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Anterior Pituitary, Sex Hormones, and Keratoconus: Beyond traditional targets

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Abstract

"The Diseases of the Horny-coat of The Eye", known today as keratoconus, is a progressive, multifactorial, non-inflammatory ectatic corneal disorder that is characterized by steepening (bulging) and thinning of the cornea, irregular astigmatism, myopia, and scarring that can cause devastating vision loss. The significant socioeconomic impact of the disease is immeasurable, as patients with keratoconus can have difficulties securing certain jobs or even joining the military. Despite the introduction of corneal crosslinking and improvements in scleral contact lens designs, corneal transplants remain the main surgical intervention for treating keratoconus refractory to

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medical therapy and visual rehabilitation. To-date, the etiology and pathogenesis of keratoconus remains unclear. Research studies have increased exponentially over the years, highlighting the clinical significance and international interest in this disease. Hormonal imbalances have been linked to keratoconus, both clinically and experimentally, with both sexes affected. However, it is unclear how (molecular/cellular signaling) or when (age/disease stage(s)) those hormones affect the keratoconic cornea. Previous studies have categorized the human cornea as an extragonadal tissue, showing modulation of the gonadotropins, specifically luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Studies herein provide new data (both *in vitro* and *in* vivo) to further delineate the role of hormones/gonadotropins in the keratoconus pathobiology, and propose the existence of a new axis named the Hypothalamic-Pituitary-Adrenal-Corneal (HPAC) axis.

Keywords

Eye; Keratoconus; Gonadotropins; Sex Hormones; Biomarkers

1. CORNEAL ANATOMY AND BIOMECHANICS

The human eye is part of the sensory nervous system, reacting to light before converting it into what we call vision. The eyes are supported by six extraocular muscles that orchestrate eye movement within the orbits, which are bony cavities where the eyes are situated. There are three main components/layers to the human eye: 1) The outermost layers: Cornea and sclera; 2) The middle layers: Choroid, ciliary body, pigmented epithelium, and iris; and 3) The innermost layer: Retina. The three layers are fined tuned to perform all the delicate actions needed for clear vision. Organization and structures are highly specific in all layers, and any disturbance/trauma can result in vision problems. Detailed reviews of the human eye have been previously published to a great extent. All the different eye structures/layers are briefly introduced, for context, in the next section and are summarized in Table 1The human cornea consists of a transparent and avascular connective tissue responsible for refracting light onto the lens (DelMonte and Kim, 2011). A healthy cornea is between 540μm and 560μm thick (Marsich and Bullimore, 2000), has a convex and spherical shape and shows midline interocular symmetry (Durr et al., 2015). The cornea is the part of the eye that is most exposed to the external environment. It is also the most innervated tissue, essential to quickly respond to any adverse external stimuli (Oliveira-Soto and Efron, 2001).

The cornea is a complex structure consisting of five layers: epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium. The epithelium is composed of four to six layers of nonkeratinized, nonsecretory, stratified squamous cells completing the first 50μm thick of the cornea (Almubrad and Akhtar, 2011; Farjo et al., 2018; Li et al., 2020). The integrity of the epithelial layer hinges on corneal epithelial stem cells that preferentially reside in the basal layer of the peripheral cornea in the limbal zone, rather than uniformly in the entire corneal epithelium. Their differentiation process requires 7–10 days before they undergo apoptosis and desquamation (Sridhar, 2018). By this well organized, and timed process, the mass of the corneal epithelium remains constant (Saghizadeh et al., 2017). The acellular Bowman's membrane, composed of type I and type V collagen fibrils is located between the epithelium and the stroma. The exact role of this membrane is unknown,

although linked to several pathologies (Wilson, 2020). The cornea's thickest layer is the stroma, about 450μm of resident cells, known as keratocytes, forming fibers interspersed with collagen type I and type V organized in flat layers called lamellae (Foster et al., 2015; Sridhar, 2018). The strength of the cornea is revealed in the complex organization of the approximately 200 lamellae which lie parallel to one another with their collagen fibrils oriented at angles to the fibrils in adjacent lamellae. The fibrils are densely packed in the anterior 2/3rds where swelling is less likely to occur than in the posterior 1/3. The anterior stromal lamellae bear the most strain when the cornea is healthy or edematous, where the posterior stromal lamellae bear the most strain when the cornea is dehydrated. Strain can be related to factors like intraocular pressure (IOP) or external mechanical factors and due to the heterogeneous biomechanical structure of the cornea, inflammation and ectatic disease can be further exacerbated. The stroma is characteristically transparent due to the extreme organization of the stromal fibers and extracellular matrix (ECM) (Meek and Boote, 2004). The stromal ECM is composed of collagen type I, III, V, and VI and glycosaminoglycans (Almubrad and Akhtar, 2011). A small population of stromal cells displays stem cell-like properties (Pinnamaneni and Funderburgh, 2012). Several lines of evidence suggest that corneal stromal stem cells to be of neural crest line-age and not from bone marrow. Localized in the anterior peripheral (limbal) stroma near to stem cells of the corneal epithelium, the role of these stem cells in stromal wound healing/regeneration is unknown.

The Descemet's membrane is a basement membrane that resides between the stroma and the endothelium and measure approximately 3μm in thickness in children, gradually growing to a thickness of 8–10μm in adults. This membrane is secreted by the endothelium, the innermost layer of the cornea, and contains Collagens Type IV and VIII. Its impairment can lead to significant corneal and visual problems. The endothelium is a thin monolayer cell layer that governs fluid and solute transport across the posterior cornea (Feizi, 2018). The endothelial cells are post-mitotic and rarely divide. Currently, there is no treatment to assist/promote wound healing and/or to confer regenerative capacity of endothelial cells.

2. KERATOCONUS

The first description of keratoconus (KCN) can be traced back to the works of Benedict Duddell, in 1736 (Grzybowski and McGhee, 2013). Duddell described the disease as "The Diseases of the Horny-coat of The Eye", which is the earliest description of what we know today as keratoconus. The study described a 14-year-old boy with "the corneas very prominent, like obtuse cones, which were sufficiently conspicuous". A search on PubMed revealed an exponential increase in manuscripts published on keratoconus (from a handful of studies in late 1800s to almost 4000 the last decade), highlighting its increasing importance.

2.1. Causes and Risk Factors

KCN is a very peculiar disease, in that sex, age, and geographic location prevalence seem to be highly variable depending on the cohort(s) examined. KCN is complex and multifactorial, with severe vision loss if left untreated. Despite decades of significant scientific and medical discoveries, kKCN onset, rate of progression, and underlying pathobiology remains largely

unknown. KCN is known to affect both males and females, with a prevalence that has been increasing steadily, going from 1:2000 back in the 1980s to 1:350 in the 2000s worldwide. Technological advancements and better diagnostics are both partially responsible for the increase seen in prevalence.

A number of contributing factors have been reported including genetics, environmental conditions, eye rubbing, and hormonal imbalances. However, the pathophysiology of KCN remains poorly understood. Though genetic studies for keratoconus have been among the earliest studies, to date, research has thus far been unsuccessful in identifying genetic markers for kKCN. Furthermore, the lack of an accurate animal model for the disease is also a great hindrance to the investigation KCN pathobiology. Several ocular disease associations have been reported, including retinitis pigmentosa (Flanders et al., 1984; Hou et al., 2018), microcornea (Li et al., 2012; Wolter, 1977), corneal degeneration (Kayasawa et al., 1984; krachmer et al., 1984), ectopia lentis (Purwar et al., 2015; Robertson, 1974; Sadiq and Vanderveen, 2013), lenticonus (Moshirfar et al., 2019), macular coloboma (Freedman and Gombos, 1971; Leighton and Harris, 1973), con-rod dystrophy (Fogla and Iyer, 2002), and floppy eyelid syndrome (Donnenfeld et al., 1991; Parunović and Ilić, 1988). Systemic disease associations have also been reported, such as Down Syndrome (Asgari et al., 2020a; Asgari et al., 2020b; Hashemi et al., 2020; Imbornoni et al., 2020; Marsack et al., 2019), Ehlers-Danlos syndrome (Kuming and Joffe, 1977; Robertson, 1974, 1975), mitral valve prolapse (Beardsley and Foulks, 1982; Kalkan Akcay et al., 2014; Lichter et al., 2000; Sharif et al., 1992; Siordia and Franco, 2020), Leber's congenital amaurosis (Hameed et al., 2000; McMahon et al., 2009; Stoiber et al., 2000), Marfan syndrome (Bass et al., 1981), and William-Beuren syndrome (Mediero et al., 2017; Pinsard et al., 2010; Viana et al., 2013). Despite these significant observations, our understanding of kKCN pathobiology remains relatively basic.

The socioeconomic impacts of the disease are harder to compute, but KCN is known to disqualify individuals from securing various jobs as well as joining the military. Worldwide, this disease remains one of the most common indications leading to corneal transplants.

2.2. Symptoms and Diagnosis

KCN is traditionally defined as a cornea disease during which the clear, dome-shaped cornea thins and gradually bulges outward into a cone-shape. As the disease progresses, several symptoms are noticeable by the patients, including: blurring of vision, increased sensitivity to bright light and glare (photophobia), need for frequent changes in eyeglass prescription due to progressive and/or irregular astigmatism, sudden worsening or clouding of vision, and double vision.

2.2.1. Symptoms—KCN progression causes insidious corneal bulging, and thinning with slow-developing symptoms in patients, primarily during adolescence, and progresses until the 4^{th} or 5^{th} decade of life. At that point in life, the disease process in most patients is known to arrest itself for reasons that remain unknown at this time. In mild-moderate stages, patients may initially present with complaints of worsening vision, including rapidly changing refractive astigmatism, both in cylindrical magnitude and axis shift. Irregular

astigmatism, which is defined as refractive astigmatism uncorrectable by glasses or soft contact lenses, is invariably present. The rate of progression also varies among individuals, with a strong association with eye rubbing, especially in unilateral and asymmetrical cases. Given the high association of KCN with atopy, floppy eyelids and allergic disease, patients may also report symptoms of ocular itching, discomfort, tearing, and occasional photophobia. Additionally, patients who use contact lenses may report a worsening history of contact lens discomfort or inadequately improved vision with soft contact lenses, prompting patients to seek out other contact lens options.

Though a near-universal clinical history finding, regardless of ethnic and geographical variance, patients may or may not endorse eye rubbing or sleeping in a prone position with habitual pressure on one eye. Thus, KCN has been classically viewed as a disease in which patients with genetic susceptibility to asymmetrical, biomechanical forces from eye rubbing develop ectatic corneal disease. This is a debatable theory, or at least an incomplete one, since validation of such a concept is almost impossible and relies heavily on patients' assertions.

In rare circumstances, a patient may have undetected KCN until it progresses to an advanced state of thinning known as corneal hydrops, wherein Descemet's membrane ruptures and the corneal stroma swells with fluid. Patients may present with a sudden onset of eye pain and decreased visual acuity. Hydrops typically develops in late-stage disease.

2.2.2. Diagnosis—Diagnosis of KCN remains a challenging and rapidly evolving process that increasingly involves diagnostic imaging modalities, especially in suspect, forme-fruste, and mild disease states. Clinical examination for classical signs of KCN, as described below, remains an invaluable component of diagnosis but may not detect significant pathology until more advanced states. In recent years, an increasing number of imaging devices and techniques have helped clinicians better screen and identify at-risk patients, especially in patients who present for vision correction by laser refractive surgery. Undeniably, KCN diagnosis can be more efficient if a biomarker/gene is discovered and readily detectable.

2.2.2.1. Clinical Refraction: For diagnostic purposes, several aspects of the manifest refraction may alert the clinician towards the diagnosis of KCN. First, a rapidly changing manifest refraction in a young patient, including sphere and cylinder power, may be present. Second, a change in the type of astigmatism corrected by glasses may also herald corneal changes; for example, a shift from with-the-rule astigmatism to oblique astigmatism. Third, a progressive KCN patient may have previously been correctable to 20/20 vision with glasses but may now have decreased vision that is no longer correctable to 20/20 or satisfactory levels. Finally, an overall subjective dissatisfaction reported by the patients, more prominent in one eye, despite multiple refractions and/or glasses prescriptions, may also indicate corneal ectasia.

2.2.2.2. Corneal Topography: Placido-disk based corneal topography, along with corneal tomography (discussed below), is an invaluable tool for detecting KCN as early disease states may demonstrate no clinically observable changes on slit lamp exam. In addition,

corneal topography allows for both qualitative and quantitative assessment of the anterior corneal surface, curvature, and presence of protrusion.

Numerous authors have proposed various topographic parameters that should arouse suspicion or help aid in diagnosis. Unfortunately, at present, there is no single abnormal topography value that absolutely confirms the diagnosis of KCN. Therefore, clinicians use a combination of topographic parameters, along with best-corrected visual acuity and clinical findings, to rule in or rule out evidence of the disease. Several important topography values that merit consideration include:

- Corneal astigmatism > 5D, especially if not aligning with refractive astigmatism.
- Keratometry values > 47D, especially in the presence of oblique astigmatism or skewed axis.
- Maximum keratometry values (Kmax) > 49D.
- Central corneal thickness < 470μm.

In addition to quantitative values, topography allows for a qualitative assessment of corneal symmetry. Patients without KCN may have mild-moderate amounts of astigmatism, including values beyond the cutoff points mentioned above, but usually demonstrate symmetrical (uniform) corneal shape known as a symmetrical bowtie, including with-the-rule, against-the-rule, and oblique astigmatism. All corneal irregularities appear on topography maps with abnormal curvature and asymmetrical appearance, including asymmetrical corneal shape (e.g., inferior > superior steepening, skewed radial axis) and irregular astigmatism, which are highly suggestive of corneal ectasia. For example, a classic criterion is increased risk of corneal ectasia if the inferior corneal steepening is > 1.50D than the superior, or if the superior corneal steepening is >2.50D greater than the inferior (Rabinowitz criteria).

Corneal topography may also help detect the location of the highest amount of corneal steepening, i.e., the location of the displaced corneal apex. This may be found either in the central 5mm or displaced to a peripheral location, most commonly in the inferotemporal quadrant.

2.2.2.3. Corneal Tomography: Corneal tomography includes various devices, using imaging strategies ranging from scanning slit to Scheimpflug imaging to provide a qualitative and quantitative assessment of both the anterior and posterior corneal surfaces. Given that posterior corneal changes often precede anterior surface changes in the early stages of KCN, corneal tomography allows clinicians to assess for early posterior elevation changes and posterior curvature abnormalities for the presence of subclinical disease.

Corneal tomography, in conjunction with corneal topography, can be used in a variety of specially designed grouped testing parameters, such as the Belin-Ambrosio Enhanced Ectasia Display (BAD) and Belin ABCD Keratoconus Display (Pentacam; Oculus GmbH). Advantages of programs like the BAD include amalgamation of imaging results to perform regression analysis, including standard deviation from the mean on measurements of interest. These additionally aid the clinician in detecting subclinical disease, including

patients that warrant close monitoring at regular intervals. The ability to predict onset, or recognize/diagnose the very early signs of KCN, remains unattainable.

Recently, an international coalition described KCN as a disease process characterized by posterior elevation and abnormal corneal thickness distribution (Gomes et al., 2015b)

2.2.2.4. Epithelial Assessment (Epithelial Thickness Mapping): In early disease states, epithelial remodeling may occur adjacent to or overlying the anterior surface changes (Belin and Duncan, 2016; Reinstein et al., 2009). For example, in early stages, epithelial thinning may be present over the apex of the conical changes, typically in the inferotemporal quadrants. This may be due to underlying stromal remodeling in the area of the steepening and protrusion as the cornea attempts to "reshape" early stages of anterior surface irregularities. In the area of the cone apex, subtle epithelial thinning can be seen as compared to the adjacent normal or slightly thickened epithelium Therefore, epithelial thickness mapping (ETM) has recently become a significant area of interest as it can detect patients with early and subclinical disease. Figure 1 demonstrates clinical presentation, and examination, of a keratoconus ETM.

Multiple studies have demonstrated a unique malleability of epithelium in keratoconic corneas. Modalities for ETM, such as using high-frequency ultrasound, may further assist clinicians in detecting abnormal epithelial thickness patterns, such as a "ring-shape" (peripheral epithelial thickening with central epithelial thinning), which may indicate the presence of a stromal protrusion and underlying cone in early disease states. In later disease states, a thicker than normal central epithelium may be present, as the epithelium thickens to "mask" the inferior thinning and steepening. Unfortunately, corneal epithelial changes are not unique to KCN, and a meticulous assessment is needed, by the clinician, on a case-by-case basis. While ETM is not a *sine qua non* modality for KCN detection, its ability to detect early disease states, in conjunction with other modalities discussed here is increasingly used by clinicians, to diagnose KCN patients. As such, developments with this modality merit observation and interest in the future from clinicians and researchers alike.

2.2.2.5. Corneal Thickness (Pachymetry): As KCN often causes corneal thinning with disease progression, corneal thickness measurements, especially in areas of the central and mid-peripheral cornea, are of significant importance both in disease monitoring and candidacy for potential treatments. Corneal thickness measurements of the entire cornea, at the location of the cone, and the thinnest point in the cornea are beneficial. As one of the hallmark findings of KCN is thinning inferior to the area of maximum protrusion, the physical location of the thinnest point is of great interest.

Though ultrasound pachymetry is considered the gold standard for measurement accuracy, its most significant limitation is sampling bias from a single-point measurement. Imaging devices can provide optical pachymetry measurements for multiple locations in the cornea to further aid in diagnosis. For example, significant thickness differences in similar superior and inferior areas in the cornea and/or thickness differences between the two eyes of a patient may be a sign of ectatic disease. Similarly, progression of thickness from the central to the peripheral cornea is helpful as well; while the normal corneal thickness increases

from the center to the periphery, this relationship demonstrates greater thickness increases in patients with ectatic disease. A number of available imaging devices can further utilize pachymetry measurements to generate additional indices that can be used by clinicians to stratify the risk of ectasia development.

2.2.2.6. Aberrometry: Aberrometry, including wavefront and ray-tracing approaches, can be used to determine the amount of higher order aberrations (HOAs), such as coma and trefoil, which may be increased in KCN. Aberrometry also helps to localize the source of HOAs as either corneal or lenticular; corneal HOAs are increased in ectatic disease. Similar to the epithelial abnormalities, a variety of pathologies may show increased HOAs, and therefore aberrometry should be used with caution. Such techniques are helpful in cases of a suspect or unclear diagnosis but cannot provide definite answers/diagnosis.

2.2.2.7. Corneal Biomechanics (Hysteresis): The cornea possesses numerous biomechanical properties, such as elasticity and hysteresis, and it is thought that some or all of these properties are altered in KCN. While the thicknesses of individual collagen lamellae are unaltered, there is a significant decrease in the number of lamellae, disrupted orientations of collagen fibrils, and reduced cross-links. All of these combining to compromised structural integrity of stromal lamellae, and biomechanical instability.

