

# Metalloproteinases as mediators of inflammation and the eyes: molecular genetic underpinnings governing ocular pathophysiology

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## Abstract

• There are many vision threatening diseases of the eye affecting millions of people worldwide. In this article, we are summarizing potential role of various matrix metalloproteinases (MMPs); the Zn (2+)-dependent endoproteases in eye health along with pathogenesis of prominent ocular diseases such as macular degeneration, diabetic retinopathy, and glaucoma via understanding MMPs regulation in affected patients, interactions of MMPs with their substrate molecules, and key regulatory functions of tissue inhibitor of metalloproteinases (TIMPs) towards maintaining overall homeostasis.

• **KEYWORDS:** age-related macular degeneration; choroidal neovascularization; diabetes; glaucoma; metalloproteinases; tissue inhibitors of metalloproteinases

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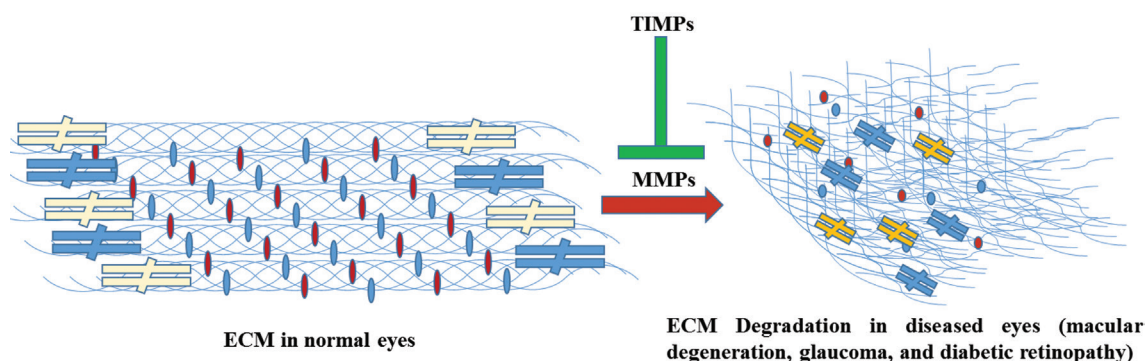
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## INTRODUCTION

Matrix metalloproteinases (MMPs) constitute a large family of secreted and membrane associated zinc-dependent proteolytic endopeptidases. They are known to perform roles in regulation of tissue morphogenesis, motility, cell growth, response to injury, and extracellular matrix (ECM) remodeling not only by degrading matrix related proteins, but also through well-controlled proteolysis of specific extracellular targets that includes receptors, cytokines, growth factors, and adhesion molecules<sup>[1-2]</sup>. Proteolytic activities of MMPs are regulated at various levels and they are produced mainly as zymogens requiring activation by dedicated proteases<sup>[3-4]</sup>. And once activated, MMPs' activities

are often controlled by their endogenous inhibitors; known as tissue inhibitors of metalloproteinases (TIMPs) (Figure 1)<sup>[5-6]</sup>. Literature survey reveals that MMPs when dysregulated can participate in a variety of medical conditions since they serve as crucial players in cell proliferation, differentiation, angiogenesis, apoptosis, and immune defense<sup>[7-8]</sup>. Thus, functional dysregulation as accompanied by excessive activation of MMPs have been associated with many human diseases<sup>[1,7,9-11]</sup>. For example, MMP-10 can degrade a broad spectrum of matrix proteins<sup>[12]</sup>, and activate MMP-1, 7, 8, and 9<sup>[13]</sup>. MMPs are present in almost all tissues impacting many aspects of their unique physiology<sup>[14-15]</sup>. Like in other organs MMPs are also responsible for maintenance and remodeling of ocular architecture by influencing a wide range of processes in eyes. Their substrates represent a variety of ECM components such as cytokines, chemokines and cell surface molecules. MMPs and TIMPs have been shown to co-localize in human interphotoreceptor matrix (IPM)<sup>[15]</sup> playing an important role in physiological reconstruction and turnover of IPM. They are also responsible during embryogenesis, angiogenesis, and ocular wound healing<sup>[16]</sup>. However, they can be detrimental in breaking apart basement membrane contributing to ulcerations such as those observed in corneal tissue<sup>[17]</sup>.

