Elastin Development-Associated Extracellular Matrix Constituents of Subepithelial Connective Tissue in Human Pterygium

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PURPOSE. We evaluated the expression of several extracellular matrix constituents implicated in the synthesis and reticulation of elastin in human pterygium, according to age and sex of the patients.

METHODS. Pterygia and normal conjunctiva samples were divided into groups according to age (<50/≥50 years) and sex. Tissue was subjected to immunohistochemical staining with anti-lysyl oxidase (LOX), lysyl oxidase-like 1 (LOXL-1), fibulin (FBLN)-5 and FBLN4, and fibrillin-1 (FBN1) antibodies. Specific primers for the same constituents were used in a quantitative real-time PCR (qRT-PCR) analysis to determine gene expression.

RESULTS. The LOXL-1 (P = 0.0002), FBLN5 (P = 0.0035), and FBN1 (P < 0.0001) mRNAs were significantly higher in pterygium than conjunctiva. No differences were found for LOX and FBLN4 mRNA. The expression of LOXL-1 was not affected by age or sex; however, pterygium from men and patients over 50 years old exhibited significantly higher FBLN5/FBN1 expression than did controls. The LOX gene expression was higher in the pathologic samples from the over 50-year-olds compared to the conjunctiva (P = 0.0396) and in men’s versus women’s pterygium (P = 0.0173). In general, the levels of LOX, LOXL-1, and FBLN5 decreased with age in healthy samples, while the pathology seemed to have increased expression of the three proteins, and even more so in older patients. The FBLN4 and FBN1 immunostaining was slight in all samples, displaying no differences between groups.

CONCLUSIONS. Several extracellular matrix constituents, LOXs, FBN1, and FBLN5, implicated in the development of elastin, are overexpressed in the subepithelial connective tissue extracellular matrix of human pterygium, supporting our hypothesis that elastic synthesis and reticulation is dysregulated in this type of pathology.

Keywords: pterygium, extracellular matrix, elastin

Pterygium, a wing-shaped, epithelial-covered fibrovascular lesion that originates from the limbus,1 involves cell proliferation, migration, angiogenesis, fibrosis, and extracellular matrix remodeling.2–4 Although the exact pathologic mechanisms underlying pterygium are uncertain, it is believed that it originates from limbal stem cells altered by chronic ultraviolet irradiation exposure with subsequent conjunctivalization of the cornea.5,5 Visual impairment can result from astigmatism and blockage of the visual axis induced by the lesion migrating toward the central cornea. Surgical excision is the definitive management of pterygium, usually with low recurrence rates and excellent cosmetic results.6,7

Histologically, a pterygium is characterized by an atrophic conjunctival epithelium and a highly vascularized mass of hypertrophic and elastotic degenerated connective tissue.8 The subepithelial connective tissue of the pterygium shows elastodysplasia (immature formation of elastic fibers) as well as elastodystrophy (degenerative changes in elastic fibers and formation of electrodense inclusions).9 Supporting this description, our preliminary investigations have shown that the pathogenesis of pterygium involves dysregulation of the elastin metabolism, since tropoelastin, fibulin (FBLN)-2, and fibrillin-3 are overexpressed in subepithelial connective tissue.10

Collagen and elastin, the major components of the extracellular matrix (ECM), are intrinsic indicators of physiologic and pathologic states. Elastin is a highly insoluble protein component of the ECM elastic fibers that provide elasticity and resilience to tissues that must deform reversibly. In adult wound healing, elastin is severely deficient and only a disorganized elastic fiber network is present after scar formation. Elastin imparts recoil and resistance, and induces a range of cell activities, including cell migration and proliferation, matrix synthesis, and protease production.11

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Elastin Constituents in Human Pterygium

Elastic fiber formation is a complex process. This protein is formed through the reticulation of tropoelastin monomers over a fibrillin-rich microfibril template. Lysyl oxidases (LOXs) are extracellular amine oxidase copper enzymes whose primary function is to catalyze the covalent cross-linking of collagen and elastin fibers in the ECM of connective tissues. Secreted from fibrogenic cells as a N-glycosylated pro-enzyme, LOXs are processed in the extracellular matrix to produce the active, mature copper-dependent enzyme that can catalyze aldehyde formation from lysine residues in collagen and elastin precursors. This results in cross-linking, which is essential for stabilization of collagen fibrils and for the integrity and functional elasticity of mature elastin. On the other hand, LOXs also are involved in tumoral (growth, migration, and invasion) and nontumoral processes, like cell adhesion, gene regulation, and differentiation of several cell types.

