Potential underlying genetic associations between keratoconus and diabetes mellitus

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A R T I C L E   I N F O

Keywords:
Keratoconus
Genetics
Diabetes
Collagen crosslinking
Oxidative stress

A B S T R A C T

Background: Keratoconus (KC) is the most common ectatic corneal disease, characterized by significantly localized thinning of the corneal stroma. Genetic, environmental, hormonal, and metabolic factors contribute to the pathogenesis of KC. Additionally, multiple comorbidities, such as diabetes mellitus, may affect the risk of KC. We reviewed candidate genes associated with KC and central corneal thickness in the literature and explored how these genes may be regulated similarly or differentially under hyperglycemic conditions and the role they play in the systemic complications associated with DM.

Main Text: Patients with diabetes mellitus (DM) have been reported to have lower risk of developing KC by way of increased endogenous collagen crosslinking in response to chronic hyperglycemia. However, this remains a debated topic as other studies have suggested either a positive association or no association between DM and KC.

To gain further insight into the underlying genetic components of these two diseases, we reviewed candidate genes associated with KC and central corneal thickness in the literature. We then explored how these genes may be regulated similarly or differentially under hyperglycemic conditions and the role they play in the systemic complications associated with DM.

Conclusions: Our comprehensive review of potential genetic factors underlying KC and DM provides a direction for future studies to further determine the genetic etiology of KC and how it is influenced by systemic diseases such as diabetes.

1. Introduction

Keratoconus (KC) is a bilateral, progressive ectatic corneal disorder characterized by localized thinning of the corneal stroma and alterations of corneal curvature. As the cornea adopts a conical shape, this results in myopia, irregular astigmatism, and eventual visual impairment. The histopathology of KC includes stromal thinning, breaks in Bowman's layer, focal fibrosis, thickening of the epithelium, and keratocyte apoptosis in the anterior stroma. Although KC is known to be multifactorial with genetic, metabolic, hormonal, and environmental influences, the exact etiology remains elusive. The risk of developing KC has been associated either positively or negatively with many systemic disorders, including but not limited to, Down syndrome, connective tissue diseases, autoimmune diseases, and diabetes mellitus (DM). However, the exact etiology of KC and its relationship to systemic diseases, such as DM, remains elusive.

DM may have multiple effects on the cornea, including keratopathy, neuropathy, inflammation, alterations in collagen fibrils, and endothelial cell loss. Multiple studies have suggested that DM is inversely associated with the risk of KC, suggesting a protective role against the development and/or severity of KC. This is in contrast to other studies that have reported either 1) a positive association in both prevalence and severity between KC and DM or 2) no significant correlation between the two diseases. This discrepancy may be reflective of the varying sample sizes, inclusion/exclusion criteria, sample ascertainment approaches, and populations analyzed (Table 1).

Our goal was to provide a comprehensive review of potential genetic associations between DM and KC. We have outlined various genes implicated in KC risk and summarized their potential roles in DM-induced corneal changes (Table 2). These genes may work through several mechanisms including alterations in corneal biomechanics and collagen crosslinking, alterations in ECM composition and proteolytic activity, as well as increased inflammation and oxidative stress.

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https://doi.org/10.1016/j.aopr.2021.100005
Received 9 June 2021; Received in revised form 18 August 2021; Accepted 29 August 2021
Available online 4 September 2021
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2. Clinical significance

By gathering information regarding the potential overlapping genetic factors in KC and DM, this review discusses potential therapeutic targets that may slow or halt the progression of KC. In other words, it might be beneficial to manage KC by utilizing the same mechanisms that strengthen the cornea in DM. These mechanisms include but are not limited to shifting the balance in the corneal microenvironment towards increased endogenous collagen crosslinking, decreased extracellular matrix (ECM) remodeling and proteolytic degradation, and decreased inflammation. By regulating local gene expression, this may serve as an alternative form of therapy for those patients in which corneal crosslinking is not an option, such as those with prior history of herpetic keratitis and history of poor epithelial wound healing. Furthermore, this may reduce the need for corneal transplantation in patients with more severe forms of KC.

3. Corneal biomechanics and collagen crosslinking in KC and DM

The cornea is naturally a viscoelastic structure, in that it must be elastic enough to expand into an aspheric half-sphere, but stiff enough to maintain its shape and resist the intraocular pressure (IOP). There is a delicate interplay between the multiple corneal layers and the composition within each layer that contributes to the overall corneal shape. This

| Study | Study size | Design | Population Characteristics | Findings | Association |
|-------|------------|--------|-----------------------------|----------|-------------|
| 13. KC patients (n=571) Non-KC controls (n=571) | Retrospective case-control study | German population | T2DM showed a protective effect against KC development (odds ratio = 0.2195) | Inverse association of KC development with DM |
| 14. KC patients without DM (n=269) KC patients with DM (n=26) | Retrospective cross-sectional study | United States population | T2DM showed a protective effect against more severe KC (odds ratio = 0.2); No difference in DM prevalence in KC population | Inverse association of DM with KC severity |
| 17. KC patients (n=1383) non-KC controls (n=1383) | Retrospective case-control study | Iranian population | T2DM showed a protective effect against KC development (odds ratio = 0.350) | Inverse association of KC development with DM |
| 18. KC patients (n=16,053) non-KC controls (n=16,053) | Retrospective longitudinal cohort study | Population-based | 20% lower odds of KC development with uncomplicated DM; 52% lower odds of KC development with DM-associated organ failure | Inverse association of KC development with DM |
| 19. KC patients (n=2679) non-KC controls (n=26,7900) | Retrospective longitudinal cohort study | Danish population | No significant difference in DM prevalence in KC patients. Total DM odds ratio=1.03, T1DM odds ratio=0.87, T2DM odds ratio=1.07 | No significant association between KC development and DM |
| 20. KC patients (n=575) non-KC controls (n=2875) | Retrospective longitudinal cohort study | Korean population | No significant difference in DM prevalence in KC patients. Multivariate odds ratio=1.02 | No significant association between KC development and DM |
| 21. 29 studies incorporating 50,358,341 subjects | Systematic review and meta-analysis | Global population; 15 countries | Odds of developing KC were 23% lower in T2DM, but relationship was not significant | No significant association between KC development and DM |
| 22. KC patients (n=2051) non-KC controls (n=12,306) | Retrospective case-control study | Netherlands population | No significant association in KC and DM, with odds ratio 1.60 (0.89-2.89) and p-value 0.149. | No significant association between KC development and DM |
| 23. KC patients (n=1377) non-KC controls (n=4131) AND T2DM KC patients (n=75) non-DM KC controls (n=225) | Retrospective case-control and Cross-sectional study | United States population | Higher prevalence of T2DM in KC population compared to controls (6.75% and 4.84%, respectively); Higher severity of KC in DM patients (odds ratio = 2.691) | Positive association of KC development with T2DM |
| 24. KC patients (n=1552) non-KC controls (n=7,760) | Retrospective cohort study | South Korean population | Higher prevalence of T2DM in KC population compared to controls (19.2% and 14.5%, respectively); Positive association of KC with DM (odds ratio = 1.35) | Positive association of KC development with DM |

Table 1

List of human studies evaluating the relationship between keratoconus and diabetes (adapted and modified from 12).
The thickness of the cornea, CH re measurements are commonly obtained: the central corneal thickness for KC. We will discuss the crosslinking mechanisms in more detail as they advanced glycation end product (AGE)-mediated crosslinking and 2) oxidase-mediated crosslinking as well as two non-enzymatic reactions: 1) lysyl collagen crosslinking important in corneal biology, as previously outlined in McKay et al., 2019. Those include an enzymatic reaction: lysyl collagen crosslinking involved in the attachment of proteoglycans and glycosaminoglycans to collagen fibers, the central corneal thickness (CCT) and corneal hysteresis (CH). While CCT is a gross measure of the overall thickness of the cornea, CH reflects the viscous damping ability of the cornea. CH combines elasticity and viscosity and provides further information on the structural integrity of the cornea. Increased edema in the cornea may give the appearance of stromal thickening and increased CCT. This must be taken into account in systemic diseases such as DM, in which glucose induces increased water retention in multiple tissues, including the cornea.