Over the last 30y our lab has been actively studying mechanisms that govern maintenance of tissues' structural integrity<sup>[18]</sup>, post-transcriptional regulation and metabolism of MMPs and TIMPs in human heart failure, atherosclerosis, microvascular permeability and vascular diseases<sup>[19-24]</sup>, involvement of MMPs in diabetes, hypertension, oxidative stress, nephropathy, and autophagy<sup>[25-28]</sup>. From our experience, we know that endogenous TIMPs regulate proteolytic activity by binding tightly to MMPs' active sites. While TIMPs can inhibit most if not all MMPs, available data also reveal tremendous heterogeneity in their affinities of different TIMP/MMP pairs, and that the structural features which differentiate stronger from weaker complexes are not well understood. Like MMPs, TIMPs represent multifunctional proteins having activities that are mediated through protein-protein interactions (PPIs) with other partners. For example, TIMP-2 has been reported to inhibit almost all MMPs that have been studied<sup>[5-6]</sup> and as listed in the MEROPS; a peptide database<sup>[29]</sup> including MMP-1, 2, 3, 7, 8, 9, 10, 13, 19, MT1-MMP, MT2-MMP, MT3-MMP, MT4-MMP, and MT6-MMP,



**Figure 1** A simple schematic highlighting the effects of MMPs on ocular ECM metabolism During disease conditions the homeostatic balance between MMPs and TIMPs gets disturbed leading to degradation of ECM normal architecture in the eyes of the affected patients.

as well as ADAM12<sup>[30]</sup>. As far as physical nature of their partnerships goes the respective inhibition constants ( $K_i$ ) for their interactions vary widely from 0.6 fmol/L for full-length MMP-2<sup>[31]</sup> to 5.8 nmol/L for MMP-10's catalytic domain<sup>[32]</sup>. Also, TIMP-2 can interact with  $\alpha 3\beta 1$  integrin molecule and regulate cell cycle progression and angiogenesis process *via* MMP-independent mechanisms<sup>[5-6,33]</sup>. The general structural basis for inhibition of MMPs by TIMPs was successfully revealed in crystal structures of MMP-3/TIMP-1<sup>[34]</sup> and MT1-MMP/TIMP-2 complexes<sup>[35]</sup>, and subsequently expanded with later structures of MMP-13/TIMP-2<sup>[36]</sup> and MMP-10/TIMP-1 complexes<sup>[32]</sup>, along with complexes of MMP-1 and MT1-MMP with N-terminal domain of TIMP-1, which makes majority of intermolecular contacts<sup>[37-38]</sup>. At a given time in healthy tissue, MMPs and TIMPs levels are found in the stoichiometric ratio of 1:1. Whenever there is an excess MMP it can lead to tissue degradation and the same is true about excess amount of TIMP which may result into ECM accumulation. Thus, the balance needs to be maintained for the proper functioning of the tissue otherwise it may result into an altered regulation of ECM remodeling (such as scarring). In short, MMPs' activities are carefully controlled by TIMPs, and ultimately balance between MMPs and TIMPs determines the final outcomes in a particular tissue (Figure 1). In glaucoma patients, there seems to be an imbalance between MMPs and TIMPs in the eye's chamber angle playing a role in the pathogenesis of the disease itself. Similarly, imbalance in TIMPs' favor can promote initiation of fibrosis leading to tissue remodeling as seen in case of MMP-9 which was shown to be important in corneal stromal remodeling in humans<sup>[39]</sup> and at the same time its involvement in corneal injury as reported in a study which was conducted on rats<sup>[40]</sup>. This study attempts to review an ever-expanding literature on molecular genetics aspects of MMPs and their related biology along with a select description in important ocular diseases such as macular degeneration, diabetic retinopathy (DR) and glaucoma that affect millions of people around the world.

### Information About Diseases in Detail

**Matrix metalloproteinases in macular degeneration** Age-related macular degeneration (AMD) leads to adverse vascular changes and is the most common cause of irreversible vision loss in elderly people globally. It may result from degeneration of rods and cones in the macular region of central retina which is responsible for high acuity vision. Death of photoreceptors appears to be a direct consequence of degeneration of neighboring retinal pigment epithelium (RPE) cells. Drusen formation; abnormal deposits in ECM, is an important hallmark of AMD disease. Typically, drusen lie between RPE basement membrane and inner collagenous layer of Bruch's membrane (BM) and contain ECM along with other molecules. It is hypothesized that drusen may result from the failure to dispose off RPE-derived molecules such as ECM, or it may be the result of dysregulated inflammatory immune mediators. Proinflammatory cytokines were recently reported to decrease the expression of genes that are critical for normal functioning of RPE<sup>[41-42]</sup>. MMP-9 has been shown to participate in the development of choroidal neovascularization (CNV) as part of AMD pathogenesis<sup>[43-45]</sup>. Although the etiology of AMD is multifactorial<sup>[44,46-47]</sup> but a significant role is played by MMP-1, 2, 9, 14 and TIMP-3. It became evident that a continuous rebuilding of ECM occurs in the early and advanced AMD disease simultaneously with the combined malfunctioning of RPE and endothelial cells. Pathological degradation or accumulation of ECM structural components are usually caused by impairment or hyperactivity of specific MMPs/TIMPs interactions, and is also influenced by genetic and environmental factors. Fiotti *et al*<sup>[48]</sup> observed a relationship between polymorphisms in MMP-9 and CNV. More recently, it was shown that MMP-9 rs3918242 (C>T) single nucleotide polymorphism (SNP) was found to play a role in AMD development, and the effect was more pronounced in patients who were less than 65 years of age (Table 1)<sup>[49-53]</sup>.