Corneal biomechanics is measured by corneal hysteresis (CH), which is the distance in pressure between the first and second applanation points, and corneal resistance factor (CRF), which describes the elastic properties of the cornea. KCN eyes have significantly reduced CH and CRF compared to non-KCN eyes. Additionally, the CH and CRF may be reduced in the absence of other clinical findings, making hysteresis measurements a potentially useful diagnostic tool for the detection of early disease states. While a variety of devices are available, one of the most commonly used instruments to measure these biomechanics is through using the Ocular Response Analyzer (ORA; Reichert Ophthalmic Instruments, Buffalo, New York, USA). ORA measures the corneal biomechanical properties in vivo using a metered air puff to indent the cornea (Vellara and Patel, 2015). Measurements of the pressures at applanation events are used to calculate CH and CRF. Although both these parameters have been reported to be significantly lower in KCN as compared to healthy corneas, the sensitivity and specificity for diagnosing KCN using ORA measurements are low. This low sensitivity may be due to variation in patients which then leads to a wide range of values obtained by the ORA. As a result, using CH and CRF to discriminate mild KCN from normal cornea may be difficult (Vellara and Patel, 2015). A more sensitive parameter would be the deformation amplitude (DA) which is determined as the amount of corneal displacement at the highest degree of concavity (Jung et al., 2019). DA refers to the movement of the corneal apex in the anterior-posterior direction and can be measured by the other commercially available instrument, the CorVis ST (Oculus, Wetzlar, Germany) (Ambrosio et al., 2017). Although a more sensitive parameter, DA still has significant overlap between ectatic and normal corneas (Masiwa and Moodley, 2020). Additionally, an ex-vivo study found that a reduction in biomechanical properties occurs first in the development of KCN (Scarcelli et al., 2014), resulting in thinning tissue that surrounds healthy areas of the cornea. It is unclear whether or not KCN

develops due to reduced CH and higher DA or CH is reduced as a consequence of the ectasia (Masiwa and Moodley, 2020). Furthermore, there is a positive correlation between central corneal thickness and central corneal hysteresis meaning reduced central corneal thickness equates to compromised corneal hysteresis (Girard et al., 2015).

Corneal biomechanics should be considered alongside other corneal assessments, such as tomography, topography, and pachymetry, in diagnosing pre-clinical KCN. Evidence in current literature suggests that biomechanics are a potentially useful and supplementary tool, but should not be used as primary determinants for clinical decision making. Early identification of KCN will result in more timely interventions being offered to patients to improve long-term outcomes and it is likely that corneal biomechanics will play a role in the diagnosis and management of KCN in the foreseeable future.

2.3 Management and Treatment Options

Management of KCN depends on the severity of the disease and its impact on the patient's visual function. Non-surgical options, such as glasses and contact lenses, can improve patients' vision but do not halt or reverse the underlying disease process. Surgical options can be considered as complementary or alternative treatment modalities in conjunction with non-surgical methods. Surgical interventions can improve visual function and potentially arrest disease progression, although significant debate exists there.

2.3.1 Eyeglasses and Contact Lenses—

Patients with forme-fruste, mild, or moderate KCN can be initially managed with spectacles and contact lenses to improve visual acuity and the ability to perform activities of daily living without surgical interventions. The following vision-correction options may be used in KCN patients.

2.3.1.1 Eyeglasses: Eyeglasses (spectacles) may provide adequate vision correction for many KCN patients. While many patients may require high spherical and cylindrical corrections and primarily depend on contact lenses for vision-intense activities, eyeglasses may be helpful for at-home use or non-vision-intense activities. For example, patients may choose to wear contact lenses to school/work during the day and use glasses, albeit with reduced vision quality, for at-home activities in the evening to avoid contact lens overuse.

Several principles should be considered when discussing eyeglasses with KCN patients. First, patients should be counseled that due to significant (and irregular) astigmatism, vision quality with glasses may be inferior to vision quality with contact lenses. Second, due to the high spherical/cylindrical refractive errors, additional considerations for factors such as lens material, lens index of refraction, lens coatings and frame type should be discussed. For example, KCN patients may prefer a lens material (such as Trivex or hi-index glass) with a higher index of refraction to decrease the lens thickness and weight. However, some of these materials, such as hi-index glass, have low Abbe values, increasing chromatic aberration and rainbow-glare noted by the patient at the edge of the lens. As a second example, using a thicker frame to mask the edge of the lens or a wrap-around style frame architecture may shift the lens edge out of the field of view and reduce these subjective complaints. A complete discussion regarding the various types of frames and lenses is beyond the scope of the article. Therefore, clinicians should work closely with a skilled optician to provide

KCN patients with the most appropriate glasses to optimize visual function. Finally, patients should be counseled regarding the additional financial costs of advanced technology and customized eyeglasses.

2.3.1.2. Contact Lenses: Depending on the contact lens used, patients with KCN can achieve excellent objective and subjective visual acuity, surpassing vision achieved with glasses. However, contact lens fitting in this patient population is a time-intensive process that often requires multiple visits with a skilled and experienced contact lens practitioner.

In the United States, contact lenses, even if used as a therapeutic treatment for corneal disease(s), may incur additional out-of-pocket expenses outside of medical insurance that may be cost-prohibitive to some patients. Both the clinician and patient may feel disappointed when a patient can achieve ~20/20 vision with an in-office trial contact lens fitting but later cannot financially afford to pay for the lenses. This is a source of frustration for both clinicians and patients: while more invasive procedures, including corneal surgery, are often covered by medical insurance, contact lenses are not; while some patients may have vision insurance covering contact lenses, many patients do not. In the future, legislative and insurance reform is sorely needed to include therapeutic contact lenses under patients' medical insurance. Financially, according to a study in 2007 (Russo, 2007), a corneal transplant typically costs $13,119 when done as an ambulatory procedure and $27,705 when performed as an inpatient surgery (likely these prices have increased since the time of the referenced publication). By contrast, contact lenses would cost significantly less, approximately $500–1000 (for lenses and professional fees), while helping patients. Additionally, this would save the patient and even insurance companies significantly more money over the ensuing decades of low-risk follow-up. This seems like an undeniable win-win-win situation where the patient, physician, and insurance company all participate in safer healthcare, provide clinical/visual benefit to the patient, and reduce costs to the health system. Patient-initiated reform is sorely needed at the administrative and bipartisan political levels.

The following types of contact lenses may be used in KCN patients:

1. Soft Contact Lenses: While soft contact lenses (SCLs) are the most commonly prescribed contact lens in the general patient population, they may not be suitable for most KCN patients. However, for mild KCN patients, SCLs offer the advantages of comfort, cost, availability, and adaptability for patient use. Recently, daily SCLs have become available at a similar price point as extended-wear SCLs, giving patients the convenience of disposability and decreasing risks associated with improper storage and disinfection.

   SCLs offer excellent oxygen permeability (Dk) and transmissibility (Dk/t), thereby allowing for greater comfort and increased ocular surface lubrication. Various SCL models from major manufacturers are available, each with variations on SCL architecture, material, and properties. Suitable KCN patients may need to be trialed on several different SCL types to find a balance of visual acuity and comfort. Most SCLs are available in select stock sizes (based on

diameter and base-curve). Keratometry measurements and assessment at the slit lamp will help the practitioner chose and adjust an appropriate SCL.

The most significant disadvantage of SCL is the lack of astigmatism correction rendering them useful only for the mildest forms of ectatic disease. Additionally, it may be challenging to fit a SCL, especially in patients with advanced disease. Additional power ranges may need to be custom ordered from a given SCL manufacturer if needed to correct extreme refractive errors.

2. Soft toric contact lenses: Soft toric contact lenses (STCLs) may be suitable for patients with mild disease (approximately 1–2.5D regular corneal astigmatism). However, as they do not correct irregular astigmatism, they may only be effectively used in early disease stages.

3. Rigid gas permeable contact lenses: Rigid gas permeable contact lenses (RGPCLs, also known as “hard contact lenses) have historically been used as the contact lens of choice in patients with KCN. RGPCLs are especially effective in correcting irregular astigmatism. Additionally, they allow for better exchange of oxygen and tears under the lens. RGPCLs are much more durable than SCLs and thus may be economically beneficial. Somewhat paradoxically, a well-fitting RGPCL can be much more comfortable than SCL, especially if a patient has worn RGPCLs for many years.

   A well-fit RGPCL will avoid mechanical contact with the corneal center, instead resting on the peripheral cornea. This is especially advantageous in KCN patients who often have significantly thin central corneas and/or corneal scarring. Additional modifications can be made for extreme powers of RGPCLs. For example, a high minus-power RGPCL may have a considerable thickness at the edge that may interfere with eyelid movement; a lenticular bevel adjustment can be made to grind the thick lens edge into a thinner shape and improve comfort.

   RGPCLs can be further customized for KCN patients. For example, an RGPCL can utilize multiple curves with incremental changes on the back surface (multiple base curves) of the lens to account for the corneal shape changes. Additional modifications, such as asymmetric lift of the lens’s inferior aspect to accommodate the inferior corneal steepening, can provide a more accurate and comfortable fitting. Some patients may benefit from custom RGPCLs with a “back surface toric” (to match the corneal surface), and a spherical anterior surface or “bitoric” RGPCLs (wherein both the anterior and posterior surfaces of the RGPCL) have cylinder power.

4. Piggyback lenses: In a piggyback contact lens (PBCL) approach, an SCL is first worn on the cornea to comfort and protect the ocular surface. Then, an RGPCL is worn on top of the SCL. This allows the patient to combine the optical benefits of the RGPCL and the comfort benefits of an SCL. The disadvantage of a PBCL is the use of two contact lenses in the eye simultaneously. This decreases oxygen transmission to the cornea and increases ocular surface susceptibility to side effects such as dry eye syndrome and infections.

5. Hybrid lenses: Hybrid lenses combine the comfort of SCLs and the visual quality of RGPCLs. These lenses have a central portion that is an RGPCL and a peripheral “skirt” around the center that is a SCL. As the hybrid lens is a single lens, it may help avoid some disadvantages of a PBCL.

6. Intralimbic contact lenses: these lenses are specialized RGPCLs with large optical zones (>11 mm), similar to scleral contact lenses (see below). They may be instrumental in cases of advanced KCN wherein significant protrusion precludes adequate fit of an RGPCL on the central cornea.

7. Reverse geometry rigid gas permeable contact lenses: Reverse geometry RGPCLs (RG-RGPCLs) are modified RGPCLs with a very flat central zone compared to the peripheral curve, allowing for a better fit with oblate corneas (wherein the corneal shape consists of excessive central flattening compared to a steep periphery). While more useful for other corneal ectasias such as pellucid marginal degeneration, RG-RGPCLs may be used in select KCN patients, including post-penetrating keratoplasty.

8. Scleral lenses: recently, scleral contact lenses (ScCLs) have become the contact lens of choice among many practitioners due to their versatility and effectiveness in the full spectrum of patients with KCN. ScCLs have several critical advantages for managing various pathologies seen in KCN patients:

    - Corrections of high amounts of regular and irregular astigmatism, including both pre and post penetrating keratoplasty.
    - Visual rehabilitation in the presence of corneal scarring, either due to mild stages of disease (apical thinning) or advanced stages (post-hydrops scarring).
    - Treatment of ocular surface disease requires persistent lubrication (e.g., advanced dry eye disease either as a result of or independent of corneal irregularities).
    - Protection of the ocular surface.
    - Management of limbal stem cell pathology by protecting the limbal niche microenvironment while providing continuous lubrication and improved vision.

ScCLs typically have a large diameter (18–24mm); smaller variants, known as semi-scleral lenses, are also available. A complete discussion of available ScCLs and nuances of the fitting is beyond the scope of this article. Effective ScCL fitting requires a skilled and experienced practitioner and usually requires multiple visits with adjustments. ScCLs remain a device to closely monitor as lens design and fitting strategies continue to evolve to improve visual quality in KCN patients and expand the population of successfully-fit candidates. Recent advancements in ScCL technology have expanded the number of KCN patients that can be successfully visually rehabilitated while avoiding surgery. One major limitation of ScCLs is the significant financial costs, which are the highest among all contact lens options. An area of future interest is making ScCLs covered by medical insurance so that

KCN patients can have access to vision rehabilitation options and avoiding invasive surgical options.

2.3.2. Therapies—Therapies for KCN can be divided into two broad categories: 1) interventions to halt progression of KCN and 2) interventions to improve visual function. For mild and moderate KCN, clinicians currently employ a combination of the following treatment strategies to avoid invasive surgical procedures such as corneal transplantation.

2.3.2.1 Corneal collagen cross-linking: Corneal collagen cross-linking (CXL) is a procedure that has fundamentally changed the treatment of progressive KCN as it can arrest or halt worsening of the disease. While performed worldwide for more than a decade, CXL gained FDA approval in the United States in April 2016.

Collagen fibrils have the potential to form strong chemical bonds (crosslinks) with adjacent collagen fibrils in the cornea. Cross-linking occurs naturally with aging in normal corneas, as evidenced by increased corneal rigidity in older patients compared to younger patients. CXL involves the use of topical riboflavin activated by ultraviolet light (UVA; wavelength of 370 nm) to form crosslinks among collagen fibrils. After activation by UVA, riboflavin generates free oxygen radicals, which induce covalent bonds within collagen molecules (Subasinghe et al., 2018). In essence, CXL stiffens the corneal collagenous extracellular matrix (ECM) to prevent further ectatic changes. Numerous studies have demonstrated the efficacy, safety, and stability of this procedure (Grisevic et al., 2020; Malik et al., 2017; Toker et al., 2017; Wu et al., 2021). CXL is especially useful in young patients with mild-moderate disease that can achieve good visual acuity through glasses and contact lenses. As this patient population is at significant risk of worsening until the 4^{th} and 5^{th} decade of life, CXL can significantly reduce the risk of patients losing vision and/or needing more advanced surgical interventions.

Several strategies have been proposed for the CXL procedure. At present, the most popular procedure is known as the “epi-off” (Dresden protocol), which has been performed since 2003 (Wollensak et al., 2003). In this procedure, the corneal epithelium is mechanically or chemically removed before topical application of 0.1% riboflavin 5-phosphate/20% dextran solution for 30 minutes. The clinician then checks the patient’s anterior chamber for the presence of “flare,” indicating that sufficient riboflavin has penetrated the corneal stroma. Next, UVA light (3 mw/cm^2) is applied for 30 minutes with additional instillation of riboflavin-dextran drops. Finally, a bandage contact lens is placed at the end of the procedure to allow for re-epithelialization over the next 3–7 days. A typical CXL procedure is shown in Video 1.

An essential requirement for the Dresden protocol is the adequate corneal thickness of ≥400 μm to prevent inadvertent endothelial damage from CXL. However, given that the KCN disease progress causes significant corneal thinning, a number of KCN patients who may still benefit from CXL may not qualify per the protocol. In addition, the CXL procedure takes considerable time to perform (90–120 minutes). As such, strategies to alter riboflavin delivery, such as epithelium-on/trans-epithelial CXL and hypo-osmolar riboflavin, have been proposed. Additionally, approaches to vary UVA delivery and reduce surgical time,

such as high-fluence UVA (with decreased treatment time, known as “accelerated CXL”) have been proposed by authors worldwide to expand the applicability of CXL for patients with advanced disease. Recently, novel treatments, such as placing a contact lens after epithelial removal or the use of oral riboflavin with sunlight exposure, have been suggested to extend treatment to patients with thin corneas and/or financial barriers to obtain treatment (Schaeffer et al., 2018).

Multiple authors worldwide have reported excellent outcomes after CXL in various disease parameters, including preventing disease progression, reducing corneal steepening, improved spherical equivalent values, and improved uncorrected and corrected visual acuity. However, as these results cannot be extrapolated to every patient, clinicians currently advise CXL for patients for the primary goal of halting disease progression.

While CXL is a minimally invasive procedure, complications may occur. For example, if the cornea fails to re-epithelialize, recurrent erosions, corneal infections, and corneal scarring may occur. Even after successful re-epithelialization, inflammatory changes such as corneal haze and edema may occur as transient or permanent changes with varying effects on visual acuity. Overall, CXL is a modality that works for some patients; however, we still do not understand the exact mechanism of it to be able to provide a personalized treatment option for every patient.

On an ongoing clinical study, our group is investigating the impact of CXL on sex hormones. Sex hormones are discussed later on; however, the preliminary findings highlight how little we know about CXL. Using blood (plasma) levels, we measured the levels of three essential sex hormones (DHEA-S, Estrone, and Estriol). In a previous study from 2019, we showed significant upregulation of adrenal-derived DHEA-S and downregulation of both gonadal-derived Estrone and Estriol levels in KCN patients, compared to their healthy counterparts (Sharif et al., 2019). Figure 1 shows the levels of DHEA-S (Fig. 2A), Estrone (Fig. 2B), and Estriol (Fig. 2C) before and 3-months after CXL, in two of our enrolled KCN patients. DHEA-S levels are lower than they were before CXL. In contrast, Estrone and Estriol levels are higher, suggesting that CXL not only affects the corneal tissue (i.e., locally), but also modulates hormonal levels in the bloodstream. This finding highlights the complex and interconnected pathophysiology of KCN and a realization that a holistic approach, one that goes beyond conventional explanations, is necessary to better understand this disease.

2.3.2.2. Intrastromal corneal ring segments: Intrastromal corneal ring segments (ICRS) were initially designed in the 1990s as a refractive procedure to correct myopia. ICRS are semi-circle ring-shaped polymethyl methacrylate (PMMA) segments placed into the mid-peripheral cornea to reshape an abnormal cornea by causing secondary flattening of the central corneal curvature, which in turn reduces refractive error, including astigmatism. At present, ICRS are now primarily used to treat corneal ectasia, especially in patients in whom glasses and contact lenses are inadequate to improve visual acuity to delay the need for corneal transplantation.

While several types of ICRSs are available worldwide, INTACS (Addition Technology Inc., Fremont, CA, USA) are the only ICRS available in the United States. An INTACS procedure is shown in Video 2. Multiple studies have demonstrated the effectiveness of ICRS in KCN to reduce topographic steepening, improve visual acuity, and reduce HOAs, especially in the short term (Fahd et al., 2015; Hashemian et al., 2014; Rho et al., 2013; Zare et al., 2007; Zare et al., 2016). However, ICRS does not halt the disease process's natural progression and may be ineffective in others, leading to an unfavorable opinion of this technique held by some clinicians. With the widespread usage of CXL, ICRS have recently gained a new wave of interest and adoption as clinicians are using a dual-therapy approach of ICRS and CXL (either combined or sequential) to flatten and stabilize the cornea. At present, most surgeons performing ICRS are using them in combination with CXL; very few surgeons are using ICRS as a standalone approach.

2.3.2.3 Refractive surgery: Historically, most corneal surgeons avoided excimer laser refractive surgery (ELRS), including LASIK and surface ablation (photorefractive keratectomy (PRK), etc.) for patients with KCN, as ELRS was known to exacerbate and accelerate the progression of corneal ectasia. While still a controversial subject among clinicians, several authors have recently described the potential usefulness of PRK to improve visual acuity when combined with CXL. The philosophy behind this approach is to use CXL to stabilize the cornea and reduce the risks of KCN progression. PRK, especially topography-guided PRK, may then be performed simultaneously or after a period of stability (e.g., 12 months) to yield a more significant improvement in uncorrected and best-corrected visual acuity.

Some clinicians have combined ICRS with CXL and PRK for further flattening and refractive improvement. It is important to emphasize that LASIK remains an absolute contraindication procedure in KCN patients.

