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REVIEW

Genetic Aspects of Keratoconus: A Literature Review Exploring Potential Genetic Contributions and Possible Genetic Relationships with Comorbidities

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ABSTRACT

Introduction: Keratoconus (KC) is a complex, genetically heterogeneous, multifactorial degenerative disorder that is accompanied by corneal ectasia which usually progresses asymmetrically. With an incidence of approximately 1 per 2000 and 2 cases per 100,000 population presenting annually, KC follows an autosomal recessive or dominant pattern of inheritance and is, apparently, associated with genes that interact with environmental, genetic, and/or other factors. This is an important consideration in refractive surgery in the case of familial KC, given the association of KC with other genetic disorders and the imbalance

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between dizygotic twins. The present review attempts to identify the genetic loci contributing to the different KC clinical presentations and relate them to the common genetically determined comorbidities associated with KC.

Methods: The PubMed, MEDLINE, Google Scholar, and GeneCards databases were screened for KC-related articles published in English between January 2006 and November 2017. Keyword combinations of "keratoconus," "risk factor(s)," "genetics," "genes," "genetic association(s)," and "cornea" were used. In total, 217 articles were retrieved and analyzed, with greater weight placed on the more recent literature. Further bibliographic research based on the 217 articles revealed another 124 relevant articles that were included in this review. Using the reviewed literature, an attempt was made to correlate genes and genetic risk factors with KC characteristics and genetically related comorbidities associated with KC based on genome-wide association studies, family-based linkage analysis, and candidate-gene approaches.

Results: An association matrix between known KC-related genes and KC symptoms and/or clinical signs together with an association matrix between identified KC genes and genetically related KC comorbidities/syndromes were constructed.

Conclusion: Twenty-four genes were identified as potential contributors to KC and 49 KC-related comorbidities/syndromes were found. More than 85% of the known KC-related genes are involved in glaucoma, Down syndrome, connective tissue disorders, endothelial dystrophy, posterior polymorphous corneal dystrophy, and cataract.

Keywords: Keratoconus comorbidities; Keratoconus genes; Keratoconus risk factors

INTRODUCTION

Keratoconus (KC) is a relatively common [1, 2] bilateral [1, 3–21] corneal disease that is accompanied by corneal ectasia [1, 3, 4, 6, 9, 10, 13–19, 21–46] which usually progresses asymmetrically [1, 5, 9, 11–13, 15, 17–19, 30, 31, 33, 38, 39, 45]. Its main clinical manifestation is thinning and protrusion of the cornea [1, 2, 4–6, 10, 13–17, 19–22, 25–34, 36–44, 47–58], which assumes a conical shape [1, 2, 4, 13, 15–19, 27, 29–31, 33, 34, 36–40, 42, 44, 47, 48, 51, 55, 56, 58, 59]. These characteristics, even in the absence of clinically manifest KC, are also major risk factors for the condition [60, 61]; depending on the stage of the disease, KC presents with a variety of clinical manifestations and combinations of these characteristics/clinical signs [15, 55, 62]. KC is characterized by refractive errors that include myopia and irregular astigmatism [1, 3–8, 10, 13, 15–22, 25–28, 31, 32, 38–40, 42, 44–46, 50, 53, 57, 58, 63, 64], vision distortion, sensitivity to light, and multiple images [13, 31, 50, 65]. It is accompanied by a loss of visual acuity [1, 3–6, 13, 15–19, 22, 31, 35, 38–40, 44–47, 58, 66, 67] because of the distortions of the corneal curvature [1, 3–8, 10, 17–19, 21, 22, 31, 32, 38–42, 45, 46, 53, 58, 63, 64], which compromise its role in vision by distorting the refraction of light and its transmission onto the retina [1]. Corneal changes in KC also include acute corneal edema and scar formation [7, 8, 42, 45, 46], as in rare cases keratoconus presents with a central dense corneal stromal edema (hydrops) with linear oblique Descemet's tears (ruptures in Descemet's membrane), followed by corneal edema and scarring [68]. Finally, KC is associated with abnormal enzymatic activity within the cornea [38, 69].

Given that KC is an insidious and irreversible disease [70], it is vital to diagnose it as soon as possible [8, 19], and to treat each patient on an individual basis because of the unique nature of the disease [8, 19].

The term "ectasia" has been broadly used by ophthalmologists, vision scientists, and optometrists to characterize different conditions that affect the shape of the cornea. Keratoconus, pellucid marginal degeneration (PMD), keratoglobus, and post-refractive surgery progressive corneal ectasia are ectatic diseases, while keratoconus and PMD are different clinical presentations of the same basic disease process. In contrast, Terrien marginal degeneration, dellen, rheumatoid/autoimmune melts, and other similar conditions are corneal thinning disorders. Against this background, ectasia progression is

considered to correspond to at least two of the following three changes: an increase in the steepness of the anterior corneal surface; an increase in the steepness of the posterior corneal surface; and a thinning or thickening of the cornea upon transversing it from the periphery to the thinnest point [71]. This means that there is an average difference of 75 μm in the central corneal thickness (CCT) between keratoconus patients and normal controls [34].

METHOD

The present review is based on a search of the PubMed, MEDLINE, Google Scholar, and GeneCards databases for articles related to KC. The keywords used were "keratoconus," "risk factor(s)," "genetics," "genes," "genetic association(s)," and "cornea," as well as all relevant/meaningful combinations of those terms. The search focused on articles written in English from January 2006 until November 2017. A total of 217 articles were identified and reviewed, and both the text and references in each paper were analyzed. The analysis revealed an additional 124 relevant articles, which were also reviewed. The review is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors. The aim of the review is to summarize current research into the genetics of KC. It also represents an attempt to correlate genes and genetic risk factors with KC characteristics and genetically related KC comorbidities based on genome-wide association studies, family-based linkage analysis, and candidate-gene approaches.

DEMOGRAPHICS

The incidence of KC is approximately 1 per 2000 [1, 2, 4, 10, 32, 49, 51–53], while the annual incidence of KC is estimated at 2 per 100,000 [1, 13, 15, 18]. However, estimates of its incidence in the general population vary widely, from 1/500 to 1/2000 per year [1, 6, 29, 31, 33–35, 39]. The prevalence of KC is estimated as between 8.8 and 54.5 per 100,000 [1, 13, 15, 18, 29, 59] and between 50 and 230 per 100,000 depending on ethnicity [25, 27, 35, 40, 56, 58, 72] and the diagnostic criteria used [1, 4, 53]. Its prevalence may rise further as the use of new technologies permits better and earlier diagnosis [2, 73].

The role of ethnicity is very important; there is a sixfold increase in the incidence of KC in Asians compared with Caucasians [74, 75]; its incidence is 25/100,000 among Asians [72] but only 3,3/100,000 in Caucasians [72], while differences have been reported in the central corneal thinning (CCT) distribution between Asian and Caucasian patients [76]. Apart from the differences between Asian and Caucasian patients, the incidence of KC is greater in Indians than in Chinese or other ethnic groups [27, 75, 77]. In the UK, the prevalence of KC in Asian (Indian, Pakistani, and Bangladeshi) subjects is 4.4–7.5 times greater than that in white Caucasians, in line with the high prevalence of KC in India [72, 75, 78] and the higher prevalence and incidence of keratoconus in Asian populations in comparison to Caucasians [72, 79]. Finally, KC appears earlier and is more severe in Chinese patients [80], requiring corneal grafting at an earlier age [81].

