• Review •

The role of elastic fibers in pathogenesis of conjunctivochalasis

Jing-Yun Gan, Qing-Song Li, Zhen-Yong Zhang, Wei Zhang, Xing-Ru Zhang

Department of Ophthalmology, Putuo Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200062, China

Correspondence to: Xing-Ru Zhang. Department of Ophthalmology, Putuo Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200062, China. zhangxingru928@163.com

Received: 2016-11-22 Accepted: 2017-03-23

Abstract

• The PubMed, MEDLINE databases and China National Knowledge Infrastructure (CNKI) were searched for information regarding the etiology and pathogenesis of conjunctivochalasis (CCh) and the synthesis and degradation of elastic fibers. After analysis of the literature, we found elastic fibers was a complex protein molecule from the structure and composition; the degradation of elastic fibers was one of the histopathological features of the disease; the vast majority of the factors related to the pathogenesis of CCh ultimately pointed to abnormal elastic fibers. By reasonably speculating, we considered that abnormal elastic fibers cause the conjunctival relaxation. In conclusion, we hypothesize that elastic fibers play an important role in the pathogenesis of CCh. Studies on the mechanism of synthesis, degradation of elastic fibers are helpful to clarify the pathogenesis of the disease and to find effective treatment methods.

• KEYWORDS: elastic fibers; conjunctivochalasis; pathogenesis

DOI:10.18240/ijo.2017.09.21

Citation: Gan JY, Li QS, Zhang ZY, Zhang W, Zhang XR. The role of elastic fibers in pathogenesis of conjunctivochalasis. *Int J Ophthalmol* 2017;10(9):1465-1473

INTRODUCTION

Conjunctivochalasis (CCh), which was first proposed by Hughes[1] in 1942, defined as a redundant, loose, non-edematous inferior bulbar conjunctiva interposed between the globe and the lower eyelid, tends to be bilateral and is more prevalent in older populations. Extracellular matrix which locates in substratum of epithelial or endothelial cells, around connective tissue cells, provides mechanical support and physical strength to the integrity of the organization, organ, and even the whole body. Under the normal physiological conditions, elastic fibers are important components of extracellular matrix, and influence the tissue flexibility and elasticity.

In 1998, Meller and Tseng[2] proposed the hypothesis which states that mechanism of CCh is *via* accumulation of degrading enzymes which resulted in elastotic degeneration and collagenolysis of bulbar conjunctiva in the tears as a result of delayed tear clearance.

Although, a lot of research has been done on it, the precise etiology of CCh remains obscure. So we summarize the previous literatures and analyze the current situation on the hypothesis of elastic fibers.

MATERIALS AND METHODS

The following electronic databases were screened: PubMed, China National Knowledge Infrastructure (CNKI). The following search equation was used: "CCh (all fields)" OR "elastic tissue (MeSH terms)", "elastic tissue (MeSH terms)" AND "2011/1/1 (PDAT):2016/5/31 (PDAT)". This equation was adapted to the characteristics of each database. In addition we selected five newly published papers, using their references to search the original literature. The last search was on July 31, 2016.

SUMMARY OF ELASTIC FIBERS

Concept of Elastic Fibers Elastic fibers is a mainly connective tissue component of extracellular matrix, produced by fibroblast, smooth muscle cells, some chondrocytes, giving their organs, such as skin, lungs, arteries, ligamentum flavum, and auricle cartilage with good flexibility.

Microscopic Behavior of Elastic Fibers Under the light microscope, the elastic fibers is presented as a fine thread, and its branches are interwoven into a net, which is between 0.2 and 1 μm in diameter. Under electron microscope elastic fibers consist of an amorphous elastin core surrounded by a mantle of longitudinally aligned microfibrils in the mature state. Oxytalan is composed of 10-16 nm diameter microtubules that are aligned along the fiber length. Elauninis formed from two components, microtubules and amorphous material, which are considered to be an immature state of elastic fibers[3].

Component of Elastic Fibers The major component of elastic fibers is elastin, which is formed following the assembly and cross-linking of its soluble precursor, tropoelastin. Human tropoelastin is encoded by a single gene that possesses 34

