase-1. Every pterygia that stained columnar epithelial cells with denatured BL have collagenase-3 and gelatinase A and B. TIMP-1 and -3 immunoreactivity was similar to gelatinases, with more staining in epithelial cells and fibroblasts without BL, however TIMP-2 was only observed weakly in pterygial epithelial cells (Di Girolamo et al., 2000).

2.4.7. Epithelial-Mesenchymal Transition

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).

EMT is critical for the disease's recurrence, according to a review of the literature on several signaling pathways in the pterygium, and several signaling pathways eventually activate EMT markers (Meshkani et al., 2019). The downstream miR-200a and ZEB1 / ZEB2 genes were thought to be part of a regulated epithelial-mesenchymal transition (EMT) linked to pterygium etiology and recurrence, P53 suppression in this condition affects miR-200a, which leads to ZEB1/ZEB2 upregulation and pterygium EMT processing (Wu et al., 2016).

The expression of the-SMA protein was found in the cytoplasm of TF-immunopositive cells with higher levels of expression, also discovered in pterygium epithelial cells. Immunocytochemistry revealed suppression of E-cadherin & intra-nuclear accumulation of b-catenin proteins in the pterygial epithelium, which regulate cell-cell adhesion. The classical b-catenin signaling pathway promotes EMT and is frequently active in cancer, and E-cadherin downregulation is a crucial last stage in the EMT process (Cárdenas-Cantú et al., 2016).

2.5. DNA Repair in Pterygium

Pterygium is a UV disease, Ultraviolet rays can damage DNA, which will be UV radiation can damage DNA, which DNA repair systems can repair, A basic excisional repair system (BER) and even a nucleotide excision repair system (NER) are two DNA repair systems.

Complementary X-ray repairing 1 (XRCC1) and human 8-oxoguanine DNA glycosylase 1 are two BER genes (hODG1), Xeroderma pigmentosum group A (XPA) & xeroderma pigmentosum group D (XPD) are the two NER genes (XPD), variation in this gene is related with changes in its interaction between DNA repair and damage and affects cancer susceptibility, Because the hODG1 polymorphism has been linked to the development of pterygium, it makes sense that it is involved in the production of XRCC1, XPA, and XPD polymorphisms (Chiang et al., 2010).

UV light is known for causing DNA oxidation (damage) and is thought to play a role in pterygium genesis. Because p53 is an important part of the DNA repair mechanism, it's probable that other parts of the system will also be abnormally expressed (Figure 2.6).

Genes involved in the repair of double-stranded DNA degradation Components of the MRN complex (RAD50 and RAD51) as well as two RAD51 paralogues (XRCC2 and XRCC3) vary in patients with pterygium, according to eyewitness reports. Increased RAD50 protein expression in pterygium tissues as compared to control conjunctival tissues demonstrates that recombinant DNA repair mechanisms are active in the pterygium. In blood cells, patients with a history of recurrent pterygium have greater levels of RAD51, XRCC2, and XRCC3 gene expression. PLK1 (pole-shaped kinase 1), that can influence the activity of RAD51, is also found in the pterygium (Figure 2.6) (Liu et al., 2013).

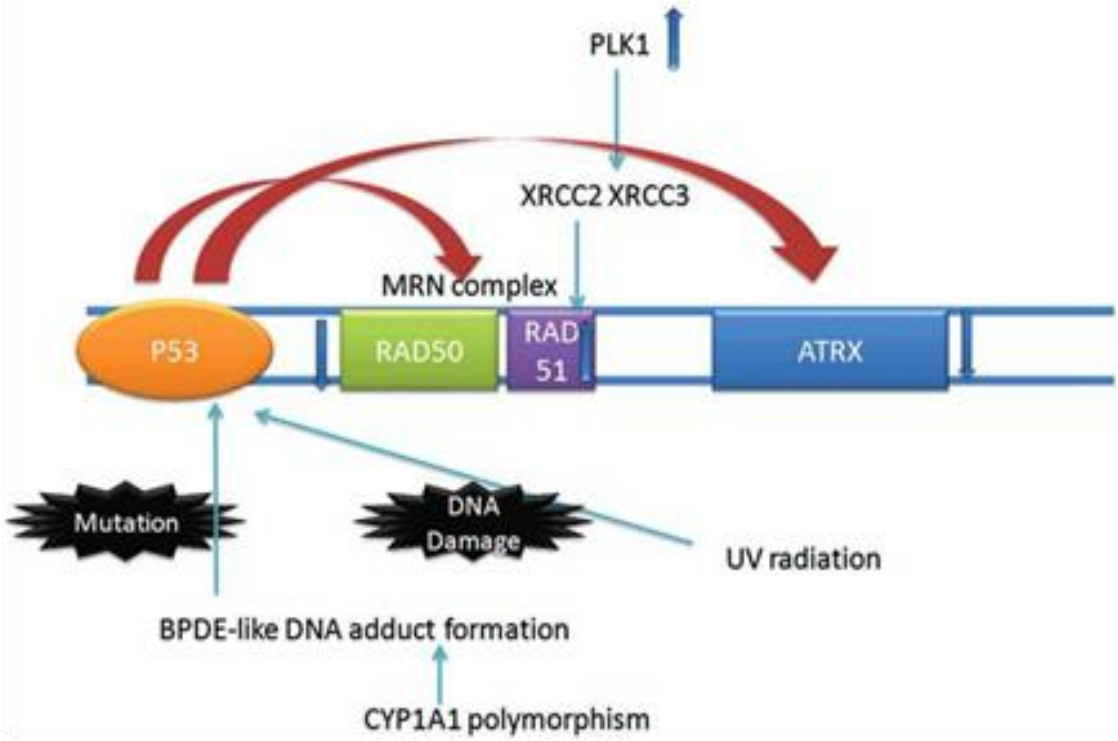

Figure 2.6: A model for the development of pterygium's double-strand DNA repair machinery.

2.6. Expression Levels of Apoptosis and Apoptosis-Related Genes in Pterygium

Pterygium is a benign pathology in which the cornea's aberrant conjunctiva expands in a triangle or winged pattern. Pterygium can be locally invasive and show a range of abnormalities, including moderate dysplasia to malignancy, chromosomal allele loss, and a low rate of microsatellite instability in slightly more than half of the samples analyzed (Tan et al., 2000; Liang et al., 2011)

In 1972, Kerr and colleagues were the first to separate apoptosis from necrotic cell death. All energy and the coordinated activity of a cascade of genes are required for cell death. It is well understood that gene expression that either inhibits or promotes apoptosis modulates progression through an apoptotic pathway. Apoptosis inhibitors include BCL-2 and BCL-xl, whereas apoptosis promoters include Bax, Bad, bak, and

BCL-xs. BCL-2, it's been discovered that apoptosis inhibitors can form heterodimers with apoptosis-promoting genes, and overexpression of BCL-2 protects cells from apoptosis.

Apoptosis cells differ from necrosis cells in several ways, including cell volume reduction, chromatin marginalization, and DNA fragmentation. When genomic DNA from cells undergoing apoptosis is examined by gel electrophoresis, it fragments as a result of enzymatic DNA breakage at the nucleosome level, resulting in a distinct DNA ladder. Specific DNA breaks produce free 3'-hydroxyl groups in DNA, which are currently thought to be apoptosis markers that may be validated using cleavage-mediated tagging. Deoxynucleotide transferase (TdT) is a type of deoxynucleotide transferase (Tan et al., 2000).

2.7. Pterygium Heterozygozity Loss and Microsatellite Instability

Although there has been new evidence of molecular changes in eye and skin illnesses, such as oncogene activation and abnormal expression of growth factors, no genetic analysis of pterygium microsatellite DNA has been undertaken, and it would be fascinating to study the association between microsatellite DNA and the disease's etiopathology.

Refer to a genetic study which found two genetic changes in pterygium microsatellite DNA:

a) loss of heterozygosity, showing the presence of TSG (s) in 53 percent of the specimens; and b) loss of heterozygosity, suggesting the presence of TSG (s).
b) microsatellite instability, which is linked to a high rate of mutation and affects 13% of cases.

Pterygium is a benign lesion that develops at the angle of the conjunctival limbus; LOH is an occurrence that occurs when diseased or precancerous cells transition into malignant cells; this fact provides solid proof that pterygoid tissue cells can transition (Spandidos et al., 1997).

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. WASP Gene

IMD2; THC; THC1; SCNX; WASPA; WAS are all synonyms for this gene. 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 WASP gene does have 12 exons and is found on the X chromosome's short arm (p) between locations Xp11.4 and p11.21. It is location found on the X chromosome between 48,427,152 and 48,434,758 base pairs (Figure 2.7). The WASP encodes a 502-amino-acid protein which is mostly produced in hematopoietic cells and has 1823 base pairs (Hara et al., 1993; Jin et al., 2004). This protein plays an important role in signal transduction from cell surface receptors to the actin cytoskeleton and has a molecular weight of around 65kD (HO., 2001; Sasahara et al., 2000).

WASP is a link for actin reorganization, platelet production/elimination, cell-cell interaction, actin polymerization, cell trafficking and motility, Signal transduction, immunological synapse development, phagocytosis, and neutrophil creation/release, as well as cytoskeleton rearrangement (Liang et al., 2011).

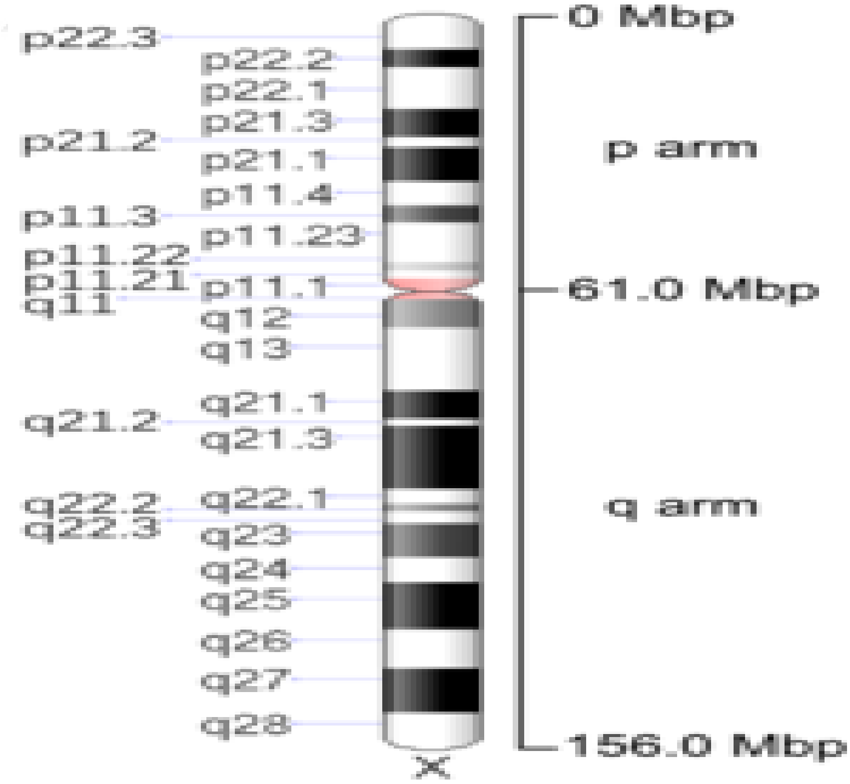

Figure 2.7: WASp gene location on X chromosome.

Exons are active sections of a gene that are "read" and actively involved in the synthesis of proteins, while introns and untranslated sections are inactive sections of a gene that are not "read" and so are not translated into proteins. WASP was difficult to understand, and its whole operation is still unknown. This complex protein is typically located in the cell's cytoplasm, especially near the cell membrane, and is hypothesized to play a role in the cell's actin cytoskeleton. WASP is mostly found in white blood cells and platelets, and it has an impact on the amount and function of these cells (scaffold).