In order to quantify differences in corneal shape and structure, two measurements are commonly obtained: the central corneal thickness (CCT) and corneal hysteresis (CH). While CCT is a gross measure of the overall thickness of the cornea, CH reflects the viscous damping ability of the cornea. CH combines elasticity and viscosity and provides further information on the structural integrity of the cornea. Increased edema in the cornea may give the appearance of stromal thickening and increased CCT. This must be taken into account in systemic diseases such as DM, in which glucose induces increased water retention in multiple tissues, including the cornea.

Inflammation and ROS production

Table 2

| Gene      | Function                                    | CHR     | KC/CCT effects                                                                 | DM effects                                                                 | References |
|-----------|---------------------------------------------|---------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------|------------|
| FOXO1     | Transcription factor                        | 13q14.1 | SNP in FOXO1 linked to CCT, FOXO1 expression/activity unknown                  | FOXO1 linked to AGE-mediated disruption of autophagic flux and vascular endothelial cell autophagic apoptosis, role in cornea unknown | 66,85,90   |
| SMAD3     | Transcription factor                        | 15q22.23| SNP in SMAD3 linked to CCT, increased pSMAD3 and increased TGF signaling in KC cells | SMAD3 linked to ECM remodeling in DN; role in cornea unknown               | 49,86,110,106 |
| TGFBI     | Transforming growth factor beta induced     | 5q31.1  | SNP in TGFBI linked to KC with decreased levels of TGFBI in KC cornea          | Unknown effect in DM cornea, shown to be upregulated in response to high glucose and TGFβ in DM proximal tubules | 124-126,132 |
| ZEB1      | Zinc finger transcription factor            | 10p11.22| Mutations in ZEB1 associated with KC and PCCD; possible genotype/phenotype correlation | Unknown effect in DM cornea, implicated in epithelial-to-mesenchymal transition under hyperglycemic conditions | 152,154,155,158 |
| MMP-9     | Matrix metalloproteinase-9                  | 20q12.2- q13.1 | Increased MMP-9 activity noted in tear sample with corresponding upregulation in MMP-9 mRNA; SNP identified in MMP-9 | Increased MMP-9 activity in tears from DM patients; SNP identified in MMP-9 associated with T2DM susceptibility | 170,177,178,181,185 |
| TIMP-1    | Tissue inhibitor of metalloproteinases-1    | 1p11.23 | Decreased TIMP-1 levels detected in KC patients, SNP in TIMP-1 associated with increased KC risk | Increased TIMP-1 levels in tears of pediatric T1DM patients, but overall role of TIMP-1 in DM remains inconclusive | 83,176,182,185 |
| MIIR184   | microRNA                                    | 15q22-q25 | Mutations in miR-184 implicated in KC pathogenesis, but extent of association with KC alone remains unclear | Unknown effect in DM cornea, implicated in epithelial-to-mesenchymal transition under hyperglycemic conditions | 187,196,200,201,310,311 |

ECM remodeling

| Gene      | Function                                    | CHR     | KC/CCT effects                                                                 | DM effects                                                                 | References |
|-----------|---------------------------------------------|---------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------|------------|
| HGF       | Hepatocyte growth factor                    | 7q21.1  | Increased HGF protein in KC corneal epithelium; increased HGF and c-Met mRNA in corneal wound healing | Increased HGF with decreased HGF receptor c-Met expression in DM cornea     | 5,234,237,238 |
| CAST      | Calpain/calpastatin, proteolytic degradation | 5q15    | SNP in CAST strongly linked to KC; CAST expression/activity unclear            | High glucose induces calpain activity, increasing ROS production and vascular endothelial dysfunction; unknown effect in cornea | 226,227,330 |
| SOD1      | Superoxide dismutase 1 cytoplasmic antioxidant enzyme | 21q22.11 | Deletion mutation in SOD1 in several cohorts; decreased SOD1 expression in KC corneal fibroblast cultures | Associated polymorphisms in SOD1 identified; decreased SOD1 expression in DM cornea with associated increase in RAGE imbalance in IL-1β to IL-1Ra in DM cornea, SNPs identified in IL1A, IL1B, and IL1RN in DM patients | 233-241,254,256 |
| IL1A/IL1B | Interleukin 1alpha/beta, inflammatory cytokine | 2q13    | Increased IL-1α expression in KC corneas, SNPs identified in IL1A and IL1B | Unknown effect in DM cornea, implicated in epithelial-to-mesenchymal transition under hyperglycemic conditions | 119,162,203,204,316,317 |

Additional genes of interest

| Gene      | Function                                    | CHR     | KC/CCT effects                                                                 | DM effects                                                                 | References |
|-----------|---------------------------------------------|---------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------|------------|
| SPRY2     | Sprouty 2                                   | 13q31.1 | SNP in SPRY2 linked to CCT and corneal epithelium proliferation; SPRY2 expression/activity in KC cornea unknown | SNP near SPRY2 linked to increased DM susceptibility; unclear effect in SPRY2 cornea | 49,278,280,282 |
| COL4A5, COL4A4 | Collagen type IV, alpha-3/4 chain, structural portion of corneal membranes | 2q36.3  | Alterations in collagen type IV reported in KC, but unclear if genetic polymorphisms play a role | Alterations in collagen type IV reported in DN and in the cornea under hypoxic conditions, unknown genetic association with DM cornea | 48,281,290,291 |

These include an enzymatic reaction: lysyl oxidase-mediated crosslinking as well as two non-enzymatic reactions: 1) advanced glycation end product (AGE)-mediated crosslinking and 2) photooxidative crosslinking mediated by riboflavin as a treatment option for KC. We will discuss the crosslinking mechanisms in more detail as they relate to the genes involved in KC and DM.

To quantify differences in corneal shape and structure, two measurements are commonly obtained: the central corneal thickness (CCT) and corneal hysteresis (CH). While CCT is a gross measure of the overall thickness of the cornea, CH reflects the viscous damping ability of the cornea. CH combines elasticity and viscosity and provides further information on the structural integrity of the cornea. Increased edema in the cornea may give the appearance of stromal thickening and increased CCT. This must be taken into account in systemic diseases such as DM, in which glucose induces increased water retention in multiple tissues, including the cornea.

Further evidence links diabetes to a higher risk of KC, with decreased levels of lysyl oxidase and increased expression of matrix metalloproteinase-9 (MMP-9) in the cornea of T1DM patients, but overall role of TIMP-1 in DM remains inconclusive.

Changes in the expression of proteins involved in ECM remodeling, such as collagen type IV, alpha-3/4 chain and collagen type V, alpha-1 chain, may contribute to the pathogenesis of KC, with decreased levels of proteoglycan core proteins and abnormal collagen synthesis. Further, increased expression of MMP-9 activity noted in tear fluid indicates a correlation between increased KC risk and the activity of MMP-9 in the cornea.

In conclusion, the relationship between diabetes and KC involves the crosslinking of collagen, with potential gene-gene and gene-environment interactions. Further research is needed to elucidate the precise mechanisms linking diabetes to KC and to develop effective therapeutic strategies for these patients.
glycosylation of corneal fibers and increases AGE-mediated crosslinking in the cornea, causing the cornea to become stiffer. 13,32–34 As a result, increased CCT and alteration in corneal endothelial cells have been observed in patients with diabetes. 73–76 Lee et al., 2006 reported that CCT was significantly correlated with the duration of diabetes after controlling for age. 77 Interestingly, Hager et al., 2009 observed a significant increase in CH in patients with diabetes, although they did not observe a significant increase in CCT after correcting for age, IOP, and gender. 78 Schler et al., 2012 also reported significantly higher CH and corneal resistance factor (CRF) in patients with poorly-controlled diabet-es as compared to healthy subjects and well-controlled diabetes. 79 Since CH and CRF are correlated to HbA1c, this suggests that the cornea biomechanics could be altered depending on extent of glucose control. 80 These findings suggest that DM may induce structural alterations in the cornea. In terms of corneal biomechanics, in this section we discussed two genes, LOX and COLSAl, and their roles in relationship to the corneal biomechanics observed in KC and DM.