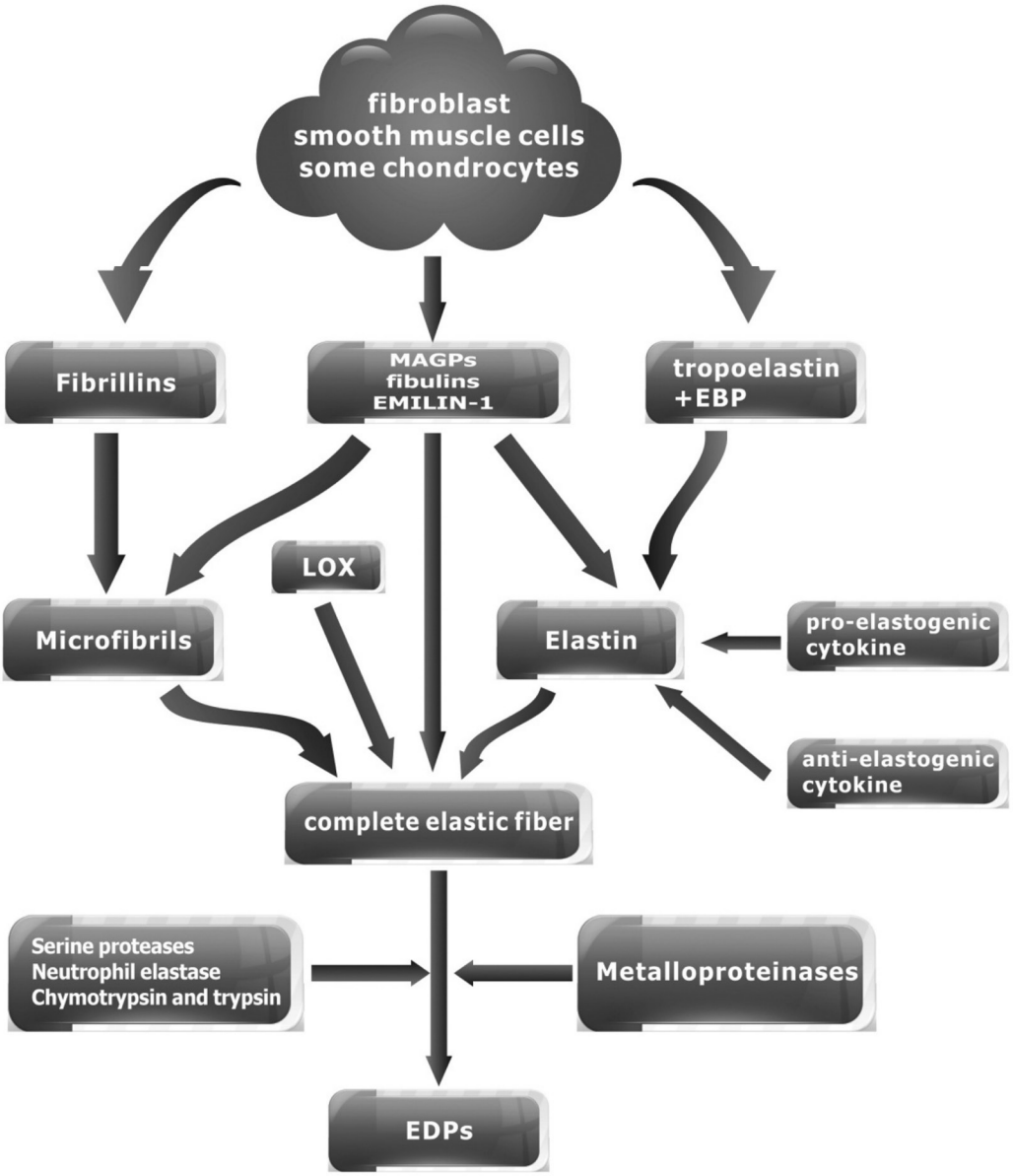

Figure 1 Summary of elastic fibers.

exons. The messenger ribonucleic acid (mRNA) encodes a polypeptide depending on the splicing pattern and removal of a signal peptide which leaves a mature protein (tropoelastin)[4]. Before it is released into the cell surface, elastin binding protein (EBP) can be specifically combined with tropoelastin to prevent it from polymerization and degradation of proteolytic enzymes in the cell[5].

The second component is visualized as small, 10-15 nm microfibrils that localize to the periphery of the fiber in adult tissues and have a more complex composition. The major structural element of microfibrils is contributed by the fibrillins[6]. Numerous other proteins associate with microfibrils or with elastin itself, including the microfibril associated glycoproteins (MAGPs), fibulins and elastin microfibril interface located protein-1 (EMILIN-1)[7]. Microfibrils is thought to provide scaffold that facilitates elastin molecular alignment and subsequent cross-linking, which is catalyzed by one or more members of the lysyl oxidase (LOX) gene family[8].

Assembly and Degradation of Elastic Fibers The formation process of the elastic fibers is not completely clear. Based on a large number of related studies, Wagenseil and Mechamt[9] proposed the model of elastic fibers assembly. First of all, tropoelastin is transported to assembly sites on the plasma membrane where it is organized into small aggregates that are cross-linked by a LOX. Secondly, the aggregates remain on the cell surface while newly secreted elastin is added to increase the size. The aggregates are then transferred to extracellular microfibrils, which interact with the cell through integrins. Thirdly, elastin aggregates on the microfibril coalesce into larger structures. At last, the elastin aggregates are further cross-linked by LOX to form the complete elastic fibers (Figure 1). Research shows that intact microfibrils are effectively catabolised *in vitro* by the serine proteases neutrophil elastase, chymotrypsin and trypsin[10]. In addition, fibrillin molecules and fibrillin-rich microfibrils are degraded by matrix metalloproteinases (MMP-2, MMP-3, MMP-9, MMP-12, MMP-13 and MMP-14) also[11].

Table 1 Histopathological changes of the CCh

Authors	Time	Country	Results	Staining	Sample sizes
Denti[15]	1930	Italy	Elastic tissue showed swelling, fragmentation, irregular course of the fibers	Weigert's elastic-tissue stain	Obscure
Hughes[1]	1942	USA	No fragments and other abnormalities of elastic fibers were found	Hematoxylin and eosin (H&E), Weigert's elastic-tissue strain	2, no control
Watanabe *et al*[16]	2004	Japan	Negligible inflammation and lymphocyte infiltration, elastic fibers fragmentation and sparsely assembled collagen fibers, 39/44 microscopic lymphangiectasia	Verhoeff-Van Gieson (VVG) staining	44, no control
Zhang *et al*[17]	2004	China	Hyperplasia of squamous epithelium with parakeratosis, pigmentation in basal cell, hemorrhage and edema of stroma, infiltration of lymphocyte and plasmocyte, decreased elastic fiber layer	H&E, VVG staining, Masson trichrome staining, Mallory phosphotungstic hematoxylin stain	17 patients and 15 cataract
Francis *et al*[18]	2005	Australia	Of 4 specimens (13.8%) had a chronic non-granulomatous inflammation and 3 specimens (10.3%) demonstrated elastosis	H&E staining, periodic acid Schiff, VVG staining	29 patients and 24 cataract
Ward *et al*[19]	2010	Japan	Decreased intercellular cohesiveness with an accumulation of elastic fibers in conjunctival stroma	VVG staining	20 patients and 22 control
Park *et al*[20]	2011	Korea	Decrease of collagen density, elastic degeneration, lymphangiectasia in conjunctiva	VVG staining, trichromestain, H&E staining	27, no control
Zhang *et al*[21]	2013	China	Elastic fibers decreased and melt of collagen fibers in lamina propria, subconjunctival mild chronic inflammatory cell infiltration, visible dilated lymphatics	H&E staining, VVG staining, Masson trichromestaining, Mallory phosphotungstic hematoxylin stain	11, no control
Bae and Park[22]	2013	Korea	Dilated lymphatic vessels, decreased goblet cell and collagen densities, degeneration of elastic fibers	H&E staining, VVG elastic staining	14, no control
Dong *et al*[23]	2014	China	Squamous epithelial hyperplasia, slight hyperkeratosis of some superficial cells, pigment calm of basal cells, lamina propria vascular congestion and expansion, interstitial infiltration of lymphoid and plasma cells	H&E staining	20 patients and 22 control
Kantaputra *et al*[24]	2014	Thailand	Hyperplasia of the conjunctival epithelium with subepithelial conjunctival mononuclear inflammatory cell infiltration, very few blood vessels with abnormal elastic fiber	Silver staining	1, no control
Yu *et al*[25]	2015	China	Obvious squamous epithelial hyperplasia, parakeratosis, basal cell pigmentation, lamina propria hemorrhage, infiltration of lymphocytes, and reduction of elastic fibers and collagen fibers	H&E staining, Masson's trichrome staining	83, no control