A phakic intraocular lens (pIOL), including toric variants, may also be used in select patients. In this procedure, an intraocular lens (IOL) implant is placed on top of the existing crystalline lens with no incisional or excisional manipulation of the cornea. Therefore, the primary advantage of pIOL is the ability to improve a patient's refraction without altering the cornea. In addition, the pIOL may be removed in the future if there are any complications. pIOLs may be an effective option for patients with mild-moderate KCN with low irregular astigmatism and good preoperative corrected distance visual acuity (Kalra et al., 2021).

2.3.2.4. Bowman's layer transplantation: While KCN is primarily a disease process of the corneal stroma, histological studies of KCN corneas have demonstrated a weakening and fragmentation of the overlying Bowman's membrane, or Bowman's layer. The actual etiology/mechanism of such weakening is rather unexplored, but excessive proteolytic degradation is plausible. The philosophy behind Bowman's layer transplantation (BLT) is to insert a donor Bowman's layer into the stroma to strengthen, flatten and restore corneal anatomy in patients with moderate-severe KCN (van Dijk et al., 2014). Therefore, BLT's goal is not to improve visual acuity through corneal flattening, but more importantly, to halt the progression ofKCN (van Dijk et al., 2015).

While BLT is a technically challenging procedure, early results published by several authors have shown promising results in terms of corneal flattening and maintenance of architectural changes. Ideal candidates for BLT include patients with good visual acuity potential in the presence of severe disease who may not qualify for ICRS and/or CXL (Sharma et al., 2018).

2.3.2.5. Corneal transplantation: In advanced cases of KCN, especially in the presence of corneal scarring after corneal hydrops or severe KCN, corneal transplantation may be the only option to improve vision. Surgical options mainly consist of deep anterior lamellar keratoplasty (DALK) and penetrating keratoplasty (PKP).

In DALK, the first three layers (epithelium, Bowman's layer, and stroma) of the keratoconic cornea are removed to preserve the host Descemet's membrane-endothelium complex leading to stronger tectonic integrity of the cornea (Figure 2)(Karamichos and Hjortdal, 2014). While DALK may incur longer surgical time and may be more technically challenging than PKP, multiple studies have demonstrated lower rates of graft rejection and overall reduced rates of postoperative complications when compared to PKP (Liu et al., 2015). DALK also eliminates the "open-sky" time wherein the intraocular contents are fully exposed, reducing the risks of visually devastating intraoperative complications such as expulsive hemorrhage (Armitage et al., 2003; Javadi et al., 2005; Reinhart et al., 2011; Shimazaki, 2000). Sutures placed during the DALK procedure may also be removed earlier than those placed during a PKP. In terms of visual acuity, studies have reported conflicting data, with multiple studies reported better, similar, and worse visual acuity with DALK as compared to PKP (Al-Torbak et al., 2006; Anwar and Teichmann, 2002; Fontana et al., 2007; Thompson et al., 2003).

In PKP, a full-thickness replacement of the cornea, including Descemet's membrane and endothelium, is performed (Figure 3)(Karamichos and Hjortdal, 2014). PKP has been the procedure of choice for more than 60 years (Lim et al., 2000). Several studies have shown BCVA after PKP is superior to DALK (Archila, 1984; Benson et al., 1993; Fogla and Padmanabhan, 2006). While the risk of endothelial rejection with PKP is greater than DALK, stromal and epithelial rejections are similar between the two procedures (Kim et al., 2011).

In recent years, femtosecond laser-assisted DALK and PKP techniques have shown promising results. However, the use of a laser for keratoplasty may incur additional costs that a patient's insurance may not cover. Additionally, logistical challenges such as the laser location in regard to its physical location to the operating room may be another barrier to widespread adoption.

With all keratoplasty procedures, most patients will require glasses and/or contact lenses, including ScCLs, to correct postoperative refractive error. In addition, many patients will have significant astigmatism even after suture removal has been completed. Therefore, additional procedures, such as astigmatic keratotomy (manual or femtosecond laser-assisted), compression sutures, wedge resection, and/or PRK may be considered in patients to further improve visual acuity.

3. PATHOBIOLOGY OF KERATOCONUS

3.1. Structural Changes in Keratoconus Cornea

3.2. Ocular Associations

Several ocular disease associations have been reported for patients with KCN. While the number of cases reported is relatively small, it is worth keeping in mind that there's never been a comprehensive study to explore similarities between KCN and the listed associations, which can help us understand its pathobiology. A summary of the most prominent associations is outlined in Table 2 and briefly discussed in the next section.

3.2.1. Floppy eyelid syndrome and obstructive sleep apnea—Multiple studies have reported a positive association between KCN floppy eyelid syndrome (FES), and obstructive sleep apnea (OSA). FES is often seen in patients with a high body mass index (BMI). Clinical features include upper eyelids that can easily be everted with minimal effort and papillary conjunctival changes. The etiology of FES likely involves a mechanical weakening of tarsal integrity, similar to mechanical weakening seen in patients with KCN. In addition, the papillary changes often lead to chronic eye rubbing and other allergic eye disease symptoms, which may further exacerbate an already weak cornea. This may also explain the asymmetric nature of KCN, as patients may habitually or subconsciously rub one eye more than the other.

While a causal relationship between OSA and KCN remains unclear, the association between OSA and KCN may follow a similar mechanical weakening pattern. OSA is a clinical condition characterized by apneic episodes during sleep with daytime somnolence. A variety of systemic comorbidities, including cardiovascular disease, have been reported. Several studies have reported that KCN patients have a higher risk and prevalence of developing OSA than the general population (Gupta et al., 2012) It is important to note that while obesity may be a common link between KCN and OSA, the prevalence of OSA in KCN patients remains higher. Patients' sleeping positions may also play a role in the development and progression of these diseases. For example, patients may sleep on their side or semi-prone (causing sustained, additional mechanical pressure on one or both eyes during nocturnal hours) to reduce snoring and the risk of apneic episodes. Sleep apnea-associated hypoxemia can be modeled *in vivo* with chronic intermittent hypoxia (CIH) in which animals are housed in chambers that allow oxygen concentrations to be manipulated from 21% room air oxygen to 10% oxygen in 6-minute cycles over 8 hours while the animal is sleeping (Shell et al., 2019; Snyder et al., 2018a; Snyder et al., 2018b; Snyder et al., 2017; Wilson et al., 2018). Upon examination of the corneas of rats exposed to CIH, we observed (Figure 4) increased expression of corneal fibrosis-related genes smooth muscle actin (SMA), thrombospondin-1, (TSP-1)(Matsuba et al., 2011; Wu et al., 2019) and cellular fibronectin (cFN)(Guo et al., 2018; McKay et al., 2019). These results indicate that OSA-associated hypoxemia could underlie the fibrosis observed in KCN patients, however, further studies are necessary. A potentially common underlying mechanism between KCN, FES, and OSA is the role of increased inflammatory mediators, such as matrix metalloproteinases (MMPs), previously reported in literature (Collier, 2001; Collier et al., 2000; Lema and Duran, 2005; Mackiewicz et al., 2006). Interestingly, serum MMPs are found to be elevated

in patients with KCN, FES, and OSA (Tazaki et al., 2004). Although these observations are inconclusive, they reinforce the multifactorial nature of the onset and progression of KCN.

3.2.3. Microcornea—While keratoconus's main characteristic is the cornea thinning, microcornea is characterized by a reduction in the cornea diameter (less than 10mm) and is usually associated with an increased risk of glaucoma. Associations between microcornea and keratoconus are rare, but some studies suggest a link between them through the Warburg Micro Syndrome, an autosomal recessive disease associated with ocular abnormalities, especially microcornea and congenital cataracts (Wheeler et al., 2012). In 2012, Li et al. introduced a novel technique called Genome-wide association studies (GWAS) to study a group of over 200 keratoconus patients and more than 3000 healthy controls (Li et al., 2012). The study found that the most significant association was with the SNP identified as rs4954218, located near the RAB2GAP1 (RAB2 GTPase activating protein subunit 1 gene). Interestingly, previously uncovered mutations in the RAB3GAP1 gene are commonly associated with the Warburg Micro Syndrome (Aligianis et al., 2005; Morris-Rosendahl et al., 2010). Microcornea has also been reported in association with keratoconus in patients with Ehlers Danlos syndrome and Laurence-Moon-Bardet-Biedl syndrome (Wolter, 1977).

3.2.2. Pellucid marginal degeneration—Pellucid marginal degeneration (PMD) is characterized by a peripheral thinning of the inferior portion of the cornea and, like KCN, is a bilateral progressive disorder but with a more prominent asymmetry within the eyes (Rabinowitz, 1998). Some studies have even suggested that PMD is a peripheral form of KCN (Fuchihata et al., 2014; Lee et al., 2007). This truly intriguing motion is probably suppressed by significant differences between the two diseases in terms of onset age (later in PMD) (Imbornoni et al., 2018; Sridhar et al., 2004), rate of progression (slower in PMD) (Kamiya et al., 2003; Martinez-Abad and Pinero, 2017), and the absence of scarring in PMD (krachmer et al., 1984).

A misdiagnosis of PMD as KCN or vice versa is not uncommon, due to its close resemblance in the clinical presentation. Although, corneal elevation and thickness evaluation using a corneal topography system can provide differentiation between PMD and KCN. Based on this technique, Tummanapalli et al., (Tummanapalli et al., 2013) developed the PMD index with great sensitivity and specificity in diagnosing PMD. The appearance of both diseases in the same patient may not be so rare. In 1984, a study conducted over 20 PMD patients showed that 17 of them also had developed KCN (Kayasawa et al., 1984). This supports the importance of a correct diagnosis of the disease in order to give the patient the correct treatment.

3.2.3. Ectopia lentis—Ectopia lentis or lens dislocation can occur with or without systemic association. Gene mutations have been identified, such as Marfan syndrome or Ehlers Danlos syndrome (Sadiq and Vanderveen, 2013). The association between ectopia lentis and KCN through Ehlers-Danlos syndrome has also been reported. A case of Jalili syndrome with KCN and ectopia lentis was not long ago (Purwar et al., 2015). Jalili syndrome is characterized by the comorbid appearance of cone–rod dystrophy and amelogenesis imperfecta. The patient presented bilateral asymmetry with KCN in the left eye and ectopia lentis in the right eye.

3.2.4. Lenticonus—Lenticonus is a rare congenital anomaly manifested as conical protrusion on the crystalline lens capsule and the underlying cortex, anteriorly or posteriorly (Drack et al., 2013). This condition commonly occurs in Alport's syndrome, as well as macula coloboma, cataracts or myopia. Even though KCN reports in these patients are limited, there is tomographic evidence of KCN in an Alport's syndrome patient with lenticonus (Moshirfar et al., 2019). When comparing topographic maps of eyes experiencing either lenticonus or KCN, several differences can be observed. Ocular spherical-like aberrations appear dominant in lenticonic eyes, whereas corneal and ocular coma-like aberrations seem to be the most prevalent in keratoconic eyes (Ninomiya et al., 2002). Irregular astigmatism is observed in both diseases; originated from the lenticular component for lenticonus, and the abnormal corneal shape for KCN (Ninomiya et al., 2002).

3.2.5. Cone-rod dystrophy—The etiology of cone-rod dystrophy (CRD) is focused on the retina, more precisely in photoreceptors, cones and rods. Symptoms of CRD include visual acuity, similar to KCN, but is often associated with photophobia and peripheral vision lost. The first report of CRD in association with KCN was in 1995, it is uncertain if this association is a random event or if it has a genetic relationship (Wilhelmus, 1995). This is not surprising given the absence of studies exploring ocular diseases in the context of the cornea-retina crosstalk.

A case study of CRD, right after a successful cornea transplant in a patient with KCN revealed the importance of preoperative evaluation of retinal photoreceptors function, particularly when the fundus is not visible due to corneal opacities (Fogla and Iyer, 2002). It is probably beneficial to start incorporating corneal topography, fundus evaluation, and ERG evaluations with KCN/CRD patients and their family members due to genetic predisposition.

3.2.8. Macular coloboma—Macular coloboma is a non-syndromic defect of the eye, although it can be associated with Down syndrome (DS) in some cases (Hayasaka and Hayasaka, 2004; Yamaguchi and Tamai, 1990) characterized by atrophic lesions in the retina, choroid, and sclera (Primo, 1990). Fundus observations show a significant defect of the central retinal with oval, or round, shape coarsely pigmented.

Keratoconus and macular coloboma and their association are uncommon. The few case studies reported are also associated with other ocular diseases and refer to compromised or null vision patients. In 1973 three cases of keratoconus and macular coloboma were reported, all three of them also associated with retinal aplasia (Leighton and Harris, 1973). Two of these three patients were blind and the other had a severe loss of vision. Macular coloboma and keratoconus in a patient with retinitis pigmentosa(Freedman and Gombos, 1971).

3.3 Systemic Associations

Systemic disease associations with KCN have also been reported. The link between systemic diseases and KCN are certainly more substantial than the ocular disease associations, however, our understanding of why or how these exist is lacking. A summary of the most prominent associations is outlined in Table 3 and briefly discussed in the next section.

3.3.1. Down Syndrome—Down Syndrome, also known as trisomy 21, is a genetic disorder associated with delayed physical growth, intellectual disabilities, and specific facial features. DS is caused by having three copies of chromosome 21 instead of the usual two chromosome copies. The trisomy of chromosome 21 is associated with other collagen disorders, including KCN because several collagen-encoding genes are located in that chromosome (Alio et al., 2018; Soeters et al., 2018). Additionally, studies have reported that patients with DS commonly rub their eyes, which is also linked to KCN (Alio et al., 2018).

The incidence of KCN in the general population worldwide is about 1 in 350 cases (Gomes et al., 2015a; Gomes et al., 2015b; Kennedy et al., 1986). The prevalence of KCN in DS patients is 10–300 times more frequent than in the general population and may affect as many as 1 in 10 cases (Alio et al., 2018; Cullen and Butler, 1963; Hashemi et al., 2020; Shapiro and France, 1985; Walsh, 1981). A recent study showed DS pediatric and young adults with thinner corneas, hence more likely to exhibit symptoms of KCN (Imbornoni et al., 2020). Despite the high frequency of this systemic association with KCN, the cellular/molecular mechanisms have not been explored.

KCN in the DS population is challenging to clinicians due to the limitations of the patient's communication and allowing clinicians access for screening. Commonly in early KCN, vision changes are not reported until later, leading to late diagnosis. Treatment can also be a challenge and is dependent primarily on the individual; for instance, due to significant eye rubbing, corneal transplantation healing and success can be at risk in the DS population. Even with CXL, by the time KCN is diagnosed on a DS patient corneal thickness may be below the 400um thickness threshold (Asgari et al., 2020a; Asgari et al., 2020b; Garzon et al., 2017; Huseynli et al., 2018; Imbornoni et al., 2020; Lopes et al., 2014; Marsack et al., 2019; Sabti et al., 2015). Clinicians monitoring DS patients are advised to consult with their immediate family members and their primary eye care doctor, and stress the importance of early age, regular eye screening. Family members need to be aware of all symptoms associated with KCN so a better, early diagnosis can be achieved.

3.3.2. Ehlers-Danlos syndrome—A positive association between Ehlers-Danlos syndrome (EDS) and KCN has been reported, (McDermott et al., 1998; Robertson, 1975) although it remains rare. EDS is an inherited disorder that affects connective tissues, including skin, joints, and blood vessels (De Paepe and Malfait, 2012). Generally, it is characterized by abnormal wound healing resulting in hypermobility and hyperextensibility of joints/tissues. Mutations of type V collagen-encoding genes, are linked to EDS manifestations, leading to less collagen produced/deposited when compared to a normal individual (De Paepe and Malfait, 2012; Malfait et al., 2010; Segev et al., 2006).

EDS's ocular features include prominent upper eyelids, kyphoscoliosis, xerophthalmia, and pathologic myopia (Gharbiya et al., 2012). EDS-related ocular complications include conjunctiva abnormalities (Whitaker et al., 2012), abnormal retinal vessels (Chikamoto et al., 2007), retinal detachment (Bodanowitz et al., 1997), and KCN (Cameron, 1993; Robertson, 1975). Some studies suggest that KCN patients have hypermobility in their joints, supporting the association with EDS (Al-Hussain et al., 2004; Cameron, 1993;

Robertson, 1975). Others argue against those findings (Street et al., 1991). The truth lies somewhere in the middle, as our knowledge of the underline mechanisms between KCN and EDS is extremely limited. Biochemical defects of connective tissues, causing weakness and wound healing dysfunction in the cornea, are probably partially responsible for the association (Cameron, 1993; Kuming and Joffe, 1977; Segev et al., 2006).

As with other systemic diseases, clinicians treating/monitoring KCN patients should be aware of their clinical history and inherited conditions that may lead to EDS diagnosis.

3.3.3. Mitral valve prolapse—Mitral valve prolapse (MVP) is associated with retinal artery embolism (Seelenfreund et al., 1988) and KCN (Beardsley and Foulks, 1982; Lichter et al., 2000; Siordia and Franco, 2020). MVP is a condition in which the two mitral valve flaps do not close smoothly, leading to partial slips backward loosely into the chamber, called the left atrium. This function occurs when the left ventricle muscle contracts during each heartbeat. The mechanism of action causing MVP is still unknown but has been associated with an abnormality of the mitral valve's connective tissue.

The first MVP report, in 1982, showed an overall KCN prevalence of 38% (Beardsley and Foulks, 1982). Advanced KCN patients have shown a more direct association with MVP (Sharif et al., 1992). The underline hypothesis for the KCN-MVP link is that collagen metabolism is abnormal in both conditions. Not surprisingly, KCN prevalence is higher in patients with MVP (Kalkan Akcay et al., 2014).

While evaluating KCN patients, it is vital to consider MVP, especially in those with severe KCN. Additional complications from MVP could be exacerbated during corneal transplant surgery, a treatment for severe KCN. Since most MVP cases do not show early symptoms, a comprehensive information-gathering approach and appropriate referral to cardiology from the eye care clinician is critical.

3.3.4. Leber's Congenital Amaurosis—Multiple studies have reported an association between Leber's congenital amaurosis (LCA) and KCN (Flanders et al., 1984; Hameed et al., 2000; Rabinowitz, 1998; Stoiber et al., 2000). LCA is a hereditary autosomal recessive eye disorder primarily affecting the retina, which typically results in severe visual impairment beginning at infancy and can slowly worsen over time. Several different mutations were identified in LCA, with at least 14 genes involved in the retina's development and function (Hameed et al., 2000; Pegoraro et al., 2003). The most commonly mutated genes in LCA cases, are CEP290, RPE65, GUCY2D, and CRB1 (Harris, 2001; McMahon et al., 2009; Ugur Iseri et al., 2010). These genes are critical to the normal development and function of the photoreceptors and phototransduction (Furukawa et al., 2002). LCA has been reported in association with other ocular symptoms, including retinitis pigmentosa, photophobia, nystagmus and hyperopia (Dagi et al., 1990; Flanders et al., 1984).

The relationship between LCA and KCN is still not completely understand, but some studies have shown an interplay in genetic mutations affecting both the retina and cornea (McMahon et al., 2009; Rabinowitz, 2003). For example, CRB1 mutation has been linked to

KCN,(McMahon et al., 2009) as well as LCA. Therefore, it would be advisable for patients with LCA and KCN to consult with both retinal and corneal specialists.