Because there are so many motifs, it's likely that they're influenced by a wide range of stimuli and interactions with other proteins. Recent research has discovered that these proteins are linked to the small GTPase, which regulates actin filament synthesis, and the Cdc42, cytoskeletal organising complex, Arp2/3, either directly or indirectly (Imai et al., 2003).

The WASP protein family act as promoter for the nucleation of the highly expressed Arp2/3 complex, 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 endolysosomal network are all regulated by Arp2/3 actin.

Members of the WASP family have recently been discovered to play a role in driving actin dynamics in the centrosome, as well as controlling nuclear structure and membrane remodel events that lead to the creation of autophagosomes. Many members of the WASP family have been discovered in the nucleus, where they act as a molecular substrate for the assembly of transcription and epigenetic machinery, directly influencing gene expression (Alekhina et al., 2017).

WASP promotes white blood cells to rebuild and travel to the area of the body where they are needed. white blood cells are programmed to switch to certain chemical attractants when needed. In the absence of this protein, the scaffold is modified and signals in the cell are disturbed, leading to early cell growth and death and contributing to immunodeficiency.

There is no control of leukocytes in individuals with WASP, which inhibits the capacity of white blood cells to choose between "self" (their own cells) and "non-self" (cells that do not belong to them, such as bacteria, viruses, differentiate, transfused cells, etc.), this can lead to lymphocytes attacking the patient's cells and causing autoimmunity. Despite the fact that the WASP protein's function is unknown, it has recently been discovered to interact with CDC42Hs and Nck, but rather two conserved motifs termed as WASP homologous 1 and 2 (WH1 and WH2) have been proposed as functional domains (Sasahara et al., 2000).

The activation of the actin-related protein (ARP2/3cytoplasmic) is required for WASP nucleation-promoting factors to reprogramme 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 depe.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).

The cytoplasm of hemopoietic cells contains WASP and CD34/ cells originating from human bone marrow, but not in nonhematopoietic cells. N-WASP, a protein with a structure similar to WASP and a number of the same functional domains, has recently been discovered in nonhematopoietic cells (Zhu et al., 1997). The Pleckstrin homology (PH) and Wiskott homology 1 (WH1) areas at the amino terminus are thought to mediate binding to membrane phospholipids, whereas the Wiskott homology 2 domain (WH2) and the actin regulatory domain (ARD) are found at the carboxy terminus (L et al., 2001).

Cells having pseudopodia in the anterior and posterior areas are required for efficient mobility. Direct compression of an active pseudo-analog catalyst with Rac maintains polarization. The position and cellular levels of active Rac are controlled by a component that promotes actin protein (WASP) nucleation in Wiskott-Aldrich syndrome, which helps preserve anteroposterior polarity. Without WASP, Dictyostelium cells activate Rac in the rear, affecting its polarity and speed. The WASP Cdc42 and Rac interaction binding motif ("CRIB") was thought to be crucial for its activation. However, it is showed that the biological significance of the CRIB motif is unexpectedly complex. WASP CRIB mutants that lack SCAR/WAVE are unable to control Rac function in the foreground and can not form new pseudopodia.

Although WASP without a structural CRIB domain normally localizes in clathrin 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).

Despite the fact that its full length nature is unknown, a transcript difference arising as a result of alternative promoter usage and containing a variant 5 'UTR sequence has been demonstrated. The WASP gene produces a cytoplasmic protein that is primarily found in hematopoietic cells, which exhibit cytoskeletal abnormalities and signaling in WAS disease, with a transcript difference resulting from the use of an alternative promoter and a variant 5 'UTR (NCBI).

2.9.1. The domains of WASP

WASP is a hematopoietic cell-specific protein that activates Arp2/3-mediated actin polymerization and binds with cytoplasmic components in signaling and cytoskeleton reconfiguration, by attaching to the hydrophobic core Cdc42's C-terminal region, which includes the verprolin homology domain, WASP produces a self-locking ring with the globular actin monomer, binding, the verprolin homology domain(VH), a cofilin homology (CH), and the acid Region (AR) (Imai et al., 2003).

Soon just after the Wiskott-Aldrich disease gene was discovered, it became clear that WASP may be connected to a wide range of proteins. Each of a protein's three domains performs a different role. The discovery of these domains was made possible by the identification of WASP homologs in the brain (N-WASP), Caenorhabditis elegans, and Saccharomyces cerevisiae. S. cerevisiae's complete genomic sequence Initially, finding a WASP homolog was simple in cerevisiae, which makes searching for homologs for a specific gene in yeast simple; however, the C.elegans database is still incomplete (Ishihara et al., 2012).

a) The first domain WH1

WH1 domain's weak similarity with the pleckstrin (PH) homology domains, which associate with phosphorylated lipids in the phosphoinositide process, was the first indication of its function. As a result, the WH1 domain-containing recombinant N-WASP fragment binds to phosphatidylinositol bisphosphate, and the corresponding WASP fragment binds to the same phosphatidylinositol bisphosphate, and over half of the mutations found in Wiskott-Aldrich disease patients are identified in the WH1 domain. Wiskott-Aldrich Interactive Protein interacts with the WH1 domain as well (WIP),a protein that regulates the affinity of WASP for one or more of its accomplices, the actin cytoskeleton reorganizes when WIP is overexpressed in transfected cells (Ishihara et al., 2012).

b) The second domain, WH2

In contrast to patients with WH1 mutations, who have a severe immunological deficit, the WH2 function has been elusive, Because WASP, which loses this domain, So because recombinant N-WASP fragment containing the WH2 domain is coupled to actin in vitro and suppresses actin polymerization in vitro, WH2 is clearly engaged in actin interaction. In vivo, overexpression of complete N-WASP in actin causes co-precipitation of N-WASP with actin in the cortical region of the cell, a behavior that is comparable but distinct from the overexpression effect of WASP co-precipitation with actin throughout the cytoplasm. The WH2 domain is mostly important for WASp or N-WASP-actin interactions (Imai et al., 2003; Ishihara et al., 2012).

c) The third domain, SH3

WASP has at least 7 SH3 binding sites in its core region, and it has been shown to interact with a number of SH3-domain-containing proteins, includes kinases like Itk and Fyn, which are also implicated in T-cell signaling, and adaptor proteins like Grb2. WASP mutations have a well-known influence on T-cell activation, but their effects on platelet formation and B-cell activities is less well-known. In response to EGF activation, the epidermal growth factor receptor (EGFR), a tyrosine kinase receptor, has been demonstrated to physically bind to WASP or N-WASP (presumably via Grb2), WASP does not appear to suppress T-cell activation, meaning that it does not inhibit T-cell activation (Ishihara et al., 2012; Sun et al., 2019)

Notably, all reported mutations in this area produce severe structural changes in the central domain, such as frameshift mutations, but point mutations have yet to be discovered. This could indicate that a mutation affecting only one or two SH3 interaction sites does not cause the Wiskott–Aldrich syndrome phenotype, and so the mutation goes unnoticed. Because the core domains of numerous WASP homologs are often not conserved.

Although the WASP study contributes to our knowledge of how the cytoskeleton is organized, There is promising evidence that WASP interacts with other cytoskeleton proteins, and that this interaction can be utilized to diagnose them early. WASP is expressed on B-lymphocytes and so all hematopoietic cells, hence the link between Wiskott-Aldrich syndrome genetic deficiency and B-cell abnormalities and eczema in patients is still unknown. Because the occurrence and severity of eczema in these patients differs between siblings, a second genetic factor is very probably at play (Castiello et al., 2014; Ishihara et al., 2012).

2.9.2. WASP-binding molecules

WASP infact is a hematopoietic cell-specific protein with many functional domains that stimulate Arp2/3-mediated actin polymerization by interacting with cytoplasmic components involved present in signaling and cytoskeleton remodelling. WASP attaches to the hydrophobic core Cdc42's C-terminal region, which includes the verprolin homology domain, GTPase interaction to globular actin monomer, verprolin homology domain(VH), cofilin homology (CH), and acid Region (AR) cancer association domain (GBD).

The activated state of Cdc42 connected to GTP stops the self-inhibition cycle, allowing the spherical actin monomer and the Arp2/3 complex to connect to the PRR and C-terminal region of the protein. Arp2/3, a seven-polypeptide complex, stimulates filamentous actin synthesis by speeding up actin polymerization, which is helped by profilin. Two WASP binding molecules, phosphatidylinositol-4,5-bisphosphate (PI (4,5) P2) and Nck, work together to activation WASP (Imai et al., 2003; Kurisu et al., 2010).

WASP phosphorylation has also been shown to increase WASP's actin polymerization activity, and Hck, a hematopoietic cell-specific Src-like tyrosine kinase, were a new partner for WASP. Hck phosphorylates and binds WASP to tyrosine 291, as does Btk, a process that enhances the activity of WASP-actin polymerization by the Ar Casein kinase phosphorylation of WASP's serine and cofilin homology domains likely improves the C-terminal region's affinity for the Arp2/3 complex (Imai et al., 2003; Ye et al., 2004).

The N-terminal domains of WASp and N-WASP have been described as a plextrine homology domain(PH), with an ELISA and a PI-specific antibody used to test contact with PI, P2. However, structural study indicated that this domain belongs to the PH domain superfamily's WH1/EVH1 domain (WASP homology 1/including VASP homology 1), which attracts proline-rich, but not proline-deficient, proteins (Hara et al., 1993; Sadhukhan et al., 2014).

Through immunoprecipitation from lymphocytes and the yeast two-hybrid technique, Volkmann and his colleagues demonstrated that WASP Interaction Protein (WIP) is a binding partner for WASP. It's found everywhere and is assumed to play a significant role in the development of filopodia, vesicle movement, and waves. A proline-rich region of WASP's C-terminal region binds to the PH/WH1/EVH1 domain. The WASP-PH/WH1/EVH1 domain contains the bulk of missense mutations observed in WAS/XLT patients, WIP binds CrkL constitutively, generating the CrkL-WIP-WASP complex, implying that it works as a ligand for the PH/WH1/EVH1 domain (Imai et al., 2003).

After activating the T cell receptor and consequent phosphorylation of ZAP-70, CrkL's SH2 domain binds to ZAP-70, enabling the CrKL-WIP-WASP complexes to be transferred to lipid rafts surrounding immunological synapses for the structure. WASP separates from WIP after serine phosphorylation by protein kinase C (PKC), and could be triggered by membrane-bound Cdc42 to induce actin-dependent polymerization of a the Arp2/3 complexes (Imai et al., 2003; Konno et al., 2007).

Despite the fact that WIP is extensively expressed and incorporated into both WASP and N-WASp, a WIP deficient phenotype has just recently been established, suggesting that persons who lack WIP are not significantly abnormal. WIP is essential for T and B lymphocyte development, as well as T lymphocyte stimulation via the T lymphocyte receptor complex (TCR)/CD3: In response to CD3 anti stimulation, T cells lacking WIP proliferated less and secreted less IL-2. When IL-2 was introduced to the culture system, the cells ability to grow in response to anti-CD3 stimulation was inhibited.

After TCR/CD3 ligation, WIP-deficient T cells appear unable to grow filamentous actin or form tubercles and pseudopodia, but react appropriately to PMA + ionomycin. WIP-deficient T cells have abnormalities that are similar to that seen in WASP-deficient patients, although they are more acute. These findings show that WIP is important for T lymphocyte activation via TCR/CD3, and that T-cell dysfunction caused by WASP deficiency could be due in part to defective WIP signaling. In response to various stimuli, B cells from WIP-deficient patients showed increased proliferation and expression of the IL-2 receptor, showing a negative WIP regulatory function. T-independent antigen antibody were normal, but T-dependent antigen antibody were deficient (Imai et al., 2003).