Among various other ethnicities, the prevalence of KC ranges from 0.3 per 100,000 in Russia [78, 82] to 2300 per 100,000 in Central India (0.0003–2.3%) [53, 78]. A relatively high prevalence has been reported in Minnesota, USA (0.054%) [2, 78] and in Jerusalem [78, 83]. Although the incidence of KC in Saudi Arabia is 20/100,000 (0.02%), its incidence and severity in specific regions of Saudi Arabia (e.g., Asir Province) are high, with an early onset and more rapid progress to the severe disease stage at a young age [78, 84]. Finally, worldwide estimates range from 1/100,000 in the United Kingdom to 2/100,000 in Minnesota (USA), 2.2/100,000 in Finland, 2.5/100,000 in Holland, and 50/100,000 in New Zealand [78]. Apparently, locations with a lot of sunshine and hot weather such as India [53, 78] and the Middle East [78, 84] tend to be associated with a higher prevalence of keratoconus than locations with cooler climates and less sunshine, such as Finland [78, 85], Denmark [51, 78], Minnesota [2, 78], Japan [78, 86], and Russia [78, 82].

GROSS AND HISTOLOGICAL PATHOLOGY

Frequent stimulation and/or damage to the corneal epithelium contribute to the pathogenesis of KC [87]. Several types of collagen are reduced in KC epithelium and stroma. It has been postulated that altered expression and/or activity of lysyl oxidase, which plays a critical role in the biogenesis of connective tissue, weakens covalent bonds between the collagen and elastin fibrils and leads to biomechanical deterioration of the cornea [43, 88]. This potentially accounts for abnormal microstructures detected in the keratoconus cone that result in slippage, folding, and even rupture of the anterior lamellae and to decreasing biomechanical strength during the progression of keratoconus. Furthermore, these alterations may encourage keratocyte activation and its conversion into fibroblasts, and even myofibroblasts [43, 88].

Inflammatory and apoptotic pathways are associated with KC [38, 44, 89, 90], and the resulting imbalance between the enhanced apoptosis and the proliferation of the corneal epithelial cells [91] is thought to play a vital role in the thinning of the cornea [38, 92]. Histologically, 75% of the normal corneal stroma thickness is lost [93], resulting in hypoplasia [6] and extensive corneal distortion characterized by severe decreases in the amount and distribution of corneal collagen fibers [6, 43, 54, 58]. The collagen fibrils appear morphologically normal but there is fragmentation of the epithelial basement membrane. There are membrane anomalies of the keratocytes, fibrillation, and disintegrations of Bowman's membrane, while electron microscopy reveals degenerative changes in the basal epithelial cells [14, 88].

Another feature is Vogt's striae, which appear as fine vertical (rarely horizontal) whitish lines in the deep stroma and Descemet's membrane that can be observed in a slit-lamp examination; they disappear on the application of gentle pressure [1, 19, 25, 26, 56, 94, 95]. Other signs are anterior stromal scars, epithelial nebulae, and increased visibility of corneal nerves [1, 4, 6, 11, 12, 19, 42, 46, 53, 96, 97]. In addition, iron deposits are frequently observed on the basal layers of the corneal epithelium, together with breaks in the continuity of Bowman's layer [1, 5, 6, 19, 96, 97].

In the more severe cases, acute stromal edema may develop, which causes breaks in Descemet's membrane and leads to aqueous leakage (hydrops) [19, 56, 94] and, ultimately, corneal scarring [19, 21, 27, 42, 45, 46, 62].

A V-shaped conformation of the lower lid when the patient's gaze is directed downwards, which is due to corneal ectasia (Munson's sign), can contribute to the diagnosis of KC [1, 6, 24, 26]. Other clinical signs that can contribute to the diagnosis of KC are Rizzuti's sign and Charleaux's sign [1, 26]. Also, the scissoring of the retinoscopic reflex with a fully dilated pupil can contribute to the diagnosis of keratoconus [26, 98, 99].

NATURAL HISTORY

Although the onset of KC is a controversial topic, most studies agree that KC appears in the teens and early twenties and progresses until the fourth decade of life [1, 6–8, 16, 18, 19, 27, 29, 31, 35, 44, 47, 48, 56, 100–103], and that it occurs in all ethnicities [6, 10, 30, 40, 74, 104–106] without a male or female predominance [6, 30, 31, 104–106]. Other studies, however, place its onset at an average age of 39.3 years [59], while others estimate the male/female ratio at 1.7 [1]. Finally, differences between populations in the biometric parameters of the eyes are attributable to environmental and interethnic factors [107, 108].

HERITABILITY

KC is a complex, genetically heterogeneous, multifactorial degenerative disorder [1, 109–114] with a sporadic distribution [1, 2, 104, 109, 115–117]. Reports indicate that proportions ranging from 5–10% [2, 31, 39, 104] to 8–10% [1, 29] or 14% [59], and even up to 23% of KC patients have a family history of KC. It follows an autosomal recessive or dominant pattern of

inheritance [1, 35, 39, 104, 118–122]. In the autosomal dominant pattern, there are many phenotypes with incomplete penetrance [39, 123]. In other words, there appears to be a genetic predisposition for KC [39, 122, 124–126], and the genes associated with KC interact with environmental, genetic, and/or other factors [1, 6, 24, 39, 49, 67, 70, 99, 109, 120, 127–129]. This underscores the potential role of genetic factors in KC pathogenesis [72, 95, 125, 128, 130], which is an important consideration in refractive surgery to treat familial KC [130, 131], given the association of KC with other genetic disorders and the imbalance between dizygotic twins [59, 120, 127, 130, 132]. Indeed, the prevalence of KC is 3.34% in first-degree relatives of KC patients (i.e., 15–67 times higher than that for the general population [125]), while its concordance in monozygotic twins adds strong support to the notion of a genetic basis for KC [1, 124, 125, 133–135].

Another heritability-related issue is that of increased corneal curvature. The heritability of this trait has been reported to range between 60 and 95% [136–140]. The Beaver Dam Eye Study (which included 715 individuals in 185 families) estimated it at 95% [137], whereas the Danish Twin Registry estimated it at 90% based on a study of 114 pairs of twins [140]. It is apparent that an increasing corneal curvature is associated with various factors aside from heredity, including age, gender, height, ethnic background, geographical area, and environmental conditions [120, 124, 136, 141–146], and is influenced by both lifestyle and genetic factors [63, 108, 141].

ASSOCIATIONS OF KC WITH DIFFERENT COMORBIDITIES

The genetic etiology of KC is far from being fully clarified, and the pathophysiological processes involved are largely unknown [24, 147–150]. Multifactorial interactions of KC with environmental and genetic factors [1, 4, 6, 31, 56, 57, 104, 121, 123–125, 128, 132, 133, 148, 151–162] have already been mentioned. The pathophysiology of keratoconus is likely to include the following components: genetic disorder, biochemical disorder, biomechanical disorder, and environmental disorder [29].