Interestingly, circulating MMPs and TIMPs have been suggested to participate in a variety of vascular remodeling and

**Table 1 Genes or variants associated with eye diseases**

| No. | Disease                      | Gene names  | References  |
|-----|------------------------------|---|---|
| 1   | AMD/Sorby's fundus dystrophy | SNPs/mutations in MMP-2, 3, 9, TIMP-2, 3, ER- $\alpha$  | MMP-9: Fiotti <i>et al</i> (2005);<br>TIMP-3: Langton <i>et al</i> (2000);<br>MMP-2, 3, 9: Liutkeviciene <i>et al</i> (2015);<br>MMP-2, TIMP-2: Ortak <i>et al</i> (2013);<br>ER- $\alpha$ , MMP-2: Seitzman <i>et al</i> (2008);<br>TIMP-3: Weber <i>et al</i> (1994);<br>TIMP-3: Chen <i>et al</i> (2010) |
| 2   | DR/optic disc anomaly        | SNPs in MMP-2, 9, VEGF, SDH and a 6-Kbp heterozygous triplication upstream of MMP-19 regulatory sequences | MMP-2, 9: Beranek <i>et al</i> (2008);<br>MMP-9: Maeda <i>et al</i> (2001);<br>MMP-18: Hazlewood <i>et al</i> (2015);<br>VEGF, SDH: Rusin and Majsterek (2007);<br>MMP-2: Yang <i>et al</i> (2010)  |
| 3   | Glaucoma                     | SNPs in MMP-1, 2, 3, 9, 12, 16, IL-1beta, TIMP-1, PTGFR   | MMP-1, 9, 12, IL-1beta and TIMP-1: Markiewicz <i>et al</i> (2013);<br>MMP-9, PTGFR: Zhang <i>et al</i> (2016);<br>MMP-1, 2, 3, 16: Vranka <i>et al</i> (2015)   |

Only select information is listed. AMD: Age-related macular degeneration; DR: Diabetic retinopathy; SNP: Single nucleotide polymorphism; MMP: Matrix metalloproteinases; TIMP: Tissue inhibitors of metalloproteinases; ER: Estrogen receptor; VEGF: Vesicular endothelial growth factor; SDH: Sorbitol dehydrogenase; PTGFR: Prostaglandin F2 $\alpha$  receptor gene.

angiogenesis processes<sup>[54]</sup> but it is still not clear whether these circulating MMPs are linked to AMD pathogenesis or not. Mutations in TIMP-3 cause Sorsby fundus dystrophy, another blinding disease with similarities to AMD<sup>[55]</sup>. TIMP-3 is a component of BM<sup>[56-57]</sup> and is found to be concentrated in drusen<sup>[58]</sup> which are cold spots for proteolysis activity. Chau *et al*<sup>[59]</sup> reported a connection between elevated plasma MMP-9 levels and AMD. By contrast, Zeng *et al*<sup>[60]</sup> reported a link between increased levels of circulating gelatinases (MMP-2 and MMP-9) and polypoidal choroidal vasculopathy (PCV)<sup>[61]</sup>; an abnormal choroidal vasculopathy distinct from typical CNV<sup>[62]</sup> but not AMD. However, in AMD lesions, it is believed that activated endothelial cells release MMPs and destroy the BM<sup>[63]</sup>. It has been reported that total levels of active MMP-2 and MMP-9 were significantly reduced in BM-choroid preparations from AMD patients<sup>[64]</sup>. Paradoxically, downregulation of MMP-2 and MMP-9 may lead to advanced pathological changes that can progress to AMD but Ahir *et al*<sup>[65]</sup> demonstrated that administration of activated MMP-2 and MMP-9 can improve fluid permeability of BM. As per their findings it appears that reactivation of MMP pathway may be an effective therapeutic modality in AMD patients. Along these lines, it is worth to mention that “nano-second” laser which uses pulse durations in the range of nanosecond restricts heat transients to <30  $\mu$ mol/L can specifically target RPE cells without any damage to photoreceptors or BM<sup>[66]</sup>. Similarly, Zhang *et al*<sup>[67]</sup> corroborated nano-second laser induced RPE-mediated release of MMP enzymes. Subsequent findings from one-year clinical trial using ultrashort-pulse laser has been reported and it is interesting to note that a single application of laser to macula of AMD patients can improve the overall macular appearance and its functions. These observations

reflected the results that unilateral laser photocoagulation can induce a bilateral MMP pathway activation by releasing a host of circulating factors. Taken together, targeting MMP activation may be a useful approach for the treatment of AMD disease which could serve as a potential therapeutic option for patients.