3.3.5. Marfan Syndrome—KCN has also been associated with Marfan syndrome (MS), which is also a genetic connective tissue disorder (Bass et al., 1981). MS is an autosomal dominant connective tissue disorder that weakens connective tissues in the musculature, cardio, and ocular systems. MS is caused by a mutation, or change, in the fibrillin-1 gene, leading to elastic fiber/microfibril defects, essential to any connective tissue in the human body.

MS can involve numerous organ systems; however in terms of ocular manifestations, common associations include ectopia lentis,(Fuchs, 1997; Maumenee, 1981; Meire et al., 1991) thinning and curvature flattening of the cornea(Heur et al., 2008; Sultan et al., 2002), early cataracts,(Biro et al., 2014) glaucoma,(Izquierdo et al., 1992; Kuchtey et al., 2013) and retinal detachment (Abboud, 1998; Loewenstein et al., 2000; Sharma et al., 2002). While very little is known on the actual MS pathobiology, it has been found that MS patients lack expression of fibrillin-1 to the superficial capsule and the ciliary epithelial surface (Kohnen et al., 2003; Nemet et al., 2006; Wheatley et al., 1995).

MS diagnosis is challenging (De Paepe et al., 1996) and can include numerous tests, including echocardiogram, electrocardiogram, cardiac magnetic resonance imaging, computed tomography, and DNA testing. Interestingly, MS has been associated with mild MVP which is also associated with KCN,(Weyman and Scherrer-Crosbie, 2004) as described above.

Therefore, clinicians treating KCN patients should be aware of additional clinical manifestations of the three organ systems associated with MS (cardiovascular, ocular, and skeletal). A multidisciplinary approach is often required for both vision and health preservation.

3.3.6. Williams-Beuren Syndrome—A rare genetic disorder called William-Beuren Syndrome (WBS), with 1:7500 to 1:10,000 prevalence worldwide (Pinsard et al., 2010; Stromme et al., 2002) is also associated with KCN. WBS is characterized by delays in development, resulting in short statures, distinctive facial features, mental deficiency, cardiovascular disease, scoliosis, and connective tissue disorders (Adams and Schmaier, 2012; Berg et al., 2007; Damasceno et al., 2014). These manifestations are caused by the deletion of the segment of chromosome 7q11.23, which includes 28 genes.(Bayes et al., 2003; Berg et al., 2007; Cusco et al., 2008; Pober, 2010)

Significant ocular manifestations have been described in patients with WBS (Ali and Shun-Shin, 2009; Offret and Laplace, 1995; Viana et al., 2015; Winter et al., 1996). Studies have shown that the entire eye development, including iris, retina, and optic nerve, are affected resulting in anterior segment dysgenesis, iris stromal hypoplasia, retinal vascular tortuosity, and nerve hypoplasia (Todorova et al., 2014; Viana et al., 2015; Winter et al., 1996). The prevalence of KCN with WBS is scarce, with only a few cases reported.(Mediero et al., 2017; Pinsard et al., 2010; Viana et al., 2013) The hypothesis on why WBS and KCN

co-exist is that the genes deleted at chromosomal 7q11.23 include the elastin gene, critical to corneal ECM, leading to KCN (Duba et al., 2002; Pinsard et al., 2010; Yue et al., 1979). Further research is warranted to determine whether this genetic origin of WBS is indeed linked to KCN. WBS is diagnosed at an early age, and therefore regular screening for KCN should be done for these patients.

3.4 Biomechanics

KCN onset and pathobiology is attributed to the disruption of the corneal collagen biomechanics. It is believed that these disruptions lead to destabilization of the cornea prior to topographic evidence of KCN, therefore serving as a biomarker and/or a diagnostic tool. In principle, this is a valid hypothesis meriting further exploration. However, several issues need to be addressed before cementing its validity. First and foremost, current technologies are unable to measure *in vivo* corneal biomechanical values/properties. This is a significant obstacle in establishing biomechanics and their role in KCN. The fact that the cornea is weakening during KCN and changes occur in its ECM is well established. Whether or not those changes can be captured before any other clinical hallmarks (i.e., topography), remains to be seen. It is possible that imaging devices or instruments may be developed, in addition to the ones discussed previously, to further enhance the role and accuracy of biomechanics in the detection and treatment of early-stage disease.

Current technologies include the Ocular Response Analyzer (ORA), the Corvis ST (CST), and Pentacam HR system (Karimi et al., 2018; Roberts, 2014; Valbon et al., 2014; Vinciguerra et al., 2016; Xu et al., 2017; Yang et al., 2019). ORA and CST are commercially available instruments that measure the cornea's dynamic behavior by a non-contact tonometer that emits an air puff *in vivo*. ORA can measure the central corneal thickness (CCT), corneal compensated intraocular pressure, corneal hysteresis (CH), and cornea resistance factor (CRF). However, the measurement lacks sensitivity and specificity depending on the measured area (McMonnies, 2012). In a study by Wolffsohn et al., authors found that that the biomechanical characteristic by ORA worked slightly better than using the traditional keratometry approach by Orbscan II, which is a 3D-slit-scanning topography system used to measures corneal thickness and anterior chamber depth to analyze the anterior and posterior surface of the cornea in KCN patients (Wolffsohn et al., 2012). The study showed that the biomechanical parameters between KCN and healthy corneas were significantly different, especially with increasing disease severity (Wolffsohn et al., 2012). Other studies have reported ORA parameters to be quite different in KCN, compared to healthy controls (Luce, 2005; Ortiz et al., 2007; Shah and Laiquzzaman, 2009; Shah et al., 2007). Unfortunately, there is a wide overlap between healthy and mild keratoconic corneas when comparing CH and CRF values, (Fontes et al., 2010; Fontes et al., 2011; Saad et al., 2010) decreasing the "strength" of these findings. A study by Shah et al., also found that biomechanical parameters, measured by ORA, were very similar when comparing KCN and post-refractive surgery eyes (Shah and Laiquzzaman, 2009). Therefore, it is uncertain whether these values have any clinical significance and can genuinely contribute to KCN diagnosis.

Likewise, the applicability of CST for the detection of KCN remains incomplete at best. CST can measure corneal deformation amplitude, IOP, and corneal thickness parameters, via a high-speed camera system that takes over 4,300 images per second, providing valuable *in vivo* information. Some studies have reported a higher deformation amplitude in the keratoconic corneas when compared to healthy counterparts (Ali et al., 2014; Brettl et al., 2018; Karimi et al., 2018; Tian et al., 2014; Yang et al., 2020). Additionally, CST has revealed that healthy corneas have a higher thickness compared to KCN corneas,(Karimi et al., 2018) however, other studies revealed healthy and KCN corneas were not comparable with IOP (Ali et al., 2014; Karimi et al., 2018) and central cornea thickness (Ali et al., 2014). However, each of these studies used different analysis characteristics to evaluate the area under the curve, making any comparisons and conclusions extremely difficult.

New corneal biomechanical parameters have been introduced to potentially better separate healthy from KCN corneas in recent years,using CST (Vinciguerra et al., 2016; Yang et al., 2020; Yang et al., 2019). Yang et al. found that keratoconic eyes have lower Ambrosio's relational thickness horizontal (ARTh), biomechanical corrected IOP, and stiffness parameter at first applanation (SP-A1), compared to healthy (Yang et al., 2019). Additionally, the authors revealed that KCN corneas had higher max inverse radius, deformation amplitude ratio max, integrated radius, and Corvis biomechanical index (Yang et al., 2020; Yang et al., 2019). Such parameters were also used to compare long-term changes in the cornea's biomechanical properties before and after CXL (4-years for patients with progressive KCN) (Sedaghat et al., 2018). Results showed that CH, CRF, ARTh, deformation amplitude ratio, and Corvis biomechanical index values were not significantly different after CXL; however, changes in dynamic corneal response were consistent with corneal stiffening (Sedaghat et al., 2018). Overall, analysis of corneal biomechanics with CST can be an excellent tool for disease management. However, understanding the cornea's biomechanics changes to assess KCN 's different stages/progression, especially in subclinical or early KCN, remains a difficult task for CST to capture.

The Pentacam provides information on the keratometry and pachymetry of the cornea using a high-resolution Scheimpflug imaging system, similar to CST (Bae et al., 2014; Karimi et al., 2018; Mihaltz et al., 2009; Ruisenor Vazquez et al., 2014; Xu et al., 2017). This system has been used extensively for corneal tomography/topography changes in the human cornea (Bae et al., 2014; Flynn et al., 2016; Hashemi et al., 2016; Ruisenor Vazquez et al., 2014; Xu et al., 2017). A study by Xu et al., looked at the entire corneal data of pachymetry and elevation of healthy, subclinical KCN and KCN patients, followed by a Zernike polynomials model to quantify the 3D distribution of corneal thickness and surface elevation (Xu et al., 2017). Results showed that elevation of the posterior surface and thickness measurements of the entire cornea distinguished preclinical KCN from a healthy cornea (Xu et al., 2017). Although these are promising studies showing relative differences in corneal biomechanical parameters, there is still the question of whether these parameters are sufficient to provide conclusive evidence of KCN progression. A recent study also looked at genetic variants' role associated with those biomechanical parameters (Khawaja et al., 2019). The study compared KCN and healthy patients to genome-wide single-nucleotide polymorphisms and ORA, and found five loci associated with corneal biomechanical parameters and KCN (Khawaja et al., 2019). This study highlights the need to look at genetic, corneal biomechanical parameters,

and corneal pathology as a whole to assess KCN progression rather than counting on only one of them.

In summary, there is significant excitement around the field of biomechanics and KCN. Unfortunately, current biomechanics cannot fully disease etiology, onset, prevalence or progression. However, future technological advancements can assist in this endeavor.

3.5 Genetics

Nearly all conditions and diseases have a genetic component. Some are a result of a single gene mutation, where others are much more complex. For example, KCN is a disease that clusters in families, yet there is no as-yet-identified gene or clear inheritance. This conclusion can be drawn based on the number studies reported on genetics in KCN over the past 70 years (690 studies on PubMed from 1950–2020). During that time, a slew of suspect genes were identified, with approximately 200 polymorphisms in more than 50 genes or loci. Unfortunately, all of these proposed genes have thus far been inconclusive, with no confirmed targets or pathways. At present, no specific therapeutic agents based on genetic information or biomarkers exists.

The main genetic components of KCN disease are found within family history. The family association rate in KCN disease is estimated at 3.34%,(McComish et al., 2020), with increasing incidence in monozygotic twins (Parker et al., 1996). This percentage is relatively low, but compared to the general population it is 15 to 67 times higher, demonstrating the familial aggregation of KCN. While there seems to be no controversy around the prevalence of positive family history of KCN, the association between the family history and the severity of the disease is still under debate. A study performed in 2016 sought to evaluate the severity of KCN compared to family history; the authors reported that patients with a higher family history had a significantly lower thinnest corneal thickness (TCT) and higher steep keratometry values (Naderan et al., 2016). Thus, while there is increased risk of ectasia based on family history, it cannot be used to estimate or assess the severity of the disease in individual family members.

Therefore, KCN is considered to be a multifactorial disease, with environmental and genetic factors involved in its onset (Abu-Amero et al., 2014; Nielsen et al., 2013; Nowak and Gajecka, 2011). Linkage mapping and association studies have been extensively used (Bisceglia et al., 2009; Brancati et al., 2004; Hutchings et al., 2005) and summarized in Table 4, finding numerous loci for KCN genes. In 2002, mutations in the VSX1 homeobox gene in the 2q11 locus were described (Heon et al., 2002). These mutations were associated with patients with KCN and posterior polymorphous dystrophy, suggesting that both are allelic variants of VSX1. However, the role of VSX1 in KCN, is supported by some authors, (Dash et al., 2010; Saee-Rad et al., 2011) and questioned by others (Aldave et al., 2006; Tanwar et al., 2010).

A more recent study, by Khaled et al. (Khaled et al., 2019) identified novel genes in familial KCN patients, using whole exome and genome sequencing in a four-generation family. Variants in the Diphosphoinositol Pentakisphosphate Kinase 2 (PPIP5K2) and Proprotein Convertase Subtilisin/Kexin Type 1 (PCSK1) genes were deemed critical for KCN, with

the gene-trap mouse model showing irregular corneal surfaces and pathological corneal thinning resembling KCN. An even more recent study by McComish et al., (McComish et al., 2020) using genome-wide association techniques, incorporated data from three different countries)United States, Australia and Northern Ireland) with nearly 7,000 participants. The association study was performed between KCN and over 6 million genetic variants. The authors identified a single genome-wide significant locus for KCN located on chromosome 11, in the region of patatin like phospholipase domain containing 2 (PNPLA2), traditionally associated with myopathy and fat accumulation in tissues throughout the body. Lastly, two studies in 2020(Gadelha et al., 2020) and one in 2021(Mok et al., 2021) have highlighted the role of lysyl oxidase (LOX) in KCN. Genetic variations/polymorphisms in LOX could be critical for corneal biomechanical integrity and function, given that LOX forms cross-links in extracellular matrix proteins. Studies like these might be a better way forward as we look to unravel the genetic aspects of KCN.

Newly developed genetic technologies, including whole exome or genome sequencing and genome-wide association, have promoted and will continue to improve our knowledge of KCN' pathogenesis. However, it appears that KCN is not a solely genetic disease.

3.6 Molecular basis of keratoconus

The molecular basis for the development of KCN is still unclear. One of the first studies ever published was from Zimmerman et al. in 1988 wherein the ECM composition in collagen and proteoglycan distribution in KCN corneas was compared to normal controls. The study found low variation in collagen I and V ratios, with elevated collagen III in KCN samples, which was directly associated with Bowman's membrane breakage. Almost a decade later, Kenney et al found altered distribution of proteoglycans and collagen-linking proteins within the stromal region near Bowman's membrane in KCN patients with fibrosis and reduced adhesions. Interestingly, the reported variations in samples within KCNs linked the structural defects to the disease severity.

Oxidative stress is also linked to KCN development and pathobiology, by numerous studies, suggesting that KCN-derived stromal cells (HKCs) have inherent structural defects that increase their susceptibility to UV-induced cell stress (Chwa et al., 2008; Chwa et al., 2006a; Kenney and Brown, 2003; Wojcik et al., 2013). Increased lipid peroxidation, sensitivity to oxidative stress, and keratocyte apoptosis in HKCs all are known to contribute to reduced response(s) to natural sources of oxidative stress (ie UV-light and environmental conditions) (Atilano et al., 2005b; Buddi et al., 2002a; Chwa et al., 2008; Chwa et al., 2006a) (Atilano et al., 2005a; Buddi et al., 2002b; Chwa et al., 2008; Chwa et al., 2006b). We identified significant upregulation of lactate-to-pyruvate levels in HKCs compared to healthy controls, suggesting that HKCs favor aerobic glycolysis over the citric acid cycle and oxidative phosphorylation in order to generate ATP (Karamichos et al., 2014). Reduced arginine levels in HKCs were also reported Such findings can be correlated with our reported findings of increased lactate:pyruvate levels in KCN tear samples when compared to healthy controls.

A popular fibrotic growth factor, the TGF-β, has also been implicated with KCN. We have found significant downregulation of SMAD6 and SMAD7 in HKCs correlated with the increased fibrotic HKC phenotype Matrix metalloproteinases (MMPs) are important

regulators of matrix remodeling during homeostasis and wound healing(Sivak and Fini, 2002; Zhou and Petroll, 2014). Studies have identified increased MMP-1 in KCN corneal buttons suggesting that matrix degradation may play a role in corneal thinning (Mackiewicz et al., 2006; Seppala et al., 2006). Not surprisingly, TGF-β1 significantly increases both MMP-1 and MMP-3 expression in HKCs, confirming its role in KCN pathobiology (Gomes et al., 2012; Zhu et al., 2014).

KCN is generally considered to be a non-inflammatory disease, since common clinical features associated with ocular inflammation, such as conjunctival injection, neovascularization, and infiltrates in the cornea, are often absent from most KCN patients (McMonnies, 2015). However, inflammatory factors such as interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α), are all found to be upregulated in the tears of KCN patients (Lema and Duran, 2005; Lema et al., 2009). A clinical study by Lema et al in 2008 found that contact lens wearers had increased concentration of TNF-α in both KCN patients and myopic patients, with KCNs showing higher IL-6 (2.9-fold) levels (Lema and Duran, 2005). Perhaps the most convincing evidence for a KCN inflammatory component role is a clinical trial published in 2015 by Shetty et al from a small cohort of 20 KCN patients who were treated with the anti-inflammatory drug, cyclosporine A (Shetty et al., 2015). The study found significant reduction in MMP-9 in tears following 6months of cyclosporine A treatment (Shetty et al., 2015). Corneal curvature reduction in KCN patients treated with cyclosporine A was also noted. Given the small population tested, more studies are needed in order to delineate the role of inflammation as a separate or complementary factor in KCN onset and progression

Autophagy is another aspect of KCN pathobiology that is currently understudied but tightly connected to mitochondria health and oxidative stress. Shetty et al. (Shetty et al., 2017) collected the corneal epithelium from 78 KCN patients undergoing corneal cross-linking or topography guided photorefractive keratectomy. Using microarray, qPCR and western blot analysis showed several autophagy-related markers being downregulated in KCN samples when compared to healthy controls, including LC3A, LC3B, ATG5, ATG7 and LAMP1. Interestingly, an earlier study by Igbal et al. (Iqbal et al., 2013) showed increased expression of SFRP-1 and LC3 using immunohistochemical assays on KCN corneal tissue. Clearly, the field is extremely understudied and further studies are needed in order to accurately determine the role of autophagy in KCN. Given the established oxidative stress and mitochondrial dysfunction in KCN, future studies on autophagy may reveal additional, critical information about KCN.

4. HORMONES

4.1 Sex Hormones

Sex hormones are steroid hormones primarily produced by the gonads, such as the ovary in females and testes in males. The two major classes of sex hormones are androgens and estrogens. Females have higher circulating levels of estrogens, whereas males have higher circulating levels of androgens. Sex hormone levels peak after puberty in both genders and then decline with aging. The profile of aging-associated decline of sex hormones is different between men and women. In men, androgen levels progressively decline after the fourth or

fifth decades of life, with a loss of approximately one-third of circulating androgen levels by 75-years-old (Deslypere and Vermeulen, 1984; Feldman et al., 2002; Vermeulen, 1991). In contrast, women experience a precipitous loss of circulating estrogens during menopause, at approximately 51 years of age (Burkard et al., 2019).. These sex hormones are involved in a multitude of physiological effects beyond reproductive health, and and influence disease risk and pathogenesis, including KCN that often exhibits a male-sex bias (Ertan and Muftuoglu, 2008; Lim and Vogt, 2002). This sex bias disappears or varies depending on the geographical location/region. Interestingly, KCN typically has a time frame of onset after puberty with stabilization at 40 to 50 years of age (Kennedy et al., 1986; Rabinowitz, 1998). This is consistent with the profile of significant hormonal changes, supporting their role in KCN pathophysiology (Karamichos et al., 2019; McKay et al., 2017). This is an area that merits further investigation and may improve our understanding of disease onset and progression.