KC has been associated with spring conjunctivitis [163], contact lens use, atopic disease, UV light, and eye rubbing [1, 31, 33, 104, 128, 151, 154, 156–160], while it can be triggered by trauma [164]. KC is also associated with several diseases, especially those belonging to the atopic diathesis. Atopic disorders affect up to one-third of the population in developed countries. They affect several epithelia, including the skin (atopic dermatitis, AD), the respiratory tract (asthma, allergic rhinitis), and the eye (allergic conjunctivitis). Atopic diseases, especially AD, which is typically the first clinical manifestation of atopy, have been associated with KC in several uncontrolled studies with conflicting data according to the type of patient recruitment [29]. KC is also associated with a constellation of genetic syndromes, including Down syndrome [32, 165–168], Marfan syndrome [169], osteogenesis imperfecta [170], GAPO syndrome [171], Ehlers–Danlos syndrome and its subtypes [56, 57, 172, 173], Noonan syndrome, pigmentary retinopathy [174], Leber congenital amaurosis (LCA) [57, 127, 175–178], Apert syndrome, mitral valve prolapse [1, 4, 104, 115, 124, 128, 132, 133, 148, 151–153, 155, 161, 165–167, 179, 180], congenital hip dysplasia [181], Rieger syndrome [182], focal dermal hypoplasia [183], Crouzon syndrome [184], floppy eyelid syndrome [185], monosomy X (Turner syndrome), Bardet–Biedl syndrome, nail-patella syndrome, ichthyosis, neurofibromatosis, xeroderma pigmentosum, collagenosis, neurocutaneous angiomatosis, pseudoxanthoma elasticum, retinitis pigmentosa [104, 124, 174], atopy, vernal keratoconjunctivitis [127, 165, 166, 176–180], and other noninflammatory connective tissue disorders such as joint hypermobility [1, 56, 167]. Finally, other disorders associated with KC are cataracts, Avellino corneal dystrophy, and granular corneal dystrophy, although there is a school of thought which insists that KC is not a feature of any specific syndrome but mostly an isolated ocular disorder [147, 148, 186, 187].

KC therefore can be divided into three broad categories: (a) KC associated with genetic

disorders such as Down syndrome, neurofibromatosis, LCA, Turner syndrome, nail-patella syndrome, etc.; (b) KC associated with eye rubbing, mitral valve prolapse, atopy, contact lens use, LCA, and a positive family history; and (c) isolated KC without any associations [188–190].

THE ROLE OF GENETIC FACTORS IN KC

Although the genetic etiology of diseases such as age-related macular degeneration and pseudoexfoliative glaucoma is almost fully known [191], much about heritable blinding diseases such as primary open-angle glaucoma, KC, and myopia is still unknown [191]. Observations regarding KC heritability and the association of KC with a multitude of genetic syndromes have prompted a shift of focus to the genetic factors involved in KC [95, 128]. A host of genetic epidemiological data, segregation analyses, and gene mapping studies have indicated that genetic factors play a significant role [95, 122, 124–126, 128], and many gene loci and chromosomal regions associated with familial KC have been mapped by linkage analysis [104, 109, 114, 117–119, 121, 192–196], especially in Caucasians, Australians, and Chinese [51]. However, no specific genes have been identified in these regions as yet [118, 121, 192–196], and reports on differentially expressed genes in KC patients are scarce [197–200].

CCT meta-analysis of a sample of more than 20,000 individuals suggested that there are 27 CCT-associated loci [201]. Eleven SNPs showed nominal associations with KC, while 6 had significant associations after correcting for multiple testing [70]. These 11 CCT-associated loci were located 100 kb upstream [202, 203] of the ZNF469 gene (zinc finger 469 gene; MIM 612078)—the gene most strongly associated with CCT [76, 201, 204–209]. The SNPs rs12447690 and rs9938149 [201] upstream of the ZNF469 gene were also identified in Australian, UK, Croatian, Scottish, Indian, Malay, Caucasian, and Latino populations [203, 205, 208], a finding that suggests the involvement of these SNPs in CCT variation. Studies from Australia and the UK revealed two genome-wide significant signals at 16q24.2 and 13q14.1 in intragenic regions that influence the genes ZNF469 and FOX01, respectively [210]. However, sequencing analysis of the gene ZNF469 in patients with KC and high myopia showed no significant variants [211].

Genome-wide association studies (GWASs) and other studies have also associated KC with more than 60 genes/loci, although the roles of these genes/loci are inconclusive, as the reported associations were inconsistent across different study cohorts. Among them are the genes HGF (Hepatocyte Growth Factor, which has well-established effects on epithelial cells), LOX (a lysyl oxidase whose copper-dependent amine oxidase activity functions in the crosslinking of collagens and plays a significant role in collagen chain trimerization), FOXO1 (a gene of the forkhead family of transcription factors, the specific function of which is yet to be determined, but which participates in the cytokine signaling pathways in the immune system), and FNDC3B (Fibronectin Type III Domain Containing 3B). GWASs have also associated KC with more than 150 polymorphisms [70, 201, 212–214]. It should be noted, however, that this number is inflated, and a significant association with KC has only been proven for some of them [70]. Thus, GWAS identified 7 genes/loci, including the LOX, FOXO1, HGF, and FNDC3B genes and the 2q21.3, 3p26, and 19q13.3 loci [70]. Again, these associations differ from study to study, so their roles remain uncertain [201, 212–214]. Moreover, the genetic association profiles of sporadic and familial keratoconus could be different [70]. For example, GWASs of KC report that rs3735520, located upstream of the HGF gene, is associated with KC in American and Australian populations [213, 215]. In addition, a relation between HGF and refractive error has been reported in Caucasian and Han Chinese populations [216]. Other studies of KC families from Australia report an association between the LOX variants (rs10519694 and rs2956540) located at 5q23.2 and KC [196, 214]. Furthermore, there are reports that rs3735520 in the promoter of HGF and rs2956540 in LOX are associated with KC in

the European population [35, 217]. In contrast, a recent meta-analysis showed that there is no significant association between KC and SNPs in ten reportedly associated genes/loci, including IL1A, IL1B, BIRC8, BHLHB2, LRRN1, KIF26B, VSX1, PPP3CA, 3q26.2, and 12p13.3 [201].

Another focus of investigations of genetic contributions to KC is their role in KC progression. This is a very complex subject because of the genetic heterogeneity of KC [195]; many of these studies focus on different chromosomal loci, have moderate sample sizes, and have difficulties in localizing regions through linkage [195]. Despite the lack of sufficient information for selecting KC susceptibility genes [212], GWASs are a very effective means for investigating KC progression [218, 219]. Two other GWASs, the Singapore Malay Eye Study (SiMES) and the Singapore Indian Eye Study (SINDI), reported two genetic regions (RXRA/COL5A1 and COL8A2) that are associated with central corneal thinning in Asians. RXRA (Retinoid X Receptor Alpha) is a protein coding gene with a well-established association with keratoconus; it participates in the apoptotic pathways in synovial fibroblasts and in organelle biogenesis and maintenance. COL5A1 encodes an alpha chain and appears to regulate the assembly of heterotypic fibers composed of both type I and type V collagen. COL8A2 encodes the alpha 2 chain of type VIII collagen; this protein is a major component of the basement membrane of the corneal endothelium and forms homo- or heterotrimers with alpha 1 (VIII)-type collagens. Defects in this gene are associated with Fuchs endothelial corneal dystrophy and posterior polymorphous corneal dystrophy (PPCD) type 2 [220]. The SiMES and SINDI studies also agreed with the results for Europeans regarding the role of ZNF469 [206] (which encodes a zinc-finger protein); it may function as a transcription factor or extranuclear regulator factor for the synthesis and/or organization of collagen fibers. Mutations in this gene cause brittle cornea syndrome.

Another GWAS, which used microarray analysis of scraped-off epithelial tissue to compare gene expression in normal corneal epithelium with that from KC patients, identified 15 keratoconus-associated SNPs from 13 loci [197–199, 221]. Other reports focus on the vital role of apoptosis in KC corneal thinning [38, 44, 200] or the associations between different SNPs and KC [24, 95, 166, 188, 201, 213, 222–230]. Meta-analysis revealed that there is an association between KC and the SNP rs4954218 located near RAB3GAP1, which encodes the catalytic subunit of the RAB3GAP enzyme (i.e., the RAB3GTPase-activating protein); this enzyme controls the exocytosis of neurotransmitters and hormones and the RAB3 cycle [231]. Alterations in RAB3GAP1 are related to the autosomal recessive Martsolf syndrome and the Warburg Micro syndrome, which are combined with ocular and neurodevelopmental dysfunctions [221, 232].