**Matrix metalloproteinases in diabetic eyes** DR is one of the most dreaded complications in diabetic patients which can lead to both severe forms of vasculopathy and neuropathy. Despite extensive progress in diabetic research, the mechanism(s) responsible for development of this chronic disease remains unknown. It is characterized by endothelial malfunctioning, enhanced permeability of vascular structures, hemostatic abnormalities, tissue ischemia, and neo-angiogenesis<sup>[68]</sup>. Several genes (MMP-2, 9, 19) and their variants have been linked in pathogenesis of DR along with epigenetic and environmental factors (Table 1)<sup>[69-73]</sup>. Several pathways are known to be involved in regulation of epigenetic changes such as micro-ribonucleic acids (microRNAs), DNA methylation, and histone acetylation<sup>[74]</sup>. In diabetic patients MMPs degrade ECM and thus dysregulate many cellular functions *via* signaling stress responses in the eye. Retina and macula are highly prone to free radical mediated damage, and this can have a devastating effect on one's vision. Oxidative stress due to excessive production of reactive oxygen species (ROS) can overwhelm intrinsic antioxidant capacity of cells and thus can induce injury to tissues<sup>[75]</sup> including cells in ocular and other compartments. Thus, oxidative stress affects the development of DR. MMP-2, a most ubiquitous member of MMP family has been shown to be a potent sensitizer for oxidative stress. When effects of mitochondrial superoxide scavenger on glucose-induced alterations in MMP-2, and its proenzyme

activator MT1-MMP and its physiological inhibitor TIMP-2 were determined in retinal endothelial cells, it was revealed that glucose induced activation of retinal capillary cell MMP-2 and MT1-MMP and a concomitant decrease in TIMP-2 suggesting a possible use of MMP-2 targeted therapy to inhibit the development of DR<sup>[76]</sup>.

Diabetes alters the blood-retinal barrier (BRB) possibly by *via* breakdown of endothelial cell tight junctions. To prove this Giebel *et al*<sup>[77]</sup> studied expression of extracellular proteinases in an animal model of early DR and MMPs' expression was investigated in retinas of rats with 12wk of diabetes. In this study role of MMPs in regulating tight junction function was investigated in retinal endothelial and RPE cells by measuring transepithelial electrical resistance (TER). Retinas of diabetic animals demonstrated elevated levels of MMP-2, MMP-9 and MMP-14 messenger RNAs. A significant increase in production of MMP-9 was seen when cells were exposed to high glucose conditions. Both cell types treated with purified MMP-2 or MMP-9 were found to have alterations of tight junction functions as shown by decreased TER. Western blot analysis of cell extracts treated with MMPs (MMP-2 or MMP-9), revealed specific degradation of tight junction protein, occludin. Results suggested that elevated expression of MMPs in retina may facilitate an increase in vascular permeability by a mechanism involving proteolytic degradation of tight junction protein occludin followed by disruption of overall tight junction complex. Similarly, when levels of both MMP-2 and MMP-9 in vitreous samples collected from proliferative diabetic retinopathy (PDR) patients were examined, ProMMP-9 and activated MMP-9 amounts were significantly increased in patients. In addition, TIMP-1 levels were also increased in PDR patients and functionally inhibited activation of MMP-9 in vitreous samples. These results clearly indicated that activated MMP-9 might be involved in hemorrhagic transformation in patients affected with PDR<sup>[78]</sup>.

Recent findings demonstrate that pathogenesis of DR involves H-Ras and MMP-9 acting in concert to accelerate apoptosis of cells such as retinal capillary cells. Using isolated retinal endothelial cells, effect of regulation of H-Ras downstream signaling cascade, Raf-1, MEK, and ERK, was investigated on glucose-induced activation of MMP-9 and the results confirmed that DR increased MMP-9 activity in retinal micro-vessels; the site associated with DR, and it was also accompanied by activated H-Ras signaling pathway (Raf-1/ERK). Together, these findings suggest that Ras/Raf-1/MEK/ERK cascade plays an important role in the activation of retinal MMP-9 resulting in apoptosis of capillary cells. Therefore, understanding the upstream mechanisms which are responsible for activation of MMP-9 should be helpful in identifying novel molecular targets for future pharmacological interventions to inhibit development and progression of DR in vulnerable

patient populations<sup>[79-80]</sup>.