The LOX gene family has five members, coding for LOX itself and for the lysyl oxidase-like 1-4 (LOXL) proteins. The LOXL sequences are closely similar to mature LOX except in the LOX peptide region. The LOX downregulation is associated with connective tissue disorders, like Ehler-Danlos syndrome and cutis laxa. The LOXL1 seems to be specifically required for tropoelastin (precursor of elastin) cross-linking and has been shown to be involved in elastic fiber formation, maintenance, and remodeling, particularly during dynamic processes, such as tissue injury, fibrosis, cancer, and development.

Fibulin-1 and FBLN5 are extracellular glycoproteins with essential roles in elastic fiber assembly. Both proteins are able to bind tropoelastin and fibrillin-1 (FBN1), the most important structural component of microfibrils, supporting a model in which these fibrils direct elastin deposition on microfibrils (Fig. 1). Some investigators have reported that strong binding between FBN4 and LOX enhances the interaction of FBN4 with tropoelastin, forming ternary complexes that may direct elastin crosslinking. In contrast, FBLN5 did not bind LOX strongly, although it did bind tropoelastin in terminal and central regions and could concurrently bind FBLN4. Both fibrils differentially bound FBN1, which strongly inhibited their binding to LOX and tropoelastin.

Fibulin-5 seems to be highly critical for elastogenesis, as it serves as an organizing molecule for some of the components of elastic fibers. Fibulin-5 deposits microfibrils, promotes aggregation of tropoelastin molecules through coacervation, and also interacts not only with LOXL-1, but also with LOXL-2 and LOXL-4, so it may tether these enzymes to microfibrils and subsequently facilitate aggregation and crosslinking of elastin itself to the microfibrils.

The formation of collagen/elastin crosslinks by LOXs leads to increased tensile strength and structural integrity, and is essential for normal connective tissue function, embryonic development, and wound healing. Consequently, aberrant LOX expression or enzymatic activity lead to disease. Increases in LOX expression contribute to the development of fibrotic diseases, such as arteriosclerosis, scleroderma, and liver cirrhosis, diseases that involve connective tissue remodeling. Unfortunately, very little information is available on LOX in human ocular tissues, whether in normal or pathologic conditions. Coral et al. have reported decreased LOX activity in the vitreous of proliferative diabetic retinopathy and rhegmatogenous retinal detachment, contributing to the inadequate collagen cross-linking that causes the ECM changes found in these diseases. Other studies have demonstrated a dysregulated expression and involvement of LOXL-1 in the formation of the aberrant fibrillar aggregates in ocular tissues of pseudoexfoliation syndrome and glaucoma eyes.

However, though LOXs have an important role in elastic fiber stabilization, to our knowledge they have never been examined in the context of pterygium pathogenesis. In this context, the current study is focused on evaluating the expression of several ECM constituents implicated in the synthesis and reticulation of elastin in the subepithelial connective tissue of human pterygium, including LOXs, FBLN4, FBLN5, and FBN1, with consideration taken of the subject’s age and sex.

METHODS

Patients

Primary nasal pterygia and conjunctiva specimens were divided in groups according to age (<50 and ≥50 years) and sex of the patients. The study protocol was approved by the Ethics Committee of the University Hospital Príncipe de Asturias and conformed to the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants. The inclusion criteria were: primary fleshy and active pterygium that invaded the cornea by more than 3 mm, no previous traumas or surgery in the affected eye, and no ocular or systemic diseases, such as diabetes, vascular disease, or reactive connective tissue diseases.

For immunohistochemical studies, specimens from 40 patients were included in the study. Control specimens (n = 20) were obtained from normal conjunctiva from subjects with a mean age ± SD of 57.15 ± 17.37 years and specimens for the pterygium group (n = 20) from patients with a mean age of 50.75 ± 17.30 years. Subjects were divided into groups according to sex and age.