4.2 Extra-Gonadal Sex Hormones

Steroid hormones are also produced outside of the gonads (i.e., extra-gonadal hormones) in tissues such as the adrenal glands, brain, adipose tissue, skin, bone, liver, and pancreas (Barakat et al., 2016). The endogenous prohormone for both androgens and estrogens is dehydroepiandrosterone (DHEA) and its 3β-sulfate conjugate (DHEA-S). DHEA and DHEA-S are primarily produced in the extra-gonadal tissue of the adrenal cortex but can also be produced in the gonads and the brain (e.g., neurosteroids) (Reddy, 2010). DHEA and DHEA-S produced in the adrenal cortex are under the control of the anterior pituitary hormone, adrenocorticotropic hormone (ACTH) (Endoh et al., 1996; Orentreich et al., 1992; Roberts and Lopez-Duran, 2019). DHEA in the circulation is mainly in the form of DHEA-S (Labrie et al., 1997; Webb et al., 2006). Specifically, circulating levels of DHEA and DHEA-S are 1–6 ng/ml and 450–3470 ng/ml, respectively (Labrie et al., 1997; Legrain et al., 2000; Orentreich et al., 1984), labeling DHEA and DHEA-S as the most abundant circulating steroids (Shealy, 1995). Production of DHEA and DHEA-S begins during puberty and peaks at 20–30 years (Flynn et al., 1999; Orentreich et al., 1992). Recent findings from our group have shown increased levels of DHEA-S in individuals with KCN compared to healthy controls (McKay et al., 2016; Sharif et al., 2019), indicating that altered levels and/or ratios of DHEA/DHEA-S may play a role in KCN pathophysiology.

4.3 Synthesis and Receptors

Steroidogenesis or synthesis of steroid hormones occurs primarily in the gonads or the adrenal cortex via conversion of cholesterol to pregnenolone by the cytochrome P450 enzyme CYP11A1, the rate limiting step (Figure 5). Pregnenolone is further metabolized to DHEA/DHEA-S via CYP17A1 (Payne and Hales, 2004). Subsequently, DHEA/DHEA-S is metabolized to androstenedione via 3β-hydroxysteroid dehydrogenases and then androgens and estrogens at target tissues via 17β-hydroxysteroid dehydrogenases and different cytochrome P450 enzymes (e.g., CYP3A4, CYP3A5, CYP3A7, CYP1A1, CYP1A2, CYP19A1-aromatase) (Kandel et al., 2017; Kitada et al., 1987b; Lu et al., 2020; Ohe et al., 2000; Shou et al., 1997; Williams et al., 2002; Yamazaki et al., 1998). These steroids can be metabolized and inactivated via different cytochrome P450 enzymes (e.g., CYP2B6,

CYP3A4, CYP3A5, CYP3A7) (Krauser and Guengerich, 2005; Lee et al., 2003; Niwa et al., 2019; Wang et al., 1997).

The prohormone DHEA can further impact steroidogenesis (Figure 5) through its actions on several cytochrome P450 enzymes to alter androgen and estrogen production (Lee et al., 2003). DHEA can increase CYP3A4, CYP3A5, (Schirra et al., 2006) and CYP2B6, (Kohalmy et al., 2007b; Singleton et al., 1999), which can hydroxylate and inactivate androgens and estrogens (Badawi et al., 2001; Cribb et al., 2006; Huang et al., 1998). In contrast, DHEA can decrease CYP1A1 and CYP1A2 (Belic et al., 2013; Ciolino and Yeh, 1999), both of which can mediate estrone and estriol synthesis (Kuhl, 2005; Lu et al., 2020). These different effects of DHEA on androgen and estrogen production may be a possible mechanism mediating the increased DHEA and decreased estrone and estriol ratios present in KCN patients (McKay et al., 2016; Sharif et al., 2019). Specifically, DHEA may suppress estrone and estriol synthesis by multiple pathways in KCN patients, such as suppression of CYP1A1 and CYP1A2 mediated estrone and estriol synthesis (Belic et al., 2013; Ciolino and Yeh, 1999; Lu et al., 2020) or increased activation of CYP3A4 and CYP2B6 mediated metabolism of estrone and estriol (Kohalmy et al., 2007a; Singleton et al., 1999). Indeed, our preliminary data show an upregulation of CYP2B6 in corneal stromal cells from KCNs (Figure 6F), consistent with increased degradation of estrone and estriol.

The biological effects of these steroid hormones are due to their actions at various receptors. Sex hormone receptors for estrogens and androgens include estrogen receptors (ER) and androgen receptors (AR), respectively. In contrast, DHEA can be metabolized into both androgens and estrogens and thus activate either AR or ER (Arnold and Blackman, 2005; Chen et al., 2005; Prough et al., 2016), although DHEA does have a higher affinity for ER-β compared to ER-α and AR (Arnold and Blackman, 2005; Chen et al., 2005; Prough et al., 2016). Thus, DHEA can have a broad range of physiological actions. AR and ER are ligand-activated transcription factors (Pawlak et al., 2012; Tenbaum and Baniahmad, 1997). AR and ER can have their biological effects by impacting transcription through classical nuclear receptor signaling (Figure7, in which hormone bound receptors dimerize, translocate to the nucleus, and bind to the hormone response element (HRE) of DNA to induce or suppress transcription (Pawlak et al., 2012). An alternative pathway for AR and ERs biological effects is through non-genomic signaling wherein these receptors initiate a rapid cellular action via intracellular signaling cascades (Björnström and Sjöberg, 2005; Duong et al., 2020; Lucas-Herald et al., 2017). ARs and ERs are expressed throughout the body, including the brain (e.g., cortex, hippocampus, amygdala, hypothalamus) (Simerly et al., 1990; Yu and McGinnis, 2001) and the cornea (Rocha et al., 2000; Suzuki et al., 2001; Wickham et al., 2000). Therefore, dysregulation of sex hormones can have direct biological actions at the receptor level in the cornea, which may be related to the observed sex dependence observed in KCN.

4.4 Sex Hormones and Cornea

While sex hormones are present in all tissues of the human body as they circulated in the bloodstream, their impact is dependent on the target cells and their related receptors.

Related physio-pathological conditions, in males and females, are reported on almost all organs/tissues, and not limited to reproductive tracks and organs.

The cornea expresses multiple receptors and enzymes for sex hormonal metabolism and cellular actions (Figure 7). Sex hormone metabolism and steroidogenesis can occur within the cornea via 3β-hydroxysteroid dehydrogenases, 17β-hydroxysteroid dehydrogenases, CYP3A4, CYP3A5, CYP1A1, CYP1A2, CYP19A1, CYP2B6, CYP20A1, (Kolln and Reichl, 2012; Mindnich et al., 2004; Nakano et al., 2014b). The metabolites of sex hormone metabolism (e.g., androgens, estrogens, progestins) can bind to their cognate receptors, such asAR, ERα, ERβ, and progesterone receptor, which are all present in the cornea (Rocha et al., 2000; Suzuki et al., 2001; Tachibana et al., 2000; Wickham et al., 2000; Wiechmann, 2003). Interestingly, and despite decades of both clinical and laboratory research studies, the exact mechanism(s) of how sex hormones regulate corneal function is not clear. For example, while ERs, PRs, and ARs have been reported in the human corneal epithelium, stroma, and endothelium, their modulation upon injury/disease is still debated.Furthermore, in the context of KCN, the data on ERs is controversial. On one hand, Yin et al., showed no significant changes in ERα/ERβ in KCN corneas, compared to healthy controls (Yin, 2017).. Ayan et al., on the other hand, showed significantly higher levels of ERα in keratoconus (Ayan et al., 2019). Thus, the role of *ERα/ERβ* in the development of KCN remains unclear at this time. One component of this problem might be that most studies focus on a small number of samples (sex or disease severity specific), leading to non-uniform results across scientific groups/studies. More studies are warranted as we attempt to unravel the role of these receptors.

During an *in vitro* study, we observed upregulation of CYP2B6 (drug metabolism and steroidogenesis), CYP19A1-aromatase, and CYP20A1 (an orphan cytochrome P450 enzyme) in KCN-derived corneal stromal cells, when compared to healthy (Figure 6A–C). In this study, we tested corneal cells derived from 1 male (between 25–35 years of age) and 1 female (between 35–45 years of age) donors from both healthy and KCN. All three CYPs showed higher levels of expression in the male donors (Figure 6D–F). Clearly, further studies are necessary to elucidate the differences between males and females. Table 5 summarizes the CYPs tested and their presence, or not, in Healthy and KCN donors. Recent studies, including those reported herein, warrant further explorations on the role of sex hormones and corneal disease(s).

4.5 Hormones and Keratoconus Treatments

It was once believed that after a corneal transplant for advanced KCN, the ectatic condition does not recur because a “healthy” donor corneal graft replaced the pathological host cornea. Though it was first reported in the mid-1990s, (Kremer et al., 1995), there are increasing reports in the recent literature supporting the recurrence of KCN. Table 6 summarizes all studies reporting KCN recurrence.

Progression or exacerbation of KCN is also known to appear after non-surgical interventions (ex. CXL). Table 7 summarizes the studies reporting KCN progression and/or exacerbation. Both tables were created and studies were filtered based on the Moher et al., model (Moher et al., 2009).

Of the 11 case reports listed in the table that had penetrating keratoplasty (PKP) as an intervention, there are 3 case reports with outlier data due to a noted interference; 2 of the cases noted the progression of KCN (as early as 3 years) with eye rubbing and 1 case noted early exacerbation within 2–4 years due to underlying Leber congenital amaurosis. With those reports omitted, the average presentation of disease recurrence post-PKP between the remaining 8 case reports is 16.31 years. This is consistent with a 2006 retrospective case series examining patients that received PKP as an intervention which showed an average presentation of recurrence at 17 years (Pramanik et al., 2006). However, a retrospective analysis in 2009 (Patel et al., 2009) showed an average post-PKP recurrence at 21.9 years and a case series in 2018 (Yoshida et al., 2018) that examined 50 eyes showed an average post-PKP recurrence at 27.2 years. Furthermore, a retrospective analysis done in 2021 found recurrence of KCN in 33–35 years with a possible association between post-PKP status and ScCL wear (Murillo et al., 2021). In comparison, patients that received DALK as an intervention had an earlier presentation of recurrence between 2–4 years. In the chart, there are 4 case reports on patients that received DALK and 2 of these case reports had secondary factors. One patient had KCN recurrence between 1–1.5 years with eye rubbing (Abad et al., 2020) and the other report was on a pregnant patient who had recurrence at 2 years (Gatzioufas et al., 2017). These numbers are consistent with a 2017 retrospective case series that examined 382 eyes with an average post-DALK recurrence rate of 4 years (Feizi et al., 2012). Of the less-utilized interventions documented in case reports, electro-surgical keratoplasty and radial keratotomy were ineffective in preventing disease recurrence.

While we do not understand why this is happening, several hypotheses may be plausible: 1) recipient keratocytes are “abnormal,” 2) peripheral/remaining tissue (cornea, conjunctiva, etc.) are responsible for the recurrence, or 3) there is a host hormonal imbalance that affects and alters the donor cornea to develop secondary ectasia While the first two hypotheses are well-described in the literature, the third hypothesis remains understudied. However, given the previous section’s discussion on the role of hormones in the cornea, if hormones are critical to KCN onset and progression, they are perhaps essential to arresting andblocking recurrence. Our group has shown that sex hormones are significantly prevalent in corneal tissue, with significant dysregulation in the keratoconic tear fluid. Hormone receptors exist in the human cornea, both in the epithelium and the stromal layer, but remain significantly understudied. Therefore, it is clear that the human cornea has the “tools” to respond to an abnormal hormone signal and altered homeostasis. The questions arising are several: *“how does the cornea respond to abnormal signaling? How is the abnormal signal processed as compared to a normal signaling process? How does this disharmony in hormone regulation affect the corneal tissue state? Finally, does this abnormal signaling affect clinical presentation and severity of* KCN*?*

As discussed previously, CXL has recently emerged as an effective treatment to halt worsening of KCN and maintain good corrected visual acuity. However, as with post-PKP patients, recurrence can still occur in patients even after CXL; while rare, such recurrences are currently an area of significant interest for clinicians and researchers alike (Maier et al., 2019). n. Mazzotta et al., reported that KCN progressed in 24% of pediatric patients after CXL with a follow-up period of 10 years (Mazzotta et al., 2018). This is highly significant if we consider the onset age of KCN and clinicians’ desire to permanently halt disease

progression with interventions such as CXL. Thus, the significant percentage of young patients that progress after CXL merits further inquiry and research.

The previously discussed hormonal component therefore merits further attention, especially when considering recurrence after corneal transplantation and progression after CXL. It would seem plausible that hormonal factors continue to influence KCN disease severity given that presence of hormones in corneal tissue and surrounding fluids (i.e., tears and aqueous humor). The interplay of hormones and CXL is not fully understood, yet several questions can be posited at this time. For example, do interventions like CXL affect hormonal balance; if so, what is the mechanism of action? As shown in Figure 1 our ongoing clinical study investigates whether CXL treatment can affect the blood (plasma) levels of three essential sex hormones (DHEA-S, Estrone, and Estriol). As mentioned previously, a combination of increased DHEA-S levels and decreased estrone and estriol levels are commonly seen in keratoconic corneal tissue. Thus, it is intriguing to see DHEA-S levels dropping following CXL, with corresponding increase in estrone and estriol levels. Thus, it is potentially underappreciated that CXL not only affects corneal tissue locally, but can modulate bloodstream hormonal levels systemically. This highlights how connected the human body is in the context of disease and/or treatment complexity, and more importantly, provides a strong rationale for the role of hormones in KCN.

5. BRAIN AND PITUITARY

5.1 Hypothalamic and Pituitary Regulation of Sex Hormones

Sex hormones are under the control of the brain and the pituitary, specifically the hypothalamus and the anterior pituitary (Plant, 2015). The primary pathway regulating sex hormones is the hypothalamic-pituitary-gonadal (HPG) axis. In this axis, gonadotropin releasing hormone (GnRH) neurons with cell bodies in the basal hypothalamus project to the median eminence to release GnRH in the anterior pituitary portal vasculature. This GnRH induces the release of two gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), from the anterior pituitary that act on the gonads to stimulate steroidogenesis (Figure 8).

The hypothalamic-pituitary-adrenal (HPA) pathway is also involved in extra-gonadal sex hormone production. Upon activation, corticotropin releasing hormone (CRH) neurons with cell bodies in the paraventricular nucleus of the hypothalamus release CRH into the portal vasculature of the anterior pituitary via the median eminence. CRH induces the release of ACTH from the anterior pituitary into the circulation, which acts on the adrenal gland to synthesize and release glucocorticoids such as cortisol(Gjerstad et al., 2018; Herman and Cullinan, 1997; Papadimitriou and Priftis, 2009). In addition to the release of glucocorticoids, ACTH can induce the synthesis and release of DHEA from the adrenal cortex (Endoh et al., 1996; Orentreich et al., 1992; Roberts and Lopez-Duran, 2019).

A negative feedback loop primarily regulates both the HPG and the HPA axes to maintain the homeostasis of steroid hormones (Gjerstad et al., 2018; Plant, 2015). Circulating steroid hormones feedback at the level of the hypothalamus and the pituitary, resulting in decreased hypothalamic releasing hormones (e.g., GnRH and CRH) and anterior pituitary stimulating

hormones (e.g., LH, FSH, ACTH) synthesis and release (Plant, 2015). Positive feedback regulation can occur in both the HPG and the HPA axes, in which hormone levels are continuously increased. In the HPG pathway, this generally occurs during the ovarian cycle, wherein estrogens can cause an LH surge (Messinis, 2006). Positive feedback regulation is also observed in the HPA axis, in which DHEA can increase hypothalamic CRH mRNA expression (Givalois et al., 1997) that can further drive DHEA synthesis and release in the adrenal cortex (Bertagna, 2017; Sirianni et al., 2005; Smith et al., 1998). Interestingly, males are more sensitive to this positive feedback effect of DHEA on CRH than females (Givalois et al., 1997). This positive feedback of the HPA can subsequently lead to chronic activation that can negatively impact neurons and glia to increase disease risk (de Kloet et al., 2005; Munck et al., 1984; Sapolsky et al., 2000). Based on our observations and the DHEA increase in KCN patients (McKay et al., 2016), this increase may be involved in a positive feedback regulation of the HPA axis, resulting in increased DHEA (Figure 8), providing a direct link to the brain that has not been previously described in the literature.

5.2 GnRH and Gonadotropic Hormones

The HPG and HPA axes do not act in isolation but can antagonistically influence each other. For example, HPG can decrease HPA activation (De Boer and Koolhaas, 2003; Spritzer et al., 2009) via an AR-mediated mechanism at the level of the CRH neurons in the hypothalamus (Cunningham and McGinnis, 2008; Kitay, 1963; Lund et al., 2006; Rosinger et al., 2019). Similarly, the HPA axis can decrease HPG activation (Retana-Márquez et al., 2003; Sapolsky et al., 2000; Whirledge and Cidlowski, 2010). This HPA suppression of HPG axis occurs through suppression of GnRH (Calogero et al., 1999; Magiakou et al., 1997; Whirledge and Cidlowski, 2010, 2017). Suppression of GnRH decreases the release of gonadotropins, LH and FSH, and subsequently decreased gonadal steroidogenesis.

DHEA is an interesting hormone as it can impact both the HPA and the HPG pathways. DHEA can increase HPA activation at the level of CRH neurons in the hypothalamus (Cleare et al., 2004; Givalois et al., 1997; Sirianni et al., 2005; Smith et al., 1998), resulting in glucocorticoid suppression of the GnRH and gonadotropic hormone release in the HPG pathway (Calogero et al., 1999; Li et al., 1995; Magiakou et al., 1997; Whirledge and Cidlowski, 2010, 2017). Since DHEA is a prohormone for androgens and estrogens, through its actions on AR and ER (Kitada et al., 1987a, b; Lu et al., 2020; Ohe et al., 2000; Shou et al., 1997; Simard et al., 2005; Williams et al., 2002; Yamazaki et al., 1998), DHEA can decrease GnRH and gonadotropic hormone release through negative feedback regulation of the HPG axis (Plant, 2015). Therefore, elevated DHEA results in the suppression of the HPG axis and overactivation of the HPA. Elevated DHEA and suppressed gonadotropins are observed in KCN (Karamichos et al., 2019; McKay et al., 2016; McKay et al., 2017; Sharif et al., 2019), indicating that dysregulation of HPG and HPA axes could be involved in KCN pathophysiology. If this theory\is correct, this may allow for a breakthrough shift in disease management and treatment through inclusion of addressing this hormonal imbalance..

5.3 Pituitary and the Human Cornea

The human cornea is known for each ability to metabolize hormones (Kaluzhny et al., 2018; Kolln and Reichl, 2012; Nakano et al., 2014a). In addition, the cornea has numerous

receptors that can to respond to androgens, estrogens, gonadotropic hormones, and even glucocorticoids. It is well-known that the human cornea expresses glucocorticoid receptors (Sulaiman et al., 2018). Activation of these glucocorticoid receptors may impact sex hormone signaling in the cornea, as glucocorticoids can influence sex hormones and even gonadotropic hormone receptor expression (Bambino and Hsueh, 1981; Saez et al., 1977; Whirledge and Cidlowski, 2010). Studies have shown corneal expression of AR, ER-α, and ER-β, which can bind androgens and estrogens to induce a biological effect within the eye (Ayan et al., 2019; Rocha et al., 2000; Sacchetti et al., 2015; Sharif et al., 2019; Suzuki et al., 2001; Wickham et al., 2000).