In another study, it was reported that active PDR patients express MMP-9 again suggesting that MMP-9 is one of the important factors in progression of PDR<sup>[81]</sup>. When fibrovascular membranes from PDR subjects were analyzed they stained positive for MMP-1, MMP-2, MMP-3, MMP-9, TIMP-1, TIMP-2, and TIMP-3 and there was a characteristic staining for MMP-9 within the perivascular matrix of PDR membranes. These and related findings indicate that MMPs are involved in degradation of fibrovascular tissue matrix, as well as TIMP-1 and TIMP-2, are found in a large proportion of membranes suggesting the existence of common pathways of ECM degradation in pathological processes leading to retinal neovascularization and fibrosis<sup>[82]</sup>. Using human donor corneal tissues researchers wanted to know whether changes in corneal structural components might be caused by decreased gene activities or increased degradation process. Expression levels of  $\alpha$ -1,  $\alpha$ -5, and  $\beta$ -1 laminin chains; nidogen-1/entactin; integrin  $\alpha$ -3 and  $\beta$ -1 chains in diabetic and DR corneal epithelium were like normal meaning that the observed basement membrane and integrin alterations were unlikely to occur because of a decreased synthesis. mRNA quantities of matrix MMP-10/stromelysin-2 were significantly increased in DR corneal epithelium and stroma, and of MMP-3/stromelysin-1 in DR corneal stroma. mRNA levels of five other proteinases and of three tissue inhibitors of MMPs were like normal in diabetic and DR corneal epithelium and stroma. The resultant data suggested that changes in laminins, nidogen-1/entactin, and epithelial integrin in DR corneas may occur because of an increased proteolytic degradation.

Overexpression of MMP-10 in diabetic corneal epithelium seemed to be the major contributor towards observed changes in DR corneas. Such changes may bring epithelial adhesive abnormalities as seen in diabetic corneas<sup>[83]</sup>. While studying effects of posterior vitreous detachment (PVD), proliferative membrane, vitreous hemorrhage, traction detachment, and cystoid macular edema on MMP activities in human vitreous samples from patients with DR and other vitreoretinal diseases Jin *et al*<sup>[84]</sup> discovered that MMP-9 may be involved in DR and that partial PVD may be related to MMP-9 activity in DR. Out of many MMPs examined in vitreous samples, only levels of MMP-2 and MMP-9 were significantly higher in PDR than control subjects. Immunohistochemical study also demonstrated localization of MMP-2 and MMP-9 in endothelial cells and glial cells of fibrovascular tissues. Here, MMP-2 was colocalized with MT1-MMP and TIMP-2, which are an activator and an activation-enhancing factor respectively for proMMP-2 clearly demonstrating that proMMP-2 is efficiently activated in the fibrovascular tissues of PDR patients, probably *via* interaction with MT1-MMP and TIMP-2. These observations suggest the possibility that activities of

MMP-2 and MT1-MMP are involved in the formation of fibrovascular tissues alterations<sup>[85]</sup>.

**Matrix metalloproteinases in glaucoma** Elevated intraocular pressure (IOP) is a primary risk factor for the etiology of glaucoma and primary open angle glaucoma (POAG) is the main cause of irreversible blindness in people all over the world. MMPs and their regulators; TIMPs and interleukins (ILs) have been extensively studied as POAG risk factors. Lowering IOP remains the only effective treatment for patients with glaucoma. Trabecular meshwork (TM) in concert with ciliary muscle contraction and relaxation provide a working control of aqueous humor outflow in our eyes. In doing so, TM plays an important physiological role in regulating IOP which is predominantly mediated by cytoskeletal and contractility mechanisms as well as signal transduction pathways. This complex system is subject to alteration as one ages and during progression of glaucoma disease. Factors such as a compromised antioxidant defense system<sup>[75]</sup> and altered ECM metabolism are known to contribute to impaired aqueous humor outflow which is common in POAG, exfoliation syndrome, and exfoliation glaucoma (XFG).

While IOP is a well-known predisposing risk factor as mentioned earlier for glaucoma, the etiology of glaucomatous optic neuropathy (GON) is currently not well understood. It appears that a variety of other potential factors particularly those of a vascular nature might be at play in GON because an unstable oxygen content supply as opposed to prevalence of chronic hypoxic conditions seems to contribute to the etiology of GON resulting from constant fluctuations in local oxygen tension leading to an unstable ocular blood flow (OBF) which in turn can fluctuate if IOP spikes or blood pressure drops. In such a scenario OBF autoregulation becomes defective because the main reason for disturbed autoregulation is a primary vascular dysregulation (PVD), particularly in the context of the so-called Flammer syndrome. This unstable oxygen tension can therefore lead to local oxidative stress with many detrimental effects such as activation of glial cells, which can alter their morphology and gene expression pattern. Because of these changes, the local concentrations of nitric oxide (NO<sub>2</sub>) and MMPs increase leading to remodeling *via* digestion of ECM components. Short-lived NO<sub>2</sub> can easily diffuse into neighboring axons, allowing a fusion with superoxide anion and thus generates a cell damaging peroxynitrite. These developments can further contribute to the development and progression of GON phenotype<sup>[86]</sup>.

Since TM in anterior chamber of the eye regulates IOP by generating resistance to aqueous humor outflow so IOP can result from reduced aqueous humor outflow. TM consists of specialized cells within a complex ECM environment. An imbalance between ECM-degrading MMPs and TIMPs within TM is thought to contribute to POAG (Figure 1).