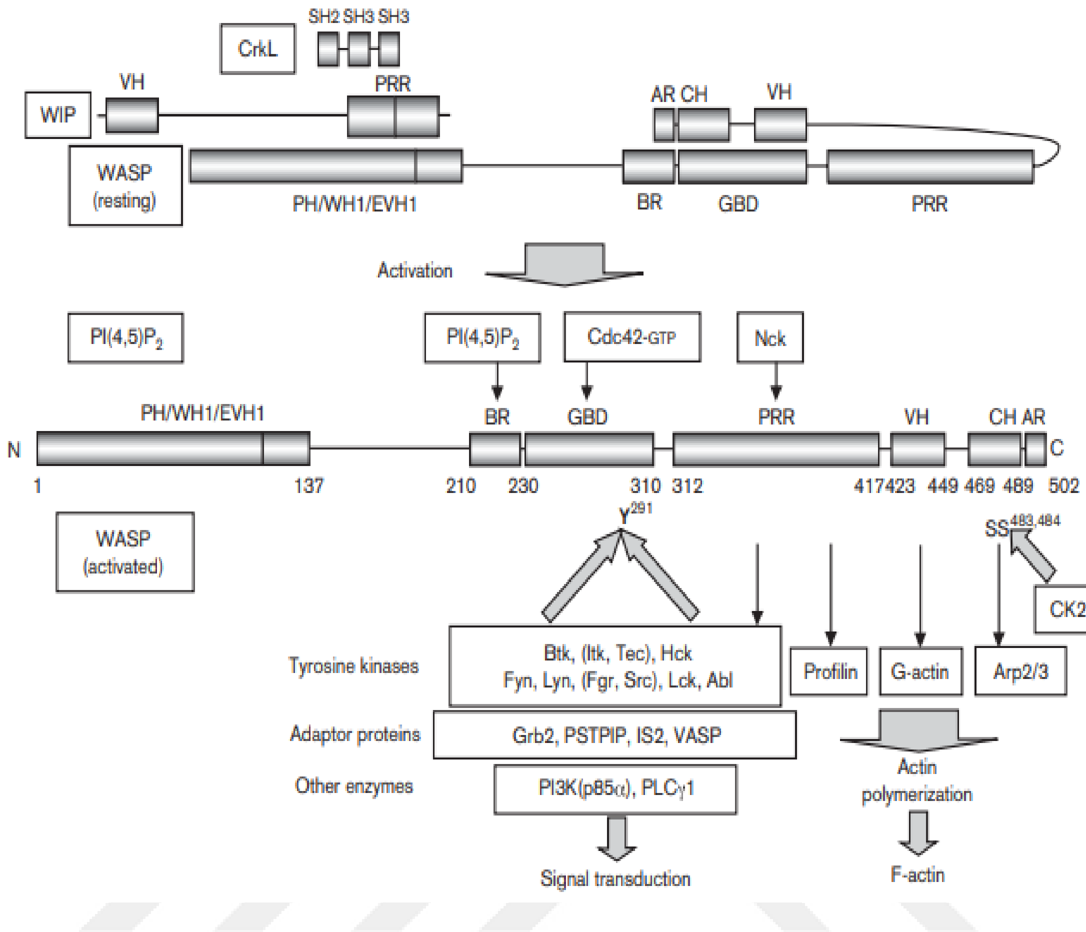

Figure 2.8: Functional domains of WASP and their binding partners (Imai et al., 2003).

The WASP 1 homology domain (WH1), the GTPase/Cdc42 binding, and also the Rac interaction binding domain (GBD/CRIB), as well as the verproline-derived C-terminal domain, central, and acid motifs, are all preserved domains in WSP1 (VCA). The WSP1 mutant, on the other hand, has problems with growth, cytokinesis, chitin dispersion, exocytosis, and exondocytosis. Additionally, the WSP1 mutant has been not able to cross genetically, form a polysaccharide capsule, or release the urease enzyme. The WSP1 mutant had a greater phagocytic index in the in vitro phagocytosis assay, and its ability to generate fatal infections in the cryptococcal mice model was likewise diminished (Shen et al., 2011).

2.9.3. Activation mechanisms of the WASP(family proteins regulate actin dynamics.)

The actin cytoskeleton reorganizes quickly in response to morphological characteristics and cell mobility, being signal-regulated molecular switches that trigger actin polymerization, WASP have gotten a lot of interest. The first member, WASP, was discovered to be the result of a gene that causes Wiskott-Aldrich syndrome, a congenital human disease, when it malfunctions. WASP, N-WASP, WAVE/Scarl, 2, and 3 are the five members of this protein family at the moment.

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 (figure 2.9), 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).

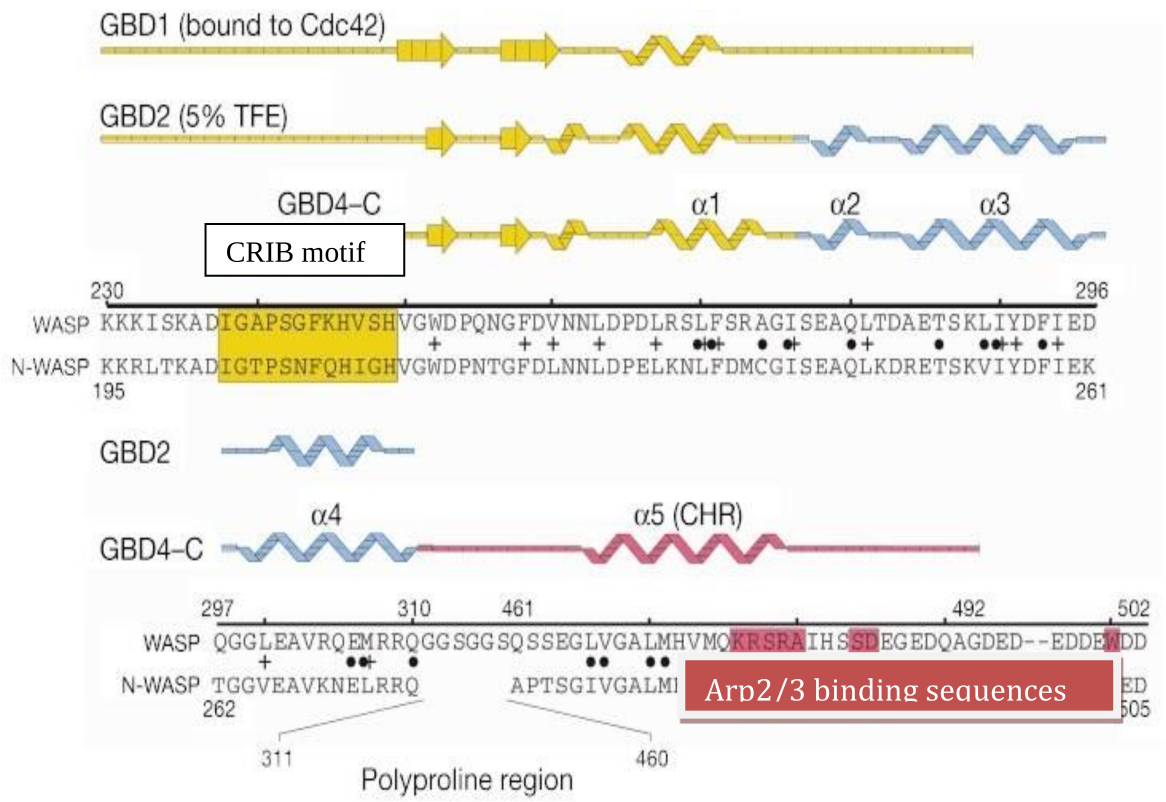

Figure 2.9: Amino-acid sequences of human WASP and N-WASP, along with the GBD constructions of WASP's consensus secondary structure, are presented above (Kim et al., 2000).

The 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 (Verproline 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.

Regardless of whether GBD is substantially unstructured in its free state, the addition of a small quantity of an organic cosolvent or even the binding of the VCA area causes the creation of a compact folded domain structure in the CRIB motif's residues that are directly C-terminally terminated. CHR has a lower affinity for actin and the Arp2/3 complex because it produces an amphipathic helix that rests on the hydrophobic surface of GBD. Surprisingly, this GBD shape is sterically incompatible with the Cdc42 complex's active state. The structural and thermodynamic foundation for WASP protein activation by Cdc42 can be seen by comparing the conformations and biochemical characteristics of these two GBD states (Kim et al., 2000).

2.9.4. Mutations in the WASp gene and phenotypic

Various researchers have separately documented a link between clinical phenotype and genetics, although others have not seen it. More than 400 patients have been identified as having the WASP gene mutation thus far. WASP base is a database that contains mutations, WASP expressions, or registered phenotypes. A simple grading system can be used to assess the severity of the clinical phenotype (Imai et al., 2003). Chronic thrombocytopenia is a frequent condition in WAS (with immunodeficiency, score 3, 4) and XLT (without immunodeficiency, score 3, 4) patients (without immunodeficiency, score 1, 2). All except four cases with WASP mutations (2X-linked neutropenia, 2 intermittent XLT) exhibit chronic thrombocytopenia. Null mutations (deletions, nonsense mutations, insertions and frameshift) account for 46% of all WASP gene mutations, missense mutations for 42%, and splice abnormalities for 12%.

A PH/EVH1/WH1 domain contains the majority of missense mutations, and individuals with splicing abnormalities and null mutations including invariant nucleotides show the characteristic WASP phenotype (86.4 percent and 89.8 percent). Though the fact that missense mutations were found in the most of XLT patients (74.6 percent, 88/118 instances), only 52.7 percent (88/167 instances) of everyone with a missense mutation had the XLT phenotype. The apparent lack of a genotype/phenotype relationship in patients with missense mutations can be due to a number of factors. Many people have been retroactively appraised in the literature based on incomplete records, for example. Many missenses cause WASP expression to be absent, resulting in the classic WASP phenotype.

The fact that WASP expression corresponds better to clinical phenotype: 74.2 percent of WAS positive patients get the XLT phenotype, while 86.5 percent of WASP-negative patients have the WASP phenotype, supports this interpretation. Arg86 (36 individuals) is the most prevalent missense mutation (Imai et al., 2003; He et al., 2017). Although 42 percent of individuals with Arg86Cys or Arg86His mutations are both WASP positive and have a WAS phenotype, just 14 percent (13/33) have a WAS phenotype, the data reported in the published papers does not reveal a strong association between protein genotype/expression and phenotype. Schindelhauer et al. also verified this mismatch. Surprisingly, Arg86 is a key interaction point for WASP and WIP (Imai et al., 2003; Zhu et al., 1997).

WASP was detected by western blot in all but two of the patients. With the exception of patients with Arg86 mutations, the connection between genotype expression and WASP phenotype has improved: 83.3 percent of those with missense mutations besides Arg86 who are also WASP-positive have the XLT phenotype (Imai et al., 2003; Boztug et al., 2010).

2.9.5. WASP in disease

The most prevalent disorders produced by mutations in the WASP gene are XLT and WAS disorder. Mutations in the WASP gene cause X-linked thrombocytopenia (XLT) & Wiskott-Aldrich syndrome (WAS). The genotype-phenotype correlation of XLT and WAS has not been fully explained (Liu et al., 2015). There is now a huge database of WASP mutations that induce classic WAS or XLT, a milder version of the disease characterized by platelet abnormalities. Various missense mutations may now be explored in relation to activated and autoinhibited WASP structures, 2 of which are included in the GBD: M307V and A236G. Helix a4 contains methionine 307, which acts as a buffer against a1. In vivo, the mutation appears to disrupt the autoinhibited structure, leading to faulty WASP activation or maybe an increase in proteolytic metabolism 23.