Elastolysis by MMPs occur in development, wound healing, and major inflammatory diseases. Elastin degradation by elastases generates elastin-derived peptides (EDPs), which are highly chemotactic and stimulating of inflammation, proliferation, and angiogenesis[12].

Cytokine Axis Regulates Elastin Formation and Degradation

The formation and degradation of elastin was regulated by the cytokine axis in which the pro-elastogenic activities of transforming growth factor β-1 (TGFβ1) and insulin-like growth factor-1 (IGF-1) are opposed by anti-elastogenic activities of basic fibroblast growth factor (bFGF/FGF-2), heparin-binding epidermal growth factor (HB-EGF)-like growth factor, epidermal growth factor (EGF)-like growth factor, platelet derived growth factor-BB (PDGF-BB), transforming growth factor-α (TGF-α), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and noncanonical TGFβ1 signaling[13]. It can be seen that the elastic fibers is a complex protein molecules.

DEGRADATION OF ELASTIC FIBERS IS A HISTOPATHOLOGICAL FEATURES OF THE CONJUNCTIVOCHALASIS

After reviewing the literature on the histopathology of the disease associated with conjunctival relaxation (Table 1). From Table 1, we conclude that the histopathological changes of the CCh are hyperplasia of squamous epithelium with parakeratosis, infiltration of Inflammatory cells, decreased collagen densities, degeneration of elastic fibers, dilated lymphatic vessels. In fact, the histopathologic data on CCh are conflicting. For example, one study showed that no significant difference in light microscopy findings between eyes with CCh and those of age-matched controls[14]. In spite of this, we can see that the degradation of elastic fibers is one of the histopathological features of the disease.

CAN THE DEGRADATION OF ELASTIN FIBERS CAUSE CONJUNCTIVOCHALASIS?

Analogical reasoning is a kind of common logic method. The

logical form of analogical reasoning is: A object and B object have attributes: a_1, a_2, …, a_n; A object yet has attributes: a_{n+1}; So, B object also has attributes: a_{n+1}.

Cutis laxa (CL) is characterized by a loose, redundant, hypoelastic skin. Typically, the skin in CL can easily be pulled away from underlying tissue and only slowly returns to its original position. Redundant skin is often most noticeable on the neck, hands, and groin, but can also be seen on the face, creating a premature aging appearance[26]. CCh is characterized by a redundant, loose, non-edematous inferior bulbar conjunctiva interposed between the globe and the lower eyelid. It can occur in the temporal, nasal, or middle of the conjunctiva.

The conjunctiva is a mucous membrane, which is composed of squamous epithelium and goblet cells. It is divided into the epithelial layer and the lamina propria, and the lamina propria is divided into the adenoid layer and the fiber layer. The epithelium of the bulbar conjunctiva is a flat type, about 2-5, and the fibrous layer is composed of collagen fibers and elastic fibers. On the view of skin embryology, skin is composed of epidermis, dermis and subcutaneous tissue and skin appendages. The dermis can be divided into papillary layer and reticular layer, which mainly consists of collagen fiber, elastic fiber and matrix. In terms of morphology, the fibrous layer of the bulbar conjunctiva is equivalent to the reticular layer of the dermis.

As mentioned before, the degradation of elastic fibers is one of the histopathological features of the CCh. In CL, microscopic findings include loss of elaunin and sparse, fragmented elastic fibers in the reticular dermis. All types of CL show some elastic abnormalities and no findings are specific for individual types of CL[26].

Markedly increased MMPs (MMP-1, MMP-2, MMP-3, MMP-9, MMP-12), tissue inhibitor of metalloproteinases-1 (TIMP-1) associated with the degradation of elastic fibers and alteration of collagen fibers were found in CL by immunohistochemistry[27]. Interestingly enough, the expression levels of MMP-1, MMP-3, TIMP-1 were higher in CCh than those in the control group, which were detected in the surgical specimen of the conjunctiva by ELISA[28].

There is no effective drug treatment for CL, and surgical excision is the main treatment method. Unlike persons with related connective tissue disorders, patients with CL generally heal well after surgery. But surgical treatment does not prevent the recurrence of skin relaxation, so patients often require repeated surgery[29]. Tamura reported botulinum toxin has been helpful in one case[30]. In the same way, surgical treatment is safe and effective for the treatment of CCh, and it is necessary to select appropriate method according to the condition of the patient and the classification of the disease. So far, the medicine treatment can alleviate the symptom only. For example, regular use of artificial tears can also improve the vision-related quality of life[31] and Pranoprofen Eye Drops can improve the patients with grade II CCh with epiphora symptoms[32].

It is now clearly that inherited forms of CL involves genetic defects which are elastin, fibulin (FBLN) 4, FBLN5, adenosine triphosphate (ATP) 6V0A2, pyrroline-5-carboxylate reductase (PYCR) 1, ATP7A, solute carrier (SLC) 2A10, latent TGF-binding protein (LTBP) 4, ras and rab interactor (RIN) 2. These mutations eventually lead to elastic fiber synthesis disorders or functional defects, causing CL. The pathogenesis of the CCh is also related to the genetic defect, it is known that FBLN5 mutations lead to the disease. Kantaputra *et al*[24] reported that a 4-year-old girl suffering from autosomal recessive CL and CCh simultaneously.