Quantification of TIMPs and MMPs levels in aqueous humor from glaucomatous and non-glaucomatous patients did reveal an imbalance among MMPs and TIMPs with a shift toward raised TIMP levels. This shift may result in the inhibition of MMPs activities, leading to an altered ECM composition in TM and thereby contributing to increased outflow resistance<sup>[87]</sup>. Researchers have also observed that aqueous humor obtained from patients with POAG display increased expression of MMP-1, MMP-9, and MMP-12 in comparison to control samples. Recent studies showed involvement of several SNPs for MMPs, TIMPs and ILs encoding genes in patients with POAG. Investigative association of -1607 1G/2G MMP-1, -1562 C/T MMP-9, -82 A/G MMP-12, -511 C/T IL-1 $\beta$  and 372 T/C TIMP-1 confirmed statistically significant increase in POAG development risk of -1607 2G/2G MMP-1 genotype and for -1607 2G MMP-1 allele, as well as for -1562 C/T MMP-9 genotype and -1562 T MMP-9 allele in patients with POAG in comparison with healthy subjects. There was also a positive association of -511 T/T IL-1 $\beta$  genotype as well as -511 T IL-1 $\beta$  allele occurrence with an increased POAG development risk. Furthermore, an association of -1607 1G/2G MMP-1, -1562 C/T MMP-9 and -511 C/T IL-1 $\beta$  gene polymorphism with decreased retinal nerve fiber layer thickness in patients with POAG group was also observed. Results also indicated an association of 372 T/C TIMP-1 polymorphism with normal range RNFL. Researchers concluded that -1607 1G/2G MMP-1, -1562 C/T MMP-9, -511 C/T IL-1 $\beta$  SNPs can be considered as important risk factors for POAG<sup>[88]</sup>. The impact of polymorphic changes in promoter regions confirmed that allele -1607 1G of MMP-1 gene had 42.91% of -1607 2G allele transcriptional activity while allele -1562 C of MMP-9 gene showed only 21.86% of -1562 T allele. These results suggest that increased expression levels of MMPs can be considered as a risk factor for development of POAG<sup>[89]</sup>. Genetic polymorphism study regarding MMP-9 gene especially in Caucasian patients confirmed that rs17576 polymorphism is not related to glaucoma condition but interestingly rs3918249 polymorphism was found to be a protective factor against glaucoma (Table 1)<sup>[90]</sup>. Prostaglandin and their analogs commonly used for lowering IOP can upregulate expression of MMPs-1, 2, 3, 9, and 17, and at the same time can lower expression levels of TIMP-1 and 2 in human non-pigmented ciliary epithelial cells<sup>[91]</sup>. Insights into molecular mechanisms of segmental aqueous outflow of TM can aid in the design and delivery of improved treatments for patients suffering from glaucoma because molecular differences between high and low outflow regions of TM are not clearly known. An examination of collagen genes such as COL16A1, COL4A2, COL6A1 and 2 and MMP-1, 2, 3 showed enrichment in high flow regions of TM, whereas COL15A1, and MMP-16 were found to be enriched in low flow regions of TM. These genes and proteins differences

across regions of TM provide evidence for a molecular basis of segmental flow routes within the aqueous outflow pathway<sup>[92]</sup>. Additionally, molecular genetic studies have helped in deciphering the potential causes of disorders in patients with a congenital optic nerve disease known as cavitory optic disc anomaly (CODA), who are born with excavation of optic nerve resembling glaucoma. Gene for the autosomal-dominant CODA was successfully mapped in a large pedigree to a chromosome 12q locus. Subsequently, in this pedigree a 6-Kbp heterozygous triplication upstream of MMP-19 gene was discovered. Further characterization of genetic sequence suggested that triplication of this sequence can lead to dysregulation of MMP-19 in the CODA patients<sup>[93]</sup>.

It is known that caveolin (CAV) mediated endocytosis process is one of the mechanisms by which TM cells can control physiological catabolism of ECM to change the composition of outflow channels inside TM. This is done to regulate aqueous outflow resistance and dysregulation of CAV functions can contribute to pathological changes in ECM that are observed in glaucoma patients often. One SNP was identified between CAV1 and CAV2 on chromosome 7 and was associated with glaucoma condition. When a CAV-silencing lentiviral vector was employed to evaluate the effects on ECM turnover by TM cells to measure the effect on outflow in anterior segment perfusion culture, outflow rates increased significantly in CAV1-silenced anterior segments, whereas outflow significantly decreased in CAV2-silenced anterior segments. MMP-2 and MMP-14, and a disintegrin and metalloproteinase with thrombospondin motifs-4 (ADAMTS4) colocalized with both CAVs in TM cells. Protein levels and enzyme activities of MMP/ADAMTS4, fibronectin protein levels, actin stress fibers, and  $\alpha$ -smooth muscle actin were all increased in CAV-silenced cells indicating that dysregulation of CAV functions may contribute to pathological changes in ECM that are commonly observed in glaucoma patients<sup>[94]</sup>.