SNPs and Loci Identification

Apart from the contributions of GWASs to known KC-related gene identification, substantial contributions to the clarification of the genetics of KC have arisen from the identification of relevant SNPs and loci. Five loci with six independent SNPs associated with KC have been reported, of which two have been confirmed in relation to KC risk in an independent cohort of patients of European origin [233]: rs1324183, upstream of the MPDZ gene (Multiple PDZ Domain Crumbs Cell Polarity Complex Component, which plays a role in nonsyndromic autosomal recessive 2 hydrocephalus and in congenital communicating hydrocephalus), and rs9938149, upstream of the ZNF469 gene. It is not known if other SNPs among those found in European patients are relevant to the SNPs found in Asians [76].

Specific CCT SNPs associated with KC are rs4894535 (FNDC3B), rs1324183 (MPDZ-NF1B), rs1536482 (RXRA-COL5A1), rs7044529 (COL5A1), rs2721051 (FOXO1), and rs9938149 (BANP-ZNF469) [201]. Furthermore, linkage analysis has shown that there are six chromosomal loci for isolated KC [201, 212]: 2p24, 3p14–q13, 5q14.3–q21.1, 13q32, 16q22.3–q23.1, and 20q12. However, no other disease-related mutation was identified for these loci [53, 118, 148, 192–194]. Of those, the chromosomal regions 2p24, 3p14–q13, 5q14.3–q21.1,

and 16q22.3–q23.1 were mapped by genome-wide scans of various resolutions in family studies, while the linkage between KC and locus 13q32 [148] was identified by linkage analysis. Also, heterozygous nucleotide substitutions were identified in c.2262A > C (exonic region of DOCK9), in c.2377-132A > C (intronic region of IPO5 which codes for a protein of the important beta family involved in nucleocytoplasmic transport), and in c.1053 + 29G > C (STK24), showing 100% segregation with the affected phenotype (in an Ecuadorian family) [148]. It should be noted that the STK24 gene encodes a serine/threonine protein kinase which is cleaved into two chains by caspases; the N-terminal fragment (MST3/N) translocates to the nucleus and promotes programmed cell death. Moreover, a Gln754His substitution (because of a variant c.2262A > C in DOCK9) might negatively affect the VSX1 (Visual System Homeobox 1)-coded protein function and structure [233]. Meta-analysis studies, therefore, have identified 8 SNPs in 6 genes/loci which are thought to be significant genetic markers for KC in whites, including FNDC3B rs4894535, BANP-ZNF469 rs9938149, RXRA-COL5A1 rs1536482, FOXO1 rs2721051, COL4A4 rs2228557 and rs2229813, and IMMP2L rs214884 and rs757219 [70]. They have also identified 10 genes/loci with suggestive evidence of associations with keratoconus [70].

Regarding 3p14–q13 and 5q14–q21 mentioned above, and 15q22–q24 (which is also involved in congenital cataract), a link has been established with autosomal dominant forms of KC [119, 195]. Moreover, the 2p24 locus has been linked with KC in families from Europe and the West Indies and the locus 16q22–q23 has been linked to KC in families from Finland [118, 192, 194, 196]. It should be noted that there are no references to these regions in other populations [117].

Also, affected-only linkage analyses related regions 4q31, 5q31, 9q34, 12p12, 14p11, 17q24, and 20q12 to KC [31, 188, 193, 194, 222, 234]. Of these, locus 9q34 has been linked with KC in families from Spain and locus 20q12 with KC in families from Tasmania. In addition, a copy number variation (deletion) of 5q31 has been reported in a family with autosomal dominant KC as well as in KC patients with other ocular and developmental abnormalities [235].

In another study, a large four-generation Caucasian pedigree was identified and the responsible gene was subsequently mapped to a novel 8.2 MB (megabases or million base pairs) genomic region located at 5q14.3–q21.1 that contains more than 50 known or predicted genes [35, 196]. Further genotyping of tightly spaced SNPs in the linkage region by the same group resulted in the narrowing down of the region to approximately 5mb (96mb–100mb) [35, 121], though this locus still needs confirmation. The 5q31.1–q35.3 linkage region overlaps with two of the other loci on 5q associated with KC, namely with 5q31 in Caucasian/Hispanic populations and 5q32–q33 in Southern Italian populations, supporting the possibility that the 5q31.1–q35.3 locus might actually be linked with KC [119, 236]. Similarly, SPARC (Secreted Protein Acidic and Cysteine Rich gene), which encodes a cysteine-rich acidic matrix-associated protein involved in extracellular matrix (ECM) synthesis [237] and the promotion of changes to cell shape) and LOX, which are located on 5q31.3–q32 and 5q23.2, respectively, are indicated as candidate genes for KC (linkage in familiar KC) [35, 121, 196].

SPECIFIC GENES INVOLVED IN KERATOCONUS

Several genes from the chromosomal regions COL6A1 (which encodes the alpha 1 subunit of type VI collagen), SOD1 (which encodes a soluble cytoplasmic protein which converts superoxide radicals to molecular oxygen and hydrogen peroxide), and COL8A1 (which encodes one of the two alpha chains of type VIII collagen, which is a major component of the basement membrane of the corneal endothelium) have been excluded as KC causal agents. Also, only a few KC patients have been reported to carry VSX1 mutations [35, 188, 193, 194, 222, 224]. VSX1 mutations cause PPCD and keratoconus (e.g., the SNP rs6050307 in a Han Chinese population) [238]. However, studies into the role of VSX1 have yielded conflicting results [149, 163, 176, 186,

188, 239–241], while there are no definitive or consistent findings about the roles of most genes [104, 118, 119, 148, 166, 180, 188, 193, 199, 200, 242, 243].

Other genes that are associated with KC are DOCK9 (Dedicator of Cytokinesis) and MIR184 (microRNA 184) [188, 228, 229, 239]. MIR184 regulates the VEGF and Akt signaling pathways, and it can inhibit corneal angiogenesis; mutations in the seed region of MIR184 cause familial keratoconus with cataract, which is known as EDICT syndrome.

THE VSX1 GENE

Despite the often conflicting results of studies focusing on the role of VSX1 in KC, there are many studies that report specifically on the role of VSX1 in the pathogenesis of KC [117, 121, 148–150, 153, 166, 186, 188, 195, 226, 228, 239–241, 244–254], as well as studies focusing on both the VSX1 and SOD1 genes [166, 188, 222, 239–241, 255], which are highly conserved across many species [188–190]. Studies that failed to associate VSX1 with KC [105, 148, 149, 166, 167, 186, 188, 190, 212, 241, 244, 245, 247, 250–252, 254, 256] most likely did so because of ethnic variation, the low frequency of changes, and the multifactorial and polygenic character of KC [167]. The VSX1 gene plays a role in corneal wound repair in the corneal stroma. This involves the development of myofibroblasts by corneal stromal cells, and is evidenced by the increased expression of VSX1 during the process of wound healing and abnormal stromal repair [257].