Otherwise, Ala 236 is found towards the N-terminus of the CRIB motif and in the structure of the Cdc42 complex above GTPase6's C-terminal helix. Two problem mutations in the VCA area, R477K and K476E, have also been found. The two changed residues will be in a conserved basic CRH motif, and hence influence the efficacy of WASP-GTPase activation. And although Arg 477 mutation may alter its interaction with Glu 285, Lys 476 extends from the autoinhibited structure's solvent-exposed surface and therefore does not create direct intramolecular interactions.

The presence of each mutation independently in GBD4 C has no effect on the melting point of the construct as assessed by CD or 1H/15N-HSQC, implying that the stability of the autoinhibited folding is not altered as well. As a result, mutations can affect how the cofilin helix interacts with another partner. Indeed, in the Dcof mutant, which does not attach to the Arp2/3complex, residues 476 and 477 are removed, implying that their alteration could influence WASP interactions with this assembly. More biochemical studies of these numerous mutant proteins, interpreted in light of activated and autoinhibited structures, would help researchers better grasp the molecular basis of Wiskott-Aldrich syndrome (Kim et al., 2000).

2.9.6. Wiskott-Aldrich syndrome

Wiskott-Aldrich disease is a X-linked primary immunodeficiency disease (PID) wherein children suffer eczema, recurrent infections, thrombocytopenia, immunodeficiency, and an increased risk of cancer at a young age. Alfred Wiskott in 1937 & Robert Aldrich in 1954 were the first to identify the condition, and it was given the term WAS after the two doctors who first characterized it. Patients having classical (WAS) frequently die before adolescence unless they are healed by bone marrow transplantation (Lemahieu et al., 1999; Brooimans et al., 2000). Mutations in the WASP gene cause the condition; the WASP gene has been found at Xp11.22 p11.23, and the gene encoding WAS protein has recently been cloned.

Despite this, missense mutations have been detected in patients with mild cases of X-linked thrombocytopenia (XLT) and WAS illness. The WASP gene has many physiological functions domain that engage with intracellular, apoptotic, signaling, and actin cytoskeleton regulatory proteins. WASP phenotypes that are severe are related with no protein expression, whereas mutations that produce lesser abnormalities are associated with detectable protein expression (Brooimans et al., 2000).

The deletion mutation is among the most common types of mutation seen in a variety of genetic diseases, one of Wiskott-Aldrich Syndrome patients originally thoughts of having a significant deletion in the WASP gene, which has recently been identified as the cause of WAS and accompanying thrombocytopenia (XLT) (Ariga et al., 1997).

2.9.7. The function of the WASP and WAVE family proteins in cancer invasion

WASP as well as WAVE (WASP family verprolin-homologous protein) family members stimulate the actin-related protein complex 2⁄3 (Arp2⁄3), the cell's main actin nucleating machinery, causing cortical actin filament networks to remodel and develop. Many elements of cell physiology, especially cell adhesion and motility, are dependent on actin regulation at the cortical level, and its disturbance has been related to cancer invasion and metastasis.

Cancer cells spread by invading nearby tissues and then traveling to various tissues via blood arteries or lymph arteries (metastasis). A metastatic cascade is a multi-step process that leads to the spread of cancer. Despite the fact that some cellular functions are altered during these processes, cancer cells, particularly those of epithelial origin, hinder cadherin-based cell adhesion and can kill them (Kurisu et al., 2010).

Knowing the mechanisms underpinning invasive progression, the first phase in metastasis, is crucial to understanding cancer-related death because cancer mortality is predominantly caused by progress to be invasive and metastatic conditions. Actin polymerization just at the point of the edge of migrating cells provides a force that push the plasma membrane forward when tumor cells become invasive. Its own main role is to maintain intercellular mechanical adhesion (cell–cell adhesion) and to govern the form of the cells, and actin polymerization at the point of the edge of migrating cells.

Recent data reveals that invasive cancer cells digest ECMs and rearrange them into specialized, actin-rich macrostructures called invadopodia or podosome macrostructures, which are thought to act as scaffolds for proteolytic enzymes. A Wiskott-Aldrich syndrome (WASP) protein superfamily contains the most critical proteins for actin polymerization in cells, with at least one homolog discovered in all eukaryotic creatures investigated to date (Kurisu et al., 2010).

WASP, which is primarily expressed in hematopoietic lineages in humans, and its homolog (N-) neuronal WASP (WASP subfamily); brain-rich WASP family verprolin-homologous protein (WAVE) 1 and WAVE3; and WAVE2 (WAVE suppressor of SCAR subfamily; WAVEs in short) are all members of the WASP superfamily, as are the junction-mediating and regulatory protein (JMY), WASP and WAVE cause fast polymerization of actin under the biological membrane, which is essential for a range of pathological and physiological activities such as immunological response, synaptogenesis and pathogen, tissue morphogenesis, cancer invasion and metastasis. WASP and WAVE appear to be critical in the progression of cancer, according to a growing body of research (Kurisu et al., 2010; Machesky et al., 1999).

While they upregulate cell migration through actin polymerization, WASP and WAVE were previously considered to promote cancer invasion and metastasis. This was later proven to be partially correct, though oversimplified. Based on patient or research condition, WASP and WAVE were believed to act as cancer suppressors or promoters. WASP and WAVE's dual activity in cancer are most likely owing to their different functions in cells.

To gain a better understanding of invasive behavior and to identify more effective cancer treatment targets, researchers must examine the WASP superfamily proteins in a holistic manner that considers both their positive and negative effects on cancer invasion. WASPs are necessary for actin assembly during Invadopodium creation and possibly for the development of spherical bubble shape movement in the 3D ECM, as previously stated. Invadopods not just serve as conspicuous cell structures, but also as a storage and secretion method for proteases, implying that WASPs are multimodal promoters of cancer cell migration into the ECM (Kurisu et al., 2010).

WAVE2 is essential for the maintenance and development of cell-cell adhesion in epithelial cells, which likely prevents the shift from the epithelial to the mesenchymal phenotype. As a result, WAVE2 appears to be a tumor suppressor in benign epithelial malignancies. WAVE, particularly WAVE2, stimulates the anterior actin network in elongated cells, which promotes tumor cell invasiveness. As a result, when cancer cells invade as a single elongated cell later in the cancer phase, WAVE2 works as an invasion promoter (Kurisu et al., 2010; Orange et al., 2011).

EMT is thought to cause carcinoma invasion and metastasis by downregulating the activity of epithelial genes like E-cadherin while activating mesenchymal genes to boost single-cell motility and, as a result, increase cancer cell proliferation. WAVE3's function is still unknown. The WAVE3 function, on the other hand, has been described as a metastatic protein. When WAVE3 expression is lost, the cell shape changes dramatically.

WAVE3 can aid in the mobility of these stem cells, which are similar to cancer cells. WAVE3 overexpression may result in a drop in WAVE2 levels and a shift in the ratio of WAVE isoforms expressed in the amounts of other proteins in the complex. The dual function of WAVE in the early phases of cancer growth, when cancer cells suppress WAVE2, may be explained by this quantitative regulation of WAVE expression levels, certain cancer cells can restore WAVE2 function to improve cancer cell motility, resulting in WAVE2 metastatic progression in invading cancer cells (Kurisu et al., 2010).

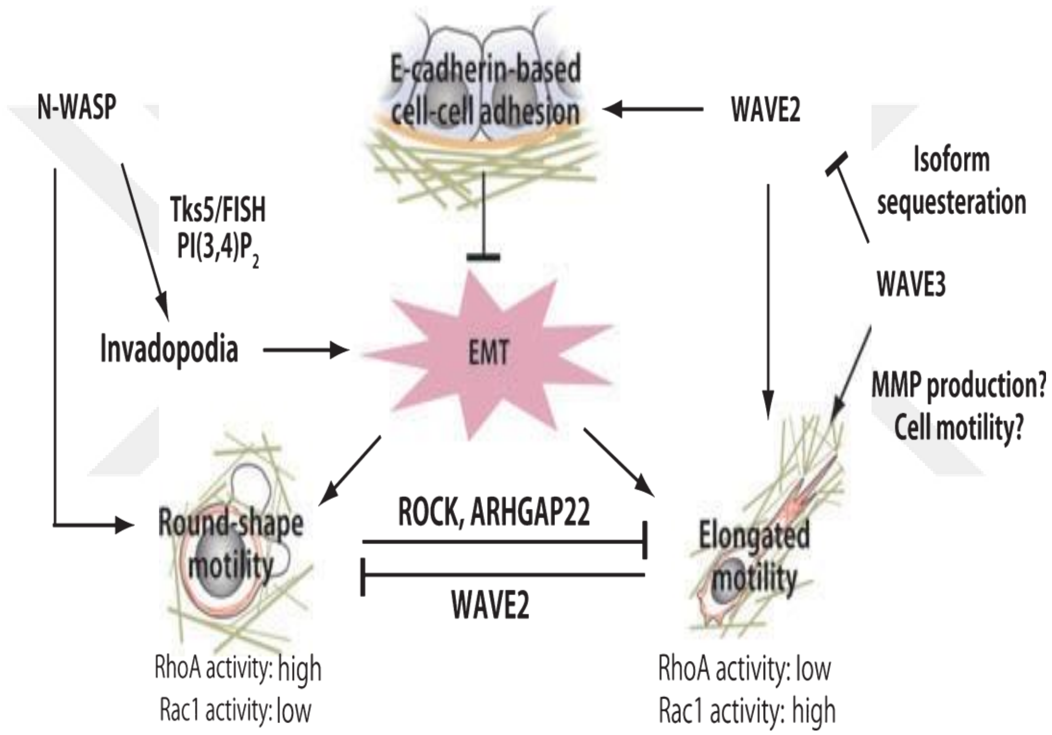

Figure 2.10: Association function of WASP and WAVE in cancer progression (Kurisu et al., 2010).

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 WASP 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

6

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.