3.1. LOX

Lysyl oxidase (LOX) is a copper amine oxidase that initiates the physiologic crosslinking of collagens and elastin by oxidizing the side chain of peptidyl lysine, thus generating reactive aldehydes on lysine residues that may cross-react with nearby groups. 26,42,46 In the eye, the LOX enzyme has been detected in the trabecular meshwork, ciliary body, lens, retina, and cornea. 44–46 In addition to LOX, there are four LOX-like proteins (LOXL1, LOXL2, LOXL3, and LOXL4) that also catalyze the oxidative deamination of lysine residues in collagen and elastin.45

Multiple genome-wide association studies (GWAS) and candidate association studies have identified the association between sequence variants in LOX to both KC risk and CCT, suggesting its potential role in KC pathogenesis. 47–49 Specifically, a particular SNP rs2956549 in LOX is associated with KC in various ethnicities as well as a meta-analysis. 50–53 Dudakova et al., 2012 reported altered distribution of LOX expression in the corneal stroma of KC patients, with a reduction in total LOX (both LOX and LOXL1) protein activity in corneal fibroblasts derived from KC corneas. 54 This was consistent with a recent study that reported reduced pre-existing LOX and collagen levels in a patient who developed ectasia after small incision lenticule extraction (SMILE), despite normal preoperative biomechanical evaluation. 54 Furthermore, ectopic LOX expression in the human corneal fibroblast induced significantly more collagen gel contraction in vitro, confirming the role that it plays in strengthening the corneal stroma. 54

Given LOX’s role in collagen crosslinking, reduced LOX activity in KC may lead to impaired cross-linking which results in corneal ectasia. 15,54 Although there is a confirmed genetic association of LOXL1 to pseudoexfoliation syndrome and open-angle glaucoma, 56,57 this is still unclear the role that LOXL proteins may play in the cornea. Dudakova et al., 2016 confirmed that LOXL1-4 enzymes were present in all layers of the cornea in cryosection samples and reported lower LOXL2 expression in KC corneas using IHC and Western blot analyses. 57 Further studies will be required to better understand the expression patterns and activity of LOX1 proteins in the cornea.

The exact role of LOX in DM-induced corneal changes remains unclear, as one study failed in observing an increase in LOX-mediated crosslinking in the cornea of DM patients. 53 However, Chronopoulous et al., 2010 revealed that hyperglycemic conditions increased LOX expression and LOX activity in rat retinal endothelial cells in vivo and in diabetic rat retinas in vivo. 81 This is consistent with the finding of increased LOX-dependent crosslinking in skin collagen in patients with diabetes. 89 Coral et al., 2013 confirmed increased LOX mRNA expression in ARPE-19 cells exposed to high glucose. 82 Further studies will be required to investigate the effects of DM on LOX activity in the cornea. The increase in LOX activity and expression in response to high glucose in retinal studies suggests that high glucose may have a similar effect in corneal stromal and endothelial cells. Given that the cornea is an avascular structure, there may be differential upstream regulation of LOX in response to high glucose concentrations as compared to the retina.

3.2. COLSAl

Since collagen is the most abundant protein in the cornea, 26 it is not surprising that various types of collagen have been implicated in association with CCT and KC, including COL1A1 and COL1A2, 61 COL8A2, 52 and COL5A1. 70–73 The genetic association of COL5A1 with CCT, in particular, reached genome-wide significance after combining data from European, Australian, and Singaporean GWAS. 74–76 COL5A1 type V is a regulatory fibril-forming collagen involved in the formation of hetero-typic fibrils with collagen type I. 77–79 Together, both collagen type I and V are the dominant collagen isoforms in the human corneal stroma. 71 Although collagen type V only comprises 2–5% of the total collagen in most tissues, it determines 10–20% of the fibrillar collagens in the cornea. 72–73 Interestingly, the most common molecular mechanism in classic Ehler-Danlos syndrome (EDS), a generalized connective tissue disorder, is a functional loss of one COL5A1 allele. 72,73,74 Segev et al., 2006 investigated the corneal phenotype in EDS patients with COL5A1 hap-loinsufficiency and COL5A1 mouse models. Both EDS patients and knockout mice exhibited consistent corneal thinning and the COL5A1 mice also exhibited a decrease in total collagen content with a 25% reduction in the number of stromal fibrils. 72 These findings suggest that alterations in COL5A1 expression serves as a strong genetic predisposition towards the development of corneal thinning and KC.

In DM, collagen is influenced by hyperglycemia primarily through nonenzymatic AGE-mediated crosslinking. This occurs by way of the Maillard reaction, in which a primary amine found on amino acid resi-dues, such as lysine or arginine, is converted to a reactive Schiff base to form the Amadori product. 70 This serves as an early-stage product that leads to the formation of AGE products. A classic example of the Maillard reaction is the glycation of hemoglobin, which gives rise to the glycated HbA1c, the established biomarker for sustained glucose levels. 76,77 Studies have identified increased end products of the Maillard reaction, including pentosidine, within the diabetic cornea. 26,33 This increase in AGE-mediated crosslinking in DM has been associated with increased tendon stiffness and higher mechanical strength, which could be inhibited by insulin supplementation. 26,76,77 As a result, the increased crosslinked collagen is resistant to enzymatic degradation in the cornea. 78 AGE-mediated crosslinked adducts can then bind to the receptor for advanced glycation end products (RAGEs), which are pro-inflammatory receptors expressed in a wide variety of tissues. Although originally described for its ability to bind AGEs, RAGEs have the capability to bind multiple other ligands and play a crucial role in homeostasis and inflammatory processes. 79,80

To our knowledge no direct associations between COL5A1 expression and DM have been established. Very recently, Ng et al., 2020 evaluated possible gene-environment interactions between genetic variants identified via GWAS, and the effect of glycemic control (indicated by HbA1c) on the risk of severe diabetic retinopathy (DR). 81 Interestingly, the SNP COL5A1 rs59126004 exhibited a protective effect against DR in patients with adequate glycemic control (HbA1c <7%), but not in patients with inadequate glycemic control (HbA1c ≥ 7%), suggesting a potential interaction between COL5A1 rs59126004 and glucose levels in the retina.

Priyadarsini et al., 2016 quantified the expression of collagen type I, III, and V in the human corneal stroma of Type 1 and Type 2 DM, and discovered elevated levels of collagen type I and type III, but not collagen type V. 27,49 Given that this was a small study with four to eight donor corneas in each category, it would be worth expanding the study with a larger sample size. Collagen type I and V are the predominant types of a healthy cornea, while collagen type III is primarily associated with fibrosis and wound healing in the setting of injury. This finding suggests that hyperglycemia may induce collagen expression in an isoform-dependent manner. 27 We expect more studies of COL5A1 to better understand DM’s direct effects on COL5A1 in the cornea.
4. Alterations in extracellular matrix (ECM) remodeling in the cornea

In line with collagen crosslinking, alterations in corneal ECM remodeling have been observed in KC and DM including variations in cellular proliferation, disruption in autophagic flux, and increased ECM fibrosis. This is complemented by the observation of an imbalance between matrix metalloproteinase (MMP) and tissue inhibitors of matrix metalloproteinase (TIMPs) in KC patients.33,34 Here, we take a close look at several KC- and CCT-associated transcription factors including FOXO1, SMAD3, TGBFβ, and ZEB1 (Table 2). We then searched the literature to determine how they may be differentially regulated under hyperglycemic conditions in the cornea. We also included ECM remodeling genes MMP-9, TIMP-1, and MIR184.

4.1. FOXO1

Transcription factor Forkhead box protein O1 (FOXO1) is ubiquitously expressed in mammalian cells.85 It mediates multiple different pathways, including regulation of metabolic homeostasis, oxidative stress, cell proliferation, and autophagy. Sequence variants near or within FOXO1 have been associated with CCT,66,85,86 but its effect on FOXO1 expression and activity is not well described in the KC cornea. FOXO1 is regulated by a variety of environmental factors, including insulin, which normally inhibits FOXO1 activity.87 In settings of insulin resistance and increased glucose, such as T2DM, increasedFOXO1 has been shown to induce glucoenolysis abnormalities, cell apoptosis, uncontrolled autophagy, and inhibition of proliferation.84,88

Although much remains to be understood about FOXO1’s role in KC and DM, some studies have analyzed its effects on vascular endothelial cells. Wilhelm et al., 2016 indicated that FOXO1 decreases the metabolic and proliferative activities of vascular endothelial cells by attenuation of glycolysis and mitochondrial respiration.84,89 This study reported that the endothelial-restricted deletion of FOXO1 in mice caused a profound increase in endothelial cell proliferation, such that it induced vascular hyperplasia and vessel enlargement. In contrast, overexpression of FOXO1 resulted in vessel thinning and reduced branching of vasculature.89 A separate study reported that FOXO1 signaling was involved in AGE-induced vascular endothelial cell autophagy through impairment of autophagosome-lysosomal fusion. This impaired autophagic flux then resulted in endothelial cell autophagic apoptosis.90 These findings are intriguing as they suggest a correlation in FOXO1 activity with AGE-induced endothelial damage and decreased endothelial cell proliferation in the vasculature. Thus, it would be interesting to perform similar studies in the microenvironment of the cornea to determine if differential regulation of FOXO1 may contribute to altered endothelial cell proliferation and/or endothelial cell apoptosis in KC and DM corneas.