In 2019, our research group was the first to find that gonadotropic hormone receptors, LH receptor (LHR) and FSH receptor (FSHR), are present in the cornea (Karamichos et al., 2019). Figure 9 shows significant upregulation of LHR, and the downregulation of FSHR in KCN - derived 3D constructs, compared to healthy controls. Interestingly, in human corneal epithelial cells, only FSHR was found as shown in Figure 10. Thus, the role and function of these extra-gonadal gonadotropic hormone receptors are unknown. It is highly likely that these corneal gonadotropic hormone receptors are involved in KCN pathophysiology. Modulation of these receptors may be therapeutically beneficial for KCN patients, as estrogens have been linked with increased corneal thickness and altered ocular function (Affinito et al., 2003). Based on these findings, we recently classified the cornea as an extra-gonadal tissue (Karamichos et al., 2019). In a current, ongoing, *in vitro* study, we are investigating LH and FSH stimulation in corneal stromal cells from both healthy and KCN donors. Figure 11 shows significant upregulation of LH receptor expression in KCN corneal stromal cells compared to healthy counterparts.

5.4. GONADOTROPIC HORMONES AND KERATOCONUS

In addition to our findings, many other authors have reported significant changes in androgens and estrogens in KCN. However, the signaling mechanisms about these hormonal imbalances remain unknown. In a recently published clinical study, our group reported the existence and modulation of gonadotropic hormones (LH and FSH) in KCN. This study placed the human cornea on the list of extragonadal tissues together with the skin, breast, uterus, and adrenals. Thus, the presence of gonadotropic hormones in the human cornea could potentially be a groundbreaking discovery.

LH and FSH are further upstream from the sex hormones (androgens and estrogens) that are affected in KCN. Further upstream on this endocrine system is the hormone GnRH, which many researchers believe is a crucial hormone that regulates numerous downstream pathways. It is possible that future research fully elucidates the status of GnRH as it relates to KCN onset and progression. If such an association can be reproducibly confirmed, this may answer numerous questions about KCN, such as geographic variability, gender inconsistencies, time of onset, and rate of disease progression. Clinicians and researchers may soon understand that KCN is actually an ophthalmic manifestation of systemic pathology, rather than a primary disease limited to the cornea. Most importantly, such an association may allow for novel, groundbreaking therapies to holistically diagnose and treat KCN patients, both at the ocular and systemic level…

6. Conclusions and Future Directions

KCN remains a significant clinical problem, with major implications to patients' visual acuity and quality of life. The first study on KCN was published in 1898 and the numbers have been growing since. It is clear that KCN is a multifactorial disease with genetic and behavioral components, thereby making it difficult to determine its exact pathophysiology. However, KCN exhibits some classical hallmarks of a multifactorial disease: 1) the risk increases due to environmental influences, 2) it is not sex-limited, 3) it is associated with multiple diseases, and 4) incidence of disease is greater among relatives.

The idea that sex hormones play a role in KCN is not new, and we are certainly not the first ones to report this. The role of sex hormones in causing changes in the cornea during pregnancy has long been documented, as hormonal fluctuations result in corneal alterations, including increases in corneal volume, central corneal thickness, and curvature (Naderan, 2018; Qin et al., 2020). Due to these hormonal changes, progression in KCN has been documented in pregnant women during pregnancy and beyond, extending to six months postpartum (Bilgihan et al., 2011). However, to the best of our knowledge, the concept that gonadal hormones play a role in KCN is novel. Previously, we were the first group to introduce the presence of LH/FSH in KCN, hinting at an unexpected pathway/mechanism.

Based on our studies, we believe that exacerbation of HPA axis, as evidenced by increased adrenal-derived DHEA in KCN patients (McKay et al., 2016), leads to the suppression of HPG axis by DHEA inhibition of GnRH, affecting the anterior pituitary/sex hormones downstream and ultimately damaging the corneal microenvironment. Systemic hormonal dysregulation is transferred onto the corneal tissue, and corneal hormone-specific receptors respond accordingly (Gonzalez-Coto et al., 2014; Karamichos et al., 2019; Suzuki et al., 2001). Long-term exposure to abnormal hormones (such as those present in the tear film and aqueous humor) leads to cellular damage, abnormal extracellular matrix (ECM) remodeling, and ultimately KCN onset. Therefore, based on the evidence presented, we propose that a new axis, the *Hypothalamic-Pituitary-Adrenal-Corneal (HPAC) axis*, exists, which may help explain the various interactions and influences between these structures Further support to this proposed mechanism is the fact that KCN onset (and progression) is highest during puberty, which is also a time for significant systemic hormonal changes and spikes in both genders. Confirmation of HPAC axis existence could answer numerous perplexing concepts in the field, including sex, race, and geographical progression differences. We posit that while the past 120 years have borne witness to remarkable advancements in KCN management, researchers and clinicians can be enthusiastic about current studies and models, especially those that incorporate a holistic approach to a disease previously viewed as one limited to a single organ system. We hope that authentication of such models may pave the way for novel and effective treatments to improve the quality of life in this challenging patient population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Keratoconus is a corneal disease that coevolves with major hormonal changes.
- Linking the keratoconus to the gonadotropins has significantly evolved the field.
- Existence of a Hypothalamic-Pituitary-Adrenal-Corneal (HPAC) axis is proposed.

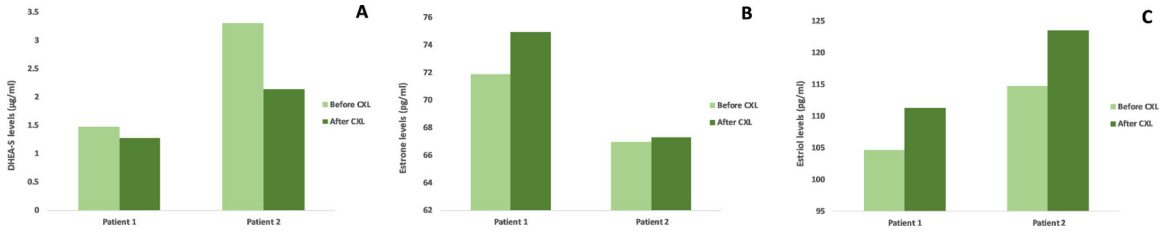

Figure 1:

Raised subepithelial nodular scars may develop over the apex of the cone in some patients with KCN (left panel). ETM (middle panel) often demonstrates epithelial thinning directly underneath the scar with a concentric region of epithelial thickening around the scar. AS-OCT (left panel) highlights the extent, location and depth of the scar. These scars may preclude the use of soft toric and rigid gas permeable contact lenses. A scleral contact lens (as shown in the AS-OCT) allows for vaulting of the lens over the scar to improve visual acuity.

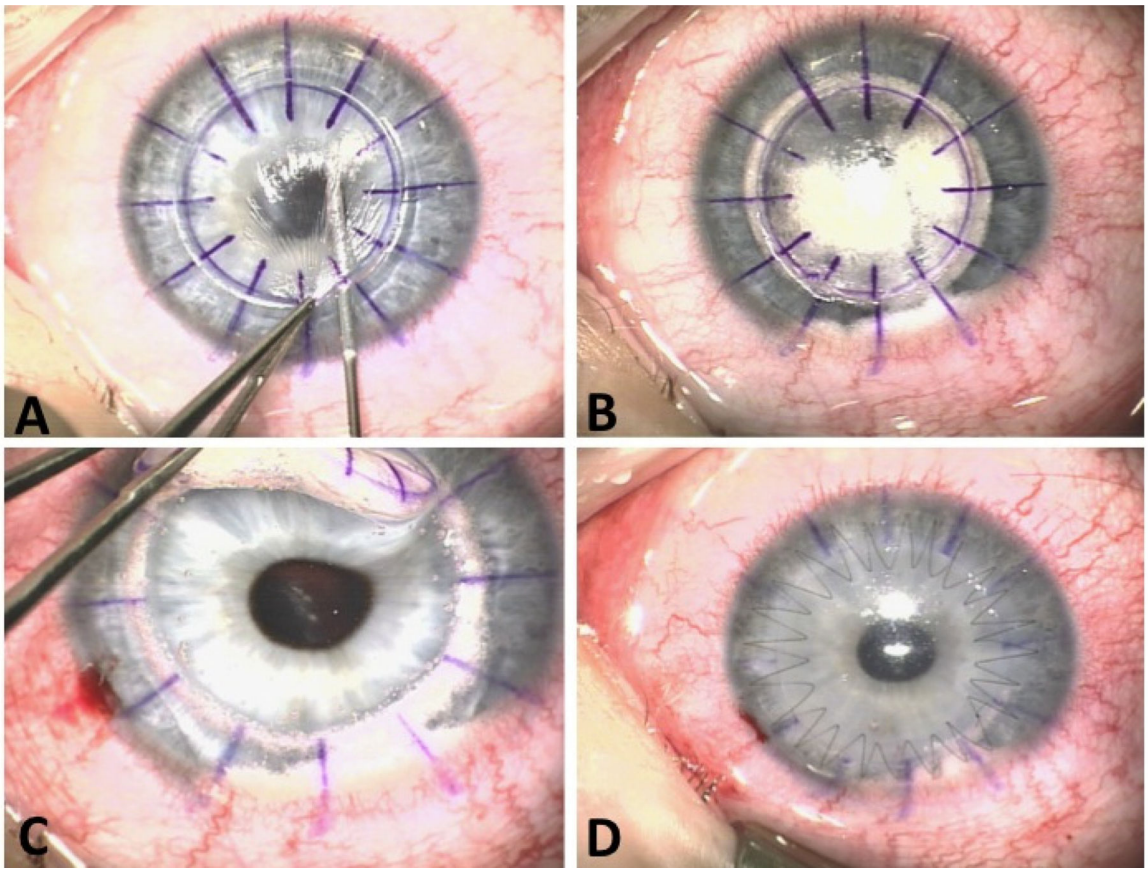

Figure 2:
Protein expression of DHEA-S (A), Estrone (B) and Estriol (C) before and 3-months after CXL treatment in two keratoconus patients. Estrone and Estriol show higher levels after treatment.

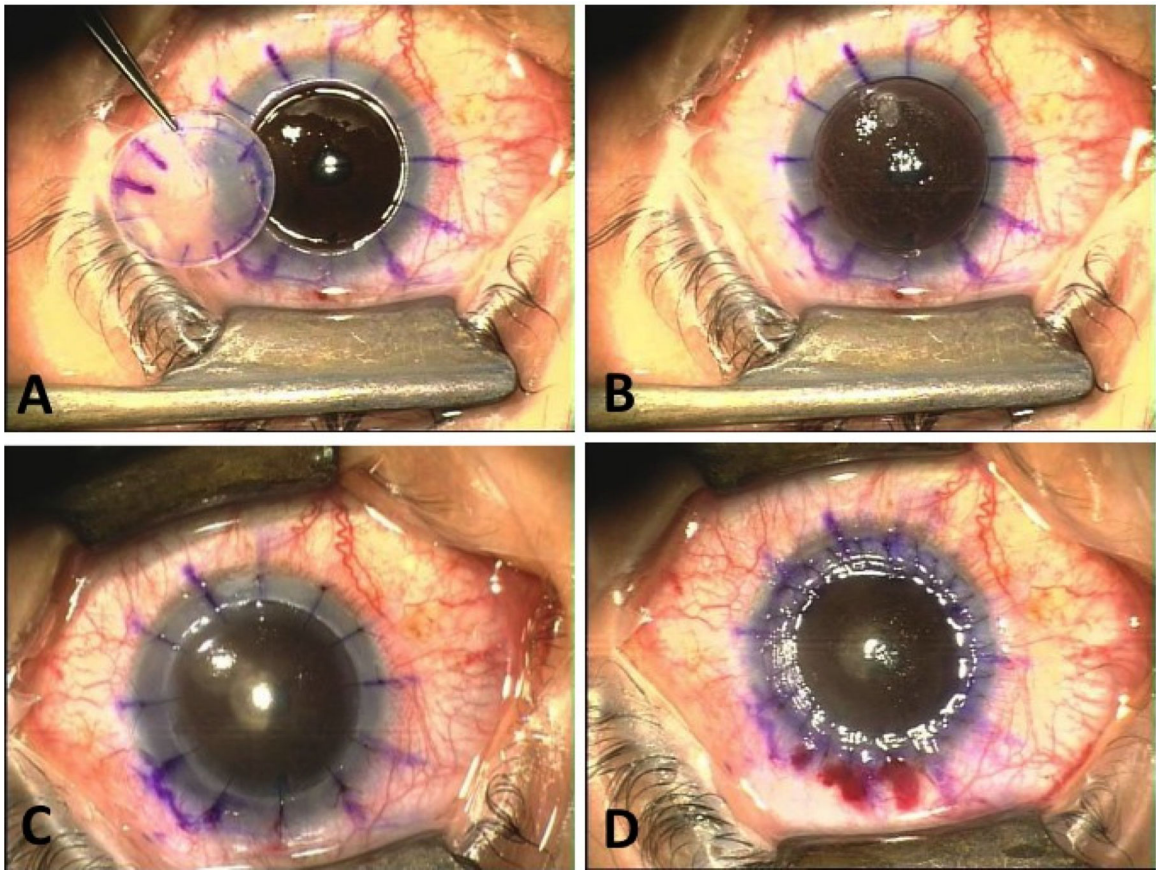

Figure 3:
Deep anterior keratoplasty (DALK) snapshots. (A) After partial trephination, a cannula is inserted deep in the corneal stroma; (B) Air injection through the cannula separates Descemets membrane from stroma; (C) Manual dissection ensures that recipient's Descemets membrane is preserved; (D) Lamellar donor graft sutured in place. Source: (Karamichos et al., 2014)

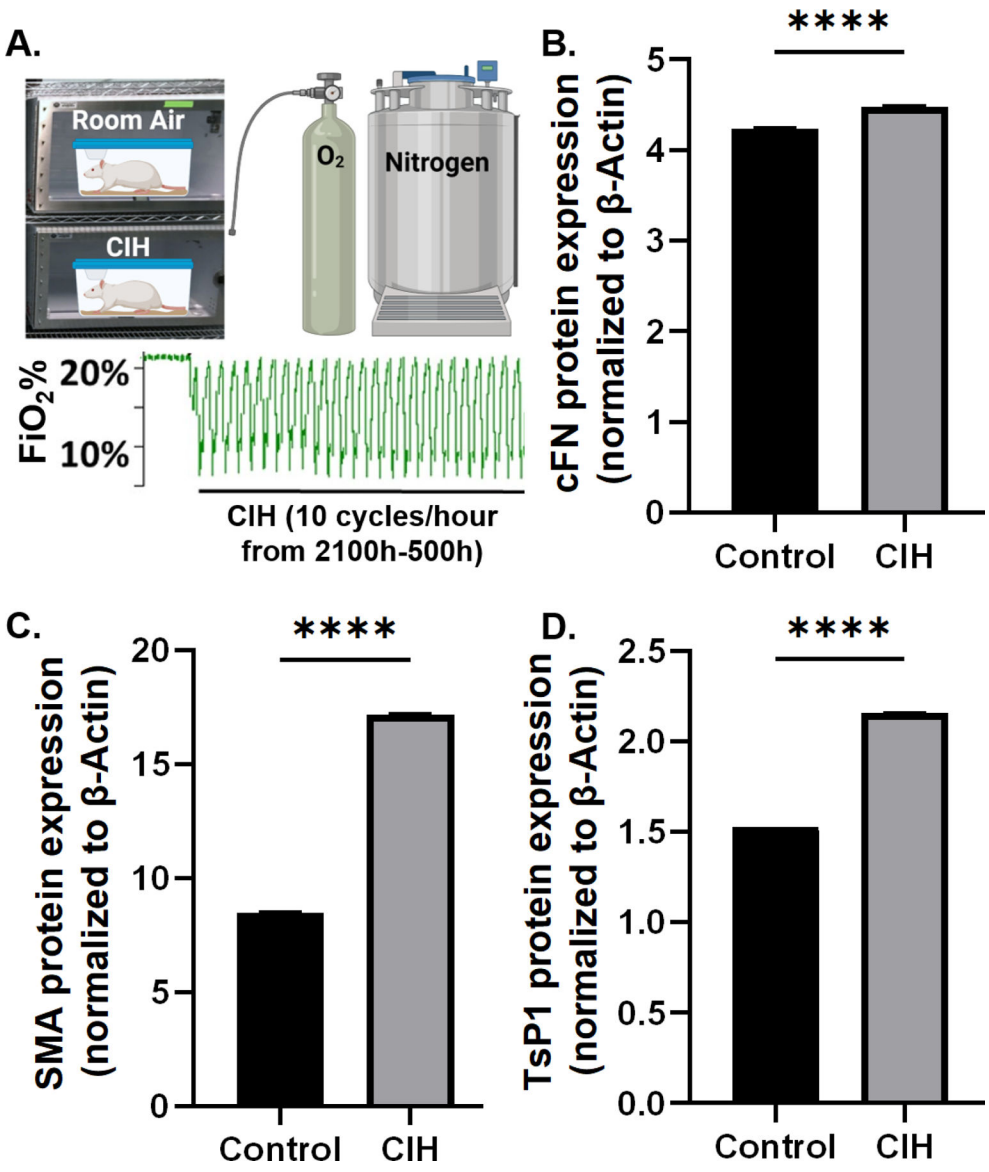

Figure 4:

Penetrating keratoplasty snapshots. (A) Distorted cornea removed; (B) Corneal graft placed in recipient bed; (C) “Stay sutures” placed; (D) Single running suture in place at end of surgery. Source: (Karamichos et al., 2014)

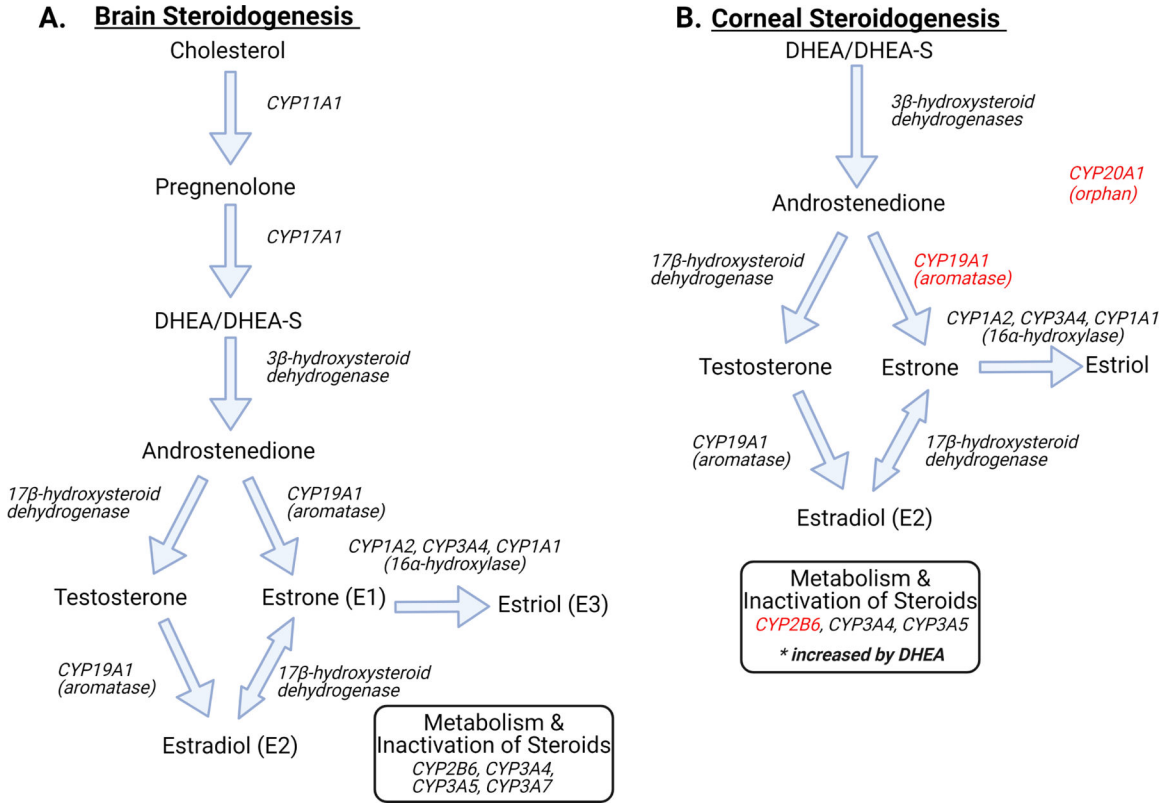

Figure 5:

(A) Representative scheme of CIH treatment on rats. Protein levels of cFN (B), αSMA (C) and TSP1 (D) normalized to β-actin in rat corneas showing a significant increase after CIH treatment. N=4, ****p<0.0001.