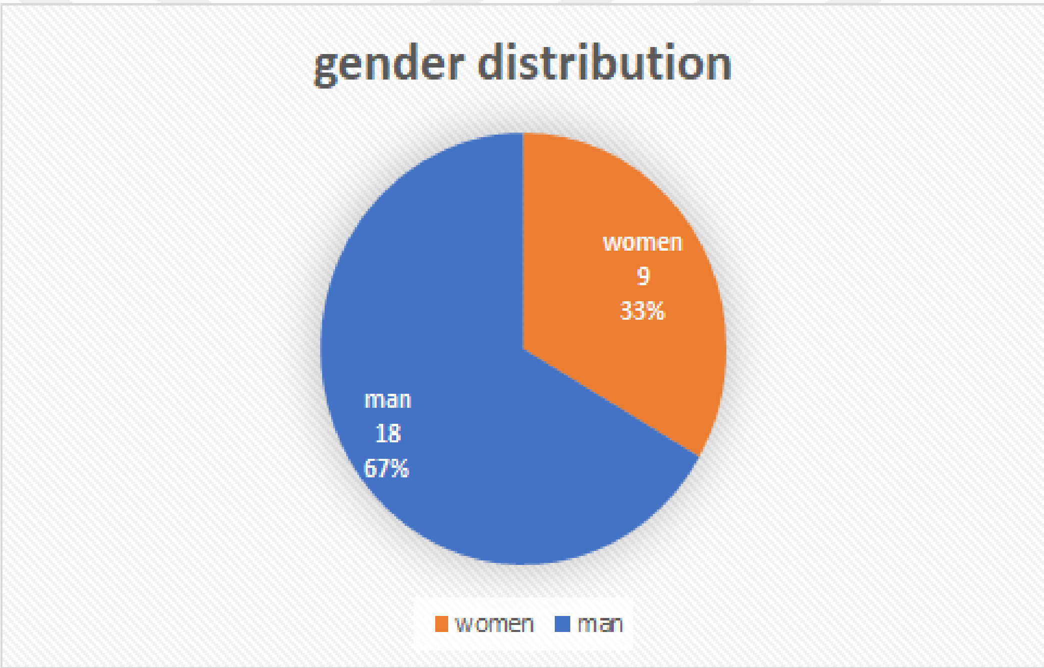

Figure 4.1: The Gender Distribution of the studied Patients

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

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

The quantitative Real-Time PCR method based on SYBR Green was used to evaluate the gene expression with WASP mRNA, for WASP 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 WASP genes observed as a result of qRT-PCR are given, respectively(Figure 4.2, Figure 4.3, Figure 4.4,Figure 4.5)

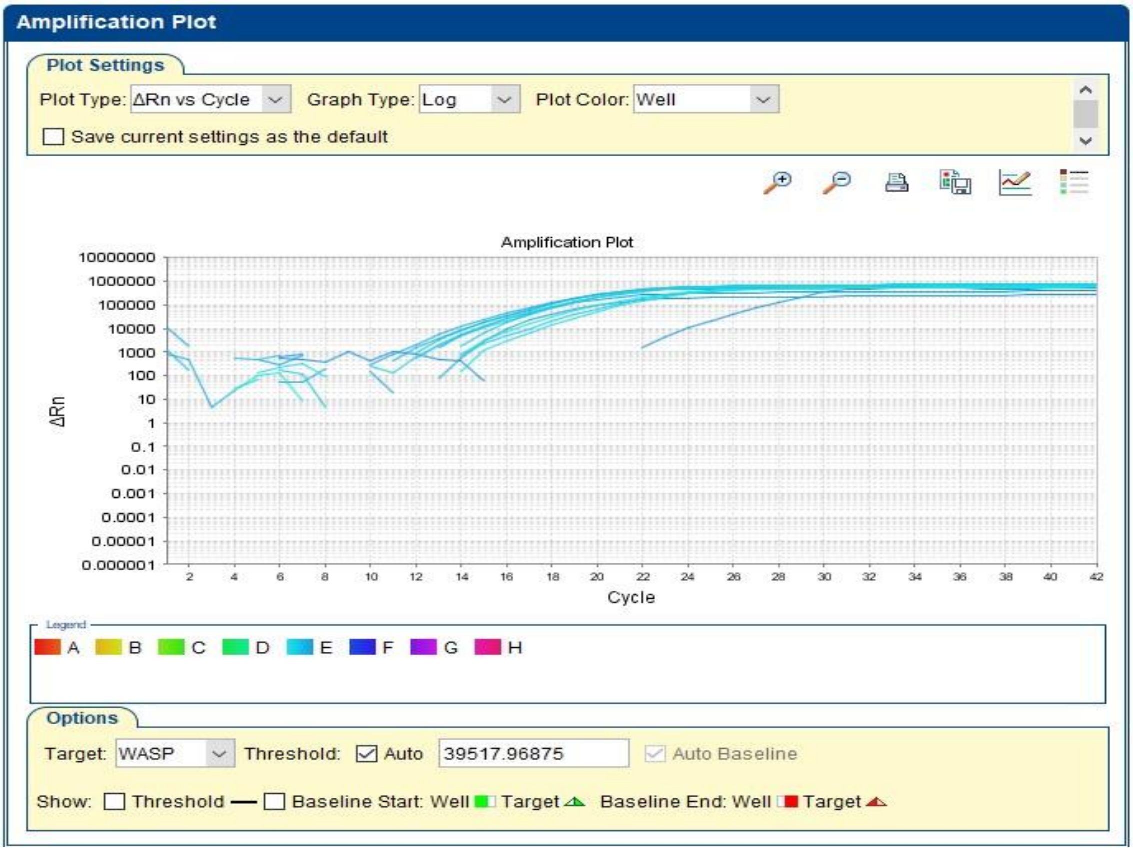

Figure 4.2: WASP gene amplification plot.

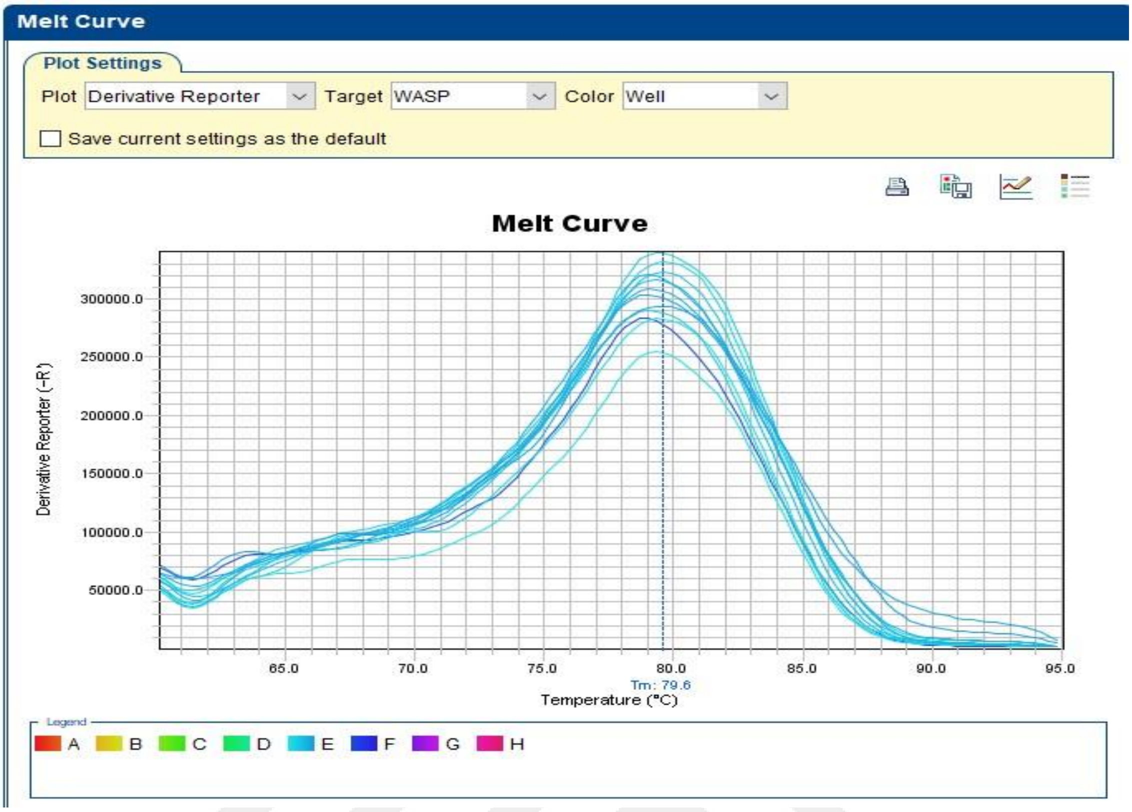

Figure 4.3: WASP gene melt curve.

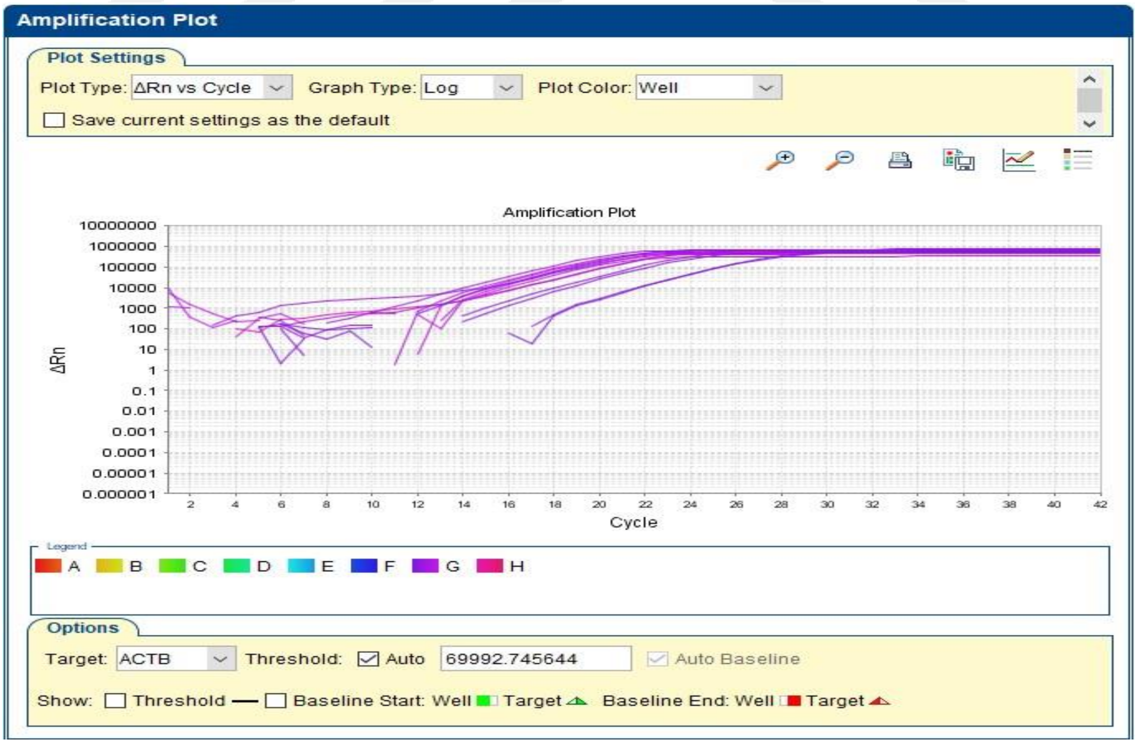

Figure 4.4: ACTB amplification plot.

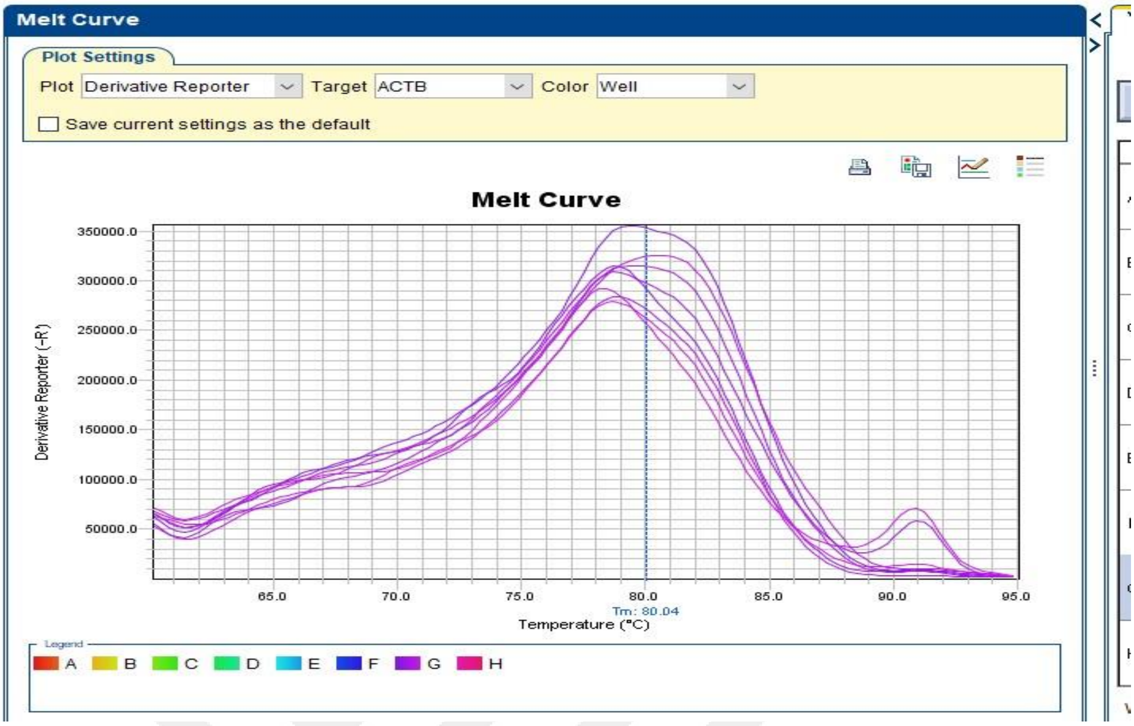

Figure 4.5: ACTB melt curve.