4.2. SMAD3

There are three functional classes of SMAD proteins, including the receptor-regulated SMAD (R-SMAD), the Co-smad (Co-SMAD), and the inhibitory SMAD (I-SMAD) proteins.1 Smad3 is an R-SMAD that is directly phosphorylated and activated by type I receptor kinases, forming activated SMAD complexes that then activate the transcription of target genes,1 specifically those involved in the TGBFβ signaling pathway. TGBFβ signaling is tightly regulated by I-SMAD proteins, SMAD6 and SMAD7, which compete for binding of SMAD3 to co-mediators.1 TGBFβ signaling is known to play an important role in ECM remodeling and MMP expression. Several studies have identified involvement of TGBFβ signaling in KC.96–99 Priyadarshini et al., 2015 observed a significant increase in pSMAD3 expression with TGFβ3 signaling in human KC cells compared to normal human corneal fibroblasts.90 This was paralleled by significant downregulation of SMAD6/7 at baseline and failure of SMAD6/7 upregulation in response to stimulation with TGBFβ in the human KC cells.98

Recently, variants near or within SMAD3 were identified to be associated with KC susceptibility in GWAS of CCT.95,100 Further reinforcing its potential role in KC pathogenesis. Taken together, it has been suggested that the lack of inhibition by SMAD6/7, along with potential alterations in SMAD3 activity, results in increased TGBFβ signaling that may promote the formation of a fibrotic ECM in KC.98

TGBFβ/SMAD signaling plays an important role in regulating glucose and energy homeostasis as well.101 The TGBFβ/SMAD3 pathway is activated downstream of the AGE/RAGE signaling pathway in the setting of hyperglycemia.102,103 This is suggested to be the main driving force in the development of diabetic nephropathy (DN) due to increased ECM deposition in mesangial cells.104,105 Ono et al., 2018 demonstrated that AGE stimulation resulted in significant activation of Smad1 and Smad3 in mesangial cells in mice, likely as a result of increased TGBFβ signaling.106 Additionally, the loss of Smad3 prevented renal dysfunction under diabetic conditions by reduced mesangial matrix accumulation and reduced GBM thickening.108 This reinforces the critical role that TGBFβ/SMAD3 plays in ECM remodeling, and shows that this pathway is also affected under hyperglycemic conditions. However, this pathway has not been analyzed in the DM cornea. Interestingly, TGBFβ/SMAD3 signaling promoted gluconeogenesis in hepatocyte cells through interaction with FOXO1, another established KC-susceptibility gene.101 It is necessary to determine if the TGBFβ/SMAD interaction with FOXO1 is present in the human cornea.

4.3. TGBFβ

The transforming growth factor beta-induced (TGBFβ) gene has been implicated in the pathogenesis of KC and a heterogeneous group of corneal dystrophies that are characterized by the progressive loss of corneal transparency.107 It encodes transforming growth factor beta-induced protein (TGBFβp), which is also known as keratoepithelin, BIGH3, or jigh3.108 In the cornea, it is expressed primarily in the corneal epithelium, stroma, and retrocorneal fibrous membranes.108–112 TGBFβp is known to interact with multiple extracellular macromolecules, including the proteoglycan decorin113 and collagen type I, II, IV, VI, and XII.114–117 It is thought to link cells to the ECM through various integrin binding sites116,118–122 and thus play important roles in corneal wound healing and maintenance of the ECM.

In a cDNA library constructed from KC corneas, TGBFβ was found to be the second most abundant transcript.123 Sequence variants in TGBFβ were also identified in Chinese and Polish KC patients.124,125 However, the exact relationship between TGBFβ, TGBFβp, and KC is still unclear. Researchers have suggested that mutations in TGBFβ could contribute to decreased mechanical stability in the cornea, thus resulting in corneal thinning as seen in KC.126 This is supported by findings of decreased TGBFβp in KC corneas.126 Conversely, elevated levels of TGBFβp have been found in areas of corneal scarring, likely due to TGBFβ1-mediated upregulation in response to corneal injury.116–122 Interestingly, two KC patients had stromal amyloid deposits that were associated with TGBFβp in the corneal buttons, but no TGBFβ mutation was present.127 It is possible that there was concurrent scarring in these two patients, or that local factors in the KC corneas predispose to development of TGBFβp amyloid deposits, thus disrupting the structural integrity of the cornea.128 TGBFβ has also been identified as a potential risk gene for the development of both T1DM and T2DM after detecting the association of several SNPs in human genetic studies.129 Aside from the cornea, TGBFβp is produced by smooth muscle cells, fibroblasts, and proximal tubular epithelial cells130,131 upon TGBFβ or high-glucose stimulation.132 In studies investigating DM, it was observed that high glucose levels increased TGBFβp expression in renal proximal tubular epithelial cells by activating TGBFβ. This coincided with findings of a high glucose-stimulated increase in collagen and fibronectin production in mesangial cells and proximal tubular cells, which is mediated by TGBFβ activation.133–135 Although the pathologic consequences of increased TGBFβp in the proximal tubules remains unclear, it may play an
important role in the degree of renal interstitial fibrosis, which is closely correlated with a progressive decline in renal function in DM.\textsuperscript{122,136,137} To date, no studies have analyzed TGF\textbeta{} 1 and TGF\textbeta{} 1p in the diabetic cornea, but TGF\textbeta{} 1 has been shown to be upregulated \textit{in vitro} in the human corneal epithelial cell line in response to TGF\textbeta{}.\textsuperscript{128} Given these findings, it is possible that TGF\textbeta{} 1p may also be increased in the cornea in response to high glucose, promoting TGF\textbeta{}-mediated corneal thickening and fibrosis. Additionally, it has been suggested that TGF\textbeta{} 1p mutations occur in a genotype-phenotype fashion, in which various mutations account for the different degrees of phenotypic severity seen in KC and corneal dystrophies.\textsuperscript{139} Thus, the high glucose environment seen in DM could result in a variation on the phenotypic spectrum of TGF\textbeta{} 1p mutations and/or epigenetic modifications.

### 4.4. ZEB1

The zinc finger E box-binding homeobox 1 (ZEB1), also known as transcription factor \textalpha{} (TCF\textalpha{})\textsuperscript{139,140} or \textalpha{}F1,\textsuperscript{141} can function as either a transcriptional enhancer or repressor for different genes.\textsuperscript{142} ZEB1 has been implicated in the regulation of type I collagen expression, particularly in osteoblasts,\textsuperscript{143} and repression of the epithelial phenotype.\textsuperscript{140,144,145} It has been identified as a potent epithelial to mesenchymal transition (EMT) activator and stimulator of angiogenesis in tumor biology studies,\textsuperscript{145-147} as well as a regulator of TGF\textbeta{} signaling with its counterpart, ZEB2.\textsuperscript{148}

Mutations in ZEB1 have been reported in posterior polymorphous corneal dystrophy (PPCD),\textsuperscript{139,140,149} Fuch's endothelial corneal dystrophy (FECD),\textsuperscript{127,150,151} and keratoconus.\textsuperscript{152} Reduced expression of COL4A3 in corneal epithelial cells in PPCD, implicating COL4A3 as a possible target of ZEB1 regulation.\textsuperscript{140} In contrast, a missense ZEB1 mutation resulted in markedly reduced COL4A1, COL4A2, and COL4A3 expression in corneal kerato-cytes.\textsuperscript{152} Reduced expression of COL4A1 and COL4A3 has also been reported in KC.\textsuperscript{29,153} However, further work is required to determine the role of COL4A1/COL4A3 and how they may be regulated by ZEB1 in KC. Based on current findings, Lechner et al.,\textsuperscript{133} suggested that missense mutations in ZEB1 are associated with FECD and KC, while protein truncating mutations result in PPCD.\textsuperscript{132} This is supported by the finding of unique mutations in a family with both KC and FECD, as well as a patient with triple corneal dystrophy consisting of KC, FECD, and epithelial basement membrane corneal dystrophy.\textsuperscript{152,154}