Further, abnormal production and accumulation of extracellular fibrillary material (XFM); a cardinal feature of exfoliation syndrome represents a pathologic matrix product made by intraocular cells, such as ciliary epithelial cells, trabecular and corneal endothelial cells, pre-equatorial epithelial cells of lens, different cell types of iris, as well as by extraocular cells (vascular cells, fibrocytes, and muscle cells). XFM composition is not fully known however biochemical analyses have shown a highly glycosylated, cross-linked, and enzymatically resistant glycoprotein/proteoglycan complex, composed of a protein core that is surrounded by glycoconjugates. Protein core includes components of basement membrane: laminin, nidogen, fibronectin, elastic fiber parts such as fibrillin-1, elastin, and latent transforming growth factor binding proteins, as well as enzymes (MMPs), extracellular chaperone clusterin, and cross-linking enzyme lysyl oxidase-like 1 (LOXL1).

LOXL1 is an important cross-linking enzyme in ECM and is required for the formation of elastic fiber and its stabilization. It is worth to remember that LOXL1 functions are dysregulated in glaucoma<sup>[95]</sup>.

Cells involved in exfoliation syndrome exhibit metabolic activation, such as increased vesicular transport to cell surface, XFM formation within infoldings of cellular surfaces, and that happens prominently *via* the rough ER structures. Also, cells involved in production of XFM display a gene expression pattern characterized by upregulation of elastic components, transient upregulation of LOXL1, and dysregulated expression of cytoprotective gene products, MMPs, and their inhibitors, possibly leading to accumulation and stable deposition of XFM<sup>[96]</sup>. Pseudoexfoliation (PEX) syndrome is a genetically determined disease of ECM and it generally leads to progressive deposition of fibrillar material in intraocular and extraocular tissues including TM. It causes open-angle glaucoma (OAG). PEX process is characterized by an excessive production and abnormal cross-linking of elastic microfibrils into fibrillar aggregates. Triggering factors include elevated fibrogenic growth factors (TGF- $\beta$ 1), reduced proteolytic enzymes, subtle inflammatory processes along with oxidative stress. Genetic studies identified association between LOXL1 gene polymorphism with PEX syndrome and glaucoma. PEX familial aggregation suggests genetic inheritance and has been strongly associated with SNPs of LOXL1 gene on chromosome 15q24.1. High risk haplotypes vary among different populations but LOXL1 risk variants occur in almost 100% PEX patients making it a principal risk factor for PEX phenotype.

## DISCUSSION

During past decade, the field of molecular genetics has become one of the fastest growing disciplines in life sciences heralding development of potential genomic markers that potentially can identify allelic variants reproducibly. These tools shall help advance research aiming at diagnosis and treatment that can ultimately improve clinical ophthalmological practice. Genomic profiling can assist in understanding genetic determinants of drugs' response in patients who could benefit clinically. Certain SNPs such as in PTGFR (prostaglandin F2 $\alpha$  receptor gene) and MMP-1 genes may determine Latanoprost's (a commonly used anti-glaucomatous drug) response as revealed in a study which identified five SNPs related to Latanoprost efficacy. For example, MMP-1 SNP; rs3753380, has already been associated with a poor response to Latanoprost in a healthy Japanese population<sup>[92]</sup>. However, it is too early to derive such parallels from research currently focusing on epigenetic centered mechanisms operating in a diseased cell. Epigenetic changes occur without alterations in the actual DNA sequences and can significantly affect gene transcription in response to environmental changes.

MMP family of enzymes plays important roles in the physiological and pathological remodeling of tissues in our body and these set of enzymes achieve this *via* an elaborate ECM metabolism<sup>[10]</sup>. Their dysregulations and subsequent malfunctioning can lead to a variety of eye diseases because of their crucial roles in many biological processes<sup>[7]</sup>. Can MMPs and their regulators (TIMPs) be used as potential markers of ocular diseases in clinics such as for treating AMD patients soon remains to be seen? There is no cure for the dry form of AMD and the treatment available for neovascular AMD is *via* photodynamic therapy (PDT), or by injections into the vitreous cavities of the affected eyes that block VEGF actions only slows down the progression of AMD in a select group of patients. Recent insights into the pathological mechanisms operating in ECM metabolism may certainly lead to the development of newer and thus improved therapies for AMD. However, detecting susceptible pool of patients early on before actual AMD disease symptoms start manifesting clinically could only be possible by having a robust biomarker tool kit based on subtle signs of degenerative changes in the photoreceptors and RPE since not much is known regarding the precise sequence of degenerative changes that happens during initiation and progression of AMD particularly in the advanced forms of neovascular AMD phenotype which is preceded by early and intermediate stages that are characterized by a significant loss of RPE coupled with associated pigmentary abnormalities<sup>[97-100]</sup>. Chorio-capillaries, in back of the eyes, are important for survival of photoreceptors, RPE and adjoining supporting structures. During AMD progression ECM serves as the area of dynamic changes connected with the activity of its specific MMPs and TIMPs. Thus, discovery of potential biochemical or genetical biomarkers based on inflammatory mediators and oxidative stress induced cellular changes arising from dysregulation of MMPs and TIMPs expression levels can help identify eye conditions, in advance, in susceptible patient populations. This can have tremendous value in clinics to predict disease risks, evaluate treatment efficacies, and to monitor AMD disease progression.