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

qRT-PCR analysis was performed for mRNA level expression of WASP gene found in pterygium tissues and control conjunctiva tissues. The qRT-PCR results of WASP and ACTB genes were quantified with step one plus software v2.3. The data belonging to the WASP 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 WAS gene and conjunctival tissues

Gene	Tissue	
	Pterygium tissue (N=27)	Normal Conjunctiva (N=27)
WASP gene (AVG±SEM)	1.910±0,367	
P	0,0001	

According to the qRT-PCR analysis results When the expression level of the WASP gene was compared with pterygium tissues, it was found that it increased WASP gene expression in the pterygium tissue (1.910±0,367). Statistically significant in terms of WASP gene mRNA expression level (p=0,0001). When the expression level of the WASP gene was compared with the pterygium and normal conjunctival tissues, it was determined that the pterygium tissue increased (1.910±0,367-Fold change) 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 WASP gene in pterygia disorder tissues and healthy conjunctival tissue ($p \leq 0.005$).

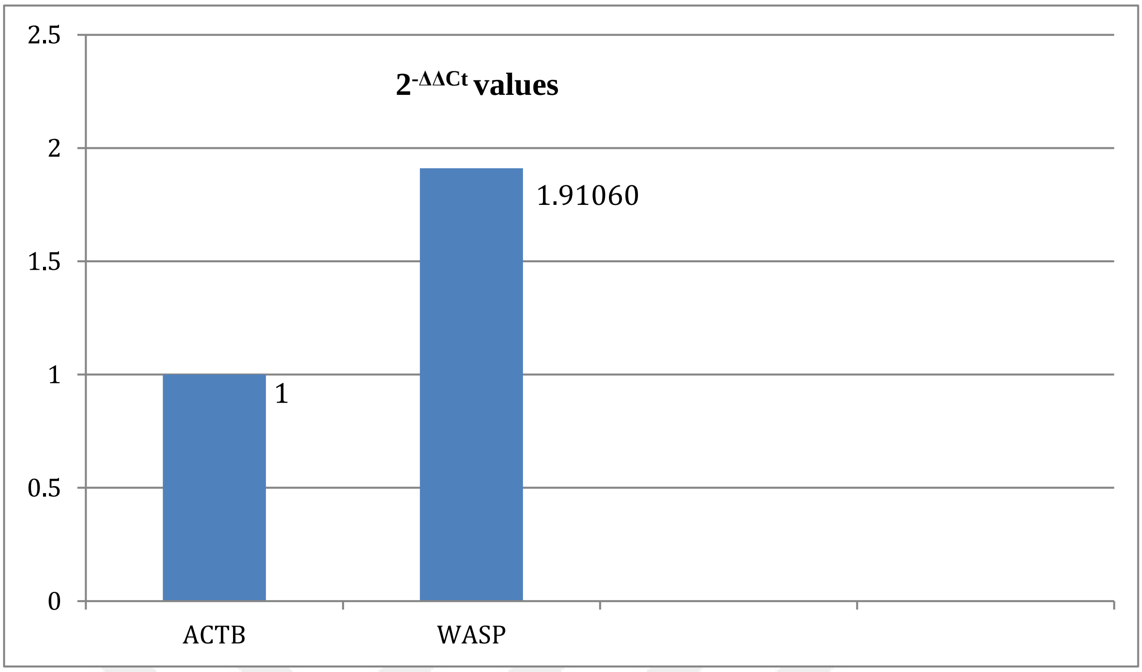

Figure: 4. 6. Average $2^{-\Delta\Delta Ct}$ values of WASP and ACTB genes.

5. DISCUSSION

The conjunctiva expands like winged along of the limbus and toward the cornea in pterygium, an invading fibrovascular development. 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).

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).

The generation of reactive oxygen species(ROS) is linked to the dynamic reorganization of the actin cytoskeleton, which is implicated in cancer cell movement and metastasis. As a result, reducing the production of reactive oxygen species (ROS) and actin polymerization in cancerous cells could be a promising anti-cancer treatment.

Intracellular ROS levels are greater in malignant cells than non-metastatic parent cell, and the intracellular ROS signal mediates melanoma cell prometastatic characteristics. Rac1 activation and WAVE2 expression are linked to ROS levels, cell migration, and melanoma cell invasiveness, implying that WAVE2 is play role in the migration of invasive melanoma cells via b (Park et al., 2012).

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).

WASP has been discovered to be a tumor suppressor in T cell lymphoma. WASP and WASP Interacting Protein (WIP) regulate signal transmission by the T cell antigen receptor (TCR) in T cells, although their significance in lymphoma is unclear. WASP and WIP expression in anaplastic large cell lymphomas (ALCL) is often low or nonexistent, in contrast to other T-cell lymphomas. ALK's oncogenic activity influences WASP and WIP via STAT3 and C/EBP-pathway mediators. CDC42 connected to active GTP increased in the absence of WASP, and genetic deletion of the CDC42 allele was enough to affect (impair) lymphoma growth.

In WASP-deficient lymphoma, greater activation of the mitogen-activated protein kinase (MAPK) pathways has been reported, which could be exploited as a therapeutic vulnerability. WASP and WIP are tumor suppressors in T lymphoma, and the data suggest that MEK inhibitors combined with ALK inhibitors may be more effective in treating ALK + ALCL (Menotti et al., 2019).

Phagocytosis and inflammatory activities Dysregulation contributes to the onset of autoinflammatory disorders. The actin cytoskeleton is critical for controlling inflammatory responses. According to new research, a lack of WASP, which regulates the actin cytoskeleton, has been linked to autophagy and inflammation. Due to the uneven production of septin cage-like cells and the development of faulty canonical autophagosomes, Bacterial clearance issues, higher activation inflammation, and host tumor growth are all symptoms of WASP deficient (Lee et al., 2017).

With 5 well-defined domains involving signal transmission, cell motility, and immunological synapses formation, WASP is a crucial regulator of actin polymerization in hematopoietic cells. Mutations in WASP gene can cause a variety of symptoms, which are mostly, if not entirely, dependent on the mutations. Proliferation, phagocytosis, immune synapses, adhesion, and direct migration are all affected by WASP deficiency, which causes cytoskeletal abnormalities.

WASP has also been demonstrated to play a role in the creation of lymphoid and monocytic myeloid cells, as well as expanded diagnostic options and therapeutic options, which range from symptomatic conventional therapy to curable hematopoietic stem cell transplantation and gene therapy. WASP facilitates nuclear translocation of the central factor kappaB and has been linked to the development and function of monocytic myeloid cells, as well as diagnostic potential and extended treatment options (Ochs et al., 2006; Blundell et al., 2017).

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.

Actin networks at intercellular junctions are actively modified to lessen cell-cell adhesion, while actin polymerization at the tip of migration cells' edges pushes the plasma membrane upward, WASP and WAVE cause fast polymerization of actin under the biological membrane, which is essential for a range of pathological and physiological activities such as immunological response, synaptogenesis and pathogens, tissue morphogenesis, and cancer invasion and metastasis, to name a few. WASP and WAVE appear to be critical in the progression of cancer, according to a growing body of research (Kurisu et al., 2010).

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

6. REFERENCES

ALEKHINA, O., BURSTEIN, E., & BILLADEAU, D. D. (2017). Cellular functions of WASP family proteins at a glance. *Journal of cell science*, 130(14), 2235–2241.

ALTAN-YAYCIOGLU R, KUCUKERDONMEZ C, KARALEZLI A, CORAK F, AKOVA YA. (2013). Astigmatic changes following pterygium removal: comparison of 5 different methods. *Indian Journal of Ophthalmology*, 61(3):104-108.

AMATO, C., THOMASON, P. A., DAVIDSON, A. J., SWAMINATHAN, K., ISMAIL, S., MACHESKY, L. M., & INSALL, R. H. (2019). WASP Restricts Active Rac to Maintain Cells' Front-Rear Polarization. *Current biology* : CB, 29(24), 4169–4182.e4.

ANGURIA, P., CARMICHAEL, T., NTULI, S., & KITINYA, J. (2013). Chronic inflammatory cells and damaged limbal cells in pterygium. *African health sciences*, 13(3), 725–730.

ARIGA, T., YAMADA, M., ITO, S., IWAMURA, M., ISEKI, M., & SAKIYAMA, Y. (1997). Characterization of a deletion mutation involving exons 3-7 of the WASP gene detected in a patient with Wiskott-Aldrich syndrome. *Human mutation*, 10(4), 310–316.

AVISAR, R., LOYA, N., YASSUR, Y., & WEINBERGER, D. (2000). Pterygium-induced corneal astigmatism. *The Israel Medical Association journal* : IMAJ, 2(1), 14–15.

BAI, H., TENG, Y., WONG, L., JHANJI, V., PANG, C. P., & YAM, G. H. (2010). Proliferative and migratory aptitude in pterygium. *Histochemistry and cell biology*, 134(5), 527–535.

BALCI, M., SAHIN, S., MUTLU, F. M., YAĞCI, R., KARANCI, P., & YILDIZ, M. (2011). Investigation of oxidative stress in pterygium tissue. *Molecular vision*, 17, 443–447.

BIANCHI, E., SCARINCI, F., GRANDE, C., PLATEROTI, R., PLATEROTI, P., PLATEROTI, A. M. ET AL. (2012). Immunohistochemical profile of VEGF, TGF-β and PGE_2 in human pterygium and normal conjunctiva: experimental study and review of the literature. *International journal of immunopathology and pharmacology*, 25(3), 607–615.

BLUNDELL, M. P., WORTH, A., BOUMA, G., & THRASHER, A. J. (2010). The Wiskott-Aldrich syndrome: The actin cytoskeleton and immune cell function. *Disease markers*, 29(3-4), 157–175.

BOZKURT, N., & ATES, O. (2020). Analysis of WWOX gene expression and protein levels in pterygium. *International ophthalmology*, 40(8), 1949–1953.

BOZTUG, K., SCHMIDT, M., SCHWARZER, A., BANERJEE, P. P., DÍEZ, I. A., DEWEY, R. A., BÖHM, M. ET AL. (2010). Stem-cell gene therapy for the Wiskott-Aldrich syndrome. *The New England journal of medicine*, 363(20), 1918–1927.

BRADLEY JC, YANG W, BRADLEY RH, REID TW, SCHWAB IR.(2010). The science of pterygia. *Br J Ophthalmol*, 94(7):815-20.

BROOIMANS, R. A., VAN DEN BERG, A. J., TAMMINGA, R. Y., REVESZ, T., WULFFRAAT, N. M., & ZEGERS, B. J. (2000). Identification of six novel WASP gene mutations in patients suffering from Wiskott-Aldrich syndrome. *Human mutation*, 15(4), 386–387.