The VSX1 gene is located within chromosome 20p11–q11 [166] and encodes a protein acting as a homeodomain transcription factor which is responsible for cone opsin expression during early ocular development and the differentiation of cells in craniofacial development [149, 249, 258–260]. Moreover, it is associated with the core of the locus region that controls the red-green visual pigment gene cluster [166]. It has also been observed that there are six transcripts and five variants which encode a truncated protein, while two of them retain the DNA-binding domain [261]. The five exons of VSX1 encode a protein consisting of 365 amino acids with a homeobox DNA-binding domain, a ceh-10 domain, and a Chx10/VSX1 domain [166, 249, 262]. The mRNA from the VSX1 gene is detected in embryonic craniofacial tissue, in the inner layer of the retina, and in the cornea [249, 257, 258]. Mutations in VSX1 trigger developmental abnormalities in retinal cells, craniofacial tissues, sella turcica, and in corneal endothelium [253].

There are 5 exons in the VSX1 gene which span 6.2 kb of the coding sequence [247, 249, 258]. Other reports mention that the exons of VSX1 span 6.7 kb of DNA on chromosome 20 with two main transcripts; transcript 1 (NM_014588) encodes protein isoform A (NP_199452.1) and transcript 2 (NM_199425) encodes protein isoform B (NP_955457) [252, 261]. Two more exons of VSX1 produce four previously unknown VSX1 transcripts [241, 249, 258, 261]. Therefore, VSX1 spans 10.65 kb of genomic DNA and has 7 exons producing 6 transcripts (via different splicing patterns). According to Genbank, these are NM_199425 (or DQ854808), NM_014588 (or DQ854807), DQ854809, DQ854810, DQ854811, and DQ854812 [241, 249, 258, 261]. Exons 2, 3, and 4 of VSX1 apparently have an increased probability of being mutated [244]. Even so, not all VSX1 mutations are causally connected to KC. Attempts to associate Q195H in VSX1 with KC were unsuccessful [153].

Variants of VSX1 such as G160D, P247R, L17P, G160V, N151S, D144E, H244R, L159M, and R166W are possibly associated with KC [153, 166, 248, 258]. For example, the G160D variant is pathogenic while P247R is not pathogenic [153, 166, 248, 258]; however, P247R seems to co-segregate with KC [188]. There is also confusion about the role of the D144E mutation in KC, as some studies mention that it is pathogenic whereas others do not [188, 248, 251, 252]. In addition, the variants H244R, L159M, and R166W are not considered sufficient to cause KC, although they have been identified in KC patients [240]. Also, the roles of mutations of H244R, L159M, and R166W in VSX1 in KC [166], as well as the role of the mutation of D144E, have been disputed [153, 186, 240, 245, 248, 251, 252, 261]. Finally,

in the Korean population, two other variants, G160V and N151S, were identified but have not been demonstrated in other populations [239].

Other VSX1 variants identified in KC patients are c.432CAG (Asp144Glu), c.475T4A (Leu159Met), c.496C4T(Arg166Trp), and c.731A4G(His244Arg) [166]. Three of these, i.e., Asp144Glu, Leu159Met and Arg166Trp, as well as Pro247Arg and Gly160Asp (also in the VSX1 gene), are possibly associated with two corneal dystrophies, KC and PPCD [166, 220]. Other mutations in VSX1 such as Leu17Pro [188], the missense mutations Asn151Ser and Gly160Val, and one intragenic polymorphism are associated with KC only [239]. In addition, susceptibility to KC is evident in patients with LCA and mutations in CRB1 (which encodes a protein that localizes to the inner segment of mammalian photoreceptors), CRX (which encodes a photoreceptor-specific transcription factor that participates in the differentiation of photoreceptor cells and is essential for normal cone and rod function), and AIPL1 (which is expressed in photoreceptors and the pineal gland; it encodes aryl-hydrocarbon-interacting protein-like 1 and is involved in nuclear transport activity) [175, 178]. Note that, apart from LCA, N mutations are also associated with a severe form of retinitis pigmentosa (RP12); CRX mutations are associated with photoreceptor degeneration, LCA type III, and autosomal dominant cone-rod dystrophy 2; while AIPL1 mutations are responsible for 20% of recessive LCA.

VSX1 and PPCD

The VSX1 gene is associated with the pathogenesis of endothelial corneal dystrophy, Fuchs, and PPCD [166, 188–190, 220, 246, 254, 263–266]. PPCD is a typically bilateral hereditary corneal dystrophy characterized by abnormalities in Descemet's membrane and the corneal endothelium, with a primarily asymmetric clinical presentation [220, 267]. PPCD is, reportedly, associated with KC [155, 220, 268, 269]; it is genotypically heterogeneous [270, 271], with one-third of the PPCD cases associated with mutations in the PPCD3 locus, in the AIPL1gene (which encodes a zinc finger transcription factor; mutations of it are associated with posterior polymorphous corneal dystrophy-3 and late-onset Fuchs endothelial corneal dystrophy) [270, 271]. PPCD is also affected by the COL4A3 gene (which encodes the collagen type IV alpha 3 chain) expression in the cornea. Similarly, mutations in COL8A2 are associated with PPCD and Fuchs [220, 266, 270, 271]. Finally, the chromosome 20p11–q11 is associated with PPCD1 (cytogenetic location 20p11.23), while VSX1 gene mutations (genetic locus VSX1; MIM#605020 and genetic locus KTCN1; MIM#148300) are involved in PPCD1 and KC, respectively [166, 220, 239, 248].

The SOD1 Gene

The SOD1 gene has a role in the pathogenesis of KC [147, 150, 166, 222], albeit without a definite association [186, 272, 273]; SOD1 mutations have also been implicated in amyotrophic lateral sclerosis [274, 275]. The SOD1 enzyme binds zinc and copper ions and destroys free superoxide radicals, thus protecting the cell from damage [147, 276, 277]. It is of note that the distribution of superoxide dismutase isoenzymes differs between the healthy cornea and the corneas of KC patients [278].

SOD1 is located on chromosome 21q22.11 and encodes superoxide dismutase enzymes [222, 274, 275]. A SOD1 variant with a seven-base deletion in intron 2 (IVS2 + 50del7 bp) is associated with KC [147, 222]; mRNA analysis showed the presence of two additional transcript splice variants coding for proteins lacking the active site of the SOD1 enzyme [222], while a deletion has been identified within intron 2 close to the 5′ splice junction of SOD1 in 3 KC patients [186, 222, 272, 273]. In addition, the skipping of SOD1 exon 2 or SOD1 exon 2 + 3 results in weak protein expression or no expression at all, since it diminishes the enzyme levels and activities [154, 186, 222, 272, 273]. It is probable that mutations on chromosome 21 may be related to the effects of oxidative stress on the cornea, while trisomy 21 is associated with an increased risk of KC [150, 255, 278].

The SOD Isoenzymes and ROS

Oxygen tension, light exposure, and high metabolic activity contribute to the production of reactive oxygen species (ROS) [277], while the human eye is particularly vulnerable to oxidative stress [277]. There are three superoxide dismutase (SOD) isoenzymes (SOD1, SOD2, and SOD3). They are compartmentalized in the mitochondrial matrix, the cytosol, and the extracellular space, and they trigger the generation of hydrogen peroxide by catalyzing the dismutation of the superoxide radicals [277]. In KC patients, these antioxidant enzymes are altered [110, 222, 278, 279], resulting in an increase in the byproducts of the nitric oxide and lipid peroxidation pathways [111, 280]. Furthermore, increased levels of reactive oxygen species (ROS) result in a decrease in mitochondrial membrane potential [281], apoptosis of corneal fibroblasts, and oxidative damage that leads to the upregulation of altered proteins, degradation of enzymes, cellular dysfunction, and DNA damage [93, 282–284].