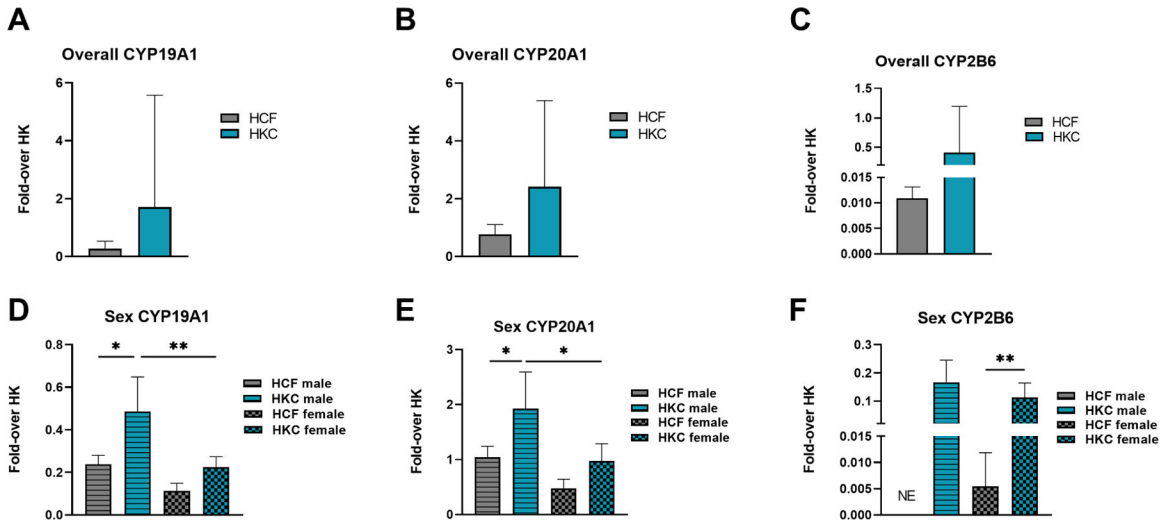

Figure 6:

Representation of steroidogenesis of steroid hormones by cytochrome P450 enzyme in the (A) brain, and (B) cornea.

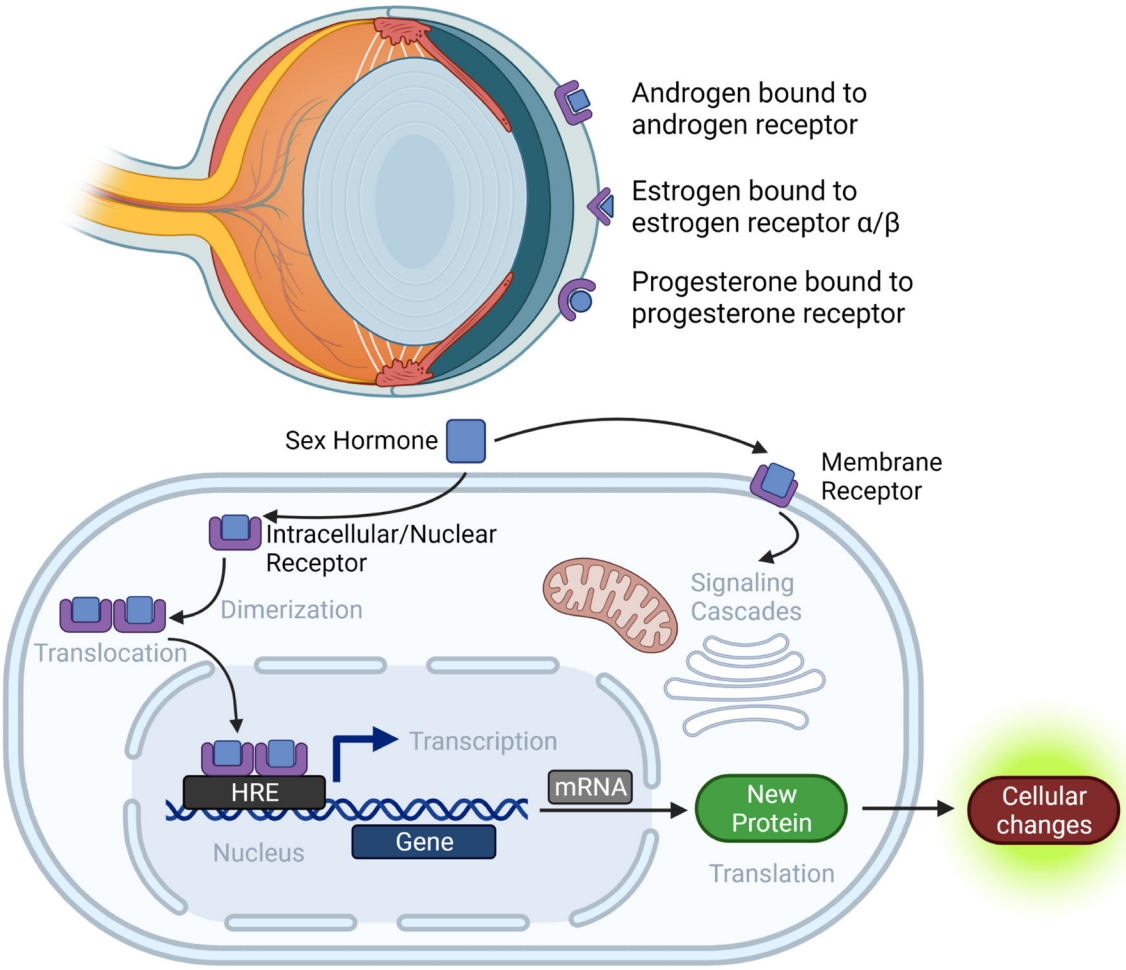

Figure 7:

Cytochrome P450 enzymes in corneal stromal cells from two (1 male and 1 female) healthy and two (1 male and 1 female) keratoconus donors. (A) Overall results for CYP19A1 in healthy and keratoconus corneal stromal cells. (B) Overall results for CYP20A1 in healthy and keratoconus corneal stromal cells. (C) Overall results for CYP2B6 in healthy and keratoconus corneal stromal cells. (D) Sex results for CY19A1 in healthy and keratoconus corneal stromal cells. (E) Sex results for CYP20A1 in healthy and keratoconus corneal stromal cells. (F) Sex results for CYP2B6 in healthy and keratoconus corneal stromal cells. N=3, *p<0.05, **p<0.01.

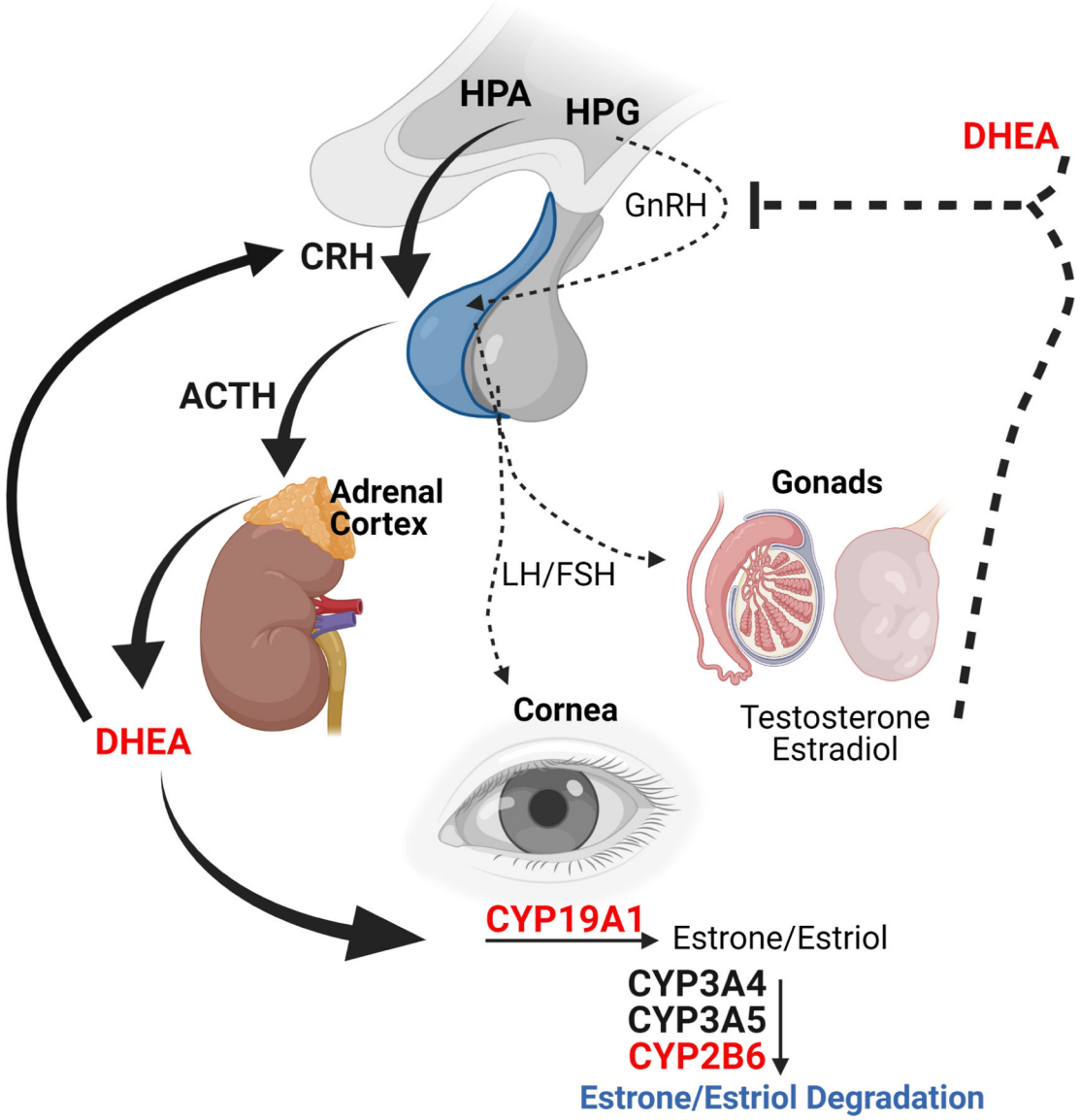

Figure 8:

The cornea expression androgen receptors, estrogen receptor alpha(a), estrogen receptor beta (b), which mediate sex hormone actions. These sex hormone receptors can have diverse cellular actions, depending on the location of the sex hormone receptor. Sex hormones are lipophilic molecules that can cross the plasma membrane of the cell and bind with intracellular/nuclear sex hormone receptor to have genomic actions. Once sex hormones binds to intracellular/nuclear receptor, the receptor dimerizes and translocates into the nucleus to bind to the hormone response element (HRE) of a gene to induce genomic transcription and translation. Sex hormones can also have non-genomic actions by binding to membrane sex hormone receptors that activate cellular signaling cascades within the cell.

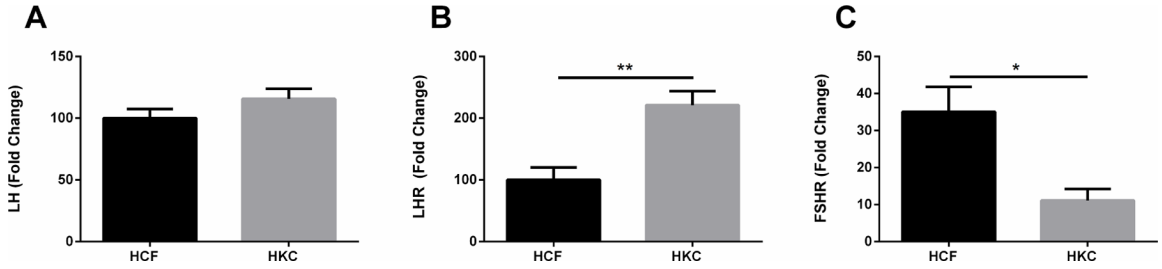

Figure 9:

Representation of steroidogenesis by GnRH from the anterior pituitary acting on the gonads to release luteinizing hormone and follicle stimulating hormone.

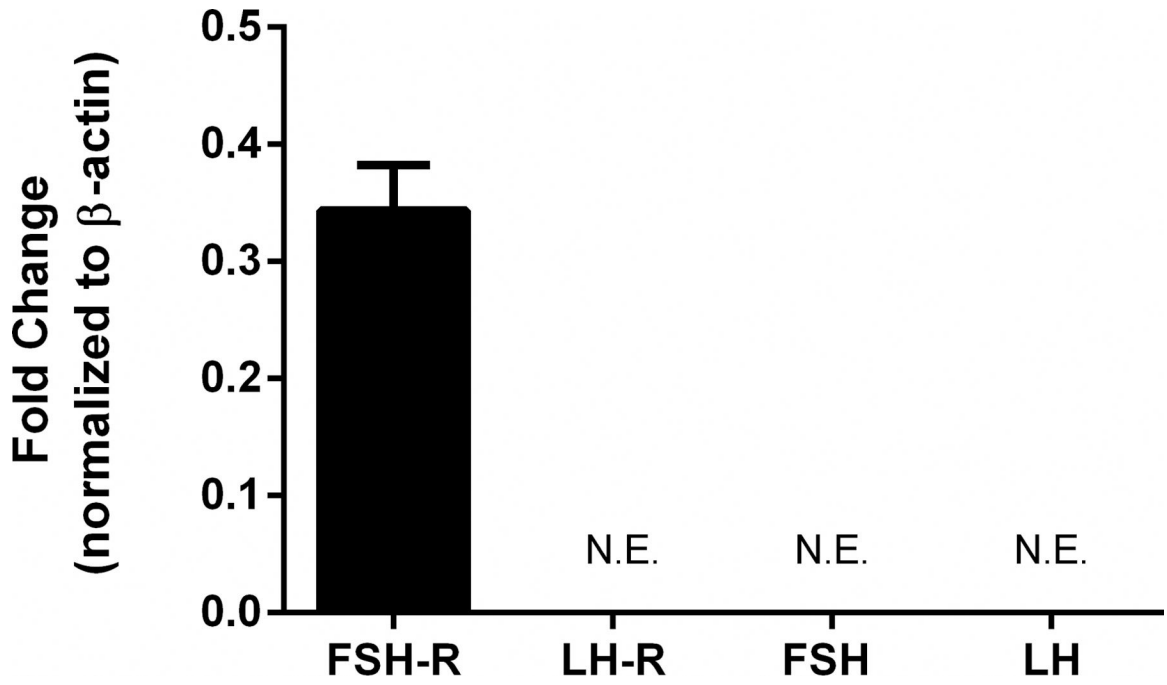

Figure 10:

Expression of LH (A), LHR (B) and FSHR (C) in healthy (HCF) and keratoconus (HKC) corneal fibroblasts grown in vitro. *p<0.05, **p<0.01. Source: (Karamichos et al., 2019)

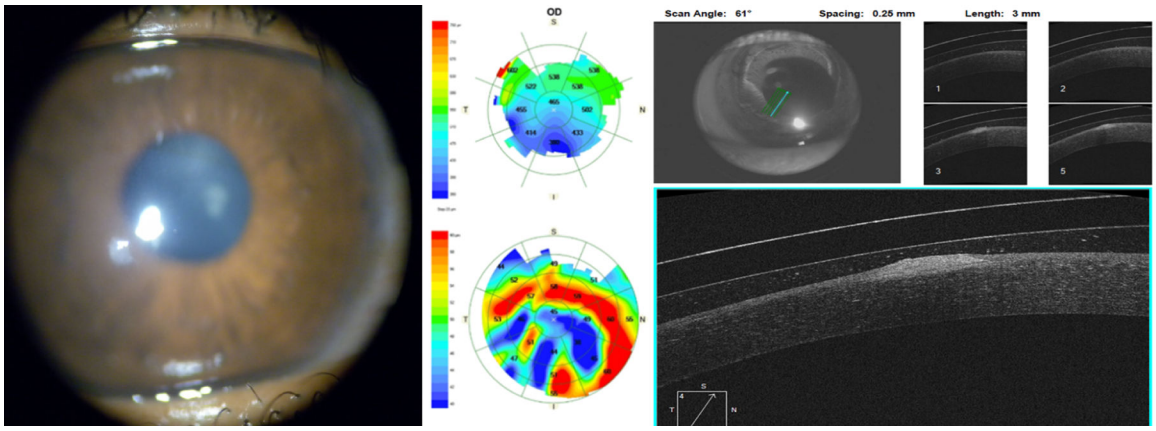

Figure 11:

Expression of FSHR, LHR, FSH, and LH in human corneal epithelial cells. FSHR was the only one expressed by the epithelial cells. Source: (Karamichos et al., 2019)

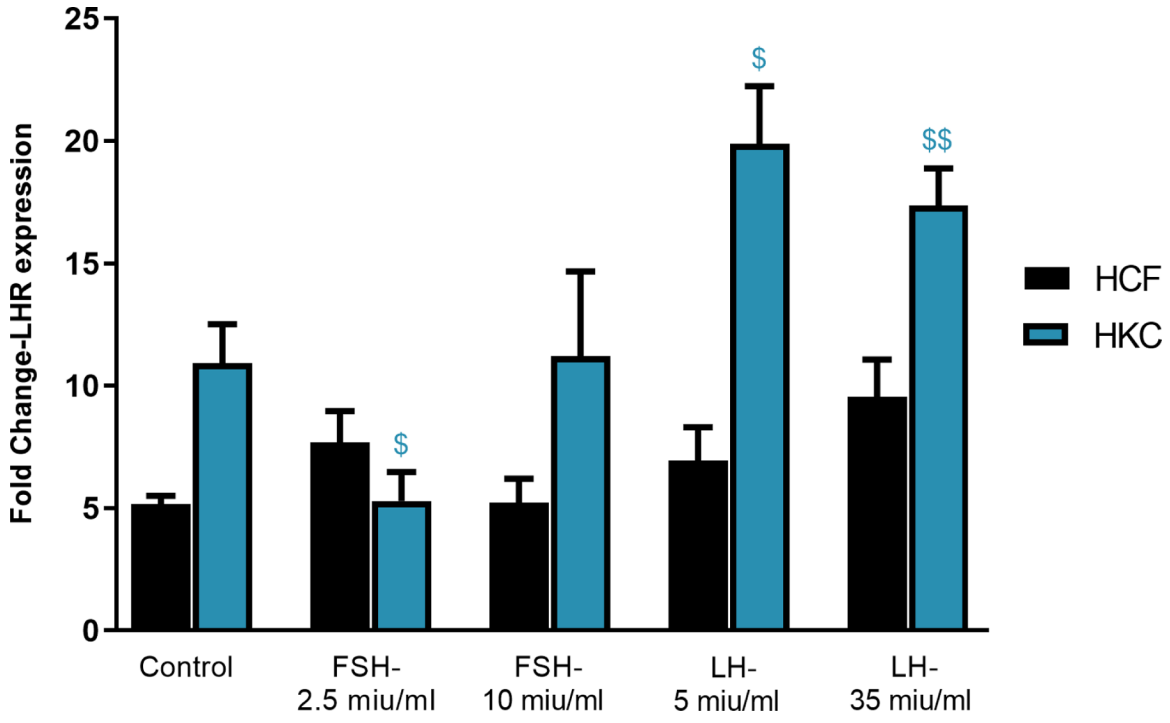

Figure 12:

Expression of LHR in corneal stromal cells from one healthy and one keratoconus donors. Cells were stimulated with two concentrations of FSH: 2.5 and 10 miU/mL and LH: 5 and 35 miU/mL. N=3, $^{\$}p<0.05$, $^{\$\$}p<0.01$.