In efforts to better understand ZEB1's role in wound healing and angiogenesis, it was recently found that persistent hyperglycemia, as seen in DM, potently induced ZEB1 expression in human dermal microvascular endothelial cells (HMEC).\textsuperscript{155} This corresponded with increased ZEB1 expression in laser capture microdissection endothelial tissue obtained from the wounded edge of diabetic wound patients.\textsuperscript{155} In Singh et al.,\textsuperscript{19} immunoprecipitation-mass spectrometry was performed to gain further mechanistic insights into the differential action of ZEB1 under normoglycemic and hyperglycemic conditions, thus revealing putative proteins that physically associated with ZEB1.\textsuperscript{155} It was found that hyperglycemia diminished the physical association of ZEB1 with E-cadherin, resulting in a loss of control over E-cadherin repression which is known to cause the microvascular endothelial dysfunction commonly observed in DM.\textsuperscript{155,156} Additionally, hyperglycemic conditions impaired the binding of several pro-inflammatory proteins, suggesting that alterations in cellular ZEB1 may contribute to the inflammation seen in DM.\textsuperscript{155}

A different study analyzed the role of long noncoding RNA (IncRNA) ZEB1 antisense 1 (ZEB1-AS1) in DM, as it has been shown to increase ZEB1 expression.\textsuperscript{157,158} By increasing ZEB1 expression, ZEB1-AS1 is thought to play an antifibrotic role in DM through modulation of EMT, which is considered to be the main pathogenic factor of renal fibrosis.\textsuperscript{158-160} Wang et al.,\textsuperscript{161} 2018 confirmed this in ZEB1-AS1 knockdown studies, which increased high glucose-induced ECM accumulation by downregulation of ZEB1 expression, resulting in renal fibrosis.\textsuperscript{161} This was investigated further by Meng et al.,\textsuperscript{2020} who reported that ZEB1-AS1 was down-regulated in kidney tissues of DM patients as well as hyperglycemic-induced HK-2 cells.\textsuperscript{162} Furthermore, ZEB1-AS1 improved the high glucose-induced EMT and fibrogenesis by mediating miR-216a-5p and BM7.\textsuperscript{163} While the exact mechanisms are beyond the scope of this review, it is clear that there is a very complex mechanism of regulation surrounding ZEB1. It is important to study the role of ZEB1 in the cornea of DM patients as it may have the potential to alter the expression of inflammatory cytokines and disrupt corneal endothelial/epithelial structure under hyperglycemic conditions.

### 4.5. MMP-9 and TIMP-1

MMPs are a family of 24 zinc-dependent proteases involved in multiple physiological processes including tissue remodeling and the degradation of ECM.\textsuperscript{164-166} The activity of the MMPs is balanced by four inhibitory proteins, the tissue inhibitors of metalloproteinases (TIMPs).\textsuperscript{164-166} Multiple MMPs have been implicated in the pathogenesis of KC, including MMP-1, MMP-2, MMP-3, MMP-7, and MMP-13.\textsuperscript{164-167} MMP-9, also known as gelatinase \textalpha{}B, is among the most well-studied in relation to KC.\textsuperscript{164} Multiple studies of tear composition have shown increased levels of MMP-9 protein in KC, including one patient with asymmetrical KC in which MMP-9 was upregulated only in the tears of the affected eye.\textsuperscript{168-170} This increase in MMP-9 was confirmed with an accompanying upregulation of MMP-9 mRNA in the corneal epithelium.\textsuperscript{170} Interestingly, the MMP-9 mRNA in KC patients was significantly higher in cells from the cone apex as compared to the corneal periphery, which may contribute to the focal structural weakness of the cornea.\textsuperscript{171} An additional study revealed an increase in MMP-9 protein level in the blood of KC patients compared to controls.\textsuperscript{172}

TIMP-1 exhibits a unique binding interaction with MMP-9, as it usually exhibits a high level of coordinated expression with MMP-9, is frequently secreted as a TIMP-1/MMP-9 complex, and binds MMP-9 with high affinity.\textsuperscript{172} Recent studies revealed a decrease in the levels of TIMP-1 in KC corneas.\textsuperscript{173,174,175} Taken together, the increased MMP-9 and decreased TIMP-1 activity seen in KC may reflect in an imbalance of proteolytic activity, thus contributing to ECM degradation and corneal thinning. This is reinforced by the analyses of genetic polymorphisms in MMP-9 and TIMP-1 in KC patients that were associated with findings of higher MMP-9 and lower TIMP-1 activity in KC tear samples.\textsuperscript{176} It is important to note that the TIMP-1 SNP was only associated with increased KC risk in females.\textsuperscript{177} Given that TIMP-1 is located on the X chromosome,\textsuperscript{177} this likely accounts for differences in gender susceptibility.

In regards to DM, studies have indicated that elevated glucose levels also disrupt the MMP/TIMP balance in macrophages and endothelial cells, primarily through an amplification in MMP expression and activity.\textsuperscript{179} Takahashi et al.,\textsuperscript{2000} reported enhanced MMP activity in human corneal epithelial cells under hyperglycemic conditions as well as increased MMP-9 activity in the cornea of diabetic rat models.\textsuperscript{180} In support of this finding, two studies reported: 1) increased MMP-9 and TIMP-1 protein levels in the tears of pediatric T1DM patients along with 2) increased MMP-9 activity in tears of T2DM patients.\textsuperscript{181,182} Additionally, genetic polymorphisms in MMP-9 have been associated with susceptibility to T2DM and diabetic nephropathy.\textsuperscript{183-185} However, no genetic association has been identified with TIMP-1 and DM in patients.\textsuperscript{186} The role of TIMP-1 in DM remains inconclusive. Taken together, differences in the imbalance of MMP-9/TIMP-1 activity may contribute to different degrees of ECM remodeling in the cornea of KC and DM patients.

### 4.6. MIR184

MicroRNAs (miRNAs) bind to the complementary sequences located in the 3'-UTR region of the target genes, resulting in degradation of the mRNA or suppression of translation.\textsuperscript{186} MIR184, in particular, encodes for...
miR-184, which is expressed in the central corneal epithelial cells and the lens epithelium.187–189 It is the most abundant miRNA in both the cornea and the lens and it is known to competitively inhibit the binding of miR-205 to the mRNA of the inositol polyphosphate phosphatase-like 1 gene (INPP1L, also known as SHIP2).185,190 This neutralizes the inhibitory activity of miR-205 on INPP1L and subsequently downregulates the Akt pathway, which has been shown to markedly increase keratinocyte apoptosis and cell death.191 Interestingly, this situation is unique to the corneal epithelium as it is the only known epithelium that exhibits overlapping expression of miR-184 and miR-205.189,190 Additionally, miR-184 has been shown to regulate differentiation of human-induced pluripotent stem cells into corneal epithelial-like cells.191,192

Given its role in the cornea, it is not surprising that mutations in miR-184 may lead to KC. To date, multiple studies have identified a heterozygous mutation in MIR184 in a Northern Ireland family with KC and cataracts,197 a family with EDICT (endothelial dystrophy, iris hypoplasia, congenital cataract, and stromal thinning) syndrome,193 and an European Spanish family with various corneal abnormalities including severe KC.194 However, the lack of mutations in KC patients from Iran,195 Turkey,195 and Saudi Arabia196 suggests a limited role of MIR184 in KC pathogenesis.196

Specific miRNAs have also been associated with T2DM cellular processes, including apoptosis, response to cytokines, and insulin secretion.197 miR-184, in particular, has been identified as an important modulator of compensatory pancreatic β-cell expansion during insulin resistance.198–200 Generally, the expression of miR-184 is increased in islet cells in periods of fasting, demonstrating an active role in pancreatic β-cells as the glucose levels decrease.201 Moreover, the miR-184 expression levels have been shown to decrease in the presence of increasing extracellular glucose.201 Together, this highlights the potential role of miR-184 in glucose metabolism. Furthermore, its expression is strongly decreased in the pancreatic islet cells of insulin-resistant mouse models and human patients with T2DM.202 miR-184 expression and activity needs to be further studied in the DM cornea. Given its regulation by glucose levels in pancreatic islet cells, it is possible that miR-184 may be similarly downregulated by high glucose levels in the DM cornea, thus inhibiting cellular apoptosis and resulting in corneal thickening.