For quantitative real-time polymerase chain reaction assay, 24 different patients were included in the study. Mean age was 47.35 ± 14.65 and 47.25 ± 12.93 years in the control (n = 12) and pterygium (n = 12) groups, respectively.

Immediately after harvesting the tissue samples, the normal conjunctiva and pterygia specimens were placed in sterile minimal essential culture medium and transferred at 4°C to the laboratory. The tissue was fixed in Bouin solution and embedded in paraffin for immunohistochemical studies or frozen at −80°C until used for RNA extraction.

Surgical Technique

Pterygium excision was combined with application of intraoperative mitomycin C (MMC) in all cases. Conjunctival anesthesia was induced by instillation of tetracaine chlorhydrate eye drops (Colirio Anestésico Doble, Alconcusi, Barcelona, Spain) with additional subconjunctival injection of 0.5 mL of 2% lidocaine with 1:200,000 epinephrine into the body of the pterygium using a 25-gauge needle. The pterygium was dissected from the cornea with a Bard-Parker 15 surgical blade and the body of the pterygium, including the adjacent Tenon capsule, was dissected and excised with Westcott scissors, leaving the bare sclera exposed. Then, MMC 0.02% (0.2 mg/
ml) was applied directly to the scleral bed for 1 minute and the ocular surface was washed copiously with a balanced salt solution. The conjunctival sutures (7-0 Vicryl; Ethicon, Inc., Somerville, NJ, USA) were removed 10 days after surgery. Postoperatively, topical tobramycin and dexamethasone eye drops (Tobrex; Alcon Cusi, Barcelona, Spain) were instilled four times daily for 1 month. Specimens of conjunctiva were removed from normal superior bulbar conjunctiva, at least to 5 mm from the sclera-corneal limbus, in subjects that underwent cataract surgery.

**Immunohistochemistry**

The sections were deparaffined, hydrated, and equilibrated in PBS (pH 7.4). Rabbit polyclonal anti-LOX and LOXL-1 antibodies (donated by Pascal Sommer), rabbit polyclonal anti-FBLN4 and FBN1, and mouse monoclonal anti-FBLN5 (Abcam, Cambridge, UK), were used as primary antibodies. For LOX and LOXL-1 antibodies, the antigen-antibody reaction was detected by a peroxidase-labeled avidin-biotin procedure. The chromogenic substrate contained diamobenzidine (DAB). For fibulins and fibrillin antibodies, the alkaline phosphatase procedure was performed. The chromogenic substrate contained 2-napthol and fast-red. Nuclei were counterstained with Carazzi’s hematoxylin. After immunostaining, the tissue sections were examined by light microscopy. A negative control of the technique was performed without the primary antibody.

Immunohistochemical staining was scored on each of the samples using the following scale. Results have been expressed as follows: 1, minimum staining (0%-25%); 2, moderate staining (25%-50%); 3, strong staining (50%-75%); and 4, maximum staining (75%-100%). Staining intensity was scored by 2 independent observers in a blinded fashion. Despite the score on each of the samples using these four-groups scale, to perform a more reliable statistical analysis of the different protein expression, the intensity of staining was joined in only two categories: (1–2) < 50% and (3–4) > 50%. A contingency Table with all the patients and the expression of each of the different antibodies for each patient has been included. Data analysis was performed using the SPSS (12th version) program (SPSS, Inc., Chicago, IL, USA). Data were compared among groups using the 2-tailed Fisher’s exact probability test. The level of significance was set at $P < 0.05$.