The ZNF 469 Gene

ZNF469 is a two-exon gene that codes a 413 kDa protein consisting of 3,925 amino acid residues [285]. It is detected in the human cornea as well as in various tissues [210]. ZNF469 shows a 30% sequence similarity to the helical parts of COL1A1 (encoding the pro-alpha1 chains of type I collagen, which is abundant in the cornea), COL1A2 (encoding the pro-alpha2 chains of type I collagen, also abundant in the cornea), and COL4A1 (which encodes a type IV collagen alpha protein, an integral component of basement membranes). The COL1A1, COL1A2, and COL4A1 genes are highly expressed in the cornea [31, 210].

There are five classical C2H2 zinc finger domains (ZNFs) in the C-terminus; these are the most important parts of the ZNF469 protein. ZNFs work as sequence-specific DNA-binding motifs that regulate some specific transcription processes [207]. Although the role of ZNF469 is not well established, evidence shows that it regulates the development and maintenance of the ECM [286]. It is also possible that ZNF469 is a transcription factor or an extranuclear regulator for the synthesis and organization of the corneal collagen fibers in humans [114, 207, 286]. This organization of the collagen fibers occurs in conjunction with the gene PRDM5 (which encodes a transcription factor of the PR-domain protein family); heterozygous mutations of PRDM5 have been linked with mildly reduced CCT (480–505 μm), KC, and blue sclera [286].

Brittle Cornea Syndrome (BCS)

BCS (also known as brittle cornea syndrome 1) can lead to blindness. It is a tissue disorder characterized by thinning and fragility of the cornea that can develop even after minor trauma, and is associated with homozygous mutations in ZNF469 [120]. It is an autosomal recessive generalized connective tissue disorder associated with extreme corneal thinning to between 220 and 450 μm, leading to a high risk of corneal rupture [286, 287]. Other common features of BCS include deafness, leading to combined sensory deprivation [287] and joint hypermobility, among other features of connective tissue disorders, including scoliosis [287, 288].

There are two types of BCS. BCS type 1 (OMIM#229200) features all of the signs mentioned above and is a result of homozygous mutations in the ZNF469 gene [210]. As previously mentioned, the gene was originally mapped to chromosome 16q24 [210], while five homozygous mutations of the ZNF469 have been reported: a homozygous frameshift mutation (c.9527delG) resulting in a premature termination codon (p.Gln3178ArgfsX23) [210]; p.Gln1392X [210]; p.Phe717SerfsX14; p.Gln1757X [286]; and the homozygous missense mutation p.Cys3339Tyr [288]. BCS type 2 is an autosomal recessive condition (like BCS type 1) results from mutations in the PRMD5 gene [286]. The wide variety of mutations in ZNF469 have been reported to be linked to an increased risk of isolated KC and BCS type 1 in patients of different ethnicities (23% of patients with KC in New Zealand, 12.5% of the European KC patients from three different cohorts and 50% of Maori or Polynesian KC patients). Among the KC patients in Polynesian populations, 23%

were reported to have a rare missense mutation in ZNF469 [203].

The TGFβ Pathway

The TGFβ pathway (transforming growth factor-β pathway) alters the modulation of the ECM, thus contributing to the pathogenesis of KC [289, 290]. TGFβ consists of three isoforms: TGFβ1, TGFβ2, and TGFβ3, and binds to TGFβ receptors (which also present three different isoforms) [291]. TGFβ1 (transforming growth factor beta 1) plays a role in myofibroblast transformation and proliferation, wound healing, keratocyte activation, chemotaxis, and corneal dystrophies [292]. TGFβ2 is increased in the aqueous humor of KC patients, although no elevation in TGFβ2 levels was observed in immunofluorescence studies [291].

THE TGFBI GENE

Another gene that may be associated with KC is TGFBI (Transforming Growth Factor β-Induced), which encodes the protein βig-h3 that binds to type I, II, and IV collagens and plays a role in cell–collagen interactions [224]. βig-h3 is involved in the development of corneal stroma, contributing to the movement, cell adhesion, and interaction with the ECM [293]. This protein is decreased in the ECM and epithelium of KC patients, implying the involvement of the TGFBI gene in KC [293]. The role of the TGFBI gene in KC was elucidated by observing that the c.1603G4T mutation located in exon 12 of TGFBI led to the Gly535Ter substitution at the protein level in a Chinese patient with sporadic KC [224]. This result has not been replicated, and an association between TGFBI and KC is yet to be established [235].

Expression of cytokine nuclear factor κβ (NF-κβ), anti-inflammatory marker transforming growth factor β (TGF-β), interleukin 6 (IL-6), and the proinflammatory marker tumor necrosis factor α (TNF-α) increases in the corneas of KC patients [294]. TGF-β is associated with corneal dystrophies [295, 296], while there is aberrant TGF-β signaling in KC [297]. The TGF-β ligand binds to TGF-βR1, which then dimerizes with TGF-βR2 and stimulates the phosphorylation of SMAD2/3 [298]. It is then translocated to the nucleus and the transcription of genes targeted by TGF-β is activated [298]. TGF-β signaling is negatively regulated by SMAD6 and SMAD7 [298, 299]. The role of SMAD 6 and SMAD7 is to compete for the binding of receptor-regulated SMAD3, to bind histone deacetylases and inhibit the transcription of TGF-β-responsive genes, and to promote the recruitment of ubiquitin E3 ligases that cause the dissolution of TGF-β receptors [298, 299]. TGF-β1, TGF-β2, and TGF-β3 are the three isoforms of TGF-β that modulate the expression of the matrix metalloproteinase, terminal differentiation to the myofibroblast, and ECM remodeling [300, 301]. In addition, TGF-β1 and TGF-β2 contribute to the stimulation of a profibrotic response after injury [302, 303], while TGF-β3 has an antifibrotic role [304, 305]. Finally, TGF-β3 can stimulate human KC cells to assemble normal stroma [304].

THE ROLE OF LONG NONCODING RNAS IN THE PATHOPHYSIOLOGY OF KERATOCONUS

Long noncoding RNAs (lncRNAs), i.e., RNAs that are at least 200 nucleotides long but do not code for proteins, participate in the complex pathophysiology of KC [306], and there are several lncRNAs that contribute to KC pathogenesis [307]. Recent surveys have accounted for more than 200,000 lncRNA transcripts in humans [308]. These are believed to be powerful regulators of transcriptomes [307], even though their function is not clear [308]. lncRNAs have been found to regulate gene expression in both pathologic and physiologic situations at the transcriptional and post-transcriptional levels [307], contributing to the cellular processes of transcription, translation, protein localization, splicing, imprinting, stem cell pluripotency, cellular structure integrity, migration, oxidative stress response, wound healing, the cell cycle, and apoptosis [309]. lncRNAs are also linked to a variety of human diseases such as cancer [306].

Microarray analyses of RNAs isolated from KC-affected epithelium and keratocytes [307] and RNA-Seq were recently used to study the expression of lncRNAs in KC and to determine the differential expression of RNAs in KC patients and normal subjects [307], as well as to assess the potential roles of lncRNAs in lncRNA–RNA duplexes [307]. These studies highlighted the role of the AQP5, lnc-WNT4-2:1, lnc-ALDH3A2-2:1, SFRP1, and CTGF genes and the WNT, TGF-β and PI3K/AKT pathways in KC [307]. In addition, many coding and noncoding RNAs that potentially contribute to KC have been identified by RNA-Seq-based analyses [307].