The results showed that the histological structure of skin and conjunctiva, and the genetic background, clinical manifestations, pathological changes and treatment methods were very similar between CCh and CL. We know that abnormal elastic fibers cause skin relaxation, so according to the principle of analogical reasoning, to determine abnormal elastic fibers also caused the conjunctival relaxation.

CAN FACTORS RELATED TO THE PATHOGENESIS OF CONJUNCTIVOCHALASIS LEAD TO ABNORMAL ELASTIC FIBERS?

Genetic Factors Kantaputra *et al*[24] reported on a 4-year-old girl with autosomal recessive CL, type IA, or pulmonary emphysema type, with loose and wrinkled skin, mitral and tricuspid valve prolapse, CCh, obstructed nasolacrimal ducts, hypoplastic maxilla, and early childhood-onset pulmonary emphysema. Histopathological study of the conjunctival biopsy showed that most blood vessels had normal elastic fibers, hyperplasia of the conjunctival epithelium with sub-epithelial conjunctival mononuclear inflammatory cell infiltration. Mutation analysis of FBLN5 showed a homozygous c.432C>G missense mutation, and heterozygosity in the parents. This is predicted to cause amino acid substitution p.Cys144Trp[24].

This case report suggests that genetic factors are involved in the pathogenesis of the CCh. FBLN5 is required to support LOX-mediated cross-linking of elastin on the microfibrillar scaffold. FBLN5 mutations lead to misfolding, decreased secretion, and a reduction of its interaction with elastin and fibrillin-1 and eventually cause structure and functional abnormalities of elastic fibers.

Mechanical Stress Clinical findings of CCh showed corneal margin type and introverted lower eyelid type or corneal limbal type and introverted lower eyelid type, so the author suggested high tension of lower eyelid is one factor of CCh pathogenesis[33]. As mentioned above, dilated lymphatic vessel is one of the histopathological features of the CCh. After the clinical epidemiological survey, some scholars believe that bulbar conjunctival lymphangiectasia may be one of the reasons for CCh[34].

Otaka and Kyu[35] hypothesize that the decrease in connective tissue (elastotic degeneration and collagenolysis) reduces the adhesion of bulbar conjunctiva to the eye, the conjunctiva is "squeezed up" by the lower eyelid margin, and the conjunctival folds are formed on the lower eyelid margin. Watanabe *et al*[16] hypothesize that mechanical forces between the lower lid and conjunctiva gradually interfered with lymphatic flow. Chronic, prolonged mechanical obstruction of lymphatic flow may result in lymphatic dilation and eventually give rise to clinical CCh[16]. High tension of lower eyelid and lymphangiectasia constitute the CCh pathogenesis hypothesis of mechanical stress.

Under the action of repeated mechanical forces, elastic fibers in the aorta appeared fatigue fracture[36]. Previous studies showed that the tensile stress can affect the extracellular matrix metabolism of the organization, including MMPs and TIMP, gene expression and protein synthesis of the matrix components. For example, under the action of cyclic tensile, the expression of MMP-3, which was secreted in bovine synovial cells seeded onto an artificial ligament scaffold, was up-regulated and the enzyme activity was enhanced[37]. It can be seen that mechanical stress lead to the destruction of elastic fibers through these two pathways.

Apoptosis Factors Apoptosis regulatory protein, apoptosis related proteins and inflammatory response associated protein was found in patients with CCh by tears for proteomic analysis and was indicated that the incidence of CCh is related to cell apoptosis and inflammation[38]. The collagen fibril is decreased and fibroblast cells are degenerated in lamina and fascia of CCh under the transmission electron microscope[39]. B cell lymphoma (Bcl)-2 and Bcl-2 associated X protein (Bax) are important gene protein in regulating apoptosis and the ratio of them decided whether cells survive after accepting the signal of apoptosis. The imbalance of expression of the two in the conjunctival relaxation also confirmed the existence of apoptosis[23].

Combined with the previous studies, we know that apoptosis occurs in fibroblasts. The physiological function of fibroblasts is the synthesis of extracellular matrix such as elastic fibers and collagen fibers. As a result the conjunctival fibroblasts can not synthesize enough extracellular matrix to compensate the degradation of its and leading to the formation of the disease.

Aging Factors Mimura *et al*[40] reported the prevalence of CCh increased dramatically with age in a consecutive case study including 1416 patients aged one to 94y. The severity of CCh affecting the temporal and nasal bulbar conjunctiva was strongly correlated with age with fourier-domain optical coherence tomography[41]. Among 2110 residents, 930 cases were confirmed as CCh, with a prevalence rate of 44.08%. The prevalence rate increased with age[42]. Data show that CCh is a common age-related eye disease.

Tendons of old compared with young rats had decreased mRNA expression levels of elastin[43]. Ageing has been shown to enhance MMP-2, -7, -9, -14 activity in the aortic walls of rodents, non-human primates, and humans[44]. The effect of aging on the human body is reflected in the increased destruction and synthesis deficiency of elastin.

Inflammatory Factors Wear contact lenses and autoimmune thyroid disease are important risk factor for CCh. Contact lenses-induced CCh is probably attributable to mechanically induced inflammation that is related to dryness and friction between the lens and conjunctiva[45]. When suffer from autoimmune thyroid disease, the body will be over express of inflammatory factors[46].

In fact, excessive inflammatory factors were detected in tears from patient. Wang *et al*[47] found that the levels of inflammatory cytokines in tear of CCh are higher than in the normal population, especially loose conjunctiva in nasal side. Erdogan-Poyraz *et al*[48] found that tear IL-6 and IL-8 levels are elevated in patients with CCh yet. Higher IL levels are observed in advanced stages of the disease, especially when punctal occlusion. What is more interesting is that tear IL levels tend to parallel the clinical severity as evaluated with the Ocular Surface Disease Index (OSDI). Inflammatory cell infiltration in tissue samples of patients also supports the pathogenesis of inflammatory factors.

Meller *et al*[49] found IL-1β and TNF-α can up-regulate mRNA and protein over-expression of MMP-1 and MMP-3 in cultured CCh fibroblasts *in vitro*. Up-regulation and activation of MMPs by inflammatory factors lead to the degradation of the elastic fibers of the conjunctiva.