**Quantitative Real-Time PCR (qRT-PCR)**

Total RNA isolated using Trizol (Invitrogen, Carlsbad, CA, USA), and cDNA synthesis by reverse transcription (RT) with M-MLV reverse transcriptase enzyme (Invitrogen) were done as described previously.29 To detect genomic DNA contamination, another RT was done without the enzyme. To quantify mRNA expression, qRT-PCR was performed with iQ SYBR Green Supermix following the manufacturer’s instructions (Bio-Rad Laboratories, Hercules, CA, USA) in a StepOnePlus Real-Time PCR System instrument (Applied Biosystems, Carlsbad, CA, USA). Quantification was done running a standard curve experiment on the instrument. A negative control with ultraPure distilled DNase-, RNase-free water (Invitrogen) was added in each reaction. The following specific human primers were amplified: LOX, 5'-GCA GAT GTC AGA GAT TAT GAT CA-3' (sense) and 5'-ATC GCC TGT GGT AGC CAT AGT AGT-3' (antisense); LOXL-1, 5'-GCA CCT CTC ATA CCC AGG GC-3' and 5'-TGG CAG TCG ATG TCG CCG CAT-3'; FBLN5, 5'-GTC TGG GAC ATG CCA GGA ATA A3' (sense) and 5'-TGG AGA TGG TGG GAG TGG CAG GGT ACG AGT-3' (antisense); FBN1, 5'-GTT GAA TGG ACA AAG ACA CAC-3' (antisense); and GAPDH, 5'-GCA GCC CAT GGA AGT TGA-3' (sense) and 5'-GTC ATT GAT GGC AAC AAT ATC CAC T-3' (antisense). The thermal cycling conditions were an initial stage at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds, 60°C (each primer) for 30 seconds, and 72°C for 1 minute. Products were subjected to 2% agarose gel electrophoresis and visualized with UV light. Gene expression was normalized against the expression of the constitutive gene glyceraldehyde 3-phosphate-dehydrogenase (GAPDH).

All data are expressed as the means±SD. Data analysis was performed using the GraphPad Prism 5 package (GraphPad Software Inc., La Jolla, CA, USA). Mean data were compared among groups using the Mann-Whitney U test. The level of significance was set at $P < 0.05$.

### RESULTS

**LOXL-1 Gene and Protein Expression**

In general, before considering patient age and sex, a significant increase ($P = 0.0002$) in LOXL-1 mRNA was observed in the pterygium samples compared to the healthy conjunctiva. The age and sex factors of the population did not affect LOXL-1 gene expression. The mRNA expression remained high in the pathologic group than in the conjunctiva from healthy older patients. At the protein level, again independently of age and sex, LOXL-1 immunostaining was stronger in the pathologic population compared to the controls when groups were separated by age or sex (Fig. 2).

At the protein level, again independently of age and sex, LOXL-1 immunostaining was stronger in the pathologic population. All the pterygium samples ($n = 20$) exhibited labeling between 50% and 100% (score 3–4) for LOXL-1, compared to 12 samples that showed this expression in the control group (see Table). Fisher’s exact probability test gave a $P$ value of 0.003.

Expression for this protein was higher in the conjunctiva of young patients than in those over 50 years old. Within the older age group, the disease significantly increased the expression of the protein (Fig. 2).

**LOX Gene and Protein Expression**

No significant differences were found in LOX mRNA levels between control conjunctiva and pterygium. When divided by age, LOX gene expression was higher ($P = 0.0396$) in the older pathologic group than in the conjunctiva from healthy older patients. Within the pathologic population, women showed...
significantly lower ($P = 0.0173$) messenger levels than men (Fig. 3).

However, when LOX protein expression was analyzed we observed that regardless of age and sex, LOX immunostaining was increased in the pathologic population. A total of 19 pterygium samples exhibited staining between 50% and 100% (score 3–4) for LOXL-1, compared to only seven samples that showed this expression in the control group (see Table). Fisher’s exact probability test gave a $P$ value $< 0.001$.

The LOX protein levels decreased with increasing age in the healthy group. Healthy groups over 50 years of age of both sexes exhibited a very light labeling for this protein. Similarly to what has just been seen with LOXL-1 protein expression, the disease significantly increased LOX expression in the connective tissue in the older age group (Fig. 3).

**FBLN5** **Gene and Protein Expression**

In general, FBLN5 mRNA levels were significantly higher ($P = 0.0035$) in the pathologic population. When age and sex were taken into account, only men ($P = 0.0061$) and patients $\geq 50$ years old ($P = 0.0025$) showed a significant increase in the pathologic populations (Fig. 4).

In general, FBLN5 showed increased protein expression in pterygia compared to the healthy tissue. A total of 16 pterygium samples exhibited strong to maximum labeling, between 50% and 100% (score 3–4) for FBLN5, compared to only five samples that exhibited this staining in the control and 15 control samples that exhibited minimum to moderate staining, between 0% and 50% (score 1–2, see Table). Fisher’s exact probability test gave a $P$ value of 0.001.