Diabetes has become a medical epidemic of 21<sup>st</sup> century and is considered a major public health concern. With over 90% patients with diabetes it is a tsunami for the risk of developing DR, nephropathy, and neuropathy in affected patients. Despite ongoing cutting edge research in diabetes field, how exactly retina and its vasculature are damaged by diabetic milieu remains quite ambiguous. Environmental factors, life style or disease process can also bring in modifications in DNA sequence itself, and these modifications either can silence or activate a specific gene. Diabetic environment can upregulate or downregulate genes in retina, and latest research shows that it can also facilitate major epigenetic modifications. Genes associated with important enzymes such as mitochondrial

superoxide dismutase, MMP-9 and thioredoxin interacting protein and transcriptional factors are epigenetically modified. Enzymes responsible for these epigenetic modifications are either activated or inhibited, and levels of microRNAs are also altered<sup>[101]</sup>. Many metabolic pathways have been implicated in DR development and thus role of epigenetics in DR is now an emerging area, and recent work has shown that histone lysine demethylase 1 (LSD1) and DNA methyltransferase are increased. Thus, a better understanding of these modifications has potential to identify novel targets to inhibit this devastating disease.

Fortunately, inhibitors and mimics targeted towards histone modification, DNA methylation, and miRNAs are now being tried for cancer and other chronic diseases and will open the door for their possible use in combating diseases such as DR<sup>[102]</sup>. Additionally, imbalance between MMPs and TIMP (Figure 1) may also promote neovascularization of retina through PKC activation hence development of novel compounds with regulatory action on MMPs and TIMPs production through inhibiting PKC activity can also lead to newer opportunities in developing therapeutics for treatment and prevention of DR<sup>[103]</sup>. Further, to understand better structural basis of TIMPs' functions and their specificities *in vivo*, we need to obtain robust structural results arising from prospective research not only of the strongest MMP and TIMP complexes but also of full spectrum of PPIs as evidenced by researchers actively pursuing such ventures. Similarly, regulation of Sirt1 along with pharmaceutical and nutritional means could also serve as a potential target to prevent or at least delay the development of DR in some patients, if not in all, because oxidative stress in diabetic individuals inhibits Sirt1 where p65 is hyperacetylated simultaneously increasing binding of p65 at MMP-9 promoter sequence. So, prevention of Sirt1 inhibition *via* modulating acetylation of p65 should in principle protect activation of MMP-9 and thus inhibit DR<sup>[104]</sup>. Genes that are differentially expressed in glaucoma patients' ocular tissue or in cultured human TM cell models are possibly implicated in disease process and include superoxide dismutase2 (SOD2), aldehyde dehydrogenases 1A1 (ALDH1A1), Microsomal glutathione S-transferase 1 (MGST1), LOX, and LOXL1, elements of transforming growth factor- $\beta$ /bone morphogenetic protein/small body size mothers against decapentaplegic (Smad) signaling pathways, connective tissue growth factor, MMP-2, TIMP-2, and endothelin-1 (ET-1). In exfoliation syndrome and XFG fibrillar phenotype, a proteinaceous extracellular material is produced in excess and accumulates in both outflow pathways. Material which is locally produced accumulates in the intertrabecular spaces, juxtacanalicular (JCT) meshwork, and in the inner wall of Schlemm's canal because of a combination of both excessive synthesis and insufficient degradation by MMPs. An increase

in JCT plaque and decreased cellularity in TM are thought to contribute to decreased outflow in glaucoma patients, but XFG patient specimens show reduced extracellular plaque material in JCT, and the structural integrity of trabecular endothelial cells is mostly retained and cellularity remains unchanged. Therefore, understanding distinctions between causes and effects of structural changes that lead to reduced outflow/elevated IOP are important for developing effective, individualized treatment strategies in glaucoma<sup>[105]</sup>.

In conclusion, irrespective of their triggers, whether it is oxidative stress<sup>[75,106]</sup> or inflammation<sup>[107]</sup>. MMPs are capable of degrading almost all components of ECM and thus play important roles in many physiological and pathological processes in the eye. On the other hand, TIMPs tend to neutralize the activities of MMPs and therefore help maintain the stability of ECM in the ocular compartment. The imbalance between the expression levels of MMPs and TIMPs has been shown to be closely associated with many ophthalmic disease conditions (Figure 1). A few were covered in this manuscript however emerging evidence suggests that MMPs are also associated with pterygium, corneal lesions and a host of other ocular injuries<sup>[108]</sup>. Thus, future design and delivery of more targeted, and thereby more effective, treatments should take into consideration the various molecular genetic aspects that we have highlighted in our work in this manuscript.

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