5. Inflammation and oxidative stress in the cornea

Although KC has traditionally been described as a noninflammatory degenerative condition, there is emerging evidence suggesting that inflammation within the epithelium and stroma are involved in the pathogenesis of KC.5 Multiple studies have observed significant increases in proinflammatory molecules such as IL-6, IL-4, IL-5, IL-8, and IL-12 in KC compared to controls.203 Additionally, KC keratoocytes have been reported to express more IL-1α receptors,204 which may trigger keratocyte apoptosis since IL-1α is a proinflammatory cytokine.204

In parallel, several studies have found that oxidative stress is involved in the development and progression of KC.5,205–207 KC corneas have exhibited abnormalities such as increased levels of inducible nitric oxide synthase, nitrotyrosine, malondialdehyde, and glutathione S-transferase,208 as well as decreased activities of extracellular superoxide dismutase.209 This is supported by multiple observations of KC corneas and fibroblasts exhibiting increased levels of ROS and relatively greater mitochondrial DNA damage as compared to controls.209,209,216 Interestingly, the combination of oxidative stress and hyperglycemia, particularly in T2DM, accelerates AGE formation.210 Thus, with increased AGE accumulation, this may create the potential for increased inflammation, enhanced production of ROS, and impairment of DNA repair mechanisms211 in the DM cornea as well.

The increase in inflammatory markers and ROS within the KC cornea may be the result of environmental stimuli such as eye-rubbing and external oxidants such as UV light, which drives the pathological thinning of the stroma.212 DM itself is an inflammatory systemic disease, so we wanted to explore its effects on the KC-associated genes. In this section, we focused on HGF, CAST, SOD1, and IL1A/IL1B and their roles in DM and KC pathophysiology (Table 2).

5.1. HGF

Hepatocyte growth factor (HGF) is a pleiotropic growth factor that binds to its receptor, mesenchymal-epithelial transition factor (c-Met/Met) and activates multiple downstream pathways, including MAPK, P38-Akt axis, and activators of transcription (JAK/STAT) pathways.213 It is primarily involved in cell proliferation and migration, particularly in corneal epithelial cells, as well as inflammatory-related signaling cascades.214,215,216 Injured corneas have exhibited increased HGF and c-Met mRNA expression during corneal wound healing.217–219

The genetic association between HGF variants and KC susceptibility was identified with several HGF SNPs in the European and Australian cohort studies.200–217,218 Given that all the currently identified SNPs are located in a noncoding region upstream of HGF, it was suggested that they regulate gene expression by way of RNA splicing, transcription factor binding, and miRNA regulation.219 Additionally, there is recent evidence of increased HGF protein in the corneal epithelium compared to control corneal epithelium.220 This suggests that poorly regulated and overexpressed HGF may have detrimental effects on the ECM due to inflammation in KC.

In diabetic corneas, both ex vivo and organ culture, HGF expression was noted to be increased with a corresponding decrease in HGF receptor c-Met expression.220 This suggests a disruption in the HGF/c-Met system, such that there is reduced cell migration and poor epithelial healing, which is characteristic of diabetic corneas.221,222 This hypothesis is supported by the finding that overexpression of c-Met in diabetic corneas resulted in restoration of nearly normal epithelial wound healing times.223 In other DM studies, HGF has been shown to play a role in the metabolic flux of glucose, manage β-cell homeostasis, and modulate the inflammatory response.223 Thus, it is possible that reduced receptor c-Met expression in DM corneas could serve a protective role against the inflammatory response mediated by HGF.

5.2. CAST

CAST encodes calpastatin, an inhibitor of calpains. CAST is a calcium-dependent cysteine protease that is involved in a variety of cellular processes, including proliferation, apoptosis, and cell migration.225 The calpain/calpastatin system is present in the corneal epithelium where it is suspected to play a role in epithelial cell turnover and wound healing.226,227 It has also been localized to corneal endothelial cells and fibroblasts.226,228 Genotyping of both Caucasian and Han Chinese patients with KC revealed a consistent association between variants near or in CAST, with KC susceptibility.228,229

In DM, calpain activity has been shown to be increased in vascular endothelial cells in response to excess glucose.127,230–232 Calpain may also play a role in mitochondrial ROS generation, such that it contributes to diabetic vascular injury by way of vascular inflammation232,234 and glucose-induced apoptosis in endothelial cells.235,236 This is supported by the finding that genetic inhibition of calpain through over-expression of calpastatin reduces vascular ROS production.236 Increased glucose-induced calpain activity has also been shown to initiate vascular endothelial dysfunction by inactivating prostacyclin (PGI2), as over-expression of endogenous calpastatin inhibited this effect.233 A similar mechanism may be observed in DM corneas, as calpain is activated by high-glucose levels. Analyzing the expression and activity levels of CAST in KC and DM corneas will give us further insight into this pathway.

5.3. SOD1

Superoxide dismutase 1 (SOD1) encodes a copper and zinc-dependent cytoplasmic enzyme that is directly involved in the antioxidative
processes associated with ROS elimination and reduction of oxidative stress in the cornea.205 It has been shown to localize to the corneal epithelium, endothelium, and keratocytes.206 Given that there have been increased levels of oxidative stress markers in the KC cornea, several studies have suggested that mutations in SOD1 might be involved in development of KC.83,237 A specific SOD1 deletion was detected in two non-related American families with an autosomal dominant form of KC as well as in the Greek and Brazilian KC population.238–240 Mutational analyses revealed that this deletion excluded the SOD1 protein active site, which suggests a loss of enzyme function.241 Although the specific SOD1 deletion has had a low frequency in cohort studies, the fact that it has been identified in various populations suggests that it might serve as a potential genetic component in the development of KC. This is supported by the finding of suppressed levels of SOD1 expression in KC corneal fibroblast cultures as compared to controls.242 However, this is a controversial topic as other studies have refuted SOD1’s involvement as there was not enough evidence in mutational analyses.243

Mutations in the SOD1 gene has also long been associated with amyotrophic lateral sclerosis (ALS). It is generally accepted that mutations in SOD1 results in conformational instability of the protein, resulting in the formation of SOD1 aggregates that exert a cytotoxic effect in motor neurons, which then results in the progressive degeneration of motor neurons observed in ALS.244 Interestingly, ALS has recently been shown to share several genetic pathways with DM.245 Additionally, several genetic variations of SOD1 polymorphism have been associated with diabetes and diabetic complications.246,247 One study implicated a role for AGE/RAGE signaling in DM-mediated vascular calcification through activation of Nox-1 and decreased expression of SOD1, which increased oxidative stress.248 Recently, a separate study investigated ocular surface damage in diabetic mice and found an accumulation of ROS, increased expression of RAGE, and decreased SOD1 expression in the cornea.249 This study also reported that topical treatment with pigment epithelium-derived factor (PEDF) was shown to improve corneal epithelial damage by decreasing RAGE and increasing SOD1 expression.250 Together, this suggests that SOD1 may play an important role in alleviating the oxidative stress seen in corneal pathology. SOD1’s role in KC pathogenesis is currently under debate, but it is possible that increased AGE/RAGE activity in the DM cornea results in decreased SOD1 expression.

5.4. IL1A and IL1B

Interleukin (IL)-1 is a proinflammatory cytokine involved in various cellular activities, including cell proliferation, differentiation, and apoptosis.251 The IL-1 family includes two proinflammatory cytokines, IL-1α and IL-1β, and the IL-1 receptor antagonist (IL-1Ra), encoded by IL1A, IL1B, and IL1RN, respectively.252 IL-1 has been shown to upregulate keratocyte expression of collagenases, metalloproteinases, and other enzymes involved in collagen remodeling during corneal wound healing.253,254 Early studies detected increased keratocyte apoptosis in KC corneas and suggested that it might be triggered by increased basal IL-1 release.240,241 This is supported by the finding of increased IL-1α binding sites in KC corneal fibroblasts compared to control corneas.203 However, genetic association analyses for polymorphisms in IL1A and IL1B in KC have remained controversial. Genetic associations with KC have been identified with variants in IL1B in the Han Chinese,255 Korean,256 and Japanese KC population,257 but the involvement of IL1A was only observed in the Han Chinese KC population.203 Furthermore, no genetic associations with variants in or near IL1A or IL1B were observed in the Turkish population.258 To date, it is still unclear how the mRNA expression levels of IL1A and IL1B relates to the expression of cytokines IL-1α and IL-1β in KC pathophysiology.