Ultraviolet Radiation Pinguecula was independently associated with CCh after adjustment for age. It has been clear that the pinguecula and the ultraviolet (UV) radiation are related, so it is speculated that there is a relationship between the conjunctival relaxation and the UV radiation[50].

The destruction of elastic fibers and UV radiation is linked. Photoaging is the process by which natural sunlight and/or artificial sources of UV radiation damage the skin. Histologically, changes can be observed in the epidermis and dermis. Dermal changes include the hallmark of photoaged skin, the so-called solar elastosis: this accumulated elastotic material in the mid- and upper-dermis is most likely a breakdown product of elastic fibers[51]. It is known that UV radiation induces damage to skin mainly by superfluous reactive oxygen species and chronic low-grade inflammation, which eventually up-regulate the expression of MMPs[52].

Oxidative Stress Specimens from patients with CCh revealed a significantly higher number of cells positively stained for hexanoyl-lysine (HEL), 8-hydroxy-2-deoxyguanosine (8-OHdG), MMP-3, and MMP-9 than the control subjects. These findings revealed lipid and DNA oxidative stress were present

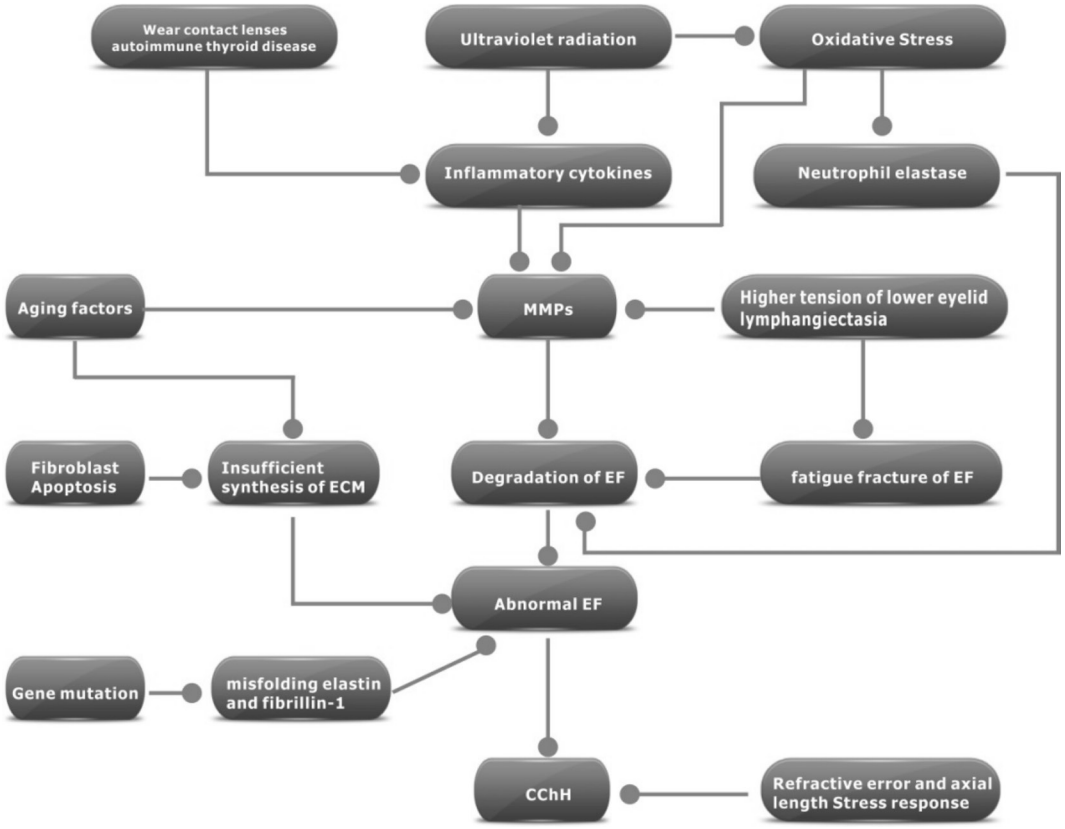

Figure 2 Elastic fibers and the pathogenesis of CCh.

in the conjunctiva in patients with CCh[19]. Acera *et al*[53] have identified a group of proteins, which is up-regulated in CCH tears. Some of them, such as calgranulin (S100) A4, S100A8, and peroxiredoxin-5, are markers of inflammation and oxidative processes. These studies suggest that the onset of the disease involves oxidative stress.

Takayasu's arteritis (TA) is an inflammatory disorder characterized by destruction of elastic fibers. Increased oxidative stress and MMPs activity were considered to play an active role in the progression of TA disease[54]. Neutrophil elastases are thought to be central players to the process of intrinsic skin aging and photoaging. Indeed, not only they directly contribute to the direct degradation of elastic fibers under oxidative stress but also, through a complex network of biochemical reactions, their interferences with collagen homeostasis in skin and contribute, to some extent, to exacerbate oxidative stress in skin[55]. Oxidative stress leads to degradation of elastic fibers through matrix metalloproteinase and neutrophil elastase.

Other Factors

Refractive error and axial length Mimura *et al*[56] reported that the prevalence and grade of CCh are dependent on refractive error and hyperopia being an important risk factor for the diseases. After two years they suggest that the severity of CCh is dependent on the axial length (AL) and a short AL contributing to the pathogenesis of CCh in another article[57].

Stress response Heat shock proteins (HSPs) are several families of proteins which are synthesized by organisms inducing of stressors. HSPs are highly conserved, and play an important role in the survival of stressed cells and stabilization of internal environment. The research showed that the expression levels of HSP27 were higher in CCh than those in the control group[58].

The relationship between refractive error and AL, stress response and elastic fibers were obscure. The vast majority of the factors related to the pathogenesis of CCh ultimately point to abnormal elastic fibers, as the saying goes: all roads lead to Rome (Figure 2).