The lncRNA–RNA duplexes affect the metabolism of the more than 50,000 transcripts that exist [306]. Bioinformatics analysis yielded 870 lncRNAs, some of which potentially affect genes putatively associated with KC [306]. There are also genes that are differentially expressed under oxidative stress. For example, the expression of lnc-ALDH3A2-2:1 increases more than threefold under oxidative stress. The lnc-ALDH3A2-2:1 sequence overlaps with the last exon of the ALDH3A1 gene and may alter protein levels without affecting mRNA [307].

The WNT signaling pathway is essential for normal corneal development, and variants of the WNT7B and WNT10A genes have been associated with CCT and KC risk [307], while dysregulation of WNT signaling in the corneal epithelium in KC was highlighted by a recent RNA-Seq-based study [307]. Also, the expression of lnc-WNT4-2:1 (a sense transcript for its overlap with exon 5 of the WNT4 gene) is increased in KC patients [307]. Finally, significant changes in WNT4 mRNA of KC corneas apparently eliminate the role of lnc-WNT4-2:1 in regulating the WNT signaling pathway [307].

MICRORNAS AND THE ROLE OF MIRNA 184 IN KERATOCONUS

Gene expression is also regulated by microRNAs (miRNAs), which are single-stranded noncoding RNAs [310]. miRNAs, which mediate mRNA degradation and the suppression of translation, consist of 19–25 nucleotides and bind to the 3′ untranslated regions (UTRs) of mRNAs [229, 311]. Many miRNA mutations can cause disease [114, 312, 313], making them possible therapeutic targets [229, 311, 314, 315]. In addition, miRNAs target the mRNAs from many genes that regulate the abundance of proteins in some organs and tissues [229]. The miRNA that is primarily expressed in the cornea and the lens is miRNA 184 [229, 311, 314, 316], which is localized in the endothelium, the basal and the immediate suprabasal cells of the epithelium of the cornea, but not in the limbus nor the conjunctival epithelia [229, 311, 314, 316]. miRNA 184 competitively inhibits the binding of miR205 to its mRNA [230, 313] so that miR205 cannot encode integrin beta 4 (ITGB4) and inositol polyphosphate like 1 (INPPL1), which it generally can [229, 311].

There are numerous miRNAs in specific chromosomal loci that are related to KC [223, 317]. Their target genes, MBNL (which encodes a C3H-type zinc finger protein that modulates alternative splicing of pre-mRNAs) and ZIC5 (which encodes a member of the ZIC family of C2H2-type zinc finger proteins that acts as a transcriptional repressor), are located on 13q32. MBNL2 (which encodes a C3H-type zinc finger protein that modulates alternative splicing of pre-miRNAs) is targeted by miRNA 548ab and miR5688, while ZIC5 is targeted by miR568 [223, 317]. The chromosome locus of miRNA 548 (which was identified in a white family from western Europe) is 8q13.1–q21.11. Finally, SMAD2 is also considered a target for miR568 [117, 297].

Another characteristic mutation that is found in KC patients is in the 5.5 Mb linkage region of miR184 on 15q25.1 (MIRN184 (MIM 613146)) [229]. This heterozygous mutation c.57 C > U was found in a Northern Irish family who had anterior polar cataract and KC [229]. Also, two KC patients were found to have two heterozygous mutations in the region of MIR184 (+3A > G and +8C > A) [291, 318].

KC mutations are associated with the region within the EDICT interval [119, 319]. EDICT is an autosomal dominant syndrome characterized by congenital cataract, KC with stromal thinning, iris hypoplasia, and endothelial dystrophy [291, 318]. Both the EDICT syndrome

Table 1 Correlation matrix between specific genes implicated in keratoconus and clinical symptoms/signs

GENE Abbreviation	GENE NAME / FUNCTION ↓ CLINICAL SIGN/SYNDROME (see legend) →	12	4	11	15	10	13	5	7	18	9	3	17	14	2	6	16	8	1	
DOCK9	Dedicator Of Cytokinesis 9																■	■	■	3
FNDC3B	Fibronectin Type III Domain Containing 3B										■						■	■	■	4
PRDM5	PR/SET Domain 5											■			■			■	■	4
MPDZ	Multiple PDZ Domain Crumbs Cell Polarity Complex Component													■		■		■	■	5
MIR184	MicroRNA 184		■								■				■			■	■	6
ZNF469	Zinc Finger Protein 469				■			■				■			■			■	■	6
RAB3GAP1	RAB3 GTPase Activating Protein Catalytic Subunit 1							■	■						■			■	■	7
COL6A1	Collagen Type VI Alpha 1 Chain								■	■		■	■	■		■	■	■	■	8
COL8A1	Collagen Type VIII Alpha 2 Chain			■						■			■	■	■	■	■	■	■	8
FOXO1	Forkhead Box O1								■	■	■	■	■	■	■	■	■	■	■	9
COL4A1	Collagen Type IV Alpha 1 Chain	■					■			■			■	■	■	■	■	■	■	9
COL4A3	Collagen Type IV Alpha 3 Chain						■	■	■	■			■	■	■	■	■	■	■	9
COL8A2	Collagen Type VIII Alpha 1 Chain			■	■		■			■	■	■	■	■	■	■	■	■	■	9
CRX	Cone-Rod Homeobox						■		■	■	■		■	■	■	■	■	■	■	9
RXRA	Retinoid X Receptor Alpha					■			■	■	■	■	■	■	■	■	■	■	■	10
CRB1	Crumbs 1, Cell Polarity Complex Component						■			■	■	■	■	■	■	■	■	■	■	11
COL5A1	Collagen Type V Alpha 1 Chain								■	■	■	■	■	■	■	■	■	■	■	11
HGF	Hepatocyte Growth Factor					■		■		■	■	■	■	■	■	■	■	■	■	12
SPARC	Secreted Protein Acidic And Cysteine Rich					■			■	■	■	■	■	■	■	■	■	■	■	12
VSX1	Visual System Homeobox 1		■	■	■		■	■	■		■	■			■	■		■	■	12
LOX	Lysyl Oxidase					■		■	■	■	■	■	■	■	■	■	■	■	■	13
TGFB1	Transforming Growth Factor Beta			■			■	■		■	■	■	■	■	■	■	■	■	■	13
COL1A1	Collagen Type I Alpha 1 Chain				■			■	■	■	■	■	■	■	■	■	■	■	■	14
SOD1	Superoxide Dismutase 1		■			■		■		■	■	■	■	■	■	■	■	■	■	14
	NUMBER OF GENE ASSOCIATIONS WITH EACH CLINICAL SYMPTOM / SIGN →	1	3	4	4	6	8	9	11	12	14	15	15	16	17	18	19	22	24	218
	CLINICAL SYMPTOM / SIGN ID (see legend below) →	12	4	11	15	10	13	5	7	18	9	3	17	14	2	6	16	8	1	

ID	SYMPTOM / SIGN	ID	SYMPTOM / SIGN	ID	SYMPTOM / SIGN
1	Central corneal thinning	7	Corneal (surface) distortion	13	Photophobia
2	Corneal curvature	8	Corneal scarring	14	Visibility of corneal nerves
3	Corneal protrusion	9	Deep stromal scarring	15	Corneal hydrops
4	Corneal steepening	10	Corneal iron deposits	16	Keratinocyte apoptosis
5	Corneal ectasia	11	Corneal guttae	17	Focal fibrosis s
6	Corneal edema	12	Scissoring sign	18	Fine parallel lines/Posterior stroma

Symptoms/signs are represented according to the number of genes they correspond to, in an ascending order from top to bottom. The correspondence between symptoms/signs and their ID in this table is presented in the columns to the left of this legent

and KC are, in some families, caused by the same mutations (c.57C > T) [291, 318]. However, although the mutations in miR184 cause congenital cataract, there are differences in the changes they inflict on the cornea. The same mutation has been found in a family from Galicia in Spain whose main symptoms were severe KC, congenital cataract, and nonectatic corneal thinning [310]. Therefore, mutations in miR184 are associated with KC regardless of the presence or absence of other lens and corneal defects.