The IL-1 family of cytokines has also been implicated in DM and DM-related complications by way of inflammation.259–262 To better understand the role of IL-1 in the diabetic cornea and corneal wound healing, a recent study utilized a genome-wide cDNA array analysis in normal and DM mouse corneas.263 This study by Yan et al., 2016 reported upregulation of IL-1β expression in the healing corneal epithelium of both normal and DM corneas, with no difference in IL-1α expression.264 There was a corresponding increase in IL1RN expression in the normal cornea, but a decrease in the DM cornea, suggesting a disturbed balance of IL-1β to IL-1Ra.265 This disruption in IL-1Ra signaling had multiple adverse effects in corneal wound healing, including suppression of proinflammatory cytokine/chemokine expression and a decrease in the overall early inflammatory response to wounding in DM mouse corneas.266 Interestingly, increased production of IL-1β has also been observed in macrophages in response to prolactin stimulation.267 Given that prolactin-induced protein has recently been suggested to be a novel biomarker for KC,268,269 this is an intriguing association between the balance of IL-1β to IL-1Ra signaling and hormonal influences.

A separate cross-sectional analysis reported increased levels of IL-1Ra in patients with prevalent DM or metabolic syndrome.262 It was suggested that the increased IL-1Ra expression levels predicted the progression of metabolic syndrome to clinically incident diabetes, independently of CRP and other risk factors.262 This study also revealed genetic variants in IL1A, IL1B, and IL1RN that may have gender-specific associations with DM.262 Further studies will be required to better understand how the genetic variants in IL1A and IL1B influence expression of IL-1α and IL-1β and whether there is differential regulation of the IL-1 family in KC and DM.

6. Additional genes of interest

Multiple other genes have been associated with CCT and KC pathophysiology, including but not limited to SPRY2 and COL4A3/ COL4A4.48,49 (Table 2). Here, we discuss how these genes may potentially have overlapping or differing roles in KC and DM pathogenesis.

6.1. SPRY2

The Sprouty family consists of four members, SPRY1-4. All are direct targets and negative feedback regulators of fibroblast growth factor (FGF) signaling,272–274 playing important roles in the early development of multiple.275-277,278 SPRY2, in particular, has been shown to modulate the apoptotic actions induced by pro-inflammatory cytokine, TNF-α.277 Kuchara et al., 2011 revealed an important role for Spry1 and Spry2 in the regulation of lens vesicle separation and corneal epithelial proliferation in mouse models.278 This study generated Spry1;Spry2 double-null mutants and observed increased corneal epithelial proliferation and an inhibition in terminal differentiation.279 A later study revealed that eyelid closure was impaired due to increased proliferation of conjunctival epithelial cells in Spry conditional knock-out mutants.279 This suggests that Spry-1 and Spry-2 normally suppress ectopic growth in the corneal epithelium. A recent GWAS identified SPRY2 as a novel candidate gene significantly associated with CCT inter-individual variation.280 Although no studies have analyzed SPRY2 in KC pathogenesis, it is possible that it may contribute to alterations in the corneal epithelium.

Recently, a variant near SPRY2 was found to be associated with increased susceptibility to T2DM in both the Han Chinese and Japanese population.285,286 SPRY2 KO in human hepatocyte cells resulted in increased glucose intake, suggesting a possible role for SPRY2 in glucose metabolism in hepatocytes.287 This same study also reported the upregulated genes in SPRY2 KO cells to be involved with DNA replication and cell cycle regulation, which is consistent with its established role in inhibition of cellular proliferation.282 Taken together, deletions in SPRY2 may have pathologic effects in both the cornea and metabolic homeostasis.

6.2. COL4A3/COL4A4

COL4A3 and COL4A4 both encode for collagen type IV, the major structural component in epithelial and endothelial basement membranes
Collagen type IV mutations have been implicated in a variety of clinical manifestations, including Alport syndrome and PPCD. Both genes have been reported to be differentially expressed in KC corneas, suggesting a role for collagen type IV in KC pathogenesis. However, in genetic analyses of COL4A3 and COL4A4, no pathogenic variants were identified in KC patients, although common polymorphisms are present in the affected and healthy populations. Thus, the role of collagen type IV mutations in KC pathogenesis is unclear.

Collagen type IV is also a major structural component of the glomerular basement membrane (GBM), and alterations in the collagen composition have been implicated in the pathogenesis of nephropathy in DM. GWAS meta-analysis of T1DM revealed a SNP rs55703767 that resulted in thinner GBM in patients with DM but was protective against renal complications. Interestingly, Onochie et al., 2020 reported that hypoxia induced a reduction in laminin and collagen type IV in the cornea, which resulted in a delay in wound healing and increased corneal stiffness. This was in parallel to the finding of impaired wound healing and a decrease in laminin along the basal lamina in the diabetic cornea. Given that diabetes can cause a transition to a hypoxic state, this suggests a potential role for collagen type IV in the induction of increased corneal stiffness that has been reported in DM. Further studies will be needed to see if collagen type IV mutations have a potential effect on the DM cornea and to better understand how hypoxia affects collagen type IV.

7. Discussion

Multiple retrospective epidemiological studies have suggested that patients with DM have reduced risk in the development and/or severity of keratoconus (Table 1). Seiler et al., 2000 was among the first to report this protective role of DM in a retrospective case-control study of the German population. This finding was later confirmed by Naderan et al., 2014 in the Iranian population, and Kuo et al., 2006 in the United States population. One limitation of the above studies is that the data was extracted from the hospital/clinic population. Woodward et al., 2016 addressed this by conducting a retrospective longitudinal population-based cohort study in the United States. With a larger...
sample size, Woodward et al., 2016 found that there were 20% lower odds of KC development with uncomplicated DM and 52% lower odds of KC development with DM-associated complications.18

A common working hypothesis in support of this observation is that chronic hyperglycemia promotes glycosylation of corneal fibers and induces endogenous collagen crosslinking within the corneal stroma, thus preventing the biomechanical weakening of the cornea.14,19,24 Although the cornea is an avascular structure, previous reports have indicated that corneas in diabetic patients are still exposed to increased glucose concentration,29,29,34 corneas are still exposed to increased glucose concentration,29,29,34 which supports this hypothesis. Another working hypothesis is that DM disrupts corneal endothelial cell function, resulting in stromal edema and increased CYP.15,17,29,29 (Fig. 1).

This remains a debated topic as other studies have either 1) suggested a positive association between KC and DM or 2) did not identify a significant correlation (Table 1).14,19,29 For example, Kosker et al., 2014 conducted a retrospective case-control and cross-sectional study in the United States population and reported a higher prevalence of T2DM in the KC population as well as greater severity of KC in DM patients.29 However, this was clinic-based, and the prevalence of DM in clinic populations as compared to the general population may account for differences in these findings. Subsequently, Moon et al., 2020 conducted a retrospective cohort population-based study in the Korean population and they also reported a higher prevalence of T2DM in the KC population with a positive association of KC and DM.21 In contrast, Bak-Nielsen et al., 2019 and Lee et al., 2020 conducted a retrospective cohort study in the Danish and Korean population, respectively, and found no significant differences in DM prevalence in KC patients. In efforts to consolidate the findings, Hashemi et al., 2020 conducted a meta-analysis and reported that although the odds of developing KC were 23% lower, this relationship was not significant. A limitation of this meta-analysis is that all studies included were either cross-sectional or case-control studies, which can be more prone to information and selection bias compared to cohort studies.29 Thus, the variations in study designs likely contribute to differences in findings.