CAMERON M. E. (1983). Histology of pterygium: an electron microscopic study. *The British journal of ophthalmology*, 67(9), 604–608.

CAMPAGNA, G., ADAMS, M., WANG, L., KHANDELWAL, S., & AL-MOHTASEB, Z. (2018). Comparison of Pterygium Recurrence Rates Among Different Races and Ethnicities After Primary Pterygium Excision by Surgeons in Training. *Cornea*, 37(2), 199–204.

CÁRDENAS-CANTÚ, E., ZAVALA, J., VALENZUELA, J., & VALDEZ-GARCÍA, J. E. (2016). Molecular Basis of Pterygium Development. *Seminars in ophthalmology*, 31(6), 567–583.

CASTIELLO, M. C., BOSTICARDO, M., PALA, F., CATUCCI, M., CHAMBERLAIN, N., VAN ZELM, M. C. ET AL. (2014). Wiskott-Aldrich Syndrome protein deficiency perturbs the homeostasis of B-cell compartment in humans. *Journal of autoimmunity*, 50(100), 42–50.

CHAO, S. C., HU, D. N., YANG, P. Y., LIN, C. Y., NIEN, C. W., YANG, S. F., & ROBERTS, J. E. (2013). Ultraviolet-A irradiation upregulated urokinase-type plasminogen activator in pterygium fibroblasts through ERK and JNK pathways. *Investigative ophthalmology & visual science*, 54(2), 999–1007.

CHEN, Q., LI, Y., XU, F., YAN, Y., LU, K., CUI, L., & LI, M. (2015). Comparison of Inferior and Superior Conjunctival Autograft for Primary Pterygium. *Current eye research*, 40(8), 786–791.

CHIANG, C. C., TSAI, Y. Y., BAU, D. T., CHENG, Y. W., TSENG, S. H., WANG, R. F., & TSAI, F. J. (2010). Pterygium and genetic polymorphisms of the DNA repair enzymes XRCC1, XPA, and XPD. *Molecular vision*, 16, 698–704.

CHUI, J., DI GIROLAMO, N., WAKEFIELD, D., & CORONEO, M. T. (2008). The pathogenesis of pterygium: current concepts and their therapeutic implications. *The ocular surface*, 6(1), 24–43.

DETORAKIS, E. T., DRAKONAKI, E. E., & SPANDIDOS, D. A. (2000). Molecular genetic alterations and viral presence in ophthalmic pterygium. *International journal of molecular medicine*, 6(1), 35–41.

DI GIROLAMO, N., CHUI, J., CORONEO, M. T., & WAKEFIELD, D. (2004). Pathogenesis of pterygia: role of cytokines, growth factors, and matrix metalloproteinases. *Progress in retinal and eye research*, 23(2), 195–228.

DI GIROLAMO, N., WAKEFIELD, D., & CORONEO, M. T. (2000). Differential expression of matrix metalloproteinases and their tissue inhibitors at the advancing pterygium head. *Investigative ophthalmology & visual science*, 41(13), 4142–4149.

DI GIROLAMO, N., WAKEFIELD, D., & CORONEO, M. T. (2006). UVB-mediated induction of cytokines and growth factors in pterygium epithelial cells involves cell surface receptors and intracellular signaling. *Investigative ophthalmology & visual science*, 47(6), 2430–2437.

DROUTSAS, K., & SEKUNDO, W. (2010). Epidemiologie des Pterygiums. Eine Ubersicht [Epidemiology of pterygium. A review]. *Der Ophthalmologe : Zeitschrift der Deutschen Ophthalmologischen Gesellschaft*, 107(6), 511–516.

DUSHKU, N., HATCHER, S. L., ALBERT, D. M., & REID, T. W. (1999). p53 expression and relation to human papillomavirus infection in pingueculae, pterygia, and limbal tumors. *Archives of ophthalmology* (Chicago, Ill. : 1960), 117(12), 1593–1599.

DUSHKU, N., REID, T W. (1997). P53 expression in altered limbal basal cells of pingueculae, pterygia, and limbal tumors. *Curr Eye Res*, 16(12), 1179-92.

GADKAR, V. Y., & FILION, M. (2014). New Developments in Quantitative Real-time Polymerase Chain Reaction Technology. *Current issues in molecular biology*, 16, 1–6.

HACIOĞLU, D., & ERDÖL, H. (2017). Developments and current approaches in the treatment of pterygium. *International ophthalmology*, 37(4), 1073–1081.

HAGEMANN, T. L., & KWAN, S. P. (1999). The identification and characterization of two promoters and the complete genomic sequence for the Wiskott-Aldrich syndrome gene. *Biochemical and biophysical research communications*, 256(1), 104–109.

HARA, T., YAMAUCHI, M., TAKAHASHI, EI. ET AL. (1993). The UDP-galactose translocator gene is mapped to band Xp11.23-p11.22 containing the Wiskott-Aldrich syndrome locus. *Somat Cell Mol* Genet 19, 571–575.

HE, X., ZOU, R., ZHANG, B., YOU, Y., YANG, Y., & TIAN, X. (2017). Whole Wiskott-Aldrich syndrome protein gene deletion identified by high throughput sequencing. *Molecular medicine reports*, 16(5), 6526–6531.

HILL, J., MASKE, R. (1989). Pathogenesis of Pterygium. *Eye* 3(Pt 2)(2), 218-226.

HILMI, MR., ZULFAEZAL, M., AZEMIN, C., KAMAL, KM., MOHD TAMRIN, MI., ABDUL GAFFUR, N. ET AL. (2017). Prediction of Changes in Visual Acuity and Contrast Sensitivity Function by Tissue Redness after Pterygium Surgery. *Curr Eye Res*, 42(6):852-856.

HO, L. L., AYLING, J., PROSSER, I., KRONENBERG, H., ILAND, H., & JOSHUA, D. (2001). Missense C168T in the Wiskott--Aldrich Syndrome protein gene is a common mutation in X-linked thrombocytopenia. *British journal of haematology*, 112(1), 76–80.

HSIAO, C. H., LEE, B. H., NGAN, K. W., CHUANG, W. Y., YEUNG, L., YEH, L. K., TAN, H. Y. ET AL. (2010). Presence of human papillomavirus in pterygium in Taiwan. *Cornea*, 29(2), 123–127.

IMAI, K., NONOYAMA, S., & OCHS, H. D. (2003). WASP (Wiskott-Aldrich syndrome protein) gene mutations and phenotype. *Current opinion in allergy and clinical immunology*, 3(6), 427–436.

ISHIHARA, D., DOVAS, A., PARK, H., ISAAC, B. M., & COX, D. (2012). The chemotactic defect in wiskott-Aldrich syndrome macrophages is due to the reduced persistence of directional protrusions. *PloS one*, 7(1), e30033.

JAMES, P., PHILIP, P. (2002). THE OFFICIAL PATIENT’S SOURCEBOOK ON PTERYGIUM. *Health Care*, 144(12).

JIAO, W., ZHOU, C., WANG, T., YANG, S., BI, H., LIU, L., LI, Y., & WANG, L. (2014). Prevalence and risk factors for pterygium in rural older adults in Shandong Province of China: a cross-sectional study. *BioMed research international*, 2014, 658648.

JIN, Y., MAZZA, C., CHRISTIE, J. R., GILIANI, S., FIORINI, M., MELLA, P. ET AL. (2004). Mutations of the Wiskott-Aldrich Syndrome Protein (WASP): hotspots, effect on transcription, and translation and phenotype/genotype correlation. *Blood*, 104(13), 4010–4019.

KASE, S., OSAKI, M., JIN, X. H., OHGAMI, K., YOSHIDA, K., SAITO, W. ET AL. (2007). Increased expression of erythropoietin receptor in human pterygial tissues. *International journal of molecular medicine*, 20(5), 699–702.

KASE, S., OSAKI, M., SATO, I., TAKAHASHI, S., NAKANISHI, K., YOSHIDA, K., ITO, H., & OHNO, S. (2007). Immunolocalisation of E-cadherin and beta-catenin in human pterygium. *The British journal of ophthalmology*, 91(9), 1209–1212.

KASE, S., TAKAHASHI, S., SATO, I., NAKANISHI, K., YOSHIDA, K., OHNO, SH. (2007). Expression of p27(KIP1) and cyclin D1, and cell proliferation in human pterygium. *Br J Ophthalmol*, 91(7):958-61.

KELISHADI, M., KELISHADI, M., MORADI, A., JAVID, N., BAZOURI, M., & TABARRAEI, A. (2015). human adenoviruses role in ophthalmic pterygium formation. *Jundishapur journal of microbiology*, 8(4), e16871.

KHEIRKHAH, A., NAZARI, R., NIKDEL, M., GHASSEMI, H., HASHEMI, H . ET AL. (2013). Postoperative conjunctival inflammation after pterygium surgery with amniotic membrane transplantation versus conjunctival autograft. *Am J Ophthalmol*, 152(5), 733-8.

KIM, A. S., KAKALIS, L. T., ABDUL-MANAN, N., LIU, G. A., & ROSEN, M. K. (2000). Autoinhibition and activation mechanisms of the Wiskott-Aldrich syndrome protein. *Nature*, 404(6774), 151–158.

KIM, K. W., PARK, S. H., & KIM, J. C. (2016). Fibroblast biology in pterygia. *Experimental eye research*, 142, 32–39.

KIM, K. W., PARK, S. H., WEE, S. W., & KIM, J. C. (2013). Overexpression of angiogenin in pterygium body fibroblasts and its association with proliferative potency. *Investigative ophthalmology & visual science*, 54(9), 6355–6362.

KONNO, A., KIRBY, M., ANDERSON, S. A., SCHWARTZBERG, P. L., & CANDOTTI, F. (2007). The expression of Wiskott-Aldrich syndrome protein (WASP) is dependent on WASP-interacting protein (WIP). *International immunology*, 19(2), 185–192.

KURISU, S., & TAKENAWA, T. (2010). WASP and WAVE family proteins: friends or foes in cancer invasion?. *Cancer science*, 101(10), 2093–2104.

LEE, P. P., LOBATO-MÁRQUEZ, D., PRAMANIK, N., SIRIANNI, A., DAZA-CAJIGAL, V., RIVERS, E., CAVAZZA, A. ET AL. (2017). Wiskott-Aldrich syndrome protein regulates autophagy and inflammasome activity in innate immune cells. *Nature communications*, 8(1), 1576.

LEMAHIEU, V., GASTIER, J. M., & FRANCKE, U. (1999). Novel mutations in the Wiskott-Aldrich syndrome protein gene and their effects on transcriptional, translational, and clinical phenotypes. *Human mutation*, 14(1), 54–66.

LEVINGER E, SORKIN N, SELLA S, TRIVIZKI O, LAPIRA M, KEREN S. (2020). Posterior Corneal Surface Changes After Pterygium Excision Surgery. *Cornea, Jul*;39(7):823-826.

LIANG, K., JIANG, Z., DING, B. Q., CHENG, P., HUANG, D. K., & TAO, L. M. (2011). Expression of cell proliferation and apoptosis biomarkers in pterygia and normal conjunctiva. *Molecular vision*, 17, 1687–1693.

LIVEZEANU, C., CRĂIŢOIU, M. M., MĂNESCU, R., MOCANU, C., & CRĂIŢOIU, S. (2011). Angiogenesis in the pathogenesis of pterygium. *Romanian journal of morphology and embryology = Revue roumaine de morphologie et embryologie*, 52(3), 837–844.