The conjunctiva showed decreased protein expression with age, something that did not occur in the pterygium. Gene expression corresponded with the protein levels found in the different groups (Fig. 4).

**FBLN4** **Gene and Protein Expression**

No differences were found in **FBLN4** gene expression and all groups showed similar values for mRNA (Fig. 5).

No differences in FBLN4 were observed for protein expression and all the different groups showed similar staining in the subepithelial connective tissue samples, very low in most cases. All the samples ($n = 20$) in control and pathologic groups displayed between 0% and 50% labeling (score 1–2) for FBLN4 (see Table). Performing the statistical test was not appropriate in the case of FBLN4.

**FBN1** **Gene and Protein Expression**

Interestingly, **FBN1** showed the same pattern of gene expression as **FBLN5**. In general **FBN1** showed a significant increase ($P < 0.0001$) in mRNA in the pathologic population. When age and sex were taken into account, only male patients ($P = 0.0025$) and patients $\geq 50$ years ($P = 0.0079$) showed a
significant increase of FBN1 mRNA in the pathologic populations (Fig. 6).

Protein levels were very low in all the different samples, 19 conjunctiva samples and 12 pterygium samples showed expression levels between 0% and 50% (score 1–2). However, we could observe a slight increase for FBN1 labeling in the extracellular matrix of all the pathologic groups, since 8 pathologic samples showed score 3–4 staining (>50%) for fibrillin-1 expression (see Table). Fisher’s exact probability test gave a \( P \) value of 0.02.

**DISCUSSION**

Pterygium is an ocular disease involving fibrovascular growth on the ocular surface with corneal tissue destruction, matrix alteration and chronic inflammation. It is characterized by elastotic degeneration of collagen, fibrovascular proliferation and accumulation of abnormal elastin. It most frequently invades the nasal limbus with an advancing portion called the head of the pterygium, which is connected to the main body of the pterygium by a neck.\(^{36}\)

Many factors,\(^{4}\) like chronic irritation or inflammation, are involved in pterygium development; however, chronic exposure to sunlight, especially UV irradiation, is considered to be the main cause of pterygia.\(^{37}\) It already has been shown that high exposure to this irradiation causes increased elastin levels in different tissues.\(^{37}\) In fact, some investigators have reported increased elastin mRNA levels and enhanced elastin promoter activity in sun-damaged skin,\(^{38}\) as demonstrated by transient transfections with a human elastin promoter/chloramphenicol acetyltransferase construct. This result correlates with our previous studies on pterygium,\(^{10}\) in which the accumulation of abnormal elastic fibers was the same as that reported in solar elastosis in photodamaged skin.\(^{37}\) Some investigators showed that there is a 4-fold increase of desmosine, a particular amino acid found in elastin, in sun-exposed skin compared to nonexposed skin in the same individual, suggesting that elastin is a major component of the extracellular matrix in sun-exposed dermis.\(^{39}\) On the contrary, other studies have shown that the elastin overexpression results from a posttranscriptional mechanism rather than increased mRNA synthesis, possibly due to mutations in the 3’ untranslated region, but not in the coding sequence of tropoelastin mRNA, which could result in errors of DNA polymerase activity and produce the massive accumulation of abnormal elastic fibers.\(^{37,40}\) Furthermore, DNA modifications in gene promoters that cause transcriptional repression by directly interfering in the binding of transcription factors to DNA or DNA methylations that attract inhibitory proteins that block access to the factors responsible for induction of the gene, within the promoter-rich cytosine guanine dinucleotide (CpG) island, could be involved.\(^{41}\) Some studies have shown that the pathogenesis of pterygium may be related to the methylated state of pivotal wound healing and developmental genes.\(^{42}\)

There is very little literature analyzing the elastic component in pterygium pathogenesis. Wang et al.\(^{37}\) reported, in agreement with our previous report,\(^{10}\) high tropoelastin protein expression in pterygium when using an immune histochemical staining technique. However, using UV-irradi-
ed fibroblasts from pterygium, they showed that this increase, rather than the result of an increase in mRNA levels, was the result of posttranscriptional modification of tropoelastin. Our previous investigations have found clear indications of elastodysplasia and the abnormal synthesis and maturation of elastic fiber precursors. We found that the elastin metabolism is dysregulated in the pathogenesis of human pterygium with tropoelastin, fibulin-2, and fibulin-3 overexpression in the subepithelial connective tissue. However, despite increased tropoelastin levels, pterygium are clinically inelastic, so the tissue has lost some of its functionality, contributing to pathologies, such as astigmatism induced by several mechanisms, such as pooling of the tear film at the leading edge of the pterygium or the mechanical traction exerted by the pterygium on the cornea.