RESEARCH STATUS OF ELASTIC FIBERS IN NORMAL CONJUNCTIVA AND LOOSE CONJUNCTIVA

Elastic Fibers in Normal Conjunctiva Scattered immature and very occasional mature elastic fibers were observed in the stroma of the bulbar conjunctiva in young subjects (1-15y). Oxytalan and lesser numbers of elaunin and mature elastic fibers intermingled with the loose collagen bundles in both structures in older subjects (over 15y). The more elderly subjects had the most mature elastic tissue[59].

Elastic Fibers in Conjunctivochalasis Elastogenesis is restricted to foetal and infancy, and mature elastin fibers remain for lifespan. Indeed, its strong reticulation makes elastin a highly stable molecule with longevity comparable with human lifespan and any proteolytic damage that does occur with age and disease is essentially irreparable[60]. Under pathological conditions, vascular and inflammatory cells can, however, produce tropoelastin, but these tropoelastin molecules fail to cross-link into mature elastic fibers[61].

Based on the above understanding, the researchers focus on the expression of MMPs in the loose conjunctiva. Li *et al*[62] reported that overexpression of MMP-1 and MMP-3 mRNA by CCh cultured fibroblasts is correlated with their increased protein levels and proteolytic activities. All conjunctival resection specimens from the patients with CCh revealed marked staining for MMP-3 and MMP-9, both in the epithelium and conjunctival stroma compared with that in specimens obtained during cataract surgery from the age- and sex-matched control subjects in the study of ward[19]. At the same time, the expression of MMPs in patients with tear also enhanced. The concentration of pro-MMP-9 was significantly higher in the CCh eyes than in the healthy controls[63]. Related studies also revealed that the activity of MMPs is regulated by the inflammatory factors. Guo *et al*[64] reported that act MMP-1 was uniquely found in cell lysates and culture media of resting CCh fibroblasts, and such expression was further augmented by IL-1β in CCh fibroblasts. It is well known that MMPs can degrade collagen and elastic fibers, so these data indirectly verified the hypothesis of the previous[2].

The description of the elastic fibers appeared only in the results of histological examination of the CCh. In addition, we have not seen the literature on the elastic fibers of the CCh. So the research on elastic fibers itself is ignored.

CONCLUSION

In conclusion, elastic fibers play an important role in the pathogenesis of CCh. In the future, the research direction of the pathogenesis and prevention and treatment of the disease should be put on the elastic fibers that have been neglected. When the body is damaged, the compensatory mechanism will play a role to repair the damage. For example, in the anemia of the body, the bone marrow can be compensated to synthesize more red blood cells to correct the anemia. It is worth to study whether there is compensatory mechanism in elastic fibers damage.

As mentioned above, the elastogenesis is restricted to foetal and infancy and mature elastic fibers remain for lifespan. When the elastic fibers is damaged by age and disease, it can't be repaired. Under pathological conditions, vascular and inflammatory cells can produce tropoelastin, but these tropoelastin molecules fail to cross-link into mature elastic fibers. The mechanism of the above phenomenon has never been clarified.

In short, there are too many problems for elastic fibers. The answers to the above questions can help us to clarify the pathogenesis of CCh and to find an effective treatment for the disease.

ACKNOWLEDGEMENTS

Foundations: Supported by the Key Medical Discipline Project of Shanghai Municipal Health Bureau- Ophthalmology (No.ZK2015A20); the Health System Independent Innovation Science Foundation of Shanghai Putuo District; Plateau Science，Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine.

Conflicts of Interest: Gan JY, None; **Li QS,** None; **Zhang ZY,** None; **Zhang W,** None; **Zhang XR,** None.

REFERENCES

1 Hughes WL. Conjunctivochalasis. *Am J Ophthalmol* 1942;25:48-51.

2 Meller D, Tseng SC. Conjunctivochalasis: literature review and possible pathophysiology. *Surv Ophthalmol* 1998;43(3):225-232.

3 Garner A, Alexander RA. Histochemistry of elastic and related fibres in the human eye in health and disease. *Histochem J* 1986;18(8):405-412.

4 Wise SG, Weiss AS. Tropoelastin. *Int J Biochem Cell B* 2009;41(3): 494-497.

5 Hinek A, Braun KR, Liu K, Wang Y, Wight TN. Retrovirally mediated overexpression of versican v3 reverses impaired elastogenesis and heightened proliferation exhibited by fibroblasts from Costello syndrome and Hurler disease patients. *Am J Pathol* 2004;164(1):119-131.

6 Sakai LY, Keene DR, Engvall E. Fibrillin, a new 350-kD glycoprotein, is a component of extracellular microfibrils. *J Cell Biol* 1986;103(6 Pt 1): 2499-2509.

7 Kielty CM, Sherratt MJ, Shuttleworth CA. Elastic fibres. *J Cell Sci* 2002;115(Pt 14):2817-2828.

8 Lucero HA, Kagan HM. Lysyl oxidase: an oxidative enzyme and effector of cell function. *Cell Mol Life Sci* 2006;63(19-20):2304-2316.

9 Wagenseil JE, Mecham RP. New insights into elastic fiber assembly. *Birth Defects Res C Embryo Today* 2007;81(4):229-240.

10 Kielty CM, Woolley DE, Whittaker SP, Shuttleworth CA. Catabolism of intact fibrillin microfibrils by neutrophil elastase, chymotrypsin and trypsin. *FEBS Lett* 1994;351(1):85-89.

11 Ashworth JL, Murphy G, Rock MJ, Sherratt MJ, Shapiro SD, Shuttleworth CA, Kielty CM. Fibrillin degradation by matrix metalloproteinases: implications for connective tissue remodelling. *Biochem J* 1999;340(Pt 1): 171-181.

12 Antonicelli F, Bellon G, Debelle L, Hornebeck W. Elastin-elastases and inflamm-aging. *Curr Top Dev Biol* 2007;79:99-155.

13 Sproul EP, Arqraves WS. A cytokine axis regulates elastin formation and degradation. *Matrix Biology* 2013;32(2):86-94.

14 Hashemian H, Mahbod M, Amoli FA, Kiarudi MY, Jabbarvand M, Kheirkhah A. Histopathology of conjunctivochalasis compared to normal conjunctiva. *J Ophthalmic Vis Res* 2016;11(4):345-349.