LIU, D. W., ZHANG, Z. Y., ZHAO, Q., JIANG, L. P., LIU, W., TU, W. W., SONG, W. X., & ZHAO, X. D. (2015). Wiskott-Aldrich syndrome/X-linked thrombocytopenia in China: *Clinical characteristic National Academy of Sciences of the United States of America*, 96(7), 3739–3744.

LI, X., DAI, Y., & XU, J. (2018). MiR-21 promotes pterygium cell proliferation through the PTEN/AKT pathway. *Molecular vision*, 24, 485–494.

MACHESKY, L. M., MULLINS, R. D., HIGGS, H. N., KAISER, D. A., BLANCHOIN, L., MAY, R. C.ET AL. (1999). Scar, a WASp-related protein, activates nucleation of actin and genotype-phenotype correlation. *Pediatric blood & cancer*, 62(9), 1601–1608.

MALIK, A., ARYA, S. K., SOOD, S., SARDA, S. B., & NARANG, S. (2014). Effect of pterygium on contrast sensitivity. *International ophthalmology*, 34(3), 505–509.

MENOTTI, M., AMBROGIO, C., CHEONG, T. C., PIGHI, C., MOTA, I., CASSEL, S. H., COMPAGNO, M. ET AL. (2019). Wiskott-Aldrich syndrome protein (WASP) is a tumor suppressor in T cell lymphoma. *Nature medicine*, 25(1), 130–140.

MESHKANI, S. E., KOOSHAN, N., MOGHADAM, A. B., FALANJI, F., ADLI, A., BAGHBANI-ARANI, F. ET AL. (2019). Signaling roadmap to epithelial-mesenchymal transition in pterygium, TWIST1 centralized. *Journal of cellular physiology*, 234(10), 18146–18155.

MIKI, H., & TAKENAWA, T. (2003). Regulation of actin dynamics by WASP family proteins. *Journal of biochemistry*, 134(3), 309–313.

MORAN, D. J., & HOLLOWS, F. C. (1984). Pterygium and ultraviolet radiation: a positive correlation. *The British journal of ophthalmology*, 68(5), 343–346.

NEJIMA, R., MASUDA, A., MINAMI, K., MORI, Y., HASEGAWA, Y., & MIYATA, K. (2015). Topographic changes after excision surgery of primary pterygia and the effect of pterygium size on topograpic restoration. *Eye & contact lens*, 41(1), 58–63.

NOLAN, T. M., DIGIROLAMO, N., SACHDEV, N. H., HAMPARTZOUMIAN, T., CORONEO, M. T., & WAKEFIELD, D. (2003). The role of ultraviolet irradiation and heparin-binding epidermal growth factor-like growth factor in the pathogenesis of pterygium. *The American journal of pathology*, 162(2), 567–574.

NUHOGLU, F., TURNA, F., UYAR, M., OZDEMIR, F. E., & ELTUTAR, K. (2013). Is There a Relation between Histopathologic Characteristics of Pterygium and Recurrence Rates. *European Journal of Ophthalmology*, 23(3), 303–308.

OCHS, H. D., & THRASHER, A. J. (2006). The Wiskott-Aldrich syndrome. *The Journal of allergy and clinical immunology*, 117(4), 725–739.

LIU, T., LIU, Y., XIE, L., HE & JI BAI X. (2013). Progress in the Pathogenesis of Pterygium. *Current Eye Research*, 38:12, 1191-1197.

LIU, L., WU, J., GENG, J., YUAN, Z., & HUANG, D. (2013). Geographical prevalence and risk factors for pterygium: a systematic review and meta-analysis. *BMJ open*, 3(11), e003787.

ORANGE, J. S., ROY-GHANTA, S., MACE, E. M., MARU, S., RAK, G. D., SANBORN, K. B. ET AL. (2011). IL-2 induces a WAVE2-dependent pathway for actin reorganization that enables WASp-independent human NK cell function. *The Journal of clinical investigation*, 121(4), 1535–1548.

OZTURK, B., YILDIRIM, M., ZAMANI, A. ET AL. (2017). K-ras oncogene mutation in pterygium. *Eye* 31, 491–498.

PARK, S. J., KIM, Y. T., & JEON, Y. J. (2012). Antioxidant dieckol downregulates the Rac1/ROS signaling pathway and inhibits Wiskott-Aldrich syndrome protein (WASP)-family verprolin-homologous protein 2 (WAVE2)-mediated invasive migration of B16 mouse melanoma cells. *Molecules and cells*, 33(4), 363–369.

PEIRETTI, E., DESSÌ, S., MULAS, C., ABETE, C., NORFO, C., PUTZOLU, M., & FOSSARELLO, M. (2007). Modulation of cholesterol homeostasis by antiproliferative drugs in human pterygium fibroblasts. *Investigative ophthalmology & visual science*, 48(8), 3450–3458.

PEIRETTI, E., MANDAS, A., COCCO, P., NORFO, C., ABETE, C. ET AL. (2010). Glucose-6-phosphate-dehydrogenase Deficiency as a Risk Factor for Pterygium. *Investigative Ophthalmology & Visual*, Vol.51, 2928-2935.

PUJOL, P., JULIO, G., DE CARVALHO, A. M., KOSTOV, B., & MARTÍ-HUGUET, T. (2014). Threshold to predict astigmatism reduction after pterygium excision.

Optometry and vision science : official publication of the American Academy of Optometry, 91(7), 747–751.

PRAJNA, N. V., DEVI, L., SEENIRAJ, S. K., & KEENAN, J. D. (2016). Conjunctival Autograft Versus Amniotic Membrane Transplantation After Double Pterygium Excision: A Randomized Trial. *Cornea*, 35(6), 823–826.

REISMAN, D., MCFADDEN, J. W., & LU, G. (2004). Loss of heterozygosity and p53 expression in Pterygium. *Cancer letters*, 206(1), 77–83.

REZVAN, F., KHABAZKHOOB, M., HOOSHMAND, E., YEKTA, A., SAATCHI, M., HASHEMI, H. (2018). Prevalence and risk factors of pterygium: a systematic review and meta-analysis. *Survey of Ophthalmology*, 63(5), 719-735.

ROMANO, V., STEGER, B., KOVACOVA, A., KAYE, S. B., & WILLOUGHBY, C. E. (2016). Further evidence for heredity of pterygium. *Ophthalmic genetics*, 37(4), 434–436.

SADHUKHAN, S., SARKAR, K., TAYLOR, M., CANDOTTI, F., & VYAS, Y. M. (2014). Nuclear role of WASp in gene transcription is uncoupled from its ARP2/3-dependent cytoplasmic role in actin polymerization. *Journal of immunology* (Baltimore, Md. : 1950), 193(1), 150–160.

SASAHARA, Y., KAWAI, S., KUMAKI, S., OHASHI, Y., MINEGISHI, M., & TSUCHIYA, S. (2000). Novel mutations, no detectable mRNA and familial genetic analysis of the Wiskott-Aldrich syndrome protein gene in six Japanese patients with Wiskott-Aldrich syndrome. *European journal of pediatrics*, 159(1-2), 23–30.

SEET, L. F., TONG, L., SU, R., & WONG, T. T. (2012). Involvement of SPARC and MMP-3 in the pathogenesis of human pterygium. *Investigative ophthalmology & visual science*, 53(2), 587–595.

SHEN, G., WHITTINGTON, A., & WANG, P. (2011). Wsp1, a GBD/CRIB domain-containing WASP homolog, is required for growth, morphogenesis, and virulence of Cryptococcus neoformans. *Eukaryotic cell*, 10(4), 521–529.

SPANDIDOS, D. A., SOURVINOS, G., KIARIS, H., & TSAMPARLAKIS, J. (1997). Microsatellite instability and loss of heterozygosity in human pterygia. *The British journal of ophthalmology*, 81(6), 493–496.

SUN, X., WEI, Y., LEE, P. P., REN, B., & LIU, C. (2019). The role of WASp in T cells and B cells. *Cellular immunology*, 341, 103919.

TAN, D. T., TANG, W. Y., LIU, Y. P., GOH, H. S., & SMITH, D. R. (2000). Apoptosis and apoptosis related gene expression in normal conjunctiva and pterygium. *The British journal of ophthalmology*, 84(2), 212–216.

TSAI, C. B., HSIA, N. Y., WANG, Y. C., WANG, Z. H., CHIN, Y. T., HUANG, T. L.ET AL. (2020). The Significant Association of MMP-1 Genotypes With Taiwan Pterygium. *Anticancer research*, 40(2), 703–707.

TSAI, Y. Y., CHANG, C. C., CHIANG, C. C., YEH, K. T., CHEN, P. L., CHANG, C. H. ET AL. (2009). HPV infection and p53 inactivation in pterygium. *Molecular vision*, 15, 1092–1097.

TSAI, Y. Y., CHENG, Y. W., LEE, H., TSAI, F. J., TSENG, S. H., & CHANG, K. C. (2005). P53 gene mutation spectrum and the relationship between gene mutation and protein levels in pterygium. *Molecular vision*, 11, 50–55.

TURAN, M., & TURAN, G. (2020). Bcl-2, p53, and Ki-67 expression in pterygium and normal conjunctiva and their relationship with pterygium recurrence. *European journal of ophthalmology*, 30(6), 1232–1237.

YUAN, C. C., PETERSON, R. J., WANG, C. D., GOODSAID, F., & WATERS, D. J. (2000). 5' Nuclease assays for the loci CCR5-+/Delta32, CCR2-V64I, and SDF1-G801A related to pathogenesis of AIDS. *Clinical chemistry*, 46(1), 24–30.

UÇAKHAN, O. O., KANPOLAT, A., ELGÜN, S., & DURAK, I. (2009). The role of oxidative mechanisms in the etiopathogenesis of pterygium: a preliminary study. Ophthalmologica. Journal international d'ophtalmologie. International journal of ophthalmology. *Zeitschrift fur Augenheilkunde*, 223(1), 41–46.

WANZELER, A., BARBOSA, I., DUARTE, B., BORGES, D., BARBOSA, E. B., KAMIJI, D., HUARACHI, D., MELO, M. B., & ALVES, M. (2019). Mechanisms and biomarker candidates in pterygium development. *Arquivos brasileiros de oftalmologia*, 82(6), 528–536.

WEINSTEIN, O., ROSENTHAL, G., ZIRKIN, H., MONOS, T., LIFSHITZ, T., & ARGOV, S. (2002). Overexpression of p53 tumor suppressor gene in pterygia. *Eye (London, England)*, 16(5), 619–621.

WHIRST, L., MBBS., MD., MPH., DO., FRACO., FRACS. (2003). The Treatment Of Pterygium. *Ophthalmology*, 48(2), 145-180.

WU, C. W., PENG, M. L., YEH, K. T., TSAI, Y. Y., CHIANG, C. C., & CHENG, Y. W. (2016). Inactivation of p53 in pterygium influence miR-200a expression resulting in ZEB1/ZEB2 up-regulation and EMT processing. *Experimental eye research*, 146, 206–211.

YE, J., SONG, Y., KANG, S. ET AL. (2004). Involvement of bone marrow-derived stem and progenitor cells in the pathogenesis of pterygium. *Eye* 18, 839–843.

ZHOU, W. P., ZHU, Y. F., ZHANG, B., QIU, W. Y., & YAO, Y. F. (2016). The role of ultraviolet radiation in the pathogenesis of pterygia (Review). *Molecular medicine reports*, 14(1), 3–15.

ZHU, Q., WATANABE, C., LIU, T., HOLLENBAUGH, D., BLAESE, R. M., KANNER, S. B., ARUFFO, A., & OCHS, H. D. (1997). Wiskott-Aldrich syndrome/X-linked thrombocytopenia: WASP gene mutations, protein expression, and phenotype. *Blood*, 90(7), 2680–2689.