These interesting results and the lack of studies regarding extracellular matrix involvement in pterygium, led us to further investigate the role of the elastic component in this type of pathology. The LOXs are considered to be the main enzymes involved in collagen or elastin crosslinking in the extracellular matrix and FBLN4/5 are some of the most important extracellular matrix proteins able to direct elastin deposition on microfibrils, but they have never been studied in the context of this disease to our knowledge.

In the literature on eye diseases related with the elastic component, the research group of Schlötzer-Schrehardt et al. have reported pseudoexfoliation syndrome eyes more vulnerable to pressure-induced optic nerve damage, and glaucoma development and progression. Other genetic studies in multiple populations have convincingly identified the \( \text{LOXL-1} \) gene as a principal contributor to the risk of developing pseudoexfoliation syndrome and pseudoexfoliation glaucoma.

Regarding pterygium, a global gene expression analysis has shown distinct differences between primary pterygium and uninvolved conjunctiva. Among the upregulated genes were those coding for proteins involved in wound healing, “mitogenic proteins” and extracellular matrix components, including different types of collagens, LOXL-1, and several structural proteins. This is consistent with our RT-PCR studies showing a significant increase of LOXL-1 mRNA in the pathologic population that was matched to a corresponding translational level of protein overexpression. The microarray data John-Aryankalayil et al. identified many new genes that were either over- or underrepresented in pterygium. In particular, several cell adhesion/extracellular matrix proteins, and proteins involved in inflammation and malignant transformation had increased expression levels.

Another important calcium-dependent enzyme involved in extracellular matrix remodeling that exhibits crosslinking activity is transglutaminase 2 (TGM2). It is involved in crosslinking several collagen types and other extracellular proteins, like fibronectin. In corneal morphogenesis it recently has been reported that two types of covalent collagen cross-links formed by catalysis involving LOXs and TGM2 are essential. The TGM2 mRNA and protein have been shown to be downreg-
ulated in pterygium, indicating that this decreased activity could facilitate the migration of abnormal pterygium tissue toward the central cornea.42

Among other eye diseases related to the elastic component, ectopia lentis and myopia are frequently observed ocular manifestations of Marfan syndrome, which involves common defects in FBN1 microfibrils causing a wide spectrum of disorders. Glaucoma also is associated with this syndrome, though the form of this pathology has not been well-characterized.49 In our present study, FBN1 levels were increased in the pterygium tissue with respect to the normal conjunctiva at the transcriptional level, but only very slight differences were observed at the protein level, possibly indicating degradation of the messenger or alterations at the translational level.

Fibulins are a family of extracellular glycoproteins that also have very important roles in elastic fiber assembly.13 Fibulins participate in diverse supramolecular structures with binding sites for several proteins, including tropoelastin, fibrillin, and proteoglycans.50 Recent studies have revealed that FBLN4 and -5 are essential for elastic fiber formation.51,52 FBLN4 is widely expressed from early embryogenesis and is necessary for normal vascular, lung, and skin development, since mice that lack FBLN4 do not form elastic fibers and die perinatally. Lack of FBLN5 causes a less severe phenotype showing irregular and fragmented elastic fibers in skin, lungs, and aorta.26,52 Mutations in both molecules can cause cutis laxa, an inheritable disorder associated with elastic fiber degeneration leading to sagging skin, vascular tortuosity, and emphysematous lungs.53,54

Genetic linkage and molecular studies also have associated several fibulin genes with various inheritable human disorders that affect a wide range of organs, including the eye.55 Here, we have found FBLN5 overexpression at the messenger and protein level in pterygium pathogenesis. However, FBLN4 did not show any type of disturbance attributable to the studied pathology.