15 Denti AV. On the development of a pleat of the bulbar conjunctiva. *Boll Spec Med Chi* 1930;4:26-32.

16 Watanabe A, Yokoi N, Kinoshita S, Hino Y, Tsuchhashi Y. Clinicopathologic study of conjunctivochalasis. *Cornea* 2004;23(3): 294-298.

17 Zhang XR, Cai RX, Wang BH, Li QS, Liu YX, Xu Y. The analysis of histopathology of conjunctivochalasis. *Zhonghua Yan Ke Za Zhi* 2004; 40(1):37-39.

18 Francis IC, Chan DG, Kim P, Wilcsek G, Filipic M, Yong J, Coroneo MT. Case-controlled clinical and histopathological study of conjunctivochalasis. *Br J Ophthalmol* 2005;89(3):302-305.

19 Ward SK, Wakamatsu TH, Dogru M, Ibrahim OM, Kaido M, Ogawa Y, Matsumoto Y, Igarashi A, Ishida R, Shimazaki J, Schnider C, Negishi K, Katakami C, Tsubota K. The role of oxidative stress and inflammation in conjunctivochalasis. *Invest Ophthalmol Vis Sci* 2010;51(4):1994-2002.

20 Park JS, Ha SW, Lew H. Histopathologic properties of eyelid skin and conjunctiva in patients with dermatochalasis. *J Korean Ophthalmol Soc* 2011;52(5):582.

21 Zhang XR, Zhang ZY, Hoffman MR, Li QS, Liu B, Zhou HM. The effect of age and conjunctivochalasis on conjunctival thickness. *Curr Eye Res* 2013;38(3):331-334.

22 Bae JB, Park WC. Histopathologic characteristics of conjunctivochalasis. *J Korean Ophthalmol Soc* 2013;54(8):1165.

23 Dong LQ, Liu D, Zhang XR. Expression of Bcl-2 and Bax of conjunctivochalasis patients. *Guoji Yanke Zazhi (Int Eye Sci)* 2014;14(2): 365-367.

24 Kantaputra PN, Kaewgahya M, Wiwatwongwana A, Wiwatwongwana D, Sittiwangkul R, Iamaroon A, Dejkhamron P. Cutis laxa with pulmonary emphysema, conjunctivochalasis, nasolacrimal duct obstruction, abnormal hair, and a novel FBLN5 mutation. *Am J Med Genet A* 2014;164A(9): 2370-2377.

25 Yu XY, Jian ZY, Wu W, Lu XH. Simultaneous treatment of pterygium complicated with conjunctivochalasis: analysis of pterygium excision and conjunctival autotransplantation combined with sclera fixation. *BMC Ophthalmol* 2015;15:100.

26 Berk DR, Bentley DD, Bayliss SJ, Lind A, Urban Z. Cutislaxa: a review. *J Am Acad Dermatol* 2012;66(5):e1-17.

27 Gu W, Liu W, Yang X, Tian Y, Meng R, Zhao Q. Cutis laxa: analysis of metalloproteinases and extracellular matrix expression by immunohistochemistry and histochemistry. *Eur J Dermatol* 2011;21(5):717-721.

28 Li QS, Zhang XR, Xiang MH, Zhang XY, Ni ZH, Zhang L, Shen JF, Zhou HM, Shi CC, Li YJ. Expression of matrix metalloproteinases in conjunctival tissues of the patients with conjunctivochalasis. *Chin J Exp Ophthalmol* 2010;46(9):838-840.

29 Banks ND, Redett RJ, Mofid MZ, Manson PN. Cutis laxa: clinical experience and outcomes. *Plast Reconstr Surg* 2003;111(7):2434-2442.

30 Tamura BM, Lourenco LM, Platt A, Pertel P, Santos LF, LevitesJ. Cutis laxa: improvement of facial aesthetics by using botulinum toxin. *Dermatol Surg* 2004;30(12 Pt 2):1518-1520.

31 Kiss HJ, Nemeth J. Isotonic glycerol and sodium hyaluronate containing artificial tear decreases conjunctivochalasis after one and three months: a self-controlled, unmasked study. *PLoS One* 2015;10(7): e0132656.

32 Zhang Y, Niu ZQ, Zhang F. Rapeutic effects analysis of Pranoprofen on tear dynamic abnormity induced by conjunctivochalasis. *Guoji Yanke Zazhi (Int Eye Sci)* 2015;15(5):850-852.

33 Yan YJ, Zhang XR, Xiang MH, Li QS, Xu Y, Zhou HM. Observation in lower eyelid position and tension of conjunctivochalasis. *Int J Ophthamol* 2009;9(3):495-497.

34 Zhang XR, Liu YX, Sheng X, Zhou HM, Han ZM, Fu ZX, Li QS, Xiang MH. Clinical observation of lymphangiectasis in conjunctivochalasis cases. *Zhonghua Yan Ke Za Zhi* 2013;49:(6):547-550.

35 Otaka I, Kyu N. A new surgical technique for management of conjunctivochalasis. *Am J Ophthalmol* 2000;129(3):385-387.

36 Duca L, Blaise S, Romier B, Laffargue M, Gavral S, El Btaouri H, Kawecki C, Guillot A, Martiny L, Debelle L, Maurice P. Matrix ageing and vascular impacts: focus on elastin fragmentation. *Cardiovasc Res* 2016;110(3):298-308.

37 Raif el M. Effect of cyclic tensile load on the regulation of the expression of matrix metalloproteases (MMPs -1, -3) and structural components in synovial cells. *J Cell Mol Med* 2008;12(6A):2439-2448.

38 Zhang XR, Xiang MH, Wu QQ, Li QS, Xu Y, Sun AG. The tear proteomics analysis of conjunctivochalasis. *Zhonghua Yan Ke Za Zhi* 2009;45(2):135-140.

39 Li QS, Yu Z, Xiang MH, Zhang XR, Li YJ. Ultrastructure change of conjunctiva and fascia tissue of conjunctivochalasis. *Chin J Exp Ophthalmol* 2012;30(7):638-640.