Table 1.

Structural layers of the human eye

Eye Structures	Size	Shape/Location	Layers	Reference
Outermost Fibrous layer				
Cornea	• In humans, it is about 10.6mm vertical and 11.7 mm horizontal in diameter. • Central radius of 7.8 mm and thickness between 0.54mm and 0.56mm.	• Convex and spherical shape position on the front of the eyeball	• Corneal epithelium: 5 to 7 layers of nonkeratinized stratified squamous cells. • Bowman's Layers: Acelluar condensation of collagen and proteoglycans. • Corneal Stroma: Transparent organized structure of keratocytes and ECM. • Descemet's Membrane: thin membrane made of type IV collagen and lamilin • Corneal endothelium: single layer of hexagonal cells, metabolically active.	Marsich, 2000 Sridhar, 2018 Funderburgh, 2003 Foster, 2015
Sclera	• Thickness of 1mm and thinnest of 0.3–0.4mm	• Continuous with the cornea to the back of the eyeball.	• Episclera: outermost layer of loose connective tissue beneath the conjunctiva. • Sclera proper: dense white tissue, responsible for the color or the sclera • Lamina fusca: innermost layer made of elastic fibers	Treuting, 2012 Sharma, 2010
Middle Uvea and Vascular Layer				
Choroid	• In Humans, it is about 0.2mm thick and narrows to 0.1mm in the peripheral. • Size can decrease to 80μm by age 90.	• Thin formation in two-thirds of the middle wall of the eye	• Haller's Layer: outer component of major blood vessels (arteries and veins). • Sattler's Layer: inner component of medium blood vessels (arterioles and venules). • Choriocapillaris: wide-bore fenestrated capillaries, 20–40μm density • Bruch's membrane: Innermost loose connective tissue (fibrous) layer 2–4μm thick. Consist of 5 layers: RPE basal lamina, inner collagenous layer, middle elastic layer, outer collagenous layer and a basement layer of endothelial cells.	Sohn, 2014 Ramrattan, 1994 Masood, 2019 Nickla, 2010
Ciliary body	• In adult eye, the length ranges from 4.5–5.2	• Circular structure between choroid posteriorly	• The ciliary muscle contains Three separate smooth muscle fibers: outer longitudinal, middle oblique and inner circular.	Rohen, 1990 Delamere, 2005 Kiel, 2011 Alm, 1976

Eye Structures	Size		Shape/Location		Layers		Reference
		nasally and 5.6–6.3mm temporally		and iris anteriorly	•	The ciliary processes consist of finger-like protrusions of anterior ciliary arteries and long posterior ciliary artery branches	
					•	Two layers of epithelium are in the inner surface of the ciliary processes: one outer pigmentated and an inner nonpigmented.	
Iris	•	In humans, it is about 10.2mm to 13.0mm in diameter and 37mm in circumference.	•	Circular disk, color layer of tissue positioned in the anterior region of the middle layer	•	Anterior border layer: Contains dense collection of fibroblasts, melanocytes and a few collagen fibers.	Rubin, 1984 Seliger, 1962 Newsome, 1971
					•	Stroma: Contains fibroblast, melanocytes, collagen fibers type I and III and Sphincter smooth muscle fibers	
					•	Radial (dilator) muscle fibers: Layer of myoepithelial cells about 4 μm thick extending 50–60μm in radius.	
					•	Posterior pigment epithelium: Two heavily pigmented epithelial layers	
Innermost Layer							
Retina	•	The area occupied is 1.094mm^2 and the estimated diameter is 32mm.	•	Oblate shape, the innermost layer tissue, positioned near the optic nerve	•	Outer retinal pigment epithelium: Monolayer of smooth hexagonal cells between 12μm in diameter and height with melanin granules.	Boulton, 2001 Panda-Jonas, 1996
					•	Inner neurosensory retina: composed of 9 layers: internal limiting membrane, nerve fiber, ganglion cell, inner plexiform, inner nuclear, outer plexiform, outer nuclear, external limiting membrane and photoreceptors (rods and cones)	

Table 2.

Ocular disease associated with KCN

Ocular disease	Description	References
Floppy eyelid syndrome	Frequently bilateral eyelid malposition that usually involves the upper eyelids.	(Donnenfeld et al., 1991; Parunović and Ilić, 1988)
Retina pigmentosa	Genetic condition that changes how the retina responds to light, generating progressive loss of vision.	(Flanders et al., 1984; Hou et al., 2018)
Microcornea	A congenital abnormality characterized by a small cornea of less than 10mm of horizontal diameter. It is a disease commonly associated to Crouzon's syndrome.	(Li et al., 2012; Wolter, 1977)
Pellucid marginal corneal degeneration	Degenerative condition of the cornea, characterized by a bilateral thinning of the inferior portion of the cornea. Sometimes confused with KCN as it shares some characteristics and prognosis.	(Kayasawa et al., 1984; krachmer et al., 1984)
Ectopia lentis	Displacement of malposition of the lens from its normal location. Associated with KCN in Ehlers Danlos syndrome.	(Purwar et al., 2015; Robertson, 1974; Sadiq and Vanderveen, 2013)
Lenticonus	Consists of the deformation of the lens of the eye in which the surface is conical, especially on the posterior side. Patients with Alport syndrome are subjected to develop eye symptoms compatible with this disease.	(Moshirfar et al., 2019)
Cone-rod dystrophy	Eye disorder that affects the retinal photoreceptors, cones and rods. The patient experiences loss vision.	(Fogla and Iyer, 2002)
Macular coloboma	Non-syndromic defect of the eye, usually unilateral, characterized by atrophic lesions of varying size presenting rudimentary of absent retina, choroid and sclera. Usually associated with down syndrome.	(Freedman and Gombos, 1971; Leighton and Harris, 1973)

Table 3.

Systemic disease associated with KCN

Systemic Diseases	Description	Correlation	References
Down Syndrome (DS)	DS is a genetic disorder caused when abnormal cellular division results in an extra copy of chromosome 21. DS visual system is inherently less sensitive than normal.	0.5–15% of people with Down Syndrome are affected with KCN. KCN is significant more prevalent in DS patients. KC is less likely to be report early because of communication limitations.	(Imbornoni et al., 2020) (Asgari et al., 2020a) (Asgari et al., 2020b) (Hashemi et al., 2020) (Krinsky-McHale et al., 2012) (Marsack et al., 2019) (Sabti et al., 2015) (Walsh, 1981) (Cullen and Butler, 1963) (Shapiro and France, 1985)
Ehlers-Danlos Syndrome (ED)	ED is a disorder associating connective tissues that support many organs and tissues including the eye. ED is an inherited genetic condition involving biosynthesis of collagen.	50% KCN patients reported hypermobility associated with ED syndrome.	(Kuming and Joffe, 1977) (Robertson, 1975) (Al-Hussain et al., 2004; Cameron, 1993; Robertson, 1974)
Mitral Valve Prolapse (MVP)	MVP is a heart mitral valves structural abnormality associated with primary connective tissue of chordae tendinea, annulus and leaflets.	Dysregulation of collagen component are a key in development of both KCN and MVP.	(Beardsley and Foulks, 1982) (Sharif et al., 1992) (Kalkan Akcay et al., 2014) (Rabbanikhah et al., 2011) (Chang et al., 2020) (Siordia and Franco, 2020) (Lichter et al., 2000)
Leber's congenital amaurosis (LCA)	LCA is an autosomal recessive disorder primarily affecting the retina. LCA results in visual impairment and reduced electroretinogram over time.	LCA association with KCN is due to genetic factors affecting both the retina and the cornea.	(McMahon et al., 2009) (Stoiber et al., 2000) (Rabinowitz, 1998) (Hameed et al., 2000)
Marfan Syndrome (MS)	MS is a genetic disorder of connective tissue resulting in ocular retinal detachment, increased axial length and myopia, flattened cornea, iris and ciliary muscle hypoplasia.	MS association to KCN is due to abnormal collagen synthesis with genetic and cellular factors.	(Bass et al., 1981)
Williams-Beuren Syndrome (WBS)	WBS is a rare genomic multisystem disorder: affecting the iris stromal hypoplasia, congenital cataracts and optic nerve disc excavation and hypoplasia.	WBS has a rare KCN association due to genetic factors.	(Mediero et al., 2017) (Viana et al., 2013) (Pinsard et al., 2010)

Table 4.

Loci identified for keratoconus genes using linkage and association methods.

Methods	Loci	References
Linkage	17p13	Hameed, 2000
	2q11	Heon, 2002
	16q22.3-q23.1	Tyynismaa, 2002
	20q12	Fullerton, 2002
	15q22.33–24.2	Hughes, 2003
	3p14-q13	Brancati, 2004
	2p24	Hutchings, 2005
	5q14.3-q21.1	Tang, 2005
	9q34	Li, 2006
	1p36.23–36.21/ 8q13.1-q21.11	Burdon, 2008
	5q21.2/ 5q32-q33/ 15q15.1	Bisceglia, 2009
	13q32	Gajecka, 2009
	14q24.3	Liskova, 2010
	5q15–5q21.1	Bykhovskaya, 2016
Association	2q14	Kim, 2008
	7q21.11/ 7q36.1/ 9q32/ 16q21	Burdon, 2010
	3p26/ 2q21.3/ 19q13.3	Li, 2012

Table 5.

Cytochrome gene targets tested.

Cytochromes Tested	Expression
CYP3A4	NONE
CYP2C9	NONE
CYP2C19	NONE
CYP2B6	EXPRESSION- HKC ONLY
CYP3A43	NONE
CYP3A7	NONE
CYP19A1	EXPRESSION- BOTH HCF +HKC
CYP17A1	NONE
CYP3A5	NONE
CYP20A1	EXPRESSION- BOTH HCF +HKC
CYP21A1P	NONE

Table 6.

Recurrence of KCN after PK and DALK

Author(s)	Year	#	Design	Intervention Prior to KCN Recurrence	Conclusion	Recurrence (Years)
Abelson et al.	1980	1 eye	Case report	PK	KCN in a graft for the same disease should be added to the list of late complications of successful keratoplasties.	16
Nirankari et al.	1983	1 eye	Case Report	PK	It can be expected that as grafts survive for longer periods of time, and there is more long-term follow up, recurrence of KCN may be seen.	22
Eiferman	1984	1 eye	Correspondence	PK	Recurrent KCN in this patient may be due to the donor cornea because only one eye was affected. If the recurrence was due to some type of intrinsic effect from the host, the "recurrent" KCN should have been bilateral.	22
Bechrakis et al.	1994	1 eye	Case report	PK	There are 2 basic possibilities that might explain recurrence: either the disorder was not apparent in the donor tissue at the time of keratoplasty, or it was a case of true recurrence from the host tissue that was not resected and had the disease originally.	19
Bechrakis et al.	1994	1 eye	Case report	PK		12
Kremer et al.	1995	1 eye	Case report	PK	The pathogenesis of this complication is still not clear and further research is needed.	7
Stoiber et al.	2000	2 eyes/1 person	Case report	PK	Recurrence could be caused by an "aggressive" genetic factor that leads to the frequent KCN in patients with Leber congenital amaurosis. This mechanism also could explain the high incidence and rapid progress of KCN in this disease.	2–4
Thalasselis et al.	2002	1 eye	Case report	PK	The etiology of recurrent KCN has yet to be clarified and few case reports appear in the literature.	40
Bourges et al.	2003	3/12 eyes	Retrospective, consecutive, interventional case series	PK	Recurrence of the KCN characteristics may result from graft repopulation by recipients' keratocytes, aging of the grafted tissue, or both.	17–28
Patel et al.	2003	1 eye	Case Report	DALK	We have reported the first case of recurrent ectasia in a relatively new treatment option. As lamellar surgery becomes increasingly popular it is important to recognize such late complications which may require further surgical intervention in the future.	3
Lim et al.	2004	10/10 eyes	Retrospective analysis	PK	Progression of KCN in the host cornea late after PK is characterized by a large astigmatic change where the flat axis of astigmatism passes through an area of host thinning visible on slit lamp examination.	13.5
Pramanik et al.	2006	6/112 eyes	Retrospective, consecutive, noncomparative case series	PK	Late recurrence of disease occurs with increasing frequency over time. Given the younger age at which KCN patients undergo corneal transplantation, these long-term findings should be incorporated into preoperative counseling.	17.9
Unal et al.	2007	1 eye	Case report	PK	Recurrence of KCN in this patient and another patient with no pre-existing KCN and in both grafts coming from the same donor support transmission of previously undiagnosed KCN in the donor instead of true of recurrence as the cause of post-keratoplasty KCN.	1.5

Author(s)	Year	#	Design	Intervention Prior to KCN Recurrence	Conclusion	Recurrence (Years)
Koenig	2008	2 eyes/1 person	Case report	PK	The findings of stromal thinning, scarring, and steepening of the right eye, and hydrops in the left eye are consistent with the clinical diagnosis of KCN in both grafts. It is very likely that these findings represent recurrent self-induced KCN and lend further support to the association between chronic eye rubbing and corneal ectasia.	12–13
Patel et al.	2009	36 eyes/25 people	Retrospective analysis	PK	Ectatic changes were often bilateral and occasionally recurred after regrafting, suggesting that host cellular and/or biochemical factors may be responsible.	21.9
Yeniad et al.	2009	2 eyes/1 person	Case report	PK	A recurrence of KCN can occur because of itch-provoked rubbing of the eyes. Patients who had PKP should be evaluated for a history of ocular allergies.	3
Hayes et al.	2010	1 eye	Case report	PK	The donor and recipient tissue remain structurally distinct and the junction between the two tissues is marked by a relative increase in collagen mass. Corneal wound healing occurs mainly in the epithelium and endothelium and a normal stromal architecture may never fully be achieved in corneal graft wounds.	13
Feizi et al.	2012	1 eye	Case report	DALK	Similar to PK, KCN can recur in the transplanted cornea after DALK. However, the time interval from transplantation to recurrence seems to be much shorter in DALK grafts.	4.1
Niziol et al.	2013	6/219 eyes	Retrospective case series	UNK	Allograft rejection is frequent in the 2 years after corneal graft for KCN. However, the 20-year probabilities of graft failure and recurrent KCN are low.	9–20
Bergmanson et al.	2014	n/a	Literature review	11 PK, 2 DALK	The host tissue most likely passes the disease to the donor tissue because the typical transplant surgery never completely eliminates the disease from the host eye. The exact mechanism of transferal has yet to be elucidated. *Re-emergence* rather than *recurrence* may better describe the pathophysiology.	PK: 19 DALK: 3–4
Feizi et al.	2017	2/382 eyes	Retrospective case series	DALK	Only 0.52% of eyes developed recurrent KCN. However, 3.7% of the total number of eyes that underwent DALK for KCN failed and required repeat keratoplasty. This rate was approximately half the rate of graft failure after PK for KCN.	4
Gatzioufas et al.	2017	1 eye	Case report	DALK	Potential risk for re-emergence of KCN during or after pregnancy particularly following lamellar keratoplasty. Physicians should be aware of this rare complication and counsel their patients accordingly.	2
Yoshida et al.	2018	18/50 eyes	Case series	PK	KCN progresses even after PK over the long term, requiring re-grafting in some cases. Risk factors for recurrent KCN after PK are increasing Ks over time and a large CYL.	27.2
Abad et al.	2020	2 eyes/1 person	Case report	OD: DALK OS: ICRS & CXL	Oftentimes, eye surgeons could have a false sense of relief after having performed DALK or CXL and might not be fastidious about controlling eye rubbing afterward. Meticulous eye rubbing control could be helpful in	1–1.5

Author(s)	Year	#	Design	Intervention Prior to KCN Recurrence	Conclusion	Recurrence (Years)
					patients with KCN after any type of surgical treatment.	
Murillo et al.	2021	3/66 people	Retrospective analysis	PK	Suggests an association between post-PK recurrent KCN and ScCl wear.	33–35

Table 7.

Progression and/or exacerbation of KCN after non-PK and non-DALK interventions

Author(s)	Year	#	Design	Intervention Prior to KCN Progression and/or Exacerbation	Conclusion	Recurrence (Years)
McDonnell et al.	1988	1 eye	Case Report	Electrosurgical Keratoplasty	The pathologic changes following electrosurgical keratoplasty in a human corneal button has not been previously described. Striking changes were present in the anterior two-thirds of the stroma after this experimental procedure. Despite the apparent contracture of the stromal collagen, the flattening induced by this procedure was short lived and the patient did require PK.	0.1
Ellis	1992	1 eye	Case Report	Radial Keratotomy	Because of the nature of the wound healing overlying the portion of the cornea affected by the KCN and because of the total regression of effect, radial keratotomy in patients with KCN does not appear to be an effective modality.	1.5
Sykakis et al.	2015	n/a	Systematic review	CXL	The evidence for the use of CXL in the management of KCN is limited due to the lack of properly controlled randomized control trials.	n/a
Abad et al.	2020	2 eyes/1 person	Case report	OD: DALK OS: ICRS & CXL	Oftentimes, eye surgeons could have a false sense of relief after having performed DALK or CXL and might not be fastidious about controlling eye rubbing afterward. Meticulous eye rubbing control could be helpful in patients with KCN after any type of surgical treatment.	1–1.5