MITOCHONDRIAL DNA

Mitochondria play an important role in the cell cycle, cell signaling, differentiation, death, and growth [320], and they are a significant endogenous source of reactive oxygen species (ROS) [321]. Mitochondria carry their own genome; mitochondrial DNA (mtDNA) is inherited maternally and has a characteristic absence of recombination and a high mutation rate. Certain mtDNA polymorphisms may predispose to certain diseases [322]. Such diseases that are focused on the eye include Leber hereditary optic neuropathy (LHON) [323], type 2 diabetes [324] and Wolfram syndrome [325], nonarteritic ischemic optic neuropathy [326], chronic progressive external ophthalmoplegia, and pigmentary retinopathy [327]. Mitochondrial haplogroups H and R have been linked to an increased risk of developing KC in Saudi patients [328].

The involvement of mitochondrial abnormalities in KC is well documented [281, 329, 330]. Compared to control subjects, KC patients have significantly lower levels of leukocyte mtDNA [331]. KC corneas also have a

Table 2 Correlation matrix between genes implicated in keratoconus and different keratoconus-related comorbidities

Comorbidity number	DOCK9	FNDC3B	MIR184	COL8A1	COL6A1	RXRA	VSX1	FOXO1	MPDZ	TGFBI	PRDM5	RAB3GAP1	ZNF469	COL4A3	CRB1	COL4A1	COL8A2	CRX	HGF	SPARC	COL1A1	LOX	COL5A1	SOD1	
1			■																						1
2										■															1
3												■													1
4						■																			1
5																			■						1
6																			■						1
7																						■		■	2
8																			■					■	2
9																	■							■	2
10				■						■															2
11															■			■							2
12							■																■		2
13														■									■		2
14										■				■		■									3
15								■											■		■				3
16																				■	■	■	■		4
17				■			■			■							■								4
18									■						■			■						■	4
19								■				■				■								■	4
20					■											■						■	■		4
21							■		■				■		■			■							5
22													■		■			■		■		■			5
23					■										■			■	■					■	5
24											■		■				■								5
25							■		■							■			■			■	■		7
26								■			■	■				■		■			■			■	7
27						■					■	■	■							■	■			■	7
28							■		■							■		■	■		■			■	7
29											■		■				■			■		■	■	■	8
30								■						■	■	■		■	■	■		■	■	■	8
31							■				■					■	■			■	■	■	■	■	8
32					■						■	■	■				■			■	■	■	■		8
33						■		■												■		■	■	■	9
34					■	■		■		■				■						■		■	■	■	9
35				■		■	■		■						■					■	■			■	9
36								■		■				■		■				■	■	■	■	■	10
37					■						■	■	■				■			■	■	■	■	■	10
38											■	■	■							■	■	■	■	■	13
39			■	■		■		■									■			■	■	■	■	■	14
40			■		■					■	■	■	■	■	■	■	■		■	■	■	■	■	■	15
41	■	■				■				■	■	■	■	■	■		■		■	■	■	■	■	■	15
42		■		■		■		■		■			■	■			■			■	■	■	■	■	15
43	■	■	■			■	■				■	■	■	■	■		■	■	■	■	■	■	■	■	17
44			■	■	■		■	■	■	■		■		■	■	■	■	■	■	■	■	■	■	■	19
45				■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	20
46	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	22
47	■	■		■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	22
48		■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	22
49		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	23
	4	6	8	11	12	13	13	14	14	15	17	17	17	17	17	18	18	19	20	20	22	24	25	30	

The comorbidities have been tabulated from top to bottom according to the number of genes that are implicated in both the comorbidity and Keratoconus. The numbers on the left column correspond to the different Keratoconus related comorbidities as they are listed in Table 3

Table 3 List of keratoconus-related comorbidities

ID#	Disease/syndrome
1	EDICT syndrome
2	Granular corneal dystrophy (GCD)
3	Martsolf syndrome
4	Xeroderma pigmentosum
5	Noonan syndrome
6	Vernal keratoconjunctivitis
7	Pseudoexfoliative glaucoma
8	Spring conjunctivitis
9	Atopy
10	Avellino corneal dystrophy
11	Bardet–Biedl syndrome
12	Turner syndrome (monosomy X)
13	Nail-patella syndrome
14	Ichthyosis
15	Neurofibromatosis
16	Osteogenesis imperfecta
17	Fuchs endothelial corneal dystrophy (FECD)
18	Leber hereditary optic neuropathy (LHON)
19	Warburg Micro syndrome
20	Floppy eyelid syndrome
21	Leber congenital amaurosis (LCA)
22	Pseudoxanthoma elasticum
23	Chronic progressive external ophthalmoplegia
24	Ehlers–Danlos syndrome
25	Nonsyndromic autosomal recessive 2 hydrocephalus
26	Rieger syndrome
27	Atopic disease
28	Congenital communicating hydrocephalus
29	Mitral valve prolapse
30	Pigmentary retinopathy
31	Marfan syndrome
32	Joint hypermobility
33	BCS (brittle cornea syndrome)
34	Focal dermal hypoplasia
35	Retinitis pigmentosa
36	Rheumatoid arthritis
37	Scoliosis
38	Congenital hip dysplasia
39	Age-related macular degeneration
40	Iris hypoplasia
41	Noninflammatory connective tissue disorders
42	Primary open-angle glaucoma
43	Eye rubbing
44	Cataract
45	Posterior polymorphous corneal dystrophy (PPCD)
46	Endothelial dystrophy
47	Connective tissue disorders
48	Down syndrome
49	Glaucoma

Numbers in the left column correspond to the numbering (comorbidity identification number) in the left column of Table 2

lower mtDNA-to-nDNA ratio, as well as a higher level of mtDNA damage when compared to normal corneas [330]. The mitochondria in KC corneal tissues appear swollen under transmission electron microscopy [329].

CONCLUSIONS

The present review focused on the genetic basis of KC and its associations with different comorbidities. Data came primarily from genome-wide association studies, SNP studies, and genetic loci identification. Emphasis was placed on the most definitively implicated genes involved in KC: the VSX1 gene, which is involved in (among other conditions) posterior polymorphous corneal dystrophy; the SOD1 gene, which determines the effects of reactive

oxygen species; the ZNF 469 gene, which is also involved in brittle cornea syndrome; the TGF8 pathway, which is involved in the regulation of the extracellular matrix composition; the TGFI gene, which plays a role in cell–collagen interactions; and the roles of microRNAs (especially miRNA 184), mitochondrial DNA, and reactive oxygen species in KC.

A total of 18 different KC symptoms and clinical signs were identified, documented, and cross-referenced to 24 different genes/genetic loci; each of the symptoms was associated with between 3 and 14 identified KC genes, as presented in Table 1. In addition, 49 diseases/syndromes that involve at least some of the KC-implicated genes were also identified and cross-referenced to the 24 identified KC genes, and each of those 49 diseases/syndromes was associated with between 1 and 23 identified KC genes, as presented in Tables 2 and 3.

The expectation underscoring the present review is that it will promote due vigilance by ophthalmologists to prevent KC, and will encourage medical practitioners to refer KC-related comorbidities to ophthalmologists so that they may prevent the further development of KC.

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