Other investigators have demonstrated that fibulins may be key proteins in supramolecular organization of the corneal stroma, affirming that these molecules and their binding partners, synthesized by corneal cells, surround fibroblasts with a tight matrix mesh that stabilizes corneal structure.56 Phenotypes previously associated with mutations in fibulin genes include Malattia Leventinese and Doyne honeycomb retinal dystrophy,55 as well as age-related macular degeneration (ARMD),57 which is the most common cause of irreversible vision loss. Variations in other fibulins (FBLN1, -2, -4, and -6) also were reported as specifically associated with ARMD patients.58

Li et al.57 showed that FBLN5 has a pleiotropic role in the pathology of ARMD. The FBLN5 may interfere with choroidal neovascularization by downregulating VEGF, CXCR4, and TGF-β1 expression, and inhibiting corneal endothelial cell (CEC) proliferation and invasion; thus, intensifying interest in FBLN5 as a target for therapeutic intervention in neovascular ARMD. Lotery et al.50 using two European cohorts of 805 ARMD patients and 279 controls, hypothesized that the missense mutations in FBLN5 may lead to susceptibility to ARMD due to reduced FBLN5 secretion.
In our case in pterygium pathology, LOXL-1 and FBLN5 mRNA were overexpressed and there was a correlation with the protein levels that also were increased in these samples. These results also agree with our previous work in which an increase in tropoelastin was observed at transcriptional and translational level. In the case of LOX, the messenger remained stable and only protein levels showed a significant increase over levels in the conjunctiva of patients with pathology. Related to this last result, we must remember that a selective role for LOXL-1 has been proposed in the elastin, but not collagen metabolism, based on desmosin and hydroxyprolin levels, which, respectively, represent elastin and collagen crosslinks. The authors of this study reported significantly lower desmosin levels in several tissues of a LOXL-1 mutant, whereas hydroxyprolin levels remained unchanged. This apparently shows that one of the major substrates for LOX is collagen I. However, LOXL-1, but not LOX, is specifically targeted to sites of elastogenesis, showing that LOXL-1 is closely related with the elastic lamina, whereas LOX is broadly distributed.

To summarize, we can say that in the subepithelial connective tissue of human pterygium, LOXL-1/FBLN5 mRNA were overexpressed and there was a correlation with the protein levels that also were increased in these samples. These results also agree with our previous work in which an increase in tropoelastin was observed at transcriptional and translational level. In the case of LOX, the messenger remained stable and only protein levels showed a significant increase over levels in the conjunctiva of patients with pathology. Related to this last result, we must remember that a selective role for LOXL-1 has been proposed in the elastin, but not collagen metabolism, based on desmosin and hydroxyprolin levels, which, respectively, represent elastin and collagen crosslinks. The authors of this study reported significantly lower desmosin levels in several tissues of a LOXL-1 mutant, whereas hydroxyprolin levels remained unchanged. This apparently shows that one of the major substrates for LOX is collagen I. However, LOXL-1, but not LOX, is specifically targeted to sites of elastogenesis, showing that LOXL-1 is closely related with the elastic lamina, whereas LOX is broadly distributed.

To summarize, we can say that in the subepithelial connective tissue of human pterygium, LOXL-1/FBLN5, and FBN1 mRNA expression is significantly increased in respect to levels in healthy conjunctiva, while the expression of LOX and FBLN4 is comparable to levels in controls. Overexpression of mRNA appears to be favored in older male patients. An increase in the age factor decreases LOX/LOXL-1 and FBLN5 protein expression in healthy tissue. The pathology results in an increased protein expression of the same constituents (LOX/LOXL-1 and FBLN5) that is more pronounced in the older age groups.

Our study is not exempt of some limitations and potential sources for variability, such as genetic and inter-individual patient differences, the limited number of patients or sample size limitations, and the reproducibility of methods. Continued research in this area is strongly recommended, since in our opinion the LOXs protein family should be considered an important target for the development of future therapies in treating diseases involving remodeling of extracellular matrix.

In conclusion, we can state that several extracellular matrix factors implicated in the assembly of elastin are overexpressed in human pterygium tissue, supporting the hypothesis of a dysregulation in the synthesis and reticulation of the elastic components in this type of pathology.

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