The multiple effects of DM on the cornea are complex and have been reviewed elsewhere.16,26,30,301 These DM effects include, but are not limited to, keratopathy, neuropathy, inflammation, alterations in collagen fibrils, endothelial cell loss, and increased glucose in the aqueous humor.16,29,29 Patients with DM are often predisposed to ocular surface complications such as dry eye, recurrent corneal erosions, and bacterial infection.26,30,304 However, the mechanism of correlation between DM and KC pathogenesis remains unclear. In efforts to better understand KC pathophysiology, multiple candidate and genome-wide association studies have identified the genetic and metabolic components involved in KC.48,49,106,305 Additionally, a recent review outlined important biological and chemical pathways related to collagen crosslinking in DM and KC.26

In terms of corneal biomechanics, KC is characterized by localized stromal corneal thinning and reduced CCT, while DM generally causes the cornea to become stiffer with increased CCT and/or CH.15,34,35,27 This is likely reflected by changes in the collagen composition of the cornea, as outlined by McKay et al., 2019. Two genes, LOX and COL5A1, may play important roles in corneal collagen crosslinking. Current evidence suggests that overall LOX activity (including LOX and LOXL1 proteins) is decreased in KC, leading to impairment of lysyl oxidase-mediated crosslinking and weakening of the cornea.15,34,27. In contrast, hyperglycemic conditions have been shown to upregulate LOX expression and activity in retinal cells.15,34,27 If a similar effect were seen in corneal cells under hyperglycemic conditions, this could explain the reduced risk of KC development in DM patients. Given that COL5A1 reached genome-wide significance in KC patients, and that patients with COL5A1 haploinsufficiency exhibited corneal thinning, we discussed whether COL5A1 shared similar genetic significance in DM corneal pathology. To our knowledge, no studies have analyzed DM’s direct effects on COL5A1 in the cornea. A recent study did find differential collagen composition with elevated levels of collagen types I and type III, but not collagen type V, in the human corneal stroma of DM patients. This finding suggests that hyperglycemia may induce collagen modulation in an isoform-dependent manner. Further studies are needed to better understand the effects of hyperglycemia and increased AGE-mediated crosslinking on the various collagen isoforms in DM corneas, and to determine whether sequence variants in or near COL5A1 are associated with KC and DM cornea pathology.

Altersations in corneal biomechanics is also characterized by disruption in the ECM composition of the cornea. Thus, we explored several genes that play a role in ECM remodeling and were identified in KC and CCT-associated GWAS. This includes several transcription factors including FOXO1, SMAD3, TGFβ1, and ZEB1. It became readily apparent that the investigation of these genes is lacking in the DM cornea, but many exhibited an association with AGE-mediated signaling and differential expression under hyperglycemic conditions in other tissues. This suggests that a similar effect could be seen in the DM cornea, but further research studies will be needed to confirm this. TGFβ signaling would be worth exploring further in the KC and DM cornea, as it has been associated with alterations in SMAD3, TGFβ1p and FOXO1 signaling. For example, increased TGFβ signaling and SMAD3 activity has been suggested to increase ECM deposition in mesangial cells, leading to the development of DN. This, in combination with an imbalance in MMP-9/TIMP-1 activity, may contribute to different degrees of ECM remodeling in the cornea of KC and DM patients. Additionally, MIR194 has a very unique role in the remodeling of the corneal epithelium. Given its downregulation by high glucose levels in pancreatic islet cells, MIR194 may potentially link elevated glucose levels and alterations in corneal epithelial cell apoptosis in the DM cornea.

Interestingly, there was genetic overlap in several inflammatory proteins and regulators of oxidative stress in both KC and DM. Although KC was previously described as a noninflammatory condition, it has been suggested that external environmental factors such as excessive eye rubbing may induce an increase in inflammatory markers, contributing to KC pathogenesis. Likewise, DM has been associated with low-grade inflammation that is thought to contribute to insulin resistance observed in T2DM.306 In this review, we closely examined the role of HGF, CAST, SOD1, and IL1A/IL1B. While HGF’s role remains unclear, the imbalance in HGF and HGF receptor c-Met expression in DM corneas is intriguing, which could lend towards a protective role against inflammatory degradation in the ECM of DM. We found that SOD1 likely plays a similar role in reducing oxidative stress, as decreased SOD1 expression has been suggested in both KC and DM. Further studies into the calcin/calpain system, as well as the ratio of IL-1α/IL-1β to IL-1α/IL-1β, will be needed before an association can be made between DM and KC. It is possible that imbalances in both systems may contribute to the differing clinical pathology observed in the cornea.

Last but not least, multiple other genes have been associated with KC pathophysiology, but their roles in DM are unclear. We highlighted a few remaining KC candidate genes, including SRP2Y2 and COL4A3/COL4A4. However, much more extensive studies are required before correlations can be made with DM. We suggest exclusively studying the DM cornea with a focus on KC-associated genes and analyzing the expression patterns of associated proteins.

From a clinical perspective, the age of onset for KC often ranges from the teenage years to the 30s and 40s while the age of onset for type 2 DM ranges from teenage years to the 70s.12,27,107,305 Type 2 DM is becoming increasingly prevalent in children and adolescents worldwide, including the US. The average age of diagnosis for children and adolescents in the US is 14 years.307 The incomplete overlap in age-onset between KC and DM may complicate efforts in interpreting the potential protective effect of DM on KC. This raises an important question to all the reported studies: what is the distribution of DM age-onset for those with KC or without KC? It will be more beneficial to only include DM patients with age-onset less than 30–40 years.
8. Conclusions

Despite the conflicting literature evidence between KC and DM, our comprehensive discussion explained whether and how DM is associated with KC and how DM may function as a protective mechanism (Fig. 1). At this time, there is more evidence to support the protective role of DM in KC patients. If true, we hope to identify potential localized therapeutic targets in KC management that act by strengthening the KC cornea through similar mechanisms that may alter the DM cornea. One such example would be targeting LOX in the KC cornea and increasing its expression such that LOX-mediated collagen-crosslinking is increased, thus resulting in corneal thickening. Additionally, by targeting inflammatory cascades such as the calpain/calpastatin system and the IL-1α/IL-1β to IL-1Ra ratio, we can then potentially halt the inflammatory-mediated corneal thinning seen in KC. To conclude, by analyzing potential genetic associations in KC and DM, this review illustrates the areas where the current literature is lacking, with the hope of providing direction for future studies in elucidating the pathophysiology of KC and DM in the cornea. Future research would have tremendous benefit in identifying potential therapeutic targets in clinical management of KC.

9. Literature search

A comprehensive literature search completed by the end of September 2020 was performed on PubMed. All selected articles were reviewed thoroughly by the authors to consolidate candidate genes that have been identified in genetic analyses and genome wide studies of keratoconus and central corneal thickness variations. We then explored how those respective genes may be similarly or differentially regulated under hyperglycemic conditions and the role they play in the systemic complications associated with diabetes.

Study Approval

Not applicable.

Author Contributions

Kristin M. Ates: Methodology, Investigation, Writing – Original Draft, Writing - Review & Editing. Amy J. Estes: Writing - Review & Editing. Yutao Liu: Conceptualization, Writing - Review & Editing, Supervision, Funding Acquisition.

Acknowledgements

We acknowledge the support of The Graduate School of Augusta University and the Medical College of Georgia.

Funding

This work was supported by the National Institutes of Health [grant numbers R01EY023242, R21EY028671, and P30EY031631]; and the startup fund from the Medical College of Georgia at Augusta University, Augusta, GA, USA.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work. reported in this paper.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ALS          | amyotrophic lateral sclerosis |
| AGE          | advanced glycation end product |
| CAST         | calpastatin |
| CCT          | central corneal thickness |
| CH           | corneal hysteresis |
| CRF          | corneal resistance factor |
| DM           | diabetes mellitus |
| DN           | diabetic nephropathy |
| DR           | diabetic retinopathy |
| ECM          | extracellular matrix |
| EDS          | Ehler-Danlos syndrome |
| EMT          | epithelial to mesenchymal transition |
| FEDD         | Fuchs’ endothelial corneal dystrophy |
| FOXO1        | forkhead box protein O1 |
| GBM          | glomerular basement membrane |
| GWAS         | genome-wide association studies |
| HGF          | hepatocyte growth factor |
| IHC          | immunohistochemistry |
| KC           | keratoconus |
| lncRNA       | long noncoding RNA |
| LOX          | lysyl oxidase |
| miRNA        | microRNA |
| MMP          | matrix metalloproteinases |
| PPCD         | posterior polymorphous corneal dystrophy |
| RAGE         | receptor for advanced glycation end products |
| SOD1         | superoxide dismutase 1 |
| TIMP         | tissue inhibitors of metalloproteinases |
| TGFBI        | transforming growth factor beta-induced |
| TGFBIp       | transforming growth factor beta-induced protein |
| VSX1         | visual system homeobox 1 |
| ZEB1         | zinc finger E box-binding homeobox 1 |

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