40 Mimura T, Yamagami S, Usui T, Funatsu H, Mimura Y, Noma H, Honda N, Amano S. Changes of conjunctivochalasis with age in a hospital-based study. *Am J Ophthalmol* 2009;147(1):171-177.e1.

41 Gumus K, Pflugfelder SC. Increasing prevalence and severity of conjunctivochalasis with aging detected by anterior segment optical coherence tomography. *Am J Ophthalmol* 2013;155(2):238-242.e2.

42 Zhang X, Li Q, Zou H, Peng J, Shi C, Zhou H, Zhang G, Xiang M, Li Y. Assessing the severity of conjunctivochalasis in a senile population: a community-based epidemiology study in Shanghai, China. *BMC Public Health* 2011;11:198.

43 Kostrominova TY, Brooks SV. Age-related changes in structure and extracellular matrixprotein expression levels in rat tendons. *Age (Dordr)* 2013;35(6):2203-2214.

44 Wang M, Kim SH, Monticone RE, Lakatta EG. Matrix metalloproteinases promote arterial remodeling in aging, hypertension, and atherosclerosis. *Hypertension* 2015;65(4):698-703.

45 Mimura T, Usui T, Yamamoto H, Yamagami S, Funatsu H, Noma H, Honda N, Fukuoka S, Amano S. Conjunctivochalasis and contact lenses. *Am J Ophthalmol* 2009;148(1):20-25.e1.

46 de Almeida SF, de Sousa LB, Vieira LA, Chiamollera MI, Barros Jde N. Clinic-Cytologic Study of conjunctivochalasis and its relation to thyroid autoimmune diseases. *Cornea* 2006;25(7):789-793.

47 Wang Y, Dogru M, Matsumoto Y, Ward SK, Ayako I, Hu Y, Okada N, Ogawa Y, Shimazaki J, Tsubota K. The impact of nasal conjunctivochalasis on tear functions and ocular surface findings. *Am J Ophthalmol* 2007;144(6):930-937.

48 Erdogan-Poyraz C, Mocan MC, Bozkurt B, Gariboglu S, Irkec M, Orhan M. Elevated tear interleukin-6 and interleukin-8 levels in patients with conjunctivochalasis. *Cornea* 2007;28(2):189-193.

49 Meller D, Li DQ, Tseng SC. Regulation of collagenase, stromelysin, and gelatinase b in human conjunctival and conjunctivochalasis fibroblasts by interleukin-1 and tumor necrosis factor-alpha. *Invest Ophthalmol Vis Sci* 2000;41(10):2922-2929.

50 Mimura T, Mori M, Obata H, Usui T, Yamagami S, Funatsu H, Noma H, Amano S. Conjunctivochalasis: associations with pinguecula in a hospital-based study. *Acta Ophthalmol* 2012;90(8):773-782.

51 Chen VL, Fleischmajer R, Schwartz E, Palaia M, Timpl R. Immunochemistry of elastotic material in sun-damaged skin. *J Invest Dermatol* 1986;87(3):334-337.

52 Zhang X, Xie YL, Yu XT, Su ZQ, Yuan J, Li YC, Su ZR, Zhan JY, Lai XP. Protective effect of super-critical carbon dioxide fluid extract from flowers and buds of chrysanthemum indicum Linnén against ultraviolet-induced photo-aging in mice. *Rejuvenation Res* 2015;18(5):437-448.

53 Acera A, Suarez T, Rodriguez-Agirretxe I, Vecino E, Duran JA. Changes in tear protein profile in patients with conjunctivochalasis. *Cornea* 2011;30(1):42-49.

54 Mahajan N, Dhawan V, Malik S, Jain S. Implication of oxidative stress and its correlation with activity of matrix metalloproteinases in patients with Takayasu's arteritis disease. *Int J Cardiol* 2010;145(2): 286-288.

55 Mahe Y, Breton L, Dalko M, Rolland A. A new pseudopeptidic elastase inhibitor with protective effects on skin elastic fibers following topical administration. *J Am Acad Dermatol* 2007;56(Suppl 2):AB86.

56 Mimura T, Usui T, Yamagami S, Funatsu H, Noma H, Toyono T, Mori M, Amano S. Relationship between conjunctivochalasis and refractive error. *Eye Contact Lens* 2011;37(2):71-78.

57 Mimura T, Yamagami S, Kamei Y, Goto M, Matsubara M. Influence of axial length on conjunctivochalasis. *Cornea* 2013;32(8):1126-1130.

58 Liu YH, Li YJ, Zhang XR, Zhang ZY, Zhang L, Li QS, Zhang WY, Lu HH. Expression of heat shock protein in conjunctival tissue of conjunctivochalasis. *Chin J Ophthalmol* 2010;46(8):743-745.

59 Alexander RA, Garner A. Elastic and precursor fibres in the normal human eye. *Exp Eye Res* 1983;36(2):305-315.

60 Shapiro SD, Endicott SK, Province MA, Pierce JA, Campbell EJ. Marked longevity of human lung parenchymal elastic fibers deduced from prevalence of D-aspartate and nuclear weapons-related radiocarbon. *J Clin Invest* 1991;87(5):1828-1834.

61 Todorovich-Hunter L, Johnson DJ, Ranger P, Keeley FW, Rabinovitch M. Altered elastin and collagen synthesis associated with progressive pulmonary hypertension induced by monocrotaline. A biochemical and ultrastructural study. *Lab Invest* 1988;58(2):184-195.

62 Li DQ, Meller D, Liu Y, Tseng SC. Overexpression of MMP-1 and MMP-3 by cultured conjunctivochalasis fibroblasts. *Invest Ophthalmol Vis Sci* 2000;41(2):404-410.

63 Acera A, Vecino E, Duran JA. Tear MMP-9 levels as a marker of ocular surface inflammation in conjunctivochalasis. *Invest Ophthalmol Vis Sci* 2013;54(13):8285-8291.

64 Guo P, Zhang SZ, He H, Zhu YT, Tseng SC. PTX3 controls activation of matrix metalloproteinase 1 and apoptosis in conjunctivochalasis fibroblasts. *Invest Ophthalmol Vis Sci* 2012;53(7):3414-3423.